ABSTRACT

DOUNGPET, MAYUREE. Environment and genetic effects on wood quality of *Populus*. (Under the direction of Dr. Ilona Peszlen)

The purpose of the research was to investigate wood properties of new poplar clones from three different environments presented in three studies as follows: (1) Impacts of the cottonwood leaf beetle (*Chrysomela scripta*) on wood properties; (2) Relationship between growth rate and wood properties in *Populus deltoides* grown under stressful site conditions; (3) Variation in wood properties of *Populus deltoides* clones under effects of growth rate and specific gravity. Specific gravity, density, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number have been investigated. Quantitative wood anatomy was conducted using an image analyzer system; meanwhile, density was measured by X-ray densitometer.

The **first study focused** on insect defoliation effect on wood properties of the two clones at six years of age with particular genetic backgrounds. The results indicated that insect defoliation caused moderate decreases in annual **growth** over the first three growing seasons for Clone NM2 and large decreases for Clone 91X. Clone NM2 had significantly higher **specific gravity** and density than Clone 91X only for the protected trees. **Density** of the wood was decreased in the first ring of unprotected Clone 91X, but otherwise, both clones showed a relative constant average wood density over the first six years regardless of beetle attack. There was no evidence that defoliation significantly affected **fiber lengths** of both clones. V**essel number** and **vessel diameter** were impacted by beetle damage and offsetting changes in vessel numbers and diameters partially cancel out changes in **vessel area**. **Ray area** was larger in both clones in

unprotected trees; Clone NM2 responded to insect attack by producing more rays; meanwhile, Clone 91X responded by producing fewer but larger rays to give an overall increase in ray area. Sample trees with high density had high vessel number and ray area and trees with high stem radius had long fiber, larger vessel diameter, but lower ray area.

The **second study dealt with** wood properties of eight six-year-old clones of two families grown under the stressful site conditions. There were no statistical differences in growth rate between two families; however, specific gravity was significantly influenced by family and by clone. Negative correlation existed between growth rate and specific gravity. Variation in fiber length was not affected by family but only by clone and position. At the second radial position (4th or 5th growth rings), clonal averages of fiber length were different suggesting that the earliest selection for fiber length should start after four or five years. Hence, it should be relatively easy to select within families for both growth rate and longer fibers.

The **third study analyzed** wood variation for four-year-old trees with exceptional specific gravity and growth rate values. There were significant differences among trees in fiber length and vessel area but not in vessel numbers, vessel diameter, and in ray numbers. The age of the cambium significantly affected all wood properties. Longer fibers tend to be associated with the faster growing trees. The fastest growing tree with high specific gravity had the longest fibers, the highest ray numbers but low vessel area, vessel number, and low vessel diameter representing an unusual combination of traits. Results indicate that trees with similar growth rates can have wide variation in vessel area and that exceptionally fast growth can be achieved by a tree with low vessel area.

ENVIRONMENT AND GENETIC EFFECTS ON WOOD QUALITY OF *POPULUS*

By

MAYUREE DOUNGPET

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DEDICATION

To My Parents, **Mr. PANG and Mrs. WIBUL DOUNGPET**, For

Their Love, Care, and Encouragement

BIOGRAPHY

The author, MAYUREE DOUNGPET, was born in Udonthani, Thailand, in February the year nineteen hundred and sixty- one. Entered elementary schools in Udonthani, and graduated from Trium Udom Suksa High School in Bangkok. She graduated with a Bachelor degree in forest products from Kasetsart University, Bangkok, Thailand in 1982. Later, she joined the Royal Forest Department and gained extensive experience in wood products and non-wood products. She pursued her graduate study at University Putra Malaysia in Selangor, Malaysia, supported by the Asian Timber Technology Center and graduated with a Master's degree in Wood Industry Technology in 1991. Subsequently, during 1993-1994, she spent two years as a graduate student at University of Gottingen, Germany. In 1997, she accepted a position as a lecturer in the Department of Forest Products at Kasetsart University, Bangkok. She enrolled as a graduated student in the wood science program at Iowa State University in the spring of 2000. In January, 2002, she transferred to pursue a Ph.D. degree in wood products at North Carolina State University.

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CHAPTER 1

INTRODUCTION

The environments probably influence variation of wood quality within a species. Several clones of hybrid poplar have been grown and distributed throughout the United States. Hybrid poplars have gained interest because they can be cultivated by both vegetative propagation and sexual reproduction, are fast growing, and can produce a relatively good stem form. Much research has been done in the interest of efficient use of hybrid poplar (*Populus spp*.) in the future.

There is a variety of hybridization and breeding programs that have been reported, the purpose of which is to improve wood quality. Early trait selection strategies include producing fast-growing, pest- resistant, and disease-resistant trees (Riemenschineider et al., 2001). Wood quality is presently determined when trees reach a commercial size. Recently, there have been many efforts to evaluate wood quality at the early age of trees. Wood properties are significant considerations in the final product quality.

Many clones are produced through breeding and selection. Wood properties of these clones need to be evaluated, in order to be the reference information for genetic selection, plantation management, and for wood products industry selection. The most important strategy is one that would allow us to know how well the properties of young wood can be used to estimate the properties of the mature wood (Zobel and Sprague, 1998).

Some discussions of wood qualities of mature poplars have been reported (Zeng et al., 1985; Ifju, 1991; and Peter et al., 2002), and some studies of young age poplar have been discussed (Chauhan et al., 2001; Matyas. and Peszlen, 1997; and Winistorfer, 1985). Many wood scientists use the terms "wood property" and "wood quality" interchangeably, but in this paper, they will have specific meanings. The term "wood property" refers to the cellular, anatomical, and chemical characteristics of wood within and among trees. The term "wood quality" refers to the cumulative effect of the wood properties on a specified product or products (Zobel and Buijitenen, 1989). Many researchers have reported on growth rates of poplars of various clones and ages (DeBell et al., 1998; DeBell et al., 2002; and Coyle et al., 2002). A few studies reported the relationship between growth rates and fiber length and density, but only very weak evidence was reported.

Primarily, poplar clones have been aimed for the raw material of the pulp and paper industry. However, another opportunity for the hybrid poplars is the solid wood market (Dickmann, 2001). The wood of hybrid poplar is successfully used in a wide range of wood products such as veneer, low grade lumber, pallets, formwork, wood composite, and fuel (Peter et al., 2002 and Phelps et al., 1985). Kretschmann et al. reported that poplar wood (Wisconsin-5) has specific gravity between 0.39 and 0.41. and it is suitable for light framing applications such as wall studs.

Peszlen and Molnar (1996) investigated three different clones of 10 to 15 years old poplars and reported that wood properties had no consistent and significant relationship with growth rate but age caused variation in wood quality. Mechanical properties were also tested and based on the results, these poplars were not recommended for solid wood structural applications.

There are at least five major *Populus* species that are indigenous to North America: one of them is *Populus deltoides*. This study concentrated on *P. deltoides and one hybrid clone (P. nigra x P. maximowczii)* from the Biofuels Development Program conducted in Iowa. Specific gravity, density, fiber length, area and size of vessels, as well as rays were measured along with growth rate.

This study investigated the wood properties of four-and six-year old poplar to clarify problems of these three categories:

1. Impact of the cottonwood leaf beetle (*Chrysomela scripta*) on wood properties.
2. Relationship between growth rate and wood properties in *Populus deltoides.*
3. Variation in wood properties of *Populus deltoides* clones in relationship to growth rate and specific gravity.

LITERATURE REVIEW

Effect of Defoliation on Wood Properties

An eight-year study of the impacts of the cottonwood leaf beetle (*Chrysomela scripta*) on the growth and yield of diverse *Populus* clones is in progress (Coyle et al., 2002). Two clones from that study were sampled for investigation of wood properties in this dissertation research. Clone 91x04-03 (Clone 91X) is one of the best clones in term of growth potential and originated from the Iowa State University breeding program. Clone 91X was also known to be very susceptible to *C. scripta* attack. Clone NM2 *(P. nigra x P. maximowczii)* is also a fast growing clone, but is less susceptible to leaf beetle attack.

In one study, tree height and diameter of three- year old of these two clones exposed to and protected from beetle attack has been reported (Coyle et al., 2002). Trees from the applied insecticide plot (or protected trees) grew significantly more in height and tree diameter. A biorational pesticide (*Bacillus thuringiensis* formulation) was used in the protected plot. Clone 91X had bigger diameter and higher stem volume than Clone NM2. However, Clone NM2 grew taller than Clone 91X. It was reported that *C. scripta* defoliation significantly influenced tree growth (Coyle et al., 2002).

In other studies, height, stem diameter, ring width, specific gravity, density, and fiber length of poplars were reported. However, the trees studied were of different ages, clones, sites (DeBell et al., 2002; Koubaa et al., 1998; Jourez et al., 2001; Phelps et al., 1984; and Zhang et al., 2003), and grown under different silvicultural and management systems (Blankenhorn et al., 1985). There has not been any report on wood properties of poplars after certain periods of defoliation.

Annual increment of three-year old *Betula pendula* Roth clones was studied for 75 to 100% defoliation in the first year. Only 100% defoliation significantly influenced annual increment reduction (Anttonen et al., 2002). Rook and Whyte (1976) studied the growth of five-year old radiata pine (*Pinus radiata* D. Don) trees after different degrees of artificial defoliation. Growth was measured up to two years after defoliation. There were significant differences of basal area increment or diameter growth between defoliated trees and controlled trees. Over two year results, trees with new leaves (new buds and up to three-year-old-leaves) defoliated were significantly lower (40 to 70%) in growth than the control trees. The most severe was 70% reduction of diameter growth from new bud and one year old leaf defoliation. No significant differences were found between control trees and trees with two- to four-year-old leaves removed.

In another investigation, the effect of defoliation of specific age leaves on five-year-old trees of *Pinus radiata* were also reported (Cown, 1979). Four treatments were performed; they were control (no defoliation), removal of new leaves, removal of one-year-old leaves, and removal of second- and third-year-old leaves. Ring width, wood density and tracheid length were measured after the following two years. Removal of the new leaves resulted in significant reductions in diameter growth. On the other hand, this treatment increased wood density and tracheid length. Removal of the first-year-old leaves gave almost as a severe reduction in diameter growth as removal of the new leaves.

Growth rate, tracheid length, and wood density of nine-year-old *Pinus radiata* were studied after spraying fungicides to control fungus (*Dothistroma pini*) infection (Harris and McConchie, 1978). Growth rate was in diameter; density was measured by densitometer, and latewood tracheid length was measured on maceration. Results of this study on 10 sample trees did not give enough evidence to make any conclusion.

Ericsson et al., (1980) studied the effects of defoliation on 15 to 20 years old Scots pines when the youngest leaves and the one-year and two-year leaves were removed. Ring width of the stem was significantly and negatively affected by defoliation. The most severe growth effects were found with the one-year and the two-year leave removal. However, the effect of the youngest and the one-year leave removal in late summer were also severe.

The effect of defoliation on wood anatomy and on wood formation of poplars has not been investigated, no information on this topic is available for plant breeding and wood utilization.

Growth Rate, Specific Gravity and Density

Growth rate is included as an important trait in selection for economic improvement. Growth rate can be expressed as stem radius, stem diameter, stem height, stem volume, and growth ring width. Specific gravity is measured by dividing the ovendry weight by the volume of wood at a specific moisture content and by the density of water at four degrees Celsius (Hoadley, 2000). In practice, specific gravity at green can be measured by dividing the ovendry weight by volume of the green wood and density of water at room temperature. Density of water at room temperature (20 ºC) is 0.99823 gram per cubic centimeters (g/cm^3). The density of wood is determined by dividing its mass by its volume, at the same moisture content. It is an important index for mechanical wood properties, strength and hardness (Hoadley, 2000).

The issue of the extent to which the growth rate influences wood density in poplars is controversial. Growth rate of nine-year-old poplars had some influence on the wood density at the breast height: there was a negative correlation (r^2= -0.46) between the growth rate and the wood density (Beaudoin et al., 1992). There was no pruning effect on the ring width and wood density at the significance level of 0.10. (DeBell et al., 2002)

Blankenhorn et al. (1992) studied specific gravity of one four-year-old poplar clone (Hybrid NE-388) at two different sites. Fertilization and site had significantly affected the specific gravity. Specific gravities were found to be higher under irrigation without fertilization. In a European study of hybrid clones, specific gravity was not affected by site, but it showed significant differences between clones (Peszlen and Molnar, 1996). No consistent trends of radial specific gravity pattern were exhibited within the species *P. deltoides* (Zobel and Sprague, 1998).

The radial variation of diffuse porous hardwoods exhibited different patterns compared to softwood density. The radial variation of density for Scots pine continuously decreased during the first 7 years, then increased outwards. Density is higher in slow-growing Scots pine trees (Lundquist, 2001). The wood density of nine-year-old poplar clones seemed constant for the first three years (0.37), to be followed by dropping values during the fourth and fifth year, and then slowly increased, all the way up to 0.43 at the ninth year (Debell et al., 2002).

Radial variation of specific gravity pattern for 15-year-old poplar clones has been reported by Peszlen, 1994). Three clones, ‘Koltay’, ‘Kopecky’ and ‘I-214’ showed different radial patterns. Specific gravity of ‘Koltay’ was the highest in the first year (0.39), then decreased to 0.35 in the third year, followed by a constant value to the year 15. ‘Kopecky’ had a pattern with higher fluctuation values such as the highest specific gravity was measured at the first year (0.38), it further decreased to the fifth year (0.31), then it abruptly increased in the sixth year (0.34), followed by a slow increase to the year 15 (0.37). ‘I-214’ had the lowest specific gravity, it increased from the first year (0.32) to the highest value at the second year (0.33), then leveled off to 0.29 in the third year before slightly varying to 0.30 in the fifteenth year.

Gartner et al., (1997) reported a slightly different pattern of wood density for a 40-year-old *Alnus rubra,* another diffuse porous species, where specific gravity did not change significantly with cambial age. Specific gravity was 0.48 in the first year, and then it continuously decreased to 0.46 in the fifth year, and remained constant at o.46 until the tenth year. After the first ten years, the specific gravity increased to 0.47, and slightly fluctuated towards the 35th year of cambial age. The specific gravity of wood near the first branch is slightly higher with a similar radial pattern

Lei et al., 1996) investigated ring porous specific gravity pattern from an 80-year-old white oak. Specific gravity was the highest in the first year (0.88) and continuously decreased to 0.65 in the 50th year. Information concerning changes after the 50th year has not been reported. Specific gravity at 4.4 m had a similar radial pattern but about 10% lower than the value measured at the breast height.

Fiber Length

Information on fiber length is important for several applications. Strength of wood and wood products are related to fiber length. Geyer et al. (2000) reported that fiber lengths of eleven four-year-old poplar clones ranged from 0.76 mm to 0.87 mm and the average fiber length was 0.84 mm. The individual fibers from maceration were measured on 20 slides. The study showed no significant difference in fiber length by clones. It was also found that fiber lengths of branches were longer than the breast height fiber lengths.

DeBell et al. (1998) reported that the growth rate of seven-year-old hybrid poplars had no consistent influence on fiber length within rings of the same age for ring two to ring six. However, there was a positive correlation between growth rate and fiber length for ring seven.

Wood samples of four-year-old poplar clones from 2.5 x 2.5 m plantation had mean fiber length of 0.86 mm and heritability of fiber length was 0.611 (Klasnja et al., 2003). DeBell et al. (2002) reported a larger variation in fiber lengths. Fiber length was the shortest in the first year and ranged from 0.52 mm to 0.63 mm, and it increased continuously through age nine to almost 1.00 mm. In addition, a negative correlation between wood density and fiber length was found within rings. Growth rate may also affected fiber length to some extend.

Fiber length was longer for nine-year-old (1.08 mm), 10-year-old (1.03 mm) and 15-year-old (1.10 mm) clones (Klasnja et al., 2001, Peszlen and Molnar, 1996). Similar results of three different poplar clones showed the shortest fiber length in the first year, rapidly increasing from the second year to the fourth year followed by a slow increase to about 1.0 mm in the ninth year (DeBell et al., 2002). There was no pruning effect on fiber length of wood samples at the significance level of 0.10.

Koubaa et al. (1998) reported a similar fiber length pattern from pith to bark for nine-year-old hybrid poplars. The fiber length increased from 0.7 mm in second growth ring to 1.1 mm in the eighth growth ring. They also found that clones, heights, and position from pith to bark had significant effect on the fiber length. The correlation between ring width and fiber length was not significant at an early age but it was slightly negative at an older age. Huang and Furukawa (2000) found that two different clones had similar fiber length patterns. Fiber length was the shortest in the first growth ring (0.42 mm), increased substantially to the seventh growth ring (0.95 mm), and followed by slight fluctuation toward the bark.

Anatomical Characteristics

Poplar is a hardwood species with diffuse-porous to semi diffuse-porous. Poplar wood is relatively soft and light color with low to moderately low specific gravity. Cross sections of vessel elements or pores are numerous and relatively uniformly distributed. Pores are very small in diameter, hardly visible to the naked eye, solitary mixed with multiples, and commonly found in radial multiples. Terminal parenchyma can be seen as a fine light yellow line along the growth ring boundary of un-dyed wood. Rays are very delicate line and hardly seen with hand lens (Hoadley, 2000). Vessel lumen diameters or vessel diameters are large in the earlywood and found decreasing close to the end of the latewood.

Formation of anatomical elements has also been studied for poplars. Chaffey et al. (2002) studied the development processes of fibers, vessels, and rays in 2-year-old hybrid aspen trees. Suzuki et al. (1996) investigated the first vessel element formation. In diffuse porous species, vessel elements were initiated two to seven weeks after the beginning of the leaf expansion. Meanwhile, in ring porous species, the first vessel elements formed two to six weeks after the beginning of the leaf expansion. In addition, secondary wall deposition of the diffuse porous species was completed within four to nine weeks after the leaf expansion. On the other hand, for ring porous species secondary wall deposition could be completed within one week before the leaf expansion to three weeks after the leaf expansion. The results indicated that the diffuse porous species had no new functional vessels at the time of the leaf expansion. The sequence of element formation made a special pattern in diffuse porous species.

Geometry of tracheid in a cross section of spruce was found to be controlled by the growth rate of the trees. The dimension of tracheid within a ring could possibly be predicted by the distance from the pith of the tree (Sirvio and Karenlampi, 2001). Few studies have been done on geometry of poplar anatomical structure (Lourez, 2001; and Peszlen, 1994).

Peszlen and Molnar (1996) investigated the fraction of vessel lumen, fiber lumen, cell wall and ray area on cross section. They found a slight change in radial distribution of these anatomical elements. Cell wall area was lower than one-third (27.4%) of the total cross section area, vessel and fiber lumen areas were found higher by 8% near the bark. Vessel lumen diameter and fiber diameter were 75.5µm and 15.2µm, respectively.

Peszlen (1994) studied vessel lumen diameter for three different clones from pith to bark. Vessel lumen diameters were small at the first year (55- 65µm), increased rapidly to the 8th year, followed by a relatively constant value toward the bark. Lei et al. (1996) found a similar vessel diameter pattern for white oak. Diameters of vessels were the smallest in the 1st year (160 µm) and rapidly changed to 250µm to 15th year, followed by a small variation toward the bark.

Anttonen et al. (2002) investigated the wood structure of three-year old *Betula pendula* Roth clones. Average vessel area was 15% and vessel lumen diameter was significantly larger by increasing age. It was reported that trees with 100% leaf defoliation had smaller vessel area (15.3%) than trees without leaf defoliation (16%) for non-fertilized area. In addition, trees with 100% leaf defoliation had larger vessel lumen diameter (32µm) than trees without leaf defoliation (34 µm) for non-fertilized area. The results of vessel lumen diameter and vessel area were not consistent on the fertilized area.

Density by X-ray Densitometry

Measurements of wood density by x-ray densitometry has been reviewed by Polge (1978) who stated that density profile data made it possible to assess wood structure, anatomy, physiology, and effects of genetic and environmental factors on wood properties more rapidly. Ellis (1971) investigated *Pinus radiata, Pinus ponderosa*, and *Pseudotsuga menziesii* by x-ray (25 kV, 300 mA, 1.2 second) to determine the contrast between earlywood and latewood in a wood core with a thickness of 5.1 mm. Though the results were not as great as expected, it was concluded that ring width measurement was more accurate and easier than using conventional methods.

Cown and Ball (2001) studied the influence of tree age, site, and genotype of 22-year old *Pinus radiata*. A total of 770 increment cores of 10 mm were collected at breast height from eight stems per each of 10 families from seven sites. Density was measured by x-ray densitometric procedure according to the method of Cown and Clement (1983). Wood density increased from pith to bark and showed significant differences by site. Heritability of ring density was report at 0.60. In another study, Bergsten et al. (2001) investigated the density of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst) by an x-ray densitometer (Woodtrax technique - 40 kV and 35 mA). Results of increment cores were significant higher than rectangular specimens at 0.05 significant level.

Density of 14-year old radiata pine was studied by indirect-reading x-ray densitometry (Zamudio et al., 2002). The x-ray images were further processed by the WinDendro software and average ring density was calculated. Density values were fluctuating from the second ring to the ninth ring and increased from the tenth ring toward the bark. Genetic control of ring density was strong at early ages such as at 2nd and 3rd rings and at 12th ring. No consistent genetic control was shown at other rings. Phenotypic effects were believed greatly dominated by local site competition for light and nutrients. There were no reports found on density of poplars measured by x-ray densitometry.

X-ray densitometry measures density of a sample based on the mass attenuation coefficient (μ_m) of x-ray. The mass attenuation coefficient is calculated based on the intensity of the initial x-ray beam and the intensity of the x-ray detected after the waves passed through the sample. Sample density can be evaluated from the sample attenuation coefficient. Tree rings can be analyzed based on the changing density regions.

Density of wood is calculated based on radiation effects. The two following equations are used to calculate the density of materials.

$$\frac{I_1}{I_2} = \exp(-\mu x) \qquad \text{(Equation 1)}$$

$$\mu_m = \frac{\mu}{\rho} \qquad \text{(Equation 2)}$$

where:

I_1 = initial x-ray intensity

I_2 = x-ray intensity at the reciever

$-\mu$ = the absorption coefficient (cm^2)

x = the thickness of material (mm)

μ_m = the the mass absorption coefficient (cm^2/g)

ρ = the density of material (g/cm^3)

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CHAPTER 2

RESEARCH OBJECTIVES

Trees with different genetic background exhibit clear differences when grown under the same site conditions (DeBell et al., 2002). Researchers are interested in the important purposes of improving wood quality and discovering the early trait selection for fast- growing, pest-resistant, and disease-resistant trees (Riemenschineider et al., 2001). Moreover, it is a great challenge to find out how well the properties of young wood can be used to estimate the wood properties of the mature wood (Zobel and Sprague, 1998). Some discussions of wood qualities of mature poplars have been reported (Zeng et al., 1985; Ifju, 1991; and Peter et al., 2002) and some studies of young age poplars have been discussed (Chauhan et al., 2002; Hall, 2001; Matyas and Peszlen, 1997; Winistorfer, 1985; and Zobel and Buijitenen, 1989). Many researchers have reported on growth rates of poplars of various clones and ages (Beaudoin et al., 1992; Coyle et al., 2001; Koubaa et al., 1998; and Zhang et al., 2003). Among them, a few studies have dealt with the correlation between growth rates and fiber length and found only weak correlations or no correlations at all (DeBell et al., 2002; Koubaa et al., 1998; and Zhang et al., 2003). Wood properties had no consistent and significant relationship with growth rate but age caused significant variation in wood quality (Peszlen and Molnar, 1996).

The overall goal of this study is to determine the influences of specific environmental factors and particular genetic backgrounds on wood properties of poplars. There is not enough evidence of impact of defoliation and neighboring competition on wood properties to guide the breeding and selection of new clones. Hence, this study investigated the wood properties of four- and six-year-old poplars to clarify problems in these three categories:

1. Impacts of the cottonwood leaf beetle (*Chrysomela scripta*) on wood properties.
2. Relationship between growth rate and wood properties in *Populus deltoides* grown under stressful site conditions.
3. Variation in wood properties of *Populus deltoides* clones under effects of growth rate and specific gravity.

Three separated situations were investigated based on samples from three different environments as follows:

1. Impacts of the cottonwood leaf beetle (*Chrysomela scripta*) on wood properties. The study design was a split plot with four clones of poplar with two different treatments: protected (applied insecticide in the plot) and unprotected (cottonwood leaf beetle were allowed to naturally feed in the plot). The study focused on two clones at six years of age from this study site. The hypothesis of this study was to test if wood properties of poplar clones were influenced by insect defoliation.

The objectives of this study were as the follows:

- Determine the effect of treatment within clones for protected and unprotected (control) samples on physical and anatomical properties: specific gravity, density, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number.
- Investigate within tree variation on wood properties (vessel area, vessel diameter, vessel number, fiber length) in relation to density and growth rate.
- Analyze the correlation between physical and anatomical properties.

` **3. Relationship between growth rate and wood properties in *Populus deltoides* grown under stressful site conditions.** Wood samples for this study were taken from six-year-old trees. A total of 25 ramets were collected from a clonal test and archive plot in Iowa (randomized design). The clones were derived from four seedlings in each of two families. The null hypothesis for this study was: there is no difference in wood properties between two families and within family growing under stressful site condition.

The objectives of this study were as follows:

- Investigate the effect of growth on specific gravity and fiber length of eight clones representing two families.
- Study within-tree radial variation of growth rate and fiber length.
- Determine correlation among wood properties.

3. Variation in wood properties of *Populus deltoides* clones under effects of growth rate and specific gravity. Five four-year-old trees were selected due to their extreme low and high specific gravity values. Two trees were from the same family, while the other three trees were from different families. One tree had a high growth rate (diameter inside bark), the other four trees had medium growth rates. The null hypothesis of this study was: there is no difference in wood formation of poplar with different growth rate and specific gravity.

The objectives of this study were as the follows:

- Study variation in anatomical properties of trees with different specific gravity but have the same growth rate, and of trees with different specific gravity and growth rate.
- Determine radial variation in anatomical properties (fiber length and vessel area, vessel number, vessel diameter) in relation to age.
- Compare wood properties of trees with similar growth rate to the high growth rate and high specific gravity.

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CHAPTER 3

IMPACTS OF THE COTTONWOOD LEAF BEETLE (*Chrysomela scripta*) ON WOOD PROPERTIES

Abstract

Wood samples were collected from two six-year-old *Populus* clones. Clone 91x04-03 (Clone 91X) had greater growth potential but is more susceptible to insect attack than Clone NM2. Half of the sixteen sample trees were exposed to natural insect defoliations (unprotected), the other half came from a plot protected by biorational insecticide spray (protected) over the first three growing seasons. Two sets of sixteen increment cores were used to investigate specific gravity, density, growth rate, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number.

Insect defoliation caused moderate decreases in annual **growth** over the first three growing seasons for Clone NM2 and large decreases for Clone 91X. Clone NM2 had significantly higher **specific gravity** and density than Clone 91X only for the protected trees. **Density** of the wood was decreased in the first ring of unprotected Clone 91X, but otherwise, both clones showed a relative constant average wood density over the first six years regardless of beetle attack. There was no evidence that defoliation significantly affected **fiber lengths** of both clones. V**essel number** and **vessel diameter** were impacted by beetle damage and offsetting changes in vessel numbers and diameters partially cancel out changes in **vessel area**. **Ray area** was larger in both clones in unprotected trees; Clone NM2 responded to insect attack by producing more rays;

meanwhile, Clone 91X responded by producing fewer but larger rays to give an overall increase in ray area.

Age effect of cambium strongly influenced the anatomical properties. Vessel area, vessel diameter, and fiber length had increasing patters; meanwhile, vessel numbers, ray numbers, and ray area showed decreasing patterns along the radius both clones.

Based on correlation analyses, sample trees with high density had high vessel number and ray area and trees with high stem radius had long fiber, larger vessel diameter, but lower ray area. However, these results may be due to the fact that all anatomical properties showed the highest variation along the radius and had increasing.

Keywords: *Populus deltoides*, poplar clones, insect defoliation, wood properties, specific gravity, density, growth rate, fiber length, vessel area, vessel number, vessel diameter, ray area, ray number, radial variation, and wood properties correlation.

INTRODUCTION

Environmental and genetic effects of wood quality have been extensively studied for years. Height, diameter, ring width of poplars were reported as growth rates by many research groups. A few reports have involved the relationship between growth rate and other wood properties such as fiber length and density, specific gravity, and strength of wood. Very few papers have presented on wood quality in terms of anatomical structures affected by environment and genetic factors.

In a study of six-month shooting of poplar, vessel elements, fibers, and rays showed very high variation (Jourez et al., 2001). The results from the older stems are important to compare with for the reliable wood quality information. Growth rate may increase without the changing of specific gravity and anatomical properties of poplar clones. However, the age of the cambium has more influence on wood properties. More variations in wood properties were commonly observed within a tree than there was variation among clones (Peszlen, 1994).

Technically, a poplar hybrid is a cross between two species or different geographic populations within species. Poplars have been grown in essentially all regions of the United States with an emphasis on the Pacific Northwest, Upper Midwest and Mississippi River valley. Fast growth and easy vegetative propagation are general characteristics of poplar clones. They had become one of the most sustainable resources for wood industries (Eckenwalder, 2001 and Riemenschneider et al., 2001,). Due to the utilization potential of poplars, many breeding programs have invested extensive efforts in developing new clones. The poplar hybrids are genetically improved by many research groups. The two sample clones in this study are part of a set of several hundreds clones that have been tested in the portion of the Biofuels Development Program conducted in Iowa ((Ferrell et al., 1995, Riemenschneider et al., 2001 and Eckenwalder, 2001). One clone is of great potential in growth rate but susceptible to insect infestation, and the other clone is moderately fast-growing and more resistant to insect attack. Defoliation by insects is an environment factor, and the competition from neighboring trees is another environment factor. The hypothesis of this study was to test if wood properties of poplar clones were influenced by insect defoliation.

Objectives of the study are as follows:

1. Determine the effect of treatment within clones for protected and unprotected (control) samples on physical and anatomical properties: specific gravity, density, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number.
2. Investigate within tree variation on wood properties (vessel area, vessel diameter, vessel number, fiber length) in relation to density and growth rate.
3. Analyze the correlation between physical and anatomical properties.

MATERIALS AND METHODS

MATERIALS

Wood samples for this study came from two poplar clones being grown in a test of the impacts of defoliation by the cottonwood leaf beetle (*C. scripta*) on stem growth. Clone 91x04-03 (Clone 91X) was selected from a controlled cross of two southern Illinois parents. It was the fastest growing *P. deltoides* clone in Iowa trials available at the time the impact study was established in 1998 (Coyle et al., 2002). As for all *P. deltoides* clones tested so far, it is highly susceptible to attack by the cottonwood leaf beetle. Clone NM2 is a sibling of the more widely known clone NM6 developed from a controlled cross of *P. nigra* and *P. maximowiczii* in Germany (Eckenwalder, 2001). It is moderately fast growing, but more resistant to cottonwood leaf beetle attack. The planting is on a bottomland site near the Iowa State University campus. A split-plot design was used with two treatments: spraying as necessary with biorational insecticides to control defoliation and control (natural levels of infestation and defoliation). Clones were randomly assigned to positions within each block of the split plots, a clone plot consisted of a 4 x 4 planting of trees at 2 m spacing within a row and 2.5 m between rows. All blocks were surrounded by one row of buffer trees (Hall et al., 2001).

The samples were selected from 16 trees and from two different clones: Clone NM2 and Clone 91X. Eight trees of two clones were grown under the insect treated plot. Pesticide sprayings were applied when beetles appeared during the first three years of growing. The other eight trees of both clones were grown under the control plot. Wood samples were 1.2 centimeter in diameter of increment cores, extracted at the breast height level from each tree. The increment cores were cut, then carefully packed and sent from Iowa State University, Ames to North Carolina State University, Raleigh, for wood properties evaluation.

There were two sets of sample cores from each tree. The first set was used for measurements of specific gravity and density profiles. The second set was used for measurements of growth rate, fiber length, and anatomical structures.

METHODS

Wood properties were measured in these categories:

- Specific Gravity by the water displacement method.
- Density by x-ray densitometry. A Density Quintek Measurement Systems (QMS) Tree Ring Analyzer (model: QTRS-01X Tree Ring Analyzer).
- Growth rate by a digital caliper, under stereoscope.
- Fiber length by the image analyzer. The image analyzer system was composed of a light microscope (Nikon model SMZ800), a stereoscope (Nikon model E200), a three charge-coupled device (3CCD) color video camera (Sony model number DXC-390), a digital camera (Nikon model Coolpix 995), and the Image Pro Plus 4.5 software.

- Vessel area, vessel number, vessel diameter, ray area, and ray number by image analyzer.

Specific Gravity

Specific gravity is measured by dividing the oven dried weight by the volume of wood, at a specific moisture content and by the density of water at four degrees Celsius. In practice, specific gravity at green can be measured by dividing the oven-dried weight by volume of the green wood and density of water at room temperature. Density of water at room temperature (20 ºC) is 0.99823 g/ml.

Increment cores, or parts of wood disks, were in green condition during the green volume measurement. The sample was first soaked/dipped in the deionized water for six hours, then the sample while soaked in water was placed under vacuum for at least four hours, before the saturated sample weight was measured.

Measurement of specific gravity

Equipment: a digital balance (model number: Mettler Toledo AB204-S), a 100 ml glass cylinder, a stand, a dissection pointer, and deionized water.

Procedure:

1. A cylinder with 75 ml deionized water was placed on the balance and then the balance was set at zero.
2. The wood sample was placed at green condition under the deionized water in the cylinder, with help of dissection pointer affixed to the stand.

3. The weight was recorded in grams, to four decimal digits, which is the volume of sample displaced in the deionized water (V_{green}).
4. The sample was taken out of the deionized water and placed at room temperature for six hours. The sample was then placed in the oven at 103 ± 2 ºC, until the constant oven-dried weight (OD) was obtained, after letting it cool in the desiccator.
5. The sample was placed at room condition for 72 hours, then the weight of sample at room condition was recorded in grams.
6. Specific gravity at green condition and moisture content at room condition were calculated.

To calculate the specific gravity (SG_{green}), the following equation was used:

$$SG_{green} = \frac{OD}{V_{green}\rho_{water}} \qquad \text{(Equation 1)}$$

where:

OD = oven dried weight in gram

V_{green} = sample volume at green in cubic centimeter

ρ_{water} = density of water in gram per cubic centimeter

To calculate the percent of moisture content (MC), the following equation was used:

$$MC(\%) = \frac{AD - OD}{OD}(100) \qquad \text{(Equation 2)}$$

Where:

AD = sample weight at room condition in gram

To calculate the specific gravity at the moisture content at room condition, the following equation was used:

$$SG_{MC_{RT}} = \frac{SG_{green}}{1+0.01\left(SG_{green}\right)\left(MC_{RT}-MC_{FSP}\right)} \qquad \text{(Equation 3)}$$

where:

MC_{RT} = the moisture content at room condition

MC_{FSP} = the moisture content at the fiber saturation point (FSP) which is approximately around 30% for wood.

Density

In this study, the radial density profile was evaluated by using an X-ray densitometer. The radial sample with 2mm thickness was measured by a Quintek Measurement Systems (QMS) Tree Ring Analyzer (model: QTRS-01X Tree Ring Analyzer). The QTRS-01X Tree Ring Analyzer measures density of a sample, based on the mass attenuation coefficient (μ_m) of X-ray. The initial x-ray waves pass through the sample, the receptor detects the intensity of the x-ray. Then, the mass attenuation coefficient is calculated, based on the intensity of the x-ray beam, after passing through the sample. Then, the sample density can be evaluated from the sample attenuation coefficient. The tree ring is analyzed, based on the change of the density regions.

In order to measure the sample density by the x-ray densitometer, it was necessary for the wood sample to be prepared as the follows:

Sample preparation: The air-dried sample was glued between two wood sticks to secure the sample so it could be cut properly, while the sample was cut into 2 mm thickness by a circular saw.

1. The increment core between two wood sticks was glued with urethane resin.
2. The resin was allowed to cure for at least 6 hours
3. The sample was cut along the radial axis, to obtain the 2 mm thickness and marked to notice the bark side and the pith side.

Measurement of density

Equipment: the QMS X-ray densitometer (model QTRX-01X), a digital caliper (model number: Mitutoyo CD-6"C)

Procedure:

1. The thickness of the sample was measured at three different positions. The average thickness of three values was used in running the densitometer at 8.7% moisture content.
2. Calibration the QMS X-ray densitometer was done by running all 16 samples, in order to get the mass absorption coefficient (μ_m). In order to run the QMS X-ray densitometer, some values need to be known such as wood moisture content, dimensions of wood samples, age of wood samples which was six years of age, and target density. Target density was derived from specific gravity at 8.7% moisture content.

3. The densitometer was run with an average μ_m (2.985), to get the density profile for each sample.
4. The growth ring boundaries (one growth ring at a time) were manually selected and marked on the density profile for each sample, to match with the microscope images.

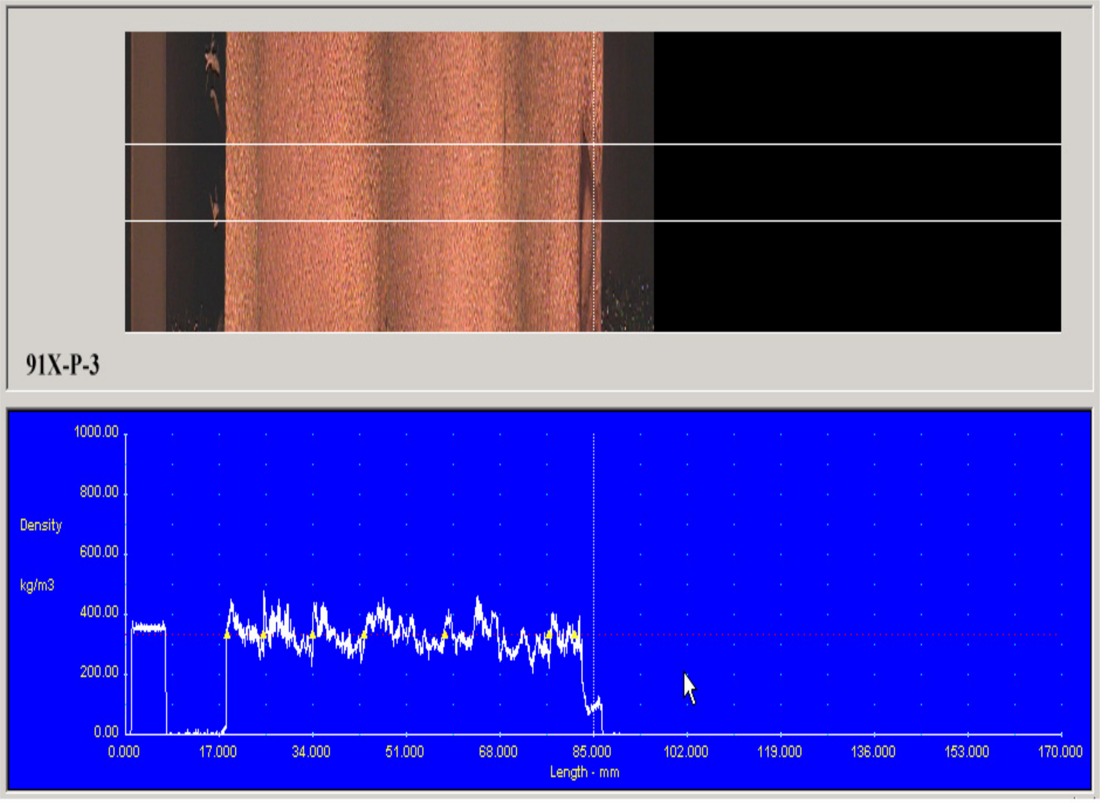

Figure 1. Density profile of wood from Clone 91X protected tree measured at 8 % moisture content. Bark side is on the left hand side, and pith side is on the right hand side. Wood image is on the top. The measured area is the area between the two horizontal white lines in the top photograph (a 2 mm distance). On the left of density profile is density profile of polyethylene, then it shows density profile of air which is very small. The density profile of wood on the right side of the profile shows much more fluctuation. Yellow triangle shapes indicate where the growth ring boundaries are.

The QMS X-ray densitometer was assembled for tree ring analysis of distinct growth rings of softwoods. Though the machine does not have ability to recognized hardwoods characteristics well, it can be used for investigating of density profiles of hardwoods including diffuse porous hardwoods.

Growth Rate

In this study, growth rate is ring width per year of increment core, ring widths of increments cores were measured by a digital caliper (Mitutoyo CD-6”C) under the stereoscope (SMZ800 Nikon) and reported in millimeters (mm). Ring width was measured at 8.7% moisture content.

Fiber Length

Fiber lengths of 16 increment cores were measured by Image Analyzer system. Fiber length was measured from pith to bark. Thirty-five observations were measured for each ring.

Fibers were measured after maceration**.** The maceration was prepared as follows: 10 mg of air-dried wood with 30 ml of 30% hydrogen peroxide solution was boiled at 80±1 ºC for 4 hours, and then filtered and the solution was washed out in a coarse filter with deionized water. Wood samples were separated/broken into fibers in 10 ml deionized water, using a glass rod. The fibers were stained with 1% safranin aqueous solution to improve contrast for the light microscope.

From each growth ring, 35 unbroken fibers were measured, using an image analyzer. The images of fibers, taken at 4x magnification, were shown on a computer monitor. Fibers were manually selected on the monitor, and a mouse was used to locate the two ends of a fiber. Then, fiber lengths were measured and recorded.

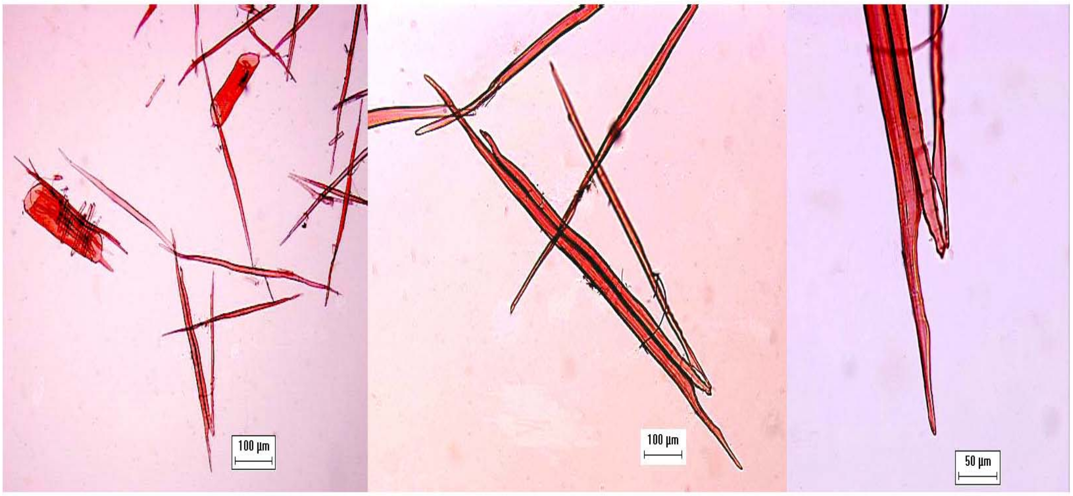

Figure 2 Fibers of Clone 91X, unprotected tree, ring six. Fiber measurement was done at 4x magnification as shown on the left side. The same fibers in the middle are at 10x magnification. There are different shapes at the end of the same fibers shown at the right at 20x magnification. There are three complete fibers; the longest fiber is at the left (1.07 mm length, 28.14 µm fiber diameter), the adjacent fiber is 0.75 mm long with 23.34 µm fiber diameter, the far right fiber is 0.69 mm long with 17.16 µm fiber diameter.

Anatomical Characteristics

Five types of wood anatomical structures were measured by image analyzer, which was the same system used for fiber length measurement but with different applications. Selected wood properties were measured from wood microscopic slides.

Slides of the cross section samples from the pith to the bark were prepared before measuring.

Slide preparation: Increment core was radial cut from the pith to the bark with 1 centimeter width. The wood samples were cut by microtome (model number 5795 by Spencer Lens Co., Buffalo N.Y.) to obtain15 µm thick cross sections. The section was stained by 1% aqueous safranin solution for a few minutes, washed with deionized water, and dried on the warm plate at 75±5 °C for a few minutes. The section was mounted on the glass slide by permount (Fisher Scientific) and xylene was used as a solvent before covering with a cover glass.

Measurement of vessel area, vessel number, vessel diameter, ray area, and ray number: The permanent slides were examined with the light microscope, five randomly selected areas were sampled from each growth ring. An individual growth ring was categorized into five zones from the pith side toward the bark side. Each zone covered 20% of the distance of the ring width and started from the pith side toward the bark side. Selected areas were covered from the earlywood zone to the latewood zone of each growth ring. Digital images of the sampling areas were taken. Later on, those images were manually adjusted and additionally marked for complete shape of elements (vessels, and rays). Images of vessels and rays were analyzed by using the images at 10x magnification. The selected areas were calculated by the Image analyzer for size, number, and area of those different elements. The total number of images taken from six rings of 16 trees was 480 images. A total area of an image was 827.288 x 1103.628

square micrometers (μm^2), which equals 0.913 mm2. Anatomical wood properties were measured per 0.913 (mm2), adjusted and reported to its equivalent 1 mm2. Percentage of an anatomical wood property of total area of the image (0.913 mm2) was reported.

***Vessel area**:* Vessel area is the cross section area of vessel lumen and it does not include the cell wall of the vessel element. Vessel diameter, vessel number and vessel area were counted and calculated by the Image Pro Plus 4.5 software. Uncounted vessel areas by the Image Pro Plus 4.5 software had been manually measured, and further added to the automatic measurement.

Vessel number: Software counted the total number of vessels, as well as the calculated vessel area, and mean vessel diameter. Software cannot count all vessel number correctly due to much variation of color intensity of each image. Vessels uncounten by the Image Analyzer were further manually counted.

***Vessel diameter*:** Vessel diameters were counted by the Image Analyzer for each image. The maximum and minimum of diameter sizes were recorded. Image analyzer was used for further analysis of the mean diameter. Mean diameter was reported in µm.

Ray number: Rays were manually counted along the tangential width of the image (827.288 µm). If rays appeared partially, they were still counted as fractions. All fractions were summed up. The rounded numbers were reported as ray number.

Ray area: The width of ray cell was manually located by mouse on the image, then Image Analyzer was used to measure the ray width in micrometers. Widths of fifteen rays were measured for each image. The average width of fifteen rays was used to calculate ray area. The average of ray width times ray number equaled ray area.

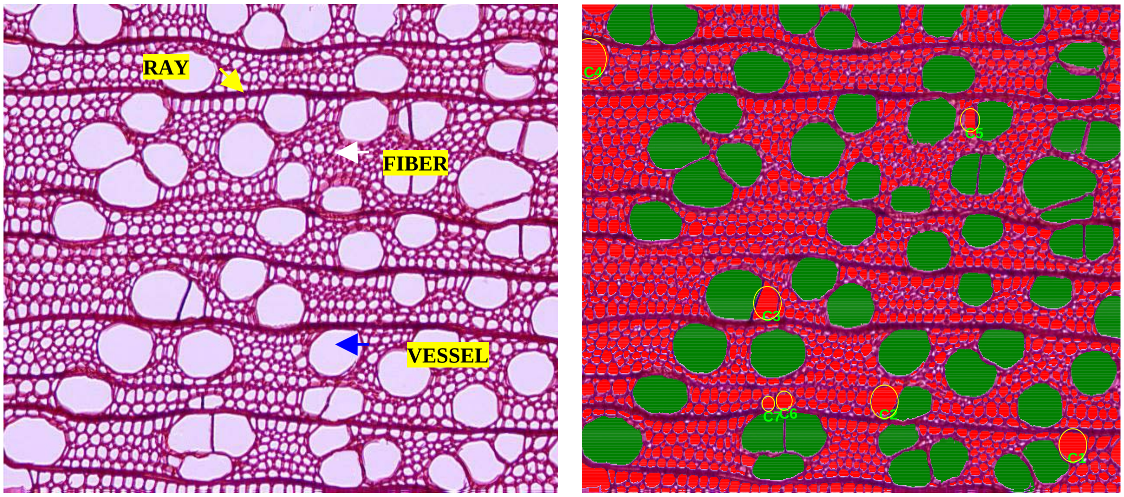

Figure 3 The image of cross section from Clone NM2 unprotected tree ring 5 from the middle ring area at 10 x magnifications before measurement (left). The left picture shows rays as horizontal lines (yellow pointer), vessels (blue pointer), and fibers (white pointer). Picture on the right shows how measurements were taken. Green areas were selected and measured by specific setting software. There were some uncounted vessels that were measured manually (yellow circles) and added to the automatic measurement. Vessel area (31.72%), mean vessel diameter (76.32 μm), and vessel number (64) were measured on this particular image. Ray number and ray area were 7 and 5.38% respectively.

Experimental Design and Statistical Analysis

Fiber length, vessel area, vessel number, ray area, and ray number from 16 trees were studied. The data were a subset of a larger ongoing experiment. That experiment is a split plot with whole plot treatments arranged in a randomized complete block design. The data for this thesis come from one block of this experiment.

This block was divided in half, the left half was sprayed, and the right half was not sprayed. The block was also divided into four strips, top to bottom; making eight sub-blocks. We measured wood properties of two trees from each sub-block, one of Clone NM2 and one of Clone 91X. Thus clone is the subplot factor in this experiment. There were a total of 2 treatments x 2 clones x 4 strips, a total of 16 trees in the study.

Dependent variable

1. Specific gravity
2. Fiber Length
3. Growth rate
4. Anatomical characteristics: vessel area, vessel number, vessel diameter, ray area, ray number

The significant difference is at alpha = 0.05, unless stated otherwise.

Data Analysis

Statistical analyses were performed using SAS v 8.2. SAS proc mixed with maximum likelihood method of estimation was used to identify significant relationships between wood properties and other factors (treatment, ring, and clone). All statistical analyses were performed in the consultation with Professor Dr. Marcia Gumpertz, Department of Statistics, NCSU. Each clone was analyzed separate with two main effects. In this study, there were four models for statistical analysis depending upon data availability as follows:

1. Specific gravity of all 16 increment cores was analyzed by using Model I. Only one measurement was taken from each tree. The study samples were 16 increment cores (1 block, 2 treatments, 2 clones, 4 trees per clone per treatment). The model was designed to determine if there was any significant difference of in specific gravity between two clones, under two different treatments, but could not test due to zero degree of freedom. Overall density is strongly related to specific gravity and may be used as a surrogate for specific gravity. Therefore, density was analyzed by model I the same way as specific gravity. Analysis of specific gravity at 8.7 % was used.

The mixed model I:

$$X_{ijkl} = \mu + T_j + \delta ij + S_k + C_l + (T \times C)_{jl} + \varepsilon_{ijkl} \qquad \text{(Equation 4)}$$

Where:

X_{ijkl} is an observation on the kth ramet or tree from the jth treatment of the lth clone.

μ = overall mean

T_j = fixed treatment effect; j=1,2

δij = random whole plot effect

S_k = random strip effect; k = 1, …, 4

C_l = fixed clone effect; l = 1, 2

$(T \times C)_{jl}$ = fixed treatment x clone interaction

ε_{ijkl} = random residual error

2. Density at age group level of 16 increment cores was analyzed by using Model II. The age group of wood samples were defined by age1 and Age 2. Age 1 was the wood from ring 1 (near the pith) to ring 3. Age 2 was the wood from ring 4 to ring 6 (bark side). Analysis of density at 8.7% was used.

The mixed model II:

$$X_{ijklm} = \mu + T_j + \delta ij + S_k + C_l + (T \times C)_{jl} + A_{m\,+} + (T \times A)_{jm} + (C \times A)_{lm} + (T \times C \times A)_{jlm}$$

$$+ \rho_{ijkl} + \varepsilon_{ijklm} \qquad \text{(Equation 5)}$$

Where:

X_{ijklm} is an observation on the kth ramet or tree from the jth treatment of the lth clone.

μ = overall mean

T_j = fixed treatment effect ; j=1,2

δij =random whole plot effect

S_k = random strip effect; k = 1, …, 4

C_l = fixed clone effect; l = 1, 2

$(T \times C)_{jl}$ = fixed treatment x clone interaction

A_m = fixed age group effect; m=1, 2

$(T \times A)_{jm}$ = treatment x age group interaction

$(C \times A)_{lm}$ = clone x age group interaction

$(T \times C \times A)_{jlm}$ = treatment x clone x age group interaction

ρ_{ijkl} =random tree effect

ε_{ijklm} = random residual error

3. Model III was used to analyze for density, growth rate, fiber length, vessel area and number, and ray area and ray number of all 16 cores. Density of each ring was obtained from density profile. One measurement was taken from each ring for density. A growth rate value was taken from each ring. Fiber lengths of 35 measurements were taken from each ring, and they were averaged before further statistical analysis. Five measurements of vessel area, vessel number, vessel diameter, ray area, and ray number were taken from each ring; furthermore they were averaged by ring before further statistical analysis. Analysis was separate for each clone. It was assumed that there was no interaction between block and treatment and clone. (Note: assumes no block x treatment x age group interaction).

The mixed model III:

$$X_{ijklm} = \mu + T_j + \delta ij + S_k + \eta_{ijk} + A_l + (T \times A)_{jm} + R(A)_{l(m)} + (T \times R(A))_{jl(m)} + \rho_{ijkl} + \varepsilon_{ijklm} \quad \text{(Equation 6)}$$

Where:

X_{ijklmn} is an observation on the kth ramet or tree from the jth treatment of the lth clone. A_m is the effect due to the mth age which m=1 is the wood from growth ring 1 to growth ring 3, where as m=2 is wood from growth ring 4 to growth ring 6.

μ = overall mean

T_j = fixed treatment effect; j=1,2

δij =random whole plot error (note: i=1)

S_k = random strip effect; k = 1, …, 4

η_{ijk} = random tree effect

R_l = fixed ring effect

$(T \times A)_{jm}$ =fixed treatment x age interaction.

$R(A)_{l(m)}$ =fixed ring (age) effect; m=1, 2, 3.

$(T \times R(A))_{jl(m)}$ =fixed treatment x ring (age) interaction

ρ_{ijkl} =random tree x age interaction

ε_{ijklm} = residual error (random error)

Random effects are considered from whole plot effect, treatment plot effect (δij), strip effect (S_k), tree effect (η_{ijk}), and tree x age group interaction (ρ_{ijkl}). Fixed effects are treatment effect, age group effect, ring within age group effect, treatment x age group interaction, and treatment x ring (age group) interaction.

4. Model IV was used to analyze density, growth rate, fiber length, vessel area, vessel number, vessel diameter, ray area and ray number of all 16 cores. The data sets of wood properties were the same one used in Model III. The model was used to determine the significant differences of wood properties among Clone NM2 unprotected trees, Clone NM2 protected trees, Clone 91X unprotected trees, and Clone 91X protected trees by ring or by age group.

The mixed model IV:

$$X_{ijklmn} = \mu + T_j + \delta ij + \eta_{ijk} + C_l + (T \times C)_{jl} + \rho_{ijkl} + R_m + (T \times R)_{jm} + (C \times R)_{lm} + (T \times C \times R)_{jlm} + \varepsilon_{ijklmn} \qquad \text{(Equation 7)}$$

Where:

X_{ijklmn} is an observation on the kth ramet or tree from the jth treatment of the lth clone.

μ = overall mean

T_j = fixed treatment effect; j=1,2

δij =random whole plot error (note: l=1)

η_{ijk} =random subplot error

C_l =fixed clone effect; l=1, 2.

$(T \times C)_{jl}$ =fixed treatment x clone interaction.

ρ_{ijkl} =random tree effect within a subplot and clone

R_m =fixed ring effect; m=1, ….., 6.

$(T \times R)_{jm}$ =fixed treatment x ring interaction

$(C \times R)_{lm}$ =fixed clone x ring interaction

$(T \times C \times R)_{jlm}$ =fixed treatment x clone x ring interaction

ε_{ijklm} = residual error (random error)

RESULTS

SPECIFIC GRAVITY AND AVERAGE DENSITY

Specific gravity and density values are numerically close and a similar pattern of variation is expected. Specific gravity and density of all 16 increment cores at 8.7% moisture content were analyzed using Model I at tree level. Specific gravity was determined and statistically analyzed at two levels of clone (Table 1). The following results can be drawn.

- The results show that there was a significant difference in specific gravity and density only by clone.
- No significant treatment by clone interaction was found.

Table 1 Analysis of variance of specific gravity and density at tree level based on model I

			Specific Gravity		Density (g/cm^3)	
Effect	**Num DF**	**Den DF**	**F Value**	**Pr**	**F Value**	**Pr**
Treatment	1	0	0	.	0	.
Clone	1	6	9.70	**0.0207****	9.72	**0.0207***
Treatment x Clone	1	6	0.83	0.3978	0.82	0.3994

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test. Specific gravity and density were at 8.7% moisture content.

Comparisons of estimated mean value of specific gravity and density can be presented as the following.

- There were significant differences in specific gravity and density between Clone NM2 and Clone 91X only for the protected trees (Table 2 and 3). Clone NM2 had a significantly higher specific gravity and density (0.3278, 0.355 g/cm^3) than Clone 91X (0.303, 0.330 g/cm^3).

Table 2 Estimated means and p-values of F-test comparisons pairs of mean specific gravity at tree level among unprotected tree Clone NM2 (NM2-U), protected tree Clone NM2 (NM2-U), unprotected tree Clone 91X (91X-U), and protected tree Clone 91X (91X-P).

Sample		**NM2-U**	**NM2-P**	**91X-U**	**91X-P**
	Lsmean	0.321	0.327	0.308	0.303
NM2-U	0.321				
NM2-P	0.327	0.5383			
91X-U	0.308	0.1691	N/A		
91X-P	0.303	N/A	**0.0293***	0.5518	

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available. Standard error (SE) =0.0085. Specific gravity was at 8.7 % moisture content.

Table 3 Estimated means and p-values of F-test comparisons pairs of mean density at tree level among unprotected tree Clone NM2 (NM2-U), protected tree Clone NM2 (NM2-U), unprotected tree Clone 91X (91X-U), and protected tree Clone 91X (91X-P).

Sample		**NM2-U**	**NM2-P**	**91X-U**	**91X-P**
	Lsmean	0.349	0.355	0.335	0.330
NM2-U	0.349				
NM2-P	0.355	0.5373			
91X-U	0.335	0.1701	N/A		
91X-P	0.330	N/A	**0.0293***	0.5501	

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available. Standard error (SE) =0.0092. Density was at 8.7% moisture content.

DENSITY

Density at age group level were statistical analyzed by model II. SAS proc mixed with maximum likelihood method of estimation was used to identify significant relationships between density and other factors (ring, age group). The statistical analysis was performed with three main effects and density was significantly affected by age group (Table 4). Mean and standard error (SE) of density at 8.7% are presented in Table 5.

Table 4 Analysis of variance of density at age level based on model II

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	0.33	.
Clone	1	6	1.67	0.2433
Treatment x clone	1	6	0.92	0.3744
Age group	1	12	8.86	**0.0323***
Treatment x age group	1	12	0.77	0.3974
Clone x age group	1	12	0.27	0.6117
Treatment x clone x age group	1	12	0.13	0.7275

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test. Density was at 8.7% moisture content.

Table 5 Means of density (g/cm^3) of 16 trees

Clone	Treatment	Age 1	Age 2
		Mean	Mean
NM2	**Unprotected**	0.369	0.331
	Protected	0.368	0.341
91X	**Unprotected**	0.364	0.329
	Protected	0.333	0.326

Notes: SE=0.0174. Density was at 8.75% moisture content.

The following analysis of variance of density results were presented based on the statistical analysis.

- Age 1 had significantly higher density than Age 2 when averaged over both treatments and both clones.
- There were no significant interactions between age group and clone and treatment.

Mean comparisons at age group level can be drawn as the following.

- No significant difference in density was observed between age group, clone and treatment.
- Clone 91X, protected, merits further study. In this experiment the mean density was the same for Age 1 and Age 2. However, no significant interaction was found.

In summary, there was a significant difference between the two age groups, which was similar for all cases except Clone 91X protected trees. This treatment might merit further study. There was no significant difference in density between the unprotected and the protected trees.

Density at ring level: For the two clones were analyzed separately by using model III and presented in Table 6. The following analysis of variance of density results were presented based on the statistical analysis. There was a significant age group effect for Clone NM2 but not Clone 91X.

Table 6 Analysis of variance of density (g/cm^3) based on model III

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	0.11	.	0.02	.
Age group	1	6	28.14	**0.0018****	0.22	0.6593
Treatment x age group	1	6	2.05	0.2023	0.21	0.6639
Ring (age group)	4	24	2.75	0.0514	2.02	0.1266
Treatment x ring (age group)	4	24	0.49	0.7404	2.37	0.0836

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test. Density was at 8.7% moisture content.

Mean density for Clone NM2 ranged from 0.331 to 0.372 g/cm^3 for unprotected trees, and from 0.347 to 0.361 g/cm^3 for protected trees (Table 7). For Clone 91X, mean density ranged from 0.320 to 0.330 g/cm^3 for unprotected trees, and at 0.323 g/cm^3 for protected trees. Standard error (SE) values of density of 16 trees are also presented.

Results of mean density comparisons by age group can be summarized as follows:

- There were significant differences for means of density between Age 1 (0.372 g/cm^3, 0.361 g/cm^3) and Age 2 (0.331g/cm^3, 0.337 g/cm^3) unprotected trees and protected trees of Clone NM2 (Table 7).

Table 7 Estimated mean density (g/cm^3), standard error of density, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	0.372	0.331	0.361	0.347	0.320	0.330	0.323	0.323
NM2-U-A1	0.0062								
NM2-U-A2	0.0062	**0.0031****							
NM2-P-A1	0.0062	0.2620	N/A						
NM2-P-A2	0.0062	N/A	0.4766	**0.0338***					
91X-U-A1	0.0127						0.5566	0.8713	N/A
91X-U-A2	0.0111							N/A	0.6834
91X-P-A1	0.0111								0.9961
91X-P-A2	0.0111								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test. Density at 8.7%

NM2-U-A1: Clone NM2 unprotected tree at Age 1
NM2-U-A2: Clone NM2 unprotected tree at Age 2
NM2-P-A1: Clone NM2 protected tree at Age 1
NM2-P-A2: Clone NM2 protected tree at Age 2
91X-U-A1: Clone 91X unprotected tree at Age 1
91X-U-A2: Clone 91X unprotected tree at Age 2
91X-P-A1: Clone 91X protected tree at Age 1
91X-P-A2: Clone 91X protected tree at Age 2

Although insect defoliation did not show significant influence on average density but it may have affected the uniformity of density within growth ring and along the radius as it can be seen on the on the density profiles (Appendix A6-13).

WOOD PROPERTIES AT RING LEVEL

Within tree variation in wood properties such as growth rate, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number were analyzed using model III. SAS V 8.2 proc mixed with maximum likelihood method of estimation was used to identify significant relationships between physical and anatomical wood properties and other factors (treatment, ring, age group).

Growth Rate

Analysis of variance of growth rate was presented in Table 8.

- There were significantly different for growth rate by ring within age group for both clones (Table 8).
- There were also significant effects by treatment x age group interaction, and treatment x ring within age group interaction for growth rate only for clone 91X (Table 8).

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 5)

Table 8 Analysis of variance of growth rate (mm) based on model III**.**

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	0.73	.	6.46	.
Age group	1	6	0.28	0.6155	5.43	0.0586
Treatment x age group	1	6	0.05	0.8321	13.31	**0.0107***
Ring (age group)	4	24	34.9	**<.0001*****	8.64	**0.0002*****
Treatment x ring (age group)	4	24	1.79	0.1636	5.22	**0.0038****

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean growth rate for Clone NM2 ranged from 9.89 to 10.88 mm for unprotected trees, and from 11.70 to 12.10 mm for protected trees (Table 9). For Clone 91X, mean growth rate ranged from 5.97 to 11.49 mm for unprotected trees, and from 10.83 to 12.05 mm for protected trees. Standard error (SE) values of growth rate of 16 trees are shown in Table 9.

Table 9 Estimated mean growth rate (mm), standard error of growth rate, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	9.89	10.88	11.70	12.10	5.97	11.49	12.05	10.83
NM2-U-A1	1.5619								
NM2-U-A2	1.5619	0.6145							
NM2-P-A1	1.5619	0.4447	N/A						
NM2-P-A2	1.5619	N/A	0.5995	0.8348					
91X-U-A1	1.0328						**0.0059****	**0.0053****	N/A
91X-U-A2	0.9847							N/A	0.6531
91X-P-A1	0.9847								0.3809
91X-P-A2	0.9847								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Mean comparisons of growth rate can be summarized as follows:

- For Clone 91X, there was significant lower in growth rate at Age 1 (5.97 mm) than at Age 2 (11.49 mm) for unprotected trees.
- For Clone 91X, there was significantly lower means of growth rate for unprotected trees (5.97 mm) than protected trees (12.05 mm) at Age 1.

Fiber Length

Analysis of variance of fiber length was presented in Table 10. Fiber length was analyzed from two trees per clone per treatment total of eight trees.

- Fiber length was significantly influenced by age group effect and ring within age group effect in both clones.
- No significant difference was observed by interaction in both clones.

Table 10 Analysis of variance fiber length (mm) based on model III.

			NM2		91X	
Effect	**Num DF**	**Den DF**	**F Value**	**Pr**	**F Value**	**Pr**
Treatment	1	0	0.11	.	1.09	.
Age group	1	2	224.61	**0.0044****	131.11	**0.0075****
Treatment x age group	1	2	1.41	0.3576	1.64	0.3291
Ring (age group)	4	6	21.44	**0.0005*****	13.46	**0.0021****
Treatment x ring (age group)	4	6	1.26	0.371	0.41	0.7953

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean fiber length for Clone NM2 ranged from 0.69 to 0.90 mm for unprotected trees, and from 0.65 to 0.90 mm for protected trees (Table 11). For Clone 91X, mean fiber length ranged from 0.68 to 0.89 mm for unprotected trees, and from 0.75 to 0.92 mm for protected trees. Standard error (SE) values of fiber length of 16 trees were also presented (Table 11).

Table 11 Estimated mean fiber length (mm), standard error of fiber length, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	0.69	0.90	0.65	0.90	0.68	0.89	0.75	0.92
NM2-U-A1	0.0370								
NM2-U-A2	0.0358	**0.0069****							
NM2-P-A1	0.0358	0.5688	N/A						
NM2-P-A2	0.0358	N/A	0.9667	**0.0112***					
91X-U-A1	0.0348						**0.0173***	0.2915	N/A
91X-U-A2	0.0333							N/A	0.6525
91X-P-A1	0.0333								**0.0131***
91X-P-A2	0.0333								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Mean comparisons of fiber length was summarized as follows:

- For both clones, there were significantly shorter in fiber length at Age 1 than at Age 2.
- For Clone NM2, there was significant shorter in fiber length for protected (0.65 mm) and unprotected trees (0.69) at Age 1 than for protected (0.90) and unprotected trees (0.90 mm) at Age 2
- For Clone 91X, there was significant shorter in fiber length for protected trees (0.75 mm) and unprotected trees (0.68 mm) at Age 1 than protected trees (0.92 mm) and unprotected trees (0.89 mm) at Age 2

Vessel Area

The results of the analyses of variance test for mean vessel area, measured by two different levels of age group, clone, and treatment can be drawn as follows:

- There were significant differences for mean of vessel area by age group, by ring within age group, and by treatment x ring within age group interaction for Clone NM2.
- There were significant differences for mean of vessel area by age group, by treatment and age group interaction, by ring within age group, and by treatment x ring within age group interaction for Clone 91X.

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 7)

Table 12 Analysis of variance of vessel area based on model III

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	2.08	.	0.04	.
Age group	1	6	10.97	**0.0162***	44.79	**0.0005*****
Treatment x age group	1	6	1.64	0.2474	7.37	**0.0349***
Ring (age group)	4	19	13.93	**<.0001*****	24.71	**<.0001*****
Treatment x ring (age group)	4	19	6.76	**0.0013****	3.22	**0.0353***

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean vessel area for Clone NM2 ranged from 24.08 to 27.19 % for unprotected trees, and from 23.67 to 25.04 % for protected trees (Table 13). For Clone 91X, mean vessel area ranged from 21.63 to 25.22 % for unprotected trees, and from 19.41 to 27.91 % for protected trees. Standard error (SE) values of vessel area of 16 trees are also presented (Table 13).

Table 13 Estimated mean vessel area (%), standard error of vessel area, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	24.08	27.19	23.67	25.04	21.63	25.22	19.41	27.91
NM2-U-A1	0.8071								
NM2-U-A2	0.7675	**0.0175***							
NM2-P-A1	0.8071	0.7315	N/A						
NM2-P-A2	0.7675	N/A	0.0958	0.2011					
91X-U-A1	1.0750						**0.0319***	0.1898	N/A
91X-U-A2	0.9905							N/A	0.1034
91X-P-A1	1.0422								**0.0005***
91X-P-A2	0.9905								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Results of mean vessel area comparisons by age group can be drawn as follows:

- Clone NM2 Age 2 (27.19 %) had significantly higher vessel area than Clone NM2 age1 (24.08 %) for unprotected trees.
- For Clone 91X, mean of vessel area of Age 2 was significantly higher than Age 1 for both unprotected trees and protected trees.

Vessel Number

Analysis of variance for vessel number resulted in the following (Table 14):

- There were significant differences for mean of vessel number by age group, by ring within age group, and by treatment x ring within age group interaction for both clones.

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 8)

Table 14 Analysis of variance of vessel number based on model III

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	8.28	.	5.47	.
Age group	1	6	66.34	**0.0002****	13.68	**0.0101***
Treatment x age group	1	6	2.15	0.1927	6.52	0.433
Ring (age group)	4	19	4.75	**0.0074****	27.13	**<.0001*****
Treatment x ring (age group)	4	19	9.78	**0.0001*****	8.16	**0.0005*****

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean vessel number per 1 mm^2 ranged from 63 to 89 for Clone NM2 unprotected trees, and from 57 to 74 for Clone NM2 protected trees. For Clone 91X, mean vessel number ranged from 55 to 77 for unprotected trees, and from 56 to 60 for protected trees (Table 15).

Table 15 Estimated mean vessel number, standard error of vessel number, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	89	63	74	57	77	55	60	56
NM2-U-A1	3.2978								
NM2-U-A2	3.0925	**0.0005***							
NM2-P-A1	3.2978	**0.0212***	N/A						
NM2-P-A2	3.0925	N/A	0.1740	**0.0033****					
91X-U-A1	3.6761						**0.0046****	**0.0148***	N/A
91X-U-A2	3.4764							N/A	0.8812
91X-P-A1	3.5901								0.4464
91X-P-A2	3.4764								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Mean comparisons of vessel number can be drawn as follows:

- Clone NM2, there was significantly higher vessel number at Age 1 (89) than at Age 2 (63) for unprotected trees, and also higher at Age 1 (74) than at Age 2 (57) for protected trees.
- Clone 91X, there was a significant higher vessel number at Age 1 (77) than at Age 2 (55) for unprotected trees.
- At Age 1, Clone NM2 and Clone 91X had significantly higher vessel number in unprotected (89 and 77) trees than in protected trees (74 and 60).

Vessel Diameter

Analysis of variance of mean vessel diameter is shown in Table 16.

- There were significant differences for mean of vessel diameter by age group, and ring within age group for both clones.
- There was significantly different for means of vessel diameter by treatment x ring within age group interaction only for Clone 91X.

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 9)

Table 16 Analysis of variance of vessel diameter (μm) based on model III

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	2.68	.	2.98	.
Age group	1	6	84.85	**<.0001*****	77.46	**0.0001*****
Treatment x age group	1	6	3.73	0.1018	0.11	0.7559
Ring (age group)	4	20	9.78	**0.0001*****	24.45	**<.0001*****
Treatment x ring (age group)	4	20	0.79	0.546	4.04	**0.0154***

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean vessel diameter for Clone NM2 ranged from 63.09 to 79.42 μm for unprotected trees, and from 69.73 to 80.40 μm for protected trees (Table 17). For Clone 91X, mean vessel diameter ranged from 64.27 to 82.28 μm for unprotected trees, and from 68.64 to 85.37 μm for protected trees. Standard error (SE) values of vessel diameter of 16 trees are presented in Table 17.

Table 17 Estimated mean vessel diameter (µm), standard error of vessel diameter, and p-values for pairwise comparisons. Estimates are based on model III

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	63.09	79.42	69.73	80.40	64.27	82.28	68.64	85.37
NM2-U-A1	2.0072								
NM2-U-A2	1.8824	**0.0002*****							
NM2-P-A1	2.0072	0.0578	N/A						
NM2-P-A2	1.8824	N/A	0.7243	**0.0021****					
91X-U-A1	2.1676						**0.0007*****	0.1979	N/A
91X-U-A2	2.0006							N/A	0.3173
91X-P-A1	2.1014								**0.0009*****
91X-P-A2	2.0006								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Results of mean vessel diameter by mean comparisons can be drawn as follows:

- For Clone NM2, unprotected (63.09µm) and protected trees (69.73µm) show significantly lower vessel diameter at Age 1 than unprotected (79.42µm) and protected trees (80.40µm) at Age 2.
- It can be assumed that for Clone NM2 at Age 1, unprotected trees showed significantly lower vessel diameter (63.09µm) than protected trees (69.73µm) because the probability is closed to 0.05 significant level.
- For Clone 91X, unprotected (64.27µm) and protected trees (85.37µm) show significantly lower vessel diameter at Age 1 than unprotected (82.28µm) and protected trees (68.64µm) at Age 2.

Ray Area

Analysis of variance for mean of ray area is shown in Table 18.

- There were significant differences for mean of vessel diameter by age group, by ring within age group, by treatment x age group interaction, and by treatment x ring within age group interaction for Clone NM2.
- There was significantly different for means of vessel diameter by age group, and by ring within age group for Clone 91X.

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 10)

Table 18 Analysis of variance of ray area based on model III.

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	13.75	.	55.82	.
Age group	1	2	616.87	**0.0016****	50.00	**0.0194***
Treatment x age group	1	2	80.21	**0.0122***	0.14	0.74719
Ring (age group)	4	7	21.69	**0.001*****	7.78	**0.0128***
Treatment x ring (age group)	4	7	89.26	**<.0001*****	1.11	0.4224

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean ray area for Clone NM2 ranged from 5.52 to 7.98 % for unprotected trees, and from 5.04 to 6.20 % for protected trees (Table 19). For Clone 91X, mean ray area ranged from 5.86 to 6.96 % for unprotected trees, and from 4.57 to 5.79 % for protected trees. Standard error (SE) values of ray area of 16 trees were also presented (Table 19).

Table 19 Estimated mean ray area (%), standard error of ray area, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	7.98	5.52	6.2	5.04	6.96	5.86	5.79	4.57
NM2-U-A1	0.2236								
NM2-U-A2	0.2206	**0.0017****							
NM2-P-A1	0.2236	**0.0300***	N/A						
NM2-P-A2	0.2206	N/A	0.2631	**0.0078****					
91X-U-A1	0.1625						**0.0464****	**0.0389***	N/A
91X-U-A2	0.1407							N/A	**0.0298***
91X-P-A1	.								**0.0303***
91X-P-A2	0.1407								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Mean comparisons of ray area can be summarized as follows:

- There were significant differences for means of ray area between age groups for both clones and both treatments.
- For Clone NM2, there was a significant higher ray area in unprotected trees (7.98 %) than protected trees (6.20 %) at Age 1.
- For Clone 91X, there were significant higher ray area in Age 1 than at Age 2 for unprotected and protected trees.
- For Clone 91X, at Age 1there were significant higher ray area in unprotected trees (6.96, 5.79 %) than protected trees (5.86, 4.57 %).

Ray Number

Analysis of variance for mean of ray number is shown in Table 20.

- There were significant differences for mean of ray number by age group, by ring within age group for both clones.
- There was a significant difference by treatment x ring within group interaction for clone NM2.

Further explanation for the interaction effects can be found under **Radial Variation** (See Figure 11)

Table 20 Analysis of variance of ray number based on model III.

Effect	Num DF	Den DF	NM2		91X	
			F Value	Pr	F Value	Pr
Treatment	1	0	0.23	.	5.72	.
Age group	1	6	57.53	**0.0003*****	39.84	**0.0007*****
Treatment x age group	1	6	0.2	0.6732	0.2	0.1937
Ring (age group)	4	24	9.48	**0.0002*****	9.48	**<.0001*****
Treatment x ring (age group)	4	24	5.29	**0.0049****	5.29	0.9628

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by F-test.

Mean ray number for Clone NM2 ranged from 9.8 to 12.4 for unprotected trees, and from 9.7 to 12.0 for protected trees (Table 21). For Clone 91X, mean ray number ranged from 9.9 to 11.6 for unprotected trees, and from 10.3 to 13.1 for protected trees. Standard error (SE) values of ray number of 16 trees were also presented (Table 21).

Table 21 Estimated mean ray number, standard error of ray number, and p-values for pairwise comparisons. Estimates are based on model III.

		NM2-U-A1	NM2-U-A2	NM2-P-A1	NM2-P-A2	91X-U-A1	91X-U-A2	91X-P-A1	91X-P-A2
	SE\MEAN	12.4	9.8	12	9.7	11.6	9.9	13.1	10.3
NM2-U-A1	0.4537								
NM2-U-A2	0.4366	**0.0011****							
NM2-P-A1	0.486	0.5608	N/A						
NM2-P-A2	0.4366	N/A	0.8485	**0.0028****					
91X-U-A1	0.3953						**0.0146***	**0.0367***	N/A
91X-U-A2	0.3649							N/A	0.4271
91X-P-A1	0.3833								**0.0014****
91X-P-A2	0.3649								

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available by t-test.

Mean comparison of ray number can be summarized as follows:

- For Clone NM2, there was significantly higher ray number for protected trees (12.0) and unprotected trees (12.4) at Age 1 than for protected trees (9.7) and unprotected trees (9.8) at Age 2
- For Clone 91X, there was significant higher ray number for protected trees (13.1) and unprotected trees (11.6) at Age 1 than protected trees (10.3) and unprotected trees (9.9) at Age 2.

RADIAL VARIATION

Radial variation of wood properties such as density, fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number are presented. In addition to describing the radial patters, radial variation in wood properties were statistically analyzed by using Model IV allowing comparisons to be made at the same age within clone between treatments (NM2-U with NM2-P, 91X-U with 91X-P) and within treatment between clones (NM2-U with 91X-U; NM2-P with 91X-P).

Density

Results of the analysis of variance for density at ring level are the following (Table 22):

- There were significant differences among rings, and a significant clone x ring interaction.

Table 22 Analysis of variance of density at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	0.09	.
Clone	1	0	15.19	.
Treatment x clone	1	0	0.00	.
Ring	5	57	2.81	**0.0247***
Treatment x ring	5	57	1.03	0.4087
Clone x ring	5	57	5.41	**0.0004*****
Treatment x clone x ring	5	57	1.23	0.3081

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

The unprotected and protected trees of **Clone NM2** had the same radial pattern (Figure 4). No significant difference was found for density between two treatments at the same ring. Clone NM2 had somewhat higher density in the first two year followed by a slight decrease and relatively constant values along the radius.

The unprotected and protected trees **Clone 91X** had the same radial pattern of density from year one to year six (Figure 4). No significant difference was observed for density between two treatments of the same age. Density values of unprotected trees were relatively lower but of the protected trees density values increased somewhat during the first three years. In general, however, density was relatively constant along the radius.

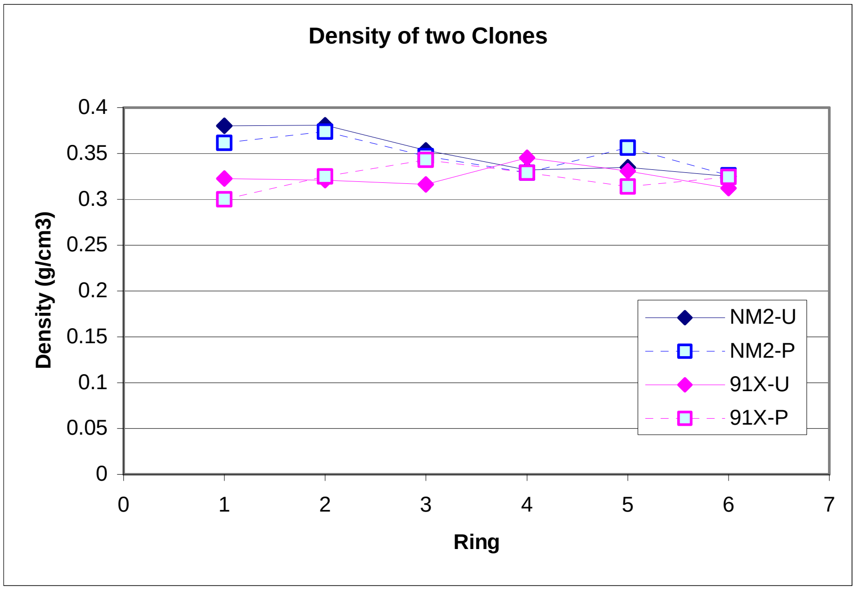

Figure 4 Density of 16 increment cores from ring 1 (near pith side) toward ring 6 (near bark side). They were two clones (Clone NM2 and Clone 91X) under two different treatments (unprotected and protected). Four trees of each clone were taken from unprotected treatment (U), and other four trees of each clone came from protected treatment (P).

Results from statistic analysis can be summarized as follows:

- For the first two growth rings, both unprotected and protected trees of Clone NM2 had significantly higher density than trees of Clone 91X (See Appendix A1).
- For the third ring, a significant difference was observed for density between two clones only for unprotected trees. On the other hand, for ring five, a significant difference was observed for density between two clones only for protected trees.
- For the fourth and six rings, no significant difference was revealed in density between clones for either treatment.

Growth Rate

Results of the analysis of variance for growth rate at ring level are the following (Table 23):

- There were significantly differences of growth rate by ring, by treatment x ring interaction, and by treatment x clone x ring interaction.

Table 23 Analysis of variance of growth rate at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	4.05	.
Clone	1	0	0.99	.
Treatment x clone	1	0	0.31	.
Ring	5	59	18.95	**<.0001*** **
Treatment x ring	5	59	6.34	**<.0001*** **
Clone x ring	5	59	0.68	0.6422
Treatment x clone x ring	5	59	2.81	**0.0243***

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Radial variations in growth rate for the two clones were different (Figure 5). **Clone NM2** had the same radial pattern from year one to year six for both unprotected and protected trees. The unprotected trees had lower growth rate than protected trees from the second ring to the fifth ring but the differences were not statistically significant. Clone NM2 had a low growth rate in the first year, followed by the maximum rate in the second year. Then it started decreasing in the third and fourth years, continued by increasing in the fifth year, and finally decreased in the sixth year.

For **Clone 91X**, there were different radial patterns between unprotected trees and protected trees (Figure 5). Unprotected trees grew slowly in the first four years, followed by a great increase to the maximum rate in the fifth year, and followed by a decrease in the sixth year to a rate slightly higher than the protected tree rate. Protected trees had a similar low growth rate as the unprotected trees in the first year, followed by a large increase in the second year, and further slight increase to the maximum rate in the third year. However, the growth rate in protected trees then started decreasing in the fourth year to the sixth year. There were significant differences in growth rate between unprotected trees and protected trees from the second ring to the fifth ring. Unprotected and protected trees grew at the same rate during the first year but after that unprotected trees had lower growth rate than protected trees till the fourth ring. However, due to an interaction, unprotected trees had higher growth rate during the last two years.

Results of mean comparisons by ring can be summarized as follows:

- For the first and six rings, no significant differences were observed for growth rate between two clones for both unprotected trees and protected trees.

- For the third ring, no significant difference was observed for growth rate between the two clones for protected trees but there was a significant difference between the clones unprotected trees
- No significant difference was observed for means of growth rate between the two clones for both unprotected trees and protected trees from the fourth ring to the sixth ring.

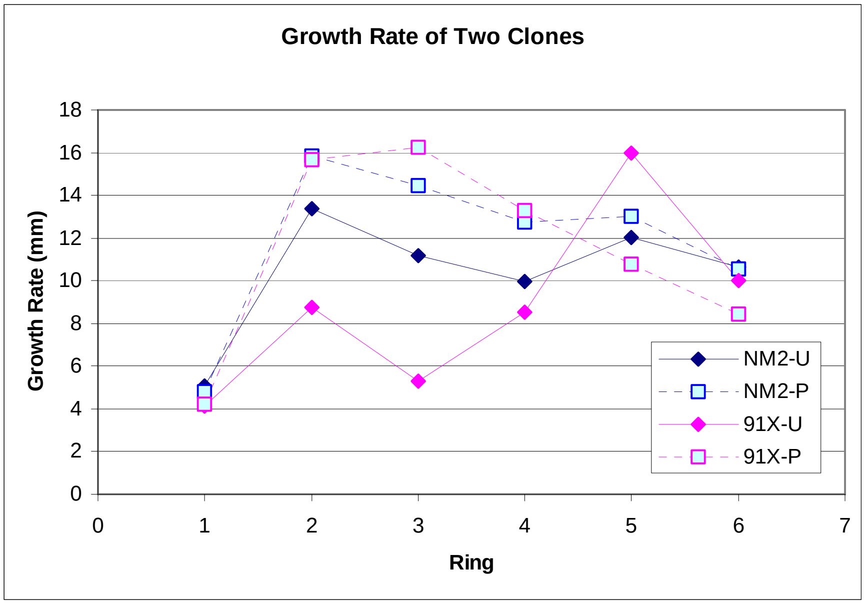

Figure 5 Growth rate of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

Fiber Length

Fiber length was analyzed from eight cores out of 16 cores (two trees per clone per treatment). Results of the analysis of variance for fiber length at ring level are the following (Table 24):

- There were significantly differences of fiber length by ring, by clone x ring interaction.

Table 24 Analysis of variance of fiber length at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	0.22	.
Clone	1	0	0.55	.
Treatment x clone	1	0	0.92	.
Ring	5	18	89.30	**<.0001*** **
Treatment x ring	5	18	1.08	0.4050
Clone x ring	5	18	3.94	**0.0137***
Treatment x clone x ring	4	18	0.89	0.5103

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Radial variation of fiber length for two clones revealed the same pattern from the first ring to the sixth ring (Figure 6). In the first two years, fiber lengths were relatively short and increased more in the third year than later from the fourth year toward the sixth year. No significant difference was observed for fiber length between unprotected and protected trees of both clones.

Results of mean comparisons can be summarized as follows:

- Besides the first growth ring, where there was a significant difference for fiber length between the two clones for protected trees, no significant differences were observed for fiber length for both unprotected and protected trees of the two clones along the radius.

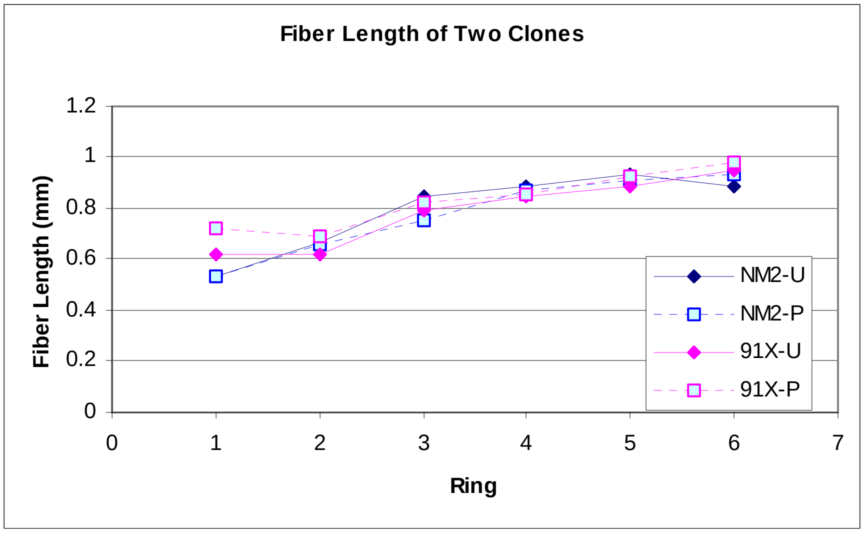

Figure 6 Fiber length along radial from ring 1 (pith side) toward ring 6 (bark side) of two clones (Clone NM2 and Clone 91X) under unprotected treatment (U) and protected treatment (P).

Vessel Area

Analysis of variance of vessel area at the ring level is presented (Table 25).

- There were significantly differences of vessel area by ring, by clone x ring interaction, and by treatment x clone x ring interaction.

Table 25 Analysis of variance of vessel area at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	1.21	.
Clone	1	0	4.32	.
Treatment x clone	1	0	1.74	.
Ring	5	51	36.89	**<.0001*****
Treatment x ring	5	51	2.06	0.0939
Clone x ring	5	51	5.05	**0.0008*****
Treatment x clone x ring	5	51	8.77	**<.0001*****

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Radial variation in vessel area of the two clones have a similar pattern (Figure 7). The radial pattern of vessel area increased to the maximum at the third year and then leveled off toward bark.

Results of mean comparisons of vessel area can be summarized as follows (Figure 7, Appendix A4):

- Protected and unprotected Clone MN2 had significantly different vessel area values only at the fourth ring. Meanwhile, protected and unprotected Clone 91X had significantly different vessel area values at the third and the fifth rings.
- The relative maximum vessel area values for protected Clone NM2 was observed at the third ring, and for unprotected trees at the fourth ring. The relative maximum vessel area values for protected Clone 91X was observed at the fourth ring, and for unprotected trees at the third ring.
- Protected trees of the two clones differed significantly in vessel area only from the third to fifth rings. Unprotected trees of the two clones differed significantly in vessel area at the fifth ring.

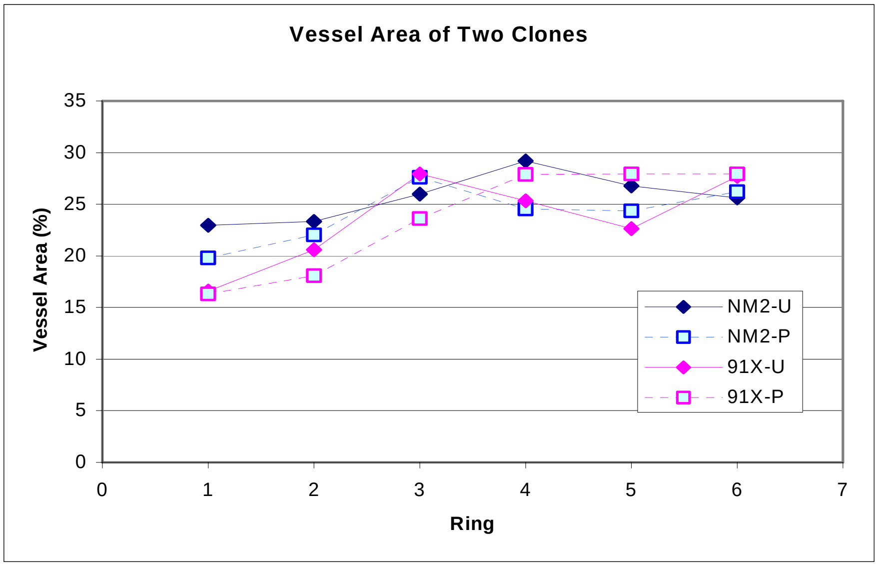

Figure 7 Vessel area of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

Vessel Number

Analysis of variance of vessel number at ring level was summarized as the followings (Table 26):

- There were significant differences in vessel numbers by ring main effect.
- Vessel number was also significantly effected by treatment x ring interaction, by clone x ring interaction, and by treatment x clone x ring interaction.

Table 26 Analysis of variance of vessel number at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	13.26	.
Clone	1	0	13.17	.
Treatment x clone	1	0	0.06	.
Ring	5	51	26.76	**<.0001*****
Treatment x ring	5	51	4.44	**0.0020****
Clone x ring	5	51	5.35	**0.0005*****
Treatment x clone x ring	5	51	5.67	**0.0003*****

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

The general radial pattern of vessel numbers decreased from pith to bark. Radial variations of vessel number of the two clones were different to some extent (Figure 8).

Results of mean comparisons at the ring level can be summarized as the followings (Figure 8 and Appendix A4):

- For Clone NM2, unprotected trees had significantly larger vessel numbers than protected trees only at the first growth ring. On the other hand, for Clone 91X, unprotected trees had significantly larger vessel numbers than protected trees only at the third ring.
- For unprotected trees in the first two years, and the fifth year Clone NM2 had significantly higher vessel number than Clone 91X. However, at the third ring Clone 91X exhibited significantly higher vessel number. At the fourth and the sixth rings, there were no significant differences found.

- For protected tree, Clone NM2 showed significantly higher vessel numbers than Clone 91X only at the second and the third rings. Meanwhile, there were no differences detected between the two clones at the other rings.

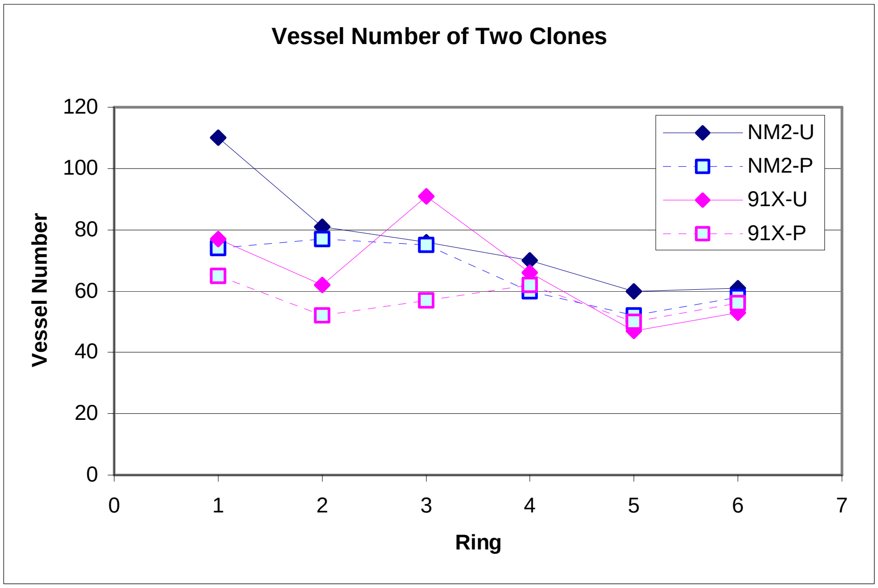

Figure 8 Vessel number of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

Vessel Diameter

Analysis of variance of vessel diameter was presented in Table 27.

- There were significant differences of vessel diameter by ring main effect.
- Vessel diameter was also affected significantly by treatment x ring interaction and by clone x ring interaction.

Table 27 Analysis of variance of vessel diameter at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	4.08	.
Clone	1	0	2.56	.
Treatment x clone	1	0	0.12	.
Ring	5	51	67.21	**<.0001*** **
Treatment x ring	5	51	2.50	**0.0427***
Clone x ring	5	51	2.77	**0.0278***
Treatment x clone x ring	5	51	0.79	0.5585

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Radial variations of vessel diameter of the two clones showed the same pattern for unprotected and protected trees from the second ring to the sixth ring (Figure 9). Mean vessel diameter was small (ranged from 55 to 66 μm) in the first year, followed by a gradual increase toward the bark (ranged from 80 to 86 μm).

Results of mean comparisons of vessel diameter at ring level can be summarized as the followings (Figure 9, Appendix A4):

- For **Clone NM2**, protected trees had larger vessel diameter than unprotected trees but the difference was significant at the first growth ring only.
- For **Clone 91X**, protected trees had larger vessel diameter than unprotected trees but with a significant difference measured at the third ring only.
- **Unprotected** trees had significant differences of vessel diameter between the two clones only at the sixth ring. However, no significant differences were observed between the **protected** trees of the two clones.

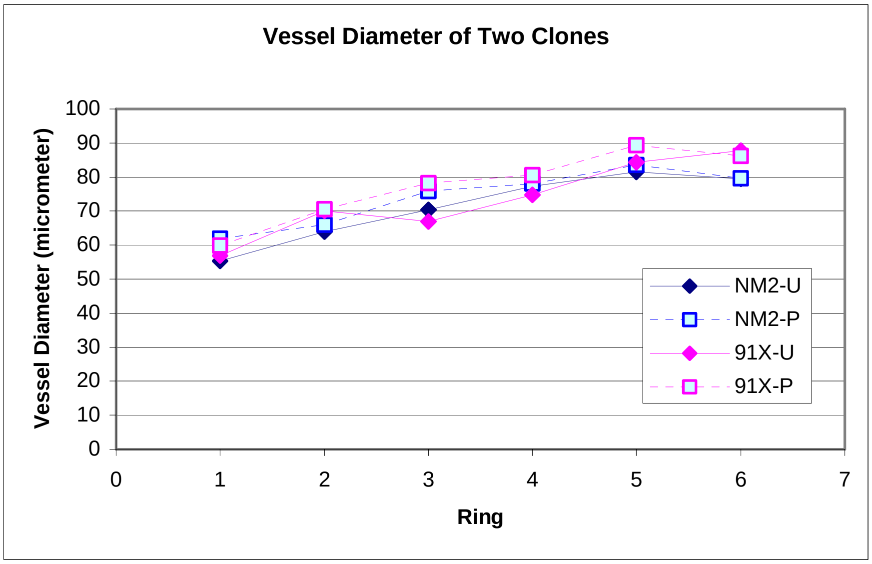

Figure 9 Vessel diameter of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

Ray Area

Analysis of variance of ray area was presented in Table 28:

- There were significant differences of ray area by ring main effect.
- There were significant differences of ray area by treatment x ring interaction, by clone x ring effect, and by treatment x clone x ring interaction.

Table 28 Analysis of variance of ray area at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	33.31	.
Clone	1	0	4.86	.
Treatment x clone	1	0	0.29	.
Ring	5	17	75.87	**<.0001*****
Treatment x ring	5	17	17.47	**<.0001*****
Clone x ring	5	17	8.06	**0.0005*****
Treatment x clone x ring	5	17	10.68	**<.0001*****

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Ray area of the two clones decreased from pith to bark and the radial variation differed by treatment (Figure 10).

Results of mean comparisons for ray number can be summarized as follows (Figure 10, Appendix A5):

- For **Clone NM2**, unprotected trees also had higher ray area than protected trees at each growth ring, except at the sixth ring, with significant differences except at the second ring.
- For **Clone 91X**, unprotected trees had significantly higher ray area than protected trees at each ring.
- For **unprotected** trees, at the first three ring Clone NM2 had higher ray area than Clone 91X but the difference was significant at the first and the third rings only. From ring forth to six, the differences between the clones were not significant.
- For **protected trees**, Clone NM2 had a significantly higher ray area than Clone 91X at the first two years and at the sixth year. Meanwhile, Clone 91X had higher ray area at the third year. No significant differences were observed at the forth and the fifth years.

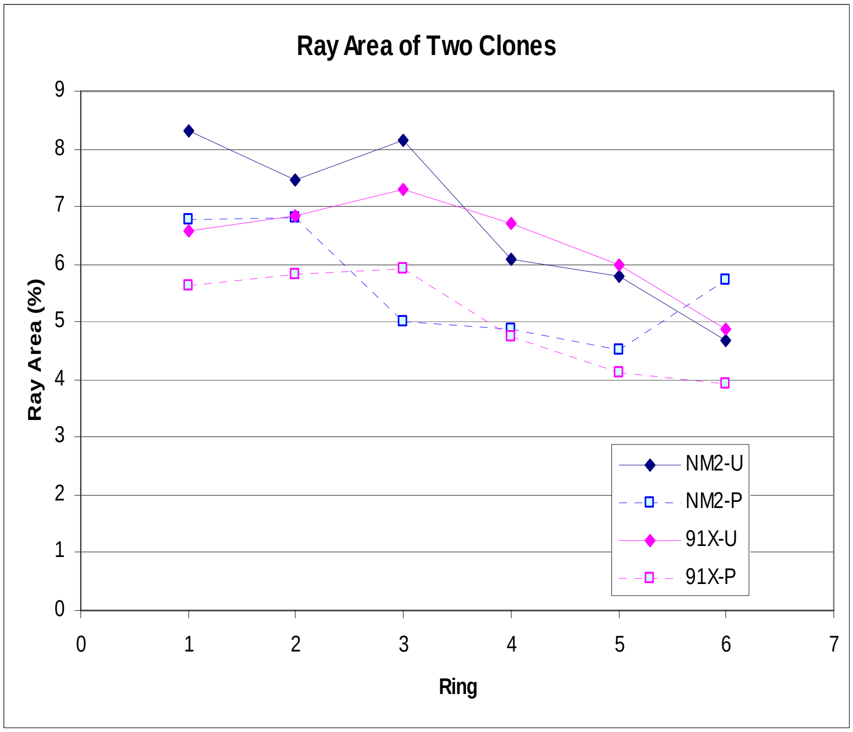

Figure 10 Ray area of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

Ray Number

Analysis of variance for ray number was presented in Table 29.

- There were significant differences of ray number by ring main effect.
- There were also significant differences of ray number by clone x ring interaction.

Table 29 Analysis of variance of ray number at ring level based on model IV

Effect	Num DF	Den DF	F Value	Pr
Treatment	1	0	0.71	.
Clone	1	0	0.50	.
Treatment x clone	1	0	2.69	.
Ring	5	50	42.97	**<.0001*****
Treatment x ring	5	50	1.81	0.1269
Clone x ring	5	50	4.77	**0.0012****
Treatment x clone x ring	4	50	2.20	0.0686

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

Radial variation of ray number of the two clones followed the same pattern; they gradually decreased from pith to bark (Figure 11).

Results of mean comparisons of ray number can be summarized as follows (Figure 11, Appendix A5):

- For Clone NM2, unprotected trees had significantly higher means than protected trees for ray number at the third ring only.
- For Clone 91X, protected trees had significantly higher ray numbers than unprotected trees at the second and the third years.
- For protected trees, Clone 91X had significantly higher ray numbers than Clone NM2 at the third and the fourth rings.
- Unprotected trees of the two clones did not show significant differences in ray numbers.

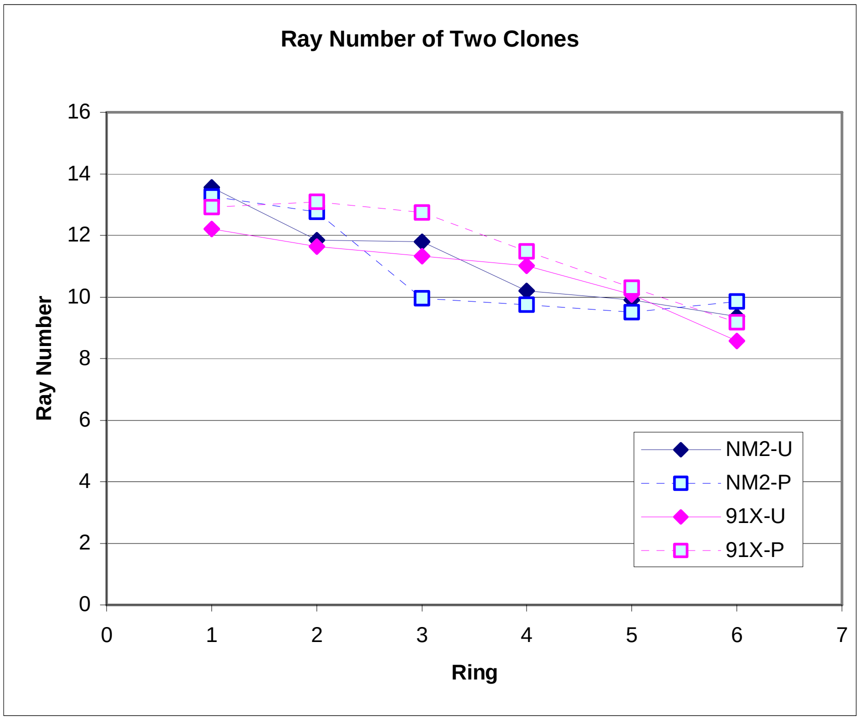

Figure 11 Ray number of two clones (Clone NM2 and Clone 91X) from ring 1 (pith side) toward ring 6 (bark side) under unprotected treatment (U) and protected treatment (P).

WOOD PROPERTY CORRELATION

The correlations between the wood properties studied in this project were analyzed. Pearson correlation coefficients (r) were calculated by using SAS proc corr procedure and results are presented in Table 30.

Table 30 Pearson correlation coefficients (the top line), probability (the middle line), and number of observations (the bottom line) among wood properties at ring level. Wood properties include density (D), growth rate (GR), stem radius (R), fiber length (FL), vessel area (VA), vessel number (VN), ray area (RA), and ray number (RN).

Wood Properties	D	GR	R	FL	VA	VN	VD	RA	RN
D	1 93								
GR	0.1684 0.1066 93	1 95							
R	-0.1493 0.1531 93	0.447 <.0001*** 95	1 95						
FL	-0.2653 0.0748 46	0.2257 0.1314 46	0.7435 <.0001*** 46	1 46					
VA	-0.006 0.9571 85	-0.1379 0.2056 86	0.4505 <.0001*** 86	0.5509 0.0001*** 43	1 86				
VN	0.2427 0.0252* 85	-0.4263 <.0001*** 86	-0.6058 <.0001*** 86	-0.6051 0.0001*** 43	0.0948 0.3855 86	1 86			
VD	-0.1607 0.1419 85	0.3385 0.0014** 86	0.811 <.0001*** 86	0.7435 <.0001*** 43	0.4623 <.0001*** 86	-0.796 <.0001*** 86	1 86		
RA	0.4145 0.0038** 47	-0.2743 0.0621 47	-0.6156 <.0001*** 47	-0.5485 0.0001*** 43	-0.0698 0.6410 47	0.7475 <.0001*** 47	-0.6764 <.0001*** 47	1 43	
RN	0.3680 0.0005*** 85	0.0600 0.5833 86	-0.5801 <.0001*** 86	-0.6438 <.0001*** 43	-0.4311 <.0001*** 86	0.3245 0.0023** 86	-0.5356 <.0001*** 86	0.8045 <.0001*** 43	1 86

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

The results of correlation analysis between density and other wood properties were summarized below:

- Correlation analysis showed positive relationships between density and the vessel number (0.24), ray area (0.40), and ray number (0.37).
- However, a negative but not significant correlation was found between density and fiber length (-0.27).

The results of correlation between **growth rate** expressed as **ring width** and other properties were summarized below:

When growth rate was expressed as, the results were the followings:

- Correlation analysis revealed a positive correlation between growth rate (ring width) and vessel diameter (0.34) but negative correlations with vessel number (-0.43) and ray area (-0.33).
- There was positive but not significant correlation between growth rate and density (0.17) and fiber length (0.23).

When **growth rate** was expressed as **stem radius**, the analysis resulted more significant correlations:

- Analysis showed positive correlations between growth rate (stem radius) and vessel diameter (0.81), fiber length (0.74), and vessel area (0.45).
- There were negative correlations between growth rate (stem radius) and vessel number (-0.61), ray area (-0.73), and ray number (-0.58).

The results of correlation between **fiber length** and other wood properties were summarized as follows:

- Correlation analysis revealed positive correlations between fiber length and vessel area (0.55) and between fiber length and vessel diameter (0.74).
- There were negative correlations between fiber length and vessel number (-0.61), ray area (-0.55), and ray number (-0.64).

Results of correlation analysis between vessel area and other wood properties were summarized below:

- There was a positive correlation between vessel area and vessel diameter (0.46).
- There was a negative correlation between vessel area and ray number (-0.43)

Results of correlation analysis between vessel number and other wood properties were summarized below:

- Correlation coefficients were relatively between vessel number and vessel diameter, and between vessel number and ray area.
- Significantly positive correlations were found between vessel number and ray area (0.75) and between vessel number and ray number (0.32).
- Significantly negative relationships were observed between vessel number and vessel diameter (-0.80).

Results of correlation analysis between vessel diameter and other wood properties were summarized below:

- Significantly negative correlations were found between vessel diameter and ray area (-0.78) and ray number (-0.54).

Results of correlation analysis between ray area and other wood properties were summarized below:

- As it was expected, significant positive correlation was found between ray area and ray number (0.80).

DISCUSSIONS

It was also found in previous studies that Clone NM2 was more resistant to insect attack than Clone 91X. Clone 91X grew to larger diameter and also was taller than Clone NM2 (Coyle et al., 2002).

Specific Gravity and Density

One measurement of specific gravity came from each tree. Specific gravity was different between the two clones. Clone NM2 had higher specific gravity than Clone 91X. In other studies, specific gravity varies significantly among trees, clones, and sites (Krestchmann et al., 2001, and Peszlen, 1994).

Specific gravity of these six year-old poplars ranged from 0.303 to 0.327, which were relatively low compared to the other studies. The 20-year-old native aspen and cottonwood in a Wisconsin study had a specific gravity (based on ovendry weight volume) ranged from 0.39 to 0.41 (Krestchmann et al, 2001). Mean specific gravity of three different clones used in Europe (10- to 15–year-old) ranged from 0.304 to 0.356 (Peszlen, 1994).

Specific gravity and density of wood sample can be used interchangeably. Density profiles of samples were measured by an x-ray densitometer at 8.7 % moisture content. Therefore, in this study both specific gravity and density were reported at 8.7% moisture content. Density profiles were analyzed between two age groups because the cottonwood leaf beetles attack only young trees. Age 1 included the first three growth rings near the pith and Age 2 included the second three growth rings near the bark.

During the first three years, new leaves were subjected to attack by cottonwood leaf beetles in the control plots; while, bio-pesticides were sprayed in the protected plot. There were no biorational pesticides applied in either plot during year four to year six. Analyzing density at age group level, age group showed significant effect on density. Age 1 has significantly higher density than Age 2 when averaged over both treatments and both clones. In one season not all the new leaves were eaten for either clone in the unprotected plots, Clone NM2 had more new leaves left than Clone 91X. However, there was no clonal difference and no significant interactions between age group x clone x treatment found for density. Thus, density was not strongly influenced by natural beetle feeding. It needs to be mentioned that although, insect defoliation did not show significant influence on average density but it may have affected the uniformity of density within growth ring and along the radius as it can be seen on the on the density profiles.

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Harris and McConchie (1978) did not found enough evidence on the effect of defoliation on wood density either when they studied the effect of fungicide on wood density. Nine-year-old *Pinus radiata* was infected by fungus (*Dothristroma*) and fungicides were sprayed. Four levels of treatment were examined; spraying with 0.05 kg Cu per liter of water when defoliation exceed 30% (3 times in 7 years), spraying with 0.08 kg Cu per liter of water when defoliation exceed 30% (3 times in 7 years), spraying with 0.08 kg Cu per liter of water when defoliation exceed 30% (every year), and no spraying in control treatment. Gartner et al (2005) also found that very small or no effect on wood density by the study of pruning the young Douglas-fir up to 50%.

In another study, wood density was significant affected by removal of 100% new leaves for two consecutive years. Cown (1977) found that 5-year-old *Pinus radiata* at four treatments had significant differences wood density and tracheid length. The four treatments were removal of new leaves for two years, removal of 1-year-old leaves for two years, removal of 2-and 3-year-old leaves for two years, and no defoliation for two years. Wood density was higher for trees with removal of new leaves for two years. Trees from this treatment decreased ring width in the first year of defoliation, and continued decreasing ring width in the second year. Due to the very small ring width, more percentage of latewood was created, which resulted in high density for this treatment. Since poplars are diffuse porous, no earlywood/latewood distinction could be made. In some cases, even the growth ring boundaries were hard to identify.

The separate analyses of the two clones also resulted that only Clone NM2 had a significant effect of density by age group. Age 1 had significantly higher density than Age 2 for both unprotected and protected trees. Although there were not enough evidences to fully assess the effect of defoliation on wood density for Clone 91X in this study, the treatment x ring within age interaction effect for density of Clone 91X was significant at 0.10 level indicating certain influence of treatment on density for this clone.

One possible reason for the finding of the significant age group effect on density only for Clone NM2 is the fact that Clone NM2 was more resistant to beetle attack. Although not all the new leaves of a particular growing season were eaten up by the beetles and thus they can continue producing food for the trees. However, NM2 had more leaves left on the trees than 91X (Hall, 2005 - personal communication). Therefore, they still could produce and had enough energy to build wood cells even after a beetle attack. Another possibility is that young leaves of NM2 developed to a different stage at which the beetles did not feed due to possible difference in chemical components of the leaves.

The above arguments were consistent with an infection experiments on six-week-old trees (three different transgenic lines) reported by Karlsson (2003). The infection in different transgenic trees started two days earlier than the control trees. There were larger areas of infection on leaf areas in the transgenic trees than in control trees. It was explained that the pathogen (*Venturia tremulae*) was able to use the phenolics as its carbon source. Moreover, it was also found that the reduction of growth may related to the synthesis and maintenance of a higher phenolic level found.

Investigating radial pattern, density was high near the pith, and low near the bark for Clone NM2 which was partially consistent with the results of Yanchuk et al. (1990), where wood density of 15 mature (80-year-old) trembling aspen clones was studied. Most of the clones had high density near the pith, and density decreased in the first 10 years. However, a few trees behaved in the opposite way. Density values were increased in the first 10 years. Variations of wood density along the radial direction among clones were substantial.

Specific gravity was found higher near the pith in another study (Peszlen, 1994) and explained by the radial variation of anatomical elements. Cell wall fraction area of 10- and 15-year-old poplars showed lower percentage in the fourth to the seventh rings than areas in the first three rings near the pith. Meanwhile, sums of vessel lumen fraction and fiber lumen fraction exhibited higher percentage in the in the fourth to the seventh rings than areas in the first three rings near the pith as well.

Growth Rate

Karlsson (2003) reported that with modifying of the part of cDNA in hybrid aspen, growth was a phenotypic trait. Environmental effect on growth rate of poplars was reported by Peszlen (1993). Although in this study, the experimental plot had a good water supply, the insect attack was considered as a specific environmental effect on growth rate.

When clones were analyzed separately, both clones showed significant ring within age group effect on growth rate expressed by ring width. However, growth rate of Clone 91X also had a significant treatment x age interaction, and a treatment x ring within age group interaction. Clone NM2 had no significant effect between Age 1 and Age 2 for both unprotected and protected trees. This exhibited that the degree of natural defoliation by insect attack did not strongly influenced diameter for clone NM2.

In contrast, growth rate of unprotected trees of Clone 91X was significantly smaller at Age 1 than at Age 2. Since Clone 91X was susceptible to insect attack, heavy leaf defoliation exhibited less food synthesis and further reflected less cells developing during defoliation years. On the other hand, for protected trees Clone 91X had higher growth at Age 1 than at Age 2. Most likely because there were more space for the trees to grow during the first few years than later when the crowns closed or at least are closer together. At Age 1, unprotected trees grew significantly less than protected trees due to the influence of insect attack.

Radial variation of growth rate

All trees had relatively similar growth rate during the first year. When this slow growth in **the first year** is considered, it was believed that the root systems were still developing in the site. In the **second year** growth of all trees increased greatly. Clone NM2 had higher growth than Clone 91X for unprotected trees; these are effects of heavy defoliation in Clone 91X and a resistance to insect attack in Clone NM2. Whereas, protected trees had very high growth in the second year.

In the **third year**, growth rate of unprotected trees showed significant difference between the two clones. Furthermore, unprotected trees decreased growth in both clones. The results were consistent with the findings by Cown and by Rook et al. Cown (1977) and Rook et al (1976) reported that removal of new leaves reduced growth in the same year and effected wood densities. In addition, removal of new leaves in the second year resulted in great reduction of growth in that year. For protected trees both clones maintained relatively the same growth rate as in the previous year.

In the **fourth year**, Clone NM2 still showed reduced growth for unprotected trees. It is believed that less food accumulation was effected by defoliation from the third year. However, Clone 91X unprotected trees grew the same rate as in the second year. Protected trees of both clones decreased their growth rate. This may be due to some defoliation that occurred in the protected plot due to large numbers of adult beetles which moved into the protected plot and to the decreased space available for crown development (Coyle et el., 2002).

In the **fifth and six year**, growth patterns were different from of the early years. Growth of protected trees of both clones leveled off. However, unprotected trees of both clones increased their growth in the fifth year due to more space for crown competition. Finally, growth was decreased in the sixth year for all samples. This finding was consistent with results studied by Coyle et al (2002), where diameter growth pattern of four different clones were measured, growths were increased in the fifth year, and reduced in the six year. Coyle et el. (2002) believed that *C. scripta* preferred feeding on leaves of young poplar trees only and growth rate were more influenced by site and climate than by insect attack in the fifth and sixth years.

Protected trees of both clones had similar growth pattern. On the other hand, growth patterns of unprotected trees were found different between Clone NM2 and Clones 91X.

During the **last three years**, although the growth rate was not always the same but there was no statistically significant difference observed for growth rate between two clones for both unprotected trees and protected trees.

Fiber Length

Fiber length was significantly influenced by age group effect and by ring within age group effect in both clones. However, no significant difference was observed by interaction for both clones. There were significant differences in fiber length between Age 1 and Age 2 for unprotected and protected trees of both clones.

Along the radius, fiber length values did not differ significantly between unprotected trees and protected trees. Fiber length was the shortest in the first year, and steadily increased by age toward the bark. The results of these two clones were consistent with other studies. Cown (1977) reported investigated the effect of defoliation on tracheid length in radiata pine. Tracheid lengths were most influenced by productions of new leaves, so did the reduction of radial growth. Gartner et al (2005) had studied the effect of pruning 13- to 18-year-old trees (Douglas-fir) on tracheid length. Tracheid length increased in the third and fourth year following pruning in those trees.

Fiber lengths of Clone NM2 and Clone 91X were effected by age and they were consistent with results with other poplar clones (Debell, J. D., et al., 1998, and Peszlen, I., 1994). DeBell et al (1998) reported that fiber lengths of two poplar clones were investigated between different growth rates (slow, medium and fast growth rates). The same radial pattern of fiber lengths was found and fiber lengths were only affected by age but not by growth rate.

Vessel Area

Analyzing the two clones separately, Vessel area of Clone NM2 showed significant age group, ring within age group effect, and treatment x ring within age group interaction effect. Vessel area of Clone 91X was significantly affected by age group, ring within age group, treatment x ring within age group, but also by interaction treatment x age group interaction.

For Clones NM2, only unprotected trees had statistically significant higher vessel area at Age 1 than at Age 2. However, unprotected trees had a higher percentage of vessel area than protected trees for both ages. It was believed that vessel area and water transport function were important factors in tree growth. When insects removed the leaves, less leaf area was involved in photosynthesis and this lack of nutrient availability may induced the production of more vessel. That is one possible reason for the result that vessel area was produced in a higher percentage in unprotected trees than trees in a normal environment (for example: protected trees). Vessel area at Age 2 was higher than at Age 1 because in general, young trees produced smaller cells than mature trees.

Clone 91X was more susceptible to insect attack than Clone NM2. Similarly to Clone NM2, vessel area of unprotected trees of Clone 91X at Age 1 was smaller than at Age 2. Furthermore, protected and unprotected trees had a higher vessel area than protected trees at Age 1.

When analyzing **radial variation** for the two clones together, vessel area had significant ring, significant clone x ring interaction, and a significant treatment x clone x ring interaction effects. Protected trees of the two clones differed significantly in vessel area only from the third to fifth rings. Unprotected trees of the two clones differed significantly in vessel area at the fifth ring.

Vessel areas were low in the first two years and increased to maximum at the third or the fourth year. Clone NM2 reached the maximum a year earlier for protected trees than unprotected trees. However, for Clone 91X, protected trees reached the maximum one year later than unprotected trees. Both clones had more constant vessel area after they reached the maximum and ranged between 25 and 28% in the sixth year.

The results of radial variation discussed above, were consistent with other studies on vessel area pattern (Gartner et el., 1997, Denne, 1999). Gartner et al. (1997) reported the vessel area increased with age at the breast height for 35-year-old red alder. Denne et al. (1999) also reported total vessel lumen area increased from the pith toward the bark in 40-year-old Rauli (*Nothofagus nervosa*) trees. Hudson et al (1998) reported vessel area also increased with age, and less vessel area was found in latewood zones than in early wood zones of seven-year-old eucalyptus trees.

Vessel Number

It is believed that higher vessel numbers help in the ease of water transport in trees (Santamaria et al, 2002). Hudson et al. (1998) also reported that vessel numbers decreased from pith to bark in seven-year-old eucalyptus trees. Denne et al. (1999) also found that vessel numbers were high at the first year and decreased toward the bark for forty-year-old eucalyptus trees.

Results in this study revealed similar trend; vessel numbers were high in the early year and decreased by age. Analyzing the two clones separately, vessel numbers were significantly influenced by the same main effects: age group, ring within age group, and by treatment x ring within age group interaction.

Both protected and unprotected trees of Clone NM2 had significantly higher vessel number at Age 1 than at Age 2. Unprotected trees of Clone 91X showed significantly higher vessel number at Age 1 than at Age 2. However, at Age 1, unprotected trees of both clones had significantly higher vessel number than protected trees.

The two clones exhibited different **radial pattern**. Results of two clones analyzed together showed there were significant effects by ring main effect, by treatment x ring interaction, clone x ring interaction, and by treatment x clone x ring interaction. For

Clone NM2, unprotected trees had significantly higher vessel numbers than protected trees at the first growth ring only. On the other hand, for Clone 91X, unprotected trees had significantly higher vessel numbers than protected trees at the third ring only.

Vessel Diameter

Vessel diameter increased from pith to bark with a maximum around 85 μm. Vessel diameter was affected significantly by age group and ring within age group effects for both clones. However, for Clone 91X treatment x ring within age interaction was also significant. For both clones, unprotected and protected trees show significantly lower vessel diameter at Age 1 than at Age 2. For Clone NM2 at Age 1, it can be assumed that unprotected trees also had significantly lower vessel diameter than protected trees because the probability was close to 0.05 significance level.

Estimated mean vessel diameter was about 60 μm in the first year. It was coincident with founding on six-month-old poplar that had mean vessel diameter of 57.1 ±14.4 μm (Jourez, B. et al, 2001).

Vessel diameter of both clones had the same radial variation pattern. Vessel diameter was significantly affected by ring, clone x ring interaction, and by treatment x clone x ring interaction for both clones. Furthermore, protected trees had larger vessel diameter than unprotected trees but the difference was statistically significant only at the first growth ring for Clone NM2 and only at the third ring for Clone 91X. Comparing unprotected trees of the two clones, Clone 91X had significantly higher vessel diameter at the sixth ring only. However, no significant differences were observed between the protected trees of the two clones.

These results were consistent with the findings of other studies on radial variation. Peszlen (1994) reported that vessel diameter of 10- and 15-year-old poplars ranged from 55 to 65 μm in the first year, and increased rapidly to the eighth year, followed by constant sizes outward. Gartner et al. (1997) reported that vessel diameter of 40-year-old red alders was low at 50 μm in the second year, and increased to 60 μm in the eighth year. Lei et al, (1996) found that 50-year-old white oaks had small vessel diameter (160 μm) in the first ring from pith and it increased to 200 μm in the eight year.

Vessel diameter is an important anatomical property and used to study the easy flow of water within a stem. It was believed that the small size of vessel diameter was the adaptation of trees to the drought or less water in the soil. In drought soil condition, plants were found to have vessels with small diameter. Santamaria et al. (2002) studied six different genotypes of avocado (*Persea Americana*). Vulnerability index was compared among six genotypes. Vulnerability index (V) was a function of the vessel diameter (VD) and the vessel number (VN), where V = VD/ VN. There were significant differences among genotypes. Trees with low vulnerability index may show a better performance under water stress conditions.

Ray Area

Ray area decreased from pith to bark. When **each clone was analyzed separately**, there were significant differences for mean of vessel diameter by age group, by ring within age group, by treatment x age group interaction, and by treatment x ring within age group interaction for Clone NM2. There was significantly different for means of vessel diameter by age group, and by ring within age group for Clone 91X.

Ray cells were relative small and more numerous at the first year than at the sixth ring. Ray area depended on ray number and the width of ray cells. There were significant differences in ray area between age groups and ring within age group for both clones and both treatments.

For Clone NM2, there was a significant higher ray area in **unprotected** trees than protected trees at Age 1. This result on ray area of unprotected trees is consistent with the vessel areas discussed before.

Analysis of **radial variation** showed that there were significant differences of ray area by ring main effect, treatment x ring interaction, clone x ring effect, and by treatment x clone x ring interaction. For **Clone NM2**, unprotected trees also had higher ray area than protected trees at each growth ring, except at the sixth ring, with significant differences except at the second ring. For **Clone 91X**, unprotected trees had significantly higher ray area than protected trees at each ring.

For **unprotected** trees, at the first three ring Clone NM2 had higher ray area than Clone 91X but the difference was significant at the first and the third rings only. From ring forth to six, the differences between the clones were not significant. For **protected trees**, Clone NM2 had a significantly higher ray area than Clone 91X at the first two years and at the sixth year. Meanwhile, Clone 91X had higher ray area at the third year. No significant differences were observed at the forth and the fifth years, however.

A significant clone by ring interactions was found for **unprotected** trees during the third year and the fourth year. This appeared one year earlier for **protected** trees. These finding are complex and the changes could be influenced by changes in either the physiological needs of the tree, or productive foods devoted to cell division.

Ray Number

Analyzing the two clones separately, there were significant differences for ray numbers by age group and by ring within age group for both clones. There was a significant difference by treatment x ring within group interaction for clone NM2.

Protected and unprotected trees of both clones showed significantly higher ray numbers at Age 1 than at Age 2. Clone 91X had significantly higher ray numbers for protected trees than for unprotected trees at Age 1.

Analysis of **radial variation** showed that there were significant differences of ray area by ring main effect and by clone x ring interaction. Ray numbers of protected and unprotected trees of both clones were decreasing from pith to bark. However, for Clone NM2, ray numbers were relatively constant during the last three years.

For Clone NM2, unprotected trees had significantly higher means than protected trees for ray area at the third ring only. For Clone 91X, protected trees had significantly higher ray numbers than unprotected trees at the second and the third years. For protected trees, Clone 91X had significantly higher ray numbers than Clone NM2 at the third and the fourth rings. It seemed that no clear evidence showed significant differences due to defoliation.

Correlations Among Wood Properties

Based on correlating all the data of two clones, two treatments, sixteen sample trees, two age groups, and six growth rings at growth ring level, there were several significant positive and negative correlations and also no correlations found among wood properties. From silvicultural and forest genetic points of view, the interest lies on the correlation between density and growth rate and between density and other wood properties.

It is surprising that d**ensity** had a significant **positive** relationship with vessel number but did not correlated with vessel area and with vessel diameter. In other words, the clones showed high vessel numbers with high wood density. Analyzing further the vessel characteristics of these poplars, vessel area did not correlate with vessel numbers but had a positive correlation with vessel diameter. Furthermore, vessel number correlated negatively with vessel diameter. Radial variation revealed that vessel number decreased and vessel diameter increased from the pith to bark. Vessel area also increased from the pith; however, it became relatively constant after the third year indicating that vessel area may be a genotypic character of the clones. No correlation was found between density and growth rate. It needs to be mentioned that the range of density was very narrow and the clones showed a relatively constant density along the radius.

There were highly significant **positive** correlations between **fiber length** and vessel area, and between fiber length and vessel diameter. On other words, it could mean that trees with high vessel area had larger vessel diameter and longer fiber length. There was a significantly positive correlation between ray area and vessel number, and a significantly negative correlation between ray area and vessel diameter. Trees with high vessel numbers had high ray area. In contrast, trees with larger vessel diameter had low ray area. However, these results may be due to the fact that all anatomical properties showed the highest variation along the radius and had increasing (fiber length, vessel area, vessel diameter) or decreasing (vessel number, ray area, ray number) values from pith toward the bark.

There was a **positive** correlation between **growth rate** as expressed as growth ring width and wood density. However; when growth rate was expressed as stem radius, it showed a negative correlation with wood density, which was consistent with results of a previous study by Hall (2005). The previous study of 72 trees of 32 poplar families also showed a negative correlation between diameter and specific gravity.

Furthermore, there were significant **positive** correlations between stem radius as **growth rate** and three wood properties such as fiber length, vessel diameter, and vessel area. Furthermore, stem radius had **negative** correlations with other three wood properties such as vessel number ray area, and ray number. However, Chen, P.Y.S. et al (1998) studied correlation between diameter growth rate and vessel lumen area of hardwoods and found no significant correlation between diameter growth rate and vessel lumen area percentages in northern red oak, black walnut, and yellow poplar.

SUMMARY

The effects of treatment on wood properties of the two clones were investigated based on 16 six-year old poplar trees of the two clones.

The effect of the environment on the characteristics of NM2 clones are summarized as the follows:

- Clone NM2 had mean **specific gravity** of 0.321 for unprotected trees and 0.327 for protected trees. Mean **density** ranged from 0.331 to 0.372 g/cm^3 for unprotected trees, and from 0.347 to 0.361 g/cm^3 for protected trees. At Age 1, unprotected and protected trees had mean density 0.369 g/cm^3 and 0.368 g/cm^3. At Age 2, unprotected and protected trees had mean density 0.331 g/cm^3 and 0.341 g/cm^3. No significant difference in density was observed within age group between treatments. Unprotected and protected trees had similar radial pattern. No significant difference was found for density between two treatments at the same ring. Clone NM2 had somewhat higher density in the first two year followed by a slight decrease and relatively constant values along the radius.

- Mean **growth rate** (ring width) ranged from 9.89 to 10.88 mm for unprotected trees and from 11.70 to 12.10 mm for protected trees. No significant difference was observed between age group and within age group between treatments. Both unprotected and protected trees had similar **radial pattern** from pith to bark, and had small growth rate in the first year, followed by the maximum rate in the second year, then it started decreasing in the third and fourth years, continued by slightly increasing in the fifth year, and finally decreased in the sixth year. The unprotected trees had lower growth rate than unprotected trees from the second ring to the fifth ring but the differences were not statistically significant.
- Mean **fiber length** ranged from 0.69 to 0.90 mm for unprotected trees, and from 0.65 to 0.90 mm for protected trees. Protected and unprotected trees showed significantly shorter fiber length at Age 1 than at Age 2. No significant difference in fiber length was observed within age group between treatments. Radial variation of **fiber length** increased from pith to bark. No significant differences were observed for fiber length between unprotected and protected trees along the radius.
- Mean **vessel area** for Clone NM2 ranged from 24.08 to 27.19 % for unprotected trees, and from 23.67 to 25.04 % for protected trees. For both unprotected trees and protected trees, vessel area was significantly smaller at Age 1 than Age 2. However, there were no significant differences in vessel area between unprotected and protected trees within age group. The **radial pattern** of vessel area increased

to the maximum at the third year and then leveled off toward bark. Unprotected and protected trees had significantly different vessel area values only at the fourth ring.

- Mean **vessel diameter** ranged from 63.09 to 79.42 µm for unprotected trees and from 69.73 to 80.40 µm for protected trees. For unprotected and protected trees, vessel diameter was significantly lower at Age 1 than at Age 2. At Age 1, unprotected trees showed significantly lower vessel diameter than protected trees but they did not differ at Age 2. **Radial variations** of vessel diameter showed increasing pattern from pith to bark. Unprotected trees had smaller vessel diameter than protected trees but the difference was significant at the first growth ring only.
- Mean **vessel numbers** ranged from 63 to 89 for unprotected trees, and from 57 to 74 for protected trees. For both unprotected and protected trees, vessel numbers were significantly higher at Age 1 (89 and 74) than at Age 2 (63 and 57). At Age 1, vessel numbers were significantly higher in unprotected trees (89) than in protected trees (74) but they did not differ significantly at Age 2. The **radial pattern** of vessel numbers decreased from pith to bark. Unprotected trees had significantly higher vessel numbers than protected trees only at the first growth ring but they did not differ later.
- Mean **ray area** ranged from 5.52 to 7.98 % for unprotected trees and from 5.04 to 6.20 % for protected trees. Unprotected and protected trees showed significantly different ray area between Age 1 and Age 2. There was significantly higher ray

area within age group between unprotected and protected trees. Ray area of the two clones decreased from pith to bark and the **radial variation** differed by treatment. Unprotected trees also had higher ray area than protected trees at each growth ring, except at the sixth ring, with significant differences except at the second ring

- Mean **ray number** ranged from 9.8 to 12.4 for unprotected trees, and from 9.7 to 12.0 for protected trees. Unprotected and protected trees showed significantly higher **ray number** at Age 1 than at Age 2. No significant difference in ray numbers was observed within age group between treatments. **Radial variation** of ray number gradually decreased from pith to bark. Unprotected trees had significantly higher means than protected trees for ray number at the third ring only.

In this study, **Clone NM2** had significant treatment effect on vessel number, vessel diameter, and ray area. Besides density and growth rate, age group effect was always significant and caused the highest variation in wood properties. At Age 1, unprotected trees showed significantly lower vessel diameter, higher vessel number, and higher ray area than protected trees. However, there were no differences found for these wood properties between unprotected and protected tress at Age 2. There were no significant differences observed within age group between treatments for density, growth rate, fiber length, vessel area, and ray number indicating that insect defoliation did not affect these properties significantly. Investigating the radial variation, vessel area, vessel diameter, and fiber length were smaller near pith and increased toward the bark. Meanwhile, decreasing radial patterns were found for vessel numbers, ray numbers, and ray area.

Comparing clonal and treatment means along the radius, wood properties seemed to differ more during the first three years than from the forth ring toward the bark, which could mean that defoliation may influenced wood properties to some extend even if not statistically significantly.

However, the clonal and treatment means of wood properties differed more during the first three years than from the forth ring toward the bark indicating that defoliation influenced wood properties to some extend.

The effect of the environment on the characteristics of Clone 91X are summarized as the follows:

- Clone 91X had mean **specific gravity** of 0.308 for unprotected trees and 0.303 for protected trees. Mean **density** ranged from 0.320 to 0.330 g/cm^3 for unprotected trees and were the same (0.323 g/cm^3) for protected trees. At Age 1, unprotected and protected trees had mean density 0.364 g/cm^3 and 0.333 g/cm^3. At Age 2, unprotected and protected trees had mean density 0.329 g/cm^3 and 0.326 g/cm^3. No significant difference in density was observed between age group and within age group between treatments. Unprotected and protected trees had similar radial pattern of density. No significant difference was found for density between two treatments at the same ring and it was relatively constant along the radius.

- Mean **growth rate** ranged from 5.97 to 11.49 mm for unprotected trees, and from 10.83 to 12.05 mm for protected trees. Only unprotected trees had significantly lower growth rate at Age 1 than at Age 2. At Age 1, unprotected trees had significantly lower growth rate than protected trees but they did not differ at Age 2. **Radial variations of growth rate** were different between unprotected trees and protected trees. **Unprotected trees** had small growth rate in the first four years, followed by a great increase to the maximum in the fifth year, and finally decreased in the sixth year. **Protected trees** had a small growth rate at the first year only, followed by a great increase showing high growth rates in the second and the third years, and then growth rate decreased toward the bark.
- Although both unprotected and protected trees had relatively small and similar growth rates during the first and the last years, they differed significantly from the second ring to the fifth ring. Unprotected trees had lower growth rate than protected trees till the fourth ring. However, due to an interaction, unprotected trees had higher growth rate during the last two years.
- Mean **fiber length** ranged from 0.68 to 0.89 mm for unprotected trees and from 0.75 to 0.92 mm for protected trees. Unprotected and protected trees had significantly shorter fiber length at Age 1 than at Age 2. No significant difference in fiber length was observed within age group between treatments. Radial variation of **fiber length** increased from pith to bark. No significant differences in fiber length were observed between unprotected and protected trees along the radius.

- Mean **vessel area** ranged from 21.63 to 25.22 % for unprotected trees, and from 19.41 to 27.91 % for protected trees. Vessel area was significantly lower at Age 1 than at Age 2 for both unprotected trees and protected trees. No significant difference in vessel area was observed within age group between treatments. The radial variations of vessel area increased from the pith, reached the maximum at the third year, and then leveled off toward bark. Unprotected and protected trees had significantly different vessel area values at the third and the fifth rings. Unprotected trees had higher vessel area at the third years; meanwhile, at the fifth ring, protected trees had higher vessel area.
- Mean **vessel diameter** ranged from 64.27 to 82.28 μm for unprotected trees, and from 68.64 to 85.37 μm for protected trees. Unprotected and protected trees show significantly lower vessel diameter at Age 1 than at Age 2. No significant difference in vessel diameter was observed within age group between treatments. **Radial variations** for unprotected and protected trees increased from pith to bark. Unprotected trees had smaller vessel diameter than protected trees but with a significant difference measured at the third ring only.
- Mean **vessel number** ranged from 55 to 77 for unprotected trees and from 56 to 60 for protected trees. Unprotected and protected trees showed significantly higher vessel numbers at Age 1 than at Age 2. At Age 1, unprotected trees had significantly higher vessel numbers than protected trees but they did not differ at Age 2. Radial variation of vessel numbers decreased from pith to bark.

Unprotected trees had significantly higher vessel numbers than protected trees only at the third ring.

- Mean **ray area** ranged from 5.86 to 6.96 % for unprotected trees and from 4.57 to 5.79 % for protected trees. Unprotected and protected trees showed significant differences in ray area between Age 1 and Age 2. Significant differences in ray area were also observed within age group between treatments. Radial variation of ray area showed a decreasing pattern from pith to bark. Unprotected trees had significantly higher ray area than protected trees at each growth ring.
- Mean **ray number** ranged from 9.9 to 11.6 for unprotected trees and from 10.3 to 13.1 for protected trees. Unprotected and protected trees showed significantly higher **ray number** at Age 1 than at Age 2. At Age 1, unprotected trees had significantly lower ray number than protected trees but they did not differ at Age2. Radial variation of **ray number** gradually decreased from pith to bark. Unprotected trees had significantly lower ray numbers than protected trees but the differences were statistically significant at the second and the third years only.

Based on this investigation, Clone 91X had significant treatment effect on growth rate, vessel number, ray area, and ray number. Besides density and growth rate, age group effect was always significant and caused the highest variation in wood properties. At Age 1, unprotected trees showed significantly lower ray numbers and higher vessel numbers than protected trees. However, there were no differences found for these wood properties between unprotected and protected tress at Age 2. There were no significant differences observed within age group between treatments for density, fiber length, vessel area, and vessel diameter indicating that insect defoliation did not affect these properties significantly.

Unlike for Clone NM2 where there were no significant differences found between age group and within age group between treatments, growth rate of Clone 91X seemed to be more complex. Growth rate of unprotected trees differed between age groups; growth rate was different between unprotected and protected trees within Age 1; but no difference was found at Age 2. Investigating the radial variation, similarly to Clone NM2, vessel area, vessel diameter, and fiber length were smaller near pith and increased toward the bark. Meanwhile, decreasing radial patterns were found for vessel numbers, ray numbers, and ray area.

Comparing clonal and treatment means along the radius, wood properties seemed to differ more during the first three years than from the forth ring toward the bark, which could mean that defoliation may influenced wood properties to some extend even if not statistically significantly.

Summarizing the correlation between wood properties

- There were several significant positive and negative correlations and also no correlations among wood properties. **Density** had a significant **positive** relationship with vessel number and with ray area and number but did not correlated with vessel area and with vessel diameter. Furthermore, there was no correlation between **growth rate** and density.

- Analyzing the **vessel** characteristics, vessel area did not correlate with vessel numbers but had a positive correlation with vessel diameter. Furthermore, vessel number correlated negatively with vessel diameter.
- There were significant **positive** correlations between **fiber length** and vessel area and vessel diameter; **negative** correlation with vessel number, ray area and with ray number. Furthermore, **ray number** positively correlated with vessel number and negatively with vessel area and vessel diameter.
- Furthermore, there were significant **positive** correlations between **growth rate** as stem radius and three wood properties such as fiber length, vessel diameter, and vessel area. Furthermore, stem radius had **negative** correlations with other three wood properties such as vessel number, ray area, and ray number.

CONCLUSIONS

Based on previous studies we know that the cottonwood leaf beetle feeds only on newly formed, succulent leaves. Plantation trees are most susceptible over their first three years when they have large amounts of succulent leaves on the leader and branch tips, growth losses can be considerable. Beetle feeding in year 4 and after is minor. In Iowa, where the sample trees are from, there are usually 3 cycles of insect attack each growing season; between cycles the trees grow new leaves. When tree leaders are heavily damaged by feeding, apical control is lost and new lateral branches are formed. The interest is, if these changes in foliage and growth rates were reflected in properties of the wood formed. Evaluated two clones, NM2, a relatively fast growing tree that is less preferred by the cottonwood leaf beetle, and 91X our fastest growing clone that is highly preferred by the beetle as a food source.

This study confirmed that:

Insect defoliation caused moderate decreases in annual ring **growth** over the first three growing seasons for Clone NM2 and large decreases for Clone 91X. In years 4-6 growth rates returned to normal in unprotected NM2. For Clone 91X growth rates actually were greater in the trees that were previously unprotected. This was probably due to stronger levels of crown competition in the protected trees that were much larger than their unprotected counterparts by age 4. In other words, the unprotected trees had more room to grow when the beetle damage was no longer significant.

This change in growth rates was accompanied by the following changes in wood properties:

Density of the wood was decreased in the first ring of unprotected Clone 91X, but otherwise, both clones showed a relative constant average wood density over the first six years regardless of beetle attack. Therefore, the beetle impact is primarily in terms of the quantity of wood formed, not in the dry matter laid down per unit of radius. Although, insect defoliation did not show significant influence on density but it may have affected the uniformity of density within growth ring and along the radius.

Fiber length tended to follow the typical pattern of increasing length with age of the growth ring in both clones. Fiber lengths were significantly shorter in the first ring of unprotected Clone 91X trees, but that was the only indication that defoliation/re-foliation and the accompanying fluctuations in carbohydrate availability and hormone levels could impact fiber development.

Vessel area did show an increase in unprotected Clone NM2 trees as might be expected in terms of more vascular area being needed to support replacement leaves and additional branching. However, this difference persisted through age 5 in Clone NM2 and it was present only in age 2 and 3 rings in unprotected trees of Clone 91X. This suggests a more complex relationship between canopy changes and vascular support than might be expected. The results of this study suggest that vessel number and vessel diameter are more clearly impacted by beetle damage and that off-setting changes in vessel numbers and diameters partially cancel out changes in vessel area.

Vessel number was markedly larger in unprotected trees of Clone 91X during the first three years when beetle damage was most severe. This was also the case the first year for Clone NM2, but not in subsequent years. **Vessel diameter** was smaller in unprotected trees of both clones over the first three years and then converged with vessel diameter in protected trees over the last three years. A logical conclusion is that defoliation/re-foliation is leading to the production of more vascular elements, but disruptions in carbohydrate supplies and hormone levels is leading to smaller sized cells.

Perhaps the most surprising and significant observations in this study involve the rays. **Ray area** is greater in both clones in unprotected trees, with the effect decaying by year 6 in Clone NM2, but persisting in Clone 91X. **Ray number** tended to be higher in unprotected trees of Clone NM2, but was consistently lower in Clone 91X. This suggests an important function of rays in responding to insect damage in the crown. Clone NM2 responded by producing more rays. Clone 91X responded by producing fewer, but larger rays to give an overall increase in ray area.

Correlations of wood properties revealed positive, negative, and no-correlations. Among statistically significant correlations, poplars in this study with high density had high vessel number and ray area. Also, tree with high stem radius had long fiber, larger vessel diameter, but lower ray area. However, these results may be due to the fact that all anatomical properties showed the highest variation along the radius and had increasing. Age effect of cambium had a strong influence on the anatomical properties. More correlations of wood properties were found in the study of the two clones, and can be further used to design for understanding of crown development effect and wood formation. It needs to be mentioned that the correlations was obviously limited by the very narrow range of density and the clones showed a relatively constant density along the radius, relatively small sample size, and limitation of anatomical measurements. Correlation between wood properties should be investigated at the same age to avoid the very strong affect of the cambium age.

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CHAPTER 4

RELATIONSHIP BETWEEN GROWTH RATE AND WOOD PROPERTIES IN *Populus deltoides* GROWN UNDER STRESSFUL SITE CONDITIONS

Abstract

Twenty-five trees from eight *Populus deltoides* clones of two families (Family 9105 and Family 9163) were sampled. Trees were six-year-old and grown at 2 x 3 meter spacing. Wood samples were cut at breast height to investigate variation in specific gravity, growth rate, and in fiber length. There were no statistical differences in growth rate between the two families; however, specific gravity was significantly influenced by family and by clone. Significant, negative correlation existed between growth rate and specific gravity. Fiber length was measured in two positions along the radius. Variation in fiber length was not affected by family but only by clone and position. At the first radial position (2nd or 3rd growth rings), no clonal differences were found and fibers were significantly shorter than at the second radial position (4th or 5th growth rings), where clonal averages of fiber length were different suggesting that the earliest selection for fiber length should start after four or five years. A positive correlation was found between stem radius and fiber length. Hence, it should be relatively easy to select within families for both growth rate and longer fibers.

Keywords: *Populus deltoides*, poplar clones, wood properties, specific gravity, growth rate, fiber length, radial variation, and wood properties correlation.

INTRODUCTION

Growth rate and wood quality can be affected by silvicultural practices and also by different natural environmental conditions. Most growth rate information was tied to the height, diameter, and stem volume. No signification differences were found in growth rate and specific gravity for four-year old poplars (Murphey et al., 1979). Fiber length was mainly influenced by genetic control and increased by age (Klasnja et al., 2003, Koubaa et al., 1998). It was reported that fiber length was not significantly affected by different growth rates (DeBell et al., 2002 and DeBell et al., 1998).

Evidences suggest that clones may differ in specific gravity under the same site conditions (Cown and Ball, 2001, DeBell et al., 2002, Klasnja et al., 2003, and McKinley et al., 2000). Specific gravity of eleven six- to eight-year old poplar clones ranged from 0.306 to 0.386 (Peter et al., 2002). Three older, 10- and15-year old poplar clones had a specific gravity range of 0.28 to 0.38 (Peszlen, 2001).

Specific gravity of 4-year old poplars was found 0.381 (Klasnja et al., 2003 and Blankenhorn, 1985). Specific gravity was lower in growing condition with fertilizer, and in a combination of fertilization and irrigation condition that the control (Blankenhorn et al., 1985).

Ramets for this study were selected from a nursery trial based on their potential for high growth rate and they grew under high competition from the neighboring trees due to narrow spacing. The hypothesis is that there are no differences in wood properties between two families and within family growing under stressful site conditions.

The objectives of this study are the followings:

1. Investigate the effect of growth on specific gravity and fiber length of eight clones representing two families.
2. Study within-tree radial variation of growth rate and fiber length.
3. Determine correlation among wood properties.

MATERIALS AND METHODS

MATERIALS

The wood samples are from two families, Family 9105 and Family 9163. Both families were open pollinated and showed fast growth. Family 9105 was represented by four clones: Clone 9105.02, Clone 9105.06, Clone 9105.08, and Clone 9105.10. Four ramets were collected from Clone 9105.02, five ramets from Clone 9105.06, three ramets from Clone 9105.08, and three ramets from Clone 9105.10. Family 9163 was represented by four clones: Clone 9163.05, Clone 9163.06, Clone 9163.09, and Clone 9163.22. Four ramets were collected from Clone 9163.09 and the other three clones were represented by two ramets each. The total samples were 25 ramets from eight clones of two families (Figure1).

The eight clones were part of a larger study conducted at Iowa State University (Hall, personal communication). Open-pollinated seeds were collected from good phenotypes in wild stands of eastern cottonwood (*Populus deltoides*). Containerized seedlings were raised in a greenhouse and then transferred to a nursery bed for one year of growth. Selection of individual trees for cloning and field testing was based on seedling diameter growth and low scores for leaf diseases. Clones from Family 9105 were planted in 1995 as part of a replicated test that included other promising clones from the North Central Region of the U.S. (Riemenschneider et al., 2001). Clones 9163.06 and 9163.09 were also planted in 1995 in a germplasm conservation orchard adjacent to the main clonal test. Clones 9163.09 and 9163.22 were added to the conservation orchard in 1996. The wood samples for this dissertation study came from trees removed from the two stands in a thinning done in the year 2001.

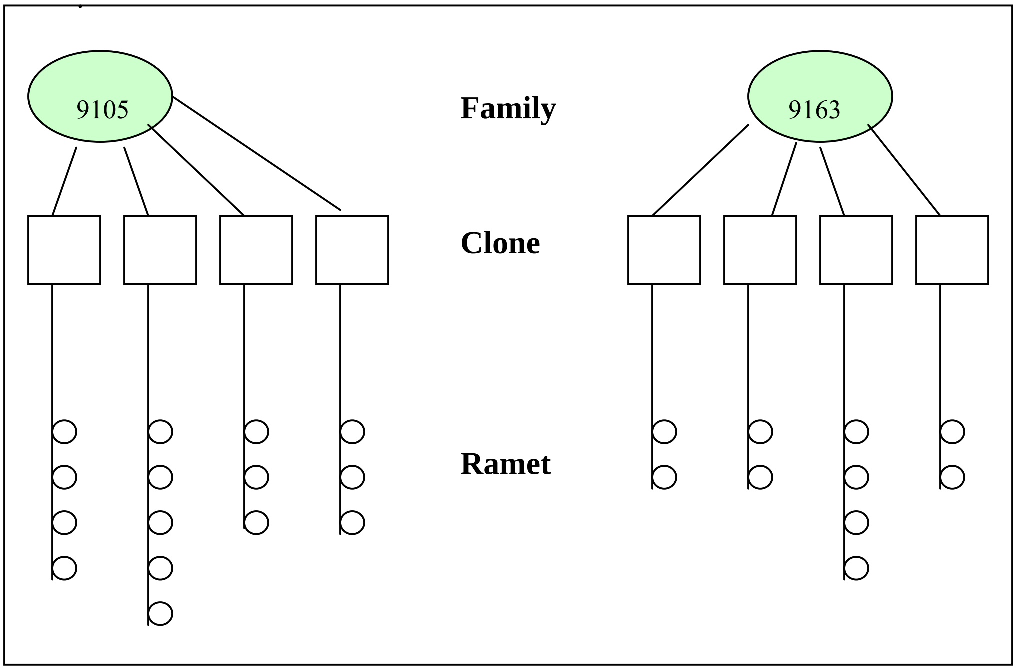
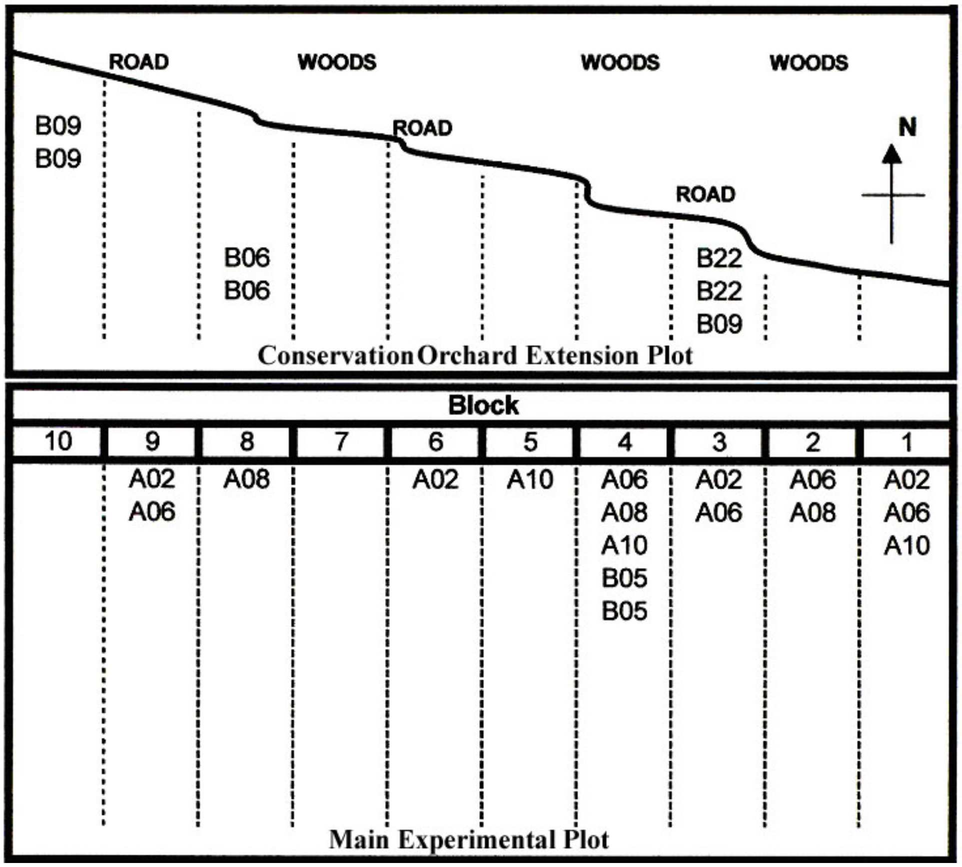


Figure 1 Samples were taken from 25 ramets of eight clones of two families.

Trees were grown in two sites near by the natural forest. One site was a main study plot, and the other site was a conservative orchard extension plot. There were woods and trail between the main study plot and the conservative orchard extension plot. Trail spacing between the sample plots and the neighbor plots was a four-meter distance. The main study plot was located in the south side. There were 10 blocks in this plot. Samples collected from this main plot were Clone 9105.02, Clone 9105.06, Clone 9105.08, Clone 9105.10, and Clone 9163.05. The other three clones (Clone 9163.06, Clone 9163.09, Clone 9163.22) were harvested from the conservative orchard extension plot (Figure 2).



Notes: **A is Family 9105.**
A02 is a ramet of Clone 9105.02.
A06 is a ramet of Clone 9105.06.
A08 is a ramet of Clone 9105.08.
A10 is a ramet of Clone 9105.10.

B is Family 9163.
B05 is a ramet of Clone 9163.05.
B06 is a ramet of Clone 9163.06.
B09 is a ramet of Clone 9163.09.
B22 is a ramet of Clone 9163.22.

Figure 2 Samples of 25 ramets of eight clones from two families are collected from nine blocks of the previous study. There were total 10 blocks in the experiment design. Woods and road were located between the main plot and the conservation orchard extension plot (located in the north). The diagram is not true scale.

` The two sites are very similar in soils and other factors; however, sample trees (B09-Clone 9163.09 and B22-Clone 9163.22) grown on the right part of the conservation orchard extension plot is one year younger.

METHODS

Three wood properties were measured and analyzed in this study: specific gravity, growth rate, and fiber length.

Specific Gravity

Wood disks were collected at 1.30 meter above the ground level. Wood disks were 2.5 cm in thickness. Specific gravity was measured by water displacement method. Two segments were cut and measured for specific gravity for each wood disk.

Specific gravity is measured by dividing the oven-dried weight by the volume of wood, at a specific moisture content and by the density of water, at four degrees Celsius. In practice, specific gravity at green can be measured by dividing the oven-dried weight by volume of the green wood and density of water at room condition. Density of water at room temperature (20 ºC) is 0.99823 g/cm^3.

Wood segments were in green condition during the green volume measurement. Soaking and dipping the sample with water and placing it under vacuum kept the sample in green condition. The sample was first soaked/dipped in the deionized water for six hours, and then sample while immersed in water was placed under vacuum for at least four hours, before the saturated sample was measured for weight.

Equipment: a digital balance (model number: Mettler Toledo AB204-S), a 100 ml glass cylinder, a stand, a disection pointer, and deionized water.

Procedure:

1. A beaker with 75 ml deionized water was placed on the balance and then the balance was set at zero.
2. The wood sample was placed at green condition under the deionized water in the beaker, with help of dissection pointer affixed to the stand.
3. The weight was recorded in grams, to four decimal digits, which is the volume of sample displaced in the deionized water (V_{green}).
4. The sample was taken out of the deionized water and placed at room condition for six hours. The sample was then placed in the oven at 103 ± 2 ºC, until the constant oven-dried weight (OD) was obtained, after letting it cool in the desiccator.
5. The sample was placed at room condition for 72 hours, and then the weight of sample at room condition was recorded in grams.
6. Specific gravity at green condition and moisture content at room condition was calculated.

To calculate the specific gravity (SG_{green}), the following equation was used:

$$SG_{green} = \frac{OD}{V_{green}\rho_{water}} \qquad \text{(Equation 1)}$$

where:

OD = oven dried weight in gram

V_{green} = sample volume at green in cubic centimeter

ρ_{water} = density of water in gram per cubic centimeter

To calculate the percent of moisture content (MC), the following equation was used:

$$MC(\%) = \frac{AD - OD}{OD}(100) \qquad \text{(Equation 2)}$$

Where:

AD = sample weight at room condition in gram

To calculate the specific gravity at the moisture content at room condition, the following equation was used:

$$SG_{MC_{RT}} = \frac{SG_{green}}{1 + 0.01\left(SG_{green}\right)\left(MC_{RT} - MC_{FSP}\right)} \qquad \text{(Equation 3)}$$

where:

MC_{RT} = the moisture content at room condition

MC_{FSP} = the moisture content at the fiber saturation point (FSP) which is approximately around 30% for wood.

Growth Rate

For this study, wood disks of 2.5 centimeters were cut at 1.30 meter above the ground level. The total growth rate was the radius of the stem. To determine the growth rate, width from one growth ring boundary to the next growth ring boundary was measured using a digital caliper under a stereoscope (Figure 3). The ring widths were measured from the inner bark toward the pith of the stem from the wood sample with the pith included. One observation was measured from each wood disk and each ring. It needs to be noted, however, that because sample disks were taken at breath height, the first growth ring near the pith can't be considered to be a full year's growth.

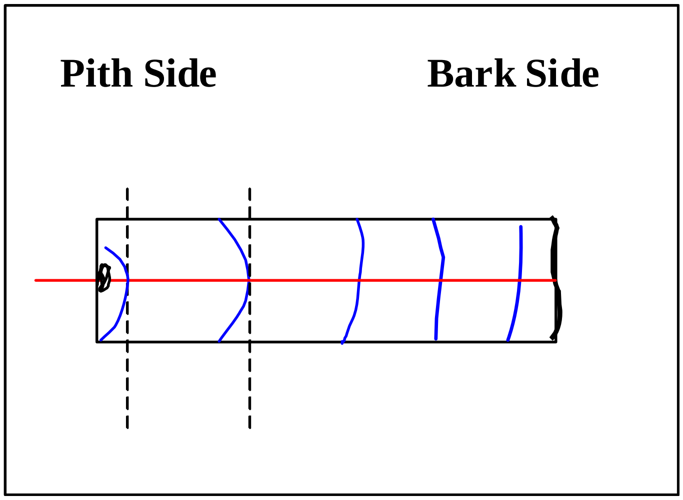


Figure 3 Ring width measurement on the wood segment. The blue lines represented growth ring boundaries. The red line was drawn from the pith toward the bark side. The dark left dash line represented the first ring boundary, while the dark right dash line indicated the second ring boundary.

Equipments: the SMZ800 Nikon stereoscope and a digital caliper (model number: Mitutoyo CD-6"C)

Procedure: The cross section of the increment cores and the wood disks were measured for the annual increment from pith to bark. Ring width was measured by the digital caliper under stereoscope. The growth rate was reported in millimeters per year.

Fiber Length

Fiber lengths of 25 trees were measured by an Image Analyzer System. Depending on the availability of wood materials, fiber length was measured from the second ring and the third ring and defined as Radial Position 1. Fibers measured from the fourth and the fifth rings were defined as fiber length from Radial Position 2.

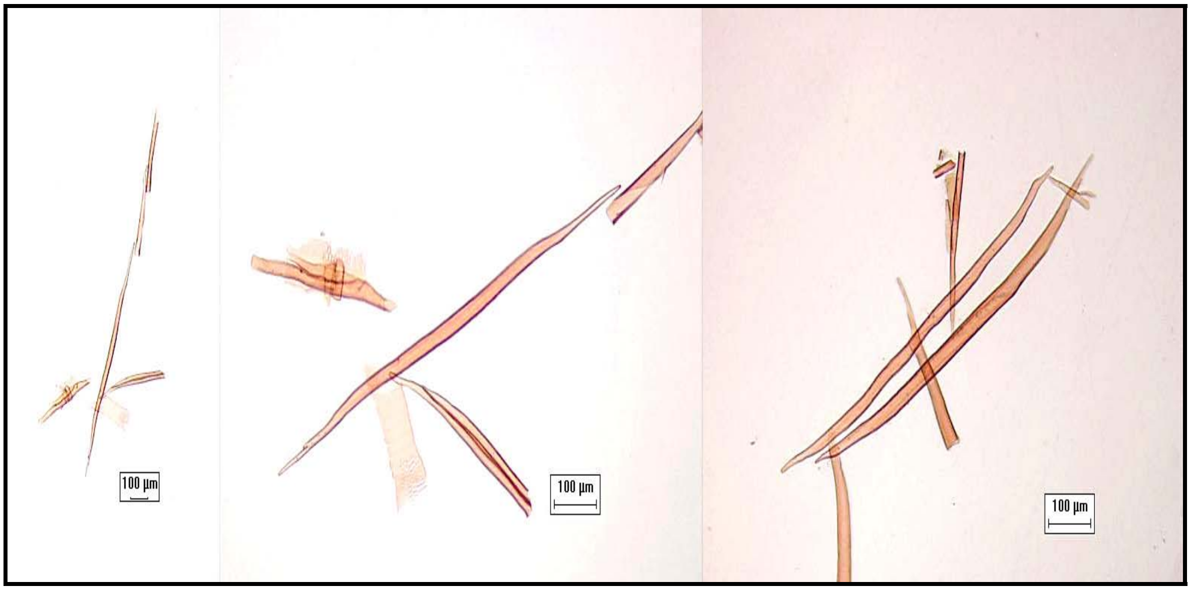


Figure 4 *Left*: Fibers from the third ring of Clone 9105.06 at 4x magnification. The length of this particular fiber was 0.92 mm. *Middle*: The same fiber at 10x magnification. *Right*: More fibers at 10x magnification, one was 0.80 mm long, the other was 0.81 mm long.

The fibers were measured on maceration**.** The maceration was prepared as follows: 10 mg of air-dried wood with 30 ml of 30% hydrogen peroxide solution at 80±1 ºC was boiled for 4 hours, then filtered and the solution washed out was in a coarse filter with deionized water. Wood samples were separated/broken into fibers in 10 ml deionized water, using a glass rod.

The fibers were stained with 1% safranin aqueous solution to improve contrast for the light microscope. From each growth ring, 35 unbroken fibers were measured using an image analyzer. The Image Analyzer System was composed of a light microscope (Nikon model SMZ800), a stereoscope (Nikon model E200), a three charge-coupled device (3CCD) color video camera (Sony model number DXC-390), a digital camera (Nikon model coolpix995), and the Image Pro Plus 4.5 software. The images of fibers, taken at 4X magnification, were shown on a computer monitor. Fibers were manually selected for measurements on the monitor and after fiber length values were recorded, the average fiber length was calculated for each ring and radial position.

Experimental Design and Statistical Analysis

The statistical analyzes used was a randomized block design for specific gravity and growth rate. For fiber length, however, a split plot design with no replication was used. Specific gravity and growth rate were analyzed by Proc GLM, while fiber length was analyzed by Proc Mixed of SAS Version 8.2. The significant difference was tested at alpha = 0.05, unless stated otherwise. Statistical analyses were based on consultation with Professor Dr. Marcia Gumpertz, Department of Statistics, NCSU.

Independent variables were:

1. Family: 2 (9105 and 9163)
2. Clone: 8 (no replication), 9105.02, 9105.06, 9105.08, 9105.10, 9163.05, 9163.06, 9163.09, 9163.22
3. Tree or ramet: 25

Dependent variables were:

1. Specific gravity
2. Fiber length (from ring 2 to ring 5)
3. Growth rate (ring 1 to ring 6)

Different computations were done as the following categories:

Specific gravity and growth rate

Two measurements of specific gravity were taken from each tree. The two measurements on each tree were averaged before further statistical analysis. One measurement of growth rate was taken from each tree. The study samples were 25 ramets. Model I was used to analyze the two families together. Tukey's studentized range test was used to compare individual clones to each other. Specific gravity at green condition was used in analysis.

The linear Model I:

$X_{ijk} = \mu + B_i + C_j + \varepsilon_{ijk}$ (Equation 4)

Where:

X_{ijk} is an observation.

μ = overall mean

B_i = random bolck effect; i = 1, …., 7

C_j = fixed clone effect; j=1,…, 4

ε_{ijk} = random error

Source	**DF**
Clone	4
Block	7
Error	5

Fiber length

The 35 measurements on each ring were averaged before further statistical analyzes. Model II was used to analyze the two families together. Tukey's studentized range test was used to compare individual clones to each other.

The linear Model II:

$$X_{ijk} = \mu + B_i + C_j + \delta ij + R_k + (C \text{ x } R)_{jk} + \varepsilon_{ijk} \qquad \text{(Equation 5)}$$

Where:

X_{ijk} is an observation on of the jth clone of the kth ring.

μ = overall mean

C_j = random clone effect ; j=1,…, 4

δij =random tree effect

R_k = fixed ring effect; k = 1, …, 5

$(C \times R)_{ik}$=ring and clone interaction

ε_{ijk} = random residual error

Source	**DF**
Block	7
Clone	4
Error1= ramet (clone*family)	5
Ring	5
Clone*ring	20
Error2	60

RESULTS

Wood properties of twenty-five ramets of eight clones from two families grown on stressful site conditions were investigated. Five clones of two families grew in the main experimental plot and the other three clones grew in the neighboring conservation orchard extension plot. Differences in wood properties between and within families among clones, correlations among wood properties, and radial variation of wood properties were analyzed.

SPECIFIC GRAVITY

The specific gravity values of all eight clones are shown in Table 1 and Figure 5.

Table 1 Mean and standard deviation (SD) of wood properties such as specific gravity, growth rate (stem diameter, mm), ring width (mm), fiber length (1- Radial Position 1, 2 - Radial Position 2) by clone.

	Specific Gravity		**Growth Rate**		**Ring Width**	**Fiber Length (1)**		**Fiber Length (2)**	
Clone	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**Mean**	**SD**	**Mean**	**SD**
' 9105.06'	0.30	0.016	45.43	9.7582	7.57	0.77	0.0438	0.77	0.0438
' 9105.08'	0.31	0.0126	34.34	1.4576	5.72	0.70	0.0271	0.70	0.0271
' 9105.02'	0.36	0.0138	44.10	12.7872	7.35	0.80	0.0838	0.80	0.0838
' 9105.10'	0.37	0.0368	33.43	3.9836	5.57	0.70	0.0377	0.70	0.0377
' 9163.05'	0.37	0.0106	39.61	9.9655	6.60	0.76	0.1395	0.76	0.7614
' 9163.06'	0.39	0.0035	45.57	0.0141	7.60	0.77	0.0574	0.77	0.7720
' 9163.09'	0.39	0.0163	33.09	5.5721	6.62	0.68	0.0555	0.68	0.0555
' 9163.22'	0.40	0.0156	28.80	8.8789	5.76	0.69	0.0194	0.69	0.0194

Notes: Specific gravity values at green condition were used.

Comparing specific gravity between two families regardless if they grown on the main experimental plot or on the neighboring conservation orchard extension plot, the following results can be drawn. Results from ANOVA are presented in Table 2. There was a significant difference of specific gravity between Family 9105, and Family 9163. Family 9163 had significantly higher specific gravity (0.39) than Family 9105 (0.33). Moreover, there were significant differences in specific gravity among clones within Family 9105.

Results of the analyses of variance within family is presented in Table 3. Clone 9105.10 and Clone 9105.02 had significantly higher specific gravity (0.37 and 0.36) than Clone 9105.08 and Clone 9105.06 (0.31 and 0.30).

Table 2 Analyses of variance for wood properties between families.

Source	**DF**	**SG**		**SR**		**FL(average)**		**FL1**		**FL2**	
		F Value	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**
Family	1	76.47	**<.0001*** **	2.33	0.1452	0.06	0.8141	2.02	0.1731	0.53	0.4774
Clone (Family)	6	9.65	**0.0001*** **	2.05	0.1149	3.93	**0.0120***	1.99	0.123	4.66	**0.0056*** **

Notes: ***, **, *: comparison significant at alpha = 0.001, 0.01, 0.05 respectively**.**
SG: Specific gravity, SR: Stem radius, FL (average): average fiber length by tree, FL 1: fiber length at Radial Position 1, and FL 2: fiber length at Radial Position 2

Table 3 Analyses of variance for wood properties within a family

Source	**DF**	**SG**		**SR**		**FL (average)**		**FL1**		**FL2**	
		F Value	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**
Clone (9105)	3	15.64	**0.0003*** **	2.01	0.1715	1.63	0.2388	3.09	0.0719	0.84	0.5005
Clone (9163)	3	1.28	0.363	2.55	0.1522	12.02	**0.0060*** **	1.12	0.4109	34.23	**0.0004*** **

Notes**:** ***, **, *: comparison significant at alpha = 0.001, 0.01, 0.05 respectively.
SG: Specific gravity, SR: Stem radius, FL (average): average fiber length by tree, FL 1: fiber length at Radial Position 1, and FL 2: fiber length at Radial Position 2

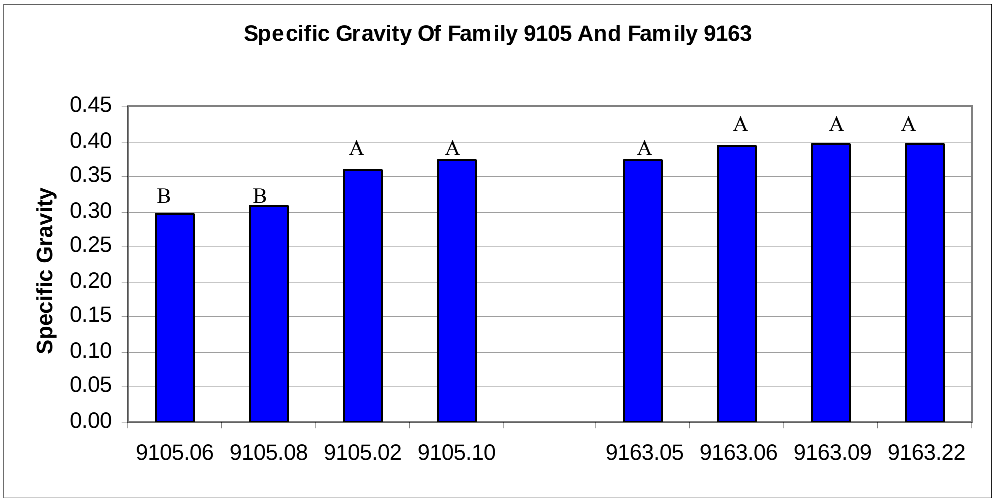


Figure 5 Specific gravity of wood disks for four clones of Family 9105 and for four clones of Family 9163. Means with the same letter were not significantly different.

By Tukey's grouping, specific gravity for the eight clones can be classified into two groups. These two groups were significantly different from each other. The higher specific gravity group included six clones namely Clone 9163.22, Clone 9163.09, Clone 9163.06, Clone 9163.05, Clone 9105.10, Clone 9105.02 and ranged from the highest specific gravity at 0.40 to 0.36. The low specific gravity group included two clones; Clone 9105.08 (0.31), and the lowest specific gravity Clone 9105.06 (0.30).

No significant difference was observed among clones for specific gravity within Family 9163. There was less variation of specific gravity within Family 9163 than in Family 9105. In summary, Family 9163 had relatively high and consistent specific gravity for all four clones.

GROWTH RATE

The total growth rate of each sample stem was the distance from the pith to the inner bark of the stem, which was the radius of the stem.

Growth rate for the eight clones are shown in Table 1 and Figure 6.

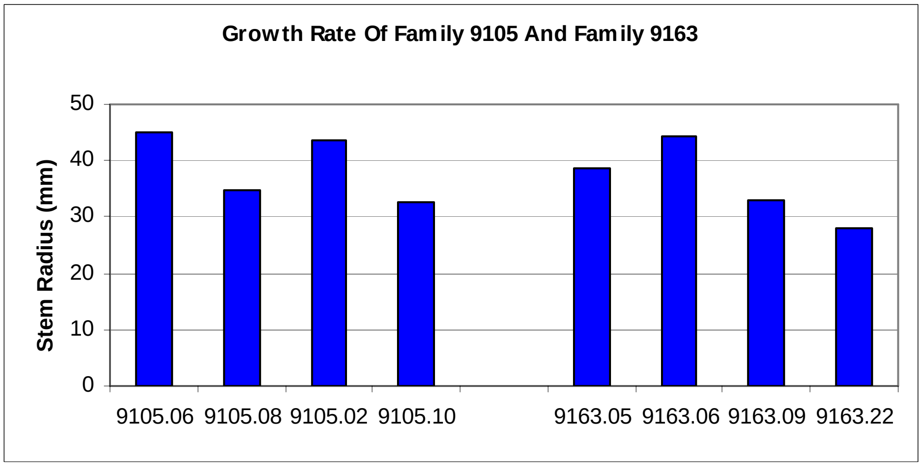


Figure 6 Growth rate as the distance from pith to bark for four clones of Family 9105, and for four clones of Family 9163.

Comparing growth rate between two families regardless if they grown on the main experimental plot or on the neighboring conservation orchard extension plot, the statistic analysis suggested that there was no significant difference in growth rate between the means of Family 9105 (40.20 mm) and Family 9163 (35.42 mm). No significant difference in growth rate was observed among clones within family (Table 3).

In summary, variation of growth rate within Family 9105 was less than variation within Family 9163. However, both families had clones with the highest growth rate close to 45 mm. The two clones with the largest stem were Clone 9105.06 from the main plot and Clone 9163.06 from the neighboring plot.

FIBER LENGTH

Beside tree averages of fiber length values, fiber length for ring 2 or ring 3 (FL1) was presented at Radial Position 1; meanwhile, fiber length for ring 4 or ring 5 (FL2) was presented at Radial Position 2 (Table 2 and Figure 7).

Comparing fiber lengths of two families regardless if they grown on the main experimental plot or on the neighboring conservation orchard extension plot, the results were summarized based on the statistical analysis in Table 2 and Table 3. There was no statistically significant difference found between families but clonal averages differed significantly. Furthermore, there was no significant difference in fiber length within Family 9105 but significant difference was found among clones within Family 9163 (Figure 9).

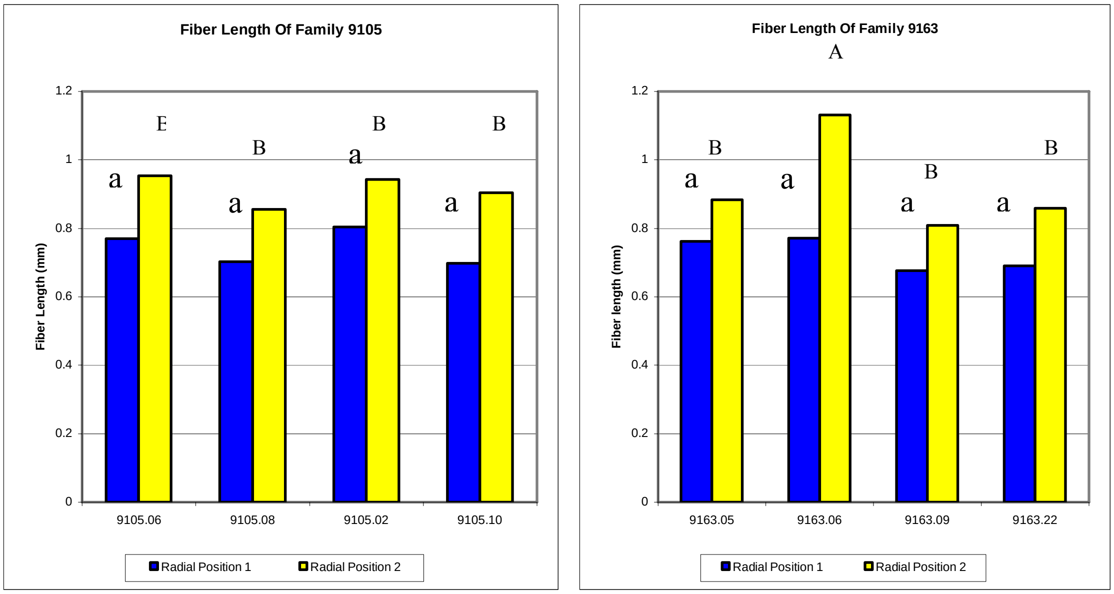


Figure 7 Fiber length measured by two different radial positions: four clones from Family 9105 (left), and four clones from Family 9163 (right). Radial Position 1 represented fiber length of ring 2 or ring 3, while Radial Position 2 represented fiber length of ring 4 or ring 5. Lower case letters are for comparing means of Radial Position 1. Capital letters are for comparing means of Radial Position 2. Clones marked with the same letter were not significantly different.

In Radial Position 1, there was no statistically significant difference in fiber length between Family 9105 (0.75 mm) and Family 9163 (0.72 mm). Furthermore, there were no statistically significant differences among clones within family.

In Radial Position 2, there was no statistically significant difference in fiber length between Family 9105 (0.92 mm) and Family 9163 (0.90 mm). There was no significant difference in fiber length within Family 9105 but significant difference in fiber length was found among clones within Family 9163. Results of the Tukey grouping for fiber length of the clones within Family 9163 was presented in Figure 8.

Mean comparisons by Tukey's studentized range test for fiber length of eight clones were conducted. For Radial Position 1, clones with the same connected line were not significantly different. Meanwhile, for Radial Position 2, clones with the same connected line were not significant differences (Figure 8). However, Clone 9163.06 had significantly longer fibers than the other clones in Radial Position 2.

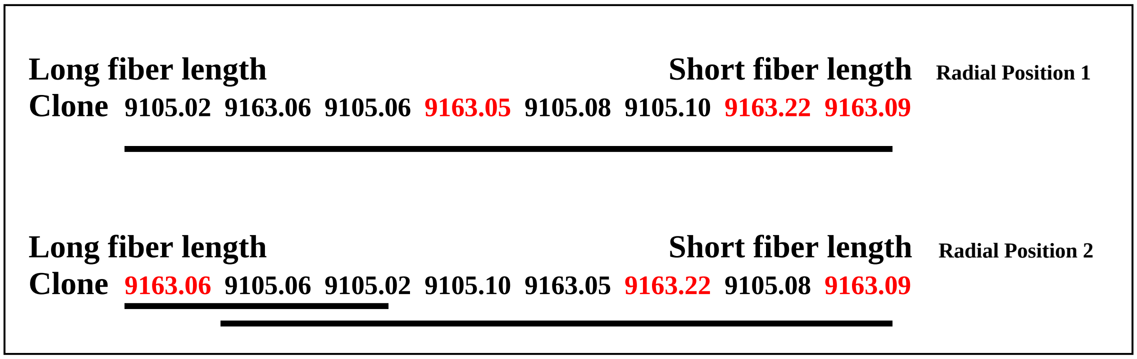


Figure 8 Mean comparison by Tukey grouping for fiber length of eight clones at Radial Position 1 and 2. Clones with the same connected line were not significantly different. Clones with red are from the conservation orchard extension plot.

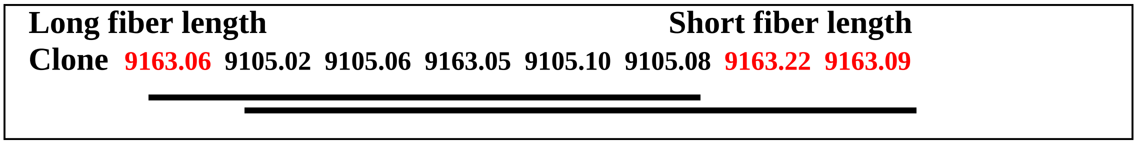


Figure 9 Mean comparison by Tukey grouping for average fiber length of eight clones. Clones with the same connected line were not significantly different. Clones with red are from the conservation orchard extension plot.

For Radial Position 2, results of fiber length by Tukey grouping show that the first three clones with the longest fiber were Clone 9163.06, Clone 9105.06, and Clone 9105.02. These three clones had growth rate above the average (38.29 mm). Clone 9163.06 and Clone 9105.02 had relatively high specific gravity (0.39 and 0.36) and high growth rate. Clone 9105.06 had low specific gravity (0.30) with high growth rate.

For average fiber length, there was significant difference between radial positions. Among the clones, Clone 9105.02 had the longest fibers (0.80 mm) and a relative high specific gravity (0.36) with a relatively fast growth rate (43.72 mm).

CORRELATION AMONG WOOD PROPERTIES

Pearson correlation analysis was conducted on the data at tree level (Table 4). The analysis revealed a negative correlation between specific gravity and growth rate (-0.38, p=0.0585, n=25) (Figure 10). Furthermore, significant correlation was found between growth rate and fiber length (Figure 11).

Table 4 Pearson correlation coefficients, and probability (in parenthesis) of four variables: specific gravity (SG), growth rate (SR), fiber length from ring 2 to 3 (FL1) and fiber length from ring 4 to 5 (FL2). N = 25.

Variable	SG	SR	FL1	FL2
SG	1			
SR	-0.3835 (0.0585)	1		
FL1	-0.2537 (0.2211)	0.6001 **(0.0015**)**	1	
FL2	-0.07124 (0.7351)	0.7310 **(<.0001***)**	0.5749 **(0.0026**)**	1

Notes: ***, **, *, NS, N/A: comparison respectively significant at alpha = 0.001, 0.01, 0.05, not significant, and not available.

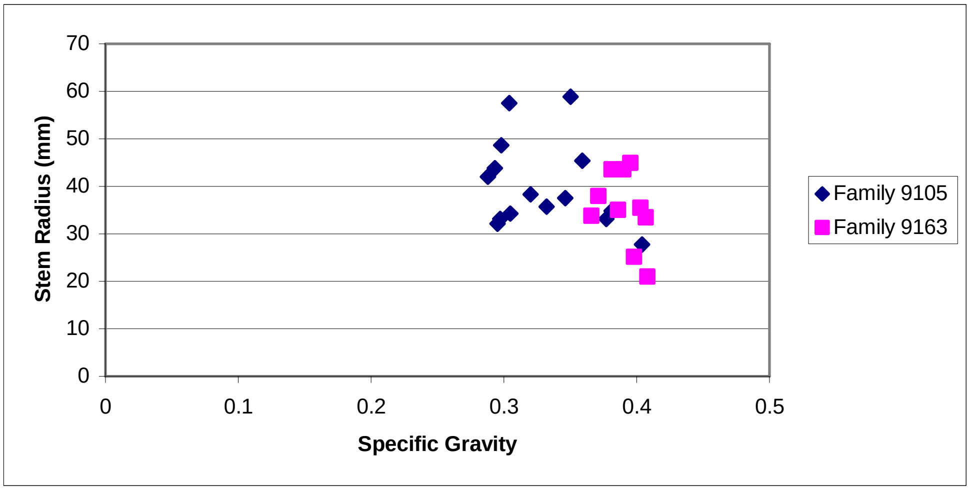


Figure 10 Specific gravity versus growth rate for Family 9105 and Family 9163.

Correlation analysis revealed a significant positive correlation between growth rate and fiber length at both radial positions (Figure 11).

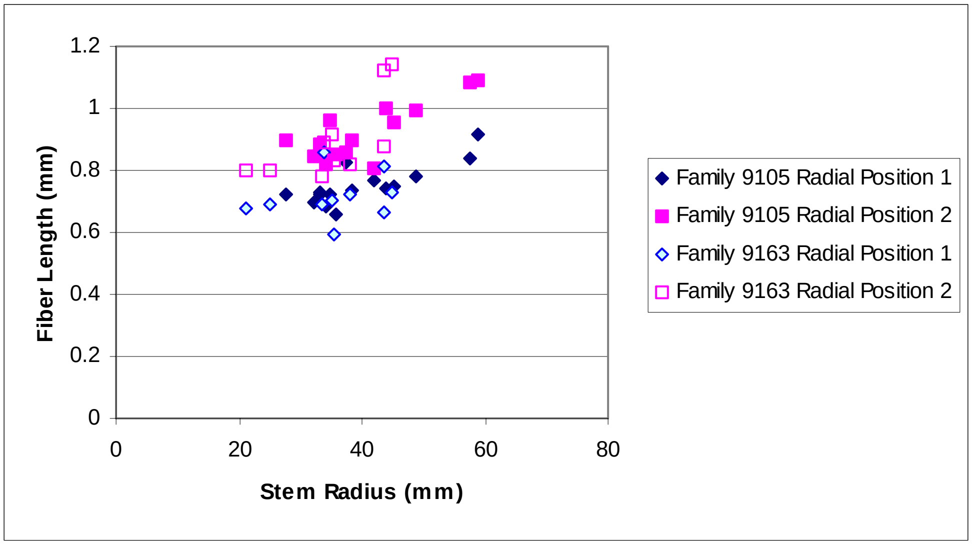


Figure 11 Growth rate versus fiber length for Family 9105 and Family 9163 at Radial position 1 (ring 2 or ring 3) and at Radial Position 2 (ring 4 or ring 5).

RADIAL VARIATION

Radial variations of **growth rate** as ring width were presented in separately for the two families (Figure 12 & Figure 13).

Similar radial patterns were found for the eight clones of two families. Growth rate of the clones increased during the first three years then decreased substantially during the fourth and fifth years but then maintained more or less the same growth during the last year.

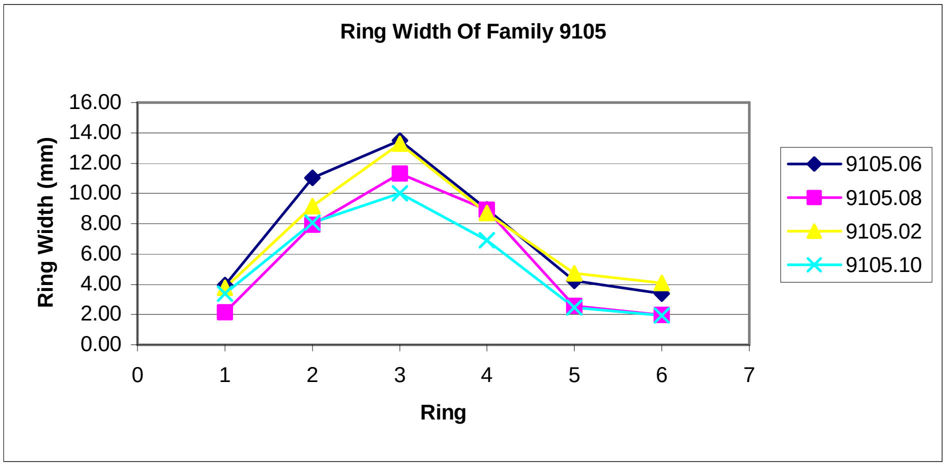


Figure 12 Radial variation of ring width for clones of Family 9105 from pith to bark.

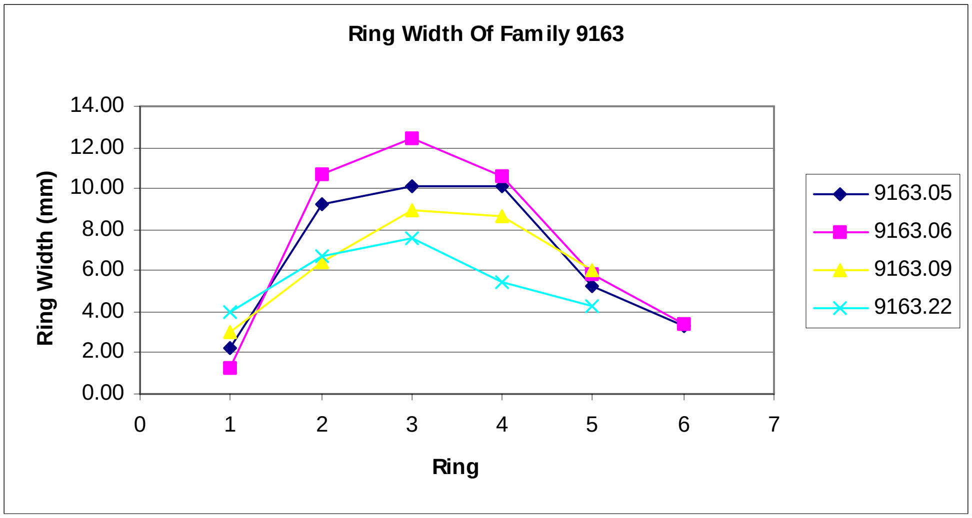


Figure 13 Radial variation of ring width for clones of Family 9163 from pith to bark.

Fiber length means for two radial positions are shown in Figure 14. Based on ANOVA analysis (Table 2), there was no significant difference of the means for fiber length between Family 9105 and Family 9163. Fibers were short at Radial Position 1 and increased up to 27% at Radial position 2 for both families (Figure 14). Fibers were significantly shorter at Radial Position 1 than at Radial position (p <.001).

According to Tukey's grouping there was no significant difference of the means for fiber length among eight clones at Radial Position 1. However, at Radial Position 2, Clone 9163.06 had the longest and significantly different fiber length values from the others (Figure 15). Clone 9105.06 and Clone 9105.02 had long fibers within Family 9105 but the differences were not statistically significant. Clone 9163.06 had the longest fibers within Family 9163.

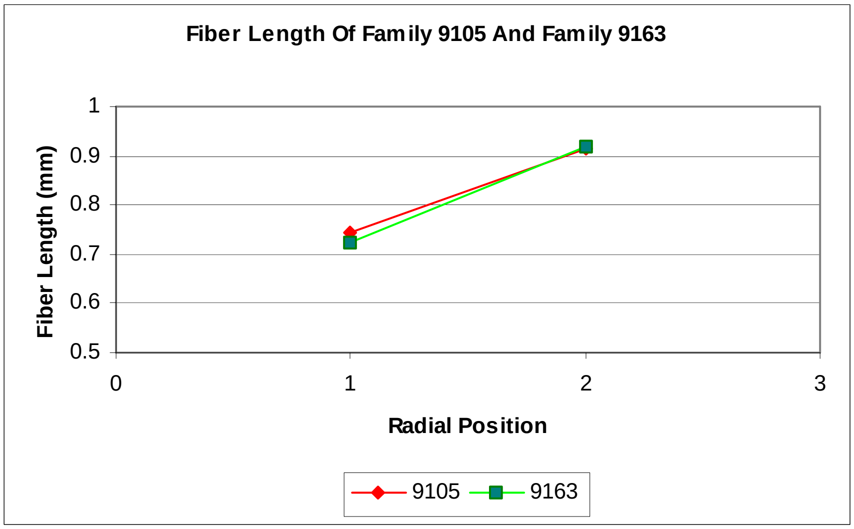


Figure 14 Mean fiber length at two radial positions for Family 9105 and Family 9163. Radial Position 1 represented ring 2 or ring 3 near the pith, while Radial Position 2 presented ring 4 or ring 5 close to the bark.

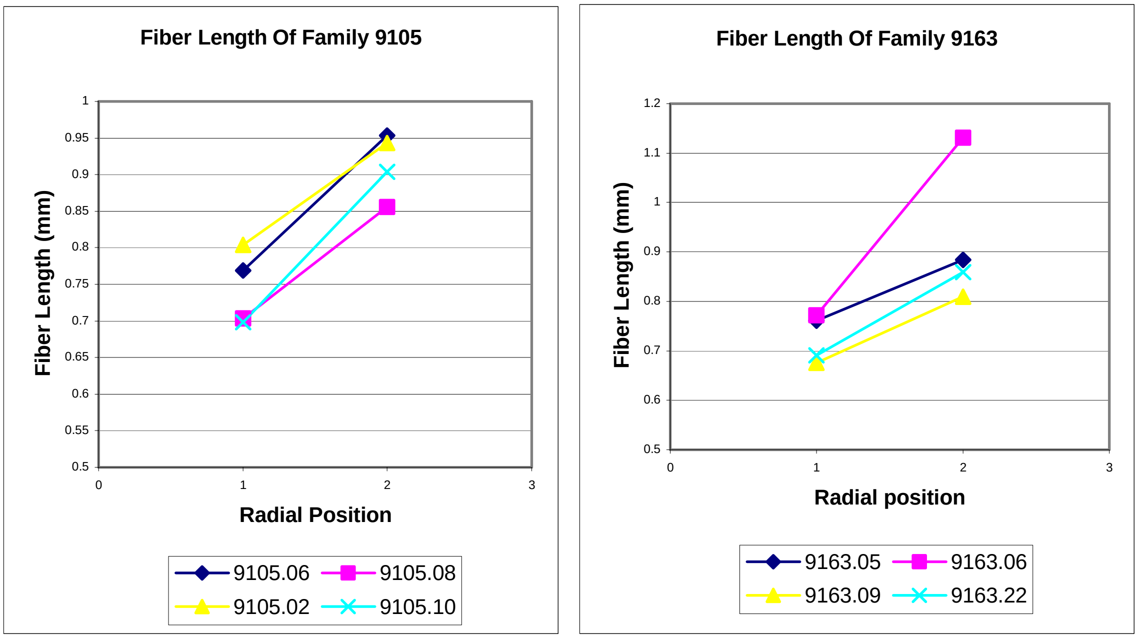


Figure 15 Mean fiber length by clone at two radial positions for Family 9105 (left) and Family 9163 (right). Radial Position 1 represented ring 2 or ring 3 near the pith, while Radial Position 2 presented ring 4 or ring 5 close to the bark.

DISCUSSIONS

Specific Gravity

There was a significant family effect on specific gravity. All four clones of Family 9163 had high specific gravity. Clone effect was significant only within Family 9105, where Clone 9105.06 and Clone 9105.08 had significantly lower specific gravity than Clone 9105.02 and clone 9105.10. Mean specific gravity of Family 9105 ranged from 0.30 to 0.37, and ranged from 0.37 to 0.39 for Family 9163. Peters et al. (2002) found that specific gravity of 6-to 8-year-old poplars ranged from 0.31 to 0.39. Peszlen

(1994) reported that specific gravity values ranged from 0.34 to 0.35 for 10-and 15-year old poplars. Kroll et al. (1992) studied specific gravity of older poplar and fund similar average value of 0.36 for 28- to 56-year old poplars in Minnesota.

Growth Rate

In this study, growth rate was stem radius or the distance from the pith to the inner bark of the stem. No significant differences of growth rate were observed by family, or within a family. Growth rates ranged from 32.76 mm (ring width of 5.57 mm) to 45.03 mm (ring width of 7.57) for Family 9105, and ranged from 28.07 mm (ring width of 5.76 mm) to 44.22 mm (ring width of 7.60 mm) for Family 9163.

Two clones (Clone 9163.09 and Clone 9163.22) were one year younger; Clone 9163.06 was the six-year of age and had the growth rate at the sixth year as three mm ring width. With an assumption that the one year younger clones can grew three mm in the sixth year. Then growth rate for Family 9163 could be ranged from 31.07 mm to 44.22 mm. The adjusted range was consistent with Family 9105 grew in the main experimental plot.

These two families had slightly lower growth rate than the findings by DeBell et al (1998). DeBell et al. (1998) reported growth rates of 7-year-old poplars ranged from 30 to 65 mm.

Clone 9105.06 was low in specific gravity (0.30) with high growth rate but Clone 9163.06 was high in both specific gravity (0.37) and growth rate. There were three clones with high specific gravity that had growth rate above the average at 38.29mm. Those were **Clone 9105.02**, Clone **9163.05,** and **Clone 9163.06**. Clone 9163.05, and Clone 9163.06 had similar codominate crown position at the planting developed. Clone 9105.02 had intermediate crown position. A wide range of crown positions were found from intermediate to dominate for different ramets of Clone 9105.06.

Comparing clones based on all wood properties measured, for Radial Position 2, Clone **9163.06**, Clone 9105.06, and Clone **9105.02** had the longest fibers. These three clones had growth rate above the average (38.29 mm). Clone **9163.06** and **Clone 9105.02** had relatively high specific gravity (0.39 and 0.36) and high growth rate. Clone 9105.06 had low specific gravity (0.30) with high growth rate.

Fiber Length

In this investigation, fiber length ranged from 0.69 mm to 0.95 mm for Family 9105, and ranged from 0.68 mm to 1.13 mm for Family 9163. In other studies, fiber length of 7-year-old hybrid poplars from extremely different growth rates ranged from 0.45 mm to 1.09 mm (Debell et al., 1998). For 10- to 15-year old poplars, mean fiber length ranged from 1.03 mm to 1.17 mm (Peszlen, 1994).

For tree average fiber length, there was significant difference between radial positions. In addition, significant difference was found among clones within Family 9163 at Radial Position 2. However, there was no significant difference among clones within family at Radial Position 1 for both families.

Comparing clones based on all wood properties measured, for Radial Position 2, Clone 9163.06, Clone 9105.06, and Clone 9105.02 had the longest fibers. These three clones had growth rate above the average (38.29 mm). Clone 9163.06 and Clone 9105.02 had relatively high specific gravity (0.39 and 0.36) and high growth rate. Clone 9105.06 had low specific gravity (0.30) with high growth rate.

Among the clones, Clone 9105.02 had the longest fibers (0.80 mm) and a relative high specific gravity (0.36) with a relatively fast growth rate (43.72 mm).

Correlations Among Wood Properties

There was a negative but statistically not significant at p=0.05 correlation between specific gravity and stem radius. However, there was a significantly positive correlation between stem radius and fiber lengths just like in the previous study on the insect effect on wood properties. This result was consistent with findings of another study. DeBell et al. (1998) also found positive correlation between growth rate and fiber length for 7-year old poplars with different growth rates.

Radial Pattern of Growth Rate

In general, similar radial patterns were found for the eight clones of two families. Growth rate of the clones increased during the first three years then decreased substantially during the fourth and fifth years but then maintained more or less the same growth during the last year.

The radial pattern was different from the result of 'Impact of the cottonwood leaf beetle (*Chrysomela scripta*) on wood formation' of the previous chapter. However, it was consistent with the radial pattern of seven- year old poplars found by DeBell et al. (1998).

SUMMARY

Based on this study on different wood property traits of eight 6-year old *P. deltoides* clones of two families growing under stressful site conditions, the following conclusions can be drawn:

- Regarding **family** differences, there was significant difference in specific gravity but not in growth rate and fiber length between the two families.
- Regarding **clonal** differences within family, there were significant differences in specific gravity among clones within Family 9105 only and fiber length among clones within Family 9163. However, no significant difference in growth rate was observed among clones within family.
- For fiber length, there was significant difference between radial positions. In addition, significant difference was found among clones within Family 9163 at Radial Position 2. However, there was no significant difference among clones within family at Radial Position 1 for both families.

- For **Family 9105** had two different **specific gravity** groups: one with a relative high specific gravity (Clone 9105.10 and Clone 9105.02), the other group had relative low specific gravity (Clone 9105.06 and Clone 9105.08). Two clones had higher but not significantly higher **growth rates** than the average of the eight clones: Clone 9105.06 with low specific gravity and Clone 9105.02 with a relative high specific gravity. Clone 9105.06 had long **fiber**s but not significantly longer than fibers of the other three clones

- **Family 9163** has relatively high and consistent **specific gravity** for all four clones. The two clones that had the **longest fiber** were Clone 9163.06, and Clone 9163.05. Both clones were high in specific gravity and large **growth rate**.
- There was a positive correlation between growth rate and fiber length. The correlation was higher at Radial Position 2 than at Radial Position 1. This suggests that either growth rate or fiber length can be an indicator for clone selection.

CONCLUSIONS

For this study, two families growing under stressful site conditions were selected based on their relative fast growth. There were no statistical differences in growth rate between two families; however, specific gravity was significantly influenced by family and by clone.

A low, but significant, negative correlation exists between growth rate and specific gravity in these poplar families just as it was found in previous studies of open-pollinated families and selected clones (See next Chapter).

Fiber length was not affected by family but only by clone and position. For these two families, fibers were significantly longer at the fourth and fifth growth rings than at the second and third rings suggesting that the earliest selection for fiber length should start after four or five years only.

The results showed a positive correlation between stem radius and fiber length. Hence, it should be relatively easy to select within families for both growth rate and longer fibers.

Selecting for both growth rate and higher specific gravity will be more difficult. More research is needed to determine the underlying reasons for this negative relationship and what can be done to improve breeding and selection for both growth rate and high specific gravity.

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CHAPTER 5

VARIATION IN WOOD PROPERTIES OF *Populus deltoides* CLONES UNDER EFFECTS OF GROWTH RATE AND SPECIFIC GRAVITY

Abstract

Eastern cottonwood (*Populus deltoides*) wood samples were taken from a four-year-old progeny test where 72 trees selected for fast growth and pest resistance exhibited a general trend of decreasing specific gravity with faster diameter growth. Five trees showing exceptions to this trend were used in this study. The trees were classified into medium and fast growth rates and further into very low, low and high specific gravity groups. For each tree fiber length; vessel number, diameter and area; and ray number, width and area were measured and analyzed across the growth rings.

There were significant differences among trees in fiber length and vessel area but not in vessel numbers, vessel diameter, and in ray numbers. The age of the cambium significantly affected all wood properties. Longer fibers tend to be associated with the faster growing trees. There was also a positive relationship between growth rate and specific gravity. However, both effects are primarily due to one exceptional tree with high specific gravity and fast growth. The fastest growing tree with high specific gravity had the longest fibers, the highest ray numbers but low vessel area, vessel number, and low vessel diameter representing an unusual combination of traits. Results indicate that trees with similar growth rates can have wide variation in vessel area and that exceptionally fast growth can be achieved by a tree with low vessel area.

Keywords: *Populus deltoides*, poplar clones, wood properties, specific gravity, growth rate, fiber length, vessel area, vessel number, vessel diameter, radial variation, and wood properties correlation.

INTRODUCTION

Many poplar research results were conducted at different clones, ages, and site conditions. A few reports had presented the weak or no correlation between growth rate and wood properties. There was no significant correlation between growth rate and specific gravity for four-year-old poplars (Murphey et al., 1979). No correlation was found by a study on six-month-old shoot stems between growth rate and wood properties (Jourez et al., 2001). However, fiber length, vessel area, and vessel lumen within the stem were reported. Fiber length was 0.57 mm at the pith side and was long 0.60 mm near the bark. Vessel number was small near the pith and increased near the bark. Vessel lumen area was two times higher near the pith than near the bark. In another study, average fiber length of 40 poplar clones (four-year-old) was 0.863 mm (Klasnja et al., 2003).

Within the frame of the Biofuels Development Program at Iowa State University, growth rate as height and stem diameter were investigated and a negative correlation between stem diameter and specific gravity were found (Hall, 2004 personal communication). For this study, five sample trees (clones) were selected for further investigation with extreme low and high density. Out of the five clones, one clone had high growth rate and the other four had medium growth rate (diameter inside bark). The null hypothesis of this study was: there is no difference in wood formation of poplars with different growth rate and specific gravity.

Objectives of the study are as follows:

1. Study variation in anatomical properties of trees with different specific gravity but having the same growth rate, and of trees with different specific gravity and growth rate.
2. Determine radial variation in anatomical properties (fiber length and vessel area, vessel number, vessel diameter) in relation to age.
3. Compare wood properties of trees with similar growth rate to the high growth rate and high specific gravity.

MATERIALS AND METHODS

MATERIALS

A four-year-old progeny test was the source of the five sample trees in this study. The test was established on a bottomland site at the Iowa State University (ISU) Moore Research farm near the main campus in Ames, Iowa. Spacing between rows was three meters, while spacing between trees was 0.3 meters. Most of the progeny represented open-pollinated families of *P. deltoides* females made in wild stands in Iowa. Ten trees of each family were planted in a row plot at a randomized location in each of three replicate blocks on the site. ISU personnel measured the heights of all 1600 trees in the test and then measured diameter at breast height on the tallest trees of the best families. Based on those measurements, 72 trees from 32 families were selected for further study and cloning. Stem disks were cut from the trees at 1.3 meters above ground and an initial survey of whole stem specific gravity was done at ISU by the water displacement method described in the next section. A plot of these data is shown in Figure 1. On that graph, the data point for each of the five trees used in this study is circled and the selected tree number is given. The trees were chosen to observe the wood properties in relatively fast-growth (Tree 702) and moderate-growth (Tree 302, 303, 1801,and 2801) trees with outlier values for high (Tree 702, 1801, and 2801) and low (Tree 302 and 303) specific gravity. Trees 302 and 303 were selections from the same family. Trees 702, 1801, and 2801 were from three other families.

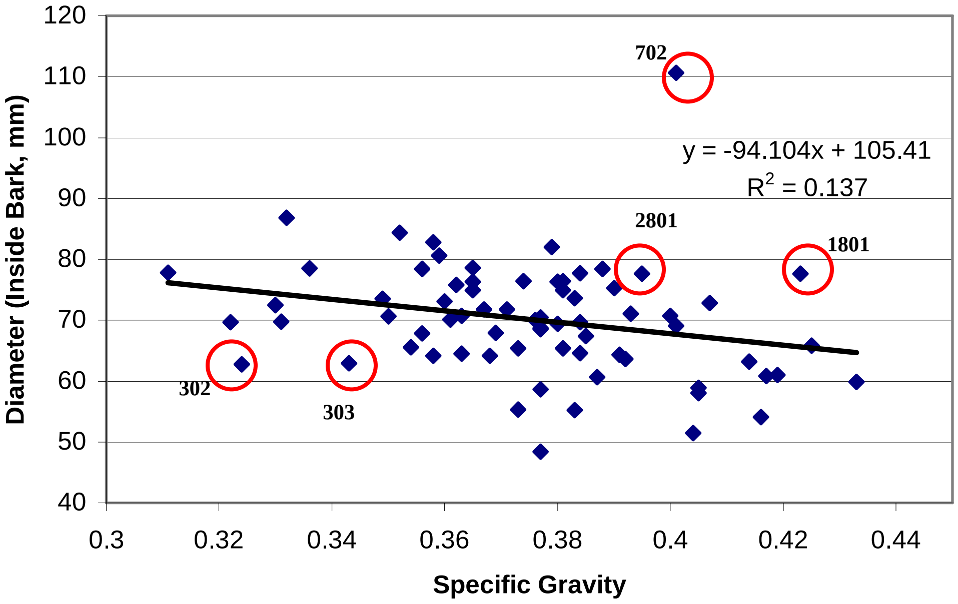

Figure 1 Specific gravity and diameter (inside bark) of 32 families and 72 ramets from the previous ISU study. Dots within the red circles were selected for this investigation.

METHODS

Wood properties of five trees were measured and analyzed in this study. Wood properties were specific gravity, growth rate, fiber length, and anatomical characteristics (vessel area, vessel number, ray area, and ray number). Measurement methods of these properties were described as the following.

Specific gravity

Wood disks were collected at 1.30 meter above the ground level, placed in a plastic bag and frozen. Wood disks were 2.5 cm in thickness. Segments of wood were cut and specific gravity was measured by water displacement method. One segment was cut and measured for specific gravity for each wood disk. Wood segment at green condition was replaced in the water at room temperature which water vessel was set on the balance. Volume of green wood was obtained from its weight in the water. The wood segment was dried at 103 ± 2° C in the oven until obtained its constant weight. Specific gravity at green can be measured by dividing the oven-dried weight by volume of the green wood and density of water at room temperature. Density of water at room temperature (20 ºC) is 0.99823 g/cm^3.

Growth Rate

In this study, inside bark diameter was determined at breast height and the average of the largest and the smallest diameters of each disk was reported in millimeter (mm).

Fiber Length

Fiber length of five trees was measured by an Image Analyzer System from pith to bark. Fibers were macerated as follows: 10 mg of air-dried wood with 30 ml of 30% hydrogen peroxide solution at 80±1 ºC was boiled for 4 hours, then filtered and the solution washed out was in a coarse filter with deionized water. Wood samples were separated/broken into fibers in 10 ml deionized water, using a glass rod.

Fibers were stained with 1% safranin aqueous solution to improve contrast for the light microscope. From each growth ring, 35 unbroken fibers were measured using an image analyzer. The Image Analyzer System was composed of a light microscope (Nikon model SMZ800), a stereoscope (Nikon model E200), a three charge-coupled device (3CCD) color video camera (Sony model number DXC-390), a digital camera (Nikon model coolpix995), and the Image Pro Plus 4.5 software. The images of fibers at 4x magnification were shown on a computer monitor. Fibers were manually selected on the monitor and a mouse was used to locate the two ends of a fiber and fiber length was calculated by Image Pro Plus 4.5 software.

Anatomical Characteristics

Four types of wood anatomical structures were measured by an image analyzer which was the same system used for fiber length measurement but with different applications. Selected wood properties were measured on cross sections mounted on microscopic slides.

Slide preparation: Samples with one centimeter width were cut out from a wood disk from the pith to the bark. Cross sections with 15 µm were cut by a microtome (serial number 5795 by Spencer Lens Co., Buffalo N.Y.). Then the sections were stained using 1% aqueous safranin solution for a few minutes, washed with deionized water, and dried on the warm plate at 75±5 °C for a few minutes. The dried sections were mounted on glass slides with Permount (Fisher Scientific) and xylene before covering with a cover glass.

Measurement of vessel area, vessel number, ray area, and ray number: The permanent slides were examined with light microscope; five randomly selected areas of each growth ring were sampled from the earlywood zone to the latewood zone. Digital images of the selected areas were taken. If needed, images were manually adjusted for automatic measurements. Images of vessels and rays were taken at 10x magnification. Size, number, and area percentage of different elements were measured on the images of selected areas by the Image Pro Plus 4.5 software. A total of 100 images were taken from four rings of five sample trees. The size of an image was 827.288 x 1103.628 square micrometers (0.913 mm^2) and measurements were recalculated to 1 mm^2 basis.

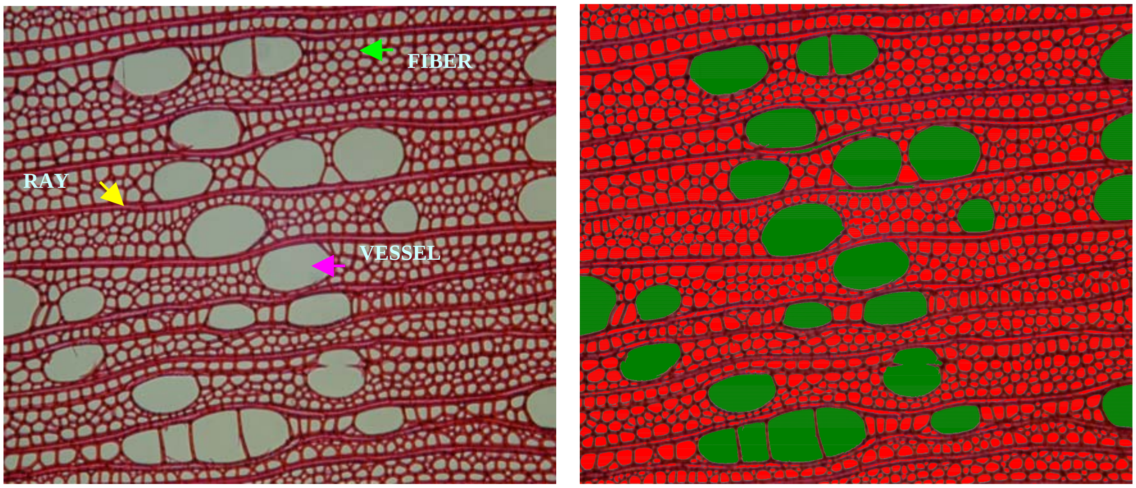

Figure 2 On the left, cross section of ring 3 from Tree 702 at 10x magnification prior to vessel measurement shows rays as horizontal lines (yellow pointer) and vessel lumens (pink pointer). The small diameter cells are fibers (green pointer). On the right image, cross section is shown after measurements where vessel area of 16.93% (green) and vessel number of 28 were recorded.

On the cross sections, vessel area (%), vessel number, vessel diameter (µm). were measured by the image analyzer on each image. Ray numbers were manually counted across the width (827.288 µm) of the image and recalculated to 1 mm^2 basis. Widths of fifteen rays were measured for each image and the average width was used to calculate ray area (%) by multiplying it with the ray numbers.

STATISTICAL ANALYSIS

Data analysis was performed by SAS program version 8.2. Data were categorized into three sets depending upon availability of observations. Different computations were done as these following categories.

1. Specific gravity and Growth rate.

Only one measurement of specific gravity and growth rate (inner bark diameter) were taken from each tree. Since there was only one measurement from each tree, no statistical analysis was done for these two properties. Specific gravity at green condition was used in this report.

2. Fiber length, vessel area, vessel number, vessel diameter, ray area, and ray number.

Since only five trees were studied, limited statistical analysis could be done on these samples. A randomized complete block analysis of variance was done, treating each tree as a block and ring as a fixed factor. Contrasts among specific trees were of interest; these were tested using Student's T-Test.

The following model (Equation 1) was used:

The linear Model I:

$$X_{ij} = \mu + T_i + R_j + \varepsilon_{ij} \qquad \text{(Equation 1)}$$

Where:

X_{ij} is an observation on the ith tree from the jth ring.

μ = overall mean

T_i = fixed tree effect; $i = 1, \ldots, 5$.

R_j = fixed ring effect; $j=1, \ldots, 4$

ε_{ij} = random residual error

Source	**Degree of freedom**
Tree sample	4
Ring	3
Error	12

A parameter was considered as a significant source of variation for a level of significance of 5%, unless otherwise stated.

Dependent variable

1. Specific gravity
2. Fiber Length
3. Growth rate
4. Anatomical characteristics: vessel area, vessel number, vessel diameter, ray area, ray number

RESULTS

Variation in wood properties such specific gravity at green condition, growth rate, fiber length, vessel area, vessel numbers, ray area, and ray numbers and correlation among wood properties were investigated for the four-year old sample trees. Results were presented in two sections. In the first section, the results of analyses of variance of wood properties using Model I are presented. In the second part, discussions of radial variation are included.

WOOD PROPERTIES BY TREE

The following results of wood properties were based on two levels of growth rate (medium and high), and three levels of specific gravity (very low, low, and high) effect in Model I. Since Tree 702 had the highest growth rate with high specific gravity, it was in the center of interest. Therefore, Tree 702 was selected to use it as a base for comparisons. In other words, all properties of Tree 702 are compared to properties of all others individually and also as a group. No comparisons for mean values of specific gravity and growth rate were given because only one measurement per tree was available.

Specific Gravity

The tree samples were selected based on specific gravity and growth rate from 32 families in the experimental plot and had an average population specific gravity of 0.375. **Tree 302** had very low specific gravity (0.324). **Tree 303** was low specific gravity

(0.343). **Tree 2801** (0.395), **Tree 702** (0.401), and **Tree 1801** (0.423) had high specific gravity values (Figure 3, Table 1).

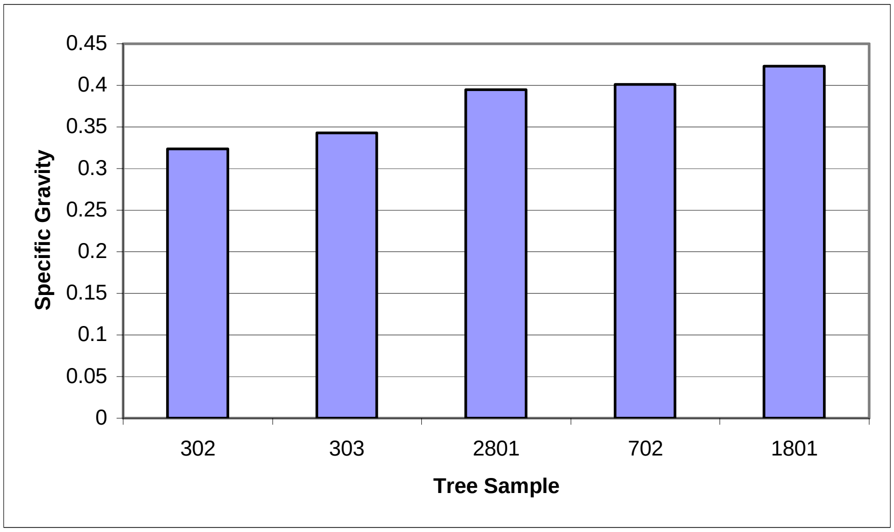

Figure 3 Mean specific gravity of the five selected trees

Growth Rate

Diameter inside bark (DIB) was computed as an average of the largest and the smallest for each tree. Growth rate as diameter inside bark was presented in Figure 4 and in Table 1. **Tree 702** had the highest growth rate with an average diameter inside bark of 110 mm. The other four trees had medium growth rates with average diameter inside bark ranged from 62.8 to 77.6 mm. Only one measurement of growth rate was taken for each tree.

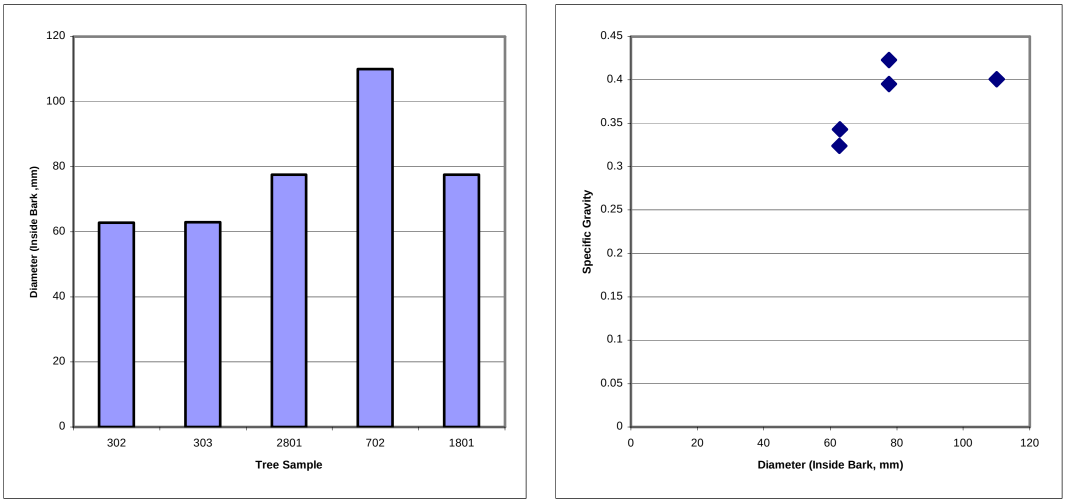

Figure 4 Growth rate as diameter inside bark (left) and relation between diameter inside bark and specific gravity (right) of the five selected trees.

Fiber Length

Mean fiber length ranged from 0.58 mm to 0.69 mm among the five trees (Figure 5, Table 1). **Tree 702** had the longest fiber length of 0.69 mm with standard deviation (SE) of 0.1148, while it had also the highest growth rate and high specific gravity. **Tree 302** had the shortest fiber length of 0.58 mm (SE=0.0783), the smallest growth rate, and very low specific gravity.

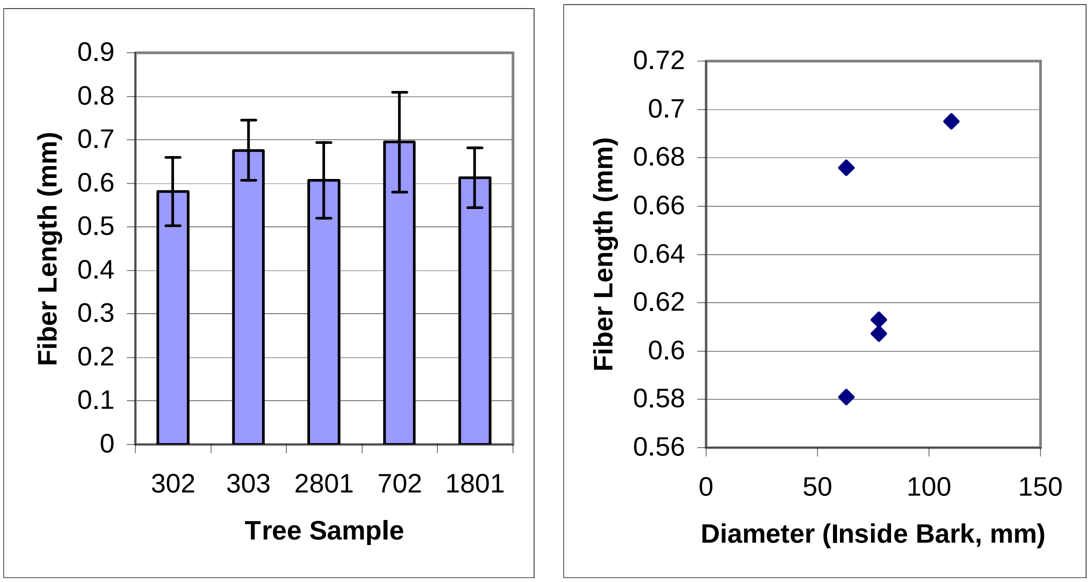

Figure 5 Fiber length and standard deviation (left) and diameter (inside bark) versus fiber length (right) of the five selected trees.

Results of the statistical analysis of mean **fiber length** computed by Model I (Table 2):

- There were significantly differences in fiber length among trees.
- There were also significantly differences in fiber length by ring.
- Tree 702 had the longest fiber (0.69 mm) and had significantly higher fiber length than Tree 302 (0.58 mm) by Tukey grouping.

Results of T-test comparison (contrast) between and among trees for **fiber length** (Appendix C1):

- For all five trees, **Tree 702** had significantly higher fiber length than the **average** fiber length of the other four trees, Tree 302, Tree 303, Tree 1801, and Tree 2801 with medium growth rate and different specific gravity.
- Comparing **Tree 702** with the other trees **individually**, it had the longest fibers and significantly higher fiber length than Tree 302, Tree 2801, and Tree 1801. However, no significant difference was observed for fiber length between Tree 702 and Tree 303.
- Comparing the **high specific gravity trees** with each other, Tree 702 with high growth rate had a significantly longer fiber length than Tree 2801 and Tree 1801with medium growth rates.
- Comparing **very low and low specific gravity** trees with medium growth rate, Tree 303 had significantly higher fiber length than Tree 302.
- Comparing trees with **medium growth rate**, only Tree 303 with low specific gravity had significantly longer fibers than Tree 2801 with high specific gravity.
- Comparing **within medium growth rate trees**, no significant differences were found for fiber length between trees with very low and low specific gravity (Tree 302 & Tree 303) and high specific gravity trees (Tree 1801 &Tree 2801).

Vessel Area

Mean vessel area of the five trees ranged from 18.43 % to 30.66 % (Figure 6, Table 1). Trees with a high percentage of vessel area were **Tree 302** (30.66 % and

SD=3.30), and **Tree 303** (24.17 % and SD=3.08). These trees were categorized as very low and low in specific gravity with the same medium growth rate. Trees with the low vessel area were **Tree 1801** (18.43 % and SD=2.25) with medium growth rate and **Tree 702** (19.55 % and SD=0.56) with the highest growth rate but both had high specific gravity.

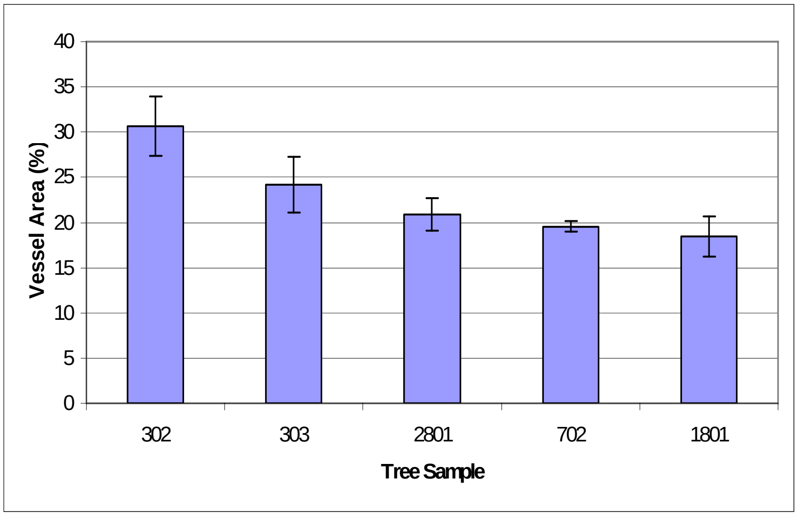

Figure 6 Mean vessel area and standard deviation of five trees.

Results of the statistical analysis of mean **vessel area** computed by Model I (Table 2):

- There were significantly different in vessel area among trees.
- There were also significantly different in vessel area by ring.
- Tree 302 had the highest vessel area (30.66 %) and significantly higher than Tree 1801 (18.43%) by Tukey grouping.

Results of T-test comparison (contrast) between and among trees for **vessel area** (Appendix C2):

- For all five trees, **Tree 702** had significantly lower vessel area than the **average** vessel area of the other four trees, Tree 302, Tree 303, Tree 1801, and Tree 2801 with medium growth rate and different specific gravity.
- Comparing **Tree 702** with the other trees **individually,** Tree 702 had significantly lower vessel area than Tree 302 and Tree 303 but did not differ significantly from Tree 1801, and Tree 2801.
- Comparing the **high specific gravity trees** with each other, there were significant differences in vessel area found between Tree 1801 and Tree 2801 but did not differ between Tree 702 and the others.
- Comparing **very low and low specific gravity** trees with medium growth rate, Tree 303 had significantly higher vessel area than Tree 302.
- Comparing trees with **medium growth rate individually**, Tree 302 with very low specific gravity had significantly higher vessel area than Tree 1801 and Tree 2801 with high specific gravity. Tree 303 with low specific gravity had significantly higher vessel area than Tree 1801 and Tree 2801 with high specific gravity.
- Comparing **within medium growth rate trees**, significant higher vessel area were found for trees with very low and low specific gravity (Tree 302 & Tree 303) than for high specific gravity trees (Tree 1801 &Tree 2801).

Vessel Number

Vessel numbers of all samples ranged from 85 to 120 (Figure 7, Table 1). **Tree 302** with very low specific gravity, the smallest growth rate, and the highest vessel area had the highest vessel numbers (120 and SD=32.76). **Tree 303** with the low specific gravity, medium growth rate, and with a relatively high vessel area had the second highest vessel numbers (103 and SD=8.58). The lowest vessel numbers (85 and SD =40.50) were found in **Tree 1801** with high specific gravity, medium growth rate, and low vessel area.

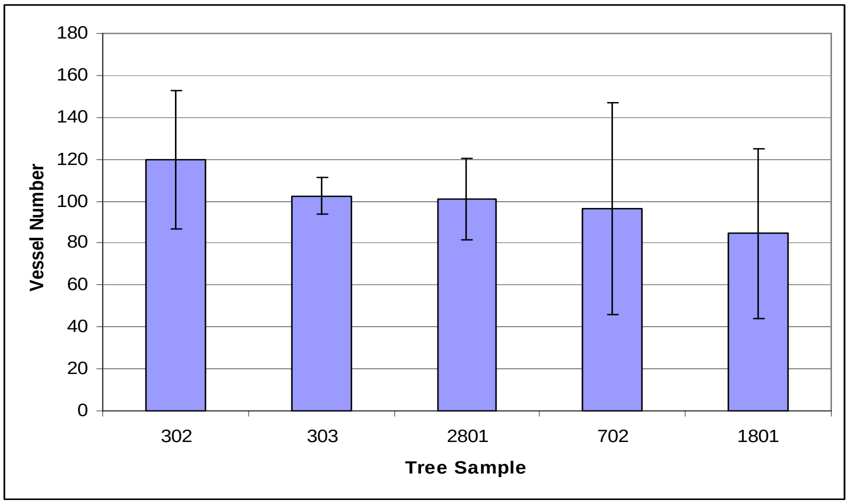

Figure 7 Mean vessel numbers and standard deviation of five trees.

Results of the statistical analysis of **vessel numbers** computed by Model I (Table 2):

- There was no significant difference in vessel numbers among trees.

- There was significant difference in vessel numbers by ring.
- Means for the five trees were not significantly different by Tukey grouping.

Results of T-test comparison (contrast) between and among trees for **vessel numbers** (Appendix C3):

- For all five trees, no significant differences in vessel numbers were found between **Tree 702** and the **average** vessel numbers of the other four trees, Tree 302, Tree 303, Tree 1801, and Tree 2801 with medium growth rate and different specific gravity.
- Comparing **Tree 702** with the other trees **individually**, no significant differences were observed for vessel numbers between Tree 702 and Tree 302, Tree 303, Tree 2801, and Tree 1801.
- Comparing the **high specific gravity trees** with each other, no significant differences were observed for vessel numbers between Tree 702 with high growth rate and Tree 2801 and Tree 1801with medium growth rates.
- Comparing **very low and low specific gravity** trees with medium growth rate, no significant differences were observed for vessel numbers between Tree 302 and Tree 303.
- Comparing trees with **medium growth rate individually**, only Tree 302 with low specific gravity had significantly higher vessel numbers than Tree 1801 with high specific gravity.

- Comparing **within medium growth rate trees**, no significant differences were found for vessel numbers between trees with very low and low specific gravity (Tree 302 & Tree 303) and trees with high specific gravity (Tree 1801 &Tree 2801).

Vessel Diameter

Mean vessel diameter of all samples ranged from 56 μm to 70 μm. **Tree 302** had the largest vessel diameter 70 μm (SD=18.31 μm) and the highest vessel area. **Tree 2801** had the smallest vessel diameter 56 μm (SD=7.39 μm), but relative low vessel area. **Tree 702** with high specific gravity, the highest growth rate, and with a relative low vessel area had moderate size vessel diameter (61 μm, SD=14.14 μm).

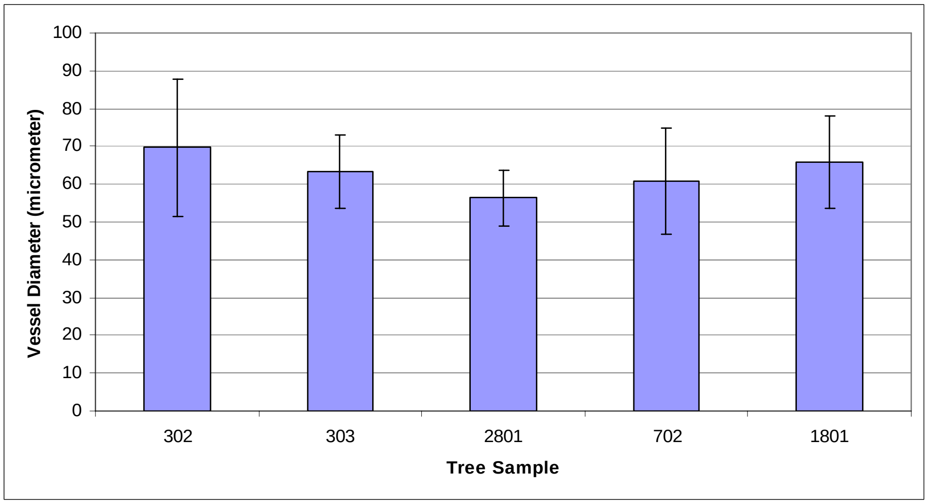

Figure 8 Mean vessel diameter and standard deviation of five trees.

Results of the statistical analysis of **vessel diameter** computed by Model I (Table 2):

- There was no significant difference in vessel diameter among trees (p=0.0829).
- There was significant difference in vessel diameter by ring.
- Means for the five trees were not significantly different by Tukey grouping.

Results of T-test comparison (contrast) between and among trees for **vessel diameter** (Appendix C3):

- For all five trees, no significant differences in vessel diameter were found between **Tree 702** and the **average** vessel number of the other four trees, Tree 302, Tree 303, Tree 1801, and Tree 2801 with medium growth rate and different specific gravity.
- Comparing **Tree 702** with the other trees **individually**, no significant differences were observed for vessel diameter between Tree 702 and Tree 302, Tree 303, Tree 2801, and Tree 1801.
- Comparing the **high specific gravity trees** with each other, there were not significant differences in vessel diameter between Tree 702 with high growth rate and Tree 2801 and Tree 1801with medium growth rates. However, Tree 1801 had significantly higher vessel diameter than Tree 2801.
- Comparing **very low and low specific gravity** trees with medium growth rate, no significant differences were found between Tree 302 and Tree 303.

- Comparing trees with **medium growth rate individually**, only Tree 302 with low specific gravity had significantly higher vessel diameter than Tree 2801 with high specific gravity.
- Comparing **within medium growth rate trees**, no significant differences were found for vessel diameter between trees with very low and low specific gravity (Tree 302 & Tree 303) and high specific gravity trees (Tree 1801 &Tree 2801).

Ray Number

Ray numbers of all samples ranged from 12 to 14 (Figure 9, Table 1). **Tree 702** had the highest ray numbers (14 and SD=1.41) as well as the highest growth rate, low vessel area, and high specific gravity. **Tree 302** had the lowest ray numbers (13 and SD=1.29) with very low specific gravity, the smallest growth rate but with high vessel area and vessel number.

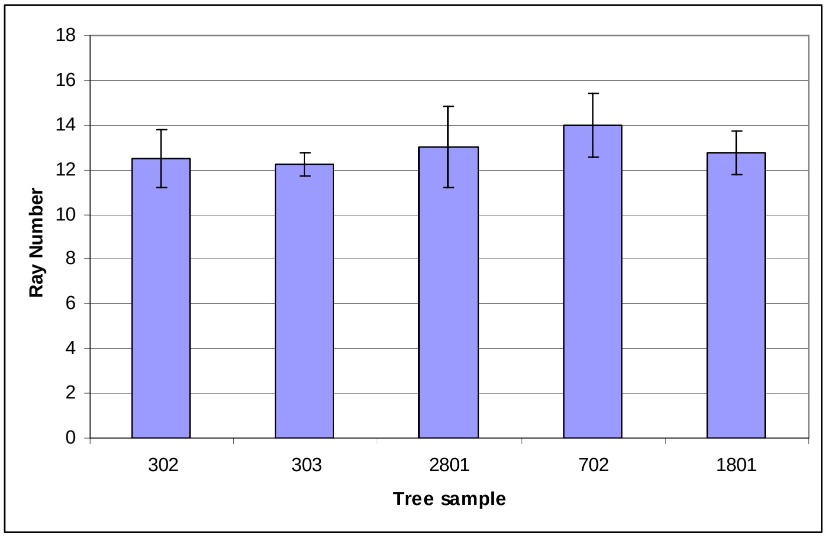

Figure 9 Ray numbers of five samples with different specific gravity and growth rate.

Results of the statistical analysis of **ray numbers** computed by Model I (Table 2):

- There were no significant differences in ray numbers among trees.
- There were significant differences in ray numbers by ring.
- Means for the five trees were not significantly different by Tukey grouping.

Results of T-test comparison (contrast) between and among trees for **ray numbers** (Appendix C3):

- For all five trees, **Tree 702** had significantly higher ray numbers than the **average** ray numbers of the other four trees, Tree 302, Tree 303, Tree 1801, and Tree 2801 with medium growth rate and different specific gravity.
- Comparing **Tree 702** with the other trees **individually**, no significant differences were observed for ray numbers between Tree 702 and Tree 2801, and Tree 1801. However, Trees 702 had significantly higher ray numbers than Tree 302, and Tree 303.
- Comparing the **high specific gravity trees** with each other, no significant differences were found for ray numbers between Tree 702 with high growth rate and Tree 2801 and Tree 1801with medium growth rates.
- Comparing **very low and low specific gravity** trees with medium growth rate, no significant differences were found between Tree 302 and Tree 303.
- Comparing trees with **medium growth rate individually**, no significant differences were found for ray numbers between Tree 302 with low specific

gravity, Tree 303 with low specific gravity, and with Tree 1801 and Tree 2801 with high specific gravity.

- Comparing **within medium growth rate trees**, no significant differences were found for ray numbers between trees with very low and low specific gravity (Tree 302 & Tree 303) and high specific gravity trees (Tree 1801 &Tree 2801).

In summary, there were significant tree effects on fiber length and vessel area (Table 2). In addition, there were significant ring effects on fiber length, vessel area, vessel numbers, vessel diameter, and on ray numbers. Characterizing wood properties of each sample tree, the following results can be summarized:

- **Tree 302** had very low specific gravity, medium growth rate, the shortest fiber length, the highest vessel area, the highest vessel numbers, large vessel diameter, and medium ray numbers.
- **Tree 303** had low specific gravity, medium growth rate, long fiber length, high vessel area, high vessel numbers, moderate vessel diameter, and the smallest ray numbers.
- **Tree 2801** had high specific gravity, medium growth rate, relative short fiber length, low vessel area, moderate vessel numbers, the smallest vessel diameter, and moderate ray number.

- **Tree 702** had high specific gravity, the highest growth rate, the longest fiber length, and the highest ray numbers. However, it had low vessel area and vessel number, and low vessel diameter.
- **Tree 1801** had the highest specific gravity with medium growth rate, moderate long fiber length, the lowest vessel area and vessel number, and moderate vessel diameter and ray number.

Table 1 Means of wood properties and standard deviation (SD) such as inside bark diameter (DIB, mm), specific gravity (SG), Fiber length (mm), Vessel area (%), vessel number, vessel diameter (μm), and ray number by tree.

			Fiber length		**Vessel Area**		**Vessel Number**		**Vessel diameter**		**Ray Number**	
Tree Sample	**DIB**	**SG**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
302	62.8	0.324	0.58	0.0783	30.66	3.3032	120	32.7554	69.63	18.3138	12.50	1.2910
303	62.9	0.343	0.68	0.0691	24.17	3.0802	103	8.5829	63.24	9.7108	12.25	0.5000
2801	77.6	0.395	0.61	0.0872	20.89	1.8295	101	19.2007	56.33	7.3914	13.00	1.8257
702	110.6	0.401	0.69	0.1148	19.55	0.5582	97	50.4546	60.76	14.1412	14.00	1.4142
1801	77.6	0.423	0.61	0.0690	18.43	2.2490	85	40.5010	82.92	40.4585	12.75	0.9574

Table 2 ANOVA (Model I) results for growth rate, fiber length, vessel area, vessel number, vessel diameter, and ray number of five samples.

Source	**DF**	**Fiber Length**		**Vessel Area**		**Vessel Number**		**Vessel Diameter**		**Ray Number**	
		F Value	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**	**F Value**	**Pr>F**
Tree	4	4.81	**0.0151***	27.07	**<.0001*****	1.52	0.2586	2.68	0.0829	2.41	0.1071
Ring	3	14.58	**0.0003*****	4.13	**0.0316***	9.39	**0.0018****	18.1	**<.0001*****	6.77	**0.0064****

Notes: ***, **, *: comparison significant at alpha = 0.001, 0.01, 0.05 respectively

RADIAL VARIATION OF WOOD PROPERTIES

Pattern of radial variation will be discussed on the following wood properties: fiber length, vessel area, vessel number, ray area, and ray number.

Fiber Length

Average fiber lengths by ring for five trees were presented in Figure 10. Radial variation of fiber length was influenced by both tree effect and ring effect. Tree 702 had the highest fiber length and significantly higher than average fibers from other four trees. The radial pattern showed a gradual increase from pith to bark. At the first year, Tree 303 had somewhat higher fiber length values; meanwhile, during the last year, Tree 302 had a relatively lower fiber length values.

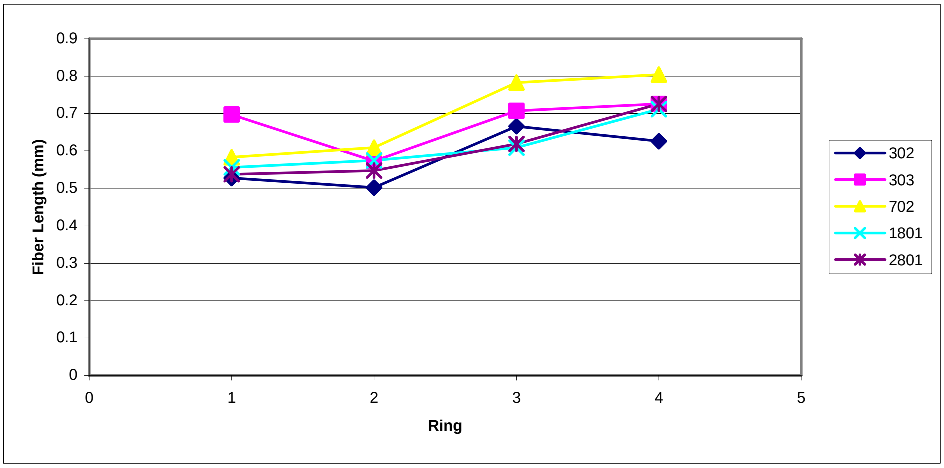

Figure 10 Fiber lengths of five sample trees with different specific gravity and growth rate from ring 1 (near pith side) toward ring 4 (near bark side).

Vessel Area

Average vessel area by ring for five trees were presented in Figure 11. Vessel area was affected by both tree and ring effects. The general pattern showed a gradual increase from pith to bark. Tree 702 with the highest growth rate and high specific gravity had low vessel area and showed more or less constant vessel area values along the radius. Tree 1801 with high specific gravity and medium growth rate had a decrease in vessel area to certain extend during the second and third year before it started to increase.

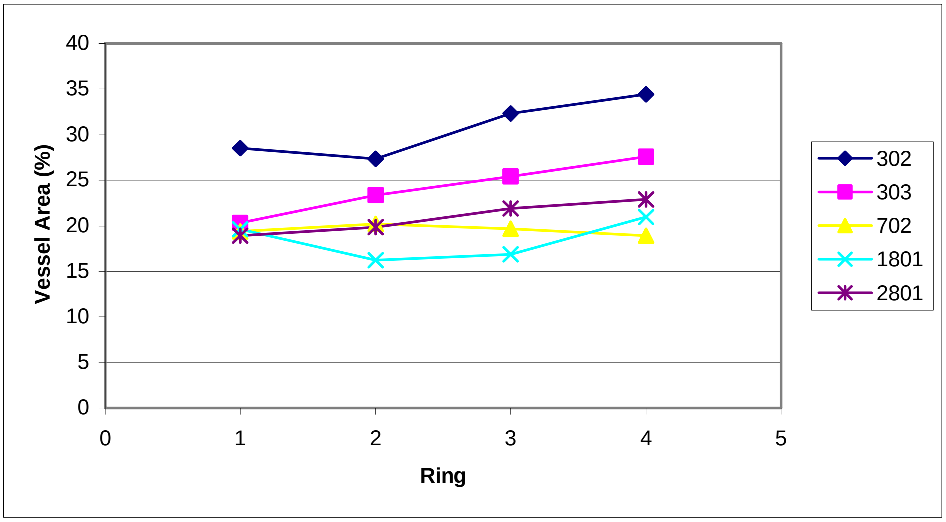

Figure 11 Vessel area of five sample trees with different specific gravity and growth rate from ring 1 (near pith side) toward ring 4 (near bark side).

Vessel Number

Average vessel number by ring for five trees were presented in Figure 12. Vessel numbers were significantly affected by ring effect but not by tree effect. In general, vessel numbers were high close to the pith and decreased toward the bark. Tree 303 with medium growth rate and low specific gravity had a relatively high vessel numbers and vessel numbers were more or less constant along the radius. Tree 702 had the highest value at the first ring, then decreased and showed the lowest values at the third and forth rings.

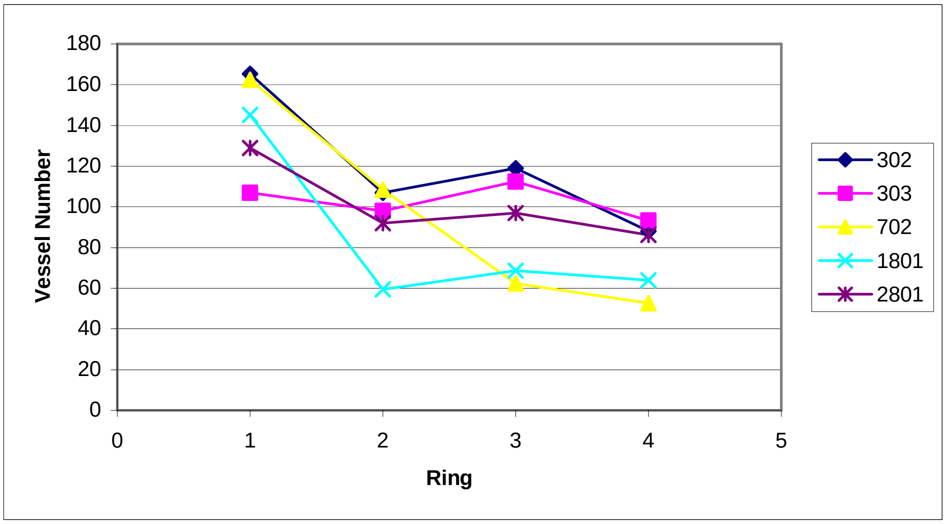

Figure 12 Vessel numbers of five sample trees with different specific gravity and growth rate from ring 1 (near pith side) toward ring 4 (near bark side).

Vessel Diameter

Average vessel diameter values by ring for five trees were presented in Figure 13. Similarly to vessel numbers, vessel diameter was significantly affected by ring effect but not by tree effect. In general, vessel diameter was small at the first year and increased toward the bark.

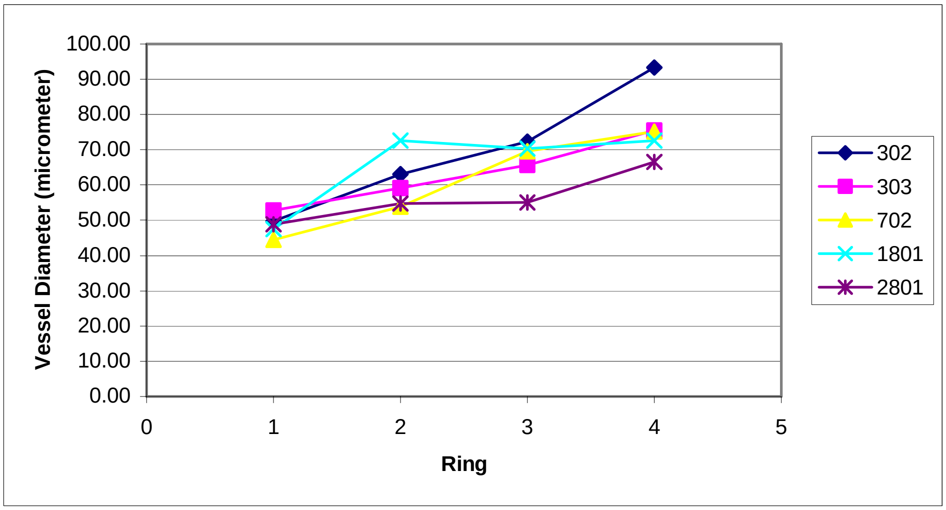

Figure 13 Vessel diameters of five sample trees with different specific gravity and growth rate from ring 1 (near pith side) toward ring 4 (near bark side).

Ray Number

Average ray numbers by ring for five trees were presented in Figure 14. Ray numbers were significantly affected by ring effect but not by tree effect. In general, ray numbers showed a slight gradual decrease along the radius.

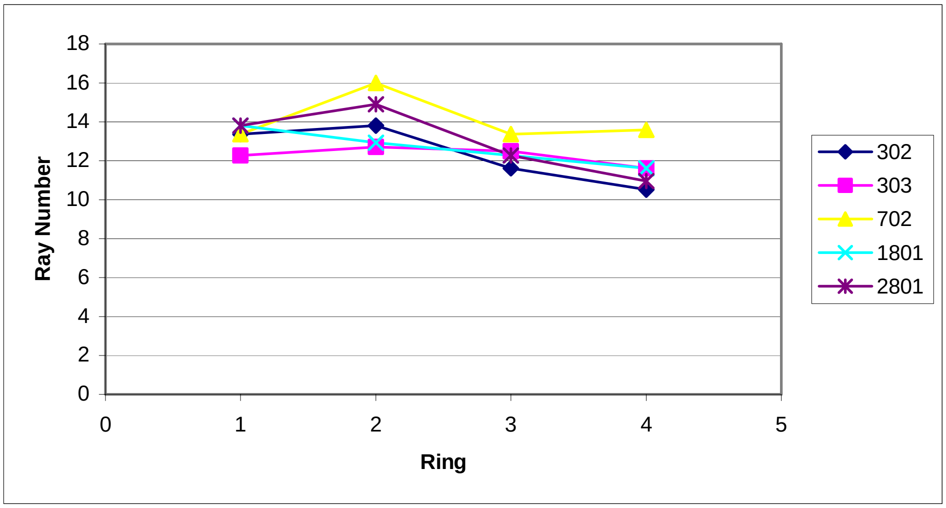

Figure 14 Ray numbers of five samples with different specific gravity and growth rate from ring 1 (near pith side) toward ring 4 (near bark side).

Tree 702 with the highest growth rate and high specific gravity had the highest ray numbers along the radius except at the first growth ring.

Table 3 Wood properties by ring of the five sample trees.

Tree Sample	Ring	FL (mm)	VA (%)	VN	VD	RN
302	1	0.53	28.50	165	49.80	13.36
303	1	0.70	20.34	107	52.73	12.27
2801	1	0.54	18.90	129	48.90	13.80
702	1	0.58	19.40	163	44.41	13.36
1801	1	0.56	19.62	145	47.53	13.80
302	2	0.50	27.35	107	63.11	13.80
303	2	0.57	23.34	98	59.11	12.71
2801	2	0.55	19.86	92	54.71	14.90
702	2	0.61	20.23	108	53.88	15.99
1801	2	0.57	16.22	60	72.60	12.92
302	3	0.67	32.34	119	72.25	11.61
303	3	0.71	25.44	112	65.65	12.49
2801	3	0.62	21.90	97	55.14	12.27
702	3	0.78	19.67	62	69.57	13.36
1801	3	0.61	16.89	69	70.33	12.27
302	4	0.63	34.44	88	93.37	10.51
303	4	0.73	27.56	93	75.47	11.61
2801	4	0.73	22.89	86	66.56	10.95
702	4	0.80	18.89	53	75.17	13.58
1801	4	0.71	20.98	64	72.65	11.61

Notes: FL: fiber length, VA: vessel area, VN: vessel number, VD: vessel diameter in μm, and RN: ray number.

In summary, ring effect was significant for all the anatomical properties such as fiber length, vessel area, vessel number, vessel diameter, and ray numbers. In general, fiber length, vessel area, and vessel diameter were gradually increasing from pith to bark. Meanwhile, vessel numbers and ray numbers decreased from pith to bark.

CORRELATION OF WOOD PROPERTIES

Although the available data were limited, Pearson correlation coefficients were calculated at tree average level and the results were presented in Table 4.

Table 4 Pearson correlation coefficients and probability (in parenthesis) between wood properties averaged by tree (N=5).

Properties	**Specific Gravity**	**Inner Bark Diameter**	**Fiber Length**	**Vessel Area**	**Vessel Number**	**Vessel Diameter**	**Ray Number**
Specific Gravity	1						
Diameter	0.6368 (0.7820)	1					
Fiber Length	0.1719 (0.7823)	0.5751 (0.3104)	1				
Vessel Area	-0.9439 **(0.0158*)**	-0.634 (0.2507)	-0.4328 (0.4667)	1			
Vessel Number	-0.9000 **(0.0374*)**	-0.4513 (0.4455)	-0.3469 (0.5673)	0.9034 **(0.0160*)**	1		
Vessel Diameter	0.2199 (0.7223)	-0.2335 (0.7054)	-0.3627 (0.5486)	-0.0665 (0.9154)	-0.3643 (0.5466)	1	
Ray Number	0.5723 (0.3133)	0.9825 **(0.0028**)**	0.4689 (0.4256)	-0.5372 (0.3505)	-0.3189 (0.6010)	-0.3200 (0.5996)	1

Notes: ***, **, *: comparison respectively significant at alpha = 0.001, 0.01, 0.05.

Based on statistical analysis the following conclusions can be summarized:

- There was a negative correlation between specific gravity and vessel area (-0.94, p=0.0158, N=5) and vessel number (-0.90, p=0.0374, N=5) (Figure 15).
- There was a positive correlation between growth rate (inner bark diameter) with ray number (0.98, p=0.0028, N=5) (Figure 16).
- There was a positive correlation between vessel number and vessel area (0.90, p=0.0160, N=5) (Figure 16).

- No correlation between fiber length and other wood properties was found.

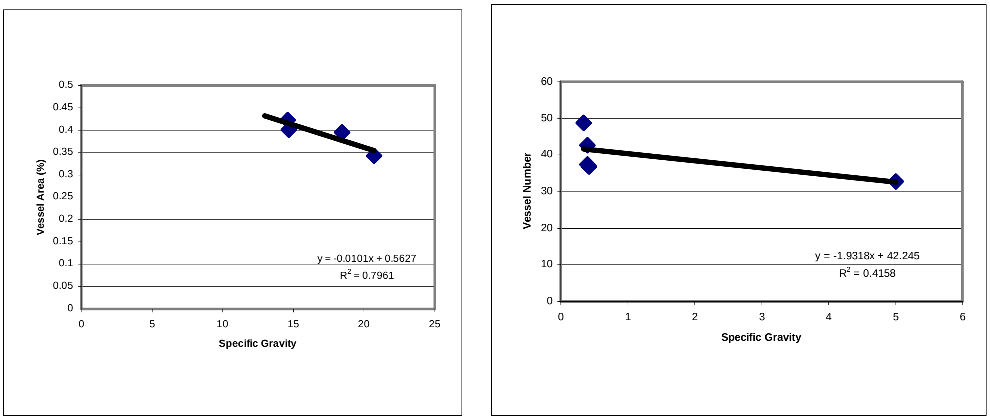

Figure 15 Specific gravity and vessel area (left) and specific gravity and vessel number (right) of five sample trees.

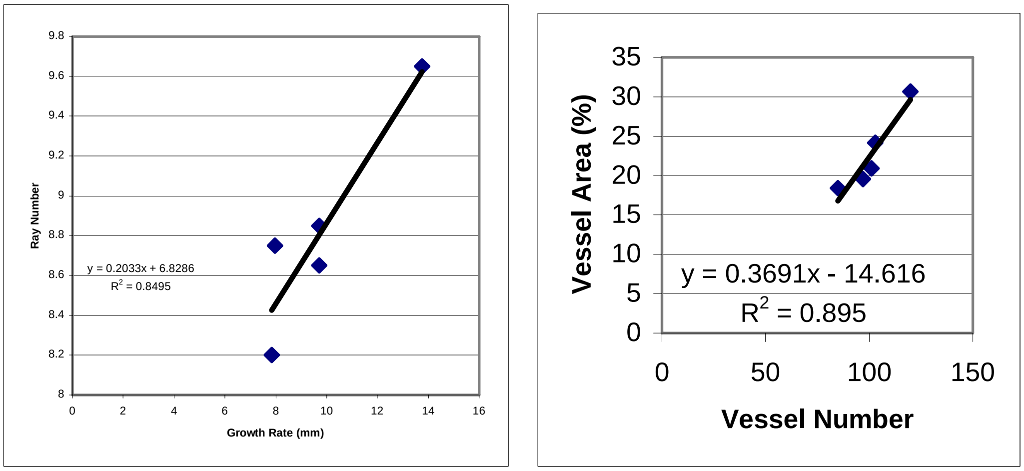

Figure 16 Growth rate and ray number (left) and vessel numbers and vessel area (right) of five sample trees.

DISCUSSION

The five trees that were analyzed in this investigation were 4-year old and they were different in genotype. They were selected from a tree population that had a negative correlation between diameter at the breast height and specific gravity. They were selected from the extreme value of diameter and specific gravity in order to compare their wood properties.

Specific gravity of five trees ranged from 0.32 to 0.42. The extremely low and high specific gravity trees were selected from a previous study with specific gravity average of 0.38 (SD=0.03). Only Tree 702 had highest growth rate with high specific gravity compared to the other four trees that were categorized in the medium growth rate.

Fiber lengths ranged from 0.53 mm in the first year to 0.80 mm in the fourth year. Fiber lengths were significantly different by tree and by ring (age). Tree 702 had the longest fiber length and Tree 302 had the shortest fiber length. These fiber length values were shorter than the results found by Klasnja et al. (2001) but fell in the same range reported by DeBell et al (2002) and DeBell et al. (1998). Klasnja, et al. (2001) reported mean fiber length of four-year-old poplars (40 clones) ranged from 0.72 mm to 1.00 mm. Fiber lengths were significantly different by clone and within clone (Klasnja, B. et al., 2001). Radial pattern of fiber length for three clones increased by age from the 0.56 mm in first year to 0.77 mm in the fourth year (DeBell et al., 2002) and ranged from 0.50 mm in the first year to 0.90 mm in the fourth year for two clones (DeBell et al., 1998).

Longer fiber length was reported from older trees by Koubaa et al., (1998). Ten 9-year old poplar clones had fiber length ranged from 0.900 mm to 1.054 mm. Another study on 21 three-year old poplar clones revealed that the arithmetic average fiber lengths ranged from 0.365 mm to 0.381 mm measured by Fiber Quality Analyzer (Zhang et al., 2003).

Vessel area percentage ranged from 18 to 31 % and was significantly affected by tree and by ring. Tree 302 with very low specific gravity had the highest vessel area; meanwhile, Tree 1801 with relative high specific gravity had the lowest vessel area. This result showed higher range of variation than the results reported on 10- and 15-year-old hybrid poplar clones, where vessel lumen area of three poplar clones were found between 18 and 25 % (Peszlen, 1994).

Vessel numbers of five trees ranged from 85 to 120. Vessel numbers were significantly affected by ring. Vessel numbers were high in the first year and gradually decreased toward the bark.

Vessel diameter of five trees ranged from 56 μm to 70 μm. Vessel diameters 50 μm in the first year and increased by ring. Tree 302 had the largest vessel diameter and Tree 2801 had the smallest vessel diameter. Tree 702 with the highest growth rate had moderate vessel diameter. These results fell in the ranged reported by Peszlen (1994), where vessel lumen diameters increased from 55 μm to 87 μm from the first to the sixth years. Somewhat similar trend was found in another study, where vessel diameters of six-month-old were 64 μm at pith side and 52 μm close to bark side (Jourez et al., 2001).

Ray numbers of five trees ranged between 12 and 14. Ray numbers were higher close to the pith and decreased with about 10% toward the bark. Tree 702 with the highest growth rate had the highest ray numbers. Tree 303 with medium growth rate showed small changes in ray numbers along the radius. Ray numbers of 10- and 15-year-old hybrid poplar clones decreased very little (about 1%) from pith to bark (Peszlen, 1994).

SUMMARY

Wood properties of five four-year old trees from four different families were investigated in this study. The five trees were selected from the population for growth rate and specific gravity. One tree, Tree 702 was exceptional in both. Investigating selected wood properties of these five trees, the following results can be summarized:

- Age of the cambium significantly affected all wood properties such as fiber length, vessel area, vessel numbers, vessel diameter, and ray numbers.
- There were significant differences among trees in fiber length, and vessel area but not in vessel numbers, vessel diameter, and ray numbers.

- **Tree 302** had very low specific gravity, medium growth rate, the shortest fiber length, the highest vessel area, the highest vessel numbers, large vessel diameter, and medium ray numbers.
- **Tree 303** had low specific gravity, medium growth rate, long fiber length, high vessel area, high vessel numbers, moderate vessel diameter, and the smallest ray numbers.
- **Tree 2801** had high specific gravity, medium growth rate, relative short fiber length, low vessel area, moderate vessel numbers, the smallest vessel diameter, and moderate ray number.
- **Tree 702** had high specific gravity, the highest growth rate, the longest fiber length, and the highest ray numbers. However, it had low vessel area and vessel number, and low vessel diameter.
- **Tree 1801** had the highest specific gravity with medium growth rate, moderate long fiber length, the lowest vessel area and vessel number, and moderate vessel diameter and ray number.
- There was a significant negative correlation between specific gravity and vessel area, and between specific gravity and vessel number. Trees with higher vessel area percentage had larger number of vessels. Significant correlation was found between growth rate and ray numbers.

Results showed that the fastest growing tree with high specific gravity had the longest fibers, the highest ray numbers but low vessel area, vessel number, and low vessel diameter. Because of this unusual combination of all these traits, this tree needs further study.

CONCLUSIONS

Wood properties of five four-year old trees from four different families were investigated in this study. The five trees were selected from the population for growth rate and specific gravity. One tree was exceptional in both. Investigating selected wood properties of these five trees, the following conclusions can be drawn:

Longer fibers tend to be associated with the faster growing trees. In this case, there was also a positive relationship between growth rate and with specific gravity. However, both effects in this study are primarily due to one exceptional tree with high specific gravity and fast growth.

A working hypothesis from the previous study of 72 trees from 32 families was that vessel area was one of the factors underlying the negative correlation between growth rate and specific gravity; i.e. high vessel area supports rapid growth, but yields low specific gravity wood. While not disproving that hypothesis in terms of general trends, this study showed that there could be wide variation in vessel area in trees with similar growth rates and that exceptionally fast growth can be achieved by a tree with low vessel area.

Vessel number is a better predictor of vessel area than vessel diameter in this study while the reverse was true in the insect study suggesting that there are different genotypic strategies and different environmental effects controlling vessel area through changes in number and diameter.

Selecting for both growth rate and higher specific gravity still remains difficult. More research is needed to determine the underlying reasons for this negative relationship and what can be done to improve breeding and selection for both growth rate and high specific gravity.

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CHAPTER 6

SUMMARY OF CONCLUSIONS

Wood properties of diverse poplars were studied and presented in three different chapters due to material and experimental design differences. The first topic dealt with the effect of cottonwood leaf beetle on wood properties of two clones. Wood properties of unprotected and protected (sprayed with insecticide) sample trees were compared and the results were presented in Chapter 3. Cottonwood leaf beetles preferred feeding on very young poplars; therefore, the investigation of wood properties was focused on differences between wood formed during the first three years and wood formed during the next three years.

In Chapter 4, wood properties of new eastern cottonwood clones of two families were evaluated. Wood properties of eight clones of two families grown under stressful site conditions were assessed and results were provided.

Wood properties of five trees with different specific gravities and growth rates were investigated in Chapter 5. Sample trees represented the extreme low and extreme high specific gravities among new cottonwood populations from the Biofuels Development Program conducted in Iowa. They were also categorized into two growth rates: medium and high growth rates.

The conclusions of this dissertation are summarized as the follows:

- Insect defoliation caused moderate decreases in annual ring **growth** for one clone over the first three growing and growth rate returned to normal during the second three years. The other clone, which was more susceptible for insect attack, had large decreases in growth rate during the first three years but had larger growth rate than the protected trees during the second three years. This may have been due to the fact that the unprotected trees had more room to grow when the beetle damage was no longer significant.
- This change in growth rates was accompanied by some changes in wood properties. Insect defoliation did not show significant influence on density but it may have affected the uniformity of **density** within growth ring and along the radius. **Fiber lengths** were significantly shorter for the unprotected trees of the clone, which was more susceptible for insect attack in the first ring. However, this was the only indication that defoliation/re-foliation and the accompanying fluctuations in carbohydrate availability and hormone levels could impact fiber development.
- **Variation in vessel area** suggests a more complex relationship between canopy changes and vascular support than might be expected. The results of this study suggest that vessel number and vessel diameter are more clearly impacted by beetle damage and that off-setting changes in vessel numbers and diameters partially cancel out changes in vessel area.

- Investigating the **number and diameter of vessels**, it can be concluded that defoliation/re-foliation is leading to the production of more vascular elements, but disruptions in carbohydrate supplies and hormone levels is leading to smaller sized cells.
- Significant observations in this study involve the **rays**. Results suggest an important function of rays in responding to insect damage in the crown. One clone responded by producing more rays, the other clone responded by producing fewer, but larger rays to give an overall increase in ray area.
- **Correlation** analyses of wood properties revealed positive, negative, and no correlations in this study. Among significant correlations, poplars with high density had high vessel number and ray area. Also, tree with high stem radius had long fiber, larger vessel diameter, but lower ray area. However, these results may be due to the fact that all anatomical properties showed the highest variation along the radius and had increasing. Age effect of cambium was strongly influenced anatomical properties.
- There were no statistical differences in growth rate between two families growing under stressful site conditions; however, specific gravity was significantly influenced by family and by clone.
- A low, but significant, negative correlation exists between growth rate and specific gravity in these poplar families just as it was found in previous

studies of open-pollinated families and selected clones. Selecting for both growth rate and higher specific gravity is still a challenge.

- Fiber length was not affected by family but only by clone and position. However, fibers were significantly longer at the fourth and fifth growth rings than at the second and third rings suggesting that the earliest selection for fiber length should start after four or five years of ages only.
- Longer fibers tend to be associated with the faster growing trees. In this case, there was also a positive relationship with specific gravity. However, both effects in this study are primarily due to one exceptional tree with high specific gravity and fast growth.
- Although a working hypothesis from the previous studies was that vessel area is was one of the factors underlying the negative correlation between growth rate and specific gravity; i.e. high vessel area supports rapid growth, but yields low specific gravity wood. While not disproving that hypothesis in terms of general trends, this study showed that there could be wide variation in vessel area in trees with similar growth rates and that exceptionally fast growth can be achieved by a tree with low vessel area.
- Wood properties such as growth rate as diameter, fiber length, vessel area, vessel number, vessel diameter, and ray number were significantly affected by ring. Age effect of cambium was strongly influenced the anatomical properties. Fiber length, vessel area, vessel diameter showed

increasing radial patterns; meanwhile, vessel numbers, ray area, and ray numbers showed decreasing variation from pith to bark.

- Besides specific individual genetic background of poplars such as family and clone, specific environmental conditions including insect defoliation and stressful site condition had complex effects on specific gravity and growth rates.
- Results showed a positive correlation between stem radius and fiber length in both the insect defoliation study and the investigation on eight clone of two families. Hence, it should be relatively easy to select within families for both growth rate and longer fibers.
- Wide variations in vessel area in trees with similar growth rates were found among different clones and exceptionally fast growth can be achieved by a tree with low vessel area. Controlling vessel area through changes in vessel number and diameter were found indicating that different genotypic strategies and different environmental effects are important for further studies and developing guidelines for breeding and new clone selection.

CHAPTER 7

FUTURE RESEARCH

In general, studying variation in wood properties of new hybrids and clones with different growth rate and specific gravity, more replications are important for reliable results. Investigating wood properties with reasonable sample size and random selection, results can be applied to predict the properties of larger population and to enhance the reliability of clonal selection.

Comparing wood properties between the insect defoliation treatment and the insecticide spraying treatment, more replications are necessary to be collected and measured the similar wood properties in order to accomplish the treatment effect test.

Correlation of wood properties within individual ring may explain developing of specific genetic backgrounds in response to particular environmental conditions.

More research is needed to determine the underlying reasons for the negative relationship between growth rate and specific gravity and what can be done to improve breeding and selection for both growth rate and high specific gravity because selecting for both growth rate and higher specific gravity is still difficult.

Wide variations in vessel area in trees with similar growth rates were found among different clones. It seems that trees can achieve exceptionally fast growth with low vessel area. Controlling vessel area through changes in vessel number and diameter were found indicating that different genotypic strategies and different environmental effects are important for further studies and developing guidelines for breeding and new clone selection.

Vessel area, vessel number, and vessel diameter are important factors for physiological study. There are options to further apply these data to relate to photosynthesis activity by leaf areas and crown areas. Vessel area, vessel number, and vessel diameter are also used for study of survival or adaptation of trees in drought or flood conditions.

For wood industry, low specific gravity of poplars with thin cell walls may be sources for wood composite products such as insulated board, core veneers for plywood. In fact, poplars are inferior grade for solid wood but it is acceptable for pulp and paper raw material.

X-ray diffraction technique is another method can be used to assess wood properties. Near Infrared Spectrum technique is used to predict density, mechanical properties, and microfibril angle of wood. It may be possible to predict some wood properties. These techniques may enhance in rapid assessment of wood properties.

APPENDICES

APPENDICES A

Analysis results related to Clone 91X and Clone NM2 were presented in appendix A.

Appendix A1 Differences of least square means for density by t-test model IV.

Effect	Versus	Effect	Ring	t-Value	Pr
91X-P		91X-U	1	-1.25	0.2168
91X-P		91X-U	2	0.22	0.8169
91X-P		91X-U	3	1.48	0.1447
91X-P		91X-U	4	-0.95	0.3476
91X-P		91X-U	5	-0.97	0.3343
91X-P		91X-U	6	0.73	0.471
NM2-P		NM2-U	1	-1.1	0.2785
NM2-P		NM2-U	2	-0.42	0.6772
NM2-P		NM2-U	3	-0.39	0.6956
NM2-P		NM2-U	4	-0.17	0.8628
NM2-P		NM2-U	5	1.27	0.2078
NM2-P		NM2-U	6	0	0.9458
91X-P		NM2-P	1	-3.65	0.0006
91X-P		NM2-U	1	-4.75	<.0001
NM2-P		91X-U	1	2.15	0.0358
91X-U		NM2-U	1	-3.17	0.0025
91X-P		NM2-P	2	-2.87	0.0058
91X-P		NM2-U	2	-3.29	0.0017
NM2-P		91X-U	2	2.9	0.0053
91X-U		NM2-U	2	-3.29	0.0017
91X-P		NM2-P	3	-0.24	0.8116
91X-P		NM2-U	3	-0.63	0.5294
NM2-P		91X-U	3	1.7	0.0943
91X-U		NM2-U	3	-2.07	0.0432

Notes: The significant difference is at alpha = 0.05. A mean represented 20 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.

Appendix A1 (continued) Differences of least square means for density by t-test model IV.

Effect	Versus	Effect	Ring	t-Value	Pr
91X-P		NM2-U	4	-0.18	0.8608
NM2-P		91X-U	4	-0.94	0.3489
91X-U		NM2-U	4	0.77	0.4439
91X-P		NM2-P	5	-2.49	0.0158
91X-P		NM2-U	5	-1.22	0.2294
NM2-P		91X-U	5	1.56	0.1352
91X-U		NM2-U	5	-0.24	0.8102
91X-P		NM2-P	6	-0.1	0.9237
91X-P		NM2-U	6	-0.03	0.9778
NM2-P		91X-U	6	0.82	0.4145
91X-U		NM2-U	6	-0.75	0.4541

Notes: The significant difference is at alpha = 0.05. A mean represented 20 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.

Appendix A2 Differences of least square means for growth rate by t-test model IV.

Effect	Versus	Effect	Ring	t-Value	Pr
91X-P		91X-U	1	0.00	0.9803
91X-P		91X-U	2	2.93	0.0048
91X-P		91X-U	3	4.64	<.0001
91X-P		91X-U	4	2.02	0.0479
91X-P		91X-U	5	-2.20	0.0321
91X-P		91X-U	6	0.66	0.5120
NM2-P		NM2-U	1	-0.14	0.8970
NM2-P		NM2-U	2	1.05	0.2986
NM2-P		NM2-U	3	1.37	0.1749
NM2-P		NM2-U	4	1.18	0.2432
NM2-P		NM2-U	5	0.42	0.6762
NM2-P		NM2-U	6	0.00	0.6939
91X-P		NM2-P	1	-0.25	0.8063
91X-P		NM2-U	1	-0.38	0.7080
NM2-P		91X-U	1	0.26	0.7991
91X-U		NM2-U	1	-0.38	0.7072
91X-P		NM2-P	2	-0.08	0.9404
91X-P		NM2-U	2	0.97	0.3342
NM2-P		91X-U	2	3.01	0.0039
91X-U		NM2-U	2	-1.96	0.0549
91X-P		NM2-P	3	0.77	0.4440
91X-P		NM2-U	3	2.14	0.0362
NM2-P		91X-U	3	3.87	0.0003
91X-U		NM2-U	3	-2.50	0.0152
91X-P		NM2-P	4	0.23	0.8177
91X-P		NM2-U	4	1.41	0.1637
NM2-P		91X-U	4	1.79	0.0788
91X-U		NM2-U	4	-0.61	0.5442
91X-P		NM2-P	5	-0.95	0.3479
91X-P		NM2-U	5	-0.53	0.6005
NM2-P		91X-U	5	-1.25	0.2164
91X-U		NM2-U	5	1.67	0.1004
91X-P		NM2-P	6	-0.89	0.3753
91X-P		NM2-U	6	-0.94	0.3517
NM2-P		91X-U	6	0.23	0.8161
91X-U		NM2-U	6	-0.28	0.7811

Notes: The significant difference is at alpha = 0.05. A mean represented 20 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.

Appendix A3 Differences of least square means for fiber length by t-test model IV.

Effect	Versus	Effect	Ring	t-Value	Pr
91X-P		91X-U	1	1.58	0.1316
91X-P		91X-U	2	1.24	0.2305
91X-P		91X-U	3	0.58	0.5644
91X-P		91X-U	4	0.10	0.9359
91X-P		91X-U	5	0.67	0.5117
91X-P		91X-U	6	0.54	0.5986
NM2-P		NM2-U	1	0.00	0.9858
NM2-P		NM2-U	2	-0.17	0.8609
NM2-P		NM2-U	3	-1.60	0.1268
NM2-P		NM2-U	4	-0.24	0.8063
NM2-P		NM2-U	5	-0.33	0.7443
NM2-P		NM2-U	6	0.71	0.4903
91X-P		NM2-P	1	3.23	0.0046
91X-P		NM2-U	1	2.87	0.0102
NM2-P		91X-U	1	-1.31	0.2069
91X-U		NM2-U	1	1.18	0.2538
91X-P		NM2-P	2	0.56	0.5857
91X-P		NM2-U	2	0.38	0.7104
NM2-P		91X-U	2	0.69	0.5015
91X-U		NM2-U	2	-0.86	0.3991
91X-P		NM2-P	3	1.20	0.2476
91X-P		NM2-U	3	-0.41	0.6896
NM2-P		91X-U	3	-0.61	0.5508
91X-U		NM2-U	3	-0.99	0.3339
91X-P		NM2-P	4	-0.27	0.7931
91X-P		NM2-U	4	-0.52	0.6127
NM2-P		91X-U	4	0.35	0.7321
91X-U		NM2-U	4	-0.60	0.5582
91X-P		NM2-P	5	0.21	0.8381
91X-P		NM2-U	5	-0.12	0.9027
NM2-P		91X-U	5	0.46	0.6495
91X-U		NM2-U	5	-0.79	0.4378
91X-P		NM2-P	6	0.89	0.3867
91X-P		NM2-U	6	1.59	0.1289
NM2-P		91X-U	6	-0.35	0.7295
91X-U		NM2-U	6	1.06	0.3025

Notes: The significant difference is at alpha = 0.05. A mean represented 70 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.

Appendix A4 Differences of least square means for vessel area (%), vessel number (per mm^2), and vessel diameter (µm) by t-test model IV.

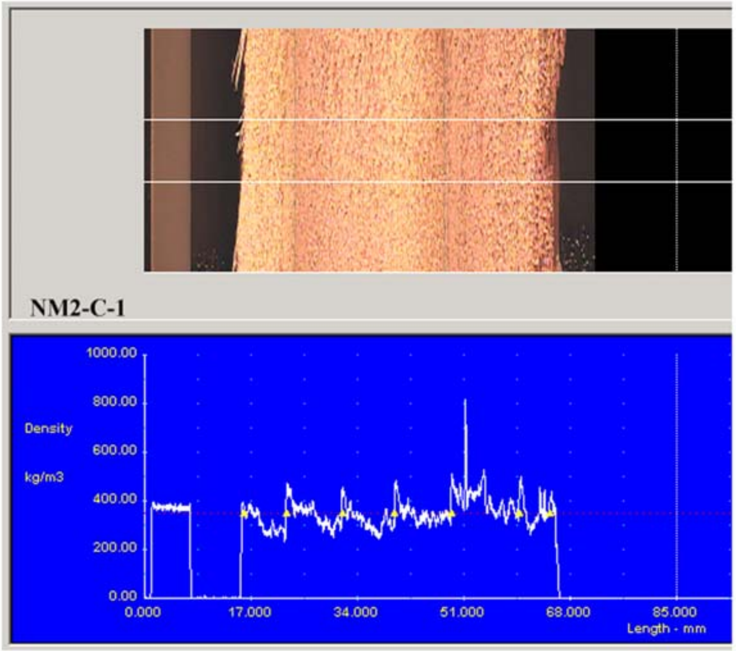
Effect	Versus	Effect	Ring	Vessel Area		Vessel Number		Vessel Diameter	
				t-Value	Pr	t-Value	Pr	t-Value	Pr
91X-P		91X-U	1	-0.17	0.8619	-1.57	0.1232	0.66	0.5112
91X-P		91X-U	2	-1.67	0.1010	-1.57	0.1242	0.14	0.8955
91X-P		91X-U	3	-3.04	0.0038	-5.75	<.0001	3.27	0.0019
91X-P		91X-U	4	1.81	0.0769	-0.68	0.5023	1.68	0.0996
91X-P		91X-U	5	3.69	0.0006	0.51	0.6145	1.45	0.1530
91X-P		91X-U	6	0.14	0.8849	0.57	0.5758	0.45	0.6560
NM2-P		NM2-U	1	-1.32	0.1916	-3.73	0.0005	0.66	0.0189
NM2-P		NM2-U	2	-0.92	0.3597	-0.71	0.4789	0.14	0.3748
NM2-P		NM2-U	3	1.16	0.2503	-0.17	0.8665	3.27	0.1122
NM2-P		NM2-U	4	-3.23	0.0022	-1.73	0.0903	1.68	0.8267
NM2-P		NM2-U	5	-1.72	0.0936	-1.28	0.2077	1.45	0.5665
NM2-P		NM2-U	6	0.45	0.6593	-0.47	0.6477	0.45	0.9544
91X-P		NM2-P	1	-1.7	0.1444	-0.94	0.3530	-0.35	0.7286
91X-P		NM2-U	1	-3.34	0.0016	-5.86	<.0001	0.99	0.3249
NM2-P		91X-U	1	1.55	0.187	-0.36	0.7200	0.94	0.3531
91X-U		NM2-U	1	-3.18	0.0025	-4.22	0.0001	0.33	0.7415
91X-P		NM2-P	2	-2.77	0.0079	-4.23	<.0001	1.34	0.1866
91X-P		NM2-U	2	-3.57	0.0008	-5.09	<.0001	1.92	0.0606
NM2-P		91X-U	2	0.91	0.3647	2.39	0.0205	-1.13	0.2644
91X-U		NM2-U	2	-1.75	0.0858	-3.17	0.0026	1.67	0.1007
91X-P		NM2-P	3	-2.72	0.0089	-3.05	0.0036	0.67	0.5043
91X-P		NM2-U	3	-1.6	0.1164	-3.23	0.0022	2.29	0.0263
NM2-P		91X-U	3	-0.21	0.8317	-2.87	0.0059	2.60	0.0122
91X-U		NM2-U	3	1.34	0.187	2.70	0.0095	-0.98	0.3306
91X-P		NM2-P	4	2.25	0.0287	0.43	0.6724	0.72	0.4767
91X-P		NM2-U	4	-0.87	0.3882	-1.35	0.182	0.94	0.3532
NM2-P		91X-U	4	-0.51	0.6149	-1.12	0.2674	0.96	0.3414
91X-U		NM2-U	4	-2.62	0.0117	-0.66	0.514	-0.74	0.4626
91X-P		NM2-P	5	2.41	0.0196	-0.46	0.6446	1.71	0.0941
91X-P		NM2-U	5	0.76	0.4512	-1.78	0.0813	2.28	0.0266
NM2-P		91X-U	5	1.15	0.2536	0.99	0.3288	-0.26	0.7995
91X-U		NM2-U	5	-2.81	0.0071	-2.30	0.0255	0.83	0.4091
91X-P		NM2-P	6	1.14	0.2583	-0.37	0.7149	1.89	0.0645
91X-P		NM2-U	6	1.57	0.1222	-0.85	0.402	1.95	0.0571
NM2-P		91X-U	6	-1.00	0.3208	0.95	0.348	-2.34	0.0234
91X-U		NM2-U	6	1.43	0.1585	-1.43	0.1602	2.39	0.0203

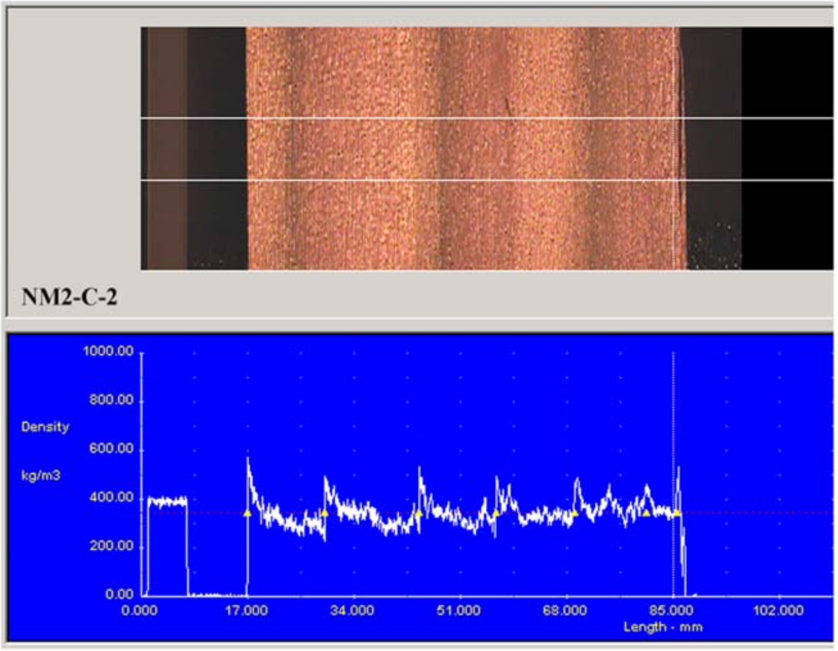
Notes: The significant difference is at alpha = 0.05. A mean represented 20 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.

Appendix A5 Differences of least square means for ray area (%), and ray number (per 1 mm^2) by t-test model IV.

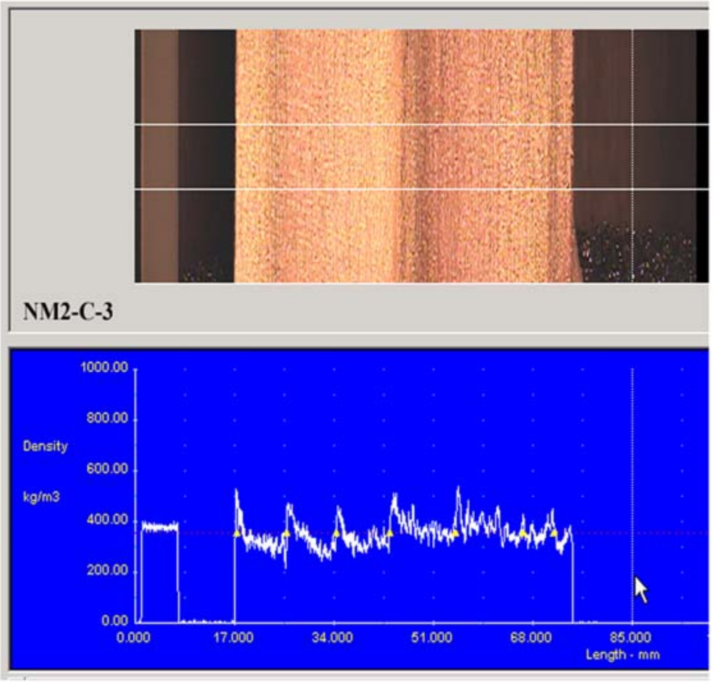
Effect	Versus	Effect	Ring	Ray Area		Ray Number	
				t-Value	Pr	t-Value	Pr
91X-P		91X-U	1	-2.18	0.0432	0.83	0.4091
91X-P		91X-U	2	-2.92	0.0096	2.07	0.0436
91X-P		91X-U	3	-3.63	0.0020	2.15	0.0360
91X-P		91X-U	4	-5.09	<.0001	0.72	0.4735
91X-P		91X-U	5	-4.83	0.0002	0.35	0.7303
91X-P		91X-U	6	-2.46	0.0250	0.93	0.3561
NM2-P		NM2-U	1	-3.10	0.0065	-0.28	0.7732
NM2-P		NM2-U	2	-1.61	0.1266	1.40	0.1692
NM2-P		NM2-U	3	-7.81	<.0001	-2.77	0.0077
NM2-P		NM2-U	4	-3.01	0.0078	-0.69	0.4931
NM2-P		NM2-U	5	-3.21	0.0051	-0.61	0.5465
NM2-P		NM2-U	6	2.64	0.0173	0.73	0.4643
91X-P		NM2-P	1	-2.62	0.0178	-0.32	0.7467
91X-P		NM2-U	1	-6.14	<.0001	-0.73	0.4669
NM2-P		91X-U	1	0.31	0.7629	1.02	0.3114
91X-U		NM2-U	1	-3.85	0.0014	-1.57	0.1239
91X-P		NM2-P	2	-2.77	0.0130	0.49	0.6241
91X-P		NM2-U	2	-3.15	0.0061	1.89	0.0648
NM2-P		91X-U	2	-0.15	0.8817	1.61	0.1146
91X-U		NM2-U	2	-1.77	0.0956	-0.29	0.7727
91X-P		NM2-P	3	3.6	0.0024	4.22	0.0001
91X-P		NM2-U	3	-5.75	<.0001	1.45	0.1542
NM2-P		91X-U	3	-6.87	<.0001	-2.07	0.0440
91X-U		NM2-U	3	-2.48	0.0249	-0.71	0.4821
91X-P		NM2-P	4	0.47	0.6415	2.65	0.0109
91X-P		NM2-U	4	-3.13	0.0064	1.95	0.0562
NM2-P		91X-U	4	-5.43	<.0001	-1.92	0.0602
91X-U		NM2-U	4	1.82	0.0877	1.23	0.2235
91X-P		NM2-P	5	-0.22	0.8284	1.21	0.231
91X-P		NM2-U	5	-4.06	0.0009	0.60	0.5479
NM2-P		91X-U	5	-4.43	0.0004	-0.87	0.3908
91X-U		NM2-U	5	0.59	0.5658	0.26	0.7973
91X-P		NM2-P	6	-4.50	0.0004	-1.02	0.3125
91X-P		NM2-U	6	-1.35	0.1974	-0.28	0.7785
NM2-P		91X-U	6	2.58	0.0201	1.95	0.0566
91X-U		NM2-U	6	0.57	0.5736	-1.21	0.2303

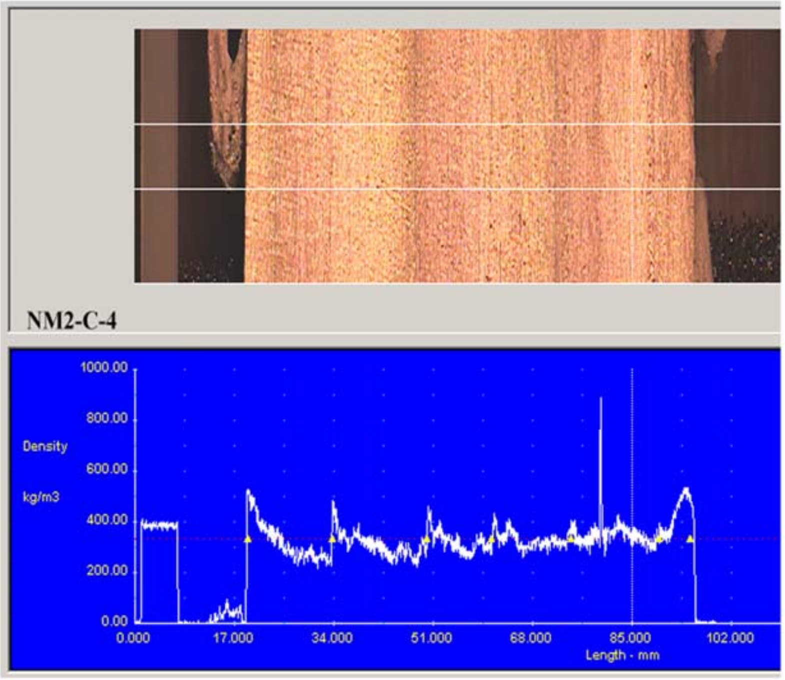
Notes: The significant difference is at alpha = 0.05. A mean represented 20 observations.
91X-P is Clone 91X protected tree. 91X-U is Clone 91X unprotected tree.
NM2-P is Clone NM2 protected tree. NM2-U is Clone NM2 unprotected tree.



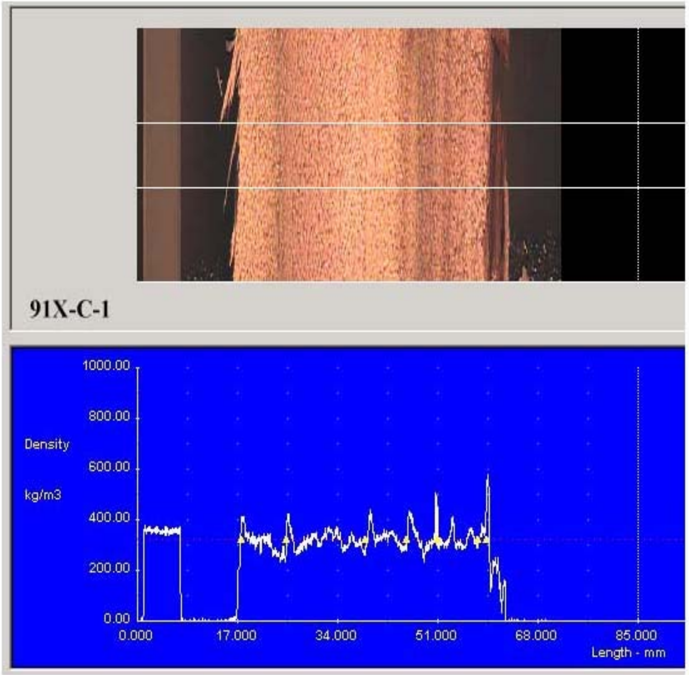


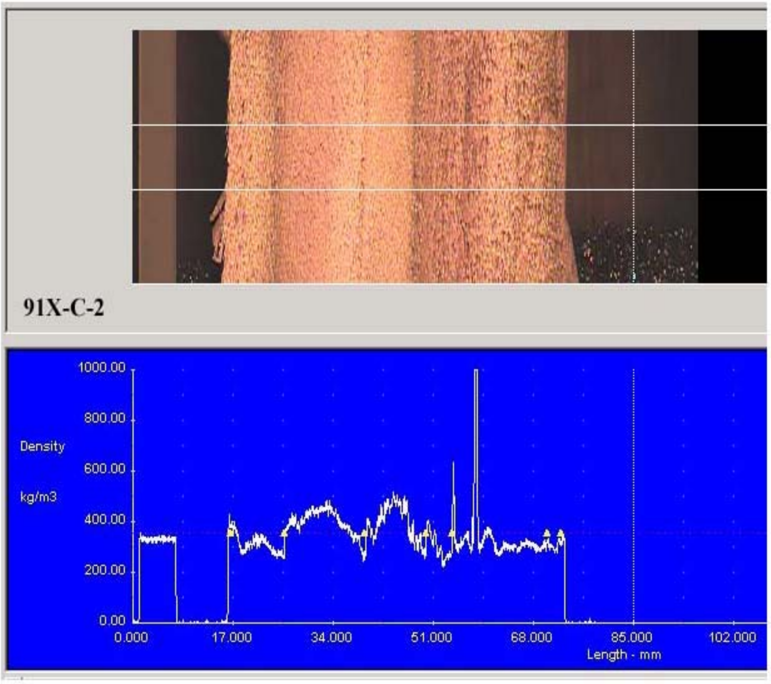
Appendix A6 Density profiles of Clone NM2 unprotected trees replication 1 and 2.



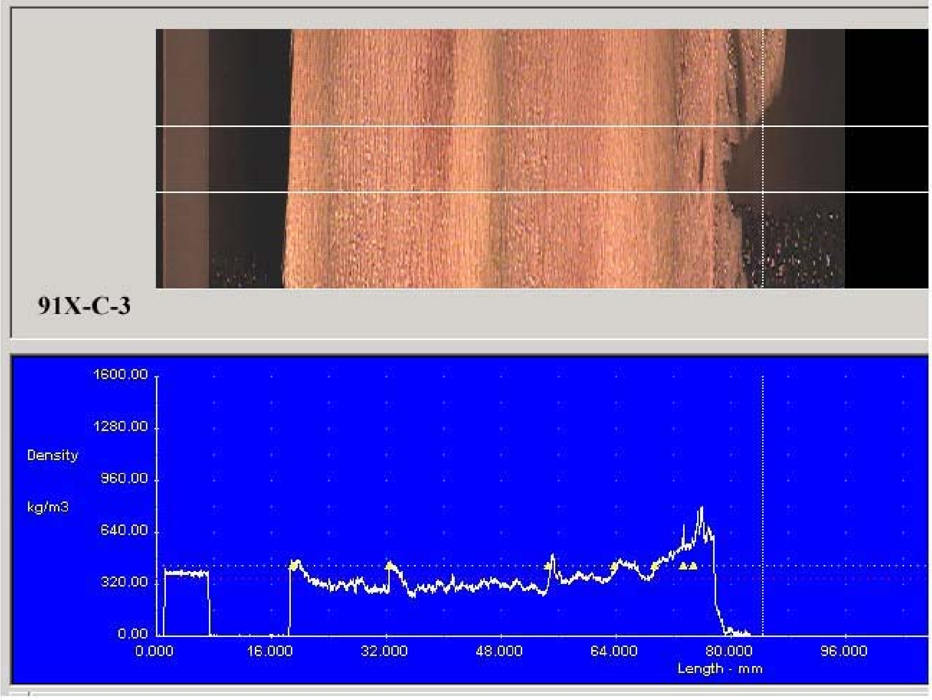


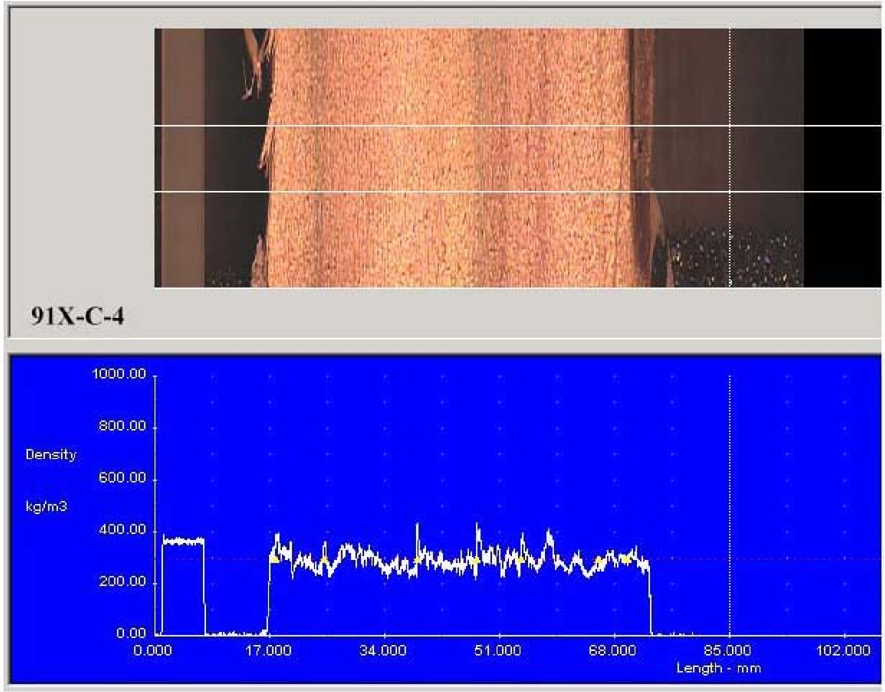
Appendix A7 Density profiles of Clone NM2 unprotected trees replication 3 and 4.



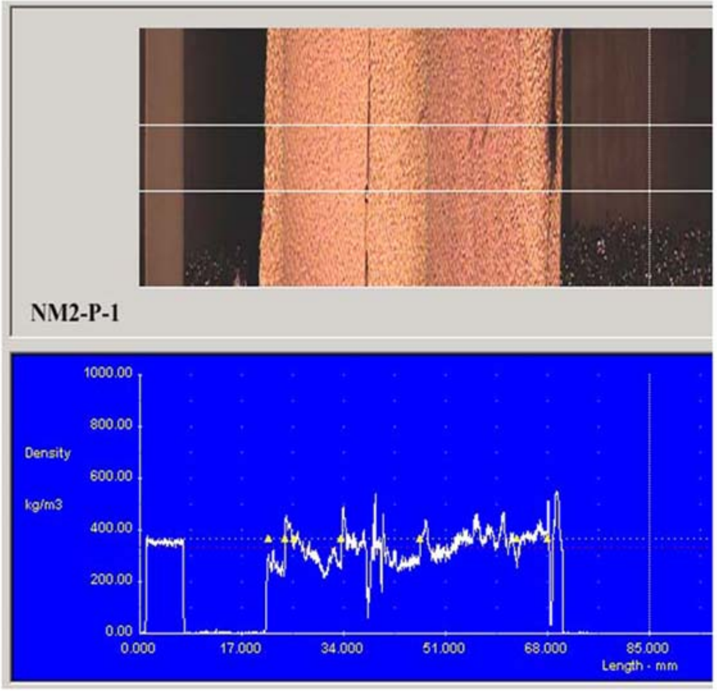


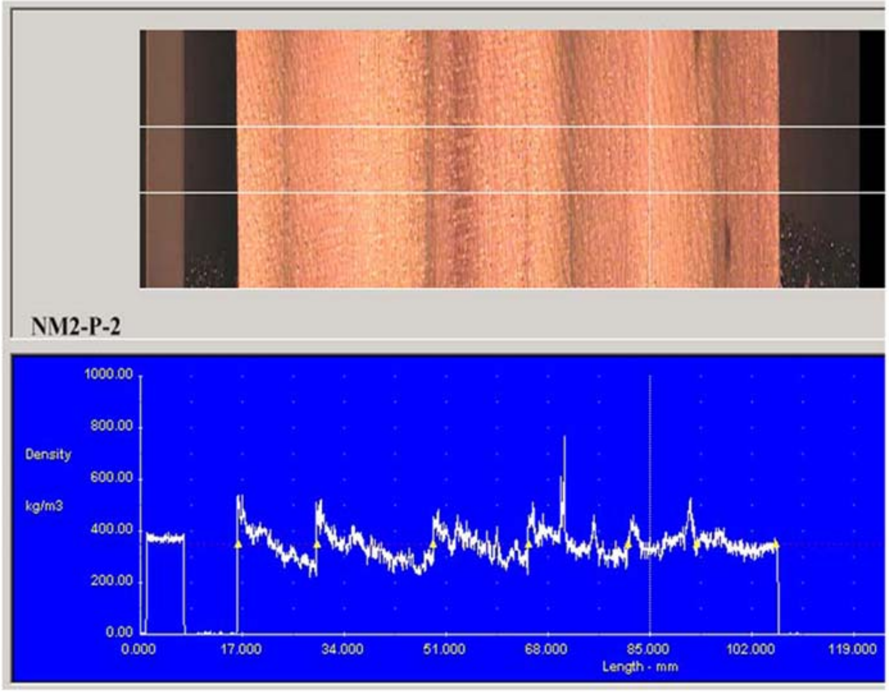
Appendix A8 Density profiles of Clone 91X unprotected trees replication 1 and 2.



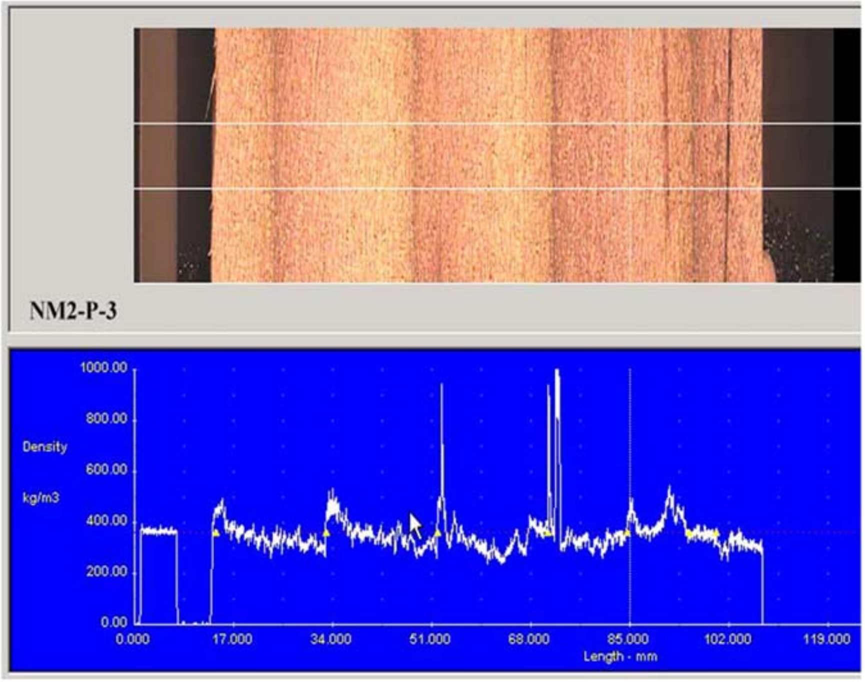


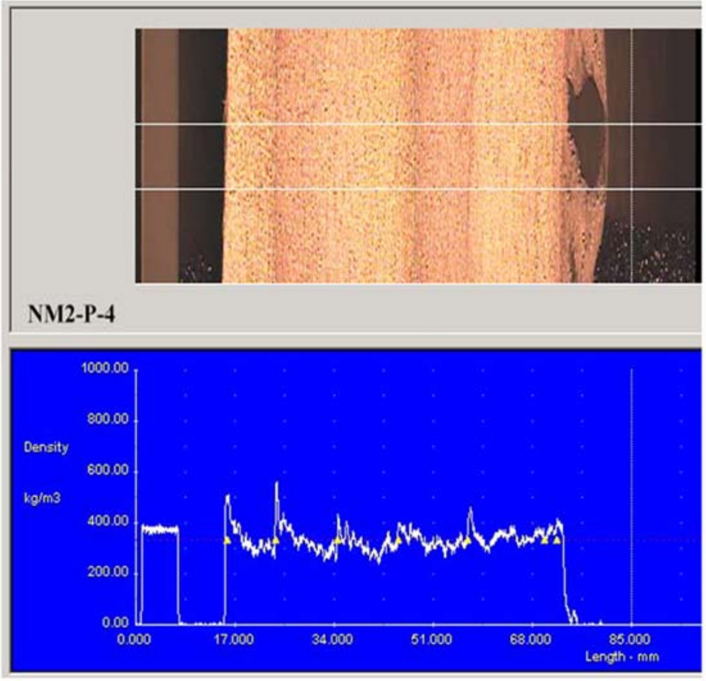
Appendix A9 Density profiles of Clone 91X unprotected trees replication 3 and 4.



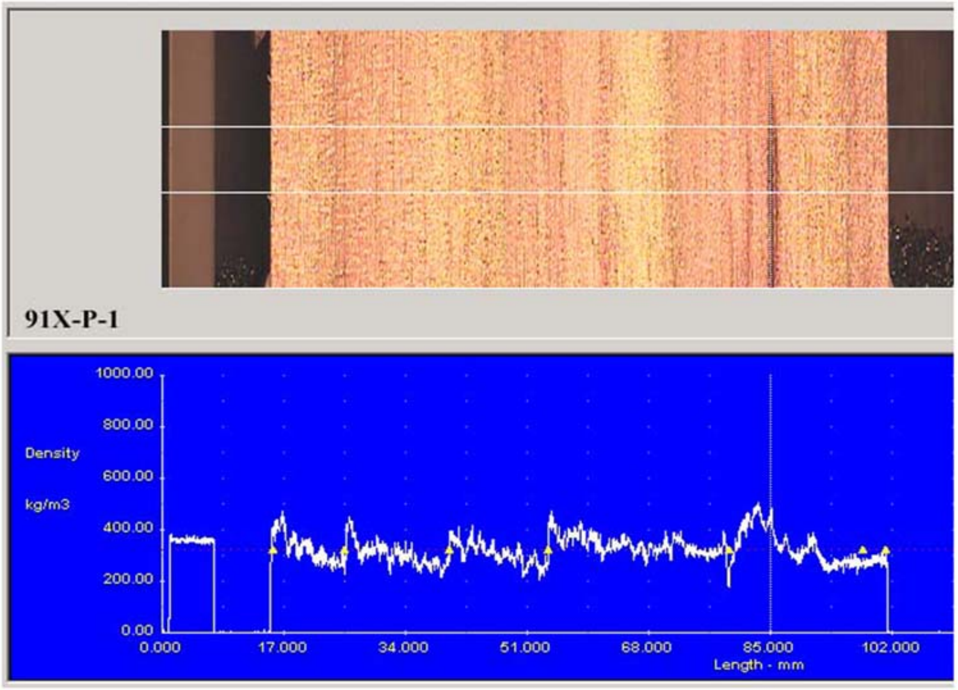


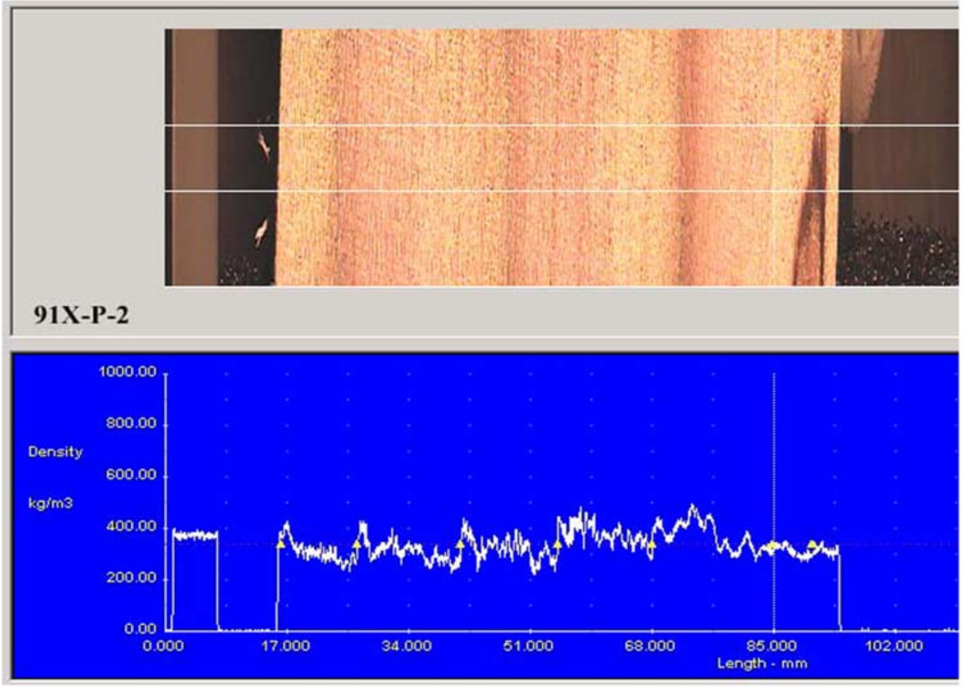
Appendix A10 Density profiles of Clone NM2 protected trees replication 1 and 2.



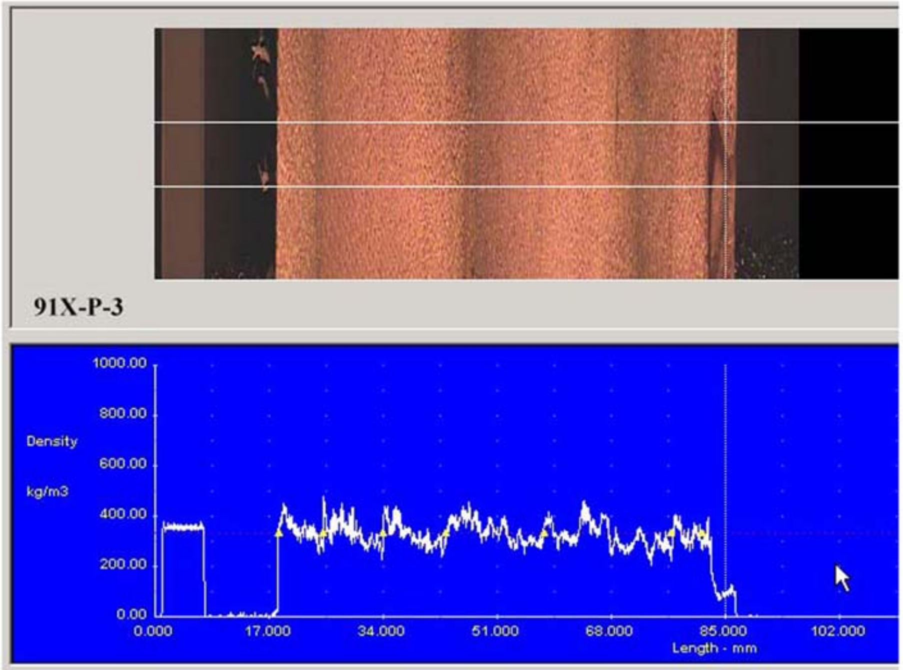


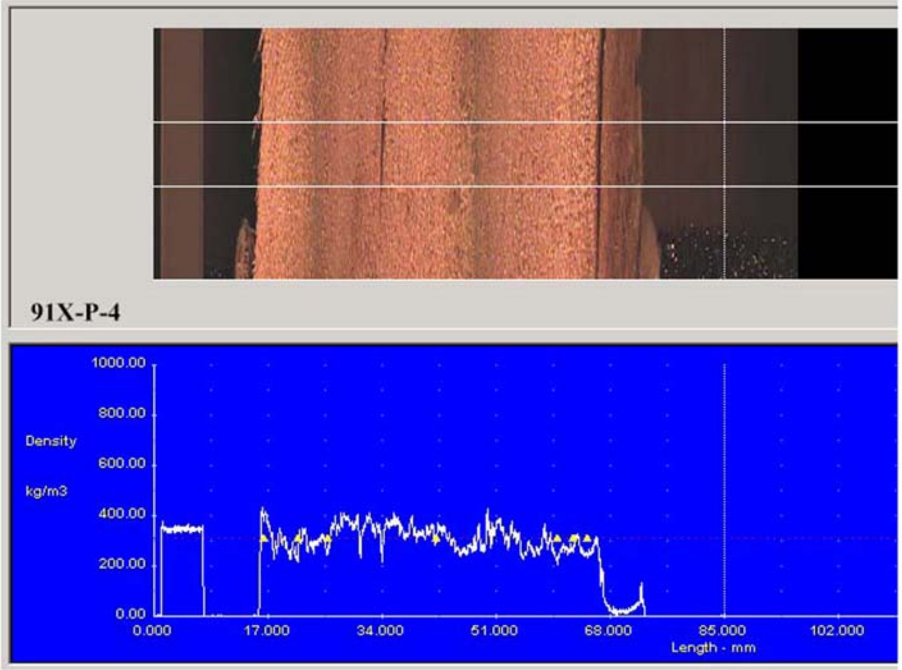
Appendix A11 Density profiles of Clone NM2 protected trees replication 3 and 4.



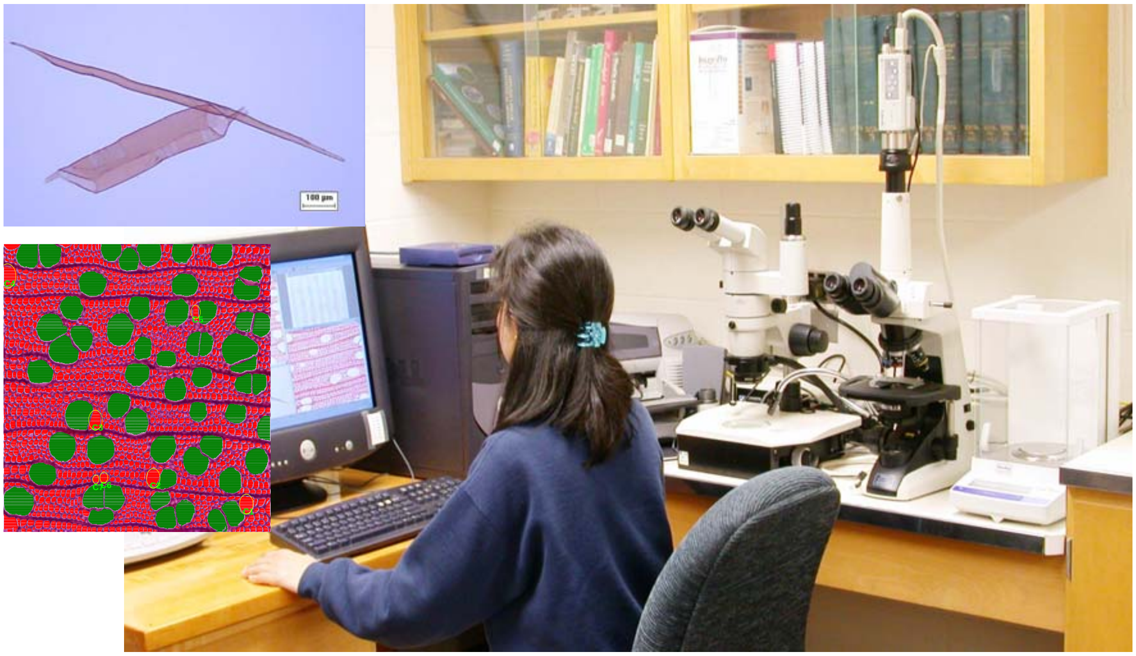


Appendix A12 Density profiles of Clone 91X protected trees replication 1 and 2.

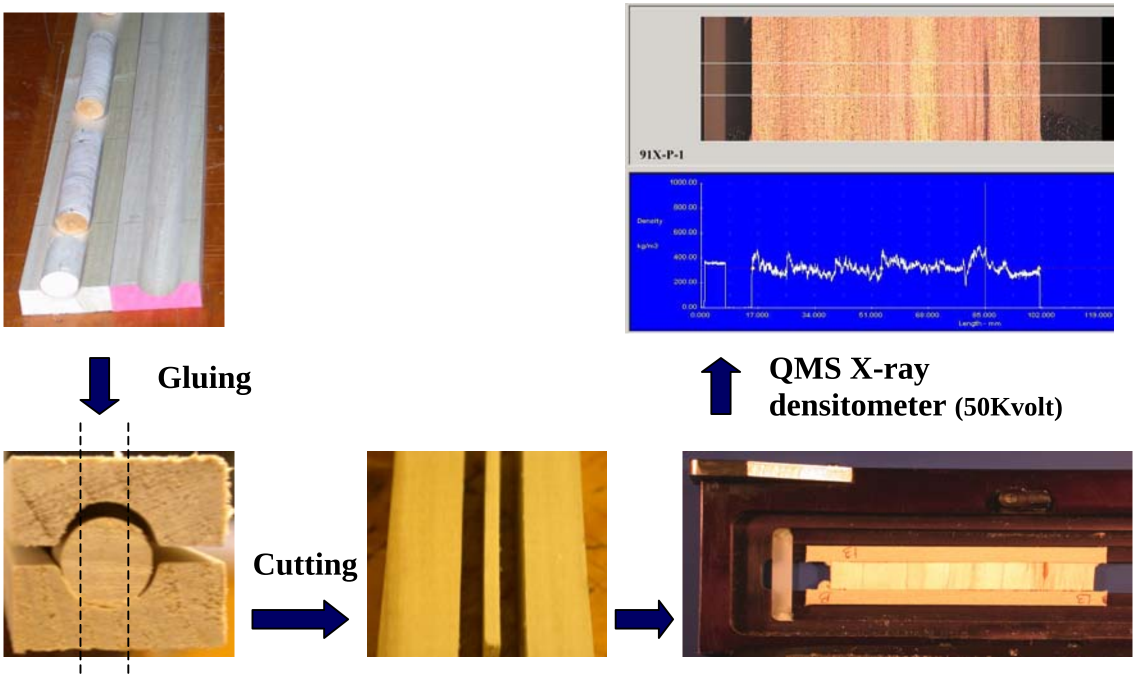




Appendix A13 Density profiles of Clone 91X protected trees replication 3 and 4.



Appendix A14 Image analysis system



Appendix A15 Sample for density profiles measurement.

Appendix B

Analysis results related to Family 9105 and Family 9163 are presented in appendix B.

Appendix B1 Analysis of variance for average fiber length of individual

Source	DF	F Value	Pr>F
Clone (family)	6	3.93	**0.0120**
Family	1	0.06	0.8141

Appendices C

Analysis results related to Tree 302, Tree 303, Tree 702, tree 1801, and Tree 2801 are presented in appendix C.

Appendix C1 Means of wood properties such as inside bark diameter (DIB), specific gravity (SG), Fiber length (FL), Vessel area (VA), vessel number (VN), vessel diameter (VD, unit: µm), and ray number (RN) by tree.

Tree Sample	DIB (mm)	SG	FL (mm)	VA (%)	VN	VD	RN
302	62.8	0.324	0.58	30.66	120	69.63	12.50
303	62.9	0.343	0.68	24.17	103	63.24	12.25
2801	77.6	0.395	0.61	20.89	101	56.33	13.00
702	110.6	0.401	0.69	19.55	97	60.76	14.00
1801	77.6	0.423	0.61	18.43	85	82.92	12.75

Appendix C2 Comparisons of fiber length by t-test

Parameter	Estimate	Standard Error	t Value	Pr>|t|
702 vs 1801	-0.0821	0.0313	-2.62	**0.0224**
702 vs all four	-0.0757	0.0248	-3.06	**0.0100**
302 vs 303	-0.0949	0.0313	-3.03	**0.0105**
302 vs 2801	-0.0263	0.0313	-0.84	0.4174
302 vs 1801	0.0319	0.0313	1.02	0.3288
303 vs 1801	-0.0630	0.0313	-2.01	0.0672
303 vs 2801	0.0686	0.0313	2.19	**0.0490**
1801 vs 2801	0.0056	0.0313	0.18	0.8617
302 vs 702	-0.1140	0.0313	-3.64	**0.0034**
303 vs 702	-0.0191	0.0313	-0.61	0.5543
2801vs 702	-0.8765	0.0313	-2.80	**0.0161**
1801vs 702	-0.0821	0.0313	-2.62	**0.0224**
302and303 vs 1801and2801	-0.0184	0.0313	-0.83	0.4232

Appendix C3 Comparisons of vessel area by t-test

Parameter	**Estimate**	**Standard Error**	**t Value**	**Pr>|t|**
702 vs 1801	-1.1225	1.2242	-0.92	0.3616
702 vs all four	3.9861	0.9678	4.12	**<.0001**
302 vs 303	6.4845	1.2242	5.3	**<.0001**
302 vs 2801	9.7695	0.0313	7.98	**<.0001**
302 vs 1801	-12.2295	0.0313	-9.99	**<.0001**
303 vs 1801	-5.7450	0.0313	-4.69	**<.0001**
303 vs 2801	3.2850	0.0313	2.68	**0.0086**
1801 vs 2801	-2.4600	0.0313	-2.01	**0.0474**
302 vs 702	11.1070	0.0313	9.07	**<.0001**
303 vs 702	4.6225	0.0313	3.78	**0.0003**
2801vs 702	1.3375	0.0313	1.09	0.2774
1801vs 702	-1.1225	0.0313	-0.92	0.3616
302and303 vs 1801and2801	-7.7573	0.8656	-8.96	**<.0001**

Appendix C4 Comparisons of vessel number by t-test

Parameter	**Estimate**	**Standard Error**	**t Value**	**Pr>|t|**
702 vs 1801	-12.0000	14.5911	-0.82	0.4269
702 vs all four	5.4375	11.5353	0.47	0.6458
302 vs 303	17.2400	14.5911	1.18	0.2600
302 vs 2801	18.7500	14.5911	1.29	0.2230
302 vs 1801	-35.2500	14.5911	-2.42	**0.0326**
303 vs 1801	-18.0000	14.5911	-1.23	0.2410
303 vs 2801	1.5000	14.5911	0.1	0.9198
1801 vs 2801	-16.5000	14.5911	-1.13	0.2802
302 vs 702	23.2500	14.5911	1.59	0.1370
303 vs 702	6.0000	14.5911	0.41	0.6882
2801 vs 702	4.5000	14.5911	0.31	0.7631
1801 vs 702	-12.0000	14.5911	0.31	0.4269
302and303 vs 1801and2801	-18.3750	10.3175	-0.82	0.1002

Appendix C5 Comparisons of vessel diameter by t-test

Parameter	Estimate	Standard Error	t Value	Pr>|t|
702 vs 1801	5.0200	4.3427	1.16	0.2702
702 vs all four	2.9868	3.4332	0.87	0.4014
302 vs 303	6.3925	4.3427	1.47	0.1668
302 vs 2801	13.3050	4.3427	3.06	**0.0098**
302 vs 1801	-3.8550	4.3427	-0.89	0.3921
303 vs 1801	2.5375	4.3427	0.58	0.5698
303 vs 2801	6.9125	4.3427	1.59	0.1374
1801 vs 2801	9.4500	4.3427	2.18	0.5020
302 vs 702	8.8750	4.3427	2.04	0.0636
303 vs 702	2.4825	4.3427	0.57	0.5781
2801 vs 702	-4.4300	4.3427	-1.02	0.3278
1801 vs 702	5.0200	4.3427	1.16	0.2702
302and303 vs 1801and2801	-5.3838	3.0707	-1.75	0.1050

Appendix C6 Comparisons of ray number by t-test

Parameter	Estimate	Standard Error	t Value	Pr>|t|
702 vs 1801	-1.2500	0.6158	-2.03	0.0651
702 vs all four	-1.3750	0.4868	-2.82	**0.0153**
302 vs 303	0.2500	0.6158	0.41	0.6919
302 vs 2801	-0.5000	0.6158	-0.81	0.4326
302 vs 1801	0.2500	0.6158	0.41	0.6919
303 vs 1801	0.5000	0.6158	0.81	0.4326
303 vs 2801	-0.7500	0.6158	-1.22	0.2466
1801 vs 2801	-0.2500	0.6158	-0.41	0.6919
302 vs 702	-1.5000	0.6158	-2.44	**0.0314**
303 vs 702	-1.7500	0.6158	-2.84	**0.0148**
2801 vs 702	-1.0000	0.6158	-1.62	0.1303
1801 vs 702	-1.2500	0.6158	-2.03	0.0651
302and303 vs 1801and2801	0.5000	0.4354	1.15	0.2732