ABSTRACT

SALAS MONTEALEGRE, MARTHA CLEMENCIA. Clonal Variation of *Eucalyptus grandis* W. Hill ex Maiden in Colombia. (Under the direction of Dr. Gary Hodge and Dr. Juan Acosta).

The aim of this work was to examine genetic variation for wood chemical traits in addition to the traditional breeding objectives of volume and wood density in a clonal population of *Eucalyptus grandis* established at Smurfit Kappa Colombia. Genetic control and genotype *x* environment interaction for growth, wood density, and several wood chemical traits for pulp production were estimated. A simulation model that integrates forestry and mill production was used to evaluate the impact of growth, wood density, pulp yield, insoluble lignin, and S/G (syringyl/guaiacyl) ratio on the cost of production of bleached kraft pulp. A number of regression models to estimate pulp yield from wood chemical traits were also developed and one of them was incorporated into the simulation model.

A wood sample subpopulation of 80 clones established in 3 sites was examined. The subpopulation was previously selected from a full population of 374 clones based on volume and wood density. The traits studied were height, DBH (diameter at breast height), volume/tree, and several near infrared (NIR) predicted variables: wood density, sugar content (glucose, xylose, galactose, arabinose, and mannose), lignin content (total, soluble, and insoluble), lignin composition (S/G ratio and S/(S+G)), and pulp yield. For those traits, linear mixed models were fitted across sites and broad-sense heritability, Type B genetic correlations, and genetic correlations between traits and ages were estimated. The clonal genetic values of volume, wood density, pulp yield, insoluble lignin, and S/G ratio were used

as inputs in a simulation model to evaluate their influence on the cost per ton of bleached kraft pulp.

Growth traits were all under moderate levels of genetic control ($\widehat{H^2}$ = 0.16 ± 0.04 to 0.33 ± 0.03), whereas wood density ($\widehat{H^2}$ = 0.39 ± 0.07) and wood chemical traits ($\widehat{H^2}$ = 0.17 ± 0.04 to 0.68 ± 0.11) were under moderate to high levels of genetic control. Estimates of Type B genetic correlations were consistently high for all kinds of traits ($\widehat{r_{Bg}}$ = 0.64 ± 0.10 to 0.75 ± 0.03 for growth traits; $\widehat{r_{Bg}}$ = 0.89 ± 0.06 for wood density; and $\widehat{r_{Bg}}$ = 0.77 ± 0.14 to 0.98 ± 0.02 for wood chemical traits) suggesting low genotype by environment interaction across sites. A moderately negative (unfavorable) correlation between growth traits and wood density was found (r_g = -0.35 to -0.43). Moderate correlations (favorable) were found between volume and pulp yield (r_g = 0.36), and for volume and insoluble lignin (r_g = -0.39). There was a moderate favorable correlation between wood density and glucose (r_g = 0.25), but a moderate unfavorable correlation between wood density and pulp yield (r_g = -0.23). The average cost per ton of pulp of the subpopulation was US $275.15. Wood biomass (product of mean annual increment and wood density) was the trait that most influenced the cost per ton of pulp (R^2 = 0.87). A ranking based on cost per ton of pulp was provided for selection of commercial clones. The average cost per ton of pulp for the 10 best clones was US$249.37.

The low genotype by environment interaction across sites found for all the traits studied suggested that a single combination of criteria can be applied to identify the best clones for the entire landbase of the company. For future generations of clonal trials, assessment of wood density and wood chemical traits using NIR is recommended; as well as a reduction in the number of trials (e.g. 3 instead of 12) and an increase in the number of clones tested in them (e.g. 800 instead of 400).

Clonal Variation of *Eucalyptus grandis* W. Hill ex Maiden in Colombia

by
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BIOGRAPHY

Martha Salas was born in 1984 in Bogotá, Colombia. She grew up in the same city where she attended Instituto Pedagógico Nacional high school and Universidad Distrital Francisco José de Caldas. Martha graduated in 2007 with a bachelor degree in forest engineering. During her college studies, she discovered an interest in tree improvement. Martha completed her first research project as part of her bachelor thesis on grafting of *Eucalyptus globulus*. She worked for the National Corporation of Forest Research and Promotion (CONIF) in 2007 as a Forest Researcher establishing progeny tests of *E. globulus* and coordinating the tree improvement program for this species along with an inter-institutional group. In 2008 Martha moved to Cali, Colombia to work for Smurfit Kappa Colombia as a Research Engineer in charge of genetic testing of several forest commercial species of eucalypts and tropical pines. In 2015, she moved to Raleigh, North Carolina in the United States to pursue a Master's degree in Forest Science at North Carolina State University sponsored by Smurfit Kappa Colombia and Camcore.

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CHAPTER 1: INTRODUCTION

Smurfit Kappa Colombia (SKC) is a vertically-integrated forest products company producing kraft pulp and paper from both pines and eucalypts. The forest plantation area of SKC covers 40,368 ha (103,784 ac), of which 15,420 ha (38,104 ac) are planted with *E. grandis* and the hybrid *E. grandis x E. urophylla*. The mills of the company demand 500,000 tons of eucalypts wood per year and produce around 150,000 tons of pulp from the wood fiber.

SKC began a tree improvement program of *Eucalyptus grandis* in 1987 with the aim of developing genetically superior clones and seeds for deployment of commercial plantations. To date, the company has completed 3 generations of improvement, and is currently executing the strategy for the fourth generation. Each generation of improvement has been carried out with two kinds of populations: a Main Population established with seedlings of around 250 families and an Elite Population established with 250 to 400 clones. The purpose of the Main Population is to select the best genotypes for seed production and conserve genetic diversity in the program; while the purpose of the Elite Population is to identify the best clones for commercial plantations (White et al. 2011). In each generation, a partial diallel mating design involving the 22 best genotypes is initiated to generate 40 full-sib families. Either 5 or 10 seeds per full-sib family are clonally replicated resulting in 250 to 400 clones for the Elite Population of the next generation (Osorio et al. 1995).

The operational clones used by the company have traditionally been selected using an index of volume and wood density at 3 years of age and genetic gains have been outstanding (White et al. 2011). However, from an industrial perspective the breeding objective for eucalypts is not just volume and wood density, but also the amount of pulp that the mill

produces per hectare planted. A characterization of wood chemical properties is relevant to address this issue. A better understanding of the wood chemical traits in the populations of *E. grandis* of SKC would contribute to identify the best clones for the specific purpose of pulp production and/or identifying alternative products of higher potential value.

Density is generally considered the most important wood property for a wide range of forest products (Zobel and Talbert 1984). It is positively correlated with wood strength, stiffness, heat transmission, and heat produced in combustion (Shmulsky and Jones 2011). Wood density also influences pulp properties and paper traits such as tear and tensile strength (Chambers and Borralho 1999). In tree improvement, whole-tree wood density is commonly estimated from wood cores taken non-destructively at breast height (Costa e Silva et al. 2009), and the oven dry weight of each sample is divided by its corresponding green volume measured by the water displacement method (Williamson and Wiemann 2010; Salas et al. 2014). Since the water displacement method can be time consuming and expensive for tree improvement programs measuring hundreds or thousands of samples, indirect estimations of wood density have been investigated, including tools or methodologies such as the Torsiometer, the Pilodyn, nail withdrawal, and Resistograph (Gao et al. 2012). The Resistograph is a motorized drill which pushes a small needle-like bit through wood while measuring the resistance to bit penetration. The Resistograph is non-destructive, and has been shown to be easy to use, and a rapid way to estimate relative wood density. These attributes make Resistograph more efficient to rank genotypes from progeny trials compared to wood cores and the other indirect methods mentioned (Isik and Li 2003; Gao et al. 2012).

Cellulose, hemicelluloses, and lignin are the main organic polymers that describe the chemical composition of wood (Stackpole et al. 2011). Cellulose is the main polymer of the

wood cell wall (Walker 2006) and is made up of glucose units (Magaton et al. 2013). The tensile strength of fibers is attributed to cellulose (Turner and Somerville 1997). Cellulose content has shown strong positive correlation with kraft pulp yield in *Eucalyptus* (Schimleck et al. 2005; Stackpole et al. 2011). Hemicelluloses are made up of six carbon sugars (glucose, galactose, and mannose), five carbon sugars (xylose and arabinose), and other sugar derivatives such as glucuronic-acid (Magaton et al. 2013; Martínez et al. 2014). Xylose is the main component of hemicelluloses in hardwoods and its retention during pulping has been studied in commercial *Eucalyptus* (Mendes et al. 2009; Magaton et al. 2013; Martinez et al. 2015). Lignin is the second-most abundant polymer of wood, and it has shown a strong negative correlation with kraft pulp yield (Walker 2006; Stackpole et al. 2011). Lignin is a complex polymer composed of phenylpropane units and is variable in composition (Santos et al. 2013). It is formed by three monolignol monomers hydroxyphenyl (H), guaiacyl (G), and syringyl (S) in varying proportions among species and individuals (Pinto et al. 2002). In *Eucalyptus* and hardwoods in general, lignin is mainly composed of S lignin and G lignin (Alves et al. 2011). The presence of methoxyl groups in the monomers determines the reactivity of lignin during pulping; S lignin has two methoxyl groups and higher reactivity than G lignin that has one methoxyl group (Pinto et al. 2002). High S/G ratios are favorable for pulp production due to higher delignification rates, lower chemical consumption and higher pulp yields in some species (Alves et al. 2011).

There are several analytical techniques for the assessment of wood chemical traits. In general, oven-dried and extractive-free ground wood samples are required. The polysaccharides of wood are glucan, xylan, galactan, arabinan, and mannan; and they constitute cellulose and hemicelluloses. Total polysaccharides can be removed from wood

by acid hydrolysis or enzymatic hydrolysis (Olsson and Salmén 1997; Miller 2013; Santos et al. 2013). For further breakdown of polysaccharides into monomeric sugars such as glucose, xylose, galactose, arabinose, and mannose, high performance liquid chromatography (HPLC) is used to measure sugar concentrations after acid or enzymatic hydrolysis (Coleman et al. 2007; Edmunds 2015; Miller 2013). The acid hydrolysis technique is also used for lignin assessment. Insoluble lignin, also known as klason lignin, can be obtained gravimetrically from the wood samples after removing polysaccharides by acid hydrolysis (Yeh 2005; Olsson and Salmén 1997; Horvath 2010; Edmunds 2015). In hardwoods, the lignin is partially dissolved during acid hydrolysis and gravimetric values are corrected using a UV spectrophotometer (Olsson and Salmén 1997, Horvath 2010, Miller 2013). Another technique for lignin assessment that has been widely used is milled wood lignin (MWL), also known as Bjorkman lignin, along with nuclear magnetic resonance NMR spectroscopy (Capanema et al. 2005; Zhang et al. 2010; Santos 2012).

Lignin monomers of wood can be determined by different methods. Thioacidolysis (Rolando et al. 1992) or a streamlined version of thioacidolysis (Robinson and Mansfield 2009) are two of them. Both techniques are applied along with gas chromatography (GC) for monomer quantification. The streamlined thioacidolysis has shown to be efficient for large phenotypic screenings of S/G ratio (Robinson and Mansfield 2009). Pyrolysis has been used as an analytical technique for S/G ratio assessment in *Eucalyptus* (Alves et al. 2011; Stackpole et al. 2011). Nitrobenzene oxidation is another technique for estimation of S/V ratio (V stands for vanillin and vanillic acid) which is subsequently converted to S/G ratio (Santos 2012).

Kraft pulp yield assessment in a laboratory is carried out by cooking wood chips in a solution of sodium hydroxide (NaOH) and sodium sulfide (NaS_2) under specified pulping conditions in a laboratory digester (Costa e Silva et al. 2009; Schimleck et al. 2005). Elevated temperature and pressure are required to dissolve lignin. In general, pulping conditions include degree of delignification (kappa number), rate of delignification (H factor), liquor to wood ratio, sulfidity, cooking temperature, and weight of the oven dry sample charged in the digester (Smook 2002).

Many of these wetlab analytical techniques are expensive, time consuming, and require highly skilled laboratory technicians. For tree breeding programs, which have an interest in evaluating a great number of genotypes, the cost of chemical analyses can be prohibitive. Near-infrared spectroscopy (NIR) is a rapid technique that can replace many types of direct analyses (Hodge and Woodbridge 2010; Schimleck et al. 2010). NIR instruments beam near-infrared light onto a wood sample and measure the reflectance across a range of wavelengths. Depending on the NIR instrument, different wavelength ranges and intervals are used. For example, the Foss 6500 scans from 400 nm to 2500 nm providing a reading every 2nm, while the microPHAZIR handheld NIR scans from 1600 nm to 2400 nm providing a reading approximately every 8 nm. NIR instruments generate hundreds of predictor variables (reflectances) which can be used to predict chemical attributes by applying partial least squares (PLS) regression (Bjorsvik and Martens 2008). The development of reliable NIR models often requires the application of mathematical pre-processing techniques on the spectra. The most commonly used pre-processing techniques are classified by Rinnan et al. (2009) into scatter correction methods and spectral derivatives. Multiplicative Scatter Correction (MSC) and Standard Normal Variate (SNV) are the most

important scatter correction methods. The most common spectral derivative tranformations are the Savitzky Golay derivative algorithm with different wavelength window sizes, and detrending (also called baseline correction) Rinnan et al. 2009).

Camcore is an international forest gene conservation and tree breeding program at North Carolina State University that works with a number of pine and eucalypt species. Camcore has successfully developed global NIR models for pines that allow the prediction of lignin and cellulose content for many pine species at many locations around the world (Hodge and Woodbridge 2010). More recently, Camcore has been working on global NIR models for *Eucalyptus* including *E. urophylla*, *E. dunnii*, *E. globulus*, and *E. nitens* (Hodge et al. 2016). The *Eucalyptus* NIR models allow for the estimation of sugars, lignins, and S/G ratio. The current NIR models for *Eucalyptus* wood chemistry can be expanded through the addition of a small number of *E. grandis* samples with wetlab wood chemistry measurements, and the expanded model could then be used to characterize the Colombian clonal population of *E. grandis*.

This thesis will evaluate the clonal genetic variation for both growth and wood properties of a population of *E. grandis* established at Smurfit Kappa Colombia. In Chapter 2, genetic control and genotype *x* environment interaction of growth, wood density, and wood chemical traits will be examined. Wood chemical traits include sugar content (glucose, xylose, galactose, arabinose, and mannose), lignin content (total, soluble, and insoluble), lignin composition assessed using S/G ratio (and S/(S+G)), and pulp yield. In Chapter 3, the impact of growth, wood density, and wood chemical traits on the cost of production of bleached kraft pulp will be analyzed by means of a simulation model that integrates forestry and mill production. Regression models to relate wood chemical composition to pulp yield

will be also developed. Chapter 4 summarizes the scope and findings of this research and presents practical implications for Smurfit Kappa Colombia.

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CHAPTER 2: GENETIC PARAMETERS FOR GROWTH, WOOD DENSITY, AND WOOD CHEMICAL TRAITS OF A CLONAL POPULATION OF *Eucalyptus grandis* IN COLOMBIA

Introduction

Eucalyptus grandis is a subtropical species native to Australia, where it grows on the east coast from Bundaberg (Queensland) to Newcastle (New South Wales) between latitudes of 16 and 34° S, and from the sea level to 1100 m altitude (Boland et al. 2006). In similar ecological conditions outside Australia (e.g., Brazil, Argentina, South Africa), *E. grandis* is recognized as an adapted fast-growing species, one of the most widely planted eucalypts species, and an important raw material for the pulp and solid-wood industries (Ugalde and Pérez 2001; Cunningham and Tamang 2014; Myburg et al. 2014). *E. grandis* naturally hybridizes with *E. urophylla* in exotic environments and can be successfully artificially crossed forming the hybrid *E. grandis x E. urophylla*, which is clonally propagated. The hybrid is also noted for being fast-growing (Hodge and Dvorak 2015).

Genetic parameters for growth traits in *Eucalyptus* have been more studied than parameters of wood traits. The main causes of the limited work on genetics of wood properties are high prices of direct assessments, and difficulties regarding time, equipment, and skills that the assessments require (Raymond 2002). This constraint exists even for wood density, the most studied wood property on trees (Zobel and Talbert 1984; Shmulsky and Jones 2011). Indirect assessments of physical and chemical wood traits using techniques like near infrared (NIR) spectroscopy, have become popular in the last two decades (Schimleck et al. 2004; Stackpole et al. 2010; Schimleck et al. 2010). NIR estimates are fast, inexpensive, and precise provided that a suitable calibration model is used. The application of NIR

chemometric methods has increased the research on genetic parameters of wood chemical traits and the commercially planted eucalypts are a target of study.

This chapter will examine the genetic control and genotype x environment interaction for growth, wood density, and wood chemical traits of an elite clonal population of *Eucalyptus grandis* planted in Colombia. We will assess the potential response to selection of key wood chemical traits as a complement to selection for growth and wood density. The wood chemical traits used in this study are NIR predictions for sugars (glucose, xylose, galactose, arabinose, and mannose), lignin (total, soluble, and insoluble), S/G ratio (also analyzed as S/(S+G)), and pulp yield. A better understanding of the genetic parameters of wood chemistry will help to optimize the selection strategy for the upcoming generations of tree improvement.

Materials and Methods

Population of Study

This study was based on measurements of growth and wood traits taken from a series of clonal trials of *E. grandis*, known as the Elite Population of the third generation of improvement at Smurfit Kappa Colombia (SKC). Clones included in these trials came from a partial diallel mating design. The mating involved 21 parents from the Elite Population of the previous (second) generation of improvement. The mating design is shown in Appendix A. From approximately 40 full-sib families created by control pollination, 374 clones were established on 12 sites with an average of 10 clones per full-sib family. Additionally, the 12 trials included 14 control lots: 7 second-generation operational *E. grandis* clones, 6 first-generation operational *E. urograndis* clones, and 1 bulk seedlings lot from a second-

generation clonal seed orchard. The 12 trials were established over a range of environmental conditions representative of the soils and climate in which *E. grandis* is planted operationally by the company. The experimental design was an alpha lattice with 5 to 9 trees per incomplete block and 10 replications per site (White et al. 2011). Each clone was represented by one ramet per replication (single-tree plot). Replications 1 to 5 were managed under operational silviculture, and replications 6 to 10 were managed under additional fertilization and intensive silviculture.

The best 77 clones out of the total 374 were chosen for this study based on a clonal selection index for volume and density at 3 years of age. The best 77 clones came from the mating of 20 parents and represent 31 controlled pollinated families. A set of 3 clonal control lots of *E. grandis* and 3 of *E. urograndis* were included in the population of study, for a total of 83 clones. Appendix B shows the selected *E. grandis* clones, and their clonal values. A subset of 3 clonal trials out of the 12 original ones was selected: Tesalia in the North Zone, Alpes in the Central Zone, and Mota in the South Zone. Table 2.1 describes general information about the clonal trials. The 3 trials contained almost all the clones of interest, had higher survival than the other 9 trials, and three different environmental exposures based on climate and soils information. In summary, 1,222 trees were selected for the wood sample subpopulation, representing 83 clones on 3 sites with approximately 5 ramets per clone per site; and the ramets were randomly selected between replicates of each site.

Table 2.1 *General information of the E. grandis clonal trials. Trials in bold were selected for this study.*

Farm	Zone	Latitude (N)	Longitude (W)	Elevation (m.a.s.l)	Rainfall (mm)	Date Planted	No. of Clones
Mediaciones	North	4° 33'	75° 36'	2114	2304	7/16/2007	372
Campania	North	5° 26'	75° 46'	2315	2739	12/20/2007	341
Balkanes	North	4° 49'	75° 37'	1779	2694	7/17/2007	373
Tesalia	**North**	**4° 48'**	**75° 36'**	**1969**	**2712**	**7/9/2007**	**374**
Claridad	South	2° 28'	76° 34'	1826	2012	6/8/2007	358
Mota	**South**	**2° 32'**	**76° 38'**	**1731**	**2221**	**6/2/2007**	**372**
San José	South	2° 35'	76° 34'	1812	2204	5/22/2007	366
Hato Frío	South	2° 17'	76° 38'	2114	2633	12/1/2007	315
Alpes	**Center**	**4° 00'**	**76° 27'**	**1614**	**1428**	**6/29/2007**	**374**
Carbonero	Center	3° 44'	76° 28'	1785	1404	5/18/2007	341
Suiza	Center	3° 51'	76° 30'	1554	1210	5/16/2007	374
Canadá	Center	4° 10'	75° 51'	2162	1825	7/26/2007	371

Traits Measured

The growth traits studied were height, diameter at breast height (DBH), and volume at 3 and 6 years. Volume/tree was calculated using equation [1] (SKC Planning Department personal communication 2017). Wood-core density at 3 and 6 years and NIR-predicted wood density at 6 years were analyzed. For NIR prediction, wood density was first determined on a sub-set of 365 samples to calibrate an NIR model, and subsequently predicted for 1,183 *E. grandis* samples at 6 years. Wood chemical traits examined were glucose, xylose, galactose, arabinose, mannose, total lignin, insoluble lignin, soluble lignin, S/G ratio, syringil percentage (as S/S+G), and pulp yield; all of them predicted for 1,183 samples at 6 years from re-calibrated global NIR models for *Eucalyptus* wood chemistry and a re-calibrated NIR model for *E. grandis* pulp yield. Further information about the used NIR models is described below.

$$\mathrm{Vol} = \pi * (\mathrm{d}/2)^2 * \mathrm{H} * \mathrm{VF} \qquad [1]$$

Where:

Vol = Tree volume in m^3

d = diameter at breast height in m

H = Total height in m

VF = Volume factor determined from the cubic spline of the corresponding diametric class

Sample Collection and Processing

Increment cores were collected from the trees at 3 and 6 years using a Haglöf Increment Borer Bit Starter. Wood cores of 5 mm diameter were taken at DBH height; from bark to bark and crossing through the pith. Gravimetric wood density determination was done in Colombia at Smurfit Kappa's laboratory. Wood cores were submerged in water for 144 hours until total water saturation. Green volume was measured using the water displacement method and dry weight was determined after oven-drying the wood cores at 103°C +/- 2 °C for 48 hours. Specific gravity was determined using equations 2 and 3.

$$\text{Volume } (cm^3) = \text{Weight in Air (g)} - \text{Weight in Water(g)} \quad [2]$$

$$\text{Specific Gravity } (g/cm^3) = \frac{\text{Dry weight (g)}}{\text{Volume } (cm^3)} \quad [3]$$

Wood discs at approximately DBH height from 1,222 9-year trees were cut. Wedges from pith to bark, of approximately 5 cm thick and 8 cm wide, were cut from the discs. Wedges were cut avoiding cracks, branches, and phytosanitary damage. For severely asymmetric shaped trees (the ones that did not have the pith at the center of the disc) the

wedge was cut representing typical wood (Figure 2.1). Wedges were oven dried at 50°C and sent to Raleigh, NC, USA and processed at North Carolina State University. Since trees are operationally harvested at 7 years and the regular age of measurement of eucalypts trials at SKC is 6 years, DBH at 9 years was measured before cutting the trees to estimate the portion of 6-year wood in each wedge of 9 years. The estimation was based on the ratio DBH6:DBH9. Wedges were cut using a band saw (model Craftsman 1/3 3.5 AMP 10'' 21400) to obtain two 6-year wood pieces: one to be ground into wood meal and scanned with a desktop NIR (Figure 2.2 piece A), and a second one for specific gravity determination (Figure 2.2 piece B). Wood pieces for desktop NIR scanning were cut removing 1 cm of wood nearest the pith, partitioned into small pieces, and ground using a spice grinder Waring WSG30 and a micro blade mill MA048 Marconi. Grinding with the micro blade mill was done with a mesh 1/16'' of diameter, 3/32'' staggered centers, 132 holes per square inch and 41% of open area. Wood meal samples were oven dried at 50°C for 12 hours before scanning. The 1,222 ground individual tree samples of approximately 5 gr each were scanned with a desktop NIR machine Foss NIRSystemsTM 6500. The spectra were collected at 2 nm intervals over the wavelength range of 400-2498 nm but only reads between 1100 and 2498 nm were used for modeling. In addition, 365 wood pieces representing the 80 *E. grandis* clones across the 3 sites were processed at N.C. State laboratory for specific gravity determination. Wood wedges were submerged in water several days until total water saturation. Specific gravity was estimated using equations 1 and 2 and the standard water displacement method to calculate green volume.

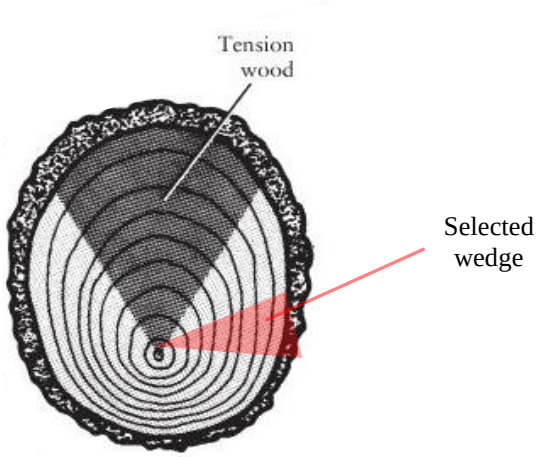

Figure 2.1 *Wedge selection for trees with tension wood (modified from Shmulsky and Jones 2011).*

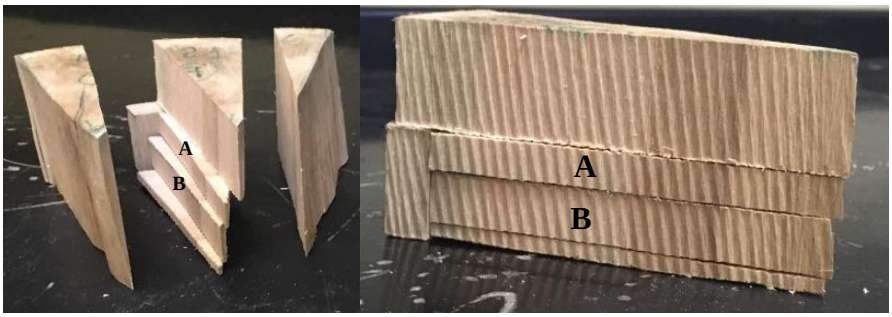

Figure 2.2 *6-year wood wedges. A: piece for desktop NIR scanning as wood meal. B: piece for specific gravity determination.*

Wood Density Estimation Using NIR Spectroscopy

A data set of 365 samples was used to build a calibration model relating specific gravity with the NIR spectral data. A pipeline (Hodge et al. 2016a) developed with the statistical software R (R Core Team 2016) and the R packages pls, DMwR, and prospectr (Mevik et al. 2016; Torgo 2010; Stevens and Ramírez 2013) was used for data analysis. Raw spectra data was analyzed and 4 outlier spectra were identified based on its Local Outlier

Factor (Breunig et al. 2000). Outlier-free spectra were used to fit the NIR calibration model using partial least squares (PLS) methods and "leave-one-out" (LOO) cross-validation. The final model retained 10 PLS factors, provided an R^2 of cross-validation of 0.66, and a standard error of cross-validation (SECV) of 0.021. The PLS calibration model was applied to 1,180 samples to eventually obtain NIR-predicted specific gravity for all the wood sample subpopulation.

Wood Chemical Traits Estimation Using NIR Spectroscopy

Global NIR models for *E. urophylla*, *E. dunnii*, *E. globulus*, and *E.nitens* wood chemistry developed by Camcore – N.C. State University (Hodge et al. 2016b) were re-calibrated including wetlab chemistry results from a subset of 36 samples of *E. grandis* from the wood sample subpopulation. The previously developed global NIR models were first used to obtain initial predictions of chemical traits for all 1,222 samples; and a principal component analysis was done for the whole spectra. Subsequently, 36 *E. grandis* samples out of 1,222 were selected for wetlab chemistry choosing the ones that better represented the variation on predicted chemical traits and the variation on the first two principal components of the spectra. Micro-analytical analyses were done at the University of British Columbia with the same 5 gr. woodmeal samples used for desktop NIR scanning. Sugar concentrations were determined by acid hydrolysis and high performance liquid chromatography HPLC (Coleman et al. 2007). Lignin content was also determined by acid hydrolysis and using a modified Klason (Coleman et al. 2007). A streamlined thioacidolysis procedure was used to determine the lignin monomer ratios (Robinson and Mansfield 2009). Re-calibrated global NIR models, one per each of the traits studied, were fitted transforming all the spectra either

with Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Savitzky-Golay 2nd derivative with 7 or 5 points in the convolution window (SG7/SG5), or a combination between them. Table 2.2 shows summary statistics for the re-calibrated NIR models used to eventually obtain NIR-predicted wood chemical traits for all the studied *E. grandis* samples of the wood sample subpopulation.

Table 2.2 *Summary statistics of re-calibrated NIR-models used for wood chemical traits prediction.*

Variable	Transformation	Factors	Cross-val R^2	SECV
Lignin	SG5	7	0.94	1.079
Insoluble lignin	SNV_SG5	6	0.96	0.935
Soluble lignin	SG7	10	0.65	0.545
S/G	MSC_SG7	9	0.86	0.551
S/(S+G)	MSC_SG7	11	0.95	1.334
Glucose	MSC_SG7	9	0.74	1.680
Xylose	SNV_SG7	9	0.89	0.754
Galactose	SG7	13	0.91	0.252
Arabinose	SG7	8	0.72	0.058
Mannose	SG7	15	0.75	0.307
Pulp yield	DT	4	0.44	1.268

Pulp Yield Estimation Using NIR Spectroscopy

In 2009 Camcore and SKC developed an NIR model to predict pulp yield for *E. grandis*. Spectra and pulp yield lab data of 99 samples from the previously developed model were re-calibrated using the R pipeline mentioned before. The re-calibrated model was constructed from transformed spectra using de-trend scatter correction and removing 3 outliers based on its LOF (Breunig et al. 2000). The final model retained 4 PLS factors and the R^2 of cross-validation, and SECV were 0.44 and 1.268 respectively (Table 2.2). The re-

calibrated model was applied to 1,180 woodmeal samples to obtain NIR-predicted pulp yield for the wood sample subpopulation.

Genetic Parameter Estimation

Linear mixed models were used to analyze different response variables. The following models were fit to the data to estimate variance components and subsequently estimate broad sense heritability $\widehat{H^2}$ [6] and Type B genetic correlations $\widehat{r_{Bg}}$ [7]. Model [4] was used for specific gravity and wood chemical variables, whereas model [5] was used for standardized growth variables. Analyses were carried out using R (R Core Team 2016) and the nmle and lme4 packages in R (Pinheiro et al. 2018; Bates et al. 2015).

$$Y_{ijkl} = \mu + F_i + R_j + (FR)_{ij} + (B_{k(i)}R)_{kj} + C_l + CF_{li} + \varepsilon_{ijkl} \quad [4]$$

Where:

$i = 1, 2, 3$ indexes the different farms

$j = 1, 2,$ indexes the different re-fertilization treatments

$k = 1, 2, 3, \ldots, 10$ indexes the different blocks

$l = 1, 2, 3, \ldots, 83$ indexes the different clones

Y_{ijkl} = any of the response variables from the ith farm, jth re-fertilization, kth block, and lth clone

μ = mean

F_i = fixed effect of farm

R_j = fixed effect of re-fertilization

$(FR)_{ij}$ = fixed effect of the interaction between farm and re-fertilization

$(B_{k(i)}R)_{kj}$ = fixed effect of the interaction between block, nested within a farm, and re-fertilization

C_l= random effect of clone, assumed $\sim N(0, \sigma^2_C)$

CF_{li} = random effect of the interaction between clone and farm, assumed $\sim N(0, \sigma^2_{CxF})$

ε_{ijkl} = experimental error, assumed $\sim N(0, \sigma^2_\varepsilon)$

$$Y_{ikl} = \mu + F_i + B_{k(i)} + C_l + CF_{li} + \varepsilon_{ikl} \quad [5]$$

Where:

i = 1, 2, 3 indexes the different farms

k = 1, 2, 3, …, 10 indexes the different blocks

l = 1, 2, 3, …, 83 indexes the different clones

Y_{ikl} = any of the response variables from the *i*th farm, *k*th block, and *l*th clone

μ = mean

F_i = fixed effect of farm

$B_{k(i)}$ = fixed effect of blocks nested within a farm

C_l= random effect of clone, assumed $\sim N(0, \sigma^2_C)$

CF_{li} = random effect of the interaction between clone and farm, assumed $\sim N(0, \sigma^2_{CxF})$

ε_{ikl} = experimental error, assumed $\sim N(0, \sigma^2_\varepsilon)$

Clonal (or broad-sense) heritability estimates were calculated as:

$$\widehat{H^2} = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2_{CxF} + \sigma^2_\varepsilon} \quad [6]$$

Where:

$\widehat{H^2}$ = broad sense heritability

σ^2_C = clonal genetic variance

σ^2_{CxF} = clone x farm interaction variation

σ^2_ε = within-site error variance attributed to microsite variation in farm and measurement error

Standard errors of clonal H^2 estimates were calculated using the standard errors of $\hat{\sigma}^2_c$ and treating $\hat{\sigma}^2_{phen}$ as a constant according to Dickerson's approximation (Dickerson 1969).

Type B clonal genetic correlations (Burdon 1977) were calculated as:

$$\widehat{r_{Bg}} = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2_{CxF}} \quad [7]$$

Where:

$\widehat{r_{Bg}}$ = Type B genetic correlation

σ^2_C = clonal genetic variance

σ^2_{CxF} = clone x farm interaction variation

Type B correlations measure the genetic correlation between the same trait expressed on two different sites (Burdon 1977). Type B correlations over multiple sites range between zero and one; an $r_B \approx 1$ indicates a near-perfect correlation between performance in different environments, or in other words, an absence of genotype (or provenance) *x* environment interaction. Standard errors of r_{Bg} estimates were calculated using the standard errors of $\hat{\sigma}^2_c$

and $\hat{\sigma}^2_{ce}$ and $Cov(\hat{\sigma}^2_c , \hat{\sigma}^2_{ce})$ using a first-order approximation of a Taylor-expansion series (Lee and Forthofer 2006).

Clonal Genetic Value Predictions and Genetic Correlations Among Traits

The linear mixed models described above and the lme4 package in R (Bates et al. 2015) were also used to estimate best linear unbiased predictions (BLUPs) of clonal effects for each of the traits. BLUPs are genetic values used by tree breeders to rank genotypes.

Genetic correlations among growth traits and wood density at ages 3 and 6, and among growth traits, wood density, and wood chemical traits at 6 years were estimated using ASREML (Gilmour et al. 2006). For each correlation of interest, a paired trait analysis was conducted using the same multiple site linear model discussed above. The clonal genetic correlation and the associated standard error were estimated directly by the program.

Results and Discussion

Growth Means

Mean growth variables did not vary substantially between sites and analyses were carried out with all the data across sites. Summary statistics of height, DBH, and volume/tree for the full population and the wood sample subpopulation are presented in Table 2.3a and Table 2.3b. In the full population height increased from 18.5 m to 27 m from 3 to 6 years age; similarly, DBH increased from 13.6 cm to 18 cm; and volume from 0.138 m^3 to 0.358 m^3 (Table 2.3a). In the wood sample subpopulation growth traits also increased substantially from 3 to 6 years. Height increased from 20.1m to 30.1 m; DBH from 15.5 cm to 21 cm; and volume from 0.180 m^3 to 0.491 m^3 (Table 2.3b). Survival in the full population decreased

from 89.1% to 84.3% from 3 to 6 years (results not shown) and survival for the wood sample subpopulation was 100% since living trees were intentionally selected for this study.

Height and volume means observed in the full population (Table 2.3a; height = 18.5 m at 3 years and 27 m at 6 years; volume = 0.138 m^3 at 3 years and 0.358 m^3 at 6 years) coincide with other results of *E. grandis* in Colombia e.g. Osorio et al. (2000, 2003) report typical heights of 15.6 m at 3 years, 26.2 m at 6 years, typical volume/tree of 0.100 m^3 at 3 years and 0.330 m^3 at 6 years. Growth means were clearly higher in the wood sample subpopulation than in the full population (Tables 2.3a and 2.3b). Height in the full population was 18.5 m at 3 years and 27 m at 6 years, while height in the subpopulation was 20.1 m at 3 years and 30.1 m at 6 years. DBH in the full population was 13.6 cm at 3 years and 18 cm at 6 years, while DBH in the subpopulation was 15.5 cm at 3 years and 21 cm at 6 years. Similarly, volume in the full population was 0.138 m^3 at 3 years and 0.358 m^3 at 6 years, while volume in the subpopulation was 0.180 m^3 at 3 years and 0.491 m^3 at 6 years. This was an expected result since the 80 clones of the subpopulation where selected out of the 374 clones of the full population based on volume and wood density. Survival of 84.3% at 6 years in the full population looks normal comparing with other *E. grandis* progeny trials that report survival between 82.2% and 89.8% at 6 years (Osorio et al. 2000; Marcó and White 2002; Osorio et al. 2003).

Table 2.3a *Full population summary statistics for growth variables across sites.*

Variable	Age	Mean	Std Dev	Min	Max
Height (m)	3	18.5	3.7	3.3	27.3
DBH (cm)	3	13.6	3.2	1.9	22.6
Volume (m^3)	3	0.138	0.071	0.001	0.431
Height (m)	6	27.0	5.7	6.6	37.8
DBH (cm)	6	18.0	5.1	4.5	31.7
Volume (m^3)	6	0.358	0.222	0.006	1.159

Table 2.3b *Wood sample subpopulation summary statistics for growth variables across sites.*

Variable	Age	Mean	Std Dev	Min	Max
Height (m)	3	20.1	3.0	8.1	27.3
DBH (cm)	3	15.5	2.5	4.6	22.6
Volume (m^3)	3	0.180	0.068	0.007	0.431
Height (m)	6	30.1	4.1	11.9	37.4
DBH (cm)	6	21.0	4.3	7.3	31.2
Volume (m^3)	6	0.491	0.220	0.028	1.158

Growth Genetic Parameters

Full population means, variance components, and genetic parameters for standardized height, DBH, and volume across sites are presented in Table 2.4a. The same information for the wood sample subpopulation is presented in Table 2.4b. In the full population, broad sense heritability was moderate ranging between 0.29 to 0.33, and Type B genetic correlations were high ranging between 0.74 and 0.81 (Table 2.4a). In the wood sample subpopulation, broad sense heritability was moderate ranging between 0.16 and 0.24, and Type B genetic correlations were moderate to high ranging between 0.59 and 0.86 (Table 2.4b). Lower estimates of broad sense heritability in the wood sample subpopulation respect the full population were an expected result. The wood sample subpopulation is a truncated set of

clones selected from the full population based on volume and wood density. For this reason, lower phenotypic variances attributed to clones (σ_C^2) are expected in the subpopulation respect the full population, which leads to lower estimates of H^2 in the subpopulation. For the same reason estimates of Type B genetic correlations where somewhat lower in the subpopulation. The moderate estimates of H^2 for volume/tree in this research were consistent with others reported for *E. grandis*. H^2 of volume at 3 years ranged from 0.18 to 0.31 in the two examined populations, and Osorio et al. (2000) report H^2 of volume at 3 years of 0.15. Similarly, H^2 of volume at 6 years ranged from 0.24 to 0.33 in the two studied populations, and Osorio et al. (2000) reports H^2 of volume at 6 years of 0.24.

In general, broad sense heritability was stable from 3 to 6 years except for DBH and volume in the subpopulation which increased over time. H^2 of DBH increased from 0.17 to 0.24, and H^2 of volume increase from 0.18 to 0.24 (Table 2.4b). An increase in broad sense heritability of vol/tree over time in *E. grandis* clones was also reported by Osorio et al. (2000). Overall, Type B genetic correlations were stable from 3 to 6 years except for height. Height r_{Bg} decreased from 0.81 to 0.75 in the full population, and decreased from 0.86 to 0.75 in the wood sample subpopulation. This result differs from the findings of Osorio et al. (2000) for *E. grandis* clones which reports that height r_{Bg} increased from 0.5 at 3 years to 0.64 at 6 years. More importantly, our estimates of r_{Bg} (0.64 to 0.75) for growth traits at 6 years (rotation age) coincide with the estimated ones for growth traits by Osorio (2000; r_{Bg}= 0.6 to 0.7) for five sites that represented the target environment of *E. grandis* at SKC in the year 2000. Osorio et al (2000) also studied two extreme sites (hot and dry "Guachicona" and cold and high-elevation "Maravillas"); none of the three sites of our study represent such extreme sites. Osorio et al (2000) did find clonal ranking differences between the target

environment and the two extreme sites. However, recently SKC has found that these two extreme environments are more suitable for other commercial species: Guachicona is being planted with the hybrid *E. grandis x E. urophylla* and Maravillas with *Pinus tecunumanii* and *Pinus maximinoi*. In other words, both studies suggest low levels of G *x* E interaction for growth at rotation age within the land where *E. grandis* is currently planted at SKC and therefore there is no pressing need to deploy clones for specific environments.

The clonal genetic standard deviation (σ_C) ranged between 8.6% and 35.4% in the full population, and between 3.8% and 21.9% in the wood sample subpopulation. The estimates of σ_C of the two populations again reflect the fact that the subpopulation is a truncated set of the full population. According to Hodge and Dvorak (2015) the clonal standard deviation (σ_C) is a genetic parameter that reflects how much genetic improvement could be made in a trait (in this case is expressed as a percentage since growth traits were standardized). Lower values of this parameter in the subpopulation suggest lower potential genetic gain compared to the full population. However, reaching the potential gain in the subpopulation will lead to larger trees than doing so in the full population since the growth means of the subpopulation are bigger as discussed previously.

Genetic parameter estimates in the two populations at rotation age indicate that growth traits are under moderate levels of genetic control. Both populations exhibited high Type B genetic correlations across sites suggesting low G *x* E interaction. Low genotype by environment interaction will enable researchers to select clones that should perform well across all the sites.

Table 2.4a *Full population means, variance components, and genetic parameters for standardized height, DBH, and volume across sites. Associated standard errors in parenthesis.*

Variable	Age	Mean	Variance components			Genetic parameters		
			σ^2_C	σ^2_{CxF}	σ^2_ε	$\widehat{H^2}$	$\widehat{r_{Bg}}$	σ_C
Height (m)	3	18.5	74.7700	17.9200	155.5200	0.30 *(0.03)*	0.81 *(0.03)*	8.6
DBH (cm)	3	13.6	155.9100	55.1600	309.3700	0.30 *(0.03)*	0.74 *(0.03)*	12.5
Volume (m^3)	3	0.138	665.1000	218.5000	1241.3000	0.31 *(0.03)*	0.75 *(0.03)*	25.8
Height (m)	6	27.0	125.3500	42.3200	262.7300	0.29 *(0.03)*	0.75 *(0.03)*	11.2
DBH (cm)	6	18.0	259.0800	93.1800	447.4700	0.32 *(0.03)*	0.74 *(0.03)*	16.1
Volume (m^3)	6	0.358	1252.9000	449.6000	2129.4000	0.33 *(0.03)*	0.74 *(0.03)*	35.4

Table 2.4b *Wood sample subpopulation means, variance components, and genetic parameters for standardized height, DBH, and volume across sites. Associated standard errors in parenthesis.*

Variable	Age	Mean	Variance components			Genetic parameters		
			σ^2_C	σ^2_{CxF}	σ^2_ε	$\widehat{H^2}$	$\widehat{r_{Bg}}$	σ_C
Height (m)	3	20.1	14.3690	2.2830	63.7540	0.18 *(0.04)*	0.86 *(0.15)*	3.8
DBH (cm)	3	15.5	36.4300	24.9600	153.6000	0.17 *(0.04)*	0.59 *(0.12)*	6.0
Volume (m^3)	3	0.180	169.7000	110.8000	659.4000	0.18 *(0.04)*	0.60 *(0.12)*	13.0
Height (m)	6	30.1	25.9220	8.6060	123.5150	0.16 *(0.04)*	0.75 *(0.14)*	5.1
DBH (cm)	6	21.0	97.6800	55.6900	259.4900	0.24 *(0.05)*	0.64 *(0.10)*	9.9
Volume (m^3)	6	0.491	478.1000	271.6000	1232.7000	0.24 *(0.05)*	0.64 *(0.10)*	21.9

Wood Density Means

Wood density did not vary substantially between sites and analyses were carried out with all the data across sites. Summary statistics of wood density for the full population and the wood sample subpopulation are presented in Table 2.5a and Table 2.5b. In the full population wood density from cores increased from 0.399 gr/cm^3 to 0.503 gr/cm^3 from 3 to 6 years age (Table 2.5a). In the wood sample subpopulation wood density from cores also increased substantially over time, from 0.415 gr/cm^3 at 3 years to 0.518 gr/cm^3 at 6 years (Table 2.5b). Though there was not substantial difference between the two examined populations in terms of wood density neither at 3 nor at 6 years. An increase of wood density over time was an expected result since the cell walls tend to get thicker as trees age (Shmulsky and Jones 2011). The similarity in wood density between the two populations might indicate that the index used to select the 80 clones of the subpopulation out of 374 clones of the full population had much higher weight on volume than in wood density.

A surprising result in the wood sample subpopulation at 6 years (Table 2.5b) was that wood density from cores (0.518 gr/cm^3) diferred substantially from wood density from wedges (0.425 gr/cm^3). One reason that could explain this difference is lack of accuracy of weighing small samples like the 5 mm diameter cores used. Small cores are more prone to absorb moisture than wedges which may affect dry weight and wood density measurements. Difficulties for wood density assessment of *Eucalyptus* with small cores were reported by Salas et al. (2014).

Wood Density Genetic Parameters

Full population means, variance components, and genetic parameters for wood density across sites are presented in Table 2.6a. The same information for the wood sample subpopulation is presented in Table 2.6b. Genetic parameters for wood density did not vary between the full population and the subpopulation. At 3 years broad sense heritability was moderate (H^2 = 0.27) and Type B genetic correlations were low (r_{Bg} = 0.29). However, at 6 years heritability was very high (H^2 = 0.89 to 0.92) and Type B genetic correlations were also very high (r_{Bg} = 0.99). All the previous estimates correspond to wood density from cores.

These results were totally unexpected and do not agree with prior reports for *E. grandis* clones. Estimates of Type B genetic correlations of 0.29 are very low for a trait like wood density that is more controlled by genetic factors than environmental factors. At 3 years Osorio et al. (2000) found high broad sense heritabilities (H^2 = 0.34 to 0.53) and high type B genetic correlations (r_{Bg} = 0.9) as opposed to our results of density from cores. At 6 years, Osorio et al. (2000) found moderate to high heritabilities for density (H^2 = 0.29 to 0.41) but not as high as the ones from wood cores of this research. Type B genetic correlations reported by Osorio et al. (2000) at 6 years were moderate to high (r_{Bg} = 0.63 to 0.88) but again not as high as the ones of this study from wood cores.

In contrast to the results from wood density from cores, genetic parameters of wood density from wedges for the wood sample subpopulation exhibited high broad sense heritability (H^2 = 0.39) and high Type B genetic correlations (r_{Bg} = 0.89) i.e. high levels of genetic control and low genotype by environment interaction. These were expected results for wood density at rotation age and are totally consistent with prior reports (Osorio et al

2000). From the previous observations, we infer that the measurements from small (5 mm diameter) wood cores were not accurate enough for wood density assessment.

The clonal genetic standard deviation (σ_C) for wood density from wedges was 0.017 gr/cm^3. This represents the achievable gain in wood density or how much the mean would increase if selection by this trait is applied without sacrificing genetic gains in other traits like volume.

Table 2.5a *Full population summary statistics for wood density across sites.*

Wood density					
Variable	Age	Mean	Std. Dev.	Min	Max
Core density (g/cm^3)	3	0.399	0.041	0.305	0.544
Core density (g/cm^3)	6	0.503	0.030	0.408	0.584

Table 2.5b *Wood sample subpopulation summary statistics for wood density and wood chemical traits across sites.*

Wood density					
Variable	Age	Mean	Std Dev	Min	Max
Core density (g/cm^3)	3	0.415	0.041	0.316	0.537
Core density (g/cm^3)	6	0.518	0.026	0.445	0.580
Wedge density (g/cm^3)	6	0.425	0.034	0.337	0.554
Wood chemical traits					
Variable	Age	Mean	Std Dev	Min	Max
Glucose (%)	6	48.0	1.9	41.3	54.0
Xylose (%)	6	12.1	1.0	9.1	15.2
Galactose (%)	6	0.6	0.3	0.0	2.9
Arabinose (%)	6	0.2	0.0	0.1	0.3
Mannose (%)	6	1.3	0.4	0.5	2.8
Total Lignin (%)	6	30.8	1.2	24.7	35.0
Insoluble Lignin (%)	6	26.9	1.2	21.2	31.7
Soluble Lignin (%)	6	3.7	0.3	2.6	4.7
S/(S+G) (%)	6	72.1	2.7	61.5	78.2
S/G	6	2.8	0.5	0.4	4.3
Pulp yield (%)	6	50.2	1.6	44.9	54.7

Table 2.6a *Full population means, variance components, and genetic parameters for wood density across sites. Associated standard errors in parenthesis.*

Wood density								
Variable	Age	Mean	Variance components			Genetic parameters		
			σ^2_C	σ^2_{CxF}	σ^2_ε	$\widehat{H^2}$	$\widehat{r_{Bg}}$	σ_C
Core density (g/cm^3)	3	0.399	0.0004	0.0009	0.0001	0.27 *(0.04)*	0.29 *(0.04)*	0.020
Core density (g/cm^3)	6	0.503	0.0009	0.0000	0.0001	0.92 *(0.07)*	0.99 *(0.00)*	0.030

Table 2.6b *Wood sample subpopulation means, variance components, and genetic parameters for wood density and wood chemical traits across sites. Associated standard errors in parenthesis.*

Wood density								
Variable	Age	Mean	Variance components			Genetic parameters		
			σ^2_C	σ^2_{CxF}	σ^2_ε	$\widehat{H^2}$	$\widehat{r_{Bg}}$	σ_C
Core density (g/cm^3)	3	0.415	0.0004	0.0010	0.0001	0.27 *(0.08)*	0.29 *(0.08)*	0.020
Core density (g/cm^3)	6	0.518	0.0006	0.0000	0.0001	0.89 *(0.14)*	0.99 *(0.01)*	0.024
Wedge density (g/cm^3)	6	0.425	0.0003	0.0000	0.0004	0.39 *(0.07)*	0.89 *(0.06)*	0.017
Wood chemical traits								
Variable	Age	Mean	Variance components			Genetic parameters		
			σ^2_C	σ^2_{CxF}	σ^2_ε	$\widehat{H^2}$	$\widehat{r_{Bg}}$	σ_C
Glucose (%)	6	48.0	1.4210	0.1410	2.0580	0.39 *(0.07)*	0.91 *(0.06)*	1.2
Xylose (%)	6	12.1	0.2386	0.0344	0.6147	0.27 *(0.05)*	0.87 *(0.10)*	0.5
Galactose (%)	6	0.6	0.0139	0.0041	0.0627	0.17 *(0.04)*	0.77 *(0.14)*	0.1
Arabinose (%)	6	0.2	0.0004	0.0001	0.0006	0.35 *(0.07)*	0.79 *(0.08)*	0.0
Mannose (%)	6	1.3	0.0214	0.0049	0.0865	0.19 *(0.04)*	0.81 *(0.13)*	0.1
Total Lignin (%)	6	30.8	0.5478	0.0937	0.5122	0.47 *(0.09)*	0.85 *(0.05)*	0.7
Insoluble Lignin (%)	6	26.9	0.6610	0.0892	0.5213	0.52 *(0.09)*	0.88 *(0.05)*	0.8
Soluble Lignin (%)	6	3.7	0.0613	0.0011	0.0331	0.64 *(0.11)*	0.98 *(0.02)*	0.2
S/(S+G) (%)	6	72.1	4.1880	0.2140	1.7650	0.68 *(0.11)*	0.95 *(0.02)*	2.0
S/G	6	2.8	0.1520	0.0087	0.0794	0.63 *(0.11)*	0.95 *(0.03)*	0.4
Pulp yield (%)	6	50.2	0.9790	0.1450	0.9950	0.46 *(0.08)*	0.87 *(0.05)*	1.0

Wood Chemistry Means

Wood chemical composition did not vary substantially between sites and analyses were carried out with all the data across sites. Summary statistics of wood chemical traits for the wood sample subpopulation are presented in Table 2.5b. Sugars in order of contribution to the total chemical composition were glucose (48.0%), xylose (12.1%), mannose (1.3%), galactose (0.6%), and arabinose (0.2%). Insoluble lignin contributed 26.9% to the total chemical composition, soluble lignin 3.7%, or total lignin 30.8%. These results are similar to other studies of *E. grandis* e.g. Kayama (2013) reports cellulose content between 46.8% and 56.9%, and lignin content between 19.4% and 25.2%. Cunningham and Tamang (2014) report glucan of 39.7%, xylan of 11.4%, manan of 0.3%, galactan of 0.9%, and arabinan of 0.3%.

In this study mean S/G ratio was 2.8 and mean S/(S+G) was 72.1% (Table 2.5b). The result of S/G ratio is within the range reported for *Eucalyptus*. e.g. *E. globulus* S/G = 3.1, *E. nitens* S/G = 2.37, *E. urograndis* S/G = 1.56 (Santos et al. 2012); *E. grandis* S/G = 1.9 (Rencoret et al. 2008). The mean pulp yield in this study was 50.2% (Table 2.5b) and this result also corresponds with *Eucalyptus* previous research e.g. *E. globulus* pulp yield = 52.2% (Costa e Silva et al. 2009).

Wood Chemistry Genetic Parameters

Subpopulation means, variance components, and genetic parameters for wood chemical traits across sites are presented in Table 2.6b. In general, wood chemical traits exhibited moderate to high levels of genetic control ranging from $H^2 = 0.17$ (galactose) to $H^2 = 0.68$ (S/(S+G). Lignin related traits had higher broad sense heritability ($H^2 = 0.46$ to 0.68)

than sugars (H^2 = 0.17 to 0.39). Pulp yield was under a high level of genetic control (H^2 = 0.46). Type B genetic correlations were generally high indicating low genotype by environment interaction (r_{Bg} = 0.77 to 0.98). Again, lignin related traits exhibited lower genotype by environment interaction (r_{Bg} = 0.85 to 0.98) than sugar traits (r_{Bg} = 0.77 to 0.91).

The clonal genetic standard deviation (σ_c) was 1.2% for glucose, 0.8% for insoluble lignin, 0.4 for S/G ratio, and 1% for pulp yield. These values represent the potential increase of the means if selection by this wood chemical traits is applied within the subpopulation. These estimates can be considered a feasible potential gain without sacrificing gains in other traits like volume.

Genetic Correlations Among Traits

Genetic correlations between growth traits and wood density at 3 and 6 years for the wood sample subpopulation are presented in Table 2.7. Correlations between growth traits and wood density (from wedges) at rotation age were moderately negative (r_g = -0.35 to -0.43). Previous studies of *Eucalyptus* report similar unfavorable correlations as the ones found in this study (r_g = -0.36 between DBH and density of *E. nitens* at 9 years, Hamilton et al. 2009). However other studies of *E. grandis* clones have found close to zero correlation between wood density and growth traits (r_g = -0.08 between wood density and MAI at 6 years, Osorio 2003). The divergence of our result and the one from Osorio et al (2003) might be attributed to the fact that he studied a different generation of clones, the second one of the program.

Strong age – age correlations between 3 and 6 years were found for all the growth traits i.e. r_g = 0.85 for volume, r_g = 0.85 for DBH, and r_g = 0.78 for height. This result agrees with the findings of Osorio et al. (2003) that reports age – age correlations between 3 and 6 years of r_{xy} = 0.84 to 0.89 for MAI, and r_{xy} = 0.51 to 0.77 for height. This confirms that genetic selection can effectively be applied at 3 years to reduce the cycles of improvement and costs.

Table 2.7 *Genetic correlations among 3- and 6-year-old growth and density traits in the wood sample subpopulation. Stronger correlations are highlighted with more intense colors (green = positive correlations, yellow = negative correlations). Standard errors are in small italic font*[1]*. C_DEN = core density; W_DEN = wedge density.*

	DBH3	VOL3	C_DEN3	HT6	DBH6	VOL6	C_DEN6	W_DEN6
HT3	0.49 *0.12*	0.69 *0.08*	-0.73 *0.13*	0.78 *0.06*	0.44 *0.12*	0.51 *0.11*	-0.29 *0.12*	-0.18 *0.14*
DBH3		0.97 *0.01*	-0.99 *0.13*	0.75 *0.07*	0.85 *0.04*	0.84 *0.05*	-0.49 *0.11*	-0.30 *0.15*
VOL3			-1.00 *B*	0.85 *0.05*	0.85 *0.04*	0.85 *0.04*	-0.48 *0.11*	-0.30 *0.14*
C_DEN3				-1.00 *0.12*	-1.00 *B*	-1.00 *B*	0.79 *0.10*	0.61 *0.13*
HT6					-0.84 *-0.05*	0.87 *0.04*	-0.45 *0.12*	-0.35 *0.14*
DBH6						0.99 *0.00*	-0.46 *0.11*	-0.43 *0.13*
VOL6							-0.44 *-0.10*	-0.41 *0.13*
C_DEN6								0.24 *0.11*
W_DEN6								

[1]*B indicates that the genetic correlation estimate was bounded near 1.00 by ASREML, therefore no standard error estimate was possible.*

Clonal genetic correlations between growth traits, wood density, and wood chemical traits at 6 years for the wood sample subpopulation are presented in Table 2.8. Correlations between growth traits and wood chemical traits were generally low to moderate, with

absolute value $|r_g| \leq 0.50$ in almost all cases. There was a slight positive correlation between growth traits and pulp yield (r_g = 0.35 to 0.50). Correlations between growth traits and wood chemical traits were not adverse for pulp production, i.e. growth correlated positively with pulp yield and negatively with lignin.

Some wood chemical traits were associated with wood density (from wedges) at a moderate level; for example, density and glucose (r_g = 0.25), and density and pulp yield (r_g = -0.23). These results suggest that at some level higher wood density interferes with pulp yield; making it difficult to find trees with high density and high pulp yield simultaneously. Wood density was positively correlated with galactose (r_g = 0.60) and mannose (r_g = -0.34).

The most relevant wood chemical traits for pulp production showed strong correlations. Glucose was strongly correlated with almost all the chemical traits either positive or negatively ($|r_g|$ = 0.61 to 0.83). This finding makes sense since glucose is the primary component of wood and as it increases the other traits must decreases and vice versa. Glucose was negatively correlated with most of the traits except with pulp yield (r_g = 0.83) and galactose (r_g = 0.65). The correlation of xylose and the minor sugars was positive with arabinose (r_g = 0.62) and mannose (r_g = 0.82), and negative with galactose (r_g = -0.56). Insoluble lignin showed negative correlation with S/G (r_g = -0.70) and S/(S+G) (r_g = -0.62). Pulp yield was positively associated with glucose (r_g = 0.83), and negatively associated with xylose (r_g = -0.48), arabinose (r_g = -0.81), total lignin (r_g = -0.82), and insoluble lignin (r_g = -0.72). Close to zero correlation was found between pulp yield and either S/G (r_g = 0.12) or S/(S+G) (r_g = 0.03).

Table 2.8 *Genetic correlations among 6-year-old growth traits, wood density and wood chemical traits in the wood sample subpopulation. Stronger correlations are highlighted with more intense colors (green = positive correlations, yellow = negative correlations). Standard errors are in small italic font. C_DEN = core density; W_DEN = wedge density.*

	DBH	VOL	C_DEN	W_DEN	GLU	XYL	GAL	ARA	MAN	TLIG	ILIG	SLIG	S/(S+G)	S/G	PY
HT	0.84 *0.05*	0.87 *0.04*	-0.45 *0.12*	-0.35 *0.14*	0.26 *0.14*	-0.08 *0.15*	-0.24 *0.16*	-0.48 *0.12*	0.18 *0.16*	-0.37 *0.13*	-0.32 *0.14*	-0.14 *0.14*	-0.07 *-0.54*	-0.01 *0.14*	0.50 *0.12*
DBH		0.99 *0.00*	-0.46 *0.11*	-0.43 *0.13*	0.06 *0.14*	0.17 *0.15*	-0.40 *0.14*	-0.19 *0.14*	0.42 *0.14*	-0.44 *0.13*	-0.41 *0.13*	-0.02 *0.14*	0.07 *0.50*	0.14 *0.13*	0.35 *0.13*
VOL			-0.44 *0.11*	-0.41 *0.13*	0.09 *0.14*	0.14 *0.15*	-0.39 *0.15*	-0.23 *0.14*	0.36 *0.15*	-0.42 *0.13*	-0.39 *0.13*	-0.02 *0.13*	0.06 *0.42*	0.12 *0.13*	0.36 *0.13*
C_DEN				0.24 *0.11*	0.05 *0.12*	-0.02 *0.12*	0.13 *0.13*	0.04 *0.12*	-0.17 *0.13*	0.09 *0.12*	0.10 *0.12*	-0.03 *0.12*	-0.04 *-0.38*	-0.07 *0.12*	-0.08 *0.12*
W_DEN					0.25 *0.12*	-0.04 *0.13*	0.60 *0.10*	-0.02 *0.14*	-0.34 *0.12*	0.16 *0.12*	0.20 *0.12*	-0.07 *0.12*	-0.19 *-1.58*	-0.19 *0.12*	-0.23 *0.12*
GLU						-0.73 *0.06*	0.65 *0.09*	-0.83 *0.04*	-0.63 *0.09*	-0.67 *0.07*	-0.61 *0.08*	-0.01 *0.13*	0.11 *0.89*	0.18 *0.12*	0.82 *0.04*
XYL							-0.56 *0.10*	0.62 *0.09*	0.82 *0.05*	0.23 *0.13*	0.34 *0.12*	-0.31 *0.12*	-0.38 *-3.44*	-0.38 *0.11*	-0.48 *0.10*
GAL								-0.29 *0.14*	-0.78 *0.07*	-0.18 *0.14*	-0.12 *0.14*	-0.13 *0.13*	-0.08 *-0.58*	-0.04 *0.14*	0.28 *0.13*
ARA									0.34 *0.13*	0.44 *0.11*	0.38 *0.11*	0.17 *0.12*	0.10 *0.83*	0.04 *0.13*	-0.81 *0.05*
MAN										0.09 *0.14*	0.16 *0.14*	-0.22 *0.13*	-0.24 *-1.87*	-0.23 *0.13*	-0.22 *0.13*
TLIG											0.96 *0.01*	-0.21 *0.12*	-0.37 *-3.56*	-0.47 *0.09*	-0.82 *0.04*
ILIG												-0.48 *0.09*	-0.62 *-8.34*	-0.70 *0.06*	-0.72 *0.06*
SLIG													0.96 *79.06*	0.93 *0.02*	-0.12 *0.12*
S/(S+G)														0.99 *0.00*	0.03 *0.12*
S/G															0.12 *0.12*
PY															

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CHAPTER 3: EFFECT OF GROWTH AND WOOD TRAITS OF A CLONAL POPULATION OF *Eucalyptus grandis* ON THE COST OF KRAFT PULPING

Introduction

Smurfit Kappa Colombia is a vertically integrated paper company with a forestry division that supplies eucalypts and pines wood for pulp, paper and packaging manufacture. The forest plantation area covers 40,368 ha (103,784 ac), of which 15,420 ha (38,104 ac) are planted with eucalypts. The mill annually receives 500,000 tons of eucalypts wood and produces around 150,000 tons of pulp from the wood fiber. The company started a tree improvement program for *Eucalyptus grandis* in 1987 and has completed three generations of improvement in 24 years achieving exceptional genetic gains in volume compared to the plant material of 1987 (White et al. 2011). In the present work, we study a subset of the series of clonal trials of third generation that were established to identify the best clones for commercial deployment.

Pulp yield refers to the amount of oven dry pulp produced per amount of oven dry wood processed in the mill. This important trait is known to be a function of the chemical composition of the wood. In practice kraft pulp yield is also influenced by pulping conditions such as degree and rate of delignification, chemical application, liquor to wood ratio, temperature cycle and even uniformity of chip size (Smook, 2002; Crane et al. 1987). In Chapter 2, pulp yield was estimated by means of an NIR model that was built from lab pulping of 99 samples. This model allows the indirect estimation of pulp yield for a particular set of pulping conditions, corresponding to the way the 99 samples were processed in the lab. Since pulping conditions might change from laboratory to industrial facilities and the mill process may change due to a variety of reasons e.g. technology, wood supplied, market

conditions, etc. it is relevant to study pulp yield as a function of wood chemical traits instead of independently. In this chapter this approach is explored. Cellulose and lignin content play an important role and correspond to biological traits that can be in fact genetically improved. Indirect selection to increase cellulose content or decrease lignin content can be applied to favor pulp yield regardless of the pulping conditions of processing. Models to estimate pulp yield from wood chemical composition might be useful for other tree improvement programs that may do not have available pulp yield data.

A good amount of research has been done in tree improvement of *Eucalyptus* related with estimation of genetic parameters (Hodge and Dvorak 2015; Costa e Silva et al. 2009; Hamilton et al. 2009). However, other than the work of Lopez et al. (2009) which studied the influence of wood properties of eucalypt clones on the financial performance of a modeled Brazilian pulp mill, there are few studies associated with economic criteria for selection of commercial genotypes (Greaves et al. 1997; Wei and Borralho 2000). The way commercial clones are selected in practice is mainly based on a selection index, a weighting of clonal values for the traits of interest e.g. volume and wood density. According to White et al. (2007, 2011) the relative selection weights of each trait on the index are determined through a Monte Carlo simulation. The simulation starts placing a weight of 1 on gain in volume and a weight of 0 on gain in density which results in the maximum gain in volume from selecting clones only for volume. The process continues successively decreasing the weight on volume by 0.1 and increasing the weight on density by 0.1 until the weight of volume reaches 0 and the weight of density reaches 1; which results in the maximum gain in wood density from selecting clones only for density. The resulting 11 indices and their corresponding gains are visually analyzed and the index that produces close to optimum gain in both traits is chosen

(White et al. 2007). Tree improvement programs of eucalypts in Chile, Brazil, and South Africa are currently using a selection index that combines 3 traits: volume, wood density, and pulp yield (Gary Hodge, Camcore, N.C. State University, personal communication 2017).

The studied clonal population was characterized in Chapter 2 where genetic control and genetic variation of growth, wood density, and several wood chemical traits were quantified. The current chapter focuses on (1) Assessing the impact of growth, wood density, pulp yield, insoluble lignin, and S/G (syringyl/guaiacyl) ratio of 80 *Eucalyptus grandis* clones on the production cost of bleached kraft pulp and (2) developing regression models to relate wood chemical composition to pulp yield of *E. grandis*. This study will use a straightforward economic model that integrates forestry and mill production.

Materials and Methods

Population of Study

A clonal population composed of 80 clones of *E. grandis* represented by 1,183 trees planted on 3 sites and approximately 5 ramets per clone per site was used for this study. The previous individuals correspond to a wood sample subpopulation derived from a full population known as the Elite Population of the third generation of improvement at Smurfit Kappa Colombia. This population was originally composed of 374 clones planted on 12 sites with 5 to 10 ramets per clone per site. The clones in these trials came from controlled full-sib crosses between 21 parents of the previous (second) generation of tree improvement. As well as in previous generations of tree improvement (first and second) the Elite Population of the third generation at Smurfit Kappa Colombia was developed with the purpose of selecting the

best clones for deployment of forest plantations that supply wood to the paper mills of the company. A more detailed description of the population of study is presented in Chapter 2.

Clonal Genetic Values

Best linear unbiased predictions (BLUPs) or clonal genetic values for tree volume, wood density, and wood chemical traits at 6 years were examined. BLUPs are genetic values used by tree breeders to rank genotypes (Wei and Borralho, 2000; White et al. 2007; Hodge and Dvorak al 2015). The clonal genetic values for this research were generated in the quantitative genetics analysis of Chapter 2 where tree volume and wood traits predicted using near infrared spectroscopy (NIR) were analyzed. Wood traits used in this chapter include density (wedges), sugars (glucose, xylose, galactose, arabinose, and mannose), lignin (total, soluble, and insoluble), and S/G ratio (also as S/(S+G)).

Conversion of Individual Tree Volume Growth to Mean Annual Increment

The calculation of mean annual increment (MAI) requires the individual tree volume. The measurement of individual tree volume is influenced by the genotype but also by the type of plot used. The clonal trials of this research were established in single tree plots (STP) which means that each tree is surrounded by other genotypes, as opposed to block plots where clones of the same genotype grow together i.e. monoclonal blocks. In this study, the calculation of MAI at 6 years from the tree volume was measured using single-tree plots (STP), which may lead to overestimation of genetic differences among clones (Kimberley et al. 2015). Trees in STPs might be growing in an uneven competition that over time increasingly favored the best genotypes at the expense of the least adapted genotypes. This

could have resulted in an inflated measure of volume per tree that was not suitable to calculate MAI at rotation age.

A conversion equation was developed to express the genetic values of individual tree volume growth into genetic values of MAI correcting the effect of the STPs. The response variable was MAI at 6 years of clones B2 and C2 (2 of the control lots of this study) estimated from 3 independent trials established at Smurfit Kappa Colombia in block plots of 16 trees and 4 to 6 replications. These 3 clonal trials were located in the same regions were the studied population was stablished. The predictor variable was the genetic value of the standardized volume per tree at 6 years of clones B2 and C2 which represented the positive and negative tails of the distribution of genetic values of this study, respectively.

Regression Models to Predict Pulp Yield from Wood Chemical Traits

A dataset of 99 observations was used for model selection corresponding to 99 *E. grandis* trees with pulp yield measured in the SKC laboratory and their NIR spectra readings measured at Camcore (data used to build the NIR pulp yield model described in Chapter 2). Re-calibrated global NIR models for *Eucalyptus* wood chemistry were applied to the 99 spectra to obtain predictions of wood chemical traits. Ultimately, a dataset of 99 observations with lab pulp yield and NIR chemical composition was used for model selection. The relationship between pulp yield and wood chemical traits was analyzed through multiple regression techniques. Different approaches were studied and most of them implemented in R (R Core Team 2016). Bayesian variable selection (BVS) was implemented using the R package BMA (Raftery et al. 2017) and models were evaluated based on their "posterior probability", a natural measure of uncertainty (Bondell and Reich 2012); the higher the

posterior probability the better the model will be to explain the association between wood chemical traits (predictors) and pulp yield (response). Stepwise selection was implemented in SAS (SAS Institute Inc. 2016) using *proc reg*; this approach provides a model in which the wood chemical traits included are significant at P = 0.15 level or better in explaining pulp yield. Akaike's Information Criterion (AIC) was implemented in R, in this approach the model with the algebraically smallest AIC statistic is selected (Quinn and Keough 2002). Finally, the adjusted R^2 approach was studied selecting the model with the highest adjusted R^2 statistic. Collinearity was detected by calculating the variance inflation factor (VIF) for each predictor. Estimates of VIF greater than 10 suggest strong collinearity (Quinn and Keough 2002); in this case the predictor was omitted to avoid redundancy and instability of the regression coefficients.

Pulp Cost Model

A straightforward model simulation developed by the Forest Biomaterials Department at N.C. State University was used to evaluate the effect of growth and wood properties of the *E. grandis* clones on the cost of pulp production. The model has both forestry and mill components. A vertically integrated paper company was assumed in which a forestry division and a mill division were already operating. No land purchase costs and no capital investment costs were considered since an existing mill and forest land was assumed. It was also assumed that the forestry division provides the wood to the mill division for a transfer price equal to the cost of wood production.

Forestry components of the model include a characterization of the clones, wood production cost, and an estimation of the wood production cost per clone based on its growth

and wood density. The characterization of clones for growth, wood density, and wood chemical traits was completed in Chapter 2; a summary of the traits included in the pulp cost evaluation is presented in Table 3.1. Wood production cost corresponds to how much the company currently spends in stumpage, harvesting, overhead and transportation of one green ton of eucalypt wood to the mill gate. The plantations have a rotation age of 7 years and all wood is destined for pulp production. A factor of 0.43 was used to convert green tons to oven dry tons of wood given that the wood loses on average 13% of its weight during transport from plantation to the mill gate (Zapata 2013), and that the oven dry weight is on average 37.3% of the green weight (data from the wood wedges measured in this study). Finally, an estimation of the wood production cost per clone was done using a factor that combines the genetic value (BLUP) of volume and that of wood density. A reference factor = 1represents the mean volume and density of the population, and corresponds to the current wood production cost. The higher the factor, the higher the amount of fiber per hectare planted, and the cost of wood production decreases. Vice versa, the lower the factor, the lower the amount of fiber per hectare, and the cost of wood production increases. Eventually, each clone has a wood production cost per oven dry ton that is accounted for in the model.

Table 3.1 *Summary statistics for growth, wood density, and wood chemical traits included in the pulp cost evaluation of 80 E. grandis clones at 6 years.*

Trait	Mean	Std. Dev.	Min	Max
MAI (m^3/ha/year)	50.3	3.1	43.8	56.3
Density (gr/cm^3)	0.430	0.016	0.391	0.470
Pulp Yield (%)	48.9	0.7	47.1	50.3
Insoluble Lignin (%)	26.9	0.8	25.6	29.0
S/G	2.8	0.4	1.4	3.4

Mill components of the model include a series of mass and energy balance assumptions and operating costs for a kraft pulping process. Technical assumptions were based on previous research of Gomide et al. (2005), De Assis et al. (2017) and Perrin et al. (2015). Figure 3.1 presents a simplified process diagram of pulp production. The process in the mill starts with debarked eucalypt and ends with bleached market pulp. Pulp yield was estimated as a linear function of wood chemical composition using the multiple regression model previously described; the selected model will be further explained later. Effective alkali was predicted as a linear function of insoluble lignin and S/G ratio (Gomide et al 2005). Recovery chemicals such as sodium hydroxide (NaOH), sodium sulfate (Na_2SO_4), and calcium oxide (CaO); along with natural gas required for pulping were calculated as a linear function of effective alkali/chemical charge (De Assis et al. 2017). Bleaching chemicals were calculated as a linear function of S/G ratio for an Elemental Chlorine Free (ECF) bleaching process (Perrin et al. 2015). The bleaching chemicals estimated were chlorine dioxide (ClO_2), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), oxygen (O_2), and magnesium sulfate ($MgSO_4$) assuming a sequence "D0 (EOP) D1" (Perrin et al. 2015). Chlorine dioxide (ClO_2) is generated in-house from sodium chlorate ($NaClO_3$), sulfuric acid (H_2SO_4), methanol (CH_3OH), and sodium hydroxide (NaOH); and during this process sodium sulfate (Na_2SO_4) is generated as byproduct. The sodium sulfate (Na_2SO_4) created during the chlorine dioxide (ClO_2) preparation is accounted for in the model i.e. it contributes to the chemical requirements. Recovery boiler steam production was estimated as a linear function of yield and effective alkali (De Assis et al. 2017). Net energy produced at the recovery boiler accounts for white liquor inorganics, oxygen delignification inorganics, and black liquor inorganics and organics. Hog fuel boiler produces additional energy from saw

dust and fines derived from the chipping (De Assis et al. 2017). Operating costs were based on market prices of chemicals, natural gas, and steam.

The main output of the model is the direct cost per ton of pulp which is partitioned into wood cost, pulping and recovery chemicals cost, bleaching chemicals cost, and steam revenue. The user of the model can change the input chemical composition, density and growth, and evaluate individual clones or a set of clones. The user can also set wood chipping yield, bleaching yield, and tons of pulp to produce, as well as change any of the technical assumptions according to particularities of production (Tiago De Assis, Forest Biomaterials Department, N.C. State personal communication 2017).

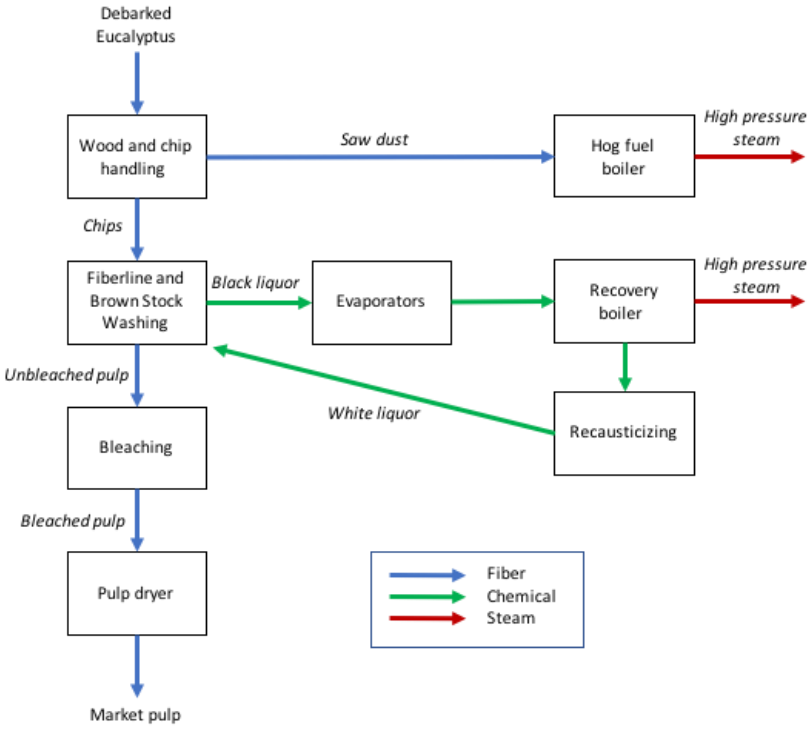

Figure 3.1 *Simplified process diagram of pulp production (De Assis et al. 2017).*

Results and Discussion

Conversion of Individual Tree Volume Growth to Mean Annual Increment

The base numbers to build the conversion equation of genetic values of standardized tree volume to genetic values of MAI are presented in Table 3.2. Clone B2 is top 4 in volume/tree and clone C2 is rank 76 which covers almost the entire distribution of the clonal subpopulation. The genetic values of standardized volume and average MAI in Table 3.2 were used to build equation [1] that was applied to the 80 studied clones to obtain their MAI genetic values.

Table 3.2 *Base genetic values of standardized tree volume and MAIs used to build the conversion equation of genetic values of standardized tree volume (%) to genetic values of MAI (m^3/ha/year) of E. grandis at 6 years.*

Clone	$\widehat{GV}$stVOL6 (%)	Rank (1 to 80)	MAI (m^3/ha/year)			
			Cabuyerita	La Estrella	Helechaux	Average
B2	30.6	4	72.3	54.9	38.7	55.3
C2	-33.5	76	55.5	47.5	31.5	44.8

$$\widehat{GV}\,\text{MAI} = 50.288 + (0.1638 * \widehat{GV}\text{stVOL6}) \quad [1]$$

Regression Models to Predict Pulp Yield from Wood Chemical Traits

Different criteria were applied for model selection using the dataset of 99 observations with lab pulp yield and NIR chemical composition. All wood chemical traits were available as possible predictors of pulp yield but some of them were omitted before model selection. The used criteria and results of model selection are presented in Table 3.3. S/G ratio was not selected as predictor in any of the models and glucose was only selected

for one model. The reason why glucose is left out of the models might be lower precision of the used global NIR model for glucose (cross-val R^2 = 0.74) compared to the models of lignin (cross-val R^2 = 0.65 to 0.96). The best regression models to predict pulp yield from wood chemical traits are presented in Table 3.4. According to the coefficients of the models, pulp yield was shown to decrease with either increasing xylose, arabinose, total lignin, insoluble lignin, or soluble lignin; or increase with increasing glucose. This agrees with the genetic value correlations found in Chapter 2 (between PY and: XYL r_g = -0.48, ARA r_g = -0.81, TLIG r_g = -0.82, ILIG r_g = -0.72, SLIG r_g = -0.12, GLU r_g = 0.83) and with previous studies that indicate that high contents of lignin in *Eucalyptus* are detrimental for pulp yield (Kien et al. 2009; Lopez et al. 2009; Stackpole et al. 2010).

The coefficients of determination (R^2) of the selected models of pulp yield from wood chemical composition were low and similar to the R^2 of the NIR model used in Chapter 2 to predict pulp yield independently. The reason of these low R^2 could be inaccuracy of the assessment of pulp yield in laboratory. Any of the 4 models presented in Table 3.4 might be useful for other tree improvement programs with available wood chemical composition but lacking pulp yield. The model with xylose and total lignin was incorporated into the simulation model for the rest of the analysis since it is simple (2 predictors) and captures almost the same variation in pulp yield than the other models of more predictors.

Table 3.3 *Criteria and outcomes of model selection. Traits shadowed were omitted before model selection. Letter x denotes that the trait was selected as predictor for the regression model. GLU = glucose (%), XYL = xylose (%), GAL = galactose (%), ARA = arabinose (%), MAN = mannose (%), TLIG = total lignin (%), ILIG = Insoluble lignin (%), SLIG = soluble lignin (%), S/G = syringyl/guaiacyl.*

Criterion before model selection	Wood chemical traits									Selected model		
	GLU	XYL	GAL	ARA	MAN	TLIG	ILIG	SLIG	S/G	No. of predictors	R^2	R
All but no TLIG		x		x			x	x		4	0.42	0.65
All but no GAL, ARA, MAN, TLIG		x					x	x		3	0.39	0.63
All but no GAL, ARA, MAN, ILIG, SLIG		x				x				2	0.36	0.60
Only GLU and ILIG	x						x			2	0.28	0.52

Table 3.4 *Best regression models to predict pulp yield from wood chemical traits and associated pulp yield means and ranges of prediction for the studied clonal population. The NIR model used in Chapter 2 is shown for comparison. PY = pulp yield (%), GLU = glucose (%), XYL = xylose (%), ARA = arabinose (%), TLIG = total lignin (%), SLIG = soluble lignin (%).*

Pulp yield as function of wood chemical traits						
Model	R^2	R	Pulp yield (%)			
			Mean	Std. Dev.	Min	Max
PY=88.83-(0.63*XYL)-(10.94*ARA)-(0.86*ILIG)-(1.27*SLIG)	0.42	0.65	51.1	0.8	48.9	52.9
PY=85.72-(0.71*XYL)-(0.85*ILIG)-(1.22*SLIG)	0.39	0.63	49.7	0.7	47.9	51.2
PY= 82.71-(0.64*XYL)-(0.85*TLIG)	0.36	0.6	48.9	0.7	47.1	50.3
PY=58.20+(0.17*GLU)-(0.70*ILIG)	0.28	0.52	47.4	0.7	45.6	48.4
Pulp yield independently						
Model	R^2	R	Pulp yield (%)			
			Mean	Std. Dev.	Min	Max
PY	0.44	0.66	50.2	0.9	47.9	52.3

Characteristics of the Clones

The top 10 clones and control lots based on MAI, wood density, and pulp yield are presented in Figures 3.2 to 3.4. In general, the clones with the highest growth rates do not exhibit the highest wood density and vice versa. A moderately negative correlation between

growth and wood density on *Eucalyptus* at 6 years was reported by Hamilton et al. (2009) and also presented in Chapter 2 (Table 2.8 r_g = -0.41). Clones D2, K3, and J2 are an exception to this trend; they are in the group of top growers and also exhibit high wood density. These three clones are excellent because they have high wood biomass growth rate (the product of MAI and wood density). None of the top clones based on pulp yield (Figure 3.4) were top clones in MAI (Figure 3.2) or wood density (Figure 3.3). This result may partially reflect the fact that selection for pulp yield has not been applied in the population. Clone C2 had the lowest MAI, wood density, and pulp yield of the control lots. Variation between the best and the worst clone, considering the entire subset of 80 clones, corresponds to 22% on MAI, 17% on wood density and 6% on pulp yield.

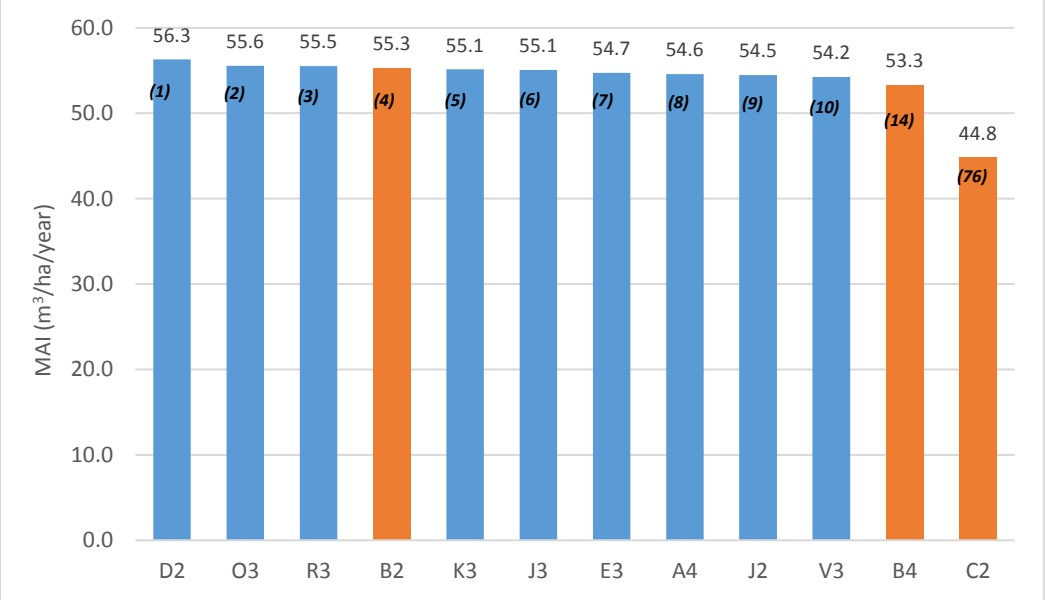

Figure 3.2 *Top 10 clones and control lots (orange) based on growth rate (mean annual increment in m^3/ha/year). Associated ranking numbers in parenthesis.*

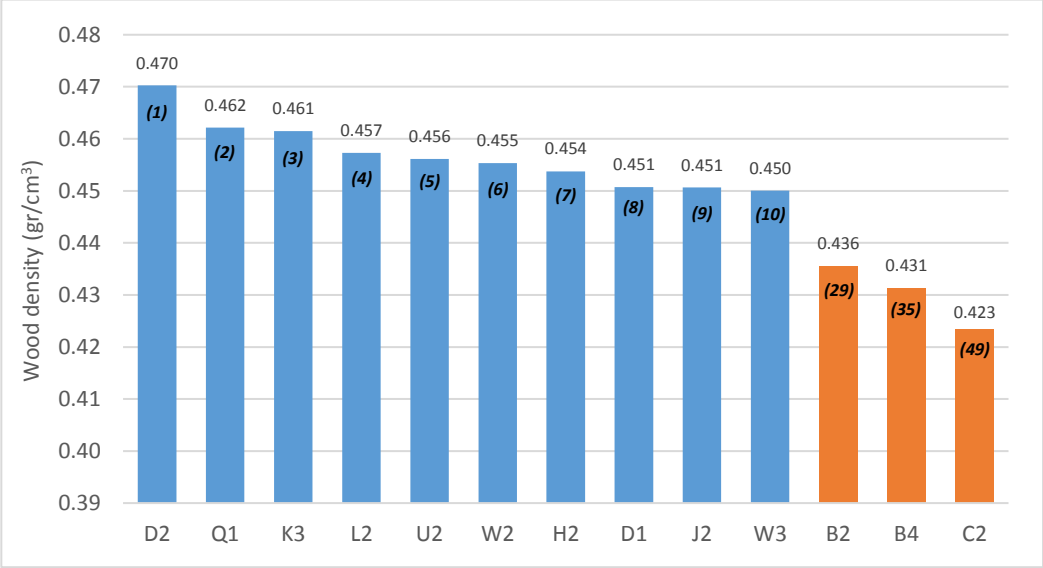

Figure 3.3 *Top 10 clones and control lots (orange) based on wood density. Associated ranking numbers in parenthesis.*

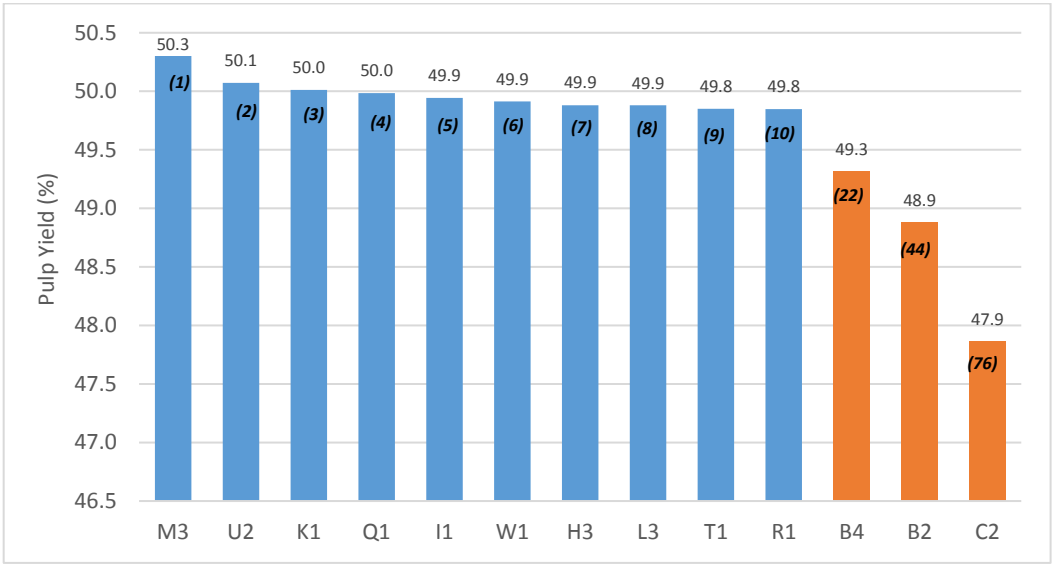

Figure 3.4 *Top 10 clones and control lots (orange) based on pulp yield. Associated ranking numbers in parenthesis.*

Cost Per Ton of Pulp

The cost per ton of pulp is the main output of the simulation model; it is a direct cost hence it is exclusively attributed to the production of pulp and does not account for other costs such as depreciation or administrative expenses of the mill (assumed to be the same for all the clones). The cost per ton of pulp is partitioned in four components: wood cost, recovery chemicals cost, bleaching chemicals cost, and steam revenue. Below is described the influence of the input traits of the model (MAI, wood density, pulp yield, insoluble lignin, and S/G) on the four components and on the total cost per ton of pulp of the clonal subpopulation. The influence of the input traits on pulp costs was measured using simple linear regressions.

Wood cost was mainly a function of wood biomass growth rate ($R^2 = 0.95$) and MAI ($R^2 = 0.67$). Wood biomass growth rate corresponds to the product of MAI and wood density. The importance of wood biomass for explaining wood cost was also described by Lopez et al. (2009) and Lopez (2015) who also highlights the importance of expressing the wood cost on an oven-dry basis for economic analysis since this allow a fair estimation of the amount of wood per unit of volume. Regarding the cost of wood itself and not as one of the components of the pulp cost the top 10 clones and control lots based on wood cost per dry ton of wood are presented in Figure 3.5. The average wood cost of the top 10 clones was US $91.89. Clones D2, K3, and J2 are US $7.57 below this average (US $84.32) because of its exceptional high wood biomass growth rate. The wood cost of the control lot C2 is US $115.30; US $23.41 above the average cost of the 10 top clones.

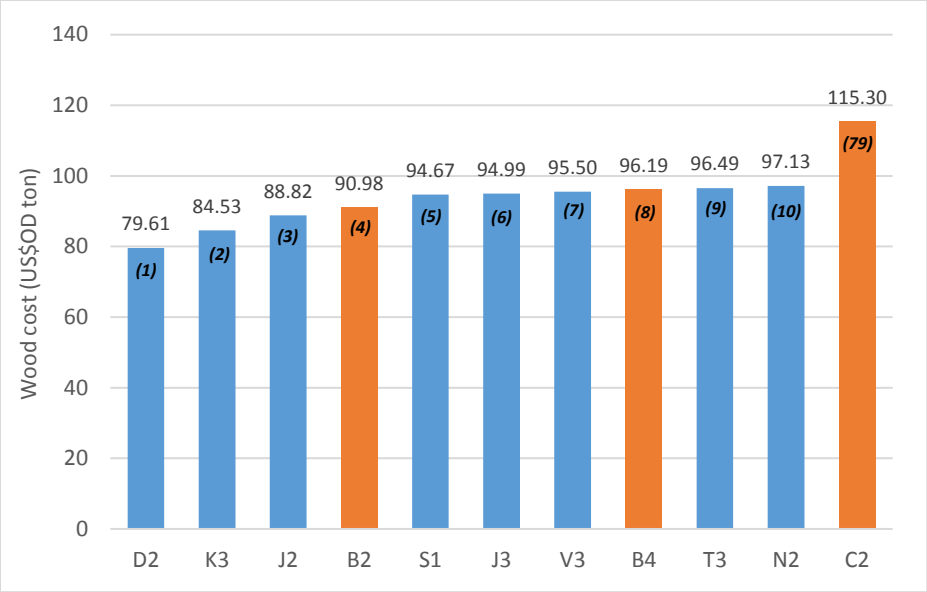

Figure 3.5 *Top 10 clones and control lots (orange) based on wood cost per oven-dry ton of wood at the gate of the mill. Associated ranking numbers in parenthesis.*

The main traits that explained the cost of recovery chemicals were pulp yield (R^2 = 0.91) and insoluble lignin content (R^2 = 0.86). The influence of insoluble lignin on recovery chemicals cost was expected since the make-up chemicals were calculated from the effective alkali which was a function of insoluble lignin and S/G ratio. However, the effect of S/G ratio on the cost of recovery chemicals was mild (R^2 = 0.16). Pulp yield is expected to influence the cost of recovery chemicals since it is strongly negatively correlated with insoluble lignin (Table 2.8 r_g = -0.72).

Bleaching chemical costs were completely attributed to S/G ratio (R^2 = 1) as expected according to one of the functions used in the model. Nevertheless, other chemical traits such as insoluble lignin (R^2 = 0.49) and pulp yield (R^2 = 0.30) moderately influenced the bleaching chemical cost.

Steam revenue was completely attributed to pulp yield ($R^2 = 1$), highly attributed to insoluble lignin ($R^2 = 0.85$), and moderately attributed to S/G ratio ($R^2 = 0.32$). The steam production in the recovery boiler and hog fuel boiler represents a by-product of the pulping process. In this model, steam production was monetized and accounted as a revenue that contributes to reduce the pulp cost. Steam production as well as pulp production is expected to vary according to the wood chemistry. For example, a low pulp yield leads to a high content of organics in the black liquor and this results in high energy production (steam) (De Assis et al. 2017). Additionally, when high chemical consumption is required the level of inorganics in the black liquor increases and this affects energy production (De Assis et al. 2017). Thus, high pulp yield is a desirable trait for pulp production, but an undesirable trait for energy production; pulp versus energy production is an important tradeoff. Nevertheless, the economic value of pulp is usually higher than that of energy and this justifies the pulp business.

The total cost per ton of pulp showed to be mostly influenced by wood biomass ($R^2 = 0.87$; Figure 3.6)**,** MAI ($R^2 = 0.65$); and moderately by insoluble lignin ($R^2 = 0.21$), and pulp yield ($R^2 = 0.20$). Wood density and S/G ratio had a mild effect on the total cost per ton of pulp. However, wood density has an important indirect effect on the cost of pulp since it takes part in the estimation of wood biomass.

The average cost per ton of pulp from the 80 studied clones is presented in Table 3.5. A main result is that 80% of the cost, excluding steam revenue, corresponds to wood cost. Any improvement in *Eucalyptus* plantations that increases productivity of wood biomass per hectare is going to have an enormous impact on reducing the production cost of pulp.

Recovery chemicals, and bleaching chemicals represents 8% and 12% of the cost respectively (Table 3.5).

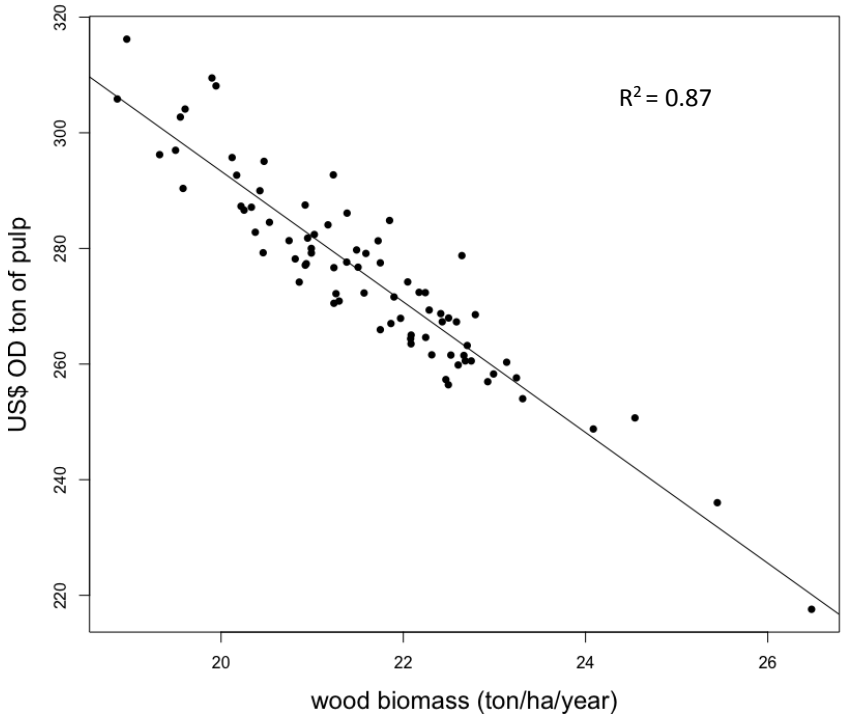

Figure 3.6 *Cost per ton of pulp as a function of wood biomass growth rate (**wood biomass ton/ha/year = MAI $m^3/ha/year$ * density gr/cm^3**).*

Table 3.5 *Summary statistics of direct cost per ton of pulp in the studied population.*

Component	Cost (US$/OD ton pulp)			
	Mean	Std. Dev.	Min	Max
Wood	237.97	15.82	182.63	272.75
Pulping and recovery chemicals	25.47	0.92	23.83	28.04
Bleaching chemicals	34.96	2.99	30.13	46.08
Steam revenue	-23.26	0.60	-24.79	-22.06
Total	275.15	16.47	217.58	316.20

Clone Recommendation

Growth, wood traits, and mill costs of the top 10 *E. grandis* clones and control lots based on cost per ton of pulp are presented in Table 3.6. Associated ranking numbers are in parenthesis for each of the traits and costs. Ranking numbers range between 1 and 80, where 1 is the most desirable and 80 the least desirable for pulp production. The cost per ton of pulp represents the main output of our model and is the proposed criteria to select commercial clones.

The ranking of total cost per ton of pulp was in general very consistent with both the ranking of wood cost and the ranking of wood biomass. The top 5 clones by total cost per ton of pulp (D2, K3, B2, J2, and S1; Table 3.6) are the same if one selects by wood cost or by wood biomass. There are a couple of aspects to consider though.

One aspect is that clones D2, K3, and J2 have the unusual advantage of being top clones in both volume (top 1 = 56.3 m3/ha/year, top 5 = 55.1 m3/ha/year, and top 9 = 54.5 m3/ha/year, respectively; Table 3.6) and wood density (top 1 = 0.47 gr/cm3, top 3 = 0.46 gr/cm3, and top 9 = 0.45 gr/cm3, respectively; Table 3.6). Thus, these three clones have almost the highest wood biomass growth rate of the subset and this characteristic makes them top clones for pulp production regardless of not being top clones by pulp yield (rank 21 = 49.4%, rank 38 = 49%, and rank 77 = 47.8%, respectively; Table 3.6), insoluble lignin (rank 12 = 26.1%, rank 60 = 27.3%, and rank 73 = 27.8%, respectively; Table 3.6), and/or S/G ratio (rank 29 = 3.0, rank 69 = 2.4, and rank 67 = 2.4, respectively; Table 3.6).

A second aspect is that favorable wood chemical traits can justify the selection of a clone that does not necessarily have high wood biomass growth rate. For example, clones Q1 and W3 are ranked 20 and 21 by wood biomass (both 22.5 ton/ha/year; Table 3.6) and are

ranked 6 and 8 by total cost per ton of pulp (US $256,41 and US $257.32, respectively; Table 3.6). These two clones exhibit desirable wood chemical traits. They have high pulp yield (top 4 = 50% and top 11 = 49.8%, respectively; Table 3.6), low insoluble lignin (top 3 = 25.8% and top 4 = 25.8%, respectively; Table 3.6), and high S/G ratio (top 4 = 3.3 and top 3 = 3.3, respectively; Table 3.6).

Clone C2, one of the control lots, consistently presents undesirable traits for pulp production within the studied subset of clones. If the mill uses this clone the cost of pulp production would increase 45% respect utilizing clone D2 (rank 80 = US $316,20 vs. top 1 = $217,58; Table 3.6). Clone C2 is top 5 for steam or energy production though (steam revenue = US $24,14; Table 3.6). Clone J2 was surprisingly suitable for both pulp and energy production (top 4 = $250.69 and top 4 = $24.26, respectively; Table 3.6).

Another criterion to consider for clone selection is the level of relatedness between them. Wei and Borralho (2000) studied the level of relatedness in an open pollinated population of *Eucalyptus urophylla* and found low coancestry ($f < 0.01$) between the selected 5% of the population based on a multi-trait BLUP. An equivalent analysis was not conducted in this research. However, Table 3.7 presents the parents of the top 10 recommended *E. grandis* clones and control lots. Clones D2 and J2 are full-sibs, clones K3 and T3 are half-sibs, and clones W3 and J3 are half-sibs. Genetic gains versus genetic diversity is a tradeoff for tree breeders. The deployment of genetically related clones increases the risk in case those related clones are susceptible to a problematic disease or climatic event. The most conservative approach would be select completely unrelated clones i.e. omit clones J2 (top 4), T3 (top 7), and J3 (top 9) from the ranking by total cost. The average cost per ton of pulp

of the 10 best clones would increase US $1.54 ($250.91 vs. $249.37) if selection of completely unrelated clones were applied.

Table 3.6 *Growth, wood traits, and mill costs per oven dry ton of pulp of top 10 E. grandis clones and control lots (bold). Associated ranking numbers in parenthesis (1 = most desirable for pulp production; 80 = least desirable for pulp production).*

Clone	Input traits							Output costs (US$/oven dry ton of pulp)				
	MAI (m3/ha/year)	Density (gr/cm3)	Wood biomass (ton/ha/year)	Wood cost US$/ODTW	Pulp yield (%)	Ins. lignin (%)	S/G	Wood	Recovery chemicals	Bleaching chemicals	Steam	Total
D2	56.3 *(1)*	0.47 *(1)*	26.5 *(1)*	79.61 *(1)*	49.4 *(21)*	26.1 *(12)*	3.0 *(29)*	182.63 *(1)*	24.67 *(19)*	33.17 *(29)*	-22.89 *(58)*	217.58 *(1)*
K3	55.1 *(5)*	0.46 *(3)*	25.4 *(2)*	84.53 *(2)*	49.0 *(38)*	27.3 *(60)*	2.4 *(69)*	195.34 *(2)*	25.40 *(43)*	38.44 *(69)*	-23.16 *(43)*	236.03 *(2)*
B2	**55.3 *(4)***	**0.44 *(29)***	**24.1 *(4)***	**90.98 *(4)***	**48.9 *(44)***	**27.0 *(46)***	**2.7 *(57)***	**210.76 *(4)***	**25.49 *(44)***	**35.78 *(57)***	**-23.26 *(37)***	**248.77 *(3)***
J2	54.5 *(9)*	0.45 *(9)*	24.5 *(3)*	88.82 *(3)*	47.8 *(77)*	27.8 *(73)*	2.4 *(67)*	210.60 *(3)*	26.58 *(71)*	37.78 *(67)*	-24.26 *(4)*	250.69 *(4)*
S1	53.4 *(12)*	0.44 *(28)*	23.3 *(5)*	94.67 *(5)*	49.5 *(16)*	26.7 *(35)*	2.7 *(51)*	216.42 *(5)*	24.87 *(24)*	35.42 *(51)*	-22.71 *(65)*	254.01 *(5)*
Q1	48.7 *(57)*	0.46 *(2)*	22.5 *(20)*	98.54 *(20)*	50.0 *(4)*	25.8 *(3)*	3.3 *(4)*	223.21 *(10)*	24.31 *(8)*	31.23 *(4)*	-22.34 *(78)*	256.41 *(6)*
T3	53.3 *(13)*	0.43 *(39)*	22.9 *(9)*	96.49 *(9)*	49.1 *(31)*	26.2 *(19)*	3.1 *(18)*	222.37 *(8)*	24.96 *(27)*	32.70 *(18)*	-23.08 *(48)*	256.95 *(7)*
W3	49.9 *(48)*	0.45 *(10)*	22.5 *(21)*	98.67 *(21)*	49.8 *(11)*	25.8 *(4)*	3.3 *(3)*	224.30 *(13)*	24.43 *(10)*	31.08 *(3)*	-22.49 *(70)*	257.32 *(8)*
J3	55.1 *(6)*	0.42 *(51)*	23.2 *(6)*	94.99 *(6)*	48.9 *(43)*	26.9 *(43)*	2.7 *(55)*	219.76 *(6)*	25.37 *(40)*	35.70 *(55)*	-23.21 *(40)*	257.62 *(9)*
B4	**53.3 *(14)***	**0.43 *(35)***	**23.0 *(8)***	**96.19 *(8)***	**49.3 *(22)***	**26.8 *(38)***	**2.8 *(50)***	**220.83 *(7)***	**25.11 *(32)***	**35.23 *(50)***	**-22.88 *(59)***	**258.28 *(10)***
C2	**44.8 *(76)***	**0.42 *(49)***	**19.0 *(79)***	**115.3 *(79)***	**47.9 *(76)***	**28.4 *(75)***	**2.1 *(78)***	**272.75 *(80)***	**26.74 *(75)***	**40.85 *(78)***	**-24.14 *(5)***	**316.20 *(80)***

Table 3.7 *Parents of the top 10 E. grandis clones and control lots (bold). Associated ranking numbers in parenthesis (1 = most desirable for pulp production; 80 = least desirable for pulp production).*

Clone	Mother	Father	Total cost (US$/ODTP)
D2	10	12	217.58 *(1)*
K3	5	6	236.03 *(2)*
B2	**0**	**0**	**248.77** ***(3)***
J2	10	12	250.69 *(4)*
S1	4	5	254.01 *(5)*
Q1	19	20	256.41 *(6)*
T3	6	8	256.95 *(7)*
W3	21	2	257.32 *(8)*
J3	1	2	257.62 *(9)*
B4	**Unknown**	**Unknown**	**258.28** ***(10)***
C2	**0**	**0**	**316.20** ***(80)***

Selection Criteria and its Influence on the Cost

Cost savings of the better clones selected by three criteria are presented in Table 3.8. The first criterion is volume and the clones are ranked by MAI. A second criterion is volume and wood density, and the clones are ranked by wood biomass growth rate. The third criterion is volume, density, and wood chemical traits (pulp yield, insoluble lignin, and S/G ratio) and the clones are ranked by cost per ton of pulp. The three criteria are compared selecting the first 5 to 10 clones of each ranking and assuming that a pulp production of 150,000 tons is divided proportionally between the number of selected clones. Costs savings decrease as the number of clones in the ranking increase (the top 5 clones are better than the top 10 clones). Higher cost savings were obtained when selecting by volume and density (US$ 3.6 to 5.1 million) compared to selecting by only volume (US$ 3.3 to 4.2 million). Equal or slightly higher cost savings were obtained when selecting by volume, density, and wood chemical traits (US$ 3.9 to 5.1 million) compared to selecting by volume and density

(US$ 3.6 to 5.1 million). These results agree with the findings of Borralho et al. (1993) that found lower cost savings on indices that included only volume compared to indices that included volume and wood traits for pulp production from *E. globulus* in Portugal.

The relatively low impact of wood chemical traits on cost savings found in this research might be caused by the genetic variation of the studied population i.e. 80 out of 374 clones are being studied currently; if for example 80 out of 800 clones were being studied this would lead to a group of clones more similar between each other in terms of high growth and high wood density. In this situation, the competition between clones would be determined by its wood chemical traits more than by its growth and wood density; and this would lead to a higher importance of the wood chemical traits. In this particular population, the impact of the wood chemical traits on cost savings might be underestimated. Other studies (Greaves and Borralho, 1996; Greaves et al. 1997) of the influence of wood properties on the cost of kraft pulping from *Eucalyptus* have not addressed this issue of the differential of selection; and they not always conclude that wood density is more economically important than pulp yield.

From the analysis of Chapter 2 we learned that the level of exploitable genetic variation of pulp yield (and the rest associated wood chemical traits) is lower than that of wood density; and that the level of inherent genetic variation of volume is higher compared to wood traits. This means that most of the genetic gains of the program are still going to come from growth and wood density even if e.g. pulp yield shows greater influence on the total cost than density.

Table 3.8 *Cost savings (US $Million) better clones selected by volume (V), volume and density (VD), and volume, density, and wood chemical traits (VDC) for production of 150,000 tons of pulp.*

# of clones	V	VD	VDC
5	$4.234	$5.060	$5.060
6	$3.966	$4.655	$4.685
7	$3.713	$4.308	$4.406
8	$3.358	$4.086	$4.189
9	$3.392	$3.935	$4.016
10	$3.275	$3.641	$3.868

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CHAPTER 4: CONCLUSIONS

Scope

In this thesis, a clonal population of *Eucalyptus grandis* established at Smurfit Kappa Colombia was studied. Genetic control and genotype *x* environment interaction among growth, wood density, and key wood chemical traits for pulp production were estimated. A simulation model that integrates forestry and mill production was developed to evaluate the impact of growth, wood density, pulp yield, lignin content, and S/G ratio on the cost of production of bleached kraft pulp. A number of regression models to estimate pulp yield from wood chemical traits were also developed and one of them incorporated into the simulation model.

In Chapter 2, genetic parameter estimation was completed on a full population as well as on a subpopulation. The full population was composed of 374 clones established on 3 sites; and the wood sample subpopulation was composed of 80 (out of the 374 clones) on the same 3 sites. The 80 clones of the wood sample subpopulation were selected based on volume and wood density at 3 years. A number of important traits for pulp production were analyzed; volume per tree, wood density, and wood chemical traits such as pulp yield, lignin content, and S/G ratio. Wood density was analyzed from wood cores at 3 and 6 years, and NIR-predicted at 6 years. All of the wood chemical traits were estimated using NIR prediction models with wood samples taken at 6 years. Genetic analyses were done using a mixed model approach, and estimates of variance components, broad-sense (clonal) heritability, and Type B genetic correlations were obtained for both populations. Also, several genetic correlations between traits and ages were calculated for the wood sample subpopulation.

In Chapter 3, a simulation model for estimation of pulp production cost from growth, wood density, pulp yield, insoluble lignin content, and S/G ratio was developed and applied to the 80 clones of the wood sample subpopulation. Genetic values of the input traits were obtained from Chapter 2 and incorporated into the simulation model along with wood production costs, mass and energy balance assumptions for a kraft pulping process, and mill operating costs. Estimation of the cost per ton of pulp and considerations about genetic relatedness between genotypes were used to recommend clones for commercial plantation. Three clonal selection criteria combining volume, wood density, and wood chemical traits were compared in terms of costs savings. Relationships between pulp yield and wood chemical traits were developed using multiple regression and model selection techniques applied to an independent data set of 99 samples with lab pulp yield and NIR wood chemical traits.

Findings

Growth traits were all under moderate levels of genetic control ($\widehat{H^2}$ = 0.16 ± 0.04 to 0.33 ± 0.03), whereas wood density ($\widehat{H^2}$ = 0.39 ± 0.07) and wood chemical traits ($\widehat{H^2}$ = 0.17 ± 0.04 to 0.68 ± 0.11) were under moderate to high levels of genetic control. Estimates of Type B genetic correlations were consistently high for all kinds of traits ($\widehat{r_{Bg}}$ = 0.64 ± 0.10 to 0.75 ± 0.03 for growth traits; $\widehat{r_{Bg}}$ = 0.89 ± 0.06 for wood density; and $\widehat{r_{Bg}}$ = 0.77 ± 0.14 to 0.98 ± 0.02 for wood chemical traits) suggesting low genotype by environment interaction across sites. Clonal genetic values at rotation age ranged from -39.3% to 36.8% for volume,

-20.0% to 16.1% for DBH, 0.391 gr/cm^3 to 0.470 gr/cm^3 for density, 47.12% to 50.3% for pulp yield, 25.6% to 29.0% for insoluble lignin, and 1.4 to 3.4 for S/G ratio. A moderately negative clonal genetic correlation between growth traits and wood density was found (r_g = -0.35 to -0.43), indicating an unfavorable relationship (better growth is associated with lower density). Correlations between growth traits and wood chemical traits were generally low to moderately low. One notable result was that moderately favorable correlations (in terms of pulp production) were found between growth traits and lignin (e.g., r_g = -0.42 for volume and total lignin), and growth traits and pulp yield (e.g., r_g, = 0.36 for volume and pulp yield). In general, wood density was not strongly correlated with any chemical traits, e.g., the clonal genetic correlation between density traits and glucose, xylose, total lignin, insoluble lignin, S/G ratio and pulp yield all had $|r_g| \leq 0.25$. As expected, there were generally strong genetic correlations between wood chemical traits, e.g., for glucose, $|r_g|$ ranged from 0.61 to 0.83 with xylose, galactose, arabinose, mannose, total lignin, insoluble lignin, and pulp yield.

The average cost per ton of pulp of the subpopulation was US $275.15 accounting for a reduction of US$23.26 from steam revenue. Wood cost represented 80% of the cost per ton of pulp, with bleaching chemicals accounting for 12% of the cost, and pulping and recovery chemicals accounting for the remaining 8%. The cost per ton of pulp was shown to decrease with increasing wood biomass (product of MAI and wood density), MAI, pulp yield, S/G ratio, and wood density; and the cost increased with increases in insoluble lignin. Wood biomass was the trait that best explained the cost per ton of pulp (R^2 = 0.87) followed in order of importance by MAI (R^2 = 0.65), insoluble lignin (R^2 = 0.21), pulp yield (R^2 = 0.20), S/G ratio (R^2 = 0.06), and wood density (R^2 = 0.05). Given the moderately negative correlation between growth traits and wood density, it was fortunate to find three clones (D2,

K3, and J2) ranked within the top 10 out of 80 for both MAI and wood density. This combination makes these three clones have exceptionally high wood biomass and low cost per ton of pulp. A ranking based on cost per ton of pulp was provided for selection of commercial clones. The average cost per ton of pulp for the 10 best clones was US$249.37 which was 21% lower than one of the control lots (clone C2 = US $316.20). Selecting clones for a combination of volume, wood density and wood chemical traits led to higher cost savings than selecting by volume and wood density and/or by volume alone.

Significance of the Results and Implications for Smurfit Kappa Colombia

The high levels of genetic control of the wood chemical traits indicate that it is possible to achieve genetic gains for these traits by applying tree improvement techniques. The low genotype by environment interaction across sites found for all the traits studied suggests that a single combination of criteria can be applied to identify the best clones for the entire landbase of the company. In other words, there is little to no benefit in selecting clones for specific environments, and therefore the same clones can be used in any region of the company. Moderate correlations (slightly favorable) were found between clonal genetic values for volume and pulp yield, and for volume and insoluble lignin, which will make it somewhat easier to select trees for both high growth and favorable wood chemical traits. Regarding wood density, there was a moderately favorable correlation between wood density and glucose (cellulose), but a moderately unfavorable correlation between wood density and pulp yield. This finding suggests that at some level higher wood density becomes detrimental for pulp yield; making it difficult to find trees with high density and high pulp yield simultaneously.

Selection of clones for wood properties might result in a genetic gain equal to one clonal genetic standard deviation for important wood traits. This would be equivalent to an increase in wood density of 0.017 gr/cm^3, and an increase in mean pulp yield of 1%. The simulation model shows that an increase of 1% in pulp yield decreases the average cost per ton of pulp by US$4.39 (US$270.76 vs. US$275.15); and that an increase of 0.017 gr/cm^3 in wood density decreases the average cost per ton of pulp by US$9.4 (US$265.75 vs. US$275.15). For a mill that produces 150,000 tons of pulp per year this represents savings of US$0.7 to US$1.4 million.

In the current generation of clonal testing for *E. grandis*, Smurfit Kappa Colombia (SKC) tested a series of 400 clones across 12 tests (4 tests per zone x 3 zones). For future generations of clonal trials, SKC should consider reducing the number of trials and increase the number of clones tested in them, e.g. a series of 4 tests (one per zone) with 800 clones. This will be significant cost savings in terms of the total number of trials and total number of trees planted and measured. The low level of genotype by environment interaction found for both growth traits ($\widehat{r_{Bg}}$ ranging from 0.64 to 0.75) and wood traits ($\widehat{r_{Bg}}$ ranging from 0.77 to 0.98) means that fewer tests are needed to identify broadly adapted clones. Increasing the number of clones in the tests would allow for an increase in selection intensity, selection differential, and ultimately produce more genetic gain.

From the studied wood traits, wood density continues to be an important trait specially to achieve high wood biomass when combined with volume. SKC should continue measuring this trait in their clonal trials, improve the accuracy of the current assessment with small wood cores, or explore more efficient sampling techniques such as indirect estimations using a resistograph. Assessment of wood chemical traits should be continued using NIR

assessments, although for the purposes of clonal ranking, this should probably be restricted to the top 10% of the clonal population for volume growth. The NIR global models for *Eucalyptus* chemistry used in the present research could be transferred to a Handheld NIR for indirect estimation of wood chemical traits using wood shavings.

Limitations of the Results

The economic importance of selecting for volume, density, and wood chemical traits instead of selecting for just volume and wood density, might be underestimated if one looks only at clonal rankings for the 80 clones in this study. It is important to note that three of the top clones based on volume growth were also top ranked clones for wood density; this should be considered a serendipitous result, and not the norm. When clones with very good volume growth and density are found, the result is exceptionally high values of wood biomass, and having desirable wood chemical traits become less important in reducing pulping costs. If a larger population of clones had been studied, it is at least possible and perhaps likely that we would have found some clones that were very highly ranked for volume and highly ranked for good wood chemistry traits, and that these would be valuable despite being more intermediate for wood density.

Recommendations

This research might be extended to the clonal population of *Eucalyptus grandis x Eucalyptus urophylla* of Smurfit Kappa Colombia. The simulation model might be adjusted to include actual mill costs of the company; also extended to make a financial assessment of the clones under potential production constraints such as limited recovery boiler capacity,

limited volumetric feed capacity, or limited pulp drying capacity as reported by Lopez et al. (2009).

APPENDICES

Appendix A. Matting Design from Which the Clonal Population Originates

		Father *E. grandis*																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Mother *E. grandis*	1		x	x																		
	2			x	x																	
	3				x	x																
	4					x	x															
	5						x	x														
	6							x	x													
	7								x	x												
	8									x	x											
	9										x	x										
	10											x	x									
	11												x	x								
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	16																	x	x			
	17																		x	x		
	18																			x	x	
	19																				x	x
	20	x																				x
	21	x	x																			

Appendix B. Studied *Eucalyptus grandis* Clones and Their Clonal Values at 3 Years

clone	cvalvol	cvalwd	cvalindex	rank
B2	83.47	1.29	25.95	1
Q2	58.18	6.74	22.17	2
D2	60.58	4.99	21.67	3
H2	45.51	8.30	19.46	4
C1	36.03	12.28	19.40	5
J2	58.25	2.41	19.16	6
B4	45.65	5.67	17.66	7
X2	46.10	4.83	17.21	8
S1	49.75	3.15	17.13	9
O3	66.66	-4.12	17.12	10
S2	55.21	0.61	16.99	11
P2	46.85	3.63	16.60	12
V2	55.73	-0.32	16.50	13
K3	40.41	5.57	16.02	14
O2	36.59	7.03	15.90	15
Z2	38.48	5.92	15.68	16
R1	45.12	2.75	15.46	17
F3	55.67	-2.22	15.15	18
C2	28.96	8.65	14.75	19
T1	54.79	-2.60	14.61	20
P3	34.65	5.76	14.43	21
X1	39.59	3.55	14.36	22
G3	48.99	-0.55	14.32	23
L3	26.04	9.29	14.31	24
O1	15.84	13.32	14.07	25
S3	40.07	2.47	13.75	26
R3	49.18	-1.46	13.73	27
A2	22.73	9.60	13.54	28
J3	59.64	-6.74	13.17	29
D1	9.28	14.71	13.08	30
F2	35.91	3.26	13.05	31
I3	38.60	2.10	13.05	32
B3	46.86	-1.55	12.97	33
Z3	47.28	-1.77	12.94	34
G2	35.29	3.35	12.93	35
L2	28.28	6.29	12.89	36
A4	44.45	-0.69	12.85	37
E3	51.48	-3.92	12.70	38
G1	27.30	6.18	12.52	39
B1	16.19	10.93	12.51	40
F1	44.42	-1.22	12.47	41
N2	28.19	5.51	12.31	42
T2	47.24	-2.68	12.29	43

clone	cvalvol	cvalwd	cvalindex	rank
H1	47.20	-2.67	12.29	44
W1	52.97	-5.25	12.21	45
M2	32.04	3.68	12.19	46
K2	21.25	8.26	12.16	47
A1	41.14	-0.64	11.89	48
L1	35.84	1.33	11.68	49
U3	45.14	-2.69	11.66	50
P1	15.02	10.20	11.64	51
Q1	19.65	8.11	11.57	52
V3	46.96	-3.63	11.55	53
C3	33.07	2.31	11.54	54
W3	36.00	0.86	11.40	55
J1	39.49	-0.81	11.28	56
T3	38.93	-0.64	11.23	57
Q3	31.09	2.64	11.17	58
U1	42.08	-2.11	11.15	59
Y1	21.46	6.61	11.06	60
E1	8.97	11.77	10.93	61
Y3	35.12	0.51	10.90	62
E2	25.68	4.49	10.85	63
U2	13.69	9.60	10.82	64
Z1	25.13	4.53	10.71	65
W2	25.51	4.34	10.69	66
M3	41.37	-2.51	10.65	67
D3	23.42	5.08	10.58	68
V1	26.85	3.61	10.58	69
M1	41.58	-2.74	10.56	70
R2	43.85	-3.72	10.55	71
N3	35.12	-0.18	10.41	72
Y2	13.00	9.05	10.23	73
X3	32.97	0.28	10.09	74
A3	39.59	-2.60	10.06	75
I2	15.32	7.79	10.05	76
H3	31.36	0.90	10.04	77
N1	34.94	-0.65	10.03	78
I1	26.99	2.52	9.86	79
K1	30.86	0.80	9.82	80