ABSTRACT

RAZOKI, MINDORO. Wood Properties of *Eucalyptus pellita*: Age-Age Genetic Correlation, Early Selection, and Economic Impact of Harvest Age (Dr. Gary R. Hodge and Dr. Juan J. Acosta).

Eucalyptus pellita F. Muell., fast-growing hardwood species, holds the second species in timber production in Indonesia in 2021, accounting for 33% (15.8 million m3). It primarily supplies the raw material for 8,66 million metric tons kraft pulpwood production for the same year, ranking the country as the 4th position as an exporter. The production is planned to gradually increase to 18 million metric tons in the near future, creating an increase in wood supply demand. Urge the needs to increase land productivity and mill efficiency by planting improved species through breeding program, including the wood properties (basic density, pulp yield, klason lignin, S/G ratio, glucose, xylose, and galactose). This efforts require knowledge in how to feasibly phenotype the wood traits, incorporating early selection to improve genetic gain and the impact of the change in pulp production cost.

The findings of Chapter 2 showed the evident of basic density, klason lignin, S/G ratio, glucose, and xylose of *E. pellita* vary significantly longitudinally up and down the stem, with longitudinal position explaining more than 70 of the variation. The most accurate single points were found at the middle part of the stem height (25 and 50 of the commercial height). Nevertheless, it is evident that sampling at breast height (1.3 m) not only practical but also provides a reliable estimate for all the assessed wood traits, particularly at the clonal level (r^2 = 77 to 94). Therefore, we recommend selecting the sampling point at 1.3 meters (breast height) as the optimal height for assessing basic density, klason lignin, S/G ratio, glucose, xylose, and galactose in *E. pellita.*

The findings of Chapter 3 highlight age-related changes in several wood properties of *E. pellita*. Basic wood density, klason lignin, and glucose demonstrated an increasing trend with age,

whereas the syringyl-to-guaiacyl (S/G) ratio and xylose exhibited a decreasing trend. Overall, the wood properties of *E. pellita* appeared to be strongly influenced genetically, with the broad-sense heritability (H^2) estimates ranging from 0.43 to 0.88. Furthermore, very high positive genetic correlations were observed between potential early selection ages (2, 3, and 4 years) and potential harvesting ages (5 and 8 years). Selection in breeding programs could be done as early as two years, primarily due to very high genetic correlations between juvenile and rotation age (R_g = 0.90 to 0.99). The efficiency of early selection was notably higher compared to direct selection. Selecting at age two has the potential to yield genetic gain efficiency = 1.5 to 3.6 times greater than direct selection at age five. Moreover, these gains increased even further when compared to direct selection at age eight (efficiency = 2.5 to 5.6).

The findings of Chapter 4 underscore the economic impact of varying wood properties in *E. pellita* harvested at two different ages (5 and 8 years). In comparison to the 5-year rotation, both basic density and pulp yield should exhibit increases when trees are harvested at the 8-year rotation. These increases should result in reduced costs for wood, decreased tonnage of wood required, and lowered specific wood consumption. Additionally, the alkali active charge should be lower at an eight-year rotation, contributing to a decrease in chemical costs for white liquor. Essentially, harvesting *E. pellita* wood at 8 years of age should lead to an important reduction in operational costs for pulping of approximately USD 8.57 per air-dried metric ton (ADT) of pulp.

Wood Properties of *Eucalyptus pellita*: Age-Age Genetic Correlation, Early Selection, and Economic Impact of Harvest Age

by
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Dr. Hasan Jameel	Insert committee member name

DEDICATION

I dedicate the fruits of my labor to honor the memory of my late father and mother, as well as my siblings – my unwavering sources of strength and inspiration. Their teachings and support instill in me the belief that dreams are not limits but rather launching pads to success.

Last but not least, to God, for surrounding me with wonderful individuals. Their kindness, support, and positivity have been a constant blessing, and I am thankful for the goodness they bring into my life. Let this achievement be a testament to God's enduring presence in my life.

BIOGRAPHY

Mindoro Razoki Sigalingging was born in Jambi, Indonesia, on January 9, 1990. He was raised in a modest household alongside his three siblings by his parents. His father, Doharman Sigalingging, served as a headmaster, while his mother, Resintauli Debataraja, worked as a teacher for a public school. Both of Mindoro's parents hailed from the Batak tribe, a tribe that is known for its enduring spirit and resilience.

Mindoro spent most of his upbringing in Jambi City until high school. Subsequently, he moved to Yogyakarta to pursue his bachelor's degree in Forest Products Technology at Gadjah Mada University. In 2014, he graduated with honors, receiving the cum laude distinction and being recognized as one of the top graduates in the Forest Products Technology Department.

In 2014, he commenced his professional career as a research officer in the Wood Technology Department, as part of the Tree Breeding Program at APRIL Asia – an integrated forestry, pulp, and paper company in Kerinci. This job served as a gateway for him to explore opportunities in the field of tree breeding. In 2018, he transitioned to Sinarmas, another integrated forestry, pulp, and paper company resides in Perawang, where he was given the opportunity to serve as the head of the Wood Technology Department. To advance his role, the company together with Camcore sponsored him to pursue a Master's Program in Forestry at North Carolina State University. In December 2021, he relocated to Raleigh, where he spent a significant portion of his life. Upon completing his program, he plans to return to Indonesia to further contribute to his role in Sinarmas and in the field of forestry in general.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction: *Eucalyptus pellita* as Pulp and Paper Material

1.1 Introduction

Eucalyptus pellita F. Muell., commonly referred to as red mahogany, is an important fast-growing hardwood species in forest plantations of Indonesia, primarily for kraft pulpwood production (Nambiar et al., 2018; Nambiar & Harwood, 2014). According to the Indonesian Central Statistics Agency (BPS), *E. pellita* holds the second position in timber production in the region, accounting for 33% (15.8 million m3) of the total timber production in 2021, an increase from 21% (8.6 million m3) in 2019 (BPS - Badan Pusat Statistik, 2023).

E. pellita plantations have been cultivated in Indonesia since the early 2000s (Hardiyanto et al., 2021). The species naturally exists in tropical regions of northern Queensland in Australia, Papua New Guinea, and the neighboring Papua Province of Indonesia, primarily growing at the edges of lowland tropical rainforests (Harwood, 1998). Some of the provenances are better adapted to lowland humid equatorial environments compared to other tropical eucalypt species (Harwood et al., 1997). This species also exhibits tolerance to many of the major pests and diseases like eucalypt rust (*Puccinia psidii*), ceratocystis wilt (*Ceratocystis fimbriata*) and leaf blight (*Cylindrocladium pteridis*) that are common in humid tropical regions (Guimarães et al., 2010; Santos et al., 2014). In Indonesia, *E. pellita* has replaced *Acacia mangium* Willd. for wood production on mineral soils (Nambiar et al., 2018). This shift occurred due to the significant decline of *A. mangium* caused by the Ceratocystis fungal wilt disease. By 2017, over 600,000 ha of area previously planted by *A. mangium* had been replaced (Figure. 1.1).

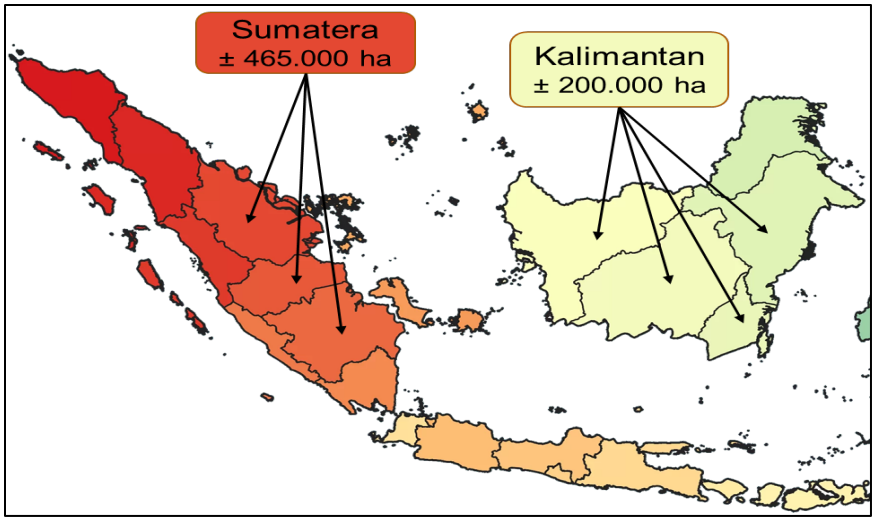

Figure 1.1. Geographical spread of *Acacia mangium* substituted with *Eucalyptus pellita* or its Hybrids in Indonesia since mid-2017. Area figures in hectares (obtained from Nambiar et al., 2018).

The success of *E. pellita* clonal forestry can be attributed to continuous efforts in genetic improvement. Genetic improvement initiatives of *E. pellita* have been documented since the 1990s where two seed orchards were established in South Kalimantan and Riau respectively in 1994 and 1996 (Leksono, 2009). A recent study demonstrated that genetic improvement and silvicultural practices have resulted in nearly a doubling of productivity, from a mean annual increment (MAI) of approximately 18 m3/ha/yr in the first rotation (Nambiar & Harwood, 2014) to the current level of 34.9 m3/ha/yr (Hardiyanto et al., 2022). The species' amenability to mass-propagation for clonal forestry establishment (Dehon et al., 2013) facilitates further genetic improvement by capturing both the additive and non-additive genetic effects.

As eucalypt breeding programs advanced, there has been an increase in the number of traits evaluated, with particular emphasis on wood quality and how the tree breeding program can contribute to the quantity and quality of the final product (Greaves et al., 1997; Raymond, 2002). As a consequence, several *E. pellita* breeding programs are focused on reducing the total cost of

production of a ton of pulp. The cost of "fiber production" per hectare this is critical, of course, and this will depend on growth rate and wood properties of the trees. Among wood properties, basic density and pulp yield are among the most crucial traits, because they have the highest potential for reducing overall pulp expenses (Magaton et al., 2009; Resquin et al., 2022; Schmidt, 2005). To maximize gains in wood traits from genetic enhancement, it is essential to assess the degree of variation of each wood quality trait attributed to genetic factors (Zobel & Talbert, 1984).

Basic density, defined as the oven dry weight of wood per unit green volume, is regarded as the single most important wood quality trait, as it influences a wide range of products including the efficiency of pulp production (Shmulsky & Jones, 2019; Zobel & Talbert, 1984). The amount of pulp produced per unit volume is directly correlated with the basic density. In modeled large-scale pulp mills, an increase in wood basic density reduces the total pulp production cost due to its influence on wood cost, which consistently constitutes the largest component of pulp expenses (Schmidt, 2005). Hence, the characterization of the basic density of raw material is always essential. The primary approach to assess basic density is through the water displacement method either using a disc, chips or a core; and multiple testing standards have been well established (ASTM D2395-17; TAPPI T258). Further development on rapidly assessing basic density for breeding purposes has been well-developed, including novel tools as the IML ResistographTM. The Resistograph has been proven as a rapid and efficient on-site tool for estimating wood density in standing trees from multiple species (Downes et al., 2018; Perek et al., 2022). This facilitates the incorporation of basic density as a selection trait for the genetic improvement programs.

Kraft pulp yield is defined as the percentage of oven-dry wood that remains after chemically removing a significant portion of lignin, called delignification process, using sodium hydroxide (NaOH) and Sodium sulfide (Na2S), referred to as white liquor, under controlled

conditions of temperature and pressure (Sixta, 2006). Kraft pulp yield consists mainly of cellulose and some hemicelluloses. Practically, it is intended to target a certain kappa number, a measure of the residual lignin content of pulp. Meanwhile, the extent of the delignification rate is monitored by the H-factor, a pulping factor that integrates both the duration and temperature of the pulping process that indicates the degree of reaction (Bajpai, 2014). To ensure profitability, kraft pulp yield needs to be monitored and maintained above acceptable minimum levels (Downes et al., 2012). In doing so, the pulping process has to be selective to lignin, meaning decreasing the lignin concentration while retaining as much cellulose and hemicelluloses as possible. Hence, kraft pulp yield depends on the chemical components of the wood - the amount of cellulose, hemicelluloses, lignin and its nature. These chemicals are the main organic polymers in the wood (Stackpole et al., 2011).

Cellulose and hemicelluloses, the primary constituents of kraft pulp, are the main polysaccharides found in the cell walls of hardwood fibers. Cellulose, having molecular formula $(C6H12O5)n$, is a carbohydrate homopolymer generated from repeating α-D-anhydro glucopyranose molecules (glucose molecules) that are linked through acetal (glycosidic) bonds between the equatorial OH group of C4 and the C1 carbon atom known as β-1,4-glucan (Asif, 2009; Klemm et al., 2005). It is the main polymer of the cell wall of the wood. The β homolinkages make the cellulose chains linear with degree of polymerization (n) more than 10,000 (Delmer, 1987). Meanwhile, hemicelluloses refer to polysaccharides in the wood cell wall other than cellulose (Pauly et al., 2013). They are co-polymers of two or more sugars and sugar acids. The sugars are mostly linked by β-1,4 glycosidic bonds as the main chain. Hemicelluloses have lower degree of polymerization than cellulose and typically fall between 50 and 300 (Patel & Parsania,

2018). In *Eucalyptus*, xylan is the major hemicellulose followed by other minor hemicelluloses such as mannan, galactan and arabinan (Vena et al., 2013; Xu et al., 2018).

Cellulose and hemicelluloses can be degraded during alkaline pulping (Johannson & Samuelson, 1975). The key degradation reactions involve a gradual breaking down of these polysaccharides, starting from the reducing end units (peeling reaction). This process is followed by the alkaline hydrolysis of glycosidic bonds and subsequent peeling reactions. The peeling reaction leads to a reduction in the degree of polymerization of these polysaccharides. However, hemicelluloses are more susceptible to the peeling reaction due to their lower degree of polymerization. This degradation is a significant factor contributing to the reduction in kraft pulp yield.

The efficiency of the delignification process in kraft pulping is influenced by the quantity and characteristics of lignin. Lignin can be described as a complex cross-linked polymer of phenylpropanoid polyphenols (Patil & Argyropoulos, 2017). The polymer consists of three lignin monomers/monolignols (Figure 2) differentiated based on the methoxyl group: sinapyl alcohol (S type, two groups), coniferyl alcohol (G type, one group), and p-coumaryl alcohol (H type without methoxyl groups). These monolignols are linked with ether bonds (β-O-4, α-O-4 and 4-O-5) and sigma bonds, bonds between two carbon atoms (5-5, β-5, β-1 and β-β) (Argyropoulos & Menachem, 1997; Awan et al., 2019). The β-O-4, the aryl ether bonds can readily be cleaved under alkaline conditions like kraft pulping. Meanwhile, the remaining carbon-carbon bonds, including 5-5 bonds, are the hardest bonds to break and are stable under alkaline conditions. Lignin in hardwood species, including *Eucalyptus*, is mainly constructed by a mixture of S and G types of monolignols and their presence is often quantified as S/G ratio. The G type monolignol possesses

aromatic units that have an unoccupied C5 position (having only one methoxyl group). Hence, it contains more biphenyl units (5-5 bond) compared to S type.

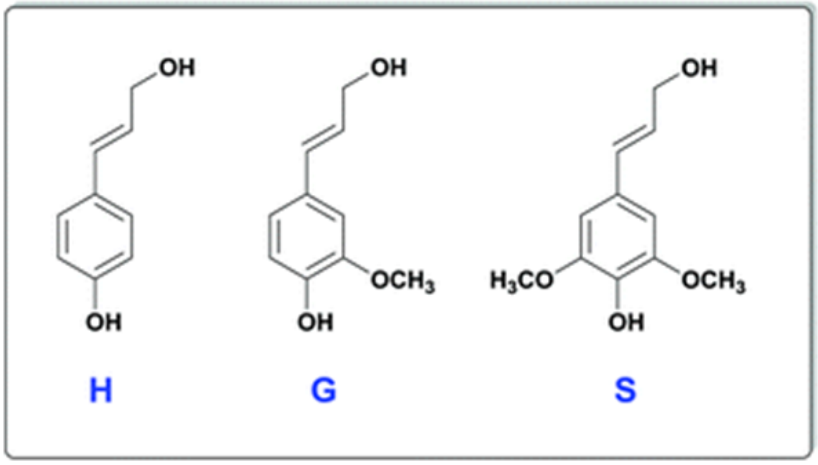

Figure 1.2. The fundamental components of lignin consist of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units (obtained from Sen et al., 2015).

The crucial role of the S/G ratio in facilitating delignification is well-documented. This association has been explored in studies involving various wood species, including eucalypt trees (Ramadevi et al., 2016; Ventorim et al., 2014). These studies concluded a higher S/G ratio proves advantageous for kraft pulping owing to increased reactivity, attributed to fewer biphenyl units in the lignin polymer. There exists a strong negative correlation between the S/G ratio and the chemical usage in both pulping and bleaching processes. Moreover, an increase in reactivity in the delignification rate reduces the degradation of polysaccharides, potentially resulting in a higher pulp yield.

Measuring wood quality traits, such as kraft pulp yield and the chemical composition of wood, is both expensive and labor-intensive. Consequently, recent research in wood quality has primarily concentrated on advancing the phenotyping of these traits. This involves the development of sampling methods, including the exploration of alternative approaches to serve as

indicators for traits with higher assessment costs (Raymond, 2002). One notable outcome is the adoption of near-infrared spectroscopy (NIRS). NIRS is known as a fast and accurate method that can substitute the expensive analysis of wood chemistry. It is well-suited for tree breeding programs that necessitate screening a large number of trees in a speedy and cost-efficient manner. Numerous studies show the utilization of NIRS to estimate the lignin and carbohydrates content of wood (Hodge et al., 2018; Yeh et al., 2005) and the kraft pulp yield (Downes et al., 2012; Terdwongworakul et al., 2005). The NIRS analysis involves reading the reflectance in the near-infrared spectrum (between 700 nm to 2500 nm) of the irradiated wood samples. This reflectance is used to build partial least square (PLS) regression models with the reference data. Fitted models are used to predict the chemical-related properties of new samples (Via et al., 2014).

There is ample evidence indicating that all the mentioned wood traits vary within a tree, both radially and longitudinally. The trajectory and magnitude of these variations can, to some extent, be attributed to genetics, signifying that they differ among various genetic materials such as species, provenance, etc (Downes et al., 1997; Stackpole et al., 2010). For instance, in *Eucalyptus*, wood density is often observed to increase both with height (longitudinally) and with age (radially), while pulp yield increases with age (Downes et al., 2012; Greaves et al., 1997; Schimleck et al., 1997). Cellulose, hemicellulose, lignin and S/G ratio change significantly with age, however the pattern varies among studies (Miranda & Pereira, 2002; Yokoi et al., 1999).

The framework to successfully improve important wood property traits through breeding programs and translate them into a reduction in production costs begins with the characterization of these variations considering both radial and longitudinal aspects as well as genetic control. The next challenge is to select outstanding genotypes to make genetic gain, and breeding programs are often focused on making selections as early as possible in order to maximize genetic gain per unit

time (Greaves et al., 1997; Zobel & Talbert, 1984). Unfortunately, the scarcity of available studies on the mentioned characterization of wood traits in *E. pellita* poses a constraint not only for the progress of breeding programs but also for further studies related to its wood property traits. Breeders working with this species often encounter the challenges outlined below:

- Identifying a consistent sampling point for multiple wood properties that highly correlates with the whole-tree value across genotype materials.
- Quantifying the genetic parameters of wood properties and their stability with age.
- Estimating the strength of the relationship of wood traits across ages and the efficiency of early selection.

To comprehensively investigate the role of wood properties in *E. pellita* for the sustainability of long-term production in integrated forestry-pulp and paper industries, a thorough examination of the economic impact of age-related variations on overall pulp expenses is essential for making informed decisions (Magaton et al., 2009). Estimating the influence of the change in both basic density and pulp yield with age on overall pulp mill production has to be taken into account when deciding the optimum rotation age. The estimates should be detailed, including the cost of wood, chemicals, and energy, considering mill capacity. Such information is not only beneficial for forest growers but especially for the financial management of pulp mill operations.

The specific objectives of this research are:

1) To examine within-tree variation of wood properties in *E. pellita*, and identify the optimum sampling point on the stem to estimate whole-tree wood properties and rank genotypes within breeding programs.
2) To estimate genetic parameters for important wood property traits, and examine the age-age genetic correlations to identify an optimum age of selection within breeding programs.

3) To examine the economic impact of age-related variation in wood properties of *E. pellita* on land productivity and overall pulp expenses for rotation ages of 5 and 8 years. This examination includes the cost of wood, chemicals, and energy, while considering mill capacity.

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CHAPTER 2

Longitudinal Variation of Wood Property Traits in *Eucalyptus pellita* F. Muell: Identifying Optimal Sampling Points for Genetic Assessment

2.1 Introduction

Eucalyptus pellita F. Muell plays a vital role in the production of kraft pulpwood in Indonesia, mainly as clonal forest plantations (Nambiar & Harwood, 2014). In 2021, *E. pellita* accounted for 33% (15.8 million m^3) of the total timber production (BPS - Badan Pusat Statistik, 2023), which became part of a reliable supply of wood for vertically integrated pulp & paper companies. Facilitated by its amenability for vegetative propagation, one of the main drivers of the success of the *E. pellita* plantation is the continuous work in genetic improvement (Nambiar et al., 2018). Considerable breeding efforts in the species have been documented in the region since the 1990s. These efforts were primarily focused on developing landraces with desirable growth rates and resistance to pests and diseases. As breeding programs progressed, the range of traits assessed increased to include wood quality traits, mainly traits of economic importance in the pulp and paper industries: basic density, pulp yield/cellulose content, fiber length, chemical consumption, and paper quality (Raymond, 2002).

The importance of basic density (the oven-dried mass of solid wood per unit volume) and pulp yield (the percentage of oven-dry weight pulp obtained from the original oven-dry weight wood) in the overall pulp expenses has been documented (Magaton et al., 2009; Resquin et al., 2022). These studies emphasize that wood quality traits are among the most crucial traits in pulp production, offering the greatest potential for reducing overall expenses. Moreover, the quantity of pulp generated per unit of wood volume demonstrates a direct correlation with basic density. A

study evaluating the cost-effectiveness of tree improvement programs in a large-scale plantation underscored that both basic density and pulp yield play pivotal roles in influencing the total cost of producing unbleached eucalypt kraft pulp, in addition to tree volume (Greaves et al., 1997).

Kraft pulp yield, consisting mainly of cellulose and some hemicelluloses, is affected by a multitude of factors as a combination of the nature of the wood and the pulping process. MacLeod (2007) studied ten factors affecting kraft pulp yield and explained that the chemical composition of wood is the main factor affecting Kraft pulp yield. Wood with higher polysaccharide content, and lower lignin and extractives content, produces higher pulp yield. In hardwood species, kraft pulp yield originates not only from cellulose but also xylan (Sjöholm et al., 2000). Hence, kraft pulp yield is reflected in the content of neutral monosaccharides (glucose, xylose, galactose and arabinose). Further studies on the effect of lignin on the kraft pulp yield showed that the ratio of the two lignin monomers in hardwoods, syringyl (S) and guaiacyl (G), plays a role in the delignification process. An improvement in the delignification process is observed when the S/G ratio increases (del Río et al., 2005; Ventorim et al., 2014).

According to Zobel and Talbert (1984), the most successful tree improvement programs include wood quality traits among the breeding objectives, and assess both the variation of each wood quality trait and the level of genetic control on those traits. Such programs depend on testing a significant number of trees, either seedling progenies or clones. The success of phenotyping techniques depends on the ability to adequately estimate the whole-tree value of the wood properties in a cost-efficient manner. Hence, research in wood quality over the past decade has been focused mostly on the advancement of the phenotyping of the wood quality traits, by developing cost-effective sampling methods for indirect selection on alternative traits that serve as indicators for traits that have higher assessment costs (Raymond, 2002).

Several studies have highlighted the significance of within-tree variations in wood properties of several eucalypt species, particularly the longitudinal variation (from the base to the top of the tree). Notable variations of basic density from the base to the top of the tree have been reported in *E. globulus* (Hamilton et al., 2007; Raymond & Muneri, 2001), the hybrid of *E. grandis* x *E. urophylla* (Silva et al., 2018), and *E. nitens* (Kube & Raymond, 2002; Raymond & Muneri, 2001). In general, these authors concluded that basic density increased longitudinally from about 10% to 70% of the tree commercial height. Longitudinal variations of cellulose content in *E. nitens* (Kube & Raymond, 2002) and total lignin content of a hybrid of *E. grandis* x *E. urophylla* (Arantes et al., 2011) have also been studied. The authors observed inverse patterns between cellulose content and total lignin content.

Due to its complexity, the longitudinal variation causes a significant logistical challenge when it comes to phenotyping several wood properties of large numbers of trees, especially when these properties need to be tested on the same individuals (Downes et al., 1997). Researchers are faced with a tradeoff between having a common sampling point to predict all traits with acceptable accuracy (commonly at breast height, 1.3 m height) and having multiple sampling points for each trait with higher cost. An additional challenge is the potential of whether the longitudinal variation differs among different genotypes of the same species (i.e., a genotype-by-height interaction). If such an interaction is present, it could potentially introduce additional bias to the whole-tree estimate.

Unfortunately, there is a scarcity of studies on within-tree variation of wood traits in *E. pellita*, both longitudinally and radially, which is a constraint not only for the progress of the breeding programs but also for further studies in wood traits of the species. Previous studies of wood traits have used the approach of collecting wood samples either at breast height (Hung et al.,

2015; Lukmandaru et al., 2016) or from solid logs (Japarudin et al., 2022). However, the strength and precision of these approaches need to be addressed. Moreover, identifying a consistent sampling point which highly correlates with the whole-tree value must be evaluated for any wood trait.

Consequently, the objective of this study is to quantify and describe the longitudinal variations of basic density, neutral monosaccharides content (glucose, xylose, galactose and arabinose), lignin content and S/G ratio in *E. pellita,* and to examine the strength of the relationship of multiple sampling points, including at breast height, with the whole-tree value. This study also identifies a consistent sampling point that is feasible for assessing these wood traits in genetic testing. Furthermore, results of this study will be used for the sampling approach in the next research endeavors.

2.2 Materials and Methods

2.2.1 Field Trial Description

We acquired wood samples from a set of *Eucalyptus pellita* clonal trials, established by the Eucalypt Breeding Program of PT. Arara Abadi (https://araraforestry.com/).

In April 2014, two adjoining clonal tests (trial 1 and trial 2) were established in District Gelombang, as part of the *E. pellita* concession area of PT. Arara Abadi, located in Siak Regency, Riau, Indonesia. The trials were established following a randomized complete block design, with 4 replicates, where the number of clones was 35 for trial 1 and 27 for trial 2, with four clones common in both trials, making a total of 58 clones tested. Trees were planted with a spacing of 4 x 1.5 meters. Trees in both trials were planted in mineral soil where the company classified the area as ‘site class I’ – a good site for establishing eucalypt species. Data on survival, tree diameter

at breast height (DBH), and total height (Ht) were assessed annually between two and five years of age. At the age of five years, clonal genetic values for volume were estimated using the best linear unbiased prediction (BLUP). Prior to the BLUP analysis, the individual volume was calculated Margules equation developed for Sinarmas *Eucalyptus* breeding program (van der Hoef et al., 2019).

Details of the number of clones selected per trial are provided in Table 2.1. In May 2022, a set of clones was selected for wood studies when the trials were eight years old. Out of the 58 clones tested, a total of 16 clones (15 unique) were selected for wood property assessment in this study. The selected clones came from the top half of the population in each test, in terms of volume growth age of five years.

Table 2.1. Details of the number of total clone, selected clones, and unique genotype per trial.

Trial	Number of established clones	Number of selected clones	Rank of selected clones[1]
Trial 1	35	9	1, 3, 5, 7, 9, 11, 13, 15, 17
Trial 2	27	7	1, 3, 5, 7, 9, 11, 13
Total	62	16	
Common clones	4	1	
Unique genotype	58	15	

[1]Rank based on clone volume growth at age 5 years

2.2.2 Sample Collection

Multiple wood samples were extracted from trees following the method shown in Figure 2.1. In June 2022, a total of 75 trees were sampled across the replicates by cutting five ramets per clone (with an exception of five clones where only four ramets per clone were available). These trees underwent visual examination to avoid stem defects and diseases. The trees were cut down,

and then the total height (Ht) and commercial height (CHt, the height with 5 cm diameter over-bark) were measured. Each tree was sectioned at six longitudinal points: 0% CHt, 1.3 m, 25% CHt, 50% CHt, 75% CHt, and 100% CHt, dividing the trees into five logs. Two discs (3 cm thick) were extracted at each longitudinal point. During sampling, data on total height, commercial height, and diameter of all sections, including at 1.3 m, were recorded to further calculate whole-tree volume

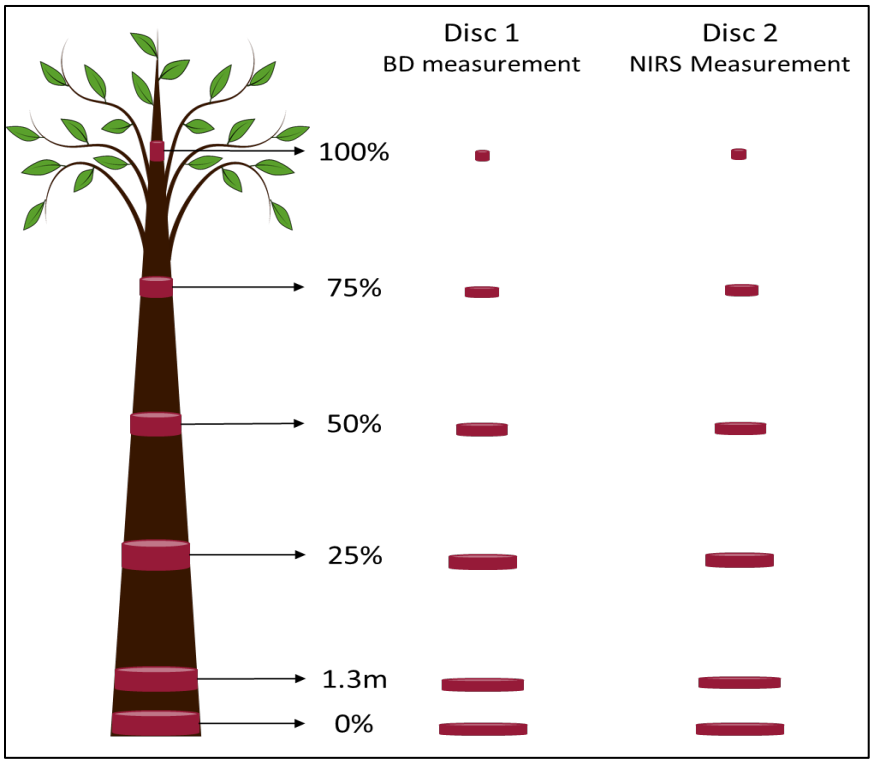

Figure 2.1. Destructive sampling scheme of discs from multiple longitudinal positions for measurement of basic density (BD) and NIR prediction of wood chemistry.

2.2.3 Laboratory Processing

For all wood discs, bark was removed and the under-bark diameter was measured. Basic density (BD) was determined in Disc 1 from all longitudinal sections. The determination used the water displacement method following TAPPI:T258 (TAPPI, 2006) and was conducted at the

Sinarmas R&D facility. Monosaccharide content (glucose, xylose, galactose, and arabinose), acid-insoluble lignin content (klason lignin), and S/G ratio were predicted from Disc 2 using NIR. Disc 2 from all longitudinal sections were immediately air dried and cut into four wedges. Two wedges from opposite sides were chipped and ground to produce wood powder samples using Fritsch Pulverisette 19. In order to standardize the size, the powder samples were later screened between 35 mesh and 50 mesh. The sample processing was conducted at Sinarmas R&D facility. Near-infrared spectra were obtained following the procedure developed by Camcore at North Carolina State University (Hodge et al., 2018). For each sample, about 4 to 5 g of wood powder was dried at 40°C for 24 hours. Before scanning, these samples were equilibrated to room temperature. A total of 450 spectra (75 trees x 6 positions) were obtained by scanning the samples in a Foss NIRsystem 6500, using a spinning sample module. For each sample, reflectance measurements were taken at wavelengths between 1100 to 2500 nm, with an interval of 2 nm reading points. For wavelength point, 32 readings were taken to produce one average reflectance.

The predictions for klason lignin, S/G ratio, glucose, xylose, and galactose were derived from spectra using a *E. pellita* NIR calibration model developed by Camcore. These models were calibrated with wet-lab data from *E. pellita* samples from Indonesia and South Africa. The Indonesia samples included a set of 50 samples selected from a larger set of 550 *E. pellita* trees. This selection was done on the spectra using Kennard-Stone algorithm in order to cover a broad range of variation in the spectra. Additionally, there was a set of 10 samples of *E. pellita* randomly selected from a species trial in South Africa. All 60 samples (50 Indonesia + 10 South Africa) were sent for wet chemistry analysis at the University of British Columbia. Monosaccharides were assessed through acid hydrolysis and high-performance liquid chromatography (HPLC), following the method outlined by Coleman et al. (2007). Similarly, the determination of lignin content was

conducted using acid hydrolysis with a modified Klason method, as detailed by Coleman et al. (2007). For the assessment of lignin monomer ratios, a thioacidolysis procedure was employed, following the approach outlined by Robinson & Mansfield (2009).

The lab analysis results served as the reference values to calibrate new *E. pellita* NIR models, following the method outlined by Acosta et al. (2020). For each studied trait, spectra underwent mathematical transformations with Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Savitzky-Golay 2nd derivative with 7 or 5 points in the convolution window (SG7 or SG5), or a combination of these methods. These transformed spectra were followed by filtering with local outlier factors (LOFs) filter out atypical spectral data.

Table 2.2. The coefficient of determination (r^2) of the independent validations based on NIR prediction and wet lab result of 50 selected samples.

Chemistry traits	r^2
Klason Lignin	0.89
S:G Ratio	0.91
Glucose	0.75
Xylose	0.73
Galactose	0.70

Although the models demonstrated strong coefficients of determination for both calibrations and validation phases ($r^2 \geq 0.85$ for all traits), independent validation of the predictions was conducted. For the validation purpose, a second set of 50 samples was intentionally selected from the spectra of the wood samples and sent to the same laboratory at the University of British Columbia for chemical analysis. In order to cover a broad range of variation in the spectra, similar method (Kennard-Stone algorithm) was used for the selection on the spectra of the 50 samples. The coefficients of determination (r^2) for these independent validations between the lab and the prediction of the 50 samples can be found in Table 2.2.

2.2.4 Data Analysis

Longitudinal variation

For the analysis, data from the two trials were treated as a single test. Besides being identical in location and silvicultural treatments, preliminary analysis of variance (ANOVA) on the common clone showed there was no significant difference in any of the wood traits between the two trials (Appendix A1). For the full analysis, positions at breast height were converted into a percentage of the commercial height (CHt), resulting value about 5 ± 0.5% of the commercial height for each tree. In order to better understand the longitudinal trends, linear regressions were performed between the wood traits and the longitudinal positions. Longitudinal position was used as a continuous variable testing linear, quadratic and cubic regressions, with clone as a discrete variable to adjust the interception of the regressions (Equation 1a, 1b, & 1c). The regressions were performed at the clonal mean level (i.e., using the average of the five ramets) for all wood traits (except for Arabinose, which accounted for no more than 0.45% of the wood dry weight). When the polynomial term is significant, an ANOVA was conducted to compare the linear, quadratic, and cubic regressions in order to avoid overfitting. The best models were selected from the ANOVA output. Since the main focus of the study was on general pattern of longitudinal variation of wood traits that can be applied across genotypes, the interaction between the clone and longitudinal position was not included in the final model.

Equation 1. $Y_{ijk} = \mu + C_i + P_j + \varepsilon_{ijk}, \qquad \varepsilon \sim iid\ (0, \sigma_\varepsilon^2)$ (a)

$$Y_{ijk} = \mu + C_i + P_j + P_k^2 + \varepsilon_{ijk}, \quad \varepsilon \sim iid\ (0, \sigma_\varepsilon^2) \tag{b}$$

$$Y_{ijk} = \mu + C_i + P_j + P_j^2 + P_j^3 + \varepsilon_{ijk}, \quad \varepsilon \sim iid\ (0, \sigma_\varepsilon^2) \tag{c}$$

Where:

- Y_{ijk} is the *k-th* observation of the *i-th* Clone and *j-th* longitudinal sampling positions;
- μ is the overall mean;
- Ci is the clone effect;
- P_j, P_j^2, and P_j^3 are the linear, quadratic and cubic of continuous longitudinal sampling positions effect;
- ε_{ijk} is the is the random error term.

Whole-tree estimates and optimum sampling point

Whole-tree estimates of wood traits were calculated as follows. First, the estimates of the mean value of each of the 5 boles were calculated by weighting the wood property values by the area of the two consecutive longitudinal points (Equations 2). Later, the whole-tree estimates were calculated by weighting the values of the bole's wood property value by their frustum volumes (Equation 3). The strength of the relationship between different sampling points and the whole-tree estimates was then quantified using the Pearson's correlation coefficient (R) between the wood traits at each longitudinal point and the whole-tree estimates. Identifying an optimum sampling point is a critical aspect for future studies to study the quantifying the genetic control of wood traits. With this in mind, the correlations between longitudinal point estimates and whole-tree estimates were examined both for clonal means (which would be appropriate in clonal trials) and individual tree values (which would be appropriate in seedling progeny tests).

Equation 2. $Bole\ mean = \frac{(value\ of\ top\ disc\ x\ area\ top\ disc) + (value\ of\ base\ disc\ x\ area\ of\ base\ disc)}{sum\ of\ area\ top\ and\ base\ disc}$

Equation 3. $Whole - tree\ estimate = \frac{(bole\ mean\ x\ bole\ volume)}{sum\ of\ all\ bole\ volume}$

2.3 Results

2.3.1 Whole-tree Value and Longitudinal Variation

The clonal means and coefficients of variation (CV) for whole-tree values of basic density, klason lignin content, S/G ratio, glucose content, xylose content, galactose content, and arabinose content are presented in Table 2.3. There is meaningful levels of variation among clones for most of the wood traits evaluated. The clonal means for basic density range from 537 to 700 kg/m³. The clonal means for klason lignin range from 25.8% to 29.3%. The majority of the lignin monomers were syringyl, with the ratio to guaiacyl (S/G) ranging from 2.40 to 3.98. Glucose is the primary monosaccharide, with the clonal means ranging from 46.4% to 50.5%, followed by xylose (9.3% to 10.9%) and galactose (1.40% to 2.66%). The coefficients of variation express the consistency or uniformity of a wood property among ramets of the same clone (i.e., among-ramet within-clone). Among-ramet CV fluctuated varied among wood traits, with smaller CV observed for the traits of basic density, klason lignin, and S/G ratio, which all had mean CVs less than 2.5%. In contrast, larger values of among-ramet CV were observed for the traits of glucose (mean CV = 7.3%) and galactose (mean CV = 9.3%), and to a lesser extent, xylose (mean CV = 3.2%). Overall, the relatively wide range of values among clones for the different wood traits is favorable for determining the optimum sampling point on the stem, as the correlations to the whole-tree estimates become more robust.

Table 2.3. Clone mean (coefficient of variation in percentage) for whole-tree values of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal).

Clone	BD (kgm^{-3})	K-Lig (%)	S/G	Glu (%)	Xyl (%)	Gal (%)
1	627 (2.2)	28.6 (2.8)	3.48 (1.6)	46.4 (7.2)	10.4 (3.2)	1.40 (10.3)
2	562 (1.1)	25.8 (1.6)	3.29 (0.5)	50.5 (3.2)	9.3 (2.4)	2.00 (7.1)
3	537 (1.2)	27.5 (3.0)	3.66 (1.2)	46.4 (4.1)	10.9 (3)	1.50 (4.5)
4	700 (1.0)	28.5 (3.0)	3.93 (1.5)	48.4 (3.0)	9.4 (2.3)	1.76 (7.2)
5	667 (1.3)	27.8 (2.8)	3.98 (2.6)	48.2 (5.9)	8.8 (7.8)	2.66 (6.6)
6	670 (0.6)	28.2 (0.8)	2.40 (0.6)	49.7 (4.7)	9.1 (0.7)	1.74 (3.3)
7	548 (1.1)	28.1 (4.6)	3.47 (3.1)	47.7 (9.8)	9.5 (2.9)	2.01 (9.0)
8	582 (3.8)	29.1 (1.8)	2.95 (1.3)	47.1 (5.0)	9.9 (3.2)	1.69 (12.4)
9	595 (1.2)	28.6 (2.1)	2.68 (1.2)	47.9 (11.5)	9.9 (4.0)	1.80 (7.8)
10	574 (0.6)	27.4 (1.5)	3.06 (1.6)	48.3 (3.9)	9.9 (2.1)	1.61 (11.6)
11	576 (0.7)	28.3 (1.6)	3.42 (1.2)	47.3 (5.6)	10.1 (1.8)	2.08 (10.3)
12	610 (1.8)	26.5 (2.4)	3.04 (2.5)	49.0 (8.0)	9.9 (5.6)	2.16 (13.7)
13	577 (3.4)	27.5 (4.1)	3.43 (0.9)	47.4 (16.2)	10.5 (3.2)	1.47 (10.0)
14	560 (2.2)	29.3 (3.1)	2.95 (1.2)	46.6 (15.4)	9.5 (3.5)	1.82 (16.0)
15	616 (2.5)	28.5 (0.9)	2.99 (1.5)	47.0 (5.9)	10.2 (2.7)	1.52 (10.2)

Figure 2.2 presents the fitted regression models and the adjusted-coefficient of determination (r^2) between wood traits and longitudinal sampling point in *E. pellita*. Additional details on the clone means for each longitudinal position and each trait are provided in Appendix A2. In addition to the variation among clones, it is clear that longitudinal position was a significant source of variation in most wood traits examined (p-value > 0.01), except for galactose. This result is strong evidence that sampling points (height) play a critical role when estimating these wood traits.

For basic density, a quadratic trend was observed, with 89 amount of variation explained. The smallest values were observed at the base of the tree, ranging from 497 to 679 kg m^{-3}. Basic density increased with height of sampling, and peaked between 50 to 75 of the commercial height with an average increase of 45 kg m^{-3} (550-713 kg m^{-3}). From this point, basic density declined as the sampling point reached the top of the commercial height (533-689 kg m^{-3}).

The klason lignin percentage and the ratio of the monomers (S/G) of the lignin in *E. pellita* both varied significantly with the height of sampling. Both longitudinal trends were found to be modeled as a cubic regression with adjusted r-squared greater than 70, however opposite trends were observed. The cubic regression for klason lignin percentage showed a tendency of decreasing with height. The highest percentage was found at the base of the tree, ranging from 27.2 to 32.1, with a rapid decline to 25.2 to 29.6 lignin at 25 of commercial height that remained stable up to 75 of the commercial height. Further declines were observed as sampling points increased from 75 to the top of the commercial height, with the percentage of klason lignin at the top ranging from 25.9 to 28.4 lignin. For S/G ratio, the regression had the opposite trend, with a tendency to increase from base to the top of the tree's commercial height. The lowest S/G ratio was observed at the base, ranging between 1.5 and 3.8. The S/G ratio gradually declined as sampling points increased to the middle portion of the tree (between 25 and 75 of commercial height) with the value ranging from 2.6 to 4.2. Further increase was shown at the sampling point at the top of the tree, where the S/G ratio ranged from 3.2 to 4.7.

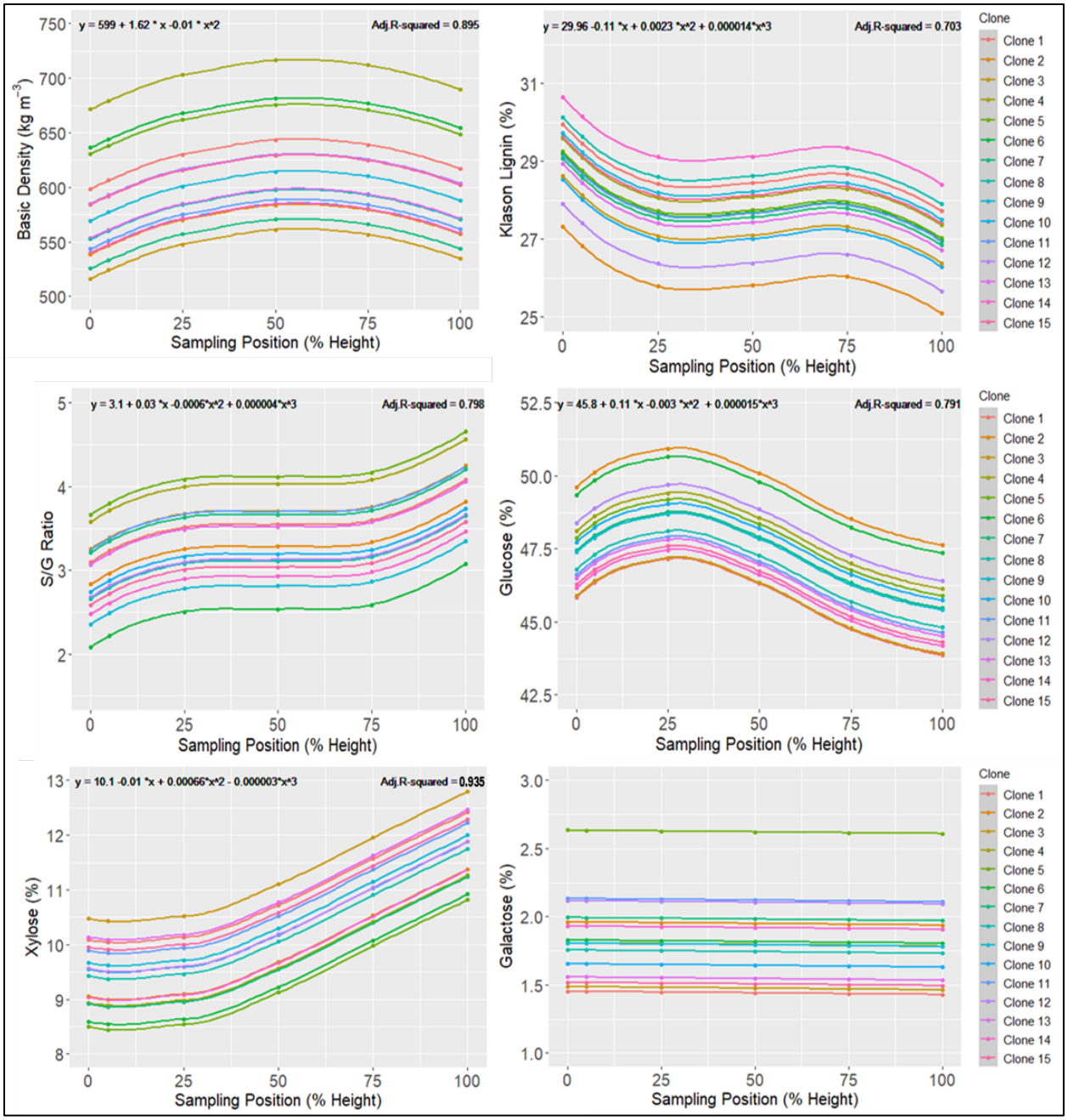

Figure 2.2. Fitted longitudinal models and the adjusted-coefficient of determination (r^2) between wood traits and longitudinal sampling point in *E. pellita*.

The results for two major neutral monosaccharides, glucose and xylose, also also demonstrate that longitudinal position is a significant source of variation. However, no

longitudinal trend was observed for percentage of galactose. The longitudinal variations of glucose and xylose were better modeled by cubic regressions with the adjusted r-squared about 80. However, the patterns of the longitudinal changes differed between the two monosaccharides. The percentage of glucose at the base of the tree ranged from 44.5 to 48.7. A rapid increase to 46.2 to 51.0 glucose was observed at 25 of the height. From this point, glucose content continually declined and reached the lowest value as the sampling point increased to the top of the tree (42.6 to 47.9 glucose). In contrast, the percentage of xylose remained relatively constant from base to 25 of height (8.4 to 10.7 xylose). Afterward, xylose content continuously increased to the top of the tree with values ranging from 10.6 to 13.1.

2.3.2 Optimum Sampling Point

Pearson's correlations (r) of the clone means for whole-tree values and the value at the different longitudinal sampling points are provided in Table 2.4. In general, most of the sampling positions demonstrated strong correlation with the whole-tree values for all the wood traits, with basic density being the strongest. In most cases, sampling points at 25 CHt, 50 CHt and breast height (5 CHt) displayed the strongest correlations across all wood traits (r = 0.877 to 0.995). It was evident that these three sampling points are good predictors for the whole-tree estimates of the clonal mean, explaining between 77% to 99% of the variation in the whole-tree estimates. Basic density, klason lignin and S/G ratio sampled at breast height exhibited similar correlations with 25 CHt and 50 CHt. Additionally, glucose, xylose and galactose sampled at breast height still demonstrated strong correlations (r = 0.877 to 0.896), although sampling points at 25 Cht and 50 CHt presented noticeable improvements on the correlations (r = 0.963 to 0.984).

Table 2.4. Pearson's correlations (r) of the clone mean of the wood traits, between the longitudinal sampling points and their whole-tree estimates of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal).

Longitudinal Positions	Whole-Tree					
	BD	**K-Lig**	**S/G**	**Glu**	**Xyl**	**Gal**
0 CHt	0.950	0.807	0.895	0.842	0.933	0.825
5CHt (1.3 m)	0.971	0.903	0.945	0.896	0.877	0.883
25 CHt	0.995	0.925	0.941	0.865	0.967	0.984
50 CHt	0.989	0.917	0.939	0.963	0.981	0.971
75 CHt	0.953	0.678	0.787	0.784	0.860	0.826
100 CHt	0.840	0.482	0.729	0.787	0.894	0.780

To examine the accuracy of these sampling points for predicting the whole-tree estimates in progenies tests, Pearson's correlations of the individual tree (or ramet) values are provided in Table 2.5. In general, the patterns for the individual tree correlations and the clonal mean correlations were similar, although, as expected, the correlations were not as strong as with clonal means. Sampling points at 25 CHt, 50 of CHt and breast height displayed the strongest correlation with whole-tree estimates in most of the wood traits (r = 0.787 to 0.982). Moreover, correlations between breast height and whole-tree estimates showed that breast height was a good predictor at the individual tree level (r = 0.787 to 0.956).

For basic density and xylose content, there was a uniform longitudinal pattern of strong correlations at all of the sampling positions, including the base and the top of the tree. This was not the case for klason lignin, S/G ratio, glucose, and galactose, where sampling points at 0 CHt, 100 CHt, and sometimes at 75 CHt had noticeably weaker correlations compared to the breast height, 25 Cht, and 50 CHt.

Table 2.5. Pearson's correlations (r) of the individual tree values of the wood traits, between the longitudinal sampling points and their whole-tree estimates of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal).

Logitudinal Position	Whole-Tree					
	BD	K-Lig	S/G	Glu	Xyl	Gal
0 CHt	0.901	0.695	0.772	0.728	0.858	0.698
5CHt (1.3 m)	0.856	0.832	0.889	0.787	0.848	0.790
25 CHt	0.982	0.862	0.858	0.892	0.922	0.932
50 CHt	0.859	0.869	0.874	0.865	0.909	0.885
75 CHt	0.895	0.594	0.684	0.742	0.814	0.754
100 CHt	0.746	0.359	0.542	0.547	0.741	0.636

2.4 Discussion

2.4.1 Whole-tree Estimates and Longitudinal Variation

This study provides a thorough examination of a set of wood properties of *E. pellita* by investigating the longitudinal patterns and the whole-tree estimates of 75 trees from 15 unique genotypes. In general, the mean whole-tree basic density of 600 kg m^{-3} found in this study corresponds to previous reports in the literature. However, several clones were identified with significantly higher values than previously reported. Clones numbers 4, 5, and 6 had whole-tree basic densities ranging from 667 to 700 kg m^{-3}, higher than reported for 6-year-old *E. pellita* from progeny trials grown in Borneo (629 kg m^{-3}) (Japarudin et al., 2022), and higher than reported for 10-year-old progeny trials in Indonesia, where the basic density ranged from 556 to 652 kg m^{-3} (Susilawati & Marsoem, 2006), and also higher than reported in Vietnam, where the basic density varied between 640 and 676 kg m^{-3} (Hung et al., 2015). In terms of the range, the basic density variation observed in this current study appeared to be broader than the ranges reported in the two

aforementioned studies, despite their relatively larger genetic sources (10 families in Indonesia and 94 families in Vietnam). Additionally, basic density of *E. pellita* in this study was noticeably higher compared to a similar study in *E. globulus* with similar age (Apiolaza et al., 2005; Raymond & Muneri, 2001).

At the whole-tree level, the percentage of klason lignin in this study was similar to a range of previous studies on the same species at a similar age conducted by (Lukmandaru et al., 2016; Sun et al., 2017; Utami et al., 2023). In addition, the S/G ratio of most of the clones fell within the values of 4-year-old *E. pellita* from Brazil (Diniz et al., 2019) which reported ranging from 2.0 to 3.5. However, there were a few exceptions among clone no. 3, 4, and 5, as these clones evidently exhibited higher S/G ratios (3.66 to 3.98, Table 2.3). Furthermore, the majority of the clones exhibited higher S/G ratios than those reported in 3-year-old *E. pellita* trees from India (Ramadevi et al., 2016). One possible explanation is that the current study involved a larger sample size of genotypes. In contrast, the materials in the mentioned studies were sampled from a smaller number of genotypes, limiting their ability to capture the variation attributed to genetics, especially considering that both lignin and the S/G ratio in eucalypt exhibit medium to high heritability (Blake Wood et al., 2012; Stackpole et al., 2011)

When compared with other important eucalypt species, the data revealed that both the percentage of klason lignin and the S/G ratio of *E. pellita* corresponded to what was discussed in several literature sources. Separate studies involving a series of hybrid clones derived from four eucalypt species (*E. urophylla, E. grandis, E. camaldulensis*, and *E. pellita* (Vieira et al., 2021)) and 9-year-old *E. globulus* (Rodrigues et al., 1998) indicated that the klason lignin percentage in *E. pellita* falls within the same range. However, these results were higher compared to those of older *E. globulus* clones with percentages of 19.8 (Rencoret et al., 2011) and 19.5 (Pereira, 1988).

Furthermore, the S/G ratio in this study had a similar range to that of 9-year-old *E. benthamii* (Diniz et al., 2019). However, it is noticeably higher than what was reported for 9-year-old *E. globulus*, which ranged from 1.5 to 2.6 (Rodrigues et al., 1999).

In *E. pellita* wood, the two most abundant monosaccharides are glucose and xylose, followed by small amounts of galactose (Table 2.3). Glucose, the monomer composition of cellulose, was the primary monosaccharide (clone means ranging from 46.4 to 50.5), while xylose represented the primary hemicellulose (ranging from 8.8 to 10.3). In hardwood, xylose can mainly be found in the form of glucuronoxylan as the primary chain of hemicellulose (Bajpai, 2014; Waldron & Faulds, 2007). The whole-tree estimates of the monosaccharides in this study were consistent with findings from two previous studies (Jang, 2018; Utami et al., 2023). Both studies concluded that glucan was the primary monosaccharide in *E. pellita*, with estimates of 42.1 and 50.9, respectively. Meanwhile, xylan was identified as the primary hemicellulose, with estimates of 8.1 and 10.8, respectively. The studies also noted small amounts of galactan and arabian (1.6 and 0.3). Furthermore, similar estimates of two most abundant monosaccharides, glucose (ranging from 43.2 to 59.5) and xylose (ranging from 9.4 to 17.8), were also reported in *E. globulus* (Rodrigues et al., 2001).

Although many studies have examined the longitudinal changes in wood properties in eucalypt species, limited literature is available on *E. pellita*. Studies have shown that these patterns vary among species. Moreover, the resolution of the sampling evidently influences the longitudinal pattern. For basic density, longitudinal patterns in other eucalypt species are often described as linear increases from the base to about 60-70 of the tree height (Hamilton et al., 2007; Raymond & Muneri, 2001; Silva et al., 2018). In this study, the wider sampling range, covering up to 100 of the CHt at a 5 cm diameter, revealed a quadratic trend for basic density (Figure 2). However, if

the sampling range is reduced to only 75 of the CHt, the trend can appear as a linear increase. A study on *E. pellita* indicated that basic density increased by about 25 kg/m³ from the base to the middle part of the tree; however, the increase was absent as the sampling point moved towards the top (Susilawati & Marsoem, 2006). The tendency of lignin to decrease from the base to the top of the tree identified in this study has also been reported in *E. globulus* (Arantes et al., 2011) and *Acacia crassicarpa* (Martins et al., 2020). The significantly lower S/G ratio (with higher G-units than S-units) at the base of the tree can potentially be attributed to the influence of xylogenesis in the roots. Marzec-Schmidt et al., (2019) detailed the difference between xylogenesis in the main stems and pioneer roots of *Populus trichocarpa*, indicating an increasing synthesis of lignin, with G-units being dominant over S-units in the primary xylem of the roots. The opposing longitudinal patterns of the two most abundant monosaccharides (glucose and xylose, Figure 2) have also been documented previously. Similar to this study, Pereira & Sardinha (1984) reported that as the sampling height increases, the percentage of cellulose on *E. globulus* declines, while pentosans increase (xylose is the main pentose monosaccharide in hardwoods). A more recent study of *E. nitens* showed a very similar trend with glucose, an overall decline in cellulose along the tree stem with an initial increase at 25 of the tree height (Kube & Raymond, 2002).

2.4.2 Optimum Sampling Point

In general, the accuracy of the sampling points for estimating whole-tree values varied among the wood traits (Tables 2.4 and 2.5). Sampling for basic density was less complex, as almost all positions provided reliable estimates, and it was also more accurate than for other traits. Meanwhile, the chemical traits were more challenging, with most of the accurate sampling points being around the 50 of the tree commercial height. Nevertheless, sampling points at 25 and 50 of the height were identified as highly reliable single positions for all traits, explaining the variation

in the whole-tree estimates between 75% to 99% at the clonal level; and 74% to 96% at the individual level. There was also evidence that sampling at breast height gave reliable estimates for all assessed traits especially at the clonal level, explaining from 77% to 94% of the whole-tree estimates. Although the accuracy slightly decreased at the tree level, it remained reliable for individual ranking (r^2 = 62 to 91). Similar results have been confirmed by previous studies in other species. However, most of these studies are limited only to basic density and cellulose. (Downes et al. (1997) summarized several studies concluding that 40 height is the most accurate point to predict whole-tree basic density of *E. nitens, E. globulus* and *E. regnans*, but sampling between 0.7 to 1.3 meters of the stem height were logistically optimum and recommended for the estimation. Another study on *E. nitens* revealed a similar trend for cellulose, sampling at 0.9 m of the stem height was enough to explain of the whole-tree variation for estimating genetic parameters (Kube & Raymond, 2002). This phenomenon was also observed in *Acacia crassicarpa* A.Cunn. ex Benth. Despite the unique trends in wood properties compared to eucalypts, a sampling point at 1.3 meters (breast height) is recommended. This height is not only the most practical but also provides reliable estimates for both basic density and wood chemistry (Martins et al., 2020).

2.5 Conclusion

After examining wood traits of 75 trees from 15 unique *E. pellita* clones, it is evident that basic density, klason lignin, S/G ratio, glucose, and xylose of *E. pellita* vary significantly longitudinally up and down the stem. The only trait that did not show a longitudinal pattern was galactose content. Some wood traits, like basic density, S/G ratio, and xylose, demonstrate a tendency to increase with tree height, while others, like glucose and klason lignin, decrease with tree height. However, these changes are not perfectly linear, they are better explained by quadratic (as with basic density) or cubic models (as with chemical compositions), with longitudinal position

in this study explaining more than 70 of the variation. The most accurate single points were found at the middle part of the stem height (25 and 50 of the commercial height), as they explain at the clonal level from 84 to 99 of the variation in whole-tree estimates. Nevertheless, it is evident that sampling at breast height (1.3 m) also provides a reliable estimate for all the assessed wood traits, particularly at the clonal level (r^2 = 77 to 94). As the average tree commercial height of the sampled trees of these clones at two years were 9.6 to 14.7 years, hence sampling at 25% of the tree commercial height is not feasible, especially for the non-destructive sampling. Given that sampling at 25% of the commercial height of *E. pellita* at 2 years is not accessible – considering the average sampled trees from these clones ranged from 9.6 to 14.7 m – this point is not feasible, especially for non-destructive methods. Therefore, it is recommended that sampling point at 1.3 meters (breast height) as the optimal height for assessing basic density, klason lignin, S/G ratio, glucose, xylose, and galactose in *E. pellita*. This height is both practical and reliable for estimating the overall value of the entire tree.

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CHAPTER 3

Age-Age Correlation and the Efficiency of Early Selection for Basic Density and Chemical Traits of *Eucalyptus pellita* F. Muell

3.1 Introduction

In recent years, the establishment of clonal plantations of *Eucalyptus pellita* as part of the integrated pulp and paper industries in tropical areas rapidly has increased. By 2017, an area exceeding 600,000 hectares, once planted with *Acacia mangium* Willd., has been replaced (Nambiar et al., 2018). With the aim of optimizing both quantity and cost-effectiveness of pulp production per hectare, breeders of *E. pellita* are now incorporating a wider variety of traits into their programs, placing particular emphasis not only on growth-related traits but also on wood quality traits. Among these wood quality traits, basic density and pulp yield are among the most crucial traits for pulp and paper industry, as they offer the highest potential for reducing overall pulp production expenses (Magaton et al., 2009; Resquin et al., 2022). Furthermore, Raymond (2002) also emphasizes the significance of wood's chemical properties in pulp and paper production in addition to basic density and pulp yield.

The framework to successfully improve important wood property traits through breeding programs and translate them into a reduction in production costs begins with the characterization of the variations in the traits of interest and at what degree these variations are controlled by genetic. The next challenge is to select outstanding genotypes to make genetic gain, and breeding programs are often focused on making selections as early as possible in order to maximize genetic gain per unit time (Zobel & Talbert, 1984). This framework applies to all traits including wood properties. However, as most of these traits change over time, breeders face challenges to decide

when and how the wood properties may be reliably assessed, with respect to predicting the breeding value at harvesting age. Hence, understanding the heritability, how it evolves over time, and the strength of genetic correlations of wood property traits at various ages (age-age genetic correlation) is essential in a breeding program. This knowledge serves as a fundamental key for evaluating the effectiveness of early selection. Accurately identifying superior genotypes at the earliest age expedites breeding efforts, ultimately leading to an increase in genetic gain per unit time (McKeand, 1988).

Efforts to assess the effectiveness of early selection for *E. pellita* have been documented, particularly in growth traits, with some studies yielding promising results. The first efforts were documented in the early 1990s with the establishment of tree seedling seed orchards (SSO) in South Kalimantan, South Sumatra and Riau. These seedling seed orchards were later studied to estimate the optimum early selection for diameter (Leksono, 2009). The study concluded that for 8-year-old *E. pellita*, selection based on diameter can be done as early as half of the rotation age. A more recent study in Colombia demonstrated strong genetic correlations for diameter and height of *E. pellita* between 1 year and 4 years (Nieto et al., 2016)

Unfortunately, information on the age-age genetic correlations and effectiveness of early selection wood property traits of *E. pellita* is limited. While efforts were made to assess the genetic parameters of basic density and pulp yield in *E. pellita* in Vietnam, the study was restricted to heritability at 10 years of age (Hung et al., 2015). In practice, breeding strategies for this species often rely on recommendations from studies of other well-studied *Eucalyptus* species, primarily temperate species such as E. *globulus* and *E. nitens*, regarding wood properties. For instance, studies in *E. globulus* and *E. nitens* suggest assessments can be done at 5 years for pulp yield and chemical properties, and at 3 years for basic density (Raymond, 2002). These recommendations

are less pertinent, considering that these suggested ages are close with the typical rotation age of *E. pellita* – rotation age is around 5-6 years of age (Hardiyanto et al., 2022; Inail et al., 2019). Therefore, the notion of assessing wood property traits as early as possible remains in question.

With that in mind, this study examines the annual changes in basic density and chemical properties of *E. pellita* clones*,* in order to evaluate the degree of genetic control to the wood properties from the age of two to eight years. The aims are to estimate heritability, understand how it changes with age, and determine the strength of genetic correlations among these ages. The results of this study will offer valuable insights to understand the effectiveness of the early selection for the wood properties traits of *E. pellita* in clonal trial and to formulate more efficient breeding programs.

3.2 Materials and Methods

3.2.1 Field Description and Material Selection

We studied genetic materials from two clonal tests of *E. pellita*, established by the *Eucalyptus* breeding program of PT. Arara Abadi, an integrated company for pulp and paper production also known as Sinarmas (https://araraforestry.com/). Test are located in District Gelombang, Siak Regency, two adjacent clonal tests (Trial 1 and Trial 2) were established in April 2014, in mineral soils classified by the company as 'site class I', indicating its suitability for establishing *Eucalyptus* species. These trials followed a randomized complete block design, with 4 replicates in each trial. Trials 1 and 2 included 35 and 27 clones, respectively, and in each replicate, five ramets per clone were planted in a line at a spacing of 4 x 1.5 meters. Assessment on the individual trees were recorded annually from ages two to five, assessing the survival, tree diameter at 1.3 meters (DBH) and total height.

Individual tree volume was calculated at five years of age using the Margules equation developed for Sinarmas *Eucalyptus* breeding program. With this volume data, Sinarmas Breeding Department calculated the clonal breeding values and the rank of the genotypes based on their best linear unbiased prediction (BLUP) estimates for both trials (van der Hoef et al., 2019). Results of the analyses were used to select a subset of genetic materials for wood sampling. All the analysis at age 5 were conducted by the Sinarmas' *Eucalyptus* breeding program. When the trials reached eight years of age, a total of 30 clones, 17 clones in Trial 1 and 13 clones in Trial 2, were selected to cover the top half of the population ranked for volume growth. From the selection, there were three clones shared between the two trials, resulting in a list of 27 unique genotypes.

3.2.2 Sample Collection and Preparation

Wood samples were obtained by destructively extracting wood discs when the trial reached 8 years of age. In June 2022, a total of 147 trees were sampled across the replicates by cutting mostly five ramets per clone, with an exception of three clones with only a total of four ramets available. The trees were cut down following the scheme presented in Figure 1. Two discs (3 cm thick) were extracted at 1.3 m height. Strong correlations between sampling at 1.3 m height and the whole estimate of assessed traits were reported in Chapter 2 of this Thesis. Each disc was earmarked for basic density and wood chemistry. All the discs were labeled properly. During sampling, data of total height (Ht), commercial height (CHt) - a height at 5 cm diameter – and the diameter at breast height (1.3 m) were measured. Following sampling, the bark was removed, and the inside bark diameter was measured. Discs designated for basic density were soaked in water, while the other two were left to air dry.

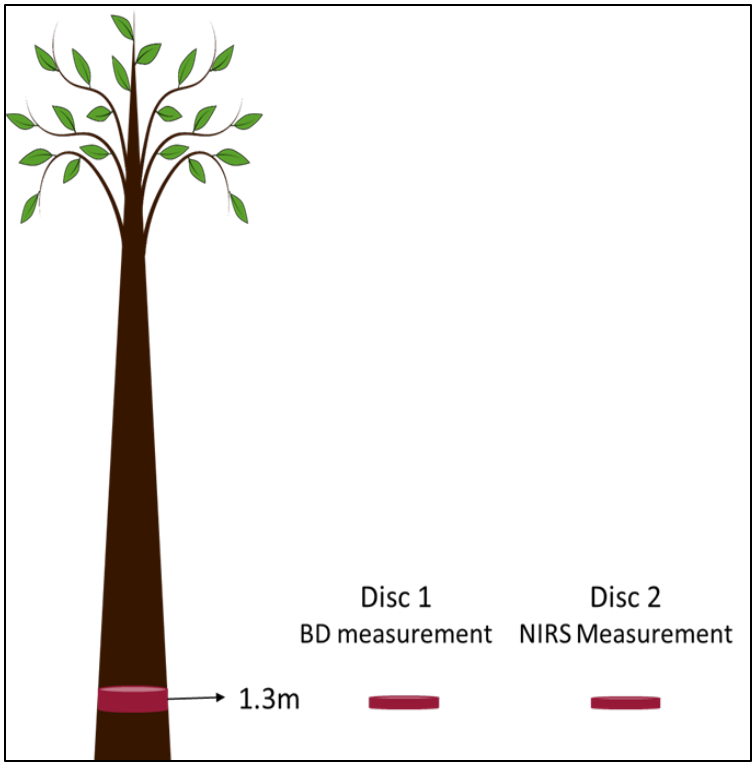

Figure 3.1. Destructive sampling scheme of discs at 1.3 m for measurement of wood traits.

The annual measurements of DBH (ages 2, 3, 4, 5, and 8) were utilized to determine the age of the wood samples. This information was then corrected for bark thickness and wood segmentation between ages was done in accordance with the scheme outlined in Figure 2. The determination process began with the measurement of the ratio of radii from the pith to the two most eccentric sides of the disc at the age of eight (Figure 2a). This ratio was then used to calculate the radii from ages five to two based on the diameter data. Segmentation between ages was achieved by drawing a circle with a compass between two points from the same age. The wood was then segmented into five increment groups (0-2 years, 2-3 years, 3-4 years, 4-5 years, and 5-8 years) based on the circles, using a chisel (Figure 2b & c). All wood segments were properly labeled to ensure traceability.

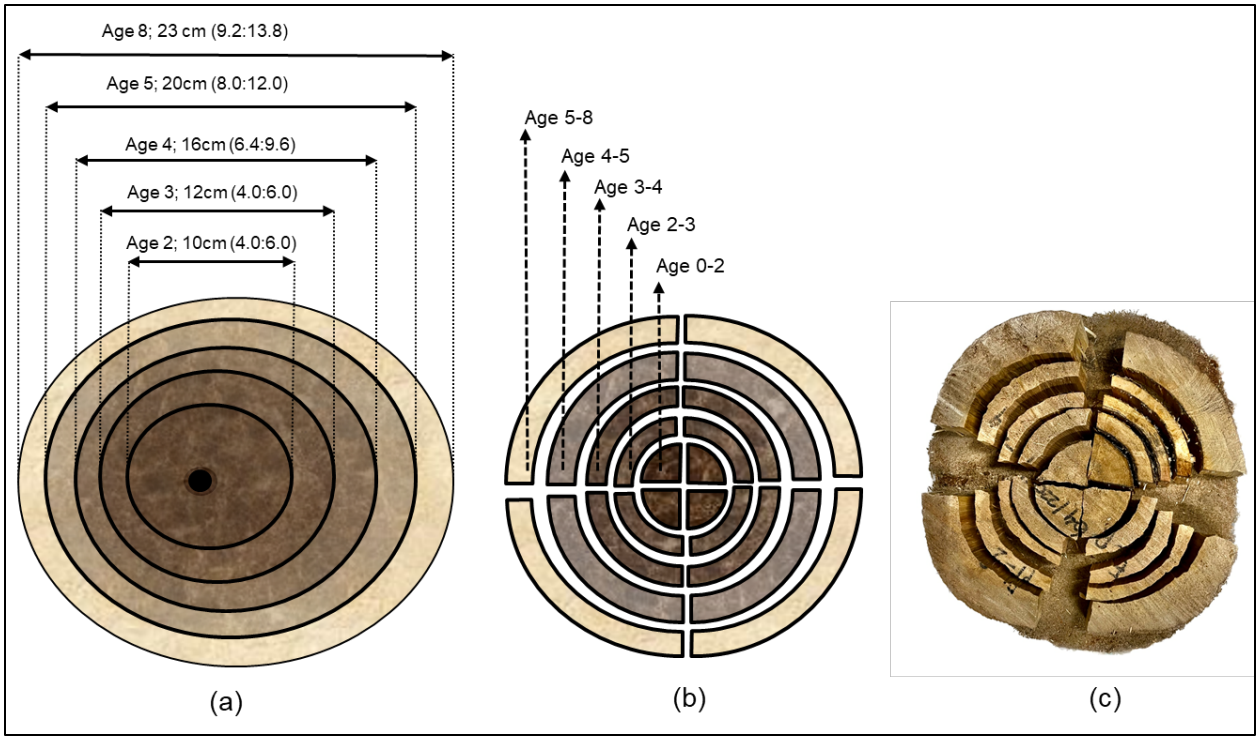

Figure 3.2. Process of (a) determining wood sample for each age based on diameter and radii ratio; (b) wood sample segmentation for each age; and (c) result of wood segmentation.

3.2.3 Laboratory Processing

Basic density was determined using wood segments from Disc 1. The determination was carried out using the water displacement method according to the TAPPI T258 standard (TAPPI - Technical Association of the Pulp and Paper Industry, 2006). Saturated volume and dry weight measurements were taken for a total of 735 wood segments belonging to the five distinct increment groups (147 trees x 5 ages: 0-2 years, 2-3 year, 3-4 year, 4-5 year, and 5-8 years). The basic density for each increment section was calculated by dividing the dry weight by the saturated volume. This process enables us to estimate the radial trend of basic density. Additionally, aggregate basic density at five ages (0-2 years, 0-3 years, 0-4 years, 0-5 years and 0-8 years) was computed by

dividing the aggregate dry weight by the aggregate volume of the corresponding segment following the equation below.

Equation 1: $BD = \frac{dry\ weight}{wet\ volume}\ x\ 100$

Where BD equals basic density at the assessed age in kg m^{-3}, dry weight is the total of the dry weight from pith to the measured ages, and wet volume is the total of the dry weight from pith to the measured ages.

The monosaccharides content (glucose, xylose, galactose, and arabinose), acid-insoluble lignin content (known as klason lignin), and the S/G ratio were measured through near-infrared (NIR) following the procedure established in the Camcore Laboratory, NCSU (Hodge et al., 2018). A total of 735 wood segments of five distinct increment groups were ground using the Fritsch Pulverisette 19 to create wood powder samples. To standardize the size, the powder samples were later sifted through meshes ranging from 35 to 50. Approximately 4 to 5 grams of wood powder from each sample were dried at 40°C for 24 hours and then brought to room temperature before scanning. Spectra of the 735 powder samples were collected by scanning them with Foss NIRsystem 6500. Utilizing a spinning sample module, reflectances were recorded at wavelengths spanning from 1100 to 2500 nm, with readings taken at 2 nm intervals. The final spectrum was generated from the average of 32 readings.

The predictions for klason lignin, S/G ratio, glucose, xylose, and galactose were derived from wood segment spectra using a *E. pellita* NIR calibration model developed by Camcore. These models were calibrated with wet-lab data from *E. pellita* samples from Indonesia and South Africa. The Indonesia samples included a set of 50 samples selected from a larger set of 550 *E. pellita*

trees. This selection was done on the spectra using Kennard-Stone algorithm in order to cover a broad range of variation in the spectra. Additionally, there was a set of 19 samples of *E. pellita* randomly selected from a species trial in South Africa. All 69 samples (50 Indonesian + 10 South Africa) were sent for wet chemistry analysis at the University of British Columbia. Monosaccharides were assessed through acid hydrolysis and high-performance liquid chromatography (HPLC), following the method outlined by Coleman et al. (2007). Similarly, the determination of lignin content was conducted using acid hydrolysis with a modified klason method, as detailed by Coleman et al. (2007). For the assessment of lignin monomer ratios, a thioacidolysis procedure was employed, following the approach outlined by Robinson & Mansfield (2009).

The lab analysis results served as the reference values to calibrate new *E. pellita* NIR models, following the method outlined by Acosta et al. (2020). For each studied trait, spectra underwent mathematical transformations with Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Savitzky-Golay 2nd derivative with 7 or 5 points in the convolution window (SG7 or SG5), or a combination of these methods. These transformed spectra were followed by filtering with local outlier factors (LOFs) filter out atypical spectral data.

Although the models demonstrated strong coefficients of determination for both calibrations and validation phases ($r^2 \geq 0.85$ for all traits), independent validation of the predictions was conducted. A second set of 50 samples were intentionally selected from the spectra of the samples and sent to the same laboratory at the University of British Columbia for chemical analysis. In order to cover a broad range of variation in the spectra, similar method (Kennard-Stone algorithm) was used for the selection on the spectra of the 50 samples. The coefficients of

determination (r^2) for these independent validations between the lab analyses and the NIRS prediction can be found in Table 3.1.

Table 3.1. The coefficient of determination (r^2) of the independent validations of 50 selected samples based on NIRS prediction and laboratory results.

Chemistry traits	**r^2**
Klason Lignin	0.89
S:G Ratio	0.91
Glucose	0.75
Xylose	0.73
Galactose	0.70

The *E. pellita* NIR model was then used to make predictions of klason lignin, S/G ratio, glucose, xylose, and galactose for all five segments of the breast height wood samples for all ramets of all clones. With the prediction, aggregate values of the chemical wood traits (klason lignin, S/G ratio, glucose, xylose and galactose) at five ages (0-2 years, 0-3 years, 0-4 years, 0-5 years and 0-8 years) were then calculated. The calculations involved weighing the NIRS predictions of corresponding segments by their dry weights obtained from the basic density measurements of the segments. Initially, the sum of the multiplication between the NIR prediction and its dry weight for each segment at the corresponding age is calculated. Then, this summation is divided by the total dry weight at the corresponding age. The calculation is showed in Equation 2 as follows:

Equation 2: $Agregate\ chemical\ content = \frac{sum\ of\ (NIR\ prediction\ x\ dry\ weight)}{sum\ of\ dry\ weight}$

3.2.4 Estimation of Genetic Parameters

The variance and covariance components of each trait were estimated through multivariate analysis using REMLF90. REMLF90 employs restricted maximum likelihood (REML) to estimate the covariance components (Misztal et al., 2010; Muñoz, 2017). The linear mixed model is denoted in equation as follows:

Equation 3: $Y_{ijk} = \mu + T_i + C_j + \varepsilon_{ijk}, \qquad \varepsilon \sim iid\ (0, \sigma_\varepsilon^2)$

Where Y_{ijk} represents the observation for the *k-th* individual tree in *i-th* trial and *j-th* clone; μ is the overall mean; T_i is the fixed effect of *i-th* trial, C_j is the random effect of the *j-th* clone, and $\boldsymbol{\varepsilon}_{ijk}$ is the residual error.

Broad-sense heritability (H^2) and the genetic correlations between the juvenile age and the rotation age ($R_{g(j\text{-}m)}$) for each trait were obtained from variance and covariance components calculated using REMLF90. The H^2 single model while $R_{g(j\text{-}m)}$ bivariate model. The calculations were done for all five ages following the equations below:

Equation 4: $H^2 = \frac{\sigma^2 C}{(\sigma^2 C + \sigma^2 \varepsilon)}$

Where the σ^2c and $\sigma^2\varepsilon$ are the variance components of clonal effect and residual effect respectively.

Equation 5: $R_{g(j-m)} = \frac{Cov_{j-m}}{(\sigma_j + \sigma_m)}$

Where $COV_{j\text{-}m}$ is the clone genetic covariance between juvenile ($_j$) and rotation age ($_m$); σ_j is the genetic standard deviation of juvenile age; σ_m is the genetic standard deviation of rotation age.

Genetic gains from both direct (G_m) and indirect ($G_{j\text{-}m}$) selection were estimated prior to determining the efficiency of gains per year (E) - ratio between the gains anticipated from juvenile selection (indirect selection) to those expected from selection at rotation age (direct selection). All calculations follow the equation provided by (McKeand, 1988). These computations were carried out for two rotation ages, specifically at five and eight years.

Equation 6: $E = \frac{G_m}{G_j}$ or $E = \frac{(i\ H_j\, H_m\, R_{g(j-m)}\, \sigma_m)/j}{(i\ H_m^2\, \sigma_m)/m}$

In this equation, *i* represents the selection intensity, and H_j and H_m denote the square roots of broad-sense heritability at the juvenile and rotation age, respectively. $R_{g(j\text{-}m)}$ stands for the genetic correlation between juvenile and rotation age, while σ_m represents the phenotypic standard deviation of clone means at the rotation age. To complete a cycle of clonal testing, an additional year (per cycle) was factored in to accommodate the time required for mass-propagation.

As the selection intensity (*i*) and the phenotypic standard deviation (σ_m) are equal in both genetic gain equations, the equation for the efficiency of gains per year can be simplified as follows:

Equation 7: $E = \frac{(H_j\, R_{g(j-m)})/j}{H_m/m}$

3.3 Results

3.3.1 Annual Trends

Table 3.2 presents the mean values and variation range (minimum and maximum) for wood properties (basic density, klason lignin, S/G ratio, glucose, xylose and galactose) as a function of age. Meanwhile, the aggregate and the annual trends of wood properties with age are illustrated in

Figure 3. In general, data shows a significant degree of variation in the tested wood properties within the samples.

Overall, as age increases, there is a trend of increasing basic density, klason lignin, and glucose for the increment value, and hence for the aggregate value (Figure. 3.3). This increase was particularly noticeable in the earlier years, peaking between ages two and four. For basic density, the increasing trend continued, while klason lignin and glucose leveled off at three and four years of age. On average, the aggregate basic density increased by about 61 kg m^{-3} (equivalent to 10 kg m^{-3} annually) from age 2 to age 8. During the same period, aggregate klason lignin increased about 0.75% (0.13% annually), while aggregate glucose increased about 0.5% (0.08% annually).

Table 3.2. Mean (minimum and maximum) of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal).

Age (Year)	BD (kg m^{-3})	K-Lig (%)	S/G	Glu (%)	Xyl (%)	Gal (%)
2	**521**	**27.4**	**3.7**	**47.6**	**9.6**	**1.76**
	(418 - 648)	(22.4-33.0)	(0.8 - 5.5)	(37.1-52.7)	(7.6 - 11.7)	(0.9 - 3.5)
3	**562**	**28.0**	**3.25**	**48.1**	**9.1**	**1.71**
	(442 - 719)	(22.8-33.7)	(0.7 - 5.1)	(38.0-54.4)	(6.2 - 11.2)	(0.7 - 3.6)
4	**581**	**28.1**	**3.08**	**48.2**	**9.1**	**1.74**
	(454 - 737)	(22.9-33.7)	(0.8 - 5.0)	(33.8-54.6)	(6.4 - 10.9)	(0.6 - 3.4)
5	**592**	**28.1**	**2.98**	**48.2**	**9.2**	**1.72**
	(468 - 739)	(23.1-33.5)	(0.5 - 4.8)	(37.3-53.3)	(6.8 - 12.1)	(0.8 - 3.9)
8	**600**	**28.2**	**2.77**	**48.1**	**9.4**	**1.71**
	(478 -727)	(23.3-33.4)	(0.2 - 4.6)	(41.2-53.3)	(7.0 - 12.5)	(0.9 - 3.4)

In contrast, an overall decreasing trend was observed for S/G ratio and xylose as age increases. Both S/G ratio and xylose decreased more rapidly at earlier ages, with the sharpest

decline occurring between ages two and four. On closer look of the annual pattern, it was found that the decline in S/G ratio occurred continuously, while for xylose it initially decreased at age of three, and then started to increase. On average, the aggregate decline for S/G ratio was 0.71 from age 2 to age 8 (-0.12 annually). As for xylose, the decline was observed between the ages of two and three (0.5%) and then leveled off. The annual and aggregate concentrations of galactose remained constant with age.

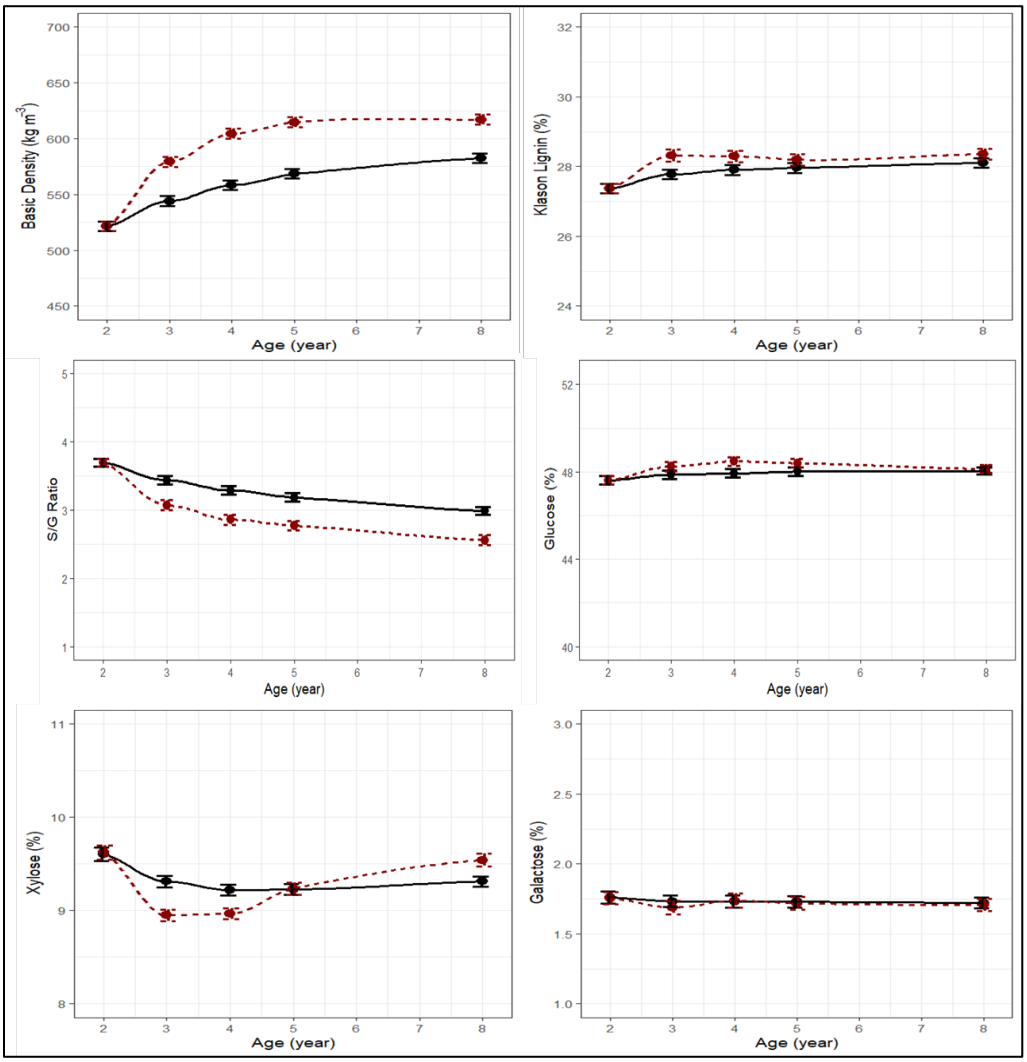

Figure 3.3. Aggregate (solid line) and annual (dashed line) change of wood properties (basic density, klason lignin, S/G ratio, glucose, xylose and galactose) with age.

An examination of the aggregate value of wood properties across age for each clone showed that more uniform trends were observed for the traits of basic density, S/G ratio and xylose content (Appendix A3). In other words, for those traits, very similar trends were observed in almost all assessed clones. However, for the traits of klason lignin, glucose and galactose, the the trends of the aggregate value vary more among clones, with changes in both magnitude and direction. For instance, in the case of lignin, some clones experienced a rapid increase that peaked at earlier ages and then leveled off, while others showed a slower but continuous increase up to older ages.

3.3.2 Heritability

The broad-sense heritability (H^2) of wood properties across various ages are presented in Table 3.3, and the variance components from the multivariate analysis are included in Appendix A4. There was large and statistically significant clonal genetic variance observed for all wood property traits across the different ages ($p<0.01$). In the majority of cases, the ratio of total genetic variance to total phenotypic variance exceeded 50%. Consequently, the H^2 estimates were notably high, ranging from 0.43 to 0.88, with standard errors of less than 0.1.

Basic density had the highest heritability, showing a slight increase with age, from 0.80 to 0.88 between age 2 to 8 years. This trend was mirrored in both the S/G ratio and galactose, where H^2 showed a slight increase, ranging from 0.64 to 0.68, and 0.43 to 0.60, respectively, over the span of two to eight years of age. In contrast, the H^2 of glucose showed a decline with age, dropping from 0.64 at two years to 0.43 at 8 years. On the other hand, the heritability of klason lignin and xylose seemed more consistent across the measured ages, with minor fluctuations ranging from 0.51 to 0.56 and 0.53 to 0.64, respectively, over the same period.

Table 3.3. The estimates of broad-sense heritability (H^2 ± standard error) of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal) across various ages.

Age (Year)	Broad-sense Heritability (H^2)					
	BD	**K-lig**	**SG**	**Glu**	**Xyl**	**Gal**
2	0.80 ±0.07	0.54 ±0.10	0.64 ±0.09	0.64 ±0.09	0.63 ±0.09	0.43 ±0.10
3	0.82 ±0.06	0.54 ±0.10	0.70 ±0.08	0.55 ±0.10	0.63 ±0.09	0.55 ±0.10
4	0.84 ±0.06	0.56 ±0.10	0.68 ±0.08	0.45 ±0.10	0.64 ±0.09	0.59 ±0.10
5	0.85 ±0.05	0.55 ±0.10	0.68 ±0.08	0.44 ±0.10	0.61 ±0.10	0.62 ±0.09
8	0.88 ±0.04	0.51 ±0.10	0.68 ±0.08	0.43 ±0.10	0.53 ±0.10	0.60 ±0.09

3.3.3 Genetic Correlation and Selection Efficiency

The genetic correlations (R_g) of wood properties between the early selection ages and two potential rotation ages (5 and 8 years) are summarized in Table 3.4. Overall, strong genetic correlations were observed across age for all wood properties. Furthermore, these correlations had tendencies to increase as the selection age approached the harvesting ages. Between the ages of two and five, the genetic correlation ranged from 0.89 to 0.98 and was stronger at closer selection ages. Even at an older harvesting age, the genetic correlations remained robust. Over the span of six years, the genetic correlations between the ages of two and eight ranged from 0.86 to 0.96.

The age-age genetic correlation exhibited slight variations among wood properties at younger ages (2 and 3 years). Around these ages, glucose and xylose displayed stronger genetic correlations. However, as the selection age increased, the correlations became more stable. This pattern is reflected in the radial trend of these wood properties, where more pronounced changes were observed at these younger ages. As the tree aged, the radial trends gradually settled.

Table 3.4. Genetic correlation (R_g ± standard error) of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal) between the potential early selection ages and two potential rotation ages (5 and 8).

Selection Age	Genetic Correlation (R_g)					
	BD	**K-lig**	**SG**	**Glu**	**Xyl**	**Gal**
5 Years Rotation						
2	0.94 ±0.03	0.90 ±0.11	0.96 ±0.05	0.98 ±0.10	0.95 ±0.09	0.89 ±0.12
3	0.98 ±0.02	0.98 ±0.12	0.99 ±0.03	0.99 ±0.09	0.99 ±0.03	0.98 ±0.04
4	0.99 ±0.15	0.99 ±0.03	0.99 ±0.03	0.99 ±0.08	0.99 ±0.03	1.00 ±0.03
5	1.00	1.00	1.00	1.00	1.00	1.00
8 Years Rotation						
2	0.90 ±0.05	0.86 ±0.11	0.90 ±0.08	0.94 ±0.09	0.96 ±0.07	0.86 ±0.12
3	0.96 ±0.03	0.95 ±0.12	0.95 ±0.06	0.99 ±0.08	0.99 ±0.07	0.97 ±0.05
4	0.98 ±0.02	0.98 ±0.07	0.97 ±0.04	0.97 ±0.13	0.99 ±0.05	0.99 ±0.04
5	0.99 ±0.01	0.99 ±0.09	0.98 ±0.04	0.99 ±0.18	0.99 ±0.05	0.99 ±0.03
8	1.00	1.00	1.00	1.00	1.00	1.00

3.4 Discussion

3.4.1 Annual Trends

The radial trend of wood properties in some *Eucalyptus* species has been explored in previous studies, especially for physical properties such as basic density. However, many of these studies had a limited range of genotype materials, so they may not fully capture the varying degrees of change across different genotypes. By incorporating a relatively wider range of genotypes in our study compared to those previously published, we aim to more precisely quantify whether the changes observed with age are influenced by genetic factors or solely driven by environmental factors.

The increasing radial trend in basic density, which our study also observed, is consistent with findings in various *Eucalyptus* species. For instance, in five-year-old *E. pellita* (Susilawati & Marsoem, 2006), seven-year-old *E. nitens* (Greaves et al., 1997), 14-year-old *E. urophylla* (Hein & Brancheriau, 2011), and five-year-old hybrids of *E. urophylla* × *E. grandis* (Pimenta et al., 2023), an increase in basic density with age was similarly noted. The increase in basic density with age is often attributed to wood maturity, which involves two potential changes in wood formation as trees mature. Firstly, the cell wall thickness increases as the cambium matures. This phenomenon is well-documented in both deciduous and coniferous trees, where the outer growth rings exhibit significantly thicker cell walls and higher density compared to the inner growth rings (Cato et al., 2006; Rungwattana & Hietz, 2018). Secondly, the proportion of earlywood, characterized by larger lumens and thinner cell walls, decreases as trees grow older. However, this decrease slows down as it approaches the bark. This phenomenon has been observed in *E. nitens* by (Salvo et al., 2017).

The increase in lignin concentration towards the older section of the wood has been previously noted in various *Eucalyptus* species. For example, radial variations in lignin content were reported in species like *E. diversicolor, E. marginata, E. regnans and E. tetrodonta*, with some showing an increase from pith to bark (Garland, 1986). More recent studies in *E. globulus* have also indicated radial variations, with some points showing an increase from pith to bark (Ona et al., 1997; Rodrigues et al., 2001). This is often accompanied by a reduction in the S/G ratio. In *E. camaldulensis* and *E. globulus*, the S/G ratio was higher on the pith side compared to other radial directions (Ona et al., 1997). A more recent study in *E. camaldulensis*, the S/G ratio gradually decreased towards the bark (Yokoi et al., 1999). Additionally, a negative correlation

between lignin content and the S/G ratio has been observed in various *Eucalyptus* species, including *E. camaldulensis* and *E. globulus* (Alves & Rodrigues, 2022; Ona et al., 1997).

The increase in glucose concentration (as the monomer of cellulose) with age, as demonstrated in this study, is consistent with findings in other *Eucalyptus* species. Research indicates a prevailing trend of increasing cellulose content from pith to bark in species like *E. nitens* (Downes et al., 2010) and *E. globulus* (Downes et al., 2012). Additionally, the authors further documented that outer wood near the cambium exhibited pulp yield values 5% to 8% higher than those at the pith in both species. A more recent study on *E. dunnii* yielded similar results. It documented an increase in glucose content with age, while xylose content showed a decrease (de los Santos Arocena, 2016).

3.4.2 Heritability

With the estimate of broad-sense heritability (H^2) ranging from 0.43 to 0.88, wood properties of *E. pellita* assessed appeared under strong level of genetic influence. The heritability of wood density is well-studied in *Eucalyptus* species, and in general, basic density is under moderate to strong levels of genetic influence. Nevertheless, the *E. pellita* broad-sense heritability in the current study are somewhat higher than generally reported for other species. Data on the heritability of several species summarized from various studies showed that the estimates of heritability for basic density range from 0.24 to 0.71 (Table 3.5).

Table 3.5. The estimates of narrow-sense (h^2) and broad-sense (H^2) heritability of wood properties for several angiosperm species summarized from various studies.

Traits	Species	Age	h^2	H^2	Reference
Basic density	*E. grandis*	6		0.39	Salas et al., 2018
	E. globulus	10	0.34-0.49		Nickolas et al., 2020
		11	0.24		Poke et al., 2006
		16	0.52		Stackpole et al., 2010
	E. nitens	7	0.53		Greaves et al., 1997
		9	0.37-0.42		Hamilton et al., 2009
		12	0.51		Kube et al., 2001
	E. pellita	10	0.25		Hung et al., 2015
		10	0.71		Susilawati & Marsoem,
	E. urophylla	14	0.61		Hein et al., 2012
		36	0.53		Denis et al., 2013
Lignin	*E. grandis*	6		0.53	Salas et al., 2018
	E. globulus	11	0.13		Poke et al., 2006
		16	0.27		Stackpole et al., 2011
	E. urophylla	14	0.76		Hein et al., 2012
		36	0.49		Denis et al., 2013
	Populus trichocarpa	9	0.66		Porth et al., 2013
S/G	*E. grandis*	6		0.63	Salas et al., 2018
	E. globulus	16	0.44		Stackpole et al., 2011
	E. urophylla	14	0.71		Hein et al., 2012
		36	0.67		Denis et al., 2013
	Populus trichocarpa	9	0.39		Porth et al., 2013
Cellulose/ glucose	*E. grandis*	6		0.39	Salas et al., 2018
	E. globulus	16	0.42		Stackpole et al., 2011
	E. nitens	9	0.34-0.51		Hamilton et al., 2009
		12	0.54		Kube et al., 2001
	E. urophylla	36	0.21		Denis et al., 2013
	Populus trichocarpa	9	0.46		Porth et al., 2013
Hemicellulose	*E. grandis*	6		0.06-0.61	Salas et al., 2018
	E. urophylla	36	0.67		Denis et al., 2013
	Populus trichocarpa	9	0.64-0.69		Porth et al., 2013

Most of the estimates summarized are narrow-sense heritability (h^2), which reflects the influence of additive genetic variance, while the current *E. pellita* broad-sense heritability (H^2) estimates reflect both additive and non-additive genetic variance. In line with that, the basic density of *E. pellita* in this study appeared to be under a very strong level of genetic influence with

the highest estimates among all wood properties (H^2: 0.80-0.88) between the age of two to eight. This finding was higher compared to most species mentioned in Table 3.6, including the broad-sense estimates for *E. grandis* (Salas, 2018). However, it was close to the result of 10-years-old *E. pellita* from Indonesia, with an estimate of 0.71 (Susilawati & Marsoem, 2006). The increase in heritability of basic density with age observed in the current study corresponds to the trend reported by Stackpole et al. (2010) in *E. globulus*, where the heritability of pilodyn (a surrogate for basic density estimates) increased from 0.35 to 0.55 between the ages of six and seventeen. This increase was larger than what was found in the current study, suggesting that for *E. globulus*, selection for basic density at older ages may lead to more accurate results.

Similar to basic density, estimates of heritability for wood chemistry across species displayed considerable variation in magnitude (Table 3.5). This variation was more pronounced in lignin, cellulose, and hemicellulose, but less so for the S/G ratio. In this study, the estimates of broad-sense heritability for both lignin content (H^2 = 0.51 to 0.56) and the S/G ratio (H^2 = 0.64 to 0.70) fall within the mid to upper range of values summarized. Upon closer examination, these findings align well with those observed in *E. grandis* (Salas et al., 2018) and *E. urophylla* (Denis et al., 2013). The estimates of broad-sense heritability for monosaccharides (glucose, xylose, and galactose) also fall within the range of the summarized values. In comparison to other *Eucalyptus* species, the results for glucose (H^2 = 0.43 to 0.64) were higher than those reported for *E. grandis* (Salas et al., 2018), *E. globulus* (Stackpole et al., 2011), and *E. urophylla* (Denis et al., 2013); they were more akin to those of *E. nitens* (Hamilton et al., 2009; Kube et al., 2001). As for hemicellulose (xylose and galactose), the estimates fell towards the upper range of the summarized values.

3.4.3 Genetic Correlation and Selection Efficiency

Studies examining the genetic correlation of wood properties across different ages are limited. When available, these studies tend to concentrate on traits that are cost-effective to assess, such as basic density. Consequently, comparing findings for chemical traits becomes challenging. In the case of basic density, the robust genetic correlations observed in this study, ranging from a very young age to the rotation age (R_g = 0.90 to 0.99), have also been demonstrated in various *Eucalyptus* species. For instance, Greaves et al. (1997) documented high genetic correlations in *E. nitens* between the ages of three and seven (R_g = 0.93). Similarly, Osorio et al. (2003) reported strong genetic correlations in *E. grandis* clones sampled across sites between the ages of three to six (R_g = 0.71 to 0.95). A recent study on *E. globulus* by Stackpole et al. (2010) also revealed a substantial genetic correlation (R_g = 0.77) between pilodyn tests at six and 17. This phenomenon has also been reported in conifer species. In *Pinus sylvestris*, the age–age genetic correlation of basic density exceeded 0.85 from ages 8 to 30 years (Hong et al., 2015).

Table 3.6 provides a summary of the selection efficiency (E) of wood properties between the early selection ages and two potential rotation ages (5 and 8). Because genetic gain is estimated per unit time, when the heritability of the juvenile age and rotation age are nearly equal, and the age-age genetic correlation is near 1.0, selection efficiency becomes primarily dependent on time (the age of selection). This phenomenon is clearly demonstrated in this study (Table 3.6). With both heritability and genetic correlations among ages showing relatively stable patterns, the selection age emerged as the most significant factor affecting efficiency, with efficiency increasing as selection age decreased. For instance, at a rotation age of five, selecting at age two showcased the highest efficiency across all wood properties, ranging from 1.5 times (for galactose) to 3.6 times (for glucose) more efficient compared to direct selection. The selection efficiency of early

ages were even higher if the rotation age of eight is assumed. The results revealed that selecting at age two was approximately 2.5 times (for galactose) to 5.6 times (for glucose) more efficient compared to direct selection.

The results suggest that accurate and efficient selections for wood properties of *E. pellita* can be made as early as age two years. Understanding the trends and strengths of correlations within wood properties and between growth traits is crucial, and prioritizing the selection of genetic material with superior wood properties will produce substantial value for the industry.

Table 3.6. The selection efficiency (E) of of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal) between the early selection ages and two potential rotation ages (5 and 8).

Selection Age	**Selection Efficiency (E)**					
	BD	**Lig**	**SG**	**Glu**	**Xyl**	**Gal**
5 Years Rotation						
2	2.2	2.2	2.3	3.6	2.5	1.5
3	1.6	1.6	1.7	2.1	1.7	1.4
4	1.2	1.3	1.2	1.3	1.3	1.2
5	1.0	1.0	1.0	1.0	1.0	1.0
8 Years Rotation						
2	3.3	3.6	3.4	5.6	4.6	2.5
3	2.4	2.7	2.6	3.4	3.1	2.4
4	1.9	2.2	1.9	2.0	2.4	1.9
5	1.5	1.7	1.6	1.6	1.8	1.6
8	1.0	1.0	1.0	1.0	1.0	1.0

3.5 Conclusion

The findings of this study highlight age-related changes in several wood properties of *E. pellita*. Basic wood density, klason lignin, and glucose demonstrated an increasing trend with age,

whereas the syringyl-to-guaiacyl (S/G) ratio and xylose exhibited a decreasing trend. Galactose, however, did not show a clear trend with age.

Overall, the wood properties of *E. pellita* appeared to be strongly influenced genetically, with the broad-sense heritability (H^2) estimates ranging from 0.43 to 0.88. The heritability of basic density and S/G ratio showed an increasing trend with age, while those of klason lignin, glucose, xylose, and galactose demonstrated minor fluctuations.

Furthermore, very high positive genetic correlations were observed between potential early selection ages (2, 3, 4, and 5 years) and potential harvesting ages (5 and 8 years). Selection in breeding programs could be done as early as two years, primarily due to very high juvenile-rotation age genetic correlations (R_g = 0.90 to 0.99). The efficiency of early selection was notably higher compared to direct selection. Selecting at age two has the potential to yield genetic gain efficiency = 1.5 to 3.6 times greater than direct selection at age five. Moreover, these gains increased even further when compared to direct selection at age eight (efficiency = 2.5 to 5.6). These findings hold significant implications for tree breeders in determining the viability of early selection for economically vital wood property traits in the pulp and paper industry.

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CHAPTER 4

Economic impacts of Age-related Changes in Wood Properties of *Eucalyptus pellita* on the Pulp Production and Plantation Area.

4.1 Introduction

The production of "wood pulp", pulp derived from wood as its raw material, has shown a notable increase in Indonesia over the past decade. According to data from the Food and Agriculture Organization (FAO), the production volume surged by approximately 1.32 million metric tons between 2016 and 2020, rising from 7,33 to 8,66 million metric tons (FAO, 2022). As of 2020, Indonesia ranked in the 8th position among the world's leading pulp producers, and in the 4th position as an exporter (FAO, 2021). Similar to the production, the Gross Domestic Product (GDP) of Indonesia from the manufacture of paper and paper products has been experiencing growth in the past five years. According to the Indonesian Central Statistics Agency (BPS), between 2017 and 2022, the GDP increased by Rp. 32,342 billion (about USD 2.2 billion), rising from Rp. 96,616 billion to Rp. 128,958 billion (BPS - Badan Pusat Statistik, 2023). Furthermore, the Ministry of Industry in Indonesia is actively seeking investments with the goal of elevating maximum wood pulp production from approximately 12 million metric tons in 2023 to 18 million metric tons in the near future (Indonesian Ministry of Industry, 2023). With these ambitious targets, there is a pressing need for pulp industry stakeholders to enhance wood fiber supplies. Closing this gap involves two major approaches: 1) expanding plantation area, and 2) improving efficiency of wood and pulp production.

First, wood production in Indonesia can be increased by expanding the plantation area. Currently, the raw material supplying wood pulp production in Indonesia is primarily sourced from

Acacia crassicarpa and *Eucalyptus pellita*. The latter species has garnered significant interest in recent years, with a rapid increase in the establishment of *E. pellita* plantations, particularly as part of integrated forestry-pulp and paper industries. This rise is evident in wood production figures, with *E. pellita* holding the second position in timber production for pulpwood in the region as of 2021, behind *A. crassicarpa*. *E. pellita* accounts for 33 (15.8 million m3) of the total timber production in 2021, marking a significant increase from 21 (8.6 million m3) in 2019 (BPS - Badan Pusat Statistik, 2023).

The second approach to increase wood production in the country is to improve the efficiency of wood-pulp and paper production processes, both in forestry and mills. This option is considered favorable as it has the potential to maximize revenue through the optimization of both land usage and efficiency in pulp and paper production. Given that the cost of wood constitutes a significant portion, at least 40, of the total production cost for hardwood pulp (Kangas et al., 2014), it is crucial to concentrate on practices and methods that increase the volume of pulp "harvested" per hectare. Therefore, the growth rate and the wood properties of the material fed to the mill need to be continuously improved through silviculture and breeding programs. Among wood properties, basic density and pulp yield are the most critical traits to focus on in breeding programs, as they hold the highest potential for reducing overall pulp expenses (Magaton et al., 2009; Resquin et al., 2022; Schmidt, 2005). Both basic density, pulp yield, and chemical properties related to pulp yield are known to increase with age in *E. pellita* (as reported in Chapter 3 of this thesis) and in other *Eucalyptus* species (Downes et al., 2012; Greaves, Borralho, & Raymond, 1997; Schimleck et al., 1997). In Indonesia, *E. pellita* is typically harvested at 5 years. However, the wood properties of trees harvested at older ages are often considered more suitable for pulp production. Therefore, when evaluating the rotation age, a comprehensive examination on the extent of the economic

impact of age-related increases in both growth and wood properties on overall pulp expenses is crucial (Magaton et al., 2009).

Estimating the influence of changes in both basic density and pulp yield with age on overall pulp mill production is crucial when deciding the optimum rotation age. Therefore, this study will aim to investigate the role of age-influenced wood properties in *E. pellita* for sustainable long-term production in integrated forestry-pulp and paper industries. The specific objective is to examine the impact of age-related variation in wood properties for 27 genotypes on overall pulp expenses. The study compares the production costs of pulp between rotation ages of 5 and 8 years, subsequently estimating the implications of the differences in these rotation ages on wood demand, wood costs, and required harvested area. Finally, these estimates will be used determent which age is more favorable for integrated pulp production.

4.2 Materials and Methods

4.2.1 Field Description and Material Selection

We studied genetic materials from two clonal tests of *E. pellita*, established by the *Eucalyptus* breeding program of PT. Arara Abadi, an integrated company for pulp and paper production also known as Sinarmas (https://araraforestry.com/). Two clonal tests (Trial 1 and Trial 2) were established adjacent to one another in April 2014. The tests are located in District Gelombang, Siak Regency, and were planted on mineral soils classified by the company as 'site class I', indicating its suitability for establishing *Eucalyptus* species. These trials followed a randomized complete block design, with 4 replicates in each trial. Trials 1 and 2 included 35 and 27 clones, respectively, and in each replicate, 5 ramets per clone were planted in a line at a spacing

of 4 x 1.5 meters. Assessment on the individual trees were recorded annually from ages 2 to 5 years, assessing the survival, tree diameter at 1.3 meters (DBH) and total height.

Individual tree volume was calculated at 5 years of age (van der Hoef et al., 2019). We calculated clonal breeding values and the rank of the genotypes based on their volume gain on both trials. Results of the analyses were used to select a subset of genetic materials for wood sampling. All the analysis at age 5 were conducted by the Sinarmas' *Eucalyptus* breeding program. When the trials reached eight years of age, a total of 30 clones, 17 clones in Trial 1 and 13 clones in Trial 2, were selected to cover the top half of the population ranked for volume growth. Data of MAI at 5 years is provided in Appendix A5. From the selection, there were three clones shared between the two trials, resulting in a list of 27 unique genotypes.

4.2.2 Sample Collection and Preparation

Wood samples were obtained by destructively extracting wood discs when the trial reached 8 years of age. In June 2022, a total of 147 trees were sampled across the replicates by cutting mostly 5 ramets per clone, with an exception of three clones with only a total of 4 ramets available. The trees were cut down following the scheme presented in Figure 4.1. Three discs (3 cm thick) were extracted at 1.3 m height. Strong correlations between sampling at 1.3 m height and the whole estimate of assessed traits were reported in Chapter 2 of this thesis. Each disc was earmarked for basic density (Disc 1) and pulp yield (Disc 2 & 3). All the discs were labeled properly. During sampling, data of total height (Ht), commercial height (CHt) - a height at 5 cm diameter – and the diameter at breast height (1.3 m) were measured. Following sampling, the bark was removed, and the inside bark diameter was measured. Discs designated for basic density (Disc 1) were soaked in water, while the other two were left to air dry.

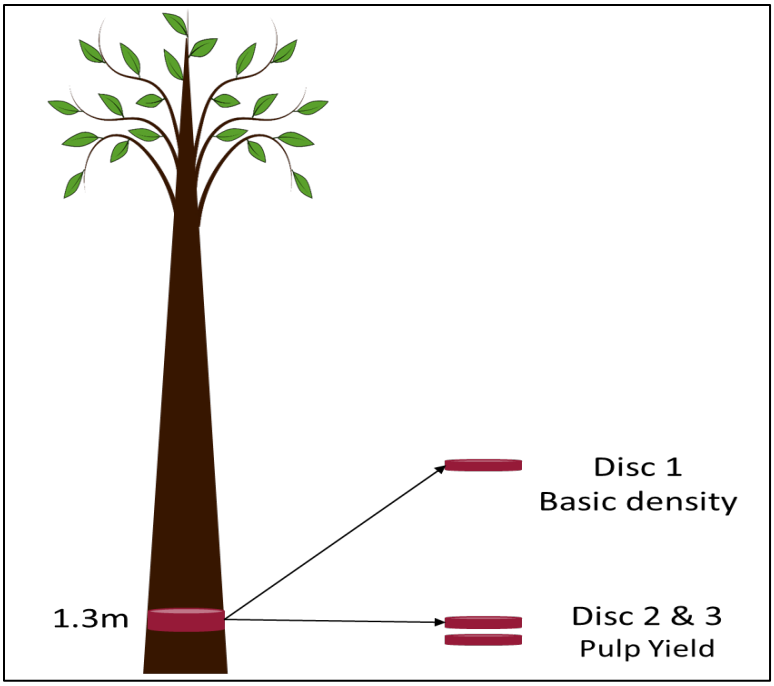

Figure 4.1. Destructive sampling scheme of discs at 1.3 m for measurement of basic density and kraft pulp yield.

The measurements of DBH at ages 5 and 8 were utilized to determine the age of the wood samples. This information was then corrected for bark thickness and wood segmentation between ages was done in accordance with the scheme outlined in Figure 4.2. The determination process began with the measurement of the ratio of radii from the pith to the two most eccentric sides of the disc at the age of eight (Figure. 4.2). This ratio was then used to calculate the radii from ages five based on the diameter data. Segmentation between ages was achieved by drawing a circle with a compass between two points from the same age. For Basic density, Disc 1 then segmented into two increment groups (0-5 years, and 5-8 years) based on the circles, using a chisel (Figure. 4.2a). For pulp yield samples at age 5, wood segments of 5-8 years were removed from the Disc 2 (Figure 4.2b). For pulp yield samples at age 8, whole wood of the Disc 3 was used (Figure 4.2c). All wood segments were properly labeled to ensure traceability.

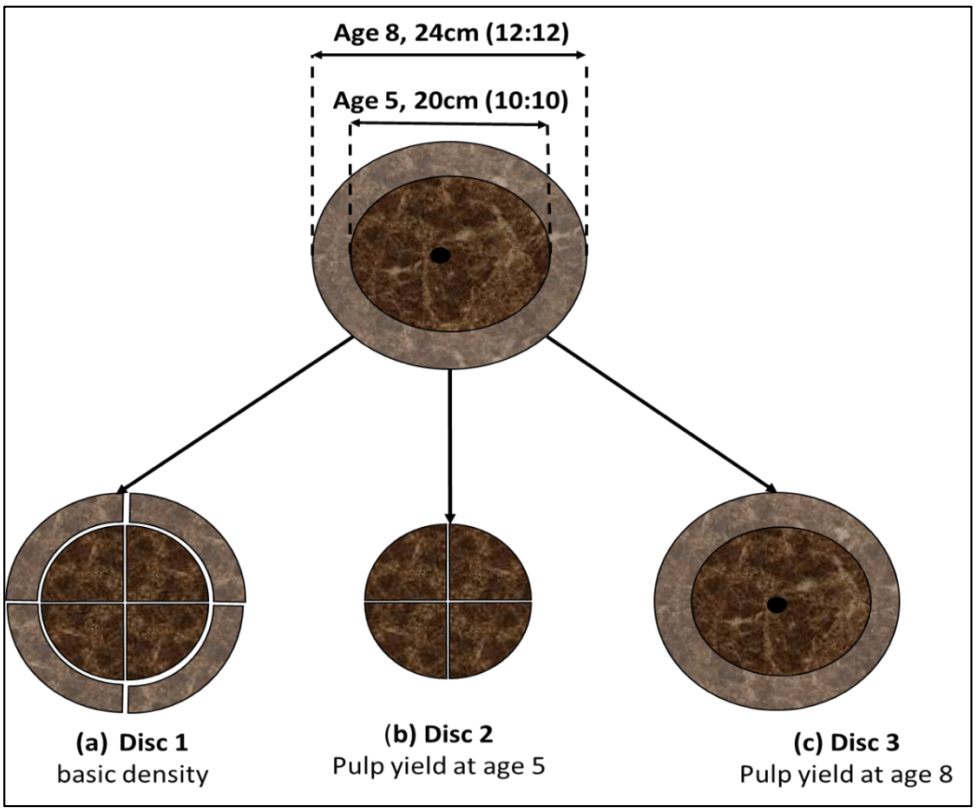

Figure 4.2. Process of determining wood sample for age of 5 and 8 based on diameter and radii ratio for: (a) Dice 1 segmentation for basic density; (b) pulp yield at age 5, by removing the part of Disc 2 from age 5 to 8; and (c) pulp yield at age 8, whole part of Disc 3.

4.2.3 Laboratory Measurements

Basic density was determined using wood segments from Disc 1. The determination was carried out using the water displacement method according to the TAPPI T258 standard (TAPPI - Technical Association of the Pulp and Paper Industry, 2006). Saturated volume and dry weight measurements were taken for a total of 294 wood segments belonging to the two distinct increment groups (147 trees x 2 ages; 0-5 years and 5-8 years). The basic density for each increment section was calculated by dividing the dry weight by the saturated volume. Additionally, aggregate basic density at age 8 (from 0-8 years) was computed by dividing the aggregate dry weight by the

aggregate volume of the corresponding sections. To better understand, basic density is calculated using the equations provided below (Equations 1 and 2).

Equation 1: $BD_5 = \frac{Dry\ weight\ sction\ 1}{Wet\ volume\ section\ 1}\ x\ 100$

Equation 2: $BD_8 = \frac{Sum\ of\ dry\ weight\ section\ 1\ and\ 2}{Sum\ of\ wet\ volume\ section\ 1\ and\ 2}\ x\ 100$

Where BD (kg m^{-3}) equals basic density at corresponding ages; section 1 is the wood segment from 0 to 5 year; and section 2 is the wood segment from 5 to 8 years.

In this study, a total of 120 kraft pulp yield (KPY) data points were utilized, derived from 120 pulps (30 clones x 2 replicates x 2 ages). To determine KPY, discs per ramet were sampled at both 5 and 8 years of age, chipped, and screened to achieve a uniform size. A total of 60 composite samples were created by equally mixing chips from 5 ramets per clone for age 5 and age 8 years (30 clones x 2 ages). Each of these composite samples then pulped go get two repetitions with kappa number = 18 ± 1. A total of 120 KPY data points (60 x 2 reps) were generated by pulping 300 g of oven-dried chips using autoclaves with an alkali active charge adjusted to achieve a kappa number of 18. The white liquor was set to 30% sulfidity with a 1:4 ratio of chemical to water. The temperature was maintained at 165°C for approximately 3 hours to reach an H-factor of 1000. After screening, the Kappa number of the pulp was determined following the test method SCAN 1:77.

4.2.4 Pulping Model and Cost Estimation

A simulation model created by the Forest Biomaterials Department at N.C. State University was employed to assess how the growth and wood properties of 27 *E. pellita* clones impact the

expenses associated with pulp production, comparing harvests at age of 5 and 8 years. The model incorporates both forestry and mill aspects, assuming the operation of a vertically-integrated paper company with existing forestry and mill divisions. The simulation excludes costs related to land acquisition and capital investments, presupposing the presence of an established mill and forest land. Additionally, it assumes that the forestry division supplies wood to the mill division at a transfer price equivalent to the cost of wood production. Figure 4.3 presents a simplified diagram of kraft pulp production (Kangas et al., 2014). The mill model starts with debarked eucalypt logs and ends with bleached market pulp, considering the operating costs for a kraft pulping process (cost of wood, chemical at digester, chemical at bleaching) and the energy balance (energy consumed and generated) using a Kamyr continuous digester. The ultimate output of the model provides estimates for the total cost in USD, and the wood tonnage required in oven-dried metric tons (ODT) to produce one air-dried metric ton (ADT) of pulp with a 10% moisture content. To derive these estimates, the model relies on measured data from samples (such as pulp yield, alkali active charge at kappa number 18) and incorporates various assumptions regarding costs, losses, and revenue associated with the pulping process. These assumptions are detailed in Appendix A6 and A7.

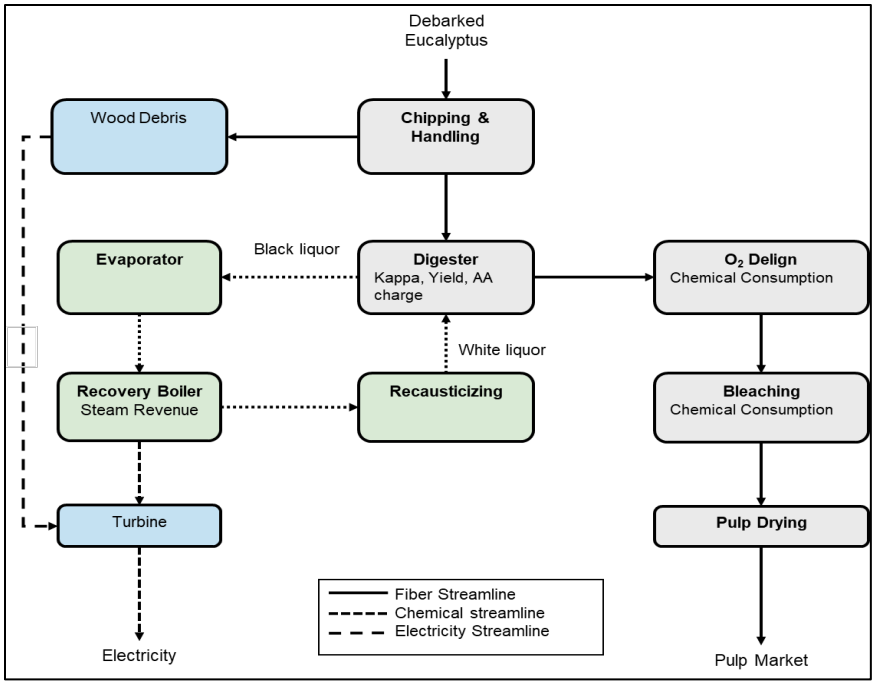

Figure 4.3. Simplified diagram of kraft pulp production (Kangas et al, 2014).

To assess the impact of age-related changes in wood properties on the harvested area, we initiated the analysis by determining the specific wood consumption (SWC) in cubic meters. The calculation, as outlined in Equation 3, involved the conversion of wood consumption in oven-dried metric tons provided by the model output using basic density. The required harvesting area was estimated by multiplying SWC with the total volume per hectare (Equation 4). For the total volume per hectare at 5-year rotation (VOL_5), mean annual increment data (MAI_5) was available directly from the test measurements of all trees from all clones. Field measurement data was not available for 8 years, so for an 8-year rotation, the total volume per hectare (VOL_8) was estimated assuming the mean annual increment data at 8-year rotation (MAI_8) equal to 90% of the MAI_5 (Equation 4). This approach is an attempt to account for potential loss in volume per hectare attributed to the rise in mortality from age 5 to 8 years. The assumption relies on research indicating

that the mortality level in *E. pellita* between 5 and 8 years is less than 10% (Bristow et al., 2006). However, it is essential to note that recent studies on MAI between 5 and 8 years have indicated a relatively stable trend for *E. pellita* (Taziwa, 2023) and *Eucalyptus* species in general (Delgado-Matas & Pukkala, 2011; Mhamdi et al., 2021). The calculations of MAI_5 and MAI_8 are outlined in Equation 5 (a & b).

Equation 3: $$SWC\ (m^3\ ADT^{-1}) = \frac{Tonnage\ wood\ (ODT\ ADT^{-1})}{Basic\ density\ ODT\ m^{-3}}$$

Equation 4: $$Harvested\ area\ (ha\ ADT^{-1}) = SWC\ (m^3\ ADT^{-1})\ x\ Vol\ (m^3 ha^{-1})$$

Equation 5: $$VOL_5\ (m^3 ha^{-1}) = MAI_5\ (m^3 ha^{-1} year^{-1})\ x\ 5\ years \quad (a)$$

$$VOL_8\ (m^3 ha^{-1}) = MAI_5\ (m^3 ha^{-1} year^{-1})\ x\ 0.9\ x\ 8\ years \quad (b)$$

To further replicate production conditions more accurately, we estimated all relevant parameters (pulping cost, wood tonnage required, specific wood consumption, and harvested area) on a daily production scale, assuming a mill output of 1000 air-dried metric tons per day.

4.3 Results

4.3.1 Wood Properties

Table 4.1 offers a summary (mean, minimum, and maximum) of wood properties of *E. pellita* and production costs observed between the ages of 5 and 8 years. Additionally, detailed wood properties categorized by clone can be found in Appendix 1. Generally, wood properties at the age of 8 seem to be more favorable for pulp production. On average, when harvested at the age of 8 years, there was an approximate increase of 14 kg m-3 in basic density and a 1 improvement in

pulp yield compared to the age of 5. Furthermore, the alkali active charge exhibited a decrease of about 1.4 from the age of 5 to 8.

The advantageous wood properties observed at the age of 8 translated into a more cost-effective production process. In essence, harvesting *E. pellita* wood at 8 years led to a reduction in operational costs for pulping by USD 8.57 per air-dried metric ton (ADT) of pulp. A closer examination of the production costs reveals significant reductions, particularly in the costs associated with wood (USD 3.85 per ADT pulp), pulping chemicals (USD 1.06 per ADT pulp), and total energy USD 3.61 per ADT pulp – the latest reduction is mostly attributed to the steam revenue generated from the recovery boiler (USD 2.41 per ADT pulp).

Table 4.1. Mean, minimum, and maximum of wood properties of 27 clones of *E. pellita* and production costs observed between 5-year and 8-year rotations.

Parameter	**5 Year**			**8 Year**		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**
			Wood Properties			
Basic Density (kg m^{-3})	562	490	670	576	501	685
Kraft Pulp Yield (% chips)	47.86	41.89	50.49	48.85	44.47	52.54
Alkali Active Charge (% chip)	17.7	15.5	20.0	16.3	14.0	19.0
			Pulping Cost (USD/ADT pulp)			
Wood	190.05	180.15	217.13	186.2	180.15	217.13
Chemical for Pulping	12.70	11.15	15.78	11.63	9.69	13.74
Chemical for Bleaching	48.17	48.11	48.3	48.13	48.05	48.22
Total Chemical	60.87	59.26	64.08	59.77	57.74	61.96
Energy Required	119.71	110.62	138.65	113.69	102.1	125.65
Steam Revenue	-106.49	-132.96	-97.68	-104.08	-127.28	-89.54
Total Energy	13.23	5.69	20.05	9.6	-13.61	23.87
Total Cost	**264.15**	**251.43**	**286.9**	**255.6**	**240.71**	**272.54**

4.3.2 Daily Production Scale

Total pulp cost in a daily production scale of *E. pellita* across genotypes is presented in Figure 4.4, assuming a mill output of 1000 ADT per day. As mentioned before, in general the production cost lower at eight-year rotation, on average USD 8,573 per day. This reduction varied across genotype and was as low as USD 29,991 per day (Clone 11). However, five out of 27 clones (No. 7, 8, 12, 15, and 16) exhibited the opposite trend. These clones required higher total pulp cost ranging from USD 213 to USD 8,694 per 1000 ADT pulp.

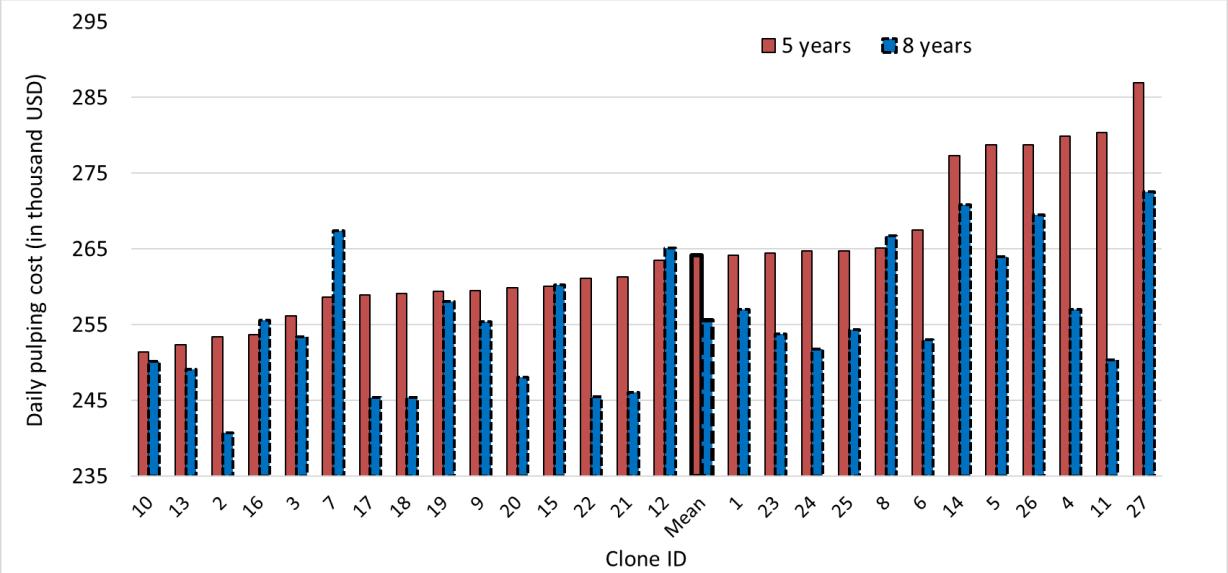

Figure 4.4. Total daily pulping costs across 27 clones of *E. pellita* in a daily production scale, assuming a mill output of 1000 air-dried metric tons per day, arranged in ascending order of specific wood consumption (SWC).

Figure 4.5 presents the wood tonnage requirements across genotypes of *E. pellita* on a daily production scale, assuming a mill output of 1000 ADT per day. On average, trees harvested at an eight-year rotation required 45.3 ODT less wood per day compared to a five-year rotation. This

reduction, of course, varied across genotypes. For some clones, there were large reductions in wood tonnage for an 8-year harvest compared to a 5-year harvest, e.g., for Clone 27, there was a reduction in 271 ODT per day. However, six out of 27 clones (No. 7, 9, 15, 21, 24, and 25) exhibited the opposite trend, requiring more wood per day.

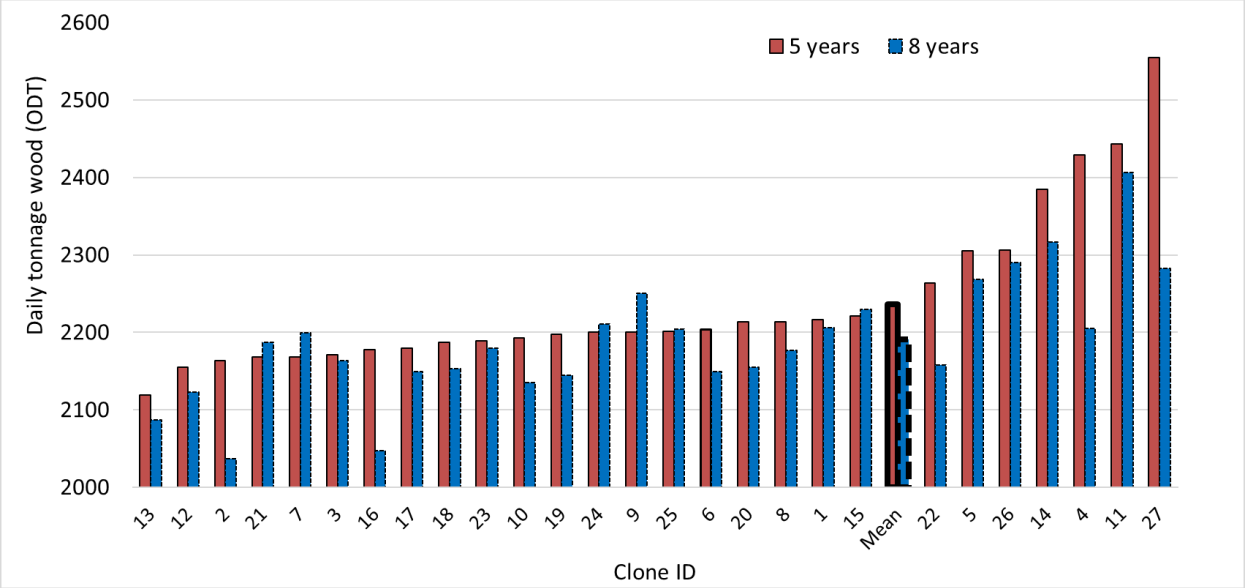

Figure 4.5. Wood tonnage required across 27 clones of *E. pellita* on a daily production scale – assuming a mill output of 1000 air-dried metric tons per day, arranged in ascending order of specific wood consumption (SWC).

Figure 4.6 presents the specific wood consumption (SWC) across genotypes of *E. pellita* on a daily production scale. At an 8-year rotation, SWC decreased compared to a 5-year rotation. On average, wood harvested at 8 years requires 176 m^3 less wood compared to when it is harvested at 5 years. The decrease in SWC varies by clone, with one clone (No. 27) showing a decrease of 604 m^3 per day. In contrast to the wood tonnage required, the trend in SWC was more uniform, with almost all clones showing a reduction at an 8-year rotation; only one clone (No. 9) had a slightly higher SWC at age 8.

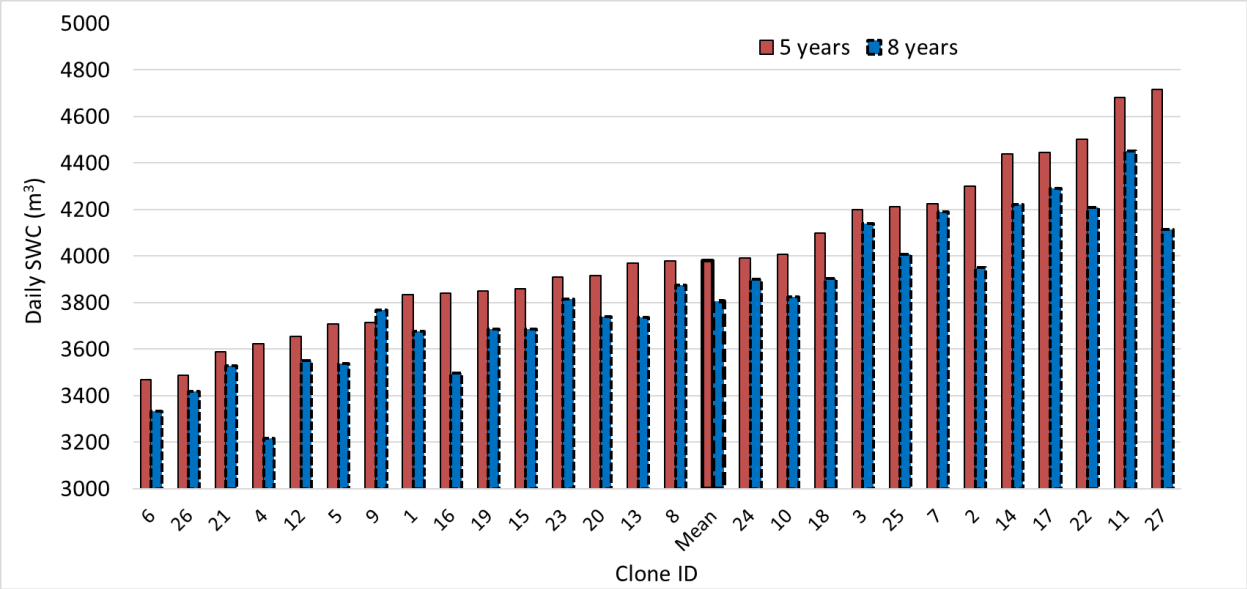

Figure 4.6. The specific wood consumption (SWC) across 27 clones of *E. pellita* on a daily production scale – assuming a mill output of 1000 air-dried metric tons per day, arranged in ascending order of specific wood consumption (SWC) at 5-year rotation.

4.3.3 Harvesting Area Required

Figure 4.7 illustrates the required harvesting area (in hectares) to meet wood demand for a daily production scale under five-year and eight-year rotations – assuming a mill output of 1000 air-dried metric tons per day. It is important to note that the standing volume per hectare for the eight-year rotation is derived from the MAI_5 with 10 reduction assumptions (90 MAI_5). In general, the eight-year rotation requires 7.75 ha less harvesting area (15.3 ha) compared to the five-year rotation (23.05 ha) to meet the mill's daily production needs. This reduction is a result of the increase in both pulp yield and basic density with age. Furthermore, similar to other parameters, the reductions in the required harvesting area from the 5-year to the 8-year rotation vary among the clones. Influenced by genotype variations in pulp yield, basic density, and mean annual

increment, the reduction in required harvesting area ranged from 3.07 hectares (Clone No. 26) to 24.51 hectares (Clone No. 27).

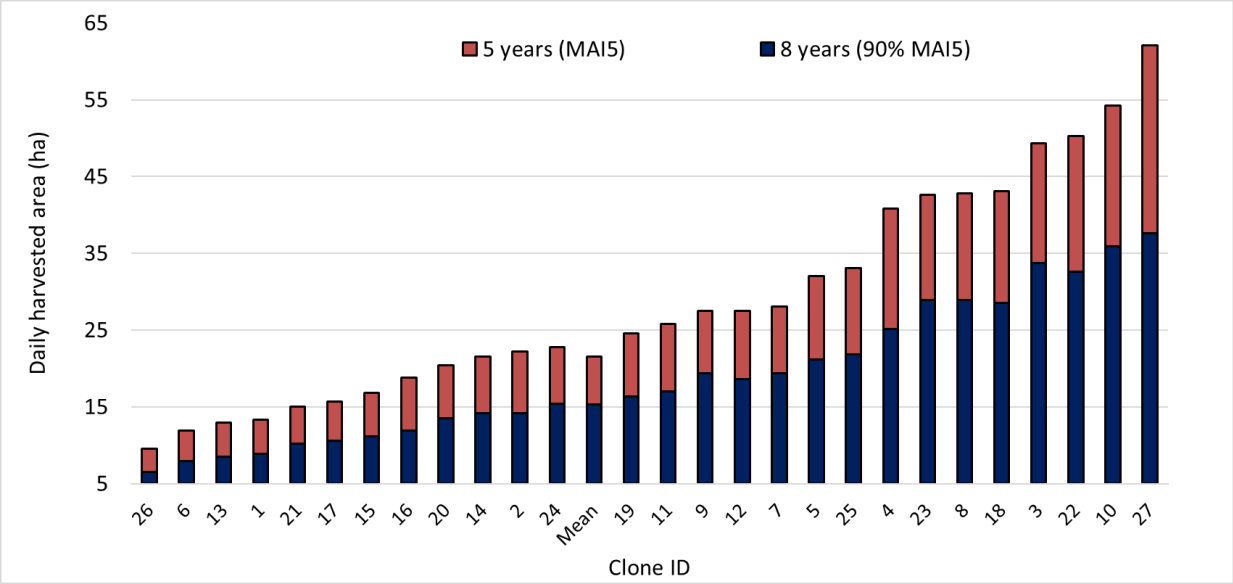

Figure 4.7. The requirement for hectares of daily harvest across 27 clones of *E. pellita* on a daily production scale – assuming a mill output of 1000 air-dried metric tons per day, arranged in ascending order of specific wood consumption (SWC) at 5-year rotation.

4.4 Discussion

The increasing trend with age of basic density has been previously documented in *Eucalyptus* species such as *E. pellita* (Susilawati & Marsoem, 2006), *E. nitens* (Greaves et al., 1997), *E. urophylla* (Hein & Brancheriau, 2011), and hybrids of *E. urophylla* × *E. grandis* (Pimenta et al., 2023). Similar results have also been found for pulp yield in *E. nitens* (Downes et al., 2010) and *E. globulus* (Downes et al., 2012). In accord with that, the results in Chapter 3 of this thesis indicate a slight increase with increasing age in glucose, the main monomer constituent of pulp yield. In comparison to a 5-year rotation, the reduced alkali active charge required for pulping trees at an

8-year rotation aligns with findings in *E. obliqua* and *E. regnans* (Clark et al., 1989). The study concluded that the active alkali necessary to pulp wood samples to a constant Kappa number decreased with age, particularly in the range of 20-59 years. However, the decreasing trend is not consistently clear, as other data for *E. grandis* suggests an increase until the age of 7, followed by a subsequent decrease (Megown et al., 2000). A possible explanation for this variation is the tendency for an increase in lignin content with tree age, as discussed in Chapter 3. Lignin content is positively correlated with alkali active demand, as lignin is a significant consumer of alkali during kraft pulping (Magaton et al., 2009).

The oposite between a higher pulp yield and lower alkali active consumption contributes to a reduction in the overall pulping cost. A higher pulp yield diminishes the need for purchasing wood – a significant cost factor in this study, constituting at least 70 of the total production cost. Simultaneously, a decrease in alkali active charge results in reduced chemical expenses for the white liquor. These findings parallel a study evaluating various rotation ages in *E. globulus* (Resquin et al., 2022), which observed that trees harvested at older ages exhibited more suitable wood properties: higher basic density, increased pulp yield, and better in efficiency of alkali active. However, that study concluded that there is no significant difference in the forest economic value between younger and older rotation ages.

Both increases in pulp yield and basic density contribute to a SWC. In conjunction with the MAI, a reduced SWC lowers the cost of pulp production per unit area of the plantation by minimizing expenses in wood production as well as reducing the harvesting area required. This finding aligns with a study examining the impact of wood properties on kraft pulp production in *Eucalyptus*, which demonstrated that both pulp yield and basic density play significant roles in reducing SWC. Notably, basic density exerts a stronger influence than pulp yield (Magaton et al.,

2009). A recent study focusing on the economic impact of wood properties in *E. grandis* on kraft pulp production revealed that wood characterized by higher basic density can effectively lower the overall cost of pulp production (Salas, 2018). The author also concluded that incorporating basic density as a criterion in material selection along with volume within breeding programs significantly contributes to reducing the total pulping cost.

As this study utilized wood properties sampled at 1.3 m both at age 5 and 8, it is crucial to establish a strong correlation between the sampling point at 1.3 m and the whole-tree to drawn a sound conclusion. At 8 years of age, the clonal mean of basic density and key wood chemical compositions relevant to kraft pulp yield, evaluated at 1.3 m height, demonstrate strong correlations with those of the whole-tree ($r = 0.88$ to 0.97), as demonstrated in Chapter Two. While this study does not directly evaluate the same correlations at age 5, existing literature consistently demonstrates the predictive capability of measurements taken at 1.3 m height for assessing whole-tree wood properties across different tree ages. Therefore, it is reasonable to assume that basic density and pulp yield assessed at 1.3 m height remain strongly correlated with the whole-tree at this age as well. Previous research involving 74 *E. pellita* trees at 5 years of age, using a similar approach to Chapter Two, found a robust correlation ($r = 0.93$) between measurements at 1.3 m height and those of the entire tree (Razoki et al., 2019). Similarly, a study on 18-year-old *E. globulus* trees revealed a strong correlation ($r = 0.79$) between predicted pulp yield at 1.3 m height and the whole-tree value (Raymond & Muneri, 1998). Another investigation on *Acacia crassicarpa* demonstrated good correlations ($r = 0.79$ to 0.88) for xylose, glucose, klason lignin, and basic density between measurements taken at 1.3 m height and those of the whole-tree at 3 years of age (Martins et al., 2022). Furthermore, A study in *Pinus taeda,* correlations between wood density, lignin, and α-cellulose at 1.3 m height and those of the whole-tree were consistently

strong (r = 0.86 to 0.93) across different sites, encompassing both juvenile and mature ages (Aspinwall et al, 2010).

4.5 Conclusion

The findings of this study underscore the economic impact of varying wood properties in *E. pellita* harvested at two different ages (5 and 8 years). In comparison to the 5-year rotation, both basic density and pulp yield should exhibit increases when trees are harvested at the 8-year rotation. These increases should result in reduced costs for wood, decreased tonnage of wood required, and lowered specific wood consumption. Additionally, the alkali active charge should be lower at an eight-year rotation, contributing to a decrease in chemical costs for white liquor. Essentially, harvesting *E. pellita* wood at 8 years of age should lead to an important reduction in operational costs for pulping of approximately USD 8.57 per air-dried metric ton (ADT) of pulp. These findings carry significant implications for the management of integrated forest-pulp and paper industries, and indicate the importance of considering wood properties when evaluating the optimal harvesting age.

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CONCLUSION

Scope of Study

This study examined the wood traits of 72 unique *E. pellita* clones planted in Indonesia. The specific objectives of this research are:

1) To examine within-tree variation of wood properties in *E. pellita*, and identify an optimum sampling point on the stem to estimate whole-tree wood properties and rank genotypes within breeding programs.
2) To estimate genetic parameters for important wood property traits in *E. pellita*, and examine the age-age genetic correlations to identify an optimum age of selection within breeding programs.
3) To compare the economic impacts (cost per ton of pulp produced) for *E. pellita* harvested at ages 5 and 8 years in the Indonesian context.

Findings

The findings of Chapter 3 highlight the longitudinal variation of wood properties of *E. pellita*. Basic density, klason lignin, S/G ratio, glucose, and xylose vary significantly longitudinally up and down the stem, with longitudinal position in this study explaining more than 70 of the variation. The most accurate single points were found at the middle part of the stem height (25 and 50 of the commercial height). Nevertheless, it is evident that sampling at breast height (1.3 m) not only practical but also provides a reliable estimate for all the assessed wood traits, particularly at the clonal level (r2 = 77 to 94).

The findings of Chapter 3 highlight age-related changes in several wood properties of *E. pellita*. Basic wood density, klason lignin, and glucose demonstrated an increasing trend with age, whereas the syringyl-to-guaiacyl (S/G) ratio and xylose exhibited a decreasing trend. Overall, the

wood properties of *E. pellita* appeared to be strongly influenced genetically, with the broad-sense heritability (H^2) estimates ranging from 0.43 to 0.88. Furthermore, very high positive genetic correlations were observed between potential early selection ages (2, 3, 4, and 5 years) and potential harvesting ages (5 and 8 years). Selection in breeding programs could be done as early as two years, primarily due to very high juvenile-rotation age genetic correlations (R_g = 0.90 to 0.99). The efficiency of early selection was notably higher compared to direct selection. Selecting at age two has the potential to yield genetic gain efficiency = 1.5 to 3.6 times greater than direct selection at age five. Moreover, these gains increased even further when compared to direct selection at age eight (efficiency = 2.5 to 5.6).

The findings of Chapter 4 underscore the economic impact of varying wood properties in *E. pellita* harvested at two different ages (5 and 8 years). In comparison to the 5-year rotation, both basic density and pulp yield should exhibit increases when trees are harvested at the 8-year rotation. These increases should result in reduced costs for wood, decreased tonnage of wood required, and lowered specific wood consumption. Additionally, the alkali active charge should be lower at an eight-year rotation, contributing to a decrease in chemical costs for white liquor. Essentially, harvesting *E. pellita* wood at 8 years of age should lead to an important reduction in operational costs for pulping of approximately USD 8.57 per air-dried metric ton (ADT) of pulp.

Impact

These findings hold significant implications for *E. pellita* wood production, especially in the pulp and paper industry. From a breeding standpoint, the findings provide recommendations regarding the determination of a feasible sampling point and the efficiency of early selection for genetic assessment of economically vital wood property traits. From a management standpoint,

the findings offer tangible information on the importance of considering wood properties when evaluating the optimal harvesting age for integrated forest-pulp and paper industries. This information can be used to make sound decisions that will maximize profits sustainably.

APPENDICES

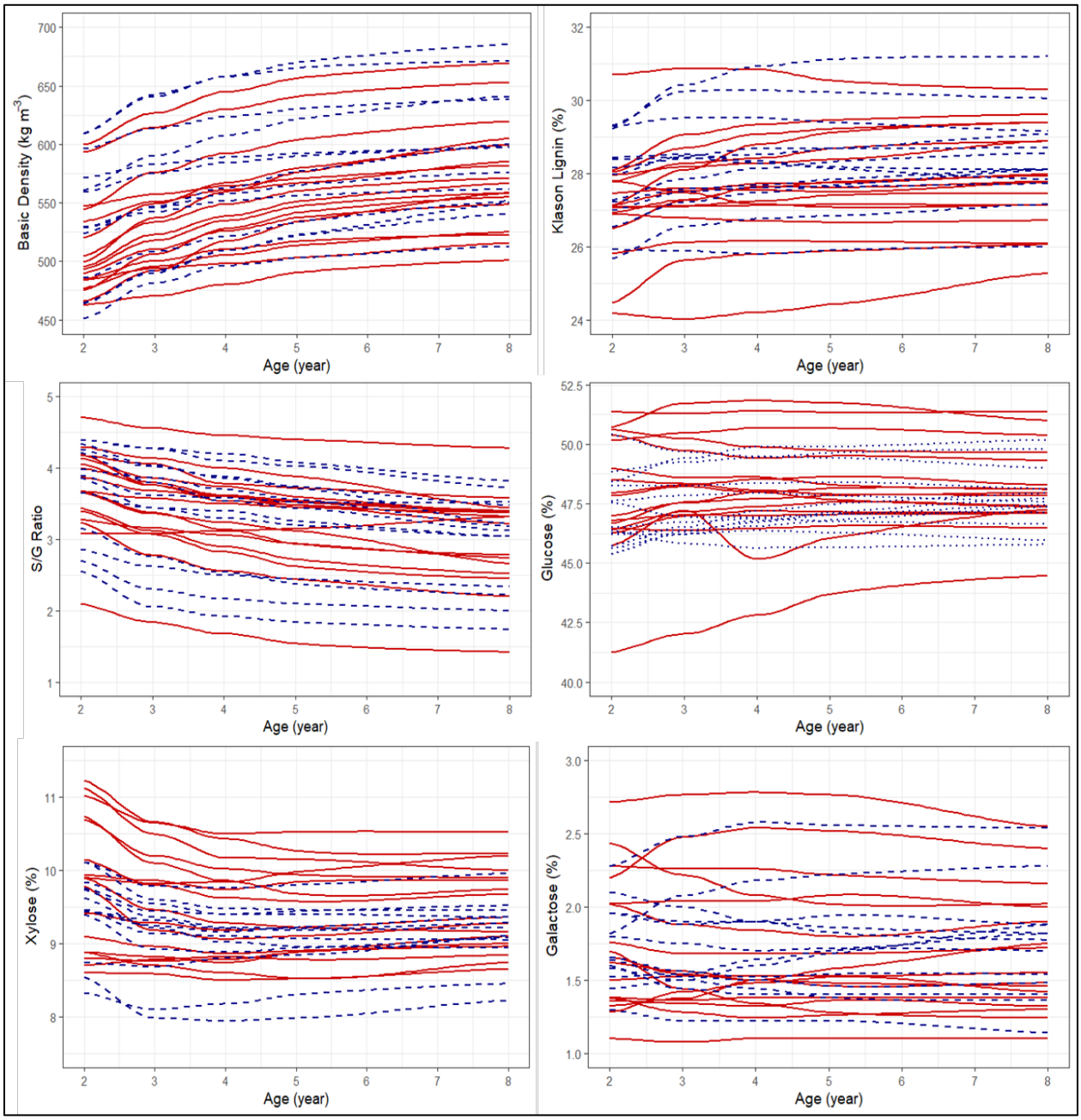
Appendix A1. Analysis of variance of between Trial 1 and Trial 2 conducted on Clone 1.

Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig. code
			Basic desnity			
Trial	1	851	851	2.927	0.093	
Position	5	43331	8666	29.8	0.000	***
Residuals	53	15413	291			
			Klason lignin			
Trial	1	2.28	2.282	3.537	0.066	
Position	5	15.83	3.167	4.91	0.001	***
Residuals	53	34.19	0.645		0	
			S/G ratio			
Trial	1	0.098	0.09761	0.841	0.363	
Position	5	1.427	0.28545	2.461	0.045	*
Residuals	53	6.148	0.116			
			Glucose			
Trial	1	1.37	1.371	1.148	0.289	
Position	5	111.34	22.268	18.64	0.000	***
Residuals	53	63.32	1.195			
			Xylose			
Trial	1	0.26	0.257	1.868	0.177	
Position	5	57.68	11.535	83.704	0.000	***
Residuals	53	7.3	0.138			
			Galactose			
Trial	1	0.0583	0.05828	1.237	0.271	
Position	5	0.9287	0.18573	3.942	0.004	**
Residuals	53	2.4973	0.04712			
	Sig. codes: '***' :0.001 , '**' :0.01, '*' 0.05					

Appendix A2. Clone mean of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal) as function of longitudinal positions.

Traits	Longitudinal Positions	Clones														
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
BD (kgm^{-3})	**0 CHt**	580	533	534	679	666	629	497	555	599	547	551	591	546	539	598
	5CHt (1.3 m)	594	514	519	683	670	647	523	558	591	554	535	588	549	539	604
	25 CHt	630	565	531	707	677	683	539	588	592	578	588	608	574	563	622
	50 CHt	648	586	550	713	664	692	577	600	604	595	604	632	599	579	628
	75 CHt	654	604	552	702	656	667	577	598	593	575	588	627	607	591	614
	100 CHt	629	577	556	689	607	649	583	558	581	533	536	608	587	572	582
K-Lig (%)	**0 CHt**	29.8	27.7	28.5	29.1	30.7	29.8	28.8	30.5	32.1	28.0	29.0	27.2	28.5	31.2	30.4
	5CHt (1.3 m)	28.8	25.8	27.5	28.9	28.3	29.3	28.2	29.7	30.5	28.0	28.3	27.1	27.3	29.7	28.8
	25 CHt	28.2	25.2	28.1	28.4	26.9	27.8	27.9	28.8	27.5	26.8	28.3	25.6	27.1	29.6	28.3
	50 CHt	28.6	25.7	26.8	28.2	27.1	27.9	28.6	28.8	27.7	27.6	28.1	26.5	27.7	29.3	28.1
	75 CHt	28.9	26.4	26.6	28.3	28.1	26.9	27.0	28.3	27.2	27.3	28.2	27.4	28.2	29.2	28.4
	100 CHt	28.4	26.1	27.1	27.6	27.0	26.4	26.8	27.6	26.4	26.4	25.9	26.6	27.8	27.8	26.8
S/G	**0 CHt**	3.38	2.87	3.11	3.78	3.21	1.79	3.28	2.57	1.53	2.86	3.55	2.86	3.38	2.58	2.20
	5CHt (1.3 m)	3.35	3.50	3.50	3.69	3.89	1.83	3.44	2.40	2.01	2.82	3.27	2.74	3.51	2.88	2.85
	25 CHt	3.60	3.42	3.47	4.08	4.22	2.56	3.46	3.06	2.99	3.27	3.29	3.32	3.50	2.76	3.02
	50 CHt	3.49	3.20	3.97	3.99	4.09	2.59	3.35	3.19	2.94	3.00	3.59	3.03	3.29	2.73	3.15
	75 CHt	3.45	2.97	4.01	3.99	3.97	3.05	3.98	3.38	3.35	3.15	3.63	3.12	3.38	2.95	3.04
	100 CHt	3.80	3.53	3.98	4.44	4.24	3.19	4.25	3.86	3.83	3.87	4.67	3.48	3.86	3.45	3.75
Glu (%)	**0 CHt**	45.7	48.7	46.1	48.5	44.8	48.6	47.1	46.0	45.7	47.9	45.4	48.6	46.9	44.5	45.8
	5CHt (1.3 m)	47.4	51.3	47.3	48.7	48.8	50.5	48.1	47.7	47.0	49.2	48.0	50.0	48.7	46.8	47.9
	25 CHt	47.1	51.0	46.2	49.1	49.6	50.3	48.4	47.6	49.1	48.6	48.5	50.0	48.0	46.7	47.7
	50 CHt	46.0	50.5	46.6	48.0	48.8	49.2	46.9	46.7	48.4	48.0	47.2	48.4	46.9	46.6	46.8
	75 CHt	44.6	48.7	45.6	47.0	46.3	48.7	47.4	46.4	47.1	47.3	45.2	46.5	44.6	45.8	44.8
	100 CHt	43.5	46.7	42.6	46.5	46.7	47.9	45.9	45.5	46.2	44.6	44.6	46.0	43.1	45.6	44.0
Xyl (%)	**0 CHt**	10.1	9.0	10.5	8.8	8.9	8.6	8.8	9.8	9.8	9.5	9.8	9.5	9.9	9.3	10.0
	5CHt (1.3 m)	9.9	8.5	10.5	8.8	8.6	9.2	8.9	9.5	9.3	9.5	9.3	9.8	9.8	9.4	9.7
	25 CHt	10.0	9.0	10.7	9.2	8.5	8.4	9.2	9.6	9.5	9.7	9.6	9.5	10.2	9.3	9.6
	50 CHt	10.8	9.7	11.0	9.8	9.1	9.4	10.0	10.2	10.4	10.1	10.5	10.3	10.9	9.7	10.6
	75 CHt	11.6	10.8	11.6	10.4	10.1	9.8	10.2	10.6	11.1	10.9	11.9	11.0	11.7	10.0	11.7
	100 CHt	12.6	11.7	13.1	11.0	10.8	10.6	10.9	11.2	12.2	12.0	12.8	11.8	12.8	11.0	12.6
Gal (%)	**0 CHt**	1.48	2.19	1.63	2.20	2.25	1.86	2.09	1.78	1.70	1.72	2.24	2.57	1.77	2.05	1.37
	5CHt (1.3 m)	1.24	2.06	1.44	1.72	2.45	1.39	2.03	1.43	1.39	1.44	1.78	2.00	1.59	1.78	1.57
	25 CHt	1.47	2.01	1.52	1.83	3.05	1.91	2.11	1.80	2.00	1.74	2.29	2.52	1.41	1.88	1.54
	50 CHt	1.44	2.06	1.51	1.66	2.71	1.77	1.96	1.65	1.95	1.60	2.09	1.98	1.37	1.84	1.49
	75 CHt	1.39	1.68	1.49	1.65	2.37	1.89	1.88	1.91	1.92	1.61	2.11	1.70	1.46	2.03	1.49
	100 CHt	1.65	1.72	1.29	1.72	2.34	2.10	1.84	1.91	1.80	1.77	2.23	1.88	1.69	1.96	1.60

Appendix A3. Change of wood properties (basic density, klason lignin, S/G ratio, glucose, xylose and galactose) with age presented as the clone's mean from Trial 1 (solid line) and Trial 2 (dashed line).



Appendix A4. The estimates of broad-sense heritability, variance components and their standard error of basic density (BD), klason lignin (K-Lig), S/G ratio (S/G), glucose content (Glu), xylose content (Xyl) and galactose content (Gal) across various ages

Age	Broad-sense Heritability & Variance Estimates					
	BD	**LIG**	**SG**	**GLU**	**XYL**	**GAL**
2 Years						
Heritability	**0.80 ±0.07**	**0.54 ±0.10**	**0.64 ±0.09**	**0.64 ±0.09**	**0.63 ±0.09**	**0.43 ±0.10**
Genetic Var.	1968.6 ±573.3	1.6 ±0.5	0.32 ±0.1	3.7 ±1.2	0.55 ±0.17	0.13 ±0.04
Residual Var.	462.5 ±59.4	1.3 ±0.2	0.17 ±0.02	2.2 ±0.3	0.30 ±0.04	0.16 ±0.02
3 Years						
Heritability	**0.82 ±0.06**	**0.54 ±0.10**	**0.7 ±0.08**	**0.55 ±0.10**	**0.63 ±0.09**	**0.55 ±0.10**
Genetic Var.	2037.5 ±591	1.7 ±0.6	0.38 ±0.11	3.3 ±1.1	0.40 ±0.12	0.16 ±0.05
Residual Var.	432.7 ±55.6	1.4 ±0.2	0.15 ±0.02	2.3 ±0.3	0.22 ±0.03	0.13 ±0.02
4 Years						
Heritability	**0.84 ±0.06**	**0.56 ±0.10**	**0.68 ±0.08**	**0.45 ±0.10**	**0.64 ±0.09**	**0.59 ±0.10**
Genetic Var.	2090.6 ±603.1	1.8 ±0.6	0.38 ±0.11	3.1 ±1.1	0.34 ±0.11	0.17 ±0.05
Residual Var.	379.7 ±48.8	1.3 ±0.2	0.17 ±0.02	3.5 ±0.4	0.18 ±0.02	0.11 ±0.01
5 Years						
Heritability	**0.85 ±0.05**	**0.55 ±0.10**	**0.68 ±0.08**	**0.44 ±0.10**	**0.61 ±0.10**	**0.62 ±0.09**
Genetic Var.	2133.8 ±612.8	1.7 ±0.5	0.38 ±0.12	2.6 ±0.9	0.31 ±0.10	0.17 ±0.05
Residual Var.	337 ±43.3	1.3 ±0.2	0.17 ±0.02	3.0 ±0.4	0.19 ±0.02	0.10 ±0.01
8 Years						
Heritability	**0.88 ±0.04**	**0.51 ±0.10**	**0.68 ±0.08**	**0.43 ±0.10**	**0.53 ±0.10**	**0.60 ±0.09**
Genetic Var.	2189.2 ±625.3	1.5 ±0.5	0.35 ±0.11	2.0 ±0.7	0.25 ±0.08	0.15 ±0.05
Residual Var.	283.1 ±36.4	1.4 ±0.2	0.16 ±0.02	2.6 ±0.3	0.22 ±0.03	0.09 ±0.01

Appendix A5. Summary of wood properties of *E. pellita* and the economic estimates across clones in for five-year and eight-year rotation

Clone ID	KPY (%)	AA Charge (%)	BD (kg m^{-3})	Pulp Cost ($)		Tonnage wood (ODT)		SWC (m^3)		MAI (m3 ha^{-1})	Volume (m3 ha^{-1})	Daily Area Required (ha)			
				ADT Pulp	Daily	ADT Pulp	Daily	ADT Pulp	Daily			MAI_5 100	MAI_5 90	MAI_5 80	MAI_5 70
							5 Years								
1	48.27	17.8	578	264.16	264,161	2.22	2217	3.8	3835	57.4	287.0	13.4			
2	49.47	16.0	503	253.37	253,367	2.16	2163	4.3	4300	38.7	193.5	22.2			
3	48.29	16.5	517	256.15	256,147	2.17	2171	4.2	4199	17.0	85.1	49.3			
4	44.05	19.3	670	279.89	279,887	2.43	2429	3.6	3624	17.8	88.8	40.8			
5	46.42	20.0	621	278.73	278,731	2.31	2305	3.7	3710	23.2	115.8	32.0			
6	48.56	18.5	635	267.49	267,490	2.20	2204	3.5	3469	58.4	292.0	11.9			
7	49.35	17.0	513	258.68	258,678	2.17	2168	4.2	4223	30.0	150.2	28.1			
8	48.34	18.0	556	265.13	265,133	2.21	2214	4.0	3980	18.6	93.0	42.8			
9	48.36	17.0	592	259.50	259,497	2.20	2200	3.7	3716	27.0	135.1	27.5			
10	48.8	15.5	547	251.43	251,431	2.19	2193	4.0	4009	14.8	73.9	54.3			
11	43.97	19.5	522	280.34	280,342	2.44	2444	4.7	4683	36.3	181.5	25.8			
12	49.66	18.0	590	263.49	263,489	2.15	2155	3.7	3653	26.6	132.8	27.5			
13	50.49	16.0	534	252.36	252,357	2.12	2119	4.0	3970	61.2	306.1	13.0			
14	44.88	19.5	537	277.33	277,327	2.38	2384	4.4	4438	41.3	206.4	21.5			
15	48.17	17.0	576	260.03	260,034	2.22	2221	3.9	3858	46.0	229.8	16.8			
16	49.14	16.0	567	253.70	253,703	2.18	2178	3.8	3842	40.9	204.3	18.8			
17	49.1	17.0	490	258.96	258,959	2.18	2179	4.4	4444	56.5	282.4	15.7			
18	48.93	17.0	534	259.15	259,153	2.19	2187	4.1	4098	19.0	95.0	43.1			
19	48.69	17.0	571	259.43	259,428	2.20	2198	3.9	3852	31.3	156.6	24.6			
20	48.34	17.0	565	259.83	259,834	2.21	2214	3.9	3917	38.4	192.2	20.4			
21	49.36	17.5	604	261.26	261,260	2.17	2168	3.6	3589	47.8	239.2	15.0			

Clone ID	KPY (%)	AA Charge (%)	BD (kg m^{-3})	Pulp Cost ($)		Tonnage wood (ODT)		SWC (m^3)		MAI (m3 ha^{-1})	Volume (m3 ha^{-1})	Daily Area Required (ha)			
				ADT Pulp	Daily	ADT Pulp	Daily	ADT Pulp	Daily			MAI_5 100	MAI_5 90	MAI_5 80	MAI_5 70
22	47.28	17.0	503	261.10	261,102	2.26	2263	4.5	4501	17.9	89.6	50.2			
23	48.88	18.0	560	264.45	264,449	2.19	2189	3.9	3909	18.3	91.7	42.6			
24	48.64	18.0	551	264.75	264,751	2.20	2200	4.0	3993	35.1	175.4	22.8			
25	48.62	18.0	523	264.78	264,777	2.20	2201	4.2	4211	25.5	127.3	33.1			
26	46.4	20.0	661	278.76	278,764	2.31	2306	3.5	3489	72.7	363.6	9.6			
27	41.89	20.0	542	286.90	286,902	2.55	2554	4.7	4717	15.2	75.9	62.1			
								8 Years							
1	48.49	16.5	600	257.01	257,008	2.21	2206	3.7	3678	57.4	459.1	8.0	8.9	10.0	11.4
2	52.54	14.0	516	240.71	240,709	2.04	2037	4.0	3950	38.7	309.6	12.8	14.2	15.9	18.2
3	49.46	16.0	523	253.38	253,378	2.16	2164	4.1	4139	17.0	136.2	30.4	33.8	38.0	43.4
4	48.53	16.5	685	256.97	256,975	2.20	2205	3.2	3218	17.8	142.0	22.7	25.2	28.3	32.4
5	47.18	17.5	641	263.94	263,939	2.27	2268	3.5	3538	23.2	185.3	19.1	21.2	23.9	27.3
6	49.78	16.0	645	253.06	253,056	2.15	2150	3.3	3333	58.4	467.2	7.1	7.9	8.9	10.2
7	48.65	18.5	525	267.37	267,371	2.20	2200	4.2	4190	30.0	240.4	17.4	19.4	21.8	24.9
8	49.16	18.5	562	266.71	266,706	2.18	2177	3.9	3876	18.6	148.8	26.1	28.9	32.6	37.2
9	47.55	16.0	597	255.39	255,387	2.25	2250	3.8	3770	27.0	216.1	17.4	19.4	21.8	24.9
10	50.11	15.5	558	250.17	250,175	2.14	2135	3.8	3824	14.8	118.2	32.3	35.9	40.4	46.2
11	44.47	14.5	541	250.35	250,351	2.41	2406	4.5	4451	36.3	290.4	15.3	17.0	19.2	21.9
12	50.14	18.5	598	265.13	265,133	2.12	2123	3.6	3551	26.6	212.4	16.7	18.6	20.9	23.9
13	51.28	15.5	559	249.11	249,107	2.09	2087	3.7	3736	61.2	489.8	7.6	8.5	9.5	10.9
14	46.19	18.5	549	270.79	270,787	2.32	2317	4.2	4223	41.3	330.2	12.8	14.2	16.0	18.3
15	47.99	17.0	605	260.25	260,247	2.23	2230	3.7	3686	46.0	367.6	10.0	11.1	12.5	14.3
16	50.27	17.0	585	255.59	255,586	2.05	2047	3.5	3498	40.9	326.9	10.7	11.9	13.4	15.3
17	49.78	14.5	501	245.34	245,343	2.15	2150	4.3	4292	56.5	451.8	9.5	10.6	11.9	13.6
18	49.7	14.5	551	245.41	245,411	2.15	2153	3.9	3905	19.0	152.0	25.7	28.5	32.1	36.7
19	49.89	17.0	582	258.08	258,078	2.14	2145	3.7	3687	31.3	250.6	14.7	16.3	18.4	21.0
20	49.66	15.0	576	248.02	248,021	2.15	2155	3.7	3740	38.4	307.5	12.2	13.5	15.2	17.4

Clone ID	KPY (%)	AA Charge (%)	BD (kg m^{-3})	Pulp Cost ($)		Tonnage wood (ODT)		SWC (m^3)		MAI (m3 ha^{-1})	Volume (m3 ha^{-1})	Daily Area Required (ha)			
				ADT Pulp	Daily	ADT Pulp	Daily	ADT Pulp	Daily			MAI_5 100	MAI_5 90	MAI_5 80	MAI_5 70
21	48.92	14.8	620	246.08	246,080	2.19	2187	3.5	3530	47.8	382.7	9.2	10.2	11.5	13.2
22	49.58	14.5	513	245.51	245,512	2.16	2158	4.2	4209	17.9	143.4	29.4	32.6	36.7	41.9
23	49.1	16.0	571	253.74	253,744	2.18	2179	3.8	3817	18.3	146.8	26.0	28.9	32.5	37.2
24	48.41	15.5	567	251.82	251,818	2.21	2210	3.9	3901	35.1	280.6	13.9	15.4	17.4	19.9
25	48.55	16.0	550	254.32	254,315	2.20	2204	4.0	4009	25.5	203.7	19.7	21.9	24.6	28.1
26	46.73	18.4	670	269.46	269,458	2.29	2290	3.4	3417	72.7	581.7	5.9	6.5	7.3	8.4
27	46.87	19.0	555	272.54	272,540	2.28	2283	4.1	4114	15.2	121.5	33.9	37.6	42.3	48.4

Appendix A6. Pulping parameters assumptions for the economic model.

Parameter	Value
Knots, % of unbleached pulp	1
Screened rejects, % unbleached	2
Knot/reject cooked yield (%)	60
Delig. in Oxygen stage (%)	40
Oxygen delig. Yield (%)	98.6824
Bleaching yield (%)	95.0876

Appendix A7. Cost assumptions for the economic model.

Description	Unit	Unit cost ($)
Raw Materials-cooking		
Wood	ton	85
Caustic make-up, liquor cycle	ton Na2O	600
Lime make-up	ton CaO	145
Raw materials-bleaching		
Sodium chlorate	ton	590
Caustic used in bleach plant	ton Na2O	600
Oxygen used in O2 delg. and bleaching	ton	143
Hydrogen peroxide used in bleaching	ton	700
Sulfuric acid used for ClO2 gen.	ton	95
Caustic used to neutralize salt cake	ton Na2O	600
- methanol	ton	450
Utilities		
Steam usage		
- for cooking	106BTU	8
-for oxygen delig.	106BTU	8
-for bleaching	106BTU	8
-for evaporation	106BTU	8
-for ClO2 generation	106BTU	8
-for caustic room	106BTU	8
Net steam from recovery boiler	106BTU	8
Lime Kiln Fuel	106BTU	13.0642106
Electrical power usage, KWH		
-cooking/washing/screening	KWH	0.026
-oxygen bleaching	KWH	0.026
-bleach plant	KWH	0.026
-ClO2 generation	KWH	0.026
-evaporation & recovery	KWH	0.026
-caustic plant	KWH	0.026
-effluent treatment	KWH	0.026
Treated mill water	106gal	129