GENETIC CONTROL OF WOOD PROPERTIES OF *PINUS PATULA* IN SOUTHERN AFRICA

by

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DECLARATION

I, hereby declare that this thesis, prepared for the degree Philosophiae Doctor, which was submitted by me to the University of the Free State, is my own original work and has not previously in its entirety or in part been submitted to any other University. All sources of materials and financial assistance used for the study have been duly acknowledged. I also agree that the University of the Free State has the right to the publication of this dissertation.

André Nel 7 October 2013

This thesis is dedicated to:

my wife Rika, and daughter Christi-Ann, thanks

for your love and support;

and the late Richard Delano Barnes (1934 - 2004), for his mentorship and guidance,

for completing the original crosses used in this study, and for the generous

donation of the sample material.

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CONTENTS

Chapter 5: Inheritance of density traits of *P. patula*

Chapter 6: Inheritance of cross-sectional tracheid traits of *P. patula* measured by image analysis

Abbreviations and Acronyms

Abbreviation	Description
BA8	Basal area at breast height at eight years (m^2)
CS	Coarseness ($\mu g\ m^{-1}$) Silviscan® derived trait
CWA	Cell wall area (μm^2)
CWT	Cell wall thickness (µm)
DBH	diameter at breast height (1.3m above ground level) in cm
DBH8	Tree diameter at breast (1.3 m) height (cm) at eight years (cm)
EWP	earlywood percentage
GCA	general combining ability
H^2	Broad-sense heritability
h^2	Narrow-sense heritability
Hgt or Ht	tree height in m
Hgt8	Tree height at eight years (m)
LD	Lumen diameter (µm)
LWP	latewood percentage
Mat	Maternal effects
MATL	Arithmetic tracheid length (µm)
MBT	Percentage break ends (%)
MCT	Percentage curl (%)
MEWD	mean earlywood density
MEWDR1	mean earlywood density ring 1
MEWDR2	mean earlywood density ring 2
MEWDR3	mean earlywood density ring 3
MEWDR4	mean earlywood density ring 4
MEWDR5	mean earlywood density ring 5
MFines	Percentage area of fines (%)
MKT	Percentage kinked tracheids (%)
MLWD	mean latewood density
MLWDR1	mean latewood density ring 1
MLWDR2	mean latewood density ring 2
MLWDR3	mean latewood density ring 3
MLWDR4	mean latewood density ring 4
MLWDR5	mean latewood density ring 5
MTC	Coarseness of tracheids (mg/m)
MTD	Mean tracheid diameter (µm)
MTnum	Number of tracheids per gram (No/g)
MTW	Tracheid width (µm)
MTWT	Tracheid wall thickness (µm)
MWDR1	mean wood density ring 1
MWDR2	mean wood density ring 2
MWDR3	mean wood density ring 3
MWDR4	mean wood density ring 4

Abbreviation	Description
MWDR5	mean wood density ring 5
MWTL	Tracheid length weighted in length (µm)
Nmat	Non-maternal effects
NoTrach	No of tracheids per mm^2 (n/ mm^2)
PCell	Percentage cell wall per mm^2 (%)
PM	Perimeter (µm) Silviscan® derived trait
RD	Radial diameter (µm)
REC	Reciprocal effects
RR	Runkel ratio
SCA	specific combining ability
SS	Specific surface (m^2 kg^{-1}) Silviscan® derived trait
TArea	Tracheid area ($µm^2$)
TD	Tangential diameter (µm)
Vol8	Tree volume at eight years (m^3)
WMWD	weighted mean wood density
WTS	Wall thickness (µm) Silviscan® derived trait

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PREFACE

This thesis consists of ten chapters and reports on a quantitative genetics study of physical wood properties for pulp and paper production of *Pinus patula*, a major South African forestry softwood tree species. **Chapter 1** provides a general introduction to the species, tree breeding, the importance of wood properties, and the objectives of this study. **Chapter 2** provides a literature review of important physical wood properties and their genetic control. It covers wood density, fibre and tracheid anatomical properties important in the pulp and paper industry, inheritance of wood properties, and rapid-screening methods to determine these properties.

The quantitative genetics study reported on in this thesis was carried out using wood samples collected from progeny trials of full-sib material. This material consisted of a five-parent diallel mating design with reciprocal crosses, and selected crosses from a 9 × 5 factorial controlled pollination mating design. **Chapter 3** provides a description of the genetic material used in this study and the sampling strategy that was followed, and **Chapter 4** provides an outline of the genetic analysis followed for wood density-, tracheid- and growth-traits discussed in Chapters 5 to 8. **Chapter 5** reports on the inheritance of wood density traits of *P. patula* grown in Southern Africa, using x-ray densitometry on pith-to-bark samples. **Chapter 6** presents a wood anatomical study of inheritance of cross-sectional tracheid properties conducted with image analysis using the same pith-to-bark samples used in Chapter 5.

Chapter 7 covers the use of relatively new technology (MorFi fibre analyser) to assess important fibre dimensions and the inheritance of various important fibre traits, again using the same samples as in Chapters 5 and 6. The growth results as assessed at eight years after planting and the inheritance of growth properties is covered in **Chapter 8**. In **Chapter 9** correlations between all the different traits assessed in chapters 5 to 8, including the original 8-year field growth data from

the progeny trials, are examined. Implications of these correlations on selection and breeding strategy are also explored. Finally, in **Chapter 10** final conclusions are drawn from this research and implications for future breeding strategies are explored.

Chapter 1

General Introduction

1.1 Historical introduction

The *Pinus* genus is the largest and most widespread conifer in the northern hemisphere; consisting of 110 species, almost exclusively occurring in the northern hemisphere (Dvorak *et al.*, 2000; Eckenwalder, 2009). It is one of the most ecologically diverse genera of woody plants, and has the greatest economic value among all conifers, with many species being utilized for commercial forestry in natural and afforested areas (Eckenwalder, 2009).

The first recorded establishment of an exotic tree species in South Africa can be traced back to 1670 when a plantation of oaks were planted at Newlands, Cape Town (Owen and Van Der Zel, 2000). The planting of various conifer species from Europe also occurred at the Cape during the late 1600's (Owen and Van Der Zel, 2000). According to Poynton (1977) the first commercial plantations consisted of *Pinus pinaster and Pinus pinea* and were established between 1825 and 1830 at Genadendal, Western Cape.

It is estimated that a total area of about 1.3 million ha of South Africa (1.1%) is used for commercial afforestation (Forestry South Africa, 2010). Round-wood sales of 18.9 million m^3 generate revenue of about R6.7 billion per annum, as assessed in the year 2009 (Forestry South Africa, 2010). Commercial companies own approximately half of the land under afforestation. The public sector (30%) and private individuals (21%) own the rest of the afforested land. Softwoods in the form of various pine species make up about 51% of the afforested area (Forestry South Africa, 2010).

1.2 Species description

Sir David Hutchins, conservator of Forests for the Cape, introduced *Pinus patula* Scheide et Deppe into South Africa in 1907 when a trial block was planted at Tokai plantation in the Western Cape Province (Poynton, 1977). Further introductions were made in 1908 when several arboretums were established at plantations near Tzaneen, Belfast and Lothair in the Mpumalanga and Northern provinces (Loock, 1977). *P. patula* is the most important softwood species in commercial forestry in South Africa. Approximately 340 000 ha is afforested with this species by the different forestry companies and it is grown for a variety of timber and pulp products (Department of Agriculture, Forestry and Fisheries, 2010). It is the most extensively planted pine species on Southern African landholdings of Sappi, a large international pulp and paper company.

P. patula belongs to the *Pinus* genus of the *Pinaceae* family. The species is placed in the section *Serotinae* subsection *Oocarpae* (Wormald, 1975). Other species included in this subsection are *Pinus tecunumanii*, *Pinus oocarpa*, *Pinus greggii*, *Pinus muricata and Pinus pringlei*. Two different varieties occur, namely, *P. patula*, var. *patula* and var. *longipedunculata*.

P. patula is indigenous to Mexico and grows at altitudes of 1500 to 3100 m and at latitudes 16^{o} N to 24^{o} N with mean annual precipitation of between 600 and 2500 mm (Wright, 1994). Figure 1.1 shows the natural distribution of *P patula* varieties across their country of origin, Mexico. Within its native range it attains a height of 35 m and diameters of up to 80 cm (Dvorak *et al.*, 2000).

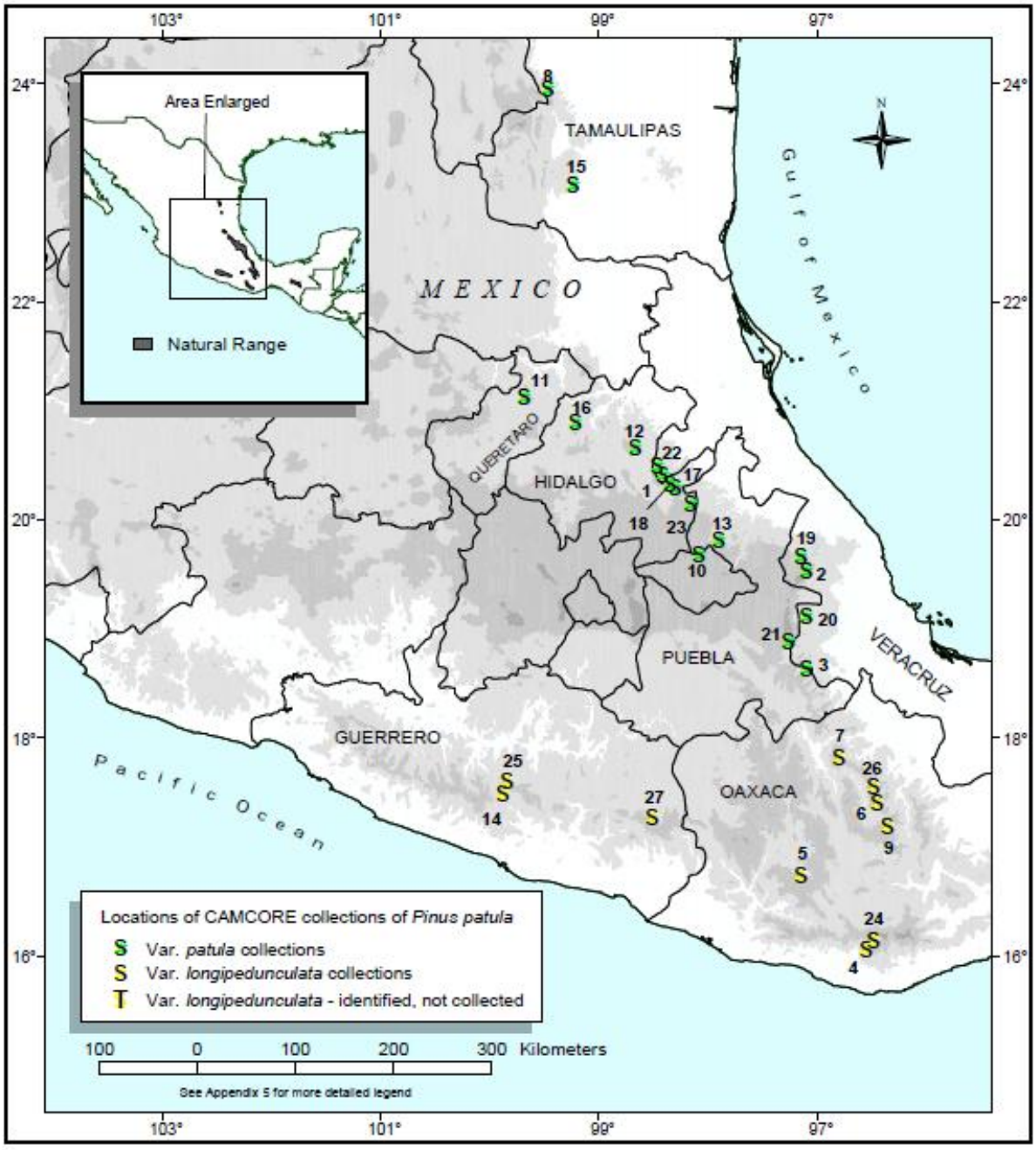

Figure 1.1 Distribution of *P. patula* varieties in the country of origin, Mexico. The numbers on the map represent different provenance seed collections made by Camcore, a gene conservation and tree improvement co-operative (Dvorak *et al*., 2000).

P. patula is the most widely planted forestry species in the *Oocarpae* subsection with an approximate 1.0 million ha established worldwide, mainly in Southern Africa. The broad growth requirements for this species in South Africa are mean annual temperature (MAT) of <18^{o}C and rainfall (MAP) of >700 mm at high altitudes and >950 mm at lower altitudes with well-drained soils (Morris and Pallett, 2000).

The wood of *P. patula* has a moderate wood-density, is low in extractives and is suitable for a number of wood and paper products (Dvorak *et al.*, 2000). These attributes and its fast growth in the summer-rainfall area make *P. patula* the most important and widely planted softwood in Southern Africa.

1.3 Tree improvement

Tree improvement programmes for forestry species (including *P. patula*) started in South Africa and Zimbabwe during the 1950's and were conducted in South Africa by the government's Department of Forestry. The main selection criteria of these early breeding programmes were restricted to growth (volume), tree form, disease resistance (tolerance) and to some extent physical lumber properties (Poynton, 1977). Private forestry companies in South Africa have initiated their own tree improvement programmes during the last three to four decades.

In the first two generations of breeding, volume improvements of between 10 and 30% have been achieved in the tree improvement programmes of various companies in Southern Africa (Kanzler and Barnes, 2004). Due to a number of factors such as food security, land restitution, increased fire damage and limited rainfall in South Africa, there are limits to future expansion of forestry land; and

there has been an actual decrease of 0.2 million ha in the planted area during the last 10 years (Forestry South Africa, 2010). This has increased the need to improve both productivity and the quality of wood and fibre on the existing land-base.

The focus on breeding for specific wood properties in breeding programmes has increased during the last 10 to 15 years. Some of the reasons that may explain the exclusion of wood properties in the initial breeding programmes are the cost of determining wood properties, deciding which properties are important for a specific product, as well as predicting which future products and properties would be important for a tree crop that takes on average 18 years to grow (Zobel and Talbert, 1984). Determining wood properties would also commonly necessitate destructive sampling which further limits its application. It is therefore critically important to identify the desirable trait to select for and to devise a non-destructive method of sampling. It is also important to note that wood properties are inter-related with pulp and paper properties (Zobel and Talbert, 1984). More recently, non-destructive methods of sampling have been introduced and have made the inclusion of wood properties as selection criteria possible.

1.4 Overall aim and objectives of study

The overall aim of this study is to gain a fundamental understanding of the inheritance of density and tracheid traits important for pulp and paper production of *P. patula* grown in Southern Africa. Understanding which properties are under strong genetic control will aid tree breeders to make gains and select for these traits and include them as part of their selection criteria.

Utilizing full-sib progeny trial material from a diallel and factorial mating design from the Zimbabwe Forest Commission's *P. patula* breeding programme, the main objectives of this study will be to determine:

1) The level of genetic control of a range of important density, anatomy and tracheid properties;

2) The general and specific combining abilities of the genetic material for the range of density, anatomy and tracheid properties;

3) Whether any reciprocal differences are evident for the studied properties;

4) The broad and narrow sense heritability for the measured and calculated properties and components;

5) The correlations between the different density, anatomy, tracheid and growth properties.

Chapter 2

Literature Review

Wood properties and their genetic control

2.1 Introduction

Wood is the principal source of cellulosic fibre used in the manufacturing of pulp and paper. Wood provides around 93% of the world's virgin fibre requirement, with the balance coming from non-wood sources such as bagasse, cereal straws and bamboo (Smook, 1986). Unlike other industrial raw materials, there is considerable variation in the wood supply driven by differences in genera, species, within tree variation, harvesting age and the sites where trees were grown prior to harvest.

The properties of wood as a raw material largely influence pulping processes and the post-pulping pulp and paper characteristics. Knowledge of these wood properties is important for optimising pulping processes and predicting pulp and paper qualities of end-products (Zobel and Talbert, 1984; Zobel and van Buijtenen, 1989). Understanding the level of genetic control of specific wood properties will allow the tree breeder to include important properties in their selection programmes.

This chapter provides an overview of basic wood structure and the different wood properties that are considered to be important in the Kraft pulp and paper process. It also provides a review of relevant research on the genetic control of wood properties considered important for pulp and paper production.

2.2 Macroscopic structure of wood

Wood is a complex biological structure which forms part of a living plant. Some of its main functions are the transport of water from the roots to the leaves, mechanical support of the plant, and storage of biochemicals (Wiedenhoeft and Miller, 2005). A cross-sectional view of a tree stem reveals three distinct areas consisting of the pith, xylem and bark. The pith is a small core of soft tissue in the centre of the trunk which is surrounded by a cylinder of wood or xylem, which in turn is surrounded by a layer of bark (Smook, 1986).

The xylem section in a tree trunk can be divided into two distinct areas consisting of sapwood and heartwood. New wood and inner bark are formed each year by the activity of a layer of dividing cells called the cambium, which is located between the inner bark and the sapwood (Society of Wood Science and Technology, 2012). Since new wood is added to the outside of existing wood the oldest wood is close to the pith, and the most recently formed wood is close to the bark. The sapwood is still active in the transport of water, provides structural support for the crown, and acts as a food storage area (Wiedenhoeft and Miller, 2005). The heartwood section on the inside of the trunk consists of dead wood cells that are no longer physiologically active, but still provides mechanical support to the tree (Smook, 1986). Heartwood is usually a darker colour due to the biochemicals and extractives that are stored in this section of the trunk.

Wood is formed annually as a sheath that extends both horizontally and vertically over the existing wood in the tree trunk. All trees produce concentric layers of wood, but not all trees have visible growth rings, for example the *Eucalyptus* species (Naidoo *et al*., 2010). In some trees seasonal changes in wood structure may be so slight that growth rings are not evident. Seasonal changes also influence the formation of growth rings; under drought conditions no annual

growth rings may be produced (SWST, 2012). When favourable conditions prevail, several growth rings may be produced in a year. Growth rings are formed due to the formation of two different types of wood; earlywood and latewood. They are visible due to the changes in the structure of wood formation through the growing season (Smook, 1986). Wood cells that are formed early in the season are larger in size and therefore appear less dense than those produced towards the end of the growth season.

2.3 Microscopic structure of wood

Forestry tree species are categorised into two main groups; angiosperms that are commonly referred to as hardwoods, and gymnosperms commonly referred to as softwoods. The main difference between these groups in terms of wood structure is the type of wood cells that they produce. Hardwoods are composed of at least four kinds of cells consisting of libriform fibres, vessel elements, longitudinal parenchyma and wood ray parenchyma (Smook, 1986). Each of these cell types also constitutes a significant portion of hardwood volume (Bowyer *et al*., 2007). Softwoods, in contrast, mainly consist of longitudinal tracheids that make up 90-95% of wood volume, and ray cells that make up the balance (Smook, 1986). Figure 2.1 provides a schematic representation of the cellular structure of the various elements of wood cells of softwoods and hardwoods.

The longitudinal tracheid cells that make up the majority of softwood volume consist of long, tapering cells. Softwood tracheids can be up to four times longer than the libriform fibres found in hardwoods (Smook, 1986). Ray cells make up a small part of softwood volume in the horizontal plane and consist of two specialized types. Ray parenchyma occurs in all softwood species, with ray tracheids only occurring in certain species (Smook, 1986).

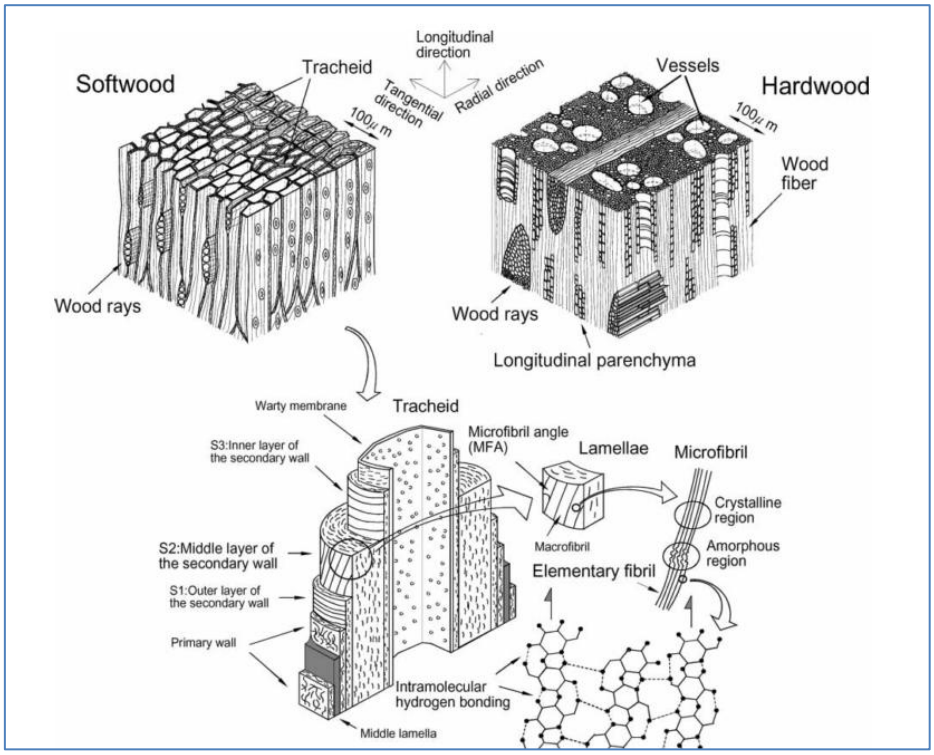

Figure 2.1 Cellular structure of softwood and hardwood and schematic structure of the various elements of the cell wall of a tracheid (from Tsuchikawa, 2007).

It is important to note that the term tracheid is not synonymous with the word fibre used by pulp and paper makers. In the context of softwood pulp and paper making, fibres refer to processed, beaten and refined fibres which are the product of pulped softwood tracheids (Stanger, 2003).

Seasonal growth is usually characterised by an earlywood section followed by a denser band of tracheids called latewood at the end of the annual growth ring. Earlywood is also referred to as springwood and latewood as summerwood in some literature sources (Corson, 1984). The latewood properties differ from those of earlywood with a density of up to four times that of earlywood (Smook, 1986). This difference in density is mainly driven by the thicker cell walls of latewood. The cell wall of a tracheid is composed of several layers (see Figure 2.1). The

middle lamella, which is very high in lignin content, separates tracheids. Each tracheid has a primary wall and a three-layered secondary wall made up of microfibrils (Smook, 1986). Microfibrils consist of bundles of cellulose molecules and their orientation and angle have an impact on the characteristics of pulp.

Apart from the inter growth-ring variation, there is also variation in wood properties from the centre to the outside of the stem, as well as along the length of the tree. This variation is a function of the age of the tree and is caused by the formation of different types of wood during the development of a tree. Juvenile wood is formed in the early years and mature wood later in the life-cycle of the tree. Juvenile wood has shorter and narrower tracheids, cell walls are thinner, a higher earlywood to latewood ratio, lower density and cellulose content, higher hemicellulose and lignin, and wider growth rings (Mimms, 1993).

2.4 Pulping process

The main aim of the pulping process is to break cell constituents such as tracheids apart into individual paper making fibres. These fibres are then further manipulated in the paper making process. This process starts with the reduction of wood to its constituent fibres, followed by the suspension of fibres in water. Suspended fibres are then beaten and refined and are blended with other additives before formation into a fibre mat. After the formation of the fibre mat, water is drained and the pulp dried before surface treatment completes the process (Bowyer *et al*., 2007).

There are different ways of producing pulp, and these can be grouped into mechanical, chemical, heat energy or combinations thereof. With chemical pulping, wood is chopped up into chips which is placed into a chemical solution

(cooking liquor) and heated in a pressurised vessel called a digester. In the Kraft chemical pulping process, pulping is based on using cooking liquor made primarily of sodium hydroxide and sodium sulphate. These chemicals separate the cells in the wood and dissolve most of the lignin contained in the cell walls (Bowyer *et al*., 2007). Fibre separation is achieved by dissolving the lignin in the middle lamella that holds tracheids together.

2.4.1 Paper and hand sheet properties

Paper quality is a function of the structure of the processed fibre network in a paper sheet. The quality is influenced by the physical dimensions of the wood cells that were used to produce the pulp fibres (Smook, 1986). A short summary of the most important paper and hand sheet properties are provided.

Draining ability or freeness refers to the resistance of pulp fibres to the flow of water in the pulping process. This refers to the ease with which water can drain from pulp through a wire mesh screen during the drying process (Smook, 1986). Freeness is expressed as Canadian Standard Freeness, or CSF. Freeness is increased with looser inter-fibre bonding and decreased with increased inter-fibre bonding. Thin-walled fibres and short fibres form a more dense fibre network which can decrease drainage.

The most important property of pulp is its papermaking potential. This can best be evaluated by beating or refining the pulp under controlled conditions, forming the pulp into standardised handsheets (Smook, 1986). The purpose of beating is to mechanically condition pulp fibres for papermaking. During the beating process, pulp fibres are mechanically flattened and unravelled, increasing their bonding potential (Bowyer *et al*., 2007).

A number of physical properties of paper are important and are influenced by the characteristics of the raw material. These are strength properties and the most important factors to consider are tensile, burst and tear strengths. Tensile strength is determined by assessing the force required to break a narrow strip of paper. Both the length of the strip and the loading rate are closely specified (Smook, 1986). Burst strength is determined by clamping a paper sample over a rubber diaphragm and applying a specified rate of pressure. The pressure value at the point of paper rupture is assessed (Smook, 1986). Tear strength is assessed by using a falling pendulum used to continue a tear made in the paper sample. The loss of energy is related to the force required to continue the tear (Smook, 1986).

Another important papermaking property is fibre coarseness. Fibre coarseness is the mass of oven-dry material per unit length of pulped fibre, and is related to papermaking properties of pulpwood fibres (Muneri and Raymond, 2001). With constant pulp fibre diameter, coarser fibres generally have thicker cell walls, are stiffer and more flexible. Coarser fibres also resist collapse during paper making and produce bulkier, more porous and rougher sheets (Muneri and Raymond, 2001).

Another property closely related to coarseness is fibre surface or specific surface. Pulping fibres with the same coarseness value can still vary in length, and such a longer fibre will have a greater surface area (Ivkovich, 2000). This will make these specific fibres more flexible and therefore increase its collapsibility. Paper strength is influenced significantly by fibre surface area and in combination with fibre coarseness, is a useful measure of its value in papermaking (Ivkovich, 2000).

2.4.2 Wood properties important for pulp and paper

The properties of wood used as raw material in pulp and paper production influence the pulping process and the properties of paper products. Wood from both hardwood and softwood groups are used in the production of pulp and paper, often in combination. Furnish from both sources are often blended together to combine the good qualities unique to each source. The properties of wood can be divided into two broad categories, chemical components and the physical properties determined by cell types such as tracheids (Mimms, 1993).Wood can be divided into four main chemical substance groups; cellulose, hemicellulose, lignin and extractives (Mimms, 1993). Cellulose forms the major part of cell walls of wood. This study will investigate the genetic control of physical wood properties, so a more detailed review of these properties will be presented.

The most important physical wood properties for softwood Kraft pulp and paper are wood density, wood cell anatomy, and tracheid dimensions (Wiedenhoeft and Miller, 2005). A critical step in any tree improvement programme is the identification of important process-specific wood property traits that are under genetic control. Pulp yield and quality in a Kraft pulp mill is affected by a number of principal factors. These factors fall into two categories; specific properties of the wood raw material, and the processing methodology used in the pulping process (Macleod, 2007). A number of physical wood properties fulfil an important role in softwood Kraft pulp. According to Barefoot *et al.* (1964), properties that are considered to be good indicators of handsheet properties are specific gravity, cell wall thickness, lumen diameter, tracheid length, the Runkel ratio (cell wall thickness/lumen diameter) and earlywood/latewood ratio.

Wood density is an expression of the quantity of wood substance in a given volume of wood. Wood density refers to the ratio of dry weight of wood to its volume and is expressed as kg per cubic meter. Wood density is a very important economic factor to consider in pulping as pulp production can be increased when a denser wood is used as more weight can be packed into the pulp digester volume (Mimms, 1993).

The major advantage of softwoods as compared to hardwoods for wood furnish is the length of their tracheids. Other important fibre properties are fibre diameter and wall thickness, which also have a close relationship with wood density (Zobel and Jett, 1995). Tracheid properties affect the formation structure of paper during the papermaking process and they are responsible for the properties of paper (Niskanen, 1998). Softwood furnish has the largest influence on the strength properties in paper products (Mimms, 1993).

Many of the physical properties of wood are interrelated. Cell wall thickness, for instance, is directly related to wood density, wood with thicker cell walls produce higher density wood. Physical wood properties also have an effect on post-pulping pulp and paper properties. Using softwood furnish with long fibres will produce pulp and paper with longer fibres that will have better bonding and strength properties (Zobel and Jett, 1995).

The wood of *P. patula* is extensively used for the production of mechanical and chemical Kraft pulp in the Southern African region. Wright (1994) and Dommisse (1994) report on the successful utilisation of *P. patula* for commercial pulp and paper production. Muneri (1994) and Naidu (2003) studied the impact of utilising *P. patula* as furnish for Kraft pulping, and they investigated physical properties such as wood density and tracheid properties.

In many of the historical tree breeding programmes around the world, the initial selection criteria consisted of growth traits such as volume, tree-form, stem-straightness and branching. This also applied to breeding programmes in Southern Africa, where wood properties were only included more recently in the 2nd or 3rd cycle of breeding. According to Zobel and Jett (1995), even after several cycles of breeding for growth traits, large variation in wood properties is still present. Further gains can therefore be made by introducing wood and fibre traits, even in advanced generations.

In order to incorporate wood properties economically into a selection programme, they need to be assessed in a convenient, cheap and rapid manner. It is preferable that sampling be done in a non-destructive manner to conserve the selected genotypes. Large scale sampling of populations is often necessary to characterise wood properties accurately. Rapid and cost efficient sampling methodology is therefore critical (Evans *et al.,* 1995). The sample properties also need to reflect those of the whole tree and a good correlation between the resource properties and the end product properties is required (Evans *et al*., 1995). Recent advances in technology have to a large extent facilitated these requirements and there has been a new emphasis on the inclusion of wood properties in breeding programmes during the last 20 years.

2.5 Wood density

Wood density has the highest economic impact on the production of pulp and paper. Wood density is therefore one of the most important and widely studied wood characteristics for all products and forestry species (Zobel and van Buijtenen, 1989). Wood density refers to the ratio of dry weight of wood to its volume and is expressed as kg per cubic meter. Pulp production can therefore be increased with denser wood as more weight can be packed into the finite digester

volume (Mimms, 1993). Higher wood density material also results in further cost benefits in wood handling and transport (Kibblewhite, 1999). Numerous studies on the genetics of wood density have been conducted worldwide, most of them on *P. taeda* and *P. radiata* (Zobel and Jett, 1995).

Wood density is not a single wood characteristic; it is a combination of different characteristics. These characteristics may have strong inheritance patterns of their own in softwoods (Zobel and Talbert, 1984). The wood properties that primarily make up wood density are cell size, cell wall thickness and amount of latewood formation (Zobel and Talbert, 1984). Wood or Kraft pulp density does not give an indication of wood tracheid dimensions or numbers, as samples with the same density and volume can have varying tracheid and cell wall dimensions (Kibblewhite, 1999).

A large amount of wood density variability occurs within each annual growth ring. This variability occurs in a consistent and predictable pattern (Megraw, 1985). Low wood density occurs at the start of the annual growth season and maximum density is reached near the middle of the latewood zone, with a definite downward trend in density at the end of the growth season (Megraw, 1985). Figure 2.2 provides an image from one of the samples of the study presented in this thesis to show the dramatic change in cell structure within a specific growth ring. Earlywood has a low density with larger cells and thin tracheid walls, while latewood has a higher density with smaller cells with thick tracheid walls.

The developmental trend of juvenile wood density in most pines is an increase in a radial direction from the centre (pith) to the outside (bark) of the tree (Zobel and Sprague, 1998). This principle generally applies within the tree regardless of tree height, providing a low density inner core for the whole length of the tree (Megraw, 1985).

This effect is directly linked to the age of the tree and is driven by increased portions of low-density earlywood versus lower levels of high density latewood in the early years, and a steady increase in the proportion of latewood with age (Megraw, 1985).

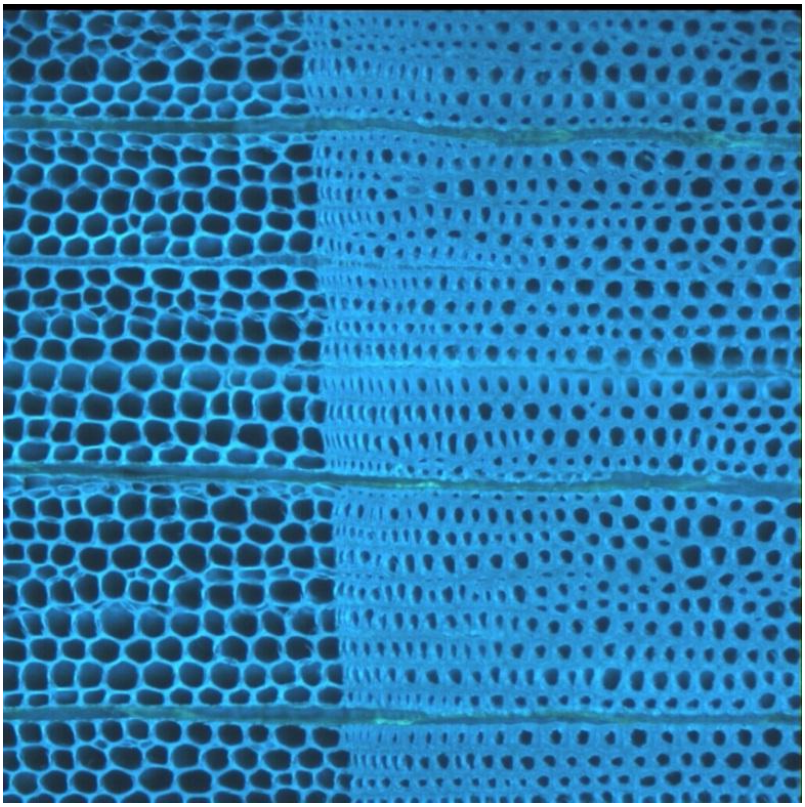

Figure 2.2 A *P. patula* growth ring from this study (scanned image of 100 × magnification). The lower wood density earlywood (towards the left of the image) has larger cells with thin tracheid walls, and the higher density latewood (towards the right of the image) has smaller cells with thicker tracheid walls.

2.5.1 Density assessment

In most cases where samples are regularly shaped, wood density can be determined easily and rapidly using dry weights and gravimetric methods (Malan and Marais, 1991). The volume of irregularly shaped samples can also be

determined using displacement methods in combination with determining weights using an electronic balance (Bowyer *et al*., 2007). These methods, however, only provide an overall density reading for the sample, whereas variation patterns within samples cannot be assessed (Malan and Marais, 1991).

The rapid and detailed assessment of wood density using radiation densitometry was first demonstrated in 1966 when X-ray radiography and an optical densitometer were used to determine density variation. Malan and Marais (1991) showed that utilising collimated soft gamma radiation, with an Americum^{241} energy source, could be used to accurately determine wood density in various wood species. Gamma ray densitometry therefore provides a rapid and highly reliable method of assessing wood density variation within a specific sample. Increment cores at breast height can be used for gamma ray densitometry and samples can therefore be collected in a non-destructive manner.

2.5.2 Genetic inheritance of wood density

In order to include any wood property into a selection programme, selected traits must be under strong genetic control. Various studies have shown that the inheritance of wood density is strong in many pine species (Birks and Barnes, 1991; Barnes *et al*., 1992b; Burdon and Low, 1992; Hodge and Purnell, 1993; Barnes *et al*., 1994; and Nyakuengama *et al*., 1999). The strong inheritance pattern for wood density in softwoods is demonstrated by a range of heritabilities of 0.5 to 0.7 (Zobel and Talbert, 1984). The relatively high heritabilities are also combined with a large variation pattern. Most of the genetic variability is of the additive type, facilitating large gains from selection within a breeding programme (Zobel and Jett, 1995). Very little work had been done on the inheritance of *P. patula* wood properties up until 1995. Zobel and Jett (1995) only reported on a

single study on *P. patula* in South Africa in 1978 in their review of the genetics of wood density.

A small number of genetic inheritance studies on Southern African grown *P. patula* wood properties were completed during the 1990's and 2000's. Birks and Barnes (1991) and Barnes *et al.* (1992b) reported on the genetic control of wood density and other growth traits in *P. patula.* They utilised samples of the same genetic material from diallel and factorial mating designs used in the study reported on in this thesis. They reported highly significant differences in pith to bark wood density and basic wood density between families and parents with additive genetic effects and without any specific or reciprocal effects.

In an open-pollinated *P. patula* family study conducted on six sites, Payn (2001) reported on the inheritance of wood density traits. In this study heritability for wood density at eight years of age ranged between 0.17 and 0.53 on single sites. Some evidence of genotype by environmental interaction was found, with type-B genetic correlations for paired sites ranging between 0.59 and 0.75, indicating that family performance was not stable across different sites.

Provenance and within provenance family effects on wood density have also been studied. In a detailed wood anatomical property study carried out by Stanger (2003) in South Africa on a single site, 972 trees from 12 provenances and 108 open-pollinated families of *P. patula* were sampled. Sampling was done in a non-destructive manner at 10.5 years by removing increment cores at breast height (1.3 m above ground level). Wood density and anatomical properties were measured using gamma ray densitometry and image analysis. This *P. patula* material represented the most complete coverage of the species in its natural range in Mexico. The results from this study indicated that provenance differences were strong for most properties including wood density traits, with large variation between families. The additive genetic control of wood properties

varied from zero to moderately strong for specific traits. The study concluded that moderate gains would be possible from direct selection on selected wood property traits, including wood density (Stanger, 2003). In this study wood density traits displayed sufficient variation and genetic control to allow for progress in a breeding programme.

In another *P. patula* open-pollinated family study across various sites in South Africa, Vermaak (2007) reported on a genetic study on wood density and tracheid traits. Significant differences were found between locations for growth traits, density and tracheid properties at eight years of age. High heritabilities for density were found for pith to bark core samples of the best performing 30 families, with an average family heritability across six sites of 0.80 and standard error of 0.10.

2.6 Tracheid cross-sectional properties

The main cell types in softwoods consist of tracheids and ray parenchyma. The majority of softwood wood volume is made up of long, slender cells called longitudinal tracheids, which are orientated parallel to the stem axis and are separated by a middle lamella (Smook, 1986; Bowyer *at al.*, 2007). Cell walls in softwoods are important structures. Unlike the lumen, which is a void space, the cell wall itself is a highly regular structure (Wiedenhoeft and Miller, 2005). The cell wall consists of three main regions; the middle lamella, the primary wall, and the secondary wall. In each of these regions, the cell wall has three major components; cellulose microfibrils, hemicelluloses, and a matrix of encrusting material, typically pectin in primary walls and lignin in secondary walls (Wiedenhoeft and Miller, 2005).

Radial variation exists in most cell characteristics. In softwoods, growth rings near the pith usually consist of a large proportion of earlywood tracheids with larger

diameters and thinner cell walls than latewood tracheids (Lachenbruch *et al*., 2011). This is followed by a gradual transition to a larger proportion of latewood tracheids with increasing ring number from pith to bark (Lachenbruch *et al*., 2011).

A number of tracheid cross-sectional properties are considered to be important factors in the pulp and paper process. Tracheids make up the principal papermaking fibres in softwood Kraft pulp (Muneri, 1994). Barefoot *et al.* (1964) list cell wall thickness, cell lumen diameter, the Runkell ratio (ratio between wall thickness and lumen diameter) and earlywood to latewood proportion as important factors in paper making. Because earlywood tracheid diameter increases with cambial age, it has an effect on the variation in wood density (Lachenbruch *et al*., 2011).

Tracheid properties directly influence the pulp fibre qualities that are produced during the Kraft pulping process. Papermaking properties are dependent on the structure of the various pulped fibres. According to Smook (1986), the two most important structural tracheid characteristics are length and cell wall thickness. A minimum tracheid length is required for inter-fibre bonding in pulp where the fibre length is proportional to the tear strength of sheets. Fibres with thinner cell walls collapse easier during sheet formation and contribute more to inter-fibre bonding than thicker walled fibres (Smook, 1986). Thicker walled fibres produce more open, absorbent bulky sheets with low burst and tensile strength, but with high tear resistance (Smook, 1986). Paper produced with thick-walled fibres tends to have poor printing surfaces and have poor burst strength (Zobel and van Buijtenen, 1989).

The relationships between different cell properties can also be an important characteristic to consider in assessing pulp quality. The Runkel ratio is the ratio of

two times the tracheid cell wall thickness divided by the tracheid lumen diameter. This is an important indicator of the suitability of tracheids for papermaking (Dinwoodie, 1965; Ogbonnaya *et al.*, 1992). The Runkel ratio gives an estimate of the collapsibility of tracheids and has been found to be the best single predictor for various paper sheet properties such as burst factor, breaking length (tensile strength) and tear (Barefoot *et al*., 1964; Evans *et al*., 1997; Saikia *et al*., 1997).

As found with wood density, large variation in cross-sectional tracheid properties within a specific tree also exists. The main source of variation is the earlywood versus latewood sections within each growth ring. Earlywood cells have thin cell walls and generally larger lumen radial diameters, while latewood cells have thick cell walls and smaller lumen radial diameters (Zobel and van Buijtenen, 1989). The tangential cell width within the growth ring is usually constant (Zobel and van Buijtenen, 1989). There are also differences between juvenile and mature wood, where cell walls are thinner in juvenile wood and increase in wall thickness in a linear direction from pith to bark (Kibblewhite, 1980; Zobel and van Buijtenen, 1989; Cown, 1992). Although there is a wide range of different cell sizes and cell wall thicknesses present in any tree, uniformity can be improved by breeding (Zobel and van Buijtenen, 1989).

2.6.1 Assessment of cross-sectional tracheid properties

Plant cell walls were first viewed microscopically during the seventeenth century when Robert Hooke viewed images of cork cell walls using a self-built compound microscope (Harris, 2006). Only in the twentieth century did the terms primary and secondary walls become known, and that cell expansion was related to the thickness and morphology of cell walls (Harris, 2006). Kerr and Bailey examined softwood tracheids in 1935 using polarised light microscopy, They discovered that the secondary walls of softwood tracheids had a three-layered structure,

which they named in the order in which they are laid down; S1, S2, and S3 (Harris, 2006).

The study of wood cell anatomy is done at the microscopic level. Rapid advances in digital image analysis have over recent years greatly aided the assessment and characterisation of anatomical properties of wood cells (Hirn and Bauer, 2006). Cross-sectional tracheid properties such as cell wall thickness and lumen diameter are assessed with an automated analysis system consisting of a microscope, fluorescence light-source, video camera and integrated image-analysis software (CSIR, 2010). Wood samples are prepared and the scanning is carried out with the samples aligned in a transverse view, looking at cross-sections of the vertically aligned tracheids. Increment cores can be used and sampling can, therefore, be carried out in a non-destructive manner. Evans *et al*. (1995) also reported on an automated wood microstructure analyser and combined gamma x-ray densitometer, SilviScan, which was developed for the rapid assessment of both cross-sectional cell properties and wood density on the same samples. To gain a reliable estimate of wood cell anatomical characteristics, measurements have to be taken on many cells per sample.

2.6.2 Genetic inheritance of cross-sectional tracheid properties

The number of studies on genetic inheritance of tracheid cross-sectional properties is limited, probably because of the high cost and difficulty of the assessment. Zobel and Jett (1995) report in their overview of tracheid properties that studies of the genetics of cell components are very scattered and many results appear to be inconclusive or contradictory. The results of many of these studies were also influenced by the limited extent of the genetic base and mating structure, and small sample size and replications.

Studies have shown moderate to strong genetic control of tracheid characteristics, however strong environmental effects were also found in some studies (Zobel and Jett, 1995). One of the earliest studies of the inheritance of tracheid cross-sectional traits was conducted by Goggans (1964), who investigated traits in *P. taeda* such as wall thickness, lumen diameter, radial and tangential cell width, tracheid length, and also wood density traits. Traits such as latewood tracheid length, percentage latewood in the core and wood density in the core were found to be highly heritable and could be selected for in breeding programmes (Goggans, 1964). Although heritabilities in this study were high, the low number of parents (6 – 8) sampled limited the value of these heritability estimates. Correlations between cross-sectional properties and wood density were also shown in this study (Goggans, 1964).

A number of studies have been carried out on *P. radiata*. In an open-pollinated *P. radiata* study, Shelbourne *et al*. (1997) reported on cross-sectional tracheid properties using the SilviScan system. Heritablilites between 0.5 and 1.0 were found for tracheid dimensions and basic density. Estimated potential genetic gains for tracheid cross-dimensional traits were between 8 and 12% per generation (Shelbourne *et al*., 1997). In another *P. radiata* study using a 4 × 4 diallel mating design, Nyakuengama (1997) conducted a study of the quantitative genetics of wood traits including tracheid cross-sectional traits and wood density. Results from this study were reported in Nyakuengama (1997) and follow-up studies in Nyakuengama *et al*. (1998; 1999; 2000). In these studies it was found that density was inversely related to fibre radial and tangential diameter, tracheid diameter and density were highly heritable traits, and that early wood properties were strongly heritable (Nyakuengama *et al*., 1998; 1999; 2000). The relatively small number of parents used in these studies should, however, be taken into account (Stanger, 2003).

A number of studies reported on heritabilities of cell characteristics for *P. taeda.* Belonger (1998) reported on a study of *P. taeda* provenances and families. There were large differences in cell wall thickness and cell diameter and relatively high narrow-sense heritability estimates of 0.34 for total cell diameter, 0.22 for lumen diameter, and 0.37 for cell wall thickness.

In the wood anatomical property study carried out by Stanger (2003) on different provenances of *P. patula* grown in South Africa, results indicated that the additive genetic control of tracheid radial diameter were moderately strong (h^2 = 0.51). The findings from this study, contrary to most reports in the literature, showed that cell wall thickness in *P. patula* were under very weak or negligible additive genetic control (Stanger, 2003). Vermaak (2007) reported high family and individual tree heritability estimates for fibre morphological characteristics of *P. patula* in South Africa, such as cell wall thickness, lumen diameter and Runkel ratio, although these were associated with large standard errors.

2.7 Tracheid length and width

The great majority of softwood volume is composed of long, slender cells called longitudinal tracheids which are oriented parallel to the stem axis. Softwood tracheids produce much longer pulp fibres than hardwoods, and have the largest influence on the strength properties in paper products (Mimms, 1993). Strong relationships between the properties of tracheids and the properties of Kraft pulp handsheets have been demonstrated (Dinwoodie 1965). Pulp and paper made from softwood contain tracheids of various dimensions and properties, which depend on the pulping process employed (Vermaak, 2007).

Tracheid length is the most important characteristic to take into account in papermaking, because long pulping fibres can make more bonds with other fibres in the handsheet network (Niskanen, 1998). Furthermore, tracheid length and cell wall thickness are two important properties determining the final product value. Tracheid length becomes particularly important in juvenile wood of some conifers (Zobel and van Buijtenen, 1989).

There is a close relationship between fibre properties and other wood properties such as density, and also with post-processing pulp and paper properties (Zobel and Jett, 1995), but Lachenbruch *et al*. (2011) report that tracheid length and shape have relatively minor effects on wood density.

Papermaking properties such as tensile strength, breaking strength, and burst strength of paper are influenced by tracheid length. In a study conducted by Lecourt *et al*. (2006) on the effect of fibre morphology on pulp properties of Norway Spruce, tracheid length had a negative effect on tensile strength and a positive effect on tear strength. Tracheid width had a negative effect on opacity; showing that the larger the width, the lower the opacity. Tracheid wall thickness also negatively influenced brightness and scattering, but positively influenced opacity.

Tracheid length varies greatly both within and among trees. Dinwoodie (1961) reports on various studies within one growth ring. Significant differences between the tracheid length of earlywood and latewood were found, with greater tracheid lengths in latewood. There is also a radial increase in tracheid length from pith to bark with tree age, with a rapid increase within the first few rings, and then a more steady increase until a maximum tracheid length is reached (Dinwoodie, 1961; Wheeler *et al*., 1966). Tracheid length also varies with increasing height up the tree; length increases upwards for a certain distance before decreasing

progressively towards the top of the tree (Dinwoodie, 1961). Ishengoma *et al*. (1995) also reported on this variation in tracheid length up the length of the tree stem in *P. patula* grown in Tanzania.

Tracheid length, unlike wood density, is influenced by the growth rate of a tree, with faster growth producing shorter tracheids. The length of a newly formed tracheid is determined by the length of the cambial initial from which it is derived (Megraw, 1985). For *P. patula* grown in Zimbabwe, Muneri and Balodis (1998) reported that age and site effects on tracheid length were substantial. It is also important to note there is always a range of different tracheid length sizes at any given point; tracheids are never of uniform size. This can be explained by the fact that tracheids at the top of every growth ring are shorter than at the start of the growth ring (Dinwoodie, 1961).

2.7.1 Assessment of tracheid length and width

Tracheids are small in size and there are a large number of tracheids per unit area. Because they are very small in size, they need to be assessed with some form of magnification. Traditionally, length assessments are carried out on whole tracheids obtained by maceration of wood samples. Typically, the Franklin maceration method would be used with equal volumes of glacial acetic acid and 30% hydrogen peroxide (Franklin, 1945; Dodd, 1986). Historically, tracheids were assessed using microscopes with a calibrated micrometer eyepiece. Projector microscopes were also used which project images onto a screen where they were measured manually (Muneri, 1994). Only intact tracheids that were not cut or damaged during the sampling or maceration procedure would be assessed (Zobel and Jett, 1995). These methods were very time consuming and limited the number of measurements per sample, thus limiting the tracheid representation of the sample material (Zobel and Jett, 1995). In many of the earlier tracheid length

studies, as few as 50 fibres per sample were assessed, and this is listed by Zobel and Jett (1995) as one of the shortcomings of earlier inheritance studies.

Assessment technology has advanced rapidly in recent years. A number of fully automated, optical and electrical devises with image analysis systems are now available. Examples of these analysers are the Kajaani FS-100 and FS-200 and MorFi Analyser, with the capacity to rapidly assess many thousands of fibres per sample (Jackson, 1988; Muneri, 1994; Robertson *et al*., 1999; Turunen *et al.*, 2005; Hirn and Bauer, 2006). Some of these systems, such as the MorFi Analyser, also assess additional traits such as fibre length distribution, fibre coarseness, width, kinks, curls, number of fibres per gram of fibres, area of fines, and surface area and length of shives (Turunen *et al.*, 2005).

Increment cores are often used for the non-destructive assessment of softwood tracheid properties. Larger diameter increment corers (10 – 12 mm) should be used to collect samples for these assessments (Zobel and Jett, 1995). This yields larger proportions of un-cut fibres for assessment of fibre properties.

2.7.2 Genetic inheritance of tracheid length and width

The inheritance of tracheid length is the most studied wood property trait after wood density. As in the case of tracheid cross-sectional properties, Zobel and Jett (1995) report that studies of the genetics of tracheid length are scattered with inconclusive or contradictory results. As in the case of tracheid cross-sectional properties, Zobel and Jett (1995) ascribe this to the limited genetic base, lack of a mating structure and limited sampling and replications that formed the basis of these studies. Despite these shortcomings, Zobel and Jet (1995) stated that the reported heritabilities in the reviewed studies are high enough and variation broad

enough to enable good genetic gains through breeding. Most tracheid properties are relatively strongly inherited, especially tracheid or fibre length; and have been widely studied (Zobel and van Buijtenen, 1989).

Studies have found that tracheid length is under genetic control. In a study conducted on *P. elliottii* and *P. taeda,* Jackson and Greene (1958) reported on tracheid length variation and inheritance. Their findings demonstrated that there was considerable variation in the length of tracheids within trees and between parent trees, and that the heritability was high enough to allow for the breeding of long or short fibres. In another study conducted with *P. radiata* clones, Nicholls (1967) found that heritabilities for tracheid length were highest closer to the pith, and there was a decline in the latter growth rings. In another of the few studies conducted on full-sib material, Barnes *et al*. (1994) reported that tracheid length, together with ring width and wood density, was under genetic control. The Stanger (2003) study on various *P. patula* provenances and families grown in South Africa showed contrary results. Tracheid length was under weak additive genetic control. Although there were statistically significant differences between provenances, he asserted that these differences were unlikely to offer any practical value (Stanger, 2003).

2.8 Sources of variation in wood properties

Wood is a widely variable raw-material used in the industrial process of pulp and paper making. Large differences occur between different species, different provenances of the same species, different families, and different trees within the same family and even within individual trees (Zobel and van Buijtenen, 1989). Variability between trees of the same species is especially large and is under genetic control (Zobel and Buijtenen, 1989).

All wood properties, including physical wood properties such as wood density and tracheid properties, have extensive levels of variation within and between trees. Zobel *et al*. (1960) and Zobel and Talbert (1984) list the following sources of variation:

a) Differences between softwood species and genera;
b) Differences between populations and sources of a species at a locality;
c) Differences between trees within the same species;
d) Differences between the same trees grown at different localities (site effects); and
e) Differences within a specific tree, from pith to bark as well as within the length of a tree.

When a wood property study is undertaken, it is vitally important to specifically take the within tree variation into consideration. The large differences in properties within a growth ring and between juvenile and mature wood has already been discussed in previous sections. The proportion of juvenile wood is largely determined by the specific species and the harvesting age. Zobel and Sprague (1998) reported on a study of *P. taeda* where the percentage juvenile wood, based on volume, varied from 85% at age 15, to 55% at 25 years and 19% at 40 years. The same trend was apparent for a study on *P. patula* grown in Kenya, where juvenile wood was found to make up 60% of the volume at 18 years and 49% at age 27 (Zobel and Sprague, 1998). Wright (1994) reported on studies conducted with *P. patula* grown in Africa that the juvenile core can persist till around 8 years of age. Other studies undertaken in Southern Africa indicated that the juvenile core can persist until the age of 12 years (Wright, 1994). Gentically improved trees are often harvested at reduced harvesting ages, but these volumes will be made up of higher proportions of juvenile wood.

2.8.1 Sampling methodology

The different levels of variation present within trees have to be taken into account when sampling is undertaken. When a wood property study is undertaken, it is important to take account of the different aspects of variation within the tree in order to reflect the properties of the whole tree. There is a large body of work available on estimating whole tree values using a single sampling point. The convention in forestry is to sample for wood properties at breast height, 1.3 m above ground level (Zobel and Jett, 1995). Evans *et al.* (1999) reports in a study using increment cores of *P. radiata* trees, that sampling at breast height gave suitable estimates of the whole tree properties. Ladrach (1984) demonstrated good relationships between sampling at breast height and the whole tree for wood density and tracheid length in *P. patula* grown in Columbia. Ringo and Klem (1980) indicated that increment cores at breast height from 25-year-old *P. patula* grown in Tanzania gave a good estimate of whole tree wood density. Zobel and Jett (1995) reported on the combined results from 33 studies on sampling tree height and concluded that the correlation between breast height sampling and whole tree properties were high. The use of a standard sampling height also enables the researcher to compare results from different studies.

In many studies, correlations between sampling point and the whole tree properties have been conducted. In these studies, trees are destructively sampled at various heights on the same trees and compared with assessment at breast height. When trees that are close to their rotation age are sampled at breast height, both juvenile and mature wood are included in the sample. Higher correlations are therefore found between whole tree and breast height sampling. Destructive sampling of trees result in the permanent loss of the genotype, and developing a reliable non-destructive method remains a challenge (Zobel and Jett, 1995).

Chapter 3

Outline of genetic material, sampling strategy and genetic analysis

3.1 Introduction

Trees that are selected for breeding purposes are usually selected based on their phenotype; usually those that appear to be superior for specific traits. Traits of interest can be under different levels of genetic control and different gene action. After selection, their genetic worth has to be determined through testing of their progeny. Genetic testing of selected, superior individuals is an important component of any aggressive and successful tree improvement programme and forms part of the breeding strategy for a specific species (Zobel and Talbert, 1984). The accepted way to test the genetic value and breeding value of selected parents is to grow and evaluate their progeny or clones in seedling or clonal tests (White *et al*., 2007). Genetic testing informs the plant breeder on what selection strategy to follow.

A proper understanding of genetic effects on important selection traits is needed to compile an optimal breeding strategy (Zobel and Talbert, 1984). Genetic testing is one of the most expensive components of a tree improvement programme, but has the biggest impact on the value of genetic improvements obtained (Wright, 1976; Zobel and Talbert, 1984). The selection of an appropriate mating design for the genetic testing of superior individuals will determine the type of genetic information that is generated by the tested progeny.

3.2 Mating designs

The main aims of selective mating are to provide the following; information to evaluate parents, estimates of genetic gain and genetic parameters, and generate a population base for future selection (Bridgwater, 1992; White *et al*., 2007). Genetic testing provides estimates of the amount of variation in specific traits and their genetic control. Genetic interrelationships among traits for one or more populations in a breeding cycle can also be determined (White *et al*., 2007).

Mating designs can be grouped into two broad categories; incomplete-pedigree designs and complete-pedigree mating designs (White *et al*., 2007). Examples of incomplete-pedigree mating designs are bulk collections, open-pollenated designs and polycross (polymix or pollen mix) designs (Zobel and Talbert, 1984). With open-pollinated designs, only the maternal parent is known. Polycross designs, on the other hand, refer to the use of a mix of known pollens applied to selected female parents. Mating designs specify exactly how parents are inter-mated to create the progeny (White *et al*., 2007). Incomplete-pedigree mating designs such as open-pollinations and polycross designs can provide estimates of additive genetic variance and heritability values for the tested population. Because only one parent is known, non-additive genetic variance estimates and specific combining ability cannot be obtained from these designs (Zobel and Talbert, 1984).

Complete pedigree designs, or full-sib designs, involve controlled pollinations in which both parents are selected and are therefore known (Zobel and Talbert, 1984). These designs are completed using artificial or controlled pollinations to create the progeny. Female flowers are isolated to exclude any unknown pollen or pollen vectors, and selected pollen is applied to isolated flowers. The specific full-sib mating design will determine the level and quality of genetic information

generated in the testing programme. For the purposes of this reported study, a short overview of factorial and diallel designs is given. An explanation of some of the terminology used in these mating designs is given first.

3.2.1 Genetic analysis terminology

The term combining ability analysis refers to a method of evaluation of a set of crosses among selected parents. This is done to determine the extent to which variances among crosses are attributable to statistically additive characteristics of the parents (Acquaah, 2007). Combining ability therefore provides an indication of the level of performance of a selection in a controlled cross and the gene action involved. An assessment of combining ability can be useful to define the contribution of each specific parent to the performance of their progeny (White *et al*., 2007). Combining ability can be subdivided into two terms, general combining ability (GCA) and specific combining ability (SCA).

GCA is defined as the additive genetic contribution of a selected parent to its progeny (Van Buijtenen, 1992). This mean performance is expressed as a deviation from the mean of all crosses in the mating design (Falconer and Mackay, 1996). A specific cross, however, may deviate positively or negatively from the expected GCA value (Falconer and Mackay, 1996). SCA is therefore defined as the deviation of a specific combination of two parents from the sum of their GCA effects (Van Buijtenen, 1992). The GCA of an individual is equal to half of its superiority over the population mean. A family with a good general combining ability has good progeny regardless of the other parent as it possesses many alleles with positive additive contributions to its breeding value (Van Buijtenen, 1992). A cross with good specific combining ability produces progeny that strongly deviates from their expected GCA and usually implies the

presence of dominance or epistasis (Van Buijtenen, 1992). The GCA of an individual is equivalent to half of the individual's breeding value.

During an analysis of variance (ANOVA), progeny results from a mating design are used to partition the variance. Variance components associated with different causes are partitioned into compartments (Van Buijtenen, 1992). The following components are of particular interest to the breeder: phenotypic and genetic variance, additive genetic variance, non-additive genetic variance and environmental variance (Van Buijtenen, 1992). Non-additive genetic variance is caused by dominance and epistasis, and environmental variance is caused by environmental or site effects.

These components provide the opportunity to calculate the strength of the inheritance for a specific trait, the heritability. Heritability is the ratio of genetic variance to phenotypic variance (Van Buijtenen, 1992). Heritability can also be seen as a concept of reliability of the phenotypic value of a plant as a guide to the breeding value of a metric trait (Acquaah, 2007). The different variance components allow for the calculation of a number of different heritabilities. Narrow sense heritability (h^2) is calculated as the ratio of the additive genetic variance to the phenotypic variance (V_a/V_p), and broad sense heritability (H^2) is the ratio of total genetic variance to the phenotypic variance (V_g/V_p) (Zobel and Talbert, 1984; Van Buijtenen, 1992; Falconer and Mackay, 1996). Heritability can also be expressed as individual tree heritability (h_i^2) when it is based on individual trees and as family heritability (h_f^2) when it is based on family means (Van Buijtenen, 1992).

It is important to note that heritabilities have to be interpreted in the correct context. Each heritability estimate is specific to the population, trait and environment on which it is based, and cannot be applied on a broader scale.

Narrow-sense heritability is used where sexual recombination occurs and where additive effects are most important. Broad-sense heritability is particularly appropriate for vegetatively propagated material (Van Buijtenen, 1992). Heritabilities generally have large errors associated with them and are affected by environmental variance caused by a variable site, poor site preparation or weed control (Van Buijtenen, 1992). The type of mating design will determine the value and quality of the estimates of heritability, general combining ability (GCA) and specific combining ability (SCA).

3.2.2 Factorial mating designs

The factorial mating design requires the mating of each member of a group of one sex with each member of a group of the other sex (Bridgwater, 1992). An example of a 5×5 factorial design would have female parents 1, 2, 3, 4 and 5 crossed with male parents A, B, C, D and E; totalling 25 specific crosses. This is an example of a square factorial where the parents are divided into two equal groups. Factorial designs can also consist of a few tester families crossed with a larger number of other families from a population (White *et al*., 2007). Factorial mating designs can estimate breeding values for all genotypes in the population and provide good estimates of GCA and SCA for the specific crosses that are made (Zobel and Talbert, 1984). One disadvantage is that the number of unrelated progeny that can serve as parents for the next generation of breeding is limited by the number of parents used.

3.2.3 Diallel mating designs

The full diallel mating design requires the mating of each parent with every other parent, including reciprocals and self-pollinations (Bridgwater, 1992). This is the most comprehensive and most costly mating design possible, but also provides the maximum amount of information on heritability within the population (Zobel and Talbert, 1984; Bridgwater, 1992; White *et al*., 2007). In certain diallel designs, where plant species have barriers to self-pollination, the self-pollinations of diallel mating designs are not carried out. Half-diallel designs where reciprocal crosses are omitted are also sometimes used (White *et al*., 2007). With half-diallels, breeders assume that reciprocal effects are not important. The performance of a parent's progeny is therefore not influenced by whether it is used as a female or male parent (White *et al*., 2007). The diallel mating design is routinely used in plant breeding research to obtain genetic information such as general combining ability (GCA) and specific combining ability (SCA) and an estimate of the genetic effects of parents (Zhang and Kang, 1997).

3.3 Genetic material used in this study

During 1965 and 1966, comprehensive *P. patula* polycross, factorial and diallel mating designs were proposed by the Zimbabwe Forest Commission (ZFC) (Burley *et al*., 1966). These designs were implemented by the ZFC from 1965 to 1967. For the purposes of this reported study, only details of the factorial and diallel designs completed in 1965 and 1966 are given. The factorial design consisted of five of the tester clones used as male parents and 9 different clones were used as female parents. A complete diallel between five of the tester clones, with reciprocals but without self-pollinations, was also completed (Barnes and Schweppenhauser, 1979). Figure 3.1 provides a schematic representation of

these two designs, with those crosses utilized in the present study, highlighted in bold.

Care was taken when clones were selected for inclusion in these mating designs. Clones were randomly selected from the plus tree population with the only proviso being adequate flowering, and were therefore representative of this population (Barnes, 1973). Full-sib seed from these controlled pollinations were used to establish progeny trials in 1968 on a number of different sites in Zimbabwe. Seed was sown during October/November 1967 in the Rupere Nursery at the John Meikle Forest Research Station near Mutare. Seedlings were grown in plastic tubing filled with uniform nursery soil mix (Barnes, 1973).

In the case of the factorial mating design, the nursery tubes were placed in plots according to a nursery experimental design. Early nursery traits such as cotyledon number and length, seedling height, and branch and whorl number were assessed while in the nursery and reported in Barnes (1973) and Barnes and Schweppenhauser (1978). The nursery plots and tree positions were carried over to the field trials so that direct nursery to field correlations could be made (Barnes, 1973). When seedlings were ready for planting, a number of field trials were established on four different sites in the three main forestry areas of the Eastern Districts of Zimbabwe.

		Male parent						
		5	**25**	**14**	**20**	**31**	**44**	**51**
Female parent	**14**				**X**	**X**	**X**	**X**
	20			**X**		**X**	**X**	**X**
	31			**X**	**X**		**X**	**X**
	44			**X**	**X**	**X**		**X**
	51	X	X	**X**	**X**	**X**	**X**	
	1	X	X	**X**	**X**	X		
	2	X	X	**X**	**X**	X		
	7	X	X	**X**	**X**	X		
	15	X	X	**X**	**X**	X		
	26	X	X	**X**	**X**	X		
	27	X	X	**X**	**X**	X		
	32	X	X	**X**	**X**	X		
	48	X	X	**X**	**X**	X		

Figure 3.1 Schematic representation of Diallel (5×5) and Factorial (9×5) mating designs of *P. patula* completed by the Zimbabwe Forest Commission and described in Barnes (1973). Only crosses highlighted in bold were included in this study.

The sites where progeny trials were planted consisted of different types of environments. The first site, Stapleford, was an optimal site for *P. patula* where it reaches its optimum development. The second site was at Martin, which is at lower altitude and hotter climate where the species has better early growth performance. The third environment was at higher altitudes at Nyangui, where *P. patula* is physiologically well adapted, but has slower growth. A map of Zimbabwe with the two trial sites at Martin and Nyangui included in this study, is presented in Figure 3.2.

Site and trial details for the progeny trial series No 7 planted in the 1968 and 1969 planting seasons are provided in Table 3.1. Trials 7A and 7B contained material from both the factorial and diallel mating designs were planted in a 8 × 8 triple lattice design with three replications of 10-tree row plots. Material from these mating designs were planted together as there was some overlap in the factorial and diallel designs as can be seen in Figure 3.1.

Trials 7C and 7D contained only the diallel design and consisted of 23 treatments. These trials were planted as randomised complete blocks with three replications and 10-tree row plots.

The progeny trials were assessed for growth traits, height and diameter at breast height (DBH) at 1.5, 5 and 8 years after planting. Growth results from these assessments were reported on by Barnes (1973), Barnes and Scweppenhauser (1979) and Barnes *et al*. (1992a; 1992b). After the 8-year assessment was completed, all trials received a 50% systematic thinning. Every other tree in the 10-tree row plot was removed, leaving the remaining trees at double-spacing and stocking of five trees per plot (Birks and Barnes, 1991). Trees that were removed during this thinning were not selected for poor growth or form. Good and poor trees were removed according to the lay-out in the plot.

Figure 3.2 Map of Zimbabwe showing the two progeny trial sites Martin and Nyangui in the Eastern Highland forestry area where samples were collected for this presented study (adapted from Ezilon.com).

Table 3.1 List of progeny trials established in 1968 with controlled pollinated material from the Zimbabwe Forest Commission *P. patula* diallel and factorial designs. Progeny tests **7B** (Martin) and **7C** (Nyangui) were included in the present study (adapted from Barnes and Schweppenhauser, 1979).

	Locality		
Site details:	**Martin**	**Stapleford**	**Nyangui**
Latitude (^{o}S)	19^{o} 45'	18^{o} 40'	18^{o} 00'
Altitude (m)	1265	1770	1880
Mean annual rainfall (mm)	1082	1750	1573
Mean monthly maximum temperature (^{o}C)	22.9	19.2	17.7
Mean monthly temperature (^{o}C)	17.7	15.1	13.0
Mean monthly minimum temperature (^{o}C)	12.6	11.1	8.3
Soil (parent material)	dolerite	granite	dolerite
Previous land use	1st rotation pine	1st rotation pine	Shifting cultivation
Trial design details:			
Trial number	**7B**	7A	**7C**, 7D
No of families	64	64	23
Mating design	5×5 diallel & 9×5 factorial	5×5 diallel & 9×5 factorial	5×5 diallel
Trial design	8×8 lattice, 3 replications	8×8 lattice, 3 replications	RCB, 3 replications

Wood sample discs 15 mm thick were taken at breast height (1.3 m above ground level) from all the thinned trees. Radial wedges were cut from the discs at a point of average radius, avoiding any abnormal wood present in the disc. Sample wedges were 15 mm thick, and ranged in length between 40 and 100 mm, depending on the diameter of the sampled trees. A study of the genetic control of pith to bark wood density was conducted using these wood samples during the late 1980's and was reported on by Birks and Barnes (1991).

During 2002, a complete set of duplicate wood samples and trial data of all these progeny trials were donated to Sappi by Dr Richard Barnes, from the Oxford

Forestry Institute. Permission was also granted to Sappi by the Zimbabwe Forest Commission to make use of these samples and data to conduct a wood properties study. A selective set of wood samples from these mating designs were utilised in the study reported on in this thesis.

The genetic structure of this material allows for the isolation of both maternal and paternal effects. This gave a unique opportunity to study the inheritance of various physical wood property traits of *P. patula* simultaneously on the same individual wedge samples.

3.4 Sampling strategy with wedge samples

One of the major considerations in any wood property study is the high cost of characterising different chemical and physical wood properties (Zobel and Talbert, 1984). Careful selection and planning therefore has to be conducted to ensure maximum value, while containing costs. With a limited budget available for this study, the sampling strategy was driven by the main objectives of this study:

1) Determine level of genetic control of a range of important density, anatomy and tracheid properties;
2) Determine the general and specific combining abilities of the genetic material for the range of density, anatomy and tracheid properties;
3) Determine if any reciprocal differences are evident for the studied properties:
4) Determine the broad and narrow sense heritability for the measured and calculated properties and components.
5) Determine the correlations between the different density, anatomy, tracheid and growth properties;

With these objectives in mind, the following strategy was followed. Two trials were selected at the extremes in terms of altitude, climatic conditions and growth potential for the species *P. patula*. Samples from trial 7B planted at Martin was selected for a full-diallel analysis. An additional 16 crosses from the factorial design was also included at this site (see Figure 3.3). The 5×5 diallel produced a limited number of crosses, which would limit the value of genetic parameters such as heritability estimates. The inclusion of these additional 16 crosses would improve the value of genetic parameters.

Another progeny trial (7C) was selected at the other extreme in altitude and climatic conditions. At the Nyangui site, samples were included from only the bottom half of the diallel design (see Figure 3.4).

		Male parent				
		14	**20**	**31**	**44**	**51**
Female parent	**14**		X	X	X	X
	20	X		X	X	X
	31	X	X		X	X
	44	X	X	X		X
	51	X	X	X	X	
	1	X	X			
	2	X	X			
	7	X	X			
	15	X	X			
	26	X	X			
	27	X	X			
	32	X	X			
	48	X	X			

Figure 3.3 Full diallel mating design with reciprocals and selected additional factorial crosses from trial 7B at Martin at altitude of 1265 m above sea level.

		Male parent				
		14	20	31	44	51
Female parent	14					
	20	X				
	31	X	X			
	44	X	X	X		
	51	X	X	X	X	
	1					
	2					
	7					
	15					
	26					
	27					
	32					
	48					

Figure 3.4 Half-diallel crosses (without reciprocals) of trial 7C at Nyangui at altitude of 1880 m above sea level.

The samples from the two trials would allow for a full diallel analysis. Effects such as combining ability, reciprocal, maternal and non-maternal effects can therefore be determined. A comparison of the two half-diallel designs at the two extreme sites would allow for a study of the effect of site on the different physical wood properties. Although not the main purpose of this study, it would also give an indication of any interaction between the genetic effects and the site effect.

Due to cost constraints, the number of samples to include per cross also had to be carefully considered. A total of between 12 and 15 radial wedge samples were available per cross for every trial. Each 10-tree plot in the three replications received a 50% (5 tree) thinning, allowing for some mortality of trees at 8 years of age. Sampling size was guided by the reasoning as set out in Stanger (2003) in determining the optimum family sampling size. With full-sib families, a coefficient of relationship (relatedness) of 0.50 is used. Heritabilities of 0.3 to 0.8 are often reported for physical wood property traits. Use was made of these theoretical heritabilities and the coefficient of relationship to calculate the optimal sample

size. A sample size of six per cross was selected for the full-diallel plus selected crosses from the factorial design from the Martin (7B) trial. At the Nyangui trial (7C), eight samples per cross was sampled for the half-diallel design. These numbers of samples were selected at random from all three replications of the original field-trial designs.

The sampling procedure followed in this reported study is outlined in Figure 3.5. The selected wedges were sent to the Council of Scientific and Industrial Research (CSIR), Forests and Forests Products laboratory in Durban, South Africa. The CSIR determined pith to bark density and cross-sectional tracheid properties on all samples. Sample components were then returned to Sappi. NIR spectroscopy was carried out on all samples. Samples were then chipped and macerated at the Shaw Research Centre near Howick, South Africa. Macerated samples were then taken to the Sappi Technology Centre in Pretoria, South Africa. The determination of tracheid properties using the MorFi fibre analysis system was carried out. The detailed procedures and methods for determining the different physical wood properties will be discussed in more detail in the following chapters.

3.5 Assumptions, constraints and limitations of this study

Certain assumptions had to be made for this study. The parents that were included in the different mating designs in this study were from a population of selections based on mature age phenotypes selected for growth, tree form and adaptive traits under Zimbabwean growth conditions. They are, therefore, not a random sample representing the species *P. patula* grown in Southern Africa. South African and Zimbabwean *P. patula* populations do, however, have similar origins (Barnes, 1973).

The parent selections were, however, randomly selected from the reference selection population. Parents included in the mating designs are also assumed to be unrelated, based on the reported selection differential of 1 out of 100 000 trees made in local stands of *P. patula* grown commercially (Barnes, 1973). Their unrelatedness could not be verified.

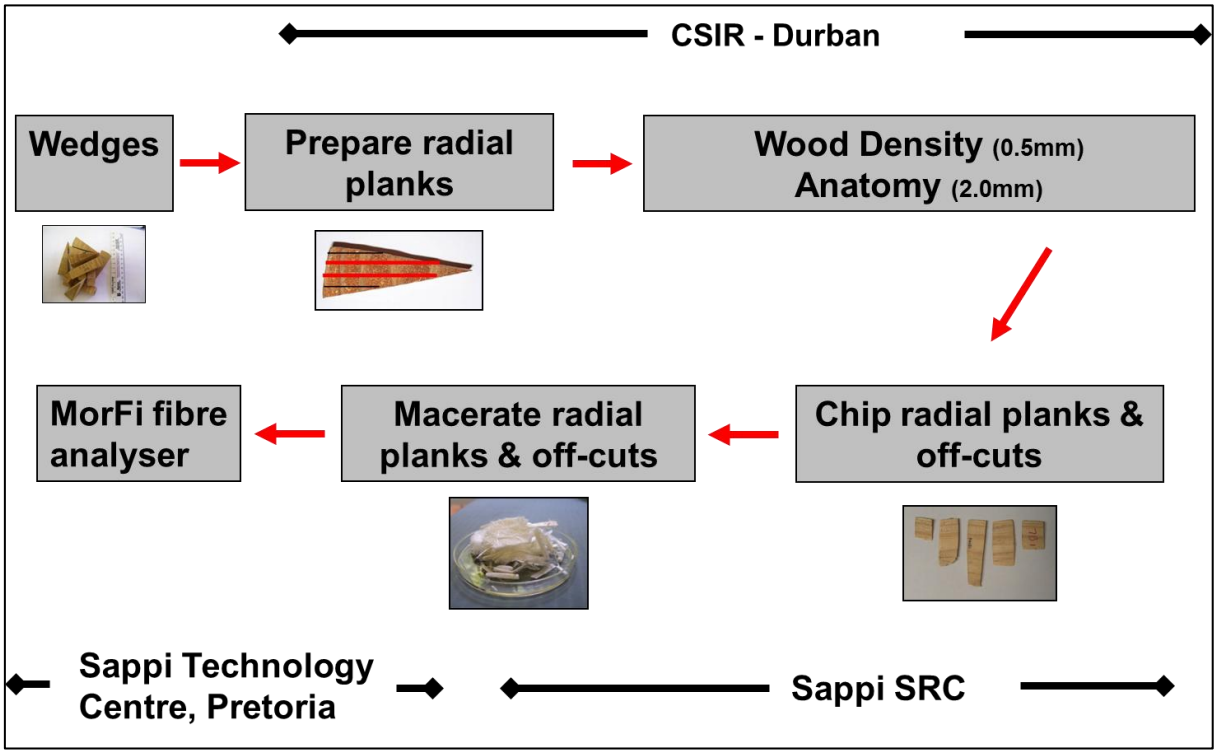

Figure 3.5 Sampling procedure with wedge samples from Zimbabwe Forest Commission diallel and factorial mating designs. All different physical wood properties were determined from the identical wedge sample.

The quantitative physical wood property traits investigated and reported in this study are based on progeny trials of *P. patula* grown on very specific sites. Results from this study stem from two trials of progeny from relatively small mating designs (diallel with five parents). The limited number of parents and sites should therefore be taken into account when results from this study are applied to

all *P. patula* or other pine species grown in Southern Africa. Factors effecting growth or wood properties such as specific site or climatic attributes at the different trial sites, were not investigated. The results from this study do provide an estimate of genetic parameters and the level of genetic control of the physical wood properties in *P. patula*. Interrelationships between different wood properties could also be explored. Cost constraints and available time also restricted the depth of the study of these properties.

A single radial wedge per tree sampled in 1976 was available for this study. The amount of macerated fibre per sample was therefore not enough to split into different growth ring sections. A bulked sample per tree was therefore used for the estimation of tracheid properties. Based on other *P. patula* studies, and the age when sampling was done, most of the sampled wood would be juvenile wood. Large differences would therefore not be expected. This is also confirmed by the pith to bark density data from this presented study which indicated low proportions of latewood in the samples. For tracheid length and width properties, only mean tree values were collected and pith to bark trends were not explored.

Chapter 4

Methods for genetic analysis of physical wood properties and growth traits

4.1 Introduction

Statistical analysis of diallel mating designs is complex and analysis methods are usually tailor-made for specific statistical software packages. Several statistical packages have been used in the analysis of genetic data from diallel designs; these include Agrobase, Genstat, SAS and ASREML (Birks and Barnes, 1991; Ericsson and Fries, 2004; Otto, 2007; Isik, 2009). Analysis approaches and programmes for the SAS statistical software package have been described by Zhang and Kang, (1997), Xiang and Li (2001), Zhang *et al*. (2005) and Isik (2009). Xiang and Li (2001) and Isik (2009) developed programmes to deal specifically with incomplete and unbalanced diallel designs. The Zhang and Kang (1997) and Zhang *et al*. (2005) diallel analysis programmes are based on the methods developed by Griffing (1956) and can be applied to various types of balanced full- and half-diallel mating designs.

The statistical analysis system (SAS-Diallel) described by Zhang and Kang (1997) and Zhang *et al*. (2005) were used for the genetic analysis of data from the various mating designs described in Chapter 3. The analysis of data of the different physical wood property and tree-growth traits covered in Chapters 5 (wood density), 6 (cross-sectional tracheid properties), 7 (tracheid dimensions) and 8 (8-year volume) was conducted in a similar manner.

The statistical models and SAS procedures used for the data analyses as outlined above, are provided in the respective sections. An outline of the analysis is given in the following sections:

Section 4.2 General descriptive statistics and analysis of variance (ANOVA) for different density and tracheid traits to determine differences and least square mean values among full-sib families from the full diallel and selected factorial crosses at Martin.

Section 4.3 ANOVA to determine differences and least square mean values for the 10 full-sib families at two sites from half-diallel designs at Martin and Nyangui.

Section 4.4 Combining ability analysis for a full-diallel at Martin, and examining site effects with a half-diallel at Martin and Nyangui.

Section 4.5 Estimation of genetic parameters using combined diallel and factorial data.

Section 4.6 Calculation of phenotypic and genetic correlations among wood property and volume traits.

4.2 General descriptive statistics and family analysis

Data were inspected with the PROC UNIVARIATE procedure in SAS ver 9.2 to examine distributions and extreme observations, and for adherence to ANOVA assumptions (SAS, 1999). The 36 full-sib families of the Martin trial were made up of the 20 families from the full-diallel and the 16 families selected from the factorial. The statistical analysis was completed using the PROC GLM function in SAS ver 9.2 (SAS, 1999).The following model was applied:

$y_{ijk} = \mu + rep_i + family_j + rep_i*family_j + e_{ijk}$ *Equation 4.2*

where:

y_{ijk} = is the k th observation of the *i* th replication for the *j* th family;

μ = is the overall mean for each trait;

rep_i = is the i th replication;

$family_j$ = is the GCA of the *j* th family; and

e_{ijk} = is the random error term.

4.3 Site effects on wood property and growth traits

An analysis was also undertaken for the half-diallel design of 10 full-sib families at the two sites, Martin and Nyangui. This analysis was undertaken to determine if there were any significant differences caused by site effects. The statistical analysis was completed using the PROC GLM function in SAS ver 9.2 (SAS, 1999). The following model was applied:

$y_{ijkl} = \mu + site_i + rep_{ij} + family_k + rep_j*family_k + site_i* family_k + e_{ijkl}$ *Equation 4.3*

where:

y_{ijkl} = is the *l* th observation of the *i* th site of the *j* th replication for the *k* th family;

μ = is the overall mean for each trait;

$site_i$ = is the i th site;

rep_{ij} = is the *j* th replication at the i th site;

$family_k$ = is the GCA of the *k* th family; and

e_{ijk} = is the random error term.

4.4 Combining ability analysis for a full-diallel and two half-diallels

During this analysis, general combining ability (GCA), specific combining ability (SCA), reciprocal and site effects were determined. The Zhang *et al*. (2005) programmes for Griffing Method III for a full diallel without selfs on one site, and Griffing Method IV for a half-diallel on two sites were used. With both these methods, the random effects model (Model II) was applied for inferences about the genetic parameters present in the parent population (Griffing, 1956). These models were valid under the assumption that parents were non-related and randomly selected from a diploid population (Cockerham, 1963).

4.4.1 Full-diallel mating design on one site at Martin

$$y_{ijkl} = \mu + rep_k + gca_i + gca_j + sca_{ij} + rec_{ij} + e_{ijkl} \qquad \textit{Equation 4.4}$$

where:

y_{ijkl} = is the *l* th observation of the *k* th replication for the *ij* th cross;

μ = is the overall mean for each trait;

rep_k = is the *k* th replication;

gca_i = is the GCA of the *i* th female parent;

gca_j = is the GCA of the *j* th male parent;

sca_{ij} = is the SCA of the *i* th and *j* th parents;

rec_{ij} = is the reciprocal effect of *i* th and *j* th parents; and

e_{ijkl} = is the random error term.

In the SAS-DIALLEL programme used for this analysis, reciprocal effects are also further subdivided into maternal and non-maternal components, by constructing maternal and non-maternal contrast matrices (Zhang and Kang, 1997).

4.4.2 Half-diallel mating design on two sites at Martin and Nyangui

$$y_{ijklm} = \mu + rep_k + gca_i + gca_j + sca_{ij} + site_l + gca.site_{il} + gca.site_{jl} + sca.site_{ijl} + e_{ijkl}$$

Equation 4.5

where:

y_{ijklm} = is the *m* th observation of the *k* th replication for the *ij* th cross at the *l* th site;

μ = is the overall mean for each trait;

rep_k = is the *k* th replication;

gca_i = is the GCA of the *i* th female parent;

gca_j = is the GCA of the *j* th male parent;

sca_{ij} = is the SCAof the *i* th and *j* th parents;

$site_l$ = is the *l* th site (environment) effect;

$gca.site_{il}$ = is the GCA by site interaction for the *i* th parent;

$gca.site_{jl}$ = is the GCA by site interaction for the *j* th parent;

$sca.site_{ijl}$ = is the SCA by site interaction for the the *i* th and *j* th parents; and

e_{ijkm} = is the random error term.

4.5 Estimation of genetic parameters utilising diallel and factorial data

After completion of the combining ability analysis described above in section 4.4, results were evaluated for each trait. Where no significant reciprocal effects were found, sample data from the full-diallel mating design were pooled together by specific cross. Data of the additional 16 crosses from the factorial mating design described in Chapter 3 were included for the estimation of genetic parameters. Genetic parameters estimated included variance components and heritabilities calculated for the different wood property traits. This combined data was structured into a re-constituted 13-parent incomplete diallel (see Figure 4.1). The increased number (26) of specific crosses would yield more representative and therefore more meaningful genetic parameters than the 10 crosses in the 5 × 5 diallel.

		Male parent												
		14	20	31	44	51	1	2	7	15	26	27	32	48
Female parent	14													
	20	X												
	31	X	X											
	44	X	X	X										
	51	X	X	X	X									
	1	X	X	-	-	-								
	2	X	X	-	-	-	-							
	7	X	X	-	-	-	-	-						
	15	X	X	-	-	-	-	-	-					
	26	X	X	-	-	-	-	-	-	-				
	27	X	X	-	-	-	-	-	-	-	-			
	32	X	X	-	-	-	-	-	-	-	-	-		
	48	X	X	-	-	-	-	-	-	-	-	-	-	

Figure 4.1 Constituted 13-parent incomplete half-diallel design (without reciprocals) by incorporation of selected factorial crosses and pooling reciprocal cross data of the full-diallel into a half-diallel for the trial at Martin.

The following statistical model was used for a constituted incomplete half-diallel incorporating selected factorial crosses on 1 site at Martin:

$y_{ijkl} = \mu + rep_k + gca_i + gca_j + sca_{ij} + e_{ijk}$ *Equation 4.6*

where:

y_{ijkl} = is the *l* th observation of the *k* th replication for the *ij* th cross;

μ = is the overall mean for each trait;

rep_k = is the *k* th replication;

gca_i = is the GCA of the *i* th female parent;

gca_j = is the GCA of the *j* th male parent;

sca_{ij} = is the SCA of the *i* th and *j* th parents;

e_{ijkl} = is the random error term

Use was made of the mixed model (PROC MIXED) procedure in SAS utilizing a programme developed by Isik (2009) which is based on methods for incomplete and unbalanced diallel designs, as described by Xiang and Li (2001). During this analysis, assuming epistatic effects were negligible, the following genetic parameters were calculated:

Additive genetic variance: $\sigma^2_A = 4(\text{cov HS}) = 4\sigma^2_{gca}$ *Equation 4.7*

The variance explained through the GCA effects of half-sib parents is a quarter of additive genetic variance (Falconer and Mackay, 1996).

Dominance genetic variance: $\sigma^2_D = 4(\text{cov FS} - 2\ \text{cov HS}) = 4\sigma^2_{sca}$

Equation 4.8

The variance explained through female by male interactions (SCA) is equal to one quarter of the dominance genetic variance (Falconer and Mackay, 1996).

Phenotypic variance: $\sigma^2_P = 2\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{error}$ *Equation 4.9*

The phenotypic variance is the sum of all observational components of variance. The variance of GCA (σ^2_{gca}) is multiplied by two because females and males contribute one quarter of additive genetic variance to the total variance (Isik, 2009).

Individual-tree broad-sense heritability (assuming no epistasis) was estimated by:

$$H^2_i = 4*(\sigma^2_{gca} + \sigma^2_{sca}) / \sigma^2_P$$ *Equation 4.10*

Individual-tree narrow-sense heritability was estimated by:

$$h^2_i = \sigma^2_A / \sigma^2_P$$ *Equation 4.11*

Standard errors (SE) of heritabilities were calculated using the Delta method (Lynch and Walsh, 1998; Isik, 2009), where:

$$Var(h_i^2) = \left(\frac{4\sigma_{GCA}^2}{\sigma_P^2}\right)\left[\frac{Var(4\sigma_{gca}^2)}{(4\sigma_{gca}^2)^2} + \frac{Var\ (\sigma_P^2)}{(\sigma_P^2)^2} - \frac{2Cov(4\sigma_{gca}^2, (\sigma_P^2)}{(4\sigma_{gca}^2\sigma_P^2)}\right]$$

Equation 4.12

$$SE\ (h_i^2) = \sqrt{Var\ (h_i^2)}$$

Equation 4.13

4.6 Phenotypic and genetic correlations for traits and age trends

Genetic correlations between different traits and growth rings were determined as follows (Falconer and Mackay, 1996):

$$r_{g\ trait1\_trait2} = \frac{cov_{f\ trait1\_trait2}}{\sqrt{\sigma^2{}_{f_{trait1}} \times \sigma^2{}_{f_{trait2}}}}$$

Equation 4.14

Genetic covariances were estimated using the MANOVA method with PROC GLM analysis in SAS.

Standard errors for genetic correlations were determined as follows (Falconer and Mackay, 1996):

$$SE_{r_g} = \frac{1 - r_g^2}{\sqrt{2}} \times \sqrt{\frac{SE_{h^2_{trait1}} \times SE_{h^2_{trait2}}}{h^2_{trait1} \times h^2_{trait2}}}$$

Equation 4.15

Predicted genetic gains from mass selection were calculated with the following method (Falconer and Mackay, 1996):

$\Delta G = i\, h^2_{trait}\, \sigma_{Phen\_trait}$ *Equation 4.16*

where i = selection intensity, h^2_{trait} is the trait heritability, and σ_{Phen_trait} is the phenotypic standard deviation for a trait.

Expected correlated responses from mass selection in secondary trait Y, affected by selection for primary trait X, were calculated using the following formula (Falconer and Mackay, 1996):

$CR_Y = i\, h_X\, h_Y\, r_A\, \sigma_{Phen\_Y}$ *Equation 4.17*

where i = selection intensity, h_X and h_Y are the square roots of heritability for trait X and Y, r_A is the genetic correlation between traits X and Y, and σ_{Phen_Y} is the phenotypic standard deviation for trait Y.

Chapter 5

Inheritance of density traits of *P. patula*

5.1 Introduction

Wood density or wood specific gravity is one of the most important and widely studied wood characteristics for nearly all products of forestry species. Wood density's importance is realized in its economic impact on wood handling and transport costs (Kibblewhite, 1999). Because wood density is under genetic control, it is possible to breed for denser wood, which makes it possible to pack more weight into the digester volume during pulp production (Mimms, 1993). Wood density refers to the ratio of dry weight of wood to its volume and is expressed as kg per cubic meter (kg/m^3) (Zobel and Van Buijtenen, 1989; Bowyer *et al.*, 2003).

The samples from the 5×5 full-diallel and selected crosses from the 5×8 factorial design outlined in Chapter 3 were used to quantify the inheritance of wood density traits in the juvenile wood of *P. patula* grown in Southern Africa. Wood density data were analysed and results are presented in the sequence outlined in Chapter 4.

5.2 Materials and Methods

5.2.1 Sample preparation

The samples used for this study were obtained in 2002 from the Oxford Forestry Institute where they had been stored since 1976. These samples were in the form

of wood wedges cut from 15 mm thick discs taken from the original thinned trees in the trials. Their sizes varied marginally, depending on the diameter of the sampled trees. The samples were then stored at the Shaw Research Centre till the start of this study in 2008, at which time they were inspected and catalogued. All samples were found to be in excellent condition with no signs of fungal or insect damage. Samples from the two progeny trials which formed part of this study were randomly selected from the three replications in each trial. A few of the selected samples contained cracks and were substituted by other randomly selected samples.

Samples were sent to the Council for Scientific and Industrial Research (CSIR) Forests and Forest Products laboratories in Durban, South Africa for measurement of wood properties. Samples were stored at 23ºC and 50% relative humidity to achieve an equilibrium moisture content of about 10%. Thereafter the samples were processed for density and cross-sectional tracheid properties. A sample jig, specially designed for this study, was used to house the sample wedges. Radial planks were prepared from the sample wedges with a specially designed electric twin-saw with tungsten, carbide-tooth surgical saw blades. The twin-saw produces radial uniform planks with a width of 2.5 mm. To obtain the radial planks, sample wedges were clamped into the moving platform of the saw with tracheids orientated vertically. Each sample wedge was then cut to produce a radial plank with dimensions of 15 mm height × 2.5 mm width from the centre of the wedge running from pith to bark. The length of the radial planks varied and depended on the diameter of the sampled trees. The outer sections of the sample wedges were retained for the determination of tracheid length and width analysis which is discussed in Chapter 7.

5.2.2 Density assessment

The radial planks prepared from the sample wedges were used to determine wood density. Wood density measurements in this study are referred to as un-extracted air-dried wood density, since extractives were not removed prior to scanning (Zboňák, 2002).

The pith ends of the radial planks were tapered and not exactly 2.5 mm in thickness, because of the wedge shape of the samples from which they were prepared. Therefore, the scanning of the radial planks for the measurement of density was undertaken from the bark end to the pith end. Also, because the uneven thickness of the radial plank samples could influence the densitometry readings, anomalous readings on the pith side of the radial plank can then be discarded.

Radial plank samples were assessed for density along the radial plane from pith-to-bark. Firstly, six radial planks were placed into a sample holder in preparation for the scanning of the density. The sample holder was fitted with a stepper motor drive, which allows for fixed incremental movements across a sample. The radial plank samples were scanned at 0.5 mm intervals using a 60 KeV collimated soft gamma ray densitometer with a Fe55 radiation source. The data acquisition system of the densitometer is fully computer controlled and automated. Weighted mean values for wood density were calculated for each radial strip to account for the larger portion of outer wood in relation to the inner wood (Zboňák, 2002). The following equation was used to calculate weighted means (Zboňák, 2002):

$$WM = \frac{\sum_{i=1}^{n}(x_i a_i)}{\sum_{i=1}^{n} a_i} \qquad \textit{Equation 5.1}$$

where*: WM* = *w*eighted mean of the wood property measured;

x_i = the wood property value of the *I* th radial interval (0.5 mm);

a = the area of the *I* th radial interval in the plank; and

n = the number of observations.

5.2.3 Earlywood and latewood

The ratio of earlywood to latewood is another important factor to consider when measuring overall wood density (Stanger, 2003). Juvenile wood contains bigger portions of lower density earlywood. Because wood density properties such as earlywood density, latewood density and latewood proportions are under strong genetic control (Zobel and Jett, 1995), wood density was partitioned into earlywood and latewood per growth ring following the criteria applied by Stanger (2003). A delineation point of 0.460 g cm^{-3} was used to differentiate between early- and latewood. This delineation point was used to allocate every 0.5 mm density reading taken across the radial plank into earlywood and latewood categories, producing a density profile of the sample. The density profile was then used to allocate density readings into growth rings following the methodology described by Stanger (2003). Growth rings were determined by the distance between two minimum density readings on the density profile. Mean wood, earlywood and latewood density traits were then calculated for each of the five growth rings of each sample. Ring 1 represents the growth that occurred in year three of each tree, up to Ring 5 which represents year seven. Growth years one and two would have occurred below the sampling height of 1.3 m. Table 5.1 provides a description of the 20 different wood density traits investigated.

Table 5.1 Description of wood density traits investigated in the present study.

Abbreviation	Description
WMWD	weighted mean wood density
MEWD	mean earlywood density
MLWD	mean latewood density
LWP	latewood percentage
MWDR1	mean wood density ring 1 (year 3)
MWDR2	mean wood density ring 2 (year 4)
MWDR3	mean wood density ring 3 (year 5)
MWDR4	mean wood density ring 4 (year 6)
MWDR5	mean wood density ring 5 (year 7)
MEWDR1	mean earlywood density ring 1 (year 3)
MEWDR2	mean earlywood density ring 2 (year 4)
MEWDR3	mean earlywood density ring 3 (year 5)
MEWDR4	mean earlywood density ring 4 (year 6)
MEWDR5	mean earlywood density ring 5 (year 7)
MLWDR1	mean latewood density ring 1 (year 3)
MLWDR2	mean latewood density ring 2 (year 4)
MLWDR3	mean latewood density ring 3 (year 5)
MLWDR4	mean latewood density ring 4 (year 6)
MLWDR5	mean latewood density ring 5 (year 7)

5.3 Results and Discussion

5.3.1 Introduction

The analysis of wood density data was conducted in the sequence outlined in Chapter 4; this sequence is also followed in the presentation of the results. All wood density traits were analysed firstly to determine the range of variation present. An analysis of variance was then carried out on the 36 full-sib families from the full-diallel and factorial mating designs at Martin to determine significant

family differences for all wood density traits. The effect of site on different wood density traits was also investigated by comparing the two half-diallel mating designs at Martin and Nyangui.

A combining ability analysis was then carried out with data from the full-diallel at the Martin site and half-diallels at Martin and Nyangui sites. Genetic parameters were estimated for wood density traits using data from both mating designs at the Martin site, allowing for estimations based on 26 full-sib families. Lastly, phenotypic and genetic correlations were calculated to compare different wood density traits, and to investigate age trends.

5.3.2 General descriptive statistics and family analysis of wood density

Results from the statistical analysis of the density data indicated a large amount of variation for most of the measured and derived traits investigated (Table 5.2). Coefficient of variation (CV) values were fairly low (< than 10%) for most density traits, except for values assessed in the first and fifth growth-rings. The calculated wood density traits (mean wood density, mean earlywood density and mean latewood density) for rings 1 and 5 generally displayed higher CV values. Density readings in growth ring 1 were influenced by the un-even thickness of the sample-plank caused by the tapered point close to the pith. Growth ring 5 also did not contain a full season's growth and therefore little latewood had formed in the last growth season when the sampling was undertaken.

Latewood percentage was the most variable trait, as relatively small amounts of late-wood were present in the juvenile wood of first five growth rings. The sample trees in the present study were eight years old at the time of sampling. At this age, *P. patula* would consist mainly of juvenile wood, with the biggest proportion consisting of earlywood (Stanger, 2003). Results confirmed this and indicated

that latewood made up only 20% (mean latewood proportion) of the density profile, while 80% was made up of earlywood (Table 5.2).

Table 5.2 Summary statistics of wood density traits investigated in this study for both trials at Martin and Nyangui.

Variable[1]	Mean	Standard Deviation	Minimum	Maximum	Range	CV%	N
WMWD	0.406	0.035	0.333	0.512	0.179	8.5	300
MEWD	0.315	0.020	0.268	0.379	0.111	6.2	300
MLWD	0.638	0.029	0.513	0.715	0.202	4.5	300
LWP	0.20	0.05	0.10	0.40	0.30	27.2	300
MWDR5	0.429	0.063	0.294	0.612	0.318	14.7	300
MWDR4	0.431	0.049	0.312	0.659	0.347	11.4	300
MWDR3	0.391	0.041	0.306	0.534	0.228	10.5	300
MWDR2	0.359	0.037	0.270	0.491	0.221	10.3	298
MWDR1	0.360	0.062	0.257	0.707	0.449	17.2	245
MEWDR5	0.352	0.030	0.272	0.445	0.173	8.4	299
MEWDR4	0.327	0.025	0.256	0.407	0.150	7.5	300
MEWDR3	0.314	0.025	0.262	0.418	0.155	8.1	300
MEWDR2	0.313	0.025	0.248	0.398	0.150	7.9	298
MEWDR1	0.317	0.038	0.241	0.452	0.211	11.9	242
MLWDR5	0.633	0.093	0.461	0.898	0.437	14.8	265
MLWDR4	0.677	0.049	0.540	0.849	0.309	7.2	300
MLWDR3	0.647	0.046	0.512	0.792	0.280	7.2	300
MLWDR2	0.627	0.043	0.522	0.764	0.242	6.8	297
MLWDR1	0.582	0.033	0.510	0.707	0.197	5.7	243

[1] Table 4.1 provide detailed descriptions of wood density traits.

The relatively low proportions of latewood present in the growth rings and fewer data points are illustrated in the density profiles of three samples displayed in Figure 5.2. The three sample profiles illustrated in Figure 5.2 represent the top (a), middle (b) and bottom (c) ranked full-sib family based on weighted mean wood density in the Martin trial (Table 5.3).

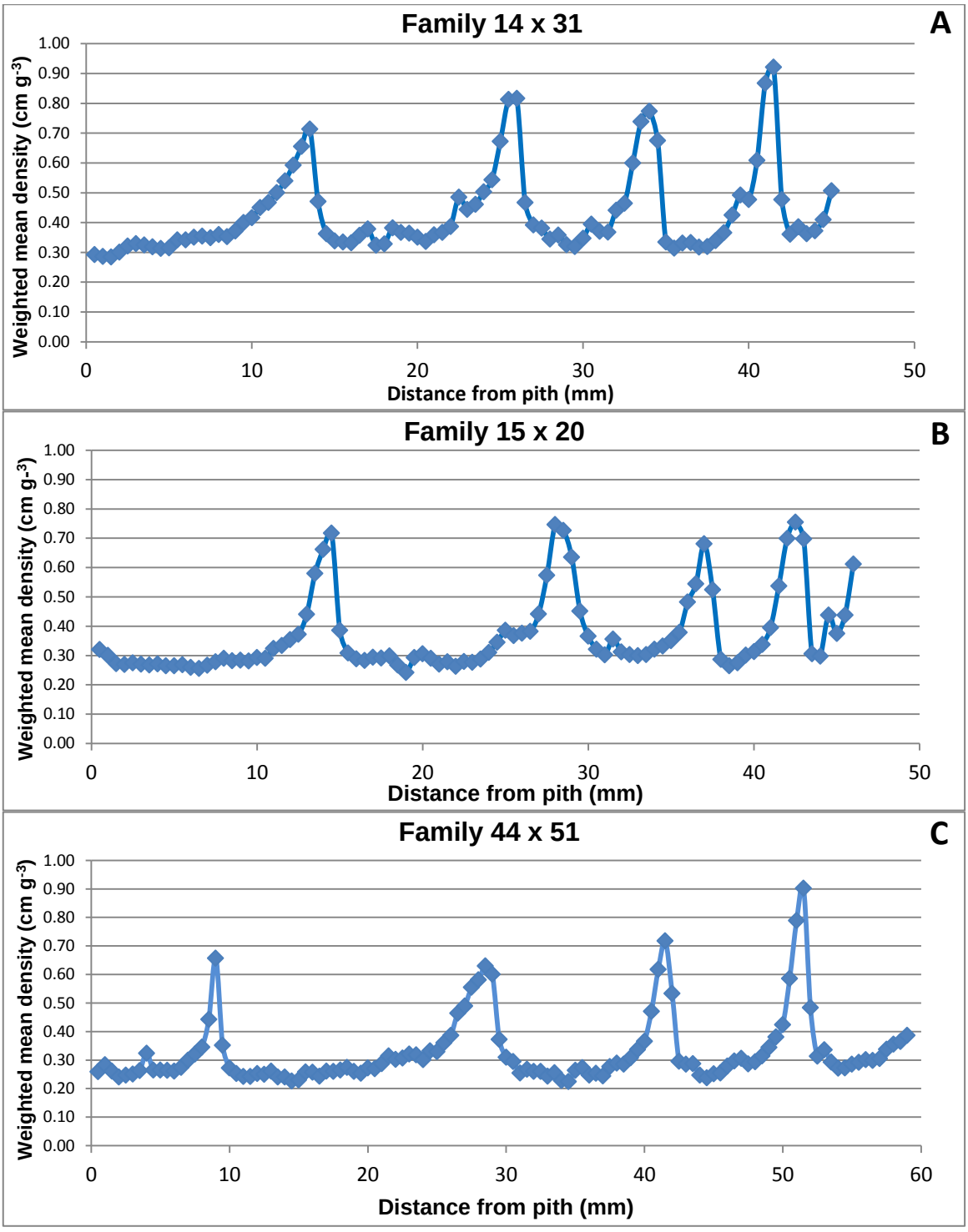

Figure 5.2 Density profiles from pith-to-bark of the top (**A**) (2 × 14), middle (**B**) (14 × 44) and bottom (**C**) (7 × 20) ranked families based on weighted mean wood density (WMWD).

5.3.2.1 Weighted mean-, mean earlywood- and latewood density

The analysis of variance indicated highly significant (p<0.001) differences among full-sib families from the diallel and factorial crosses for most of the wood density traits investigated (Appendix 1). The weighted mean wood density (WMWD), mean earlywood density (MEWD), latewood proportion (LWP) and earlywood proportion (EWP) showed highly significant differences (p<0.0001) among families. Differences in mean latewood density (MLWD) among families were significant (p<0.05). Mean density trait values are summarised in Table 5.3.

WMWD ranged from 0.359 g cm^{-3} for family 44 × 51 to 0.467 g cm^{-3} for family 14 × 31, with a trial mean of 0.403 g cm^{-3} for the Martin trial (Table 5.3). The range in family mean density was 0.108 g cm^{-3}, which is 27% of the trial mean value of 0.403 g cm^{-3}. This range is much larger than values reported by Stanger (2003) for a wild population of *P. patula* planted in South Africa. Payn (2001) and Vermaak (2007) also reported smaller ranges in mean wood density for *P. patula* planted in South Africa. In the Stanger (2003) study, a much larger range in density between individual trees in the unimproved provenance study was reported. These differences between studies are likely due to different sampling ages of these studies, and the different levels of improved material used.

MEWD ranged from 0.351 g cm^{-3} for family 14 × 31 to 0.288 g cm^{-3} for family 7 × 20, with a trial mean of 0.315 g cm^{-3}. The rankings of MEWD were very similar to the rankings of WMWD. MLWD ranged from 0.660 g cm^{-3} for family 51 × 31 to 0.596 g cm^{-3} for family 27 × 14, with a trial mean of 0.634 g cm^{-3}. Differences in MLWD among families were significant (p<0.05). Standard deviations for all traits were relatively low. LWP also followed similar rankings as WMWD and MEWD, with the highest LWP of 0.29 for family 14 × 31, and the lowest for family 44 × 51 of 0.13, and a trial mean of 0.19. EWP was the inverse of LWP, with the lowest proportion of 0.71 for family 14 × 31, and the highest proportion of 0.87 for 44 × 51.

Table 5.3 Mean values per family ranked on weighted mean wood density (WMWD) for selected wood density traits for the full-diallel and selected factorial crosses from the Martin trial.

Family	WMWD (g cm^{-3})	MEWD (g cm^{-3})	Rk[1]	MLWD (g cm^{-3})	Rk	LWP	Rk	EWP	RK
14 × 31	0.467	0.351	1	0.636	15	0.29	1	0.71	36
2 × 14	0.447	0.326	9	0.644	8	0.26	2	0.74	35
32 × 14	0.445	0.343	3	0.643	10	0.26	3	0.74	34
51 × 31	0.438	0.322	12	0.660	1	0.23	7	0.77	30
51 × 14	0.431	0.325	11	0.639	12	0.21	10	0.79	27
14 × 51	0.431	0.331	5	0.651	4	0.22	8	0.78	29
7 × 14	0.428	0.330	6	0.635	18	0.23	6	0.77	31
20 × 14	0.426	0.326	10	0.634	19	0.22	9	0.78	28
1 × 14	0.422	0.333	4	0.648	5	0.18	23	0.82	14
15 × 14	0.421	0.330	7	0.644	9	0.21	11	0.79	26
31 × 14	0.420	0.329	8	0.645	7	0.19	17	0.81	20
27 × 14	0.415	0.350	2	0.596	36	0.19	19	0.81	18
14 × 20	0.412	0.320	13	0.634	21	0.20	15	0.80	22
20 × 31	0.412	0.310	23	0.637	14	0.24	5	0.76	32
44 × 31	0.407	0.316	17	0.654	3	0.19	18	0.81	19
32 × 20	0.405	0.316	18	0.600	35	0.25	4	0.75	33
2 × 20	0.403	0.306	27	0.645	6	0.18	22	0.82	15
15 × 20	0.401	0.311	22	0.623	28	0.21	12	0.79	25
31 × 51	0.400	0.317	16	0.625	26	0.20	16	0.80	21
14 × 44	0.399	0.318	14	0.641	11	0.16	28	0.84	9
31 × 44	0.397	0.317	15	0.623	27	0.21	13	0.79	24
31 × 20	0.396	0.309	26	0.637	13	0.20	14	0.80	23
48 × 14	0.390	0.309	25	0.631	24	0.18	21	0.82	16
44 × 14	0.389	0.312	20	0.658	2	0.15	33	0.85	4
27 × 20	0.387	0.311	21	0.617	33	0.16	27	0.84	10
44 × 20	0.386	0.309	24	0.632	23	0.17	25	0.83	12
51 × 20	0.386	0.298	32	0.633	22	0.18	24	0.82	13
48 × 20	0.381	0.299	29	0.630	25	0.16	29	0.84	8
51 × 44	0.377	0.303	28	0.634	20	0.15	31	0.85	6
1 × 20	0.377	0.312	19	0.616	34	0.15	32	0.85	5
20 × 44	0.375	0.298	31	0.635	16	0.17	26	0.83	11
7 × 20	0.374	0.288	36	0.620	30	0.19	20	0.81	17
20 × 51	0.371	0.292	35	0.635	17	0.14	34	0.86	3
26 × 20	0.370	0.295	33	0.620	31	0.15	30	0.85	7
26 × 14	0.365	0.299	30	0.622	29	0.13	35	0.87	2
44 × 51	0.359	0.294	34	0.620	32	0.13	36	0.87	1
Trial Mean	0.403	0.315		0.634		0.19		0.81	
SD	0.036	0.021		0.028		0.05		0.05	

[1] Family ranking for respective wood density traits.

The low proportion of latewood is expected due to the young age of the study material, when mostly juvenile wood consisting mainly of earlywood, is produced. Zobel and van Buijtenen (1989) described the importance of latewood as the source of variation in wood density in many pines. Stanger (2003) suggested that the short pulpwood rotations used for *P. patula* in Southern Africa may limit the importance of latewood in determining wood density. Latewood proportion only increases dramatically for Southern African grown *P. patula* in material over 20 years of age (Burley *et al*., 1970; Burley *et al*., 1972). When comparing the family rankings of WMWD and MEWD in the present study (Table 5.3), there are similar trends with slight rank changes. At this young age, most of the wood consists of earlywood, as can be seen from the high earlywood proportions of the samples. Earlywood would therefore have a large influence on the mean wood density of 8-year old trees.

MEWD and MLWD mean values were very similar to results from a study conducted by Payn (2001). In this study on *P. patula* open-pollinated families, sampling was also conducted on 8-year old trees at six different trial sites. The MEWD ranged from 0.3270 g cm^{-3} to 0.3461 g cm^{-3}, and MLWD ranged between 0.622 g cm^{-3} and 0.680 g cm^{-3}. Birks and Barnes (1991) conducted a comprehensive wood density study using identical wedge samples compared to the present study. This study by Birks and Barnes (1991) included six trial sites, two of which are used (Martin and Nyangui) in the present study. Very similar mean density results to the present study were reported for these two trials.

In a study conducted on 12-year old *P. taeda* open-pollinated families on different sites, similar MEWD and MLWD values were reported to results from the present study. This study reported MEWD values of 0.307 – 0.337 g cm^{-3} and MLWD values of 0.678 and 0.730 g cm^{-3} on two sites in Alabama and Florida (Belonger, 1998). LWP values were higher, ranging from 0.36 to 0.49 for the two sites, and WMWD values ranged from 0.446 to 0.548 for Alabama and Florida respectively.

5.3.2.2 Wood density traits for growth rings 1 to 5

Weighted mean wood density (WMWD), mean earlywood density (MEWD) and mean latewood density (MLWD) were calculated for each of the five growth rings of each density profile (Tables 5.4, 5,5, 5.6). These measurements were taken at 0.5 mm intervals along the radial wood sample. Mean values were calculated for each of the three traits for every growth ring. Due to missing values in growth rings 1 and 5, some WMWD, MEWD and MLWD values could not be estimated for some families. Highly significant (p<0.01) differences were found between full-sib families for WMWD at all growth rings, and for MEWD at all but growth ring 1 (see Appendix 1). Sampling error in the first growth ring was encountered due to the tapered point of the wedge sample. MLWD at all growth rings were found to be not significant. This non-significance is most probably due to the relatively young age of the sample trees used in this study. Relatively small proportions of latewood density (> than 0.460 g cm^{-3}) are visible in the density profiles of the samples in Figure 5.2.

Each of the WMWD, MEWD and MLWD values were plotted for each of the five growth rings (Figure 5.3). It is evident that there was a radial increase in all three wood density traits from growth ring 1 to 5. The only deviation from this trend was the WMWD and MLWD values for growth ring 5. As previously explained in Chapter 3, the trees used in this study were felled in the middle of the last growth season, before much latewood had formed. This also had an effect on WMWD, which is a combination of earlywood and latewood. The trend of gradual increase in wood density traits with age corresponds very well with the results from Birks and Barnes (1991) for WMWD, and with Stanger (2003) for WMWD, MEWD and MLWD. This trend is also common in many other species, including *P. radiata* and *P. taeda*.

Table 5.4 Mean wood density values for growth rings 1 to 5 per family ranked on mean wood density at ring 2 (MWDR2) for the full-diallel and selected factorial crosses from the Martin trial.

Family	MWDR1	Rk	MWDR2	Rk	MWDR3	Rk	MWDR4	Rk	WMDR5	Rk
14 × 31	0.399	2	0.422	1	0.457	1	0.502	2	0.438	8
2 × 14	0.337	22	0.398	2	0.451	2	0.507	1	0.481	1
7 × 14	0.358	10	0.396	3	0.418	8	0.462	8	0.405	21
32 × 14	0.540	1	0.394	4	0.432	3	0.468	6	0.469	3
27 × 14	0.368	6	0.387	5	0.405	13	0.435	16	0.415	19
15 × 14	0.362	8	0.385	6	0.425	6	0.441	13	0.395	25
31 × 44	0.396	3	0.383	7	0.385	23	0.428	20	0.409	20
20 × 31	0.362	9	0.380	8	0.387	19	0.422	26	0.402	22
32 × 20	0.391	4	0.378	9	0.389	17	0.422	24	0.418	15
1 × 14	0.337	21	0.378	10	0.407	11	0.453	11	0.445	7
51 × 31	0.350	15	0.378	11	0.419	7	0.479	4	0.457	4
51 × 14	0.342	17	0.376	12	0.431	4	0.488	3	0.479	2
44 × 31	0.353	13	0.375	13	0.406	12	0.461	9	0.419	14
14 × 51	0.381	5	0.374	14	0.430	5	0.471	5	0.451	5
31 × 20	0.355	12	0.367	15	0.385	22	0.424	23	0.394	27
44 × 14	0.323	28	0.366	16	0.387	20	0.439	14	0.395	24
14 × 20	0.338	20	0.365	17	0.389	18	0.467	7	0.424	13
20 × 14	0.352	14	0.364	18	0.414	9	0.454	10	0.434	9
14 × 44	0.331	26	0.362	19	0.387	21	0.431	17	0.417	16
31 × 14	-[1]	35	0.360	20	0.413	10	0.430	19	0.434	10
1 × 20	0.334	25	0.358	21	0.365	33	0.412	30	0.372	32
48 × 14	0.348	16	0.355	22	0.389	16	0.425	22	0.396	23
15 × 20	0.358	11	0.354	23	0.393	15	0.448	12	0.415	18
7 × 20	0.340	19	0.350	24	0.360	34	0.430	18	0.357	36
27 × 20	0.335	24	0.349	25	0.375	28	0.436	15	0.416	17
20 × 44	0.336	23	0.349	26	0.368	32	0.399	34	0.367	34
2 × 20	0.322	30	0.344	27	0.403	14	0.428	21	0.448	6
31 × 51	-[1]	36	0.341	28	0.382	24	0.407	32	0.433	11
44 × 20	0.363	7	0.339	29	0.375	27	0.411	31	0.377	31
48 × 20	0.324	27	0.338	30	0.373	29	0.413	29	0.385	30
51 × 20	0.322	29	0.337	31	0.379	25	0.404	33	0.425	12
26 × 20	0.314	32	0.333	32	0.376	26	0.413	28	0.395	26
26 × 14	0.317	31	0.332	33	0.355	35	0.381	36	0.369	33
51 × 44	0.341	18	0.329	34	0.372	30	0.419	27	0.392	28
44 × 51	0.300	34	0.328	35	0.369	31	0.399	35	0.364	35
20 × 51	0.300	33	0.328	36	0.349	36	0.422	25	0.387	29
Trial Mean	0.414		0.437		0.395		0.363		0.352	
SD	0.059		0.052		0.043		0.036		0.062	

[1] Mean values not estimated due to missing data values for ring 1

Table 5.5 Mean earlywood density mean values for growth rings 1 to 5 per family ranked on mean earlywood density at ring 2 (MEWDR2) for the full-diallel and selected factorial crosses from the Martin trial.

Family	MEWDR1	Rk	MEWDR2	Rk	MEWDR3	Rk	MEWDR4	Rk	MEWDR5	Rk
14 × 31	0.343	1	0.348	1	0.357	1	0.367	2	0.391	1
27 × 14	0.332	4	0.343	2	0.350	2	0.368	1	0.386	2
32 × 14	-[1]	34	0.340	3	0.344	4	0.351	5	0.372	8
15 × 14	0.319	7	0.338	4	0.335	9	0.334	13	0.356	18
7 × 14	0.316	9	0.336	5	0.331	11	0.331	14	0.367	12
2 × 14	0.297	25	0.334	6	0.345	3	0.338	10	0.382	3
1 × 14	0.303	17	0.333	7	0.344	5	0.360	3	0.375	6
14 × 51	0.341	2	0.329	8	0.332	10	0.349	6	0.370	9
51 × 14	0.304	16	0.329	9	0.343	6	0.356	4	0.376	5
20 × 14	0.311	12	0.325	10	0.330	12	0.345	7	0.351	20
14 × 20	0.301	19	0.322	11	0.318	16	0.339	9	0.364	13
44 × 31	0.304	15	0.321	12	0.316	17	0.325	18	0.368	11
31 × 44	0.340	3	0.320	13	0.312	19	0.329	16	0.356	19
44 × 14	0.294	27	0.320	14	0.320	14	0.328	17	0.360	16
14 × 44	0.299	21	0.319	15	0.325	13	0.338	12	0.349	21
1 × 20	0.297	24	0.319	16	0.310	20	0.329	15	0.346	23
31 × 14	-[1]	35	0.319	17	0.336	8	0.341	8	0.363	14
51 × 31	0.314	11	0.317	18	0.338	7	0.338	11	0.375	7
32 × 20	0.329	5	0.317	19	0.302	29	0.321	23	0.369	10
20 × 31	0.308	14	0.314	20	0.306	23	0.308	33	0.329	34
15 × 20	0.317	8	0.311	21	0.305	25	0.320	24	0.341	28
31 × 20	0.315	10	0.309	22	0.303	27	0.308	34	0.330	33
48 × 14	0.303	18	0.307	23	0.307	22	0.312	28	0.345	24
27 × 20	0.310	13	0.306	24	0.315	18	0.324	21	0.359	17
44 × 20	0.326	6	0.305	25	0.308	21	0.311	30	0.338	29
31 × 51	-[1]	36	0.305	26	0.319	15	0.322	22	0.336	31
51 × 44	0.301	20	0.302	27	0.297	33	0.324	20	0.341	27
20 × 44	0.299	22	0.301	28	0.296	34	0.305	35	0.325	35
2 × 20	0.292	28	0.299	29	0.306	24	0.325	19	0.381	4
26 × 14	0.287	30	0.297	30	0.303	28	0.313	27	0.343	26
26 × 20	0.279	32	0.295	31	0.298	32	0.318	25	0.363	15
20 × 51	0.277	33	0.295	32	0.288	35	0.317	26	0.338	30
48 × 20	0.294	26	0.293	33	0.301	30	0.308	32	0.333	32
7 × 20	0.298	23	0.292	34	0.287	36	0.300	36	0.324	36
44 × 51	0.282	31	0.292	35	0.300	31	0.312	29	0.348	22
51 × 20	0.291	29	0.292	36	0.304	26	0.309	31	0.344	25
Trial Mean	0.355		0.328		0.318		0.315		0.309	
SD	0.031		0.026		0.027		0.024		0.034	

[1] Mean values not estimated due to missing data values for ring 1

Table 5.6 Mean latewood density mean values at growth rings 1 to 5 per family ranked on mean latewood density at ring 2 (MLWDR2) for the full-diallel and selected factorial crosses from the Martin trial.

Family	MLWDR1	Rk	MLWDR2	Rk	MLWDR3	Rk	MLWDR4	Rk	MLWDR5	Rk
44 × 31	0.596	12	0.681	1	0.680	5	0.695	4	0.628	7
44 × 14	0.585	19	0.681	2	0.664	8	0.699	2	0.679	1
1 × 14	0.584	21	0.659	3	0.633	26	0.687	10	0.663	4
51 × 31	0.600	9	0.653	4	0.692	1	0.684	14	0.604	14
31 × 20	0.573	31	0.651	5	0.652	17	0.687	11	0.586	22
14 × 44	0.605	4	0.649	6	0.640	23	0.689	9	0.601	16
7 × 14	0.573	28	0.646	7	0.642	21	0.685	12	0.540	32
15 × 14	0.593	13	0.645	8	0.685	2	0.675	22	0.577	26
26 × 20	0.571	33	0.642	9	0.630	29	0.646	33	0.598	19
48 × 14	0.584	22	0.635	10	0.658	12	0.684	15	0.571	28
20 × 51	0.626	2	0.633	11	0.623	32	0.666	26	0.579	24
14 × 51	0.596	11	0.632	12	0.645	19	0.698	3	0.667	3
51 × 44	0.576	27	0.631	13	0.656	13	0.690	7	0.605	13
20 × 44	0.591	14	0.631	14	0.662	11	0.675	21	0.543	31
51 × 14	0.605	5	0.631	15	0.653	15	0.694	5	0.578	25
15 × 20	0.586	18	0.629	16	0.642	20	0.651	30	0.597	20
27 × 14	0.587	17	0.629	17	0.599	36	0.616	36	0.524	33
14 × 31	0.597	10	0.627	18	0.665	7	0.677	19	0.582	23
20 × 14	0.580	25	0.626	19	0.630	28	0.677	18	0.608	11
31 × 44	0.585	20	0.623	20	0.652	16	0.648	32	0.626	8
1 × 20	0.571	34	0.622	21	0.617	33	0.650	31	0.560	29
44 × 51	0.588	15	0.621	22	0.628	31	0.672	24	-[1]	35
44 × 20	0.600	7	0.618	23	0.631	27	0.672	23	0.600	18
2 × 14	0.604	6	0.617	24	0.685	3	0.689	8	0.586	21
31 × 14	-[1]	35	0.616	25	0.662	10	0.666	27	0.636	5
32 × 14	0.632	1	0.616	26	0.663	9	0.663	28	0.674	2
26 × 14	0.573	30	0.615	27	0.629	30	0.654	29	-[1]	34
20 × 31	0.616	3	0.612	28	0.651	18	0.669	25	0.630	6
51 × 20	0.600	8	0.612	29	0.671	6	0.676	20	0.621	9
14 × 20	0.573	29	0.612	30	0.636	24	0.678	17	0.601	17
32 × 20	0.577	26	0.612	31	0.615	34	0.625	35	0.555	30
2 × 20	0.587	16	0.611	32	0.683	4	0.693	6	0.602	15
27 × 20	0.583	23	0.608	33	0.607	35	0.682	16	0.573	27
31 × 51	-[1]	36	0.605	34	0.635	25	0.643	34	0.616	10
7 × 20	0.572	32	0.603	35	0.654	14	0.704	1	-[1]	36
48 × 20	0.581	24	0.597	36	0.640	22	0.685	13	0.607	12
Trial Mean	0.601		0.674		0.648		0.629		0.588	
SD	0.083		0.050		0.048		0.044		0.032	

[1] Mean values not estimated due to missing data values for rings 1 and 5

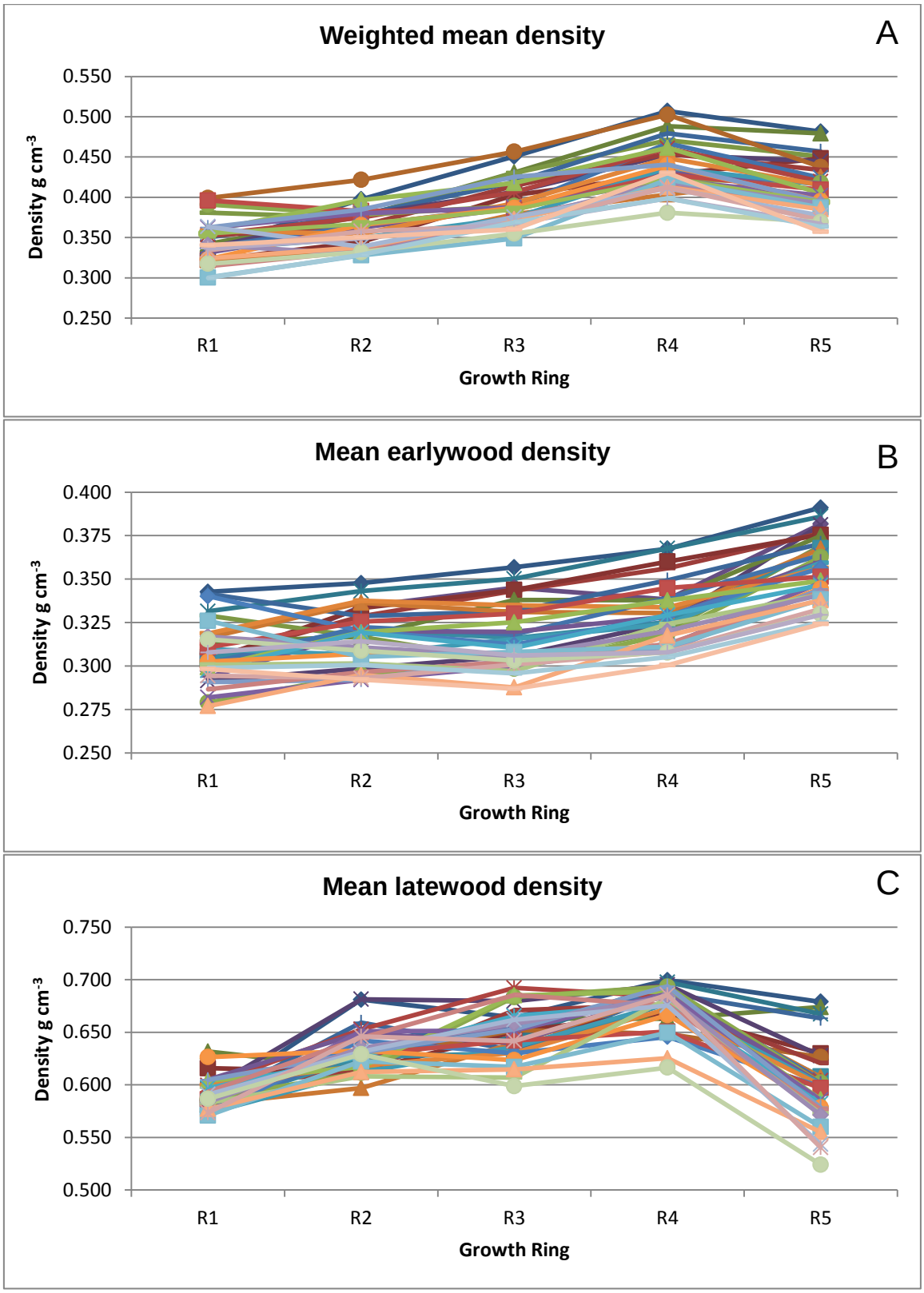

Figure 5.3 Weighted mean density(WMWD) (**A**), mean earlywood density (MEWD) (**B**) and mean latewood density (MLWD) (**C**) per growth ring for full-sib families from the Martin trial. Results for families with missing values are not displayed.

5.3.3 Site effects on wood density traits

The effect of site (based on altitude) was also investigated in the present study. Results from the half-diallel design at the Martin and Nyangui trial sites, established at different altitudes, were compared. Only 10 full-sib families were common at the two sites. Analysis of variance (ANOVA) indicated that significant differences were present between the two sites for some of the wood density traits (Appendix 2). When comparing the main wood density traits WMWD, MEWD, MLWD and LWP, only LWP showed significant differences (p<0.0119) between sites. The difference between sites for the other main wood density traits was found to be non-significant. No family by site interactions were present. The main wood density trait mean values for the 10 full-sib families from the half-diallel at the two sites at Martin and Nyangui are presented in Figure 5.4.

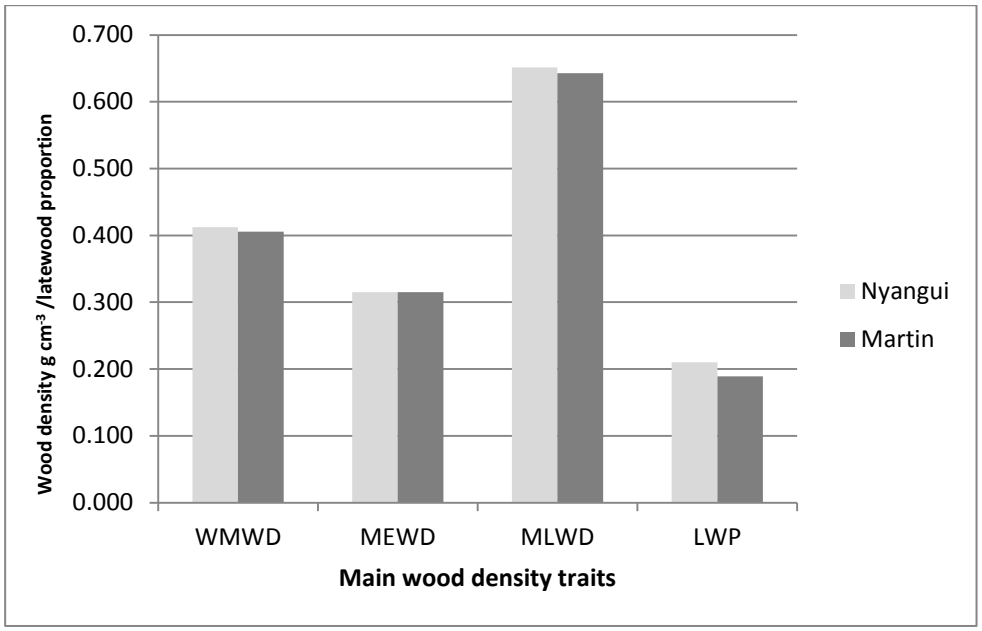

Figure 5.4 Weighted mean wood density (WMWD), mean earlywood density (MEWD), mean latewood density (MLWD) and latewood proportion (LWP) for 10 full-sib families of a half-diallel mating design at two sites at Martin and Nyangui.

When wood density traits at the different growth rings at the two sites were compared, significant differences between sites were found for MWD at rings 5, 4, 3 and 1. MWD values at ring 2 did not differ significantly. Differences for MEWD were significant for rings 5, 3, 2 and 1, with ring 4 being non-significant. Differences between MLWD values at rings 5, 2 and 1 were significant, with rings 4 and 3 being non-significant. It should be noted that, differences at the two sites were not consistent across years, environmental differences at the two sites could have produced different density profiles for different growth rings.

5.3.4 Combining ability analysis of a full-diallel and two half-diallels

5.3.4.1 Full-diallel mating design on one site at Martin

An analysis of variance for combining ability from the full-diallel mating design at Martin was undertaken for the main wood density traits, as well as density traits at the different growth rings. The combining ability results for the main wood density traits indicated highly significant effects (p<0.01) for general combining ability for all traits, except MLWD (Table 5.7). Family effects were highly significant for all traits except MLWD (p<0.01). Specific combining ability effects were not significant for all main density traits (p>0.05). Reciprocal effects were not significant for WMWD, MEWD and MLWD (p>0.05), but highly significant for LWP (p<0.01). There was also a significant maternal effect for WMWD (p<0.05). Maternal and non-maternal effects give an indication of the genetic causes of reciprocal effects. It can indicate whether these effects are caused by cytoplasmic DNA (maternal effect) or the interaction between nuclear and cytoplasmic DNA (non-maternal effect) (Wu and Matheson, 2001).

Table 5.7 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for weighted mean wood density (WMWD), mean earlywood density (MEWD), mean latewood density (MLWD) and latewood proportion (LWP) for a full-diallel at Martin.

Source of variation	df	WMWD MS		MEWD MS		MLWD MS		LWP MS	
Families	19	0.00421	**	0.00124	**	0.00067	ns	0.00904	**
GCA	4	0.01459	**	0.00473	**	0.00042	ns	0.02832	*
SCA	5	0.00121	ns	0.00038	ns	0.00051	ns	0.00315	ns
Rec	10	0.00151	ns	0.00024	ns	0.00083	ns	0.00430	**
Mat	4	0.00210	*	0.00026	ns	0.00092	ns	0.00645	ns
NMat	6	0.00055	ns	0.00023	ns	0.00081	ns	0.00283	ns
Error	102	0.00087		0.00023		0.00066		0.00169	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

The wood density at individual growth rings revealed similar results to the mean values. Mean wood density at the family level was highly significant at rings 1-4, but not significant for ring 5 (Tables 5.8). General combining ability effects were significant for rings 2, 3, 4 and 5. No significant reciprocal, maternal or non-maternal effects were found for mean wood density for rings 1-5.

Table 5.8 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for mean wood density for growth rings 1 to 5 (MWDR1 to MWDR5) for a full-diallel at Martin.

Source of variation	df	MWDR1 MS		MWDR2 MS		MWDR3 MS		MWDR4 MS		MWDR5 MS	
Families	19	0.00514	**	0.00329	**	0.00417	**	0.00618	**	0.00570	ns
GCA	4	0.01451	ns	0.01015	*	0.01329	*	0.01552	*	0.01987	*
SCA	5	0.00323	ns	0.00105	ns	0.00179	ns	0.00272	ns	0.00341	ns
Rec	10	0.00250	ns	0.00170	ns	0.00170	ns	0.00389	ns	0.00122	ns
Mat	4	0.00315	ns	0.00253	ns	0.00269	ns	0.00648	ns	0.00245	ns
NMat	6	0.00198	ns	0.00114	ns	0.00102	ns	0.00224	ns	0.00038	ns
Error	102	0.00232		0.00115		0.00141		0.00237		0.00358	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

Highly significant family effects were found for mean earlywood density for rings 1-4 (Table 5.9). General combining ability effects were also highly significant for rings 2, 3 and 4. No significant SCA, reciprocal, maternal or non-maternal effects

were found for mean earlywood density for rings 1-5. No significant family, GCA, SCA, reciprocal, maternal or non-maternal effects were found for mean latewood density for rings 1-5 (Table 5.10), probably due to the incomplete ring formation.

Table 5.9 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for mean earlywood density for growth rings 1 to 5 (MEWDR1 to MEWDR5) for a full-diallel at Martin.

Source of variation	df	MEWDR1 MS		MEWDR2 MS		MEWDR3 MS		MEWDR4 MS		MEWDR5 MS	
Families	19	0.00224	**	0.00125	**	0.00200	**	0.00200	**	0.00430	ns
GCA	4	0.00437	ns	0.00448	**	0.00761	**	0.00759	**	0.00430	ns
SCA	5	0.00178	ns	0.00037	ns	0.00061	ns	0.00054	ns	0.00451	ns
Rec	10	0.00163	ns	0.00034	ns	0.00047	ns	0.00043	ns	0.00340	ns
Mat	4	0.00214	ns	0.00049	ns	0.00067	ns	0.00049	ns	0.00645	ns
NMat	6	0.00121	ns	0.00024	ns	0.00032	ns	0.00040	ns	0.00222	ns
Error	102	0.00088		0.00047		0.00051		0.00046		0.00454	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

Table 5.10 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for mean latewood density for growth rings 1 to 5 (MLWDR1 to MLWDR5) for a full-diallel at Martin.

Source of variation	df	MLWDR1 MS		MLWDR2 MS		MLWDR3 MS		MLWDR4 MS		MLWDR5 MS	
Families	19	0.00081	ns	0.00266	ns	0.00207	ns	0.00134	ns	0.00655	ns
GCA	4	0.00033	ns	0.00330	ns	0.00240	ns	0.00158	ns	0.00492	ns
SCA	5	0.00101	ns	0.00200	ns	000051	ns	0.00081	ns	0.00828	ns
Rec	10	0.00099	ns	0.00272	ns	0.00274	ns	0.00147	ns	0.00841	ns
Mat	4	0.00191	ns	0.00195	ns	0.00456	ns	0.00210	ns	0.00284	ns
NMat	6	0.00042	ns	0.00328	ns	0.00157	ns	0.00109	ns	0.01254	ns
Error	102	0.00092		0.00194		0.00187		0.00229		0.00689	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

5.3.4.2 Half-diallel mating design on two sites at Martin and Nyangui

An analysis of variance (ANOVA) for combining ability from the half-diallel mating design at Martin and Nyangui was undertaken for the main wood density traits, as well as for density traits at the different growth rings. Family effects were highly significant for MEWD and LWP, and site effects were significant for LWP (Table 5.11). There were no significant family-by-site interactions. General combining ability effects were highly significant for MEWD and LWP. SCA and interactions between GCA and site, and SCA and site were not significant.

Table 5.11 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for weighted mean wood density (WMWD), mean earlywood density (MEWD), mean latewood density (MLWD) and latewood proportion (LWP) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	WMWD MS		MEWD MS		MLWD MS		LWP MS	
Families	9	0.00056	ns	0.00104	**	0.00088	ns	0.00683	**
Site	1	0.00180	ns	0.00000	ns	0.00156	ns	0.01502	*
Family*Site	9	0.00109	ns	0.00020	ns	0.00060	ns	0.00128	ns
GCA	4	0.00007	ns	0.00196	**	0.00077	ns	0.01153	**
SCA	5	0.00091	ns	0.00020	ns	0.00106	ns	0.00275	ns
GCA*Site	4	0.00083	ns	0.00015	ns	0.00048	ns	0.00166	ns
SCA*Site	5	0.00139	ns	0.00024	ns	0.00069	ns	0.00091	ns
Error	118	0.00093		0.00024		0.00076		0.00221	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

Similar results were found for MWD, MEWD and MLWD at rings 1 to 5 (Table 5.12, 5.13 and 5.14). For MWD (Table 5.12), family effects were significant or highly significant for rings 2 to 5, and site effects were significant or highly significant for rings 1, 3, 4 and 5. There were no significant family by site interactions. GCA effects were significant for rings 1, 2, 3 and 5. SCA, GCA by site interaction and SCA by site interaction were not significant.

Table 5.12 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean wood density for growth rings 1 to 5 (MWDR1 to MWDR5) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	MWDR1 MS		MWDR2 MS		MWDR3 MS		MWDR4 MS		MWDR5 MS	
Families	9	0.00508	ns	0.00412	**	0.00275	*	0.00382	**	0.00691	**
Site	1	0.02979	**	0.00199	ns	0.00711	*	0.02506	**	0.08585	*
Family*Site	9	0.00188	ns	0.00092	ns	0.00165	ns	0.00258	ns	0.00285	ns
GCA	4	0.00758	*	0.00662	**	0.00481	**	0.00282	ns	0.01218	**
SCA	5	0.00343	ns	0.00210	ns	0.00033	ns	0.00253	ns	0.00197	ns
GCA*Site	4	0.00297	ns	0.00172	ns	0.00107	ns	0.00289	ns	0.00272	ns
SCA*Site	5	0.00143	ns	0.00047	ns	0.00221	ns	0.00293	ns	0.00282	ns
Error	118	0.00259		0.00113		0.00111		0.00148		0.00294	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

Results for MEWD (Table 5.13) followed similar trends, with significant family effects for rings 2 to 5, and significant site effects at rings 1, 3, and 5. There were no significant family by site interactions. General combining ability effects were highly significant for rings 2 to 5. SCA, GCA by site interaction and SCA by site interaction were not significant.

Table 5.13 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean earlywood density for growth rings 1 to 5 (MEWDR1 to MEWDR5) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	MEWDR1 MS		MEWDR2 MS		MEWDR3 MS		MEWDR4 MS		MEWDR5 MS	
Families	9	0.00156	ns	0.00168	**	0.00163	**	0.00202	**	0.00182	**
Site	1	0.02916	**	0.00198	ns	0.00770	**	0.00033	ns	0.00479	**
Family*Site	9	0.00039	ns	0.00053	ns	0.00083	ns	0.00054	ns	0.00070	ns
GCA	4	0.00207	ns	0.00242	**	0.00301	**	0.00407	**	0.00337	**
SCA	5	0.00180	ns	0.00101	ns	0.00034	ns	0.00021	ns	0.00016	ns
GCA*Site	4	0.00089	ns	0.00103	ns	0.00075	ns	0.00050	ns	0.00051	ns
SCA*Site	5	-0.00024	ns	0.00019	ns	0.00102	ns	0.00061	ns	0.00096	ns
Error	118	0.00102		0.00051		0.00031		0.00038		0.00056	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

MLWD (Table 5.14) showed fewer significant effects due to the small amount of latewood formation in 8-year old trees. Family effects were significant for only ring 2, and site effects for rings 1, 2 and 5. There were no significant family by site

interactions. GCA effects were significant at ring 2, while SCA effects were significant for ring 3. GCA by site interaction and SCA by site interaction were not significant.

Table 5.14 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean earlywood density for growth rings 1 to 5 (MLWDR1 to MLWDR5) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	MLWDR1 MS		MLWDR2 MS		MLWDR3 MS		MLWDR4 MS		MLWDR5 MS	
Families	9	0.00064	ns	0.00411	*	0.00246	ns	0.00154	ns	0.00550	ns
Site	1	0.01210	**	0.01403	**	0.00485	ns	0.00011	ns	0.25461	**
Family*Site	9	0.00088	ns	0.00411	ns	0.00223	ns	0.00128	ns	0.00783	ns
GCA	4	0.00037	ns	0.00472	*	0.00218	ns	0.00271	ns	0.00266	ns
SCA	5	0.00105	ns	0.00339	ns	0.00358	*	0.00136	ns	0.00950	ns
GCA*Site	4	0.00051	ns	0.00112	ns	0.00207	ns	0.00159	ns	0.00995	ns
SCA*Site	5	0.00137	ns	0.00104	ns	0.00252	ns	0.00096	ns	0.00701	ns
Error	118	0.00080		0.00178		0.00150		0.00216		0.00499	

df, degrees of freedom; * Significant at P ≥ 0.05; ** Significant at P ≥ 0.01; ns, not significant

5.3.5 Estimation of genetic parameters utilising diallel and factorial data

Results reported in the previous sections clearly demonstrate the lack of reciprocal effects in the density traits studied. Data from the reciprocal section of the full-diallel were therefore pooled together to create a larger data set for the 10 specific crosses in the half-diallel. The 16 additional crosses from the factorial were also added to constitute a larger incomplete half-diallel with 26 different specific crosses (see Figure 4.1). Detailed genetic parameters were calculated from this data for the main density traits, as well as the density traits at each growth ring.

The individual broad-sense heritability (H^2) values ranged from 0.27 for MLWD to 1.12 for MEWD (Table 5.15). This was due to a very low phenotypic variance value for this trait. Individual narrow-sense heritabilities (h^2) ranged between 0.20 for MLWD to 0.90 for MEWD. The low estimates for MLWD are probably due to

imprecise measurement of the relatively small amounts of latewood present in the young sample material. These heritability values are much higher than typical heritability estimates for growth traits, and are in agreement with values that have been reported for pines by other authors (Zobel and Jett, 1995). The heritability values from the present study are higher than those reported by Birks and Barnes (1991) in the comparative study utilising the same genetic material. This result may be due to more precise assessment of density in the present study. Two other southern African studies using open-pollinated *P. patula* families reported much lower heritability estimates (Payn, 2001; Stanger 2003).

Table 5.15 Genetic effects and heritabilities for weighted mean wood density (WMWD), mean earlywood density (MEWD), mean latewood density (MLWD) and latewood proportion (LWP) for a constituted half-diallel at Martin.

Parameters	WMWD	MEWD	MLWD	LWP
Additive variance	0.001176	0.004200	0.000164	0.002148
Dominance variance	0.000148	0.000096	0.000052	0.000280
Phenotypic variance	0.001445	0.000462	0.000795	0.003519
Individual H^2	0.92	1.12	0.27	0.97
Individual h^2	0.81	0.90	0.20	0.89
SE of h^2	0.25	0.26	0.19	0.31

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

The heritability estimates and combining ability analysis from the present study indicate high levels of additive variance and that significant progress could be made with some of the density traits studied. Higher gains are likely to be achieved if density traits are included in the selection process.

Heritability estimates for mean wood density and mean earlywood density traits at each growth ring were lower compared to the main density traits, but still substantial (Tables 5.16 and 5.17). Although the heritability values were higher in

the present study, this trend corresponds well with the study done by Stanger (2003). The lower heritability estimates for mean latewood density at the individual ring level is probably also due to the imprecise measurement of the relatively small amounts of latewood present in the young sample material (Table 5.18).

Table 5.16 Genetic effects and heritabilities for mean wood density for growth rings 1 to 5 (MWDR1 to MWDR5) for a constituted half-diallel at Martin.

Parameters	MWDR1	MWDR2	MWDR3	MWDR4	MWDR5
Additive variance	0.003372	0.000804	0.000928	0.000656	0.015880
Dominance variance	0.000368	0	0.000140	0.000692	0.000408
Phenotypic variance	0.004779	0.001390	0.001939	0.002763	0.003808
Individual H^2	0.78	0.58	0.55	0.48	0.52
Individual h^2	0.46	0.58	0.48	0.24	0.42
SE of h^2	0.31	0.22	0.22	0.18	0.23

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

Table 5.17 Genetic effects and heritabilities for mean earlywood density for growth rings 1 to 5 (MEWDR1 to MEWDR5) for a constituted half-diallel at Martin.

Parameters	MEWDR1	MEWDR2	MEWDR3	MEWDR4	MEWDR5
Additive variance	0.000424	0.000404	0.000472	0.000424	0.000340
Dominance variance	0.000001	0.000029	0.000064	0.000204	0.000280
Phenotypic variance	0.001285	0.000601	0.000712	0.000708	0.001001
Individual H^2	0.33	0.72	0.75	0.89	0.62
Individual h^2	0.33	0.67	0.66	0.60	0.34
SE of h^2	0.22	0.25	0.23	0.26	0.22

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

Table 5.18 Genetic effects and heritabilities for mean latewood density for growth rings 1 to 5 (MLWDR1 to MLWDR5) for a constituted half-diallel at Martin.

Parameters	MLWDR1	MLWDR2	MLWDR3	MLWDR4	MLWDR5
Additive variance	0.0000004	0.000164	0.000716	0	0.000136
Dominance variance	0	0	0	0.000092	0
Phenotypic variance	0.001005	0.001936	0.002464	0.002448	0.007021
Individual H^2	0	0.08	0.29	0.04	0.02
Individual h^2	0	0.08	0.29	0	0.02
SE of h^2	0	0.08	0.21	0	0.06

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

5.3.6 Phenotypic and genetic correlations for traits and age trends

5.3.6.1 Main Density traits

Phenotypic correlations among main density traits at the individual tree level were highly significant, except between MLWD and MEWD, and MLWD and LWP (Table 5.19). The strongest correlations were found between WMWD and MEWD (0.80), and WMWD and LWP (0.86). MLWD correlated poorly with WMWD (0.37), MEWD (0.07) and LWP (0.13). Family mean phenotypic correlations were slightly higher, but of similar magnitude, with MEWD and MLWD and MLWD and LWP not being significantly correlated. The correlation results indicate that MEWD and LWP are the main determinants of WMWD in the 8-year old trees sampled. Increases in MEWD and LWP will result in an increase in WMWD. These results are very similar to correlation values determined by Birks and Barnes (1991) and Stanger (2003).

Table 5.19 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among wood density traits. Significant phenotypic correlations in bold with *p*-values in brackets.

	WMWD	**MEWD**	**MLWD**	**LWP**
WMWD	-	**0.80 (0.000)**	**0.37 (0.000)**	**0.86 (0.000)**
MEWD	**0.87 (0.000)**	-	0.07 (0.303)	**0.64 (0.000)**
MLWD	**0.40 (0.015)**	0.17 (0.333)	-	0.13 (0.057)
LWP	**0.89 (0.000)**	**0.69 (0.000)**	0.17 (0.325)	-

Additive genetic correlations were slightly higher than the individual phenotypic correlations and displayed similar relationships with relatively low standard errors (Table 5.20). A strong genetic correlation between WMWD and MEWD (0.91) and

WMWD and LWP (0.89) was demonstrated. The main determinants of WMWD are MEWD (0.91) and LWP (0.89).

Table 5.20 Additive genetic correlations with standard errors among wood density traits.

	WMWD	MEWD	MLWD	LWP
WMWD	-			
MEWD	0.91 ± 0.07	-		
MLWD	0.47 ± 0.31	0.25 ± 0.36	-	
LWP	0.89 ± 0.08	0.70 ± 0.19	0.27 ± 0.64	-

5.3.6.2 Density trait age trends

Phenotypic and genetic correlations were also calculated for each growth ring for MWD, MEWD and MLWD. Individual phenotypic correlations for MWD were highly significant between most growth ring comparisons (Table 5.21). The strongest correlations were found between the growth rings 2, 3 and 4. As explained in previous sections, growth ring 1 and 5 presented sampling errors due to the tapered end in growth ring 1 and the incomplete growth in ring 5. Family phenotypic correlations were higher, but demonstrated similar relationships.

Additive genetic correlations were of higher magnitude, with the weakest correlation between growth rings 1 and 5 (Table 5.22). Some genetic correlation values higher than 1 were estimated, probably due to sampling methodology. These results nevertheless correspond well with results from Birks and Barnes (1991) and Stanger (2003).

Table 5.21 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among mean wood density traits for different growth rings. Significant phenotypic correlations are indicated in bold with *p*-values in brackets.

	MWDR1	MWDR2	MWDR3	MWDR4	MWDR5
MWDR1	-	**0.16 (0.028)**	**0.20 (0.005)**	0.04 (0.590)	**0.27 (0.000)**
MWDR2	**0.52 (0.001)**	-	**0.66 (0.000)**	**0.62 (0.000)**	**0.18 (0.007)**
MWDR3	**0.48 (0.003)**	**0.82 (0.000)**	-	**0.72 (0.000)**	**0.40 (0.000)**
MWDR4	0.31 (0.069)	**0.77 (0.000)**	**0.86 (0.000)**	-	**0.41 (0.000)**
MWDR5	**0.42 (0.010)**	**0.55 (0.001)**	**0.80 (0.000)**	**0.75 (0.000)**	-

Table 5.22 Additive genetic correlations with standard errors among mean wood density traits for different growth rings.

	MWDR1	MWDR2	MWDR3	MWDR4	MWDR5
MWDR1	-				
MWDR2	0.97 ± 0.02	-			
MWDR3	0.78 ± 0.19	0.92 ± 0.07	-		
MWDR4	0.76 ± 0.26	0.88 ± 0.14	1.13 ± 0.14	-	
MWDR5	0.59 ± 0.34	0.88 ± 0.14	1.11 ± 0.12	1.16 ± 0.18	-

MEWD phenotypic correlations were generally higher than MWD at individual growth rings, and all correlations were significant other than for ring 1 and 5 (Table 5.23). The strongest correlation was again found between rings 2, 3 and 4.

Family phenotypic correlations were of higher magnitude and were all highly significant, other than for correlations between rings 1 and 5. Additive genetic correlations for MEWD at different growth rings were high with relatively small standard errors (Table 5.24).

Table 5.23 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among mean earlywood density traits for different growth rings. Significant phenotypic correlations are indicated in bold with *p*-values in brackets.

	MEWDR1	MEWDR2	MEWDR3	MEWDR4	MEWDR5
MEWDR1	-	**0.23 (0.001)**	**0.28 (0.000)**	**0.19 (0.009)**	0.14 (0.07)
MEWDR2	**0.64 (0.000)**	-	**0.64 (0.000)**	**0.66 (0.000)**	**0.49 (0.000)**
MEWDR3	**0.50 (0.003)**	**0.90 (0.000)**	-	**0.67 (0.000)**	**0.56 (0.000)**
MEWDR4	**0.46 (0.007)**	**0.84 (0.000)**	**0.90 (0.000)**	-	**0.64 (0.000)**
MEWDR5	0.34 (0.050)	**0.68 (0.000)**	**0.78 (0000)**	**0.81 (0.000)**	-

Table 5.24 Additive genetic correlations with standard errors among earlywood density traits for different growth rings.

	MEWDR1	MEWDR2	MEWDR3	MEWDR4	MEWDR5
MEWDR1	-				
MEWDR2	1.16 ± 0.15	-			
MEWDR3	0.96 ± 0.03	1.01 ± 0.01	-		
MEWDR4	0.90 ± 0.09	0.91 ± 0.08	1.01 ± 0.01	-	
MEWDR5	0.84 ± 0.16	0.82 ± 0.19	0.91 ± 0.10	0.96 ± 0.04	-

MLWD phenotypic correlations were generally poorer than MWD and MEWD at individual growth rings, and most correlations were not significant (Table 5.25). Again, the strongest correlation was again found between rings 2, 3 and 4. The strongest correlation was again found between rings 2, 3 and 4. Family phenotypic correlations were of higher magnitude, but were also mostly not significant.

Table 5.25 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among mean latewood density traits for different growth rings. Significant phenotypic correlations are indicated in bold with *p*-values in brackets.

	MLWDR1	**MLWDR2**	**MLWDR3**	**MLWDR4**	**MLWDR5**
MLWDR1	-	**0.19 (0.008)**	**0.31 (0.000)**	0.05 (0.052)	0.12 (0.156)
MLWDR2	-0.07 (0.684)	-	**0.34 (0.000)**	**016 (0.018)**	0.04 (0.573)
MLWDR3	0.29 (0.112)	0.27 (0.146)	-	**0.24 (0.000)**	-0.06 (0.445)
MLWDR4	0.11 (0.550)	0.31 (0.086)	**0.55 (0.001)**	-	-0.07 (0.375)
MLWDR5	0.30 (0.096)	0.23 (0.213)	0.31 (0.088)	**0.40 (0.025)**	-

Genetic correlations were not calculated between latewood densities at growth rings 1-5. Calculated additive genetic variance and heritability values were at very low levels (see Table 5.18) for these traits and would yield imprecise genetic correlations, which would have little practical value (Stanger, 2003).

5.4 Conclusions

Highly significant differences between families were demonstrated for density traits such as weighted mean wood density, mean earlywood density and latewood proportion. Large ranges in values for these properties also indicate that good opportunities exist to improve these properties through selection and breeding. A strong age effect in weighted mean density, mean earlywood density and mean latewood density was also demonstrated. A steady increase in all three properties was observed outwards from growth ring 1 to 5.

The diallel analysis indicated that no reciprocal effects were present in any of the studied density traits, other than latewood percentage. This may be due to a Type II error in the analysis, and as stated, could be due to the imprecise measurement of latewood. Maternal effects can therefore be ignored and future studies could be conducted with less labour-intensive half-diallel mating designs. The combining ability analysis indicated that general combining effects are highly significant and that weighted mean wood density, mean earlywood density and latewood proportion are under strong additive genetic control. Mean latewood density was not significantly correlated to any of the effects, most probably due to the small quantities of latewood present in the juvenile sample material. Specific combining ability effects were not significant, indicating that dominance effects are absent. These effects were generally similar when studied at individual growth rings.

The half-diallel analysis at two sites indicated that site effects were mostly not significant for the studied density traits, other than latewood proportion. There was also no site- by- family interaction or GCA by site or SCA by site interaction. These effects were again generally similar when studied at individual growth rings.

Individual heritability estimates show that wood density is under strong additive genetic control. Gains can therefore be made with classical breeding for general combining ability and recurrent selection. Mean earlywood density was under the strongest genetic control and it was also demonstrated that earlywood had the strongest influence on weighted mean wood density. Latewood proportion also had a strong influence on weighted mean wood density. Breeding for these wood density traits for a short-rotation crop such as pulpwood will likely yield an increase in the weighted mean wood density of the furnish used for pulp production. The wood density traits assessed for the individual growth rings also indicate that early predictions could be made with relatively high heritabilities.

Phenotypic and genetic correlations indicate that many of the important wood density traits are highly correlated. High correlations were also calculated for wood density properties between growth rings, further highlighting the ability to select for density traits at an earlier age. Latewood proportion had a strong genetic correlation with weighted mean wood density, and also had a high heritability. This suggests that latewood proportion may be a key trait to include for selection and breeding, but could be indirectly bred for by selecting on weighted mean wood density. Higher density wood is more desirable for the Kraft pulping process, allowing for higher throughputs in the pulping process.

An important factor to consider when studying wood density is the effect of tracheid cross-sectional properties and tracheid dimensions on wood density. From published literature it is known that tracheid properties play an important role in determining density traits. This will be further investigated in the following chapters.

Chapter 6

Inheritance of cross-sectional tracheid traits of *P. patula* measured by image analysis

6.1 Introduction

The main cell types in softwoods consist of tracheids and ray parenchyma. Radial variation exists in most cell characteristics. In softwoods, growth rings near the pith usually consist of a greater proportion of earlywood tracheids with larger diameters and thinner cell walls than latewood tracheids (Lachenbruch *et al*., 2011). A number of tracheid cross-sectional properties are considered to be important factors in the pulp and paper process. Tracheids make up the principal papermaking fibres in softwood Kraft pulp (Muneri, 1994). Barefoot *et al.* (1964) list cell wall thickness, cell lumen diameter, the Runkell ratio (ratio between wall thickness and lumen diameter) and earlywood- to- latewood proportion as important factors in paper making. Earlywood tracheid diameter increases with cambial age, and it therefore has an effect on the variation in wood density (Lachenbruch *et al*., 2011).

Tracheid properties directly influence the pulp fibre qualities that are produced during the Kraft pulping process. Papermaking properties are dependent on the structure of the various pulped fibres. Two of the most important structural tracheid characteristics are length and cell wall thickness (Smook, 1986). A minimum tracheid length is required for inter-fibre bonding in pulp where the fibre length is proportional to the tear strength of sheets. Fibres with thinner cell walls collapse easier during sheet formation and contribute more to inter-fibre bonding than thicker walled fibres (Smook, 1986). The relationships between different cell properties can also be an important characteristic to consider in assessing pulp quality.

The Runkel ratio is the ratio of two times the tracheid cell wall thickness divided by the tracheid lumen diameter. The Runkel ratio gives an estimate of the collapsibility of tracheids and has been found to be the best single predictor for various paper sheet properties such as burst factor, breaking length (tensile strength) and tear (Barefoot *et al*., 1964; Evans *et al*., 1997; Saikia *et al*., 1997).

The number of studies on genetic inheritance of tracheid cross-sectional properties is limited, probably because of the high cost and difficulty of assessment. Zobel and Jett (1995) report in their overview of tracheid properties that studies of the genetics of cell components are limited and many results appear to be inconclusive or contradictory. The results of many of these studies were also influenced by the limited extent of the genetic base and mating structure, and small sample size and number of replications. These studies have shown moderate to strong genetic control of tracheid characteristics (Zobel and Jett, 1995). In a wood anatomical property study carried out by Stanger (2003) on different provenances of *P. patula* grown in South Africa, results indicated that the additive genetic control of tracheid radial diameter were moderately strong. Contrary to most reports in the literature, cell wall thickness in *P. patula* was under very weak or negligible additive genetic control (Stanger, 2003). Vermaak (2007) reported high heritability estimates for tracheid cross-sectional characteristics of *P. patula* in South Africa.

This chapter reports on results from a tracheid cross-sectional trait study using pith to bark wedge samples. Samples from a 5 × 5 full-diallel and selected crosses from a 5 × 8 factorial design were used as outlined in Chapter 3. The objective of this chapter is to quantify the inheritance of tracheid cross-sectional traits in the juvenile wood of *P. patula* grown in Southern Africa. Correlations with wood density traits and other tracheid characteristics will also be investigated in this and following chapters.

6.2 Material and Methods

6.2.1 Assessment of tracheid cross-sectional characteristics

The same radial planks used for the determination of wood density properties in Chapter 5, were utilised for assessing cross-sectional characteristics. After completion of the densitometry assessments, the radial planks were soaked in water to soften the wood. A sliding microtome was used to slice off a thin layer of the surface to expose a clean and smooth surface on the transverse plane in preparation for image analysis. A Leica DMLB light microscope fitted with fluorescent illumination in the UV range was used to capture images along the radial plank. Up to 12 radial planks were mounted in a jig on a motorised stage to facilitate rapid assessment. Measurement of cross-sectional tracheid characteristics was performed automatically every 2 mm along the radial surface. The measurements were done using 10× objective magnification, providing measurement frames with an area of 1 mm × 0.8 mm. For each frame, weighted mean values for each of the cross-sectional tracheid characteristics were calculated automatically.

The Leica image analysis system identifies and measures the area of the cell lumen, assuming it has a circular shape. Lumen diameter (LD) is derived from this measurement. The image analysis system then captures the boundaries between cells and calculates cell area within these boundaries, which includes the lumen. Cell wall thickness (CWT) is then derived by subtracting the lumen diameter from the cell diameter. Measurements of the tangential (TD) and radial (RD) cell diameters are also taken and a mean tracheid diameter (MTD) was calculated from these two readings.

Weighted mean values for cross-sectional tracheid characteristics were also calculated for each radial strip in order to account for the bigger portion of outer

wood in relation to the inner wood (Zboňák, 2002). The following equation by Zboňák (2002) was used to calculate weighted means:

$$WM = \frac{\sum_{i=1}^{n}(x_i a_i)}{\sum_{i=1}^{n} a_i} \qquad \textit{Equation 6.1}$$

where*: WM* = *w*eighted mean of the wood property measured;

x_i = the wood property value of the *I* th radial interval (2 mm);

a = the area of the *I* th radial interval in the disc; and

n = the number of observations.

A number of additional tracheid cross-sectional characteristics which play an important role in Kraft pulping, were derived from the traits measured with image analysis (Stanger, 2003). These traits were mean tracheid area (TArea), mean number of tracheids per square millimeter (NoTrach), mean percentage of cell wall per square millimeter (PCell) and Runkel Ratio (RR). They were derived as follows:

TArea = RD × TD × *pi*, where *pi* = 3.141592654 *Equation 6.2*

NoTrach = 1000000 / TArea *Equation 6.3*

PCell = ((WArea × NoTrach)/1000000) ×100

where: WArea = wall area

Equation 6.4

RR = (2 × WT)/LD *Equation 6.5*

A number of wood studies report on physical properties assessed with the Silviscan® analysis system (Shelbourne *et al*., 1997; Nyakuengama *et al*., 1999). The Silviscan® system combines x-ray wood densitometry and image analysis to assess tracheid cross-sectional traits in a single operation, using increment core wood samples (Evans, 1994). Evans *et al.* (1995) provide details of assumptions and formulae to derive Silviscan® traits from image analysis traits. These traits are coarseness (CS), specific surface (SS), perimeter (PM) and wall thickness (WTS). Including the Silviscan® traits enables comparisons with other published results by Nyakuengama *et al*. (1999), Shelbourne *et al*. (1997), Stanger (2003), Donaldson *et al.* (2004) and Vermaak (2007). A summary of all cross-sectional tracheid traits with their abbreviations investigated in the present study is provided in Table 6.1.

Table 6.1 Summary of tracheid cross-sectional properties investigated in this study.

Abbreviation	Description	Source
RD	Radial diameter (µm)	Image analysis
TD	Tangential diameter (µm)	Image analysis
MTD	Mean tracheid diameter (µm)	Calculated
LD	Lumen diameter (µm)	Image analysis
CWA	Cell wall area (µm²)	Image analysis
CWT	Cell wall thickness (µm)	Image analysis
TArea	Tracheid area (µm²)	Calculated
NoTrach	No of tracheids per mm² (n/ mm²)	Calculated
PCell	Percentage cell wall per mm² (%)	Calculated
RR	Runkel ratio	Calculated
CS*	Coarseness (µg m^{-1})	Calculated
SS*	Specific surface (m^2 kg^{-1})	Calculated
PM*	Perimeter (µm)	Calculated
WTS*	Wall thickness (µm)	Calculated

* Silviscan® derived traits

6.3 Results and Discussion

6.3.1 Introduction

The analysis of cross-sectional tracheid trait data was conducted in the sequence outlined in Chapter 4, this sequence is also followed in the presentation of these results. All cross-sectional tracheid traits were analysed firstly to determine the range of variation present. An analysis of variance was then carried out on the 36 full-sib families from the full-diallel and factorial mating designs at Martin to determine significant family differences for all cross-sectional tracheid traits. The effect of site on different cross-sectional tracheid traits was also investigated by comparing the two half-diallel mating designs at Martin and Nyangui.

Pith-to-bark age trends were not investigated, as the larger radial measurement intervals (every 2 mm) would not yield accurate growth-ring values. This study only investigated the weighted mean values of the various assessed and derived cross-sectional tracheid traits. Stanger (2003) showed that there was a steady increase in tracheid diameter traits as the age of a tree increases. Cell wall area and cell wall thickness initially increased steadily, with a rapid increase during the last three years of the 10-year old sampled trees (Stanger, 2003).

A combining ability analysis was then carried out with data from the full-diallel at the Martin site and half-diallels at Martin and Nyangui sites. Genetic parameters were estimated for cross-sectional tracheid traits using data from both mating designs at the Martin site, allowing for estimations based on 26 full-sib families. Lastly, phenotypic and genetic correlations were calculated for comparison of different cross-sectional tracheid traits.

6.3.2 General descriptive statistics and family analysis of cross-sectional tracheid traits

The results from the statistical analysis of the cross-sectional tracheid data indicated a large amount of variation for most of the measured and derived cross sectional traits (Table 6.2). Coefficient of variation (CV) values were fairly low (< than 10%) for most of the traits, except for some of the calculated traits. There was a large amount of variation present among sampled trees for all of the cross-sectional tracheid traits, indicating scope for selection and improvement for traits under additive genetic control. This large range of variation was similar to the outcome of the study of different provenances of *P. patula* reported by Stanger (2003).

Table 6.2 Summary statistics of cross-sectional tracheid traits investigated in this study for both trials at Martin and Nyangui.

Variable	Description	Mean	SD	Min	Max	Range	CV%	N
RD	Radial diameter (µm)	42.51	3.92	34.30	54.17	19.88	9.22	300
TD	Tangential diameter (µm)	39.47	1.92	33.58	44.75	11.17	4.85	300
MTD	Mean tracheid diameter (µm)	40.99	2.74	34.66	49.22	14.56	6.69	300
LD	Lumen diameter (µm)	28.39	2.66	21.23	35.63	14.40	9.37	300
CWA	Cell wall area (µm²)	557.06	61.72	383.93	727.54	343.61	11.08	300
CWT	Cell wall thickness (µm)	5.45	0.54	4.21	7.21	3.00	9.90	300
TArea	Tracheid area (µm²)	5289.36	702.33	3770.43	7534.14	3763.71	13.28	300
NoTrds	No of tracheids per mm² (n/ mm²)	192.32	25.00	132.73	265.22	132.49	13.00	300
PCell	Percentage cell wall per mm² (%)	10.61	1.08	8.16	13.55	5.39	10.20	300
RR	Runkel ratio	0.40	0.07	0.26	0.58	0.32	16.67	300
CS*	Coarseness (µg m^{-1})	679.53	82.37	476.90	979.50	502.60	12.12	300
SS*	Specific surface (m^2 kg^{-1})	243.34	19.87	188.03	317.53	129.50	8.16	300
PM*	Perimeter (µm)	163.97	10.96	138.64	196.88	58.24	6.69	300
WTS*	Wall thickness (µm)	2.98	0.26	2.23	3.87	1.64	8.64	300

* Silviscan® derived traits

6.3.2.1 Cross-sectional tracheid image analysis traits

Results from the analysis of variance indicated that differences between families were highly significant (p<0.001) for all tracheid cross-sectional properties assessed by image analysis (Appendix 3). There is limited published information available on cross-sectional tracheid properties of *P. patula.* The variation in sample age, site effects and different assessment methods in published studies make direct comparisons between studies difficult. Few studies have been conducted on multiple families of a specific species at the same sampling age and sampling site. Results of mean values for tracheid cross-sectional traits from the present study were very similar to those in publications cited by Stanger (2003) and reported in the Stanger (2003) and Vermaak (2007) *P. patula* studies. The mean family values calculated in the present study were 42.28 µm (RD), 39.56 µm (TD), 40.92 µm (MTD), 28.30 µm (LD), 561.19 µm² (CWA) and 5.48 µm (CWT) (Table 6.3). Family mean results for all traits are provided in Table 6.3. Families are ranked according to RD, in descending order. There were relatively small rank changes among families between the different cross-sectional tracheid traits RD, TD, MTD, LD and CWA (Table 6.3). CWT values were negatively correlated with all the other cross-sectional tracheid traits.

The mean values of tracheid diameter for *P. patula* in this study were generally larger than those published for *P. radiata* (Shelbourne *et al*., 1997; Nyakuengama *et al*., 1999), but smaller than those of *P. taeda* (Belonger, 1998).

Table 6.3 Mean values per family for tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT). Families are ranked on means for RD for the full-diallel and selected factorial crosses from the Martin trial.

Pedigree	RD (µm)	TD (µm)	RK	MTD (µm)	RK	LD (µm)	RK	CWA (µm²)	RK	CWT (µm)	RK
44 × 51	49.90	41.41	4	45.66	2	32.90	1	610.38	3	5.21	30
51 × 44	49.04	43.37	1	46.21	1	32.72	2	667.96	1	5.67	12
44 × 20	47.60	40.68	7	44.14	4	31.23	4	598.63	5	5.39	25
20 × 44	47.15	41.17	5	44.16	3	31.24	3	597.41	6	5.44	21
44 × 14	46.65	40.09	11	43.37	6	30.31	7	591.58	7	5.48	18
27 × 20	46.38	41.71	2	44.04	5	30.55	6	636.27	2	5.74	5
14 × 44	45.89	39.81	14	42.85	8	30.14	8	583.40	9	5.37	27
20 × 51	44.69	41.60	3	43.14	7	30.95	5	573.29	14	5.23	29
44 × 31	44.33	40.54	8	42.43	9	29.08	10	598.84	4	5.72	7
26 × 14	43.29	38.70	28	41.00	14	28.91	13	539.79	27	5.20	32
27 × 14	43.11	39.56	18	41.33	11	28.57	15	573.99	13	5.44	22
15 × 14	42.77	39.16	23	40.96	16	27.76	24	582.10	10	5.73	6
31 × 44	42.75	39.23	22	40.99	15	28.04	19	562.25	18	5.60	15
14 × 51	42.48	39.78	15	41.13	13	28.07	18	580.36	11	5.67	13
51 × 20	42.16	40.31	9	41.24	12	29.19	9	539.81	26	5.21	31
14 × 20	42.15	38.92	24	40.53	20	27.56	25	572.44	15	5.68	11
48 × 14	42.09	38.45	30	40.27	24	27.96	20	542.71	25	5.35	28
15 × 20	42.09	40.72	6	41.41	10	29.03	11	564.20	16	5.41	23
1 × 20	42.01	39.31	19	40.66	19	28.90	14	523.73	32	5.06	34
1 × 14	41.92	39.57	17	40.74	18	27.88	22	578.90	12	5.63	14
26 × 20	41.54	39.28	21	40.41	21	28.96	12	511.21	34	4.98	35
31 × 20	41.33	40.19	10	40.76	17	28.14	17	560.17	19	5.55	17
51 × 14	40.77	38.86	27	39.82	26	27.07	28	549.90	21	5.56	16
31 × 51	40.68	40.07	12	40.38	22	28.18	16	549.57	22	5.39	24
51 × 31	40.52	40.07	13	40.30	23	26.97	30	585.66	8	5.91	2
32 × 20	40.21	39.65	16	39.93	25	27.81	23	538.86	28	5.37	26
2 × 20	40.12	39.30	20	39.71	27	27.46	26	538.34	29	5.44	19
48 × 20	40.10	37.61	35	38.86	30	27.22	27	496.76	35	5.08	33
7 × 14	39.54	38.87	26	39.20	28	26.99	29	535.90	30	5.44	20
7 × 20	39.24	38.30	31	38.77	32	27.90	21	462.97	36	4.75	36
20 × 14	39.24	38.47	29	38.85	31	25.95	32	555.51	20	5.75	4
20 × 31	39.04	38.88	25	38.96	29	26.25	31	544.32	24	5.69	8
32 × 14	38.95	38.06	32	38.50	33	25.73	33	546.48	23	5.69	9
14 × 31	37.91	37.72	33	37.81	34	24.15	36	562.70	17	6.14	1
2 × 14	37.53	37.31	36	37.42	36	24.52	35	534.03	31	5.81	3
31 × 14	37.34	37.65	34	37.49	35	24.83	34	522.46	33	5.68	10
Trial Mean	42.28	39.56		40.92		28.30		561.19		5.48	
SD	3.92	1.98		2.78		2.71		61.33		0.53	

6.3.2.2 Cross-sectional tracheid derived traits

Four additional cross-sectional tracheid property traits were calculated from the cross-sectional tracheid properties assessed with image analysis, as described in section 6.2.1. The four traits are tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall per mm² (PCell) and the Runkel ratio (RR). These four traits provide an indication of the paper-making ability of a particular pulp (Kibblewhite, 1999). Results from the analysis of variance indicated that differences between families were highly significant (p<0.001) for all calculated tracheid cross-sectional properties (Appendix 3).

Family mean values for calculated cross-sectional tracheid traits are presented in Table 6.4. Families are ranked according to TArea values. All three other traits are negatively correlated with TArea. Family 51 × 44 had the highest mean TArea, but was ranked among the lowest for NoTrach, PCell and RR. Family 2 × 14 had the lowest mean TArea, and was among the highest ranked for NoTrach, PCell and RR. These calculated traits are the product of tracheid traits assessed by image analysis, and therefore follow similar trends to tracheid and lumen diameters.

Mean calculated cross-sectional tracheid trait values of *P. patula* differed from those reported by Stanger (2003). Area mean values were generally higher that those found in the Stanger (2003) study, while NoTrach, PCell and RR values were lower. The RR mean values were lower than found in another study of open-pollinated *P. patula* families (Vermaak, 2007), but higher than those from a provenance study of *P. patula* (Stanger, 2003). These differences are probably because of the different sampling ages of *P. patula* in the various studies, and different populations and provenances used in these studies.

Table 6.4 Mean values per family for tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR). Families are ranked on means for TArea for the full-diallel and selected factorial crosses from the Martin trial.

Pedigree	TArea (µm²)	NoTrach (n/ mm²)	Rk	PCell %	Rk	RR	Rk
51 × 44	6686.26	149.97	36	10.00	30	0.35	32
44 × 51	6502.61	154.76	35	9.39	36	0.32	36
20 × 44	6108.83	165.03	34	9.81	35	0.35	30
44 × 20	6097.21	165.76	32	9.89	32	0.35	31
27 × 20	6083.42	165.40	33	10.48	23	0.38	22
44 × 14	5883.99	171.11	31	10.10	29	0.36	25
20 × 51	5852.57	172.73	30	9.83	34	0.34	35
14 × 44	5742.11	174.74	29	10.16	26	0.36	28
44 × 31	5646.57	177.64	28	10.61	21	0.39	17
27 × 14	5386.85	190.73	23	10.75	17	0.38	20
15 × 20	5385.21	186.10	27	10.47	24	0.37	24
51 × 20	5351.30	188.88	26	10.13	28	0.36	27
14 × 51	5318.12	189.86	25	10.96	13	0.41	11
31 × 44	5273.41	190.91	21	10.70	19	0.40	14
26 × 14	5267.32	190.71	24	10.25	25	0.36	26
15 × 14	5265.17	190.80	22	11.12	8	0.42	8
1 × 14	5226.83	193.65	19	11.10	9	0.41	12
31 × 20	5223.33	192.52	20	10.77	16	0.40	16
1 × 20	5209.68	195.82	17	10.15	27	0.35	29
14 × 20	5156.06	194.62	18	11.07	12	0.42	10
26 × 20	5133.00	195.99	16	9.98	31	0.34	33
31 × 51	5131.23	196.35	15	10.73	18	0.39	19
51 × 31	5104.55	196.50	14	11.48	6	0.44	6
48 × 14	5090.79	197.95	13	10.66	20	0.38	21
32 × 20	5011.06	200.59	12	10.81	15	0.39	18
51 × 14	4986.57	202.09	11	11.07	10	0.42	9
2 × 20	4960.57	202.80	10	10.89	14	0.40	15
7 × 14	4830.23	207.58	9	11.07	11	0.40	13
20 × 31	4771.18	210.12	8	11.41	7	0.44	7
20 × 14	4751.93	212.46	6	11.74	5	0.45	4
48 × 20	4740.50	211.42	7	10.48	22	0.37	23
7 × 20	4727.88	213.00	5	9.83	33	0.34	34
32 × 14	4664.78	216.54	4	11.74	4	0.44	5
14 × 31	4498.82	224.05	3	12.51	1	0.51	1
31 × 14	4428.31	228.31	2	11.87	3	0.46	3
2 × 14	4404.04	228.79	1	12.09	2	0.48	2
Trial Mean	5272.54	193.05		10.73		0.39	
SD	713.23	25.57		1.07		0.07	

6.3.2.3 Silviscan® traits calculated from image analysis

Results from the analysis of variance indicated that differences between families were highly significant (p<0.001) for all calculated Silviscan® traits (Appendix 3). The four calculated traits are Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS). Mean family values for the calculated Silviscan® traits are presented in Table 6.5, with families ranked on mean CS values. Family 51 × 44 had the highest CS value of 803.05 µg m^{-1}, was also ranked among the highest families for PM (184.83 µm) and WTS (3.10 µm), and was ranked 33rd for SS (230.97 m^2 kg^{-1}). All traits were correlated, with SS having a negative correlation with CS, PM and WTS. Family rankings were fairly stable across the four Silviscan® traits.

Results were similar to those reported by Stanger (2003) in the *P. patula* provenance study, but with maximum values being slightly higher for all traits. When the Silviscan® traits were compared to those of *P. radiata*, CS, PM and WTS were found to be greater, while the mean SS values were smaller (Shelbourne *et al*., 1997; Nyakuengama *et al*., 1999).

Table 6.5 Mean values per family for calculated Silviscan® traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS). Families are ranked on means for CS for the full-diallel and selected factorial crosses from the Martin trial.

Pedigree	CS ($\mu g\ m^{-1}$)	SS ($m^2\ kg^{-1}$)	Rk	PM (µm)	Rk	WTS (µm)	Rk
51 × 44	803.05	230.97	33	184.83	1	3.10	5
27 × 20	750.29	236.33	25	176.16	5	3.05	12
44 × 20	745.16	237.59	24	176.55	4	3.02	15
44 × 51	741.65	246.66	16	182.64	2	2.89	26
44 × 31	730.59	232.83	32	169.74	9	3.10	7
14 × 44	728.90	235.98	26	171.41	8	3.05	11
14 × 51	728.18	228.71	34	164.53	13	3.20	2
20 × 44	727.65	244.16	17	176.64	3	2.94	20
44 × 14	725.71	239.30	22	173.49	6	3.00	17
51 × 31	710.04	227.94	35	161.19	23	3.19	3
27 × 14	707.59	235.75	27	165.33	11	3.07	10
15 × 14	702.17	233.85	30	163.85	16	3.10	8
1 × 14	699.91	233.72	31	162.98	18	3.09	9
20 × 51	690.41	251.55	9	172.58	7	2.85	28
15 × 20	688.76	241.50	19	165.62	10	2.99	18
51 × 14	684.39	234.16	29	159.27	26	3.10	4
14 × 20	678.26	241.47	20	162.13	20	3.01	16
14 × 31	670.39	227.72	36	151.26	34	3.22	1
31 × 44	667.04	247.90	15	163.96	15	2.92	21
7 × 14	659.37	239.21	23	156.82	28	3.04	14
32 × 14	658.69	234.51	28	154.01	33	3.10	6
31 × 20	656.09	248.70	14	163.03	17	2.89	25
51 × 20	654.72	253.48	8	164.95	12	2.84	29
31 × 51	653.40	248.71	13	161.51	22	2.90	22
32 × 20	643.54	249.33	12	159.71	25	2.90	23
20 × 14	640.65	243.75	18	155.42	31	2.98	19
2 × 20	634.27	250.86	11	158.85	27	2.87	27
48 × 14	630.72	256.70	6	161.08	24	2.80	31
2 × 14	627.99	240.07	21	149.67	36	3.04	13
20 × 31	624.95	251.48	10	155.84	29	2.89	24
1 × 20	622.89	262.90	5	162.64	19	2.73	32
26 × 14	612.32	268.87	3	163.99	14	2.66	34
26 × 20	604.47	268.68	4	161.64	21	2.67	33
31 × 14	589.90	254.56	7	149.98	35	2.83	30
48 × 20	574.93	271.58	2	155.43	30	2.65	35
7 × 20	561.95	277.40	1	155.07	32	2.59	36
Trial Mean	673.00	245.22		163.68		2.95	
SD	80.23	20.20		11.12		0.26	

6.3.3 Site effects on cross-sectional tracheid traits

The effect of site (altitude) on cross-sectional tracheid properties was also investigated. Results from the 10 full-sib families from the half-diallel designs at the two sites at Martin and Nyangui were used in this analysis. Analysis of variance (ANOVA) indicated significant differences for site for some of the assessed and calculated cross-sectional tracheid properties (Appendix 4). For the traits assessed by image analysis, site effects for TD and CWA were highly significant (p<0.01), site effects for CWT were significant (p<0.05), and for RD, MTD and LD were non-significant (p>0.05) (Table 6.6). There was no significant site by family interaction for any of the cross-sectional tracheid traits (Appendix 4).

Table 6.6 Mean values for sites (Martin and Nyangui) for tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT).

Site	RD (µm)	TD (µm)	MTD (µm)	LD (µm)	CWA (µm²)	CWT (µm)
Nyangui	43.28	39.32	41.30	28.69	547.42	5.37
Martin	42.89	40.03	41.46	28.56	577.01	5.59
P-values	0.326	0.004	0.590	0.667	0.001	0.016

For calculated cross-sectional tracheid traits, there were highly significant (p<0.01) site effects for PCell, while the effects for TArea, NoTrach and RR were not significant (p>0.05) (Table 6.7). All calculated Silviscan® traits demonstrated no significant site effects (p>0.05) (Table 6.8).

Table 6.7 Mean values for sites (Martin and Nyangui) for derived traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR).

Site	TArea (µm²)	NoTrach (n/ mm²)	PCell %	RR
Nyangui	5363.54	189.51	10.28	0.38
Martin	5415.90	188.53	10.77	0.40
P-values	0.487	0.721	0.003	0.064

Table 6.8 Mean values per family for calculated Silviscan® traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS).

Site	CS (µg m^{-1})	SS (m^2 kg^{-1})	PM (µm)	WTS (µm)
Nyangui	701.66	237.43	165.21	3.05
Martin	694.08	240.30	165.84	3.01
P-values	0.465	0.282	0.590	0.209

Results from this study indicated that site did not have a statistically significant effect on many of the studied cross-sectional tracheid traits. The analysis showed that the traits TD, CWA and PCell displayed highly significant (p<0.01) site differences, while for CWT the differences were significant (p<0.05). These traits were generally greater in value at the lower altitude site planted at Martin, and could be due to more favourable growing conditions at the lower site. PCell is derived from CWA, so it is closely associated with this trait. In another study consisting of 30 open pollinated *P. patula* families conducted over six different sites in South Africa, highly significant site effects were found for CWT, RR and LD (Vermaak, 2007). There were also highly significant (p<0.01) site by family interaction for CWT and RR, but site by family interaction for LD was non-significant (Vermaak, 2007). In another study on *P. taeda* provenances and families in the south-east United States of America tested on two sites, no site effects were found for cross-sectional tracheid traits tracheid diameter (equivalent of MTD in the present study), LD and CWT (Belonger, 1998).

6.3.4 Combining ability analysis of a full-diallel and two half-diallels

6.3.4.1 Full-diallel mating design on one site at Martin

An analysis of variance for combining ability from the full-diallel mating design at Martin was undertaken for all cross-sectional tracheid traits (RD, TD, MTD, LD, CWA and CWT). The combining ability analysis results indicated highly significant effects for families and general combining ability (GCA) for all image analysis traits, except for CWT (see Table 6.9). The effect of specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects were all non-significant for all image analysis traits (p>0.05).

Table 6.9 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for cross-sectional traits tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT) for a full-diallel at Martin.

Source of variation	df	RD MS		TD MS		MTD MS		LD MS		CWA MS		CWT MS	
Families	19	81.628	**	11.293	**	35.761	**	36.319	**	6218.6	**	0.348	ns
GCA	4	360.158	**	44.930	**	156.030	**	158.342	**	17681.9	*	0.817	ns
SCA	5	6.058	ns	1.389	ns	3.229	ns	4.034	ns	2910.8	ns	0.185	ns
Rec	10	8.408	ns	3.067	ns	4.307	ns	4.045	ns	3163.6	ns	0.223	ns
Mat	4	10.210	ns	0.260	ns	2.731	ns	4.683	ns	2492.8	ns	0.376	ns
NMat	6	7.149	ns	4.911	ns	5.279	ns	3.573	ns	3605.7	ns	0.125	ns
Error	102	6.699		2.593		3.795		3.950		2814.7		0.256	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

The combining ability analysis results indicated highly significant (p<0.01).effects for families and general combining ability (GCA) for the calculated traits TArea, NoTrach, PCell and RR (Table 6.10). SCA, REC, Mat and NMat effects, on the other hand, were all found to be non-significant (p>0.05).

Table 6.10 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for calculated cross-sectional traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR) for a full-diallel at Martin.

Source of variation	df	TArea MS		NoTrach MS		PCell MS		RR MS	
Families	19	2369108	**	2784.0	**	4.013	**	0.014	**
GCA	4	10305769	**	12048.9	**	16.185	**	0.057	**
SCA	5	244244	ns	241.7	ns	0.900	ns	0.003	ns
Rec	10	282689	ns	384.5	ns	0.674	ns	0.003	ns
Mat	4	153983	ns	274.9	ns	1.122	ns	0.004	ns
NMat	6	362572	ns	454.3	ns	0.387	ns	0.001	ns
Error	102	257069		312.7		0.826		0.003	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

The combining ability analysis for Silviscan® traits indicated that family and GCA effects were highly significant ($p<0.01$) for CS, SS and PM, while WTS demonstrated a significant ($p<0.05$) effect for family only (Table 6.11). All other effects (SCA, REC, Mat and NMat) were non-significant ($p>0.05$).

Table 6.11 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for calculated Silviscan® cross-sectional tracheid traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS) for a full-diallel at Martin.

Source of variation	df	CS MS		SS MS		PM MS		WTS MS	
Families	19	14964	**	485	ns	10884	**	0.092	*
GCA	4	51051	**	656	ns	2499	**	0.117	ns
SCA	5	1970	ns	227	ns	72	ns	0.048	ns
Rec	10	6777	ns	513	ns	69	ns	0.097	ns
Mat	4	8739	ns	913	ns	44	ns	0.184	ns
NMat	6	5582	ns	258	ns	85	ns	0.041	ns
Error	102	4427		295		61		0.053	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

There is very little published information available on comprehensive combining ability studies of growth and tracheid properties of softwood species. To the best of the author's knowledge, this is the first comprehensive full-sib genetic analysis study on cross-sectional tracheid properties of *P. patula*. The only other comprehensive study found was a study on wood density, tracheid dimensions and Silviscan® tracheid traits of *P. radiata* conducted in Australia (Nyankuengama, 1997; Nyankuengama *et al*., 1999). The results from the *P. radiata* study concurred with the present study, finding that GCA was the only significant effect (p<0.05) in the genetic analysis of a 4 × 4 diallel design. The other combining ability components (SCA, REC, Mat and NMat effects) were all found to be non-significant (p>0.05) (Nyankuengama, 1997).

6.3.4.2 Half-diallel mating design on two sites at Martin and Nyangui

An analysis of variance for combining ability from the half-diallel mating design at Martin and Nyangui was undertaken for all cross-sectional tracheid traits (RD, TD, MTD, LD, CWA and CWT). The combining ability analysis results indicated highly significant (p<0.01) effects for families for all the traits, except for CWT (see Table 6.12). The combining ability analysis results were more varied than the full-diallel design on a single site. For RD, the GCA effect was highly significant (p<0.01), and all other effects (SCA, REC, Mat, NMat) were non-significant (p>0.05). For TD, the family and site effects were highly significant (p<0.01), GCA, SCA and GCA- by- site interaction was significant (p<0.05).

MTD yielded highly significant (p<0.01) family and GCA effects, and significant (p<0.05) SCA and GCA- by- site effects. The combining ability effects family and GCA were highly significant (p<0.01) for LD, while CWA yielded highly significant (p<0.01) effects for family and site only. For CWT, only families were significant (p<0.05), all other effects were non-significant (p>0.05).

Table 6.12 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean square values for cross-sectional traits tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	**df**	**RD MS**		**TD MS**		**MTD MS**		**LD MS**		**CWA MS**		**CWT MS**	
Families	9	174.7	**	21.3	**	76.7	**	60.6	**	18499	**	0.286	ns
Site	1	2.3	ns	21.2	**	2.4	ns	0.1	ns	32205	**	1.677	*
Family*Site	9	3.7	ns	2.8	ns	2.7	ns	4.2	ns	1541	ns	0.147	ns
GCA	4	385.5	**	40.6	*	166.0	**	132.2	**	33012	ns	0.338	ns
SCA	5	8.7	ns	5.0	*	5.7	*	2.7	ns	6344	ns	0.224	ns
GCA*Site	4	4.3	ns	5.9	*	5.1	*	6.4	ns	1502	ns	0.031	ns
SCA*Site	5	2.3	ns	0.6	ns	0.9	ns	2.3	ns	2136	ns	0.245	ns
Error	118	5.8		2.3		3.3		3.9		2643		0.274	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

The analysis of variance for combining ability from the half-diallel mating design at Martin and Nyangui for all calculated cross-sectional tracheid traits also yielded varying results. Apart from highly significant (p<0.01) family effects for all four traits (TArea, NoTRach, PCell and RR), GCA effects were highly significant (p<0.01) for the two traits TArea and NoTrach (Table 6.13). Both these traits also demonstrated significant (p<0.05) effects for SCA, while TArea displayed a significant (p<0.05) GCA- by- site interaction.

Table 6.13 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for square values for calculated cross-sectional traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	TArea MS		NoTrach MS		PCell MS		RR MS	
Families	9	5076505	**	5843	**	4.917	**	0.015	**
Site	1	218934	ns	114	ns	6.984	**	0.011	ns
Family*Site	9	181736	ns	264	ns	0.755	ns	0.003	ns
GCA	4	10885751	**	12702	**	10.409	ns	0.031	ns
SCA	5	416095	*	453	*	0.505	ns	0.001	ns
GCA*Site	4	355395	*	461	ns	0.396	ns	0.002	ns
SCA*Site	5	59404	ns	80	ns	0.889	ns	0.002	ns
Error	118	218430		287		0.896		0.003	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

The results of analysis of variance for combining ability from the half-diallel mating design at Martin and Nyangui for calculated Silviscan® cross-sectional tracheid traits was also varied. Family effects were highly significant for all traits, while CS and PM demonstrated highly significant (p<0.01) GCA effects (Table 6.14). PM also showed significant (p<0.05) SCA and GCA- by- site interaction effects.

Table 6.14 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for square values for calculated Silviscan® cross-sectional tracheid traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	CS MS		SS MS		PM MS		WTS MS	
Families	9	48736	**	874	**	1229	**	0.135	**
Site	1	466	ns	149	ns	38	ns	0.045	ns
Family*Site	9	3332	ns	271	ns	44	ns	0.052	ns
GCA	4	104516	**	1443	ns	2659	**	0.207	ns
SCA	5	7772	ns	485	ns	92	*	0.088	ns
GCA*Site	4	4172	ns	221	ns	82	*	0.042	ns
SCA*Site	5	2286	ns	281	ns	15	ns	0.053	ns
Error	118	3856		248		53		0.047	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

6.3.5 Estimation of genetic parameters utilising diallel and factorial data

The combining ability analysis of the full-diallel indicated, as was the case of wood density in Chapter 5, that there were no reciprocal effects. As in the case of wood density, data from the reciprocal crosses were pooled together and the 16 additional crosses from the factorial design added to constitute a larger incomplete half-diallel (see Figure 4.1). This increased the number of crosses to 26, which would yield more robust genetic parameters for the cross-sectional tracheid traits.

The individual broad-sense heritability (H^2) values ranged from 0.38 to 1.13 for the basic cross-sectional tracheid traits (Table 6.15). Individual narrow-sense heritabilities (h^2) ranged from 0.38 to 1.04, with standard error (SE) values ranging from 0.21 to 0.38. In the case of CWT, the dominance variance was zero, so H^2 and h^2 values were the same.

Table 6.15 Genetic effects and heritabilities for cross-sectional traits tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT) for a constituted half-diallel at Martin.

Parameters	RD	TD	MTD	LD	CWA	CWT
Additive variance	9.306	2.940	9.306	8.135	2650	0.124
Dominance variance	0.551	0	0.551	0.196	557	0
Phenotypic variance	8.728	4.197	8.728	7.998	4290	0.304
Individual H^2	1.13	0.70	1.13	1.04	0.74	0.38
Individual h^2	1.04	0.70	1.04	1.02	0.48	0.38
SE of h^2	0.24	0.23	0.24	0.23	0.28	0.21

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

The calculated cross-sectional tracheid properties TArea, NoTrach, PCell and RR individual H^2 ranged from 1.13 to 0.74 (Table 6.16). Individual h^2 ranged from 1.04 to 0.74, with SE's of between 0.23 and 0.25. In the case of PCell and RR, the dominance variance was zero, thus H^2 and h^2 values were the same.

Table 6.16 Genetic effects and heritabilities for calculated cross-sectional traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR) for a constituted half-diallel at Martin.

Parameters	TArea	NoTrach	PCell	RR
Additive variance	610500	733	0.958	0.003
Dominance variance	38445	57	0	0
Phenotypic variance	572989	735	1.241	0.005
Individual H^2	1.13	1.07	0.76	0.74
Individual h^2	1.04	0.95	0.76	0.74
SE of h^2	0.24	0.25	0.23	0.23

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

The values of calculated Silviscan® cross-sectional tracheid properties CS, SS, PM and WTS individual H^2 ranged from 1.13 to 0.63 (Table 6.17). Individual h^2 ranged from 1.04 to 0.56, with SE's of between 0.24 and 0.27. In the case of CS, the dominance variance was zero, thus H^2 and h^2 values were the same.

Table 6.17 Genetic effects and heritabilities for calculated Silviscan® cross-sectional tracheid traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS) for a constituted half-diallel at Martin.

Parameters	CS	SS	PM	WTS
Additive variance	6296	324	149	0.046
Dominance variance	0	17	9	0.002
Phenotypic variance	7592	477	140	0.075
Individual H^2	0.66	0.71	1.13	0.63
Individual h^2	0.66	0.58	1.04	0.56
SE of h^2	0.27	0.27	0.24	0.25

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

Heritability estimates from this study were fairly high and indicate that cross-sectional tracheid properties are under strong control. Much higher individual heritability estimates for cross-sectional tracheid traits were found than those calculated for a *P. patula* provenance study (Stanger, 2003). These heritability estimates are also much higher than those from a study of open-pollinated *P. taeda* conducted in south-eastern United States of America (Belonger, 1998). The range of calculated standard errors for h^2 is also higher compared to the *P. patula* provenance study (Stanger, 2003).

Similarly high h^2 estimates for cross-sectional tracheid traits have been found in studies of other *Pinus* species. In a study of a 4 × 4 diallel of *P. radiata* conducted in Australia, very high h^2 estimates were found for common cross-sectional tracheid properties (Nyakuengama, 1997). Some of the h^2 estimates from this study were greater than one, the theoretical maximum value (Nyakuengama, 1997). In this *P. radiata* study using Silviscan®, the h^2 estimates ranged from

0.46 for SS, to 2.04 for PM, and SE for h^2 were also much higher than the present study, ranging from 0.31 for SS to 1.29 for PM (Nyakuengama, 1997). In another published *P. radiata* study conducted on 13-year old trees from open-pollinated families, h^2 estimates also ranged between 0.53 (TD) to 1.09 (RD) (Shelbourne *et al*., 1997). CWT was found to be lower for *P. radiata* with a h^2 estimate of 0.50 (Nyankuengama *et al*., 1999).

The heritability estimates, although higher than reported elsewhere, confirm that cross-sectional tracheid traits are under strong genetic control. In this study, heritability estimates ranged between 0.48 and 1.04, with standard errors (SE) ranging from 0.23 to 0.28. These values were similar, but not as extreme as those found in the *P. radiata* study (Nyakuengama, 1997), and the SE values were also smaller. Nevertheless, results found in this *P. patula* study do need to be viewed with some caution. Genetic parameters that are associated with large errors are probably due to large differences between trees within a family (Nyakuengama, 1997), or the relatively small number of parents used in this study. Heritability estimates exceeding one reflect genetic sampling error of family variation, which is multiplied by a factor of four to calculate additive genetic variance in the heritability ratio (Shelbourne *et al*., 1997).

As for heritability estimates, caution should also be applied to the interpretation of genetic parameters of some of the calculated cross-sectional tracheid traits. When viewing the heritability estimates, the traits that were independently assessed (RD, TD, LD) had some of the highest values. The calculated or derived traits were more intermediate and were the product of the independently assessed traits, and their values depended on the traits they were derived from (Shelbourne *et al*., 1997).

6.3.6 Phenotypic and genetic correlations between cross-sectional tracheid traits

The relationship between different cross-sectional tracheid traits was also investigated. Phenotypic and genetic correlations were calculated for the various traits that were assessed. It must be pointed out that many of the calculated traits that were investigated are products of the independently assessed cross-sectional tracheid traits. There would therefore be a level of auto-correlation between the independently assessed traits and other calculated traits (Shelbourne *et al*., 1997).

For the cross-sectional tracheid traits assessed by image analysis, strong individual tree and family correlations were established (Table 6.18). Individual tree correlations between cross-sectional tracheid dimensions (RD, TD, MTD and LD) were strong (0.75 to 0.97), while correlations with CWT were generally weak and negative. Correlations between these dimensional traits and CWA were weaker, ranging from 0.35 to 0.72. Family phenotypic correlations were generally stronger than the individual tree correlations for all trait comparisons.

Table 6.18 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among cross-sectional traits tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT). Significant phenotypic correlations are indicated in bold with *p*-values in brackets.

	RD	TD	MTD	LD	CWA	CWT
RD	-	**0.75 (0.000)**	**0.97 (0.000)**	**0.91 (0.000)**	**0.61 (0.000)**	-0.08 (0.226)
TD	**0.82 (0.000)**	-	**0.88 (0.000)**	**0.77 (0.000)**	**0.72 (0.000)**	**-0.15 (0.024)**
MTD	**0.98 (0.000)**	**0.91 (0.000)**	-	**0.91 (0.000)**	**0.69 (0.000)**	-0.00 (0.957)
LD	**0.95 (0.000)**	**0.87 (0.000)**	**0.96 (0.000)**	-	**0.35 (0.000)**	**-0.40 (0.000)**
CWA	**0.75 (0.000)**	**0.77 (0.000)**	**0.78 (0.000)**	**0.59 (0.000)**	-	**0.71 (0.000)**
CWT	-0.17 (0.307)	-0.06 (0.727)	-0.15 (0.392)	**-0.42 (0.012)**	**0.49 (0.003)**	-

Genetic correlations were generally strong with relatively small standard errors (Table 6.19). The genetic correlations ranged from 0.70 (LD and CWA) to 0.98 (RD and MTD). Correlations between the dimensional traits and CWT were negative and also much weaker, ranging from -0.25 (RD and CWT) to -0.47 (LD and CWT). The genetic correlation between CWA and CWT was weak (0.29) with a large standard error.

Individual tree and phenotypic correlations for the calculated traits TArea, NoTRach, PCell and RR were all found to be significant and were generally strong (Table 6.20). Individual tree correlations ranged from 0.57 to 0.98, and family correlations from 0.74 to -0.99. As expected, TArea was negatively correlated to the other traits NoTrach, PCell and RR. Genetic correlations were stronger than the phenotypic correlations, and ranged from -0.80 to 0.99, with relatively small standard errors (Table 6.21).

Table 6.19 Additive genetic correlations with standard errors among cross-sectional traits tracheid radial diameter (RD), tangential diameter (TD), mean tracheid diameter (MTD), lumen diameter (LD), cell wall area (CWA) and cell wall thickness (CWT).

	RD	TD	MTD	LD	CWA	CWT
RD	-					
TD	0.86 ± 0.09	-				
MTD	0.98 ± 0.01	0.93 ± 0.06	-			
LD	0.95 ± 0.03	0.92 ± 0.06	0.97 ± 0.02	-		
CWA	0.84 ± 0.10	0.77 ± 0.16	0.84 ± 0.10	0.70 ± 0.17	-	
CWT	-0.25 ± 0.32	-0.27 ± 0.38	-0.26 ± 0.49	-0.47 ± 0.26	0.29 ± 0.49	-

Table 6.20 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among calculated cross-sectional traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR). Significant phenotypic correlations are indicated in bold with p-values in brackets.

	TArea	NoTrach	PCell	RR
TArea	-	**-0.98 (0.000)**	**-0.57 (0.000)**	**-0.52 (0.000)**
NoTrach	**-0.99 (0.000)**	-	**0.57 (0.000)**	**0.52 (0.000)**
PCell	**-0.74 (0.000)**	**0.77 (0.000)**	-	**0.98 (0.000)**
RR	**-0.71 (0.000)**	**0.74 (0.000)**	**0.99 (0.000)**	**-**

Table 6.21 Additive genetic correlations with standard errors among calculated cross-sectional traits tracheid area (TArea), number of tracheids per mm² (NoTrach), percentage cell wall area (PCell) and Runkel Ratio (RR).

	TArea	NoTrach	PCell	RR
TArea	-			
NoTrach	-0.99 ± 0.01	-		
PCell	-0.82 ± 0.11	0.85 ± 0.10	-	
RR	-0.80 ± 0.12	0.84 ± 0.11	0.99 ± 0.01	**-**

The correlations among the calculated Silviscan® cross-sectional tracheid traits generally reflected their auto-correlation with the independently assessed dimensional traits described earlier. Phenotypic correlations among the Silviscan® calculated traits were statistically significant (p<0.05) and were very strong (Table 6.22). The additive genetic correlations (Table 6.23) between the Silviscan® calculated traits were generally strong and were similar to estimates pubished for *P. patula* (Stanger, 2003) and for *P. radiata* (Shelbourne *et al*., 1997; Nyakuengama, 1997; Nyakuengama *et al.*, 1999).

In summary, there were very strong phenotypic correlations among most of the cross-sectional tracheid traits investigated in the present study. This gives an indication that not all traits have to be assessed to determine their level of genetic control and to make gains through selection and breeding.

Table 6.22 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among calculated Silviscan® cross-sectional tracheid traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS). Significant phenotypic correlations are indicated in bold with *p*-values in brackets.

	CS	SS	PM	WTS
CS	-	**-0.84 (0.000)**	**0.76 (0.000)**	**0.79 (0.000)**
SS	**-0.77 (0.000)**	-	**-0.31 (0.000)**	**-0.99 (0.000)**
PM	**0.80 (0.000)**	-0.24 (0.158)	**-**	**0.22 (0.001)**
WTS	**0.71 (0.000)**	**-0.99 (0.000)**	0.14 (0.416)	**-**

Table 6.23 Additive genetic correlations with standard errors among calculated Silviscan® cross-sectional tracheid traits Coarseness (CS), Specific Surface (SS), Perimeter (PM) and Wall Thickness (WTS).

	CS	SS	PM	WTS
CS	-			
SS	-0.72 ± 0.22	-		
PM	0.84 ± 0.14	-0.21 ± 0.46	-	
WTS	0.63 ± 0.27	-0.99 ± 0.01	0.09 ± 0.33	**-**

6.4 Conclusions

Results from this cross-sectional tracheid property study indicated that large individual tree variation was present for all traits. Highly significant differences between full-sib families were also found for most of the assessed and derived traits. Site effects were found to be not significant for the assessed traits radial tracheid diameter, mean tracheid diameter and lumen diameter. Tangential tracheid diameter, cell wall area and cell wall thickness were found to have significant site effects. Most of the calculated tracheid traits and Silviscan® calculated traits did not have significant site effects, except for percentage cell wall area.

The full-diallel analysis indicated that general combining ability was the most important and significant effect for the image analysis assessed traits. Highly significant differences were also found between families. Only cell wall thickness was found to also have non-significant effects for families and general combining ability. No specific combining ability, reciprocal, maternal or non-maternal effects were found for these traits. The same applied for the calculated traits tracheid area, number of tracheids per mm^2, percentage cell wall area and the Runkel ratio. For the Silviscan® calculated traits, general combining ability effects were highly significant for coarseness and perimeter, but none of the effects were significant for specific surface and wall thickness.

The results of half-diallel analysis consisting of 10 full-sib families at two sites indicated that site effects were mostly not significant for all cross-sectional tracheid traits. Some exceptions were cell wall area and cell wall thickness, and percentage cell wall area. Significant general combining ability effects were found for most of the traits, with tangential tracheid diameter and mean tracheid diameter, tracheid area and number of tracheids per mm^2 and perimeter also displaying some significant specific combining ability effects. The latter were generally weaker than the general combining ability effects. Some significant

general combining ability by site interaction was also found for a few traits, namely tangential tracheid diameter, mean tracheid diameter, tracheid area and perimeter. These results indicate that there is a high level of additive control for cross-sectional tracheid traits, and that non-additive effects play a minor role.

The genetic parameter analysis yielded heritability estimates that were generally much higher than reported for growth traits. Many of the heritability estimates were greater than one, and was also associated with moderately high standard errors. This may be an indication of large within family variation, caused by the limited number of trees per family included in this study. The heritability estimates were larger than reported for open pollinated *P. patula* families (Stanger, 2003; Vermaak, 2007), but were similar to those reported for *P. radiata* (Shelbourne *et al*., 1997; Nyakuengama, 1997 and Nyakuengama *et al.,* 1999). Although the high heritability estimates may be associated with high standard errors, they are in line with published data for other *Pinus* species, indicating a high level of genetic control of the studied traits.

The phenotypic and genetic correlation results show that there are high levels of correlation among the different cross-sectional tracheid traits. These correlations are also due to genetic control of the contrasted traits. There is a high level of auto-correlation among the assessed and calculated traits present, and the correlation results should be interpreted accordingly. The strong correlations among certain traits indicate that assessment of all traits may not be necessary, and fewer traits can be assessed in future studies.

Tracheid wall thickness was assessed in two different ways in the present study. With the image analysis assessment of cross-sectional tracheid traits, cell wall thickness is determined by subtracting the lumen diameter from the mean tracheid diameter (Stanger, 2003). The calculated Silviscan® cell wall thickness is also derived, and not assessed directly (Evans *et al.*, 1995). These two

methods produced very different results, with the image analysis assessment returning values more than two times that of the Silviscan® values. These different values for the two methods were also found in a provenance study of *P. patula* (Stanger, 2003). In this latter study, the Silviscan® method yielded higher heritability estimates (0.56 versus 0.38) than the image analysis assessment (Stanger, 2003). In the following chapter (Chapter 7), the use of the MorFi® analyser will be investigated to assess both tracheid length and assessing cell wall thickness. With the MorFi® analyser, the cell wall thickness of a large number of macerated tracheids is assessed directly.

The large ranges in cross-sectional tracheid trait values, strong general combining ability effects, high heritability estimates and strong correlations between investigated traits indicate that good opportunities exist for tree improvement through tree breeding and selection.

Chapter 7

Inheritance of tracheid dimension traits of *P. patula* measured by MorFi fibre analysis

7.1 Introduction

The great majority of softwood volume is composed of long, slender cells called longitudinal tracheids which are oriented parallel to the stem axis. Softwood tracheids produce much longer pulp fibres than hardwoods, which have the greatest influence on the strength properties in paper products (Mimms, 1993). Strong relationships between the properties of tracheids and the properties of Kraft pulp handsheets have been demonstrated (Dinwoodie 1965). Tracheid length is the most important characteristic to take into account in papermaking, because long pulping fibres can make more bonds with other fibres in the handsheet network (Niskanen, 1998). Furthermore, tracheid length and cell wall thickness are two important properties determining the final product quality. Tracheid length becomes particularly important in juvenile wood of some conifers as shorter tracheids are produced in the juvenile core, which may fall below the required level (Zobel and van Buijtenen, 1989). There is a close relationship between fibre properties and other wood properties such as density, and also with post-processing pulp and paper properties (Zobel and Jett, 1995).

Tracheid length, unlike wood density, is influenced by the growth rate of a tree. The length of a newly formed tracheid is determined by the length of the cambial initial from which it is derived (Megraw, 1985). For *P. patula* grown in Zimbabwe, age and site effects on tracheid length were substantial (Muneri and Balodis, 1998). Also, there is always a range of different tracheid length sizes at any given point; tracheids are never of uniform size. This can be explained by the fact that tracheids at the top of every growth ring are shorter than at the start of the growth ring (Dinwoodie, 1961).

The inheritance of tracheid length is the most studied wood property trait after wood density. Most tracheid properties are relatively strongly inherited, especially tracheid or fibre length; and have been widely studied (Zobel and van Buijtenen, 1989). As in the case of tracheid cross-sectional properties, Zobel and Jett (1995) report that studies on the genetics of tracheid length are scattered with inconclusive or contradictory results. This is ascribed to the limited genetic base, lack of a mating structure and limited sampling and replications that formed the basis of these studies (Zobel and Jett, 1995). Despite these shortcomings, the reported heritabilities in the reviewed studies are high enough and variation broad enough to enable good genetic gains through breeding (Zobel and Jett, 1995).

Tracheids are small in size and there are a large number of tracheids per unit area and require sensitive assessment methods. Assessment technology has advanced rapidly in recent years. A number of fully automated, optical and electrical devises with image analysis systems are now available to assess anatomical features. Examples of these analysers are the Kajaani FS-100 and FS-200 and the MorFi® Analyser, have the capacity to rapidly assess many thousands of fibres per sample (Jackson, 1988; Muneri, 1994; Robertson *et al*., 1999; Turunen *et al.*, 2005; Hirn and Bauer, 2006). Some of these systems, such as the MorFi® Analyser, also assess additional traits such as fibre length distribution, fibre coarseness, width, kinks, curls, number of fibres per gram, area of fines, and surface area and length of shives (Turunen *et al.*, 2005).

This chapter reports on results from a tracheid dimension study using the MorFi® fibre analyser system. In cited literature, tracheids that have been separated through pulping or maceration, is also referred to as fibres. In this chapter, the words tracheid and fibre will be used interchangeably. Samples from the 5 × 5 full-diallel and half-diallel designs as well as selected crosses from a 5 × 9 factorial design were used as outlined in Chapter 3. The objective of this chapter is to quantify the inheritance of tracheid dimensions in the juvenile wood of *P. patula* grown in Southern Africa.

7.2 Materials and Methods

After completion of the wood density and cross-sectional tracheid trait assessments on the radial-plank samples, the samples as well as the off-cut sections from the original sample wedges were returned by the CSIR. These sample sections were manually chipped with vertically mounted Stanley® knife blades into match-stick sized wood chips of approximately 2 mm × 2 mm × 15 mm in size. A single sample of wood chips was produced per tree from the wedge sections. In this study radial or age trends were not investigated, as the samples were deemed too small to produce enough macerated fibre to warrant radial subdivision. Therefore, no attempt was made to subdivide a sample into radial sections. The wood chips were macerated in preparation for tracheid dimension assessment using methods first described by Franklin (1945) with some adaptations (Dodd, 1986). The purpose of the maceration process is to chemically separate the cell walls and loosen the bonds between tracheids (Dodd, 1986).

A maceration solution was made up consisting of 30% hydrogen peroxide and glacial acetic acid in a 1:1 ratio at the wood laboratory at the Shaw Research Centre near Howick, South Africa. Wood chips were placed in labelled bottles and were completely submerged in the maceration solution. Sample bottles were placed in a water-bath at a constant 60°C temperature for 48 hours inside a fume-hood. The maceration process was deemed complete when the samples were completely pale and partly retained the shape of the original wood chips. The macerated samples were then decanted into filter-paper lined funnels of 15 cm diameter to drain off the maceration solution. Distilled water was used to rinse the samples 10 times with a laboratory wash bottle until all the maceration solution was washed out. The optimum number of rinses was determined by testing a batch of samples and measuring the pH level of the run-off water repeatedly till it measured neutral (following Mansfield *et al*., 2009).

After allowing the rinsed samples to dry over-night in the fume-hood, the macerated wood chips were removed from the filter paper and placed inside labelled petri dishes. The samples were then dried in a laboratory oven at 45°C for 24 hours till bone dry, i.e. till there was no further weight loss of samples. The samples were then heat-sealed inside each petri dish with plastic tubing and stored at room temperature (approximately 15°C) in air-tight containers.

7.2.1 Assessment of tracheid dimensions

Samples were transported under controlled conditions in a cool box at between 5 and 10°C to the Sappi Technology Centre in Pretoria, South Africa for assessment of tracheid dimensions. An accurately weighed sub-sample of 0.4 g was taken from each sealed petri dish and was re-suspended in 400 ml of distilled water. The re-suspended solution was agitated with a hand-held cyclone mixer to ensure that tracheids were completely separated and not clumped together in bundles, and was freely suspended in the distilled water.

A MorFi® LB-01 (Morphological Fibre Analysis Apparatus) fibre analyser and MorFi® WT cell wall thickness analyser manufactured by TECHPAP in France were used to determine the tracheid dimensions of the macerated samples. The MorFi® system consists of a measuring cell, hydraulic pump and a computer interface. Fibre dimensions were assessed automatically by a computer analysis of images of suspended fibres flowing through a flat cell under observation of a digital CCD (charge-coupled device) video camera. The analysis is completed on a fibre network, so that assessments are done in the natural unrestrained environment, allowing for rapid, reliable and accurate statistical assessment of thousands of fibres (Sawoszczuk *et al.*, 2004). The optical resolution was set at 10 µm and morphological characteristics of tracheids, shives and fine elements were assessed.

A total of eight samples at a time were loaded onto a sampling carousel. The assessment procedure and capturing of data happened automatically. Each assessment cycle, consisting of hydraulic uptake of the sample suspension, image analysis, data generation and storage, and clean-out of the system, took approximately 15 minutes per sample. A sub-sample of 25 ml was taken from the prepared fibre suspension and diluted by making up the solution to 500 ml of water. For the measurement of cell wall thickness, successive images of a fibre was taken with the MorFi® WT cell wall thickness analyser CCD camera set at 1 µm intervals solution (Palmer, 2009). A minimum of 75 fibre images were assessed. Cell wall thickness assessments took approximately 8 minutes per sample.

In the MorFi® system tracheids, also referred to as fibres after maceration, are defined as being 200 to 10 000 µm in length, and between 5 and 75 µm in width (Palmer, 2009). Shives are defined as elements with a width that is greater than the maximum value of 75 µm of a fibre, while fine elements have dimensions that are smaller than those of fibres, i.e. shorter than 200 µm and narrower than 5 µm (Palmer, 2009). A minimum of 10 000 fibres, excluding fine elements, were assessed for length and width properties for each sample. Width measurements were undertaken at approximately 75 000 measuring points along the cell walls in the 75 images.

The different traits assessed with the two MorFi® analyser systems consisted of tracheid length, tracheid width and cell wall thickness traits. Table 7.1 provides a list of all the traits and definitions that were considered. Apart from the dimensional traits, the percentage fibres which displayed defects (curl, kink, broken ends) were also calculated by the MorFi® analyser system. Fibres with defects will translate into defects in quality and strength properties in the paper produced when high percentages of these defects are present.

Table 7.1 Summary of tracheid dimension traits investigated in this study.

Abbreviation	Description
MATL	Arithmetic tracheid length (µm)
MWTL	Tracheid length weighted in length (µm)
MTW	Tracheid width (µm)
MCWT	Tracheid wall thickness (µm)
MTnum	Number of tracheids per gram (No/g)
MTC	Coarseness of tracheids (mg/m)
MKT	Percentage kinked tracheids (%)
MCT	Percentage curl (%)
MBT	Percentage break ends (%)
MFines	Percentage area of fines (%)

Longer tracheids provide better bonding and therefore better strength properties of Kraft pulp paper products. The two tracheid dimension traits MATL and MWTL provide an estimation of the mean tracheid length of a sample (Palmer *pers. comm*., 2013). Length-weighted tracheid length (MWTL) is a more meaningful trait, as it is corrected for the natural bias present in size distributions toward shorter tracheids, which are usually over represented (Schimleck *et al.*, 2004). MTW is important because wider tracheids increase the bulk of a paper sheet, with fewer tracheids per gram. MCWT is another important trait as tracheids with thick cell walls are usually strong and rigid, which will translate into a higher tear index of a paper sheet, but requires more refining energy to develop flexibility and tensile properties. MTnum indicates how many million tracheids are present per gram of pulp, generally the more tracheids the better the paper strength and optical properties. MTC is important as high tracheid width usually have high coarseness and a low number of tracheid per gram. Pulp with low coarseness usually shows better strength properties. Large percentages of the tracheid defects MKT, MCT and MBT indicate damaged and weaker pulp. A high percentage of MFines result in slow drainage of a pulp, but can also contribute to strength development till it reaches a critical level and cause drainage problems (Palmer, *pers. comm*., 2013).

7.3 Results and Discussion

7.3.1 Introduction

The analysis of tracheid dimension data was conducted in the sequence outlined in Chapter 4. This sequence is also followed in the presentation of the results. Tracheid dimension traits were analysed firstly to determine the range of variation present. An analysis of variance was then carried out on the 36 full-sib families from the full-diallel and factorial mating designs at Martin to determine significant family differences for all tracheid dimension traits. The effect of site was also investigated by comparing the two half-diallel mating designs at Martin and Nyangui.

Macerated pulp samples are heterogeneous in nature and the distribution of tracheids or fibres in size classes are also an important consideration. Some of these distributions are also presented in addition to the analysis of variance of average trait values. Pith-to-bark age trends were not investigated, as the relatively small original wedge samples did not produce sufficient macerated fibre to enable sub-division into age classes. A single value for each tree sample was therefore assessed.

A combining ability analysis was then carried out with data from the full-diallel at the Martin site and half-diallels at Martin and Nyangui sites. Genetic parameters were estimated for tracheid dimension traits using data from both mating designs at the Martin site, allowing for estimations based on 26 full-sib families. Lastly, phenotypic and genetic correlations were calculated for comparison of different tracheid dimension traits.

7.3.2 General descriptive statistics and family analysis of tracheid dimensions

Tracheid dimension property data was examined for deviations from normality to comply with the standard ANOVA assumptions. Tracheid coarseness (MTC) data displayed a high skewness level (above 3) as well as a high coefficient of variation, indicating the presence of outliers. Basic statistics were calculated for all the tracheid dimension traits (Table 7.2). Coefficient of variation levels were low for most of the traits, indicating that sampling for the particular traits were done effectively. With most of the traits, a large amount of variation was present, indicating that there was opportunities for selection for those traits.

The most investigated tracheid trait of *P. patula* is tracheid length. Tracheid dimension results from this study compare well with other *P. patula* studies conducted in Southern Africa (Wright and Sluis-Cremer, 1992; Barnes *et al*., 1994; Ishengoma *et al*., 1995; Muneri and Balodis, 1998; Stanger, 2003; Vermaak, 2007). Many of these cited studies compared tracheid dimensions across growth rings, but mean values for similar aged material are comparable with results from this study. Many studies on various *Pinus* species also report a dramatic increase in tracheid length in a radial direction during the first 10 years of growth (Stanger, 2003).

Table 7.2 Summary statistics of cross-sectional tracheid traits investigated in this study for both trials at Martin and Nyangui.

Variable	Mean	SD	Minimum	Maximum	Range	CV	N
MATL (µm)	1415.2	94.6	1164	1731	567	6.7	300
MWTL µm)	2222.4	160.6	1749	2670	921	7.2	300
MTW (µm)	40.5	1.0	36.3	43.5	7.2	2.5	300
MCWT (µm)	5.0	0.3	4.1	6.2	2.0	6.0	299
MTnum (No/g)	3.2	0.5	1.2	4.5	3.3	14.8	300
MTC (mg/m)	0.2	0.04	0.16	0.61	0.44	17.7	300
MKT (%)	13.0	2.0	7.2	18.4	11.2	15.6	300
MCT (%)	5.5	0.6	4.0	7.3	3.2	10.4	300
MBT (%)	36.3	1.8	31.3	42.9	11.6	5.1	300
Mfines (%)	1.800	0.386	1.118	4.2	3.082	21.4	300

Results from the analysis of variance indicated that differences between families were significant (p<0.05) for most of the traits examined (Appendix 5). The traits MWTL and MKT did not show any significant family differences. Family mean results for all tracheid dimension traits are provided in Tables 7.3 and 7.4. In Table 7.3, families are ranked according to MATL in descending order, and in Table 7.4, families are ranked according to MKT. There does not appear to be a good relationship between MATL and MWTL, indicating that there are a large proportion of shorter tracheids present in the samples. There is also a large proportion of broken fibres present in the samples (with a mean of 36.17%), but this could be linked to the sample preparation process.

Table 7.3 Mean values per family for MorFi® traits arithmetic tracheid length (MATL, µm), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum) and tracheid coarseness (MTC). Families are ranked on means for MATL from the Martin trial.

Family	MATL (µm)	MWTL (µm)	Rk	MTW (µm)	Rk	MCWT (µm)	Rk	MTnum (No/g)	Rk	MTC (mg/m)	Rk
1 × 14	1497.5	2307.5	28	41.2	2	5.09	11	3.11	28	0.239	19
32 × 20	1481.8	2334.3	32	40.6	18	4.84	29	3.30	14	0.227	29
51 × 31	1479.0	2350.2	33	40.5	21	4.94	23	3.30	16	0.227	28
20 × 31	1478.0	2330.5	31	39.7	33	4.91	26	3.15	24	0.237	22
15 × 20	1471.3	2350.5	34	41.1	3	5.13	9	2.99	35	0.249	10
20 × 51	1469.7	2328.7	30	41.2	1	4.81	30	3.27	17	0.232	26
51 × 20	1468.4	2305.6	27	40.9	6	4.68	35	3.17	23	0.240	18
7 × 20	1467.7	2260.0	21	40.7	12	4.76	31	3.76	2	0.201	36
14 × 31	1457.7	2238.2	17	40.3	23	5.18	2	3.08	32	0.250	8
32 × 14	1452.8	2228.3	12	39.5	34	5.17	5	3.06	33	0.249	9
31 × 44	1452.7	2350.8	35	39.9	30	5.12	10	2.99	36	0.255	6
1 × 20	1449.8	2293.7	26	40.6	17	4.96	21	3.46	7	0.221	32
31 × 51	1449.3	2271.0	25	40.2	25	5.17	4	3.19	20	0.242	16
2 × 14	1438.0	2195.3	9	40.6	15	5.23	1	3.19	21	0.246	12
26 × 20	1434.7	2235.5	14	41.0	5	4.69	34	3.74	4	0.209	34
48 × 20	1427.7	2158.2	3	40.6	14	4.75	32	3.82	1	0.202	35
2 × 20	1426.7	2230.0	13	40.4	22	5.09	12	3.31	13	0.234	25
51 × 14	1419.7	2193.7	8	40.6	16	5.07	13	3.17	22	0.246	13
7 × 14	1419.3	2247.0	19	40.2	27	5.15	6	3.30	15	0.235	24
15 × 14	1418.3	2240.8	18	40.9	7	5.06	15	3.08	30	0.258	4
27 × 20	1418.2	2359.8	36	40.5	19	4.94	24	3.11	27	0.255	7
14 × 51	1418.1	2236.1	15	40.8	10	4.98	19	3.22	19	0.242	14
26 × 14	1409.7	2176.7	6	41.0	4	4.72	33	3.74	3	0.212	33
20 × 14	1391.3	2192.0	7	39.7	32	5.06	14	3.34	11	0.236	23
31 × 14	1389.5	2123.2	2	40.0	29	5.00	18	3.50	6	0.225	30
44 × 31	1389.0	2263.0	22	39.5	35	5.18	3	3.01	34	0.262	2
31 × 20	1388.7	2319.8	29	39.4	36	4.89	27	3.24	18	0.240	17
14 × 20	1388.5	2237.0	16	39.9	31	5.14	8	3.42	8	0.231	27
20 × 44	1387.2	2268.2	23	40.7	13	4.85	28	3.14	25	0.248	11
44 × 14	1386.8	2270.5	24	40.2	24	4.94	22	3.33	12	0.237	21
27 × 14	1361.5	2164.7	4	40.2	26	5.03	17	3.37	10	0.242	15
44 × 20	1348.2	2171.8	5	40.5	20	5.04	16	3.12	26	0.258	5
14 × 44	1339.7	2197.2	10	40.1	28	5.15	7	3.09	29	0.261	3
51 × 44	1337.5	2223.8	11	40.8	8	4.93	25	3.08	31	0.262	1
44 × 51	1334.2	2251.5	20	40.8	9	4.65	36	3.38	9	0.238	20
48 × 14	1326.1	2110.1	1	40.7	11	4.96	20	3.62	5	0.225	31
Trial Mean	1418.9	2250.6		40.4		4.98		3.28		0.238	
SD	94.6	160.6		1.0		0.3		0.5		0.040	

Table 7.4 Mean values per family for MorFi® traits kinked tracheids (MKT), percentage curl (MTC), percentage broken ends (MBT) and percentage area of fines (Mfines). Families are ranked on means for MATL for the full-diallel and selected factorial crosses from the Martin trial.

Family	MKT (%)	MCT (%)	Rk	MBT (%)	Rk	Mfines (%)	Rk
26 × 14	14.56	6.19	3	36.95	5	1.65	29
20 × 51	14.53	6.03	4	36.43	17	1.60	33
7 × 20	14.39	6.23	1	36.04	20	1.44	36
48 × 20	14.24	5.94	7	34.37	34	1.56	34
51 × 20	14.16	6.02	5	36.70	8	1.80	21
44 × 51	13.77	6.20	2	38.36	2	1.98	7
26 × 20	13.44	5.99	6	35.63	26	1.62	32
32 × 14	13.37	5.27	31	34.26	36	1.95	8
51 × 14	13.36	5.50	19	36.11	19	2.01	6
27 × 20	13.34	5.75	9	37.34	4	1.79	23
1 × 20	13.31	5.64	14	35.03	32	1.63	31
32 × 20	13.26	5.73	10	35.65	24	1.81	18
2 × 14	13.04	5.45	20	36.65	11	2.14	1
7 × 14	13.00	5.45	21	35.98	22	1.70	28
31 × 20	12.97	5.51	18	34.83	33	1.79	22
44 × 14	12.96	5.76	8	36.65	10	1.76	24
51 × 44	12.90	5.64	13	38.59	1	1.89	14
15 × 14	12.76	5.55	16	36.65	12	1.75	25
31 × 44	12.69	5.67	11	35.60	28	1.91	10
44 × 20	12.57	5.63	15	36.69	9	1.89	13
20 × 31	12.55	5.35	24	34.35	35	1.88	15
20 × 44	12.55	5.64	12	36.57	13	1.91	11
15 × 20	12.54	5.53	17	36.49	16	1.64	30
14 × 44	12.53	5.33	25	38.02	3	1.87	16
2 × 20	12.50	5.32	27	36.43	18	1.81	20
51 × 31	12.45	5.44	22	35.65	25	1.54	35
1 × 14	12.34	5.28	29	35.80	23	1.70	27
20 × 14	12.22	5.29	28	35.37	31	2.04	4
27 × 14	11.92	5.21	32	36.51	15	2.09	3
48 × 14	11.83	5.42	23	36.84	7	1.94	9
14 × 51	11.80	5.33	26	36.88	6	2.12	2
31 × 51	11.75	5.19	33	35.55	30	1.85	17
31 × 14	11.71	5.09	35	35.98	21	1.91	12
44 × 31	11.62	5.27	30	35.62	27	1.72	26
14 × 20	11.54	5.13	34	35.57	29	1.81	19
14 × 31	11.33	4.86	36	36.52	14	2.03	5
Trial Mean	12.83	5.55		36.17		1.82	
SD	2.0	0.6		1.8		0.39	

Pulp samples are never homogeneous, but contain a large distribution of different dimensions for fibre length, width and cell wall thickness. The end product in the Kraft pulping process is a mat of fibres, and the quality of the product is determined by how well these different sized fibres bond together in a paper sheet (Smook, 1986). A large range in fibre dimensions aid in the paper making process and ensure that a uniform surface is formed. Although the mean values for the different traits form the main focus of data analysis in this chapter, Figures 7.1, 7.2 and 7.3 show the distribution of arithmetic tracheid length, tracheid width and cell wall thickness respectively for the different families.

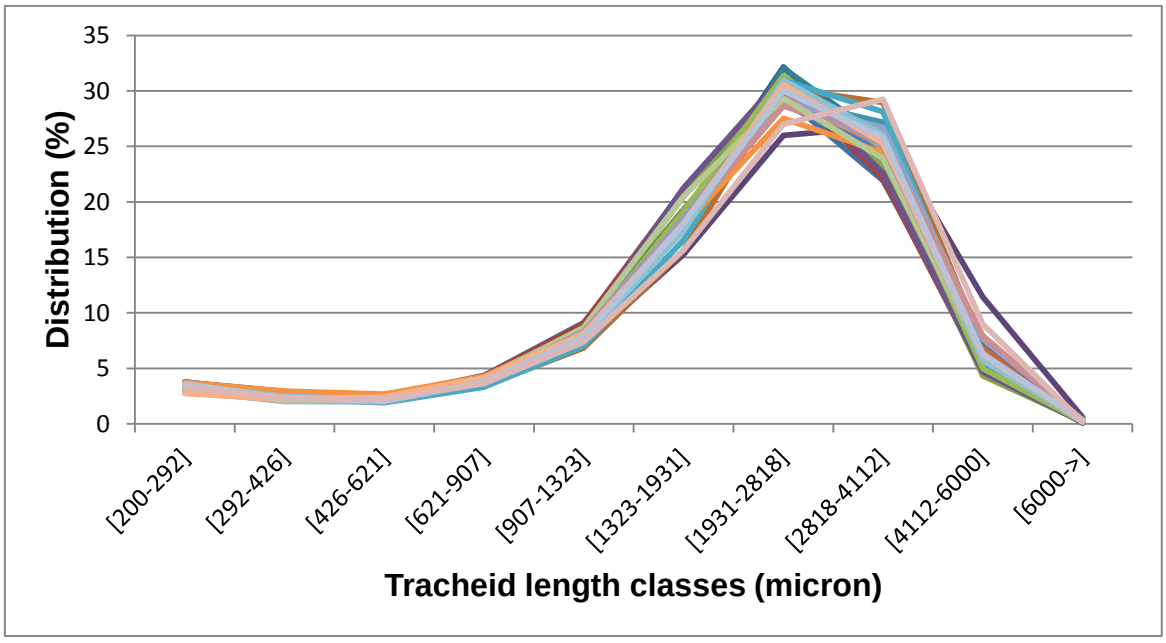

Figure 7.1 Mean tracheid distribution of arithmetic tracheid length classes assessed with the MorFi® fibre analyser for the 26 families at Martin.

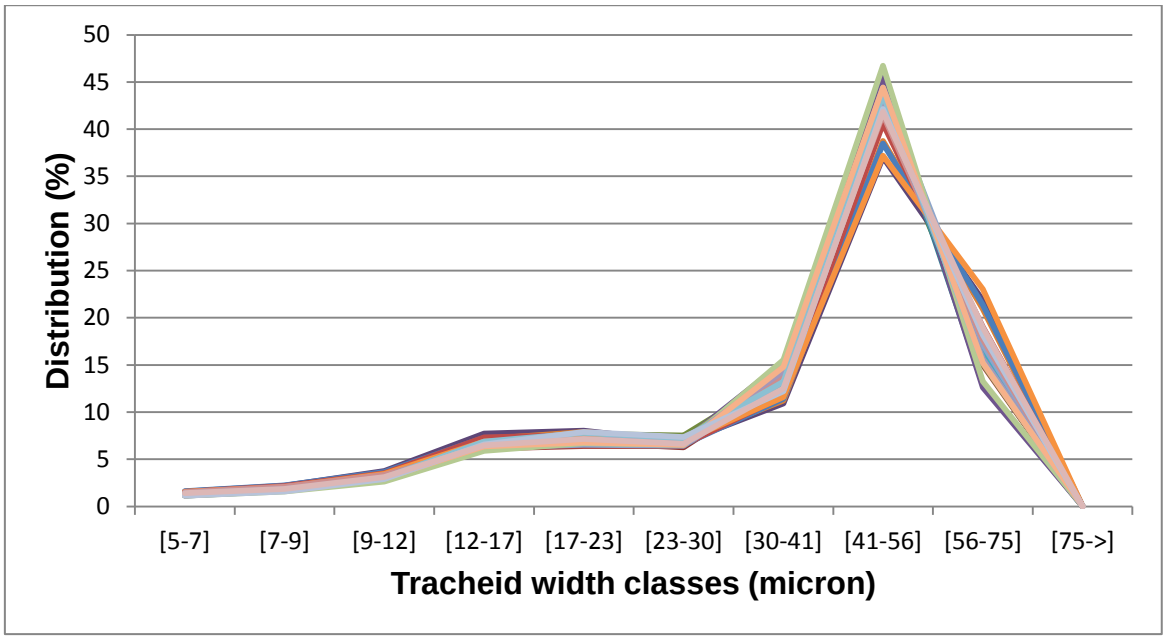

Figure 7.2 Mean distribution of tracheid width classes assessed with the MorFi® fibre analyser for the 26 families at Martin.

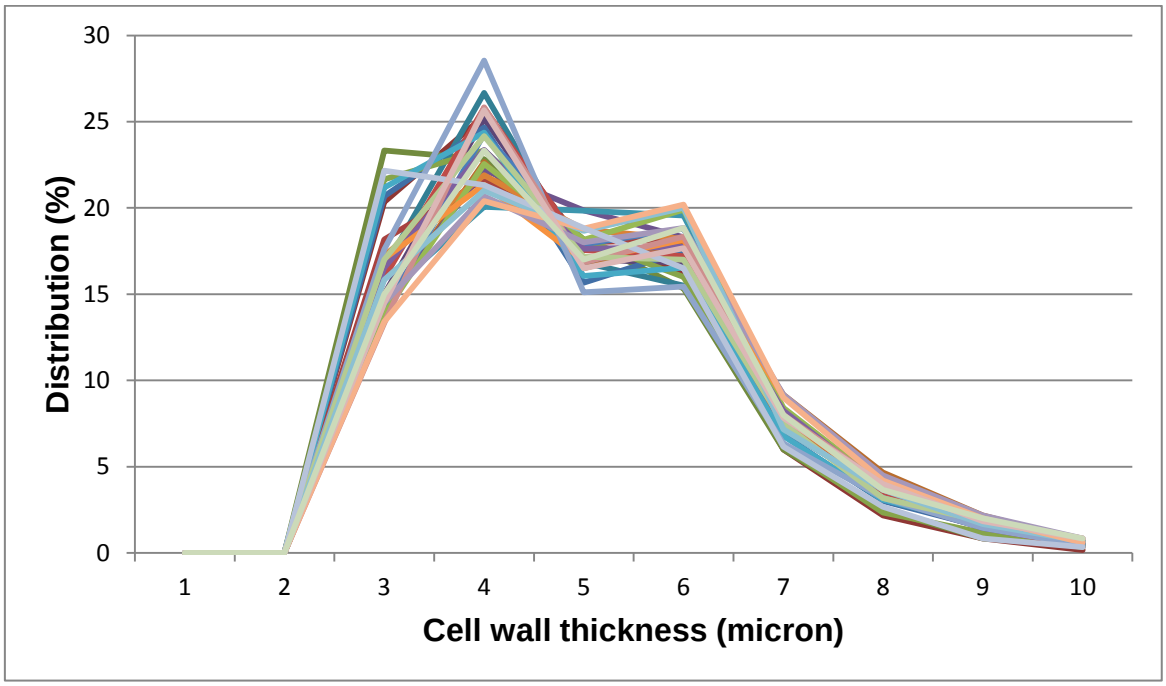

Figure 7.3 Mean distribution of tracheid cell wall thickness classes assessed with the MorFi® fibre analyser for the 26 families at Martin.

7.3.3 Site effects on tracheid dimensions

The effect of site or altitude on tracheid dimension traits was also investigated. Results from the 10 full-sib families from the half-diallel designs at Martin and Nyangui were used for this analysis. Analysis of variance (ANOVA) indicated that for six of the ten tracheid dimension traits investigated, site effects were significant (Appendix 6). Mean values for the two studied sites and *p*-values are presented in Tables 7.5 and 7.6. MATL values were not significant, but MWTL site effects were highly significant ($p < 0.01$). Site effects for MTW, MCWT, MTnum, MTC and MKT were also significant. Site effects were also found for tracheid length in other studies conducted on *P. patula* in Southern Africa (Barnes *et al*., 1994; Muneri and Balodis, 1998). There was significant site by family interaction effects for MTW and MBT, but not for the rest of the 10 traits.

Table 7.5 Mean values for sites (Martin and Nyangui) for MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum) and tracheid coarseness (MTC).

Site	MATL (µm)	MWTL (µm)	MTW (µm)	MCWT (µm)	MTnum (No/g)	MTC (mg/m)
Nyangui	1407	2150	40.9	5.07	2.98	0.270
Martin	1400	2242	40.2	4.97	3.23	0.243
p-values	0.571	0.001	0.001	0.024	0.001	0.003

Table 7.6 Mean values for sites (Martin and Nyangui) for MorFi® traits kinked tracheids (MKT), percentage curl (MTC), percentage broken ends (MBT) and percentage area of fines (Mfines).

Site	MKT (%)	MCT (%)	MBT (%)	Mfines (%)
Nyangui	13.55	5.5	36.5	1.72
Martin	12.71	5.5	36.2	1.84
p-values	0.014	0.729	0.294	0.109

7.3.4 Combining ability analysis of a full-diallel and two half-diallels

7.3.4.1 Full-diallel mating design on one site at Martin

An analysis of variance for combining ability from the full-diallel mating design at Martin was undertaken for all tracheid dimension traits (MATL, MWTL, MTW, MCWT, MTnum, MTC, MKT, MCT, MBT and Mfines). The combining ability analysis indicated significant effects for families for the traits MATL, MTW, MCWT, MCT, MBT and Mfines (Tables 7.7 and 7.8). Traits MWTL, MTnum, MTC and MKT did not show significant family effects. In the diallel analysis, a reduced number of families are compared, in contrast with the ANOVA reported for the 36 families in section 7.3.2.

Significant GCA effects were found for MATL, MTW, MCWT, MTC, MKT, MCT, MBT and Mfines (Tables 7.7 and 7.8). There were no significant SCA, Rec, Mat and Nmat effects for all the studied tracheid traits.

Table 7.7 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum) and tracheid coarseness (MTC) for a full-diallel at Martin.

Source of variation	df	MATL MS		MWTL MS		MTW MS		MCWT MS		MTnum MS		MTC MS	
Families	19	14996	*	24811	ns	1.52	*	0.16	**	0.115	ns	0.0008	ns
GCA	4	38965	**	54644	ns	5.09	**	0.35	**	0.178	ns	0.0018	*
SCA	5	11849	ns	23084	ns	1.30	ns	0.13	ns	0.072	ns	0.0002	ns
Rec	10	6495	ns	13621	ns	0.20	ns	0.08	ns	0.116	ns	0.0007	ns
Mat	4	5604	ns	5707	ns	0.17	ns	0.03	ns	0.126	ns	0.0005	ns
NMat	6	7118	ns	18885	ns	0.22	ns	0.12	ns	0.109	ns	0.0008	ns
Error	102	7854		22170		0.83		0.07		0.147		0.0005	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

Table 7.8 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for mean square values for MorFi® traits kinked tracheids (MKT), percentage curl (MTC), percentage broken ends (MBT) and percentage area of fines (Mfines) for a full-diallel at Martin.

Source of variation	df	MKT MS		MCT MS		MBT MS		Mfines MS	
Families	19	5.23	ns	0.74	**	0.74	**	7.18	**
GCA	4	12.93	**	12.93	**	2.37	**	25.86	**
SCA	5	5.28	ns	5.28	ns	0.35	ns	4.94	ns
Rec	10	1.94	ns	1.94	ns	0.26	ns	0.95	ns
Mat	4	2.07	ns	2.07	ns	0.25	ns	1.72	ns
NMat	6	1.76	ns	1.76	ns	0.26	ns	0.41	ns
Error	102	3.21		0.28		0.28		2.42	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

There is little published information available on combining ability studies of growth and physical wood property traits of *Pinus* species. One of the other comprehensive studies published was the studies on wood density and tracheid cross-sectional properties of *P. radiata* in Australia (Nyankuengama 1997, Nyankuengama *et al.*, 1999). None of the properties reported on in this chapter was, however, included in these studies. In another study on *P. pinaster*, using a 12×12 half-diallel mating design, significant GCA effects were also found for arithmetic tracheid length, weighted tracheid length, tracheid width and coarseness (Pot *et al*., 2002). As in the case with results from this study, SCA effects were found to be not significant (Pot *et al*., 2002). To the best of the author's knowledge, the current study is the first comprehensive combining ability analysis study on tracheid dimension traits of *P. patula*.

7.3.4.2 Half-diallel mating design on two sites at Martin and Nyangui

An analysis of variance for combining ability from the half-diallel mating design at Martin and Nyangui was undertaken for all tracheid dimension traits (MATL, MWTL, MTW, MCWT, MTnum, MTC, MKT, MCT, MBT and Mfines). The combining ability analysis results indicated significant effects for families for most of the tracheid dimension traits, except for MTC, MKT and Mfines (Tables 7.9 and 7.10). In this analysis, only 10 families from the half-diallel design are included. Site effects were significant for MWTL, MTW, MCWT, MTnum, MTC and MKT. GCA effects were significant for MATL, MWTL, MTW, MCWT, MTnum, MTC, MKT, MCT and MBT. There were also significant SCA effects for MATL and MWTL, although at lower levels as compared to GCA. There was also a significant GCA by Site interaction for MTW.

Table 7.9 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean square values for MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum) and tracheid coarseness (MTC) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	MATL MS		MWTL MS		MTW MS		MCWT MS		MTnum MS		MTC MS	
Families	9	26079	**	55873	**	3.30	**	0.175	*	0.426	*	0.004	ns
Site	1	317	ns	335420	**	15.25	**	0.304	*	1.459	**	0.019	**
Family*Site	9	1987	ns	11070	ns	2.21	**	0.102	ns	0.109	ns	0.001	ns
GCA	4	42117	**	70137	**	8.00	**	0.306	**	0.886	**	0.009	**
SCA	5	13381	*	48694	*	0.15	ns	0.074	ns	0.194	ns	0.002	ns
GCA*Site	4	231	ns	8465	ns	3.29	**	0.111	ns	0.157	ns	0.001	ns
SCA*Site	5	2645	ns	12085	ns	1.42	ns	0.079	ns	0.048	ns	0.001	ns
Error	118	5764		17033		0.74		0.069		0.192		0.002	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

Table 7.10 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean square values for MorFi® traits kinked tracheids (MKT), percentage curl (MTC), percentage broken ends (MBT) and percentage area of fines (Mfines) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	MKT MS		MCT MS		MBT MS		Mfines MS	
Families	9	6.893	ns	0.909	**	20.156	**	0.137	ns
Site	1	20.667	*	0.001	ns	3.212	ns	0.411	ns
Family*Site	9	3.727	ns	0.352	ns	5.400	*	0.130	ns
GCA	4	13.568	**	1.893	**	45.555	**	0.065	ns
SCA	5	3.107	ns	0.258	ns	2.745	ns	0.237	ns
GCA*Site	4	5.317	ns	0.423	ns	9.941	**	0.235	ns
SCA*Site	5	1.846	ns	0.254	ns	2.087	ns	0.042	ns
Error	118	3.631		0.231		2.179		0.166	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

7.3.5 Estimation of genetic parameters utilising diallel and factorial data

The combining ability analysis of the full-diallel design indicates, as in the case of wood density and tracheid cross-sectional traits reported in Chapters 5 and 6, that there were no reciprocal effects. Data from the reciprocal crosses were therefore pooled and the 16 additional crosses from the factorial design were added to constitute a larger incomplete half-diallel (see Chapter 4). This increase number of crosses would yield more robust genetic parameters for tracheid dimension traits.

The individual broad-sense heritability (H^2) values for tracheid dimension traits ranged from 0.13 to 0.66 (Tables 7.11 and 7.12). Individual narrow-sense heritabilities (h^2) ranged from 0.13 to 0.64, and were associated with standard error (SE) values ranging from 0.10 to 0.28. With the traits MWTL, MTnum and MTC the dominance variance estimate was zero, therefore H^2 and h^2 values were the same.

Table 7.11 Genetic effects and heritabilities for MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum) and tracheid coarseness (MTC) for a constituted half-diallel at Martin (7B).

Parameters	MATL	MWTL	MTW	MCWT	MTnum	MTC
Additive variance	2741.6	3343.2	0.361	0.034	0.112	0.0005
Dominance variance	46.6	0	0.109	0.010	0	0
Phenotypic variance	10053.8	26240.6	0.944	0.099	0.215	0.0009
Individual H^2	0.28	0.13	0.50	0.45	0.52	0.64
Individual h^2	0.27	0.13	0.38	0.35	0.52	0.64
SE of h^2	0.17	0.10	0.22	0.20	0.26	0.28

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

Table 7.12 Genetic effects and heritabilities for MorFi® traits kinked tracheids (MKT), percentage curl (MTC), percentage broken ends (MBT) and percentage area of fines (Mfines) for a constituted half-diallel at Martin (7B).

Parameters	MKT	MCT	MBT	Mfines
Additive variance	0.462	0.161	1.344	0.038
Dominance variance	0.557	0.039	0.711	0.015
Phenotypic variance	3.794	0.370	3.137	0.111
Individual H^2	0.27	0.54	0.66	0.48
Individual h^2	0.12	0.44	0.43	0.34
SE of h^2	0.12	0.22	0.22	0.25

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

The heritability estimates for tracheid dimension traits were lower than the cross-sectional tracheid traits reported in Chapter 6. The standard error (SE) estimates were also higher than those reported in Chapter 6. Heritability estimates for tracheid dimension properties in published literature vary greatly, but generally exhibit strong heritability within several conifer species. In a study of 14 to 16-year old *Picea engelmanni*, individual h^2 estimates for tracheid length ranged from 0.30 (SE of 0.15) to 0.59 (SE of 0.15) (Ivkovich, 2000; Ivkovich *et al*., 2002). For *P. sylvestris*, the narrow sense heritability for tracheid length ranged from 0.30 to 0.50 (Hannrup and Ekberg 1998). Studies on *P. radiata* and *P. taeda*

report h^2 estimates for tracheid length that range from 0.55 to 0.97 (Nicholls *et al*., 1964; Matziris and Zobel, 1973; Belonger, 1998).

Very little genetic information on tracheid dimension traits is available for *P. patula* due to the small number of genetic studies conducted on this species. In a *P. patula* provenance study of open-pollinated families conducted in South Africa on ten-year old material, individual h^2 estimates ranged from 0.04 to 0.09 for tracheid length (Stanger, 2003). This difference could be ascribed to the less precise estimates from open-pollinated provenance material.

7.3.6 Phenotypic and genetic correlations between tracheid dimensions

Tracheid dimension trait phenotypic correlations were calculated on individual tree and family mean values (Table 7.13). There was a strong and highly significant correlation between MATL and MWTL (0.80 for individual, 0.62 for family), but tracheid length (both MATL and MWTL) correlated poorly with MTW and MCWT. Some of the tracheid defect properties such as MBT and Mfines correlated negatively with the tracheid dimension traits MATL, MWTL and MTW. The tracheid length traits (MATL and MWTL) correlated negatively with MTnum, the number of tracheids per gram; there were fewer long tracheids per gram of fibre. Many of the phenotypic correlations were low and also not significant, and many of the significant correlations seem to be of little selection value.

The additive genetic correlations were generally lower than the phenotypic correlations, especially between MATL and MWTL (Table 7.14) where the correlation was 0.16 ± 0.55. Many of the genetic correlations were also associated with large standard errors. There was generally poor correlation between different tracheid dimension traits, except for a negative correlation between MTW and MCWT (-0.82 ± 0.17).

Table 7.13 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among all MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum), tracheid coarseness (MTC), kinked tracheids (MKT), percentage curl (MCT), percentage broken ends (MBT) and percentage area of fines (Mfines).

	MATL	**MWTL**	**MTW**	**MCWT**	**MTnum**	**MTC**	**MKT**	**MCT**	**MBT**	**Mfines**
MATL	-	**0.81 (0.001)**	**0.21 (0.001)**	0.02 (0.729)	**-0.45 (0.001)**	0.04 (0.597)	**0.38 (0.001)**	**0.25 (0.001)**	**-0.35 (0.001)**	**-0.42 (0.001)**
MWTL	**0.62 (0.001)**	-	0.00 (0.966)	-0.04 (0.581)	**-0.58 (0.001)**	**0.24 (0.001)**	**0.33 (0.001)**	**0.25 (0.001)**	**-0.35 (0.001)**	**-0.42 (0.001)**
MTW	0.17 (0.334)	0.04 (0.826)	-	-0.12 (0.087)	0.01 (0.925)	-0.10 (0.160)	0.07 (0.271)	**0.27 (0.001)**	**-0.29 (0.001)**	**-0.40 (0.001)**
MCWT	0.00 (0.994)	-0.09 (0.596)	**-0.47 (0.004)**	-	**-0.34 (0.001)**	**0.33 (0.001)**	**-0.47 (0.001)**	**-0.61 (0.001)**	0.05 (0.458)	**0.22 (0.001)**
MTnum	-0.10 (0.577)	**-0.43 (0.009)**	0.23 (0.180)	**-0.58 (0.001)**	-	**-0.89 (0.001)**	0.01 (0.827)	**0.19 (0.004)**	-0.03 (0.685)	**-0.15 (0.025)**
MTC	-0.26 (0.123)	0.16 (0.351)	-0.25 (0.139)	**0.58 (0.001)**	**-0.92 (0.001)**	-	-0.16 (0.021)	**-0.30 (0.001)**	**0.20 (0.003)**	**0.38 (0.001)**
MKT	0.23 (0.170)	0.17 (00327)	**0.44 (0.007)**	**-0.68 (0.001)**	0.38 (0.023)	**-0.43 (0.009)**	-	**0.89 (0.001)**	**-0.20 (0.003)**	**-0.41 (0.001)**
MCT	0.05 (0.789)	0.18 (0.284)	**0.55 (0.001)**	**-0.82 (0.001)**	**0.44 (0.008)**	**-0.42 (0.010)**	**0.88 (0.001)**	-	-0.07 (0.299)	**-0.44 (0.001)**
MBT	**-0.53 (0.001)**	-0.17 (0.330)	**0.47 (0.003)**	-0.12 (0.499)	-0.16 (0.360)	**0.37 (0.028)**	0.03 (0.868)	0.22 (0.196)	-	**0.25 (0.001)**
Mfines	**-0.40 (0.016)**	**-0.37 (0.025)**	-0.29 (0.083)	**0.39 (0.019)**	**-0.39 (0.020)**	**0.52 (0.001)**	**-0.48 (0.003)**	**-0.49 (0.002)**	0.24 (0.155)	-

Table 7.14 Additive genetic correlations with standard errors among MorFi® traits arithmetic tracheid length (MATL), weighted tracheid length (MWTL), tracheid width (MTW), cell wall thickness (MCWT), number of tracheids per gram (MTnum), tracheid coarseness (MTC), kinked tracheids (MKT), percentage curl (MCT), percentage broken ends (MBT) and percentage area of fines (Mfines).

	MATL	**MWTL**	**MTW**	**MCWT**	**MTnum**	**MTC**	**MKT**	**MCT**	**MBT**	**Mfines**
MATL	-									
MWTL	0.16 ± 0.55	-								
MTW	0.13 ± 0.55	0.11 ± 0.61	-							
MCWT	-0.08 ± 0.56	-0.23 ± 0.59	-0.82 ± 0.17	-						
MTnum	0.35 ± 0.49	-0.41 ± 0.51	0.42 ± 0.44	-0.70 ± 0.28	-					
MTC	-0.60 ± 0.36	0.28 ± 0.57	-0.37 ± 0.47	0.68 ± 0.29	-0.94 ± 0.06	-				
MKT	-0.46 ± 0.44	-0.06 ± 0.62	1.02 ± 0.03	-1.09 ± 0.10	0.75 ± 0.22	-0.68 ± 0.25	-			
MCT	-0.09 ± 0.56	0.17 ± 0.60	0.94 ±0.07	-1.00 ± 0.01	0.51 ± 0.37	-0.40 ± 0.39	0.94 ± 0.05	-		
MBT	-0.70 ± 0.28	0.04 ± 0.62	0.46 ± 0.42	-0.30 ± 0.49	-0.26 ± 0.47	0.48 ± 0.36	0.36 ± 0.62	0.50 ± 0.38	-	
Mfines	-0.54 ± 0.40	-0.52 ± 0.45	-0.42 ±0.44	0.59 ± 0.35	-0.60 ± 0.32	0.67 ± 0.26	-0.68 ± 0.38	-0.63 ± 0.30	0.25 ± 0.47	-

7.4 Conclusions

Results from the tracheid dimension trait study indicated that large individual tree variation was present for all traits. Highly significant differences between full-sib families were also found in the ANOVA for most of the assessed tracheid dimension traits, except for weighted tracheid length. Significant site effects were found for the MorFi® assessed traits weighted tracheid length, tracheid width, cellwall thickness, number of tracheids per gram, and tracheid coarseness. Arithmetic mean tracheid length and most of the tracheid defect properties had no significant site effects.

The full-diallel analysis indicated that, as in the case of wood density and cross-sectional tracheid traits, general combining ability was the most important and significant effect for tracheid dimension traits. Significant differences were also found between the reduced number of families for some of the dimension traits. Weighted tracheid length again did not have any significant effects in the combining ability analysis, indicating that it was not under genetic control. This trait is weighted according to the length distribution of the sample, adjusting for fibre damage during maceration. This adjustment seems to introduce non-genetic effects. No significant specific combining ability, reciprocal, maternal or non-maternal effects were found for any of the tracheid dimension traits.

The half-diallel analysis consisting of 10 full-sib families at two sites indicated that there were significant site effects for the traits weighted tracheid length, tracheid width, cellwall thickness, number of tracheids per gram, tracheid coarseness and kinked tracheids. There was no significant family- by- site interactions found. Highly significant general combining ability effects were also found for most of the tracheid dimension traits, except for percentage fines. There were also some significant specific combining ability effects for arithmetic and weighted tracheid length, but this is probably related to the less precise estimation in the half-diallel analysis. Highly significant general combining ability by site interaction effects

were found for tracheid width and percentage broken tracheids. No specific combining ability by site interactions was found. This analysis was, however, restricted to only two sites.

The genetic parameter analysis yielded heritability estimates that were lower than reported for other *Pinus* species in the few studies reported in literature (Nicholls *et al*., 1964; Matziris and Zobel, 1973; Belonger, 1998; Hannrup and Ekberg 1998). The heritability estimates were, however higher than those reported for other *P. patula* studies (Stanger, 2003; Vermaak, 2007). The heritability estimates were lower than those for wood density and cross-sectional tracheid traits in this study, but were similar to reported values for growth traits.

The phenotypic correlation results show that there was a strong correlation between arithmetic and weighted mean tracheid length, but these correlated poorly with tracheid width and cellwall thickness. The genetic correlation between arithmetic and weighted mean tracheid length, however, was poor. Some of the tracheid dimension defect traits correlated negatively with the dimension traits. The tracheid length traits correlated negatively with the trait number of tracheids per gram, there were fewer long tracheids per gram of fibre. Many of the phenotypic correlations were low and also not significant, and many of the significant correlations seem to be of little selection value. Most of the genetic correlations were associated with large standard errors. There was generally poor correlation between different tracheid dimension traits, except for a negative correlation between tracheid width and cellwall thickness.

Many of the studies reported in literature used older technology to assess tracheid dimension traits, often on as little as 30 tracheids per sample. MorFi® technology enabled the study of many thousands of tracheids per tree, assessed in only a few minutes per sample. This study is the first detailed genetic inheritance study utilizing the MorFi® fibre analyser technology.

Chapter 8

Inheritance of eight year growth traits of *P. patula*

8.1 Introduction

Volume growth is the most important and most studied trait that has been the focus of tree improvement programmes around the world. Growth traits were also the first focus when applied tree improvement started in the 1950's (Zobel and Talbert, 1984). Some of the reasons for the focus on growth traits are the economic importance of increased yield and shorter rotations, and the relative ease of assessing growth traits. Only more recently, has the focus shifted to also include wood quality traits, and understanding the relationship between growth traits and wood properties (Zobel and Jett, 1995). Tree growth is usually expressed as utilizable volume, which is a function of the tree height, diameter and stem-taper. Specific volume equations have been developed by forest mensurationists to accurately calculate volume for various species and growth conditions (Bredenkamp, 2012). In some cases a volume index is also used to estimate growth, especially with the assessment of relatively young trees (Hodge and Dvorak, 1999).

Growth results at various ages of the material used in this study have been reported in detail, including results from all the progeny trials in the series, including the two sites used in this study (Barnes, 1973; Barnes *et al*., 1978; 1979; 1992a; 1992b). Detailed genetic parameters, based on these studies, have also been reported (Barnes, 1973; Barnes *et al*., 1978; 1979; 1992a; 1992b). In this chapter, growth trait results and genetic parameters of the 300 trees that were selected for the study of physical wood properties, are reported. It is not the intension to duplicate work already reported on, but rather to generate genetic

parameters for growth traits for use in following chapters where growth traits will be correlated with wood property traits.

8.2 Materials and Methods

8.2.1 Assessment of growth traits

All trees in the progeny trials from this diallel designs were assessed at the age of 7.5 years (referred to as 8-year traits hereafter) just prior to the first thinning being carried out. Heights (Hgt8) were assessed in the standing trees with a hypsometer, to the nearest 0.1 m. Diameter (DBH8), a standardised forestry measurement, was determined at breast height (1.3 m above ground level) with a diameter tape measured to the nearest 0.1 cm. A volume index was used to calculate individual 8-year tree volumes using the following equation (Hodge and Dvorak, 1999):

Tree volume (Vol8) (m^3) = 0.00003 × DBH^2 (cm) × Height (m) *Equation 8.1*

Basal area would account for the larger amounts of the outer wood formed on the stems of trees. The basal area of each sample tree was calculated using the following formula:

Basal area (BA8) (m^2) = $(DBH8/200)^2 \times \pi$ *Equation 8.2*

8.3 Results and Discussion

8.3.1 Introduction

The analysis of growth trait data was conducted in the sequence as outlined in Chapter 4. This sequence was also followed to present the results. All growth trait data was examined for deviations from normality to comply with the standard ANOVA assumptions. All growth traits were analysed firstly to determine the range of variation present. An analysis of variance was then carried out on the 36 full-sib families from the full-diallel and factorial mating designs at Martin to determine significant family differences for growth traits. The effect of site was also investigated by comparing the two half-diallel mating designs at Martin and Nyangui.

A combining ability analysis was then carried out using data from the full-diallel at the Martin site and half-diallels at Martin and Nyangui sites. Genetic parameters were estimated for growth traits using data from both mating designs at the Martin site, allowing for estimations based on 26 full-sib families. Lastly, phenotypic and genetic correlations were calculated for comparison of the different growth traits.

8.3.2 General descriptive statistics and family analysis of growth traits

The results from the statistical analysis of growth trait data indicated a large amount of variation for all traits (Table 8.1). The coefficient of variation (CV) values for the assessed traits was low for Hgt8 and DBH8, and rather high for the calculated traits BA8 and Vol8.

Table 8.1 Summary statistics of growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8) investigated in this study for both trials at Martin and Nyangui.

Variable	Mean	SD	Minimum	Maximum	Range	CV	N
Hgt8	14.8	1.4	8.9	18.6	9.7	9.3	300
DBH8	16.7	2.8	8.3	23.5	15.2	16.8	300
BA8	0.022	0.007	0.005	0.043	0.038	32.1	300
Vol8	0.1	0.0	0.0	0.3	0.3	37.8	300

Results from the analysis of variance indicated that differences between families were significant (p<0.01) for all growth traits, except for Hgt8 (Appendix 7). Family mean results for all growth traits are presented in Table 8.2. Families are ranked according to Hgt8 in descending order. There appears to be a positive relationship among the traits, with similar rankings for the different growth traits.

Table 8.2 Mean values per family for 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8). Families are ranked on means for DBH8 for the full-diallel and selected factorial crosses from the Martin trial.

Family	Hgt8	DBH8	Rk	BA	Rk	Vol8	Rk
51 × 44	16.4	20.3	1	0.0326	1	0.206	1
15 × 14	15.2	18.5	2	0.0270	4	0.158	6
14 × 44	15.6	18.5	3	0.0272	3	0.163	3
44 × 51	14.7	18.5	4	0.0273	2	0.157	7
44 × 14	15.8	18.3	5	0.0268	5	0.164	2
14 × 20	15.7	18.2	6	0.0266	6	0.162	4
27 × 20	14.9	18.2	7	0.0261	7	0.146	12
44 × 31	16.2	18.1	8	0.0260	8	0.161	5
48 × 14	15.9	17.7	9	0.0247	9	0.151	8
44 × 20	14.9	17.3	10	0.0239	11	0.138	14
7 × 14	15.6	17.2	11	0.0235	13	0.142	13
14 × 51	15.7	17.2	12	0.0237	12	0.146	11
27 × 14	14.9	17.0	13	0.0245	10	0.149	9
32 × 14	14.6	17.0	14	0.0227	15	0.127	20
15 × 20	15.1	16.9	15	0.0226	16	0.131	15
31 × 20	16.0	16.9	16	0.0232	14	0.146	10
20 × 44	14.7	16.8	17	0.0226	17	0.128	19
51 × 31	15.3	16.6	18	0.0219	18	0.130	16
20 × 14	15.6	16.6	19	0.0217	19	0.129	18
2 × 14	14.7	16.4	20	0.0215	20	0.122	22
51 × 20	15.4	16.1	21	0.0212	21	0.129	17
1 × 20	14.8	16.0	22	0.0205	23	0.117	24
51 × 14	15.3	16.0	23	0.0206	22	0.124	21
32 × 20	14.6	16.0	24	0.0203	24	0.114	26
1 × 14	14.8	15.9	25	0.0200	25	0.114	25
31 × 51	13.9	15.6	26	0.0200	26	0.111	27
14 × 31	15.5	15.6	27	0.0194	27	0.118	23
26 × 14	14.8	15.4	28	0.0192	28	0.110	28
26 × 20	15.1	15.3	29	0.0187	29	0.109	29
2 × 20	14.5	15.3	30	0.0186	30	0.103	30
20 × 51	14.4	14.4	31	0.0169	31	0.094	32
48 × 20	14.6	14.3	32	0.0165	32	0.094	31
31 × 14	14.1	14.1	33	0.0159	34	0.086	36
20 × 31	14.5	14.1	34	0.0158	35	0.088	34
7 × 20	14.8	14.0	35	0.0155	36	0.088	35
31 × 44	14.2	13.9	36	0.0160	33	0.089	33
Trial Mean	15.1	16.5		0.02198		0.129	15.1
SD	1.3	2.8		0.007		0.050	1.3

8.3.3 Site effects on growth traits

The effect of site or altitude on 8-year growth traits was also investigated. Results from the 10 full-sib families from the half-diallel designs at Martin and Nyangui were used for this analysis. Analysis of variance (ANOVA) indicated that for all traits investigated, except for Hgt8, site effects were not significant (p>0.05) (Appendix 8). Mean values for the two studied sites and p-values are presented in Tables 8.3. The site effect for Hgt8 was highly significant (p<0.01), as has been found in numerous studies with many *Pinus* species (Zobel and Talbert, 1984). There was no significant site by family interaction effects for any of the growth traits.

Table 8.3 Mean values per family for 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8)

Site	Hgt8 (m)	DBH8 (cm)	BA8 (m^2)	Vol8 (m^3)
Nyangui	13.9	17.1	0.024	0.128
Martin	15.5	17.0	0.023	0.141
P-values	0.001	0.831	0.865	0.101

8.3.4 Combining ability analysis of a full-diallel and two half-diallels

8.3.4.1 Full-diallel mating design on one site at Martin

An analysis of variance for combining ability from the full-diallel mating design at Martin was undertaken for all growth traits (Hgt8, DBH8, BA8 and Vol8). The combining ability analysis indicated significant (p<0.05) effects for families for all the growth traits (Tables 8.4). In the diallel analysis, a reduced number of families are compared, in contrast with the ANOVA reported for the 36 families in section 8.3.2.

Significant GCA effects were found for DBH8, BA8 and Vol8. All the other effects SCA, Rec, Mat and Nmat for these traits were not significant. Hgt8 was not significant for GCA and SCA, but had significant Rec and Mat effects. This could be related to the relatively small number of trees included in this analysis.

Table 8.4 General combining ability (GCA), specific combining ability (SCA), reciprocal (REC), maternal (Mat) and non-maternal (NMat) effects for 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8) for a full-diallel at Martin.

Source of variation	df	Hgt8 MS		DBH8 MS		BA8 MS		Vol8 MS	
Families	19	3.12	*	18.10	**	1.5453	**	0.0058	**
GCA	4	1.80	ns	38.81	**	3.3778	**	0.0106	**
SCA	5	1.96	ns	15.07	ns	1.3096	ns	0.0046	ns
Rec	10	4.24	**	11.60	ns	0.9565	ns	0.0045	ns
Mat	4	5.30	*	14.15	ns	1.1918	ns	0.0056	ns
NMat	6	3.63	ns	9.99	ns	0.8073	ns	0.0038	ns
Error	102	1.51		7.87		0.6594		0.0025	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

8.3.4.2 Half-diallel mating design on two sites at Martin and Nyangui

An analysis of variance for combining ability from the half-diallel mating design at Martin and Nyangui was undertaken for all growth traits (Hgt8, DBH8, BA8 and Vol8). The combining ability analysis results indicated significant (p<0.05) effects for families for all the growth traits (Tables 8.5). In this analysis, only 10 families from the half-diallel design are included. Site effects were not significant for DBH8, BA8 and Vol8, but Hgt8 had a highly significant (p<0.01) site effect. GCA effects were highly significant (p<0.01) for all growth traits (Hgt8, DBH8, BA8 and Vol8) . There was also a significant SCA effect for Hgt8, but there were no significant SCA effects for all other growth traits. There was no GCA or SCA by Site interactions.

Table 8.5 General combining ability (GCA), specific combining ability (SCA), site, family by site, GCA by site and SCA by site interactions for mean square values for 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8) for a half-diallel at two sites at Martin and Nyangui.

Source of variation	df	**Hgt8 MS**		**DBH8 MS**		**BA8 MS**		**Vol8 MS**	
Families	9	4.544	**	30.954	**	2.7487	**	0.010	**
Site	1	85.208	**	0.053	ns	0.0064	ns	0.006	ns
Family*Site	9	1.616	ns	5.583	ns	0.4898	ns	0.002	ns
GCA	4	6.071	**	57.308	**	5.2196	**	0.018	**
SCA	5	3.027	*	11.552	ns	0.9146	ns	0.004	ns
GCA*Site	4	2.118	ns	8.798	ns	0.7229	ns	0.003	ns
SCA*Site	5	1.642	ns	1.832	ns	0.2130	ns	0.001	ns
Error	118	1.304		6.535		0.5596		0.002	

df, degrees of freedom; * Significant at $p \geq 0.05$; ** Significant at $p \geq 0.01$; ns, not significant

8.3.5 Estimation of genetic parameters utilising diallel and factorial data

The combining ability analysis of the full-diallel design indicates, as in the case of wood density and tracheid cross-sectional and dimension traits reported in Chapters 5, 6 and 7, that there were no reciprocal effects. Data from the reciprocal crosses were therefore pooled and the 16 additional crosses from the factorial design were added to constitute a larger incomplete half-diallel (see Chapter 4). This increased number of crosses would yield more robust genetic parameters for tracheid dimension traits.

The individual broad-sense heritability (H^2) values for growth traits ranged from 0.01 for Hgt8 to 0.32 for Vol8, 0.38 for DBH8 and 0.41 for BA8 (Table 8.6). Individual narrow-sense heritabilities (h^2) ranged from 0.01 for Hgt8 to 0.22 for Vol8, 0.23 for DBH8 and 0.26 for BA8 with standard error (SE) values ranging from 0.01 to 0.17. With Hgt8, the dominance variance estimate was zero, therefore H^2 and h^2 values were the same.

Table 8.6 Genetic effects and heritabilities for 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8) for a constituted half-diallel at Martin.

Parameters	Hgt8	DBH8	BA8	Vol8
Additive variance	0.025	1.854	0.1799	0.0006
Dominance variance	0	1.212	0.0969	0.0002
Phenotypic variance	1.686	8.077	0.6801	0.0025
Individual H^2	0.01	0.38	0.41	0.32
Individual h^2	0.01	0.23	0.26	0.22
SE of h^2	0.01	0.16	0.17	0.15

H^2 – broad-sense heritability, h^2 – narrow-sense heritability

The heritability estimates from this study are in agreement with many other studies including various *Pinus* species (Zobel and Talbert, 1984). Specific studies on *P. patula* on material of similar age also report individual tree heritability estimates of similar magnitude for growth traits (Barnes *et al*., 1992b; Stanger, 2003; Vermaak, 2007). Actual values for the more detailed study of 8-year growth traits at two sites at Martin and Stapleford, using the same material as in this study, found individual tree heritability estimates of 0.04 for height, 0.22 for circular area at breast height, and 0.18 for over-bark volume (Barnes *et al*., 1992b). These values are very similar, considering that the current study only utilised 300 trees.

8.3.6 Phenotypic and genetic correlations between growth traits

Eight-year growth trait phenotypic correlations (individual tree and family) were calculated on individual tree and family mean values (Table 8.7). There were strong and highly significant (p<0.01) correlations for growth traits Hgt8, DBH8, BA8 and Vol8, ranging from 0.67 to 0.99. The additive genetic correlations were generally lower than the phenotypic correlations for all correlations with Hgt8, and

were associated with large standard errors (Table 8.8). Other genetic correlations were of similar magnitude, or slightly higher, with smaller standard errors.

Table 8.7 Individual tree (above diagonal, n=220) and family mean (below diagonal, n=36) phenotypic correlations among 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8). Significant phenotypic correlations are indicated in bold with p-values in brackets.

	Hgt8	DBH8	BA8	Vol8
Hgt8	-	**0.76 (0.001)**	**0.75 (0.001)**	**0.82 (0.001)**
DBH8	**0.68 (0.001)**	-	**0.99 (0.001)**	**0.97 (0.001)**
BA8	**0.67 (0.001)**	**0.99 (0.001)**	-	**0.98 (0.001)**
Vol8	**0.77 (0.001)**	**0.98 (0.001)**	**0.99 (0.001)**	**-**

Table 8.8 Additive genetic correlations with standard errors among 8-year growth traits height (Hgt8), diameter at breast height (DBH8), basal area (BA8) and individual tree volume (Vol8).

	Hgt8	DBH8	BA8	Vol8
Hgt8	-			
DBH8	0.51 ± 0.52	-		
BA8	0.55 ± 0.49	0.99 ± 0.00	-	
Vol8	0.66 ± 0.40	0.99 ± 0.01	1.00 ± 0.01	**-**

8.4 Conclusions

Results from the 8-year growth trait study indicated that large individual tree variation was present for all traits. There were also highly significant family differences for growth traits diameter at breast height, basal area and tree volume. Tree height did not display any significant family differences. Tree height showed significant site effects, but variation in all the other traits were not significant for site.

The full-diallel analysis indicated that tree height did not have any significant general or specific combining ability effects, but it did display significant reciprocal and maternal effects. The other growth traits diameter at breast height, basal area and tree volume all had significant general combining ability effects, and all other combining ability effects were not significant. The half-diallel analysis indicated again that only tree height had significant site effects. General combining ability effects for all growth traits were significant, and tree height also had a significant specific combining ability effect. No significant combining ability by site interactions was found for any growth traits. The combining ability analyses could have been affected by the relatively small number of trees that were included. A much larger number of trees are usually assessed for growth traits.

Heritability estimates for growth traits were much lower than the estimates for wood density and cross-sectional tracheid traits, but were in line with other reported studies (Kanzler, 2002; Stanger, 2003; Vermaak, 2007). Heritability estimates from the relatively small sample of 300 trees were very similar to those of the detailed study on multiple sites conducted with the same genetic material used in the present study. Strong phenotypic and genetic correlations were found among the growth traits, especially for diameter at breast height, basal area and tree volume.

Chapter 9

Correlations between physical wood properties and growth traits, predicted genetic gains and correlated responses

9.1 Introduction

In advanced tree improvement programmes, the need arises for the inclusion of more than one trait for selection and breeding. In forest tree improvement programmes around the world, the most common selection trait historically has been volume growth (Zobel and Talbert, 1984). The emphasis during the last couple of decades has moved to the inclusion of important wood properties such as wood density, in addition to volume growth (Zobel and Jett, 1995). With any multi-trait breeding programme, knowledge of the association between different selection traits is of the utmost importance. If a breeder is unaware of an interrelationship between two different traits, the related trait could be negatively affected by selection for the primary trait (Shelbourne *et al*., 1997; Nyakuengama *et al*., 1998; Atwood *et al*., 2002; Pot *et al*., 2002).

The association between any two traits that is directly observable is referred to as phenotypic correlation. This association can be determined from assessments of those traits in a number of individuals of a specific population (Falconer and Mackay 1996). The phenotypic correlation is made up of two components, an environmental component and genetic component (Falconer and Mackay, 1996). For plant breeders, the additive genetic correlation between traits is important as it will identify those traits that are inter-dependent. With artificial selection, pleiotropic effects are important. Pleiotropy refers to the simultaneous variation in more than one trait caused by the same gene action (Falconer and Mackay, 1996). Strong additive correlations between traits will allow the tree breeder to construct selection indexes for multiple traits. Difficult- to-measure traits that have

strong additive genetic correlations with easy-to-measure traits, can be selected for indirectly by selecting for the easy-to-measure traits (Atwood *et al*., 2002; Pot *et al*., 2002; Stanger, 2003; White *et al*., 2007).

Many studies have investigated the correlation of growth traits with various wood property traits of various species. The most studied relationship is that of growth and wood density (White *et al.*, 1993; Zobel and Jett, 1995, Atwood *et al*., 2002). Results from these studies show conflicting results, ranging from positive to negative relationships, but most studies show zero to very low, and sometimes negative correlation (Zobel and van Buijtenen, 1989). Many studies lack genetic structure and rely on phenotypic correlation only, which can be influenced by environmental or silvicultural influences. The number of studies of other wood properties such as tracheid dimension and cross-sectional characteristics are very limited (Via *et al*., 2004). Little information is available on interrelationships among these traits, and their relationships with growth traits (Zobel and van Buijtenen, 1989; Atwood *et al*., 2002; Via *et al*., 2004).

This chapter will present and discuss phenotypic and genetic correlations between wood density, cross-sectional tracheid, tracheid dimension and growth traits covered in Chapters 5 to 8. A summary of all the traits investigated is given in Table 9.1. These traits were selected based on their importance in determining pulp and paper quality as outlined in Chapter 2, and the genetic parameters determined in Chapters 5 to 8. Predicted genetic gains for the various traits are also presented and correlated responses to selection for multiple traits will be discussed. The statistical methods used for correlation between traits and predictions of gain and correlated responses are presented in Chapter 4. Predicted genetic gains are based on the genetic parameter results from the preceding chapters. Correlated responses to selection for multiple traits are also explored, combining genetic parameters for those traits and the genetic correlation values among those specific traits. This chapter follows a similar sequence, lay-out and methodology as presented in chapters 6 and 7 of Stanger

(2003). Following similar methodology as Stanger (2003) for predicted gains and correlated responses to selection will enable direct comparisons with the findings of this provenance study of wood properties of *P. patula*.

Table 9.1 Abbreviations and descriptions for all wood property and growth traits investigated for interrelationships.

Trait	Description	Category
WMWD	weighted mean wood density	Density
MEWD	mean earlywood density	Density
MLWD	mean latewood density	Density
LWP	latewood percentage	Density
RD	Radial diameter (µm)	Cross-sectional
TD	Tangential diameter (µm)	Cross-sectional
MTD	Mean tracheid diameter (µm)	Cross-sectional
LD	Lumen diameter (µm)	Cross-sectional
CWA	Cell wall area (µm²)	Cross-sectional
CWT	Cell wall thickness (µm)	Cross-sectional
TArea	Tracheid area (µm²)	Cross-sectional
NoTrach	No of tracheids per mm² (n/ mm²)	Cross-sectional
PCell	Percentage cell wall per mm² (%)	Cross-sectional
RR	Runkel ratio	Cross-sectional
CS	Coarseness (µg m^{-1}) Silviscan® derived trait	Cross-sectional
SS	Specific surface (m^2 kg^{-1}) Silviscan® derived trait	Cross-sectional
PM	Perimeter (µm) Silviscan® derived trait	Cross-sectional
WTS	Wall thickness (µm) Silviscan® derived trait	Cross-sectional
MATL	Arithmetic tracheid length (µm)	Tracheid dimension
MWTL	Tracheid length weighted in length (µm)	Tracheid dimension
MTW	Tracheid width (µm)	Tracheid dimension
MCWT	Tracheid wall thickness (µm)	Tracheid dimension
MTnum	Number of tracheids per gram (No/g)	Tracheid dimension
MTC	Coarseness of tracheids (mg/m)	Tracheid dimension
MKT	Percentage kinked tracheids (%)	Tracheid dimension
MCT	Percentage curl (%)	Tracheid dimension
MBT	Percentage break ends (%)	Tracheid dimension
MFines	Percentage area of fines (%)	Tracheid dimension
Hgt8	Tree height at eight years (m)	Growth
DBH8	Tree diameter at breast (1.3 m) height at eight years (cm)	Growth
BA8	Basal area at breast height at eight years (m²)	Growth
Vol8	Tree volume at eight years (m³)	Growth

9.2 Correlations between growth and wood density, tracheid cross-sectional and dimension properties

9.2.1 Phenotypic correlations

Results from this study indicated that individual tree and family phenotypic correlations between growth and wood density traits were weak and mostly non-significant (Table 9.2). There was a weak but significant positive individual tree correlation between growth trait Hgt8 and MLWD, and weak negative correlations among DBH8, BA8, Vol8 and LWP. In contrast, moderate and significant (p < 0.05), phenotypic individual tree and family correlations were found between growth traits and assessed and derived tracheid cross-sectional traits (Table 9.3). The correlations between growth traits DBH8, BA8 and Vol8 and the assessed tracheid cross-sectional traits were positive and ranged in magnitude from 0.42 to 0.55. Correlations between Hgt8 and the cross-sectional traits were weaker, but still significant. As expected, the derived traits were also well correlated, as the assessed traits form their basis.

Table 9.2 Individual tree (n=220) and family mean (n=36) phenotypic correlations among growth and wood density traits. Bold figures indicate significant phenotypic correlations.

	Individual tree				**Family mean**			
	Hgt8	**DBH8**	**BA8**	**Vol8**	**Hgt8**	**DBH8**	**BA8**	**Vol8**
WMWD	0.10 (0.157)	-0.05 (0.428)	-0.07 (0.288)	-0.04 (0.521)	0.10 (0.549)	0.05 (0.792)	0.06 (0.741)	0.08 (0.650)
MEWD	0.04 (0.544)	0.06 (0.343)	0.07 (0.329)	0.07 (0.275)	0.06 (0.712)	0.07 (0.703)	0.08 (0.629)	0.10 (0.570)
MLWD	**0.23 (0.001)**	0.11 (0.115)	0.09 (0.188)	0.11 (0.095)	0.06 (0.715)	0.10 (0.547)	0.09 (0.594)	0.08 (0.626)
LWP	0.02 (0.716)	**-0.15 (0.029)**	**-0.16 (0.015)**	**-0.14 (0.041)**	0.06 (0.728)	-0.04 (0.808)	-0.03 (0.867)	0.00 (0.987)

Table 9.3 Individual tree (n=220) and family mean (n=36) phenotypic correlations among growth and tracheid cross-sectional traits. Bold figures indicate significant phenotypic correlations.

	Individual tree				Family mean			
	Hgt8	**DBH8**	**BA8**	**Vol8**	**Hgt8**	**DBH8**	**BA8**	**Vol8**
RD	**0.28 (0.000)**	**0.54 (0.000)**	**0.55 (0.000)**	**0.52 (0.000)**	0.28 (0.104)	**0.41 (0.014)**	**0.40 (0.016)**	**0.38 (0.024)**
TD	**0.24 (0.000)**	**0.36 (0.000)**	**0.37 (0.000)**	**0.36 (0.000)**	0.28 (0.093)	**0.41 (0.013)**	**0.40 (0.016)**	**0.39 (0.018)**
MTD	**0.28 (0.000)**	**0.51 (0.000)**	**0.52 (0.000)**	**0.50 (0.000)**	0.29 (0.088)	**0.42 (0.010)**	**0.41 (0.013)**	**0.39 (0.018)**
LD	**0.18 (0.006)**	**0.42 (0.000)**	**0.44 (0.000)**	**0.41 (0.000)**	0.20 (0.235)	0.32 (0.055)	0.31 (0.066)	0.29 (0.086)
CWA	**0.34 (0.000)**	**0.44 (0.000)**	**0.45 (0.000)**	**0.45 (0.000)**	**0.40 (0.016)**	**0.52 (0.001)**	**0.51 (0.002)**	**0.49 (0.002)**
CWT	**0.18 (0.009)**	0.07 (0.284)	0.06 (0.345)	0.09 (0.180)	0.23 (0.185)	0.22 (0.191)	0.23 (0.181)	0.23 (0.173)
TArea	**0.28 (0.000)**	**0.51 (0.000)**	**0.52 (0.000)**	**0.50 (0.000)**	0.29 (0.091)	**0.42 (0.011)**	**0.41 (0.014)**	**0.39 (0.02)**
NoTrach	**-0.29 (0.000)**	**-0.50 (0.000)**	**-0.51 (0.000)**	**-0.49 (0.000)**	-0.30 (0.079)	**-0.44 (0.008)**	**-0.43 (0.010)**	**-0.41 (0.014)**
PCell	-0.01 (0.927)	**-0.18 (0.007)**	**-0.20 (0.004)**	**-0.17 (0.014)**	-0.03 (0.883)	-0.14 (0.441)	-0.13 (0.467)	-0.11 (0.536)
RR	-0.01 (0.948)	**-0.19 (0.004)**	**-0.21 (0.002)**	**-0.18 (0.009)**	-0.02 (0.916)	-0.12 (0.490)	-0.11 (0.525)	-0.09 (0.595)
CS	**-0.39 (0.000)**	**0.52 (0.000)**	**0.52 (0.000)**	**0.52 (0.000)**	**0.44 (0.008)**	**0.57 (0.000)**	**0.57 (0.000)**	**0.56 (0.000)**
SS	**-0.33 (0.000)**	**-035 (0.000)**	**-0.34 (0.000)**	**-0.35 (0.000)**	**-0.40 (0.016)**	**-0.48 (0.003)**	**-0.48 (0.003)**	**-0.48 (0.003)**
PM	**0.28 (0.000)**	**0.51 (0.000)**	**0.52 (0.000)**	**0.50 (0.000)**	0.29 (0.088)	**0.42 (0.010)**	**0.41 (0.013)**	**0.39 (0.018)**
WTS	**0.32 (0.000)**	**0.31 (0.000)**	**0.30 (0.000)**	**0.32 (0.000)**	**0.39 (0.019)**	**0.45 (0.006)**	**0.46 (0.005)**	**0.46 (0.005)**

Correlations between growth traits and tracheid dimension traits were generally weaker than correlations with tracheid cross-sectional traits (Table 9.4). The tracheid length and width traits MATL and MTW had weak to moderate significant negative correlations with the growth traits. Tracheid coarseness MTC had moderate positive correlations with the growth traits, slightly weaker, but similar to the Silviscan® trait CS (Table 9.3). Some of the tracheid defect traits such as MKT, MCT and MBT also correlated weakly with the growth traits, some positively (MBT) and others negatively (MKT, MCT). Generally Hgt8 did not correlate well with the tracheid dimension traits than DBH8, BA8 and Vol8.

Table 9.4 Individual tree (n=220) and family mean (n=36) phenotypic correlations among growth and tracheid dimension traits. Bold figures indicate significant phenotypic correlations.

	Individual tree				**Family mean**			
	Hgt8	**DBH8**	**BA8**	**Vol8**	**Hgt8**	**DBH8**	**BA8**	**Vol8**
MATL	**-0.18 (0.007)**	**-0.41 (0.000)**	**-0.41 (0.000)**	**-0.39 (0.000)**	-0.24 (0.152)	-0.19 (0.263)	-0.20 (0.252)	-0.22 (0.200)
MWTL	0.06 (0.362)	-0.05 (0.443)	-0.06 (0.411)	-0.04 (0.532)	0.04 (0.810)	0.18 (0.282)	0.18 (0.289)	0.15 (0.382)
MTW	**-0.22 (0.001)**	**-0.19 (0.005)**	**-0.19 (0.005)**	**-0.21 (0.002)**	0.03 (0.856)	-0.01 (0.948)	-0.03 (0.844)	-0.04 (0.839)
MCWT	0.12 (0.085)	0.12 (0.085)	0.10 (0.144)	0.10 (0.138)	-0.11 (0.533)	0.06 (0.719)	0.06 (0.715)	0.03 (0.866)
MTnum	**-0.17 (0.011)**	-0.11 (0.110)	-0.11 (0.117)	-0.13 (0.065)	-0.14 (0.427)	-0.27 (0.108)	-0.26 (0.122)	-0.23 (0.174)
MTC	**0.27 (0.000)**	**0.29 (0.000)**	**0.29 (0.000)**	**0.31 (0.000)**	0.19 (0.273)	**0.34 (0.046)**	0.33 (0.052)	0.30 (0.078)
MKT	-0.11 (0.092)	**-0.14 (0.037)**	-0.13 (0.063)	-0.13 (0.060)	-0.10 (0.573)	-0.16 (0.362)	-0.17 (0.329)	-0.17 (0.323)
MCT	**-0.14 (0.032)**	**-0.14 (0.038)**	-0.13 (0.064)	**-0.14 (0.044)**	-0.03 (0.863)	-0.04 (0.817)	-0.05 (0.776)	-0.05 (0.756)
MBT	0.07 (0.303)	**0.21 (0.002)**	**0.22 (0.001)**	**0.20 (0.002)**	0.19 (0.271)	0.26 (0.128)	0.25 (0.147)	0.23 (0.171)
MFines	0.01 (0.939)	0.02 (0.736)	0.03 (0.637)	0.04 (0.520)	0.14 (0.424)	-0.02 (0.905)	-0.02 (0.911)	0.01 (0.940)

9.2.2 Additive genetic correlations

For the calculation of genetic correlations, only traits with individual heritabilities above 0.10 were considered. Traits with heritability estimates lower than 0.10 are not likely to be included in a selection programme (Stanger, 2003). In this study, all traits except Hgt8 had heritability estimates above 0.10 and were therefore considered. Genetic correlations between growth and wood density traits were weak, ranging from -0.08 to -0.33, and were also associated with large standard errors (Table 9.5). Correlations between growth traits and WMWD, MLWD and LWP were negative, while correlations with MEWD were positive.

Table 9.5 Additive genetic correlations with standard errors among growth and wood density traits.

	DBH8	BA8	Vol8
WMWD	-0.08 ± 0.33	-0.34 ± 0.28	-0.12 ± 0.32
MEWD	0.15 ± 0.31	0.15 ± 0.30	0.14 ± 0.31
MLWD	-0.03 ± 0.57	-0.05 ± 0.56	-0.05 ± 0.57
LWP	-0.27 ± 0.32	-0.30 ± 0.31	-0.33 ± 0.31

Genetic correlations between growth traits and assessed tracheid cross-sectional traits were strong and positive, ranging from 0.62 to 0.88 (Table 9.6). Tracheid diameter traits were positively correlated with DBH8, BA8 and Vol8 increased diameter dimensions were found with increased growth traits. The traits NoTrach, PCell, RR and SS were negatively correlated with growth traits. As tracheid diameters increase, a smaller number of tracheids per mm² are present, influencing paper properties such as tear, burst and coarseness. The Silviscan® derived trait WTS was poorly correlated, in contrast with the cross-sectional trait CWA.

Table 9.6 Additive genetic correlations with standard errors among growth and tracheid cross-sectional traits.

	DBH8	BA8	Vol8
RD	0.79 ± 0.11	0.81 ± 0.09	0.84 ± 0.08
TD	0.75 ± 0.15	0.79 ± 0.12	0.77 ± 0.14
MTD	0.79 ± 0.13	0.82 ± 0.11	0.83 ± 0.10
LD	0.62 ± 0.17	0.65 ± 0.16	0.67 ± 0.15
CWA	0.88 ± 0.10	0.89 ± 0.09	0.88 ± 0.10
CWT	0.10 ± 0.43	0.09 ± 0.42	0.09 ± 0.43
TArea	0.72 ± 0.14	0.74 ± 0.12	0.75 ± 0.12
NoTrach	-0.68 ± 0.16	-0.71 ± 0.15	-0.72 ± 0.14
PCell	-0.27 ± 0.30	-0.32 ± 0.28	-0.32 ± 0.29
RR	-0.01 ± 0.33	-0.03 ± 0.32	-0.01 ± 0.33
CS	0.87 ± 0.09	0.88 ± 0.08	0.88 ± 0.08
SS	-0.65 ± 0.23	-0.65 ± 0.23	-0.64 ± 0.24
PM	0.72 ± 0.14	0.74 ± 0.13	0.76 ± 0.12
WTS	0.09 ± 0.39	0.09 ± 0.38	0.09 ± 0.39

Growth traits correlated poorly with tracheid dimension traits, except for MATL and MKT (Table 9.7). MATL correlated negatively with growth traits DBH8, BA8 and Vol8 with r values of -0.69 to -0.83. This indicated that increased tree-growth resulted in shorter tracheids. Tracheid width and cell wall thickness correlated poorly with growth traits, ranging from 0.05 to 0.08 for MTW, and 0.03 to 0.04 for MCWT. MKT, the proportion of kinked tracheids, was negatively correlated with growth traits, with r values ranging from -0.62 to -0.78. MBT showed high correlations with the growth traits, associated with large standard errors.

Table 9.7 Additive genetic correlations with standard errors among growth and tracheid dimension traits.

	DBH8	BA8	Vol8
MATL	-0.69 ± 0.25	-0.71 ± 0.22	-0.83 ± 0.15
MWTL	-0.11 ± 0.51	-0.11 ± 0.50	-0.19 ± 0.49
MTW	0.08 ± 0.45	0.07 ± 0.43	0.05 ± 0.44
MCWT	0.04 ± 0.44	0.04 ± 0.43	0.03 ± 0.44
MTnum	-0.11 ± 0.41	-0.11 ± 0.40	-0.10 ± 0.41
MTC	0.01 ± 0.39	0.01 ± 0.38	0.01 ± 0.39
MKT	-0.62 ± 0.36	-0.62 ± 0.35	-0.78 ± 0.23
MCT	-0.01 ± 0.42	-0.01 ± 0.40	-0.01 ± 0.41
MBT	2.05 ± 1.34	2.07 ± 1.35	2.16 ± 1.53
MFines	0.10 ± 0.50	0.10 ± 0.49	0.11 ± 0.49

The general consensus of many published studies of growth and wood density traits indicates a weak to moderate negative genetic correlation (Zobel and van Buijtenen, 1989; Birks and Barnes, 1991; Barnes *et al*., 1994; Atwood *et al*., 2002). There is, however, large variability among different species and even different sources of the same species. A study of wood density and growth using the identical genetic material used in this study conducted on several sites in Zimbabwe indicated negative genetic correlations between these traits (Birks and Barnes, 1991). A provenance study of *P. patula* conducted on a single site in South Africa also showed weak to moderate negative genetic correlations ranging from -0.12 to -0.46 between growth and wood density traits (Stanger, 2003). Another study of 100 *P. patula* open-pollinated families at eight years showed a negative genetic correlation between growth and earlywood percentage, and weighted density, and a positive correlation for growth and latewood percentage (Vermaak, 2007). Negative genetic correlation for growth and wood density was also found for *P. taeda* in a study of four southern provenances (Belonger, 1998).

Interrelationships between growth traits and tracheid cross-sectional and dimension traits have only been reported in a limited number of studies. In a study of *P. radiata*, phenotypic correlations of 0.47 and 0.37 were found between DBH and radial and tangential tracheid diameters (Nyakuengama *et al*., 1998). A *P. taeda* study reported genetic correlations of -0.43 and -0.44 for tracheid diameter and lumen diameter respectively (Belonger, 1998). In the *P. patula* provenance study conducted in South Africa, generally weak negative genetic correlations were found for growth and tracheid cross-sectional and dimension traits (Stanger, 2003).

9.3 Correlations between wood density and tracheid cross-sectional and tracheid dimension properties

9.3.1 Phenotypic correlations

Phenotypic correlations between wood density and tracheid cross-sectional traits were generally significant ($p< 0.05$), and were both positive and negative and were moderate to strong (Table 9.8). MLWD displayed much weaker relationships with the cross-sectional traits. The phenotypic correlations between wood density (excluding MLWD) and the tracheid diameter traits RD, TD, MTD and LD were all negative and ranged from -0.29 to -0.70. The traits CWA and CWT were positively correlated with wood density traits, with r values ranging from 0.13 to 0.30 for CWA, and 0.39 to 0.68 for CWT. The derived tracheid cross-sectional traits displayed similar correlations as the assessed traits, with WTS having positive moderate to strong correlations between 0.49 for MLWD and 0.75 for WMWD. Correlations between growth and tracheid dimension traits were generally weaker than the cross-sectional traits, ranging from 0.02 to -0.57 (Table 9.9).

Table 9.8 Individual tree (n=220) and family mean (n=36) phenotypic correlations among wood density and tracheid cross-sectional traits. Bold figures indicate significant phenotypic correlations.

	Individual tree				Family mean			
	WMWD	**MEWD**	**MLWD**	**LWP**	**WMWD**	**MEWD**	**MLWD**	**LWP**
RD	**-0.51 (0.000)**	**-0.39 (0.000)**	0.09 (0.200)	**-0.55 (0.000)**	**-0.62 (0.000)**	**-0.41 (0.013)**	-0.08 (0.633)	**-0.71 (0.000)**
TD	**-0.31 (0.000)**	**-0.33 (0.000)**	0.14 (0.064)	**-0.29 (0.000)**	**-0.50 (0.002)**	**-0.41 (0.014)**	-0.08 (0.648)	**-0.71 (0.000)**
MTD	**-0.47 (0.000)**	**-0.39 (0.000)**	0.11 (0.105)	**-0.49 (0.000)**	**-0.61 (0.000)**	**-0.42 (0.010)**	-0.09 (0.624)	**-0.68 (0.000)**
LD	**-0.70 (0.000)**	**-0.57 (0.000)**	-0.06 (0.378)	**-0.67 (0.000)**	**-0.78 (0.000)**	**-0.60 (0.000)**	-0.22 (0.213)	**-0.79 (0.000)**
CWA	**0.16 (0.015)**	**0.13 (0.049)**	**0.30 (0.000)**	0.06 (0.393)	-0.04 (0.798)	0.09 (0.588)	0.18 (0.284)	-0.20 (0.231)
CWT	**0.68 (0.000)**	**0.51 (0.000)**	**0.39 (0.000)**	**0.56 (0.000)**	**0.77 (0.000)**	**0.70 (0.000)**	**0.48 (0.003)**	**0.63 (0.000)**
TArea	**-0.47 (0.000)**	**-0.39 (0.000)**	0.11 (0.110)	**-0.49 (0.000)**	**-0.60 (0.000)**	**-0.42 (0.011)**	-0.09 (0.617)	**-0.67 (0.000)**
NoTrach	**0.46 (0.000)**	**0.40 (0.000)**	-0.11 (0.093)	**0.49 (0.000)**	**0.62 (0.000)**	**0.44 (0.007)**	0.08 (0.645)	**0.68 (0.000)**
PCell	**0.83 (0.000)**	**0.69 (0.000)**	**0.20 (0.002)**	**0.73 (0.000)**	**0.92 (0.000)**	**0.78 (0.000)**	0.33 (0.050)	**0.85 (0.000)**
RR	**0.82 (0.000)**	**0.65 (0.000)**	**0.28 (0.000)**	**0.74 (0.000)**	**0.91 (0.000)**	**0.75 (0.000)**	**0.38 (0.023)**	**0.85 (0.000)**
CS	**0.20 (0.003)**	**0.15 (0.030)**	**0.38 (0.000)**	0.08 (0.261)	-0.01 (0.954)	0.12 (0.460)	0.20 (0.249)	-0.18 (0.293)
SS	**-0.69 (0.000)**	**-0.54 (0.000)**	**-0.49 (0.000)**	**-0.52 (0.000)**	**-0.62 (0.000)**	**-0.65 (0.000)**	**-0.42 (0.012)**	**-0.42 (0.012)**
PM	**-0.47 (0.000)**	**-0.39 (0.000)**	-0.11 (0.105)	**-0.49 (0.000)**	**-0.61 (0.000)**	**-0.42 (0.010)**	-0.08 (0.624)	**-0.68 (0.000)**
WTS	**0.75 (0.000)**	**0.59 (0.000)**	**0.49 (0.000)**	**0.59 (0.000)**	**0.70 (0.000)**	**0.70 (0.000)**	**0.43 (0.009)**	**0.50 (0.002)**

Table 9.9 Individual tree (n=220) and family mean (n=36) phenotypic correlations among wood density and tracheid dimension traits. Bold figures indicate significant phenotypic correlations.

	Individual tree				Family mean			
	WMWD	**MEWD**	**MLWD**	**LWP**	**WMWD**	**MEWD**	**MLWD**	**LWP**
MATL	**0.19 (0.004)**	**0.04 (0.586)**	**0.19 (0.005)**	**0.26 (0.000)**	0.27 (0.109)	0.06 (0.724)	0.01 (0.966)	**0.41 (0.013)**
MWTL	0.02 (0.778)	-0.09 (0.171)	**0.25 (0.000)**	0.07 (0.283)	-0.08 (0.638)	-0.19 (0.271)	-0.05 (0.784)	0.08 (0.633)
MTW	**-0.21 (0.002)**	**-0.18 (0.008)**	**-0.17 (0.013)**	**-0.17 (0.012)**	**-0.39 (0.020)**	**-0.40 (0.016)**	-0.19 (0.276)	**-0.40 (0.015)**
MCWT	**0.56 (0.000)**	**0.58 (0.000)**	0.14 (0.040)	**0.47 (0.000)**	**0.72 (0.000)**	**0.73 (0.000)**	0.33 (0.051)	**0.58 (0.000)**
MTnum	**-0.36 (0.000)**	**-0.24 (0.000)**	-0.35 (0.000)	**-0.32 (0.000)**	**-0.41 (0.013)**	**-0.39 (0.017)**	-0.28 (0.095)	**-0.38 (0.024)**
MTC	**0.30 (0.000)**	**0.25 (0.000)**	0.26 (0.000)	**0.23 (0.001)**	0.32 (0.061)	**0.39 (0.020)**	0.25 (0.149)	0.22 (0.193)
MKT	**-0.36 (0.000)**	**-0.36 (0.000)**	-0.06 (0.369)	**-0.27 (0.000)**	**-0.55 (0.000)**	**-0.62 (0.000)**	-0.27 (0.114)	**-0.44 (0.008)**
MCT	**-0.57 (0.000)**	**-0.57 (0.000)**	-0.15 (0.024)	**-0.43 (0.000)**	**-0.78 (0.000)**	**-0.80 (0.000)**	-0.33 (0.047)	**-0.63 (0.000)**
MBT	-0.11 (0.080)	-0.08 (0.254)	-0.07 (0.270)	**-0.18 (0.008)**	-0.27 (0.111)	-0.17 (0.309)	-0.05 (0.783)	**-0.39 (0.018)**
MFines	**0.33 (0.000)**	**0.34 (0.000)**	0.01 (0.829)	**0.23 (0.000)**	**0.45 (0.006)**	**0.53 (0.001)**	0.02 (0.913)	**0.37 (0.024)**

9.3.2 Additive genetic correlations

The additive genetic correlations between wood density and tracheid cross-sectional traits were mostly stronger than the phenotypic correlations and were associated with small standard errors (Table 9.10). Tracheid diameter traits were also negatively correlated as with the phenotypic correlations. MLWD again displayed weak correlations with the cross-sectional traits. The derived traits RR and WTS displayed very weak genetic correlations, indicating environmental effects included in the phenotypic correlations.

Table 9.10 Additive genetic correlations with standard errors among wood density and tracheid cross-sectional traits.

	WMWD	MEWD	MLWD	LWP
RD	-0.69 ± 0.10	-0.43 ± 0.15	-0.25 ± 0.31	-0.81 ± 0.07
TD	-0.82 ± 0.08	-0.6 ± 0.14	-0.54 ± 0.28	-0.85 ± 0.07
MTD	-0.73 ± 0.09	-0.47 ± 0.15	-0.32 ± 0.30	-0.83 ± 0.06
LD	-0.88 ± 0.04	-0.65 ± 0.10	-0.42 ± 0.27	-0.92 ± 0.03
CWA	-0.17 ± 0.29	0.06 ± 0.29	-0.05 ± 0.53	-0.36 ± 0.28
CWT	0.20 ± 0.28	0.20 ± 0.27	0.12 ± 0.51	0.16 ± 0.30
TArea	-0.65 ± 0.11	-0.42 ± 0.15	-0.29 ± 0.30	-0.75 ± 0.09
NoTrach	0.67 ± 0.11	0.44 ± 0.16	0.28 ± 0.32	0.76 ± 0.09
PCell	1.07 ± 0.03	0.91 ± 0.04	0.51 ± 0.28	1.01 ± 0.00
RR	0.04 ± 0.22	0.03 ± 0.21	0.02 ± 0.38	0.04 ± 0.23
CS	-0.17 ± 0.24	0.07 ± 0.24	-0.06 ± 0.44	-0.36 ± 0.23
SS	-0.48 ± 0.21	-0.61 ± 0.16	-0.26 ± 0.44	-0.26 ± 0.27
PM	-0.66 ± 0.11	-0.43 ± 0.15	-0.29 ± 0.30	-0.76 ± 0.09
WTS	0.09 ± 0.26	0.11 ± 0.25	0.04 ± 0.46	0.05 ± 0.28

Genetic correlations between wood density and tracheid dimension traits were also mostly weaker than the phenotypic correlations (Table 9.11). Tracheid length and width was negatively correlated with wood density traits, while MCWT showed a weak positive correlation. The implication of this correlation is that denser wood will contain shorter narrower tracheids, with a higher percentage of cell-walls increasing the density. Tracheid defect traits MKT and MBT had strong negative genetic correlations with wood density traits.

Table 9.11 Additive genetic correlations with standard errors among wood density and tracheid dimension traits.

	WMWD	MEWD	MLWD	LWP
MATL	0.17 ± 0.30	0.00 ± 0.30	-0.18 ± 0.53	0.30 ± 0.30
MWTL	-0.22 ± 0.33	-0.25 ± 0.31	-0.43 ± 0.49	-0.06 ± 0.36
MTW	-0.27 ± 0.28	-0.27 ± 0.27	-0.13 ± 0.52	-0.32 ± 0.29
MCWT	0.13 ± 0.29	0.12 ± 0.28	0.10 ± 0.52	0.10 ± 0.31
MTnum	-0.07 ± 0.28	-0.08 ± 0.27	-0.06 ± 0.49	-0.05 ± 0.29
MTC	0.00 ± 0.26	0.01 ± 0.25	0.01 ± 0.46	0.00 ± 0.28
MKT	-1.73 ± 0.78	-1.90 ± 0.99	-1.19 ± 0.29	1.41 ± 0.41
MCT	-0.30 ± 0.25	-0.31 ± 0.24	-0.18 ± 0.47	-0.25 ± 0.28
MBT	-0.87 ± 0.07	-0.56 ± 0.19	0.00 ± 0.49	-1.35 ± 0.25
MFines	0.09 ± 0.33	0.10 ± 0.32	0.05 ± 0.59	0.07 ± 0.36

Correlations between wood density traits and tracheid diameter traits (RD and TD) were of similar magnitude and also negative in a *P. radiata* study conducted in New Zealand (Shelbourne *et al*., 1997). Silviscan® traits SS and PM were also comparable with the *P. radiata* study, but WTS and coarseness values were very different (Shelbourne *et al*., 1997). In a *P. taeda* study conducted in west-central Georgia and Louisiana, USA, genetic correlations between wood density and radial tracheid width and lumen diameter were also negative, but of smaller magnitude (Goggans, 1964). Genetic correlations between wood density and radial tracheid width and lumen diameter in another *P. taeda* study of four southern provenances were however, positive and ranged between 0.31 and 0.42 (Belonger, 1998). In the South African *P. patula* provenance study, genetic correlations between cross-sectional tracheid traits were similar to the present study, but of smaller magnitude (Stanger, 2003).

Genetic correlations between tracheid dimension traits such as length and width and wood density have been reported in few studies. In the aforementioned *P. taeda* study conducted in the USA, genetic correlations between tracheid length and width with wood density were very small and no relationship could be established (Goggans, 1964). In the South African *P. patula* provenance study, similar correlations were found with some values being of higher magnitude than the present study (Stanger, 2003). In most of these studies, different methods of determining tracheid dimension traits were used, with varying number of tracheids being assessed.

9.4 Phenotypic correlations between tracheid cross-sectional and tracheid dimension properties

9.4.1 Phenotypic and genetic correlations

Phenotypic correlations between tracheid cross-sectional and tracheid dimension traits were very variable, but with many significant ($p < 0.05$) family and individual correlations (Table 9.12 and 9.13). Generally, MATL correlated better with cross-sectional traits than MWTL. Genetic correlations were generally of higher magnitude than phenotypic correlations (Table 9.14). MATL correlated negatively with tracheid diameter traits RD, TD, MTD, LD and CWA, while NoTrach correlated positively with MATL. Interestingly, the cross-sectional trait CWT did not correlate well with the MorFi® assessed trait MCWT. These two traits differ in that CWT is assessed *in situ* in the wood profile, whereas MCWT is assessed after much of the lignin present in the cell wall is removed through the maceration process. MCWT correlated very well with PCell (0.89), while MTW correlated negatively (-0.74) with PCell. Tracheid dimension defect properties (MKT, MCT and MBT) correlated well with many of the tracheid cross-sectional properties. MTC (MorFi tracheid coarseness) correlated well with the Silviscan derived coarseness (CS).

Table 9.12 Individual tree (n=220) phenotypic correlations among tracheid cross-sectional and tracheid dimension traits. Bold figures indicate significant phenotypic correlations.

	Individual tree									
	MATL	**MWTL**	**MTW**	**MCWT**	**MTnum**	**MTC**	**MKT**	**MCT**	**MBT**	**MFines**
RD	**-0.24 (0.000)**	**0.17 (0.013)**	**0.14 (0.039)**	**-0.23 (0.001)**	**-0.20 (0.004)**	**0.30 (0.000)**	0.11 (0.092)	**0.26 (0.000)**	**0.34 (0.000)**	-0.13 (0.061)
TD	0.02 (0.816)	**0.38 (0.000)**	**0.17 (0.011)**	**-0.15 (0.027)**	**-0.38 (0.000)**	**0.37 (0.000)**	0.09 (0.120)	**0.18 (0.007)**	**0.28 (0.000)**	**-0.14 (0.040)**
MTD	**-0.16 (0.016)**	**0.25 (0.000)**	**0.16 (0.018)**	**-0.21 (0.002)**	**-0.27 (0.000)**	**0.35 (0.000)**	0.11 (0.099)	**0.25 (0.000)**	**0.34 (0.000)**	**-0.14 (0.040)**
LD	**-0.18 (0.008)**	**0.20 (0.004)**	**0.18 (0.007)**	**-0.35 (0.000)**	-0.08 (0.257)	**0.14 (0.034)**	**0.24 (0.000)**	**0.41 (0.000)**	**0.28 (0.000)**	**-0.23 (0.001)**
CWA	-0.07 (0.334)	**0.23 (0.001)**	0.06 (0.387)	**0.14 (0.035)**	**-0.49 (0.000)**	**0.55 (0.000)**	**-0.18 (0.006)**	**-0.18 (0.009)**	**0.30 (0.000)**	0.09 (0.167)
CWT	0.10 (0.127)	0.12 (0.084)	-0.09 (0.196)	**0.38 (0.000)**	**-0.44 (0.000)**	**0.43 (0.000)**	**-0.34 (0.000)**	**-0.45 (0.000)**	0.07 (0.305)	**0.24 (0.000)**
TArea	**-0.16 (0.016)**	**0.25 (0.000)**	**0.16 (0.018)**	**-0.21 (0.002)**	**-0.27 (0.000)**	**0.35 (0.000)**	0.11 (0.097)	**0.25 (0.000)**	**0.34 (0.000)**	**-0.13 (0.048)**
NoTrach	**0.14 (0.038)**	**-0.28 (0.000)**	**-0.16 (0.016)**	**0.21 (0.002)**	**0.28 (0.000)**	**-0.34 (0.000)**	-0.11 (0.117)	**-0.24 (0.000)**	**-0.32 (0.000)**	0.16 (0.016)
PCell	**0.14 (0.043)**	-0.09 (0.178)	**-0.16 (0.014)**	**0.46 (0.000)**	**-0.19 (0.005)**	**0.15 (0.022)**	**-0.36 (0.000)**	**-0.54 (0.000)**	-0.12 (0.081)	**0.31 (0.000)**
RR	**0.16 (0.019)**	-0.05 (0.501)	**-0.17 (0.012)**	**0.44 (0.000)**	**-0.23 (0.001)**	**0.19 (0.005)**	**-0.34 (0.000)**	**-0.51 (0.000)**	-0.11 (0.092)	**0.30 (0.000)**
CS	-0.04 (0.596)	**0.29 (0.000)**	0.03 (0.624)	**0.16 (0.015)**	**-0.56 (0.000)**	**0.60 (0.000)**	**-0.14 (0.040)**	**-0.16 (0.045)**	**0.29 (0.000)**	0.07 (0.275)
SS	-0.09 (0.197)	**-0.24 (0.000)**	0.09 (0.179)	**-0.43 (0.000)**	**0.62 (0.000)**	**-0.61 (0.000)**	**0.30 (0.000)**	**0.42 (0.000)**	**-0.14 (0.039)**	**-0.24 (0.000)**
PM	**-0.16 (0.016)**	**0.25 (0.000)**	**0.16 (0.018)**	**-0.21 (0.002)**	**-0.27 (0.000)**	**0.35 (0.000)**	0.11 (0.099)	**0.25 (0.000)**	**0.34 (0.000)**	**-0.14 (0.040)**
WTS	0.10 (0.124)	**0.22 (0.001)**	-0.10 (0.126)	**0.45 (0.000)**	**-0.60 (0.000)**	**0.59 (0.000)**	**-0.32 (0.000)**	**-0.44 (0.000)**	0.12 (0.084)	**0.24 (0.000)**

Table 9.13 Family mean (n=36) phenotypic correlations among tracheid cross-sectional and tracheid dimension traits. Bold figures indicate significant phenotypic correlations.

	Family mean									
	MATL	**MWTL**	**MTW**	**MCWT**	**MTnum**	**MTC**	**MKT**	**MCT**	**MBT**	**MFines**
RD	**-0.57 (0.000)**	0.10 (0.567)	0.27 (0.105)	-0.29 (0.092)	-0.20 (0.232)	**0.39 (0.019)**	0.14 (0.414)	**0.39 (0.017)**	**0.65 (0.000)**	0.00 (0.988)
TD	-0.24 (0.153)	**0.44 (0.007)**	0.26 (0.125)	-0.25 (0.149)	**-0.39 (0.017)**	**0.43 (0.008)**	0.10 (0.561)	0.29 (0.085)	**0.52 (0.001)**	-0.11 (0.533)
MTD	**-0.49 (0.002)**	0.21 (0.225)	0.28 (0.097)	-0.28 (0.093)	-0.27 (0.111)	**0.42 (0.011)**	0.13 (0.437)	**0.38 (0.023)**	**0.64 (0.000)**	-0.03 (0.859)
LD	**-0.43 (0.008)**	0.19 (0.256)	**0.38 (0.023)**	**-0.44 (0.007)**	-0.08 (0.646)	0.21 (0.217)	0.31 (0.069)	**0.55 (0.001)**	**0.58 (0.000)**	-0.19 (0.271)
CWA	**-0.43 (0.0009)**	0.20 (0.236)	0.01 (0.944)	0.16 (0.350)	**-0.61 (0.000)**	**0.74 (0.000)**	-0.28 (0.098)	-0.14 (0.417)	**0.57 (0.000)**	0.30 (0.077)
CWT	0.01 (0.948)	0.07 (0.666)	**-0.41 (0.013)**	**0.64 (0.000)**	**-0.63 (0.000)**	**0.61 (0.000)**	**-0.64 (0.000)**	**-0.73 (0.000)**	-0.01 (0.972)	**0.52 (0.001)**
TArea	**-0.49 (0.002)**	0.20 (0.236)	0.28 (0.095)	-0.28 (0.093)	-0.27 (0.107)	**0.42 (0.010)**	0.13 (0.437)	**0.38 (0.024)**	**0.65 (0.000)**	-0.02 (0.903)
NoTrach	**0.46 (0.005)**	-0.25 (0.143)	-0.28 (0.094)	0.29 (0.092)	0.27 (0.112)	**-0.41 (0.014)**	-0.14 (0.432)	**-0.39 (0.020)**	**-0.61 (0.000)**	0.08 (0.647)
PCell	0.30 (0.078)	-0.14 (0.416)	**-0.43 (0.008)**	**0.61 (0.000)**	-0.22 (0.189)	0.13 (0.456)	**-0.51 (0.002)**	**-0.74 (0.000)**	**-0.39 (0.018)**	**0.40 (0.015)**
RR	0.28 (0.093)	-0.11 (0.515)	**-0.46 (0.005)**	**0.61 (0.000)**	-0.26 (0.120)	0.17 (0.318)	**-0.52 (0.001)**	**-0.74 (0.000)**	**-0.37 (0.025)**	**0.42 (0.011)**
CS	**-0.40 (0.015)**	0.21 (0.221)	0.06 (0.723)	0.19 (0.255)	**-0.65 (0.000)**	**0.76 (0.000)**	-0.26 (0.129)	-0.12 (0.489)	**0.59 (0.000)**	0.28 (0.097)
SS	0.14 (0.410)	-0.13 (0.453)	0.22 (0.207)	**-0.62 (0.000)**	**0.79 (0.000)**	**-0.81 (0.000)**	**0.56 (0.000)**	**0.60 (0.000)**	-0.28 (0.103)	**-0.50 (0.002)**
PM	**-0.49 (0.002)**	0.21 (0.225)	0.28 (0.097)	-0.28 (0.093)	-0.27 (0.111)	**0.42 (0.011)**	0.13 (0.437)	**0.38 (0.023)**	**0.64 (0.000)**	-0.03 (0.86)
WTS	-0.08 (0.643)	0.11 (0.518)	-0.23 (0.185)	**0.65 (0.000)**	**-0.76 (0.000)**	**0.77 (0.000)**	**-0.57 (0.000)**	**-0.63 (0.000)**	0.22 (0.194)	**0.50 (0.002)**

Table 9.14 Additive genetic correlations with standard errors among tracheid cross-sectional and tracheid dimension traits.

	MATL	MWTL	MTW	MCWT	MTnum	MTC	MKT	MCT	MBT	MFines
RD	-0.88 ± 0.06	0.09 ± 0.30	0.38 ± 0.22	-0.38 ± 0.22	-0.29 ± 0.22	0.52 ± 0.16	0.24 ± 0.32	0.50 ± 0.18	0.82 ± 0.08	0.13 ± 0.29
TD	-0.48 ± 0.25	0.19 ± 0.15	0.50 ± 0.23	-0.55 ± 0.21	-0.64 ± 0.17	0.67 ± 0.15	0.39 ± 0.34	0.65 ± 0.17	0.88 ± 0.06	0.00 ± 0.35
MTD	-0.80 ± 0.10	0.34 ± 0.26	0.41 ± 0.22	-0.43 ± 0.21	-0.37 ± 0.21	0.56 ± 0.15	0.28 ± 0.31	0.54 ± 0.17	0.85 ± 0.07	0.1 0± 0.29
LD	-0.63 ± 0.16	0.44 ± 0.24	0.58 ± 0.17	-0.64 ± 0.15	-0.16 ± 0.23	0.31 ± 0.20	0.54 ± 0.24	0.75 ± 0.10	0.79 ± 0.09	-0.18 ± 0.28
CWA	-0.75 ± 0.18	0.18 ± 0.46	-0.02 ± 0.41	0.12 ± 0.40	-0.67 ± 0.21	0.83 ± 0.11	-0.29 ± 0.50	-0.04 ± 0.38	0.66 ± 0.22	0.57 ± 0.31
CWT	-0.06 ± 0.42	-0.06 ± 0.46	-0.19 ± 0.39	0.23 ± 0.38	-0.19 ± 0.36	0.20 ± 0.33	-0.27 ± 0.49	-0.24 ± 0.35	-0.01 ± 0.38	0.24 ± 0.42
TArea	-0.72 ± 0.13	0.30 ± 0.27	0.37 ± 0.22	-0.39 ± 0.22	-0.34 ± 0.21	0.51 ± 0.17	0.25 ± 0.32	0.49 ± 0.18	0.77 ± 0.10	0.10 ± 0.29
NoTrach	0.66 ± 0.16	-0.39 ± 0.27	-0.37 ± 0.24	0.39 ± 0.23	0.33 ± 0.23	-0.48 ± 0.18	-0.26 ± 0.34	-0.51 ± 0.19	-0.72 ± 0.13	-0.03 ± 0.31
PCell	0.39 ± 0.26	-0.46 ± 0.27	-0.74 ± 0.13	0.89 ± 0.06	-0.21 ± 0.26	0.10 ± 0.25	-0.87 ± 0.09	-1.03 ± 0.02	-0.61 ± 0.18	0.61 ± 0.21
RR	0.01 ± 0.31	-0.02 ± 0.35	-0.03 ± 0.30	0.03 ± 0.30	-0.01 ± 0.28	0.01 ± 0.26	-0.03 ± 0.39	-0.04 ± 0.28	-0.02 ± 0.28	0.02 ± 0.34
CS	-0.74 ± 0.16	0.12 ± 0.39	0.08 ± 0.34	0.15 ± 0.33	-0.70 ± 0.16	0.84 ± 0.09	-0.29 ± 0.41	-0.03 ± 0.32	0.71 ± 0.16	0.57 ± 0.26
SS	0.41 ± 0.32	0.12 ± 0.42	0.29 ± 0.34	-0.70 ± 0.19	0.83 ± 0.11	-0.88 ± 0.07	0.78 ± 0.19	0.62 ± 0.21	-0.33 ± 0.31	-0.84 ± 0.12
PM	-0.72 ± 0.13	0.31 ± 0.27	0.37 ± 0.22	-0.39 ± 0.22	-0.34 ± 0.21	0.51 ± 0.17	0.25 ± 0.32	0.50 ± 0.18	0.77 ± 0.10	0.09 ± 0.29
WTS	-0.06 ± 0.37	-0.03 ± 0.41	-0.05 ± 0.36	0.12 ± 0.35	-0.13 ± 0.33	0.13 ± 0.31	-0.13 ± 0.46	-0.11 ± 0.33	0.04 ± 0.34	0.14 ± 0.40

Only one other reported study of tracheid cross-sectional and tracheid dimension traits could be located. The South African *P. patula* provenance study found positive but smaller magnitude genetic correlations between tracheid length for growth rings 6 and 10, and similar tracheid cross-sectional traits (Stanger, 2003). There were also marked differences between correlations at growth rings 6 and 10, with greater magnitude correlations for the older sample material in ring 10 (Stanger, 2003).

9.5 Predicted genetic gains for physical wood properties

An important part of a selection and breeding programme is to gain an understanding of expected gains for specific traits. These predicated gains are usually based on the genetic parameters gained from progeny testing selected parents in the population. During the current study, genetic parameters were calculated for the specific breeding population from the diallel and factorial mating designs. Typical gains that are achieved per generation in tree breeding programmes for pine species range from 10 to 30% (White *et al*., 1993; Atwood *et al*., 2002; Stanger, 2003). To enable direct comparisons between studies, similar methodology was used for the South African *P. patula* provenance wood property study (Stanger, 2003).

A selection intensity of 2% (one in 50) was used and gain predications were calculated for the various wood property traits investigated in this study (Table 9.15). Wood property traits with heritabilities over 0.10 were used, as traits with lower than 0.10 heritability estimates would not usually be used in breeding programmes (Stanger, 2003). The predicted gains for wood property traits from this study ranged from 2 to 57% (Table 9.15). Due to the high heritability estimates calculated for traits in this study, predicted gains for most of the traits are substantial and higher than those predicted for the *P. patula* provenance study (Stanger, 2003). The predicted gains reported in Table 9.15 indicate that

many of the wood density and tracheid cross-sectional and dimension properties could be improved in a selection and breeding programme.

Table 9.15 Prediction of trait gains for direct selection based on wood property traits using a selection intensity of 2% (i = 2.421).

Trait	Trait h^2	Trait σ^2_{phen}	Trait mean	Actual gain	% Gain
WMWD	0.81	0.001445	0.403	0.075	18%
MEWD	0.90	0.000462	0.315	0.047	15%
MLWD	0.20	0.000795	0.634	0.014	2%
LWP	0.89	0.002519	0.19	0.108	57%
RD	1.04	8.728	42.28	7.439	18%
TD	0.70	4.197	39.56	3.472	9%
MTD	1.04	8.728	40.92	7.439	18%
LD	1.02	7.998	28.3	6.984	25%
CWA	0.48	4290	561	76.11	14%
CWT	0.38	0.304	5.48	0.51	9%
TArea	1.04	572989	5273	1905.9	36%
NoTrach	0.95	735	193	62.35	32%
RR	0.74	0.005	0.39	0.127	32%
CS	0.66	7592	673	139.23	21%
SS	0.58	477	245	30.67	13%
PM	1.04	140	164	29.79	18%
WTS	0.56	0.075	2.95	0.371	13%
MATL	0.27	10053.8	1418.9	65.54	5%
MWTL	0.13	26240.6	2250.6	50.99	2%
MTW	0.38	0.944	40.4	0.89	2%
MCWT	0.35	0.099	4.98	0.27	5%
MTnum	0.52	0.215	3.28	0.58	18%
MTC	0.64	0.0009	0.238	0.046	20%
MKT	0.12	3.794	12.83	0.566	4%
MCT	0.44	0.37	5.55	0.648	12%
MBT	0.43	3.137	36.17	1.84	5%
MFines	0.34	0.111	1.82	0.274	15%

9.6 Correlated responses to selection with multiple traits

Tree growth traits have been the main selection criteria in most of the tree improvement programmes around the world. The recent focus on wood properties will most probably necessitate the use of more than one trait in tree

improvement programmes of the future. An important question that needs to be addressed with multiple traits is the interrelationship between traits and the positive or negative effect that selection pressure for one trait has on other traits (Zobel and van Buijtenen, 1989). In order to address this, correlated responses for multiple traits can be calculated (Falconer and Mackay, 1996). Correlated responses can be calculated for traits if genetic correlations between traits are reasonably strong and heritabilities are available for the traits (Pot *et al*., 2002; Stanger, 2003; White *et al*., 2007).

In order to establish the level of correlated responses to selection for specific traits, a number of important primary traits were selected. The effect on other secondary traits was calculated, using heritability estimates, phenotypic variance estimates and additive genetic correlations between traits. These genetic parameters were used to calculate expected responses from the trait means for the breeding population. The same selection intensity (i = 2.421) was used as in section 9.5 (predicted gains) and was also used by Stanger (2003). Predicted correlated responses were estimated for growth and wood property traits with direct selection for diameter at breast height (DBH8), weighted mean wood density (WMWD), mean tracheid diameter (MTD) and MorFi® arithmetic tracheid length (MATL). These traits had high heritability estimates, and are important in determining pulp and paper products. The MorFi® calculated trait weighted tracheid length, although deemed more meaningful by processors, was not used as this trait had a low heritability estimate, and seem to have been affected by the maceration process.

In the first comparison, DBH8 was chosen for direct selection and the response on wood density and tracheid traits were calculated (Table 9.16). The response on wood density and tracheid traits ranged from -1% to 12%. Wood density traits ranged from a slight decrease of -1% for WMWD to a -8% decrease in LWP. The impact on tracheid cross-sectional traits ranged from -11% (NoTrach) to 12% (TArea). The results for these two traits can be expected, since these two

calculated traits represent transverse properties determined by tracheid cross-sectional dimensions. The effect on tracheid dimension traits ranged from -4% (MKT) to 8% (MBT), and tracheid length and width traits remained largely unaffected. An increase in the growth trait DBH8 will therefore likely produce the same length tracheids, but tracheid cross-sectional traits radial and tangential tracheid diameter and lumen diameter will increase. This will also increase the coarseness of the fibres in paper products.

Table 9.16 Predicted correlated responses of wood property traits with direct selection on tree diameter growth (10% predicted gain).

Trait 1	h^2 Trait 1	Trait 2	h^2 Trait 2	r_A	Trait 2 σ^2_{phen}	Trait Mean	Actual Response	% of Mean Trait 2
DBH8	0.23	**WMWD**	0.81	-0.08	0.0014	0.403	-0.0032	-1%
	0.23	**MEWD**	0.90	0.15	0.0005	0.315	0.0036	1%
	0.23	**MLWD**	0.20	-0.03	0.0008	0.634	-0.0004	0%
	0.23	**LWP**	0.89	-0.27	0.0025	0.19	-0.0148	-8%
DBH8	0.23	**RD**	1.04	0.79	8.73	42.28	2.76	7%
	0.23	**TD**	0.70	0.75	4.20	39.56	1.49	4%
	0.23	**MTD**	1.04	0.79	8.73	40.92	2.76	7%
	0.23	**LD**	1.02	0.62	8.00	28.3	2.06	7%
	0.23	**CWA**	0.48	0.88	4290	561	46.37	8%
	0.23	**CWT**	0.38	0.10	0.3040	5.48	0.0395	1%
	0.23	**TArea**	1.04	0.72	572989	5273	645.33	12%
	0.23	**NoTrach**	0.95	-0.68	735.00	193	-20.87	-11%
	0.23	**RR**	0.74	-0.01	0.0050	0.39	-0.0007	0%
	0.23	**CS**	0.66	0.87	7592	673	71.50	11%
	0.23	**SS**	0.58	-0.65	477.00	245	-12.55	-5%
	0.23	**PM**	1.04	0.72	140.00	164	10.09	6%
	0.23	**WTS**	0.56	0.09	0.0750	2.95	0.0214	1%
DBH8	0.23	**MATL**	0.27	-0.69	10053.80	1419	-41.7402	-3%
	0.23	**MWTL**	0.13	-0.11	26240.60	2251	-7.4595	0%
	0.23	**MTW**	0.38	0.08	0.9440	40.4	0.0556	0%
	0.23	**MCWT**	0.35	0.04	0.0990	4.98	0.0086	0%
	0.23	**MTnum**	0.52	-0.11	0.2150	3.28	-0.0427	-1%
	0.23	**MTC**	0.64	0.01	0.0009	0.238	0.0003	0%
	0.23	**MKT**	0.12	-0.62	3.7940	12.83	-0.4857	-4%
	0.23	**MCT**	0.44	-0.01	0.3700	5.55	-0.0047	0%
	0.23	**MBT**	0.43	2.05	3.1370	36.17	2.7644	8%
	0.23	**MFines**	0.34	0.10	0.1110	1.82	0.0226	1%

In the second comparison, WMWD was chosen for direct selection and the response on wood density, tracheid and growth traits were calculated (Table 9.17). Selecting for WMWD will increase the other wood density traits by a magnitude ranging from 2% (MLWD) to 48% (LWP). The effect on tracheid cross-sectional properties is largely negative, ranging from a decrease of -19% in LD to a 1% increase in RR and WTS, and a 20% increase in NoTrach.

Table 9.17 Predicted correlated responses of wood property traits with direct selection on weighted mean wood density (18% predicted gain).

Trait 1	h^2 Trait 1	Trait 2	h^2 Trait 2	r_A	Trait 2 σ^2_{phen}	Trait Mean	Actual Response	% of Mean Trait 2
WMWD	0.81	**MEWD**	0.90	0.91	0.0005	0.315	0.0404	13%
	0.81	**MLWD**	0.20	0.47	0.0008	0.634	0.0129	2%
	0.81	**LWP**	0.89	0.89	0.0025	0.19	0.0918	48%
WMWD	0.81	**RD**	1.04	-0.69	8.73	42.28	-4.53	-11%
	0.81	**TD**	0.70	-0.82	4.20	39.56	-3.06	-8%
	0.81	**MTD**	1.04	-0.73	8.73	40.92	-4.79	-12%
	0.81	**LD**	1.02	-0.88	8.00	28.3	-5.48	-19%
	0.81	**CWA**	0.48	-0.17	4290	561.19	-16.81	-3%
	0.81	**CWT**	0.38	0.20	0.3040	5.48	0.1481	3%
	0.81	**TArea**	1.04	-0.65	572989	5272.5	-1093	-21%
	0.81	**NoTrach**	0.95	0.67	735.00	193.05	38.58	20%
	0.81	**RR**	0.74	0.04	0.0050	0.39	0.0053	1%
	0.81	**CS**	0.66	-0.17	7592	673	-26.22	-4%
	0.81	**SS**	0.58	-0.48	477.00	245.22	-17.40	-7%
	0.81	**PM**	1.04	-0.66	140.00	163.68	-17.35	-11%
	0.81	**WTS**	0.56	0.09	0.0750	2.95	0.040	1%
WMWD	0.81	**MATL**	0.27	0.17	10053.80	1418.9	19.30	1%
	0.81	**MWTL**	0.13	-0.22	26240.60	2250.6	-27.99	-1%
	0.81	**MTW**	0.38	-0.27	0.9440	40.4	-0.35	-1%
	0.81	**MCWT**	0.35	0.13	0.0990	4.98	0.052	1%
	0.81	**MTnum**	0.52	-0.07	0.2150	3.28	-0.051	-2%
	0.81	**MTC**	0.64	0.00	0.0009	0.238	0.0000	0%
	0.81	**MKT**	0.12	-1.73	3.7940	12.83	-2.54	-20%
	0.81	**MCT**	0.44	-0.30	0.3700	5.55	-0.263	-5%
	0.81	**MBT**	0.43	-0.87	3.1370	36.17	-2.201	-6%
	0.81	**MFines**	0.34	0.09	0.1110	1.82	0.0381	2%
WMWD	0.81	**DBH8**	0.23	-0.08	8.077	15.1	-0.2376	-2%
	081	**Vol8**	0.23	-0.12	0.0025	0.129	-0.0061	-5%

Tracheid dimension traits were largely unaffected, except for a reduction in the tracheid defect traits MKT, MCT and MBT. There was also a negative correlation with DBH8 and Vol8 with r values ranging from -2% to -5% respectively.

The cross-sectional trait MTD was selected for the third comparison and the effect on other wood density, tracheid cross-sectional and dimension and growth traits were assessed (Table 9.18). Wood density traits were negatively affected ranging from -2% for MLWD to -51% for LWP. This could have a negative effect on tear properties, and a positive effect on burst. The other tracheid cross-sectional traits (RD, TD, LD and CWA) were positively affected (10% to 24%) by selection for MTD.

Coarseness also increased by 20%, and RR and NoTrach was negatively affected by -20% and -31% respectively. Tracheid dimension traits were affected ranging from -9% for MTnum to 14% for MTC. Again, there were similar responses for the calculated cross-sectional trait CS and the MorFi® trait MTC (20% and 14% respectively). Growth traits DBH8 and Vol8 were increased by 10% and 37% respectively.

In the last comparison, the tracheid dimension trait MATL was chosen for direct selection and the response on wood density, tracheid and growth traits was calculated (Table 9.19). Wood density traits were not affected to a major degree; except for LWP (9%). Tracheid cross-sectional diameter traits were negatively affected ranging from -3% for TD to -8% for RD, LD and CWA. A decline in these traits also caused a decline in coarseness (CS) and TArea. Tracheid dimension traits were not affected greatly, but tracheid defect traits were reduced. MTC again was closely related with the calculated tracheid cross-sectional trait CS. There was a large effect on growth traits, -8% for DBH8 and -19% for Vol8.

The correlated responses from this study were very similar to the South African *P. patula* provenance study (Stanger, 2003), but responses were mostly of larger magnitude due to higher heritability and genetic correlations. Similar trends were also reported for *Pinus pinaster* (Pot *et al*., 2002). No other detailed studies could be located in the literature for further comparisons in any other *Pinus* species.

Table 9.18 Predicted correlated responses of wood property traits with direct selection on mean tracheid diameter (18% predicted gain).

Trait 1	h^2 Trait 1	Trait 2	h^2 Trait 2	r_A	Trait 2 σ^2_{phen}	Trait Mean	Actual Response	% of Mean Trait 2
MTD	1.04	**WMWD**	0.81	-0.73	0.0014	0.403	-0.0617	-15%
	1.04	**MEWD**	0.90	-0.47	0.0005	0.315	-0.0237	-8%
	1.04	**MLWD**	0.20	-0.32	0.0008	0.634	-0.0100	-2%
	1.04	**LWP**	0.89	-0.83	0.0025	0.19	-0.0970	-51%
MTD	1.04	**RD**	1.04	0.98	8.73	42.28	7.2897	17%
	1.04	**TD**	0.70	0.93	4.20	39.56	3.9356	10%
	1.04	**LD**	1.02	0.97	8.00	28.3	6.8403	24%
	1.04	**CWA**	0.48	0.84	4290	561	94.1109	17%
	1.04	**CWT**	0.38	-0.26	0.3040	5.48	-0.2182	-4%
	1.04	**TArea**	1.04	0.92	572989	5273	1753.43	33%
	1.04	**NoTrach**	0.95	-0.93	735.00	193	-60.6736	-31%
	1.04	**RR**	0.74	-0.52	0.0050	0.39	-0.0781	-20%
	1.04	**CS**	0.66	0.77	7592	673	134.5712	20%
	1.04	**SS**	0.58	-0.31	477.00	245	-12.7305	-5%
	1.04	**PM**	1.04	0.92	140.00	164	27.4082	17%
	1.04	**WTS**	0.56	0.22	0.0750	2.95	0.1113	4%
MTD	1.04	**MATL**	0.27	-0.8	10054	1419	-102.91	-7%
	1.04	**MWTL**	0.13	0.34	26241	2251	49.03	2%
	1.04	**MTW**	0.38	0.41	0.9440	40.4	0.6063	2%
	1.04	**MCWT**	0.35	-0.43	0.0990	4.98	-0.1976	-4%
	1.04	**MTnum**	0.52	-0.37	0.2150	3.28	-0.3054	-9%
	1.04	**MTC**	0.64	0.56	0.0009	0.238	0.0332	14%
	1.04	**MKT**	0.12	0.28	3.7940	12.83	0.4665	4%
	1.04	**MCT**	0.44	0.54	0.3700	5.55	0.5379	10%
	1.04	**MBT**	0.43	0.85	3.1370	36.17	2.4374	7%
	1.04	**MFines**	0.34	0.10	0.1110	1.82	0.0480	3%
MTD	1.04	**DBH8**	0.23	0.79	8.08	15.1	2.66	18%
	1.04	**Vol8**	0.22	0.83	0.0025	0.129	0.0481	37%

Table 9.19 Predicted correlated responses of wood property traits with direct selection on MorFi® arithmetic tracheid length (5% predicted gain).

Trait 1	h^2 Trait 1	Trait 2	h^2 Trait 2	r_A	Trait 2 σ^2_{phen}	Trait Mean	Actual Response	% of Mean Trait 2
MATL	0.27	**WMWD**	0.81	0.17	0.0014	0.403	0.0073	2%
	0.27	**MEWD**	0.90	0.00	0.0005	0.315	0.0000	0%
	0.27	**MLWD**	0.20	-0.18	0.0008	0.634	-0.0029	0%
	0.27	**LWP**	0.89	0.30	0.0025	0.19	0.0179	9%
MATL	0.27	**RD**	1.04	-0.88	8.73	42.28	-3.34	-8%
	0.27	**TD**	0.70	-0.48	4.20	39.56	-1.04	-3%
	0.27	**MTD**	1.04	-0.8	8.73	40.92	-3.03	-7%
	0.27	**LD**	1.02	-0.63	8.00	28.3	-2.27	-8%
	0.27	**CWA**	0.48	-0.75	4290	561	-42.81	-8%
	0.27	**CWT**	0.38	-0.06	0.3040	5.48	-0.0257	0%
	0.27	**TArea**	1.04	-0.72	572989	5273	-699.20	-13%
	0.27	**NoTrach**	0.95	0.66	735.00	193	21.94	11%
	0.27	**RR**	0.74	0.01	0.0050	0.39	0.0008	0%
	0.27	**CS**	0.66	-0.74	7592	673	-65.90	-10%
	0.27	**SS**	0.58	0.41	477.00	245	8.5789	3%
	0.27	**PM**	1.04	-0.72	140.00	164	-10.93	-7%
	0.27	**WTS**	0.56	-0.06	0.0750	2.95	-0.0155	-1%
MATL	0.27	**MWTL**	0.13	0.16	26241	2251	11.76	1%
	0.27	**MTW**	0.38	0.13	0.9440	40.4	0.0979	0%
	0.27	**MCWT**	0.35	-0.08	0.0990	4.98	-0.0187	0%
	0.27	**MTnum**	0.52	0.35	0.2150	3.28	0.1472	4%
	0.27	**MTC**	0.64	-0.60	0.0009	0.238	-0.0181	-8%
	0.27	**MKT**	0.12	-0.46	3.7940	12.83	-0.3905	-3%
	0.27	**MCT**	0.44	-0.09	0.3700	5.55	-0.0457	-1%
	0.27	**MBT**	0.43	-0.70	3.1370	36.17	-1.0227	-3%
	0.27	**MFines**	0.34	-0.54	0.1110	1.82	-0.1320	-7%
MATL	0.27	**DBH8**	0.23	-0.69	8.077	15.1	-1.1831	-8%
	0.27	**Vol8**	0.22	-0.83	0.0025	0.129	-0.0245	-19%

9.7 Conclusions

Results from this study have indicated that weak and mostly non-significant individual and family phenotypic correlations exist between growth and wood density traits. Additive genetic correlations between growth and wood density traits were also low and were associated with large standard errors. Individual tree and family phenotypic correlations between growth and tracheid cross-sectional traits were higher and mostly significant, and genetic correlations were moderate to high. Solid wood tracheid diameter traits were positively correlated with growth traits. Macerated tracheid dimension traits and growth traits were both negatively and positively correlated, phenotypic correlations were low to moderate with few correlations being statistically significant. Genetic correlations between tracheid length and growth traits were moderate and negative. Tracheid width and cell-wall thickness was poorly correlated with growth traits.

Phenotypic correlations between wood density and solid wood tracheid cross-sectional traits were generally significant and moderate to strong. Tracheid diameter traits were negatively correlated with wood density traits. Mean latewood density was poorly correlated with tracheid cross-sectional traits, due to the relatively low levels present in the juvenile wood of this study. Genetic correlations were generally moderate to strong, and the tracheid diameter traits were again found to be negatively correlated with wood density. Tracheid width and cell-wall thickness was also poorly correlated with wood density traits. Phenotypic correlations between wood density and tracheid dimension traits were low to moderate, and were mostly statistically significant. Genetic correlations between wood density and tracheid length, width and MorFi® calculated cell-wall thickness were weak.

Individual tree and family phenotypic correlations between tracheid cross-sectional and dimension traits were generally low to moderate and statistically significant. The correlation matrix between tracheid cross-sectional and

dimension traits is complex, and represent similar traits from two different processes. With the MorFi® traits, tracheids are separated and part of the cell-wall is removed during the maceration process. This probably accounts for the low correlation between similar tracheid traits such as radial and tangential tracheid diameter, and MorFi® assessed cell-wall thickness. The best and most useful correlations were between tracheid cross-sectional traits and MorFi® assessed tracheid coarseness. The latter trait also correlated well with the Silviscan® derived trait coarseness. Genetic correlations generally reflected similar relationships between traits compared to the phenotypic correlations, but some with higher magnitude. Mean arithmetic tracheid length displayed strong negative genetic correlations with tracheid diameter traits. Tracheids with broken ends as assessed with MorFi® also correlated positively and strongly with tracheid diameter traits.

Predicted gains for all wood property traits investigated in this study ranged from 2 - 57%. A selection intensity of 2% was applied in the predictions of genetic gain for these traits. Wood density traits ranged from 2% for mean latewood density, to 15% for mean earlywood density, 18% for weighted mean wood density and 57% for latewood proportion. Tracheid cross-sectional trait gains ranged from 9% to 36%, and the most meaningful gains were for mean tracheid diameter and associated traits such as tracheid Area, number of tracheids per unit area, Runkel ratio and coarseness. Tracheid dimension trait gains ranged from 2 to 20%, with the most meaningful being mean arithmetic tracheid length, tracheid width, and tracheid wall thickness. Number of tracheids per unit area and coarseness also had high predicted gains of 18 and 20% respectively.

Correlated responses between traits considered as multiple traits for selection were also investigated. Selecting diameter growth (at breast height) at eight years as the primary trait produced correlated responses in wood property traits. These ranged from -11 to 8%. There was a -1% predicted change for weighted mean wood density, while most of the tracheid cross-sectional traits would show

improvement from selecting diameter at breast height as the primary selection trait. Tracheid dimension traits showed either no response, or a small negative response. Selecting weighted mean wood density as the primary trait caused reductions in most tracheid cross-sectional and dimension traits, as well as a decline in growth traits. When mean tracheid diameter was selected as the primary selection trait, other tracheid diameter traits benefited, and there were substantial benefits for growth traits. Wood density traits and tracheid dimension traits were, however, negatively influenced. Selecting mean arithmetic tracheid length as the primary trait caused wood density traits to improve slightly or remain unchanged, but reduced tracheid cross-sectional traits such as tracheid diameter, cell-wall area and coarseness. Growth traits were also influenced negatively.

The range of wood density and tracheid properties evaluated in this study can be influenced positively or negatively, depending on the primary selection traits. Wood property traits can therefore be influenced favourably, depending on the end product of the Kart pulp produced from this wood source. Negative effects on growth traits and wood density do, however, have to be limited as these still form the most important aspects for increased pulp production. Several generations of breeding for growth properties may have caused an unintended reduction of wood density and tracheid length in Southern African breeding populations of *P. patula*.

Chapter 10

Final conclusions and recommendations

10.1 Introduction

This study reported on a comprehensive quantitative genetics study of physical wood property traits of *P. patula* grown in Zimbabwe. Results from this study indicate that wood density and some important tracheid characteristics are under strong genetic control, and that additive gene action is the most important factor in the control of these traits. This chapter will provide a short summary of the key findings of this study, as well as a discussion of their implications for a *P. patula* tree improvement programme. Recommendations are also given for further research in areas highlighted by this study.

10.2 Key findings from study

The combining ability analysis for wood density and tracheid traits indicated that general combining ability effects were the most important to consider. Specific combining ability was almost completely absent, except for a few traits. There was also very little significant evidence of any reciprocal, maternal or non-maternal effects for the studied traits. Although the aim of this study was not to investigate site effects on wood properties in detail, there was some indication that site (altitude) affected certain of the wood property traits. Heritability estimates from this study were generally higher than previously reported for *P. patula*, but were comparable with studies on other species such as *P. radiata* and *P. taeda (*Nyankuengama, 1997; Belonger, 1998; Stanger, 2003; Vermaak, 2007). Inter-trait correlations also highlighted that some of the traits are

negatively correlated, which can have a major impact on multi-trait selection and breeding.

The study of wood density, as assessed through x-ray densitometry, indicated that there was large family variation for all wood density traits. The combining ability analysis indicated that general combining ability effects were highly significant and that weighted mean density, mean earlywood density and latewood proportion are under strong additive control. Reciprocal or maternal effects were found to be not significant for wood density traits and future studies could be simplified and be conducted with less labour-intensive half-diallel mating designs. Wood density traits also displayed a radial increase in magnitude from pith-to-bark, which is a characteristic of juvenile wood of many *Pinus* species. Site effects or site- by-family interaction effects were found to be mostly not significant for the studied wood density traits, other than for latewood proportion. The results per growth ring were generally similar to the mean values. Heritability estimates from this study were high, indicating that gains could be made with classical breeding for general combining ability and recurrent selection. The heritability estimates from this study were higher than those reported in other studies of wood density of *P. patula* (Stanger, 2003; Vermaak, 2007)*, P. radiata* and *P. taeda* (Belonger, 1998; Wu *et al*., 2008). Correlations between density traits were generally high, also between the different growth rings, indicating that early selection could be made for these traits.

Large family variation was found for most cross-sectional tracheid traits assessed through image analysis. The full-diallel analysis indicated again that general combining ability was the most important and significant effect, with specific combining ability, reciprocal, maternal and non-maternal effects not being significant. Site effects were mostly not significant for cross-sectional tracheid traits. Heritability estimates were generally much higher than those for growth traits, and were also higher than reported for the same traits in other studies of *P. patula*, but similar to other species such as *P. radiata and P. taeda*

(Nyankuengama, 1997; Belonger, 1998; Stanger, 2003; Vermaak, 2007). There were strong correlations among traits, indicating that the assessment could be restricted to a selection of the traits.

Results from the study of tracheid dimension traits using the MorFi® fibre analyser system showed large family and individual tree variation. The combining ability analysis indicated, as in the case of wood density and cross-sectional tracheid traits, that general combining ability effects were the most significant effect. Significant site effects were found for weighted tracheid length, tracheid width, cellwall thickness and other derived traits. The heritability estimates for the tracheid dimension traits were lower than the other wood density and cross-sectional tracheid traits reported in this study. These estimates were, however, similar to other studies conducted on *P. patula* (Stanger, 2003; Vermaak, 2007). Correlations between different tracheid dimension traits were weaker than those of the other traits, and genetic correlations were also associated with large standard errors. The estimation of genetic parameters of tracheid dimensions was generally less precise than for wood density and cross-sectional tracheid traits. Weighted tracheid length is a more accepted trait in the interpretation of pulping data by processors, but in this study arithmetic length was a more heritable trait. Although phenotypic correlations between these traits were strong, the genetic correlation was very weak.

An in-depth analysis of growth traits was not attempted in this study. Instead, genetic parameters were calculated to enable inter-trait relationships and correlations. The study of growth traits were restricted to the 300 trees included in the wood property study, a much lower number than what would normally be considered for growth traits. The results did, however, correspond very well with the published study on multiple sites of growth properties at the same age utilising the same genetic material used in the current study (Barnes *et al*. 1992b). These calculated genetic parameters for growth traits allowed for a tree-

by-tree investigation of inter-trait correlations for all the physical wood property traits investigated in this study.

Inter-trait correlations between wood density, tracheid cross-sectional and dimension properties and growth traits were complex, often negative and of varying magnitude. Gain predictions were influenced by correlated responses between different traits, depending on which primary trait was selected. This analysis again highlighted the complex nature of multi-trait selection and breeding reported in many other studies (Verryn, 2008).

The importance of physical properties of wood in the production of pulp and paper products has become increasingly important in a more competitive, low-cost producing world market. More specialised paper and packaging products are being produced, requiring specific wood properties. Softwoods fulfil an important function in the production of paper and packaging products as their long pulp fibres contribute to the strength and quality properties of these products. A fundamental understanding of the inheritance of important physical properties will allow tree breeders to select and breed for these specific traits. A complicating factor is the rapid rate of development in the pulp and paper industry. Deciding which specific wood property traits will be important in the future for inclusion in a long-generation turnover crop such as trees, remains a major challenge (Sorensson, 2008).

Results from this study of physical wood properties cannot be evaluated in isolation. The species *P. patula* has been the most important and preferred softwood species used as a furnish for various solid wood and pulping processes in Southern Africa. Recent problems with post-planting mortality caused by the pitch canker fungus *Fusarium circinatum* has impacted on the deployment of the species. This has spurred the development of inter-specific hybrids between *P. patula* and other more tolerant species such as *P. tecunuma*nii and *P. oocarpa*.

The wood properties of *P. patula* do still, however, remain important in the inter-specific hybrid. One possible breeding response is to structure genetic material into sub-populations based on specific properties. The number of selection traits within sub-populations can then be restricted to ensure rapid progress. Top selections from the different sub-populations and other species could be combined via controlled pollination and tested and deployed through vegetative propagation.

The Kraft pulping process can utilise both hardwood and softwood furnish. Some of the most important wood properties contributed by softwoods are wood density and increased strength properties. Longer average tracheid length and lower fibre coarseness contribute positively to the strength properties and bonding of Kraft paper. This study indicated that these two properties are negatively correlated, therefore with increased tracheid length, fibre coarseness declines. Selecting genotypes with increased tracheid length would thus also result in a decrease in fibre coarseness. Increased tracheid width also increases fibre coarseness, which negatively impacts on strength properties. Wood density is further influenced by the fibre properties, since increased density can be linked with an increased number of fibres, or fewer fibres with thicker cellwalls. Careful consideration will have to be given to selecting the correct tracheid properties for the future end product of a specific breeding programme. One of the most important contributions of softwoods in Kraft pulp furninsh is an improvement of paper strength properties, influenced by tracheid length and diameter traits. The challenge is to incorporate some of these tracheid traits without compromising volume growth and wood density. Wood scientists and processors need to provide guidance to tree breeders on this matter.

A unique set of genetic sample material formed the basis of this quantitative genetics study. This study utilised a complete 5 × 5 full-diallel and associated factorial mating design with progeny trials planted on several sites. This material provided the opportunity to investigate the inheritance of physical wood properties

of *P. patula*. Controlled pollination of *P. patula* is problematic with high abortion rates during the two year pollination and seed ripening phase, and few large and comprehensive mating designs have been reported in literature. Although the specific genetic material used in this study is not available for future selection and breeding, it has provided a platform to characterise the wood properties for the species and other populations currently in South African breeding programmes. The *P. patula* populations in breeding programmes in Southern Africa share common origins in the central parts of Mexico where original seed collections were made (Stanger, 2003).

This study also provided the opportunity to evaluate a range of physical wood properties using the identical radial wedge sample. This enabled accurate and unbiased comparisons and correlations between the different wood density and tracheid characteristics. Although the samples utilised in this study were collected in a destructive manner, the use of radial planks cut from these wedge samples resembled the non-destructive method utilising pith-to-bark increment cores. Future studies investigating similar wood property traits as this study could therefore be completed in a non-destructive manner from selected trees in other studies and populations. The current study was limited to an investigation of physical wood properties at a specific location on a tree, but many studies have found that properties at breast height can be representative of the entire tree (Ringo and Klem, 1980; Ladrach, 1984; Zobel and Jett, 1995; Evans *et al.*, 1999).

The age of the sample material used in this study was eight years old, which is currently considered to be half-rotation for pulping rotations. There is an increasing trend worldwide to reduce the age of rotations, with an increase in the proportion of juvenile wood. It is also in the juvenile part of the wood where the most radial variation occurs for many wood properties (Lachenbruch *et al*., 2011). Improved genetic material grows faster and can be harvested earlier, but there is an impact on wood properties. This study found an upward trend in wood density traits with radial growth. Although not explored for tracheid properties in this

study, other studies have found an increase in tracheid dimension properties with increasing age (Megraw, 1985; Muneri and Balodis, 1998; Stanger, 2003). This study has provided detailed further insight into the physical wood properties of juvenile wood.

10.3 Recommendations

This study has shown that a range of important physical wood properties are under strong, additive genetic control. When applying the results from this study on proprietary genetic material, a large number of individuals will have to be phenotyped. Current technology exists to achieve this aim in a rapid, cost-effective and non-destructive manner. New technology such as MorFi® enables rapid and comprehensive tracheid property studies. MorFi® analysis can also be viewed as a surrogate for micro-pulping, which is very slow and costly, and cannot be done in a non-destructive manner. The use of Near Infrared spectroscopy (NIR) as a rapid screening tool can also be considered to assess large numbers of progeny (Schimleck, 2008; Hodge and Woodbridge, 2004; Hodge and Woodbridge, 2010).

There are also some limitations with the completed study. In long rotation crops such as forest trees, early selection is crucial. This study has not provided any insight into the juvenile mature correlations for wood properties. Other studies have been done on juvenile mature correlations, showing that early selection can be done (Barnes, 1973; Birks and Barnes, 1991; Zobel and Sprague, 1998). It will be important to study this specifically for the wood properties of *P. patula* reported on here. Future studies should also be completed using a larger number of parents. A larger number of parents will allow for more robust results, and would increase the confidence of the combining ability results. The diallel mating design is ideal for combining ability analysis, and in the absence of reciprocal effects,

half-diallel designs can be utilised. This will allow for larger mating designs with an increased number of parents for the same amount of pollination resources.

This study has increased the current knowledge of wood density and tracheid properties of *P. patula,* and has contributed to the study of growth properties completed by Barnes (1973) on the same genetic material. This study has also supplemented the study of basic wood density properties completed by Birks and Barnes (1991) conducted on the same genetic material. It has provided insight into the combining ability for physical wood properties of *P. patula*, and has highlighted the inter-relationships and associations between different wood properties such as wood density, tracheid cross-sectional and tracheid dimension properties.

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Summary

Pinus patula is the most widely planted softwood species in Southern Africa and is utilised for various solid wood and pulp and paper products. Tree improvement programmes for forestry species started in Southern Africa during the 1950's, with an initial focus on volume improvements. The focus for many advanced tree improvement programmes has moved to the improvement of wood properties.

This quantitative genetics study utilized half-rotation age *P. patula* progeny material from a 5 × 5 full diallel mating design and additional factorial crosses. A radial wood sample at 1.3 m above ground level from each of 300 trees was used to study a range of wood density, tracheid cross-sectional and -dimension characteristics. A large range of family variation was found for all wood properties. The combining ability analysis indicated that general combining ability was the most predominant effect and that specific combining ability effects were absent for nearly all the investigated wood density and tracheid traits. Reciprocal, maternal and non-maternal effects were also not significant for all but a few traits. Some of the wood properties were influenced by the specific site where trees were grown.

Heritability estimates for many of the important wood density and tracheid traits were moderate to high, indicating strong additive genetic control of these properties. Wood density traits were under strong genetic control, with a pith-to-bark increase in wood density traits. Latewood proportion and earlywood density had a strong effect on weighted mean wood density. There were also strong positive correlations for density traits between growth rings, indicating that early selection would be possible. Tracheid cross-sectional properties were also strongly inherited, and strong correlations were found between the cross-sectional traits and calculated pulp and paper traits. Tracheid dimension traits such as tracheid length, width and cellwall thickness had lower heritability

estimates than those found for wood density and tracheid cross-sectional traits. These were, however, of higher magnitude than growth trait heritabilities.

Several strong positive and negative correlations were found between growth, wood density and tracheid property traits. These negative correlations would make multi-trait selections very problematic. Predicted gains for some of the studied wood properties were substantial, but correlated responses between primary and secondary selection traits were often negative. The structuring of genetic material into wood property specific sub-populations would ensure the improvement of selected important wood properties. These different properties can then be combined by means of controlled pollinations, and be deployed via vegetative propagation. This study has provided novel information on the genetic inheritance of physical wood properties of *P. patula* grown in Southern Africa, and will allow tree breeders to include some of these properties in breeding programmes.

Keywords: *Pinus patula*, physical wood properties, wood density, tracheid properties, general and specific combining ability, heritability, correlations

Opsomming

Pinus patula is die mees aangeplante sagtehout spesie in Suider-Afrika en word vir verskeie soliede hout, pulp en papier produkte benut. Boom-veredelingsprogramme vir bosbou spesies is in die 1950's in Suider-Afrika begin met die fokus op verhoogde boomvolume. Vir baie van die gevorderde boomveredelingsprogramme het die toekomstige klem verskuif na die verbetering van houtkwaliteit-eienskappe.

Hierdie kwantitatiewe genetika studie het van half-rotasie ouderdom *P. patula* nageslagsmateriaal, afkomstig van 'n 5 × 5 volle dialleel ontwerp, asook bykomende faktoriale kruisings gebruik gemaak. Die studie het 'n pit- tot- bas radiale houtmonster, wat geneem is op 'n hoogte van 1.3 m van grondvlak af, benut om houtdigtheid en trageïed eienskappe te ondersoek. Al die houtkwaliteite wat ondersoek is het 'n groot hoeveelheid familie-variasie getoon. Verdere ondersoek het aangetoon dat algemene kombineervermoë betekenisvol was vir die oorgrote meerderheid houtdigtheid- en trageïed-eienskappe. Spesifieke kombineervermoë was nie 'n betekenisvol faktor vir die meeste eienskappe gewees nie. Resiproke, moederlike en nie-moederlike effekte was ook in die meeste gevalle nie betekenisvol gewees nie, behalwe vir enkele hout-eienskappe. Die spesifieke groeiplek het 'n betekenisvolle effek op sekere van die houteienskappe gehad.

Heelwat van die belangrike houtdigtheid- en trageïed-eienskappe het gemiddeld tot hoë oorerflikheidswaardes in hierdie studie getoon. Hierdie hoë waardes en die algemene kombineervermoë was 'n aanduiding dat die eienskappe deur sterk additiewe geen-aksie beheer word. Die studie het ook bevind dat houtdigtheid, met 'n pit- tot- bas verhoging in digtheidswaarde, onder 'n hoë vlak van additiewe genetiese beheer was. Najaarshout-persentasie en voorjaarshout-digtheid het die grootste bydra tot beswaarde gemiddelde houtdigtheid gemaak. Daar was ook

hoogs betekenisvolle positiewe korrelasies tussen houtdigtheidswaardes vir die verskillende jaarringe, wat aandui dat vroeë seleksie moontlik is. Die studie het ook bevind dat trageïed dwars-snit eienskappe onder ‘n hoë vlak van additiewe genetiese beheer was, met hoogs betekenisvolle positiewe korrelasies tussen sekere van hierdie eienskappe en berekende pulp en papier eienskappe. Trageïed-afmetingseienskappe, insluitend trageïed lengte, breedte en selwand-dikte was onder ‘n laer vlak van additiewe genetiese beheer as houtdigtheid- en trageïeddwars-snit eienskappe, maar was steeds hoër as boomgroei-eienskappe.

Die studie het bevind dat daar verskeie sterk positiewe en negatiewe korrelasies tussen boomgroei-, houtdigtheid-, trageïed dwars-snit- en afmetings-eienskappe was. Hierdie negatiewe korrelasies tussen eienskappe gaan die keuse vir meervoudige eienskappe bemoeilik. Voorspelde teelwinste vir sekere van die houteienskappe was aansienlik hoog, maar korrelasies tussen die primêre en sekondêre eienskappe was in baie gevalle negatief. Dit het tot gevolg dat genetiese teelmateriaal in sub-populasies met spesifieke houteienskappe verdeel sal moet word, om verbetering van die belankrikste gekose eienskappe te verseker. Verskillende houteienskappe kan dan gekombineer word deur kruisbestuiwings uit te voer en plantmateriaal vegetatief voort te plant. Hierdie studie het nuwe inligting verskaf oor die genetiese oorerflikheid van fisiese houteienskappe van *P. patula* wat in Suider Afrika verbou is, en sal boom-telers in staat stel of van die belangrike eienskappe in te sluit in boomveredelings-programme.

Sleutelwoorde: *Pinus patula*, fisiese houteienskappe, houtdigtheid, trageïed eienskappe, algemene en spesifieke kombineervermoë, oorerflikheidswaarde, korrelasies

Appendix 1

Analysis of variance tables for density traits, family analysis at Martin

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Weighted Mean Wood Density (WMWD)					
Rep	2	0.00057539	0.00028770	0.35	0.7023
Family	35	0.14159390	0.00404554	4.99	<.0001
Rep*Family	70	0.05131735	0.00073310	0.90	0.6740
Error	112	0.09087661	0.00081140		
Corrected Total	219	0.28444691			
Mean Earlywood Density (MEWD)					
Rep	2	0.00032306	0.00016153	0.74	0.4776
Family	35	0.05225553	0.00149302	6.88	<.0001
Rep*Family	70	0.01681038	0.00024015	1.11	0.3141
Error	112	0.02431975	0.00021714		
Corrected Total	219	0.09374029			
Mean Latewood Density (MLWD)					
Rep	2	0.00051394	0.00025697	0.34	0.7102
Family	35	0.04251909	0.00121483	1.62	0.0301
Rep*Family	70	0.04031587	0.00057594	0.77	0.8817
Error	112	0.08385277	0.00074869		
Corrected Total	219	0.16641923			
Latewood proportion (LWP)					
Rep	2	0.00083978	0.00041989	0.24	0.7871
Family	35	0.30238161	0.00863947	4.94	<.0001
Rep*Family	70	0.11914803	0.00170211	0.97	0.5451
Error	112	0.19606607	0.00175059		
Corrected Total	219	0.61739226			
Earlywood proportion (EWP)					
Rep	2	0.00083978	0.00041989	0.24	0.7871
Family	35	0.30238161	0.00863947	4.94	<.0001
Rep*Family	70	0.11914803	0.00170211	0.97	0.5451
Error	112	0.19606607	0.00175059		
Corrected Total	219	0.61739226			
Mean wood density Ring 5 (MWDR5)					
Rep	2	0.00419665	0.00209832	0.65	0.5219
Family	35	0.21947887	0.00627082	1.95	0.0044
Rep*Family	70	0.18703856	0.00267198	0.83	0.7947
Error	112	0.35927807	0.00320784		
Corrected Total	219	0.77276683			
Mean wood density Ring 4 (MWDR4)					
Rep	2	0.00503475	0.00251737	1.05	0.3526
Family	35	0.18707053	0.00534487	2.23	0.0008
Rep*Family	70	0.12757813	0.00182254	0.76	0.8903
Error	112	0.26798428	0.00239272		
Corrected Total	219	0.59469209			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean wood density Ring 3 (MWDR3)					
Rep	2	0.00237343	0.00118671	0.76	0.4686
Family	35	0.14697734	0.00419935	2.70	<.0001
Rep*Family	70	0.08544062	0.00122058	0.78	0.8627
Error	112	0.17415564	0.00155496		
Corrected Total	219	0.41048159			
Mean wood density Ring 2 (MWDR2)					
Rep	2	0.00409559	0.00204779	2.17	0.1192
Family	35	0.11042102	0.00315489	3.34	<.0001
Rep*Family	70	0.06794116	0.00097059	1.03	0.4434
Error	110	0.10388069	0.00094437		
Corrected Total	217	0.28552820			
Mean wood density Ring 1 (MWDR1)					
Rep	2	0.00339405	0.00169702	0.52	0.5958
Family	35	0.25789388	0.00736840	2.26	0.0014
Rep*Family	68	0.17729200	0.00260724	0.80	0.8263
Error	80	0.26049138	0.00325614		
Corrected Total	185	0.72051566			
Mean earlywood density Ring 5 (MEWDR5)					
Rep	2	0.00139989	0.00069994	0.83	0.4405
Family	35	0.07021915	0.00200626	2.37	0.0004
Rep*Family	70	0.04229016	0.00060415	0.71	0.9363
Error	111	0.09407316	0.00084751		
Corrected Total	218	0.21049121			
Mean earlywood density Ring 4 (MEWDR4)					
Rep	2	0.00013367	0.00006684	0.18	0.8320
Family	35	0.06746624	0.00192761	5.31	<.0001
Rep*Family	70	0.04047263	0.00057818	1.59	0.0138
Error	112	0.04062984	0.00036277		
Corrected Total	219	0.15001020			
Mean earlywood density Ring 3 (MEWDR3)					
Rep	2	0.00087212	0.00043606	0.91	0.4039
Family	35	0.07440384	0.00212582	4.46	<.0001
Rep*Family	70	0.02988514	0.00042693	0.89	0.6899
Error	112	0.05343899	0.00047713		
Corrected Total	219	0.15933455			
Mean earlywood density Ring 2 (MEWDR2)					
Rep	2	0.00196470	0.00098235	2.67	0.0740
Family	35	0.05365026	0.00153286	4.16	<.0001
Rep*Family	70	0.03011812	0.00043026	1.17	0.2314
Error	110	0.04053685	0.00036852		
Corrected Total	217	0.12562378			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean earlywood density Ring 1 (MEWDR1)					
Rep	2	0.00009168	0.00004584	0.04	0.9638
Family	35	0.06024865	0.00172139	1.39	0.1173
Rep*Family	67	0.05655436	0.00084409	0.68	0.9467
Error	78	0.09683319	0.00124145		
Corrected Total	182	0.21630068			
Mean latewood density Ring 5 (MLWDR5)					
Rep	2	0.00304109	0.00152055	0.20	0.8194
Family	35	0.26624664	0.00760705	1.00	0.4861
Rep*Family	67	0.39810569	0.00594188	0.78	0.8514
Error	80	0.60902553	0.00761282		
Corrected Total	184	1.27599813			
Mean latewood density Ring 4 (MLWDR4)					
Rep	2	0.00819690	0.00409845	1.52	0.2235
Family	35	0.08950923	0.00255741	0.95	0.5584
Rep*Family	70	0.13930105	0.00199002	0.74	0.9154
Error	112	0.30226644	0.00269881		
Corrected Total	219	0.53785853			
Mean latewood density Ring 3 (MLWDR3)					
Rep	2	0.00184364	0.00092182	0.37	0.6890
Family	35	0.10994289	0.00314123	1.27	0.1721
Rep*Family	70	0.10994851	0.00157069	0.64	0.9787
Error	112	0.27620833	0.00246615		
Corrected Total	219	0.49890494			
Mean latewood density Ring 2 (MLWDR2)					
Rep	2	0.00069740	0.00034870	0.18	0.8349
Family	35	0.08103732	0.00231535	1.20	0.2362
Rep*Family	70	0.12021849	0.00171741	0.89	0.6976
Error	109	0.21029874	0.00192935		
Corrected Total	216	0.41267462			
Mean latewood density Ring 1 (MLWDR1)					
Rep	2	0.00011197	0.00005599	0.05	0.9511
Family	35	0.03406558	0.00097330	0.87	0.6675
Rep*Family	68	0.06178829	0.00090865	0.81	0.8067
Error	78	0.08706651	0.00111624		
Corrected Total	183	0.18209398			

Appendix 2

Analysis of variance tables for density traits, family analysis at Martin and Nyangui

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Weighted mean wood density (WMWD)					
Site	1	0.00156525	0.00156525	2.08	0.1528
Rep	2	0.00113604	0.00056802	0.75	0.4735
Family	9	0.03173673	0.00352630	4.68	<.0001
Rep*Family	18	0.02349531	0.00130530	1.73	0.0455
Site*Family	9	0.00637590	0.00070843	0.94	0.4948
Error	102	0.07692779	0.00075419		
Corrected Total	141	0.13663111			
Mean earlywood density (MEWD)					
Site	1	0.00000010	0.00000010	0.00	0.9822
Rep	2	0.00020317	0.00010158	0.50	0.6101
Family	9	0.01031082	0.00114565	5.60	<.0001
Rep*Family	18	0.00815029	0.00045279	2.21	0.0067
Site*Family	9	0.00183375	0.00020375	1.00	0.4482
Error	102	0.02086621	0.00020457		
Corrected Total	141	0.04066439			
Mean latewood density (MLWD)					
Site	1	0.00265303	0.00265303	3.80	0.0539
Rep	2	0.00080595	0.00040297	0.58	0.5629
Family	9	0.00999346	0.00111038	1.59	0.1274
Rep*Family	18	0.01699545	0.00094419	1.35	0.1717
Site*Family	9	0.00576351	0.00064039	0.92	0.5126
Error	102	0.07112851	0.00069734		
Corrected Total	141	0.10540046			
Latewood proportion (LWP)					
Site	1	0.01448808	0.01448808	6.57	0.0119
Rep	2	0.00017434	0.00008717	0.04	0.9613
Family	9	0.06160378	0.00684486	3.10	0.0025
Rep*Family	18	0.04219963	0.00234442	1.06	0.4002
Site*Family	9	0.00842787	0.00093643	0.42	0.9193
Error	102	0.22503669	0.00220624		
Corrected Total	141	0.35277492			
Earlywood proportion (EWP)					
Site	1	0.01448808	0.01448808	6.57	0.0119
Rep	2	0.00017434	0.00008717	0.04	0.9613
Family	9	0.06160378	0.00684486	3.10	0.0025
Rep*Family	18	0.04219963	0.00234442	1.06	0.4002
Site*Family	9	0.00842787	0.00093643	0.42	0.9193
Error	102	0.22503669	0.00220624		
Corrected Total	141	0.35277492			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean wood density Ring 5 (MWDR5)					
Site	1	0.08950558	0.08950558	33.25	<.0001
Rep	2	0.01397616	0.00698808	2.60	0.0795
Family	9	0.07369037	0.00818782	3.04	0.0029
Rep*Family	18	0.06969187	0.00387177	1.44	0.1299
Site*Family	9	0.01655759	0.00183973	0.68	0.7222
Error	102	0.27453414	0.00269151		
Corrected Total	141	0.53789240			
Mean wood density Ring 4 (MWDR4)					
Site	1	0.02993955	0.02993955	23.26	<.0001
Rep	2	0.01336625	0.00668312	5.19	0.0071
Family	9	0.03931555	0.00436839	3.39	0.0011
Rep*Family	18	0.04945483	0.00274749	2.13	0.0093
Site*Family	9	0.01696117	0.00188457	1.46	0.1714
Error	102	0.13128506	0.00128711		
Corrected Total	141	0.26452680			
Mean wood density Ring 3 (MWDR3)					
Site	1	0.00845570	0.00845570	8.22	0.0050
Rep	2	0.00313852	0.00156926	1.53	0.2224
Family	9	0.02603295	0.00289255	2.81	0.0054
Rep*Family	18	0.03352463	0.00186248	1.81	0.0336
Site*Family	9	0.01221876	0.00135764	1.32	0.2358
Error	102	0.10490822	0.00102851		
Corrected Total	141	0.18578604			
Mean wood density Ring 2 (MWDR2)					
Site	1	0.00206162	0.00206162	1.97	0.1638
Rep	2	0.00060271	0.00030135	0.29	0.7507
Family	9	0.04023950	0.00447106	4.27	0.0001
Rep*Family	18	0.03617728	0.00200985	1.92	0.0222
Site*Family	9	0.00785810	0.00087312	0.83	0.5874
Error	102	0.10691018	0.00104814		
Corrected Total	141	0.19444192			
Mean wood density Ring 1 (MWDR1)					
Site	1	0.02440717	0.02440717	9.21	0.0034
Rep	2	0.00321658	0.00160829	0.61	0.5478
Family	9	0.03460282	0.00384476	1.45	0.1839
Rep*Family	18	0.03532928	0.00196274	0.74	0.7576
Site*Family	9	0.01409004	0.00156556	0.59	0.8001
Error	69	0.18277506	0.00264891		
Corrected Total	108	0.32629101			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean earlywood density Ring 5 (MEWDR5)					
Site	1	0.00623612	0.00623612	12.85	0.0005
Rep	2	0.00355943	0.00177971	3.67	0.0290
Family	9	0.01662625	0.00184736	3.81	0.0004
Rep*Family	18	0.01650108	0.00091673	1.89	0.0250
Site*Family	9	0.00539951	0.00059995	1.24	0.2819
Error	101	0.04903442	0.00048549		
Corrected Total	140	0.09439707			
Mean earlywood density Ring 4 (MEWDR4)					
Site	1	0.00068102	0.00068102	2.21	0.1405
Rep	2	0.00165889	0.00082945	2.69	0.0729
Family	9	0.02057525	0.00228614	7.41	<.0001
Rep*Family	18	0.01455344	0.00080852	2.62	0.0012
Site*Family	9	0.00435915	0.00048435	1.57	0.1345
Error	102	0.03148580	0.00030868		
Corrected Total	141	0.07031479			
Mean earlywood density Ring 3 (MEWDR3)					
Site	1	0.00860512	0.00860512	31.67	<.0001
Rep	2	0.00028860	0.00014430	0.53	0.5896
Family	9	0.01553495	0.00172611	6.35	<.0001
Rep*Family	18	0.00972448	0.00054025	1.99	0.0167
Site*Family	9	0.00610980	0.00067887	2.50	0.0126
Error	102	0.02771200	0.00027169		
Corrected Total	141	0.06782718			
Mean earlywood density Ring 2 (MEWDR2)					
Site	1	0.00221669	0.00221669	4.71	0.0323
Rep	2	0.00020529	0.00010265	0.22	0.8044
Family	9	0.01596447	0.00177383	3.77	0.0004
Rep*Family	18	0.01570194	0.00087233	1.85	0.0285
Site*Family	9	0.00504314	0.00056035	1.19	0.3090
Error	102	0.04800118	0.00047060		
Corrected Total	141	0.08704700			
Mean earlywood density Ring 1 (MEWDR1)					
Site	1	0.02427308	0.02427308	22.65	<.0001
Rep	2	0.00103445	0.00051722	0.48	0.6193
Family	9	0.01125055	0.00125006	1.17	0.3305
Rep*Family	18	0.01139262	0.00063292	0.59	0.8944
Site*Family	9	0.00326015	0.00036224	0.34	0.9591
Error	68	0.07287758	0.00107173		
Corrected Total	107	0.14240154			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean latewood density Ring 5 (MLWDR5)					
Site	1	0.29326397	0.29326397	56.71	<.0001
Rep	2	0.00314987	0.00157493	0.30	0.7382
Family	9	0.05527579	0.00614175	1.19	0.3119
Rep*Family	18	0.07703708	0.00427984	0.83	0.6640
Site*Family	9	0.05484541	0.00609393	1.18	0.3177
Error	95	0.49129459	0.00517152		
Corrected Total	134	0.96628179			
Mean latewood density Ring 4 (MLWDR4)					
Site	1	0.00000762	0.00000762	0.00	0.9547
Rep	2	0.01097028	0.00548514	2.33	0.1020
Family	9	0.01445657	0.00160629	0.68	0.7222
Rep*Family	18	0.01803935	0.00100219	0.43	0.9791
Site*Family	9	0.00990568	0.00110063	0.47	0.8927
Error	102	0.23967496	0.00234975		
Corrected Total	141	0.29348572			
Mean latewood density Ring 3 (MLWDR3)					
Site	1	0.00358068	0.00358068	2.47	0.1191
Rep	2	0.00180251	0.00090125	0.62	0.5390
Family	9	0.02478048	0.00275339	1.90	0.0602
Rep*Family	18	0.04185780	0.00232543	1.60	0.0727
Site*Family	9	0.02139429	0.00237714	1.64	0.1138
Error	102	0.14784892	0.00144950		
Corrected Total	141	0.24158818			
Mean latewood density Ring 2 (MLWDR2)					
Site	1	0.00935948	0.00935948	5.51	0.0208
Rep	2	0.00359266	0.00179633	1.06	0.3508
Family	9	0.04522297	0.00502477	2.96	0.0037
Rep*Family	18	0.03883244	0.00215736	1.27	0.2230
Site*Family	9	0.00943452	0.00104828	0.62	0.7795
Error	101	0.17141085	0.00169714		
Corrected Total	140	0.27443454			
Mean latewood density Ring 1 (MLWDR1)					
Site	1	0.01227582	0.01227582	14.78	0.0003
Rep	2	0.00127592	0.00063796	0.77	0.4678
Family	9	0.00594028	0.00066003	0.79	0.6221
Rep*Family	18	0.01576318	0.00087573	1.05	0.4151
Site*Family	9	0.00750190	0.00083354	1.00	0.4457
Error	68	0.05646637	0.00083039		
Corrected Total	107	0.10150852			

Appendix 3

ANOVA tables for cross-sectional tracheid traits, family analysis at Martin

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean radial tracheid diameter (RD)					
Rep	2	4.345233	2.172616	0.28	0.7548
Family	35	2124.282513	60.693786	7.88	<.0001
Rep*Family	70	382.104051	5.458629	0.71	0.9399
Error	112	862.942493	7.704844		
Corrected Total	219	3373.652636			
Mean tangential tracheid diameter (TD)					
Rep	2	3.4351442	1.7175721	0.56	0.5726
Family	35	359.7374908	10.2782140	3.35	<.0001
Rep*Family	70	147.3146207	2.1044946	0.69	0.9549
Error	112	343.3155823	3.0653177		
Corrected Total	219	860.9063196			
Mean tracheid diameter (MTD)					
Rep	2	3.4978479	1.7489240	0.39	0.6752
Family	35	979.5187496	27.9862500	6.31	<.0001
Rep*Family	70	212.3087143	3.0329816	0.68	0.9568
Error	112	496.971340	4.437244		
Corrected Total	219	1693.901322			
Mean lumen diameter (LD)					
Rep	2	0.9005410	0.4502705	0.10	0.9011
Family	35	895.9099121	25.5974261	5.92	<.0001
Rep*Family	70	221.6981320	3.1671162	0.73	0.9196
Error	112	484.024048	4.321643		
Corrected Total	219	1604.902670			
Mean Cell Wall Area (CWA)					
Rep	2	7057.5706	3528.7853	1.15	0.3209
Family	35	305762.5912	8736.0740	2.84	<.0001
Rep*Family	70	162881.1259	2326.8732	0.76	0.8953
Error	112	344188.7356	3073.1137		
Corrected Total	219	823625.7658			
Mean Cell Wall Thickness (CWT)					
Rep	2	0.49067918	0.24533959	0.94	0.3932
Family	35	16.52613598	0.47217531	1.81	0.0103
Rep*Family	70	15.42148033	0.22030686	0.85	0.7751
Error	112	29.19023075	0.26062706		
Corrected Total	219	61.93842000			
Mean tracheid area (TArea)					
Rep	2	195421.47	97710.74	0.34	0.7154
Family	35	64511971.90	1843199.20	6.34	<.0001
Rep*Family	70	13994033.41	199914.76	0.69	0.9544
Error	112	32572091.7	290822.2		
Corrected Total	219	111404831.1			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean number of tracheids (NoTrach)					
Rep	2	490.92341	245.46171	0.61	0.5475
Family	35	78779.68281	2250.84808	5.55	<.0001
Rep*Family	70	18419.50493	263.13578	0.65	0.9740
Error	112	45395.2326	405.3146		
Corrected Total	219	143163.0116			
Mean (PCell)					
Rep	2	1.1341490	0.5670745	0.69	0.5043
Family	35	108.7481928	3.1070912	3.77	<.0001
Rep*Family	70	47.6811115	0.6811587	0.83	0.8034
Error	112	92.2152300	0.8233503		
Corrected Total	219	251.1118875			
Mean Runkel Ratio (RR)					
Rep	2	0.00237229	0.00118614	0.36	0.6961
Family	35	0.38318735	0.01094821	3.35	<.0001
Rep*Family	70	0.18537189	0.00264817	0.81	0.8268
Error	112	0.36549374	0.00326334		
Corrected Total	219	0.94054797			
Mean Coarseness (CS)					
Rep	2	6655.9576	3327.9788	0.70	0.4968
Family	35	624000.6672	17828.5905	3.77	<.0001
Rep*Family	70	252576.5442	3608.2363	0.76	0.8886
Error	112	529441.731	4727.158		
Corrected Total	219	1409839.861			
Mean Specific Surface (SS)					
Rep	2	446.40954	223.20477	0.72	0.4907
Family	35	34952.75943	998.65027	3.21	<.0001
Rep*Family	70	19159.38082	273.70544	0.88	0.7189
Error	112	34893.93856	311.55302		
Corrected Total	219	89350.05513			
Mean Perimeter (PM)					
Rep	2	55.96557	27.98278	0.39	0.6752
Family	35	15672.29999	447.78000	6.31	<.0001
Rep*Family	70	3396.93943	48.52771	0.68	0.9568
Error	112	7951.54144	70.99591		
Corrected Total	219	27102.42115			
Mean Wall Thickness (WTS)					
Rep	2	0.06922646	0.03461323	0.68	0.5085
Family	35	5.50704408	0.15734412	3.09	<.0001
Rep*Family	70	3.18558514	0.04550836	0.89	0.6903
Error	112	5.69770541	0.05087237		
Corrected Total	219	14.42626947			

Appendix 4

Analysis of variance tables for cross-sectional tracheid traits, family analysis at Martin and Nyangui

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean radial tracheid diameter (RD)					
Site	1	5.098882	5.098882	0.97	0.3261
Rep	2	11.822174	5.911087	1.13	0.3275
Family	9	1596.353760	177.372640	33.86	<.0001
Rep*Family	18	140.048864	7.780492	1.49	0.1106
Site*Family	9	38.576977	4.286331	0.82	0.6005
Error	102	534.241801	5.237665		
Corrected Total	141	2313.365601			
Mean tangential tracheid diameter (TD)					
Site	1	16.6693121	16.6693121	8.54	0.0043
Rep	2	0.7854179	0.3927090	0.20	0.8180
Family	9	201.8295758	22.4255084	11.49	<.0001
Rep*Family	18	68.2434007	3.7913000	1.94	0.0201
Site*Family	9	29.2358688	3.2484299	1.66	0.1072
Error	102	199.0447781	1.9514194		
Corrected Total	141	508.8205455			
Mean tracheid diameter (MTD)					
Site	1	0.8324158	0.8324158	0.29	0.5898
Rep	2	4.6556665	2.3278333	0.82	0.4442
Family	9	711.1310955	79.0145662	27.77	<.0001
Rep*Family	18	89.9204776	4.9955821	1.76	0.0415
Site*Family	9	30.0660555	3.3406728	1.17	0.3196
Error	102	290.242510	2.845515		
Corrected Total	141	1112.769129			
Mean lumen diameter (LD)					
Site	1	0.6757269	0.6757269	0.19	0.6666
Rep	2	8.9264671	4.4632336	1.23	0.2957
Family	9	576.8878109	64.0986457	17.71	<.0001
Rep*Family	18	89.7719593	4.9873311	1.38	0.1590
Site*Family	9	42.6392324	4.7376925	1.31	0.2416
Error	102	369.244901	3.620048		
Corrected Total	141	1051.924409			
Mean Cell Wall Area (CWA)					
Site	1	29279.5688	29279.5688	11.58	0.0010
Rep	2	369.9821	184.9911	0.07	0.9295
Family	9	164061.8302	18229.0922	7.21	<.0001
Rep*Family	18	58496.9290	3249.8294	1.29	0.2132
Site*Family	9	11366.4571	1262.9397	0.50	0.8717
Error	102	257873.2802	2528.1694		
Corrected Total	141	531224.7570			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean Cell Wall Thickness (CWT)					
Site	1	1.62554286	1.62554286	6.00	0.0160
Rep	2	0.24903024	0.12451512	0.46	0.6326
Family	9	2.76885742	0.30765082	1.14	0.3445
Rep*Family	18	5.25155049	0.29175280	1.08	0.3852
Site*Family	9	1.03779117	0.11531013	0.43	0.9185
Error	102	27.61575815	0.27074273		
Corrected Total	141	38.50587425			
Mean tracheid area (TArea)					
Site	1	91675.95	91675.95	0.49	0.4870
Rep	2	351521.46	175760.73	0.93	0.3966
Family	9	46957419.75	5217491.08	27.70	<.0001
Rep*Family	18	6014520.81	334140.05	1.77	0.0387
Site*Family	9	2027666.73	225296.30	1.20	0.3056
Error	102	19211780.45	188350.79		
Corrected Total	141	73614281.12			
Mean number of tracheids (NoTrach)					
Site	1	32.11755	32.11755	0.13	0.7206
Rep	2	307.84208	153.92104	0.62	0.5420
Family	9	54679.82402	6075.53600	24.32	<.0001
Rep*Family	18	7271.99695	403.99983	1.62	0.0693
Site*Family	9	2748.17364	305.35263	1.22	0.2896
Error	102	25476.37244	249.76836		
Corrected Total	141	89418.31273			
Mean (PCell)					
Site	1	7.85464497	7.85464497	9.31	0.0029
Rep	2	2.15424160	1.07712080	1.28	0.2833
Family	9	47.78666443	5.30962938	6.30	<.0001
Rep*Family	18	19.81905158	1.10105842	1.31	0.2003
Site*Family	9	6.30014100	0.70001567	0.83	0.5901
Error	102	86.0298508	0.8434299		
Corrected Total	141	164.9916776			
Mean Runkel Ratio (RR)					
Site	1	0.01138555	0.01138555	3.50	0.0641
Rep	2	0.00539447	0.00269724	0.83	0.4390
Family	9	0.14488354	0.01609817	4.95	<.0001
Rep*Family	18	0.07074726	0.00393040	1.21	0.2679
Site*Family	9	0.02288647	0.00254294	0.78	0.6329
Error	102	0.33148872	0.00324989		
Corrected Total	141	0.56992284			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean Coarseness (CS)					
Site	1	1922.5084	1922.5084	0.54	0.4651
Rep	2	863.8407	431.9203	0.12	0.8864
Family	9	460243.8181	51138.2020	14.30	<.0001
Rep*Family	18	93786.3111	5210.3506	1.46	0.1220
Site*Family	9	25089.8139	2787.7571	0.78	0.6356
Error	102	364793.6340	3576.4082		
Corrected Total	141	952856.2111			
Mean Specific Surface (SS)					
Site	1	275.294911	275.294911	1.17	0.2823
Rep	2	153.930138	76.965069	0.33	0.7221
Family	9	9333.576558	1037.064062	4.40	<.0001
Rep*Family	18	5957.474142	330.970786	1.40	0.1455
Site*Family	9	1576.655798	175.183978	0.74	0.6683
Error	102	24035.84244	235.64551		
Corrected Total	141	41266.98658			
Mean Perimeter (PM)					
Site	1	13.31865	13.31865	0.29	0.5898
Rep	2	74.49066	37.24533	0.82	0.4442
Family	9	11378.09753	1264.23306	27.77	<.0001
Rep*Family	18	1438.72764	79.92931	1.76	0.0415
Site*Family	9	481.05689	53.45077	1.17	0.3196
Error	102	4643.88016	45.52824		
Corrected Total	141	17804.30606			
Mean Wall Thickness (WTS)					
Site	1	0.07145133	0.07145133	1.60	0.2086
Rep	2	0.01743933	0.00871966	0.20	0.8228
Family	9	1.48437270	0.16493030	3.70	0.0005
Rep*Family	18	1.22879722	0.06826651	1.53	0.0948
Site*Family	9	0.30096486	0.03344054	0.75	0.6629
Error	102	4.55159116	0.04462344		
Corrected Total	141	7.61935047			

Appendix 5

Analysis of variance tables for tracheid dimension traits, family analysis at Martin

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean MorFi arithmetic tracheid length (MATL)					
Rep	2	23696.0784	11848.0392	1.46	0.2370
Family	35	458483.8577	13099.5388	1.61	0.0319
Rep*Family	70	705840.5445	10083.4364	1.24	0.1530
Error	112	909955.583	8124.603		
Corrected Total	219	2115512.632			
Mean MorFi weighted tracheid length (MWTL)					
Rep	2	30351.446	15175.723	0.62	0.5419
Family	35	909742.374	25992.639	1.06	0.4034
Rep*Family	70	1947887.614	27826.966	1.13	0.2797
Error	112	2758705.750	24631.301		
Corrected Total	219	5691562.109			
Mean MorFi tracheid width (MTW)					
Rep	2	12.36315323	6.18157662	9.22	0.0002
Family	35	51.41792594	1.46908360	2.19	0.0010
Rep*Family	70	64.72339702	0.92461996	1.38	0.0643
Error	112	75.0875000	0.6704241		
Corrected Total	219	201.9370909			
Mean MorFi cell wall thickness (MCWT)					
Rep	2	0.11079193	0.05539596	0.74	0.4806
Family	35	5.75812559	0.16451787	2.19	0.0011
Rep*Family	70	5.62070484	0.08029578	1.07	0.3724
Error	111	8.33732576	0.07511104		
Corrected Total	218	20.06221186			
Mean MorFi number of tracheids per gram (NTpg)					
Rep	2	2.12971342	1.06485671	7.78	0.0007
Family	35	10.66782398	0.30479497	2.23	0.0008
Rep*Family	70	13.89279763	0.19846854	1.45	0.0397
Error	112	15.33573650	0.13692622		
Corrected Total	219	42.30924878			
Mean MorFi tracheid coarseness (MTC)					
Rep	2	0.01348441	0.00674220	13.57	<.0001
Family	35	0.05315532	0.00151872	3.06	<.0001
Rep*Family	70	0.04971780	0.00071025	1.43	0.0457
Error	112	0.05565773	0.00049694		
Corrected Total	219	0.17229099			
Mean MorFi kinked tracheids (MKT)					
Rep	2	28.7672060	14.3836030	4.61	0.0120
Family	35	163.5517203	4.6729063	1.50	0.0590
Rep*Family	70	292.7813735	4.1825910	1.34	0.0835
Error	112	349.7283057	3.1225742		
Corrected Total	219	849.9360802			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean MorFi percentage curl (MCT)					
Rep	2	0.98517987	0.49258994	1.75	0.1784
Family	35	23.89359559	0.68267416	2.43	0.0002
Rep*Family	70	19.52690409	0.27895577	0.99	0.5094
Error	112	31.51573483	0.28139049		
Corrected Total	219	76.72287793			
Mean MorFi broken tracheids (MBT)					
Rep	2	1.9484431	0.9742215	0.48	0.6182
Family	35	212.0431389	6.0583754	3.00	<.0001
Rep*Family	70	204.2815967	2.9183085	1.45	0.0405
Error	112	225.9268785	2.0172043		
Corrected Total	219	640.5831674			
Mean MorFi percentage area of fines (Mfines)					
Rep	2	2.32681515	1.16340758	15.09	<.0001
Family	35	6.19324123	0.17694975	2.30	0.0005
Rep*Family	70	7.50111827	0.10715883	1.39	0.0597
Error	112	8.63409625	0.07709015		
Corrected Total	219	24.89878764			

Appendix 6

Analysis of variance tables for tracheid dimension traits, family analysis at Martin and Nyangui

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean MorFi arithmetic tracheid length (MATL)					
Site	1	1724.2992	1724.2992	0.32	0.5712
Rep	2	7286.7149	3643.3575	0.68	0.5079
Family	9	247763.8293	27529.3144	5.15	<.0001
Rep*Family	18	108426.2524	6023.6807	1.13	0.3373
Site*Family	9	26033.5841	2892.6205	0.54	0.8410
Error	102	544846.4799	5341.6322		
Corrected Total	141	951575.1831			
Mean MorFi weighted tracheid length (MWTL)					
Site	1	279951.4170	279951.4170	18.38	<.0001
Rep	2	42529.7426	21264.8713	1.40	0.2522
Family	9	548108.4711	60900.9412	4.00	0.0002
Rep*Family	18	402514.1413	22361.8967	1.47	0.1174
Site*Family	9	119217.7271	13246.4141	0.87	0.5548
Error	102	1553530.009	15230.686		
Corrected Total	141	3046613.775			
Mean MorFi tracheid width (MTW)					
Site	1	15.55891595	15.55891595	23.18	<.0001
Rep	2	6.90895003	3.45447502	5.15	0.0074
Family	9	29.31617620	3.25735291	4.85	<.0001
Rep*Family	18	18.31846003	1.01769222	1.52	0.0995
Site*Family	9	20.84541341	2.31615705	3.45	0.0010
Error	102	68.4747903	0.6713215		
Corrected Total	141	164.1588732			
Mean MorFi cell wall thickness (MCWT)					
Site	1	0.32775111	0.32775111	5.24	0.0242
Rep	2	0.17427349	0.08713675	1.39	0.2531
Family	9	1.70653956	0.18961551	3.03	0.0030
Rep*Family	18	2.01739154	0.11207731	1.79	0.0362
Site*Family	9	0.98090451	0.10898939	1.74	0.0889
Error	102	6.38175291	0.06256620		
Corrected Total	141	11.44626437			
Mean MorFi number of tracheids per gram (NTpg)					
Site	1	2.07955681	2.07955681	11.29	0.0011
Rep	2	0.53776685	0.26888343	1.46	0.2372
Family	9	3.68704119	0.40967124	2.22	0.0262
Rep*Family	18	3.94424264	0.21912459	1.19	0.2839
Site*Family	9	1.00477024	0.11164114	0.61	0.7894
Error	102	18.79082702	0.18422379		
Corrected Total	141	30.64878718			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean MorFi tracheid coarseness (MTC)					
Site	1	0.02510241	0.02510241	9.58	0.0025
Rep	2	0.01431667	0.00715834	2.73	0.0698
Family	9	0.03573894	0.00397099	1.52	0.1522
Rep*Family	18	0.03401744	0.00188986	0.72	0.7820
Site*Family	9	0.00872693	0.00096966	0.37	0.9468
Error	102	0.26717543	0.00261937		
Corrected Total	141	0.39679645			
Mean MorFi kinked tracheids (MKT)					
Site	1	23.32578532	23.32578532	6.29	0.0137
Rep	2	11.18083315	5.59041657	1.51	0.2263
Family	9	60.90341451	6.76704606	1.82	0.0725
Rep*Family	18	85.71808821	4.76211601	1.28	0.2140
Site*Family	9	32.21104903	3.57900545	0.97	0.4732
Error	102	378.2235620	3.7080741		
Corrected Total	141	587.1197480			
Mean MorFi percentage curl (MCT)					
Site	1	0.02774340	0.02774340	0.12	0.7292
Rep	2	0.58879616	0.29439808	1.28	0.2828
Family	9	8.32212863	0.92468096	4.02	0.0002
Rep*Family	18	5.44277231	0.30237624	1.31	0.1953
Site*Family	9	2.99201477	0.33244609	1.44	0.1793
Error	102	23.47955625	0.23019173		
Corrected Total	141	41.27060287			
Mean MorFi broken tracheids (MBT)					
Site	1	2.2585340	2.2585340	1.11	0.2939
Rep	2	15.4976801	7.7488401	3.82	0.0251
Family	9	164.0970285	18.2330032	8.99	<.0001
Rep*Family	18	50.9023639	2.8279091	1.39	0.1507
Site*Family	9	45.4732374	5.0525819	2.49	0.0129
Error	102	206.9330760	2.0287556		
Corrected Total	141	525.6636846			
Mean MorFi percentage area of fines (Mfines)					
Site	1	0.45204693	0.45204693	2.62	0.1087
Rep	2	2.27235116	1.13617558	6.58	0.0020
Family	9	1.37675468	0.15297274	0.89	0.5402
Rep*Family	18	2.39615421	0.13311968	0.77	0.7283
Site*Family	9	1.20631984	0.13403554	0.78	0.6382
Error	102	17.60361823	0.17258449		
Corrected Total	141	25.22198479			

Appendix 7

Analysis of variance tables for growth traits, family analysis at Martin

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean Individual tree height at 8 years (Hgt8)					
Rep	2	11.17507510	5.58753755	3.31	0.0401
Family	35	77.91369558	2.22610559	1.32	0.1404
Rep*Family	70	97.00562000	1.38579457	0.82	0.8128
Error	112	189.0291167	1.6877600		
Corrected Total	219	376.4662232			
Mean Individual tree diameter at breast height at 8 years (DBH8)					
Rep	2	3.6476332	1.8238166	0.25	0.7812
Family	35	493.6807300	14.1051637	1.91	0.0057
Rep*Family	70	380.8151643	5.4402166	0.74	0.9145
Error	112	825.290000	7.368661		
Corrected Total	219	1707.831091			
Mean Individual tree basal area at 8 years (BA8)					
Rep	2	0.00003865	0.00001932	0.41	0.6675
Family	35	0.00334556	0.00009559	2.01	0.0032
Rep*Family	70	0.00250482	0.00003578	0.75	0.9015
Error	112	0.00533425	0.00004763		
Corrected Total	219	0.01124378			
Mean Individual tree volume at 8 years (Vol8)					
Rep	2	0.00560718	0.00280359	1.22	0.2991
Family	35	0.15481393	0.00442326	1.93	0.0053
Rep*Family	70	0.12049789	0.00172140	0.75	0.9037
Error	112	0.25735113	0.00229778		
Corrected Total	219	0.53918056			

Appendix 8

Analysis of variance tables for growth traits, family analysis at Martin and Nyangui

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Mean Individual tree height at 8 years (Hgt8)					
Site	1	79.76176605	79.76176605	57.21	<.0001
Rep	2	10.53885601	5.26942801	3.78	0.0261
Family	9	37.85259348	4.20584372	3.02	0.0031
Rep*Family	18	13.32649185	0.74036066	0.53	0.9371
Site*Family	9	15.81220537	1.75691171	1.26	0.2677
Error	102	142.1964384	1.3940827		
Corrected Total	141	299.0864993			
Mean Individual tree diameter at breast height at 8 years (DBH8)					
Site	1	0.3241243	0.3241243	0.05	0.8313
Rep	2	15.6817223	7.8408611	1.10	0.3356
Family	9	235.4086681	26.1565187	3.68	0.0005
Rep*Family	18	78.0887247	4.3382625	0.61	0.8840
Site*Family	9	57.2718638	6.3635404	0.90	0.5320
Error	102	724.617222	7.104090		
Corrected Total	141	1123.334155			
Mean Individual tree basal area at 8 years (BA8)					
Site	1	0.00000138	0.00000138	0.03	0.8649
Rep	2	0.00010282	0.00005141	1.08	0.3428
Family	9	0.00165056	0.00018340	3.86	0.0003
Rep*Family	18	0.00052509	0.00002917	0.61	0.8814
Site*Family	9	0.00040054	0.00004450	0.94	0.4970
Error	102	0.00484654	0.00004752		
Corrected Total	141	0.00761489			
Mean Individual tree volume at 8 years (Vol8)					
Site	1	0.00599352	0.00599352	2.74	0.1006
Rep	2	0.00662875	0.00331437	1.52	0.2241
Family	9	0.07719183	0.00857687	3.93	0.0003
Rep*Family	18	0.02378007	0.00132112	0.61	0.8883
Site*Family	9	0.02037153	0.00226350	1.04	0.4164
Error	102	0.22272383	0.00218357		
Corrected Total	141	0.35735617			