Genetic variation for growth, wood and fibre properties of *Pinus patula* families grown on six sites in South Africa.

by

JA Vermaak

Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Forest Science (M. Sc)

at

The University of Stellenbosch

Date: **March 2007**

DECLARATION

I, the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

SIGNATURE:…….. DATE: ……

JA Vermaak

ABSTRACT

This study evaluates the variation that exists between six sites and between more than 200 *Pinus patula* families established across the sites for various traits utilised in a tree improvement programme. The traits utilised were growth at ages five and eight years, gravimetric densitometry of a sub-sample of the top 100 families at age eight, and micro-densitometry and fibre morphological characteristics as determined by image analysis of increment cores, extracted from a sub-sample of the top 30 families. Significant differences were found between locations for growth at ages five and eight, density (both gravimetric and micro densitometry) and fibre properties. For each of the site combinations, utilising Type B- genetic correlations, the interaction between families and locations were evaluated in order to determine which locations could be grouped together in order to determine the effect various sites will have on the deployment of material. Significant differences were also found between families for the various traits investigated, which would indicate that desired trait or trait combinations can be selected for in a tree improvement programme. Heritability estimates for growth varied across sites, ranging from 0,32 to 0,57 at five years and 0,34 to 0,59 at eight years for family heritability. The individual tree estimates ranges from 0,08 to 0,27 at five years and from 0,09 to 0,26 at eight years. The standard errors associated with the heritability estimates for growth however indicate that the estimates, especially those of the individual trees should be used with caution. The heritability estimates for density and fibre morphological characteristics on the family and individual tree level are on a number of sites very high, although this is associated with large standard errors. Indications were that the traits can be combined effectively into a multi-trait selection index, since the phenotypic and genotypic correlations indicated mostly favorable or slight negative correlations between traits.

OPSOMMING

Hierdie studie evalueer die variasie tussen ses groeiplekke, en die variasie tussen meer as 200 *Pinus patula* families gevestig op hierdie groeiplekke. Die verskille word ge-evalueer in terme van verskeie eienskappe van toepassing in `n boomveredelingsprogram. Die eienskappe wat gebruik is, is volumeproduksie op vyf jaar en agt jaar, hout digtheid van die beste 100 families, gebaseer op die vyf-jaar groeimeting, en veseleienskappe van die top dertig families. Beduidende verskille is gevind tussen groeiplekke vir volumegroei op vyf jaar en agt jaar, digtheid en vesel eienskape. Met behulp van Tipe B – genetiese korrelasies is die interaksie tussen families en groeiplekke gekwantifiseer vir elk van die groeiplek kombinasies. Hierdie interaksies bepaal die noodsaaklikheid vir individuele teel programme of die saamgroepering van groeiplekke wat dan deur `n enkele program bedien kan word. Beduidende verskille is ook gevind tussen families vir alle eienskappe ge-evalueer. Dit dui daarop dat al die eienskappe in `n boomveredelingsprogram gebruik kan word, en dat die eienskappe verbeter kan word deur middel van seleksie. Oorerfbaarheidsyfers vir groei varieer met groeiplek, en waardes vir vyf jaar groei strek vanaf 0,32 tot 0,57, en vir agt jaar groei vanaf 0,34 tot 0,59 vir oorerfbaarheid op die familie vlak. Individuele boom skattings varieer vanaf 0,08 tot 0,27 en vanaf 0,09 to 0,26 vir vyf jaar en agt jaar groei metings respektiewelik. Die standaard fout wat met die oorerfbaarheidsyfers geassosieer word is egter groot, veral op die individuele boom vlak, en dus moet skattings met sorg gebruik word. Die oorerfbaarheid syfers vir die meeste van die hout en vesel eienskappe was hoog, maar ook met hoë standaard foute ge-assosieer. Na aanleiding van gunstige fenotipiese en genotipiese korrelasies is getoon dat die eienskappe in `n seleksie indeks gekombineer kan word, wat seleksies vir die volgende generasie van veredeling meer effektief maak.

ACKNOWLEDGEMENTS

I express my sincere thanks and appreciation to:

1. **Prof Gerrit van Wyk** for his guidance, comments and patience during the course of the study, even when retired.

2. **Eric Kietzka** for his assistance, advice and constructive discussions while trying to explain the results. His vision in establishing a trial series of this magnitude is inspiring.

3. **Mondi Business Paper** for allowing me to utilise the data and the time required for completing this study.

4. My family and friends for the constant encouragement and support.

5. Finally, Brenda and Alana for everything.

TABLE OF CONTENTS

Chapter 3 Assessment of growth in *Pinus patula*

Chapter 4 Assessment of wood and fibre properties in *Pinus patula*

LIST OF FIGURES

LIST OF TABLES

Chapter 5

LIST OF APPENDICES

Chapter 1
Introduction

1. *Pinus patula* in its natural habitat

Pinus patula, a closed-cone pine is native to Mexico, and occurs in a narrow but long distribution between 16^{o} N to 24^{o} N latitude. Two varieties of *P. patula* have been described, *P. patula* Schiede ex Schlect. & Cham. var. *patula*, which occurs in the Sierra Madre Oriental from Tamaulipas to north-eastern Oaxaca, and *P. patula* Schiede ex Schlect. & Cham. var. *longipedunculata* Loock ex Martinez, which overlaps with var. *patula* in north-eastern Oaxaca and then extends along the Sierra Madre de Sur to the west, as far as Guerrero. The difference between the two varieties was attributed to the fact that the cones of the var. *longipedunculata* were borne on peduncles (Dvorak *et al.*, 2000).

P. patula occurs on fertile, deep clay soils across a variety of climatic regions ranging from humid, tropical to temperate, where the mean annual precipitation ranges from 1000 to 2500mm, with additional moisture being provided in the form of heavy mists, cloud and fog. Most of the distributions occur between 2100 and 2800 metres altitude, although some populations occur in the altitudinal range from 1500 to 3100 m. Within Mexico, *P. patula* can withstand heavy frosts and dry periods up to 5 months, but is best suited to warm, humid conditions. Heights of up to 35 metres and diameters of up to 90 centimetres have been recorded from its native range (Perry, 1991).

2. *Pinus patula* as plantation species in South Africa

Pinus patula was first introduced to Southern Africa in 1907, but was initially established in the winter rainfall area at Tokai plantation near Cape Town. It reached the summer rainfall area of South Africa in 1908, which was the first of three importations which could be seen as the start of the establishment of *P. patula* in the summer rainfall area. The 1908 introduction, received from Meehan and Sons, USA was known as seed stock A1. Seed stock A1 seems to have been collected from the Tamaulipas, Queretaro or Hidalgo provenances (Burgers, 1975). A second

introduction in 1911/ 1913, known as seed stocks 307, 529 and 637 was received from Vilmorin, France. It would appear that seed stock 529 was collected around the Oaxaca provenance (Burgers, 1975), and the third introduction was received from Sociedad Forestal Mexicana, Mexico in 1926/ 1928. These were known as seed stocks 9185, 9338 and 9400. These seed lots were established in various blocks and showed potential as a commercial species, because of its superior growth, stem form and wood properties (Poynton, 1977).

The majority of breeding programmes within companies in South Africa includes a number of selections made in commercial stands of *P. patula*. The origin of the seed from which these compartments were generated was not always known, but it would probably be erroneous to assume that these would have originated from the three seed introductions listed above.

Of the total plantation area in South Africa of approximately 1,351 million hectares (Forestry South Africa, 2002) situated mainly in the summer rainfall region of the eastern seaboard and interior, fifty two percent (± 705 000 hectares) have been established to pines or softwoods, of which forty eight percent (± 330 000 hectares) have been established to *Pinus patula* (Stanger, 2003).

During 2002, a total of 16,8 million cubic metres of round wood was produced in the pulp industry (Forestry South Africa, 2002), of which approximately fifty five percent or 7,3 million cubic metres originated from softwood.

Pinus patula is a preferred species for the production of pulp by the pulpwood industry in South Africa, and in a comparative study with other pine species such as *P. ayacahuite, P. elliotii, P. greggii, P. kesiya, P. leiophylla, P. pseudostrobus, P. radiata, P. taeda* and *P. tecunumannii*, its suitability as a preferred plantation species has been proven. The suitability of *P. patula* is summarised below under a few characteristics that could be of interest (Dommisse, 1994):

Wood properties:

Characteristics such as long fibres, high fibre coarseness, and low lignin content and lowest extractives of all species evaluated were found for *P. patula*.

Specific energy requirements:

P. patula had some of the lowest energy requirements for both beating and refining, only bettered by *P. radiata* and *P. greggii* to attain certain levels of freeness.

Strength properties:

P. radiata was the only species that was superior to *P. patula* in all strength properties, while *P. greggii* had higher tear strength.

Optical properties:

Excellent opacity values achieved, because of good distribution in long fibre and fines content leading to a balance between strength and optical properties. Only the pulp produced from *P. leiophylla*, was superior in brightness.

Yield:

P. patula gave the highest yields, in conjunction with low lignin and extractives content, resulting in *P. patula* being "an excellent pine species with regards to mechanical pulping". Although *P. radiata* seems more suited for a number of criteria, it is highly susceptible to attack from *Sphaeropsis sapinea* after hail storms, which are very typical in the summer rainfall region.

The conditions considered optimum for the establishment of *Pinus patula* in South Africa have formed the subject of various studies. Work done by Esterhuyse (1985), Schönau and Grey (1987), Morris and Pallett (2000) and TPCP (2004) is summarised in Table 1.1.

TABLE 1.1: Optimum site conditions for the growing of *Pinus patula* in South Africa.

Climatic parameters.	
MAT (mean annual temperature)	< 18 ^{0}C
MAP (mean annual precipitation)	>700 mm/annum at high altitudes >950 mm/annum at lower altitudes Optimum growth at 1000 mm/annum
Altitude	> 800 m in Eastern Cape > 1100 m in KwaZulu – Natal > 1400 m in Northern Transvaal Altitude not exceed 2000m
Hail	Sensitive due to thin bark.
Snow	Least prone of commercial pines in SA, especially at older ages.
Frost	Shows reasonable resistance to frost damage.
Soil parameters.	
Effective rooting depth (ERD)	> 600 mm
Soil drainage	Best growth on well drained, dystrophic soils. Poor growth on wet soils.
Soil texture	Best growth on loamy, clayey sub soils with >35% clay.
Stones	Growth affected when stone in subsoil >20%
Insects	
	Attacked by *Sirex notilus* (woodwasp)
	Attacked by *Euproctis terminalis* (Emperor moth), especially on Mpumalanga Highveld.
Disease	
	Susceptible to *Sphaeropsis sapinea* after hail.
	Susceptible to *Fusarium circinatum*, especially in nurseries.

3. Tree Improvement

Prior to commencing with a tree improvement programme, it is essential to determine which trait, could be of interest, not only to the productivity of the species, but also for the production of softwood pulp and paper. Where an industry is solely responsible for a certain aspect of fibre production, such as the growing of trees, it is often found that only traits of interest to this aspect are considered important. Thus traits that directly affect the productivity of trees such as superior growth, stem- and crown form and disease resistance are incorporated into breeding programmes, while traits of interest to the end-user of the product is neglected. If any of these criteria has a negative correlation to any of the traits that could be of interest to the end-user, the end-user will have an inferior product. It is thus essential to evaluate the traits in terms of their effect on the end product rather than evaluating them in isolation.

Once these traits have been defined, it is recommended that the following steps should be followed, when incorporating a trait into a tree improvement programme (Zobel and Talbert, 1984):

1) Determination of the species, or geographic sources (provenances) within a species exhibiting the properties or characteristics of interest,
2) Determination of the amount, kind and causes of variation for the trait or combination of traits required, within the species,
3) Combining all the desired traits into improved individuals, such as selected families or individuals within families,
4) Mass producing improved individuals for reforestation purposes in the form of seed or vegetative propagated material,
5) Developing and maintaining a genetic base population broad enough for needs in advanced generations.

In order to incorporate a trait successfully into a tree improvement programme, Evans *et al.* (1997) recommend a few criteria that should be met prior to incorporation:

1) sampling should be non-destructive
2) sampling size should be small
3) sampling rate should be high
4) measurement rate should be high and repeatable
5) the sample properties should be representative of the whole resource
6) the properties of the resource should control the properties of the product.

The traits incorporated into a breeding programme are usually determined by an organization's goals. Growth traits, such and volume, form and disease resistance have always readily been incorporated into breeding programme, since these traits easily conforms to be criteria specified above, and are key to the production of fibre in the form of trees. The improvement of wood and fibre properties, until recently has been seen as a secondary element to be improved, after characteristics such as growth, stem form and disease resistance, due to the fact that until recently these traits have been difficult and expensive to measure.

With technological advances in methods of assessing wood and fibre properties, and realisation of the importance that these properties play in the end product, the need to incorporate wood and fibre properties into breeding programmes is becoming a point of focus.

With the use of specialized equipment, such as motorized increment corers and SilviScan® or Kajaani® fibre analysis equipment, the first four criteria are met.

A non-destructive representative sample is essential for the sampling of especially softwood trees such as pines, since in most cases the tree to be sampled is a selected tree or in a seed production area, thus can not be felled for whole tree sampling. Various studies have found adequate correlations between single point sampling for certain criteria, usually at breast height, and whole tree predictions (Einspahr *et al*., 1962; Smith, 1966; Evans *et al.,* 1997).

Although variability is essential to the success of any tree improvement programme, variable raw material, in the form of trees or fibre, is not preferred by any processor since the process can not readily be modified to incorporate the variability. It will thus necessitate the weighting of the various criteria in terms of their importance to the end product, which will enable a tree breeder to select for the traits desired from a population and improve this trait by means of a breeding programme. Since it has been shown that most growth, wood and fibre properties are moderately to highly heritable (Zobel and Talbert, 1984), rapid gains can be made by means of tree breeding to improve the fibre resource available.

4. Wood and tracheid properties important to the pulp and paper industry

Wood and tracheid properties play an integral part in determining the pulp or paper quality. To realise the role each of these properties play in the formation of the end-product, it is essential that the characteristics of the wood and tracheid properties be correlated to properties utilised during the production of paper, and properties of the final product. These correlations will then determine the suitability of the tracheids. In order to determine the suitability of tracheids, hand sheets are produced from the tracheids, and various relationships between the wood, tracheid, pulp and sheet properties are defined. These relationships not only determine the effect of various tracheid and wood properties on pulp and paper produced from those tracheids, but can also be used to determine the effect of various stand management practises on end-product to predict the pulp properties of improved planting stock. It has been found that very few characteristics can be utilised as single indicators of hand sheet properties, and these properties are usually an interactive influence of a number of properties (Kibblewhite and Uprichard, 1997).

Few characteristics acting as multiple indicators are also desired from a breeding point of view, since the higher the number of characteristics required the more difficult it is to meet all the requirements in a breeding programme.

Utilizing *P. elliottii* for the production of Kraft pulp, Barefoot *et al.*, (1964), investigated a number of correlations and ratios to determine the effect various properties have on the production of paper. Utilising multiple regression, they

indicate the best predictor i.e. the trait that accounted for the greatest amount of variation in the paper property and also indicated other characteristics that had significant correlations on hand sheet properties. A summary of their multiple regression results are presented in Table 1.2. Where available the actual correlation has been indicated.

Using Table 1.2 it can be seen that certain characteristics are frequently mentioned as "Best single predictor' or "Best fibre dimension ratio", and could therefore be considered as being correlated with the end-product. Although some of these are negatively correlated, these correlations should be considered when these characteristics are being incorporated into a breeding programme. It would appear that cell wall thickness, especially of the latewood, specific gravity and the Runkell ratio are best indicators of a number of hand sheet properties and could therefore be considered as traits in a breeding programme. The Runkell ratio is a commonly used indicator of the collapsibility of tracheids (Evans *et al.*, 1997), which refers to the ratio between double the wall thicknesses and lumen diameter. In studies utilising *P. radiata* and *P. elliottii* for Kraft pulp production, it was found that the Runkell ratio was the best fibre dimension ratio, and accounted for 80 to 85% (Barefoot *et al.*, 1964; Kibblewhite, 1982) of the variation in the hand sheet tear. It was found that where this ratio is less than 1, the collapsibility is most desirable.

TABLE 1.2: Correlation between fibre properties/ ratios and hand sheet properties (Barefoot *et al.*, 1964).

Paper property	*Best single predictor*	*Other significant predictor*	*Best fibre dimension ratio*
Beating time	Cell wall thickness (-0.88)		Runkell ratio (-0.92)
Apparent sheet density	Extracted specific gravity (-0.88)	Latewood cell wall thickness Percentage compression wood (+) Fibre length (-)	Runkell ratio (-0.74)
Burst factor	Latewood cell wall thickness (-0.87)	Fibre length (+) Summerwood percentage Summerwood cell wall thickness (-)	Runkell ratio (-0.87) Fibre length / diameter (+)
Breaking strength	Latewood cell wall thickness (-0.91)	Fibre length (+)	Runkell ratio (-0.87)
Tear ratio	Extracted specific gravity (+0.94)	Latewood cell wall thickness (+0.86) Cell lumen (+) Percentage latewood(+)	Runkell ratio (+0.76)

5. Objectives of the study

Utilizing second generation material from Mondi Business Paper's *Pinus patula* breeding programme, the main objectives of this study will be aimed at determining the following:

1) Are there significant differences, or is there adequate variation between families and individuals within families for growth, wood and fibre properties that can be utilised within a breeding programme?

2) What is the effect of different sites on the ranking of families, and should different breeding populations be developed for different regions?

3) What is the correlation between growth, wood and fibre properties, and could these be combined in a selection index?

References: Chapter 1

Barefoot, A.C., Hitchings, R.G. and Ellwood, E.L.(1964). Wood characteristics and Kraft paper properties of four selected Loblolly Pines. Effect of fibre morphology under identical cooking conditions. *Tappi* **47 (6),** 343-355.

Burgers, T.F. (1975). Mexican origins of *Pinus patula* seeds introduced in South Africa. *Forestry in South Africa* **16,** 31-43.

Dommisse, E.J. (1994). Thermomechanical pulping (TMP) and Chemithermomechanical pulping (CTMP) of South African grown pine species – a comparative study. MSc thesis. University of Stellenboch, 175 pp.

Dvorak, W.S., Hodge, G.R., Kietzka, J.E., Malan, F., Osorio, L.F. and Stanger, T. K. (2000). *Pinus patula*. In: Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU. Raleigh, NC.USA, 149-173.

Einspahr, D.W., van Buijtenen, J.P. and Thode, E.F. (1962). Wood and pulp properties as determined from Slash pine increment core and whole tree measurements. *Silvae Genetica* **11,** 68-77.

Esterhuyse, C.J. (1985). Site requirements of the most important commercial trees planted in South Africa. *South African Forestry Journal* **133,** 61-66.

Evans, R., Kibblewhite, R.P. and Stringer, S. (1997). Kraft pulp fibre property prediction from wood properties in eleven radiata pine clones. *Appita Journal* **50 (1),** 25-33.

Forestry South Africa (2003). Website: http://forestry.co.za/fsa/downloads SA Forest Industry 2002.

Kibblewhite, R.P. (1982). The qualities of radiata pine papermaking fibres. *Appita* **35 (4)**, 289 – 298.

Kibblewhite, R.P. and Uprichard, J.M. (1997). Kraft pulp qualities of eleven radiata pine clones. *Appita* **49 (4)**, 243-250.

Morris, A. and Pallett, R. (2000). Site requirements and species matching – Pines. In: South African Forestry Handbook volume 1. The Southern African Institute of Forestry, 79-84.

Perry, J.P. (1991). The pines of Mexico and Central America. Timber Press, Portland, 231pp.

Poynton, R.J. (1977). Report to the Southern African Regional Commission for the conservation and utilization of the soil (SARCCUS) on tree planting in South Africa, volume 1 – The Pines. South African Forestry Research Institute. Department of Forestry, Republic of South Africa, 576 pp.

Schönau, A.P.G. and Grey, D.C. (1987). Site requirements of exotic tree species. In: Forestry Handbook. The Southern African Institute of Forestry, 82-94.

Smith, W.J. (1966). The heritability of fibre characteristics and its application to wood quality Improvement in Forest Trees. *Silvae Genetica* **16 (2)**, 41-50.

Stanger T. K (2003). Variation and genetic control of wood properties in the juvenile core of *Pinus patula* grown in South Africa. Ph.D – Department of Forestry. North Carolina State University, 188 pp.

TPCP - Tree Protection Co-operative Programme (2004). Website: http://fabinet.up.ac.za/tpcp/pamphlets. Forestry and Agricultural Biotechnology Institute (FABI) – University of Pretoria

Zobel, B.J. and Talbert, J.T. (1984). Applied forest tree improvement. John Wiley and Sons, Inc., 505pp.

Chapter 2

The mode of variation in forest trees

1. Introduction

The existence of variation for a trait is essential in order for a trait to be utilised within a tree improvement programme. The genetic variation that exists within a population for the trait of interest should be estimated in order to evaluate the suitability of the trait for incorporation into a breeding programme. Without sufficient genetic variation the use of genetics to improve forest trees will be unsuccessful. It is therefore essential to determine the causes, nature and amount of variation present in the population of interest (Zobel and Talbert, 1984).

2. Causes and nature of variation

The phenotypic variation of any trait i.e. that which is observed, is a combination of mainly three components, namely a) the genetic variation b) the environmental variation and c) the interaction between the genetic and the environmental factors affecting the trait (Zobel and Talbert, 1984).

2.1 Genetic variation

A phenotype (P), defined as the characteristic that is observed, is as a result of a combination of its genetic constitution, called the genotype (G), and the environment (E) and a component attributed to the interaction between the genetic and environmental components (GxE). This is usually expressed as follows (Wright, 1976; Zobel and Talbert, 1984):

$$\text{Phenotype} = \text{Genotype} + \text{Environment} + \text{GxE}$$

From the above, it would thus follow that any variation seen in the phenotype would be due to variation in the factors resulting in the phenotype. This relationship could then be presented as follows (Falconer, 1989; Zobel and Talbert, 1984):

$$V_P = V_G + V_E + V_{GxE}$$

Where V_P = Phenotypic variation

V_G = Genotypic variation

V_E = Variation as a result of the environment

V_{GxE} = Variation due to genotype x environment interaction

Genotypic variation is generally divided into two components i.e. additive and non-additive variation. Additive variation is due to the cumulative effect of alleles on all gene loci influencing a trait, and is usually of most value in an improvement programme. Non-additive variation is divided into dominance variation, caused by the interaction of specific alleles at a gene locus, and epistatic variation, caused by the interaction among gene loci. In most improvement programmes, the non-additive variation is given little attention, since only the additive component of genetic variation is heritable, except where clonal programmes are combined with breeding programmes.

2.2 Environmental variation

Environmental variation is usually associated with environmental conditions prevailing on the site where the trees are grown. Some of these conditions, such as tree-to-tree competition, stocking levels etc. can be controlled by use of silvicultural practices, where others, such as rainfall, wind etc. are uncontrollable. Environmental variation is normally difficult to control because it is non-heritable.

Various parameters are used to describe the suitability of a site for tree growth (Boden, 1982; Grey, 1985; Louw, 1999; Pallett, 2000; Schönau and Schulze, 1984; Zwolinski *et al.*, 1998). The parameters most generally used to describe a site are listed below in Table 2.1.

TABLE 2.1: Parameters used to describe sites used for afforestation.

Category	Parameter
Climatic factors.	Mean annual precipitation (MAP)
	Mean annual temperature (MAT)
	Mean monthly temperature
	Mean monthly precipitation
	Minimum and maximum temperature and precipitation
Edaphic factors	Geology of parental material
	Soil type
	Effective rooting depth
	Clay percentage of top soil
	Soil chemistry
Location parameters	Altitude
	Longitude
	Latitude
	Slope position
	Slope (degrees)
	Aspect

Several of the criteria listed in Table 2.1 have been mentioned as having a possible effect on tree growth. It was however concluded that none of the individual environmental variables used, affected tree growth significantly on its own, but that growth rather occurred in reaction to a combination or interaction of factors (Kanzler, 2000).

Silviculture also plays a major role in determining the growth, wood and fibre properties of various forestry crops. A number of silvicultural treatments have been investigated and are described by Zobel and Van Buijtenen (1989) with numerous examples. Some additional, but mainly concurring findings have been summarised in Table 2.2.

TABLE 2.2: Studies investigating the environmental effect of silviculture on growth, wood and fibre properties.

Factor	Author	Species	Findings
Age	Clarke *et al.*, 2003	*Pinus patula, P. elliottii, P.taeda, P. kesiya, P. maximinoi*	Age has effect on density, pulp yield and pulp strength properties.
Stand density	Malan *et al.*, 1997	*P. patula*	Adverse espacement, such as those in CCT, has effect on wood density, but "normal" stand density has no adverse effect on wood density and pulp and paper properties.
Thinning	Cown, 1973	*P. radiata*	Thinning increases volume increment, and reduced wood density and tracheid length.
Fertilizing	Shupe *et al.*, 1996	*P. taeda*	Fertilizing increased growth rate, but lead to thinner cell walls, lower specific gravity and shorter fibres.

From the studies listed by Zobel and Van Buijtenen (1989) and those mentioned in Table 2.2, it can be concluded that the effect of these practices and parameters can most adequately be summarised by stating that "anything that changes the growth pattern of a tree affects its wood properties" (Zobel and Talbert, 1984).

2.3 Genotype x environment interaction (GxE).

Genotype x environment interaction indicates that the performance of clones, families, provenances or species differs as they are grown in different environments. It is defined as the significant change in ranking of genotypes across sites or the change in relative performance of genotypes, or a change in the relative differences between genotypes, and has been recognised in agricultural crops as well as forestry crops. The change of ranking across sites has serious implications for a breeding programme, and if significant, could result in the need to develop separate breeding

populations for different regions. Most breeding programmes aim at the development of widely adapted families or clones, but when significant genotype x environment interaction exists, it could have significant implications on the deployment of material in environments to which it is not best suited (Zobel and Talbert, 1984).

In South Africa where forestry areas cover a multitude of climatic and edaphic conditions, it is essential to be able to determine the genetic material best suited to these conditions. In order to determine the presence of GxE it is essential to establish progeny trials across a number of sites and evaluate the possibility of significant rank changes.

Various methods can be used to evaluate the presence of interaction (Falkenhagen, 1985; Kanzler, 2002):

- Ranking of genotype means in the different environments.
- Phenotypic and genetic correlations between genotypes in different environments. One such genetic correlation is the so-called Type B- genetic correlation (r_{Bg}), which measures the genetic correlation for the same trait on two different sites. It can be used to calculate correlations on various levels such as the family level (r_{Bg}) (Burdon, 1977) or provenances level (r_{Bprov}) (Hodge and Dvorak, 1999).
- Estimation of the GxE component of variance in an ANOVA and calculation of the contribution of the different genotypes, blocks and sites to the interaction sums of squares.
- Regression of the individual genotype means on the trial means taken as site index, when more than two trials exist. The regression ratio can be confined to the ratio between the over sites GxE variance of a clone or family and the mean GxE variance of all the clones or families on the same site (Robbertse, 1989).
- Stability analysis by assigning stability measures to genotypes.
- Multivariate analysis.

Methods of eliminating GxE are (Falkenhagen, 1985):

1) To stratify large growing regions into sub-regions with similar growing conditions and thus minimal interaction.
2) Select genotypes that do well across a variety of growing conditions.
3) Eliminate the unstable genotypes.
4) In the trial scenario, the trial causing the interaction can be omitted from the analysis, although this is not an option when commercial sites are concerned.

GxE does not affect the formation of breeding populations on a regional level only. From an across country evaluation of provenances of *P. patula* established in Brazil, Colombia and South Africa, moderately low (r_{Bp} = 0.40) Type-B provenance correlations for volume growth was found when comparing volume growth between provenances established in Brazil and South Africa. These correlations were even lower (r_{Bp} = 0.29 and 0.00) when comparing provenances established in Colombia with Brazil and South Africa (Dvorak *et al.*, 2000). This would indicate significant changes in the performance of provenances across countries, and lead to regionalization where populations selected for breeding differs from country to country. When looking at within country provenance performance, Dvorak *et al.*, (2000), found high Type-B provenance correlations for the countries of Colombia and Brazil (0.6 and 0.71 respectively), but these high correlations were not prevalent in South Africa (0.42). This was attributed to the variability between the sites in South Africa.

Genotype x environment interaction in South Africa has indicated that certain species are more susceptible to interaction (Falkenhagen, 1985). Later studies looking at trial series within species indicated significant interaction between areas of testing for families within species. From these studies it was concluded that regionalization was necessary within their breeding strategies, since certain families had to be deployed in specific regions (Kanzler *et al.*, 2003; Malan, 1998; Snedden and Verryn, 1999; Wright *et al.*, 1991).

The most recent, and probably most comprehensive, study on GxE for *P. patula* would be the study conducted by Kanzler (2002). Evaluating 81 provenance and / or

progeny tests over 54 sites in Southern Africa, it was concluded that moderate levels of genotype x environment interaction occurred across most sites. Theoretical gains of 1.5 – 2.7 percent were calculated, and even with regionalisation of the breeding populations, it was concluded that these gains would not make this an attractive option. It was felt that regionalisation would rather be driven by disease or product.

3. Sources of variation

Variation in forest trees is usually described in terms of various categories, levels or sources. Variation in a breeding programme is usually defined at a number of levels or sources (Zobel and Talbert, 1984):

- Species
- Geographic (provenance) variation
- Variation among sites within provenances
- Differences between families within provenances
- Differences between trees within families
- Within trees

3.1 Species

Due to large differences which occasionally occur between the natural environment of a species and the environment in which the species will be grown as an exotic, it is essential to evaluate a number of species, not only for their ability to grow within the exotic environment, but also to determine the effect this environment will have on the wood and fibre properties.

Determining the differences between species and making recommendations about species with commercial potential based on characteristics of interest has been the first step in many tree improvement programmes. In South Africa a number of species were identified as having commercial potential based on growth across a number of sites (Loock, 1947; Poynton, 1975), which would have been used as directives for improvement programmes. With improvements in methodology for the determination of wood and fibre characteristics, differences between species have

been investigated in terms of wood and fibre morphological characteristics. A summary of some studies conducted is listed in Table 2.3.

TABLE 2.3: A summary of publications indicating differences between species.

Author (country of test)	Number of species	Trait (s)	Findings
Uprichard and Grey, 1973 (New Zealand)	10	Specific gravity	Distinct differences between species for specific gravity of inner and outer wood. Species however of different age and from different sites.
De Villiers, 1974 (South Africa)	6	Density	Differences between species.
Du Plooy, 1981 (South Africa)	2	Specific gravity	Differences between species and varieties within species across sites. Material of different age, but age difference small.
Robertson, 1991 (South Africa)	3	Specific gravity	Difference between species for weighted density.
Wright and Malan, 1991 (South Africa)	3	Several	Significant differences between species for growth, wood and tracheid properties, not ring width.
Malan, 1994	4	Several	Significant differences between species for early wood density, ring structure, tracheid length. Also differences across sites within species.
Clarke *et al.*, 2003	5	Several	Differences between species mainly attributed to differences in altitude, age.

In all the studies listed in Table 2.3 the variation found between species, enabled the researchers to determine the species best suited to their needs for the traits of interest.

In this study, the study of variation will be limited to the investigation of a single species, i.e. *Pinus patula.*

3.2 Geographic (provenance) variation

Provenance variation is defined as variation caused by segments of differing environments within a species range (Zobel and Van Buijtenen, 1989). Thus although it is the same species, it exhibits different characteristics due to its adaptation to a different environment. This tends to complicate the assessment of

properties from differing provenances, since in most cases genotypes and the environment tend to play a significant role. Provenance testing is done in order to determine the genetic variability that exists between sources or origins from the natural distribution of a species, to determine the best origins available for reforestation or further breeding work (Wright, 1976).

A comprehensive summary of provenance testing of various tropical and subtropical pine species can be found in the publication by the CAMCORE Cooperative (2000) where the results from provenance testing within the co-operative is presented. The publication contains detailed descriptions of all species that the cooperative has an interest in, and the findings from testing provenances and families within provenances across the landholdings of their members. In most of the studies, growth traits such as volume increment, height growth, diameter growth, basal area increment, stem form or survival are used to illustrate differences between provenances.

Apart from the above-mentioned publication, various other studies have been conducted to determine the provenance(s) within species, best suited to required criteria and growing conditions (Table 2.4). Where stated, growth refers to height growth at early ages or volume growth at later stages where both height and diameter at breast height (dbh) were assessed. The number of families per provenances was not always the same for all provenances.

The study conducted by Stanger (2003) was the first to investigate provenance differences for tracheid length and cross-sectional properties in *P. patula*. Utilising 972 trees from 108 families across 12 provenances from one site in KwaZulu- Natal, South Africa, significant differences were detected for individual ring tracheid length, mean tracheid length at different ages and all other cross-sectional properties investigated, which included radial diameter, tangential diameter, lumen diameter, wall area and wall thickness.

TABLE 2.4: Summary of studies investigating provenance differences within species.

Author (Country of study)	Species	Number of provenances	Trait(s)	Findings
Falkenhagen, 1978 (South Africa)	*Pinus elliottii and P. taeda*	6 and 11	Growth at various ages.	No significant differences between provenances for *P. elliottii*. Significant differences detected at most trials for *P. taeda.*
Falkenhagen, 1979 (South Africa)	*P. patula*	4	Growth, stem and crown form (6 years).	Differences between provenances across sites, performance of provenances not constant across sites. Selections made from different provenances at different sites.
Eguiluz-Piedra and Zobel, 1986 (Guatemala)	*P.tecunumanii*	4	Wood properties (age unknown)	No significant differences between locations for specific gravity. Significant differences between locations for measured traits (cell wall thickness, lumen width and tracheid diameter) but not for tracheid length.
Wright, 1990 (Various sites)	*P. caribaea var. hondurensis*	11	Wood density	Significant differences between provenances for density.
Burdon and Low, 1992 (New Zealand)	*P. radiata*	7	Wood properties (8-9 years)	Significant differences between populations within sites for wood density, spiral grain variables, tracheid length.
Wright and Osorio, 1992 (Colombia)	*P. tecunumanii +P. oocarpa*	24	Growth (3 year height, 5,8 year volume), gravimetric density (8 years)	Significant differences between provenances for volume and density at 8 years, volume at 5 years and height at 3 years.
Dvorak *et al.*, 1995 (Brazil, Colombia and South Africa)	*P. patula*	13	Height and diameter growth (3 years)	Significant differences across countries for best provenance, and provenances within countries.
Nyoka and Barnes, 1995 (Zimbabwe)	*P. oocarpa* and *P. patula* subsp. *tecunumanii*	6, 2	Height, dbh, stem form. (2,5, 8 years)	Significant differences between provenances for all traits at all ages.

Table 2.4: Continue

Dvorak *et al.*, 1996 (Brazil, Colombia and South Africa)	*P. greggii*	3 Southern, 6 Northern	Survival and height growth (3 years)	Significant differences between populations and provenances within populations.
Kariuki, 1998 (Kenya)	*P. patula*, *P.patula* subsp. *tecunumanii* and *P.oocarpa*	7 families, 5 provenances, 3 provenances	Height, dbh (8 years)	Significant differences in some traits for *P.oocarpa*. Differences non-significant for *P. patula*.
Moura and Dvorak, 1998 (Brazil)	*P. tecunumanii*	13	Growth, survival, stem form, branch diameter, forking, broken tops (12 years).	Significant differences between sources and provenances within sources for traits assessed.
Mugasha *et al.*, 1998 (Tanzania)	*P. oocarpa*	16	Survival, growth, yield, stem form and basic density (22 years).	Significant differences between provenances for all traits except survival and stem form.
Munthali and Stewart, 1998 (Malawi)	*P. tecunumanii*	5	Growth (9 years)	Significant differences for dbh between provenances.
Burdon *et al.*, 1999 (New Zealand)	*P. radiata*	4	Wood density (31-32 years)	Significant provenance differences detected.
Hodge and Dvorak, 1999 (Brazil, Colombia, South Africa and Venezuela)	*P. tecunumanii*	40 (24 high and 16 low elevation)	Growth, stem form and breakage, forking (3,5,8 years)	Significant differences between provenances within elevation groups and across trial sites for growth and top breakage. Provenance effects not great, but still offers potential.
Gapare *et al.*, 2001 (Brazil, Colombia and South Africa)	*P. maximinoi*	22	Growth (3, 5, 8 years)	Some significant provenance x site interaction (30%) at ages 3 and 5. Non – significant for sites within countries.
Stanger, 2003 (South Africa)	*P. patula*	12	Wood and fibre properties (11 years)	Provenance differences significant between varieties for most wood properties especially wood density and cross sectional traits of tracheids.

Significant gains can be made by selecting the best suited provenance(s) for the criteria of interest. As an example the differences in the performance of provenances between countries can be seen from Table 2.5 (Dvorak *et al.*, 2000).

TABLE 2.5: The performance (Percentage gain above controls) for volume growth of nineteen provenances of *Pinus patula* established in Colombia and South Africa (Dvorak *et al.*, 2000).

Provenance	Colombia	South Africa
Potrero de Monroy	-3.2	14.8
Ingenio del Rosario	-6.7	-0.2
Corralitla	24.2	8.6
El Manzanal	19.3	-10.9
El Tlacuache	15.9	-12.2
Ixtlàn	11.5	-8.9
Santa Marìa Pàpalo	19.2	-4.9
Conrado Castillo	-51.5	-7.6
Cuajimoloyas	0.4	-9.1
Tlacotla	-23.3	-13.2
Pinal de Amoles	-10.5	6.0
Zacualtipàn	-2.5	5.4
Llano de las Carmonas	-16.7	-1.3
El Cielo	6.3	6.2
La Encarnaciòn	2.5	9.3
La Cruz	5.2	6.6
Cumbre de Muridores	4.0	9.9
Cruz Blanca/ Manz	8.0	1.2
Calcahualco	-1.8	0.9

By selecting the best provenance for South Africa (Potrero de Monroy) as opposed to the worst provenance (Tlacotla), a volume gain of 28 percent on a provenance level can be made. Table 2.5 seems to confirm the statement made by Zobel and Van Buijtenen (1989) that “in order to determine the effect of the environment on a tree’s wood and fibre properties, it is essential to grow that tree within that specific environment”.

3.3 Provenances / species within and between sites

The large differences seen in Table 2.5 are not always due to genetics, but are usually the effect of varied environmental conditions under which the provenances are tested, resulting in conflicting results from studies conducted to investigate the performance of provenances or families across sites (Zobel and Van Buijtenen, 1989; Cown and Ball, 2001).

Differences due to environmental factors have been attributed to factors such as low temperatures (Dvorak *et al.,* 1995*;* Falkenhagen, 1979*),* rainfall and temperature (Barnes *et al.,* 1994) or the interaction between these terms (Wright, 1990) which played a significant role in the growth performance of a provenance across sites within a country.

Some studies have however indicated that different provenances not necessarily imply variation. Gapare *et al.,* (2001), studying *P. maximinoi,* indicated no significant differences for growth, and Robertson (1991), evaluating the average weighted density values of *P. tecunumanii* indicated no significant differences for provenances established across a number of sites

Investigating the within provenance variation within *P. radiata,* Cown and Kibblewhite (1980) found highly significant differences in density within and between geographic regions in New Zealand. They also found the same trend for tracheid length, with differences of up to 0.75 mm. The trend was however not as clear as that for density, and they found major site-to-site variation. The results from this study enabled them to optimise the utilisation of material from certain regions for certain products.

3.4 Differences between families within provenances

Most studies involving the use of material from different provenances have indicated significant differences between families within provenances. A summary of some publications is given in Table 2.6.

As with the performance of provenances established across a number of sites, the same can be found for families within provenances or species established across a number of sites. Significant differences were found for families tested across a number of sites.

TABLE 2.6: A summary of publications indicating differences between families.

Author	**Species**	**Number of families**	**Trait(s)**	**Findings**
Bannister and Vine, 1981	*P. radiata*	26	Wood density	Highly significant differences between families.
Wright and Malan, 1991	*P. patula, P. maximonoi and P. pseudostrobus*	4 / 10	Various wood and tracheid properties	Highly significant differences among trees within species for most traits investigated.
Barnes *et al.*, 1992	*P. patula*	Various families from different mating designs.	Various growth and quality traits.	Significant differences between families for certain traits.
Dvorak *et al.*, 1995	*P. patula*	282 within 13 provenances	Growth at 3 years of age	Differences between families for growth.
Shelbourne *et al.*, 1997	*P. radiata*	25	Tracheid cross section dimensions	Highly significant differences between families for all dimensions.
Malan, 2001	*P. chiapensis*	10	Wood and saw timber properties.	Significant differences between families for density.

The majority of this study focuses on differences between families of *P. patula* for growth, wood and fibre properties.

3.5 Individual trees within families

Variation among individual trees is usually one of the greatest sources of genetic variation utilised within a breeding programme. A number of general observations can be made concerning between tree variation (Zobel and Van Buijtenen, 1989):

- "Usually between tree variability within a species or provenance within a species is large and of importance to all wood and fibre properties.

- This large variation necessitates the sampling of at least 30 trees to get a valid estimate of properties for a particular family or provenance.
- The large variation makes it difficult to assess site, environmental and silvicultural effects on wood and fibre properties.
- The amount of between tree variation differs considerably between species.
- Much of the between tree variation is genetically controlled.
- Vegetative propagation can produce similar properties within clones, but between clone differences can be large.
- The large between tree variation and strong genetic control makes breeding for wood properties possible.
- Tree to tree variability in juvenile wood is less than in mature wood".

The publications listed below (Table 2.7) make use of trees within families tested within trials to evaluate the differences between trees within specific families.

Table 2.7: Studies indicating differences between trees within specific families.

Author	**Species**	**Number of individual trees**	**Trait(s)**	**Findings**
Malan, 2001	*P. chiapensis*	5 trees from 10 families	Wood density	Differences between trees within families accounted for 69% of variation
Stanger, 2003	*P. patula*	9 trees from each of 108 families	Various wood anatomical properties	Individual tree variation was large for all wood properties.

Few articles report on the differences between individual trees within the same family, but these differences are usually reflected in individual tree heritability estimates, when the article reports on genetic variation of traits.

This study will also report on individual tree heritability estimates, which reflects differences between trees within families for growth, wood and fibre properties of *Pinus patula*.

3.6 Within trees

Within tree variation is in the majority of cases the largest source of differences in wood and fibre properties due to the fact that various factors within the tree have significant impacts on the fibres produced. Various patterns of variability exist within a tree (Zobel and Van Buijtenen, 1989):

- Within – ring differences
- Changes from the centre (pith) to the outside (bark)
- Differences due to different heights.

3.6.1 With-in ring differences - early wood vs. latewood

In most softwood such as the pines, the growth rings are divided into two easily distinguishable parts. The first, so-called early wood refers to the fibres laid down in the early part of the growing season, and the second, so-called latewood, refers to the fibres laid down in the latter part of the growing season. The tracheids associated with each of these categories display marked differences in terms of wall thickness and fibre length in that the fibres from the early wood are shorter and have thinner walls than those of the latewood. The proportion of early wood to latewood, referred to as the early wood/latewood ratio, can be used as an indication of wood specific gravity or density since there is a high correlation between the traits (Dadswell *et al.*, 1959; Zobel and Van Buijtenen, 1989). High latewood content is usually associated with high density and thus pulp yield per unit volume. The proportion of latewood to early wood could increase from 10% near the pith to 50% in the mature wood for *P. radiata* (Corson, 1999).

Most characteristics exhibit distinct differences between early wood and latewood. It has been stated that “the greatest variability in specific gravity occurs in each annual ring due to the presence of early and latewood” (Zobel and Van Buijtenen, 1989).

In his review, Dinwoodie (1961), quotes a number of authors having found significant differences between the tracheid length of spring (early) wood and summer (late) wood. The general consensus is that the length of latewood tracheids

is greater than the length of early wood tracheids. Variation across the ring is seldom linear, and the transition from summerwood to springwood in consecutive growth rings is gradual rather than abrupt.

Great differences have also been found in the majority of cases for chemical composition of certain species, although there have been instances where no significant differences for lignin concentration in early wood and latewood were found (Donaldson, 1985).

3.6.2 Changes in wood properties from the centre (pith) to the outside (bark) - juvenile vs. mature wood (the effect of age)

The greatest source of variation in softwoods could possibly be due to the presence of juvenile wood (core wood), and its relative proportion to mature wood (slab wood/ outer wood). Juvenile wood is formed by a juvenile or immature cambium as opposed to a mature cambium resulting in the formation of mature wood. Since the top of a tree consists of mainly juvenile cambium, and the juvenile wood resembles a cylinder formed around the pith, the wood near the pith and at the top is mainly juvenile. Thus juvenile wood occurs both at the top and the base of the tree, but the top is primarily juvenile, and the base has a larger proportion of mature wood (Ishengoma *et al.*, 1995) (Figure 2.1). It would thus be of benefit to differentiate and utilise the tops and bases of trees for different products and thereby utilise the variation that exists within the resource as has been done in studies by Kibblewhite (1980; 1984). The term juvenile wood is sometimes replaced by the term “core wood” in New Zealand, since it is felt that the phenomenon persists beyond the “juvenile” stage of tree growth, is experienced at all heights in the stem, and thus refers to the central core of the stem.

It would seem that the juvenile wood is characterized by rapid changes in most fibre and tracheid properties. In general, for conifers, wood fibres produced in the juvenile stage have the following characteristics:

- Fibre length: fibres are usually short, one third or half the length of fibres in mature wood. Fibre length increases rapidly from the pith outwards until

about the 10^{th} - 15^{th} ring, where after the increase was less marked (Chikamai, 1987; Cown and McConchie, 1980; Muneri and Balodis, 1998).

- Density: low near the pith, increase for 10 – 15 years and then level off (Ishengoma *et al.*, 1995; Muneri and Balodis, 1998). Utilising 52 year old trees, it was however found that the density increased from the centre outwards (Cown and McConchie, 1980). Diffuse, porous wood tends to have lowest density near the pith.
- Cellulose yields: rapid increases for first 10 years, then levels off.
- Fibril angle: large fibril angle near the pith that decreases towards bark. Angle differences in the S2 layer have been found to vary from 15^0 to 70^0 in juvenile wood, and from 0^0 to 60^0 in mature wood for *P. radiata*. It has also been shown that the angle for latewood fibres tend to be 2^0 to 7^0 higher than for early wood (Donaldson and Burdon, 1995).
- Cell walls are thinner for juvenile wood, ranging between 6.2 and 7.7 µm versus 6.8 to 9.9 µm for mature *P. radiata* kraft pulps (Kibblewhite, 1980).
- The increase in wall thickness increases is almost linear from pith to bark (Chikamai, 1987).

A comparison of the fibre characteristics found in core wood (juvenile) and outer wood (mature) is given in Figure 2.1 (Cown, 1992).

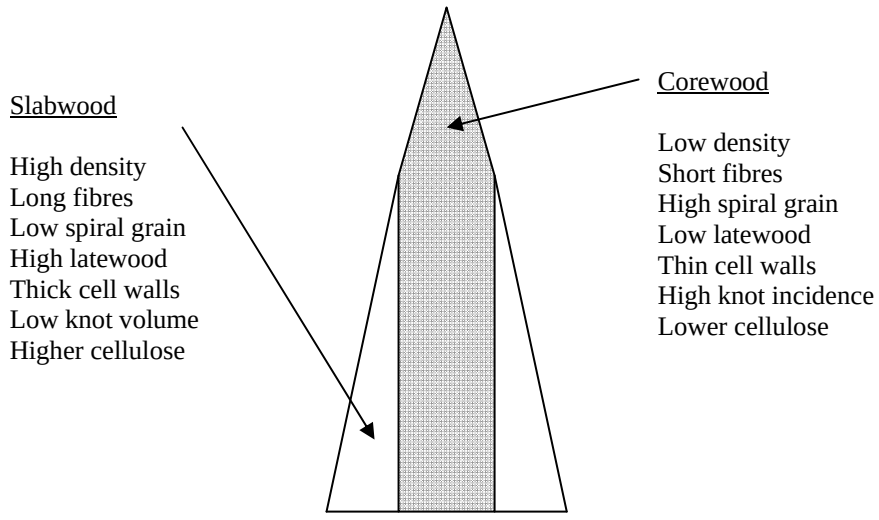

FIGURE 2.1: The location and characteristics of corewood and outerwood (slabwood) (Cown, 1992).

As mentioned above, the wood properties within the juvenile zone are characterized by rapid changes. There then seems to be a zone where the changes are reduced and levelled off, creating a so-called transition zone into the mature wood where changes are less rapid (Harris, 1981) (Figure 2.2).

The transition age associated with the transition zone is defined as the age where mature wood is first produced, and this age seems to be heritable (Hodge and Purnell, 1993; Loo *et al.*, 1985). Various studies have been aimed at defining the age or distance from the pith of transition from juvenile to mature wood, and the answer varied from study to study (Cown, 1992; Gwaze *et al*., 2001; Hodge and Purnell, 1993).

It would thus seem that the transition of juvenile to mature wood varies with the genetic constitution of the tree, the site on which it is grown, the climate and the silviculture practised on that site. It would however appear that there is a negative correlation between age of transition and growth rate (Cown, 1992).

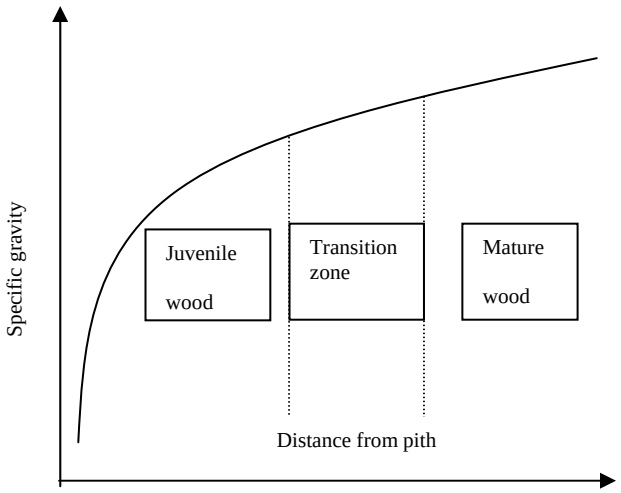

FIGURE 2.2: The change in specific gravity from pith to bark (Zobel and Van Buijtenen, 1989).

Where forestry operations are aimed at producing fast growing trees, i.e. by production of such individuals through breeding, wider espacement to reduce competition between trees, weed control and thinning at early ages, all of these would prevent the transition from juvenile to mature wood, thus result in a variable resource to be utilised by pulp and paper manufacturers.

The effect of age on wood and fibre properties is also closely related to the proportion of juvenile wood present. Thus characteristics such as density increase over age, mainly due to an increase in the proportion of mature wood relative to juvenile wood. In the study of Chikamai (1987) a drop in the density near the pith was noted, before the density steadily increased. This was attributed to the increase in tracheid diameters rather than tracheid wall thickness near the pith. Site and wood age, as a function of the position in the stem, have significant effects on the tear index of a hand sheet (Cown and Kibblewhite, 1980). It is true up to a point, where after it seems that the values for specific gravity seem to become constant or only increase slightly, irrespective of age. This is attributed to the presence of heartwood, which is associated with high concentrations of resin or other extractives, which needs to be extracted prior to the assessment of wood or fibre properties. For tracheid length Dinwoodie (1961) concluded that the relationship between length, age and distance from pith is much more involved than anticipated, and that both factors are involved in determining the length, but that the relative significance of these factors seems to vary with distance from the pith.

The effect of juvenile wood seems to vary among wood characteristics within the same tree. It can not be assumed that all properties are transferred from juvenile to mature wood at the same time, as has been shown by Loo *et al.*, (1985). They found no correlation between the transition for specific gravity and tracheid length. This is also apparent from work done by Muneri and Balodis (1998) who found that a rapid increase in density occurred until age 14, but this increase only occurred until age 10 for tracheid length.

It is however of utmost importance to realize that juvenile wood is different wood, and not necessarily poor wood. It is excellent for use in the mechanical processes, for the production of writing paper, some tissues and newsprint. This is due to the large surface caused by fibre collapse of the thin-walled cells, close chemical bonding, printability and ability to bend of juvenile material. In the production of Kraft pulp, utilizing *P. radiata*, Kibblewhite (1982), concluded that the thin walls of core wood attributed to greater collapsibility and flexibility than slab wood. Hand sheets prepared from the core wood fibres had high density, high burst and tensile indexes, but a low tearing index. The paper from the slab wood however had low

densities, low burst and tensile indexes, but high tear index. Thus slab wood fibres would be excellent for the use in packaging materials where high tear strength is required (Uprichard, 1980).

All of the fibre characteristics associated with core wood is different to fibre characteristics from outer (mature) wood, but it has been found that in the most of the fast growing softwoods, core wood comprises the bulk of logs harvested on short rotations. From a processor's point of view, core wood affects the yield and productivity during conversion, as well as the quality of the end product (Cown, 1992).

It would be perceived that the majority of the samples utilised in this study would mainly consist of juvenile wood, or the majority of juvenile wood with a little wood from the transition zone (Figure 2.2).

3.6.3 Differences due to different heights

Differences correlated with the increase in height, is mainly associated with differences in the juvenile and mature wood proportions in the tree, since the proportion of juvenile wood increases with an increase in height (Zobel and Van Buijtenen, 1989). Due to the high proportion of juvenile wood in the tops of trees, they usually exhibit the characteristics associated with juvenile wood. Generally, in conifers, there seems to be a decrease in specific gravity and tracheid length with an increase in height (Ishengoma *et al.*, 1995; Kibblewhite, 1984; Malan, 1989; Muneri and Balodis, 1998), although some contradicting findings have been reported for fibre length (Cown and McConchie,1980; Zobel and Van Buijtenen, 1989). Significant differences have been found at different sampling heights for ring width, latewood width and latewood percentage (Malan 1989). Although Malan (1989) found a decrease in tracheid length with height, he did find an increase from the ground level to about 15% of the tree height before it decreased. This trend was also shown by Muneri and Balodis (1998).

As is often the case, some contradicting evidence to that listed above has been found for *P. greggii* in the study by Malan (1994), who found that height had no relationship with the density or bark to pith variation.

Paper made from wood of *P. radiata* sampled at the top of the tree had a higher scattering coefficient, but a lower fraction long fibres and tear index, than wood sampled from the outer part of the tree base (Corson, 1999).

The impact of variation associated with height is of the utmost importance when it comes to sampling of softwoods to determine fibre and wood properties. These methods usually have to be non-destructive, cost effective and representative of the properties of the whole tree. The most commonly used method for sampling of softwoods is sampling at breast height, by means of extracting increment cores from the tree. This is possible since it has been shown that whole tree values can be predicted from values determined from increment cores (Chikamai, 1987; Zobel and Van Buijtenen, 1989; Evans *et al.*, 1997). These correlations between properties assessed at breast height and whole tree properties have also been confirmed by measuring properties with a SilviScan–1®. Some correlations for a number of area weighted breast height properties are indicated in Table 2.8. It was however indicated that these correlations are not significant for tracheid perimeter (diameter) and wall thickness (Evans *et al.*, 1997).

It can be seen from Table 2.8 that for all the properties utilised, high correlations were found between the breast height property values and the whole tree values. Some of the standard errors associated with the correlations would however suggest that the correlations should be used with caution, since they are large.

Table 2.8: Correlations (and standard errors (SE)) for prediction of whole tree properties from area weighted breast height properties (Evans *et al.*, 1997)

Property	**r**	**SE**
Density	0,915	12,30
Coarseness	0,956	10,20
Wall thickness	0,903	0,06
Perimeter	0,958	2,18
Radial diameter	0,956	0,64
Tangential diameter	0,947	0,52
Aspect ratio	0,932	0,01

References: Chapter 2.

Bannister, M.H. and Vine, M.H. (1981). An early progeny trial in *Pinus radiata*. 4: Wood density. *New Zealand Journal of Forestry Science* **11 (3)**, 221-243.

Barnes, R.D., Mullin, L.J. and Battle, G. (1992). Genetic control of eight year traits in *Pinus patula* Schiede and Deppe. *Silvae Genetica* **41 (6)**, 318 – 326.

Barnes, R.D., Birks, J.S., Battle, G. and Mullin, L.J. (1994). The genetic control of ring width, wood density and tracheid length in the juvenile core of *Pinus patula*. *South African Forestry Journal* **169,** 15-20.

Boden, D.I. (1982). The relationship between timber density of the three major pine species in the Natal midlands and various site and tree parameters. *Wattle Research Institute – Annual Report*: 1981 – 1982, 120-126.

Burdon, R.D. (1977). Genetic correlations as a concept for studying Genotype-Environment Interaction in Forest Tree Breeding. *Silvae Genetica* **26 (5-6),** 168 – 175.

Burdon, R.D., Kibblewhite, R.P. and Riddell, M.J. (1999). Wood density and kraft fibre and pulp properties of four *Pinus radiata* provenances. *New Zealand Journal of Forestry Science* **29 (2),** 214-224.

Burdon, R.D. and Low, C.B. (1992). Genetic survey of *Pinus radiata*. 6: Wood properties: variation, heritabilities, and interrelationships with other traits. *New Zealand Journal of Forestry Science* **22 (2/3),** 228-245.

CAMCORE Cooperative (2000). Conservation and testing of tropical and subtropical forest tree species by the CAMCORE Cooperative. College of Natural Resources, NCSU. Raleigh, NC.USA, Grow Graphics, South Africa, 234 pp.

Chikamai, B.N. (1987). Variation in the wood quality of *Pinus patula* grown in Kenya. *East African Agriculture and Forestry Journal* **52 (3),** 176-183.

Clarke C.R.E., Morris, A.R., Palmer, E.R, Barnes, R.D., Baylis, W.B.H, Burley, J., Gourlay, I.D., O'Brien, E., Plumtre, R.A and Quilter, A.K. (2003). Effect of environment on wood density and pulp quality of five pine species grown in Southern Africa. *Tropical Forestry Papers* **43**, Oxford Forestry Institute, University of Oxford, 162 pp.

Corson, S.R. (1999). Tree and fibre selection for optimal TMP quality. *Appita Journal* **52(5),** 351-357.

Cown, D.J. (1973). Comparison of the effect of two thinning regimes on some wood properties of radiata pine. *New Zealand Journal of Forestry Science* **4,** 540-551.

Cown, D.J. (1992). Corewood (juvenile wood) in *Pinus radiata* – should we be concerned? *New Zealand Journal of Forestry Science* **22 (1),** 87-95.

Cown, D.J. and Ball, R.D. (2001). Wood densitometry of 10 *Pinus radiata* families at seven contrasting sites: influence of tree age, site and genotype. *New Zealand Journal of Forestry Science* **31(1),** 88-100.

Cown, D.J. and Kibblewhite, R.P. (1980). Effects of wood quality variation in New Zealand radiata pine on kraft paper properties. *New Zealand Journal of Forestry Science* **10 (3),** 521 - 532.

Cown, D.J. and McConchie, D.L. (1980). Wood property variations in an old-crop stand of radiata pine. *New Zealand Journal of Forestry Science* **10(3),** 508-520.

Cown, D.J., McKinley, R. B and Ball, R.D (2002). Wood density variation in 10 mature *Pinus radiata* clones. *New Zealand Journal of Forestry Science* **32 (1)**, 48-69.

Dadswell, H.E., Watson, A.J. and Nicholls, J.W.P. (1959). What are the wood properties required by the Paper Industry in the trees of the future? *Tappi* **42 (7),** 521-526.

De Villiers, A.M. (1974). Observations on the timber properties of certain tropical pines grown in South Africa and their improvement by tree breeding. *Forestry in South Africa* **15,** 57-64.

Dinwoodie, J. M. (1961). Tracheid and fibre length in timber – A review of literature. *Forestry* **34 (2),** 125 -144.

Donaldson, L.A. (1985). Within- and between-tree variation in lignin concentration in the tracheid cell wall of *Pinus radiata*. *New Zealand Journal of Forestry Science* **15 (3),** 361-369.

Donaldson, L.A. and Burdon, R.D. (1995). Clonal variation and repeatability of microfibril angle in *Pinus radiata*. *New Zealand Journal of Forestry Science* **25**, 164-174.

Du Plooy, A.B.J. (1981). Growth rate and pulp quality of South African grown *P. caribaea* and *P. elliottii*. *Appita* **35(3),** 229-236.

Dvorak, W.S., Donahue, J.K. and Vasquez, J.A. (1995). Early performance of CAMCORE introductions of *Pinus patula* in Brazil, Colombia and South Africa. *South African Forestry Journal* **174,** 23-33.

Dvorak, W.S., Kietzka, J.E. and Donahue, J.K. (1996). Three-year survival and growth of provenances of *Pinus greggii* in the tropics and subtropics. *Forest ecology and management* **83,** 123-131.

Dvorak, W.S., Hodge, G.R., Kietzka, J.E., Malan, F., Osorio, L.F. and Stanger, T. K. (2000). *Pinus patula*. In: Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU. Raleigh, NC.USA, 149-173.

Eguiluz-Piedra, T. and Zobel, B.J. (1986). Geographic variation in wood properties of *Pinus tecunumannii*. *Wood and Fibre Science* **18 (1),** 68-75.

Evans, R., Kibblewhite, R.P. and Stringer, S. (1997). Kraft pulp fibre property prediction from wood properties in eleven radiata pine clones. *Appita Journal* **50 (1),** 25-33.

Falconer, D.S. (1989). Introduction to Quantitative Genetics – Third edition. Longman Scientific and Technical, 340 pp.

Falkenhagen, E.R. (1978). Thirty five year results from seven *Pinus elliottii* (Engelman.) and *Pinus taeda* (L.) provenance trials in South Africa. *South African Forestry Journal* **107,** 22-33.

Falkenhagen, E.R. (1979). Early assessment of four half-sib family trials of *Pinus patula* (Schiede et Deppe in Schlechtendal et Chamisso) in South Africa. *South African Forestry Journal* **109,** 7-13.

Falkenhagen, E.R. (1985). Genotype by environment interaction in South African Pine progeny trials: implications for tree breeding. *South African Forestry Journal* – December 1985, 53-60.

Gapare, W.J., Hodge, G.R. and Dvorak, W.S. (2001). Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia and South Africa. *Forest Genetics* **8(2),** 159 – 170.

Grey, D.C. (1985). Classification of forestry land. *South African Forestry Journal* – March 1985, 54-57.

Gwaze, D.P., Bridgwater, F.E., Byram, T.D and Lowe, W.J. (2001). Genetic parameter estimates for growth and wood density in loblolly pine (*Pinus taeda* L.). *Forest genetics* **8(1),** 47-55.

Harris, J.M. (1981). Wood quality of radiata pine. *Appita* **35 (3),** 211 – 215.

Hodge, G.R. and Dvorak, W.S. (1999). Genetic parameters and provenance variation of *Pinus tecunumanii* in 78 international trials. *Forest Genetics* **6(3),** 157-180.

Hodge, G.R. and Purnell, R.C. (1993). Genetic parameter estimates for wood density, transition age and radial growth of slash pine. *Canadian Journal of Forestry Research* **23,** 1881-1891.

Ishengoma, R.C., Gillah, P.R. and Iddi, S. (1995). Basic density, tracheid length and strength properties of juvenile and mature wood of *Pinus patula* grown in Tanzania. *South African Forestry Journal* **172,** 19-23.

Kanzler, A. (2000). Genotype x environment interaction in a series of provenance/ progeny trials of *Pinus patula* in South Africa. In: Proceedings: Forest genetics for the next millennium. IUFRO Working Party 2.08.01, Durban, South Africa, October 2000, 151-157.

Kanzler, A. (2002). Genotype x environment interaction in *Pinus patula* and its implications in South Africa. Ph.D – Department of Forestry North Carolina State University, 229 pp

Kanzler, A., Hagedorn, S.F., Hodge, G.R. and Dvorak, W.S. (2003). Genotype by environment interaction for volume growth at 6 years of age in a series of five *Pinus patula* progeny trials in southern Africa. *Southern African Forestry Journal* **198,** 3-15.

Kariuki, J.G. (1998). Provenance and family-within-provenance variation in *Pinus patula, Pinus patula* subspecies *tecunumanii* and *Pinus oocarpa* planted at Turbo, Kenya. *Forest Ecology and Management* **107**, 127-133.

Kibblewhite, R.P. (1980). Radiata pine corewood and slabwood, and their relationship with pulp and handsheet properties. *New Zealand Journal of Forestry Science* **10 (3),** 533-550.

Kibblewhite, R.P. (1982). The qualities of radiata pine papermaking fibres. *Appita* **35 (4),** 289 – 298.

Kibblewhite, R.P. (1984). *Pinus radiata* wood residue qualities and some utilisation options. *New Zealand Journal of Forestry Science* **14 (3),** 382-394.

Loo, J.A., Tauer, C.G. and McNew, R.W. (1985). Genetic variation in the time of transition from Juvenile to Mature wood in Loblolly Pine (*Pinus taeda* L.). *Silvae Genetica* **34 (1),** 14-19.

Loock, E.E.M. (1947). The pines of Mexico and British Honduras. A report on a reconnaissance of Mexico and British Honduras during 1947. Bulletin **35** Department of Forestry, Union of South Africa, 238-239

Louw, J.H. (1999) A review of site-growth studies in South Africa. *Southern African Forestry Journal* **185**, 57 – 65.

Malan, F.S. (1989). Wood property variation in six *Pinus radiata* (D.Don) trees grown in the Jonkershoek State Forest. *South African Forestry Journal* **151,** 39-45.

Malan, F.S. (1994). The wood properties and quality of *Pinus pringlei* Shaw and *P. greggii* Engel. compared with that of *P. patula* and *P. elliottii* grown in South Africa. *South African Forestry Journal* **171,** 43-52.

Malan, F.S., Retief, R.J. and Male, J.R. (1997). The influence of planting espacement on the wood density and pulping properties of *Pinus patula*. *Southern African Forestry Journal* **180**, 23 – 32.

Malan, F.S. (1998). Variation in the incidence of resin-filled shakes and wood density among open-pollinated families of *Pinus elliottii* planted at four different sites. *Southern African Forestry Journal* **182**, 21-26.

Malan, F.S. (2001). The wood quality of *Pinus chiapensis* (Mart.) Andresen grown in the Mpumalanga forest region. *Southern African Forestry Journal* **192**, 51-57.

Moura, V.P.G and Dvorak, W.S. (1998). Provenance and family performance of *Pinus tecunumanii* at 12 years of age in the Cerrado region of Brazil. *Forest Genetics* **5(3)**, 137-145.

Mugasha, A.G., Mgalla, H.A., Iddi, S., Nshubemuki, L., Chamshama, S.A.O and Malimbwi, R.E. (1998). Survival, growth, yield, stem form and wood basic density of *Pinus oocarpa* provenances at Buhindi, Mwanza, Tanzania. *Silvae Genetica* **47 (2-3)**, 102-107

Munthali, C.R.Y. and Stewart, M. (1998). Growth of nine-year-old provenance and taxonomy trials of *Pinus tecunumanii* at Zomba and Chongoni, Malawi. *Southern African Forestry Journal* **181**, 13-19.

Muneri, A. and Balodis, V. (1998). Variation in wood density and tracheid length in *Pinus patula* grown in Zimbabwe. *Southern African Forestry Journal* **182,** 41-50.

Nyoka, B.I. and Barnes, R.D. (1995). Genetic parameters and productivity of *Pinus oocarpa* and *Pinus patula* ssp. *tecunumanii*. *South African Forestry Journal* **173**, 1-7.

Pallett, R. (2000). Growth and fibre yield of *Pinus patula* and *Pinus elliottii* pulpwood plantations at high altitude in Mpumalanga. *Southern African Forestry Journal* **187**, 11-17.

Poynton, R.J. (1975). Pine species of potential economic importance in South Africa. *South African Forestry Journal* **92,** 6-12.

Robbertse, P.J. (1989). The role of genotype-environment interaction in adaptability. *South African Forestry Journal* **150,** 18-19.

Robertson, P.E. (1991). The pulping characteristics of *P. tecunumanii, P. patula* and *P. taeda*. *CSIR Report* **FOR-I 83**, CSIR.

Schönau, A.P.G and Schulze, R.E (1984). Climatic and altitudinal criteria for commercial afforestation with special reference to Natal. *South African Forestry Journal* - September 1984, 10-18.

Shelbourne, T., Evans, R., Kibblewhite, P. and Low, C. (1997). Inheritance of tracheid transverse dimensions and wood density in radiata pine. *Appita Journal* **50 (1)**, 47 - 50.

Shupe, T.D., Choong, E.T., Stokke, D.D. and Gibson, M.D. (1996). Variation in cell dimensions and fibril angle for two fertilized even-aged loblolly pine plantations. *Wood and Fibre Science* **28 (2)**, 268-275.

Snedden, C.L. and Verryn, S.D. (1999). An investigation into the occurance and nature of genotype by environment interaction in *Pinus patula*. *South African Forestry Journal* **186,** 67-75.

Stanger T. K. (2003). Variation and genetic control of wood properties in the juvenile core of *Pinus patula* grown in South Africa. Ph.D – Department of Forestry. North Carolina State University, 188 pp.

Uprichard, J.M. (1980). Effects of wood age on the papermaking properties of radiata pine kraft pulps. *New Zealand Journal of Forestry Science* **10 (3),** 558-576.

Uprichard, J.M. and Grey, J.T. (1973). Papermaking properties of kraft pulps from New Zealand grown softwoods. *Appita* **27 (3)**, 185-191.

Wright, J.A. (1990). Provenance variation in wood properties of *Pinus caribaea* var. *hondurensis*. *New Zealand Journal of Forestry Science* **20 (1),** 111-121.

Wright, J.A. and Malan, F.S. (1991). Variation in wood and tracheid properties of *Pinus maximinoi, P. pseudostrobus* and *P. patula*. *IAWA Bulletin* **12,** 467-475.

Wright, J.A. and Osorio, L. F. (1992). Results of provenance and family within provenance trials of *Pinus tecunumannii* in Colombia, South America. *Forest Ecology and Management* **55,** 107-116.

Wright, J.A., Shaw, M.J.P and Hadebe, W. (1991). Genotype x environment interaction in pine hybrid families at four sites in South Africa. *Forest Ecology and Management*, **40,** 93-99.

Wright, J.W. (1976). Introduction to Forest genetics. Academic Press, 463 pp.

Zobel, B.J. and Talbert, J.T. (1984). Applied forest tree improvement. John Wiley and Sons, Inc., 505pp

Zobel, B.J. and Van Buijtenen, J.P. (1989). Wood variation: its causes and control. Springer-Verlag, Germany, 363 pp

Zwolinski, J.B., Hensley, M and Monnik, K.A. (1998) Site conditions and growth of pines at the North East Cape Forests. *South African Forestry Journal* **183**, 1-16.

Chapter 3

Assessment of growth in *Pinus patula*

1. Introduction

The use of growth parameters to express differences between the various sources of variation has readily been used in most studies listed in Chapter 2, since these assessments easily conform to the criteria specified by Evans *et al.,* (1997) (Chapter 1). This also explains why most breeding programmes, until recently, have been utilising growth, rather than wood or fibre properties, as the main trait to improve.

Growth is usually expressed as a function of height and diameter growth, which is then converted to a volumetric unit by using a volume equation (Bredenkamp and Loveday, 1984) or volume index (Hodge and Dvorak, 1999). The equations are developed for a number of species based on observations across a number of sites, based on growth and yield modelling. Height growth and survival are usually the preferred measure used to express growth, where studies are being assessed at early ages (Dvorak *et al.*, 1996), while parameters such as basal area per hectare and quality traits such as stem and crown form are also used to indicate growth differences.

In this chapter, the differences for volume growth will be evaluated. The differences will be determined in terms of the effect of site, and parameters associated with site or trial design, and differences due to different genetic entities or families. A comparison of the relative performance of families across sites will also be made in order to establish the magnitude of differences in the performance of families across sites, thus the existence of genotype x environment interaction (GxE). This will be done by means of Type B- genetic correlations (Burdon, 1977). In all instances growth will be expressed as volume increment with unit of measure being cubic metres (m^3) (Bredenkamp and Loveday, 1984).

2. Material and methods

2.1 Description of the *Pinus patula* trial series

The material used in the study comprises second generation breeding material from Mondi Business Paper's Pine programme, i.e. a second generation *P. patula* progeny trial series established across various sites. The material is the progeny of selections made in the South African landrace, and material from Zimbabwean and Malawian origin. Based on the origin of the parental material, the families were grouped into seven sets. Due to a shortage of trees not all the sets are represented at all the sites.

The trial series consists of 237 second generation open-pollinated families, but due to differences in germination some of the families are not represented in all the trials, but all trials have at least 205 families in common (including control lots). The trial series was established on seven sites, but due to a severe hailstorm, which affected the growth of one of the sites, it was decided not to use this site for the purpose of this study. Detail about the location and design of the trials is given in Table 3.1.

TABLE 3.1: The location and design of the six *Pinus patula* trials in South Africa in detail.

Site	Location (nearest town)	Number of families	Number of replications	Number of sets	Date established
Site 1	Sabie	205	5	6	November 1992
Site 2	Graskop	237	5	7	December 1992
Site 3	Lothair	233	5	7	January 1993
Site 4	Melmoth	205	4	6	January 1993
Site 5	Underberg	205	5	6	December 1992
Site 6	Ugie	205	5	6	February 1993

The most noteworthy difference between the sites is the fact that the trial at Site 4 was established with only four replications, due to a shortage of trees.

The design used is a randomised complete block where each family was established in a line plot of six trees per replication, with the position of the family within the set, and the position of the set within the replication, completely at random. Due to the number of replications, sets and families not being consistent across all sites, the

series can be deemed unbalanced. All trials were established at an espacement of 3m x 3m.

Climatic information for the trial sites is given in Table 3.2. MAP depicts the mean annual precipitation, while MAT is an indication of the mean annual temperature. This data was obtained from the Computing Centre for Water Research (CCWR) climatic model currently managed by the University of KwaZulu Natal's School of Bioresources Engineering and Environmental Hydrology in Pietermaritzburg, South Africa. This model predicts these variables using the latitude and longitude co-ordinates, and the altitude above mean sea level.

TABLE 3.2: Climatic information of the *P. patula* trial sites.

Site	Latitude	Longitude	Altitude (m)	MAP (mm)	MAT (^{o}C)
Site 1	25 ^{o} 16' S	30 ^{o} 45' E	1328	999	17,6
Site 2	24 ^{o} 53' S	30 ^{o} 49' E	1480	1450	15,7
Site 3	26 ^{o} 16' S	30 ^{o} 38' E	1680	851	15,3
Site 4	28 ^{o} 34' S	31 ^{o} 18' E	1091	985	17,1
Site 5	29 ^{o} 55' S	29 ^{o} 22' E	1700	870	13,7
Site 6	31 ^{o} 07' S	28 ^{o} 10' E	1360	742	15,0

The various methods of site preparation used, and the dominant soils present on the trial site are listed in Table 3.3. All the sites were soil surveyed on a 50m x 50m grid, and it was attempted to have a complete replication on the same soil type, in order to reduce within replication differences.

TABLE 3.3: The dominant soils and site preparation of the trial sites.

Site	Dominant soil type (family)	ERD* (m)	Site preparation
Site 1	Hutton 1100	1,5	Chopper rolled + pitted
Site 2	Hutton 1200 / Griffin 1200	0,9 –1,5	Pitted
Site 3	Hutton 1200 / Clovelly 1200	0,9 – 1,5	Ripped – ameliorated to 1.5m, and pitted
Site 4	Hutton 1100	1,5	Pitted
Site 5	Hutton 1100	1,3	Ripped – ameliorated to 1.5m and pitted
Site 6	Hutton / Griffin	1,0 – 1,5	Ripped – ameliorated to 1.5m, ridged and pitted

* ERD = effective rooting depth i.e. the soil depth to which roots will grow unrestricted without any amelioration to the soil.

2.2 Growth assessments

2.2.1 Height

All the trials were assessed during 1998, when approximately five years of age for various growth traits and repeated during 2001, then eight years of age. Heights were assessed using Suunto® hypsometers, to the nearest 0,1 m. All trees within the trial were measured, except trees that were broken, fillers (not of the same species), or clearly runts (noticeably smaller than the other trees within the trial).

2.2.2 Diameter at breast height (DBH)

Breast height diameter was assessed at 1,3 meter above ground level, using diameter tapes rather than callipers, to compensate for stem eccentricity, which could lead to incorrect measurement of diameter.

2.2.3 Volume determination

Using the assessments of height and DBH, the individual tree volume was determined, using the volume equation developed by Bredenkamp and Loveday (1984):

$$\log V : b_0 + b_1 \log (D + d) + b_2 \log H$$

Where:	log	=	common logarithm to base 10
	V	=	stem volume (m^3)
	D	=	diameter at breast height (mm)
	d	=	correction factor
	H	=	tree height (m)
And for *Pinus patula*:	b_0	=	- 8,28929
	b_1	=	2,43963
	d	=	80
	b_2	=	0,99634

2.3 Statistical procedure

Prior to the analysis of variance (ANOVA), the data was standardised using the within replication standard deviation for volume. Adjusting the volumes decreases the bias of the genotype x environment interaction variance associated with the heterogeneous variance due to scale effects (Hodge and Dvorak, 1999).

For the analysis of individual sites, individual standardised tree values were used, while in the case of the across site analysis, in order to reduce the processing time, the plot mean standardised volume was used.

2.3.1 Evaluating site differences

2.3.1.1 Individual site analysis

For an individual site analysis at ages five and eight years, using PROC GLM in SAS® (1999), an adaptation of the model used by Kanzler and Hodge (2000) was utilized:

$$y_{ijklm} = \mu_i + R_j + S_k + R*S_{ik} + F(S)_{kl} + R*F(S)_{jkl} + \varepsilon_{ijklm}$$

Where: y_{ijklm} = phenotypic value of the ijklmth tree

μ_i = overall mean

R_j = the random effect of the jth replication

S_k = the random effect of the kth set

$(R*S)_{jk}$ = the random interaction effect of the jth replication and the kth set

$(F(S))_{kl}$ = random effect of the lth family within the kth set

$(R*F(S))_{jkl}$ = the random interaction between the lth family within the kth set and the jth replication

ε_{ijklm} = random error associated with ijklmth tree

The ANOVA format for the calculation of expected means squares for a single site is presented in Table 3.4.

TABLE 3.4: Format for the calculation of expected mean squares for an individual site analysis of variance for volume growth using individual tree data.

Source of variance	df	MS	Expected mean squares
Replication	r-1	MS_1	$\sigma^2_w + n\sigma^2_p + nf\sigma^2_{rs} + nfs\sigma^2_r$
Set	s-1	MS_2	$\sigma^2_w + n\sigma^2_p + nr\sigma^2_{f(s)} + nf\sigma^2_{rs} + nrf\sigma^2_s$
Replication x set	(r-1)(s-1)	MS_3	$\sigma^2_w + n\sigma^2_p + nf\sigma^2_{rs}$
Family (set)	s(f-1)	MS_4	$\sigma^2_w + n\sigma^2_p + nr\sigma^2_{f(s)}$
Rep x fam(set)	sr(f-1)	MS_5	$\sigma^2_w + n\sigma^2_p$
Sampling error	rsf(n-1)	MS_6	σ^2_w

Where:

MS_1 = Mean square for replications

MS_2 = Mean square for sets

MS_3 = Mean square for sets x replication interaction

MS_4 = Mean square for family within set

MS_5 = Mean square for the replication x family within set interaction

MS_6 = Mean square for within plot error

r = number of replications

s = number of sets

f = number of families per set

n= mean number of trees per plot

And:

σ^2_w = Within plot variation

σ^2_p = Between plot variation

$\sigma^2_{f(s)}$ = Variation associated with the families within sets

σ^2_{rs} = Variation due to the interaction between replications and sets

σ^2_s = Between set variation

σ^2_r = Variation associated with differences between replication

In order to test the significance of some of the main effects, mean squares other than the error mean square was used. In order to test the significance of set effects, it was necessary to pool some of the mean squares. This makes use of the so-called Satterthwaite's quasi- F ratio (Steel and Torrie, 1980).

According to the quasi- F ratio the F-statistic, and associated degrees of freedom p and q, are calculated as follows:

$$F_{p,q} = \frac{(M_r + ... + M_s)}{(M_m + ... + M_y)}$$

With

$$p = \frac{\left(M_r + ... + M_s\right)^2}{\frac{M_r{}^2}{f_r} + ... + \frac{M_s{}^2}{f_s}}$$

And

$$q = \frac{\left(M_m + ... + M_v\right)^2}{\frac{M_m{}^2}{f_m} + ... + \frac{M_v{}^2}{f_v}}$$

Where each M_i represents any mean square, and f_i represents the associated degrees of freedom.

For sets:

$$F_{sets} = \frac{(MS_2 + MS_5)}{(MS_3 + MS_4)}$$

With associated degrees of freedom p and q where

$$p = \frac{\left(MS_2 + MS_5\right)^2}{\frac{MS_2{}^2}{(s-1)} + \frac{MS_5{}^2}{sr(f-1)}}$$

And

$$q = \frac{\left(MS_3 + MS_4\right)^2}{\frac{MS_3{}^2}{(r-1)(s-1)} + \frac{MS_4{}^2}{s(f-1)}}$$

For replications:

$$F_{reps} = \frac{(MS_1)}{(MS_3)}$$

For families (sets):

$$F_{fam(sets)} = \frac{(MS_4)}{(MS_5)}$$

2.3.1.2 Multiple site analysis

The purpose of doing this analysis would be mainly to determine whether differences exist for volume growth across the different locations. In order to run an across site analysis the plot mean standardised volume was calculated in order to reduce the data processing time.

An analysis of variance (ANOVA) for growth at five years and eight years was conducted utilizing PROC GLM in SAS® (1999). The model utilised by Kanzler and Hodge (2000), was adapted as follows:

$$y_{ijkl} = \mu + L_i + R(L)_{ij} + S_k + L*S_{ik} + S*R(L)_{ijk} + F(S)_{kl} + L*F(S)_{ikl} + \varepsilon_{ijkl}$$

Where: y_{ijkl} = value for the plot mean of the l^{th} family in the k^{th} set in the j^{th} replication on the i^{th} location

μ = overall mean

L_i = random effect of the i^{th} location

$(R(L))_{ij}$ = random effect of the j^{th} replication within the i^{th} location

S_k = random effect of the k^{th} set

$(L * S)_{ik}$ = random effect of the interaction of the k^{th} set with the i^{th} location

$(S* R(L))_{ijk}$ = the random effect interaction effect of the j^{th} replication within the i^{th} location and the k^{th} set

$(F(S))_{kl}$ = random effect of the l^{th} family within the k^{th} set

$(L*F(S))_{ikl}$ = the random effect of the interaction between the l^{th} family within the k^{th} set and the i^{th} location

ε_{ijkl} = random error associated with the plot from the i^{th} location, j^{th} replication, the k^{th} set and the l^{th} family

The analysis of variance is schematically presented in Table 3.5. The table indicates the calculation of the various components in order to evaluate the significance of the means. Due to unbalanced data, the degrees of freedom calculated are not in accordance with the theoretical degrees of freedom, as indicated in Table 3.5.

TABLE 3.5: The calculation of expected mean squares for across site analysis of variance for volume growth using plot means, for a balanced trial series.

Source of variation	df	MS	Expected mean squares
Location	ℓ -1	MS_1	$\sigma^2_p + r\sigma^2_{lf(s)} + f\sigma^2_{sr(l)} + rf\sigma^2_{ls} + sf\sigma^2_{r(l)} + sfr\sigma^2_l$
Replication (Location)	ℓ (r-1)	MS_2	$\sigma^2_p + f\sigma^2_{sr(l)} + sf\sigma^2_{r(l)}$
Set	s-1	MS_3	$\sigma^2_p + r\sigma^2_{lf(s)} + rs\sigma^2_{f(s)} + f\sigma^2_{sr(l)} + rf\sigma^2_{ls} + rf\ell\sigma^2_s$
Location x Set	(ℓ -1)(s-1)	MS_4	$\sigma^2_p + r\sigma^2_{lf(s)} + f\sigma^2_{sr(l)} + rf\sigma^2_{ls}$
Set x Replication (Location)	ℓ (s-1)(r-1)	MS_5	$\sigma^2_p + f\sigma^2_{sr(l)}$
Family (Set)	s(f-1)	MS_6	$\sigma^2_p + r\sigma^2_{lf(s)} + rs\sigma^2_{f(s)}$
Location x Family(Set)	s(ℓ -1)(f-1)	MS_7	$\sigma^2_p + r\sigma^2_{lf(s)}$
Residual	ℓ s(r-1)(f-1)	MS_8	σ^2_p

Where:

MS_1 = Mean square for location

MS_2 = Mean square for replications within location

MS_3 = Mean square for sets

MS_4 = Mean square for location x set interaction

MS_5 = Mean square for set x replication (location)

MS_6 = Mean square for family within set

MS_7 = Mean square for location x family within set

MS_8 = Mean square for between plots

ℓ = number of locations

r = number of replications per location

s = number of sets

f = number of families per set

And:

σ^2_l = Variation associated with differences between locations

$\sigma^2_{r(l)}$ = Variation due to differences between replications within locations

σ^2_s = Variation due to differences between sets

σ^2_{ls} = Variation due to the interaction between locations and sets

$\sigma^2_{sr(l)}$ = Variation associated with the interaction between sets and replications within locations

$\sigma^2_{f(s)}$ = Variation due to differences between families within sets

$\sigma^2_{lf(s)}$ = Variation associated with the location x families within sets interaction

σ^2_p = Between plot variation / residual

Again in order to test the significance of some of the main effects, some of the mean squares had to be pooled, making use of the Satterthwaite's quasi- F ratio (Steel and Torrie, 1980).

For location:

$$F_{locations} = \frac{(MS_1 + MS_5)}{(MS_2 + MS_4)}$$

With associated degrees of freedom *p* and *q* where

$$p = \frac{\left(MS_1 + MS_5\right)^2}{\frac{MS_1{}^2}{(\ell - 1)} + \frac{MS_5{}^2}{\ell s(r-1)}}$$

And

$$q = \frac{\left(MS_2 + MS_4\right)^2}{\frac{MS_2{}^2}{\ell(r-1)} + \frac{MS_4{}^2}{(\ell - 1)(s-1)}}$$

For sets:

$$F_{sets} = \frac{(MS_3 + MS_7)}{(MS_4 + MS_6)}$$

With associated degrees of freedom *p* and *q* where

$$p = \frac{\left(MS_3 + MS_7\right)^2}{\frac{MS_3{}^2}{(s-1)} + \frac{MS_5{}^2}{\ell s(f-1)}}$$

And

$$q = \frac{\left(MS_4 + MS_6\right)^2}{\frac{MS_4{}^2}{(\ell-1)(s-1)} + \frac{MS_6{}^2}{(s)(f-1)}}$$

For location x set interaction:

$$F_{locxsets} = \frac{(MS_4 + MS_8)}{(MS_5 + MS_7)}$$

With associated degrees of freedom *p* and *q* where

$$p = \frac{\left(MS_4 + MS_8\right)^2}{\frac{MS_4{}^2}{(\ell-1)(s-1)} + \frac{MS_8{}^2}{\ell s(r-1)(f-1)}}$$

And

$$q = \frac{\left(MS_5 + MS_7\right)^2}{\frac{MS_5{}^2}{\ell s(r-1)} + \frac{MS_7{}^2}{\ell s(f-1)}}$$

The calculation of the F-ratio for factors other than those mentioned above would not require the use of Satterthwaite's quasi F – ratio, thus were calculated as follows:

For replications (locations):

$$F_{rep(loc)} = \frac{(MS_2)}{(MS_5)}$$

For set x replication (location):

$$F_{setxrep(loc)} = \frac{(MS_5)}{(MS_8)}$$

For families within sets:

$$F_{fam(set)} = \frac{(MS_6)}{(MS_8)}$$

For location x family (set):

$$F_{locxfam(set)} = \frac{(MS_7)}{(MS_8)}$$

2.3.2 Evaluating family differences

2.3.2.1 Ranking families for growth

Ranking the families based on the average family volume was done per site using PROC RANK in SAS® (1999). The families were ranked in descending order, with tied ranks assuming the higher rank value.

2.3.2.2 Comparison of performance/ ranking of families with age

In order to evaluate the change in family performance with age a Type-A genetic correlation was used (Burdon, 1977). This type of correlation was used, since the assessments at different ages can be seen as different traits, but assessed on the same individuals.

The Type-A genetic correlation was calculated as follows (Tibbits and Hodge, 2003):

$$r_{Ag} = \sigma_{Fxy} / \left(\sigma_{Fx} * \sigma_{Fy}\right)^{1/2}$$

Where: r_{Ag} = the Type-A genetic correlation

σ_{Fxy} = the family covariance component of traits x and y

σ_{Fx} = the family variance component for trait x

σ_{Fy} = the family variance component for trait y

The covariance component was calculated using a dummy variable (x+y) for each of the trait pairs. The covariance was calculated using the following (Tibbits and Hodge, 2003):

$$Var(x+y) = Var(x) + Var(y) + 2Cov(x,y)$$

Thus:

$$Cov(x,y) = \frac{Var(x+y) - Var(x) - Var(y)}{2}$$

Where:

$Cov(x,y)$ = the covariance between variable x and y

$Var(x+y)$ = the variance for dummy variable (x+y)

$Var(x)$ = the variance for variable x

$Var(y)$ = the variance for variable y

2.3.3 Genotype x environment interaction (GxE)

The magnitude of GxE can be evaluated in various ways (Chapter 2). In this study, the so-called Type B- genetic correlation (r_{Bg}) was used, which measures the genetic correlation for the same trait on two different sites. Standardised data from individual sites were pair wise compared and Type B- genetic correlations (r_{Bg}) were calculated as follows (Tibbits and Hodge, 2003):

$$r_{Bg} = \frac{\sigma_{F1F2}}{\sqrt{\left(\sigma^2_{F1} * \sigma^2_{F2}\right)^{1/2}}}$$

Where: σ_{F1F2} = the co-variance of family means for trait1 and trait2

σ_{F1} = the single-site family variance for trait1

σ_{F2} = the single-site family variance for trait2

The standard errors associated with the genetic correlation were calculated as follows (MacDonald *et al*., 1997):

$$\sigma_{r_G} = \frac{\left(1 - r_G{}^2\right)}{\sqrt{2}} \sqrt{\frac{\sigma_{h^2_x} \sigma_{h^2_y}}{h^2{}_x h^2{}_y}}$$

Where: σ_{r_G} = Standard error of the genetic correlation

$r_G{}^2$ = The genetic correlation between traits

$\sigma_{h^2_x}$ = The standard error of the heritability estimate for trait1 (Chapter 5)

$\sigma_{h^2_y}$ = The standard error of the heritability estimate for trait2 (Chapter 5)

$h^2{}_x$ = Heritability estimate for trait1

$h^2{}_y$ = Heritability estimate for trait2

It has been proposed that a correlation of 0,67 is the point at which the GxE variance represents 50% of the total additive variance, and that correlations lower than 0,67 should be of concern (Shelbourne, 1972).

3. Results and discussion

3.1 Site differences

3.1.1 Differences within individual sites

The effect of replications on the five year assessment data varies from site to site (Appendix 1). On only one site (Site 5), are the differences highly significant, with the remainder of the sites having significant or non-significant differences. When looking at the design of the trial, it could be reasoned that when testing such a large number of families in one replication, it could be expected that changes in especially the soil type would occur. These differences are usually only detected once trials have been established and assessed. In order to resolve such differences, extensive soil sampling and analysis should be undertaken, as was the case in this series, and it would seem that in some sites, this seemed successful.

For evaluating such a large number of families, alternate designs such as single tree plots can be utilised in order to reduce the size of the individual replications. A further method of negating the effect of replication differences when analysing the data, is using techniques such as data standardising, which is used to accommodate the differences between replications.

The results for replication differences have changed for the eight year assessments from those found for the five year assessment (Appendix 1). The significant replication differences that occurred at Site 1 at the five year assessment have disappeared, but still occurred at Site 2 and Site 3. The other significant differences and interactions prevailed.

When comparing the results of the five versus the eight year assessment, it would seem that at most sites the impact of the replications at the five year assessment seem to have reduced, where highly significant differences were reduced to significant differences, and significant differences were reduced to non-significant differences. This is noticed at most sites, with the exception of Site 2, where non-significant differences at the five year assessment were changed to highly significant differences at the eight year assessment.

When looking at the analysis of variance (ANOVA) for sets for the five and eight year growth data of each trial in more detail (Appendix 1), the most noteworthy would be the non-significant differences between sets detected for most sites with the exception of Site 1. It would seem that based on the five year results that dividing the families into sets based on the origin of the parental material had little benefit, although blocking families into sets enables the researcher to remove more variation associated with environmental factors, in order to increase the sensitivity of evaluating the differences between families.

The effect of sets however seems to increase with age, since significant differences can be detected for Site 1, Site 2, Site 4 and Site 5 based on the eight year assessment, thus it would seem that blocking the families into sets could be of value at later ages.

Significant replication x family within set interaction (REPxFAM(SET)) is also not desirable, since it would imply that the performance of families across replications is not constant, thus the best families in one replication is not necessarily the best in the next replication. Again this occurred in all trials analysed, and was highly significant at both ages.

3.1.2 Across site differences

In order to investigate the growth differences between the various trials, the average tree volume per trial was determined. This was done prior to standardizing the data, to give an indication of growth, and the ranking of sites for tree growth. The average tree volume per location for the five year growth data is presented in Figure 3.1 and for the eight year growth data in Figure 3.2.

Looking at the climatic data presented in Table 1.1 of Chapter 1, and Table 3.2 of this chapter, the results are what could be expected, with the exception of the growth at Site 2. The growth conditions are in accordance to the recommendations specified in Table 1.1, but this site had a history of the presence of frost pockets and severe weed competition from the onset. The effect of this can clearly be seen in the results.

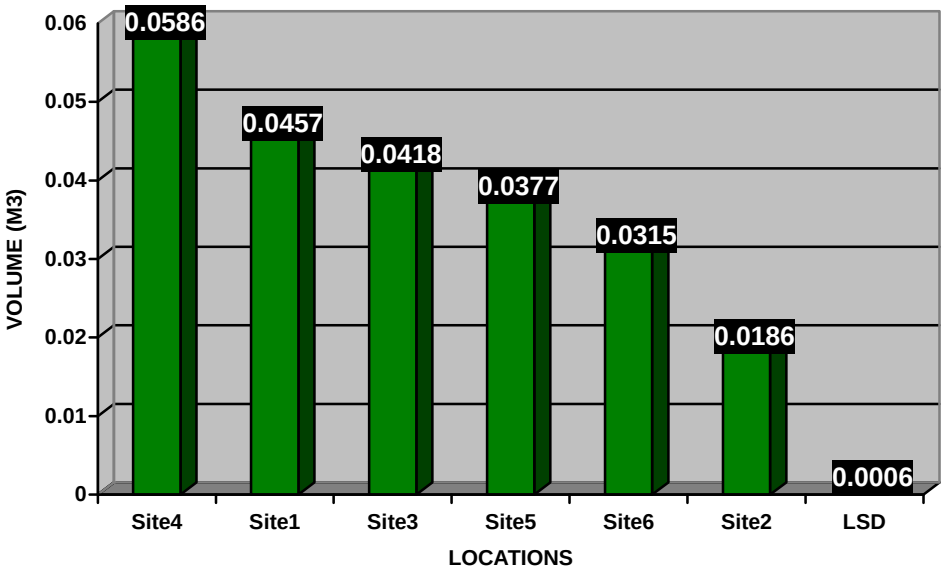

FIGURE 3.1: Average tree volume per location for *Pinus patula* at five years of age, established across six sites (non-standardized data).

From Figure 3.1 and Figure 3.2 it can be seen that there are definite differences between the sites. This is confirmed by the analysis of variance (ANOVA) presented in Table 3.6.

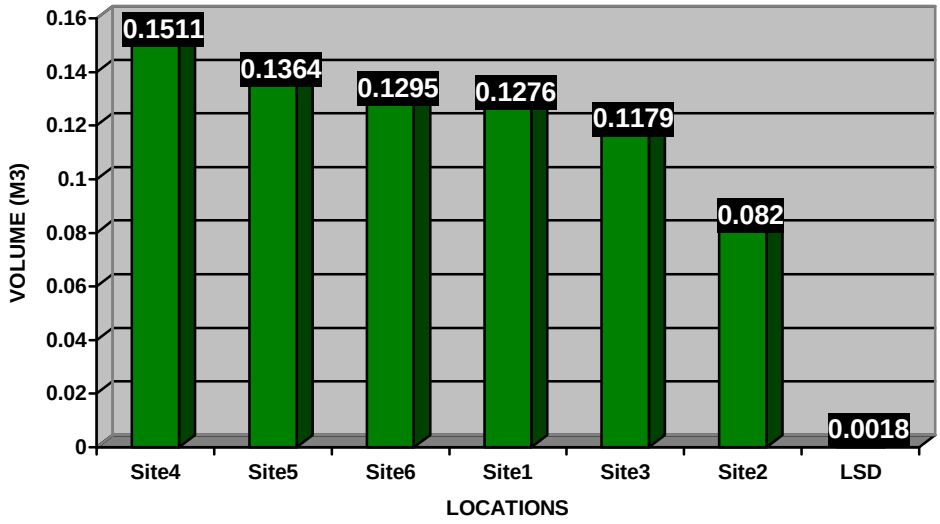

FIGURE 3.2: Average tree volume per location for *Pinus patula* at eight years of age, established across six sites (non-standardized data).

When comparing Figure 3.1 and Figure 3.2, it can be seen that the ranking of the sites have changed. While Site 4 still has the best growth, and Site 2 still shows the effect of the problems encountered during and after establishment, the improvement in growth on the more "marginal" Site 5 and Site 6 is noteworthy. Based on climatic information, it would have been expected that the good growth found on Site 1 at the earlier age should have been maintained, but the greater growth occurred on Site 5 and Site 6.

From the analysis of the five year growth data, the differences between means for all sources of variation were highly significant (Table 3.6), except for sets. For sets, the tabulated F-value (Ott, 1988) exceeds the calculated value, thus the null hypothesis, that the means are the same, is not rejected, and the differences between sets were not significant. These differences between sets did however become significant by age eight, indicating that the origin of the material could have an impact on the performance of material when evaluated across locations.

TABLE 3.6: Summarized analysis of variance (ANOVA) for five and eight year volume growth of a *Pinus patula* trial series established across six sites in South Africa with unbalanced data.

		Five year		Eight year	
Source of variation	**df**	**MS**	**F-value**	**MS**	**F-value**
Loc	5	235,21**	20,87	89,32**	15,40
Rep(Loc)	23	8,64**	32,96	3,84**	2,75
Set	6	4,82ns	1,30	8,32*	2,76
Loc*Set	26	2,72**	1,42	2,05**	1,34
Rep*Set(Loc)	123	1,78**	6,80	1,40**	5,77
Fam(Set)	217	1,22**	4,66	1,08**	4,45
Loc*Fam(Set)	921	0,32**	1,22	0,31**	1,29
Error	4339	0,26		0,24	

** - highly significant differences $\alpha < 0.001$
* - significant difference $0.05 < \alpha < 0.01$
ns – non – significant differences

Significant replication within location (REP(LOC)) differences, are not preferred in breeding trials, since it would indicate that the site is not uniform. In all the sites, with the exception of Site 2 highly significant differences occurred.

The interactions between the replications and sets (REP*SET(LOC)) being highly significant, is concerning. This would imply that the performance of sets across different replications is not constant within the same location. Thus the best sets are not consistently the best.

3.2 Family differences

3.2.1 Family differences across ages

Significant family within sets differences (FAM(SET)) is however the most sought after difference from a tree improvement point of view, since it would imply that there are differences/ variation between families, which will make the selection of the better performing families possible. This was present in all trials for both ages (Table 3.6). Descriptive statistics for all the trials for average family volume at age five are presented in Table 3.7 and for age eight presented in Table 3.8.

TABLE 3.7: Descriptive statistics for mean family volume at five years of age for six trial sites of *Pinus patula*.

Statistic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Trial mean (m^3)	0,045	0,019	0,042	0,058	0,037	0,314
Maximum (m^3)	0,064	0,028	0,062	0,084	0,049	0,041
Minimum (m^3)	0,031	0,009	0,033	0,041	0,029	0,023
Range (m^3)	0,033	0,019	0,029	0,043	0,020	0,018
Std dev (m^3)	0,006	0,003	0,005	0,007	0,003	0,003

For both ages, the greatest differences and spread of values occurred on the site with the greatest growth (Site 4). Due to the superior growth, this would then also be the site with the highest maximum and minimum values and thus the widest range of family means, and spread of values around the mean. This could possibly be attributed to the expression of differences between families being amplified on sites with high growth potential, while on "poorer" sites the expression is not as pronounced.

TABLE 3.8: Descriptive statistics for mean family volume at eight years of age for six trial sites of *Pinus patula*.

Statistic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Trial mean (m^3)	0,126	0,081	0,120	0,150	0,136	0,130
Maximum (m^3)	0,179	0,127	0,177	0,217	0,177	0,157
Minimum (m^3)	0,081	0,051	0,087	0,100	0,105	0,100
Range (m^3)	0,098	0,076	0,090	0,117	0,073	0,062
Std dev (m^3)	0,020	0,013	0,014	0,021	0,014	0,013

The relative performance of the families across sites is of interest, but with such a large number of families being tested, difficult to present, but can be obtained from the author if required.

3.2.2 Comparison of performance/ ranking of families with age

By utilising a Type-A genetic correlation to compare the ranking of families on the same site, across the various age groups, it is shown that at all the sites a degree of change in the performance of the families occurred between ages five and eight (Table 3.9). It can however be seen that the values are high, indicating good correlations, thus it would follow that the performance of families with age do change, but these changes are small, especially when considering the number of families.

When evaluating the correlation between the two ages across the sites, it can be seen that on all sites except Site 5, the performance of families at age 5 correlates well with the performance of the same families at age 8. For Site 5 the standard error would also indicate that the correlation should be used with caution since it is large. For the other sites however, it can thus be reasoned that if families were selected at an early age, such as age 5 based on the growth of the family, that those families with a few changes would still be valid at age 8.

TABLE 3.9: Type-A genetic correlation coefficient (r) and standard error of the correlation (σ_{r_G}) for mean family volume growth at five and eight years of age.

Site	r	σ_{r_G}
Site1	0,95	0,03
Site2	0,96	0,03
Site3	0,83	0,16
Site4	0,91	0,04
Site5	0,59	0,34
Site6	0,82	0,10

3.3 Family x site interaction

When evaluating the ranking of the families across sites (available from author), it is observed that there are several changes in the ranking of families across sites and that there are very few families that constantly perform well across all trials. This would indicate that, per definition, genotype x environment interaction (GxE) exists between the various sites.

When the genotype by environment interaction (GxE), is large, it could necessitate the development of different breeding populations for zones with similar genetic correlations, so-called regionalisation. It would thus follow that the magnitude of the change in ranking for performance of families across different sites should be quantified, and the possibility of grouping sites together to form separate breeding populations investigated.

In order to determine the magnitude of rank changes of families between sites, Type B- genetic correlations were used. The Type B- genetic correlations between sites for the ranking of families are presented in Table 3.10. Implementing Shelbourne's prescribed value of 0, 67 (Shelbourne, 1972), the acceptable correlations can be highlighted (Table 3.10). When a correlation is greater than 0, 67 it would indicate that the change in ranking for the performance of the families between the sites being compared, could be considered small enough to not adversely affect the deployment of the families across the two sites.

TABLE 3.10: Type B- genetic correlation (and standard error) between sites for volume growth of all families at five years.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site1		0,69 (0,06)	0,44 (0,09)	0,61 (0,06)	0,22 (0,12)	0,64 (0,06)
Site2			0,32 (0,10)	0,34 (0,09)	0,39 (0,12)	0,39 (0,09)
Site3				0,53 (0,08)	0,85 (0,04)	0,69 (0,06)
Site4					0,10 (0,11)	0,41 (0,08)
Site5						0,37 (0,11)
Site6						

As with the five year volume growth, Type B- genetic correlations were calculated for the eight year assessment between sites using the standardised volume data. These values can be seen in Table 3.11. The acceptable correlations (Shelbourne, 1972) have been highlighted.

TABLE 3.11: Type B- genetic correlation (and standard error) between sites for volume growth of all families at eight years.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site1		0,77 (0,04)	0,54 (0,08)	0,78 (0,04)	0,08 (0,12)	0,63 (0,07)
Site2			0,43 (0,09)	0,63 (0,06)	0,16 (0,13)	0,40 (0,10)
Site3				0,53 (0,08)	0,47 (0,10)	0,67 (0,07)
Site4					0,07 (0,11)	0,37 (0,09)
Site5						0,27 (0,12)
Site6						

Based on the highlighted correlations for the five year assessment (Table 3.10) and the eight year assessment (Table 3.11) it would follow that some of the sites could be grouped together based on the Type B-genetic correlations between the sites. It should however be mentioned that these groupings are by no means the only options available. By more in depth investigation of the family rankings, outliers can be eliminated, causing correlations to improve, thus resulting in more acceptable site groupings.

Since growth as expressed by volume is one of the major criteria, though not the only, to be incorporated into a breeding programme, these interactions should not be seen in isolation. It would be necessary to also evaluate the implications of the genotype x environment interaction of other criteria, such as wood and fibre

properties, that will be incorporated into a breeding programme. This will form the topic of Chapter 4.

References: Chapter 3.

Bredenkamp, B.V. and Loveday, N.C. (1984). Research Note: Volume equations for diameter measurements in millimetres. *South African Forestry Journal* **130:** 40.

Burdon, R.D. (1977). Genetic correlations as a concept for studying Genotype-Environment Interaction in Forest Tree Breeding. *Silvae Genetica* **26 (5-6),** 168 - 175.

Dvorak, W.S., Kietzka, J.E. and Donahue, J.K. (1996). Three-year survival and growth of provenances of *Pinus greggii* in the tropics and subtropics. *Forest ecology and management* **83**: 123-131.

Evans, R., Kibblewhite, R.P. and Stringer, S. (1997). Kraft pulp fibre property prediction from wood properties in eleven radiata pine clones. *Appita Journal* **50 (1):** 25-33.

Hodge, G.R. and Dvorak, W.S. (1999). Genetic parameters and provenance variation of *Pinus tecunumanii* in 78 international trials. *Forest Genetics* **6(3):** 157-180.

Kanzler, A. and Hodge, G.R. (2000). Genotype x environment interaction in a series of provenance/ progeny trials of *Pinus patula* in South Africa. In: Proceedings: Forest Genetics for the next millennium. IUFRO Working party 2.08.01 Tropical Species Breeding and genetic Resources. Durban, South Africa: 151-157.

MacDonald, A.C., Borralho, N.M.G and Potts, B.M. (1997). Genetic variation for growth and wood density in Eucalyptus globulus ssp. globulus in Tasmania (Australia). *Silvae Genetica* **46, 4**: 236-241.

Ott, L. (1988). An introduction to statistical methods and data analysis – 3rd edition. PWS–Kent Publishing Company – Boston, 835pp.

SAS 1999. Statistical Analysis System – SAS Users Guide – 1999 Edition – version 9. SAS Institute Inc., Box 8000, Cary, N.C, 27511.

Shelbourne, C.J.A (1972). Genotype – Environment Interaction: Its study and its implications in forest tree improvement. In: Proceedings of IUFRO Genetics – Sabrao joint symposia, Tokyo, Japan, 1-27.

Steel, R.D.G. and Torrie, J.H. (1980). Principles and procedures of statistics: a biometrical approach – second edition. International student edition. McGraw - Hill International Book Company: 633 pp.

Tibbits, W.N. and Hodge, G.R. (2003). Genetic parameters for cold hardiness in Eucalyptus nitens (Deane & Maiden) Maiden. *Silvae Genetica* **52, 3-4**: 89-97.

Chapter 4

Assessment of wood and fibre properties in *Pinus patula*

1. Introduction

Wood and fibre properties have been utilised as selection criteria in breeding programmes, but due to the large expenses associated with the assessment of such properties, the use has been limited. Developments in the techniques available for the measuring of these properties have however made the assessment of wood and fibre properties more readily available, and can therefore be incorporated into breeding programmes.

In chapter one (Table 1.2), it has been shown that a number of wood and fibre properties can be used to predict hand sheet properties. Since these properties impact on the properties associated with the end product, they should be of importance to a breeding programme. The properties considered to be good indicators of hand sheet properties are the following (Barefoot *et al.*, 1964):

1) Specific gravity
2) Cell wall thickness – particularly of the latewood cells.
3) Runkell ratio: the ratio between cell wall thickness and cell lumen diameter
4) Tracheid length
5) Summerwood / latewood percentage
6) Cell lumen diameter

1.1 Specific gravity

Specific gravity is a complex characteristic, and is the average result of a number of properties such as the specific gravity and micro porosity of the cell wall, the amount of early wood (springwood) and latewood (summerwood), tracheid length, cell wall thickness and level of extractives in the wood (Einspahr *et al.*, 1967).

Wood density usually refers to the mass of dry wood per unit of green volume, and is an important measure of wood quality, since it is a useful indicator of various other properties, apart from the fact that it is relatively easy to determine (Wright and Sluis-Cremer, 1992). Density is used as an indicator of the amount of wood tracheids present

and is a measure of tracheid wall thickness, since higher density usually indicates a higher proportion of thick walled tracheids. It could also indicate a higher proportion of latewood.

A distinction between specific gravity and wood density is made as follows (Zobel and Talbert, 1984):

$$Specific\ gravity = \frac{Weight\ of\ \ given\ volume\ of\ \ wood}{Weight\ of\ \ equal\ volume\ of\ \ water}$$

$$Wood\ \ density = Weight\ of\ \ wood\ \ per\ unit\ volume\ such\ as\ kg/m^3$$

Density normally increases from the pith outwards in softwoods. The increase is rapid up to a certain age or distance away from the pith, after which the density remains constant or the increase is only slight. The age or distance where this happens however seems to be a controversial point and differs between species, sites and silvicultural treatment (De Villiers, 1974; Cown and McConchie, 1980).

Pulp made from high density wood is usually characterised by high resistance to beating, high bulk, high tearing strength, low tensile and bursting strengths, and low folding endurance (Joransen, 1960). Care should however be taken that the high density is not due to the presence of high concentrations of extractives, which contributes to high density, but not pulp yield.

When evaluating studies concerning specific gravity cognisance should be taken whether the data mentioned reflects extracted or non-extracted densities. Extracted densities refer to the removal of resins by means of chemicals, usually an alcohol-benzene extraction, prior to the determination of the density. It has been shown that non-extracted densities are usually higher than those of extracted densities, and it seems in most studies extraction is done prior to specific gravity determination (Kromhout, 1966; Malan, 2001). It has however been indicated in other studies that the extracted and non-extracted densities were highly correlated, such that extraction was deemed unnecessary (Eguiluz-Piedra and Zobel, 1986; Koch and Fins, 2000).

1.2 Cell wall thickness

Cell wall thickness plays a major role in determining the papermaking characteristics of tracheids. Thicker walls tend to give:

- higher volume pulp yield
- coarse, bulky sheets
- increase in tear resistance
- rougher sheet surfaces
- decrease in burst, tensile and fold (Joransen, 1960).

Thinner walls are desirable since they collapse easier, becoming ribbon-like and therefore providing a larger surface for bonding. When comparing sheets made from thin walled tracheids and thick walled tracheids of equivalent length it was found that the sheets from thinned walled material, although inferior in tensile was superior in other properties, such as high burst strength (Dadswell *et al.*, 1959; Wright and Sluis-Cremer, 1992).

1.3 Runkell ratio

A commonly used indicator of the collapsibility of tracheids is the so-called Runkell ratio (Kibblewhite, 1980), which refers to the ratio between wall thickness and lumen diameter.

$$Runkell\ ratio = \frac{2 * (wall\ thickness)}{lumen\ diameter}$$

In studies utilising *Pinus radiata* and *P. elliottii* for kraft pulp production, it was found that the Runkell ratio was the best fibre dimensions ratio, and accounted for 80 to 85% of the variation in the hand sheet tear. It was found that where this ratio was less than 1, the collapsibility was most desirable (Barefoot *et al.*, 1964; Kibblewhite, 1982).

1.4 Tracheid length

Softwood pulps contain tracheids of various dimensions and properties, depending on the pulping process deployed. Tracheid length influences most of the pulp strength properties, and positive correlations have been found between tracheid length and tear index for *Pinus radiata* and *P. elliottii* (Wright and Sluis-Cremer, 1992).

It is however essential to also consider tracheid flexibility when determining paper strength. Where unrefined tracheids are utilised, the distribution of tracheid lengths is important during the process of washing and screening, where longer tracheids wash more easily, but can cause problems during screening. The properties associated with the pulp are interdependent on the fibre and fines component (see below). The tracheids usually form the network that gives paper its bulk and strength, while fines assist in the consolidation of this network, provide tracheid-bond reinforcement, increase opacity, and provide a capillary structure in the dry sheet (Corson, 1993).

The proportion of each of these classes is assessed by screening the suspension through a screen with a defined mesh width or slot width. These are mainly classified into one of the following categories i.e. shives, long tracheids, short tracheids or fines:

Shives are tracheid bundles, which have not been separated, and wood particles that can be defined without a microscope. Shives are usually non-defibrated, between 1 and 4 mm long and thicker than tracheids. The shive fraction should be kept as small as possible, and the majority should be removed during the screening process or be refined further in the refiner since it has very little bonding ability.

Long tracheids are important for tear strength and wet strength, except where it influences sheet formation. Longer tracheids usually lead to an increase in tear resistance, burst and tensile strength and fold, since long tracheids provide a greater area for stress distribution. The length and distribution of the tracheids are important, although these can be altered by the process of blending or refining, depending on the product required.

The high speeds at which paper machines are operating require higher wet strength and thus higher fraction long tracheids. The long tracheid fraction is determined by:

- Temperature in the grinding zone.
- Specific energy
- The grindstone used.

The higher the spray water temperature, the easier the tracheids detach, resulting in longer tracheids. This could however also increase the number of shives.

Short tracheids usually represent all tracheids not classified as long tracheids or fines and are of less importance in the manufacture of mechanical pulp.

Fines fill the cavities between the tracheids and thus contribute to the bonding capabilities of the pulp for tensile strength, and the development of a smooth surface for printing quality. Fines consist of fibrils and flour, which can only be distinguished by means of a microscope. Fibrils increase the bonding capabilities and flour the absorbency. Fines can determine the strength properties, since the paper strength increases with increased fine content, but only up to a point, where after it drops. The maximum level depends on the tracheid structure and the paper grade required.

The quality of the fines also plays a significant role in the paper properties achieved. Fines from mature wood consists primarily of fine, fibrillar elements, while the fines from top logs are much more heterogeneous with fibrillar elements, short and long fibre fragments. This could partially explain the high hand sheet densities and strengths obtained from the longer, stiffer fibres of mature wood, while the heterogeneous nature of the fines from the top logs would explain the lower hand sheet densities but higher optical properties (Kibblewhite, 1984; Corson, 1999).

There is a rapid increase in tracheid length from the pith outwards, but after a few years a constant value is reached. It would seem that this levelling off is associated with the transition from juvenile to mature wood (Dadswell *et al.*, 1959).

Mechanical pulp quality can be improved by means of refining. Refining improves the quality of the fibres in terms of their ability to comply with processing, flexibility and surface fibrillation, and an increase in the fine fraction. The danger associated with the development of the long fibre fraction, would be that this would happen at the expense of the medium and short fibres, which could lead to bulking and linting of the sheets.

1.5 Summerwood/ latewood percentage

The impact of the percentage summerwood / latewood is mainly due to the effect it has on the specific gravity. Summerwood or latewood is usually characterised by thick cell walls, which leads to an increase in the specific gravity. The latewood percentage will be determined by the seasonal period of latewood formation, which seems to be influenced by both environmental and genetic factors (Zobel and Van Buijtenen, 1989).

1.6 Cell lumen diameter

Cell lumen and wall thickness can collectively be seen as cell diameter. It would thus follow, that these two criteria would be inversely related to each other for a given cell diameter. Since tracheid diameter influences the behaviour of pulp during washing, screening and refining, and plays a significant role in sheet formation, bonding between tracheids, tracheid rigidity and mobility, it would follow that each of the individual components that comprises tracheid diameter will also influence the bonding between fibres to result in the strength properties of the sheets formed. As expressed by the Runkell ratio, it can be seen that the effect of the lumen diameter is of greater importance, and as has been stated under point 1.3 above, the greater the lumen diameter, the greater the collapsibility of the fibres, and thus the resulting strength properties. It would seem that small-diameter; thin walled tracheids, and thus relative large cell lumens are more desirable for paper formation and strength.

2. Material and methods

During 1998, Professor Tim White, University of Florida, was used as a consultant to determine the across site breeding values for the families in the trial series described in Chapter 3, based on the five year volume calculations. From the analysis done by Prof. White, utilising the Best Linear Unbiased Prediction (BLUP) method, the top 100 families based on the across site breeding values for volume growth at five years was determined. These 100 families constitute the material used by K.G. Payn in his study and for a complete description on the sampling strategy implemented the reader should refer to Payn (2001).

In this study, six trees were sampled per site from each of the top 100 families, resulting in a total of 3500 samples. Trees were sampled at 1,4 meters above ground level, extracting a 12 mm bark-to-bark increment core, using a powered increment corer. From all the sites, 600 samples were collected, with the exception of Site 4 where only five samples per family were collected, since the trial had only four replications (Chapter 3).

In retrospect, following the reasoning of Stanger (2003) and looking at the heritability figures calculated by Payn (2001), even with a relationship coefficient of 1/3, six trees per family would not be adequate to get a valid estimate of the properties for a particular family, thus in future studies it would be recommended that at least ten trees per family should be sampled. Payn (2001) calculated the across site family heritability estimate for density to be 0,38 and using the reasoning of Stanger (2003) the optimum family size to estimate heritability values for wood properties require at least eight individuals, using the relationship coefficient of 1/3.

It would seem that the method of sample treatment for the calculation of density is not consistent, and tends to differ from study to study. In one study the trees were sampled at 1,37 m and oven-dried at 103 ^{0}C for an unspecified time period (Burdon and Maddern Harris, 1972), while for another study trees were sampled at 1,3 m and increment cores oven dried for 50 hours at 85 ^{0}C prior to density calculations (Eguiluz-Piedra and Zobel, 1986). Sampling height of 1,2 m above ground was used by Stanger (2003), while Shelbourne *et al.,* (1997) and Evans *et al.,* (1997) sampled at 1,4 meters above ground level, extracting ten and twelve-millimetre thick cores respectively.

In this study, each core was divided in two, one half used for density determination and image analysis. The other half of the core will be used for fibre length determination. Unfortunately the fibre length determination is not complete, and will thus not form part of this study.

2.1 Gravimetric density

The differences between the gravimetric densities at the various sites formed the topic of Payn's thesis (Payn, 2001). Some analyses were repeated for comparison and interpretation of the results.

Using the core, the gravimetric density of the top 100 families was determined using the water displacement method (Eguiluz-Piedra and Zobel, 1986; Payn, 2001). All densities were determined using non-extracted cores, and usually density is expressed in kg/m^3, but for the purpose of this study, the gravimetric and micro densitometry data will be expressed in g/cm^3. In order to determine the dry weight, the cores were dried in an oven at 50 ^{0}C, for about 48 hours until the weight remained constant.

2.2 Micro densitometry

Further to the gravimetric densities utilised by Payn (2001), the cores from the trees sampled for the top 30 families based on the breeding values for volume production were used for micro densitometry determination. Each core was divided in two, one half used for density determination and image analysis and the other half will be macerated for fibre length determination using a Kajaani® fibrelab, which will not form part of this study.

In order to ensure uniform air-dry moisture conditions, samples from the same sites were stored in batches at room temperature for a number of weeks prior to measurement. Pith-to-bark strips were cut using a densitometer saw, producing samples with dimensions 12 mm deep and 2 mm thick along the radial plane. The strips were mounted on a moveable densitometer tray, and scanned along the radial plane at 0,5 mm intervals using a gamma ray densitometer with Fe^{55} radiation source. Approximately 35–40 measurements were made per growth ring, from which the density profile can be

produced. From these measurements, the fluctuations of density from early wood to latewood can also be determined.

On a number of the cores, the first two to three rings were not available for micro densitometry, due to the fact that the pith was missed during the core extraction process. Situations where the pith is missing, has also occurred in other studies, which was rectified by eliminating incomplete rings from the datasets. In a study using micro densitometry, Shelbourne *et al.*, (1997) confined their study to the outermost rings (rings 8 to 13) of the cores.

2.2.1 Division into growth rings and into early and latewood

The ring width was determined as the distance between the minimum densities that defines the beginning and end of a growth ring, similar to the method used by Stanger (2003) (Figure 4.1).

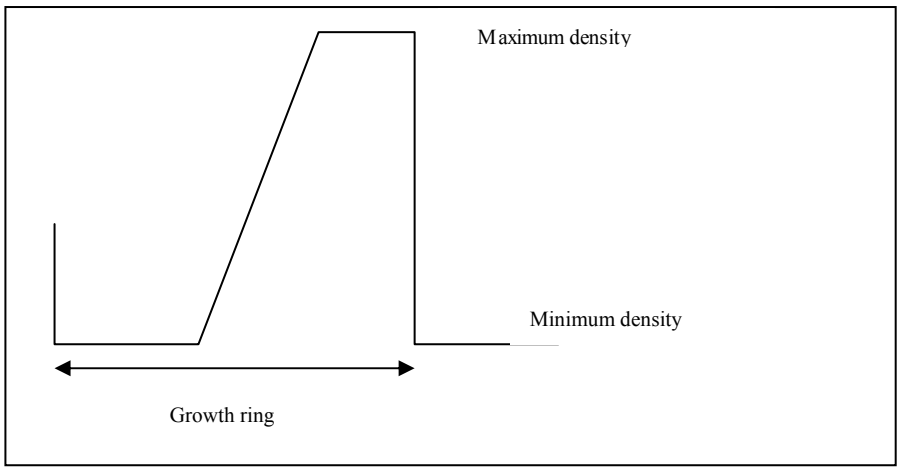

FIGURE 4.1: The determination of growth ring width (Stanger, 2003).

In addition to the micro densitometry data, using customized software developed by the Council for Scientific and Industrial Research (CSIR), various within-ring variables were also determined. These variables are corresponding to those mentioned by Cown and Clement (1983), and include variables such as ring width, early wood and latewood width, densities and percentages that were generated. This was used to verify and, if need be, adjust the ring width allocation. This also enabled the calculations of the various ring components. This data is however only available for a number of rings, mainly rings four to eight.

Another technique that has been used for the partitioning of densitometry data into early wood and latewood is the definition of a density value that determines the separation of measurements into early- and latewood. This value however seems to differ according to study and author, which could make this technique a bit controversial. A value of 0,48 g/cm^3 was used by Hodge and Purnell (1993), Cown *et al*., (2002) set the boundary at 0,500 g/cm^3, while Stanger (2003) defined this value as 0,460 g/cm^3. Payn (2001) determined the transition between early and latewood by calculating the midpoint of the minimum and maximum density values for each growth ring.

In this study, once the data for the density and fibre morphology was aligned (Point 2.3.2), the data generated by the CSIR was used to divide the growth ring into early and latewood rings, which in turn was used to determine the early and latewood proportions. As mentioned, this was however only available for rings four to eight of the cores, and the study was limited to these rings only.

2.2.2 Weighting of densitometry and image analysis data

The micro densitometry data and fibre characteristics were weighted using the area represented by the distance from the pith. Area weighting considers the position of measurement within the radius and thus more accurately represents the value to be expected from a whole disc. This implies that density further away from the pith has a greater influence on the density since it represents a greater area. This method assumes that the stem of a tree is circular, which is not always correct, and the error associated with this assumption is difficult to determine (Stanger, 2003).

If the core could be presented as a disk taken at the same height, the area of the disk would be calculated as:

Area of disk = Πr^2

Where: r would represent the length of the core taken from the disk from pith to bark.

In order to calculate the area represented by an individual sample point along the core, the area would be calculated as the difference between the area of two consecutive sample points, which in the case of densitometry is 0,5 mm apart, and in the case of fibre morphology is 1,0 mm apart. By expressing these areas as a portion of the area

represented by the whole core, the observed value was weighted. Thus for fibre morphology measurements this can be expressed as (Stanger, 2003):

$$\overline{p} = \frac{\sum_{i}^{n}(2x_i - 1)p}{\sum_{i}^{n}(2x_i - 1)}$$

Where: p would represent any of the tracheid morphological characteristic measured at every 1 mm using image analysis. In the case of densitometry the difference would be 0, 5 mm

And x_i would represent the distance from the bark, thus working from bark to pith.

2.3 Fibre morphology

2.3.1 Image analysis

Image analysis was done by the CSIR, Durban to provide information on the fibre diameter, lumen diameter and cell wall thickness, utilizing the samples that were used for the micro densitometry. After the densitometry was completed, the samples were softened, by soaking in water. A sliding microtome was used to expose a clean transverse cut on the transverse plane of the strip. This gives a smooth surface that allows for clearer image analysis. Up to 12 strips were mounted in a holder (jig) and placed upon the motorized stage utilizing a Leica® DMLB light microscope. The air-dried surfaces were examined from bark to pith using UV fluorescent illumination.

Measurement of anatomical features was performed automatically every 2 mm, controlled by macros designed by Dr Anton Zbonak, CSIR, Forests and Forest Products, Durban, within the image analysis software.

The properties of tracheids were measured using 10x objective magnification after appropriate calibration of the software to the magnification. For each measured frame area (1mm x 0,8mm) averaged values and standard deviation for each of the features

measured were automatically inserted and compiled in an electronic spreadsheet (Microsoft Excel®) for subsequent analysis.

In order to avoid erroneous measurements i.e. to exclude the measurements of unwanted objects such as resin canals and pith rays (middle lamella spaces), the operator can impose restrictions, such as that of roundness, a shape factor. The user can define a certain minimum and maximum value for roundness, and values falling out of this range can be excluded from the measurements. Hence, limitations on size and shape ensure that desired objects are measured, while unwanted objects are discarded.

The sequence of operations performed by the image analyser system:

Image recording: Capturing of an optimum image includes various adjustments in the settings, such as colour, filters and suitable magnification. In digitised images, objects are represented by pixels, which after calibration can be converted to units of measurement, such as micrometers. Calibration for the 20x magnification yielded a measurement of 0,528 μm for every pixel.

Grey level: The next step involves conversion of images acquired in colour to greyscale. This step is recommended because subsequent image processing becomes faster and more time-efficient and the threshold for detection and recognition between features becomes easier.

Segmentation: Allows for the conversion of the greyscale image into a binary image (black and white). Segmentation operates on the basis of detection, enabling distinction between e.g. the lumen and cell wall. Threshold controls are adjusted to the desired detection level, manually or using a macro, based on grey levels. Achieving suitable segmentation involves a trade-off between a set with all lumen pixels included and a set where few non-lumen objects are included. Enhancement assists in detection of segmentation by sharpening the image and creating greater contrast between the two features being measured.

At this stage, the image displays two separate features, the lumen and the cell wall. Watershed segmentation can be used at this stage to separate individual cells in the grey-

level image. The aim is that most of the objects after the watershed segmentation should correspond to a single feature in the image.

Characteristics measured: The operator needs to define the desired features of the processed image and the measurement frame. The following wood anatomical parameters were measured in this investigation:

area of entire object,
area of coincidence parameter (lumen),
feret 0 (radial dimension of tracheid),
feret 90 (tangential dimension of tracheid)
lumen diameter.

Subsequently, it was possible to calculate cell wall area, cell wall thickness, and equivalent circle diameter (tracheids) by means of simple equations based on the acquired measurements.

2.3.2 Alignment of data from densitometry and image analysis

In order to make any inferences on data generated by the densitometry and the image analysis, it was necessary to align the data. For the alignment, the data generated for micro densitometry and cell wall thickness were used, since both displays the same pattern when plotted against distance from bark (Figure 4.2). In order to align the data, the maximum points were used, thus the alignment was done based on the assumption that the maximum density and the maximum wall thickness occurs at the same distance from the bark. The alignment was done using Microsoft Excel®.

Once the data from densitometry and image analysis is aligned, the within ring variables such as ring width, early wood and latewood width, densities and percentages will also be aligned, allowing for the classification of the image analysis data into early wood and late wood.

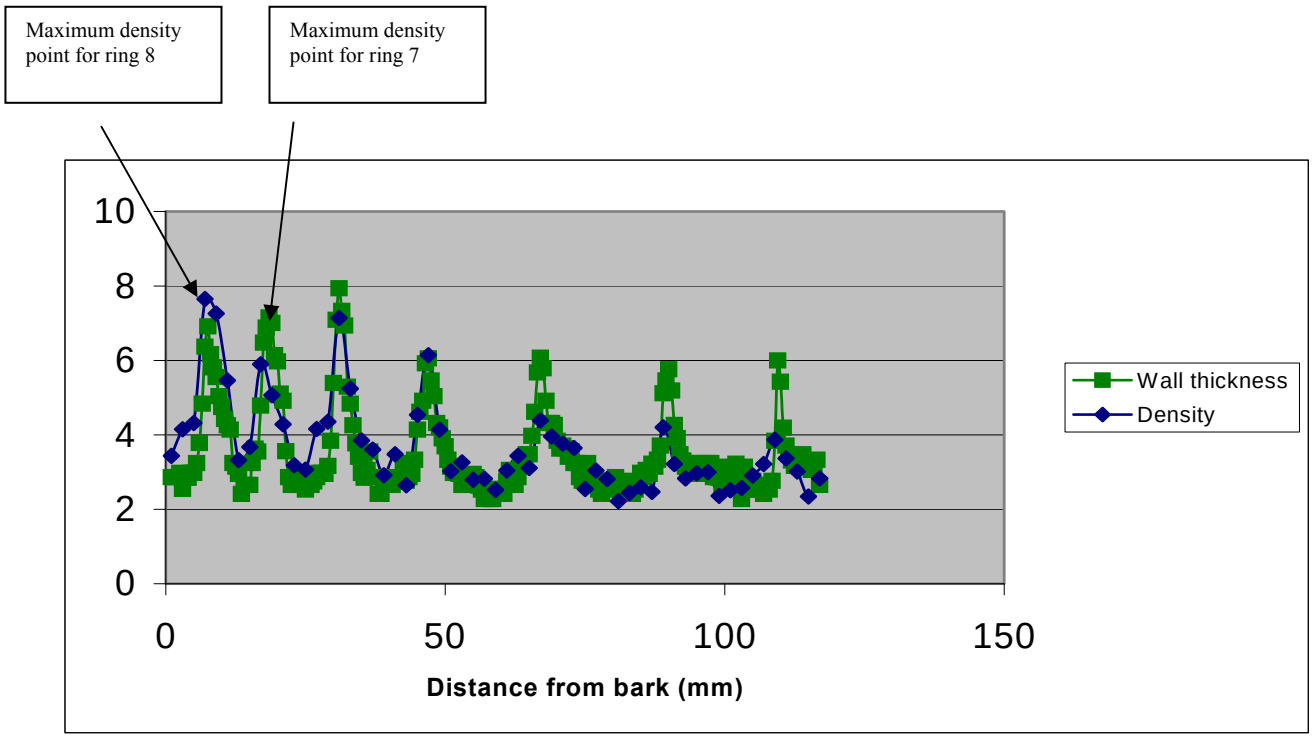

FIGURE 4.2: Alignment of data from densitometry and image analysis, using cell wall thickness and density.

2.4 Summary of traits measured and derived

1) Density – gravimetric and micro densitometric

2) The anatomical characteristics measured included:

Fibre diameter (radial and tangential),

Fibre lumen diameter,

Cell wall thickness,

Latewood percentage: data generated by the CSIR per ring was utilised.

3) Traits derived:

Runkell ratio – the cell lumen and cell wall thickness was determined by area weighting the parameters, prior to determining the ratio.

2.5 Analysis of variance (ANOVA).

The analysis of variance, utilising the individual data for gravimetric density and area weighted data for micro density and fibre morphological data was done using PROC GLM in SAS® version 9.3 (SAS, 1999).

2.5.1 Single site analysis

Since the analysis of an individual site will basically be limited to the families as source of variance, the analysis for gravimetric density, micro density and the fibre morphological data was done using the following model:

$y_{ij} = \mu + fam_i + \varepsilon_{ij}$

Where: y_{ij} = value associated with the trait of the j^{th} tree in i^{th} family

μ = overall mean

fam_i = random effect associated with the i^{th} family, i =1,..100

ε_{ij} = random error associated with j^{th} tree from i^{th} family

The format used for the determination of the mean squares associated with the various sources of variation, is presented in Table 4.1.

TABLE 4.1: Format for the calculation of expected mean squares for an individual site from a *Pinus patula* trial series for the analysis of variance of gravimetric density.

Source	df	MS	Expected mean square
Family	f-1	MS_1	$\sigma^2_w + n\sigma^2_f$
Error	f(n-1)	MS_2	σ^2_w

Where:

MS_1 = Mean square for families

MS_2 = Mean square for error

n = number of trees per family

And:

σ^2_f = variance component for families

σ^2_w = error variance component

2.5.2 Across site analysis

An across site analysis of variance for the gravimetric density, micro density and fibre morphological data were done utilizing PROC GLM in SAS® using the following linear model:

$$y_{ijkl} = \mu + loc_i + fam_j + (loc* fam)_{ij} + \varepsilon_{ijk}$$

Where:

y_{ijk}	=	weighted mean for the trait of the k^{th} tree in j^{th} family in i^{th} location
μ	=	overall mean
loc_i	=	random effect associated with the i^{th} location, i = 1,…6
fam_j	=	random effect associated with the j^{th} family, j =1,..100
$(loc*fam)_{ij}$	=	the interaction between the j^{th} family and the i^{th} location
ε_{ijk}	=	random error associated with the k^{th} tree from the i^{th} location and j^{th} family

The calculation of mean squares for the various sources of variation, the following format was used (Table 4.2).

TABLE 4.2: Format for the calculation of expected mean squares for across site analysis of variance for wood and fibre properties of a *Pinus patula* series established across six sites.

Source	df	MS	Expected mean square
Location	l-1	MS_1	$\sigma^2_w + n\sigma^2 + nf\sigma^2_l$
Fam	f-1	MS_2	$\sigma^2_w + n\sigma^2 + n\sigma^2_f$
Loc*Fam	(l-1)(f-1)	MS_3	$\sigma^2_w + n\sigma^2_{lf}$
Error	lf (n-1)	MS_4	σ^2_w

Where:

MS_1 = Mean square for location

MS_2 = Mean square for family

MS_3 = Mean square for location x family interaction

MS_4 = Mean square for error

l = number of locations

f = number of families

n = number of trees per family

And:

σ^2_l = Variation due to different locations

σ^2_f = Variation between families

σ^2_{lf} = Variation associated with the interaction between location and family

σ^2_w = error variation

2.6 Ranking families for density and fibre morphology

Ranking the families based on the average family gravimetric density and mean area weighted morphological data was done per site using PROC RANK in SAS® (1999). The families were ranked in descending order, with tied ranks assuming the higher rank value.

2.7 Correlation between gravimetric density and micro densitometry

In order to determine whether there is a good correlation between the values generated for density between the value calculated by means of gravimetric density determination and area weighted micro densitometry, a Spearman correlation coefficient (r_s) between the two values for each site was calculated as follows (Ott, 1988):

$$r_s = \frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}$$

Where S_{xy} = covariance between paired observations

S_{xx} = variance for factor x

S_{yy} = variance for factor y

With factor x the gravimetric density per sample, and factor y the area weighted micro densitometry density per sample.

2.8 Genotype x environment interaction (GxE)

The magnitude of GxE was evaluated as discussed in Chapter 3, Section 2.3.3. Type B-genetic correlations (r_{Bg}) (Burdon, 1977) were calculated using the method described by Tibbits and Hodge, (2003), and the standard error associated with the Type-B correlation using the method described by MacDonald *et al*.,(1997).

3. Results and discussions

3.1 Differences between sites

3.1.1 Density

The differences between sites for mean gravimetric density of the top 100 families (100 fams gr), the mean gravimetric density for the top 30 families (30 fams gr) and the area weighted mean density for the top thirty families (30 fams aw) are depicted in Figure 4.3. The ranking of sites has been done according to the average gravimetric density of the top 100 families.

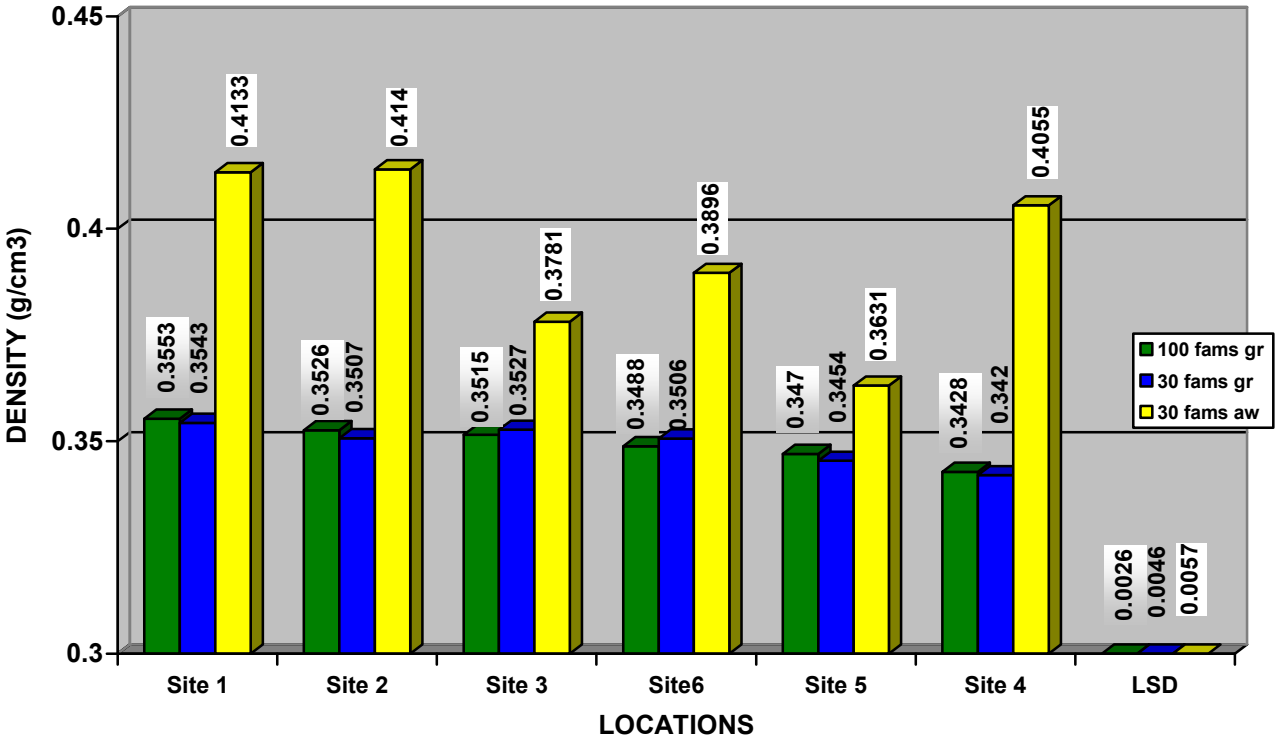

FIGURE 4.3: Average gravimetric and area weighted micro density of *Pinus patula* families established across six sites.

When comparing the mean values for the area weighted micro density and the mean gravimetric density of the same site (Figure 4.3), it can be seen that not only is there a difference in the ranking of the sites, but the micro density is higher than the gravimetric density. These differences are attributed to the difference in moisture content between

the various methods. Gravimetric density was determined using saturated samples, while micro densitometry was done using samples at ambient moisture content, thus with less volume (Payn, 2001). The higher values for area weighted density could also be attributed to the fact that the micro density was determined utilising rings four to eight, discarding rings one to three, that usually contains a higher percentage early wood, and thus leads to a reduction in density.

When comparing the gravimetric density values for the top one hundred and the top thirty families on the same sites, it can be seen that the ranking of sites based on the gravimetric density of the top thirty families is, with the exception of one rank change, consistent with the ranking based on the density for the top hundred families. It can also be observed that the differences between the density for the top thirty and the top one hundred families are very small. A possible explanation for the change in ranking of the sites could be attributed to the relationship between volume production and density. In the case of a positive correlation, increased volume production would lead to an increase in density, and the opposite true for a negative correlation.

Based on an analysis of variance for the top 100 families, it was found that significant differences exist between sites (Table 4.3). Significant differences also existed between families, and a significant location x family interaction was found (Table 4.3), which would indicate that the performance of families across sites is not constant.

TABLE 4.3: Summarized analysis of variance (ANOVA) for gravimetric density of the top 100 families of a *Pinus patula* trial series established across six sites in South Africa.

Source of variation	df	MS	F-value
Loc	5	0,010	13,52**
Fam	99	0,003	3,89**
Loc*Fam	495	0,001	1,19**
Error	2885	0,001	

** - highly significant differences $\alpha < 0.001$
* - significant difference $0.05 < \alpha < 0.01$
ns – non – significant differences

A summary of the analysis of variance (ANOVA) for the gravimetric density and mean area weighed micro density of the top thirty families is presented in Table 4.4.

TABLE 4.4: Summarized analysis of variance (ANOVA) for gravimetric and area weighted micro density of the top thirty families in a *Pinus patula* trial series established across six sites in South Africa.

		Gravimetric density		Area weighted micro density	
Source of variation	**df**	**MS**	**F-value**	**MS**	**F-value**
Loc	5	0,004	5,13**	0,059	48,15**
Fam	29	0,003	3,52**	0,006	5,01**
Loc*Fam	145	0,001	1,36**	0,001	1,36**
Error	870	0,001		0,001	

** - highly significant differences $\alpha < 0.001$
* - significant difference $0.05 < \alpha < 0.01$
ns – non – significant differences

For both the variables, significant differences were detected between sites and families. Again there was also significant location x family interaction for both variables, indicating inconsistent performance of families across sites.

From a Waller-Duncan mean comparison (Ott, 1988), it can be observed that the grouping for sites differs between variables (Table 4.5).

TABLE 4.5: Waller-Duncan means comparison for the gravimetric and area weighted micro density of the top families in a *Pinus patula* trial series established across six sites in South Africa.

Top 100 gravimetric density			Top 30 gravimetric density			Top 30 micro density		
Group	Site	Mean	Group	Site	Mean	Group	Site	Mean
A	Site1	0,355	A	Site1	0,354	A	Site2	0,414
AB	Site2	0,352	A	Site3	0,353	A	Site1	0,413
B	Site3	0,351	A	Site2	0,351	B	Site4	0,405
C	Site6	0,349	A	Site6	0,351	C	Site6	0,390
C	Site5	0,347	B	Site5	0,345	D	Site3	0,378
D	Site4	0,343	B	Site4	0,342	E	Site5	0,363

For the gravimetric density of the top 100 families, four groupings can be distinguished, indicating meaningful differences between sites. This could probably be attributed to the large number of families used, because for the gravimetric density of the top thirty

families, only two groupings occur. For the micro density of the same families, five groupings can be observed, although the grouping and order of sites based on the mean values do not correspond with the grouping for the gravimetric density.

3.1.2 Fibre morphology

The average area weighted values across sites for the various fibre morphological characteristics is presented in Figures 4.4 to 4.7. When looking at Figure 4.4 and the values for density as indicated in Figure 4.3, it is noticeable that Site 4 has the lowest gravimetric density, but the highest wall thickness (Figure 4.4), as can be expected from the high percentage late wood (Figure 4.6). This would then also explain the small lumen diameters as can be observed from Figure 4.7. The opposite reasoning can be followed for Site 5.

When comparing Figure 4.4 and Figure 4.6, the trend is almost as expected, since late wood cells are usually associated with thick cell walls, thus it could be expected that sites with thick cell walls should have high latewood percentages. This is true for Site 4, Site 1 and Site 2, but not for the other three sites.

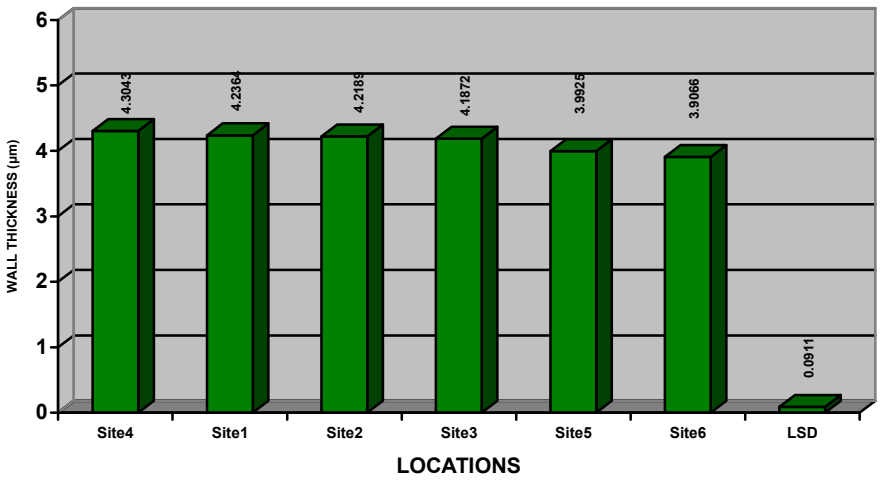

Figure 4.4: Average area weighted wall thickness across sites.

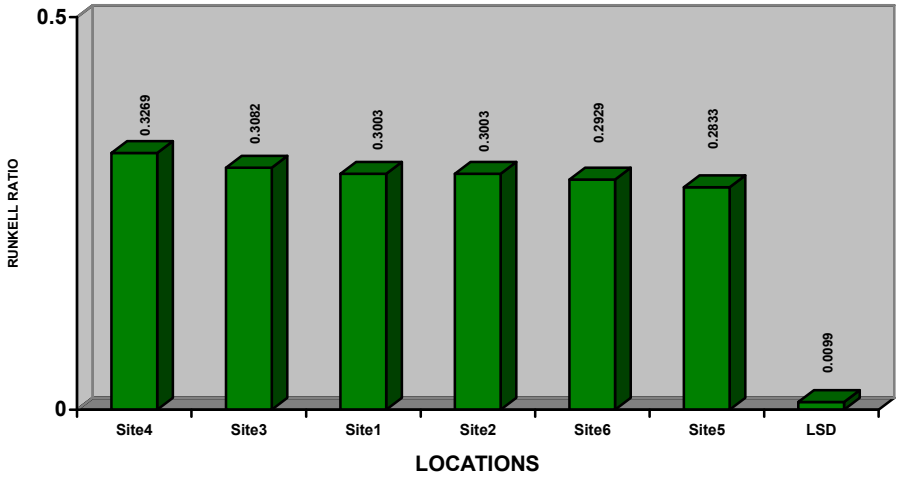

Figure 4.5: Average area weighted Runkell ratio across sites.

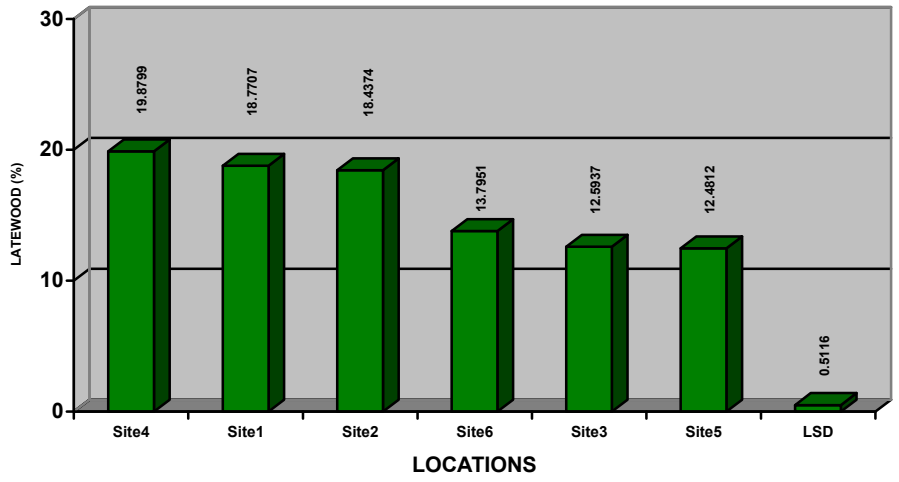

Figure 4.6: Average area weighted latewood percentage across sites.

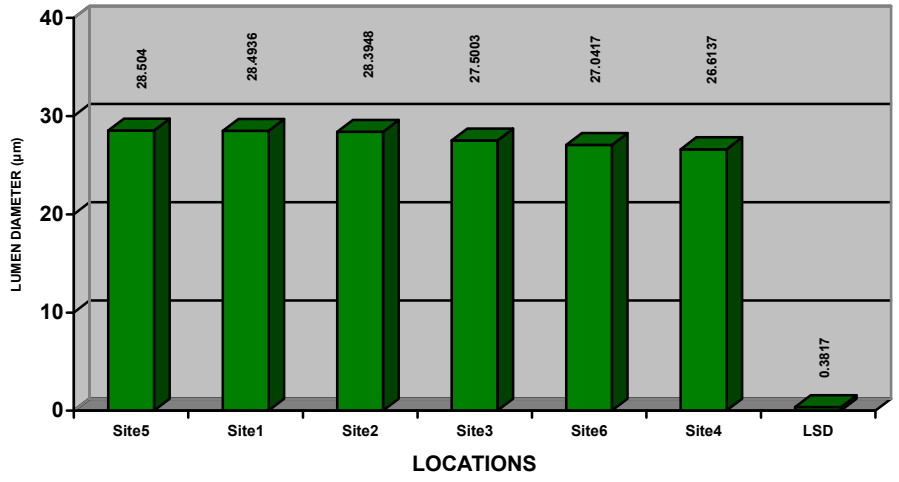

Figure 4.7: Average area weighted lumen diameter across sites.

Summarised analysis of variance (ANOVA) for each of the variables is indicated in Table 4.6. For each of the area weighted variables, significant differences between locations and families existed, while the location by family interaction also proofed significant for all variables, except lumen diameter.

TABLE 4.6: Summarized analysis of variance (ANOVA) for area weighted fibre properties of the top thirty families in a *Pinus patula* trial series established across six sites in South Africa.

Cell wall thickness				
	Source of variation	**df**	**MS**	**F-value**
	Loc	5	2,740	6,22**
	Fam	29	0,790	0,01 *
	Loc*Fam	143	0,442	2,08**
	Error	817	0,212	
Runkell ratio				
	Source of variation	**df**	**MS**	**F-value**
	Loc	5	0,026	5,84**
	Fam	29	0,011	2,55**
	Loc*Fam	143	0,004	1,80**
	Error	817	0,003	
Latewood percentage				
	Source of variation	**df**	**MS**	**F-value**
	Loc	5	1954,290	165,33**
	Fam	29	48,001	4,06**
	Loc*Fam	143	11,820	1,49**
	Error	856	7,950	
Lumen diameter				
	Source of variation	**df**	**MS**	**F-value**
	Loc	5	103,904	23,74**
	Fam	29	23,780	5,43**
	Loc*Fam	143	4,377	1,09ns
	Error	817	4,008	

** - highly significant differences $\alpha < 0.001$
* - significant difference $0.05 < \alpha < 0.01$
ns – non – significant differences

The Waller-Duncan mean comparison (Ott, 1988) between sites for each variable is indicated in Table 4.7. As seen from the analysis of variance, a number of significant differences do occur between the various sites, but there is also a reasonable degree of similarity between sites since there are a number of sites that are grouped together for certain characteristics.

Table 4.7: Waller-Duncan means comparisons for area weighted fibre morphological characteristics of *Pinus patula* established across six sites in South Africa.

Cell wall thickness		Runkell ratio		Late wood percentage		Lumen diameter	
Group	Site	Group	Site	Group	Site	Group	Site
A	Site4	A	Site4	A	Site4	A	Site5
AB	Site1	B	Site3	B	Site1	A	Site1
AB	Site2	BC	Site1	B	Site2	A	Site2
B	Site3	BC	Site2	C	Site6	B	Site3
C	Site5	CD	Site6	D	Site3	C	Site6
C	Site6	D	Site5	D	Site5	D	Site4

3.2 Differences between families

3.2.1 Density

There were also highly significant differences ($\alpha < 0.001$) between the top 100 families for gravimetric density across sites (Table 4.3), confirming Payn's results (Payn, 2001), which is always of value in a breeding programme. On an individual site basis however, non-significant differences were detected at Site 3. The individual site analysis and the ranking of the individual families across sites for mean gravimetric density can be obtained from the author. Descriptive statistics for the individual sites and the distribution of mean family values of the top 100 families are given in Table 4.8. Although the mean values are fairly similar, the spread of data varies from site to site. Site 3 has the greatest spread of data, according to the range, due to the maximum value being substantially greater than on any other site, while Site 2 has the narrowest spread of data. The lowest minimum density however occurred at Site 1.

The ranking of families across sites are not constant, and there are seldom families that ranks constant across all sites. This will by definition, indicate the presence of genotype x environment interaction.

TABLE 4.8: Descriptive statistics for mean family gravimetric density of the top 100 families established across six trial sites of *Pinus patula*.

Statistic	Site1	Site2	Site3	Site4	Site5	Site6
Trial mean (g/cm^3)	0,355	0,353	0,352	0,343	0,347	0,349
Maximum (g/cm^3)	0,487	0,440	0,544	0,506	0,433	0,456
Minimum (g/cm^3)	0,224	0,296	0,262	0,279	0,270	0,281
Range (g/cm^3)	0,263	0,145	0,282	0,227	0,163	0,175
Std dev (g/cm^3)	0,027	0,025	0,027	0,027	0,024	0,027

Significant differences between the top 30 families were also observed for gravimetric density and area weighted density (Table 4.4). The ranking for the top thirty families based on average gravimetric density and average area weighted micro density across sites can be obtained from the author. It can be seen that for both variables there are changes in ranking occurring across sites. It can also be observed that for the different variables on the same site, there are changes in the ranking.

Descriptive statistics for the gravimetric density is indicated in Table 4.9 and for area weighted micro density in Table 4.10.

TABLE 4.9: Descriptive statistics for average family gravimetric density for the top thirty families established across six trial sites of *Pinus patula*.

Statistic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Trial mean (g/cm^3)	0,354	0,351	0,353	0,342	0,345	0,351
Maximum (g/cm^3)	0,418	0,419	0,486	0,412	0,416	0,420
Minimum (g/cm^3)	0,286	0,299	0,301	0,282	0,270	0,286
Range (g/cm^3)	0,131	0,120	0,185	0,130	0,146	0,134
Std dev (g/cm^3)	0,024	0,024	0,024	0,024	0,024	0,027

The statistics in Table 4.9 indicates that the greatest spread in the density values occurred at Site 3. The highest density was found at Site 3, with the lowest value occurring at Site 4. Little differences in the standard deviations would indicate that the spread of the values around the means were very similar across sites.

TABLE 4.10: Descriptive statistics for average area weighted family micro density for the top thirty families established across six *Pinus patula* trial sites.

Statistic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Trial mean (g/cm^3)	0,413	0,414	0,378	0,406	0,363	0,39
Maximum (g/cm^3)	0,503	0,538	0,495	0,513	0,447	0,492
Minimum (g/cm^3)	0,317	0,324	0,280	0,319	0,311	0,322
Range (g/cm^3)	0,186	0,214	0,216	0,194	0,136	0,17
Std dev (g/cm^3)	0,036	0,035	0,031	0,036	0,026	0,034

As with the gravimetric density, the greatest value for area weighted micro density were found at Site 2 (Table 4.10). Since most of the values are greater than the gravimetric density values described in Table 4.9, it can be seen that the data has a greater spread, judged by the range, although the standard deviations across sites are very similar, with the exception of Site 5, where the spread is reduced. The lowest value for area weighted density was found at Site 3.

From the Spearman correlation coefficient calculated between the gravimetric and micro densitometry density for the top 30 families, the following correlations were found per site (Table 4.11). It would seem that the correlation of 0,93 would indicate that gravimetric and area weighted density determination can be used as alternates for determining the density of samples. It should be mentioned that the "Across sites" value is not the mean correlation value, but rather the correlation between all data on all sites.

Table 4.11: Spearman correlations between gravimetric and densitometry density for the top 30 families across sites.

Site	r_s
Site 1	0,92
Site 2	0,83
Site 3	0,72
Site 4	0,82
Site 5	0,75
Site 6	0,95
Across sites	0,93

3.2.2 Fibre morphology

From the analysis of variance (Table 4.6), it can be concluded that the differences between families for all of the fibre morphology characteristics evaluated were significant. Various descriptive statistics for the family mean values per site can be evaluated from Table 4.12 to Table 4.15.

TABLE 4.12: Descriptive statistics for mean area weighted family cell wall thickness of the top thirty families in a *Pinus patula* trial series established across six sites.

Statistic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Trial mean (μm)	4,236	4,219	4,187	4,304	3,993	3,907
Maximum (μm)	5,587	6,098	6,243	5,449	6,151	5,928
Minimum (μm)	3,063	3,067	2,795	3,095	3,042	3,028
Range (μm)	2,525	3,031	3,448	2,353	3,109	2,900
Std dev (μm)	0,508	0,547	0,569	0,448	0,482	0,497

TABLE 4.13: Descriptive statistics for the mean area weighted Runkell ratio of the top thirty families in a *Pinus patula* trial series established across six sites.

Statistic	Site1	Site2	Site3	Site4	Site5	Site6
Trial mean	0,3001	0,3002	0,3082	0,3269	0,2833	0,2929
Maximum	0,4424	0,5270	0,5960	0,4640	0,5638	0,5750
Minimum	0,1992	0,1956	0,1775	0,2093	0,1908	0,1992
Range	0,2432	0,3314	0,4185	0,2547	0,3730	0,3758
Std dev	0,0504	0,0547	0,0608	0,0519	0,0519	0,0586

Looking at all the descriptive statistics for the Runkell ratio, none of the statistics are greater than 1, thus it would seem that the collapsibility of the fibres are desirable on all sites (Section 1.3). This would however not imply that the ratio can not be improved. Care should be taken when the individual parameters, namely wall thickness and lumen diameter, are being manipulated, that the ratio is not negatively affected.

TABLE 4.14: Descriptive statistics for mean area weighted latewood percentage for the top thirty families in a *Pinus patula* trial series established across six sites.

Statistic	Site1	Site2	Site3	Site4	Site5	Site6
Trial mean (%)	18,7707	18,4374	12,5937	19,8799	12,4812	13,7951
Maximum (%)	30,4246	28,6912	20,0758	31,6166	21,5596	23,0318
Minimum (%)	10,9140	9,6936	7,6694	11,9336	7,7425	8,2068
Range (%)	19,5106	18,9976	12,4064	19,683	13,8171	14,825
Std dev (%)	3,9719	3,4327	2,3657	3,4981	2,2669	2,7539

TABLE 4.15: Descriptive statistics for mean area weighted family lumen diameter for the top thirty families in a *Pinus patula* trial series established across six sites.

Statistic	Site1	Site2	Site3	Site4	Site5	Site6
Trial mean (μm)	28,4936	28,3984	27,5003	26,6137	28,5040	27,0417
Maximum (μm)	34,7318	33,7657	33,2831	32,5537	34,8269	32,2364
Minimum (μm)	20,8654	23,0844	20,9737	22,5560	21,8223	20,6129
Range (μm)	13,8664	10,6813	12,3094	9,9977	13,0046	11,6234
Std dev (μm)	2,0104	2,0640	2,1628	2,1908	2,2358	2,2798

The ranking of the families for the fibre morphological criteria across the various sites can be obtained from the author. When evaluating the ranking of families across the various sites for the various characteristics, it can be seen that there are numerous changes in the ranking of families between sites for the various criteria. These changes would per definition indicate the presence of genotype x environment interaction.

3.3 Genotype x environment interaction (GxE)

3.3.1 Density

Based on the ANOVA, the location x family interaction is significant (Table 4.3 and Table 4.4) for all density assessments. When evaluating the ranking of families across sites it is very noticeable how the ranking of families changes across sites. With very few exceptions are there families that constantly perform among the top families across all the sites.

These interactions were described by means of Type B- genetic correlations by Payn (2001), who concluded that there were a number of site combinations where the correlations were lower than the prescribed value of 0.67 (Shelbourne, 1972) which could warrant the need for regionalisation or separate breeding populations. Based on the Type B- correlations for the top 100 families, he recommended grouping the sites into two separate breeding populations which coincides with the rainfall, temperature and longitudinal coordinates of the sites.

Due to the re-sampling of a few samples, Payn's results have been reproduced, but with little change to his results. The results still follow the trends that Payn has indicated in that the sites with high correlations still have acceptable correlations (Table 4.16). The acceptable correlations have been shaded.

TABLE 4.16: Type B- genetic correlations for gravimetric wood density between sites for the top 100 families of a *Pinus patula* trial series established across six sites in South Africa (standard error in parenthesis).

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		**0,82 (0,07)**	**0,59 (0,14)**	**0,96 (0,07)**	**0,73 (0,07)**	**0,87 (0,06)**
Site 2			**0,38 (0,33)**	**0,73 (0,10)**	**0,90 (0,01)**	**0,68 (0,11)**
Site 3				**0,71 (0,08)**	**0,88 (0,09)**	**0,54 (0,12)**
Site 4					**0,53 (0,13)**	**0,56 (0,13)**
Site 5						**0,96 (0,08)**
Site 6						

For the analysis of variance for both gravimetric density and area weighted density of the top thirty families, significant interactions were found between the locations and the families on those sites. This is also visible from the ranking of families across sites, which can be obtained from the author. Type B- genetic correlations gave the following correlations between the various sites for the various characteristics investigated (Table 4.17 and Table 4.18).

TABLE 4.17: Type B- genetic correlations between sites for the area weighted micro density of the top thirty families in a *Pinus patula* progeny trial series.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		**0,82 (0,05)**	**0,51 (0,22)**	**0,66 (0,12)**	**0,69 (0,11)**	**0,51 (0,14)**
Site 2			**0,69 (0,19)**	**0,81 (0,09)**	**0,91 (0,06)**	**0,51 (0,18)**
Site 3				**0,70 (0,16)**	**0,80 (0,11)**	**0,81 (0,10)**
Site 4					**0,46 (0,19)**	**0,48 (0,17)**
Site 5						**0,77 (0,10)**
Site 6						

TABLE 4.18: Type B- genetic correlations between sites for the gravimetric density of the top thirty families in a *Pinus patula* progeny trial series.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		**0,91 (0,05)**	**0,46 (0,36)**	**0,65 (0,10)**	**0,70 (0,18)**	**0,49 (0,26)**
Site 2			**0,57 (0,23)**	**0,45 (0,19)**	**0,98 (0,01)**	**0,65 (0,14)**
Site 3				**0,53 (0,34)**	**0,76 (0,19)**	**0,45 (0,52)**
Site 4					**0,60 (0,25)**	**0,52 (0,34)**
Site 5						**0,54 (0,16)**
Site 6						

From the highlighted cells in Tables 4.16, 4.17 and 4.18, it can be seen that based on gravimetric density for the top hundred families and gravimetric density and area weighted density for the top thirty families, there are families that could successfully be deployed across other sites. Further investigation of the sites should however be done in order to determine the families that tend to be the most susceptible to GxE. These families can possible be removed from the dataset, which could result in higher correlations. The high standard errors obtained on some of the sites would also suggest that these correlations should be used with caution.

3.3.2 Fibre morphology

When examining the ranking of families across sites for the various fibre morphological characteristics, it can be observed that the ranking of the families are not constant, thus it can be expected that some genotype x environment interaction is present for the fibre morphology variables. These interactions were evaluated by means of Type B- genetic correlations, and the results presented in Table 4.19 to Table 4.22. In all the tables the favourable correlations have been highlighted as per Shelbourne's definition.

TABLE 4.19: Type B- genetic correlations between sites for the area weighted cell wall thickness of the top thirty families in a *Pinus patula* progeny trial series.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		**0,64 (0,11)**	**0**	**0**	**0**	**0,11 (0,13)**
Site 2			**0**	**0,22 (0,18)**	**0**	**0,53 (0,13)**
Site 3				**0,84 (0,13)**	**0**	**0**
Site 4					**0**	**0,26 (0,13)**
Site 5						**0**
Site 6						

TABLE 4.20: Type B- genetic correlations between sites for the average Runkell ratio of the top thirty families in a *Pinus patula* progeny trial series.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1		**0,73 (0,11)**	**0**	**0,49 (0,17)**	**0,91 (0,05)**	**0,87 (0,04)**
Site 2			**0**	**0,42 (0,21)**	**0,92 (0,03)**	**0,73 (0,11)**
Site 3				**0**	**0**	**0**
Site 4					**0,51 (0,21)**	**0,56 (0,13)**
Site 5						**0,82 (0,09)**
Site 6						

TABLE 4.21: Type B- genetic correlations between sites for the average latewood percentage of the top thirty families in a *Pinus patula* progeny trial series.

	Site1	Site2	Site3	Site4	Site5	Site6
Site1		**0,68 (0,13)**	**0**	**0,52 (0,19)**	**0**	**0,67 (0,16)**
Site2			**0**	**0,31 (0,21)**	**0,95 (0,02)**	**0,80 (0,09)**
Site3				**0**	**0**	**0**
Site4					**0,76 (0,12)**	**0,49 (0,21)**
Site5						**0,86 (0.08)**
Site6						

TABLE 4.22: Type B- genetic correlations between sites for the area weighted cell lumen diameter of the top thirty families in a *Pinus patula* progeny trial series.

	Site1	Site2	Site3	Site4	Site5	Site6
Site1		**0,70 (0,10)**	**0,13 (0,32)**	**0,09 (0,41)**	**0,46 (0,16)**	**0,34 (0,11)**
Site2			**0**	**0,28 (0,19)**	**0**	**0,64 (0,10)**
Site3				**0**	**0,77 (0,16)**	**0,49 (0,14)**
Site4					**0,94 (0,11)**	**0,34 (0,12)**
Site5						**0,73 (0,22)**
Site6						

From Tables 4.19 to Table 4.22, it can be seen that from the highlighted Type-B genetic correlations, there are indications that some site combinations are favourable. A number of correlations were not calculated due to negative variance and co-variance components. These were indicated with "0". The favourable correlations should however be considered with caution, since a number of them are associated with large standard errors. Further evaluation of the Type-B genetic correlations is warranted in order to determine the families that are most sensitive for GxE. By eliminating these outliers, the correlations might improve sufficiently to be able to make more concrete recommendations in terms of the deployment of material across sites.

References: Chapter 4.

Barefoot, A.C., Hitchings, R.G. and Ellwood, E.L.(1964). Wood characteristics and Kraft paper properties of four selected Loblolly Pines. Effect of fibre morphology under identical cooking conditions. *Tappi* **47 (6),** 343-355.

Burdon, R.D. (1977). Genetic correlations as a concept for studying Genotype-Environment Interaction in Forest Tree Breeding. *Silvae Genetica* **26 (5-6),** 168 - 175.

Burdon, R.D. and Maddern Harris, J. (1972). Wood density in radiata pine clones on four different sites. *New Zealand Journal of Forestry Science* **3 (3),** 286-303.

Corson, S.R. (1993). Direction in New Zealand mechanical pulping research Part 2 – Fundamental characteristics of pulp components; long fibre enhancement; refining energy; optical properties; size distribution model of refining process; bleaching; TMJP environmental impact and opportunities for improved products. *Appita* **46 (3),** 214-219.

Corson, S.R. (1999). Tree and fibre selection for optimal TMP quality. *Appita Journal* **52(5),** 351-357.

Cown, D.J. and Clement, B.C. (1983). A wood densitometer using direct scanning with X- rays. *Wood Science and Technology* **17**, 91-99.

Cown, D.J. and McConchie, D.L. (1980). Wood property variations in an old-crop stand of radiata pine. *New Zealand Journal of Forestry Science* **10(3),** 508-520.

Cown, D.J., McKinley, R.B. and Ball, R.D. (2002). Wood density variation in 10 mature *Pinus radiata* clones. *New Zealand Journal of Forestry Science* **32 (1),** 48-69.

Dadswell, H.E., Watson, A.J. and Nicholls, J.W.P. (1959). What are the wood properties required by the Paper Industry in the trees of the future? *Tappi* **42 (7),** 521-526.

De Villiers, A.M. (1974). Observations on the timber properties of certain tropical pines grown in South Africa and their improvement by tree breeding. *Forestry in South Africa* **15,** 57-64.

Eguiluz-Piedra, T. and Zobel, B.J. (1986). Geographic variation in wood properties of *Pinus tecunumannii*. *Wood and Fibre Science* **18 (1):** 68-75.

Einspahr, D.W., van Buijtenen, J.P. and Peckham, J.R. (1967). Pulping characteristics of ten year Loblolly Pine selected for extreme wood specific gravity. *Silvae genetica* **18 (3),** 57-61.

Evans, R., Kibblewhite, R.P and Stringer, S. (1997). Kraft pulp fibre property prediction from wood properties in eleven radiata pine clones. *Appita Journal* **50(1)**, 25-33.

Hodge, G.R. and Purnell, R.C. (1993). Genetic parameter estimates for wood density, transition age and radial growth of slash pine. *Canadian Journal of Forestry Research* **23,** 1881-1891.

Joransen, P.N. (1960). Pulpwood properties: response of processing and of paper quality to their variation. Forest biology subcommittee no 2 on tests ad quality objectives. *Tappi Journal* **43 (11),** 40-64.

Kibblewhite, R.P. (1980). Radiata pine corewood and slabwood, and their interrelationship with pulp and handsheet properties. *New Zealand Journal of Forestry Science* **10 (3)**, 533-550.

Kibblewhite, R.P. (1982). The qualities of radiata pine papermaking fibres. *Appita* **35 (4)**, 289 – 298.

Kibblewhite, R.P. (1984). *Pinus radiata* wood residue qualities and some utilisation options. *New Zealand Journal of Forestry Science* **14 (3)**, 382-394.

Koch, L. and Fins, L. (2000). Genetic variation in wood specific gravity from progeny tests of Pondorosa Pine (*Pinus ponderosa* Laws.) in Northern Idaho and Western Montana. *Silvae Genetica* **49**, 174-180.

Kromhout, C.P. (1966). "The effect of resin content on the specific gravity of three pine species". (In Afrikaans with English summary). *Forestry in South Africa* **6**: 53-61.

Malan, F.S. (2001). The wood quality of Pinus chiapensis (Mart.) Andresen grown in the Mpumalanga forest region. *Southern African Forestry Journal* **192**, 51-57.

MacDonald, A.C., Borralho, N.M.G and Potts, B.M. (1997). Genetic variation for growth and wood density in Eucalyptus globulus ssp. globulus in Tasmania (Australia). *Silvae Genetica* **46, 4**: 236-241.

Ott, L. (1988). An introduction to statistical methods and data analysis – 3rd edition. PWS–Kent Publishing Company – Boston, 835pp.

Payn, K.G. (2001). Heritability of wood density and the occurrence of genotype x environment interaction in a progeny trial series of *Pinus patula*. M.Sc thesis. University of Natal – Durban. 69pp.

SAS (1999). Statistical Analysis System – SAS Users Guide – 1999 Edition – version 9. SAS Institute Inc., Box 8000, Cary, N.C, 27511.

Shelbourne, C.J.A (1972). Genotype – Environment Interaction: Its study and its implications in forest tree improvement. In: Proceedings IUFRO Genetics – Sabrao joint Symposia. Tokyo.

Shelbourne, T., Evans, R., Kibblewhite, P. and Low, C. (1997). Inheritance of tracheid transverse dimensions and wood density in radiata pine. *Appita Journal* **50 (1)**, 47-50.

Stanger T. K. (2003). Variation and genetic control of wood properties in the juvenile core of *Pinus patula* grown in South Africa. Ph.D – Department of Forestry. North Carolina State University. 188pp

Tibbits, W.N. and Hodge, G.R. (2003). Genetic parameters for cold hardiness in Eucalyptus nitens (Deane & Maiden) Maiden. *Silvae Genetica* **52, 3-4**: 89-97.

Wright, J.A. and Sluis-Cremer, H.J. (1992). Tracheid morphology and pulp and paper strength traits of *Pinus taeda* and *P. patula* at age 17 years in South Africa. *Tappi Journal* – May 1992, 183-187.

Zobel, B.J. and Talbert, J.T. (1984). Applied forest tree improvement. John Wiley and Sons, Inc., 505pp

Zobel, B.J. and Van Buijtenen, J.P. (1989). Wood variation: its causes and control. Springer-Verlag, Germany. 363pp

Chapter 5

The improvement of growth, wood and fibre properties

1. Introduction

In Chapter 2 it was shown that the phenotype (P) of a tree can be expressed as follows:

$$\text{Phenotype} = \text{Genotype} + \text{Environment} + \text{GxE}$$

It was also indicated that any variation seen in the phenotype would be due to variation in the factors resulting in the phenotype. This relationship would then be presented as:

$$V_P = V_G + V_E + V_{GxE}$$

Where:
- V_P = Phenotypic variation
- V_G = Genotypic variation
- V_E = Environmental variation
- V_{GxE} = Variation due to genotype x environment interaction

Genotypic variation (V_G) is divided into two components i.e. additive (V_A) and non-additive variance (V_{NA}) (Chapter 2). Since non-additive variation can also be divided into two components, dominance variation (V_D) and the variation associated with the interaction among loci (V_I), the relationship between the various components can then be described as:

$$V_P = V_A + V_D + V_I + V_E + V_{GxE}$$

Where:

V_P = Phenotypic variation

V_A = Additive/ Breeding variation

V_D = Dominance variation

V_I = Interaction variation

V_E = Environmental variation

V_{GxE} = Variation due to genotype x environment interaction

Since the additive variance is mainly attributed to differences between families, the relationship between the additive variance and the family variance depends on the relationship between the relatives as tabled below in Table 5.1 (Falconer, 1989).

TABLE 5.1: The relationship between family and additive variance.

Relatives	**Variance**
Offspring and one parent	½ V_A
Offspring and mid-parent	½ V_A
Half sibs	¼ V_A
Full sibs	½ V_A + ¼ V_D + V_{EC}

V_{EC} would indicate environmentally induced variance effects

It would thus follow that in the case of half sib progeny being tested the family variance would account for one-quarter of the additive variance, although in certain cases exceptions are being made when some relatedness are expected among the progeny being evaluated, where the family variance are being taken as accounting for one-third of the additive variance (Squillace, 1974).

1.1 Heritability

The relative genetic and environmental variability of traits used in an improvement programme is usually expressed by means of heritability. Different types of heritability i.e. narrow-sense or broad-sense are distinguished, and is an indication of the relative importance of the various components to the total value obtained. The

ratio between the genotypic variance and the total phenotypic variance i.e. V_G/V_P is known as the broad-sense heritability and indicates the extent to which an individual's phenotypic variance is determined by its total genetic make-up. The ratio V_A/V_P, known as the narrow-sense heritability, indicates the extent to which phenotypes are determined by the genes transmitted from the parents. Of most importance is the additive variance, which is an indication of the variation of breeding values, which in turn is the major cause of resemblance between relatives, and thus the major contributor to the observed genetic properties of a population (Falconer, 1989).

Various studies have been done to determine the heritability of various wood and fibre properties. The "ease" with which certain characteristics for pines can be improved by means of genetic selection has been indicated as follows (Koch, 1973):

Easiest:

- Tracheid length – latewood
- Percentage latewood
- Specific gravity of tree
- Tracheid diameter of early wood
- Tracheid length of early wood
- Bark thickness

Intermediate:

- Specific gravity – latewood
- Wall thickness latewood
- Tracheid diameter of latewood
- Ratio of wall thickness to radial diameter – early wood

Difficult:

- Ratio of wall thickness to radial diameter – late wood
- Wall thickness early wood
- Specific gravity – early wood

From the above, there are strong indications that characteristics could be genetically manipulated.

1.1.1 Genetic variablity of growth properties

The genetic control of growth properties has formed the subject of numerous studies, mainly since these traits are easily measured, and as mentioned, have been the major traits of interest in various breeding programmes. Growth traits frequently assessed are quantitative traits such as height and/or diameter, used for volume calculations, and quality traits such as stem form, branching characteristics, pest or disease resistance and traits such as fox tailing, forking, broken tops and survival.

Various techniques are available to use in the determination of heritability estimates (Wright, 1976), but the most widely used would be growing progeny in field tests and then deriving the estimates within and between parents based on the performance of the progeny. Heritability values vary with age (Hodge and White, 1992), although the pattern of variation is not always predictable (Zobel and Talbert, 1984; Gapare *et al*., 2001).

Heritability estimates for growth properties have been determined for various traits at various levels of genetic source. Estimates can be determined for a species on a country basis (Hodge and Dvorak, 1999; Gapare *et al*., 2001) or provenance level (Moura and Dvorak, 1998). Estimates indicated that differences can be found at both individual and family level, and that great gains can be made by selecting the best suited provenance or from selecting best families or individuals within the best provenance within a specific country.

Noticeable differences have also been indicated for heritability estimates for the same families and traits across a number of sites (Barnes *et al*., 1992a and b; Gwaze *et al*., 2001). This would serve to confirm that due to environmental variation, estimates from a geographic region would not apply to other areas.

Quantitative growth traits, such as height and diameter, usually exhibit higher values than qualitative traits, mainly due to method of assessment used. Quantitative traits

are usually measured by means of measuring equipment resulting in values, while qualitative traits are usually evaluated by means of a subjective point scale, resulting in less variability and thus lower heritability estimates (Hodge and Dvorak, 1999).

1.1.2 Genetic variability of specific gravity / density

The majority of the work has been done on specific gravity since although it is a fairly complex trait, it shows great between tree variation, strong heritability, low genotype x environment interaction (Barnes *et al*., 1994; Muneri and Balodis, 1998), and has a major effect on yield and quality (Zobel and Talbert, 1984), which makes it an ideal trait to improve by means of selection. Although it is accepted that specific gravity is a complex characteristic it has been shown by Hodge and Purnell (1993) that several of the individual components show reasonable strong genetic control. They concluded that the transition age, for ring density, from juvenile to mature density values, latewood density and latewood percentage showed heritability values ranging from 0,16 to 0,22 and stated that selection could possibly reduce the transition age by up to one year. They also showed heritability figures for core density, mature wood density and juvenile wood density of 0,16, 0,33 and 0,15 respectively, indicating genetic variability that could be utilised by means of selection. The high heritabilities of the individual components of density has been confirmed by Zobel and Jett (1995), but they did conclude that none of the components had higher value than specific gravity, and thus concluded that specific gravity could be utilised as a composite characteristic.

Various heritability figures for specific gravity of various softwood species are listed by Zobel and Van Buijtenen (1989) and Payn (2001), ranging from 0,13 to 0,9, depending on the level of improvement. The low heritability figures were found for *Pinus virginiana* working on a provenance level, while the high figures were found utilizing controlled cross progeny and grafts of *P. taeda*, and was attributed by the authors to working with a species with low density in contrast with the high density or hard pines (Hodge and Purnell, 1993). They however concluded that the narrow sense heritability of wood specific gravity for individual softwood trees ranges from 0,4 – 0,7.

1.1.3 Genetic variability of fibre properties

In a summary of various publications, Smith (1966) concluded that the heritability for tracheid length seems to be moderate to high, at least for the first nine years of growth. He also found that latewood tracheid length tends to be more heritable than early wood tracheid length.

Using rings 8-13 of 13 year old *P. radiata* in New Zealand the following narrow sense (h^2) and mean family heritability (h^2_f) figures were estimated for various tracheid characteristics (Shelbourne *et al*., 1997) (Table 5.2). Some of the variables were measured using Silviscan® , while others were derived from those measured.

Table 5.2: Narrow sense (h^2) and mean family heritability (h^2_f) estimates for a number of tracheid characteristics (Shelbourne *et al.*, 1997).

Variable	**h^2**	**h^2_f**
Radial diameter	1.09	0.75
Tangential diameter	0.53	0.55
Density	0.87	0.69
Coarseness	0.82	0.67
Wall thickness	0.77	0.66
Specific surface	0.79	0.66
Perimeter	0.97	0.72

It can be seen that these estimates are moderate to high, making improvement by means of selection possible and the achievement of gains extremely easy. Although no standard errors are given for the estimates, the authors mention that the high heritability figures obtained for the narrow sense heritability could be attributed to genetic sampling error (Shelbourne *et al*., 1997).

Heritability figures are also available for various other wood and fibre properties, as has been indicated by Payn (2001). As can be seen from the sources he quotes, most of the properties exhibit the same wide range of values. These findings merely indicate that the majority of traits are under genetic control and can thus be improved by means of tree improvement programmes.

1.2 Correlation between traits

Two types of correlations i.e. phenotypic and genotypic correlations are distinguished in the field of trait improvement by means of breeding. Phenotypic correlations would be the correlation between measured values for two traits of importance. Genotypic correlations measure the correlation between breeding values for different traits, and are caused by genes influencing more than one trait (Falconer, 1989).

1.2.1 Correlations between growth rate, wood and fibre properties

Growth rate, expressed as the number of growth rings per measure unit, has always been considered to be useful, since it has been believed to be closely correlated with density. With more data becoming available, this relationship is proving to be less reliable, and the usefulness is becoming suspect. The relationship is however of extreme importance in most breeding programmes, since most programmes are driven by the selection of superior growth trees, and should a negative relationship exist, this selection could be to the detriment of other characteristics. It would however seem that the more studies are done on the effect of growth rate on fibre and wood properties, the more controversial the conclusions. The relationship is confusing, mainly due to the complexity caused by the various factors that impact on both wood and tree growth (Zobel and Van Buijtenen, 1989).

Early work indicated that a radial increase in density occurred irrespective of growth rate, but it was shown that faster growing trees formed lighter wood compared to slow growing trees, except near the pith where the weights were similar irrespective of species or growth rate (Banks and Schwegmann, 1957). The same authors also concluded that the aggregate density of faster growing trees was higher than slower

growing trees due to a higher proportion of mature wood. These finding were confirmed by Joransen (1960), although he expressed concern about the generalization of the statement.

These findings were again confirmed by Zobel (1971), utilizing *P. taeda*, when it was concluded that it was possible to have high growth rate with either high or low specific gravity, since there was no close genetic correlation between growth rates or specific gravity. Working with *P. radiata* Burdon and Maddern Harris (1972) and Nicholls and Wright (1976) found a negative correlation between diameter growth and density, but found a positive correlation between height growth and density, when neglecting the first five growth rings. Utilising 52 year old *P. radiata* an inverse relation between tree volume and basic density was found, and an indication that up to 40% of the variation in tree density could be attributed to size (Cown and McConchie, 1980). They compiled a correlation matrix for the variables studied, which is presented in Table 5.3.

In Table 5.3 the negative correlation between the growth parameters and the wood properties confirm most of the findings from other studies, as mentioned. The positive correlation between tracheid length and the growth parameters is however encouraging, since most breeding programmes have been focussing on the improvement of growth rate, thus could have been indirectly improving tracheid length. These findings should however be seen in the context of the age of the material and species being utilised, and should not be seen as a general trend.

Table 5.3: Wood properties correlation matrix for 52 year old *Pinus radiata* (Cown and McConchie, 1980).

Variable	Density			Tracheid length	Heartwood	Moisture content	Yield
	Green	Air-dry	Basic				
DBH	-0.25	-0.50	-0.53	0.30	-0.12	0.42	-0.41
Volume	-0.25	-0.6*	-0.63*	0.17	-0.23	0.53	-0.52
Green density		0.48	0.41	-0.28	-0.70*	0.14	-0.16
Air dry density			0.99**	-0.11	0.38	-0.86**	0.85**
Basic density				-0.10	0.35	-0.84**	0.83**
Tracheid length					0.26	-0.04	0.08
Heartwood						-0.79**	0.81**
Moisture content							-0.99**

* - significant at 5% level ** - significant at 1% level

In his review of literature, Dinwoodie (1961) concluded that although the opinions differ, the majority tends to lean towards a negative correlation between tracheid length and ring width, but there seems to be a positive correlation between length and growth rate.

Findings of Barnes *et al*., (1994), working with *P. patula* indicated a number of genetic correlations between various parameters assessed at breast height across two sites. These are presented in Table 5.4.

Table 5.4: Summary of correlations between various parameters assessed at breast height (Barnes et al., 1994).

	Ring width		Basic density		Tracheid length	
	Site 1	**Site 2**	**Site1**	**Site2**	**Site 1**	**Site 2**
Ring width			-0,45	-0,51	-0,34	-0,07
Basic density					-0,34	0,06
Tracheid length						

It can be seen from Table 5.4 that the correlations vary from site to site, not only in magnitude, but also in relationship. No standard errors have however been indicated. These findings have also been confirmed by King *et al*., (1998) from work done on *Tsuga heterophylla.*

The same findings were confirmed by Boden (1982) working with *P. patula*, *P. taeda* and *P. elliottii* where he found both height and diameter growth negatively correlated with density.

From the above, it can be seen that the findings are controversial, and Zobel and Talbert (1984) attribute the controversy to an inability to recognise within-tree variability, in that when comparisons are made, distinctions are not made between the juvenile wood and the mature wood, resulting in comparisons of juvenile wide ringed wood and mature narrow ringed wood.

This has however been done in one of the most comprehensive study on correlations between various growth and wood and fibre characteristics done by Hodge and Purnell (1993). The authors estimated genetic and phenotypic correlations between various parameters, after distinguishing between juvenile and mature portions. Some of the correlations found are reflected in Table 5.5. Only the correlations with growth have been presented where:

Coreden= density of the whole core

Matden= density of the "mature" portion of increment core i.e. rings greater than transition age.

Juvden= density of the "juvenile" portion of increment core i.e. rings less than transition age.

Radius= average annual radial growth increment.

Radmat= average annual radial growth increment of "mature" rings.

Table 5.5: Estimated genetic correlations (above diagonal) and tree phenotypic correlations for selected wood quality and growth traits for *Pinus elliottii* (Hodge and Purnell, 1993).

	Coreden	***Matden***	***Juvden***	***Radius***	***Radmat***	***Radjuv***
Coreden	1	0.36 (0.51)	1.13 (1.38)	0.47 (0.90)	0.10 (0.50)	1.12 (2.13)
Matden	0.71	1	0.45 (0.53)	0.26 (0.48)	-0.54 (0.44)	0.26 (0.73)
Juvden	0.91	0.68	1	0.39 (0.80)	-0.08 (0.50)	1.16 (2.17)
Radius	-0.07	-0.05	-0.04	1	0.84 (0.65)	0.96 (1.21)
Radmat	0.14	-0.21	-0.00	0.69	1	1.29 (1.64)

* The standard errors for the genetic correlations are given in parentheses.

It is interesting to observe the high correlation between the juvenile core density and the whole core density. This can however be attributed to a higher proportion of juvenile wood and the picture might change when the proportions of juvenile and mature wood are equal. The large standard errors indicated however justifies further investigation since such large errors makes the estimates questionable.

Utilising eleven 16-year old *P. radiata* clones, it was concluded that growth rate had little influence on the cross-sectional dimensions of tracheids. The authors also found that the genetic control of tracheid dimensions was far greater than the environmental influence of the site studied (Evans *et al.*, 1997).

It has been postulated that by controlling the growth rate, the relative proportion of thick walled latewood fibres will be influenced (Corson, 1999). It was found that by increasing the growth rate, the proportion of latewood decreased.

Although phenotypic and genotypic correlations are essential to determine the effect of selection on various traits, it would seem that these correlations differ from study to study, and that it would be difficult to make general assumptions about the correlations between traits.

1.3 Selection index

In order to determine the value or worth of individuals or families within a breeding population that would be suitable for utilisation and incorporation into advanced generation breeding populations, the individuals or families must be evaluated in terms of their suitability for further breeding. A method of defining this suitability is by means of selection indices, which gives a value for the individual or family relative to the other individuals or families from which the selections will be made. These values are commonly referred to as breeding values.

A selection index can be compiled for a single trait or for multiple traits, and can incorporate both the individual and family information, so-called combined index. The indices can also be adapted to incorporate similar traits across a number of sites (Burdon, 1979). In its simplest form for a single trait, calculating the breeding value for an individual tree, and incorporating family information, these indices look as follows (Cotterill and Dean, 1990):

$$I = \hat{P} + b\overline{F}_x$$

Where: I = combined index value

$\hat{P}$ = individual tree block (replication) adjusted value for trait of interest

b = index or weighting coefficient

$\overline{F}_x$ = mean value for the x^{th} family for the trait of interest to which the individual tree belongs

The index or weighting coefficient, used to weigh the family mean is calculated as follows (Cotterill and Dean, 1990):

$$b = \frac{r(1-h^2)}{1-r} * \frac{n}{1+(n-1)r} * h^2$$

Where: r = the coefficient of relationship between progeny

h^2 = individual heritability

n = number of progeny per family

Various more complex indices are available for the determination of breeding values such as Best Linear Prediction (BLP) and Best Linear Unbiased Prediction (BLUP) (White and Hodge, 1989), but for the purpose of this study, the so-called Smith-Hazel indices, will be used. These indices has the characteristic and advantage that it takes into account the heritability, phenotypic and genotypic correlations among traits, in such a way that it would maximize efficiency of selection.

The Smith-Hazel index could then be defined as follows (Cotterill and Dean, 1990):

$$[P]b = [A]a ,$$

Where: $[P]$ = the phenotypic variance and covariance matrix

b = vector of index coefficient

$[A]$ = the genotypic variance and covariance matrix

a = vector of economic weights

The index coefficients (b) are calculated in order to maximize the genetic gain for a variable H, which is the total genetic merit.

$$H = a_1G_1 + a_2G_2 + ...a_nG_n$$

Where: a = economic weight

G = The breeding value for each trait

By means of a linear model, the genetic gain in H is aimed at maximising the correlation (r_{IH}) between the index I and the breeding merit (Cotterill and Jackson, 1985).

Various methods for the determination of the economic weights associated with the selection indices are available, such as (Cotterill and Jackson, 1985; Cotterill and Dean, 1990):

a) Equal emphasis
b) Desired gain
c) Partial regression
d) Juvenile – mature correlation

Each of these methods has advantageous and disadvantageous, mainly depending on the information available to the breeder. In this study equal emphasis was used, mainly for illustrative purposes, but the economic weighting of parameters should receive much emphasis when selection indices are applied.

Utilising the Smith-Hazel indices, a single trait selection index, incorporating individual and family information, can be expanded to include multiple traits, as follows (Cotterill and Dean, 1990):

$$I = b_1\hat{P}_1 + b_2\hat{P}_2 + b_3\overline{F}_1 + b_4\overline{F}_2 \ldots$$

Where:
I = combined index value
b_x = weighting coefficients
$\hat{P}_1$ = block adjusted individual value for trait 1
$\hat{P}_2$ = block adjusted individual value for trait 2
$\overline{F}_1$ = family mean for trait 1
$\overline{F}_2$ = family mean for trait 2

As mentioned, the use of the Hazel-Smith equation is merely for illustrative purposes to evaluate the use of a selection index.

2. Materials and methods

2.1 Heritability for growth on a single site

In Section 1.1 it has been indicated that the ratio between the genotypic variance and the total phenotypic variance i.e. V_G/V_P is known as the broad-sense heritability and indicates the extent to which an individual's phenotype is determined by its genetic make-up. The ratio V_A/V_P, known as the narrow-sense heritability, indicates the extent to which phenotypes are determined by the genes transferred from the parents.

The determination of the various variance components, using the expected mean squares calculated from the analysis of variance (ANOVA) for an individual site (Table 3.4, Chapter 3), is done as follows:

$$\sigma^2_{\text{error}} = \sigma^2_{\text{w}} = MS_6$$

$$\sigma^2_{\text{rep x fam(set)}} = \sigma^2_{\text{r*f(s)}} = \frac{MS_5 - MS_6}{n}$$

$$\sigma^2_{\text{fam(set)}} = \sigma^2_{\text{f(s)}} = \frac{MS_4 - MS_5}{nr}$$

$$\sigma^2_{\text{set x rep}} = \sigma^2_{\text{sxr}} = \frac{MS_3 - MS_5}{nf}$$

$$\sigma^2_{\text{set}} = \sigma^2_{\text{s}} = \frac{MS_2 + MS_5 - MS_3 - MS_4}{nrf}$$

$$\sigma^2_{\text{reps}} = \sigma^2_{\text{r}} = \frac{MS_1 - MS_5}{nfs}$$

Where:

MS_1	=	Mean square for replications
MS_2	=	Mean square for sets
MS_3	=	Mean square for sets x replication interaction
MS_4	=	Mean square for families within sets
MS_5	=	Mean square for the replication with families within set interaction
MS_6	=	Mean square for within plot error
r	=	number of replications
s	=	number of sets
f	=	number of families within set

And:

σ^2_{error}	=	variance associated with the within plot error
$\sigma^2_{r*f(s)}$	=	variance associated with the interaction between the replication and families within sets
$\sigma^2_{f(s)}$	=	variance associated with the families within sets
σ^2_{sxr}	=	variance due to the interaction between sets and replications
σ^2_{s}	=	variance due to sets
σ^2_{r}	=	variance due to replications

Since some of the families originated from the same trials, and therefore could be related, the coefficient of relationship for the estimation of heritability figures were used as ⅓ (Squillace, 1974) as apposed to ¼, usually recommended for use with open-pollinated (half-sib) progeny tests (Falconer, 1989). This would thus result in the additive variance being three times the family variance.

The formula used for the calculation of heritability values for a **single site** was as follows:

Individual tree heritability:

$$h^2_i = \frac{\sigma^2_A}{\sigma^2_P} = \frac{3 * \sigma^2_f}{\sigma^2_w + \sigma^2_{r*f(s)} + \sigma^2_{f(s)}}$$

Where:
- σ^2_f = family variance
- σ^2_p = total phenotypic variance
- σ^2_A = additive variance
- σ^2_w = within plot variance
- $\sigma^2_{r*f(s)}$ = replications * family within set variance / plot variance
- $\sigma^2_{f(s)}$ = family within set variance

The family heritability on a **single site** was calculated using the following formula:

$$h^2_{fam} = \frac{\sigma^2_f}{\sigma^2_P} = \frac{\sigma^2_{f(s)}}{\sigma^2_w + \frac{\sigma^2_{r*f(s)}}{nr} + \frac{\sigma^2_{f(s)}}{r}}$$

Where:
- σ^2_f = family variance
- σ^2_p = total phenotypic variance
- σ^2_w = within plot variance
- $\sigma^2_{r*f(s)}$ = replications * family within set variance / plot variance
- $\sigma^2_{f(s)}$ = family within set variance
- n = number of trees per family
- r = number of replications

2.2 Heritability for growth across sites

Taking into account that the plot means for growth was used, rather than between plot error and variance, the mean squares from the analysis of variance (ANOVA) (Table 3.5, Chapter 3) for the across site analysis, are utilised to determine the variance components as follows:

$$\sigma^2_{\text{error}} = \sigma^2_{w} = MS_8$$

$$\sigma^2_{\text{fam(set) x location}} = \sigma^2_{f(s)l} = \frac{MS_7 - MS_8}{r}$$

$$\sigma^2_{\text{fam(set)}} = \sigma^2_{f(s)} = \frac{MS_6 - MS_7}{rl}$$

$$\sigma^2_{\text{set x rep(loc)}} = \sigma^2_{sr(l)} = \frac{MS_5 - MS_8}{f}$$

$$\sigma^2_{\text{set x location}} = \sigma^2_{ls} = \frac{MS_4 - MS_5 - MS_7 + MS_8}{rf}$$

$$\sigma^2_{\text{set}} = \sigma^2_{s} = \frac{MS_3 - MS_4 - MS_6 + MS_7}{rfl}$$

$$\sigma^2_{\text{reps(location)}} = \sigma^2_{r(l)} = \frac{MS_2 - MS_5}{fs}$$

$$\sigma^2_{\text{location}} = \sigma^2_{l} = \frac{MS_1 - MS_2 - MS_4 + MS_5}{fsr}$$

Where: MS_1 = Mean square for location

MS_2 = Mean square for replications within location

MS_3 = Mean square for sets

MS_4 = Mean square for sets x location interaction

MS_5 = Mean square for replication (location) x set

MS_6 = Mean square for family within set

MS_7 = Mean square for family within set x location

MS_8 = Mean square for between plots

l = number of locations

r = number of replications per location

s = number of sets

f = number of families per set

Once the variance components have been calculated, the individual tree heritability value, using plot mean volume, was calculated as:

Individual tree heritability:

$$h^2_i = \frac{\sigma^2_A}{\sigma^2_P} = \frac{3 * \sigma^2_f}{\sigma^2_w + \sigma^2_{l*f(s)} + \sigma^2_{f(s)}}$$

Family heritability value, using plot means, was however calculated using the following formula (Wright, 1976; Zobel and Talbert, 1984):

$$h^2_{fam} = \frac{\sigma^2_f}{\sigma^2_P} = \frac{\sigma^2_{f(s)}}{\frac{\sigma^2_w}{nrl} + \frac{\sigma^2_{l*f(s)}}{l} + \sigma^2_{f(s)}}$$

Where: σ^2_f = family variance

σ^2_A = additive variance

σ^2_p = total phenotypic variance

$\sigma^2_{l*f(s)}$ = variance due to interaction between location and families within sets

σ^2_w = within plot variance

$\sigma^2_{r*f(s)}$ = replications * family within set variance / plot variance

$\sigma^2_{f(s)}$ = family within set variance

n = number of trees per family

r = number of replications

ℓ = number of locations

2.3 Heritability for wood and fibre properties

2.3.1 Individual site

The calculation of the variance component from the analysis of variance (ANOVA, Table 4.1, Chapter 4) is done as follows:

$$\sigma^2_{error} = \sigma^2_w = MS_2$$

$$\sigma^2_{fam} = \sigma^2_f = \frac{MS_1 - MS_2}{n}$$

Where: MS_1 = Mean square for family

MS_2 = Mean square for error

n = number of trees per family

After the determination of the variance components, the following formula were used for the determination of the heritability estimates for the gravimetric density, area weighted density and area weighted fibre morphological characteristics.

For individual tree heritability:

$$h^2_{ind} = \frac{\sigma^2_A}{\sigma^2_P} = \frac{3 * \sigma^2_f}{\sigma^2_w + \sigma^2_f}$$

And for family heritability:

$$h^2_{fam} = \frac{\sigma^2 f}{\sigma^2 P} = \frac{\sigma^2 f}{\frac{\sigma^2 w}{n} + \sigma^2 f}$$

Where: σ^2_A = additive variance

σ^2_p = total phenotypic variance

σ^2_w = error variance

σ^2_f = family variance

n = number of trees per family

2.3.2 Across sites

Prior to the calculation of heritability estimates the various variance components from the analysis of variance (ANOVA) as depicted in Table 4.2 was done as follows:

$$\sigma^2_{error} = \sigma^2_w = MS_4$$

$$\sigma^2_{loc*fam} = \sigma^2 = \frac{MS_3 - MS_4}{n}$$

$$\sigma^2_{fam} = \sigma^2_f = \frac{MS_2 - MS_3}{nl}$$

$$\sigma^2_{loc} = \sigma^2_l = \frac{MS_1 - MS_3}{nf}$$

Where: MS_4 = Mean square for error

MS_3 = Mean square associated with the interaction between family and location

MS_2 = Mean square for family

MS_1 = Mean square for locations

n = number of trees per family

f = number of families per locations

l = number of locations

Once the variance components were calculated, the heritability estimates were calculated as follows:

For individual tree heritability:

$$h^2_{ind} = \frac{\sigma^2_A}{\sigma^2_P} = \frac{3 * \sigma^2_f}{\sigma^2_w + \sigma^2 + \sigma^2_f}$$

And for family heritability:

$$h^2_{fam} = \frac{\sigma^2_f}{\sigma^2_P} = \frac{\sigma^2_f}{\frac{\sigma^2_w}{nl} + \frac{\sigma^2}{l} + \sigma^2_f}$$

Where:

σ^2_A = additive variance

σ^2_p = total phenotypic variance

σ^2_w = error variance

σ^2 = plot variance (location x family interaction variance)

σ^2_f = family variance

σ^2_l = variance due to location differences

n = number of trees per family

ℓ = number of locations

The standard error associated with the individual tree and family heritability estimates for all properties were calculated using the following (Wright, 1976):

$$\sigma_{h^2} = \frac{\left(1 - h_x{}^2/3\right)\left[1 + (nrl)h_x{}^2/3\right]}{nrl\left[(f-1)/2\right]^{1/2}}$$

Where: σ_{h^2} = Standard error of the individual tree/ family heritability estimate

$h_x{}^2$ = The individual tree/ family heritability estimate

n = The number of trees per plot

r = The number of replications per site

ℓ = The number of sites

f = The number of families

The above equation was adapted depending on single site or across site heritability estimates being calculated.

2.4 Phenotypic and genotypic correlations

The phenotypic correlations (r_p) were determined using the same method as the Spearman correlation coefficient used in Chapter 3 and 4:

$$r_p = \frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}$$

Where S_{xy} = covariance between paired observations

S_{xx} = variance for factor x

S_{yy} = variance for factor y

The genetic correlations were determined in the same manner, but instead of phenotypic covariance and variance components, genetic covariance and variance components were utilised. In order to determine the genetic covariance and

variances, PROC IML in SAS® was used. Thus the genetic correlations (r_G) were determined as follows (Hodge and Purnell, 1993):

$$r_G = \frac{\sigma_{g_{xy}}}{\sqrt{\sigma^2{}_{g_x}\sigma^2{}_{g_y}}}$$

Where $\sigma_{g_{xy}}$ = genetic covariance between paired traits

$\sigma^2{}_{g_x}$ = genetic variance for trait x

$\sigma^2{}_{g_y}$ = genetic variance for trait y

2.5 Selection indices

In Section 1.3 it has been shown that the Smith-Hazel index is defined as follows (Cotterill and Dean, 1990):

$$[P]b = [A]a ,$$

Where: $[P]$ = the phenotypic variance and covariance matrix

b = vector of index coefficient

$[A]$ = the genotypic variance and covariance matrix

a = vector of economic weights

Thus solving the equation, the weighting coefficients (b), would be determined as follows:

$$[b] = [P]^{-1}[A][w]$$

The aim of solving this equation would be to calculate the weighting coefficients for individual tree breeding value calculation. For each site, the individual tree information was adjusted by standardising the individual tree value with the trial mean for the variable of interest. This aims at removing some of the site effect, and

can be seen as a site adjustment (Cotterill *et al*., 1983). The following parameters were determined (Cotterill and Dean, 1990):

a) Phenotypic standard deviations, with the following notation:

$\sqrt{P_{11}}$ = phenotypic standard deviation for trait1

$\sqrt{P_{22}}$ = phenotypic standard deviation for trait2

$\sqrt{P_{33}}$ = phenotypic standard deviation for trait3

b) Genotypic variance components:

As determined in Section 2.1 and Section 2.3 above. The heritability values can also be expressed as:

$$h^2_1 = \frac{A_{11}}{P_{11}}$$

Where: h^2_1 = the individual heritability value for trait1

A_{11} = genotypic variance for trait1

P_{11} = phenotypic variance for trait1

Thus for trait1:

$$A_{11} = h^2_1 * P_{11}$$

c) Phenotypic correlations:

Utilising the phenotypic correlation calculated in Section 2.4 above, the phenotypic covariance between traits was calculated as follows:

$$r_{p_{12}} = \frac{P_{12}}{\sqrt{P_{11}} * \sqrt{P_{22}}}$$

Where: $r_{p_{12}}$ = phenotypic correlation between trait1 and trait2

P_{12} = phenotypic covariance between trait1 and trait2

P_{11} = phenotypic variance for trait1

P_{22} = phenotypic variance for trait2

Thus for the calculation of the phenotypic correlation between trait1 and trait2:

$$P_{12} = r_{p_{12}} * \sqrt{P_{11}} * \sqrt{P_{22}}$$

d) Genotypic covariance:

Following the same reasoning as for the phenotypic correlation between traits, it can be shown that the genotypic correlation between traits can be calculated as:

$$G_{12} = r_{g_{12}} * \sqrt{G_{11}} * \sqrt{G_{22}}$$

Where: $r_{g_{12}}$ = genotypic correlation between trait1 and trait2

G_{12} = genotypic covariance between trait1 and trait2

G_{11} = genotypic variance for trait1

G_{22} = genotypic variance for trait2

In some instances, no genetic correlation was calculated, due to family variance components being zero. In these instances, the genetic correlation was also set equal to zero.

e) Economic weights:

In this study equal emphasis (Cotterill and Jackson, 1985) weights were used, expressed as the inverse of the phenotypic standard deviation for the various traits. This method was used, mainly due to the lack of more exact quantitative economical data (Costa E Silva *et al*., 1998), and due to the absence of data to utilise other methods such as juvenile-mature correlations.

Thus: $W_x = \frac{1}{\sigma_x}$

Where: W_x = the economic weight associated with trait x

σ_x = the phenotypic standard deviation of trait x

By means of matrices, the selection index coefficients (b_x) were then determined by solving the following:

$$\begin{pmatrix} b_1 \\ b_2 \\ b_3 \end{pmatrix} = \begin{pmatrix} P_{11} & P_{12} & P_{13} \\ P_{21} & P_{22} & P_{23} \\ P_{31} & P_{32} & P_{33} \end{pmatrix}^{-1} \begin{pmatrix} G_{11} & G_{12} & G_{13} \\ G_{21} & G_{22} & G_{23} \\ G_{31} & G_{32} & G_{33} \end{pmatrix} \begin{pmatrix} W_1 \\ W_2 \\ W_3 \end{pmatrix}$$

In the cases where the phenotypic or genotypic correlations were not calculated due to negative variance components, the correlations were set to zero.

For the calculation of family coefficient the following was used (Cotterill and Dean, 1990):

$$b = \frac{r(1-h^2)}{1-r} * \frac{n}{1+(n-1)r} * h^2$$

Where: r = the coefficient of relationship between progeny

h^2 = individual heritability

n = number of progeny per family

The following traits were incorporated as an example to evaluate the use of a selection index for the individual trees within the top thirty families:

1) Density – gravimetric density

2) Growth – expressed as individual tree volume growth

3) Runkell ratio – this ratio combines the lumen diameter and wall thickness

Latewood percentage was omitted from the analysis due to the co-linearity with density.

The equation used for the calculation of breeding values, incorporating individual tree and family information, are then as follows:

$$I = b_1\hat{P}_D + b_2\hat{P}_G + b_3\hat{P}_{RR} + b_4\overline{F}_D + b_5\overline{F}_G + b_6\overline{F}_{RR}$$

With:

I = the combined index value

b_1 = the selection coefficient for density of an individual tree

b_2 = the selection coefficient for growth of an individual tree

b_3 = the selection coefficient for Runkell ratio of an individual tree

b_4 = the selection coefficient for density of the family

b_5 = the selection coefficient for growth of the family

b_6 = the selection coefficient for Runkell ratio of the family

$\hat{P}_D$ = the adjusted value for the density of the individual tree

$\hat{P}_G$ = the adjusted value for the growth of the individual tree

$\hat{P}_{RR}$ = the adjusted value for the Runkell ratio of the individual tree

$\overline{F}_D$ = the family mean for gravimetric density

$\overline{F}_G$ = the family mean growth

$\overline{F}_{RR}$ = the family mean Runkell ratio

By substituting the values into the equation, the index value per individual tree can be calculated, utilising individual and family information.

3. Results

3.1 Heritability

3.1.1 Heritability of growth

Heritability estimates were determined for the growth assessments at ages five and eight (Table 5.6). From Table 5.6 it can be observed that the family heritability estimates are higher than those for the individual tree. This is usually the case, since greater gains can be achieved by selecting the best family, which is based on a number of observations rather than an individual tree, which is based on a single observation.

Table 5.6: Family (fam) and individual tree (ind) heritability estimates for volume at five and eight years of a *Pinus patula* series established across six sites (standard errors presented in parenthesis).

	Five year growth		**Eight year growth**	
	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)
Site1	0,44 (0,03)	0,14 (0,08)	0,54 (0,03)	0,19 (0,08)
Site2	0,35 (0,03)	0,14 (0,09)	0,34 (0,03)	0,12 (0,09)
Site3	0,43 (0,03)	0,14 (0,08)	0,37 (0,02)	0,11 (0,07)
Site4	0,57 (0,03)	0,27 (0,08)	0,59 (0,03)	0,26 (0,08)
Site5	0,32 (0,03)	0,08 (0,08)	0,37 (0,03)	0,09 (0,07)
Site6	0,54 (0,02)	0,18 (0,07)	0,44 (0,02)	0,11 (0,07)
Across sites	0,71 (0,05)	0,07 (0,14)	0,68 (0,05)	0,06 (0,14)

When comparing the heritability figures for the two age groups it is interesting to note that for some of the sites, such as Site 2, Site 3 and Site 6 there is a decrease in the heritability estimates with an increase in age. This is somewhat in contradiction with general believe, where there is usually an increase in estimates with age, since it would be expected that the differences between families at an early age would become more pronounced as age increases, since trees inferior in growth at an early age are usually suppressed by superior trees, thus making the differences between families greater.

The standard errors, especially for the individual tree heritability figures would suggest that some care should be taken when using these estimates for further evaluations, and that large differences are possible between individual trees. For the family heritability estimates the standard errors are acceptable and small.

3.1.2 Heritability of density

Heritability for the various density determinations are depicted in Table 5.7. In Section 1.1.2 it has been indicated that the genetic control of specific gravity and density tend to be high, which can also be seen from the heritability estimates listed in Table 5.7. It however has to be stated that some of the estimates are very high, which will be attributed to large differences between families. This can be observed in the high standard errors of the estimates, especially those for the individual tree estimates of the top thirty families.

Table 5.7: Family (fam) and individual tree (ind) heritability estimates for density at eight years of age of the top families in a *Pinus patula* series established across six sites (standard errors in parenthesis).

	Gravimetric density				**Area weighted density**	
	Top 100 families		**Top 30 families**		**Top 30 families**	
	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)
Site1	0,87 (0,04)	0,47 (0,12)	0,90 (0,08)	0,62 (0,23)	0,94 (0,08)	0,91 (0,25)
Site2	0,89 (0,04)	0,53 (0,12)	0,86 (0,07)	0,46 (0,21)	0,87 (0,08)	0,49 (0,23)
Site3	0,12 (0,08)	0,01 (0,03)	0,73 (0,06)	0,22 (0,18)	0,77 (0,06)	0,27 (0,19)
Site4	0,80 (0,04)	0,41 (0,13)	0,89 (0,09)	0,75 (0,26)	0,89 (0,09)	0,72 (0,26)
Site5	0,85 (0,04)	0,40 (0,11)	0,85 (0,07)	0,54 (0,21)	0,86 (0,09)	0,57 (0,28)
Site6	0,84 (0,04)	0,39 (0,11)	0,67 (0,06)	0,20 (0,17)	0,83 (0,08)	0,44 (0,24)
Across site	0,71 (0,06)	0,25 (0,19)	0,65 (0,14)	0,22 (0,25)	0,80 (0,10)	0,43 (0,22)

Looking at the individual tree estimates (narrow sense heritability) it would seem that these values, although high, are with a few exceptions within the range suggested by Hodge and Purnell (1993).

Evaluating the estimates for the various methods of density determination, in general, from the across site heritability figures, there are very little differences between the estimates for the three methods. When evaluating the individual sites however, the most noticeable difference would be found when comparing the estimates of Site 3.

For the analysis of variance for the gravimetric density of the top 100 families, the differences between families are non-significant, which would explain the low heritability values obtained at that site. For the gravimetric and area weighted density of the top 30 families, the differences however proof significant, thus the increase in the heritability values. Based on the heritability estimates it can be concluded that density is highly heritable on a family level and on an individual level on the majority of sites.

3.1.3 Heritability for fibre morphological characteristics of the top thirty families

The heritability figures for the fibre morphological characteristics of the top 30 families are presented in Table 5.8. Due to non-significant differences between families for area weighted wall thickness at Site 5, and lumen diameter and latewood percentage at Site 3, heritability estimates are zero. On an individual site basis it can be seen that significant gains can be made by selecting on a family and individual tree within family basis.

Table 5.8: Family (fam) and individual tree (ind) heritability estimates for area weighted fibre morphological characteristics of the top 30 families at eight years of age in a *Pinus patula* trial series established across six sites (standard errors in parenthesis).

	Wall thickness		**Lumen diameter**		**Runkell ratio**		**Latewood percentage**	
	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)	h^2 (fam)	h^2 (ind)
Site 1	0,95 (0,09)	1,00 (0,26)	0,92 (0,08)	0,34 (0,24)	0,95 (0,09)	0,98 (0,26)	0,91 (0,02)	0,16 (0,06)
Site 2	0,92 (0,08)	0,73 (0,25)	0,86 (0,07)	0,45 (0,22)	0,89 (0,08)	0,59 (0,23)	0,93 (0,04)	0,38 (0,11)
Site 3	0,55 (0,06)	0,10 (0,17)	0	0	0,05 (0,05)	0,01 (0,15)	0	0
Site 4	0,93 (0,09)	1,00 (0,28)	0,86 (0,09)	0,60 (0,26)	0,91 (0,09)	0,85 (0,27)	0,71 (0,02)	0,05 (0,06)
Site 5	0	0	0,57 (0,08)	0,15 (0,24)	0	0	0,84 (0,02)	0,10 (0,05)
Site 6	0,92 (0,09)	0,87 (0,28)	0,91 (0,09)	0,78 (0,27)	0,92 (0,09)	0,87 (0,28)	0,88 (0,02)	0,13 (0,06)
Across site	0,44 (0,02)	0,13 (0,05)	0,81 (0,03)	0,34 (0,10)	0,59 (0,02)	0,22 (0,07)	0,75 (0,03)	0,33 (0,10)

For the across sites estimates, it can be seen that heritability figures on the family level are higher than the individual level. This would indicate that greater gains can be made by selecting on a family basis. It is however a concern that the values are so variable on an individual site basis. This could be attributed to the number of samples taken not being adequate as can be seen that the standard errors, especially for the individual tree heritability estimates are large, thus these values should be interpreted as such. Certain estimates could not be calculated due to negative variance components, and were indicated with "0".

3.2 Correlations between traits

3.2.1 Phenotypic and genotypic correlations between growth and specific gravity

The phenotypic and genotypic correlations between growth and gravimetric density for the top 100 families are presented in Table 5.9. On some sites (0) the genotypic correlations were not calculated due to negative family variance component, since on some sites, non-significant differences between families occurred. This could be due to the fact that the sample size was inadequate to explain the differences between families, resulting in large sampling and standard errors of the estimates.

Table 5.9: Phenotypic and genotypic (in brackets) correlations between growth and gravimetric density of the top 100 families in a *Pinus patula* series established across six sites.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
	Growth	Growth	Growth	Growth	Growth	Growth
Dens	-0,05 (0,04)	-0,13 (-0,04)	-0,02 (0)	-0,11 (-0,32)	-0,13 (-0,12)	-0,24 (-1,18)

Where the correlations were determined, it can be seen that in most cases, with the exception of Site 1, the phenotypic and genotypic correlations between gravimetric density and growth, is negative. The phenotypic correlation reveals a negative correlation. This would imply that on the majority of sites, when phenotypic selections are made based on volume growth, the density would be negatively influenced.

3.2.2 Phenotypic and genotypic correlations between growth, specific gravity and fibre morphological characteristics

The individual site phenotypic and genotypic correlations can be obtained from the author. The results for the across site phenotypic and genotypic correlations are presented in Table 5.10.

Most of the correlations indicate the expected trends, especially for the phenotypic correlations. The greatest differences between correlations occur for the correlations with growth. This would indicate that when selecting for growth only, the effect on other characteristics is difficult to predict.

Table 5.10: Across site phenotypic and genotypic (in brackets) correlations between growth, density and fibre morphological characteristics for the top thirty families in a *Pinus patula* trial series established across six sites.

	GROWTH	RR	EWPERS	LWPERS	BDENS	WDENS	GDENS
GROWTH		0,11 (0,21)	-0,20 (-0,26)	0,20 (0,25)	-0,23(-0,04)	-0,51 (-0,15)	-0,63 (-0,18)
RR			-0,77 (-0,55)	0,77 (0,55)	0,78 (0,96)	0,75 (1,14)	-0,64 (0,84)
EWPERS				-1,00 (-1,00)	-0,78 (-0,74)	-0,64 (-0,75)	0,55 (-0,68)
LWPERS					0,77 (0,74)	0,64 (0,75)	-0,55 (0,68)
BDENS						0,82 (1,15)	-0,46 (1,14)
WDENS							-0,16 (0,98)
GDENS							

RR = area weighted Runkell ratio, EWPERS = early wood percentage, LWPERS = latewood percentage, BDENS= gravimetric density of top 30 families, WDENS = area weighted density of top 30 families, GDENS= gravimetric density of the top 100 families

Looking at the results from Table 5.9 and Table 5.10 it can be concluded, in general, that selecting for growth will lead to a slight decrease in density, which will lead to an increase in lumen diameter and early wood percentage, and a decrease in wall thickness and late wood percentage. This will in turn improve the Runkell ratio which should improve the collapsibility of the fibre.

3.3 Index coefficients

The weighting coefficients (b_x) for the three traits including the individual tree and family information for the various sites are listed in Table 5.11.

Table 5.11: Weighting coefficients for selection indices for a *Pinus patula* trial series established across six sites.

	Individual tree coefficients			Family coefficients		
	Density (b_1)	Growth (b_2)	Runkell ratio (b_3)	Density (b_4)	Growth (b_5)	Runkell ratio (b_6)
Site 1	1,390	-0,317	1,259	0,091	0,171	0,022
Site 2	-0,722	1,947	3,346	0,274	0,116	0,266
Site 3	0,162	-0,085	-0,089	0,218	0,108	0,011
Site 4	4,284	3,129	2,391	0,213	0,204	0,135
Site 5	0,647	0,090	-0,226	0,249	0,083	0,000
Site 6	2,997	1,474	1,465	0,258	0,103	0,118

By substituting the above coefficient for individual tree and family mean values, gains were calculated. From the gains calculated, it can be seen that large genetic gains can be achieved by selecting certain individual trees.

From an analysis of variance on the breeding values calculated, it can be seen that significant differences occurs between sites and between families for the breeding values calculated. It is evident that in order to maximise the gains possible, the forward selections should mainly be done on Site 4, Site 6, Site 2 and that selections at Site 1, Site 5 and Site 3 should be done with caution, since using this scenario very low breeding values were obtained on these sites.

Due to the significant differences between families for breeding values, it is envisaged that especially for the family selections and selections within family (Zobel and Talbert, 1984), that more selections will be taken from specific families, rather than a number of selections from a number of families. It can therefore be seen that combining traits into a multi-trait selection index is feasible, but this is by no means the only option available. Other methods of determining the breeding value of an individual or family is available and probably more suitable, such as Best Linear Prediction (BLP) (White and Hodge, 1989), but the scope of these applications did not form part of this study.

References: Chapter 5

Banks, C.H. and Schwegmann, L.M. (1957). The physical properties of fast – and slow – grown *Pinus patula* and *P. taeda* from South African sources. *The Journal of the South African Forestry Association* **30,** 45-59.

Barnes R.D., Mullin, L.J. and Battle, G. (1992a). Genetic control of fifth year traits in *Pinus patula* Schiede and Deppe. *Silvae Genetica* **41(4-5)**, 242-248.

Barnes R.D., Mullin, L.J. and Battle, G. (1992b). Genetic control of eigth year traits in *Pinus patula* Schiede and Deppe. *Silvae Genetica* **41(4-5)**, 242-248.

Barnes, R.D., Birks, J.S., Battle, G. and Mullin, L.J. (1994). The genetic control of ring width, wood density and tracheid length in the juvenile core of *Pinus patula*. *South African Forestry Journal* **169,** 15-20.

Boden, D.I. (1982). The relationship between timber density of the three major pine species in the Natal midlands and various site and tree parameters. *Wattle Research Institute – Annual report 1981-1982*, 120-126.

Burdon, R.D. (1979). Generalisation of multi-trait selection indices using information from several sites. *New Zealand Journal of Forestry Science* **9**, 145-152.

Burdon, R.D. and Maddern Harris, J. (1972). Wood density in radiata pine clones on four different sites. *New Zealand Journal of Forestry Science* **3 (3),** 286-303.

Corson, S.R. (1999). Tree and fibre selection for optimal TMP quality. *Appita Journal* **52(5),** 351-357.

Costa E Silva, J., Wellendorf, H. and Pereira, H. (1998). Clonal variation in wood quality and growth in young sitka spruce (*Picea sitchensis* (BONG.)CARR): Estimation of quantitative genetic parameters and index selection for improved pulpwood. *Silvae Genetica* **47 (1)**, 20-33.

Cotterill, P.P, Correll, R.L and Boardman, R. (1983). Methods of estimating the average performance of families across incomplete open-pollinated progeny tests. *Silvae Genetica* **32 (1-2)**, 28-32.

Cotterill, P.P. and Dean, C.A. (1990). *Successful tree breeding with index selection*. CSIRO Publications, Australia. 80 pp.

Cotterill, P.P and Jackson, N. (1985). On index selection 1. Methods of determining economic weight. *Silvae Genetica* **34 (2-3)**, 56-63.

Cown, D.J. and McConchie, D.L. (1980). Wood property variations in an old-crop stand of radiata pine. *New Zealand Journal of Forestry Science* **10(3),** 508-520.

Dinwoodie, J. M. (1961). Tracheid and fibre length in timber – A review of literature. *Forestry* **34 (2),** 125 -144.

Evans, R., Kibblewhite, R.P. and Stringer, S. (1997). Kraft pulp fibre property prediction from wood properties in eleven radiata pine clones. *Appita Journal* **50 (1),** 25-33.

Falconer, D.S. (1989). Introduction to Quantitative Genetics – 3rd edition. Longman Scientific and Technical: 340 pp.

Gapare W.J., Hodge, G.R. and Dvorak, W.S. (2001). Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia and South Africa. *Forest Genetics* **8(2),**159-170.

Gwaze, D.P., Bridgwater, F.E., Byram, T.D. and Lowe, W.J. (2001). Genetic parameter estimates for growth and wood density in loblolly pine (*Pinus taeda* L.). *Forest Genetics* **8(1)**, 47-55.

Hodge, G.R. and Dvorak, W.S. (1999). Genetic parameters and provenance variation of *Pinus tecunumanii* in 78 international trials. *Forest Genetics* **6(3),** 157-180.

Hodge, G.R. and Purnell, R.C. (1993). Genetic parameter estimates for wood density, transition age and radial growth of slash pine. *Canadian Journal of Forestry Research* **23,** 1881-1891.

Hodge, G.R. and White, T.L. (1992). Genetic parameter estimates for growth traits at different ages in slash pine and some implications for breeding. *Silvae Genetica* **41(4-5)**, 252-262.

Joransen, P.N. (1960). Pulpwood properties: response of processing and of paper quality to their variation. Forest biology subcommittee no 2 on tests ad quality objectives. *Tappi Journal* **43 (11),** 40-64.

King, J.N., Cartwright, C., Hatton, J. and Yanchuk A.D. (1998). The potential of improving western hemlock pulp and paper quality – genetic control and interrelationships of wood and fibre traits. *Canadian Journal for Forest Research* **28**, 863-870

Koch, P. (1973). Utilization of the Southern pines. Volume 1 – The raw material. Agriculture Handbook no. 420. US Department of Agriculture Forest Service.US Government Printing Office, Washington.

Moura, V.P.G. and Dvorak, W.S. (1998). Provenance and family performance of *Pinus tecunumanii* at 12 years of age in the Cerrado region of Brazil. *Forest Genetics* **5(3)**, 137-145.

Muneri, A. and Balodis, V. (1998). Variation in wood density and tracheid length in *Pinus patula* grown in Zimbabwe. *Southern African Forestry Journal* **182**, 41-50.

Nicholls, J.W.P. and Wright, J.P. (1976). The effect of environmental factors on wood characterisitics – The influence of climate and site on young *Pinus radiata* material. *Canadian Journal of Forestry Research* **6**, 113-121.

Payn, K.G. (2001). Heritability of wood density and the occurrence of genotype x environment interaction in a progeny trial series of *Pinus patula*. M.Sc thesis. University of Natal – Durban. 69 pp.

Shelbourne, T., Evans, R., Kibblewhite, P. and Low, C. (1997). Inheritance of tracheid transverse dimensions and wood density in radiata pine. *Appita Journal* **50 (1)**, 47-50.

Smith, W.J. (1966). The heritability of fibre characteristics and its application to wood quality Improvement in Forest Trees. *Silvae Genetica* **16 (2)**, 41-50.

Squillace, A.E. (1974). Average genetic correlations among offspring from open-pollinated forest trees. *Silvae Genetica* **23 (5)**, 149-156.

White, T. L. and Hodge, G.R. (1989). Predicting breeding values with application in forest tree improvement. Kluwer Academic publishers.367pp.

Wright, J.W. (1976). Introduction to Forest genetics. Academic Press. 463 pp.

Zobel, B. (1971). Genetic manipulation of wood of Southern pines including chemical characteristics. Wood Science and Technology 5. Springer-Verlag 1971, 255-271.

Zobel, B.J. and Jett, J.J. (1995). Genetics of Wood Production. Springer-Verlag. 338pp.

Zobel, B.J. and Talbert, J.T. (1984). Applied forest tree improvement. John Wiley and Sons, Inc., 505pp.

Zobel, B.J. and Van Buijtenen, J.P. (1989). Wood variation: its causes and control. Springer-Verlag, Germany. 363pp

Chapter 6

Conclusions

In this study it has been shown that there are differences for all traits assessed or derived for all sources of variance. Significant differences were found between families for all growth, wood and fibre traits utilised within this study, indicating that these traits can be improved by means of selection. The heritability figures for the various traits also indicate that significant gains can be obtained by selecting on a family and individual tree level. Heritability estimates for growth varied across sites, ranging from 0,32 to 0,57 at five years and 0,34 to 0,59 at eight years for family heritability. The individual tree estimates ranges from 0,08 to 0,27 at five years and from 0,09 to 0,26 at eight years. The standard errors associated with the heritability estimates for growth however indicate that the estimates, especially those of the individual trees should be used with caution. The heritability estimates for density and fibre morphological characteristics on the family and individual tree level are on a number of sites very high, although this is associated with large standard errors.

Significant differences were also indicated between sites for all the growth, wood and fibre properties included in the study. By means of Type-B genetic correlations, it was concluded that certain sites can be grouped together in terms of deployment of families. More in depth analysis of the correlations are however required, since by evaluating and eliminating the families most sensitive to genotype x environment interaction, a number of correlations could be improved. The actual need for regionalisation was not calculated.

The phenotypic and genotypic correlations between the various traits indicated that different correlations exist for various traits across various sites. General conclusions indicated that a positive correlation existed between density, latewood percentage and wall thickness and negative correlation were found between growth and density and density and lumen diameter. This conforms to most findings from other studies. The

correlations between growth and the fibre properties are not conclusive, and vary from site to site.

As an option, utilising a Smith-Hazel selection index, it was shown that a multi-trait selection index could be constructed for growth, density and the Runkell ratio. This was used to calculate breeding values incorporating both individual tree and family information. From the values calculated it can be seen large gains can be made by selecting individual trees and families. Significant differences for the gain values however indicate that certain sites should rather be ignored when selecting since low genetic gains are evident on these sites.

Appendix 1: Summarised analysis of variance (ANOVA) for the individual sites of the *Pinus patula* trial series for five and eight year volume growth.

Table A1.a: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site1.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	4	19.16	4.04 *	10.18	2.08 ns
Set	5	16.72	2.46 *	17.92	3.66 *
Replication x set	20	4.74	5.77 **	4.90	5.81 **
Family (set)	181	2.64	1.88 **	2.85	2.33 **
Rep x fam(set)	724	1.41	1.72 **	1.22	1.45 **
Sampling error	4244	0.82		0.84	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns – non-significant differences

Table A1.b: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site2.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	4	3.7449	0.63 ns	13.13	4.48 **
Set	5	7.4401	1.06 ns	14.46	4.94 **
Replication x set	24	5.9634	8.21 **	2.93	3.63 **
Family (set)	209	2.5819	1.58 **	2.17	1.56 **
Rep x fam(set)	804	1.6327	2.25 **	1.39	1.73 **
Sampling error	4080	0.7263		0.81	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns - non-significant differences

Table A1.c: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site3.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	4	128.4505	10.56 **	45.95	3.36 *
Set	6	19.0383	1.40 ns	16.00	1.17 ns
Replication x set	24	12.1598	14.87 **	13.66	17.02 **
Family (set)	205	2.2937	1.86 **	2.21	1.67 **
Rep x fam(set)	820	1.2332	1.51 **	1.33	1.65 **
Sampling error	4619	0.8176		0.8021	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns - non-significant differences

Table A1.d: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site4.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	3	14.609	1.25 ns	3.78	0.68 ns
Set	5	8.3743	0.65 ns	16.56	2.97 *
Replication x set	15	11.6572	14.67 **	5.58	6.54 **
Family (set)	181	3.1124	2.52 **	2.87	2.75 **
Rep x fam(set)	543	1.235	1.55 **	1.04	1.22 **
Sampling error	3411	0.7945		0.85	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns - non-significant differences

Table A1.e: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site5.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	4	23.5827	3.51 *	13.20	14.53 ns
Set	5	13.0733	1.67 ns	13.39	14.74 *
Replication x set	20	6.7228	7.71**	4.76	5.25 **
Family (set)	181	1.8421	1.53 **	1.86	1.69 **
Rep x fam(set)	724	1.2008	1.38 **	1.10	1.22 **
Sampling error	3815	0.8724		0.91	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns - non-significant differences

Table A1.f: Summarised analysis of variance for five and eight year volume growth of *Pinus patula* at Site6.

		Five year		Eight year	
Source	**df**	**MS**	**F- value**	**MS**	**F- value**
Replication	4	61.4927	4.33 *	21.97	1.96 ns
Set	5	33.1486	2.03 ns	19.62	1.75 ns
Replication x set	20	14.1866	17.52 **	11.22	12.86 **
Family (set)	181	2.6787	2.33 **	2.10	1.90 **
Rep x fam(set)	724	1.1486	1.42 **	1.10	1.26 **
Sampling error	4454	0.8098		0.87	

** - highly significant differences between means x>1%
* - significant differences between means 1% < x < 5%
ns - non-significant differences