# Breeding of advanced generation of *Eucalyptus macarthurii* – growth parameters and development of a near infrared (NIR) calibration model to predict whole tree pulp yield using non destructive cores

**By**

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# GENERAL ABSTRACT

*Eucalyptus macarthurii* is one of the cold tolerant eucalypt species grown in South Africa for pulp and paper. However, little research has been done on this species' growth performance. A study was therefore initiated to: i) analyse growth characteristics of *Eucalyptus macarthurii* at two sites and to calculate genetic parameters (genetic and phenotypic correlations, heritabilities and genetic gains), ii) develop a non-destructive near infrared calibration model to predict whole tree pulp yield of *Eucalyptus macarthurii*, and iii) screen a second generation *Eucalyptus macarthurii* breeding population, using the developed near infrared calibration model on core samples, to predict screened pulp yield and to rank and identify families with superior pulping properties.

*Eucalyptus macarthurii* population growth data (diameter under bark, diameter over bark, bark thickness, bark stripping, height, basic wood density and stem form) were measured at Pinewoods and Vlakkloof sites and their respective genetic parameters calculated. Genotype by environment interaction was found in this population, indicating that different populations should perhaps be developed independently of each other for the two sites. Genetic and phenotypic correlations between diameter over bark and diameter under bark were, 0.96 and 0.98 for Pinewoods and 0.98 and 0.99 for Vlakkloof, respectively. These correlations indicated that selection of diameter over bark would lead to a positive indirect selection for diameter under bark. The heritability estimates also ranged from 0.03 to 0.23 at both sites, which indicated a reasonable response to selection. The predicted gains for all traits found at Pinewoods were higher than those at Vlakkloof for progeny trials E76/P1, except height for progeny trial E76/P2, which was 2.09m at Pinewoods site and 3.52m at Vlakkloof site which showed that, selection for taller trees will be more effective at Vlakkloof site.

A preliminary study was undertaken from eleven second generation trees (2007 tree collection) to investigate if the radial strip core taken at breast height predicts the whole tree wood properties. Correlations found between laboratory Kraft pulping of whole tree wood discs and whole tree NIR spectra with that of the radial strip core NIR spectra were 0.9472 and 0.9506, respectively. These results confirmed that NIR spectra of the radial strip core at breast height predict the whole tree wood properties.

A non-destructive near infrared calibration model using wood samples was obtained from *Eucalyptus macarthurii* felled trees. The wood samples were chipped into wood chips, pulped using Kraft pulping (reference method) and a sub-sample of wood chips of the same trees were ground into sawdust samples and analysed through near infrared spectroscopy for screened pulp yield. The screened pulp yield values obtained from both processes had a narrow screened pulp yield range of 40 to 48%. The *Eucalyptus macarthurii* screened pulp yield values obtained from both processes, as well as from values obtained from other eucalypt species, were subjected to Vision® Software for calibration and validation of the near infrared calibration model.

The results indicated a strong calibration correlation coefficient of 94%, between Kraft pulping and near infrared spectroscopy with a validation coefficient of 89%. The strong correlation and validation coefficient indicated that a reliable non-destructive near infrared model to predict screened pulp yield was successfully developed. The successful development of the valid calibration model required a wider range of other eucalypts species, which improved the development of the model.

The developed calibration model was applied to the second generation breeding population planted in KwaZulu-Natal and Mpumalanga provinces, using wood core samples obtained from standing trees for the prediction of screened pulp yield. The highest screened pulp yield achieved was 48%, which compared well to that found for Kraft pulping, which confirmed the success of the development of the calibration model.

There was a wide scope of growth variation found amongst traits, which will be useful in selecting superior trees for the next generation. The development of the non-destructive near infrared calibration model was a success due to the strong correlation coefficients found between the screened pulp yields obtained from Kraft pulping and near infrared spectroscopy processes, which was achieved by the inclusion of other eucalypt species in the dataset.

The calibration model can be used to select the top performing individual and family trees for the next generation based on screened pulp yield. Tree improvement trials can now be conserved for further breeding, without felling the trees for determination of pulping properties.

# DECLARATION

I, Zama Thandekile Laureen Ndlovu, declare that:

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As research supervisor's we agree to submission of this dissertation for examination:

Signed: …… Date: ……..

**Professor Pangirayi Tongoona (Principal supervisor)**

Signed: …… Date: ……..

**Ms Tammy Swain (Co-supervisor)**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **BSO** | Breeding seed orchard |
| **CSIR** | Council for Scientific and Industrial Research |
| **CTE** | Cold tolerant species |
| **Dbh** | Diameter at breast height |
| **ICFR** | Institute for Commercial Forestry Research |
| **$h^2$** | heritability estimates |
| **MAP** | Mean annual precipitation |
| **m.a.s.l.** | Metres above sea level |
| **MAT** | Mean annual temperature |
| **NIR** | Near infrared spectroscopy |
| **NSW** | New South Wales |
| **$R^2$** | Correlation coefficient |
| **$r_g$** | Genetic correlation coefficient |
| **$r_p$** | Phenotypic correlation coefficient |
| **$r_B$** | Type B genetic correlation coefficient |
| **SAMTMA** | South African Mining Timber Manufacturer's Association. |
| **SEC** | Standard error of calibration |
| **SECV** | Standard error of cross validation |
| **SEP** | Standard error of prediction |
| **SPY** | Screened pulp yield |

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# GENERAL INTRODUCTION

*Eucalyptus* is a large genus of plants which includes over 700 species native to Australia. Many of the eucalypt species are fast growing and produce high value timber of good quality (Darrow, 1996). In South Africa, *Eucalyptus* species are commonly known as gum trees. This genus is important to the South African mining and forestry industries, as they contribute 66.5% of timber grown for pulpwood (Department of Water Affairs, 2007). In order to meet the increasing demand for timber and pulpwood from this source, forestry companies need to increase their outputs. This may be done either by increasing the amount of timber attainable from the existing land base or through the acquisition of additional land (Little and Gardner, 2003; Pallett and Sale, 2004).

In South Africa, present and future land use policies are likely to restrict the conversion of non-afforested land to plantations. Therefore, factors that may contribute to an increase in yield and pulpwood from an existing land base include the use of site-species matching (planting the correct species for the site for maximum growth), tree breeding, clonal propagation, inter-specific hybrids and improved silvicultural practices (Little and Gardner, 2003). Another option may be to extend the planting of trees into areas previously considered unsuitable for forestry due to unfavourable climatic conditions (Schönau and Gardner, 1991; Swain, 2001).

Various eucalypt species are planted all over the world, and there are five main commercial species that make up the bulk of eucalypt plantations in South Africa; *Eucalyptus grandis*, *Eucalyptus nitens*, *Eucalyptus smithii*, *Eucalyptus macarthurii* and *Eucalyptus dunnii* (Swain and Gardner, 2003; 2004). The genetic improvement of commercial eucalypt species in South Africa began with *E. grandis* in the early 1960s (Schönau, 1991). However, the expansion of hardwoods for the pulp and paper industry led to the expansion of eucalypts onto colder sites which are not suitable for growth of *E. grandis*.

In the mid 1980s, the Institute for Commercial Forestry Research (ICFR) established a series of site species interaction trials and provenance/progeny trials investigating eucalypt species for growth on high altitude sites (Swain, 2001). The results from these trials have confirmed the superiority of *E. macarthurii* and *E. nitens,* both of which grew very well on high altitude sites (sites prone to extreme cold and frost conditions) on the Highveld plateau, as well as identifying *E. dunnii* and *E. smithii* as species of good growth potential over a range of mid-altitude sites (Swain and Gardner, 2003; Pallett and Sale, 2004).

*Eucalyptus macarthurii* has been found to be one of the most frost hardy of the commercial eucalypt species in South Africa. Due to the species tolerance to frost, cold and drought, *E. macarthurii* has traditionally been planted on low productivity sites (unfavourable growth conditions), where other species have failed to grow. The species is now mostly grown on these sites for pulp and paper production, although there is presently some controversy regarding the pulping properties of the species (Swain and Gardner, 2003; 2004), as historically, pulping properties of *E. grandis* and *E*. *smithii* have been more desirable than those of *E. macarthurii.* Selection and breeding of this species has been undertaken at the ICFR over two generations, and has greatly improved the species for other characteristics (Swain *et al*., 1999).

An earlier study was undertaken in 2002 by the CSIR, in collaboration with the ICFR and other forestry sponsor companies, to develop a near infrared (NIR) calibration model for predicting screened pulp yield (SPY) of *E. macarthurii.* This was done using disc samples obtained from felled trees, that is, a destructive sampling method (Sefara *et al.*, 2002). However, the results showed that the trees sampled displayed a narrow SPY range, thus limiting the predictive power of the developed NIR calibration model for use in future screening (Sefara *et al.*, 2002).

Thus, the purpose of this investigation was to:

1. Analyse the growth measurements and characteristics of *E. macarthurii* second generation progeny trials at two sites. This also allowed a number of genetic parameters to be calculated, which could be used in future selection in the breeding programmes;

2. Develop a non-destructive *E. macarthurii* NIR calibration model that could be used to screen the ICFR's *E. macarthurii* second generation breeding population, and

3. Screen the ICFR's *E. macarthurii* second generation breeding population using the developed *E. macarthurii* NIR calibration model on sawdust obtained from core samples extracted from standing tree.

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# Chapter 1

# Literature Review

## 1.1 Introduction

This chapter provides a review of literature to cover different areas of cold tolerant eucalypt species (CTEs) mainly *Eucalyptus macarthurii*. This review looked at the background of main CTEs grown in South Africa, and specifically the distribution and growth of *E. macarthurii*. It also looked at some of the wood properties, which are important in tree breeding and further reviewed the assessment methods for wood properties. Thus, this chapter provides an overall frame work of the study.

## 1.2 Background on cold tolerant eucalypt species

*Eucalyptus* is a diverse genus of trees; rarely shrubs, the members of this genus dominate the tree flora of Australia. There are more than 700 species of *Eucalyptus*, mostly native to Australia, occupying a range of different habitats (Darrow, 1996; Pallett and Sale, 2004). A large variety of *Eucalyptus* species can be found in almost every part of the Australian continent, adapted to the range of Australia's climatic conditions (Schönau and Gardner, 1991).

*Eucalyptus* plantations cover approximately 600 000 ha of South African land with 75% being *Eucalyptus grandis (*Department of Water Affairs and Forestry, 2007*).* Historically, *E. grandis* has been the most important hardwood for the South African forestry industry (Pallett and Sale, 2004). *Eucalyptus grandis* is a relatively fast growing species with good rooting ability, most commonly used as a source of pulpwood, fuel and timber. However, *E. grandis* is generally an average to poor pulper, and it has low drought and frost tolerance (Pallet and Sale, 2004). In the last three decades, the increasing demand for hardwoods, particularly for the pulp and paper industry, has led to the expansion of hardwoods into the colder sites where *E. grandis* does not survive (Little and Gardner 2003; Pallet and Sale, 2004).

From the early 1980s, the major timber companies expanded their planted areas to include the colder, frost-prone highland areas of western KwaZulu-Natal, the north-eastern Cape and south-eastern Mpumalanga Highveld. As *E. grandis* was found not to be tolerant of frost and snow, a range of cold tolerant eucalypt species (CTEs) were planted in these areas as alternatives namely; *Eucalyptus nitens, E. smithii, E. dunnii* and *E. macarthurii* (Schönau and Gardner, 1991). Since then, selective breeding combined with site-species matching has resulted in a significant improvement in tree volume, stem straightness and pulping yield in these species (Gardner, 2001).

Of the alternative species listed previously, *E. nitens* is most suited to cooler sites in the summer rainfall regions of South Africa; i.e. those areas with a mean annual temperature (MAT) of 14°C to 16°C and minimum mean annual precipitation (MAP) above 825 to 950mm (Herbert, 2000; Swain and Gardner, 2003). The species is classified as the most cold tolerant of the eucalypts grown in South Africa, is frost tolerant, although not as hardy as *E. macarthurii*, and has good snow tolerance. *Eucalyptus nitens* is susceptible to various forms of *Mycosphaerella nobilosa* leaf-spot disease in its juvenile state, and it does not coppice well, its ability to coppice decreasing with age (Swain and Gardner, 2003). The bark of the species strips relatively easily and the species has good pulping properties (Clarke, 2000a). This is the species of choice for cold, high altitude sites, or for sites where there is a risk of snow (Herbert, 2000).

*Eucalyptus smithii* is suited to deep, well drained soils on cool sites in the summer rainfall regions of South Africa, with an MAT not greater than 15°C to 18°C (Schönau and Gardner, 1991) and MAP above 830 to 950mm for optimum growth (Herbert, 2000; Swain and Gardner, 2003). The species is relatively cold tolerant but not frost hardy, with moderate snow tolerance. The species coppices well, and is ideal for second rotation coppice crops (Herbert, 2000). The bark strips easily during summer and the species has excellent pulping properties (Herbert, 2000; Swain and Gardner, 2003). This species is the most favourable of the CTEs due to its favourable pulping properties, but is often limited by site conditions which are; shallow soils,

stony or gravel soils or soils with soil-water drainage problems as the species is susceptible to *Phytophthora* root rot (Swain and Gardner, 2003).

*Eucalyptus dunnii* grows better than *E. grandis* on cooler sites and has better frost tolerance than the latter species. It is ideally suited to sites in the summer rainfall regions of South Africa with an MAT of greater than 15.5°C (Schönau and Gardner, 1991; Herbert, 2000) and MAP of 822 to 925mm for optimum growth (Herbert, 2000). *Eucalyptus dunnii* is drought tolerant but is sensitive to snow and is susceptible to frost on the valley bottoms. The species coppices well but can be susceptible to wind damage. The species has excellent stem form, with very little taper, the bark strips easily, and has very good pulping properties for Kraft pulping (Schönau and Gardner, 1991; Herbert, 2000; Swain and Gardner, 2003).

Few *Eucalyptus* species do well on low productivity (marginal) sites in South Africa, these sites being “unproductive” generally due to unfavourable environmental conditions. *Eucalyptus macarthurii* is commonly grown in these areas for pulp and paper production, because other eucalypt species have failed to meet reasonable production standards in these low productivity areas (Swain and Gardner, 2003). Although there is presently some debate about the pulping properties of this species, it is capable of fast growth, is frost tolerant, and has much improved stem form and strippable bark during the rainy season (Swain *et al*, 1999; Swain and Gardner, 2003).

*Eucalyptus macarthurii* requires an MAP of 780 to 925mm for optimum growth, but this may be reduced at the cooler end of its range (Swain and Gardner, 2003; 2004). Where there is an extended dry season with minimal rainfall, or high variation in MAP, these limits should be conservatively applied on shallow soils with a low to moderate water storage capacity. Thus sites must have a continuous supply of water, without long periods of drying out (Swain and Gardner, 2003). *Eucalyptus macarthurii* suffers little from insect attacks and is considered to be resistant to root damage from termites (Herbert, 1993). A comparative summary of the commercial CTE species in South Africa is presented in Table1.1.

**Table 1.1 Comparative summary of the commercial CTE species grown in South Africa, with relative tolerance ranking for growth characteristics (Swain and Gardner, 2004)**

| Species | Optimum MAT range (°C) | Minimum MAP range (mm) | Comparative tolerance ranking | | | |
|---|---|---|---|---|---|---|
| | | | Cold tolerance | Frost tolerance | Snow tolerance | Drought tolerance |
| ***E. dunnii*** | 15.0 – 19.0 | 800 – 950 | 4 | 4 | 5 | 1 |
| ***E. macarthurii*** | 14.0 – 18.0 | 780 – 925 | 2 | 1 | 5 | 3 |
| ***E. nitens*** | 14.0 – 16.0 | 825 – 950 | 1 | 2 | 1 | 4 |
| ***E. smithii*** | 15.0 – 18.0 | 830 – 950 | 3 | 3 | 4 | 2 |
| Note: 1 = very tolerant; 2 = tolerant; 3 = moderate; 4 = slightly sensitive; 5 = sensitive | | | | | | |

## *1.3 Eucalyptus macarthurii*

*Eucalyptus macarthurii* is often the only *Eucalyptus* species that can be commercially grown on low productivity (marginal) sites in South Africa for pulp and paper production (Swain and Gardner, 2003). Although there is ongoing debate about the pulping properties of this species, it is still important to the South African forestry industry because of its ability to meet reasonable production standards in these low productivity areas. Thus studies into improving the growth and pulping properties of this species are necessary.

### 1.3.1 Origin and Adaptation

The natural occurrence of *E. macarthurii* is restricted to the central and southern tablelands of New South Wales (NSW), from the Blue Mountains to Goulburn in Australia, Figure 1.1 (Boland *et al*., 1992).

The altitude varies from 500 to 1200 metres above sea level (m.a.s.l), with latitude ranging from 33ºS to 35ºS. The species is found in undulating topography along stream banks and flood plains on heavy soils derived from shales and basalts, but can also grow on light sandy-loam soils. When found on better sites, the species is considered as dominant amongst various eucalypt species (Swain *et al*., 1999; Swain, 2001; Swain and Gardner, 2003; 2004).

The mean maximum and minimum temperatures of the hottest and coolest months of NSW are 25ºC and -1ºC, respectively. Rainfall is relatively evenly distributed throughout the year, with a slight summer maximum in the southern part of the range, and a definite summer maximum in the more central range (Brooker, 2000).

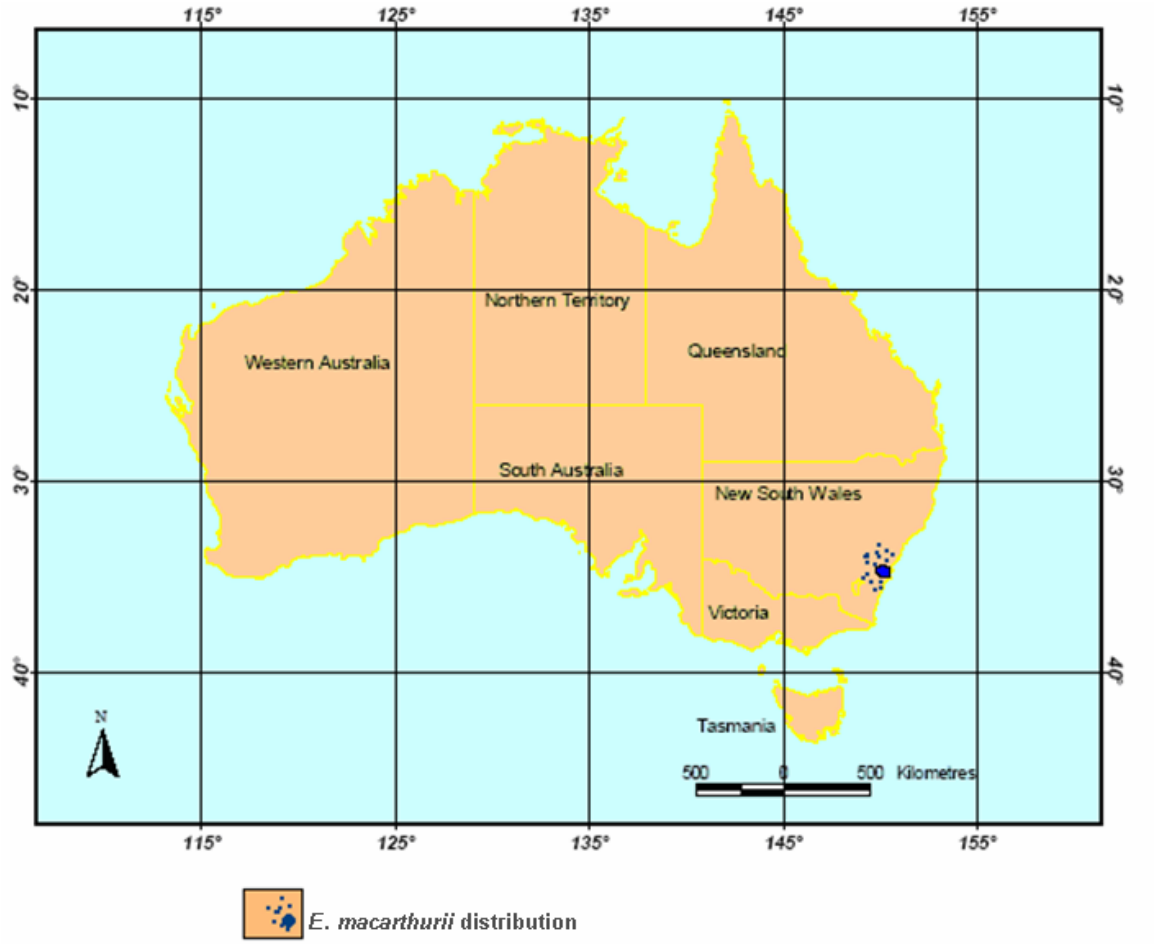


**Figure 1.1** **Natural distribution map of *Eucalyptus macarthurii* in Australia** (Boland *et al*., 1992)

Frosts are severe and frequent with regular light snowfalls in the higher areas (Boland *et al*., 1992; Herbert, 1993). The areas where the species is found are cooler and wetter in winter than surrounding hillsides. The soils may vary in texture from clay to sandy loams, with best growth occurring on well drained, but moist, sites (Herbert, 1993).

### 1.3.2 Description in Australia

*Eucalyptus macarthurii* is a medium sized tree which grows up to a height of 18 to 24m and 60 to 90cm in diameter in its natural environment. When grown under favourable conditions, it grows up to 30 to 40m tall. It has moderate stem form which is heavily branched, spreading and growing as a dense crown. Wood that is yielded by the species is pale in colour. It flowers well in September and October (Poynton, 1979; Swain and Gardner, 2003).

The species is mostly used for shelter, shade and ornaments. It is also suitable for planting in parks and playing grounds. Under favourable conditions, it grows rapidly and produces a dense head of dark green foliage. It also coppices well, and responds well to shaping by pruning (Swain and Gardner, 2003; 2004).

### 1.3.3 Growth in South Africa

In South Africa (Figure 2.2), *E. macarthurii* has proved to be one of the most frost tolerant of the CTE species. However, young trees may still be damaged by severe frost, especially if the species is planted late in the season (Swain *et al.*, 1999). Although it survives well in cold areas, the MAT should be at least 15.5ºC for optimum growth. The species does not survive well in warm conditions and optimum growth is unlikely where the MAT exceeds 18ºC. Within its optimal temperature range, *E*. *macarthurii* is one of the hardiest of the CTEs, producing commercially viable yields with relatively low risk on low productivity (marginal) sites (Swain *et al.*, 1999; Swain, 2001; Swain and Gardner, 2003; 2004).

Breeding of *E*. *macarthurii* has been undertaken by the Institute for Commercial Forestry Research (ICFR) since 1984. In this programme wood characteristics such as yield, diameter and genetic gains were assessed (Swain *et al*., 1999). Unimproved *E. macarthurii* has poor stem form when grown in South Africa. However, the ICFR breeding programme for *E. macarthurii* has produced significant improvements in stem form in the second generation (Swain, 2001).

Wood and pulping properties of *E. macarthurii* are generally not as desirable as those of other CTEs such as *E. smithii* and *E. nitens* (Swain, 2001). However, studies have revealed that second generation *E. macarthurii,* when grown on higher productivity sites than the species is normally grown on, has wood and pulping properties that are more comparable to *E. smithii* and *E. nitens* (Swain, 2001). Nevertheless, as the importance of the species lies in it being commercially viable on low productivity sites, often with resultant poor pulping properties, improvements in this trait still need to be made.

An evaluation of wood and pulping properties of *E. macarthurii* would be of great value to the South African Forestry Industry breeding programmes and would facilitate the selection of genotypes with acceptable wood and pulping properties in the ICFR breeding programme.

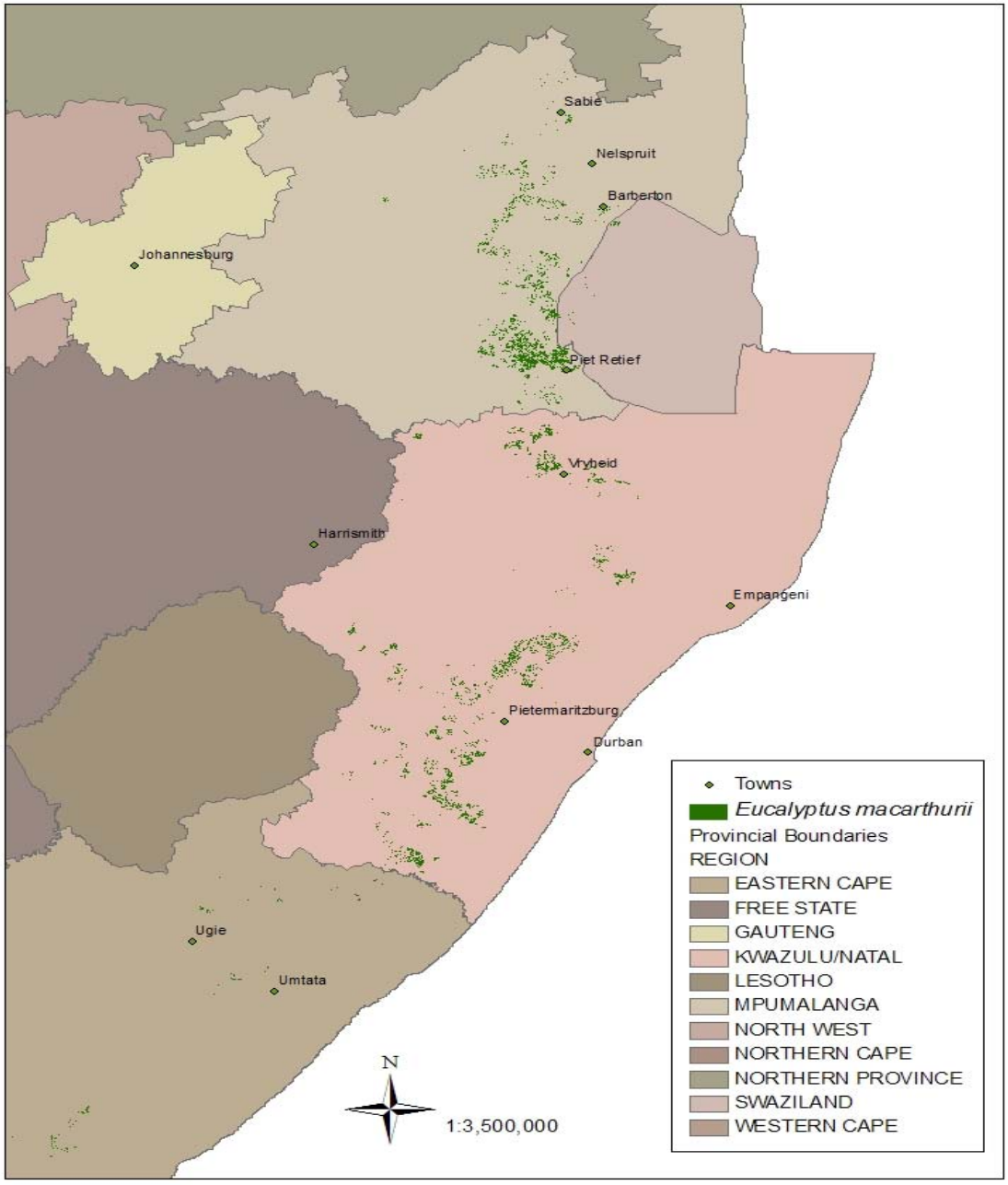


**Figure 1.2 Distribution of *E. macarthurii* in South Africa**

## 1.4 Wood properties important for breeding

### 1.4.1 Introduction

The primary source of raw material for paper making in South Africa is wood fibre from plantation forests. Wood is categorised in two types, softwoods and hardwoods. Softwoods are cone bearing trees called Gymnosperms, such as pine trees, whereas hardwood species are seed bearing trees called

angiosperm, such as eucalypts (gum trees) (Treacy *et al*., 2001; So *et al.*, 2004). The fibres from softwoods and hardwoods differ considerably in their nature and use.

Softwood fibres are approximately 2.1 mm to 2.5mm long, wider and stronger than hardwood fibres, which is about 1mm in length. Softwood fibres are used in applications of strength and bulk, which are the qualities required in sheets of paper, and are used extensively in newsprint, magazine and packaging grades (Treacy *et al*., 2001). On the other hand, hardwood fibres are packed closely into a sheet of paper producing a smooth finished printing surface. Fine paper manufacturers make extensive use of hardwood fibres, mainly because they are stiffer than softwood fibres. It has been found that hardwoods have lower lignin content and higher hemicellulose content than softwoods, and this is reflected by a higher pulp yield and lower kappa number under similar pulping conditions in hardwoods (Clarke, 1995).

In the past two decades there has been an increased global focus on wood quality of eucalypt species. Wood quality had previously not been an important component for many tree improvement programmes because of the inability to measure wood chemistry, fibre morphology, mechanical and physical properties in a breeding programme. Rather, improvements of growth, stem straightness, removal of reaction wood and increasing disease resistance have been the major targets in genetic improvement. However, wood properties play an important role in the quality of wood (Little *et al*., 2003; So *et al.*, 2004), and development of techniques where these can be measured and used in tree improvement programmes is ongoing.

Wood properties vary greatly within and between trees and are categorised into physical and chemical properties. The main physical properties of wood are basic wood density and the angle of microfibrils which are situated in the cell wall, the latter being the major determinants of strength of solid wood products (Sandercock *et al.*, 1995). Chemically, wood consists of cellulose, hemicellulose, lignin and variable quantities of non-structural components called extractives (Clarke, 2000b). These physical and chemical properties are important determinants in pulp and paper production and they play a very

important role in determining the quality, pulp yield and rate of delignification in eucalypt pulpwood (Sandercock *et al.*, 1995; So *et al.*, 2004; Clarke, 2000b).

## 1.4.2 Physical Composition of Wood

### 1.4.2.1 Basic wood density

Basic wood density is defined as an oven dried mass divided by green volume (Clarke, 1995). Wood density determines the mass of pulp available for paper production for a given volume of wood (Sandercock *et al.*, 1995; Bergander, 2001) and varies with species, provenance, site location and within each tree, depending on the point at which a tree is measured (Treacy *et al*., 2001). Despite this variation, wood density remains the main indicator of wood quality since it is a good indicator of wood strength, which is the ability of wood to resist applied stress and compression. In addition, it has been shown in previous studies that, as wood density increases, so does wood strength (Treacy *et al*., 2001 and Lundgen, 2004). Traditionally, density is also considered a useful indicator of pulping potential and is therefore used as an important selection criterion in many tree breeding programmes (Clarke, 1995).

### 1.4.2.2 Microfibrils

Wood cells are made up of multiple layers; the middle lamella, primary layer and three secondary layers; S1, S2 and S3 (Figure 1.3) (Koch, 1985). The secondary layers consist of helically arranged cellulose microfibrils orientated towards the long axis of the tracheids. Microfibrils are highly ordered bundles of cellulose chains that give strength and stiffness to the cell wall (Booker and Sell, 1998; French *et al.*, 2000). These microfibrils have a significant impact upon paper properties in that, as the length of microfibrils increases, wood stiffness decreases. Consequently, small microfibrils are associated with high tensile strength in paper (Treacy *et al.*, 2001; Barnet and Bonham, 2004)) and larger microfibrils produce paper with low strength (Lundgen, 2004; Treacy *et al*., 2001).

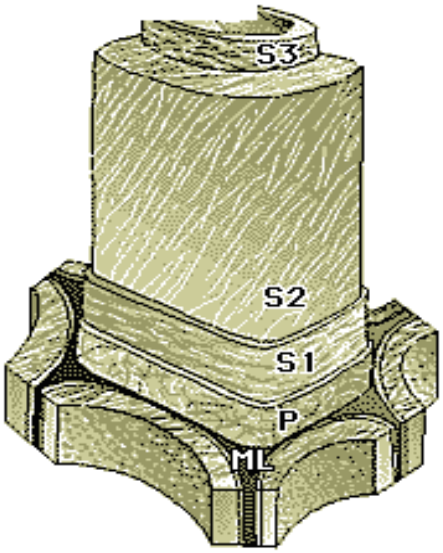


**Figure 1.3 Cell wall structure (Koch, 1985)**

**Layers: middle lamella (ML), Primary wall (P), Outer secondary wall (S1), middle secondary wall (S2) and inner secondary wall (S3)**

Thus, microfibrils are important in that they form part of a wall structure that influences fibre strength, flexibility and conformation. They are extensively used as an indicator of fibre stiffness, wood shrinkage and other properties used in paper-making (Downes *et al.*, 1997).

## 1.4.3 Chemical composition of wood

### 1.4.3.1 Cellulose

Cellulose is a major chemical component of wood, constituting approximately 50% of wood substances by weight. It is a high molecular weight, linear polymer found in all higher plants where it acts as a structural material in their cell walls, and is built up of chains of 1 to 4 β-D-linked glucose monomers (Bergander, 2001). During growth of a tree, cellulose molecules are arranged into ordered strands called microfibrils, which are organised into large structural elements that make up the cell wall of wood fibres (Sandercock *et al*., 1995 and Bergander, 2001). Cellulose content is strongly correlated with pulp yield and can be used as an alternative selection criterion to pulp yields in tree breeding programmes (Schimleck *et al*., 2004).

#### 1.4.3.2 Hemicellulose

Hemicelluloses are branched hetero-polysaccharide polymers which are made up of two to six different monosaccharides. Hemicellulose chemical structure is more dependent on a particular genus whilst cellulose has the same structure in both softwoods and hardwoods (Bergander, 2001). Hemicellulose plays an important role in fibre to fibre bonding in the papermaking process, and thus high hemicellulose and cellulose contents in eucalypts results in increased pulp yield. Also, if the hemicellulose is maintained after Kraft cooking, burst and pulp strength properties increases (Sandercock *et al.*, 1995).

#### 1.4.3.3 Lignin

Lignin is a three-dimensional natural polymer consisting of three different alcohol monomers called monolignols, and its structure and distribution in wood is still not fully understood (Bergander, 2001). Lignin constitutes about 23 to 33% of wood substance in softwoods and 16 to 25% in hardwoods. Although lignin occurs in wood throughout the cell wall, it is mostly concentrated towards the outside and between the cells, where it acts as a cementing agent that binds individual cells together.

On a commercial scale, lignin is removed during the pulping process so as to produce pulp that will result in high grade paper and other pulp products (Clarke, 1995; Michell, 1995). Varying quantities of lignin are removed by chemical dissolution in the Kraft pulping process, to release the cellulose-rich fibres (Clarke, 1995).

#### 1.4.3.4 Extractives

Extractives are natural products that are extraneous to a lignin-cellulose cell wall. They are found within cell walls but are not chemically attached to it. Extractives are known to enhance durability of timber against bio-degradation agents such as termites (Bergander, 2001). In eucalypt, there are both water soluble and polar soluble extractives. Water soluble extractives are mainly polyphenols and carbohydrates, compounds that are undesirable in the pulping process because they discolour pulp and consume chemicals that are contained in the cooking liquor, and which adversely affects chemical recovery.

Sandercock *et al*. (1995) have found that large amounts of extractives are highly correlated to lower pulp yields and high chemical consumption in *E. globulus*. Extractives can be removed from wood using solvents such as water, alcohol, acetone, benzene or ether (Bergander, 2001). However, little is known about the genetic and environmental influence on extractives in wood (Clarke, 1995).

## 1.5 Assessment of wood properties

### 1.5.1 Introduction

There has been increased focus by the pulp and paper industries on improving pulp yield of plantation grown trees through tree breeding programmes (Raymond, 2002). Clarke (1995) has defined pulp yield as the proportion of wood that is converted to pulp, and it is quoted as a percentage at a specific kappa number or a state of delignification (Clarke, 1995). Schimleck *et al.* (1998) have defined pulp yield as the percentage of the original mass of wood fibre remaining after pulping, to a set content of residual lignin. Basically, the main goal of pulping is to increase the amount of cellulose and decrease the lignin and extractives contained in wood fibres so as to produce high grade quality paper (Schimleck *et al.*, 1998). Pulp is made up of wood fibres that have been separated from each other either by chemically dissolving the lignin that binds them together, or by mechanically tearing them apart and fracturing the cell wall. Previous studies (Schimleck *et al.*, 1998; 1999; 2000; 2006) have revealed that higher amounts of cellulose and lower lignin and extractives content are highly correlated to higher screen pulp yield, which is the total pulp yield minus the rejects after Kraft pulping process.

The value of making improvements in pulp yield through selection and breeding is large, giving rise to a number of benefits (Schimleck *et al.*, 1998; 2004):

1. reduction in raw wood requirement,
2. increase in pulp production capacity for a given level of wood intake,
3. lower chemical cost and improved pulp strength, and
4. increased plantation profitability.

As wood properties are affected by site conditions, position of wood in the tree, as well as genetic background, tree breeders still need to assess the effects that wood properties have on pulp yield (Schimleck *et al.*, 1998). Estimation of pulp yield within a breeding population is an integral tool in a breeding programme of a pulpwood forestry species (Raymond, 2002).

## 1.5.2 Review of wood sampling methods

There are two sampling methods that can be used to obtain wood samples for the determination of SPY; these are traditional and coring sampling methods which are destructive and non-destructive, respectively. The traditional method requires trees to be felled and further processed in the laboratory, and the SPY calculated after the pulping process has been undertaken. Conversely, wood core samples can be obtained from standing trees using a non-destructive core sampling method. These cores are then ground into sawdust and the SPY predicted using near infrared (NIR) spectroscopy.

### 1.5.2.1 Traditional method (Destructive sampling)

Sampling using the traditional method involves felling of selected trees. Either the butt log of the tree is used, or discs are cut at a prescribed distance along the length of the tree. The butt log or discs are chipped and bulked and a sample taken for chemical pulping (Raymond and Apiolaza, 2004). Although this is a successful technique, an important requirement for screening of material in breeding programmes for the estimation of screened pulp yield is that the method should be non-destructive and should enable the rapid screening of a large number of samples (Schimleck *et al.*, 2001; 2004).

As whole trees are felled using this method, this is a destructive procedure which renders the tree unavailable for future breeding. Apart from the loss of material, it is also recognised that such a destructive procedure is costly, time consuming and requires refinement so that the procedure is more non-destructive in nature (Muneri *et al*., 2005; Schimleck *et al.*, 2006). Therefore, this traditional method is not suitable for tree improvement programmes as felled trees cannot be used for further selection and breeding.

#### 1.5.2.2 Coring method (Non-destructive sampling)

In the early 1990s, a motor driven coring system for removing 12mm wood core samples (radially-orientated cylinders) from standing trees was developed. This development allowed non-destructive sampling of a large number of core samples from the stems of standing trees at breast height for the assessment of wood properties, whilst still conserving genotypes for later experimentation and research (Schimleck *et al.*, 2005).

The ability to accurately estimate whole tree pulp yield based on NIR spectra collected from cores extracted at breast height would greatly reduce the cost of plantation assessment and facilitate non-destructive sampling. In order to estimate whole tree pulp yield using NIR spectra from breast height cores, calibration between whole tree pulp yield (obtained from destructively sampled trees) and NIR spectra collected from breast height cores need to be developed (Schimleck *et al*., 2006). Schimleck *et al*. (2006) examined this approach based on several eucalypt species and hybrids. It was shown that wood pulp yield calibrations based on whole tree data and NIR spectra collected from whole tree wood discs and cores taken at breast height provided similar statistics. It was found that, *E. nitens* had correlations obtained from whole tree pulp yield and NIR spectra from wood cores of 0.92 and 0.88, respectively (Schimleck *et al*. 2006). Hybrid poplar was also found with a correlation of 0.96 for whole tree pulp yield and 0.90 for NIR spectra from wood cores (Schimleck *et al*., 2005).

The wood core samples are ground into sawdust and SPY estimated rapidly using NIR spectroscopy analysis. NIR spectroscopy analysis is a technique that is currently used to determine chemical composition of wood and prediction of screened pulp yield. When developing an effective and efficient infield sampling strategy, several key issues need to be addressed (Schimleck *et al*., 1999):

i) the change in wood properties up the stem in relation to site and tree age;
ii) suitable height at which to remove a core sample together with the side of the tree to be sampled;
iii) how well the core sample predicts the whole tree value, and
iv) the number of trees to be sampled.

The sampling strategy should be rapid and easy to use in the field, as a large number of trees would be required to be sampled, and there should be very little resultant damage to sampled trees (Muneri *et al.*, 2005 and Schimleck *et al.*, 2006). Identification of a suitable sampling height is very important in that the core sample must represent the whole tree as accurately as possible. In *E. grandis* this has been identified as at breast height (1.3m above the ground) (Michell, 1995; Michell and Schimleck, 1995). To address these issues, a detailed knowledge of pulp yield variation of the wood within plantation trees is required (Schimleck *et al.*, 1998).

### 1.5.3 Review of screening methods

Wood samples require a number of processing steps before calculation of SPY can be undertaken. Wood samples obtained from felled trees are chipped and thereafter subjected to the pulping process. Pulping processes are generally classified as chemical, mechanical and semi-chemical. The chemical pulping process is further categorised into Kraft, sulphite and soda pulping processes, the Kraft pulping process being most commonly used. On the other hand, wood core samples extracted non-destructively from standing trees can be ground into sawdust for NIR spectroscopy analysis.

#### 1.5.3.1 Pulping process

In the Kraft pulping process, chipped wood disc samples are cooked in an alkaline solution at prescribed temperatures and pressure to dissolve lignin, which is a cementing agent, leaving cellulose and hemicellulose intact (Clarke, 1995). The resultant pulp is washed to remove dissolved lignin and other undesirable chemicals. During the washing process, the pulp is passed through a series of washers and screens, separating the solid pulp from a liquid containing lignin as well as the chemicals used to separate lignin from cellulose (Clarke, 1995; 2000a). The pulp product is primarily used to make writing, printing and tissue paper because of its desirable sheet properties that include high bulk, good strength and smooth sheet formation (Clarke, 1995; 2000a). This pulp product is what is used for estimation of SPY values. However, as this process requires the destruction of selected trees by felling, it is not ideal

for use in tree improvement programmes where the selected trees are still required for production of seed, and cuttings of scion material for grafting.

#### 1.5.3.2 Near infrared (NIR) spectroscopy analysis

Near Infrared spectroscopy analysis is a fast, cost-effective, environment-friendly and non-destructive analytical technique that has gained widespread acceptance in recent years, as a rapid technique for predicting pulp yield and basic density in *Eucalyptus* plantations. Near infrared is part of the electromagnetic spectrum, defined as the region of wavelengths ranging from 780nm to 2500nm, and the technique is based on vibrational spectroscopy that monitors changes in molecular vibrations associated with changes in molecular structure (Barton, 2002; So *et al*., 2004).

Spectra within the NIR region consist of an overtone and combination bands of fundamental stretching vibrations of functional groups that occur in the middle infrared region, mainly C-H, O-H and N-H, which represent the backbone of biological compounds (Baillères *et al.*, 2002). Functional groups that are observed in the mid-infrared region (2500-10000nm) contain chemical and physical information of wood samples under study (Schimleck *et al.*, 2000; 2004).

The NIR spectroscopy technique involves measuring the spectra of a large number of samples and developing a regression calibration model that associates the spectra with the pulpwood trait of interest (Muneri *et al.*, 2005 and Schimleck *et al.*, 2006). The NIR spectroscopy system is calibrated on the basis of a set of fully characterised samples and mathematical models with high prediction accuracy (Baillères *et al.*, 2002). Calibration models that are developed are compared using different sampling intensities and using trees sampled from different sites. Then genetic gains can be estimated and compared in for example cellulose content from a developed model (Schimleck *et al.*, 2004).

There is a broad range of analytical applications of NIR spectroscopy in the agriculture, food, pharmaceuticals, polymer and textile industries. In forestry, this technology is being used for rapid prediction of pulp yield and other pulping

characteristics (Birkett and Gambino, 1988). Several recent studies have used NIR spectroscopy to estimate pulping parameters such as Kappa number (Muneri *et al.*, 2005 and Schimleck *et al.*, 2006). Kappa number is defined as a degree of delignification, measured by the amount of lignin remaining after pulping (So *et al*., 2004). NIR spectroscopy technology is now also being developed and calibrated to replace classical wet chemical methods for wood applications. In addition, a few studies have used NIR spectroscopy to assess physical and mechanical properties such as basic density, stiffness and strength (Baillères *et al.*, 2002).

Forestry companies are focusing more on pulp quality through selection and breeding. Pulp yield has been identified as a characteristic of major economic long-term importance for pulp and paper producers in increasing their end-product profitability.

### 1.5.4 Wood properties of *E. macarthurii*

Very little research appears to have been done on wood properties of *E. macarthurii.* A study was undertaken in 2002 to predict SPY with NIR spectral features of *E. macarthurii* disc samples, obtained using a destructive sampling method (Sefara *et al.*, 2002). This study revealed firstly that the calibration model had a low predictive power of $R^2 = 0.3$, which was too low to be used as a calibration model, the low predictive power being due to the narrow range in SPY. Secondly, the SPY of the *E. macarthurii* samples tested was considered low, ranging from 43 to 48% (Sefara *et al.*, 2002).

### 1.5.5 Conclusion

In summary, *E. macarthurii* is an important cold tolerant eucalypt species in South Africa due to the fact that it is the most frost hardy of the cold tolerant eucalypts grown in this country, and is widely grown on low productivity sites for pulp and paper production. Growth and stem form have been improved through the ICFR breeding programme, and improvements in the 2$^{nd}$ generation need to be quantified. However, there is still some debate about the

suitability of the pulping properties of *E. macarthurii* as there is very little literature on the wood properties of this species. As assessing pulp properties requires destructive sampling of trees in the breeding population, it is necessary to develop and use a non-destructive process, to conserve trees for later experimentation. The initial calibration that was developed by Sefara *et al*. using trees obtained non-destructively (2002) however, had a predictive power which was too low to be used as a calibration model, and thus still needs to be better calibrated. Once re-developed, it will be very useful to the ICFR's tree improvement programme, as well as to the forestry industry as a whole.

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# Chapter 2

## Analysis of growth characteristics and basic wood density of *Eucalyptus macarthurii*


## Abstract

*Eucalyptus macarthurii* is an important species in forestry in South Africa, as it is the most frost tolerant of the eucalypts grown in this country. In this investigation, growth characteristics (diameter under bark, diameter over bark, bark thickness, bark stripping, stem form, height and basic wood density) of second generation *Eucalyptus macarthurii* families were evaluated at Pinewoods and Vlakkloof sites in South Africa. Genetic and phenotypic correlations, heritabilities and genetic gains were calculated. Results showed that increased diameter and improvements in stem form have been made in this species through selection and breeding. Genotype by environment interaction was found indicated that different populations should perhaps be developed independently of site types. Genetic and phenotypic correlations of diameter under bark and diameter over bark were (0.91 and 0.96; 0.98 and 0.99 at Pinewoods and Vlakkloof sites respectively, which indicated that selecting for increased diameter over bark will result in increased diameter under bark. These correlations indicated that, selection of one trait could lead to a positive indirect selection for the other traits. At Vlakkloof, stem form improved with increasing diameter. Heritability estimates ranged from 0.03 to 0.23 at both sites for progeny trial E76/P1, and 0.09 to 0.12 for progeny trial E76/P2 at both sites which was within the normal *Eucalyptus* range, which will results in good response in selecting for best performing individuals with good traits for the next generation. The predicted gains for all traits found at Pinewoods were higher than those at Vlakkloof for progeny trials E76/P1, except height for progeny trial E76/P2, which was lower at Pinewoods (2.09m) and higher at Vlakkloof (3.52m), therefore, selecting for taller trees can be achieved at both sites but better genetic gains can highly achieved at Vlakkloof site. The overall, predicted and actual gains will result in moderate to high genetic gains in the next generation. These results gave a wide scope of increased in traits variation within and between sites, which can be used in selecting for future traits in the forestry breeding programmes.

## 2.1 Introduction

*Eucalyptus* is one of the most widely cultivated hardwood genera in tropical and subtropical regions of the world because of its economic importance (Santos *et al*., 2004). This success largely reflects the adaptability of the genus to a variety of climatic and edaphic conditions, as does its fast growth, versatility and usefulness of its wood to forestry industries. However, despite the numerous advantages, eucalypts are prone to suffer strong growth stresses within a living tree, which reduces the general quality of an end product and may also limit the usefulness of wood (Santos *et al*., 2004).

Plantation forestry with eucalypts can be highly productive and sustainable by increasing effective risk management strategies such as site-species matching, maintaining genetic diversity and reducing growth stresses such as drought, frost, snow, pest and diseases. Thus, it is important to have an early evaluation of the performance of the species and suitability of wood grown in newly adapted plantation environments for anticipated end uses before progressing with breeding programmes. One species which showed potential in South African forestry in the early 1980s was *Eucalyptus macarthurii* (Swain and Gardner, 2003).

*Eucalyptus macarthurii*, commonly known as Camden woollybutt, is native to New South Wales (NSW) in Australia. The species was first brought to South Africa in about 1892 to produce wood for fuel, poles, shelter and ornaments. In 1923, farmers started to recognise the species value for where it was used extensively for mining timber (Poynton, 1979). *Eucalyptus macarthurii* is the most frost tolerant of the eucalypt species grown in South Africa and is commonly referred to as a cold tolerant eucalypt (CTE). The species is also cold and drought tolerant, but is susceptible to stem breakage by heavy snowfalls. Because of its frost tolerance, *E. macarthurii* is widely planted in the Highveld regions of Mpumalanga (MPU) province and in certain areas of KwaZulu-Natal (KZN) where frost damage is severe, especially in valleys and drainage areas (Swain and Gardner, 2003). Although young plants of this species may be damaged by frost, most frost damage occurs in winter following late plantings, and is in the form of tip scorching (dried out or dead),

depending on the frequency and severity of frost. Although the species may be completely scorched and drop leaves, it has the capacity to recover in spring (Herbert, 2000). As the emphasis in the forestry industry shifted from mining timber to pulp and paper production in the early 1980s, *E. macarthurii* was investigated as a pulping species on temperate and frost-prone forestry sites (Herbert, 2000; Swain, 2001).

Historically, pulping properties of CTEs such as *Eucalyptus smithii* and *Eucalyptus nitens* have been more desirable than those of *E. macarthurii* (Herbert, 2000; Swain, 2001; Swain and Gardner, 2003). Due to the frost, cold and drought tolerance of *E. macarthurii*, the species has traditionally been planted on low productivity sites (unfavourable environmental conditions and shallow soils) where it is not economically viable to grow other species. However, if planted on good sites, *E. macarthurii* is capable of fast growth and improved stem form, both of which improve strippability of the bole and pulping properties (Swain and Gardner, 2003).

In this investigation, growth characteristics (diameter under bark, diameter over bark, bark thickness, bark stripping, stem form, height and basic wood density) measurements of the 2$^{nd}$ generation *E. macarthurii* families were evaluated, as to determine the species growth performance of progeny trials on two trial sites in South Africa. A number of genetic parameters (genetic and phenotypic correlations, heritabilities and genetic gains) were also investigated which could act as support tools for future selection and improvement in the breeding programme.

## 2.2 Materials and Methods

### 2.2.1 Origin of trial material

Improved seed from *E. macarthurii* was collected from families in the ICFR Jessievale and Seven Oaks Breeding Seed Orchards (BSOs) during the early 1990s. A minimum of 500 seeds were collected from each family, and seed from the 164 families in the Seven Oaks BSO were established in two progeny trials (E76/P1) at Pinewoods in KwaZulu-Natal (KZN) and Vlakkloof in Mpumalanga (MPU) at the end of 1993 and early in 1994, respectively

(Table 2.1). The remaining 29 families from Seven Oaks BSO and 56 families from the ICFR Jessievale BSO were established at the same sites at the end of 1994 (E76/P2), with five families in common with the E76/P1 trials series (Table 2.1). Five commercial controls were included in all of the progeny trials. Appendices Tables 2.1 and 2.2 provide details on the families included in the E76/P1 and E76/P2 progeny trials. The progeny trials were established in single row plots (seven or eight trees) of 1667 stems $ha^{-1}$, with four replications in various balanced and unbalanced lattice designs. Details of the trial designs are provided in Table 2.2.

**Table 2.1 Site details of *E. macarthurii* progeny trials**

| Trial number | Location | Planting date | Latitude (S) | Longitude (E) | MAP[1] (mm) | MAT[2] (°C) | Altitude (masl) | Soil depth (mm) |
|---|---|---|---|---|---|---|---|---|
| E76/P1 | Pinewoods | 09/12/93 | 29°39' | 30°04' | 890 | 15,3 | 1380 | >1200 |
| E76/P1 | Vlakkloof | 18/01/94 | 26°54' | 30°36' | 827 | 16,1 | 1360 | >1200 |
| E76/P2 | Pinewoods | 09/12/94 | 29°40' | 30°03' | 884 | 15,3 | 1385 | 800-1000 |
| E76/P2 | Vlakkloof | 14/12/94 | 26°54' | 30°36' | 827 | 16,1 | 1360 | >1200 |

[1]Mean annual precipitation, and [2]Mean annual temperature.

**Table 2.2 Trial design information of *E. macarthurii* progeny trials**

| Trial number | Location | Planting date | No. of families | Trial Design | Reps | No. trees/ plot* |
|---|---|---|---|---|---|---|
| E76/P1 | Pinewoods | 09/12/93 | 169 | 13x13 lattice | 4 | 8 |
| E76/P1 | Vlakkloof | 18/01/94 | 169 | 13x13 lattice | 4 | 6 |
| E76/P2 | Pinewoods | 09/12/94 | 90 | 9x10 lattice | 4 | 7 |
| E76/P2 | Vlakkloof | 14/12/94 | 90 | 9x10 lattice | 4 | 7 |

* all single row plots.

The improved material used in these progeny trials originated, two generations back, from selections made in local commercial, open-pollinated *E. macarthurii* plantations during the early 1980s by members of the Hardwood Working Group (which later became the South African Mining Timber Manufacturers' Association, SAMTMA), in collaboration with the Institute for Commercial Forestry Research (ICFR). Selections were based on growth, and stem form (straightness), the latter being particularly important as unimproved *E. macarthurii* had extremely poor stem form in South Africa (Swain *et al*., 1999). The seed collected from a single mother tree was referred to as a family. These commercial trees were referred to as generation 0 (Gen 0), as in Figure 2.1.

A series of trials were established to test this open-pollinated material, and these were called progeny trials. The trials were established at five sites in Mpumalanga Province (MPU) in 1984 namely; Jessievale (26°14'S, 30°31'E) Helvetia (25°31'S, 30°20'E), Vlakkloof (85: 26 °54'S, 30 °36'E; 86: 26°53'S, 30°37'E), Groenfontein (26°57'S, 30°50'E) and Jaglust (26°07'S, 30°27'E). A BSO was established at Seven Oaks in KZN at the same time. Due to poor initial survival at Jaglust, this trial was converted to a BSO 18 months after planting. This trial series was regarded as generation 1 (Gen 1), Figure 2.1.

Between 1991 and 1993, diameter at breast height (Dbh) measurements were done in Gen 1 and the data analysed. Based on these measurements, the top 70% of families were selected, and the Seven Oaks and Jaglust BSOs were thinned to include only the best tree per plot of each of these families. The trial at Jessievale was also converted to a BSO at this time, in the same manner. Seed was collected from the Seven Oaks and Jessievale BSOs and used to establish the next generation, generation 2 (Gen 2), of progeny trials and seed orchards as presented in Figure 2.1.

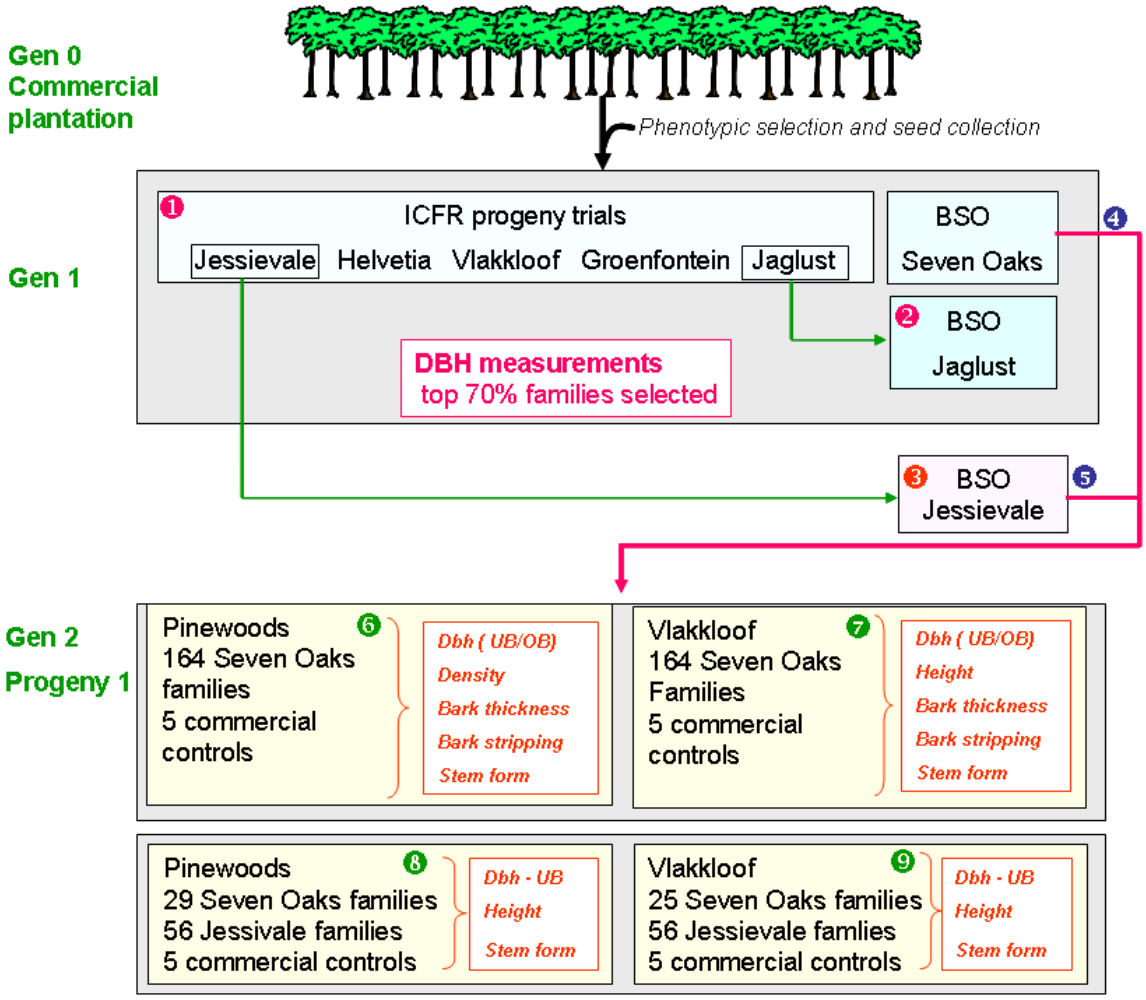


**Figure 2.1 Establishment of *E. macarthurii* trial material in South Africa**

Comprehensive measurements and sampling of the 2nd generation *E. macarthurii* trials was done in 2002 at both sites, when the E76/P1 trials were 8.5 years of age (102 months) and the E76/P2 trials were 7.5 years of age (90 months). The traits measured were as follows:

1. **Growth** – diameter at breast height over bark (Dbh-OB) was measured with a diameter tape at 1.3 m above ground level. This is a standard measure of growth/yield in forestry tree species. Once the bark had been stripped, diameter under bark (Dbh-UB) was measured,
2. **Height** – was measured using a Vertex hypsometer, at Vlakkloof only,

3. **Basic wood density** – was measured by water displacement method (Santos *et al*., 2004) at Pinewoods only,
4. **Stem form** – stem straightness was measured using a subjective scoring system of 1 to 4, where 1 indicated good stem form and 4 poor stem form,
5. **Bark thickness** – this was calculated using the formula: [diameter at breast height-over bark (Dbh-OB) – diameter at breast height-under bark (Dbh- UB)] / 2, and
6. **Bark stripping** – the measurement of how easily bark is physically removed from a tree using a "panga" or axe is referred to as "bark stripping". Measurements were done using a subjective score where 1 indicated ease of bark stripping and 4 indicated great difficulty in bark stripping.

### 2.2.2 Analysis of data

Genotype by environment interaction, individual site and combined site family means were calculated for progeny trials 1 (E76/P1) and 2 (E76/P2). Thereafter, estimates of heritabilities and genetic and phenotypic correlations among traits were calculated for the individual sites, which is fundamental to determining breeding strategies and for selection purposes (White, 1987; Falconer and Mackay, 1996). In this investigation, these parameters were calculated using Genstat® for Windows™, Release 10.2 procedures. The data from the trials were analysed using Restricted Maximum Likelihood (REML) analysis in Genstat®, using the model (Lane and Payne 1996):

$$y = X_a + Z_b + V_f + W_c + Q_s + T_{sf} + e$$

Where;

$y$ = trait or data vector

$X_a$ = replication effects (random),

$Z_b$ = block effects (random),

$V_f$ = family effects (fixed),

$W_c$ = plot effects (random),

$Q_s$ = site effect (combined site analysis),

$T_{sf}$ = site by family interaction coefficient, and

$e$ = random error effects.

### 2.2.3 Genetic parameter estimations

In this investigation, heritabiities based on the ratios of variance components of each trait were estimated, and used for selection purposes and predicting genetic gains. Phenotypic and genetic correlations for the various traits measured were also estimated. To this end, genetic and environmental variances and covariances were calculated.

#### *i. Heritability*

Heritability is a useful statistical tool used in selection and describes relative contributions of the genotype and the environment to the phenotype (Namkoong, 1979). The heritability of a particular trait is a measure of how strongly the observed variation of a trait is influenced by the genetic and environmental components in a particular population, thus allowing tree breeders to develop selective breeding programmes and to predict genetic gain (Namkoong, 1979; Falconer and Mackay, 1996).

Heritabilities ranging from 0.10 to 0.30 are considered intermediate and generally indicate moderate genetic gains, which are usually expected from individual tree selection. This is the range most commonly found in eucalypts. On the contrary, heritabilities less than 0.10 are considered low, resulting in poor genetic gains from selection. However, heritabilities ranging from 0.30 to 1.00 are considered high, with resultant good genetic gains (Namkoong, 1979; Cotterill and Dean, 1990; Falconer and Mackay, 1996).

Narrow sense heritability ($h^2$) is generally expressed mathematically as $h^2 = \sigma^2_a / \sigma^2_p$, which is the ratio of additive genetic variance ($\sigma^2_a$) to phenotypic variance ($\sigma^2_p$) (Falconer and Mackay, 1996). Thus, in order to calculate $h^2$,

the additive variance ($\sigma^2_a$) has to be estimated. However, Squillace (1994) noted that relatedness among individuals can bias the estimate of the additive variance component in open pollinated populations of forest trees. Namkoong (1966) found that a coefficient of relationship of 0.25 usually resulted in an overestimation of additive genetic variance which then biases the estimation of heritability and of genetic gains. Thus Verryn (2007) recommended that the coefficient of relationship between half-sibs be increased from 0.25 to 0.3 in open-pollinated forest trees, under the assumption of a 20% increase in relatedness. This assumption was implemented to take into account any additive genetic variance and heritability overestimations that may have occurred due to relatedness between parents. Therefore, as it was assumed that some inbreeding would have occurred in the *E. macarthurii* 2nd generation breeding population, a coefficient of relationship of 0.3 was used for this study.

Prior to the estimation of heritability, variance components were determined from REML in Genstat. The estimation of variance components was based on residuals calculated after fitting the fixed model by generalised linear mixed model. REML has proved to have better properties for unbalanced data in forestry genetic tests than other estimators (Huber *et al*., 1994).
These variance components were calculated using the following formulae (Verryn, 2007):

Phenotypic variance ($\sigma^2_p$) was estimated as,

$$\sigma^2_p = \sigma^2_f + \sigma^2_e$$

Where;

$\sigma^2_f$ = family variance

$\sigma^2_e$ = error variance

The family variance was estimated as,

$$\sigma^2_f = \tfrac{1}{3}\,\sigma^2_a$$

Hence, the additive genetic variance was calculated as,

$$\sigma^2_a = 3\,\sigma^2_f$$

Thus, the heritability estimates of each trait were calculated as the ratio of $\sigma^2_a$ and $\sigma^2_p$, according to the following formula,

$$h^2 = \frac{3\sigma^2_f}{\sigma^2_p}$$

Thus, the heritability estimates of each trait were calculated as the ratio of $\sigma^2_a$ and $\sigma^2_p$, according to the following formula,

$$h^2 = \frac{3\sigma^2_f}{\sigma^2_p}$$

### *ii. Genetic and phenotypic correlations*

The phenotypic correlation ($r_p$) (Pearson's Correlation Coefficient), is the statistical association between two measured traits in a population based on individual trees (Verryn, 2007). The phenotypic correlations between two traits were estimated using the following formula:

$$r_{p\,(xy)} = \frac{cov_{p}\,(xy)}{\sqrt{\sigma^2}_x \times \sqrt{\sigma^2}_y,}$$

Where;

$r_{p\,(xy)}$ = phenotypic correlation coefficient of traits x and y,

$cov_{p\,(xy)}$ = phenotypic covariance of two traits, x and y, and

$\sigma^2_x$, $\sigma^2_y$ = respective variance components for traits x and y.

The genetic correlation ($r_a$) is estimated as the correlation of breeding values for different traits (Cotterill and Dean, 1990; Falconer and Mackay, 1996). The genetic correlation is important in breeding, in that it allows the estimation of correlated gains in one trait as a consequence of selection in the second trait (Namkoong, 1979). Additive genetic correlations were calculated using the following formula:

$$r_{a\,(xy)} = \frac{cov_{a\,(xy)}}{\sqrt{\sigma^2}_x \times \sqrt{\sigma^2}_y,}$$

Where;

$r_{a\ (xy)}$ = genetic correlation coefficient of traits x and y,

$cov_{a\ (xy)}$ = genetic covariance of two traits, x and y, and

$\sigma^2_{ax}$, $\sigma^2_{ay}$ = respective variance components for traits x and y.

### *iii. Genotype by environment interaction*

The concept of genetic correlation between environments has major advantages in research strategies. It is directed at the role of environments in generating interactions, so as to define sites within a particular grouping which are optimal for phenotypic selection or progeny testing (Burdon, 1977). Based on the premise that in certain instances, performance of genotypes relative to each other depends mainly on the environment, this phenomenon has been formulated in the concept of genotype by environment interaction (GEI). When two traits are measured in different individuals within genetic groups, a special case being genetic correlation between trees of the same family grown in different environments, the correlation is designated a Type B genetic correlation ($r_B$) (Burdon, 1977). Or, in other words, an expression of the same trait in two environments can be considered as two different characters, and the genetic correlation between them can be estimated by a Type B genetic correlation (Falconer, 1952). It was suggested by Robertson (1959), that the GEI is of biological and agricultural importance if the genetic correlation for the same traits in different environment is less than 0.80; that is, the further the genetic correlation deviates from 1, the larger the interaction (Robertson, 1959). For tree breeding, a Type B genetic correlation of 0.67 is the level at which the GEI variance represents 50% of the total additive variance, and is the point where it is postulated that the GEI variance may be a cause for concern among tree breeders (Shelbourne, 1972).

Type B genetic correlations were estimated for Pinewoods and Vlakkloof site pairs on growth characteristics within the species using the following formula (Burdon, 1977):

$$r_B = \frac{\sigma^2_{family}}{\sigma^2_{family} + \sigma^2_{family\ X\ site,}}$$

Where;

$r_B$ = Type B genetic correlation coefficient of traits x and y,

$\sigma^2_{family}$ = family variance, and

$\sigma^2_{family\ X\ site}$ = family-site variance components for traits x and y.

### *iv. Genetic gains*

Although gains can be predicted for any one population using heritabilities, it is always useful to determine whether those gains were actually realised in the following generation. Actual genetic gains that were realised for Gen 2 were calculated by comparing the average of the top 15 families in Gen 2 with the Jessievale best and SAMTMA commercial controls, and these were expressed as a percentage improvement over the commercial controls. Genetic gains were also predicted from Gen 2, for Gen 3, according to Falconer and Mackay (1996) as follows:

$$\Delta G = ih^2\sigma_p$$

Where;

$\Delta G$ = genetic gain or response to selection,

$i$ = selection intensity of 1.196

$h^2$ = narrow-sense heritability estimates, and

$\sigma_p$ = phenotypic standard deviation.

## 2.3 RESULTS

The estimates of the Type B genetic correlation coefficients for all growth characteristics ranged from 0.152 to 0.522 (Table 2.3), which was less than the value of 0.67, where it is postulated that GEI variance may be of some concern (Shelbourne, 1972). Thus the low $r_B$ values indicated that genotype by environment interactions exist, which means that different populations for different sites have to be developed independently of each other. It is therefore valid to analyse the data for Pinewoods and Vlakkloof separately for family means.

**Table 2.3 Genotype-environment interaction from Type B genetic correlation estimates of various growth traits of *E. macarthurii* for E76/P1 progeny trials at Pinewoods and Vlakkloof**

| Growth characteristics | Type B genetic correlation ($r_B$) |
|---|---|
| Dbh-OB | 0.522 * |
| Dbh-UB | 0.538 * |
| Stem form | 0.172 * |
| Bark thick | 0.537 * |
| Bark Strip | 0.152 * |

* represents data that is significant at $p \leq 0.05$ (95%).

**Table 2.4 Genotype-environment interaction from Type B genetic correlation estimates of various growth traits of *E. macarthurii* for E76/P2 progeny trials at Pinewoods and Vlakkloof**

| Growth Characteristics | Type B genetic correlation ($r_B$) |
|---|---|
| Dbh-OB | 0.545* |
| Height | 0.306* |
| Stem form | 0.410* |

* represents data that is significant at $p \leq 0.05$ (95%).

An across site analysis to calculate mean squares was undertaken to investigate whether there were any significant family differences amongst and within sites. Homogeneity of errors was tested for prior to an across site analysis. The mean square values found across sites for all the growth traits for progeny trials E76/P1 and E76/P2 were highly significant ($p \leq 0.001$) at both site and family level (Tables 2.5 and 2.6, respectively), the former confirming the presence of GEI.

**Table 2.5 Across site mean square values of various growth traits of *E. macarthurii* for E76/P1 progeny trials at Pinewoods and Vlakkloof**

| Source of variation | D.F. | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | Dbh-OB | Dbh-UB | Stem form | Bark thick | Bark Strip |
| Site | 1 | 1098.55*** | 4176.72*** | 403.46*** | 72.67*** | 48.32*** |
| Rep/site | 6 | 30.89$^{NS}$ | 30.76*** | 8.00*** | 0.46*** | 30.61*** |
| Family | 169 | 21.25*** | 14.47*** | 0.84*** | 0.25*** | 0.65*** |
| Site*family | 166 | 11.55$^{NS}$ | 8.30$^{NS}$ | 0.70*** | 0.13$^{NS}$ | 0.50*** |
| Error (residual) | 4419 | 15.24 | 7.42 | 0.46 | 0.11 | 0.34 |
| Total | 4761 | 15.57 | 8.78 | 0.57 | 0.13 | 0.41 |

*, **, ***, represents data that is significant at $p \leq 0.05$ (95%), $p \leq 0.01$(99%), $p \leq 0.001$ (99.99%), respectively,
$^{NS}$not significant and D.F. = Degrees of freedom.

**Table 2.6 Across site mean square values of various growth traits of *E. macarthurii* for E76/P2 progeny trials at Pinewoods and Vlakkloof**

| Source of variation | D.F. | Mean squares | | |
|---|---|---|---|---|
| | | Dbh-OB | Height | Stem form |
| Site | 1 | 5089.07*** | 10137.53*** | 72.94*** |
| Rep/site | 6 | 26.52$^{NS}$ | 81.94*** | 1.80*** |
| Family | 89 | 23.70*** | 12.34*** | 1.25*** |
| Site*family | 89 | 9.74$^{NS}$ | 8.05*** | 0.57*** |
| Error (residual) | 3736 | 11.81 | 5.22 | 0.38 |
| Total | 3921 | 13.35 | 8.15 | 0.42 |

*, **, ***, represents data that is significant at $p \leq 0.05$ (95%), $p \leq 0.01$(99%), $p \leq 0.001$ (99.99%), respectively,
$^{NS}$ not significant and D.F. = Degrees of freedom.

At an individual family level, there were significant differences among various traits for the E76/P1 progeny trials planted at Pinewoods and Vlakkloof, which are presented in Tables 2.7 and 2.8, respectively. All growth traits were significant ($p \leq 0.001$) at a family level, except for Dbh-OB at Vlakkloof (Table 2.8).

**Table 2.7 Mean square values of various growth traits of *E. macarthurii* for the E76/P1 progeny trial at Pinewoods**

| Source | D.F. | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dbh-OB | Dbh-UB | Stem form | Bark thick | Bark strip | Density [1] |
| Rep | 3 | 60.83 | 60.65 | 14.14 | 0.71 | 12.10 | 114.306 |
| Block/Rep | 48 | 9.05 | 6.84 | 1.16 | 0.20 | 0.48 | 53.22 |
| Family | 167 | 8.18*** | 5.50*** | 0.80*** | 0.21*** | 0.36*** | 50.22*** |
| Error (residual) | 1716 | 5.29 | 3.74 | 0.60 | 0.11 | 0.27 | 31.99 |
| Total | 1934 | 5.72 | 4.06 | 0.65 | 0.12 | 0.30 | 35.88 |

*, **, ***, represents data that is significant at $p \leq 0.05$ (95%), $p \leq 0.01$(99%), $p \leq 0.001$ (99.99%), respectively
$^{NS}$not significant, D.F.= Degrees of freedom, and [1] Density = Basic Wood Density.

**Table 2.8 Mean square values of various growth traits of *E. macarthurii* for the E76/P1 progeny trial at Vlakkloof**

| Source | D.F. | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dbh-OB | Dbh-UB | Stem form | Bark thick | Bark strip | Height |
| Rep | 3 | 0.94 | 0.87 | 1.87 | 0.21 | 49.12 | 147.73 |
| Block/Rep | 48 | 25.32 | 12.96 | 0.57 | 0.14 | 0.59 | 20.22 |
| Family | 168 | 24.81 $^{NS}$ | 17.76*** | 0.78*** | 0.15*** | 0.78*** | 15.15*** |
| Error (residual) | 1967 | 21.71 | 10.45 | 0.35 | 0.10 | 0.39 | 10.39 |
| Total | 2187 | 21.93 | 11.05 | 0.38 | 0.11 | 0.49 | 10.98 |

*, **, ***, represents data that is significant at $p \leq 0.05$ (95%), $p \leq 0.01$(99%), $p \leq 0.001$ (99.99%), respectively, $^{NS}$not significant and D.F.= Degrees of freedom.

All growth traits for progeny trials E76/P2 were highly significant (P≤0.001) at a family level at both sites (Tables 2.9 and 2.10).

**Table 2.9 Mean square values of various growth traits of *E. macarthurii* for the E76/P2 progeny trial at Pinewoods**

| Source of variation | D.F. | Mean squares | | |
|---|---|---|---|---|
| | | Dbh-OB | Height | Stem form |
| Rep | 3 | 88.96 | 148.24 | 10.74 |
| Block/Rep | 32 | 47.35 | 53.62 | 2.42 |
| Family | 89 | 79.70*** | 71.64*** | 2.70*** |
| Error (residual) | 2395 | 40.01 | 35.76 | 1.55 |
| Total | 2519 | 41.56 | 37.39 | 1.62 |

*, **, ***, represents data that is significant at P≤ 0.05 (95%), P≤ 0.01(99%), P≤0.001 (99.99%), respectively, $^{NS}$not significant and D.F.= Degrees of freedom.

**Table 2.10 Mean square values of various growth traits of *E. macarthurii* for E76/P2 progeny trials at Vlakkloof**

| Source of variation | D.F. | Mean squares | | |
|---|---|---|---|---|
| | | Dbh-OB | Height | Stem form |
| Rep | 3 | 282.55 | 488.83 | 7.82 |
| Block/Rep | 36 | 30.02 | 45.31 | 1.16 |
| Family | 89 | 94.31*** | 97.45*** | 2.04*** |
| Error (residual) | 2391 | 47.41 | 45.21 | 1.06 |
| Total | 2519 | 49.12 | 47.61 | 1.11 |

*, **, ***, represents data that is significant at $p \leq 0.05$ (95%), $p \leq 0.01$(99%), $p \leq 0.001$ (99.99%), respectively, $^{NS}$not significant and D.F.= Degrees of freedom.

The top 20 and worst 10 performing families at Pinewoods E76/P1 were ranked for all traits, according to Dbh-UB performance, as presented in Table 2.11. These ranked results are very useful as they provide all the trait information required for each family, and are particularly useful for selection in field.

The top 20 best and 10 worst performing families for each measured trait at each site in the E76/P1 and E76/P2 trial series were ranked and are

presented in Tables 2.11 to 2.18. Their respective correlations, heritabilities and genetic gains were also calculated and are presented in Tables 2.19 to 2.22.

The ranking of the top 20 and worst 10 performing families planted at Pinewoods E76/P1 showed that families performed differently for the various traits, as presented in Table 2.11. Only three families, 58, 143 and 195, were found having the same values amongst the best performing families for Dbh-UB and Dbh-OB. None of the families found in the top 10 ranking families for these two traits were in the top 10 for any other traits. In contrast, several of the poorest performing families had the same values for Dbh-UB and Dbh-OB, that is, 10, 44, 51, 71, 54 and 138, although they were still different for other traits. These relationships were investigated further when genetic and phenotypic correlations were calculated.

The top performing family for both Dbh-UB and Dbh-OB, family 58, was significantly higher ($p \leq 0.05$) than all other families for these two traits. Other top performing families for Dbh-UB and Dbh-OB, such as families 143, 195, 161, 16, 68 and 168, were not significantly different from each other, but were generally significantly different ($p \leq 0.05$) from all other families ranked 10 positions below them. The *E. macarthurii* control of the bulk of Jessievale seedlots (family 268) performed in the top 10 families for both Dbh-UB and Dbh-OB, and performed significantly higher than two of the controls, family 267 (bulk of best families from Jessievale) and 270 (SAMTMA commercial control).

The top performing families were found to be significantly higher ($p \leq 0.05$) than the worst performing families for bark thickness, bark stripping and stem form, but were not significantly different for basic wood density ($p \geq 0.05$). Although there were significant differences between the four *E. macarthurii* controls for these first three traits, there were rank changes for each trait. There were no significant differences between the controls for basic wood density.

**Table 2.11 Detailed ranking of the top 20 and 10 poorest performing families for each trait in the E76/P1 trial at Pinewoods at 102 months.**

| Ranking[1] | Dbh-UB (cm) | Fam no. | Dbh-OB (cm) | Fam no. | Basic wood density (kg m$^{-3}$) | Fam no. | Bark thickness (cm) | Fam no. | Stem form[3] | Fam no. | Bark stripping[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 18.23 | 58 | 22.15 | 17 | 625.45 | 129 | 0.93 | 44 | 1.40 | 3 | 1.00 |
| **2** | 17.05 | 143 | 19.95 | 67 | 594.27 | 53 | 1.01 | 35 | 1.67 | 37 | 1.00 |
| **3** | 16.78 | 68 | 19.90 | 72 | 592.25 | 170 | 1.09 | 63 | 1.67 | 41 | 1.00 |
| **4** | 16.74 | 161 | 19.76 | 101 | 590.52 | 54 | 1.10 | 10 | 1.69 | 63 | 1.00 |
| **5** | 16.58 | 195 | 19.59 | 128 | 589.36 | 202 | 1.10 | 9 | 1.71 | 95 | 1.00 |
| **6** | 16.58 | 182 | 19.58 | 48 | 585.19 | 37 | 1.12 | 108 | 1.73 | 115 | 1.00 |
| **7** | 16.56 | 174 | 19.54 | 18 | 583.55 | 34 | 1.13 | 48 | 1.75 | 135 | 1.00 |
| **8** | 16.53 | 268 | 19.51 | 173 | 581.78 | 185 | 1.14 | 164 | 1.78 | 163 | 1.00 |
| **9** | 16.49 | 9 | 19.49 | 180 | 581.52 | 122 | 1.14 | 110 | 1.79 | 167 | 1.00 |
| **10** | 16.45 | 109 | 19.48 | 99 | 579.78 | 156 | 1.15 | 187 | 1.79 | 173 | 1.00 |
| **11** | 16.43 | 75 | 19.46 | 66 | 576.77 | 163 | 1.16 | 27 | 1.80 | 182 | 1.00 |
| **12** | 16.39 | 42 | 19.41 | 139 | 576.55 | 45 | 1.18 | 195 | 1.80 | 189 | 1.00 |
| **13** | 16.35 | 16 | 19.40 | 54 | 575.71 | 172 | 1.18 | 22 | 1.81 | 78 | 1.06 |
| **14** | 16.30 | 168 | 19.32 | 195 | 574.09 | 138 | 1.18 | 34 | 1.82 | 77 | 1.07 |
| **15** | 16.27 | 88 | 19.29 | 78 | 571.77 | 46 | 1.19 | 97 | 1.82 | 122 | 1.07 |
| **16** | 16.20 | 188 | 19.27 | 95 | 571.71 | 177 | 1.19 | 117 | 1.82 | 128 | 1.07 |
| **17** | 16.19 | 63 | 19.23 | 143 | 570.55 | 204 | 1.21 | 61 | 1.83 | 134 | 1.07 |
| **18** | 16.19 | 158 | 19.19 | 32 | 570.11 | 82 | 1.22 | 24 | 1.85 | 17 | 1.08 |
| **19** | 16.16 | 108 | 19.15 | 169 | 569.35 | 176 | 1.22 | 42 | 1.85 | 79 | 1.08 |
| **20** | 16.15 | 98 | 19.13 | 53 | 568.30 | 4 | 1.23 | 55 | 1.85 | 87 | 1.08 |
| **160** | 14.16 | 10 | 16.55 | 165 | 509.06 | 99 | 1.60 | 75 | 2.67 | 268 | 1.57 |
| **161** | 14.06 | 44 | 16.52 | 3 | 506.03 | 188 | 1.60 | 88 | 2.67 | 156 | 1.60 |
| **162** | 14.04 | 34 | 16.52 | 118 | 504.43 | 145 | 1.61 | 15 | 2.75 | 25 | 1.63 |
| **163** | 13.99 | 51 | 16.47 | 170 | 503.35 | 24 | 1.65 | 68 | 2.75 | 39 | 1.64 |
| **164** | 13.96 | 53 | 16.42 | 40 | 499.57 | 128 | 1.65 | 171 | 2.75 | 184 | 1.67 |
| **165** | 13.95 | 71 | 16.32 | 58 | 497.83 | 9 | 1.65 | 202 | 2.82 | 186 | 1.69 |
| **166** | 13.88 | 37 | 16.20 | 15 | 494.83 | 68 | 1.66 | 205 | 2.82 | 124 | 1.70 |
| **167** | 13.81 | 129 | 16.09 | 201 | 491.31 | 184 | 1.69 | 16 | 2.83 | 36 | 1.73 |
| **168** | 13.60 | 138 | 15.96 | 63 | 477.56 | 183 | 1.72 | 37 | 2.86 | 53 | 1.85 |
| **169** | 13.60 | 54 | 15.80 | 177 | 466.44 | 58 | 1.96 | 50 | 2.86 | 54 | 1.89 |
| **10** | 16.49 | | 19.51 | | 533.74 | | 1.51 | | 2.29 | | 1.57 |
| **27** | 16.05 | | 19.00 | | 545.90 | | 1.48 | | 2.25 | | 1.43 |
| **36** | 15.92 | | 18.56 | | 518.11 | | 1.32 | | 2.30 | | 1.20 |
| **60** | 15.70 | | 18.67 | | 564.05 | | 1.49 | | 2.30 | | 1.20 |
| **Trial Mean[2]** | **15.35** | | **18.12** | | **545.15** | | **1.38** | | **2.24** | | **1.29** |
| **Trial Min[2]** | **13.60** | | **15.80** | | **466.44** | | **0.93** | | **1.40** | | **1.00** |
| **Trial Max[2]** | **18.23** | | **22.15** | | **625.45** | | **1.96** | | **2.86** | | **1.89** |
| **Trial SD[2]** | **0.74** | | **0.90** | | **23.03** | | **0.14** | | **0.27** | | **0.18** |
| **Trial SE[2]** | **0.21** | | **0.32** | | **190.00** | | **0.01** | | **0.03** | | **0.02** |
| **Trial LSD$^2_{0.05}$** | **0.42** | | **0.63** | | **372.40** | | **0.02** | | **0.05** | | **0.03** |

[1]Only top 30 and bottom 10 families presented, [2]Calculated for total number of families (n =169),
[3]scores (1=good, 4= poor), [4]scores (1=good, 4=difficult), and Fam no.=Family numbers.
Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

**Table 2.12 Ranking of the 20 top and 10 worst performing families according to Dbh-UB for each trait in the E76/P1 trial at Pinewoods at 102 months**

| Fam no. | Dbh-UB (cm) | Dbh-OB (cm) | Basic wood density (kg m$^{-3}$) | Bark thickness (cm) | Stem form[1] | Bark stripping[2] |
|---|---|---|---|---|---|---|
| 58 | 18.23 | 22.15 | 497.83 | 1.96 | 2.17 | 1.33 |
| 143 | 17.05 | 19.95 | 570.55 | 1.45 | 2.23 | 1.23 |
| 195 | 16.78 | 19.59 | 574.09 | 1.41 | 1.80 | 1.27 |
| 161 | 16.74 | 19.76 | 531.94 | 1.51 | 2.13 | 1.38 |
| 16 | 16.58 | 19.40 | 517.89 | 1.41 | 2.83 | 1.17 |
| 68 | 16.58 | 19.90 | 512.92 | 1.66 | 2.75 | 1.25 |
| 168 | 16.56 | 19.32 | 556.50 | 1.38 | 2.31 | 1.08 |
| 109 | 16.53 | 19.48 | 563.65 | 1.48 | 2.08 | 1.08 |
| 268 | 16.49 | 19.51 | 533.74 | 1.51 | 2.29 | 1.57 |
| 182 | 16.45 | 19.58 | 559.33 | 1.57 | 2.40 | 1.00 |
| 88 | 16.43 | 19.29 | 549.83 | 1.43 | 2.67 | 1.22 |
| 75 | 16.39 | 19.46 | 534.85 | 1.53 | 2.67 | 1.44 |
| 174 | 16.35 | 19.54 | 532.69 | 1.59 | 2.18 | 1.18 |
| 106 | 16.30 | 19.01 | 556.73 | 1.35 | 2.33 | 1.18 |
| 42 | 16.27 | 19.41 | 542.26 | 1.57 | 1.85 | 1.54 |
| 158 | 16.20 | 19.19 | 536.91 | 1.50 | 2.23 | 1.08 |
| 9 | 16.19 | 19.49 | 533.86 | 1.65 | 1.71 | 1.14 |
| 201 | 16.19 | 19.08 | 491.31 | 1.45 | 2.45 | 1.36 |
| 84 | 16.16 | 18.82 | 530.26 | 1.33 | 2.15 | 1.15 |
| 108 | 16.15 | 19.15 | 555.11 | 1.50 | 1.73 | 1.45 |
| 41 | 14.16 | 16.55 | 509.06 | 1.60 | 2.67 | 1.57 |
| 10 | 14.06 | 16.52 | 506.03 | 1.60 | 2.67 | 1.60 |
| 44 | 14.04 | 16.52 | 504.43 | 1.61 | 2.75 | 1.63 |
| 101 | 13.99 | 16.47 | 503.35 | 1.65 | 2.75 | 1.64 |
| 37 | 13.96 | 16.42 | 499.57 | 1.65 | 2.75 | 1.67 |
| 200 | 13.95 | 16.32 | 497.83 | 1.65 | 2.82 | 1.69 |
| 51 | 13.88 | 16.20 | 494.83 | 1.66 | 2.82 | 1.70 |
| 71 | 13.81 | 16.09 | 491.31 | 1.69 | 2.83 | 1.73 |
| 54 | 13.60 | 15.96 | 477.56 | 1.72 | 2.86 | 1.85 |
| 138 | 13.60 | 15.80 | 466.44 | 1.96 | 2.86 | 1.89 |
| **Commercial controls ranked according to Dbh-UB** | | | | | | |
| 268 | 16.49 | 19.51 | 533.74 | 1.51 | 2.29 | 1.57 |
| 269 | 16.05 | 19.00 | 545.90 | 1.48 | 2.25 | 1.43 |
| 267 | 15.92 | 18.56 | 518.11 | 1.32 | 2.30 | 1.20 |
| 270 | 15.70 | 18.67 | 564.05 | 1.49 | 2.30 | 1.20 |
| **[2]Trial Mean** | **15.35** | **18.12** | **545.15** | **1.38** | **2.24** | **1.29** |
| **[2]Trial Min** | **13.60** | **15.80** | **466.44** | **0.93** | **1.40** | **1.00** |
| **[2]TrialMax** | **18.23** | **22.15** | **625.45** | **1.96** | **2.86** | **1.89** |
| **[2]Trial SD** | **0.74** | **0.90** | **23.03** | **0.14** | **0.27** | **0.18** |
| **[2]Trial SE** | **0.21** | **0.32** | **190.00** | **0.01** | **0.03** | **0.02** |
| **[2]Trial $LSD_{0.05}$** | **0.42** | **0.63** | **372.40** | **0.02** | **0.05** | **0.03** |

[1]Only top 30 and bottom 10 families presented, [2]Calculated for total number of families (n =169),
[3]scores (1=good, 4= poor), [4]scores (1=good, 4=difficult), and Fam no.=Family numbers.
Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

The top 20 and 10 poorest performing families planted at Vlakkloof E76/P1 were ranked for all traits and presented in Tables 2.13 and 2.14. As at Pinewoods, families performed differently for the various traits. Families 143, 80 and 195 were found to be the best performing families for Dbh-UB and Dbh-OB, 143 and 195 having been amongst the top performers for these two traits at Pinewoods. As found at Pinewoods, none of the families found in the top 10 ranking families for these two traits were in the top 10 for any other traits. Families 124, 121 and 201 were common to the 10 poorest performers for Dbh-UB and Dbh-OB, but were different to the poorest families for other traits. These relationships were investigated further when genetic and phenotypic correlations were calculated. There was very little difference between Dbh-UB and Dbh-OB in the *E. nitens* control (Family 271) (17.03cm and 17.43cm, respectively).

Although the top performing families for Dbh-UB, families 271, 143 and 80, were not significantly different ($p \geq 0.05$) from each other, they were significantly higher ($p \leq 0.05$) than all families ranked five positions below them. The *E. nitens* control (family 271) was the best performing family for both Dbh-Ub and Dbh-OB, and was significantly different ($p \leq 0.05$) from the *E. macarthurii* control families 267, 268, 269 and 270. None of the four *E. macarthurii* controls were in the top 30 performing families for Dbh-UB and Dbh-OB at Vlakkloof. Control 268 (bulk of Jessievale seedlots) was significantly lower than 267 (bulk of best Jessievale seedlots) for both Dbh-UB and Dbh-OB, which was contradictory to what was found at Pinewoods.

With regards to height, family 80 was significantly taller ($p \leq 0.05$) than all families, with the exception of family 143. The *E. nitens* control, family 271, was not significantly different ($p \geq 0.05$) from family 267, but was significantly higher ($p \leq 0.05$) than control families 270, 269 and 268 for height. The remaining traits, bark stripping, bark thickness and stem form, also showed significant differences ($p \leq 0.05$), with top performing families being higher that the worst performers. The bark thickness measurements showed that *E. nitens* has notably thinner bark than *E. macarthurii*, as the *E. nitens* control differed significantly ($p \leq 0.05$) from the *E. macarthurii* control families 267, 269 and 270. Control family 268 also had significantly thinner bark ($p \leq 0.05$) than

the other three *E. macarthurii* controls at Vlakkloof, although this was not the case at Pinewoods.

The top 20 and 10 poorest performing families planted at Vlakkloof E76/P1 were ranked according to Dbh-UB, for all traits, as presented in Table 2.14. These ranked results are very useful as they provide all the trait information required for each family, especially for selection in field.

In the E76/P2 trial at Pinewoods (Table 2.15), the top 30 and worst 10 performing families for Dbh-UB were also those for height, with the exception of families 27 and 3. These top families were 271 (*E. nitens* control), 250, 222, 229, 262 and 267 (bulk best Jessievale families).

**Table 2.13 Detailed ranking of the 20 top and 10 worst performing families for each trait in the E76/P1 trial at Vlakkloof at 102 months**

| Ranking[1] | Fam No. | Dbh-UB (cm) | Fam No. | Dbh-OB (cm) | Fam No. | Bark thickness (cm) | Fam No. | Height (m) | Fam No. | Stem form[3] | Fam No. | Bark stripping[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 271 | 17.03 | 143 | 20.51 | 271 | 0.87 | 80 | 22.74 | 179 | 1.10 | 98 | 1.00 |
| **2** | 143 | 16.29 | 80 | 20.17 | 160 | 0.89 | 143 | 22.31 | 128 | 1.24 | 99 | 1.00 |
| **3** | 80 | 16.00 | 120 | 20.10 | 268 | 0.89 | 99 | 21.98 | 61 | 1.24 | 120 | 1.00 |
| **4** | 195 | 15.49 | 195 | 20.03 | 139 | 0.89 | 8 | 21.97 | 43 | 1.25 | 179 | 1.00 |
| **5** | 146 | 15.34 | 88 | 19.85 | 177 | 0.90 | 155 | 21.96 | 90 | 1.25 | 148 | 1.05 |
| **6** | 48 | 15.31 | 89 | 19.47 | 34 | 0.91 | 90 | 21.93 | 10 | 1.26 | 175 | 1.07 |
| **7** | 103 | 15.26 | 99 | 19.46 | 117 | 0.91 | 128 | 21.89 | 143 | 1.26 | 207 | 1.08 |
| **8** | 22 | 15.20 | 146 | 19.27 | 144 | 0.91 | 36 | 21.87 | 99 | 1.27 | 195 | 1.10 |
| **9** | 36 | 15.19 | 179 | 19.25 | 112 | 0.92 | 111 | 21.77 | 182 | 1.27 | 187 | 1.13 |
| **10** | 128 | 15.17 | 90 | 19.16 | 264 | 0.92 | 207 | 21.74 | 48 | 1.28 | 77 | 1.15 |
| **11** | 187 | 15.14 | 188 | 19.11 | 97 | 0.93 | 31 | 21.73 | 80 | 1.31 | 271 | 1.15 |
| **12** | 99 | 14.97 | 22 | 19.07 | 133 | 0.94 | 208 | 21.72 | 105 | 1.32 | 7 | 1.17 |
| **13** | 37 | 14.83 | 48 | 19.06 | 72 | 0.95 | 146 | 21.67 | 51 | 1.33 | 16 | 1.17 |
| **14** | 90 | 14.74 | 103 | 19.02 | 93 | 0.95 | 185 | 21.64 | 68 | 1.33 | 97 | 1.17 |
| **15** | 185 | 14.69 | 207 | 18.96 | 197 | 0.96 | 75 | 21.61 | 103 | 1.33 | 111 | 1.18 |
| **16** | 88 | 14.69 | 189 | 18.84 | 180 | 0.96 | 179 | 21.60 | 38 | 1.35 | 78 | 1.19 |
| **17** | 189 | 14.68 | 187 | 18.74 | 175 | 0.97 | 135 | 21.56 | 98 | 1.36 | 8 | 1.21 |
| **18** | 89 | 14.65 | 36 | 18.72 | 171 | 0.97 | 22 | 21.55 | 195 | 1.36 | 104 | 1.21 |
| **19** | 111 | 14.65 | 130 | 18.56 | 201 | 0.97 | 78 | 21.53 | 87 | 1.37 | 128 | 1.21 |
| **20** | 207 | 14.64 | 37 | 18.53 | 161 | 0.97 | 172 | 21.41 | 95 | 1.37 | 24 | 1.24 |
| **161** | 117 | 11.35 | 124 | 15.13 | 188 | 1.28 | 41 | 18.57 | 33 | 2.00 | 80 | 2.00 |
| **162** | 180 | 11.30 | 200 | 14.99 | 88 | 1.29 | 176 | 18.30 | 72 | 2.00 | 93 | 2.00 |
| **163** | 41 | 11.15 | 44 | 14.96 | 37 | 1.29 | 124 | 18.29 | 201 | 2.00 | 110 | 2.00 |
| **164** | 124 | 11.06 | 63 | 14.95 | 207 | 1.30 | 203 | 18.26 | 206 | 2.05 | 129 | 2.00 |
| **165** | 60 | 11.04 | 268 | 14.94 | 9 | 1.31 | 50 | 18.19 | 138 | 2.06 | 197 | 2.00 |
| **166** | 203 | 11.03 | 117 | 14.84 | 36 | 1.33 | 73 | 18.15 | 40 | 2.08 | 115 | 2.08 |
| **167** | 44 | 10.88 | 114 | 14.80 | 80 | 1.34 | 44 | 18.12 | 35 | 2.12 | 73 | 2.09 |
| **168** | 121 | 10.82 | 203 | 14.41 | 143 | 1.35 | 160 | 18.02 | 161 | 2.17 | 58 | 2.22 |
| **169** | 51 | 10.50 | 121 | 14.38 | 184 | 1.36 | 65 | 18.00 | 44 | 2.18 | 56 | 2.25 |
| **170** | 201 | 10.50 | 201 | 14.29 | 128 | 1.38 | 268 | 17.71 | 203 | 2.18 | 60 | 2.80 |
| **Commercial control ranked according to Dbh-UB** | | | | | | | | | | | | |
| **1** | 271 | 17.03 | | 17.43 | | 0.87 | | 20.65 | | 1.62 | | 1.15 |
| **65** | 267 | 13.65 | | 17.68 | | 1.16 | | 20.66 | | 1.63 | | 1.47 |
| **104** | 270 | 12.93 | | 17.06 | | 1.12 | | 19.62 | | 1.74 | | 1.80 |
| **136** | 269 | 12.38 | | 15.87 | | 1.04 | | 19.30 | | 1.74 | | 1.53 |
| **152** | 268 | 11.62 | | 14.94 | | 0.89 | | 17.71 | | 1.83 | | 1.50 |
| **[2]Trial mean** | | **13.28** | | **17.14** | | **1.11** | | **20.27** | | **1.63** | | **1.52** |
| **[2]Trial Min** | | **10.50** | | **14.29** | | **0.87** | | **17.71** | | **1.10** | | **1.00** |
| **[2]Trial Max** | | **17.03** | | **20.51** | | **1.38** | | **22.74** | | **2.18** | | **2.80** |
| **Trial SD** | | **1.17** | | **1.24** | | **0.11** | | **0.99** | | **0.21** | | **0.28** |
| **Trial SE** | | **0.57** | | **0.48** | | **0.004** | | **0.22** | | **0.017** | | **0.014** |
| **Trial $LSD_{0.05}$** | | **1.12** | | **0.95** | | **0.008** | | **0.44** | | **0.034** | | **0.028** |

[1]Only top 30 and 10 poorest families presented, [2]Calculated for total number of families (n =169),
[3]scores (1=good, 4= poor), [4]scores (1=good, 4=difficult), and Fam no.=Family numbers, SD= standard deviation.
Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

**Table 2.14 Ranking of the 20 top and 10 poorest performing families according to Dbh-UB for each trait in the E76/P1 trial at Vlakkloof at 102 months**

| Fam no. | Dbh-UB (cm) | Dbh-OB (cm) | Bark thickness (cm) | Height (m) | [1]Stem form | [2]Bark stripping |
|---|---|---|---|---|---|---|
| 271 | 17.03 | 17.43 | 0.87 | 20.65 | 1.62 | 1.15 |
| 143 | 16.29 | 20.51 | 1.35 | 22.31 | 1.26 | 1.27 |
| 80 | 16.00 | 20.17 | 1.34 | 22.74 | 1.31 | 2.00 |
| 195 | 15.49 | 20.03 | 1.27 | 21.36 | 1.36 | 1.10 |
| 146 | 15.34 | 19.27 | 1.27 | 21.67 | 1.44 | 1.25 |
| 48 | 15.31 | 19.06 | 1.25 | 21.31 | 1.28 | 1.29 |
| 103 | 15.26 | 19.02 | 1.21 | 20.86 | 1.33 | 1.64 |
| 22 | 15.20 | 19.07 | 1.16 | 21.55 | 1.73 | 1.33 |
| 36 | 15.19 | 18.72 | 1.33 | 21.87 | 1.78 | 1.32 |
| 128 | 15.17 | 18.35 | 1.38 | 21.89 | 1.24 | 1.21 |
| 187 | 15.14 | 18.74 | 1.27 | 20.42 | 1.50 | 1.13 |
| 99 | 14.97 | 19.46 | 1.05 | 21.98 | 1.27 | 1.00 |
| 37 | 14.83 | 18.53 | 1.29 | 21.11 | 1.55 | 1.25 |
| 90 | 14.74 | 19.16 | 1.15 | 21.93 | 1.25 | 1.31 |
| 185 | 14.69 | 17.94 | 1.13 | 21.64 | 1.50 | 1.31 |
| 88 | 14.69 | 19.85 | 1.29 | 21.15 | 1.82 | 1.43 |
| 189 | 14.68 | 18.84 | 1.19 | 21.29 | 1.67 | 1.50 |
| 89 | 14.65 | 19.47 | 1.23 | 20.77 | 1.40 | 1.27 |
| 111 | 14.65 | 18.39 | 1.10 | 21.77 | 1.53 | 1.18 |
| 207 | 14.64 | 18.96 | 1.30 | 21.74 | 1.41 | 1.08 |
| 117 | 11.35 | 15.13 | 1.28 | 18.57 | 2.00 | 2.00 |
| 180 | 11.30 | 14.99 | 1.29 | 18.30 | 2.00 | 2.00 |
| 41 | 11.15 | 14.96 | 1.29 | 18.29 | 2.00 | 2.00 |
| 124 | 11.06 | 14.95 | 1.30 | 18.26 | 2.05 | 2.00 |
| 60 | 11.04 | 14.94 | 1.31 | 18.19 | 2.06 | 2.00 |
| 203 | 11.03 | 14.84 | 1.33 | 18.15 | 2.08 | 2.08 |
| 44 | 10.88 | 14.80 | 1.34 | 18.12 | 2.12 | 2.09 |
| 121 | 10.82 | 14.41 | 1.35 | 18.02 | 2.17 | 2.22 |
| 51 | 10.50 | 14.38 | 1.36 | 18.00 | 2.18 | 2.25 |
| 201 | 10.50 | 14.29 | 1.38 | 17.71 | 2.18 | 2.80 |
| Commercial control ranked according to Dbh-UB | | | | | | |
| 267 | 13.65 | 17.68 | 1.16 | 20.66 | 1.63 | 1.47 |
| 268 | 11.62 | 14.94 | 0.89 | 17.71 | 1.83 | 1.50 |
| 269 | 12.38 | 15.87 | 1.04 | 19.30 | 1.74 | 1.53 |
| 270 | 12.93 | 17.06 | 1.12 | 19.62 | 1.74 | 1.80 |
| 271 | 17.03 | 17.43 | 0.87 | 20.65 | 1.62 | 1.15 |
| **Trial mean[2]** | **13.28** | **17.14** | **1.11** | **20.27** | **1.63** | **1.52** |
| **Trial Min[2]** | **10.50** | **14.29** | **0.87** | **17.71** | **1.10** | **1.00** |
| **Trial Max[2]** | **17.03** | **20.51** | **1.38** | **22.74** | **2.18** | **2.80** |
| **Trial SD[2]** | **1.17** | **1.24** | **0.11** | **0.99** | **0.21** | **0.28** |
| **Trial SE[2]** | **0.57** | **0.48** | **0.004** | **0.22** | **0.017** | **0.014** |
| **Trial $LSD^2_{0.05}$** | **1.12** | **0.95** | **0.008** | **0.44** | **0.034** | **0.028** |

[1]Only top 30 families presented, [2]Calculated for total number of families (n =169), [3]scores (1=good, 4= poor), [4]scores (1=good, 4=difficult), and Fam no.=Family numbers.
Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

The top performing family for Dbh-OB, the *E. nitens* control (271), was found not to be significantly different ($p \geq 0.05$) from other top performing families 250, 229, 132 and 267 (control bulk of best Jessievale families), but was significantly different ($p \leq 0.05$) to the remaining families (Table 2.15). The top 28 best performing families were significantly higher ($p \leq 0.05$) than the worst performers. The *E. nitens* control 271 was found not to be significantly higher ($p \geq 0.05$) than control family 267, but was significantly better ($p \leq 0.05$) than the remaining three *E. macarthurii* controls 268, 269 and 270. With regards to heights, the top 13 best performing families were not significantly different ($p \geq 0.05$) from each other, but were significantly different ($p \leq 0.05$) from the remaining families, with the exception of the control families 271 and 267. Control 269 was significantly higher than control family 268 and significantly lower than family 270. There were significant differences ($p \leq 0.05$) between top and poor performing families for stem form.

At the E76/P2 progeny trial at Vlakkloof (Table 2.17), family ranking differed markedly for the various traits. For example, only four families were found in common in the top 30 families for Dbh and height; 81and 92, 220 and 229, which was somewhat different to what was found in E76/P2 at Pinewoods. With regards to the poorest performing families, only families 47, 74, 28 and 140 were not common for Dbh-OB and height at Vlakkloof. The poor families for Dbh-OB and height were not in common with those that had poor stem form.

The top 14 performers for Dbh-OB were not significantly different ($p \geq 0.05$) from each other, although showed significantly higher mean values than other families ranked more than 15 positions below them. The control families 267, 268 and 269 were significantly higher than families 270 and 271 for all the traits. The same trend was found for heights, with the first 20 best performing families not differing significantly ($p \geq 0.05$) from each other, but differing from lower ranking families. With regards to stem form, the top 30 families were not significantly different from each other but they were significantly higher ($p \leq 0.05$) than the lower ranked families, as well as the controls.

**Table 2.15 Detailed ranking of the 20 top and 10 worst performing families for each trait in the E76/P2 trial at Pinewoods site at 90 months**

| Ranking | Fam no. | Dbh-OB (cm) | Fam no. | Height (m) | Fam no. | Stem form[2] |
|---|---|---|---|---|---|---|
| **1** | 271 | 13.77 | 271 | 13.03 | 30 | 1.11 |
| **2** | 250 | 12.49 | 62 | 12.36 | 123 | 1.11 |
| **3** | 229 | 12.39 | 222 | 12.26 | 16 | 1.21 |
| **4** | 132 | 12.37 | 267 | 12.13 | 74 | 1.32 |
| **5** | 222 | 12.35 | 250 | 12.08 | 211 | 1.39 |
| **6** | 267 | 12.29 | 229 | 12.00 | 265 | 1.50 |
| **7** | 62 | 12.07 | 262 | 11.97 | 134 | 1.54 |
| **8** | 262 | 12.05 | 9 | 11.91 | 28 | 1.57 |
| **9** | 140 | 12.05 | 269 | 11.85 | 264 | 1.61 |
| **10** | 269 | 11.96 | 140 | 11.75 | 96 | 1.61 |
| **11** | 52 | 11.80 | 259 | 11.67 | 266 | 1.61 |
| **12** | 248 | 11.79 | 263 | 11.60 | 225 | 1.61 |
| **13** | 259 | 11.76 | 230 | 11.56 | 92 | 1.68 |
| **14** | 214 | 11.58 | 214 | 11.52 | 268 | 1.68 |
| **15** | 230 | 11.58 | 137 | 11.48 | 29 | 1.71 |
| **16** | 137 | 11.51 | 213 | 11.48 | 254 | 1.71 |
| **17** | 213 | 11.50 | 252 | 11.39 | 255 | 1.75 |
| **18** | 252 | 11.44 | 132 | 11.27 | 85 | 1.79 |
| **19** | 217 | 11.34 | 253 | 11.27 | 86 | 1.79 |
| **20** | 263 | 11.32 | 248 | 11.25 | 126 | 1.79 |
| **72** | 221 | 8.13 | 221 | 8.23 | 222 | 2.25 |
| **73** | 202 | 8.13 | 260 | 8.20 | 251 | 2.25 |
| **74** | 134 | 7.93 | 134 | 8.13 | 261 | 2.25 |
| **75** | 96 | 7.66 | 96 | 8.05 | 262 | 2.29 |
| **76** | 266 | 7.66 | 266 | 7.72 | 19 | 2.32 |
| **77** | 123 | 7.64 | 123 | 7.53 | 230 | 2.32 |
| **78** | 16 | 6.03 | 225 | 6.35 | 137 | 2.36 |
| **79** | 225 | 5.96 | 265 | 6.05 | 271 | 2.39 |
| **80** | 265 | 5.54 | 16 | 6.03 | 62 | 2.46 |
| **81** | 30 | 4.01 | 30 | 4.06 | 267 | 2.46 |
| **Commercial control ranked according to Dbh-OB** | | | | | | |
| **1** | 271 | 13.77 | | 13.03 | | 2.39 |
| **6** | 267 | 12.29 | | 12.13 | | 2.46 |
| **10** | 269 | 11.96 | | 11.85 | | 2.18 |
| **58** | 268 | 9.53 | | 9.45 | | 1.68 |
| **64** | 270 | 9.13 | | 9.29 | | 2.04 |
| **Trial mean[2]** | | **10.13** | | **11.66** | | **11.53** |
| **Trial Min[2]** | | **4.01** | | **10.79** | | **10.83** |
| **Trial Max[2]** | | **13.77** | | **13.77** | | **13.03** |
| **Trial SD[2]** | | **1.71** | | **0.64** | | **0.52** |
| **Trial SE[2]** | | **0.83** | | **0.74** | | **0.03** |
| **Trial $LSD^2_{0.05}$** | | **1.63** | | **1.45** | | **0.06** |

[1]Calculated for total number of families (n =81), [2]scores (1=good, 4= poor), and Fam no.=Family numbers, SD=standard deviation.

Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

The top 20 performing and 10 poorest families at Pinewoods and Vlakkloof (E76/P2) were ranked according to Dbh-UB as in Tables 2.16 and 2.18. These ranked results are very useful as they provide all the trait information required for each family, especially for selection in field.

**Table 2.16 Summary ranking of the top 20 and 10 worst performing families according to Dbh-UB for each trait in the E76/P2 trial at Pinewoods at 90 months**

| Fam no[1]. | Dbh-OB (cm) | Height (m) | Stem form[2] |
|---|---|---|---|
| 271 | 13.77 | 13.03 | 2.39 |
| 250 | 12.49 | 12.08 | 2.18 |
| 229 | 12.39 | 12.00 | 2.18 |
| 132 | 12.37 | 11.27 | 2.07 |
| 222 | 12.35 | 12.26 | 2.25 |
| 267 | 12.29 | 12.13 | 2.46 |
| 62 | 12.07 | 12.36 | 2.46 |
| 262 | 12.05 | 11.97 | 2.29 |
| 140 | 12.05 | 11.75 | 2.11 |
| 269 | 11.96 | 11.85 | 2.18 |
| 52 | 11.80 | 11.91 | 2.18 |
| 248 | 11.79 | 11.25 | 1.93 |
| 259 | 11.76 | 11.67 | 1.96 |
| 214 | 11.58 | 11.52 | 2.25 |
| 230 | 11.58 | 11.56 | 2.32 |
| 137 | 11.51 | 11.48 | 2.36 |
| 213 | 11.50 | 11.48 | 2.18 |
| 252 | 11.44 | 11.39 | 1.86 |
| 217 | 11.34 | 10.85 | 2.25 |
| 263 | 11.32 | 11.60 | 2.18 |
| 221 | 8.13 | 8.23 | 2.25 |
| 202 | 8.13 | 8.20 | 2.25 |
| 134 | 7.93 | 8.13 | 2.25 |
| 96 | 7.66 | 8.05 | 2.29 |
| 266 | 7.66 | 7.72 | 2.32 |
| 123 | 7.64 | 7.53 | 2.32 |
| 16 | 6.03 | 6.35 | 2.36 |
| 225 | 5.96 | 6.05 | 2.39 |
| 265 | 5.54 | 6.03 | 2.46 |
| 30 | 4.01 | 4.06 | 2.46 |
| **Commercial control ranked according to Dbh-OB** | | | |
| 267 | 12.29 | 12.13 | 2.46 |
| 268 | 9.53 | 9.45 | 1.68 |
| 269 | 11.96 | 11.85 | 2.18 |
| 270 | 9.13 | 9.29 | 2.04 |
| 271 | 13.77 | 13.03 | 2.39 |
| **Trial mean[3]** | **10.13** | **11.66** | **11.53** |
| **Trial Min[3]** | **4.01** | **10.79** | **10.83** |
| **Trial Max[3]** | **13.77** | **13.77** | **13.03** |
| **Trial SD[3]** | **1.71** | **0.64** | **0.52** |
| **Trial SE[3]** | **0.83** | **0.74** | **0.03** |
| **Trial $LSD^3_{0.05}$** | **1.63** | **1.45** | **0.06** |

[1]Family numbers, [2]scores (1=good, 4= poor), and [3]Calculated for total number of families (n = 81), SD=standard deviation. Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

**Table 2.17 Detailed ranking of the 30 top and 10 poorest performing families for each trait in the E76/P2 trial at Vlakkloof at 90 months**

| **Ranking**[1] | Fam no. | Dbh-OB (cm) | Fam no. | Height (m) | Fam no. | Stem form[4] |
|---|---|---|---|---|---|---|
| **1** | 81 | 16.16 | 233 | 16.50 | 30 | 1.21 |
| **2** | 234 | 15.76 | 81 | 16.44 | 16 | 1.25 |
| **3** | 224 | 15.41 | 92 | 16.19 | 265 | 1.32 |
| **4** | 92 | 15.39 | 234 | 16.10 | 237 | 1.32 |
| **5** | 244 | 15.01 | 229 | 15.99 | 271 | 1.38 |
| **6** | 91 | 14.88 | 220 | 15.79 | 140 | 1.46 |
| **7** | 253 | 14.80 | 244 | 15.76 | 243 | 1.46 |
| **8** | 230 | 14.79 | 231 | 15.72 | 29 | 1.50 |
| **9** | 220 | 14.76 | 266 | 15.58 | 74 | 1.50 |
| **10** | 229 | 14.67 | 253 | 15.55 | 102 | 1.50 |
| **11** | 233 | 14.48 | 252 | 15.41 | 211 | 1.50 |
| **12** | 223 | 14.44 | 239 | 15.17 | 134 | 1.54 |
| **13** | 218 | 14.37 | 269 | 15.15 | 191 | 1.56 |
| **14** | 132 | 14.37 | 246 | 15.10 | 28 | 1.57 |
| **15** | 268 | 14.24 | 212 | 14.93 | 85 | 1.57 |
| **16** | 231 | 14.23 | 268 | 14.90 | 96 | 1.57 |
| **17** | 239 | 14.17 | 245 | 14.88 | 123 | 1.61 |
| **18** | 245 | 14.13 | 224 | 14.83 | 232 | 1.61 |
| **19** | 260 | 14.12 | 119 | 14.81 | 242 | 1.61 |
| **20** | 215 | 14.05 | 218 | 14.78 | 248 | 1.61 |
| **72** | 47 | 10.43 | 242 | 10.93 | 213 | 2.21 |
| **73** | 28 | 10.29 | 74 | 10.93 | 215 | 2.21 |
| **74** | 140 | 10.28 | 243 | 10.68 | 222 | 2.21 |
| **75** | 242 | 10.16 | 16 | 10.44 | 229 | 2.21 |
| **76** | 16 | 9.89 | 102 | 10.08 | 239 | 2.21 |
| **77** | 237 | 8.88 | 191 | 9.75 | 262 | 2.21 |
| **78** | 191 | 8.85 | 270 | 9.46 | 238 | 2.25 |
| **79** | 270 | 8.45 | 237 | 9.44 | 266 | 2.36 |
| **80** | 265 | 7.19 | 265 | 8.29 | 244 | 2.36 |
| **81** | 30 | 6.83 | 30 | 7.66 | 252 | 2.43 |
| **Commercial controls ranked according to Dbh-OB** | | | | | | |
| **29** | 269 | 15.15 | | 13.70 | | 1.86 |
| **15** | 268 | 14.90 | | 14.24 | | 2.04 |
| **37** | 267 | 14.51 | | 13.21 | | 2.07 |
| **75** | 271 | 10.97 | | 10.89 | | 1.38 |
| **58** | 270 | 9.46 | | 8.45 | | 1.71 |
| **Trial mean**[3] | | **14.43** | | **15.14** | | **1.55** |
| **Trial Min**[3] | | **16.16** | | **16.50** | | **1.75** |
| **Trial Max**[3] | | **16.16** | | **16.50** | | **1.75** |
| **Trial SD**[3] | | **0.63** | | **0.67** | | **0.14** |
| **Trial SE** | | **0.97** | | **0.94** | | **0.02** |
| **Trail LSD** | | **1.90** | | **1.84** | | **0.04** |

Calculated for total number of families (n = 81), [4]scores (1=good, 4= poor), and Fam no. = Family numbers. Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

**Table 2.18 Summary ranking of the top 30 and 10 worst performing families according to Dbh-OB for each trait in the E76/P2 trial at Vlakkloof at 90 months**

| Fam no. | Dbh-OB (cm) | Height (m) | Stem form[1] |
|---|---|---|---|
| 81 | 16.16 | 16.44 | 2.04 |
| 234 | 15.76 | 16.10 | 2.14 |
| 224 | 15.41 | 14.83 | 2.00 |
| 92 | 15.39 | 16.19 | 1.82 |
| 244 | 15.01 | 15.76 | 2.36 |
| 91 | 14.88 | 14.68 | 2.07 |
| 253 | 14.80 | 15.55 | 2.18 |
| 230 | 14.79 | 14.37 | 1.64 |
| 220 | 14.76 | 15.79 | 1.96 |
| 229 | 14.67 | 15.99 | 2.21 |
| 233 | 14.48 | 16.50 | 2.11 |
| 223 | 14.44 | 14.13 | 1.75 |
| 218 | 14.37 | 14.78 | 2.00 |
| 132 | 14.37 | 14.54 | 1.86 |
| 268 | 14.24 | 14.90 | 2.04 |
| 231 | 14.23 | 15.72 | 2.00 |
| 239 | 14.17 | 15.17 | 2.21 |
| 245 | 14.13 | 14.88 | 2.04 |
| 260 | 14.12 | 13.40 | 1.86 |
| 215 | 14.05 | 14.48 | 2.21 |
| 47 | 10.43 | 10.93 | 2.21 |
| 28 | 10.29 | 10.93 | 2.21 |
| 140 | 10.28 | 10.68 | 2.21 |
| 242 | 10.16 | 10.44 | 2.21 |
| 16 | 9.89 | 10.08 | 2.21 |
| 237 | 8.88 | 9.75 | 2.21 |
| 191 | 8.85 | 9.46 | 2.25 |
| 270 | 8.45 | 9.44 | 2.36 |
| 265 | 7.19 | 8.29 | 2.36 |
| 30 | 6.83 | 7.66 | 2.43 |
| **Commercial control ranked according to Dbh-OB** | | | |
| 269 | 15.15 | 13.70 | 1.86 |
| 268 | 14.90 | 14.24 | 2.04 |
| 267 | 14.51 | 13.21 | 2.07 |
| 271 | 10.97 | 10.89 | 1.38 |
| 270 | 9.46 | 8.45 | 1.71 |
| **[3]Trial mean** | **14.43** | **15.14** | **1.55** |
| **[3]Trial Min** | **16.16** | **16.50** | **1.75** |
| **[3]Trial Max** | **16.16** | **16.50** | **1.75** |
| **[3]Trial SD** | **0.63** | **0.67** | **0.14** |
| **Trial SE** | **0.97** | **0.94** | **0.02** |
| **Trial $LSD_{0.05}$** | **1.90** | **1.84** | **0.04** |

Fam no. = Family numbers; and [1]scores (1=good, 4= poor).
Controls: 267*Bulk Jessievale BSO best families, 268*Bulk Jessievale BSO all families, 269*Bulk Seven Oaks BSO all families, 270*SAMTMA bulk, unimproved ex-commercial and 271**E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016.

Genetic parameters; genetic ($r_g$) and phenotypic ($r_p$) correlations, heritabilities, and predicted gains are presented in Tables 2.19 to 2.22. Tables 2.19 and 2.20 provide genetic and phenotypic correlations for various trait combinations found at Pinewoods and Vlakkloof in the E76/P1 and E76/P2 series, respectively. The $r_g$ and $r_p$ values for the E76/P1 series ranged between -1 and 1, which is the standard correlation range (Falconer and Mackay, 1996). The $r_g$ and $r_p$ values found at Pinewoods for the E76/P2 progeny trial series were similar to those at Vlakkloof, ranging between 0.72 to 0.98, with the exception of $r_g$ of 1.46 (Table 2.20) for Dbh-OB and height at Pinewoods.

**Table 2.19 Genetic ($r_g$) and phenotypic correlations ($r_p$) for various trait combinations in 2nd generation E. macarthurii progeny trials (E76/P1) at Pinewoods and Vlakkloof**

| Traits | Trial sites | | | |
|---|---|---|---|---|
| | Pinewoods | | Vlakkloof | |
| | $r_g$ | $r_p$ | $r_g$ | $r_p$ |
| Dbh-OB / Dbh-UB | 0.91 | 0.96 | 0.98 | 0.99 |
| Dbh-OB / bark thick | 0.67 | 0.64 | 0.25 | 0.80 |
| Dbh-OB / basic wood density | -0.10 | 0.041 | - | - |
| Dbh-OB / stem form | 0.09 | -0.08 | -0.25 | -0.24 |
| Dbh-OB / bark strip | -0.52 | -0.17 | -0.63 | -0.42 |
| Dbh-UB / bark thick | 0.31 | 0.42 | 0.06 | 0.72 |
| Dbh-UB / basic wood density | -0.12 | 0.03 | - | - |
| Dbh-UB / stem form | 0.02 | -0.07 | -0.23 | -0.24 |
| Dbh-UB / bark strip | -0.47 | -0.16 | -0.74 | -0.42 |
| Stem form / bark thick | 0.21 | -0.07 | -0.05 | -0.19 |
| Stem form /density | -0.30 | -0.07 | - | - |
| Stem form / bark strip | -0.42 | 0.02 | 0.17 | 0.15 |
| Bark strip / basic wood density | -0.43 | 0.01 | - | - |
| Bark thick / basic wood density | 0.00 | 0.04 | - | - |
| Bark thick / bark strip | -0.38 | -0.11 | -0.08 | -0.29 |
| Dbh-OB / height | - | - | 0.73 | 0.82 |
| Dbh-UB / height | - | - | 0.74 | 0.83 |
| Stem form / height | - | - | -0.46 | -0.33 |
| Stem form / bark strip | - | - | 0.17 | 0.15 |
| Bark strip / height | - | - | -0.58 | -0.38 |
| Height / bark thick | | | 0.08 | 0.59 |

A correlation coefficient of 1 would indicate a perfect correlation between the behaviour of genotypes on two traits being assessed. Due to the nature of the variance component estimates, the correlation may in practice exceed unity, although in theory, this is not possible (Hettasch *et al*., 2007)

**Table 2.20 Genetic ($r_g$) and phenotypic correlations ($r_p$) for various traits combinations in 2nd generation *E. macarthurii* progeny trials (E76/P2) at Pinewoods and Vlakkloof**

| Traits | Progeny trial sites | | | |
|---|---|---|---|---|
| | Pinewoods | | Vlakkloof | |
| | $r_g$ | $r_p$ | $r_g$ | $r_p$ |
| Dbh-OB / height | 0.98 | 0.98 | 0.97 | 0.94 |
| Dbh-OB / stem form | 1.46 | 0.74 | -0.69 | -0.72 |
| Height / stem form | 0.86 | 0.79 | 0.72 | 0.79 |

$r_g$=genetic correlation, and $r_p$=phenotypic correlation.

The heritabilities and predicted gains for each site are presented in Table 2.21. The $h^2$ estimates for the E76/P1 and E76/P2 trials at Pinewoods ranged from 0.12 (12%) to 0.24 (24%), and at Vlakkloof from 0.03 (3%) to 0.16 (16%).

**Table 2.21 Heritability ($h^2$) and predicted gains (ΔG) for various traits in 2nd generation *E. macarthurii* E76/P1 and E76/P2 progeny trials at two sites**

| Traits | Pinewoods | | Vlakkloof | |
|---|---|---|---|---|
| | **Heritability ($h^2$)** | **Predicted gain (ΔG)** | **Heritability ($h^2$)** | **Predicted gain (ΔG)** |
| | **Progeny trial site E76/P1** | | | |
| Dbh-OB | 0.14 | 1.37 cm | 0.03 | 0.16 cm |
| Dbh-UB | 0.12 | 3.55 cm | 0.16 | 0.60 cm |
| Bark thickness | 0.24 | 0.10 cm | 0.11 | 0.04 cm |
| Basic wood density | 0.15 | 10.77 $kgm^{-3}$ | - | - |
| Height | - | - | 0.09 | 0.40 m |
| | **Progeny trial site E76/P2** | | | |
| Dbh-OB | 0.12 | 2.72 cm | 0.11 | 1.02 cm |
| Height | 0.12 | 2.09 m | 0.12 | 3.52 m |

The actual, or realised, genetic gain that was made in Gen 2 over Gen 1 in the trial series was calculated from the average of the top 15 families in Gen 2, compared to the SAMTMA commercial control, and Jessievale BSO best families control respectively, and expressed as a percentage improvement or gain (Table 2.22). The genetic gains found at Pinewoods were notably higher than those at Vlakkloof.

**Table 2.22 Actual gains (ΔG) for various traits in 2nd generation *E. macarthurii* E76/P2 and E76/P1 progeny trials at two sites in relation to the SAMTMA and Bulk Jessievale BSO best families controls**

| Traits | Pinewoods | | Vlakkloof | |
|---|---|---|---|---|
| | SAMTMA[1] (ΔG) (%) | Jess best[2] (ΔG) (%) | SAMTMA[1] (ΔG) (%) | Jess best[2] (ΔG) (%) |
| **E76/P1 Progeny trial sites** | | | | |
| Dbh-OB | 5.57 | 6.20 | 17.06 | 14.30 |
| Dbh-UB | 5.92 | 4.46 | 17.71 | 11.50 |
| Basic wood density | 3.76 | 12.96 | - | - |
| Height | - | - | 11.62 | 6.00 |
| **E76/P2 Progeny trial sites** | | | | |
| Dbh-OB | 33.07 | - | 43.29 | 12.79 |
| Height | 22.26 | - | 65.86 | 8.13 |

[1] South African Mining Timber Manufacturer's Association, unimproved commercial; and
[2] Bulk Jessievale BSO best families, improved

## 2.4 Discussion

Overall growth was better at Pinewoods than Vlakkloof for the E76/P1 trial series, but the opposite was true for the E76/P2 series. The Pinewoods site is marginally cooler and wetter than the Vlakkloof site and may have been more suitable for growth of *E. macarthurii* than the Vlakkloof site. The reason for the markedly poorer growth at Pinewoods in the P2 trial series may be that this site suffered damage by heavy snow 18 months after establishment.

The genetic and phenotypic correlations revealed that trees planted at the Pinewoods site had highly significant $r_p$ and $r_g$ for Dbh-OB with Dbh-UB. These correlations suggest that selection for one trait could lead to strong positive indirect response to selection for the other trait, which is what would be expected as Dbh-OB and Dbh-UB are dependent traits that is, Dbh-UB is a function of Dbh-OB and bark thickness. It was also found that Dbh-OB and bark thickness were highly correlated ($r_g$ = 0.67), which was confirmed by the Dbh-UB with bark thickness correlation. These results indicated that as diameter increases, so does bark thickness. Although forestry industries would prefer big trees with thinner bark, this suggests that it is not possible to breed for thinner bark on bigger trees without breaking the linkage between these two traits. However, the correlations for bark thickness and stripping revealed that, as bark thickness and Dbh-OB increased, stripping of bark became easier. The correlations also indicated that stripping of bark became easier with improved stem form. A very slight negative trend was noted between diameter and basic wood density ($r_g$=-0.10), which was supported by $r_g$ of -0.07 and $r_p$ of 0.064 found for the same traits in *E. grandis* and *E. urophylla* hybrids (Retief and Stanger, 2007a). The positive correlations found between basic wood density and bark thickness for this study ($r_p$ =0.42 and 0.64), for Dbh-UB and Dbh-OB respectively, were similar to those of *E. grandis* and *E. urophylla* hybrids ($r_p$ =0.39) (Retief and Stanger, 2007b). Interestingly, it was also found that, as basic wood density increased, stripping of bark became easier, and stem form improved. There is no clear explanation as to why basic wood density should be correlated to these two traits in this manner.

The correlations found at Vlakkloof compared favourably with those at Pinewoods in most cases. The $r_g$ and $r_p$ of Dbh-OB with Dbh-UB found at the Vlakkloof site were strongly positive, as was found at Pinewoods. It was also found that height increased with increasing diameter as shown by strong $r_g$ and $r_p$ of Dbh-OB and Dbh-UB with height. Bark thickness was found to increase with increasing diameter, as was found at Pinewoods. Increasing height was also correlated with increased bark thickness. Similarly, correlations were strong for diameter, height and stripping, which indicated that as diameter and height increased, stripping of bark became easier. Also, as height increased, stem form improved, which indicated that taller trees had good stem form.

At Vlakkloof, there was a slight trend of stem form improving with increasing diameter. On the contrary, correlations between these two traits at Pinewoods were close to zero, although it did appear that stem form improved with diameter in the field. As stem form had fairly strong correlations with all other traits at Pinewoods, it may be that there were some confounding effects between diameter and stem form at this site. There was a strong genotype by environment interaction amongst all the growth characteristics, which was indicated by a low $r_B$. Genotype by environment interaction was found in this population, indicating that different populations should perhaps be developed independently of each other for the two sites/site types

The E76/P2 trials planted at Pinewoods and Vlakkloof also revealed good correlations amongst the traits. The results showed that as Dbh-OB increased, so did height, which compared favourably with results found in *E. camaldulensis* (Mahmood *et al*., 2003). At Vlakloof, as Dbh-OB and height increased, stem form improved, however, there was no correlation of diameter with stem form at Pinewoods. In similar studies in *E. camaldulensis*, stem form was negatively correlated with height and Dbh-OB (Mahmood *et al*., 2003).

The heritability ($h^2$) estimates calculated for traits in the E76/P1 and E76/P2 progeny trials fell within the normal range for eucalypts (Cotterill and Dean, 1990). The $h^2$ estimates calculated in this study for both sites for Dbh (E76/P1: 0.12 to 0.16 and E76/P2: 0.11 to 0.12) and height (E76/P1: 0.09 and E76/P2: 0.12) compared well to those of *E. camaldulensis*, which had $h^2$ estimates of 0.11 and 0.14 for Dbh and height, respectively (Mahmood *et al*., 2003). The $h^2$ estimates for basic wood density in this study (0.15) were lower than those found in another study on *E. dunnii*, (0.42) (Arnold *et al*., 2004). *Eucalyptus grandis* and *E. urophylla* hybrids had $h^2$ estimates of 0.04 and 0.06 for heights, respectively (Retief and Stanger, 2007), which were lower than the $h^2$ estimates for height found in this study (0.09 and 0.12 for E76/P1 and E76/P2 respectively). The $h^2$ estimates found for this investigation revealed that there would be a good probability of producing the desired response to selection, which agrees with the results found for Shimizu *et al.* (2002).

Predicted gains for the offspring of the E76/P1 trials at Pinewoods and Vlakkloof differed in that the predicted gains for Pinewoods were much greater than at Vlakkloof, with the exception of basic wood density and height, which were not measured at both sites for the E76/P1 progeny trials. This was probably related to the higher $h^2$ at the Pinewoods site in this trial series. Interestingly, the predicted increases in bark thickness were very small, which is suitable for the forestry industries. These results were slightly different for E76/P2 progeny trials which had comparable gains at both sites for Dbh-OB and height.

The actual gains realised in Gen 2 (E76/P1 progeny trials) were calculated for Dbh-UB and Dbh-OB, basic wood density and height, whereas progeny trials E76/P2 actual gains were calculated for Dbh-Ob and height only. These gains were based on the mean of the top 15 families, as this is ideally what would be used in a commercial seed bulk, and compared to two of the controls; SAMTMA (unimproved commercial) and a bulk mix of the Jessievale BSO best families (improved) (Table 2.21). It was found that a 6% increase in diameter was made from generation 2 over generation 1 at Pinewoods, and

11 to 17% at Vlakkloof when comparing with the Jessievale best families bulk and the SAMTMA commercial control, respectively. The results revealed that, much higher gains were made which ranged from 8% to 66%. These gains will have resulted in substantial returns over large plantation areas. Actual gains were better at Vlakkloof than Pinewoods, although growth was better at Pinewoods, which might be due to different climatic conditions.

## 2.5 Conclusion

Growth characteristics of the second generation *E. macarthurii* were evaluated. Significant family differences were found for various growth traits at both sites, indicating that improvements in growth (as measured by Dbh-OB and Dbh-UB) and stem form were achieved. Correlations between Dbh-OB and Dbh-UB at both sites indicated that it was sufficient to select for increased Dbh-OB to achieve increased Dbh-UB. It was also found that selection for Dbh-OB and Dbh-UB resulted in increased in height. The heritabilities were also found to be in the normal range of eucalypt species, indicating that moderate improvement can be made for the traits measured in the following generation. Predicted and actual gains achieved ranged from moderate to high, which will results in substantial returns in genetic gains for the next generation, which is extremely useful to the South African forestry industry. The information generated from this study will be extremely valuable when making selections for, and decisions regarding, the following generation of breeding in *E. macarthurii*.

## References

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## 2.5 Appendices

**Appendix Table 2.1 Family composition of second generation *E. macarthurii* E76/P1 progeny trials**

| Gen 2 Families | Family no. | Gen 2 Families | Family no. | Gen 2 Families | Family no. | Gen 2 Families | Family no. | Gen 2 Families. | Family no. |
|---|---|---|---|---|---|---|---|---|---|
| 1* | MO1010 | 44* | MO513 | 82* | MO114 | 124* | MO8 | 171* | MO501 |
| 2* | MO519 | 45* | MO307 | 83* | MO42 | 125* | MO666 | 172* | MO330 |
| 3* | MO1003 | 46* | MO625 | 84* | MO35 | 128* | MO647 | 173* | MO340 |
| 4* | MO2 | 48* | MO109 | 87* | MO5512 | 129* | MO545 | 174* | MO334 |
| 6* | MO552 | 49* | MO705 | 88* | MO570 | 130* | MO308 | 175* | MO518 |
| 7* | MO88 | 50* | MO104 | 89* | MO43 | 133* | MO509 | 176* | MO410 |
| 8* | MO655 | 51* | MO585 | 90* | MO568 | 134* | MO6 | 178* | MO1004 |
| 9* | MO636 | 53* | MO315 | 93* | MO727 | 135* | MO511 | 179* | MO22 |
| 10* | MO1012 | 54* | MO70 | 94* | MO657 | 136* | MO316 | 180* | MO543 |
| 13* | MO564 | 55* | MO648 | 95* | MO5516 | 138* | MO86 | 181* | MO339 |
| 14 | MO565 | 56* | MO301 | 97* | MO69 | 139* | MO65 | 182* | MO504 |
| 15* | MO67 | 57* | MO1019 | 98* | MO5522 | 142* | MO317 | 183* | MO409 |
| 16* | MO1020 | 58* | MO306 | 99* | MO577 | 143* | MO94 | 184* | MO329 |
| 17* | MO1013 | 59* | MO645 | 101* | MO633 | 144* | MO85 | 185* | MO93 |
| 18* | MO103 | 60* | MO704 | 103* | MO664 | 145* | MO7 | 186* | MO39 |
| 22* | MO5508 | 61* | MO634 | 104* | MO717 | 146* | MO327 | 187* | MO522 |
| 24* | MO584 | 63* | MO111 | 105* | MO719 | 148* | MO516 | 188* | MO338 |
| 25* | MO662 | 64* | MO413 | 106* | MO722 | 153* | MO523 | 189* | MO16 |
| 26* | MO1 | 65* | MO134 | 107* | MO582 | 155* | MO1002 | 190* | MO526 |
| 27* | MO512 | 66* | MO1001 | 108* | MO706 | 156* | MO1021 | 192* | MO593 |
| 31* | MO11 | 67* | MO139 | 109* | MO5509 | 158* | MO309 | 194* | MO1009 |
| 32* | MO66 | 68* | MO108 | 110* | MO546 | 159* | MO411 | 195* | MO591 |
| 33* | MO669 | 69 | MO1022 | 111* | MO594 | 160* | MO533 | 197* | MO507 |
| 34* | MO312 | 70* | MO129 | 112* | MO303 | 161* | MO19 | 200* | MO631 |
| 35* | MO5507 | 71* | MO127 | 113* | MO63 | 162* | MO32 | 201* | MO745 |
| 36* | MO630 | 72* | MO252 | 114* | MO1015 | 163* | MO532 | 202* | MO754 |
| 37* | MO1011 | 73* | MO125 | 115* | MO62 | 164* | MO525 | 203* | MO757 |
| 38* | MO514 | 75* | MO317 | 116* | MO5 | 165* | MO530 | 204* | MO748 |
| 39* | MO10 | 76* | MO1017 | 117* | MO72 | 166* | MO337 | 205* | MO732 |
| 40* | MO404 | 77* | MO5517 | 118* | MO726 | 167* | MO38 | 206* | MO755 |
| 41* | MO402 | 78* | MO5520 | 120* | MO559 | 168* | MO539 | 207* | MO759 |
| 42* | MO135 | 79* | MO5502 | 121* | MO5514 | 169* | MO509 | 208* | MO753 |
| 43* | MO302 | 80* | MO670 | 122* | MO567 | 170* | MO326 | 209* | MO736 |
| **Commercial controls included in E76/P1 trials, but not in sub-populations** | | | | | | | | | |
| 267* | Bulk Jessievale BSO best families | | | | | | | | |
| 268* | Bulk Jessievale BSO all families | | | | | | | | |
| 269* | Bulk Seven Oaks BSO all families | | | | | | | | |
| 270* | [1]SAMTMA bulk, unimproved ex-commercial | | | | | | | | |
| 271* | *E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016 | | | | | | | | |

Gen 2 Families = Second generation Families, Family no.= Family number,
MO = seed collected from Seven Oaks BSO, MJ= seed collected from Jessievale BSO,
* Common families with E76/P2 trial series; and [1] South African Mining Timber Manufacturer's Association.

## Appendix Table 2.2 Family composition of second generation *E. macarthurii* (E76/P2 trials)

| Gen 2 Families | Family no. | Gen 2 Families. | Family no. | Gen 2 Families | Family no. |
|---|---|---|---|---|---|
| 2 | MO519 * | 211 | MJ406 | 240 | MJ125 |
| 16 | MO1020 * | 212 | MJ127 | 241 | MJ1014 |
| 19 | MO68 | 213 | MJ1004 | 242 | MJ1018 |
| 28 | MO642 | 214 | MJ323 | 243 | MJ1017 |
| 29 | MO637 | 215 | MJ1002 | 244 | MJ139 |
| 30 | MO557 | 216 | MJ317 | 245 | MJ322 |
| 47 | MO313 | 217 | MJ308 | 246 | MJ306 |
| 52 | MO654 | 218 | MJ1005 | 247 | MJ314 |
| 62 | MO406 | 219 | MJ507 | 248 | MJ135 |
| 69 | MO1022 | 220 | MJ305 | 249 | MJ510 |
| 74 | MO44 | 221 | MJ326 | 250 | MJ103 |
| 81 | MO649 | 222 | MJ85 | 251 | MJ1009 |
| 85 | MO653 | 223 | MJ324 | 252 | MJ5 |
| 86 | MO5506 | 224 | MJ1021 | 253 | MJ526 |
| 91 | MO540 | 225 | MJ117 | 254 | MJ307 |
| 92 | MO586 | 226 | MJ110 | 255 | MJ513 |
| 96 | MO1005 | 227 | MJ512 | 256 | MJ61 |
| 102 | MO314 | 228 | MJ413 | 257 | MJ121 |
| 119 | MO583 | 229 | MJ112 | 258 | MJ1020 |
| 123 | MO1016 | 230 | MJ7 | 259 | MJ315 |
| 126 | MO1025 | 231 | MJ1012 | 260 | MJ108 |
| 132 | MO9 | 232 | MJ66 | 261 | MJ130 |
| 134 | MO6 * | 233 | MJ316 | 262 | MJ1022 |
| 137 | MO321 | 234 | MJ132 | 263 | MJ129 |
| 140 | MO4 | 235 | MJ1013 | 264 | MJ138 |
| 156 | MO1021 | 236 | MJ63 | 265 | MJ72 |
| 191 | MO23 | 237 | MJ1016 | 266 | MJ327 |
| 199 | MO304 | 238 | MJ62 | | |
| 202 | MO754* | 239 | MJ1008 | | |
| **Commercial controls included in E76/P2 trials, but not in sub-populations** | | | | | |
| 267* | Bulk Jessievale BSO best families | | | | |
| 268* | Bulk Jessievale BSO all families | | | | |
| 269* | Bulk Seven Oaks BSO all families | | | | |
| 270* | [1]SAMTMA bulk, unimproved ex-commercial | | | | |
| 271* | *E. nitens* commercial bulk ex Sappi Kalmoesfontein SN 10016 | | | | |

Gen 2 Families= Second generation treatment numbers, Family no. = Family number, MO = seed collected from Seven Oaks BSO, MJ = seed collected from Jessievale BSO, * Common families with E76/P1 trial series, and

[1] South African Mining Timber Manufacturer's Association.

# Chapter 3

## Development of a near infrared (NIR) model to predict whole tree pulp yield of *Eucalyptus macarthurii* using non-destructive cores

Current measurement of pulping properties in *Eucalyptus macarthurii* is a destructive process, and thus a non-destructive process is necessary to prevent destroying selected trees in a breeding population. The objective of this study was therefore to develop and calibrate a near infrared model for use in measurements of pulp yield in *Eucalyptus macarthurii* using cores. Wood chips samples were obtained from felled trees and pulped through a Kraft pulping process, which was used as a reference method. A sub-sample of wood chips of the same trees were ground into sawdust and analysed through near infrared spectroscopy as to develop a non-destructive calibration model for screened pulp yield. A trial study was undertaken to investigate if breast height core predicts the whole tree pulp yield. Calibration correlations found between whole tree wood chips and its near infrared spectra as well as that of breast height core were 0.9472 and 0.9506, respectively. The screened pulp yield values obtained from both processes as well as from values obtained from other eucalypt species were subjected to Vision® Software, and the dataset was partitioned into four different combinations for calibration and validation. The outcomes obtained from the Vision® Software indicated a strong correlation coefficient of 94% between Kraft pulping and near infrared spectroscopy with a validation coefficient of 89%.The successful development of the valid calibration model required an inclusion of a wider range of other eucalypts species, which improved the robustness of the model. This model was used to estimate the screened pulp yield of *E. macarthurii* breeding population.

## 3.1 Introduction

*Eucalyptus macarthurii* is one of the few cold and frost tolerant species commercially planted on colder, high altitude and low productivity sites in South Africa (Swain, 2001). This species has vigorous growth and good coppicing ability and is mostly used for pulp and paper production (Swain and Gardner, 2003). This species also has the ability of recovering from fire. However, the pulping properties of this species are not considered desirable. To breed for improved pulp yield, it is essential to determine screened pulp yield (SPY) of individual trees, which conventionally involves destruction of trees. It would be of great value to a breeding programme if pulping properties of advanced generation selections could be determined in an efficient, rapid and, most importantly, non-destructive manner.

The traditional method of obtaining an estimation of SPY is through laboratory Kraft pulping, which requires trees to be felled, chipped and then cooked in an alkaline solution at an elevated temperature and pressure to dissolve lignin, leaving cellulose and hemicellulose intact (Schimleck *et al*., 2004). However, this method is destructive, time consuming and expensive. An alternative non-destructive method would be of great advantage (Michell, 1995; So *et al.*, 2004).

Near infrared (NIR) spectroscopy is a fast analytical method that can be implemented in a non-destructive manner. It is based upon vibrational spectroscopy that recognises changes in molecular vibrations which are associated with changes in molecular structure. Spectral graphs are generated within the NIR region, which represent the foundational compounds CH, OH and NH of biological systems. The application of NIR spectroscopy first requires calibration with a standard laboratory reference, which is developed from regression equations based on the NIR spectra of known reference data (Schimleck *et al.*, 2000; Baillères *et al.*, 2002; Kelley, 2004).

An earlier study was undertaken in 2002 to predict SPY with NIR spectral features of *E. macarthurii* disc samples, obtained using a destructive sampling method (Sefara *et al.*, 2002). In this 2002 study, 80 *E. macarthurii* trees were felled and assessed for their pulping properties as part of an investigation to develop a calibration model using Kraft pulping and NIR spectroscopy. The outcome of the initial 2002 study revealed that the calibration model had a low predictive power due to the narrow range in SPY (Sefara *et al.*, 2002). The purpose of this investigation was therefore to develop a calibration model for NIR spectroscopy, with a wider variation of sample base, that could be used for non-destructive measurements of SPY in *E. macarthurii.*

## 3.2 Materials and Methods

There were two processes that were undertaken for the development of a NIR calibration model for a more accurate prediction of SPY; Kraft pulping and NIR spectroscopy. Kraft pulping requires wood chips, whereas NIR spectroscopy requires sawdust for the analyses. A number of steps were undertaken to collect and prepare the wood samples required for each of the processes. The wood chips prepared for Kraft pulping were cooked to produce pulp, which was then washed and SPYs determined. A sub-sample of wood chips of the same trees was ground into sawdust, which was subjected to NIR spectroscopy to predict SPYs. The SPYs obtained from both processes were then used to develop a calibration model. The SPY values obtained from the Kraft pulping process were used as a reference guide for those obtained from NIR spectroscopy.

### 3.2.1 Materials

The SPY values previously obtained in the 2002 study (Sefara *et al.*, 2002) were included in the new dataset for the re-development of the calibration model.

To improve the predictive power of the model, further trees were felled in 2006, disc samples were chipped and then pulped, and the SPY values estimated and further used in the development of the calibration model (Table 3.3). The details of the 2006 tree collection are provided in Table 3.1. In an attempt to widen the SPY variation, the inner and outer regions of the whole discs were separated from each other, chipped and then pulped, as it is known that, in eucalypts, outer wood has a higher SPY than inner wood (Clarke, 2000).

**Table 3.1 Numbers and site origins of *E. macarthurii* trees selected for Kraft pulping process (2006 tree collection)**

| **Pinewoods-KwaZulu-Natal (KZN)** | | **Piet Retief-Mpumalanga (MPU)** | |
|---|---|---|---|
| Family no. | No. of trees[1] | Family no. | No. of trees[1] |
| 19 | 3 | U019C | 4 |
| 29 | 3 | U011 | 3 |
| 39 | 3 | R029 | 4 |
| 199 | 3 | - | - |
| 211 | 3 | - | - |
| Total no. of trees | 11 | Total no. of trees | 9 |

[1] individual family trees

The initial SPY values determined for the 2006 trees were found to be similar to those of the 2002 study. As these values did not broaden the range of the SPY values as was expected, it was then decided to include an additional seven *E. macarthurii* trees from second generation families that had not yet been tested, as well as four trees from a commercial plantation, again in anticipation that the range of the SPY values would be increased, the details of which are presented in Table 3.2. These trees were referred to as the 2007 collection.

The 2007 tree collection was also used to investigate whether radial strip cores extracted from wood disc samples taken at 1.3 metres above ground level (representative of breast height) predict the whole tree pulp yield.

**Table 3.2 Numbers and site origins of *E. macarthurii* trees selected for correlating whole tree pulp yield and breast height through Kraft pulping and NIR processes (2007 tree collection)**

| Pinewoods- KZN Second generation | | Sarsden, Balgowan - KZN Commercial plantation | |
|---|---|---|---|
| Family no. | No. of trees[1] | Family no. | No. of trees[1] |
| 11 | 1 | Comm 1 | 1 |
| 61 | 1 | Comm 2 | 1 |
| 29 | 1 | Comm 3 | 1 |
| 102 | 1 | Comm 4 | 1 |
| 113 | 1 | - | - |
| 123 | 1 | - | - |
| 174 | 1 | - | - |
| Total no. of trees | 7 | Total no. of trees | 4 |

[1] individual family trees and comm = commercial plantation.

## 3.2.2 Methods

Thirty-six wood disc samples obtained from the whole trees collected in both the 2006 and 2007 populations were chipped into wood chips of smaller sizes to fit into the electrical rotating digester, and thereafter, wood chips were subjected to Kraft pulping. Sawdust ground from a sub-sample of the wood chips from the same trees were analysed through NIR spectroscopy.

### 3.2.2.1 Processing of the wood

The different steps for the processing of the 2006 wood are described in Table 3.3, which was the same procedure used for the 2002 study.

**Table 3.3 Steps to prepare wood samples required for the prediction of SPY (Tappi, 1996)**

| Wood processing steps |
|---|
| ***Step 1: Identification and felling of trees***<br>Twenty six *E. macarthurii* trees were selected and felled. Half of these were from second generation progeny trials and sub-populations in KwaZulu-Natal (KZN) and half from commercial *E. macarthurii* plantations near Piet Retief. |
| ***Step 2: Cutting of discs***<br>Whole discs were cut from the felled trees. Four 20 mm thick discs were cut per tree at 1.3 metres from ground level, which was representative of breast height. A further four 20 mm thick discs were cut at 1-metre intervals along the length per tree using a handheld chainsaw. |
| ***Step 3: Separation of inner and outer wood***<br>The discs were separated into inner and outer wood using a "wood cutting bandsaw" in the laboratory. The separation was undertaken with the expectation of broadening the SPY range. The inner wood fractions obtained from the different discs of each tree were placed in a single brown paper bag and clearly labelled. The outer wood fractions were treated in the same manner and placed in a brown paper bag. |
| ***Step 4: Chipping of wood***<br>The inner and outer wood fractions were then chipped separately into wood chips using a Guillotine laboratory chipper. |
| ***Step 5: Screening of wood chips***<br>The inner and outer wood chips were screened using a chip screener to eliminate oversized wood pieces. This was achieved through shaking of the wood chips in the screener, the latter comprising several sieves with different hole sizes. The screened wood chips were then placed on racks for drying, where they were left at room temperature for four weeks to dry thoroughly. A small fraction of these air dried wood chips were used to determine moisture content. The remaining chips were sealed in plastic bags to maintain the moisture content until processed further. |
| ***Step 6: Grinding of screened wood chips***<br>A sub-sample of chips, approximately a quarter of the dried screened wood chips, was then crushed into smaller pieces with a Hammer Mill crusher. These pieces were ground into rough sawdust for NIR spectroscopy. The remaining wood chips were used for Kraft pulping. |
| ***Step 7: Shaking of ground wood sawdust***<br>The rough sawdust was further separated into three different sized sawdust fractions using a Wiley Mill shaker; rough (420 µm mesh), moderately fine (340 µm mesh), and very fine sawdust (250 µm mesh). The Wiley Mill shaker consists of three levels of separation; the top container that collects the rough sawdust, the middle container that collects moderately fine sawdust suitable for chemical analyses, and the bottom container that collects very fine sawdust suitable for NIR spectroscopy analysis. The fine sawdust was stored in a room under controlled conditions to maintain wood moisture content and thereafter used for NIR spectroscopy analysis. |

#### 3.2.2.2 Moisture content determination

Before the Kraft pulping of wood chips could be undertaken, the moisture content was measured to estimate a gram weight of material and volume of cooking liquor needed in the Kraft pulping process. The following formula was applied to determine moisture content (Tappi, 1996):

$$\text{Moisture} = \frac{\text{wet wood chips weight (g)} - \text{dry wood chips weight (g)}}{\text{wet wood chips weight (g)}} \times 100\%$$

To be able to apply the formula for the determination of the moisture content of the wood, weights of wet wood and dry wood had to be determined. This was undertaken by following these steps (Tappi, 1996):

1. A scoop of the air dried wood chips prepared in step 5 (Table 3.3) in the wood processing phase, referred to here as wet wood chips, was placed in a 500 or 1 000 ml glass beaker and weighed. This weight represented the combined wet weight of the wood chips and glass beaker. The wet wood chip weight was calculated by subtracting the beaker weight from the combined weight, which was determined prior to the addition of the chips to the beaker. These measurements were made in duplicate for the inner wood and outer wood fraction of each tree.
2. These wood chips were oven dried at 110°C for 24 hours.
3. The oven dried wood chips were weighed and the mass recorded. This represented the dry wood weight. These chips were not processed further and discarded.
4. The moisture content was calculated by incorporating the wet wood chip weights and dry wood chip weights into the moisture content formula.

### 3.2.2.3 Kraft pulping

Because the SPY values of the 2002 investigation (Sefara *et al.*, 2002) were included in this study, the same conditions used in the 2002 study were applied in the processing of the 2006 and 2007 tree collections.

Sefara *et al.* (2002) cooking conditions included:

1. Sulphidity (Sodium sulfate, $Na_2S$) of 25%.
2. Active alkalinity charge of 18, (Sodium Hydroxide, NaOH).
3. Liquor to wood ratio of 4 : 5 (based on weight), which indicates the total liquid amount compared to completely dry wood which includes all liquids involved in cooking process.
4. Pulping cycle: Ambient temperature of 170°C and time varied to achieve desired Kappa of 18 to 20. Kappa number is defined as the amount of delignification, that is, the amount of lignin that is remaining in the pulp after Kraft pulping.
5. Degassing was carried out at 120°C and 140°C to remove gases that were not condensable in water.
6. Blow-down (cooling) to atmospheric pressure inside the digester at the end of the cooking cycle before opening for 20 minutes.
7. Cooking liquor was prepared according to Tappi methods (Tappi, 1996).
8. The amount of wet wood chips required was calculated using the following formulae,

   i. Solids contents = $\frac{\text{dry wood chips (g)} \times 100\%}{\text{wet wood chips (g)}}$

   ii. Amount of wet wood chips for cooking (g) = $\frac{\text{charge (OD)} \times 100}{\text{solids contents}}$

   Where;

   charge used = 600 grams OD

*Cooking process* (Tappi, 1996)

1. Wet wood chips of the calculated gram were weighed.
2. Cooking liquor of predetermined volume was weighed.
3. Electrically heated rotating cooking digester was adjusted for the required temperature, pressure and time prior to pulping as specified by Sefara *et al.* (2002).
4. Wood chips, as well as cooking liquor, were inserted into the electrically heated rotating cooking digester.
5. The electrically heated rotating cooking digester was closed tightly, after which the pulping process continued until the end of the cooking cycle of varied times.
6. The pulp was then washed.

*Washing of pulp* (Tappi, 1996)

1. Pulp (cooked chips) was removed from the digester and placed on a washing screen (mesh sieve) consisting of two separate meshes of 0.1 m and 0.5 m in diameter.
2. Water was flushed over the pulp to flush the pulp onto a second screen while keeping the reject material (knots and uncooked chips) on the upper screen.
3. Washed pulp was placed into pillow bags and closed tightly with string, and then spin dried for 10 minutes.
4. Spin-dried pulp was transferred into plastic bags and wet mass (g) recorded.
5. The pulp and reject material was oven-dried for 24 hours at 170°C and their oven-dried masses recorded.
6. Oven-dried pulp was packed into small plastic bags in duplicate for Kappa determination.

7. Finally, SPY of the samples were calculated as the mass of wet pulp produced per mass of oven-dried wood, and expressed as a percentage using the following formulae:
   i) Consistency calculation:
   Consistency (C) = (100 – moisture content) %

   ii) SPY (%) = [(C /100) /charge (OD) x M] x 100

   Where;
   M = mass of wet pulp
   charge = 600 grams OD ( oxygen delignification)

*Kappa determination* (Tappi, 1996)

During the pulping process, delignification occurs, the amount of lignin left in the pulp being determined by calculating the kappa number. Kappa number was determined in the following manner:

1. Approximately two grams of oven-dried pulp was measured in duplicate, which represented the test samples.
2. The test sample was transferred to a disintegrator and disintegrated into 300 ml of deionised water until free of fibre clots.
3. The disintegrated sample was transferred into a 2 000 ml reaction beaker and the disintegrator rinsed with 400 ml of deionised water to remove the remains of the sample. The washed remains were added to the reaction beaker up to a total volume of 700 ml.
4. The reaction beaker was then placed on an electric stirrer to mix the sample thoroughly.
5. A potassium permanganate solution of 100 ml together with 100 ml of sulphuric acid were pipetted and mixed in a 250 ml conical flask.

6. The mixture in step 5 was immediately added to the disintegrated pulp sample (step 4) and left for ten minutes, timed with a stop watch. During the ten minute period, the conical flask containing the mixture was rinsed with 100 ml of deionised water and the rinse solution added to the 2 000 ml reaction beaker.
7. The temperature of the mixture was recorded after 5 minutes.
8. The reaction was stopped at exactly 10 minutes by adding 20 ml of 1.0 N potassium iodide solution using a measuring cylinder.
9. Free iodide was titrated with 0.2 N sodium thiosulphate into the mixture in step 8 and stirred, without filtering out fibres, until a pale yellow colour was obtained. Thereafter, a few drops of 0.2% starch solution were added, whilst stirring very slowly, until a cloudy white colour was obtained.
10. Readings required for kappa number calculation were recorded.
11. Prior to sample testing, a blank test was undertaken using the same kappa determination procedure, but omitting the pulp sample to determine the background.
12. The kappa number was calculated using the following formulae:

i) $p = \frac{(b - a)\,N}{0.1}$

ii) $K = \frac{p \times f}{w}$ (used when temperature of reaction mixture is 25ºC)

iii) $K = \frac{p \times f}{w}\,[1 + 0.013\,(25 - t)]$ (used when temperature of reaction mixture is not equal to 25ºC)

Where;

a = volume of thiosulphate consumed by the test sample, measured in ml,

b = volume of thiosulphate consumed in the blank determination, measured in ml,

N = normality of sodium thiosulphate,

p = volume of the 0.1N potassium permanganate consumed by the sample,

w = mass of dried pulp, measured in grams,

f = correction factor,

t = temperature of reaction mixture, and

K = kappa number.

#### 3.2.2.4 NIR spectroscopy

Near infrared spectroscopy analysis requires sample preparation (steps 6 and 7 in the wood processing phase), spectra collection and thereafter, development of a calibration model. The suitable calibration model was validated by Vision® Software and further used to screen second generation *E. macarthurii* breeding populations, the results of which are presented in Chapter 4.

*NIR spectra collection* (Tappi, 1996)

1. Each sawdust sample (step 7 in the wood processing phase) was mixed thoroughly, and approximately five grams removed with a spatula and placed in a small quarter cup.
2. The spectra were measured at 2nm intervals over the spectral range of 1 100 to 2 500nm using NIR Systems Inc Model 6500 scanning spectrophotometer.
3. After the first measurement, the cup was emptied and then refilled with another sawdust sample to obtain a second spectrum for the same sample material. The average of the two spectral values was determined and used for subsequent analyses.
4. Steps 1 to 3 were repeated for all samples.

*Development of calibration model*

A calibration model was created in a stepwise manner by utilising data groups 1 to 4 (Table 3.4), different data sets after each calibration attempt. Data analysis was conducted using Vision® Software (Version 3.0.5.0) supplied by Foss NIR Systems. Prior to analysis, spectra were transformed into the second derivative

mode to remove baseline shift. The calibration for SPY was developed using Partial Least Squares (PLS) regression, a commonly used linear regression method for highly co-linear data which extracts variation in NIR spectra (Schimleck *et al*., 1998; 2000; 2006).

Vision® Software has a cross-validation method consisting of four cross-validation segments that verifies the predictive power of the PLS model. Partial least squares (PLS) regressions were performed on trees with screened pulp yield values of greater than 43% to generate a NIR calibration model. The Vision® Software splits the calibration set randomly into four segments, each with the same number of samples. One segment was excluded and the remaining three segments were used to develop a PLS model with different numbers of factors. This was then validated against the samples in the fourth segment. The process repeats automatically until each segment has been used and validated (Michell, 1995; Raymond and Schimleck, 2002). Statistical summary data was then generated for each factor, and the factor with the best summary statistics was selected to generate the calibration model with actual values.

**Table 3.4 Data groups used for NIR calibration model development**

| Data group description |
|---|
| *Group 1:*<br>Combination of all *E*. *macarthurii* SPY values obtained from both the 2002 and 2006 investigations. The data were split using Mahalanobis* distance into a calibration (70%) and validation set (30%). |
| *Group 2:*<br>Comprised Group 1 plus SPY values from a variety of *Eucalyptus* species. The data were split using Mahalanobis distance into a calibration (70%) and validation set (30%). The validation set consisted of *E. macarthurii* and a variety of other eucalypt species. |
| *Group 3:*<br>Comprised Group 2 plus fifteen *E. macarthurii* samples covering the whole range of SPY values, and these were selected into the validation set. All remaining samples of *E. macarthurii,* plus a variety of eucalypt species were used to develop the calibration model, which was then validated on these 15 samples. |
| *Group 4:*<br>This group was comprised of Group 2 plus eleven of the 2007 second generation *E. macarthurii* samples. The data were split using Mahalanobis distance into a calibration (70%) and validation set (30%). |

*splitting data set randomly into four segments. One segment is excluded and the remaining three segments are used to develop a PLS model with different numbers of factors. This is validated against the samples in the fourth segment. The process is repeated until each segment is used and validated.

The strength and accuracy of the models were expressed by the square of the correlation coefficient ($R^2$). The standard error of calibration (SEC) was used to evaluate how well a calibration of the linear regression fitted the data set (So *et al.*, 2004). In addition, standard error of cross validation (SECV) was also used to evaluate the PLS calibration performance, which is an indication of how well an equation would predict SPY (Schimleck *et al.*, 1998; 2000; 2006). The accuracy of calibration performance was determined by the standard error of prediction (SEP).

The SPY values of the inner and outer wood fractions as well as those of the 2007 tree collection were then determined after pulping, while a sub-sample was used for NIR spectroscopy. The four different data combinations were tested for their suitability for the development of a calibration model using Vision Software®.

#### 3.2.2.5 Correlation of breast height cores with whole tree pulp yield

Four breast height wood discs were taken from the seven trees in the 2007 collection at 1.3 metres above ground, to correlate both the breast height pulp yield and breast height core NIR spectra to the whole tree pulp yield. An additional twelve samples of wood chips were taken at 1 metre intervals throughout each tree. A radial strip of 12mm in diameter from bark to bark from breast height discs in each tree were cut, chipped and thereafter ground into sawdust for NIR analyses. Wood discs taken throughout the tree were processed in the same manner as the 2002 and 2006 tree collections for Kraft pulping and NIR analyses (Table 3.3). For the trial study, wood discs were not separated into inner and outer wood fractions, but chipped whole discs were prepared to estimate the SPY for the Kraft pulping and NIR processes.

## 3.3 Results

Values for SPYs and Kappa numbers obtained from pulping for different lengths of time for the 2006 inner and outer wood fractions were compared using statistical summary values obtain from the Vision Software®. A comparison was also done between these and the SPY values predicted from NIR spectroscopy from the four different groups that had been composed for the development of the calibration model, as well as that of the SPYs predicted from NIR spectroscopy in an earlier study (Sefara *et al.*, 2002), and the SPYs from the additional eleven 2007 second generation trees.

### 3.3.1 Screened pulp yield (SPY)

The SPY values obtained after pulping for inner and outer wood fractions revealed that the ranges of SPY of inner wood sections were slightly narrower than those of the outer wood fractions (Table 3.5). For inner wood, the SPY values ranged from 42.19 to 46.47%, with a mean value of 44.17%, while for outer wood fractions, values ranged from 43.46 to 49.71% with a mean value of 46.54%. Similarly, the range for whole discs were similar to that of the outer wood, SPY values found from the whole disc samples ranged from 44.43% to 49.12%, with a mean value of 46.87% (Table 3.7).

**Table 3.5 Screened pulp yield (%) values for the 2006 *E. macarthurii* samples**

| Family | Inner wood mean SPY (%) | Outer wood mean SPY (%) |
|---|---|---|
| 29 | 42.19 | 46.81 |
| 39 | 45.23 | 47.07 |
| 199 | 44.97 | 49.71 |
| 211 | 42.85 | 47.88 |
| R029 | 46.47 | 43.46 |
| U011 | 42.26 | 46.54 |
| U019C | 45.23 | 44.31 |
| **Mean** | 44.17 | 46.54 |
| **Min** | 42.19 | 43.46 |
| **Max** | 46.47 | 49.71 |
| **Std dev** | 1.71 | 2.11 |

Std dev = standard deviation' SPY = Screened Pulp Yield

These results showed that outer wood fractions and whole disc samples had slightly higher SPYs than inner wood, and that these values were similar to those found in the 2002 study. The SPY values of the 2002 study ranged from 44.91% to 49.95%, with a mean SPY value of 47.69% (Sefara *et al.*, 2002).

### 3.3.2 Kappa number

The Kappa number after pulping the inner wood fractions ranged from 17 to 31, with a mean of 23.89 (Table 3.6). Most tree samples had a Kappa number of 22 to 31, which was higher than the target Kappa number range of 18 to 20. On the contrary, the outer wood fractions had Kappa numbers ranging from 18 to 20, with the mean of 18.8. Tree R029 had the lowest Kappa number of 15.29 (Table 3.6).

**Table 3.6 Mean Kappa numbers for the 2006 *E. macarthurii* tree collection**

| Family | Mean Kappa number (inner wood) | Mean Kappa number (outer wood) |
|---|---|---|
| 29 | 26.07 | 20.92 |
| 39 | 22.59 | 19.05 |
| 199 | 24.88 | 19.76 |
| 211 | 31.27 | 19.60 |
| R029 | 17.79 | 15.29 |
| U011 | 23.87 | 18.96 |
| U019C | 20.73 | 19.28 |
| **Mean** | 23.89 | 18.98 |
| **Min** | 17.79 | 15.29 |
| **Max** | 31.27 | 20.92 |
| **Std dev** | 4.26 | 1.75 |

Std dev = standard deviation

The inner wood fractions took approximately 45 minutes to cook, and even when this time was increased gradually up to a maximum time of 99.95 minutes at a cooking charge of 20, the target Kappa number was still not achieved. With regards to the outer wood fractions, cooking time ranged from 45 to 70 minutes at a cooking charge of 18, which was an appropriate time to reach the target Kappa number (Table 3.6). The additional 2007 tree collections, using whole discs, had a Kappa number range of 17.77 to 20.67 with a mean of 19.63. However, of the 2007 trees, eight trees took 60 minutes to cook and two

commercial trees took 75 minutes, whereas one commercial tree took 45 minutes to achieve the Kappa target (Table 3.7).

**Table 3.7 Screened pulp yields (SPY) (%) and Kappa numbers obtained from the 2007 *E. macarthurii* tree collection**

| Family | SPY (%) | Kappa number | Coking time (minutes) |
|---|---|---|---|
| comm. 1 | 49.12 | 19.76 | 75 |
| comm. 2 | 47.75 | 20.37 | 45 |
| comm. 3 | 47.39 | 18.61 | 60 |
| comm. 4 | 47.07 | 19'66 | 60 |
| 11 | 46.62 | 19.91 | 60 |
| 61 | 45.25 | 17.77 | 60 |
| 29 | 44.43 | 19.93 | 75 |
| 102 | 47.30 | 20.67 | 60 |
| 113 | 48.10 | 18.90 | 60 |
| 123 | 45.70 | 20.37 | 60 |
| 174 | 46.89 | 20.04 | 60 |
| **Mean** | 46.87 | 19.63 | 61 |
| **Min** | 44.43 | 17.77 | 45 |
| **Max** | 49.12 | 20.67 | 75 |
| **Std dev** | 1.34 | 0.92 | 8.1 |

SPY = Screened pulp yield; comm. = commercial plantation

### 3.3.3 Correlation of breast height cores with whole tree pulp yield

The SPY obtained form the laboratory Kraft pulping and NIR for both whole tree wood discs and that of the radial strip core were not significantly different, although the NIR SPYs were generally slightly higher than those SPYs obtained from Kraft pulping (Table 3.8).

**Table 3.8 Screened pulp yields (SPY) (%) obtained from the 2007 *E. macarthurii* tree collection**

| Family | SPY from Kraft pulping of whole tree wood discs (%) | NIR predicted SPY from whole tree wood discs (%) | NIR predicted SPY from breast height radial strip core (%) |
|---|---|---|---|
| comm. 1 | 49.12 | 49.35 | 49.50 |
| comm. 2 | 47.75 | 48.31 | 48.11 |
| comm. 3 | 47.39 | 47.21 | 47.74 |
| comm. 4 | 47.07 | 47.69 | 47.93 |
| 11 | 46.62 | 46.97 | 46.90 |
| 61 | 45.25 | 44.89 | 45.21 |
| 29 | 44.43 | 44.78 | 44.99 |
| 102 | 47.30 | 47.23 | 47.11 |
| 113 | 48.10 | 48.85 | 48.83 |
| 123 | 45.70 | 45.43 | 45.61 |
| 174 | 46.89 | 46.99 | 47.37 |
| **Mean** | 46.87 | 47.06 | 47.21 |
| **Min** | 44.43 | 44.78 | 44.99 |
| **Max** | 49.12 | 48.85 | 48.83 |
| **Std dev** | 1.34 | 1.55 | 1.48 |

SPY = Screened pulp yield; comm. = commercial plantation,

Calibration correlations of laboratory Kraft pulping for whole tree wood chips with both whole tree NIR and NIR of the breast height radial strip core within each tree were strong with $R^2$ of 0.947 and 0.951, respectively, which was not significantly different (Figure 3.1). These results thus revealed that the wood core sample taken at breast height in a tree, accurately predicts the wood properties of the whole tree.

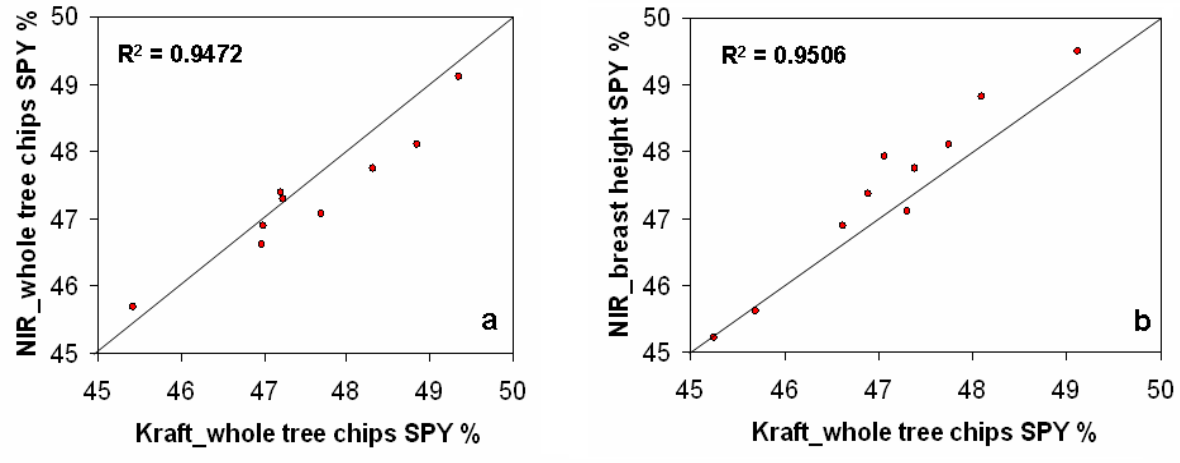


**Figure 3.1** **Calibration correlation graphs of SPYs of whole tree wood chips with breast height radial strip core (2007 tree collection)**

**a. Correlation graph of whole tree Kraft SPYs with whole tree NIR SPYs**

**b. Correlation graph of whole tree Kraft SPYs with NIR SPYs from breast height radial strip cores**

### 3.3.4 Comparison of the four calibration/validation groups

The four data groups used to develop the calibration model differed in their ranges of SPY values (Table 3.9). As was expected, the SPY range of Group 1 (8.55%), which contained only *E. macarthurii* trees, was much narrower than the ranges of Groups 2, 3 and 4 which also included SPY values of other *Eucalyptus* species; 15.48, 17.78 and 11.90% respectively. These data thus revealed that the inclusion of values of other *Eucalyptus* species broadened the range of the values notably.

**Table 3.9 Summary of NIR spectroscopy predicted SPY values of the four data groups**

| Group | Calibration set | | | | Validation set | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean | Min-Max | SD | N | Mean | Min-Max | SD |
| **1. Combined *E. macarthurii* (2002 + 2006)** | **77** | **47.44** | **41.22– 49.77** | **1.45** | **22** | **47.45** | **42.26 – 50.64** | **1.74** |
| **2. Group 1 + other eucalypt species** | **156** | **49.92** | **41.22– 56.70** | **3.27** | **66** | **49.71** | **41.96 – 57.00** | **3.32** |
| **3. Calibration: Group 2 - 15 *E. macarthurii* Validation: 15 *E. macarthurii*** | **209** | **50.07** | **41.22 – 59.00** | **3.32** | **15** | **47.07** | **42.26 – 49.53** | **2.04** |
| **4. Group 3 + 2007 eleven 2nd generation *E. macarthurii* trees** | **159** | **49.83** | **42.57– 57.47** | **3.28** | **52** | **49.23** | **42.65 – 55.91** | **3.33** |

N = number of samples, min = minimum, max = maximum and SD = standard deviation.

### 3.3.5 Calibration model

The calibration models developed for the four data groups in this study displayed $R^2$ that were notably different (Table 3.10). Group 1, consisting of only *E. macarthurii* SPY values, displayed the lowest $R^2$ of 0.670, while Groups 2, 3 and 4, which contained values of other eucalypt species, produced $R^2$ above 0.9. This reflected a stronger relationship between the SPY values obtained after Kraft pulping and the NIR spectra values for Groups 2, 3 and 4.

**Table 3.10 Summary of NIR calibration and validation statistics**

| Group | Calibration set | | | | Validation set | |
|---|---|---|---|---|---|---|
| | Factor | $R^2$ | SEC | SECV | $R^2$ | SEP |
| 1. Combined *E. macarthurii* (2002 +2006) | 4 | 0.666 | 0.870 | 1.099 | 0.490 | 1.263 |
| 2. Group 1 + other eucalypt species | 8 | 0.908 | 1.018 | 1.112 | 0.931 | 0.875 |
| 3. Calibration: Group 2 - 15 *E. macarthurii* Validation: 15 *E. macarthurii* | 8 | 0.923 | 0.935 | 1.038 | 0.833 | 1.050 |
| 4. Group 2 + 2007 eleven 2nd generation *E. macarthurii* trees | 8 | 0.944 | 0.800 | 0.973 | 0.887 | 1.194 |

$R^2$= correlation coefficient, SEC= standard errors of calibration, SECV=standard errors of cross-validation, SEP= standard errors of prediction.

The validation results of the current study displayed the same trends as those of the calibration results (Table 3.10); Group 1 had lower $R^2$ values than Groups 2, 3 and 4, this was due to the latter three groups consisting of a variety of eucalypts species.

The calibration and validation graphs provide a visual perspective of the outcomes of this investigation (Figures 3.2 and 3.3). These graphs also show that the Group 1 data set (Figure 3.2 a, b) does not provide a suitable model, as the $R^2$ was substantially less, and SEP greater, than those obtained for Groups 2, 3 and 4 (Figures 3.2 c, d; 3.2 e, f; 3.2 g, h) respectively.

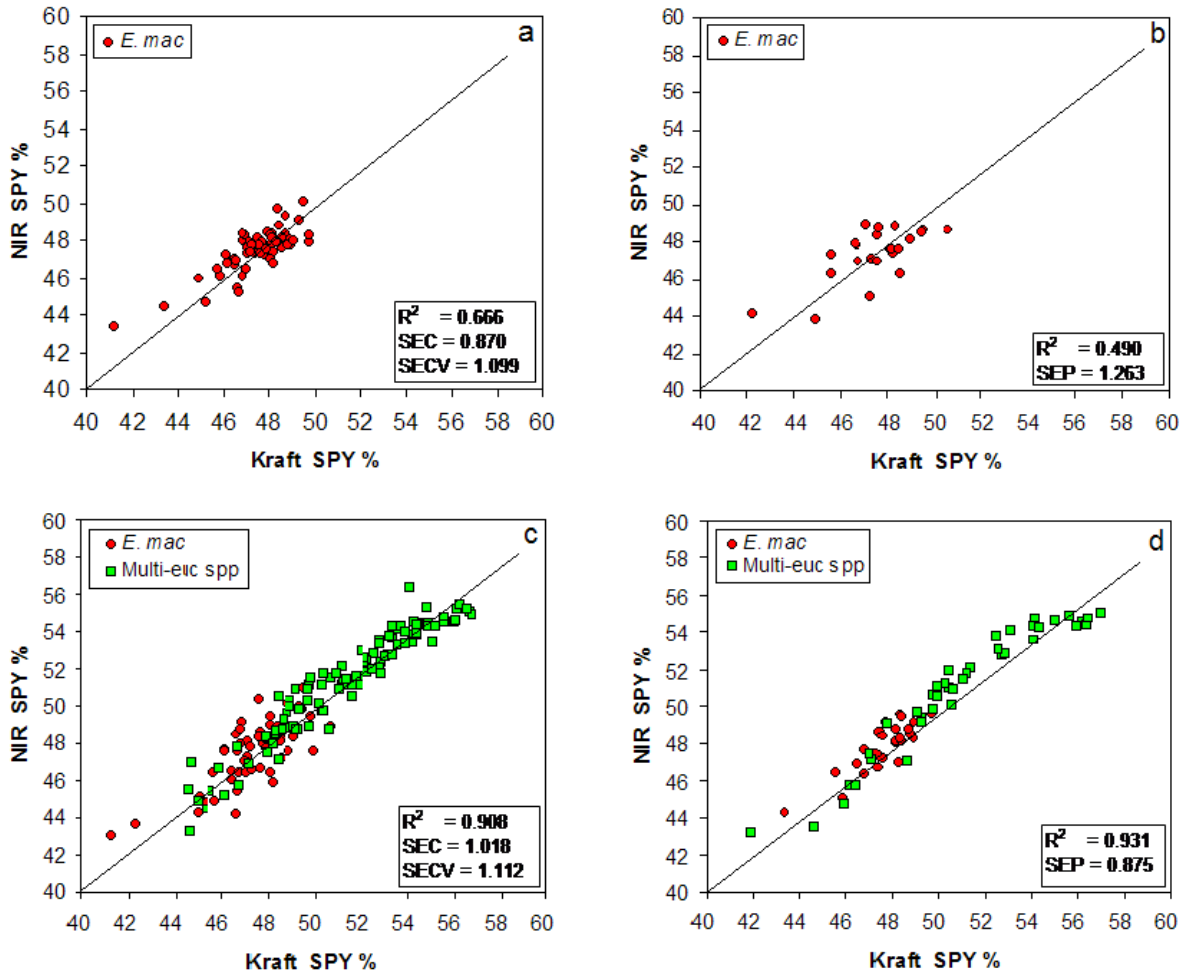


**Figure 3.2** **Calibration and validation graphs of SPY of Kraft pulping versus NIR spectroscopy.**

**a. Calibration graph for Group 1; b. Validation graph for Group 1;**

**c. Calibration graph for Group 2; d. Validation graph for Group 2.**

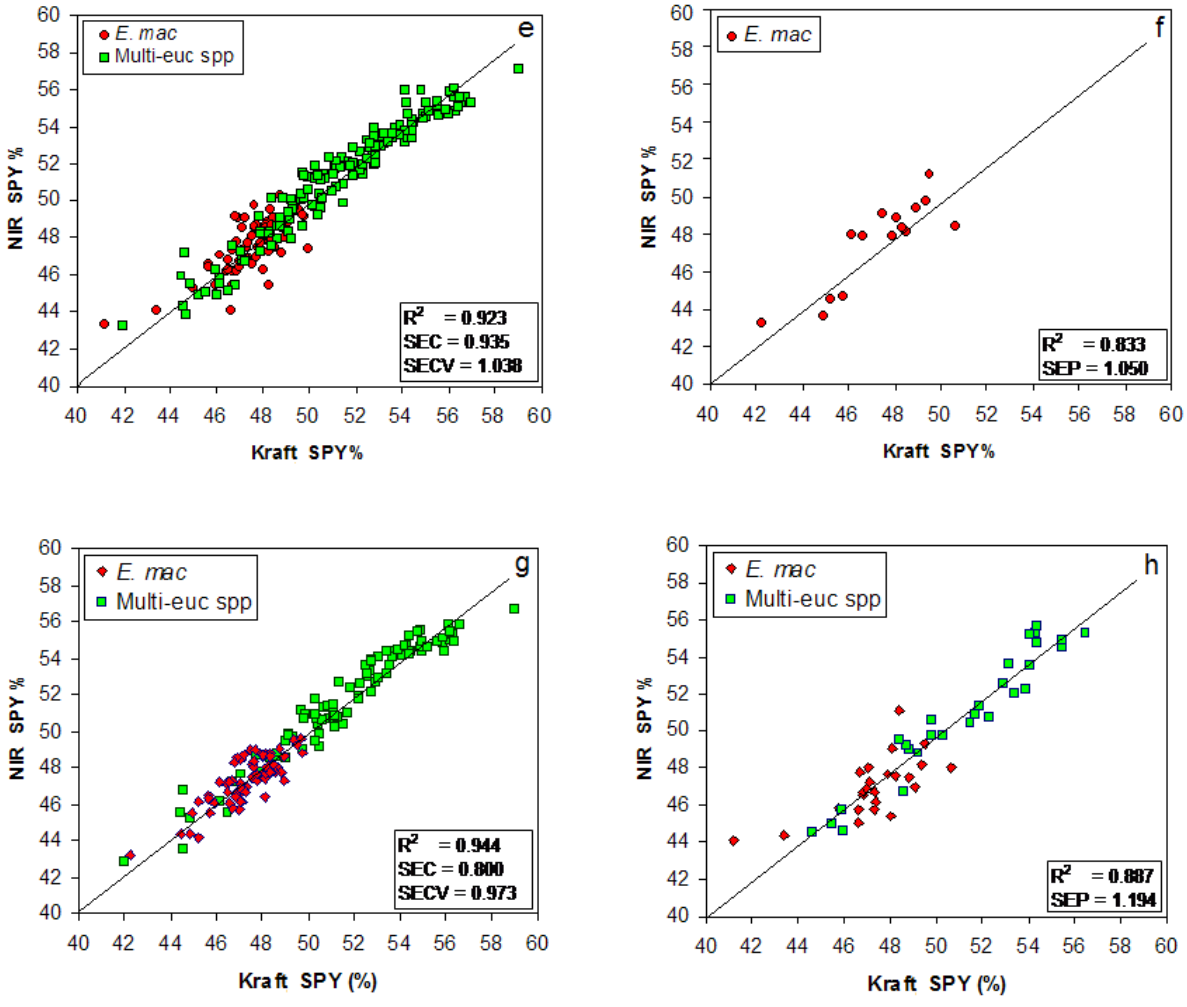


**Figure 3.3** **Calibration and validation graphs of SPY of Kraft pulping versus NIR spectroscopy**

**e. Calibration graph for Group 3; f. Validation graph for Group 3**

**g. Calibration graph for Group 4; h. Validation graph for Group 4.**

## 3.4 Discussion

A further attempt to broaden the range of the *E. macarthurii* NIR model was undertaken by incorporating SPY values of other eucalypt species with the SPY values of the 2002, 2006 and 2007 *E. macarthurii* tree collections. It had been found, from other CSIR cooperative projects, that multiple eucalypt species showed a strong correlation between SPY and NIR spectra (CSIR, 2005). Therefore, the inclusion of multiple eucalypt species into the pure *E. macarthurii* group in this investigation was to artificially force the *E. macarthurii* SPYs to lie within a regression line.

The SPY values of the *E. macarthurii* samples and the other eucalypt species were partitioned into four different groups in an attempt to find the best range of SPY values for calibration. The range of the SPY values of the group containing only *E. macarthurii* (8.55%) was similar to that found in other *Eucalyptus* species; *E. dunnii* (7.2%), (Muneri *et al.,* 2005), *E. grandis* (7.0%) (Sefara *et al.,* 2000) and *E. nitens* (8.9%) (Schimleck *et al.,* 2005). The pure *E. macarthurii* group also produced the lowest calibration and validation correlation coefficients of 0.670 and 0.490 respectively. Conversely, the three groups containing SPY values of other eucalypt species as well as those of *E. macarthurii,* displayed correlation coefficients greater than 0.900. These results compared well with those found for other *Eucalyptus* species; $R^2$ values of 0.907 and 0.912 for *E. grandis* (Sefara *et al.*, 2000) and values of 0.935 and 0.920 for one provenance of *E. globulus* (Schimleck *et al.,* 1998) and *E. nitens* (Schimleck *et al.*, 2005), respectively.

Furthermore, the standard errors of calibration (SEC) for the four groups, were 0.870, 1.018, 0.935 and 0.800 respectively, which were markedly higher than those found in previous studies, where the SEC for *E. dunnii* was 0.690 (Muneri *et al.,* 2005), for *E. grandis* 0.528 and 0.516 for two different provenances, respectively (Sefara *et al.,* 2000), and *E. nitens* 0.620 and 0.750

for two different provenances (Schimleck *et al.,* 2005). The exception was *E. globulus,* with SEC values of 0.901 and 0.811 for two different provenances (Schimleck *et al.,* 1998). These outcomes revealed that the NIR calibration model developed for this investigation was successfully developed.

The validation correlation coefficients of the three mixed groups were also relatively high, greater than 0.800, which were comparable to those found in *E. dunnii* (0.890) (Muneri *et al.,* 2005) and for one provenance of *E. grandis* (0.836) (Sefara *et al.*, 2000). In contrast, these coefficients were greater than those found for *E. nitens* (0.770) (Schimleck *et al.,* 2005) and an *E. grandis* provenance (0.787) (Sefara *et al.,* 2000). These outcomes supported the calibration results found for this investigation.

It was possible to select, from the mixed groups, the best group(s) for calibration based on the highest calibration and validation correlation coefficients. Two groups displayed values that were suitable for calibration. Both these groups contained SPY values that had been specifically compiled for the purposes of this model. One group (Group 3) contained selected *E. macarthurii* SPY values that covered the entire range of values in addition to the multiple species eucalypts, while the other group (Group 4) contained an additional set of *E. macarthurii* SPY values with the multiple species eucalypts.

In the 2007 tree study, rapid non-destructive estimation of whole tree pulp yield through NIR was investigated by correlating whole tree wood disc SPYs with those of the breast height, radial strip wood core within the same tree. The results revealed a very high correlation of 0.950 between whole tree wood discs and that of breast height wood core SPYs obtained from Kraft pulping and NIR processes. Schimleck *et al*. (2006) showed similar results for *E. nitens* with a strong correlation of 0.900 for whole tree pulp yield and core samples taken at 1.3 metres above ground level. A study on *Pinus taeda* investigated other wood properties such as basic wood density, microfibril angle and stiffness using NIR,

and also showed strong correlations of 0.830, 0.900 and 0.930, respectively, of whole trees with breast height core samples (Schimleck *et al*., 2005).

## 3.5 Conclusions

A NIR spectroscopy calibration model that can be used for the accurate prediction of whole tree SPYs using non-destructive breast height cores in *E. macarthurii* has been successfully developed through this study. This objective was achieved by the inclusion of the SPY values of other eucalypt species from a CSIR co-operative project (CSIR, 2005), which broadened the SPY range and thus improved the development of the calibration model. The outcome of this investigation showed that a successful calibration model required a wider base range of other eucalypts species SPY values to be included in the dataset so as to widen the variation of the calibration model developed.

The newly developed calibration model was then used to predict SPY values of advanced generation *E. macarthurii* selections from the ICFR progeny trials and sub-populations planted in the provinces of KwaZulu-Natal and Mpumalanga in South Africa. The wood material was obtained non-destructively from a 12mm bark to bark breast height core that was extracted from standing trees at 1.3 metres above ground level, which will be a true representative of the whole tree pulp yield, as has been proved through this study. The results that will be obtained will greatly assist with selection and improvement in the ICFR *E. macarthurii* breeding programme.

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# Chapter 4

## Screening of a second generation *Eucalyptus macarthurii* population using core samples and a developed NIR calibration model to predict screened pulp yield


## Abstract

*Eucalyptus macarthurii* is an important cold tolerant species grown widely on low productivity sites for pulp production in South Africa. However, there is ongoing controversy about the pulping properties of this species, and it was thus necessary to assess the screened pulp yields of trees in an advanced generation breeding population. A predictive model using near infrared was applied to the Institute for Commercial Forestry's second generation *Eucalyptus macarthurii* breeding population, with the aim of predicting the screened pulp yield of the top 70% of families using non-destructive wood core samples obtained from standing trees, and thus identifying those families and individuals with the best pulping properties. The screened pulp yield results revealed a narrow screened pulp yield range, with the highest screened pulp yield value of 48%. Families 85 (Maxwell sub-population 4), 52 (Maxwell sub-population 3) and 106 (Sutton sub-population 6) were the top performing families with a screened pulp yield of 48%. However, progeny trials 1 and 2 planted at Pinewoods and Vlakkloof had the highest screened pulp yield for families 48 and 77 respectively, as well as for family 29 from progeny trial 2 (Pinewoods) with screened pulp yields of 47%. The Mpumalanga sites were found to be significantly different ($p \leq 0.001$) (site mean of between 43 to 45%) from each other, although no significant differences were found between the KwaZulu-Natal sites. The developed near infrared calibration model enabled the screening of large population successfully without felling the trees, as the core samples predicts the whole tree pulp yield. The Kraft pulping

results reported in Chapter 3 of this thesis compared well to those of this investigation, which confirmed the success of the application of the technique.

## 4.1 Introduction

*Eucalyptus macarthurii* is a frost hardy, cold tolerant species that is widely grown on colder, high altitude and low productivity sites for pulp production in South Africa. Pulp yield is an important factor in determining pulpwood quality and plantation profitability (Schimleck *et al*., 2000; 2005). Schimleck and co–workers (2000) have defined pulp yield as the percentage of the original mass of wood fibre remaining after Kraft pulping, to a given content of lignin.

Traditional pulp yield estimation requires trees to be felled, cut into disc samples, chipped into wood chips and taken for chemical pulping. The method is time consuming, costly and most importantly, destructive, all of these limiting the number of samples that can be tested. An alternative is to use the coring method which removes a 12 mm (in diameter) bark to bark core (a radially orientated cylinder of wood) at 1.3 metres above ground level from a standing tree. The extraction of core samples is non-destructive and has the advantage of conserving trees for later experimentation, research and breeding. The core sample extracted at breast height predicts the whole tree pulp yield (Schimleck *et al*., 2006). The core samples can then be milled and pulp yields predicted using near infrared (NIR) spectroscopy (Schimleck *et al*., 1998).

Near infrared spectroscopy analysis involves measuring the NIR spectra of a large number of samples, and thereafter developing a model linking the spectra to the constituent of interest (Schimleck *et al*., 1998; Yamada *et al.*, 2006). The purpose of this investigation was to use a developed NIR calibration model to predict SPYs of the Institute for Commercial Forestry Research (ICFR) second generation *E. macarthurii* breeding population from non-destructive core

samples, which are representative of the whole tree pulp yield, so as to identify families with the best pulp yield across and within sites.

## 4.2 Materials and Methods

### 4.2.1 Sample origin

Open-pollinated seed was collected from two ICFR first generation seed orchards at Jessievale (26°14'S, 30°31'E) and Seven Oaks (29°11'S, 30°39'E) during the early 1990s. Seed collected from a single mother tree was referred to as a family, and a minimum of 500 seeds were collected per tree. This material was established into two progeny trials (E76/P1 and E76/P2) and 10 sub-populations in each of the KwaZulu-Natal (KZN) and Mpumalanga (MPU) provinces during December 1993 and January 1994. Family composition of the E76/P1 trial series and the sub-populations are presented in Table 4.1, and the E76/P2 series in Table 4.2. The progeny trials were aimed at testing the open-pollinated material, and the sub-populations comprised discrete groups of families which could be bred for different environmental conditions and produce seed accordingly.

The family number prefixes, MO and MJ, denoted seed collected from the Seven Oaks and Jessievale BSOs, respectively. The progeny trials were established in single row plots of 1667 stems $ha^{-1}$, with four replications in various balanced and unbalanced lattice designs, and details of the site information and trial designs are provided in Tables 4.3 and 4.4 respectively. The sub-populations comprised 25 families each in a 5x5 balanced lattice design, planted in single row plots of 1667 stems $ha^{-1}$, with six replications (Tables 4.3 and 4.4).

## Table 4.1 Family composition of second generation *E. macarthurii* (progeny trials E76/P1 and sub-populations)

| Pop 1 : | Comrie Woodstock | Pop 2 : | Comrie Woodstock | Pop 3 : | Maxwell The Brook | Pop 4 : | Maxwell The Brook | Pop 5 : | Sutton Dorstbult |
|---|---|---|---|---|---|---|---|---|---|
| Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. |
| 1* | MO1010 | 27* | MO512 | 52 | MO654 | 77* | MO5517 | 102 | MO314 |
| 2* | MO519 | 28 | MO642 | 53* | MO315 | 78* | MO5520 | 103* | MO664 |
| 3* | MO1003 | 29 | MO637 | 54* | MO70 | 79* | MO5502 | 104* | MO717 |
| 4* | MO2 | 30 | MO557 | 55* | MO648 | 80* | MO670 | 105* | MO719 |
| 5 | MO510 | 31* | MO11 | 56* | MO301 | 81 | MO649 | 106* | MO722 |
| 6* | MO552 | 32* | MO66 | 57* | MO1019 | 82* | MO114 | 107* | MO582 |
| 7* | MO88 | 33* | MO669 | 58* | MO306 | 83* | MO42 | 108* | MO706 |
| 8* | MO655 | 34* | MO312 | 59* | MO645 | 84* | MO35 | 109* | MO5509 |
| 9* | MO636 | 35* | MO5507 | 60* | MO704 | 85 | MO653 | 110* | MO546 |
| 10* | MO1012 | 36* | MO630 | 61* | MO634 | 86 | MO5506 | 111* | MO594 |
| 11 | MO575 | 37* | MO1011 | 62 | MO406 | 87* | MO5512 | 112* | MO303 |
| 13* | MO564 | 38* | MO514 | 63* | MO111 | 88* | MO570 | 113* | MO63 |
| 14 | MO565 | 39* | MO10 | 64* | MO413 | 89* | MO43 | 114* | MO1015 |
| 15* | MO67 | 40* | MO404 | 65* | MO134 | 90* | MO568 | 115* | MO62 |
| 16* | MO1020 | 41* | MO402 | 66* | MO1001 | 91 | MO540 | 116* | MO5 |
| 17* | MO1013 | 42* | MO135 | 67* | MO139 | 92 | MO586 | 117* | MO72 |
| 18* | MO103 | 43* | MO302 | 68* | MO108 | 93* | MO727 | 118* | MO726 |
| 19 | MO68 | 44* | MO513 | 69 | MO1022 | 94* | MO657 | 119 | MO583 |
| 20 | MO132 | 45* | MO307 | 70* | MO129 | 95* | MO5516 | 120* | MO559 |
| 21 | MO716 | 46* | MO625 | 71* | MO127 | 96 | MO1005 | 121* | MO5514 |
| 22* | MO5508 | 47 | MO303 | 72* | MO252 | 97* | MO69 | 122* | MO567 |
| 23 | MO401 | 48* | MO109 | 73* | MO125 | 98* | MO5522 | 123 | MO1016 |
| 24* | MO584 | 49* | MO705 | 74 | MO44 | 99* | MO577 | 124* | MO8 |
| 25* | MO662 | 50* | MO104 | 75* | MO317 | 100 | MO599 | 125* | MO666 |
| 26* | MO1 | 51* | MO585 | 76* | MO1017 | 101* | MO633 | 126 | MO1025 |
| **Pop 6 :** | **Sutton Dorstbult** | **Pop 7 :** | **Pinewoods Helvetia** | **Pop 8 :** | **Pinewoods Helvetia** | **Pop 9 :** | **Mistley Helvetia** | **Pop 10 :** | **Mistley Helvetia** |
| Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. | Gen 2 Tmt no. | Family no. |
| 128* | MO647 | 162* | MO32 | 187* | MO522 | 214 | MJ323 | 239 | MJ1008 |
| 129* | MO545 | 163* | MO532 | 188* | MO338 | 215 | MJ1002 | 240 | MJ125 |
| 130* | MO308 | 164* | MO525 | 189* | MO16 | 216 | MJ317 | 241 | MJ1014 |
| 132 | MO9 | 165* | MO530 | 190* | MO526 | 217 | MJ308 | 242 | MJ1018 |
| 133* | MO509 | 166* | MO337 | 191 | MO23 | 218 | MJ1005 | 243 | MJ1017 |
| 134* | MO6 | 167* | MO38 | 192* | MO593 | 219 | MJ507 | 244 | MJ139 |
| 135* | MO511 | 168* | MO539 | 193 | MO403 | 220 | MJ305 | 245 | MJ322 |
| 136* | MO316 | 169* | MO509 | 194* | MO1009 | 221 | MJ326 | 246 | MJ306 |
| 137 | MO321 | 170* | MO326 | 195* | MO591 | 222 | MJ85 | 247 | MJ314 |
| 138* | MO86 | 171* | MO501 | 196 | MO322 | 223 | MJ324 | 248 | MJ135 |
| 139* | MO65 | 172* | MO330 | 197* | MO507 | 224 | MJ1021 | 249 | MJ510 |
| 140 | MO4 | 173* | MO340 | 199 | MO304 | 225 | MJ117 | 250 | MJ103 |
| 142* | MO317 | 174* | MO334 | 200* | MO631 | 226 | MJ110 | 251 | MJ1009 |
| 143* | MO94 | 175* | MO518 | 201* | MO745 | 227 | MJ512 | 252 | MJ5 |
| 144* | MO85 | 176* | MO410 | 202* | MO754 | 228 | MJ413 | 253 | MJ526 |
| 145* | MO7 | 177* | MO15 | 203* | MO757 | 229 | MJ112 | 254 | MJ307 |
| 146* | MO327 | 178* | MO1004 | 204* | MO748 | 230 | MJ7 | 255 | MJ513 |
| 148* | MO516 | 179* | MO22 | 205* | MO732 | 231 | MJ1012 | 256 | MJ61 |
| 153* | MO523 | 180* | MO543 | 206* | MO755 | 232 | MJ66 | 257 | MJ121 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 155* | MO1002 | 181* | MO339 | 207* | MO759 | 233 | MJ316 | 258 | MJ1020 |
| 156* | MO1021 | 182* | MO504 | 208* | MO753 | 234 | MJ132 | 259 | MJ315 |
| 158* | MO309 | 183* | MO409 | 209* | MO736 | 235 | MJ1013 | 260 | MJ108 |
| 159* | MO411 | 184* | MO329 | 211 | MJ406 | 236 | MJ63 | 261 | MJ130 |
| 160* | MO533 | 185* | MO93 | 212 | MJ127 | 237 | MJ1016 | 262 | MJ1022 |
| 161* | MO19 | 186* | MO39 | 213 | MJ1004 | 238 | MJ62 | 263 | MJ129 |

Gen 2 Families = second generation family, Family no. = Family number, MO = seed collected from Seven Oaks BSO, MJ = seed collected from Jessievale BSO, and * Common families with E76/P2 trial series

**Table 4.2 Family composition of second generation *E. macarthurii* (progeny trial E76/P2)**

| Gen 2 Family no. | Family no. | Gen 2 Family no. | Family no. | Gen 2 Family no. | Family no. |
|---|---|---|---|---|---|
| 2 | MO519 | 211 | MJ406 | 240 | MJ125 |
| 16 | MO1020 | 212 | MJ127 | 241 | MJ1014 |
| 19 | MO68 | 213 | MJ1004 | 242 | MJ1018 |
| 28 | MO642 | 214 | MJ323 | 243 | MJ1017 |
| 29 | MO637 | 215 | MJ1002 | 244 | MJ139 |
| 30 | MO557 | 216 | MJ317 | 245 | MJ322 |
| 47 | MO313 | 217 | MJ308 | 246 | MJ306 |
| 52 | MO654 | 218 | MJ1005 | 247 | MJ314 |
| 62 | MO406 | 219 | MJ507 | 248 | MJ135 |
| 69 | MO1022 | 220 | MJ305 | 249 | MJ510 |
| 74 | MO44 | 221 | MJ326 | 250 | MJ103 |
| 81 | MO649 | 222 | MJ85 | 251 | MJ1009 |
| 85 | MO653 | 223 | MJ324 | 252 | MJ5 |
| 86 | MO5506 | 224 | MJ1021 | 253 | MJ526 |
| 91 | MO540 | 225 | MJ117 | 254 | MJ307 |
| 92 | MO586 | 226 | MJ110 | 255 | MJ513 |
| 96 | MO1005 | 227 | MJ512 | 256 | MJ61 |
| 102 | MO314 | 228 | MJ413 | 257 | MJ121 |
| 119 | MO583 | 229 | MJ112 | 258 | MJ1020 |
| 123 | MO1016 | 230 | MJ7 | 259 | MJ315 |
| 126 | MO1025 | 231 | MJ1012 | 260 | MJ108 |
| 132 | MO9 | 232 | MJ66 | 261 | MJ130 |
| 134 | MO6 | 233 | MJ316 | 262 | MJ1022 |
| 137 | MO321 | 234 | MJ132 | 263 | MJ129 |
| 140 | MO4 | 235 | MJ1013 | 264 | MJ138 |
| 156 | MO1021 | 236 | MJ63 | 265 | MJ72 |
| 191 | MO23 | 237 | MJ1016 | 266 | MJ327 |
| 199 | MO304 | 238 | MJ62 | | |
| 202 | MO754 | 239 | MJ1008 | | |

Gen 2 Family no. = Second generation families, MO = seed collected from Seven Oaks BSO, and MJ = seed collected from Jessievale BSO.

**Table 4.3 Site information of second generation *E. macarthurii* progeny trials (E76/P1 and E76/P2) and sub-populations**

| Trial number | Trial type | Location | Planting date | Latitude (S) | Longitude (E) | MAP[1] (mm) | MAT[2] (°C) | Altitude (m) | Soil depth (mm) |
|---|---|---|---|---|---|---|---|---|---|
| E76/P1 | Progeny | Pinewoods | 09/12/93 | 29°39' | 30°04' | 890 | 15,3 | 1380 | >1200 |
| E76/P1 | Progeny | Vlakkloof | 18/01/94 | 26°54' | 30°36' | 827 | 16,1 | 1360 | >1200 |
| E76/P2 | Progeny | Pinewoods | 09/12/94 | 29°40' | 30°03' | 884 | 15,3 | 1385 | 800-1000 |
| E76/P2 | Progeny | Vlakkloof | 14/12/94 | 26°54' | 30°36' | 827 | 16,1 | 1360 | >1200 |
| E76/S(N)1 & 2 | Sub-pop | Comrie | 24/11/93 | 29°54' | 29°56' | 840 | 16,0 | 1190 | 300-700 |
| E76/S(N)3 & 4 | Sub-pop | Maxwell | 26/11/93 | 30°02' | 29°56' | 800 | 15,1 | 1360 | >1200 |
| E76/S(N)5 & 6 | Sub-pop | Sutton | 30/11/93 | 30°09' | 30°02' | 820 | 16,8 | 1100 | 900-1200 |
| E76/S(N)7 & 8 | Sub-pop | Pinewoods | 30/11/93 | 29°39' | 30°04' | 890 | 15,1 | 1380 | >1200 |
| E76/S(N)9 & 10 | Sub-pop | Mistley | 15/12/93 | 29°11' | 30°39' | 740 | 16,8 | 1060 | >1200 |
| E76/S(T)1 | Sub-pop | Woodstock | 09/12/93 | 26°22' | 30°38' | 900 | 15,1 | 1655 | 600-1200 |
| E76/S(T)2 | Sub-pop | Woodstock | 06/12/93 | 26°22' | 30°38' | 900 | 15,1 | 1655 | 600-1200 |
| E76/S(T)3 & 4 | Sub-pop | The Brook | 27/01/94 | 26°11' | 30°39' | 922 | 14,5 | 1760 | >1000 |
| E76/S(T)5 | Sub-pop | Dorstbult | 07/12/93 | 26°07' | 30°20' | 841 | 15,6 | 1600 | >1000 |
| E76/S(T)6 | Sub-pop | Dorstbult | 10/12/93 | 26°08' | 30°20' | 742 | 14,6 | 1640 | >1000 |
| E76/S(T)7, 8 & 9 | Sub-pop | Helvetia | 08/12/93 | 25°32' | 30°20' | 775 | 14,8 | 1700 | >1000 |
| E76/S(T)10 | Sub-pop | Helvetia | 08/12/93 | 25°31' | 30°24' | 775 | 14,8 | 1700 | >1000 |

[1]minimum mean annual precipitation, and [2]mean annual temperature, and Sub-pop=sub-population.

**Table 4.4 Trial design information of second generation *E. macarthurii* progeny trials (E76/P1 and E76/P2) and sub-populations**

| Trial number | Trial type | Plantation | Planting date | No. of families | Trial Design | Reps | No. trees/ plot* |
|---|---|---|---|---|---|---|---|
| E76/P1 | Progeny | Pinewoods | 09/12/93 | 169 | 13x13 lattice | 4 | 8 |
| E76/P1 | Progeny | Vlakkloof | 18/01/94 | 169 | 13x13 lattice | 4 | 8 |
| E76/P2 | Progeny | Pinewoods | 09/12/94 | 90 | 9x10 lattice | 4 | 7 |
| E76/P2 | Progeny | Vlakkloof | 14/12/94 | 90 | 9x10 lattice | 4 | 7 |
| E76/S(N)1 & 2 | Sub-pop | Comrie | 24/11/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(N)3 & 4 | Sub-pop | Maxwell | 26/11/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(N)5 & 6 | Sub-pop | Sutton | 30/11/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(N)7 & 8 | Sub-pop | Pinewoods | 30/11/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(N)9 & 10 | Sub-pop | Mistley | 15/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)1 | Sub-pop | Woodstock | 09/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)2 | Sub-pop | Woodstock | 06/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)3 & 4 | Sub-pop | The Brook | 27/01/94 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)5 | Sub-pop | Dorstbult | 07/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)6 | Sub-pop | Dorstbult | 10/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)7, 8 & 9 | Sub-pop | Helvetia | 08/12/93 | 25 | 5x5 lattice | 6 | 8 |
| E76/S(T)10 | Sub-pop | Helvetia | 08/12/93 | 25 | 5x5 lattice | 6 | 8 |

sub-pop=sub-population *= single row plots

When the trees in the progeny trials and sub-populations had reached 14 years of age, wood core samples were extracted from standing trees from the majority of the trials. The site of origin and details of the 302 second generation trees used in this investigation are presented in Figure 4.1 and Table 4.5. Although cores have since been extracted from more trees in all progeny trials and sub-populations, making up a total of approximately 500 trees, only the data from the original 302 trees are presented in this study.

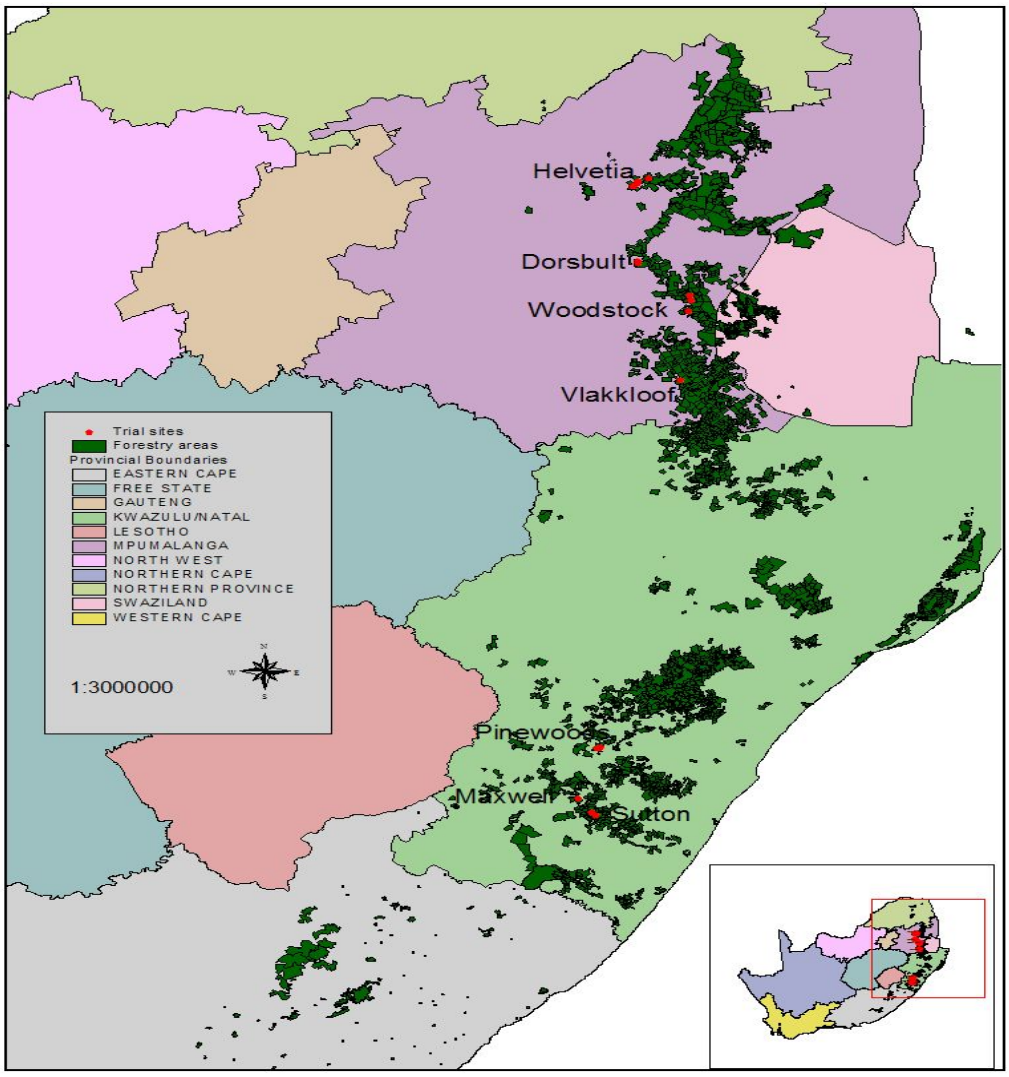


**Figure 4.1 Distribution of ICFR second generation *E. macarthurii* progeny trials and sub-populations in South Africa**

**Table 4.5 Numbers and site origins of second generation *E. macarthurii* trees selected for screening using NIR predicted pulp yield**

| 2nd generation trials | No. of trees* |
|---|---|
| Pinewoods, KZN (Progeny trial, E76/P1) | 34 |
| Pinewoods, KZN (Progeny trial, E76/P2) | 22 |
| Maxwell, KZN (Sub-populations 3 and 4) | 26 |
| Sutton, KZN (Sub-populations 5 and 6) | 14 |
| Pinewoods, KZN (Sub-populations 7 and 8) | 16 |
| Vlakkloof, MPU (Progeny trial, E76/P1) | 85 |
| Woodstock, MPU (Sub-populations 1 and 2) | 10 |
| The Brook, MPU (Sub-populations 3 and 4) | 16 |
| Dorstbult, MPU (Sub-populations 5 and 6) | 14 |
| Helvetia, MPU (Sub-populations 7 and 8) | 44 |
| Mistley, MPU (Sub-populations 9 and 10) | 21 |
| Total no. of trees | 302 |

*number of individual trees from within and between families,
KZN=KwaZulu-Natal, and MPU= Mpumalanga.

### 4.2.2 Methods

Two wood core samples were extracted at 1.3 metres above ground level (representative of breast height) from each standing tree, using a hand-held corer from bark to bark. The hand-held corer is a hollow metal tube, with an internal bore which extends to an exterior borer, called a drill bit, and is used for cutting radially orientated tubes (wood core samples) of about 12mm in diameter and varying length, as shown in Figure 4.2. The extracted core samples were then labelled and stored in a cooler box, preventing the cores from breaking before further processing.