GENETIC AND ENVIRONMENTAL FACTORS AFFECTING ROOTING IN *EUCALYPTUS GRANDIS* X *EUCALYPTUS LONGIROSTRATA* HYBRID CUTTINGS

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Thesis Abstract

In clonal deployment programmes of plantation species, there is frequently the need to deploy *Eucalyptus* species and interspecific hybrids as rooted cuttings. However, the rooting ability of a particular species or hybrid is a major, and usually limiting, factor affecting the economics of commercial deployment. There is also significant between-species and between-family variation for this trait. Recently, *Eucalyptus longirostrata* was crossed with *E. grandis* in an effort to combine its desirable wood properties, drought tolerance and disease resistance with the latter's vigorous growth. Should progeny with these suitable characteristics be identified, there is the possibility of extending plantations to more marginal areas and increasing the quality and volume from existing plantations.

An investigation was undertaken to study the rooting ability of *E. grandis* x *E. longirostrata* hybrid cuttings. The plant material was sourced from five families in seedling derived hedges at two nurseries, as well as five families coppiced from an unreplicated progeny trial planted in the midlands of KwaZulu-Natal. Their rooting ability was assessed by determining the percentage of cuttings that developed roots using two different rooting methods.

The variation between and within families and clones is presented. The study shows that root strike is under moderate genetic control, with an estimate of broad-sense rooting heritability of 0.197 (S.E. = 0.070). The two nurseries used in this study used different rooting technologies, with cuttings rooted either in media (traditional method) or in air (aeroponically). The aeroponics technology was highly significantly ($p<0.0001$) superior, in terms of rooting success. Rooting was also highly significantly affected ($p<0.0001$) by the temperature at the time of cutting. Rooting success of cuttings from seedling-derived parental hedges was similar to cuttings from coppiced stumps. The consequences of the low repeatability of measurements of rooting ability, 0.187 (S.E. 0.067) by clone and 0.340 (S.E. 0.072) by ramet, which was influenced by the response to age of material and nursery conditions, is discussed.

This study demonstrated that large improvements in rooting success can be made by the optimization of rooting protocols and selecting for superior genotypes, as long as the performances of genotypes are accurately assessed.

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Finally, I would like to thank my colleagues at CSIR Tree Improvement.

Declaration

I, Nuveshen Naidoo, declare that:

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Thesis Introduction

Commercial forestry is a major and competitive global industry. With demand for timber and other wood products growing significantly over the last half century, sustainable and profitable commercial forests have become increasingly popular, reducing over-reliance on natural forests. Commercial forestry requires species that have certain desirable characteristics which include fast growth rates, high quality wood, physiological fitness, disease and pest resistance and adaptability to a wide range of habitats. These qualities make some exotic species very suitable for commercial use.

Eucalypts cover approximately 480 000 ha of South African land, with 75% being *Eucalyptus grandis* Hill ex Maiden (DWAF, 2008). *E. grandis* is a relatively fast growing species with good ‘rootability’. It is most commonly used as a source of pulpwood, fuel and timber (Retief and Stanger, 2009). However, *E. grandis* generally has low drought tolerance and is very susceptible to disease (Denison and Kietzka, 1993). These shortcomings led to the search for alternative eucalypt species for use in South African commercial plantation forests. One particular species, *E. longirostrata* (Blakely) Johnson and Hill, exhibits many desirable attributes such as drought resistance, disease tolerance and high pulp yield. In 2002, crosses between superior *E. grandis* selections and pollen from *E. longirostrata* were made and F_1 hybrids were produced by the CSIR to make use of the complimentary traits of the parent species.

Genetic gains from hybrids are short-lived as maximum heterosis is only observed in the first generation and subsequently declines. Yet hybrid vigour may be maintained if the resultant hybrids are vegetatively propagated (Warrag *et al.*, 1990). Rooted cuttings are the most cost effective and widely used method of propagating clones. However, they are significantly more costly than seedlings. A major part of this higher cost is due to poor rooting of cuttings, and high level of cutting mortality. Rooting is believed to be under strong genetic control (Borralho and Wilson, 1994), and thus there exists the potential to select for superior families or clones. Environmental factors also play a large role in the survival and rooting of cuttings, allowing room for optimization of nursery productivity. Different nurseries have different rooting regimes, and these may impact on rooting success.

Therefore, in order to make recommendations on how to increase rooting success and nursery productivity, this study had the following explicit goals:

1. To investigate the genetic control of rooting of some hybrids of *E. grandis* x *E. longirostrata;*
2. To examine some of the environmental factors affecting rooting of its cuttings;
3. To compare different nursery practices.

Should the *E. grandis* x *E. longirostrata* hybrids meet expectations of high pulp yield and be tolerant of marginal areas, then increasing the rooting success of cuttings would help decrease the costs of commercial clonal deployment of the best hybrid genotypes.

Note 1. All unreferenced graphics presented in this thesis were generated by the author.

Note 2. The format of the thesis was prescribed by the supervisor, in terms of the accepted formats for theses at UKZN.

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Chapter One: Literature Review

1.1 Introduction

Trees play a major role in supporting life on earth and make up a significant proportion of the earth's biomass. Tree coverage varies greatly throughout the world (Figure 1.1). Trees provide shelter, food and fuel for humans and animals. Forests are of immense importance in soil stabilization and erosion control. Trees are also vital in atmospheric carbon reduction through carbon fixation, and thus serve as carbon reservoirs. Throughout the world, forests consist of naturally occurring tree species or trees planted to meet man's need for tree resources and are termed indigenous and plantation forests, respectively.

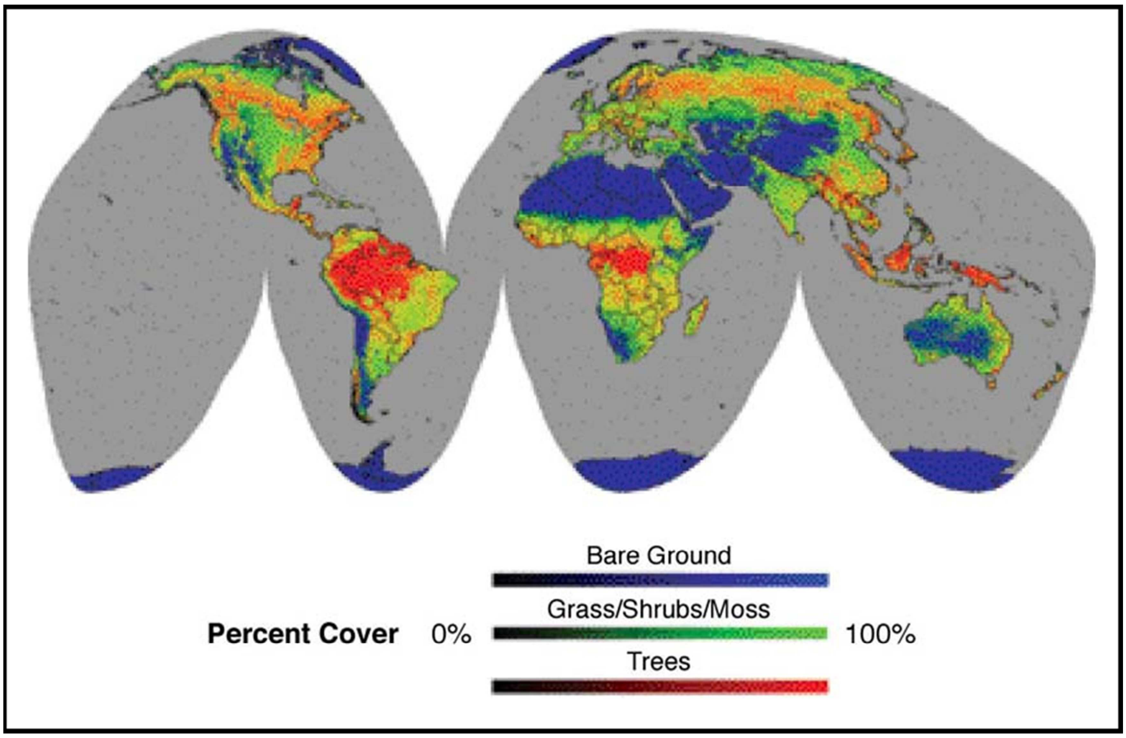


Figure 1.1 Global land-cover estimates of percentage tree cover, herbaceous cover, and bare ground (DeFries *et al.*, 2000)

Indigenous forests have traditionally provided for man's forestry needs. Indigenous forest ecosystems consist of many species that have co-adapted to the environment, and thus are maintained in equilibrium. However, the felling of selected tree species by man for timber, food, fuel and to make room for agriculture threatens the survival of these systems (Cummings *et al*., 1997). The United Nations Food and Agriculture Organization (2001) estimated that the world lost about 16.1 million hectares of natural forests annually in the 1990s (FAO, 2001). It has thus become increasingly important to supplement indigenous forests with commercial plantations.

With demand for timber and other wood products growing significantly over the last half century, sustainable and profitable commercial forests have become increasingly important in order to reduce reliance on natural forests. It is estimated that 30% of the earth's land was forested in 2001 (FAO, 2001). Global forest distribution is shown in Table 1.1 at a continental level.

Table 1.1 Global forest cover by region (FAO, 2001)

Region	Total Forest (M ha)	Total Forest (%)
Africa	650	17
Asia	542	14
Oceania	201	5
Europe	1040	27
North and Central America	539	14
South America	874	23
World Total	3856	100

South Africa is a country of low rainfall. As a result, most of the vegetation is non-woody, with natural forests consisting of a narrow broken belt of closed canopy forest along the southern and eastern seaboards and open canopy savannah woodlands in the north-eastern interior of the country. Compared with a world mean in excess of 30%, in South Africa natural closed canopy forest covers only roughly

0,5% and savannah woodlands covers roughly 19,0% of the total land area (Owen and Zel, 2000). Furthermore, indigenous species have slow growth rates, making them less suitable for commercial exploitation (Sedjo, 1999).

Commercial forestry requires species that have certain desirable characteristics. Some of these important characteristics include fast growth rates, high quality wood, physiological fitness, disease and pest resistance and adaptability to a wide range of habitats (FAO, 2001). These qualities make many exotic species suitable for commercial use, whereas most South African indigenous species do not have these attributes (Sedjo, 1999).

Exotic species used in the South African forestry industry mainly originate from Australia, Southeast USA and Mexico. These include species of *Pinus* (pines), *Eucalyptus* (eucalypts) and *Acacia* (wattles). Pines and other softwoods comprise approximately 54% of South Africa's plantation land whilst hardwoods, including eucalypts and wattle, occupy about 46% (FSA, 2007). The species and provincial distribution is shown in Table 1.2.

Table 1.2 Distribution of timber species in South Africa (DWAF, 2008)

[redacted]

1.2 The genus *Eucalyptus*

Eucalyptus is a diverse genus of trees and shrubs, consisting of more than 800 different species (Hopper, 1997). *Eucalyptus* is native to Australia, with a small number of species found adjacently in New Guinea, Indonesia and Philippine Islands. The genus is one of a group of three similar genera, together with *Corymbia* and *Angophora*, commonly known as ‘eucalypts’ (Ladiges and Humphries, 1983) (Figure 1.2). *Eucalyptus* has been widely spread around the world for commercial purposes, and is now grown extensively in Brazil, China, Spain, South Africa and the United States (Booth and Pryor, 1991). While eucalypt plantations benefit many developing economies, serious problems such as invasiveness and excessive water use have been noted (Richardson and van Wilgen, 2004).

Eucalypts are usually assigned to one of two habit categories (tree or mallee) and are described by Brooker and Kleinig (1994) as:

Forest trees	“...are single-stemmed and have a crown forming a minor proportion of the whole tree height, with combined crowns occupying at least 30% of the ground cover.”
Woodland trees	“...are single-stemmed, although they may branch a short distance above ground level, with the combined crowns occupying less than 30% of the ground cover.”
Mallees	“...are multi-stemmed from ground level, usually less than 10 metres in height, often with the crown predominantly at the ends of branchlets, and individual plants may combine to form either an open or closed forest formation.”

While these categories help in the description of many eucalypt species, they are not completely distinct because form can often be affected by environmental factors such as fire, physical damage or competition.

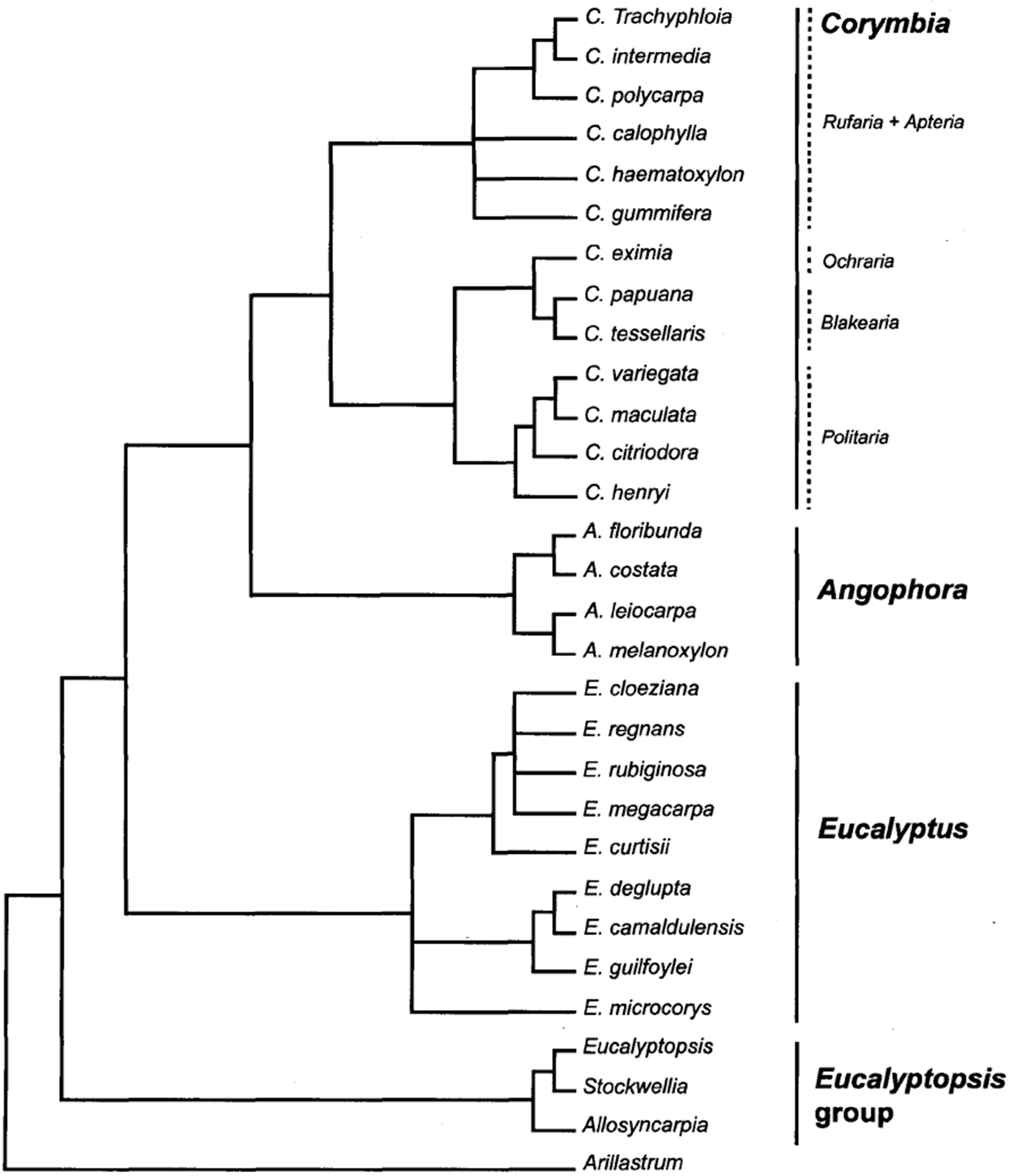


Figure 1.2 **Phylogenic relationship between members of the eucalypts (adapted from Parra-O *et al*., 2006)**

Eucalyptus has many different uses and end products. Eucalypts are used as wind breaks, for soil erosion control, as ‘forage’ for the bee industry and for carbon sequestration (Christie and Scholes, 2005; Henson *et al*., 2007). Furthermore, many products are derived from eucalypts (Figure 1.3).

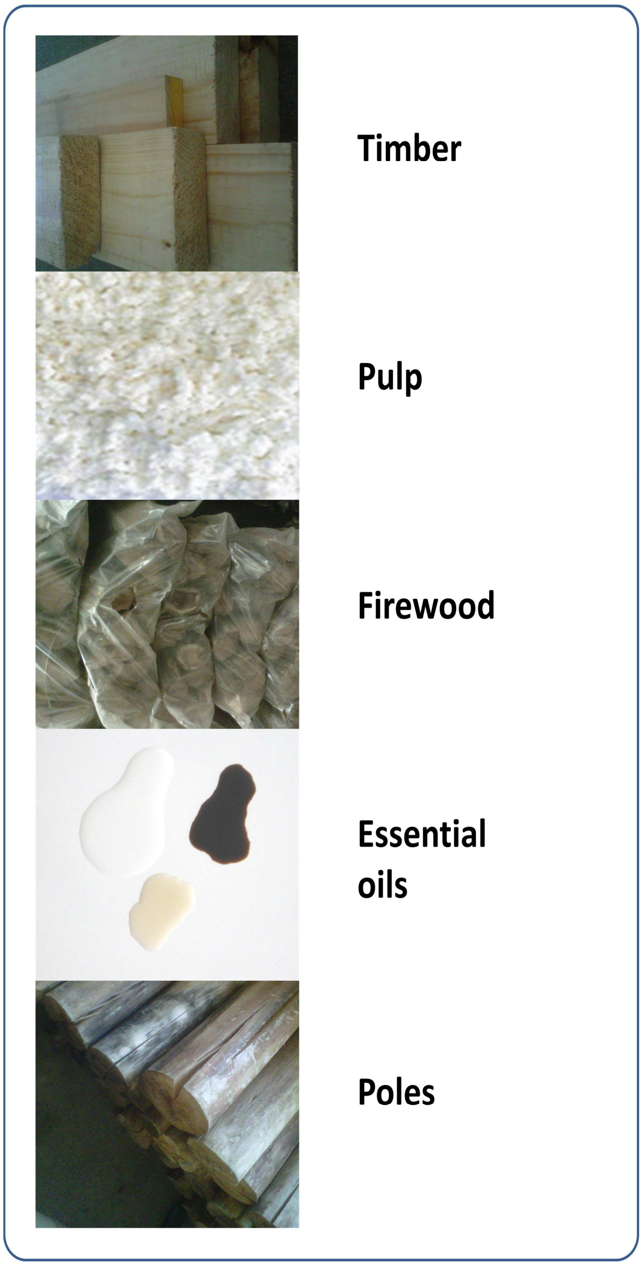


Figure 1.3 Common products derived from eucalypts

1.2.1 *Eucalyptus grandis*

Eucalyptus grandis Hill ex Maiden is commonly known as Flooded Gum or Rose Gum (Brooker and Kleinig, 1994). One of the most commonly grown commercial forestry species in South Africa, *E. grandis* covers approximately 27% of the country's plantation forests (FSA, 2007).

Native to the Australian States of Queensland and New South Wales (Meskimen and Francis, 1990), *E. grandis* grows in coastal and subcoastal ranges (Brooker and Kleinig, 1994). Found mostly on flats or lower slopes, *E. grandis* prefers moist, well drained, deep loamy soils of alluvial or volcanic origin (Boland *et al*., 2006). The natural distribution of *E. grandis* is shown in Figure 1.4.

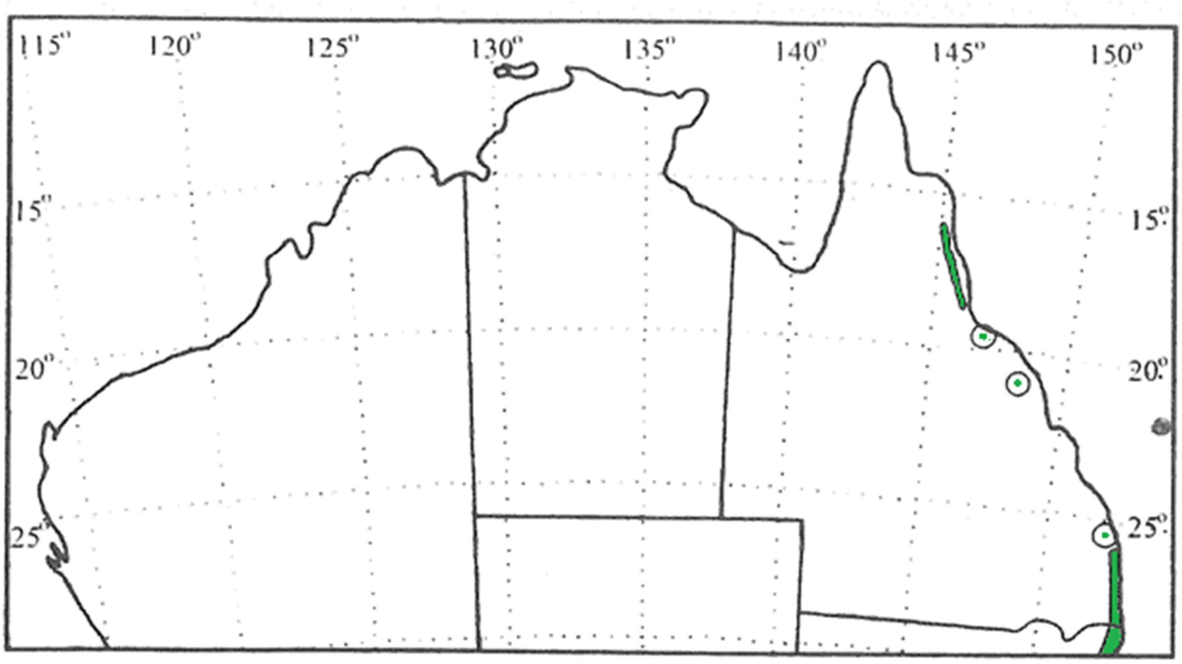


Figure 1.4 The natural distribution *of Eucalyptus grandis* in Australia (from Brooker and Kleinig, 1994)

E. grandis has a tall and straight form, with Meskimen and Francis (1990) describing its bark as thin and deciduous, shedding in strips to expose a smooth surface (Figure 1.5).

Figure 1.5 ***Eucalyptus grandis*** **progeny trial in KwaZulu-Natal, South Africa**

In South Africa, *E. grandis* is well known for its fast growth (Schönau, 1984), stem straightness (Meskimen and Francis, 1990) and good pulp yields (Campinhos, 1999). In addition, its good fertility and rooting ability make *E. grandis* a good ‘all rounder’ and an attractive hybrid partner (Retief and Stanger, 2009).

1.2.2 *Eucalyptus longirostrata*

Eucalyptus longirostrata (Blakely) Johnson and Hill is commonly known as Grey Gum (Brooker and Kleinig, 1994). Although newly introduced to South Africa, it has shown the potential to become an important species for commercial plantations (Gardner, 2001; Snedden *et al*., 2007).

Native to Australia, *E. longirostrata* is widespread in south-eastern Queensland, the Expedition Range, and the Blackdown Tableland (Brooker and Kleinig, 1994). *E. longirostrata* grows on a range of sites on low hills and ridges in shallow soils of sandstone origin (Boland *et al*., 2006). The natural distribution of *E. longirostrata* is shown in Figure 1.6.

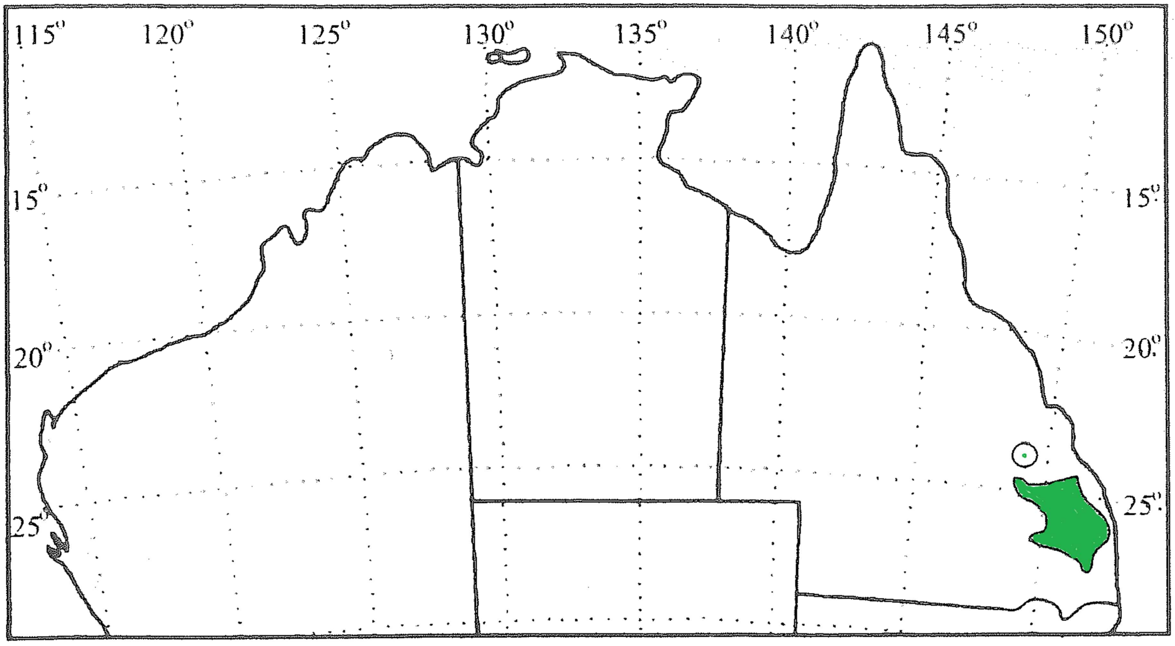


Figure 1.6 The natural distribution *of Eucalyptus longirostrata* in Australia (from Brooker and Kleinig, 1994)

E. longirostrata is a medium-sized tree with bark shedding to ground level, with a granular and mottled surface (Brooker and Kleinig, 1994) (Figure 1.7).

Figure 1.7 ***Eucalyptus longirostrata*** **progeny trial in KwaZulu-Natal, South Africa**

In South African trials, unimproved *E. longirostrata* has compared favourably with commercial hybrid clones for growth yield, pulp yield and ‘pulp ability’ (Gardner, 2001).In addition, its high density and reported high disease resistance (Gardner *et al.*, 2007) make it a suitable hybrid partner candidate (Snedden *et al*., 2007).

1.3 Hybrids

Hybridisation is the crossing of genetically diverse parents. In forestry, the term is generally accepted by default to mean the crossing of different species, i.e., inter-specific hybridisation. In recent years, and for the reasons explained below, hybrid breeding in forestry has become an increasingly popular method of genetic improvement.

Three important reasons for the development of hybrids in forestry are better parent complementarity, adaptability and heterosis (Kain, 2003). Complementarity is the combination of favourable traits from different species. Due to the increased genetic variability associated with hybrids, they may be more adaptable to diverse environments than their parent species. Better parent heterosis is the superior performance in a specific trait of a hybrid over either of its parents (Dungey, 2001).

The term "heterosis" is an often controversial in genetic terminology (Virmani, 1994). It has been proven in many species crosses, but has also been claimed in a many more (Wright, 1962). Virmani (1994) defines heterosis as the biological phenomenon in which an F_1 hybrid of two genetically dissimilar parents shows increased vigour at least over the mid-parent value (P_1+P_2/2). From a tree breeding perspective, the term heterosis is frequently used to describe better parent heterosis.

Three hypotheses have been proposed to explain heterosis (Moll and Stuber, 1974):

i. Dominance: Favourable, dominant vigour genes are manifested in the F_1 from the two parents. It assumes that dominant genes contributed by one parent may complement the genes contributed by the other parent, resulting in a more favourable combination than either parent in the F_1.
ii. Over-dominance: Rare loci exist in which the F1 hybrid with the greatest number of heterozygous alleles will be the most vigorous compared to the two homozygote parents for the same gene.
iii. Epistasis: Heterosis is due to the interaction between more than one gene affecting the trait.

Furthermore, complementarity and adaptability of F_1 can be confused for heterosis if the trait is affected by many loci or if the F_1 is better adapted to the site, respectively. Hybrid superiority may rely on one or more of these factors, and it has been suggested that in forestry, hybrids are usually bred for their complementary and adaptive characteristics (Kain, 2003).

The presence of natural hybrids in *Eucalyptus* was suspected as early as 1810 but verification by experimentation was only attempted in the 1930s (Wright, 1962). Since then, eucalypt hybrids have become significant parts of breeding programmes in South Africa (Table 1.3) and the rest of the world. Most eucalypt hybrids deployed are created through controlled pollinations (Figure 1.8). However, not all hybrid combinations are possible, with the general trend being that hybridisation occurs more readily with closely related species, and becomes more difficult as genetic/taxonomic distance increases (Potts and Dungey, 2004). Some authors (e.g. Potts *et al*., 1987) have proposed an optimum degree of genetic/taxonomic divergence for crossing success and the expression of hybrid vigour (Figure 1.9). Furthermore, several reproductive barriers prevent hybridisation (Table 1.4).

Table 1.3 Some *Eucalyptus* hybrids used in South Africa

Cross	**Features**
Eucalyptus grandis x Eucalyptus nitens	Cold tolerance
Eucalyptus grandis x Eucalyptus urophylla	Superior growth, disease tolerance
Eucalyptus grandis x Eucalyptus camaldulensis	Tolerates dry conditions
Eucalyptus grandis x Eucalyptus tereticornis	Tolerates hot and dry conditions

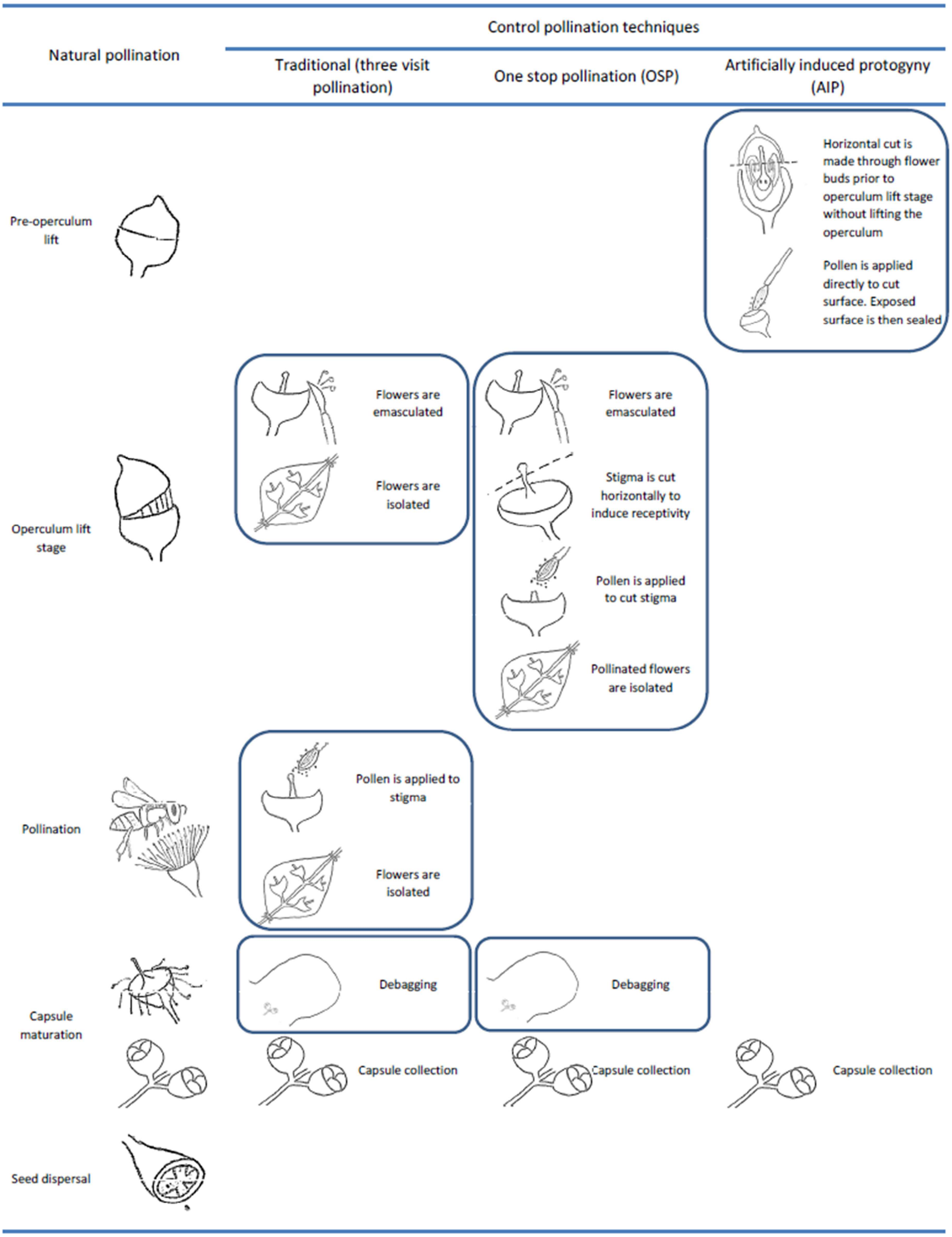


Figure 1.8 Different methods of cross pollination in *Eucalyptus*

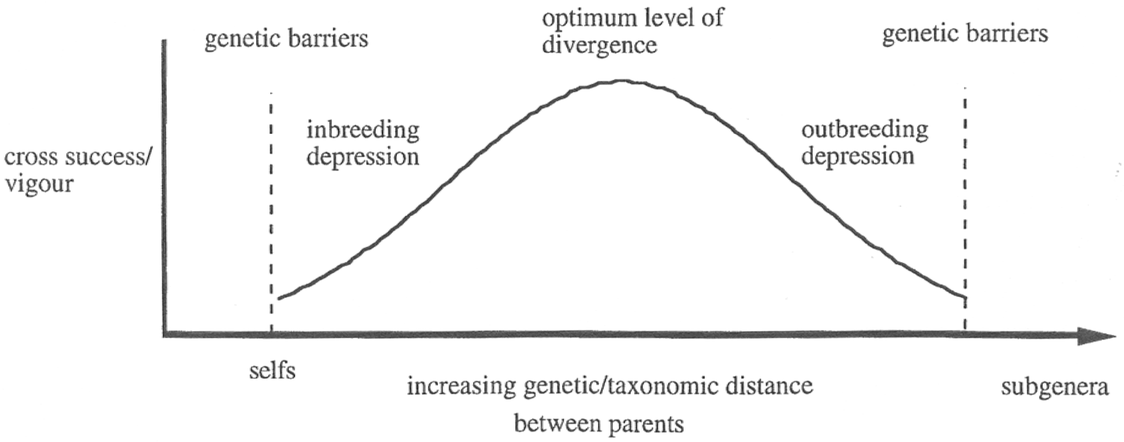


Figure 1.9 **The hypothetical relationship between cross success and the genetic distance between parents (from Potts and Gore, 1995)**

Table 1.4. **Reproductive barriers to hybridisation in *Eucalyptus***

Reproductive barriers in *Eucalyptus*		**Examples**	**Observed in *Eucalyptus***
Pre-mating		Flowering time	While common, these are overcome by pollen storage and control pollinations
		Pollinators	
Post-mating	Pre-zygotic	Flower structure	Gore *et al*., 1990
		Barriers in stigma or style	Ellis *et al*., 1991
	Post-zygotic	Embryo abortion	Potts *et al*., 1987
Post-dispersal		F_1 vigour, sterility	Myburg *et al*., 2004
		Advanced generation breakdown	Potts and Dungey, 2004

Even when the barriers to the creation of many hybrids are overcome, their deployment poses a few problems:

i. Low fecundity: Controlled crosses often yield low numbers of viable seed (Potts and Dungey, 2004).
ii. Variablity: The progeny from inter-specific crosses are highly variable, compared to intra-specific crosses (Potts and Reid, 1985).
iii. Poor economics: Controlled crosses are expensive, labour intensive and time-consuming (Suitor *et al*., 2007; Horsley *et al*., 2010).

All the previously listed factors can potentially limit the deployment of inter-specific hybrids. Cloning F_1 hybrids is often the only economically feasible method to deploy them on a large scale (Campinhos, 1999).

1.4 Clones

In forestry, clones are a group of genetically identical individuals (ramets) derived asexually from a single individual (ortet) (Venkatesh, 1986; de Assis, 2001). Although vegetative propagation has been used for centuries, it has become an increasingly important tool in many tree improvement programmes. Vegetative propagation can bring many benefits to both tree improvement research as well as operational deployment (Wright, 1962).

While vegetative propagation has numerous uses in research (Gaudin *et al*., 2008), its applicability to commercial plantations yields the most rewards (Libby and Rauter, 1984). The use of clones allows the capture of both additive and non-additive genetic effects of specific genotypes (of particular usefulness with hybrids), and for them to be multiplied for large scale deployment (Libby and Rauter, 1984). Furthermore, an advantage to the use of clones is the quicker deployment of superior individual plants than via traditional seed production. Operationally, the use of clones is a great advantage when uniformity of the resource is desirable (Mitchell, 1992).

There are many different methods of vegetative propagation, some of which are presented in Table 1.5.

Table 1.5 Methods of vegetative propagation

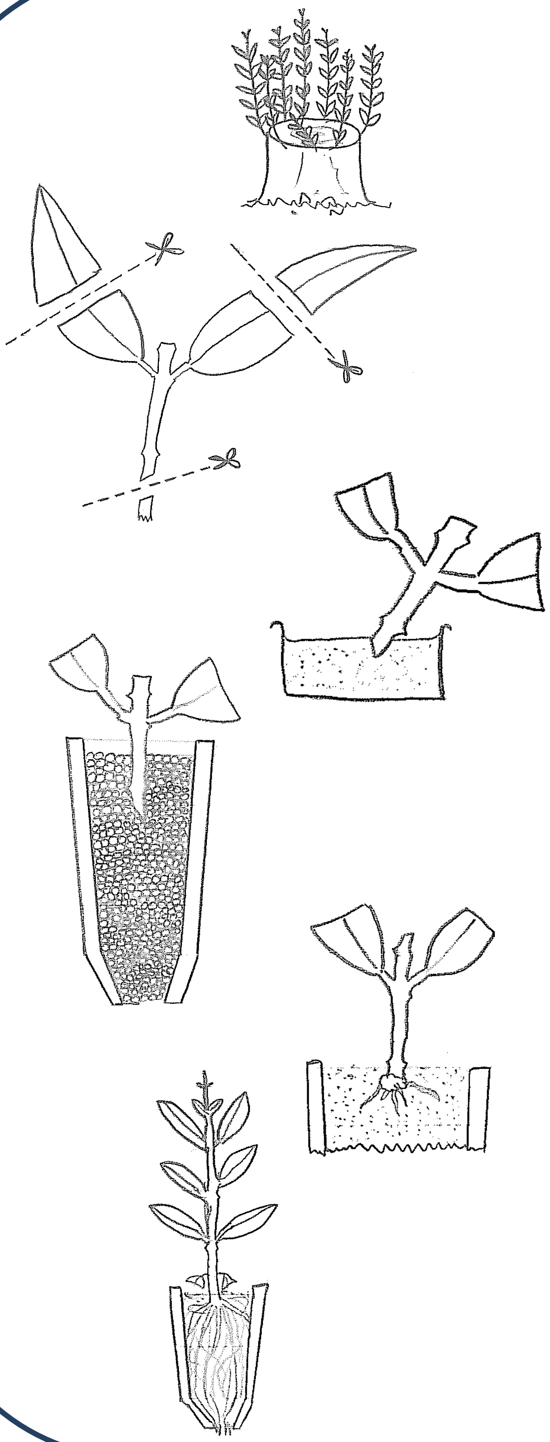
Cloning method	Description	Uses	Advantages	Disadvantages
Grafting	Branches are cut from parent tree and attached to a compatible root stock.	Clone preservation Seed orchard establishment	Early flowering Non-destructive	Expensive Scion-rootstock incompatibility
Rooted cuttings	Stem cuttings are induced to form roots.	Commercial deployment to plantations	Low cost	Poor rooting in some species
Air layering	Rooting is induced (by girdling or hormone treatment) on branches that are still attached to parent tree.	Obtaining propagules from trees that do not coppice from stumps.	Non-destructive	Time consuming
Tissue culture	*In vitro* regeneration of propagules from cells or tissue in a medium providing nutrients, growth hormones and energy	Research Production of hedges	The potential to increase material exponentially	Expensive Requires skilled labour Requires sophisticated facilities

While both tissue culture and rooted cuttings are both suitable for large scale deployment, the high costs of tissue culture limit its current use.

The most widely used method of vegetative propagation in eucalypts is rooted cuttings (Figure 1.10). The method usually includes the following steps, but vary greatly between different deployment programmes (Venkatesh, 1986):

1. A tree with desirable traits is selected.
2. The tree is felled and allowed to coppice.
3. The coppice shoots are harvested and subdivided into a few cuttings per shoot, with each having two leaves. The leaves are cut shorter to decrease moisture loss through transpiration.
4. The cuttings may be treated with a growth hormone to induce root formation.
5. The cuttings are then placed in planters; examples of different planting media include sand, charcoal fines, composted pine bark, and vermiculite.
6. The planters are kept in a greenhouse that is misted frequently.
7. Roots generally appear within a few weeks of setting.
8. Cuttings are then transferred to new planters with a growing medium, if necessary, and hardened before being transferred to the nursery.

Many different factors affect rooting success of stem cuttings in *Eucalyptus*, some of which are presented in Table 1.6.



Selected tree is felled and allowed to coppice

Shoots are harvested and sub-divided

Cuttings may be treated with rooting hormones

Cuttings are placed in planters

Callus forms at stem base, roots grow from it

Cuttings are hardened off

Figure 1.10 Reproduction of *Eucalyptus* clones as rooted cuttings

Table 1.6 Factors affecting rooting of eucalypt stem cuttings

Type	Factor	Observed effect	e.g. Studies
Genetic	Additive	Rooting is under genetic control	Borralho and Wilson, 1994
	Non-additive	Low specific combining abilities reported for rooting ability	Lemos *et al*., 1997
Environmental	Nutrition	Optimization of macro- and micro-nutrient fertilization can significantly increase rooting success	McNabb *et al*., 2002
	Age of ramet	Induction of juvenility increases rooting ability	Xavier and Comerio, 1996
	Auxin application	Application of rooting hormones can increase rooting ability	Luckman and Menary, 2002
	Temperature	Optimizing temperature of hedge and cuttings can increase rooting	Corrêa and Fett-Neto, 2004
	Dissolved oxygen	Increasing dissolved oxygen concentration at stem cutting base increases rooting success	Gaudin *et al.*, 2008
Genotype x environment interaction		Typically low in *Eucalyptus*	Borralho and Wilson, 1994

While vegetative propagation is indispensible to modern tree improvement programs, a few concerns exist that limit its application:

i. *Topophysis* is the phenomenon that occurs when rooted cuttings maintain for some time the branchlike growth habit (plagiotropic growth) that they had as shoots on the ortet (Olesen, 1978).
ii. *Cyclophysis* is the process of maturation of the apical meristem (Olesen, 1978). This results in cuttings from older ortets usually rooting less easily than from young ortets (Wright, 1962).

Economic feasibility is often the most important criterion when choosing a vegetative propagation deployment strategy (Lal, 1994). For deployment to be economically feasible the genetic benefits of clonal deployment must outweigh its additional costs. Because most material and labour costs are fixed for a particular propagation method, the ultimate variable to the economic feasibility is the inherent rooting ability of the specific clone.

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Chapter Two: Genetic control of rooting in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrid

2.1 Abstract

Many factors affect the success of a clone in forestry, particularly its rooting ability. An investigation was undertaken to determine the genetic control of rooting in *E. grandis* x *E. longirostrata* hybrid cuttings. The plant material was sourced from five families in hedges at two nurseries in KwaZulu-Natal, South Africa. The rooting ability was assessed by determining the percentage of cuttings that developed roots, using two different rooting methods, i.e., either in air or media.

The mean rooting ability between the five families were significantly different ($p<0.05$) and ranged from 23% to 34%. The mean rooting ability between the 25 clones tested were significantly different ($p<0.0001$) and ranged from 12% to 52%. The broad-sense heritability was estimated to be 0.197 (S.E. 0.070). The results of this study suggest that the independent culling of poor rooting clones prior to their field testing may be a fair compromise between the cost of the programme and genetic gains.

2.2 Introduction

Eucalypts are an important species for the South African forestry industry. *Eucalyptus grandis* Hill ex Maiden is commonly grown for pulp, and accounts for approximately 263 000 ha (27%) of plantation area in the country (FSA, 2007). Demand for pulp wood worldwide has increased rapidly in recent years, but planted area in South Africa is in decline (FSA, 2007).

A newly introduced species, *Eucalyptus longirostrata* (Blakely) Johnson and Hill, has shown potential for commercial forestry application in South Africa (Gardner *et al*., 2007). In 2002, *E. grandis* x *E. longirostrata* hybrids were produced as part of Project Pulp, an ongoing collaboration between NCT and the CSIR. The identification of individuals that combine the desirable wood properties, disease resistance and drought tolerance of *E. longirostrata* with the vigorous growth of *E. grandis* is anticipated (Snedden *et al*., 2007). This would allow for increasing quality and volume from existing plantations, extending plantations to more marginal areas, or both.

Hybridisation affords the opportunity to make significant short term gains, although hybrid seed is very variable and the seed production is costly (Zobel and Talbert, 1984). Vegetative production of superior individuals or families can reduce costs and increase uniformity (Wright, 1962). Although eucalypt clones are most often deployed as rooted cuttings, the rooting ability of a particular species or hybrid is a major, and sometimes limiting, factor affecting the economic viability of commercial deployment.

Two concurrent clonal deployment strategies feature in Project Pulp, namely the “traditional” deployment of superior individuals, as well as a “family forestry” model, in which families are cloned. The “family forestry” model has the potential to decrease the intensity of management at the nursery as individual clone identities are not needed and the risks associated with monoculture are mitigated.

The aim of this study was to quantify the variation in rooting between families and clones, to investigate the genetic control of rooting ability in *E. grandis* x *E. longirostrata* adventitious cuttings. The results of these studies would be used to inform the deployment strategy of the best hybrid clones.

2.3 Materials and Methods

Genetic material

The genetic material consisted of full-sib *E. grandis* x *E. longirostrata* families. Seedlings were bulked up by rooting vegetative cuttings at two commercial nurseries in Pietermaritzburg, KwaZulu-Natal. Five full-sib families, each consisting of five individuals were selected for a hedge rooting investigation. Four ramets per clone were planted in a randomized complete block design, and trained as hedges, between both nurseries.

Vegetative propagation

Adventitious cuttings were made periodically in batches when hedges were ready for harvesting (selective harvests) which ranged from 15-44 days over a period of nine months at both nurseries. Cuttings were rooted in a perlite/vermiculite/coir medium after dipping in 0.6% (w/w) 4-(indoyl)-butyric acid (IBA) at Nursery A. In contrast, Nursery B did not use any rooting hormones, and rooted the cuttings in the air, suspended in upturned seedling trays, using a form of aeroponics. Cuttings were misted in rooting tunnels for 30-40 days and heated during cold weather. Further differences in the conditions between both nurseries are detailed in Chapter 3.

Observations and measurements

The first two batches of cuttings were discarded because they were used to standardise the size of the ramets. Hedge productivity was measured as the number of cuttings harvested per ramet per batch. Cuttings were assessed for root strike after being set for approximately 3 to 3.5 months because the rooted cuttings were used to create more hedges. The presence or absence of roots was recorded, with a cutting being considered as ‘rooted’, if it had at least one root longer than 1mm. Rooting was recorded as the percentage of cuttings rooted per ramet, provided that more than ten cuttings were made. If a batch yielded cuttings from less than eight clones, then it was discarded.

Data analysis

Analyses were conducted in SAS® for Windows ver. 9.1 (SAS, 2003). Statistical analysis was conducted on log-transformed data. An analysis of variance was performed on the unbalanced data using Type III sums of squares method of the generalised linear models, after correction for fixed site (nursery) and random batch (replicate) effects. The following statistical model was used:

$$y_{ijk} = \mu + f_i + c_{j(i)} + e_{ijk}$$

where:

μ is the overall mean

f_i is the effect of family i;

$c_{j(i)}$ is the effect of clone j nested in family i;

e_{ijk} is the error

In these analyses, family, clone and batch were treated as random effects.

The least significant differences (LSD) multiple comparison test (α=0.05) was used to detect significant differences in rooting success between clones (Fisher, 1935).

Heritability was estimated from hedge clone results using a Model Least-Squares and Maximum Likelihood program (LSMLMW & MIXMDL PC-2 Version) developed by Harvey (1990).

2.4 Results

Family and clone effects on rooting

The mean rooting ability across the entire trial was 29.4% (S.E. 15.8). An analysis of variance (ANOVA) was performed to determine the family and clone effects on rooting, after correcting for nursery and batch (significant fixed) effects (Table 2.1). The Least Square Means for family and clone rooting ability throughout the trial, and across both nurseries, are presented in Table 2.2.

Table 2.1 Analysis of Variance for log transformed root strike percentage of cuttings from hedges

Source	Degrees of Freedom	Sum of Squares (type 3)	Mean Square	F value	P
Family	4	5.11	1.27	3.06*	<0.05
Clone within family	20	25.71	1.28	3.08***	<0.001
Error	203	84.85	0.41		

The ANOVA detected significant differences between the rooting success of families and clones within families.

Table 2.2 Mean family and clone rooting (% root strike) of cuttings from hedges at both nurseries

Family				Clone				LSD*
Family	Mean Rooting	Std Err	n	Clone	Mean Rooting	Std Err	n	
G050 X L09	27.03	2.53	57	PP207	31.07	5.1	9	bcdefg
				PP280	21.12	3.8	16	defgh
				PP284	32.26	4.8	10	bcde
				PP295	31.25	4.8	10	bcdefgh
				PP299	26.36	4.4	12	bcdefgh
G075 X L03	34.34	3.05	52	PP353	31.24	4.8	10	bcdefgh
				PP359	41.96	4	15	ab
				PP360	30.8	5.4	8	bcdefg
				PP371	25.53	4.3	13	bcdefgh
				PP373	37.59	6.3	6	abc
G075 X L14	23.14	3.04	35	PP429	24.49	4.6	11	cdefgh
				PP465	38.59	5.8	7	bcd
				PP486	11.9	7.7	4	gh
				PP501	23.57	7.7	4	defgh
				PP504	19.26	5.1	9	fgh
G107 X L09	27.24	3.65	42	PP746	19.99	4.8	10	defgh
				PP754	52.15	4.4	12	a
				PP836	24.25	6.3	6	efgh
				PP838	16.41	5.8	7	efgh
				PP845	16.62	5.8	7	h
G113 X L09	32.68	2.72	55	PP862	31.42	3.7	17	bcdef
				PP872	23.58	4	15	bcdefgh
				PP873	33.87	7.7	4	bcdefg
				PP875	30.72	5.1	9	bcdefgh
				PP878	40.63	4.8	10	abc

*The least significant differences (LSD) multiple comparison test (α=0.05) declares no significant differences between clones with the same letter (Fisher, 1935).

A much larger difference in rooting between clones within the same family than between families was observed.

Heritability

The broad-sense heritability was calculated using clone-means, disregarding the relationship between families. The broad-sense heritability was 0.197 (S.E. 0.070).

2.5 Discussion

Significant differences ($p<0.05$) were observed between the different full-sib families, with mean rooting ranging from 23% to 34%. Although the number of families was limited, the differences in mean rooting between families were clear. A similar outcome can be predicted for a wider source of families.

Much larger differences were observed within families, more specifically the clone within family effects ($p<0.0001$) with mean rooting between clones ranging from 12% to 52%.These large differences in rooting were of a magnitude that can be the deciding factor in the feasibility of commercial deployment of particular clones.

Because of the small number of crosses, too few families were available for a reliable family estimate of heritability (Klein *et al*., 1973). The moderate broad-sense heritability value of 0.197 (S.E. 0.070) suggests that improvement of rooting ability during commercial deployment could be achieved by using it as a trait in the selection of clones. Although results are indicative, a larger study with more families would provide a better estimate of the genetic effects.

The large variation observed amongst clones within families suggests that screening pre-commercial clones would be more effective than screening their families for rooting ability. However, this traditional strategy is resource-expensive at the pre-commercial stage, as the identities of many clones need to be kept discrete. The “family forestry” model might offer a fair compromise between costs and benefits in this regard (Snedden *et al*., 2007). Furthermore, the lower rooting gains post-screening of families, as opposed to clones, in the “family forestry” model should increase during the pre-commercial stage as the successive rooting of ramets will act as an artificial selection pressure for genotypes that root better, such that the poor rooting ones would be “diluted” thereby being less well represented during commercial deployment.

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Chapter Three: A comparison of the effects of two different rooting methods on ramet productivity and cutting rootstrike in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrids

3.1 Abstract

The deployment of hybrids as rooted cuttings allows for both additive and non-additive variance of superior genotypes to be captured in commercial plantations. Although the use of rooted cuttings is usually the most affordable cloning method, the productivity of different programmes is highly variable. The aim of this analysis was to examine the effect of nursery conditions, protocols and practices at two commercial nurseries in KwaZulu-Natal, South Africa on the growth of clonal hedges and rooting performance of their cuttings from *Eucalyptus grandis* x *E. longirostrata* clones. Large differences in hedge growth and root strike were observed between the nurseries, the relationship between hedge productivity and rooting ability is reported on, and their implications on the propagation methods are discussed.

3.2 Introduction

Increased yield gains are realised in eucalypts when superior genotypes are deployed as clones (Ruaud, 1999). The deployment of superior genotypes, particularly interspecific hybrids, as clones have several advantages over seedlings. In addition to the capture of both additive and non-additive effects, the increased uniformity of clones is highly desirable for silvicultural practices.

Different methods exist to clonally propagate eucalypts, but the induced rooting of adventitious stem cuttings is by far the most widely used (Eldridge *et al*., 1993). Although the basic principles are similar, there is variation in the protocols, equipment and practices employed by nurserymen. Amongst different propagation operations, both research and commercial, variation between the sources, conditioning, chemical treatment, rooting material and raising conditions of the cuttings exist (Venkatesh, 1986).

The present paper examines the rooting success levels of cuttings at two commercial nurseries in KwaZulu-Natal, South Africa, the different protocols they follow and the effects these have on their productivity.

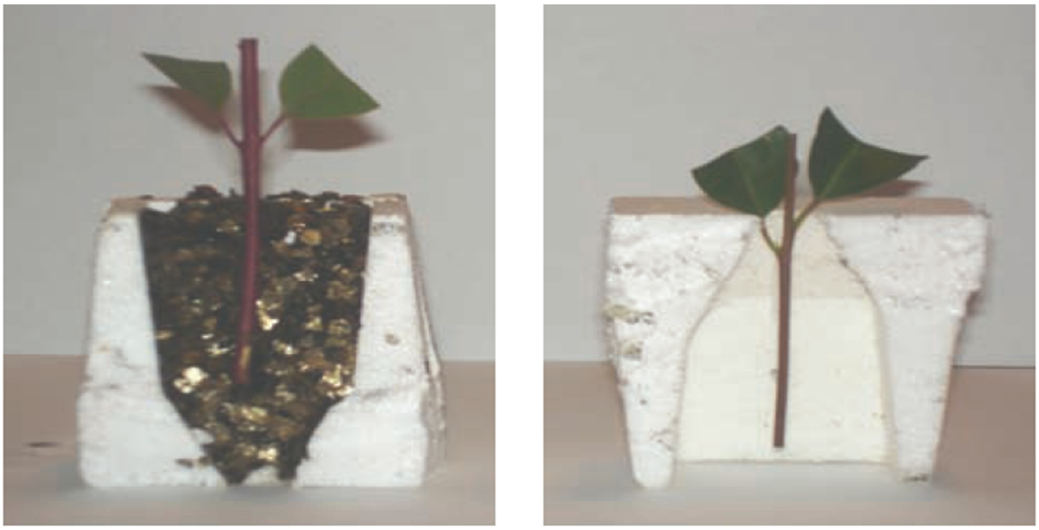
3.3 Materials and Methods

Genetic material

The genetic material consisted of full-sib *E. grandis* x *E. longirostrata* families. It is discussed elsewhere (Chapter 2).

Vegetative propagation

The harvesting of cuttings was as described in Chapter 2. The different practices employed at Nursery A (Traditional) and Nursery B (Aeroponic) are listed in Table 3.1 and the different rooting methods are shown in Figure 3.1. At both nurseries cuttings were misted in rooting tunnels for 30-40 days and heated during cold weather.



Nursery A **Nursery B**

Figure 3.1 Different rooting methods were employed at both nurseries; cuttings were dipped in IBA and placed in perlite/vermiculite/coir media (Traditional) at Nursery A, and not treated with IBA and suspended in the air (Aeroponic) at Nursery B.

Table 3.1 **The different practices in the production of clones employed at both nurseries**

Nursery Conditions		Nursery A	Nursery B
Hedges	Growing area	Tunnel	Tunnel
	Media	Perlite/vermiculite/coir	Composted pine bark
	Shading	± 20 %	± 20 %
	Water	200 ml/ ramet/ week	On demand
	Fertilizer	1000uc NPK (3:1:3)/ weekly	500uc NPK (3:1:5)/ daily
Cuttings	Rooting area	Tunnel	Tunnel
	Chemicals	0.6% (w/w) IBA	None
	Media	Perlite/vermiculite/coir	Air
	Heating	Floors > 15℃	28-30℃
	Misting	Netafilm coolnet mister	
	Misting freq.	10 seconds/ 5 minutes	On demand
	Humidity	80-90%	~100%

Observations and measurements

These followed the same procedures as documented in Chapter 2. Hedge productivity was measured as the number of cuttings harvested per ramet per batch. Cuttings were assessed for root strike after 3 to 3.5 months. A cutting was considered to be ‘rooted’, if it had at least one root longer than 1mm. Rooting was recorded as the percentage of cuttings rooted per ramet, provided that more than ten cuttings were made.

Data Analysis

Analyses were conducted in SAS® for Windows ver. 9.1 (SAS, 2003). Statistical analysis was conducted on log-transformed data. An analysis of variance was performed on the unbalanced data using Type III sums of squares method of the generalised linear model procedure, after correction for fixed batch (replicate) effects. The following statistical model was used:

$$y_{ijk} = \mu + n_i + e_{ijk}$$

where:

μ is the overall mean

n_i is the effect of nursery i;

e_{ijk} is the error

In these analyses, nursery was treated as the random effect.

Pearson correlation coefficients were used to determine the strength of the relationship between rooting ability and growth.

3.4 Results

Effects of nursery conditions on rooting

Mean rooting for each nursery is presented in Figure 3.2. An analysis of variance was performed to determine whether there were significant differences in the rooting ability of adventitious cuttings between the two nurseries (Table 3.2).

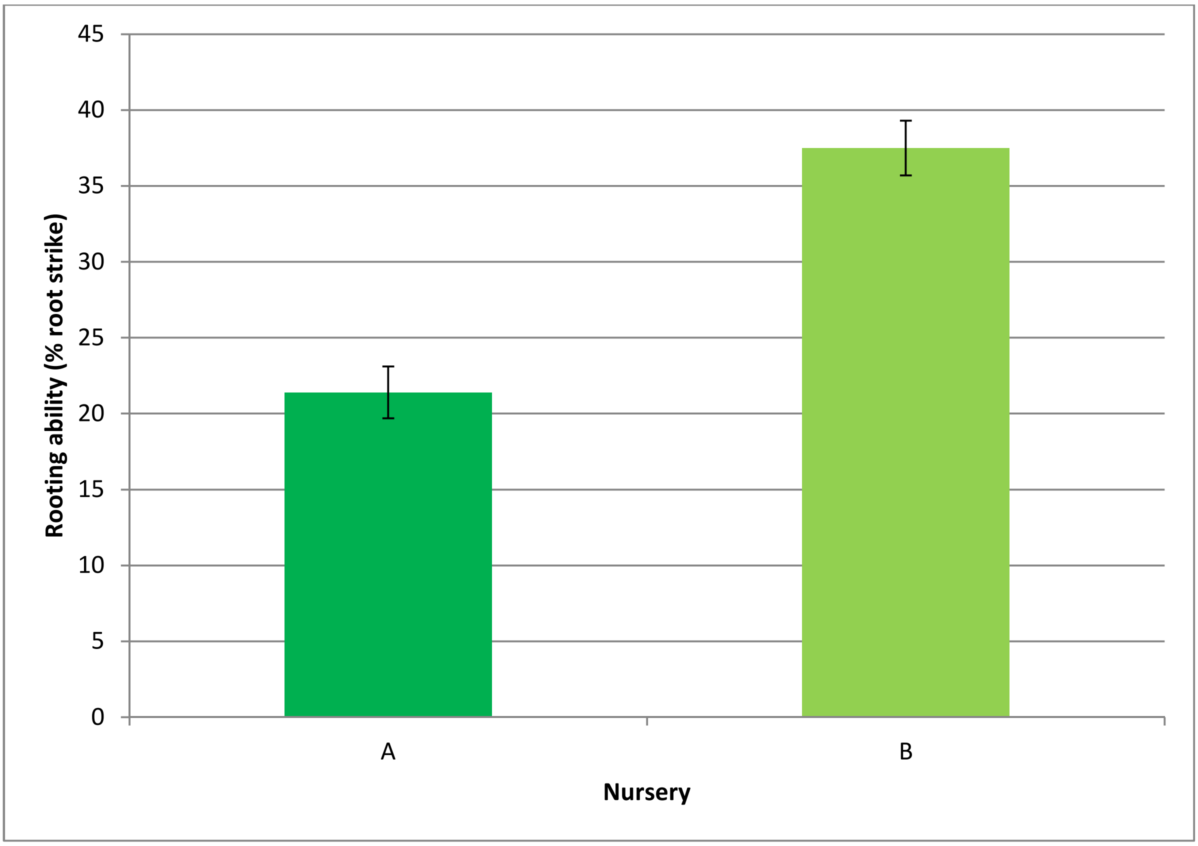


Figure 3.2 **Comparison of mean rooting (with standard error bars) between *E. grandis* x *E. longirostrata* hybrid cuttings at two different nurseries over a period of 320 days**

Mean rooting at Nursery B was much higher than at Nursery A.

Table 3.2 **Analysis of Variance for log transformed root strike percentage of cuttings from hedges at nurseries**

Source	Degrees of Freedom	Sum of Squares#	Mean Square	F value
Nursery	1	32.30	32.30	48.01***
Error	231	155.44	0.67	

* $p<0.05$ ** $p<0.01$ ***$p<0.0001$ # Type III Sum of Squares

Root strike between the two nurseries was significantly different.

Effects of nursery conditions on ramet growth

Mean growth (number of cuttings per ramet per harvest) for each nursery is presented in Figure 3.3. An analysis of variance was performed to determine whether there were significant differences in the rooting between the two nurseries (Table 3.3).

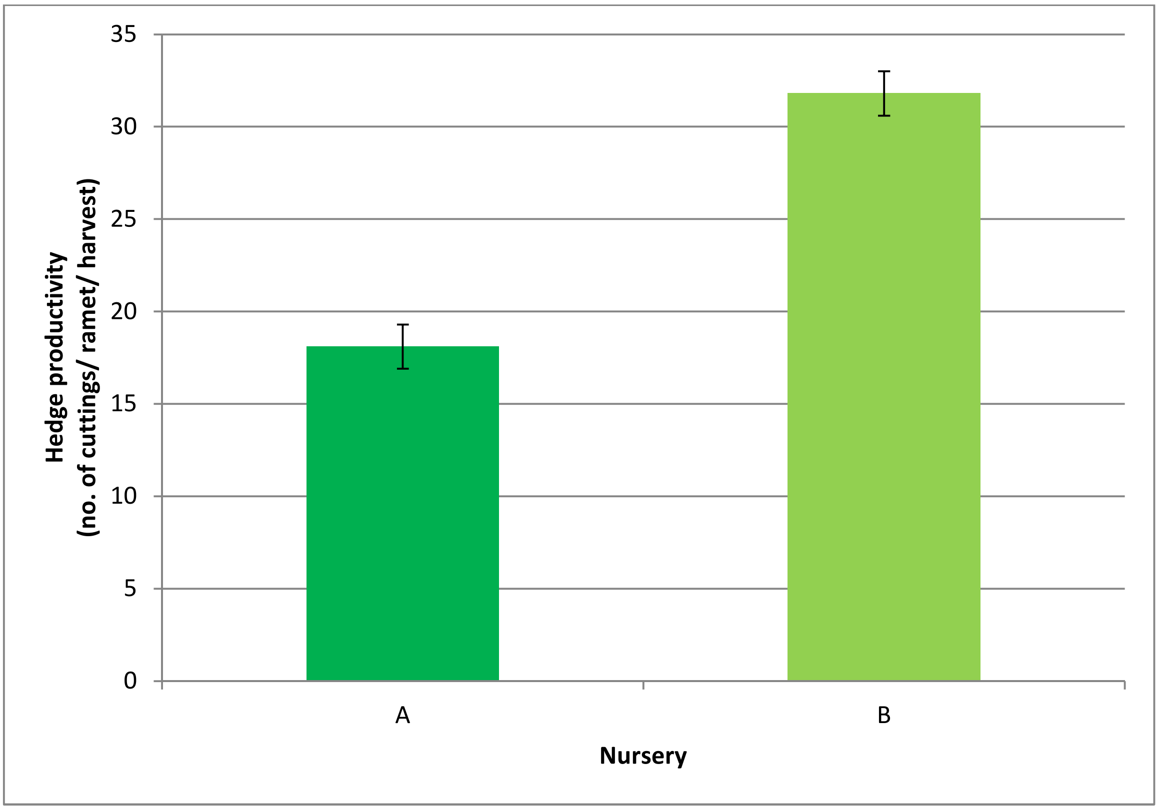


Figure 3.3 **Comparison of mean hedge productivity (with standard error bars) of *E. grandis* x *E. longirostrata* hybrids at different nurseries over a period of 320 days**

Mean productivity at Nursery B was much higher than at Nursery A.

Table 3.3 **Analysis of Variance for hedge productivity (mean no. of cuttings/ ramet/ harvest) between nurseries**

Source	Degrees of Freedom	Sum of Squares#	Mean Square	F value
Nursery	1	11266.29	11266.29	63.53***
Error	239	42386.46	177.35	

* p<0.05 ** p<0.01 ***p<0.0001 # Type III Sum of Squares

Hedge productivity between nurseries was significantly different.

Relationship between ramet growth and rooting ability of adventitious cuttings

The Pearson correlation coefficient between the number of cuttings harvested and the percentage rooted was -0.03 (p=0.56), i.e, it was non-significant.

3.5 Discussion

Significant differences in rooting ability were observed between nurseries (p<0.0001) with a mean rooting across batches of 21% at Nursery A and 38% at Nursery B. The large difference observed between these two nurseries is of a magnitude that could determine the economic feasibility of the clonal deployment of the germplasm used in this study, at each nursery. Although Nursery A treated cuttings with IBA, an auxin which has been shown to increase root-strike in eucalypt cuttings (Blakesley, 1994; Corrêa and Fett-Neto, 2004; Fogaça and Fett-Neto, 2005), Nursery B was almost twice as successful. Thus the much better performance at Nursery B can be attributed specifically to the aeroponic system. Soffer and Burger (1988) showed that aeroponic rooting of cuttings allows more dissolved oxygen at the stem surface-to-water interface than traditional rooting in media, thereby promoting oxygen-intensive respiration required for root initiation and growth. The large effect that the aeroponic technique at Nursery B had on rooting success must have eclipsed any beneficial effects of the auxin applied at Nursery A.

Significant differences were observed in hedge productivity between nurseries ($p<0.0001$) with means and 18 and 32 cuttings per ramet per harvest for Nursery A and B respectively. This large difference in productivity, just as rooting ability, can affect the feasibility of clonal deployment. Because ramets were received in similar condition at both nurseries, the differences observed between the nurseries, can probably be attributed to the specific growing medium, watering, fertilisation and pesticide application regimes as well as temperature, light, humidity within the greenhouses and hedge management regimes however these were not quantified but should be in further work.

The Pearson correlation coefficient of -0.03 showed no significant ($p=0.56$) relationship between hedge productivity and rooting ability. While this result is not advantageous, it implies no penalty in breeding for growth on rooting.

For research purposes, as in the ranking of clones for rooting ability, the aeroponic system is highly desirable due to the facility to non-destructively assess root-strike of cuttings (Gaudin *et al.,* 2008). This study has clearly shown a difference in rooted cutting productivity between traditional and aeroponic rooting methods. While the traditional method does work, and the medium acts as a buffer against drying, the higher dissolved oxygen available to aeroponically rooted cuttings and the resultant enhancement of rooting of cuttings show the aeroponic system of Nursery B to be a superior technology.

Further research is needed to identify the specific factors within the aeroponic rooting protocol that make it superior. The potential value of IBA used in this system could also be investigated.

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Chapter Four: Root-strike success of cuttings harvested from hedges and coppice stumps of *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrids

4.1 Abstract

The source of adventitious cuttings in clonal deployment programmes plays a major role in their rooting success. Factors such as cyclophysis and topophysis can have detrimental effects on the rooting ability of adventitious cuttings. Adventitious cuttings are most often derived from hedges or coppiced stumps. Both these sources have different strengths and weaknesses with regards to their use in different breeding strategies. The aim of this study was to determine whether there was a difference in the rooting ability of cuttings sourced from hedges and coppiced stumps. *Eucalyptus grandis* x *E. longirostrata* cuttings were sourced from hedges and coppiced stumps and rooted at two different nurseries. The differences in root-strike between rooting from the different sources were not significantly different (p>0.05). Since both methods for producing shoots for cuttings are to be equally productive, breeders can choose which form of cuttings material to employ, based on their practical applicability, without suffering any rooting success penalty for the 'clone first' strategy.

4.2 Introduction

The vegetative propagation of eucalypts as rooted cuttings has allowed breeders to capture the performance gains of superior genotypes for commercial deployment (Ruaud, 1999). While rooted cuttings are usually more expensive to produce than open pollinated seed, provided the rooting ability of a genotype is high enough, deployment of clones in this manner can be cost competitive with seed from controlled pollinations.

The rooting ability of adventitious cuttings is known to be under strong genetic control (Borralho and Wilson, 1994), but is also affected by temporary environmental effects (Lemos *et al*., 1997). Furthermore, the source of the cutting (the ortet) can also play a major role in the rooting success (Eldridge *et al*., 1993) and can be treated as a permanent environmental effect.

Many conditions affecting the ortet can influence the rooting success of eucalypt cuttings (Eldridge *et al*., 1993), including cyclophysis, when cuttings are taken from an older part of the ortet, and topophysis, where the position of the cutting in the crown can influence the rooting ability (Olesen, 1978; Wilson, 1993), primarily due to auxin concentration variation in the ortet (Fogaça and Fett-Neto, 2005).

There are many different sources of adventitious cuttings in eucalypts. The most common sources are coppiced stumps and hedge plants. Mature superior trees can often be felled and the coppice shoots collected to be rooted. The growing of seedlings or clones in hedges can also provide a source of cuttings (Bachelard and Stowe, 1963).

Conventional hybrid deployment programmes involve the coppicing of superior trees, followed by the rooting of the cuttings. Project Pulp is an ongoing research collaboration between NCT[1] and the CSIR[2]. In this project, the goal is to accelerate the commercial deployment of hybrids as clones (Snedden *et al.*, 2007). In addition to conventional coppicing of parental hedges, the researchers have simultaneously grown F_1 seedling-derived hedges, in order to produce an alternative source of cuttings.

The aim of this study was to determine whether there was any difference in the rooting ability of *Eucalyptus grandis* x *E. longirostrata* cuttings sourced from hedges versus coppiced stumps.

4.3 Materials and Methods

Genetic material

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[1] NCT (Pty) Ltd, Pietermaritzburg, South Africa.

[2] Council for Scientific and Industrial Research, Pretoria, South Africa

The genetic material consisted of full-sib *E. grandis* x *E. longirostrata* families. Some seedlings were bulked up by rooting vegetative cuttings at two commercial nurseries and the remainder were planted as an unreplicated block planting in Pietermaritzburg, KwaZulu-Natal. Five full-sib families, each consisting of five individuals, were selected for planting as hedges. Four ramets per clone were planted as hedges in a randomized complete block design at both nurseries (Hedge Trial) (Figure 4.1a). The ramets were planted in a perlite/vermiculite/coir medium at Nursery A, and in composted pine bark at Nursery B. The block planting was felled at the age of 26 months, leaving stumps with heights of 150 to 200 mm. The stumps of the felled trees were allowed to coppice to facilitate the collection of material for cuttings. Coppiced stumps were treated with capsaicin to repel foraging wild animals. From this trial, five families, each represented by ten trees, were selected for the coppice rooting investigation (Coppice Trial) (Figure 4.1b).

Figure 4.1 **Cuttings from (a) hedges and (b) coppiced stumps were evaluated for rooting ability**

Vegetative propagation

The production and harvesting of cuttings from hedges was as described in Chapter 2. Coppice shoots were collected from stumps at 176 days (Nursery A) and 225 days (Nursery B) after felling, and rooted. Nursery B was affected by a field staff shortage following the harvest, resulting in the harvested shoots coppice not being cut or set for as much as 72 hours. Because of the increased stress on those cuttings, the harvest rooted at Nursery B was not used in this analysis.

Observations and measurements

These followed the same procedures as documented in Chapter 2. Hedge productivity was measured as the number of cuttings harvested per ramet per batch. Cuttings were assessed for root strike after 3 to 3.5 months. A cutting was considered to be ‘rooted’, if it had at least one root longer than 1mm. Rooting was recorded as the percentage of cuttings rooted per ramet, provided that more than ten cuttings were made.

Data Analysis

Analyses were conducted in SAS® for Windows ver. 9.1 (SAS, 2003). Statistical analysis was conducted on log-transformed data. An analysis of variance was performed on the unbalanced data using Type III sums of squares method of the generalised linear models, after correction for fixed nursery and batch (replicate) effects. The following statistical model was used:

$$y_{ijk} = \mu + s_i + e_{ijk}$$

where:

μ	is the overall mean
s_i	is the effect of cutting source i;
e_{ijk}	is the error

In this analysis, the cutting source was treated as a random effect.

4.4 Results

An analysis of variance was performed to determine whether there were significant differences in the rooting ability of adventitious cuttings from hedges grown in tunnels and coppiced stumps of mature trees (Table 4.1) after correcting for fixed batch effects (Chapter 2). Mean rooting of cuttings from hedges and coppice, rooted at Nursery A is presented in Table 4.3 and Figure 4.2.

Table 4.1 Analysis of Variance for log transformed root strike percentage of cuttings at Nursery A from different sources

Source	Degrees of Freedom	Sum of Squares#	Mean Square	F value
Cutting source	1	0.86	0.86	0.91^{NS}
Error	186	176.42	0.95	

* $p<0.05$ ** $p<0.01$ ***$p<0.0001$ $^{NS}p>0.05$ # Type III Sum of Squares

The percentage of root strike of cuttings from coppiced stumps and hedges was not significantly different.

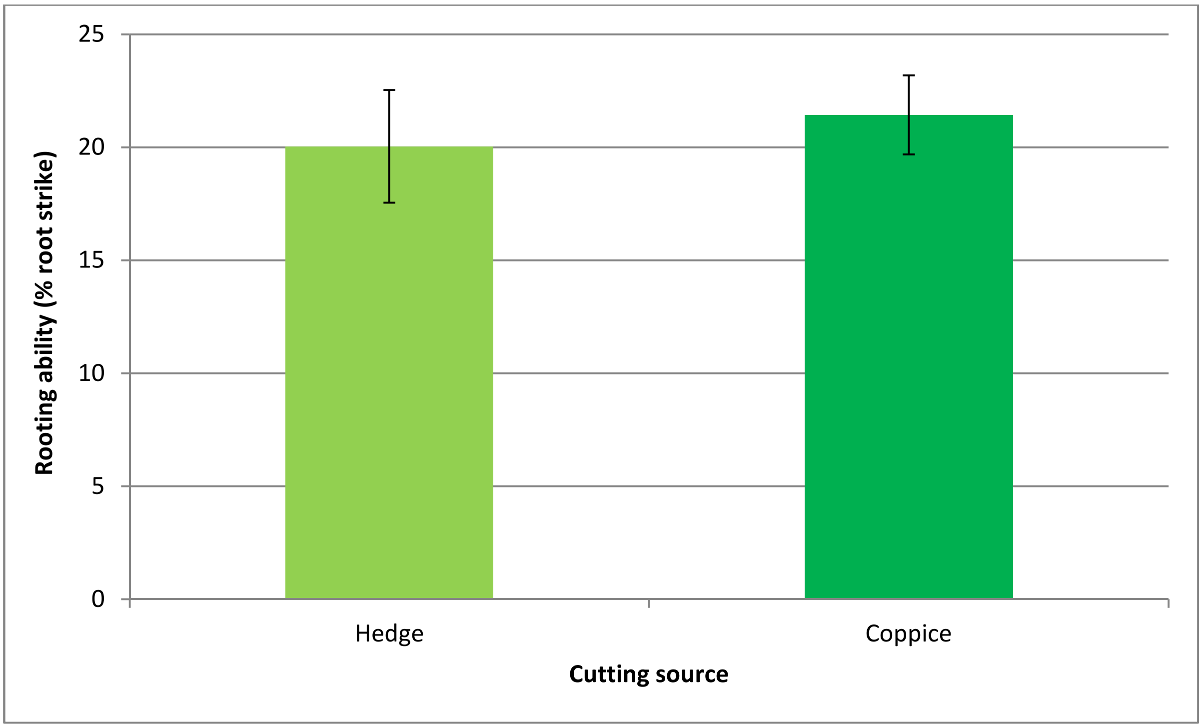

Figure 4.2 Comparison of mean rooting (with standard error bars) between cuttings made from coppice or hedge cuttings at Nursery A

4.5 Discussion

The mean rooting of cuttings harvested from the coppiced stumps at Nursery A at 176 days after felling was 20.0% (S.E. 2.5%) (Figure 4.2). The low rooting observed in the first flush of coppice is expected because many authors, including Eldridge *et al*. (1993), have noted poor rooting success of cuttings taken from the first flush of tissue taken from coppiced trees.

No significant differences ($p>0.05$) were detected in the level of successful rooting of adventitious cuttings shoots harvested from between hedges and the coppiced stumps at Nursery A. The rooting success of cuttings at Nursery A was very similar between those from the hedges and coppice (1.4% difference). This study indicates that the success of rooting of adventitious cuttings of both hedges and coppiced stumps in E*. grandis* x *E. longirostrata* is similar. However, only two flushes from the stumps were rooted, and previous work (e.g. Copes, 1992) has shown that rooting decrease after the first few flushes. Both sources of cuttings, hedges and coppices, have both advantages and disadvantages. Seedling derived hedges allow for multiple harvests, but the germplasm is often untested. Coppicing stumps to produce clones allows for superior trees to be selected, but the genotype can be lost if the stump does not coppice.

Since both methods to producing shoots for cuttings seem to be equally productive, breeders can decide on which cutting sources to use, based on their applicability to their breeding strategies, without suffering any rooting success penalty for the ‘clone first’ strategy.

Because of the field staff shortages experienced at Nursery B, further research will have to reattempt to quantify the differences in rooting success between coppice flushes using the aeroponics technology. This approach is so different that different results might be found.

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Chapter Five: Seasonal effects on root-strike in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrids

5.1 Abstract

The effects of temperature during harvest, and seasonal effects on the root-strike of cuttings, were examined in a study on *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrid clones, periodically harvested from ramets of 25 clones over a period of nine months and rooted at two commercial nurseries in KwaZulu-Natal, South Africa. The temperature during harvesting had a significant effect ($p<0.0001$) on root-strike, with reduced performance when cuttings were harvested at temperatures above 32°C. Seasonally, hedge productivity increased during the warmer months. Root-strike varied greatly, depending on the prevailing conditions when the cuttings were set. Different nursery practices are needed during the warmer months to mitigate some of the adverse seasonal effects.

5.2 Introduction

Interspecific hybrids allow tree breeders to combine the desirable traits of different species. Deployment of superior hybrid genotypes often allow for increased genetic gains (Zobel and Talbert, 1984). The most common method of vegetative propagation for commercial deployment is as rooted cuttings (Venkatesh, 1986; de Assis, 2001). However, the rooting ability of a species, hybrid type and genotype are often the limiting factors affecting the usefulness of this reproductive method (Thirunavoukkarasu and Gurumurti, 1998).

Rooting ability is a complex trait, influenced significantly by both genetic (Tibbets *et al*., 1997; Puri and Swamy, 1999; Borralho and Wilson, 1994) and environmental (Stape *et al*., 2001; Metaxas *et al*., 2008) effects. The effects of genetic factors (Chapter 2), nursery practices (Chapter 3) and source of cuttings (Chapter 4) have been discussed previously. However, both temperature and season also have an important role in survival and rooting success of cuttings (de Assis, 2001; Kibbler *et al*., 2004).

The aim of this investigation was to determine the effect of temperature during harvesting, and the effect of season during the setting period on the root-strike in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrids.

5.3 Materials and Methods

Genetic material

The genetic material consisted of full-sib *E. grandis* x *E. longirostrata* families. It is discussed elsewhere (Chapter 2).

Vegetative propagation

The harvesting of cuttings was as described in detail in Chapter 2. Nursery A used a traditional approach of growing cuttings in a pine bark medium, whereas Nursery B employed an aeroponics technology. At both nurseries cuttings were misted in the rooting tunnels for 30-40 days, and were heated during cold weather.

Observations and measurements

These followed the same procedures as documented in Chapter 2. Hedge productivity was measured as the number of cuttings harvested per ramet per batch. Cuttings were assessed for root strike after 3 to 3.5 months. A cutting was considered to be ‘rooted’, if it had at least one root longer than 1mm. Rooting was recorded as the percentage of cuttings rooted per ramet, provided that more than ten cuttings were made.

Temperature at the time of harvest and mean monthly maximum and minimum temperatures were obtained from the South African Weather Service (Weather station No. 685810). The temperatures at time of harvest were grouped into five different temperature classes.

Data analysis

Analyses were conducted in SAS® for Windows ver. 9.1 (SAS, 2003). Statistical analysis was conducted on log-transformed data. An analysis of variance was performed on the unbalanced data using Type III sums of squares method of the generalised linear models, after correction for fixed site (nursery) and clone effects.

The following statistical model was used:

$$y_{ijk} = \mu + t_i + e_{ijk}$$

where:

μ is the overall mean

t_i is the effect of temperature class *i*;

e_{ijk} is the error

In this analysis, temperature classes were treated as random effects.

The least significant differences (LSD) multiple comparison test (α=0.05) was used to detect significant differences in rooting success between clones (Fisher, 1935).

5.4 Results

The effect of temperature at time of harvesting on root-strike

An analysis of variance (ANOVA) was performed to determine effect of temperature at time of harvest on rooting for both nurseries, after correcting for clone (significant fixed) effects (Table 5.1 and 5.2). The mean rooting ability across the different temperature ranges is presented in Table 5.3.

Table 5.1 **Analysis of Variance for log transformed root strike percentage of cuttings harvested at different temperature ranges at Nursery A**

Source	Degrees of Freedom	Sum of Squares$^{\#}$	Mean Square	F value
Temperature	3	1.51	0.50	1.04^{NS}
Error	105	50.86	0.48	

* $p<0.05$ ** $p<0.01$ $^{NS}p>0.05$ # Type III Sum of Squares

The mean rooting for cuttings harvested at different temperature ranges was not significantly different at Nursery A.

Table 5.2 **Analysis of Variance for log transformed root strike percentage of cuttings harvested at different temperature ranges at Nursery B**

Source	Degrees of Freedom	Sum of Squares$^{\#}$	Mean Square	F value
Temperature	3	5.66	1.89	7.21**
Error	115	30.12	0.26	

* $p<0.05$ ** $p<0.01$ ***$p<0.0001$ # Type III Sum of Squares

The mean rooting for cuttings harvested at different temperature ranges was significantly different at Nursery B.

Table 5.2 Mean rooting ability (% root strike) of cuttings harvested different temperatures at two different nurseries

Temperature (℃)	Rooting ability (% rootstrike)							
	Nursery A				Nursery B			
	Mean	Std Err	n	LSD*	Mean	Std Err	n	LSD*
16-19	24.3	3.3	14	a	45.7		1	a
20-23	19.2	1.9	50	a b	36.5	1.8	75	a
24-27	20.8	1.9	50	a b	49.4	3.2	24	a
28-31	34.2	7.7		b				
32-35					25.8	1.9	19	a

*The least significant differences (LSD) multiple comparison test (α=0.05) declares no significant differences between treatments with the same letter.

The percentage of successful root strikes was highest as a result of a harvest temperature range of 28-31°C, but there was a sharp decline as a result of harvesting cuttings at higher temperatures.

Seasonal variation in hedge productivity

Ramet growth was measured from August 2006 to March 2007. Ramet growth, measured indirectly as the number of cuttings per hedge per harvest, at both nurseries, are presented in Figure 5.1.

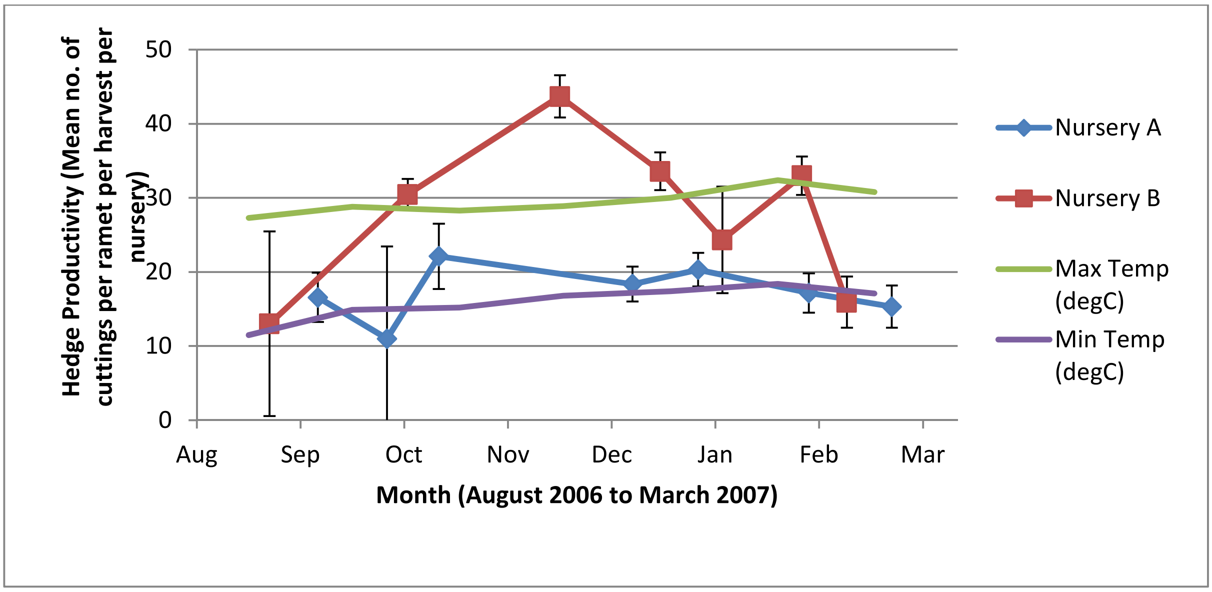

Figure 5.1 **Seasonal effect on hedge productivity (mean no. of cuttings/ hedge/ harvest with standard error bars) of *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrids at two different nurseries over a period of nine months. Mean minimum and maximum monthly temperatures are shown.**

Hedge productivity varied, particularly in early batches, with high standard errors being observed. More variation between batches was observed at Nursery B than Nursery A. Hedge productivity peaked at different times at each nursery.

Seasonal variation in rooting success of cuttings

Root-strike of cuttings was measured from August 2006 to March 2007. Root strike at both nurseries is presented in Figure 5.2.

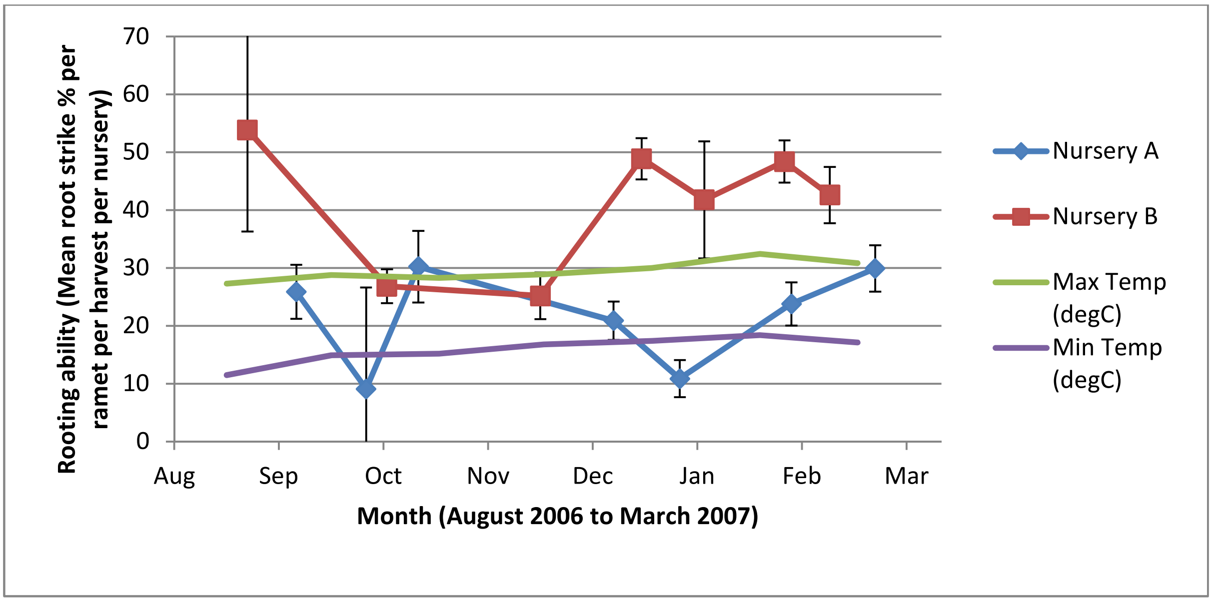

Figure 5.2 **Seasonal effect on rooting (mean root strike (%)/ ramet/ harvest with standard error bars) of *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrid cuttings at two different nurseries over a period of nine months. Mean minimum and maximum monthly temperatures are shown.**

Root strike varied, particularly in early batches, with a high standard error being observed. Root strike peaked at different times at each nursery.

5.5 Discussion

Temperature at time of harvest had a significant ($p<0.0001$) effect on the root-strike of cuttings over the trial period (Table 5.1). Mean root strike was the highest at a harvest temperature range of 28-31°C and was followed by a sharp decline at higher temperatures (Table 5.2). Although too few measurements were available at the peak temperature range to rule out error, it is apparent that temperatures above 32°C affect the rooting ability of cuttings negatively (Seiler, 1998). This decrease in rooting ability may be caused by heat stresses either on the ramet or on the recently harvested cutting, induced by the high temperature (Corréa and Fett-Neto, 2004).Given the limited water content of the cuttings, and their limited ability to absorb water in the absence of roots, their heat stress cannot be relieved by transpiration and evaporative cooling. While both nurseries are careful to harvest cuttings before noon, particularly on warm days, it is unavoidable that the ambient temperature at the time of harvesting plays a major role in rooting success (Corréa and Fett-Neto, 2004). Therefore, harvesting of cuttings should stop when temperatures reach a critical level of >32°C. Cutting viability may be increased by ensuring ramets are well watered before harvesting events.

Hedge productivity patterns changed between seasons at Nursery B but not at Nursery A. Measurements began in late winter and growth of the parental hedges increased in the warmer months. Ramet performance peaked at the beginning of summer and then declined steadily until the end of the investigation in mid-autumn. While these results are in parallel with increased plant metabolism at warmer temperatures, underlying cause of the significant difference ($p<0.001$) between the two nurseries needs to be investigated further.

Root-strike of cuttings varied greatly between seasons. The initially high level of rooting of cuttings observed at the end of winter decreased in spring (Nursery B) and summer (Nursery A). Rooting in cuttings rose again by midsummer (Nursery B) or late summer (Nursery A). The increased rooting was maintained until the study ended in mid autumn. The decline in rooting observed in the warmer months seemed to be inversely related to hedge productivity, but there was no significant correlation ($p>0.05$). The decline in rooting during the warmer months may be due to the increased stress on the cuttings during these periods (Corréa and Fett-Neto, 2004). While the high humidity in the misting tunnels prevents the cuttings from drying out, it limits the evapo-transpiration that is essential to removing heat from the leaf surface of cuttings, thus allowing them to suffer from thermal damage. Manipulating the temperature by shading the growing- and misting- tunnels, or cooling them to a greater extent during warmer periods, should reduce cutting mortality (Benz *et al*., 1996).

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Chapter Six: A note on the measurement of root-strike in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrid cuttings

6.1 Abstract

Breeding or clonal selection for increased rooting ability requires the accurate ranking of families and clones. Due to the many different environmental (permanent and temporary) factors affecting root-strike in eucalypt cuttings, a single phenotypic assessment may not accurately represent a family or clone. This analysis investigated the repeatability of root-strike measurements *of Eucalyptus grandis* x *Eucalyptus longirostrata* cuttings across two different sites over a period of eight months. Repeatability measurements of 0.024 (S.E. 0.031), 0.187 (S.E. 0.067) and 0.340 (S.E. 0.072) were observed for families, clones and ramets, respectively. These low to medium repeatablities suggest that more than one measurement of rooting ability is required to get reliable estimates of a family or clones rooting ability. However, high rank order (0.418-0.666) and phenotypic correlations (0.564-0.959) within each site show that clones can be ranked reliably with just a few assessments.

6.2 Introduction

While the clonal deployment of superior genotypes can translate into higher yields in forest plantations, the higher cost of the production of cuttings compared to seedlings can deter their use. Increasing rooting ability can improve the economics of the clonal deployment of eucalypt cuttings (Zobel and Talbert, 1984). Due to the importance of the trait, the inclusion of rooting ability as a selection trait in difficult-to-root species and hybrid types, may allow for significant savings on the cost of reproducing them.

It has been shown that rooting ability, while under strong genetic control (Borralho and Wilson, 1994; Lemos *et al*., 1997), is also significantly affected by temporary (Chapter 5; Corrêa and Fett-Neto, 2004) and permanent environmental (Chapters 3 and 4; Eldridge *et al*., 1993; Fogaça and Fett-Neto, 2005) effects. Thus, while rooting ability can be improved through breeding, the usefulness of rooting ability as a selection trait is also dependant on the accuracy of the phenotypic assessment of families and clones.

The aim of this analysis was to estimate the reliability of ranking families and clones on rooting ability.

6.3 Materials and Methods

Genetic material

The genetic material consisted of full-sib *E. grandis* x *E. longirostrata* families. It is discussed elsewhere (Chapter 2).

Vegetative propagation

Cuttings were made periodically in batches when hedges were ready for harvesting (selective harvests), which ranged from 15-44 days, harvested over a period of nine months at both nurseries. Cuttings were rooted at two commercial nurseries in KwaZulu-Natal, using two different rooting technologies (conventional and aeroponics) (Chapter 3).

Observations and measurements

These followed the same procedures as documented in Chapter 2. Hedge productivity was measured as the number of cuttings harvested per ramet per batch. Cuttings were assessed for root strike after 3 to 3.5 months. A cutting was considered to be ‘rooted’, if it had at least one root longer than 1mm. Rooting was recorded as the percentage of cuttings rooted per ramet, provided that more than ten cuttings were made.

Data analysis

Analyses were conducted in SAS® for Windows ver. 9.1 (SAS, 2003).

Repeatability of rooting was calculated by family, clone and ramet, as per Becker (1992), with each batch being regarded as a repeated measurement:

$$R = \frac{\sigma_w^2}{\sigma_w^2 + \sigma_e^2}$$

Where: $\sigma_e^2 = MS_e$

$$\sigma_w^2 = \frac{MS_w - MS_e}{k_1}$$

MS_e	Is the mean sum of squares for repeated measurements within individual
MS_w	is the mean sum of square between individuals
k_1	is the number of repeated measurements

The Spearman rank-order correlation was used to compare the ranking of clones by rooting ability across different batches of cuttings. If the ranks of the values of the two variables are given by *r* and *s*, then the Spearman correlation coefficient is given by the following equation from Snedecor and Cochran (1989):

$$\theta = \frac{\sum (r_i - \bar{r})(s_j - \bar{s})}{\sqrt{\sum (r_i - \bar{r})^2 \sum (s_j - \bar{s})^2}}$$

Where:

θ	is the rank correlation between sites
r_i	is the rank of the ith family
$\bar{r}$	is the rank mean at the first site
s_j	is the rank of the jth family
$\bar{s}$	is the rank mean at the second site

6.4 Results

The repeatability estimates for root strike at the family, clone and ramet level are presented in Table 6.1. The Spearman rank order correlation coefficient between both nurseries was 0.52 and the phenotypic correlation was 0.34. The Spearman rank order correlation coefficients between each batch and the mean performance within each nursery is presented in Table 6.2.

Table 6.1 **Repeatability estimates for root strike of family, clone and ramets in *Eucalyptus grandis* x *Eucalyptus longirostrata* hybrid cuttings**

Level	Repeatability	Standard Error
Family	0.024	0.031
Clone	0.187	0.067
Ramet	0.340	0.072

Repeatability estimates of root strike ranged from very low at the family level to moderate at the ramet level.

Table 6.2 **Spearman rank order and phenotypic correlation coefficients between each batch and the mean clone performance at each nursery**

Nursery	Batch	r (batch-nursery mean)	
		Rank	Phenotype
A	1	0.666	0.959
	2	-	-
	3	-	-
	4	-	-
	5	0.511	0.576
	6	0.547	0.596
	7	0.641	0.714
	8	0.596	0.786
B	9	-	-
	10	0.548	0.564
	11	0.773	0.847
	12	0.884	0.793
	13	-	-
	14	0.610	0.568
	15	0.418	0.590

'-' insufficient data to calculate correlation coefficient

Moderate to high rank-order and phenotypic correlations between the mean rooting of single batches and the mean rooting across all batches within each nursery were observed.

6.5 Discussion

Repeatability was calculated to measure the degree of agreement between repeated measurements of families, clones and ramets. The low repeatability estimates, particularly at the family level, indicate that several repeated measurements are required to accurately assess rooting ability. These results concur with those of Shepherd *et al*. (2005), who noted low repeatability in studies of hybrid pine rooting during the first year after hedge establishment. Technical inexperience in raising micro-cuttings may have also contributed to the low repeatability estimates.

Spearman's rank order coefficient tests for broad agreement between ranks (Snedecor and Cochran, 1989). The moderate rank order correlation coefficient of 0.52 and the low phenotypic correlation of 0.34 suggest that, in addition to the nursery effect discussed previously (Chapter 3), there may be a significant genotype-by-nursery interaction. While these results differ from those of Borralho and Wilson (1994) and Lemos *et al.* (1997), who worked on *Eucalyptus globulus,* and Tibbits *et al*. (1997), who used *E. nitens*. Both of these parties reported low levels of genotype-by-test interactions. However, the different results reported here can be attributed to the larger variability in testing conditions because this experiment used very different rooting protocols, and there was greater genetic diversity in the interspecific hybrid use here, compared to the pure species used in the previous studies.

At Nursery A, the rank order correlation coefficients ranged from 0.511 to 0.666, and the phenotypic correlation coefficients ranged from 0.576 to 0.959. At Nursery B, the rank order correlation coefficients ranged from 0.418 to 0.884, and the phenotypic correlation coefficients ranged from 0.564 to 0.847. Since rank correlations do not consider actual family performance, the implications of a significant result have to be considered carefully (Matheson and Raymond, 1984). However, most importantly, they do show that a selection index can be reliable.

The moderate to high correlations observed between clone performance per batch and the mean performance for all batches at each nursery, considering that the different batches tested the clones multiple times, over a significant time frame and range of seasons, suggest that clones can be reliably ranked on rooting ability with relatively few (three to four) rooting experiments.

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Thesis Overview

Eucalyptus grandis x *E. longirostrata* is a promising interspecies cross in the genus *Eucalyptus*. However, as with most hybrids, their production through controlled pollinations is expensive. While deployment of hybrids as rooted cuttings allows for the capture of additive and non-additive genetic variation of superior genotypes, poor rooting can invalidate the perceived cost competitiveness of clones. In a study on this new hybrid, the CSIR and an industry partner, NCT, attempted to quantify the influences of genetic and the environmental effects on the rooting success of cuttings.

Root-strike was shown to be moderately heritable, allowing for its inclusion as a selection trait to improve productivity. Rooting cuttings aeroponically is far more productive than the traditional method of rooting cuttings in media, enough to justify the higher infrastructural costs. There appears to be no correlation between hedge productivity and rooting of cuttings, such that both traits can be selected for concurrently. Cuttings from seedling hedges and coppiced stumps had similar rooting success, meaning that there is no rooting penalty to the “clone-first” strategy. Rooting success of cuttings vary between seasons and is affected by temperature. Clones can be reliably ranked, based on just a few rooting trials.

Based on this study, some recommendations can be made to improve root-strike in shoot cuttings. While overall root-strike can be improved by its inclusion as a selection trait, other traits that affect yield play a much larger role in the entire value chain, to justify selection for rooting to dilute their gains. For new and existing production facilities, aeroponic rooting systems are a far superior choice and worth the extra investment and management required. If sufficient heat mitigation through preclusion or extraction, in misting tunnels is unavailable, it may be necessary to suspend the production of cuttings during periods of excessively high temperatures.

Further research could address research questions beyond the scope of this study. Comparing the rooting of the hybrids to their parents may determine the inheritance patterns. Studying a larger number of families would allow for the estimation of narrow-sense heritabilities. Determining the optimal operating temperatures or temperature ranges in misting tunnels for the rooting of cuttings would also be valuable. This study raised a genotype x environment interaction concern which needs to be addressed. Rooting protocols could to be optimised for specific species and hybrid types.