Investigation of rootstocks for seed production in *Eucalyptus nitens*

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THESIS ABSTRACT

Rootstock age and size, combined with nutrients, temperature, and humidity are major factors thought to play an important role in grafting success in eucalypts. Research was conducted on the grafting success of interspecies and sub-species rootstocks, and three scions were chosen for their differential flowering ability, combined with six different rootstocks, chosen for their precocity of flowering, or as representatives of *Eucalyptus nitens* by itself or in a hybrid. The agro-meteorological conditions found inside the greenhouse tunnel were monitored and grafted plants were placed in three positions at different distances from the wet wall. The grafting environment was optimized by controlling the temperature and humidity of the greenhouse tunnel to achieve optimum grafting conditions. Overall, 44% of the grafted plants survived. The best rootstock host for grafting *E. nitens* scion was the hybrid with a strong *E. nitens* appearance (R5) at 67% survival rate. The two grafting periods had no significant effect on the grafting success, nor did the position of the plants in the greenhouse tunnel.

For flower initiation, environmental factors such as day length (light and dark periods), temperature, and topography may affect the flowering characteristics of *E. nitens*, factors which are often geographically specific. Research was also conducted on the use of interspecies and sub-species rootstocks for early flowering in *E. nitens* and to monitor the impact of light, cold temperatures (via chilling units) and site location on floral induction. Six rootstocks were selected from *Eucalyptus* taxa and provenances, including three species and two hybrids. Three scions were selected from three *Eucalyptus* provenances: Tallaganda, Barren Mountain, and Barrington Tops. Exposed sites with good air drainage and low winter day/night temperature amplitudes were considered ‘good flowering sites’. Of the six rootstocks selected, amongst the ungrafted trees, the hybrid with a strong *E. nitens* appearance (R5) had the highest budding percentage while amongst the grafted trees, the hybrid rootstock with a strong *E. grandis* appearance (R2) induced the highest budding percentage on the *E. nitens* scions. This study provides further insight into the selection of rootstocks and site conditions that yielded good flowering results within the subtropical climatic conditions found in South Africa.

Overall, the study showed that grafting on to dwarfing and precocious rootstocks, as well as suitable planting sites are effective in inducing early flowering in *E. nitens*. The optimized methods developed in this study will be important for *E. nitens* breeding and future flowering research.

DECLARATION

I, declare that:

(i) The research reported in this thesis, except where otherwise indicated, is my original research.

(ii) This thesis has not been submitted for any degree or examination at any other university.

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GENERAL INTRODUCTION

Importance of early flowering trees

Eucalyptus nitens is a cold tolerant Eucalyptus species that grows in high altitude areas of South Africa. It is endemic to Australia in areas where the mean maximum and minimum temperatures of the hottest and coolest months are 26°C and -5°C. In South Africa, they are planted in high altitude areas which are considered low productivity sites due to the temperatures and have annual mean temperatures on between 13 – 16.5°C (Gardner and Bertling, 2005).

Eucalyptus nitens is naturally a late flowerer in its native environment - Australia (Moncur and Hasan, 1994), usually taking five to six years before first flowering. In South Africa, time to first flowering is even longer, ten to twelve years, due to the warmer climate (Barrington, 2005). This poses economic issues for seed collectors and plant breeders. Seed collectors are unable to collect an adequate number of good quality seeds for planting every year, and the late and irregular flowering patterns set a lower limit on the rate at which the plant breeder can turn over generations.

In apple production, dwarfing rootstocks have been used to limit excessive growth, increase yield per tree size, increase precocity, and regular flowering (Atkinson and Else, 2001). One of the advantages of using dwarfing rootstocks is that it induces precocious scion flowering. This has aided breeding efforts and increased income for apple producers over the past few decades. We hope the same can be accomplished by *E. nitens* growers and breeders. By reducing the time it takes to first flowering, this will have major economical up rise for the forestry industry in South Africa.

Aim

To reduce the time to first flowering in *Eucalyptus nitens* by using dwarfing and precocious rootstocks.

Objectives

To investigate the usage of interspecies and sub-species rootstocks to induce early flowering in *E. nitens* and to select rootstocks for early flowering in the South African environment.

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CHAPTER 1: LITERATURE REVIEW

1.1. The development of manipulated forest seed orchards

The relatively long time demanded to accomplish breeding cycles or generation turnover (selection, testing, recombination and multiplication or delivering) in *E. nitens*, has been one of the main reasons manipulated flowering research is important. Manipulated flowering research is a way to increase the speed of breeding programs by allowing breeding and reforestation programs to identify genetically superior individuals and breed them more quickly (Philipson, 1990). It allows for the production of small quantities of high quality control-pollinated seeds for commercial mass vegetative propagations of hybrid trees. Greater efforts have been directed to them, in many cases than those dedicated to the development of classical breeding programs (Moncur, 1998).

1.2. Cultural techniques for orchard management

Cultural techniques for orchard management have always been used for increased flowering, increased seed production and better harvesting. A well-managed orchard is generally in a better competitive situation when cultural practices such as pruning, girdling, root restriction, grafting, and chemical manipulation are carried out efficiently.

1.2.1. Physical Constraints (manipulation)

1.2.1.1. Pruning

The two main objectives of pruning are to improve vegetative growth and reproductive growth (Therios, 2008). Successful pruning of an eucalyptus tree improves the reproductive growth by adjusting tree shape, alters source-sink relationships, and changes the carbohydrate status of the tree (Pinkard and Beadle, 1998)

Eucalyptus tree growth is partly controlled by indole-3-acetic acid (IAA), an auxin which is produced in the upper shoot tips. It is then transported downward via the phloem and affects the growth of shoots by the following: the inhibition of lateral bud growth, decrease in growth rate in length of lateral branches, and increase in the angle between side branches and trunks. Pruning of the upper shoot tips decreases the concentration of IAA and increases lateral bud growth, growth rate in length of lateral branches, and decreases the angle between side branches and trunks (Pinkard and Beadle, 1998).

The rate of CO_2 assimilation per unit leaf area is important for tree growth. Pruning leads to the removal and reduction of the photosynthetic area. But a large decrease of leaves may negatively affect eucalyptus tree growth. On the contrary, the lack of pruning, or pruning high up, leads to shading of the tree canopy (Pinkard and Beadle, 1998).

If shading occurs, the floral bud formation in eucalyptus will decrease due to a dense canopy, because light penetration is often less than 10% light intensity. Photosynthesis requires opens stomata and therefore CO_2 assimilation is concurrent with the transpiration which leads to water loss. That means that pruning removes a proportion of leaf area, reducing water consumption and improving water use efficiency (Boland, 2006).

In olive plantations, pruning represents 20% - 30% of the annual cultivation costs. This is due to the high cost of labour and the amount of time required per tree (Therios, 2008). The developing trend today in orchard management is to reduce pruning to a minimum, hence the timing of pruning to induce floral bud initiation is very important. The best time for pruning eucalypts is between the end of winter and the initiation of flowering. It can also be performed after harvesting of capsules.

1.2.1.2. Girdling

Girdling refers to the controlled cutting of a tree bark to interrupt phloem translocation of sugars and various amino acids – a complete cut is thus used to kill trees but an incomplete ‘C’ cut is used to slow translocation of assimilates to roots in order to keep resources in the upper portion of the tree to improve fruit set and fruitlet growth. It is a technique used for floral induction in *E. nitens* (Williams *et al.*, 2003).

Girdling is also used to force floral induction in mangoes. The theory is that the axillary buds which normally remain dormant during the flowering period, and some of which would later produce vegetative shoots, can be caused to differentiate into inflorescences by girdling. Girdling a branch interrupts the inhibiting effect of the terminal bud and forces the flower-inducing hormone to move proximally in one branch and distally into an adjacent branch of a forked pair, acting upon the most distal buds of the second branch and inducing flowering (Shu and Sheen, 1987).

1.2.1.3. Root restriction

The use of root restriction in Eucalypt seed orchards is not well documented. Root restriction is of importance for productivity in both forestry and agriculture (Tombesi *et al.*, 2010). It is done by limiting root growth by growing a plant or tree in a small container (Richards and Rowe, 1977) or by restricting tree root volume by using a plastic barrier in the soil (Winer, 2007). Root restriction and container size (barrier size) affects root and shoot growth, biomass accumulation and partitioning, photosynthesis, leaf chlorophyll content, plant water relations, nutrient uptake, respiration, flowering and yield (NcSmith and Duval, 1998).

Plant responses to reduced soil volume have been reported for a wide range of crops, with some conflicting data among them. There are differences in responses reported between species and even between cultivars within a species. When roots are confined in a container that restricts their growth, the roots compete for essential resource (Van Lersel, 1997).

The effect of container size and root restriction on canopy volume and fruit yield is well documented in a study by Myers (1992). The author investigated the effect of root restriction and container volume on the canopy volume and total fruit per tree in apple and peach over three growing seasons. The author found that root restriction reduced canopy volume in apple and peach; demonstrated by using different container sizes. The author also found growth control increased linearly with decreasing container volume. In general, as container size increases plant leaf area, shoot biomass and root biomass increase (Daniel, 1993). During the third growing season, there was no treatment difference in fruit number per tree, total fruit weight per tree, or mean fruit size in peach. A mean 44% reduction in tree size resulted in an increase in yield efficiency in root-restricted peach trees. In the third season of growth, apple trees grown in fabric containers had a higher flower cluster number and percent fruit set than control trees. Within container treatments, flower cluster and fruit number per limb increased linearly with decreasing container volume (Myers, 1992).

Contradictory evidence and differing responses between species and cultivars in response to rooting volume suggest a need for further experimentation. In tomato, the time from sowing to anthesis was decreased as rooting volume increased (Kemble *et al.*, 1994). Also, a delay in fruit maturation was shown for root restricted tomatoes (Ruff *et al.*, 1987). In contrast, root restriction resulting from small containers did not have an influence on duration of flowering or time to anthesis in summer squash (NeSmith, 1993). In bell pepper increased root restriction decreased the time necessary to begin and halt flowering (NeSmith *et al.*, 1992). Root restriction has been viewed as a possible means to accelerate flowering and harvest of cotton (*Gossypium hirsutum*) (Ruff *et al.*, 1987).

1.2.1.4. Grafting

Grafting refers to the unity of a shoot or bud (scion) with a growing plant by insertion or by placing in close contact. Grafting is most commonly used in agriculture and forestry in asexual propagation to produce clonal orchard tree. In most cases, one plant is selected for its root and is known as a rootstock while the other plant is selected for its leaves, stem, and canopy properties and is known as a scion (Barrington, 2005). The two most commonly used grafting techniques in Eucalypt grafting are cleft grafting and bud grafting. In cleft grafting, the shoot of a selected, desired tree is grafted onto the rootstock of another established seedling. In bud grafting, a dormant side bud is grafted onto the stem of an established seedling, and when it has inosculated successfully, it is encouraged to grow by pruning off the upper shoots of the stock plant just above the newly grafted bud (Lewis and Alexander, 2009). A successful graft is determined by a contact of the vascular cambium tissues of the rootstock and the scion plants, and keeping both tissues alive for up to 3 weeks after grafting. A grafted plant needs to be watered on a regular basis and fertilised so it has enough nutrients to carry out cellular repairs and form new cells for a successful union (Gardner, 1996). Grafting has the advantage of being a non-destructive method of capturing a selection, maintaining its maturity and providing a flexible system with which to add or remove selections from the breeding population (Moncur, 1998).

In Eucalypts, grafted trees generally become reproductive and produce flowers and seeds much earlier than seedling trees which have to pass through the juvenile stage (Gardner, 1995). However, this method is logistically tedious with additional costs of a conventional grafting being incurred. Also, the level of success (often less than 50%) and ultimate success in compatibility cannot be predicted because of the genetic variation that exist within a seedling rootstock population (Gardner, 1996).

According to Gardner (1996), grafting usually takes place under shade in a moist, relatively cool workroom. Grafted plants are subsequently removed shortly after grafting to a shaded greenhouse with controlled conditions. The air temperature is controlled to maintain day-time temperatures below 25^{o}C within the greenhouse and the humidity is controlled by misting so as to avoid a case of “too wet” or “too dry” conditions because this can result in a rapid onset of disease or rotting symptoms, or desiccation of the scions, respectively. The overall level of success of *E. nitens* grafting can reach 74% under the conditions described by Gardner (1996). This approach has been documented to improve precocious flowering in *E. nitens*. It is already considered essential to the commercial fruit growing industry.

1.2.2. Chemical manipulation

Plant growth retardants have been used successfully in Eucalypts to reduce unwanted longitudinal shoot growth without lowering plant productivity and enhance flowering (Moncur, 1998).

Efforts on chemical manipulation on *E. nitens* have been largely focused on the use of paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol) which is capable of inducing precocious and abundant flowering in mature *E. nitens* plants without degrading seed quality. Paclobutrazol is a triazole derivative, which inhibits the Gibberellic Acid (GA) biosynthesis pathway between *ent*-kaurene and *ent*-kaurenoic acid (Gardner and Bertling, 2005). Paclobutrazol application on *E. nitens* acts on the apical tissue and enhances the reproductive levels of mature *E. nitens* by reducing the levels of endogenous GA. Williams *et al.* (1999) initially tested paclobutrazol activity on 11 months old *E. globulus* and *E. nitens*, to control vegetative growth in an attempt to induce precocious flowering. It was found that paclobutrazol was effective in reducing growth but was not effective in inducing precocious flowering in juvenile *E. nitens* but it was effective in reducing growth and inducing precocious flowering in *E. globulus* plants. Paclobutrazol was later found to enhance flowering in mature grafted *E. nitens* trees at less than two years of age, three years ahead of their normal reproductive development (Moncur and Boland, 2000).

Griffin *et al.* (1993) reported that paclobutrazol greatly suppressed *E. nitens* and *E. globulus* growth on trees between 2 and 17 years old. The trees were subjected to high levels of trunk injection and collar drenching which persisted for up to six growing seasons. This yielded an increase in the frequency of flowering and heaviness of bud crop. For both crops, growth responses were expressed in the immediate growing season, but flowering responses were not evident for another year. Foliar spray treatments reduced vegetative growth in young trees of both species for one growing season, but only the *E. globulus* showed an associated flowering response. Paclobutrazol application was found to have no detrimental effect on seed quality. This research showed that collar drenching was the most promising technique for treating large numbers of seed orchard trees because application time is substantially independent of tree size and weather conditions.

Moncur *et al.* (1994) also used the collar drench method to induce flowering in *E. nitens*. The collar drench method enhanced flower bud production for 3 years. A similar experiment, using trunk injection only proved effective for with regards to flower bud production for 1 year. Trees which were untreated with paclobutrazol did not produce any flower buds until Year 3, after the treated trees had already started producing buds. Results such as these suggest that the effects of paclobutrazol treatment are affected by the application technique used.

Despite the positive flowering effect of paclobutrazol in several fruit crops, Trewavas (1981) indicates that paclobutrazol can persist in the soil for several years as it is generally resistant to degradation and mass movement. The impact of a high dose of paclobutrazol on *E. globulus* trees has been detected for up to 69 months after treatment. The long-term effects on plants of a chemical with such a high residual activity can be difficult to regulate. This poses a high risk of overdosing and the potential for deleterious inhibition of growth and development (Curry and Reed, 1989). This has led to on-going research into alternative methods of flowering and seed production stimulation in *E. nitens.*

1.3. The timing of cultural practices to stimulate floral induction

Eucalyptus nitens takes at least five years to start flowering from a seedling under ideal conditions. It is known as an infrequent and a light flowerer (Moncur and Hasan, 1994). Understanding the mechanism controlling floral induction is considered essential for the development of flowering management practices in South Africa (Barrington, 2005).

Several environmental factors known to cause reduced flowering initiation include: the length of light and dark periods, and temperature (Chiaperro and Swain, 2003). Floral induction of *E. nitens* is comparatively prolific at high altitude sites in the South African summer rainfall region (Gardner and Bertling, 2005). It is thought that the amount of winter chilling has an effect on flower initiation by triggering the formation of a hypothetical hormone known as florigen. However, attempts to isolate this hormone have failed so far leading to further hypotheses that the environmental triggers such as light divert nutrient such as sucrose within the plant, which in turn leads to flower initiation (Salisbury and Ross, 1992). Whatever the nature of the biological triggers, it results in changes in the levels of growth substances (gibberellins, cytokinins, auxins, abscisic acid, ethylene, etc.), as well as changes in levels of metabolites and cofactors (Chiaperro and Swain, 2003). These changes lead to the synthesis of substances that stimulate floral induction (Chiaperro and Swain, 2003), given suitable conditions such as high levels of accumulated winter chilling units (Gardner and Bertling, 2005). Being able to understand exactly how accumulated winter chilling and reproductive and vegetative growth interact is essential for the development of practices to stimulate floral induction.

1.4. Floral bud induction

Flower bud inflorescences are borne in the axils of small young leaves (Tibbits, 1989). Flower bud initiation sets in motion the process of floral differentiation, anthesis, pollination, and fruit maturation (Barrington, 2005). According to a phenological model developed by Barrington (2005), in the southern hemisphere flower initiation takes place between October and March, and flower parts develop during December and October. For flower induction, chilling is a prerequisite.

1.4.1. The formation of a floral bud

Eucalyptus nitens belongs to a genus in which heterochrony appears to be significant. Heterochrony is the quantitative genetic control of the timing of developmental processes (Jordan *et al.*, 1999). Since *E. nitens* is a heteroblastic plant, flowers are in fact modified shoots and floral organs are modified leaves. The change in morphology is a result of meristem identity genes. Small genetic changes in these genes can result in alterations to the phenotype of mature plants, resulting in the differentiation of meristems. Meristems or stem cells are induced to form either floral or vegetative structures at the direction of the meristem identity genes (Weigel and Clark, 1996).

Flowering can be separated into three sequential component processes: it is important to differentiate between floral induction, floral evocation, and floral initiation (Meilan, 1997).

1.4.1.1. Floral induction

The first stage of flowering is bud induction. Floral induction may be defined as the process by which stimuli originating outside the shoot apex induce the formation of flower primordial (Hempel *et al.*, 2000). During the process of floral induction, photoperiod-induced floral stimulus moves from the leaves to the shoot apex. Once this stimulus reaches the shoot apex it acts on the apical meristem or it might act on the developing primordium (Hempel *et al.*, 2000). In *E. nitens,* floral bud induction may occur six to eight weeks before macroscopic appearance of buds, based on the assumption that the species in South Africa behaves the same way as it does in Australia (Gardner, 2003).

1.4.1.2. Floral evocation

Floral evocation can be defined as the process whereby the assignment of flowering fate is irreversible even when the flower-inducing conditions no longer exist. Initially the induction of sepals takes place and is followed by petals, stamens and carpels (Meilan, 1997).

1.4.1.3. Floral initiation

Floral initiation refers to the morphological changes that leads to the development of the central axis and side branches of the inflorescence and the meristems destined to develop into flowers. Floral evocation and floral initiation refers specifically to the inception of floral structures, whereas floral induction refers more to the induction processes occurring in macro-structures such as leaves and shoots (Gardner, 2003).

The flowering process in *E. nitens*, which is very similar to *Olives*, is summarised below from floral induction, through evocation, to initiation. The stages of flowering bud induction and differentiation was divided up into the following: the stage of *flowering bud induction*, the state of *morphological changes*, the state of *differentiation of flowers*, and the stage of *completion of growth*. The stages are described below.

- “The stage of *flowering bud induction,* which starts in August to early September and lasts up to the end of January. The vegetative buds are subjected to winter chilling, which causes physiological changes necessary for flowering bud induction. However, these buds remain unaltered morphologically” (Therios, 2008; Gardner, 2003).

- “The stage of *morphological changes,* which leads to the development of a central axis and side branches of the inflorescence and the meristems destined to develop into flowers. The duration of this state for the climatic conditions of *Olives* is about 40 days. For this stage low temperatures exert a beneficial influence” (Therios, 2008).

- “The stage of *differentiation of flowers*. Initially the induction of sepals takes place and is followed by petals, stamens and carpels. The induction takes place in the first 15 days of April, while that of carpels after 16 to 20 days” (Therios, 2008).

- “The stage of *completion of growth* of various parts of flowers. The full bloom happens from the end of May to the first days of June. The duration of the third and fourth stage is 45 to 60 days” (Therios, 2008).

Although the change in morphology of shoots to flowers and leaves to floral organs are a result of meristem identity genes (Weigel and Clark, 1996), it is critical to note that aspects of flowering depends on environmental conditions. Although the hormonal stimuli for flowering remains to be identified, it is nevertheless clear that all of them are formed in the leaves in response to photoperiod, temperature, and moisture (Gardner, 2003).

1.5. Cultural Conditions (environmental stimuli of floral bud induction)

Studies on flowering time control have shown that plants integrate several environmental signals. Predictable factors such as photoperiod, temperature, and water availability are regarded as primary factors and clearly interact with each other and the less predictable factors such as mineral nutrition. In the case of floral induction by photoperiod, the signalling for floral induction occurs between the leaves and the shoot apical meristem via the phloem. In some plants, such as *Sinapis alba*, photoperiod signalling has been shown to involve the root system and to include sucrose, nitrate, glutamine and cytokinins, but not gibberellins (Bernier and Périlleux, 2005). Temperature is shown to be perceived by all plant parts, although low temperature (vernalization) is often perceived mainly by the shoot apical meristem. Water availability is shown to involve the root system (Bernier, 1993).

From the information available, it is necessary to establish which factors play a role in floral bud induction in *E. nitens*. The following factors will be discussed in further details below: photoperiod, mineral nutrients, temperature, and moisture stress.

1.5.1. Photoperiod

Photoperiod is the period of a plant's daily exposure to light. It is considered especially with regard to the effect of growth and flowering of plants that are sensitive to seasonal changes in day length (Barrington, 2005).

Photoperiod does not promote flowering in *E. nitens*. In an experiment to test the effect of photoperiod on flowering ability, Moncur and Hasan (1994) concluded that photoperiod is not a strong stimulus of flowering in *E. nitens* due to the inability of changing day length to induce flowering in combination with paclobutrazol. This result contrasts with the results of Bolotin (1975) for *E. occidentalis.* This may indicate that photoperiod is a species-specific response within the genus.

1.5.2. Mineral Nutrition

Fertiliser application is an important cultural practice in orchard management. Its effect on levels of tree growth and fruit quality of 'Hakuho' Peaches (*Prunus persica*) reveals that fertiliser level affects fruit quality and yield (Huijuan *et al.*, 1999).

Fertilising at planting of seedling and clonal seed orchards is common practice at Sappi Forest South Africa, Mondi, and the ICFR, but no subsequent nutritional additions are usually made (Barrington, 2005). Studies by Chambers *et al.* (1997) has shown how fertilisation induced first flowering in a late flowering *E. globulus* after five years in plantations in Australia (unfertilised stands tends to flower when more mature). Campion *et al.* (2006) showed how soil fertilisation helped increase timber yield in *E. grandis* in a study conducted in South Africa.

Most fertilisation studies done on *E. nitens* have been done outside South Africa. In these studies, there is a disagreement as to the length of time fertiliser should be applied to enhance tree growth. Carlson *et al.* (2000) recommended the application of a general fertiliser for the improvement of the nutritional status of seed orchards. These authors placed emphasis on the use of high rates of copper, iron, and potassium based on the nutrition present in the flowering and non-flowering branches of 10 year old *E. nitens* breeding seed orchards. Copper and iron were found in higher concentrations in foliage of flowering branches compared to foliage of non-flowering branches while potassium was found to be almost depleted in the foliage of flowering branches as compared to that of non-flowering branches where potassium was abundant. This is because copper and iron were not as utilised in flowering as compared to Potassium which was used up. Potassium plays a role in protein synthesis and the translocation of photosynthates (Pettigrew, 2008), a further indication that it may have been used in flowering.

Williams *et al.* (2003) showed that an increase in nitrogen affected flower bud production at two levels. The first level was accelerating growth; this caused reproductive maturity to be reached much earlier, creating sites on the tree suitable for flower bud development. The second was that nitrogen may cause a biochemical effects on flower bud initiation. Williams *et al.* (2003) further showed the efficacy of a combination of paclobutrazol and nitrogen in stimulating the flowering of the reproductively mature trees by applying both nitrogen fertilizer and paclobutrazol at several rates to 4 years old *E. nitens* trees. Application of both nitrogen fertilizer and paclobutrazol substantially increased the occurrence of precociously flowering trees over that of either treatment applied alone.

The timing of the application of fertilisers, in terms of root and shoot growth, may have a significant bearing on the efficacy of the added nutrients (Barrington, 2005). In a study by Huijuan *et al.* (1999), it was shown that excessive fertiliser application for peach trees significantly diminishes fruit quality production but stimulates vigorous vegetative growth. Therefore, fertiliser added at the wrong phenological stage will stimulate vegetative growth but at the cost of reproductive growth in fruit trees.

1.5.3. Temperature

Several climatic factors are apparently critical for *E. nitens* performance with respect to floral induction. Winter chilling promotes floral induction in *E. nitens* (Moncur and Hasan, 1994).

Moncur and Hasan (1994) found that only grafts exposed to cold temperature flowered, regardless of the level of paclobutrazol applied. This response may resemble the vernalization response in annual plants, though its function in a woody perennial is not clear. A period of cold could result in destruction of a flowering inhibitor, a change in inter-organ competition, or both.

In addition, (Gardner and Bertling, 2005) showed that high levels of accumulated winter chill (96 Chilling Portions) stimulated a high percentage of seedlings (25–50%) and grafts (55–64%) to produce flower buds. However, considerable variation in precocity and chilling requirement (for floral induction) is evident within the South African *E. nitens* breeding population.

A chilling period is required prior to flowering in *E. nitens* but it is possible that the chilling requirement of *E. nitens* can be decreased by using a rootstock from another eucalyptus species or a eucalyptus hybrid that has *E. nitens* genes but requires little or no chilling requirement. He *et al.* (2007) showed that rootstocks have a significant effect on the chilling requirements of dormant buds in grapes. The chilling requirement of grapes increased when a rootstock with a high chilling requirement was used, but decreased when a rootstock with a low chilling requirement was employed.

1.5.4. Moisture stress

Since soil water availability is the most common limitation to growth in South African forestry plantations (Schonau and Grey, 1987), it is possible that irrigation will have a greater effect on productivity than the application of fertiliser.

In a study conducted by Campion *et al.* (2006) on *E. grandis* trees, irrigation and fertiliser applications were tested. At 4 years old, the respective totals of the above- and below- ground biomass pools for irrigated and fertilised tree treatments were 82 and 78 t ha^{-1}. The faster growing irrigated trees accumulated more nitrogen and phosphorous than trees that did not receive additional water. However, according to Williams *et al.* (2003), accelerated growth decreases time to reproductive maturity and the speedy uptake of nitrogen may have had a biochemical effect on flower bud initiation in *E. nitens.* Hence, timing of irrigation is very critical for flower bud initiation.

In an attempt to quantify the effect of the water stress on flowering in eucalypts, Moncur (1998) subjected 18 month old grafts of *E. nitens* in pots to mild and moderate water stress, and found that water stress delayed leaf initiation. Since these are the sites of floral initiation, flower bud development was also delayed.

However, proper timing of drought events can have positive effects. Controlled drought (Boot *et al.*, 1986), and accumulated chilling units (Gardner and Bertling, 2005) can promote flowering in *E. nitens*. Moderate drought, combined with accumulated chill units may be beneficial for floral bud initiation.

1.5.5. Interactions between photoperiod, temperature, mineral nutrition, and moisture stress

Eucalyptus nitens starts to produce flower buds at about five years under natural conditions in Australia, where it is are endemic (Moncur and Hasan, 1994). Although the mechanism for induction is unknown, it is known that to achieve reproductive success, a plant must select the most favourable season to initiate reproductive development. This selection requires the existence of molecular mechanisms to continuously monitor environmental factors and to properly respond to the adequate conditions. Many environmental factors influence flowering time (Bernier and Périlleux, 2005).

The predictable changes in the year, such as light and temperature, are the most relevant factors in terms of the selection of flowering season. However, less predictable factors such as nutrient or wind can also modulate flowering time. Woody perennials are able to perceive all this environmental variation and modulate their growth and development in the short term such as growth response to ambient temperature or in the long term as in flowering responses to vernalization (Ausin *et al.*, 2005).

According to Gardner (2003), in order to successfully induce a flowering response in *E. nitens* in South Africa, a selection of a planting site above 1400 meters, deep rich soils, with a record of exceptionally cold winters to achieve the level of cumulative cold requirement implicated for floral induction, plus good rainfall (>950mm). Gardner (2003) further explained the importance of temperature amplitudes relative to site selection: "The 'good flowering' sites are always in exposed positions on crests or southwest to east-facing up-slopes where air drainage is good and winter day/night temperature amplitudes are low, whereas the 'poor flowering' sites occur on flatter terrain where winter day/night temperature amplitudes are relatively high. This phenomenon suggests that uniform cool conditions in winter promote flowering in *E. nitens* more than extremely varying temperature conditions.

1.6. Management of flowering using dwarfing rootstocks

The use of dwarfing rootstocks to control excessive shoot growth to enhance flowering has been in practice for many centuries. An example is in apple production, where rootstocks have been used for many years to limit excessive growth, increase yield per tree size, and increase precocity. The accomplishment of this goals has enabled the world's fruit production to intensify through the use of rootstocks for trees that crop early, have high yields, and can be planted in large numbers per unit land area (Atkinson and Else, 2001)

One of the advantages of using dwarfing rootstocks is that it induces precocious scion flowering. Not only does it reduce time taken to flower but also a large numbers of flowers are produced per unit tree compared to a normal seedling tree (Long and Kaiser, 2010). However, by decreasing the tree size, dwarfing rootstocks produce less vegetative growth and greater yield on a per tree basis when compared to trees on vigorous rootstocks (Wheaton *et al.*, 1995). However smaller trees allow for a lot of trees to be planted at higher densities per unit of planted area. Moreover, yielding efficiency of the trees increases with the extent of scion dwarfing. Measurements that account for differences in tree weight, canopy volume, light interception and trunk cross-sectional area all show an increased yield efficiency associated with dwarfing rootstocks (Whiting *et al.*, 2005). The effect of dwarfing rootstocks has been documented to improve crop production and it should be considered essential as a technique for enhance seed production in *E. nitens.*

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CHAPTER 2: OPTIMIZING CONDITIONS FOR THE GRAFTING OF *E. NITENS* USING VARIOUS SCIONS AND ROOTSTOCKS

Abstract

Rootstock age and size, combined with nutrients, temperature, and humidity are major factors thought to play an important role in grafting success in eucalypts. Research was conducted into the grafting success of interspecies and sub-species rootstocks, and three scions chosen based on their differential flowering ability, combined with six different rootstocks, chosen for their precocity of flowering, or as representatives of *E. nitens* by itself or in a hybrid. The agro-meteorological conditions found inside the greenhouse tunnel were monitored, and grafted plants were placed in three positions at different distances from the wet wall, which provided the highest humidity and cooling potential. The grafting environment was optimized by controlling the temperature and humidity of the greenhouse tunnel to achieve optimum grafting conditions. Overall, 44% of the grafted plants survived. The best rootstock host for grafting *E. nitens* scion was the hybrid with a strong *E. nitens* appearance (R5) at 67% survival rate. The two grafting periods had no significant effect on the grafting success, nor did the position of the plants in the greenhouse tunnel.

2.1. Introduction

2.1.1. Grafting for accelerated seed production

A tree orchard is usually characterised by its early flowering and/or high seed production. However, *E. nitens* has shown itself to be a reticent flowerer in South Africa. According to Eldgridge and Griffin (1983), *E. nitens has* a slow onset to reproductive age, even under natural conditions in Australia. The current seed-producing age in un-manipulated seedling trees is approximately 12 years. Yet even in these trees, the flowering rate is around 50%, with the final mature seed production being even lower due to poor cross-pollination. Poor cross-pollination occurs due to the cool-season flowering period, which is usually from April to September in South Africa, when natural pollinators such as bees, flies, and other insects are least active. The poor flowering of the selected trees of *E. nitens* in seed orchards cause downstream problems such as generation turnover delays in various forestry breeding programmes, delays in development of improved seed production, and ultimately less gain in commercially produced South African seeds of *E. nitens*.

A hypothesis was developed at the ICFR by Gardner et al. (2003) that this problem could be solved by the use of grafted trees of *E. nitens*, using precocious scions, grafted onto compatible rootstocks that promoted precocity. These trees would then be planted at sites that optimized flower initiation. However, early research by the ICFR was plagued by a low take of grafted plants. Subsequent preliminary studies showed that keeping the humidity high could enhance the take of grafts.

A successful graft is measured by monitoring the growth and development of the scion because its growth would be determined by the transport of water, nutrients and signalling molecules from the rootstock to the scion (Nisra et al. 2012). Various grafting configurations exist, including the “root-shoot”, where the hypocotyls of the rootstock of one particular genotype and the scion of another genotype are joined together; the “two shoot Y-graft”, in which a shoot is maintained by a rootstock of the same genotype and a second shoot of a different genotype is grafted; and “shoot-shoot”, which has an inflorescence stem of a rootstock grafted to an inflorescence stem of a scion, where the basal part of the shoot is the same genotype as the rootstock and the apical part is of a different genotype (Nisra et al. 2012).

In grafting, the growth and development of the scion is largely dependent on the rootstock. Rootstocks are capable of altering physiological processes in the scion, including those related to fruit quality, biomass accumulation, and response to abiotic stresses such as water deficits (Cookson and Ollat, 2013). This in turn is affected by the environmental factors in which the rootstock is exposed to. For example, variations in biomass accumulation are essentially related to temperature as well as to minerals and water availability (Cookson and Ollat, 2013). In conditions of water deficit, an excess of lignin is deposited, which is associated with the stiffening of cell walls that lead to a reduction in scion growth (Fan et al., 2006; Bomfim et al. 2011).

Temperature regulation during grafting is extremely important. Temperatures that are too low may inhibit callus growth, while temperatures that are too high may cause tissue death or excessive callus growth, which depletes the carbohydrate reserves of the plant (Trinchera et al. 2013). It has been observed that callus formation is promoted in grafted plants by maintaining constant optimum temperatures (in accordance with the plant type) for a period of several months. Sufficient moisture and high relative humidity must also be maintained in order to minimise water loss and desiccation (Trinchera et al. 2013).

The research in this chapter covers a study into optimizing the grafting of three scions onto six rootstocks, in an environmentally controlled greenhouse tunnel. The environmental conditions in the tunnel were monitored to determine whether these conditions affected the incidence of success of grafts.

2.2. Materials and Methods

2.2.1. Rootstock Selection

Six treatments were selected, consisting of various *Eucalyptus* taxa and provenances, including three different species and 2 different hybrids (see Table 2.1). The treatments were selected for their unique properties, which are defined below:

E. grandis (R1): This species is reported to be a vigorous grower (Myers et al., 1996) with good rooting properties, and is a precocious flowerer (15 to 20 months) that flowers well in tropical regions.

E. nitens x *E. grandis* (R2): This is a natural hybrid clone, which has an *E. nitens* mother and a *Grandis*-like appearance. It was selected for its excellent rooting properties, early flowering abilities (+2 years), and having an *E. nitens* parent meant it would be more compatible for grafting to an *E. nitens* scion.

E. globulus (R3): The seeds for the *E. globulus* plants were imported from Australia. The seeds were collected from a small region known as Wilson Promontory provenance. Trees of *E. globulus* growing in the region are unimproved, have a slow vegetative growth (dwarfed) and flower in 15 to 20 months. *E. globulus* was selected due to its high precocity and slow rate of growth.

E. nitens (R4): This *E. nitens* is a commercial seedling. It is a vigorous grower and but a slower flowerer: time to first flower is between ten and twelve years. It was selected as a Control treatment.

E. grandis x *E. nitens* (R5): This is a hybrid clone. The seeds for this clone are from an *E. grandis* female parent. This clone is more phenotypically similar to *E. nitens* than it is to *E. grandis*. This clone has both *E. nitens* and *E. grandis* genes, which should facilitate better grafting results. It is also a precocious flowerer (+3 years).

E. nitens (R6): This E. *nitens* seedling was collected from flowering trials held at Gilboa and Willowmere, Kwazulu-natal, South Africa. It is most likely selfed because it was the only tree crop to flower in the trial at the time of flowering. This *E. nitens* provenance was chosen because it produced better flowering results compared to other *E. nitens* rootstocks in past grafting and paclobutrazol trials. It was therefore predicted to be a good flowering *E. nitens* rootstock to graft onto.

Table 2.1: Eucalyptus accessions used as rootstock plants for grafting

Treatment Number	**Species/ Hybrid**	**Seedlot / Clone Number.**	**Estimated time to First Flower**	**Description**	**Source of seedlot or clone**
R1	*E. grandis*	EG6 (G175)	15 - 20 months	Commercial clone	Nseleni Nurseries (JDM Keet), Kwazulu-natal, South Africa
R2	*E. nitens* x *E. grandis*	NH58	+2 years	Commercial clone	Mondi Mountain Home TTC, Kwazulu-natal, South Africa
R3	*E. globulus*	17609	15 – 20 months	Unimproved	CSIRO (ATSC),

				seedling	Canberra, Australia
R4	*E. nitens*	M9594	10 – 12 years	Commercial seedling	Mondi Mountain Home TTC, Kwazulu-natal, South Africa
R5	*E. grandis x E. nitens*	GN155	+3 years	Commercial clone	Mondi Mountain Home TTC, Kwazulu-natal, South Africa
R6	*E. nitens*	EN47	10 – 12 years	Seedling (probably selfed)	Mt Gilboa & Willowmere trials, Kwazulu-natal, South Africa

2.2.2. Scion Selection

Three parent trees were selected as scions. The scions were 20 years old (physiologically) at the time of collection, and consisted of three different *Eucalyptus* provenances found in Australia. The scion source for this experiment was from a trial of the Institute of Commercial Forestry Research (ICFR) at Gowan Brae, Kwazulu-natal, South Africa. The three provenances chosen were Barrington Tops (47-BT), Tallaganda (62-T), and Barren Mountain (136-BM). These provenances were chosen to represent the different regions in which most *E. nitens* currently planted in South Africa originate from, in order to give a more conclusive result of the effect of rootstocks on *E. nitens* flowering, since these tree crops exhibit different precocities in South Africa. Figure 2.1 illustrates the level of flowering at the same time of the year for all scions collected. The strongest flowerer is 47-BT, followed by 136-BM, then 62-T. Their information is available in Table 2.2 and the selection properties of the scions are explained below:

Barren Mountain (136-BM): this provenance is from the northern part of New South Wales, Australia and this is the most widely planted provenance in South Africa. The trees have a medium flowering level compared to the other two provenances.

Barrington Tops (47-BT): The site of origin is in Mount Royal Range of mountains, 200km north of Sydney, also in New South Wales, Australia. This provenance is renowned as a precocious and strong flowerer. It is documented by the ICFR as the most precocious *E. nitens* in all of ICFR's flowering trials.

Tallaganda (62-T): The provenance was found at site in the mountainous region of the Great Dividing Range, close to Canberra but still in New South Wales. This is the least precocious provenance and weakest flowerer of the three scions. From ICFR and South African Pulp and Paper Industries (SAPPI) orchard records, it is a shy flowering provenance, and sometimes produces no flowers.

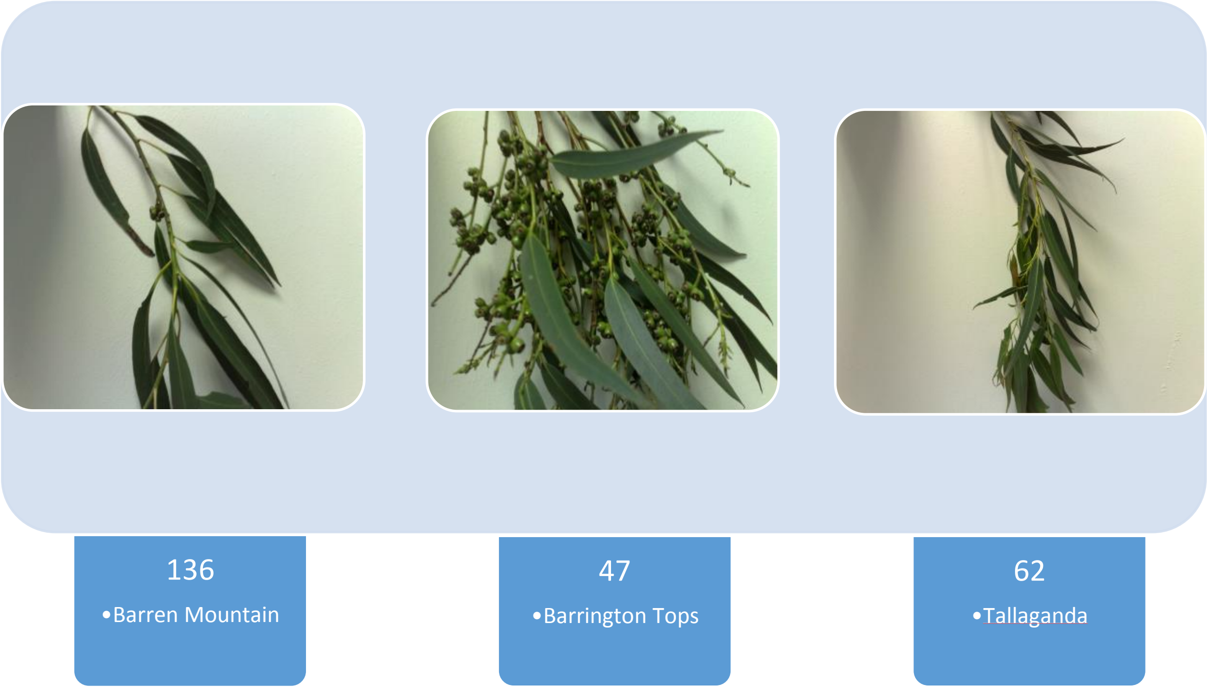

Figure 2.1: Selected scion types for grafting onto the selected rootstocks

Table 2.2: Information on the three treatments selected for scions

Treatment Number	**Species**	**ICFR Selection/ Clone Number.**	**Family Number**	**Provenance**	**Source of scion material**
47-BT	*E. nitens*	EN47	34838	Barrington Tops	ICFR Gowan Brae CSO, Kwazulu-natal, South Africa
62-T	*E. nitens*	EN62	37255	Tallaganda	ICFR Gowan Brae trial, Kwazulu-natal, South Africa
136-BM	*E. nitens*	EN136	32091	Barren Mountain	ICFR Gowan Brae trial, Kwazulu-natal, South Africa

2.2.2.1. Scion collection technique

The scion collections were conducted by a team of four people with unique skills: two expert tree climbers and two tree climbing supervisors from the ICFR. The materials used for tree climbing were: aluminium ladders, climbing ropes, tree saws, cooler boxes, ice, newspaper, and plastic bags. The scions were collected and grafted on the same day.

2.2.2.2. Tunnel

The grafting tunnel used was accessed from the Department of Plant Pathology at the University of KwaZulu-Natal (UKZN), Kwazulu-natal, South Africa. This is a 30m x 8m plant tunnel, cooled with fan and pad technology, and with an 80 bar fogging system to enhance humidity control. Blocks of plants were placed in three positions: (1) close to the wet wall (but further than 1.5 m of the wet wall); (2) in the middle of the tunnel; and (3) at the far end, near the door, which is furthest from the wet wall. The plants were arranged in blocks of 4 by 5, with walkways of 75-80 cm between plants to facilitate working with the plants. The automated plant tunnel temperature control was set at 24ºC, and the humidity at 80%. The humidity in the tunnel was kept high by the automatic fogging system coming on for 3 minutes at a time and going off for a minimum of two minutes. The fogging system assisted with keeping the ambient temperature low, so it was turned off on cool days due to the naturally high humidity that occurs on cooler days. Three Hobo temperature and humidity loggers (HOBO RH Temp Logger H08-003-02) were placed in the plant tunnel to measure temperature and humidity every 20 minutes. The Hobo data loggers were placed 2m from the wet wall, in the middle of the tunnel, and 2m from the tunnel doors, furthest from the wet wall.

2.2.3. Grafting

Grafting of the eucalyptus plants was conducted by two highly experienced grafters from the ICFR. The grafting method used was the offset cleft graft (Figure 2.2 and 2.3). Grafting commenced on the 27th August 2009 and continued until the 21st September 2009. The process was divided into two periods (Figure 2.4). Two grafting period were used in order to evaluate the impact of environmental factors (weather conditions) on grafting success rates. Period One was from 27/08/09 until 11/09/09 (early spring in South Africa), and Period Two from 12/09/09 until 21/09/09 (late spring in South Africa).

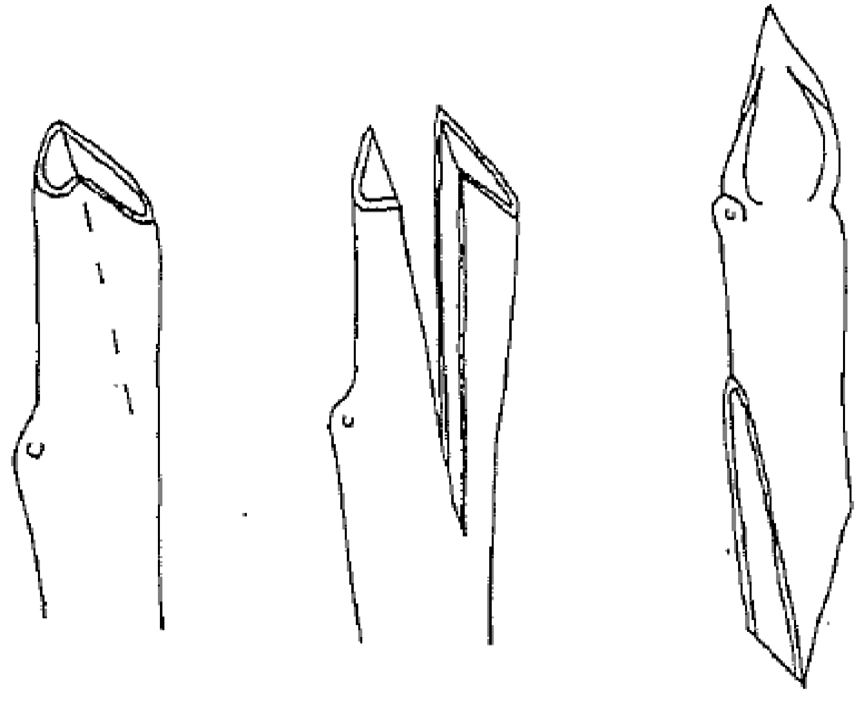

Figure 2.2: Preparation of the rootstock (left) and scion (right) for an offset wedge graft.

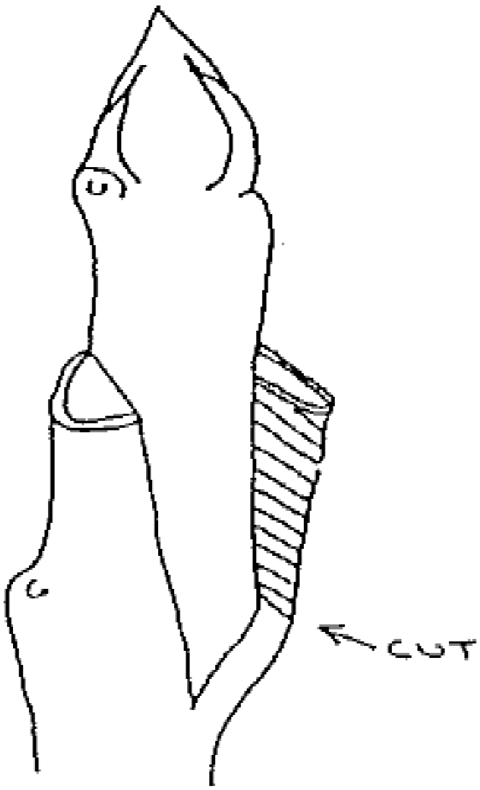

Figure 2.3: Insertion of the scion into the rootstock.

The plants were labelled as follows: Rootstock Treatment number/Scion treatment number /Grafting batch number. i.e., R1/47/P1.

2.2.3.1. Care of grafted plants

The grafted plants were initially watered every second day, which was in accordance with the rate of drying out. Watering was conducted using a hosepipe fitted with an adjustable rose head located in the tunnel. When the plants were watered, care was taken not to excessively wet the foliage, in order to prevent diseases. The count of successful graft takes were taken 3 months after grafting.

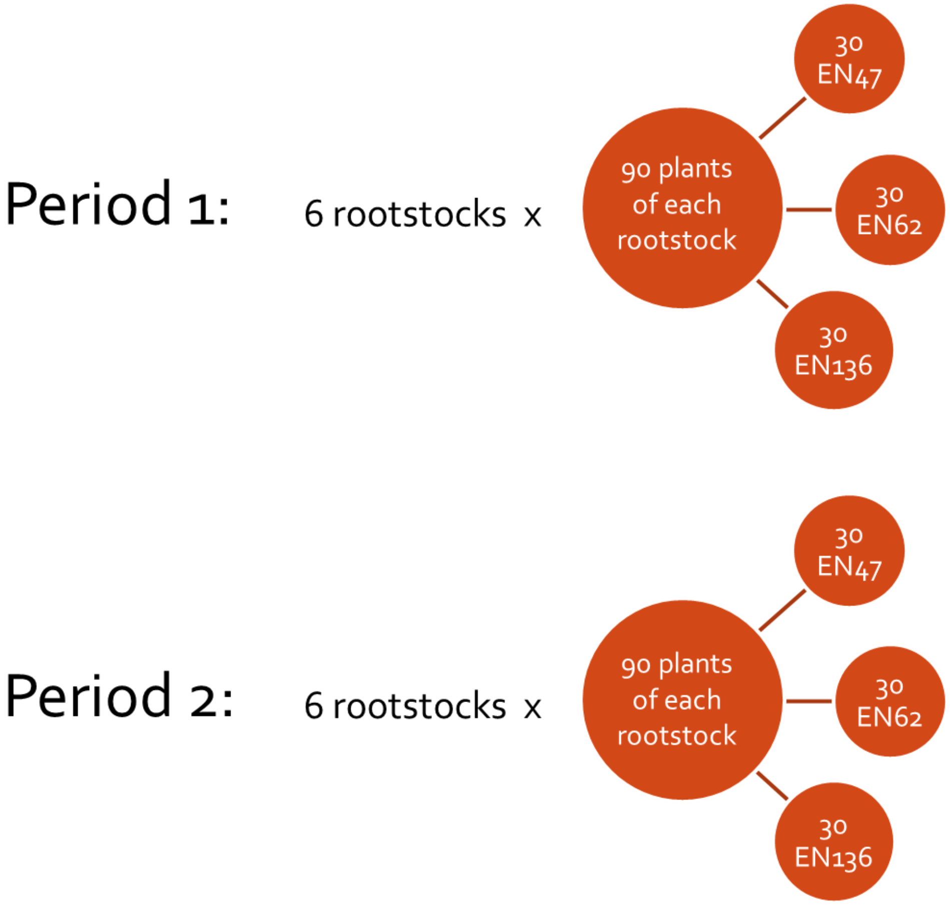

Figure 2.4: Grafting periods for Eucalyptus grafting experiment

2.3. Results

Time to first budding was from September 2009 to October 28, 2011, approximately 20 months after field planting.

2.3.1. Grafting success

Overall 44% of the grafted plants survived. Of the 44% that survived, Scion 62-T had the most survival at 66%, followed by Scion 136-BM at 47 % and Scion 47-BT at 30% (see Figure 2.5).

Results of different scions on the rootstocks indicated that Rootstock R5 provided the best rootstock host for grafting (67%). This is interesting given that R5 is a hybrid with a strong *E. nitens* appearance. The next most compatible rootstocks were the two *E. nitens* accessions, R4

(44%) and R6 (40%), respectively. Rootstocks R3 and R2 were both equal at 37% and Rootstock R1 was the least suitable host at 34% successful grafts (Figure 2.6).

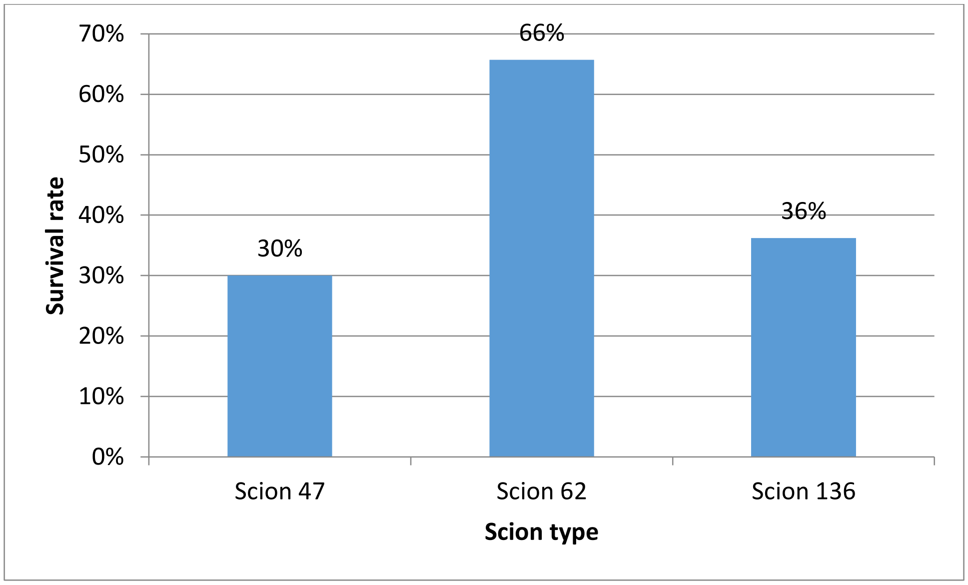

Figure 2.5: Comparative grafting success of the different scions

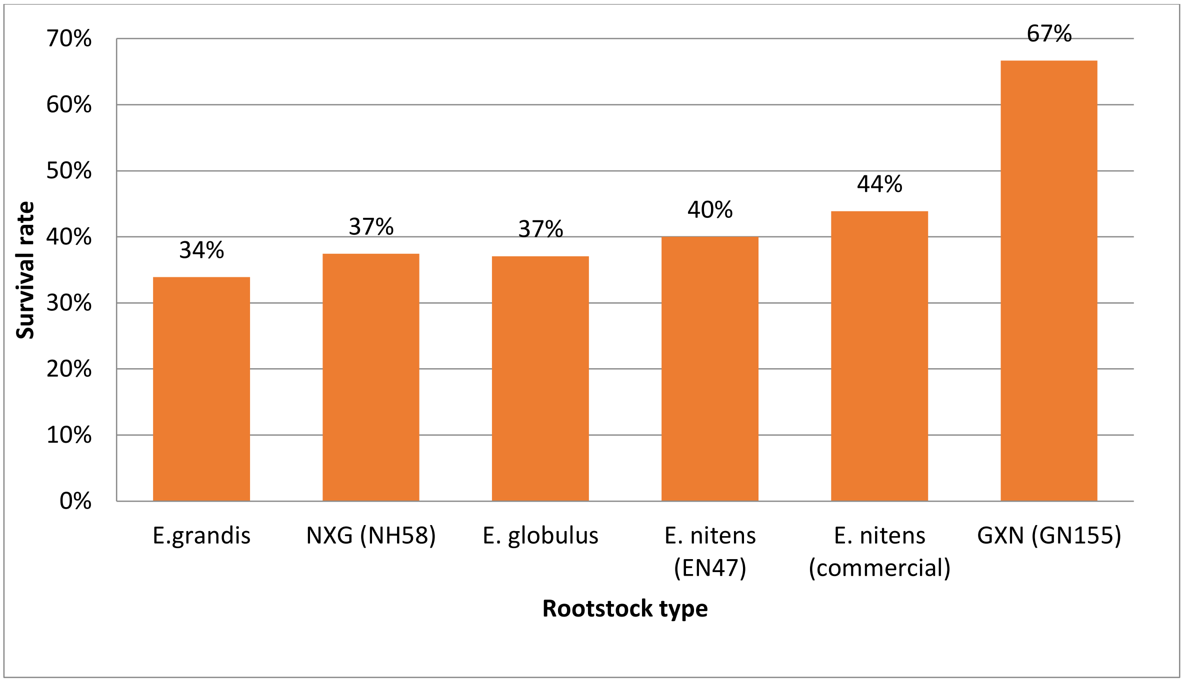

Figure 2.6: The comparative grafting success of the different rootstocks, where R1 is *E. grandis*, R2 is N x G (NH58), R3 is *E. globulus*, R6 is *E. nitens* (EN47), R4 is *E. nitens* (commercial), and R5 is G x N (GN155)

The two grafting periods (See Figure 2.7), revealed a 1% difference in survival rate, where 44% of the grafted plants survived in the second period and 43% survived in the first period. The data collected was insufficient to perform a statistical analysis. Figure 2.8 shows the comparative grafting success of the grafted plants kept in three distinct three zones in the greenhouse tunnel, where 1 is near the wet wall, 2 is in the middle and 3 is near the door. According the results Plant kept at zone 3's relative humidity and temperature (see Table 2.3) had the highest survival rate. However, Zone 2 had the coolest temperature and highest relative humidity (Table 2.3) Which was thought to be preferential for higher grafting success.

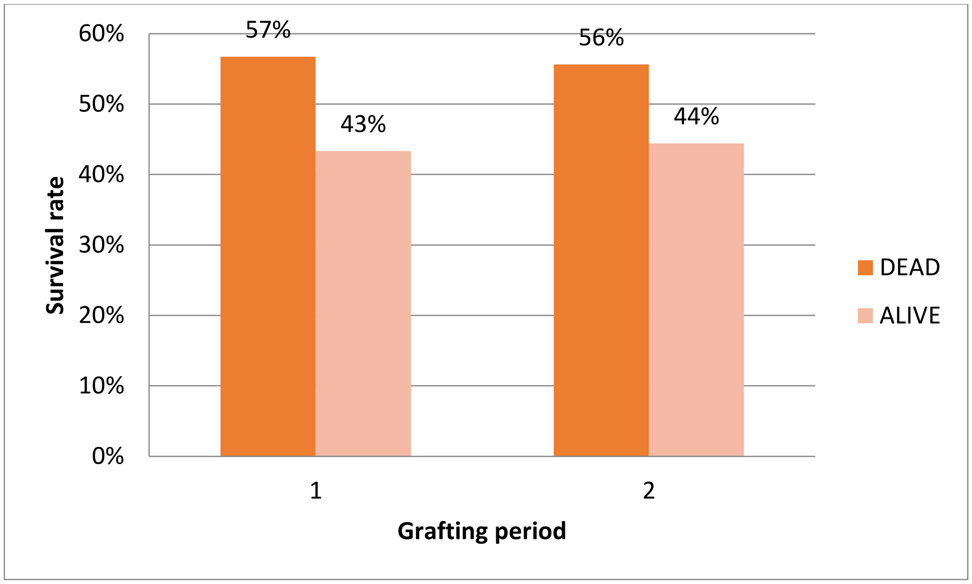

Figure 2.7: The comparative grafting success of the two grafting periods

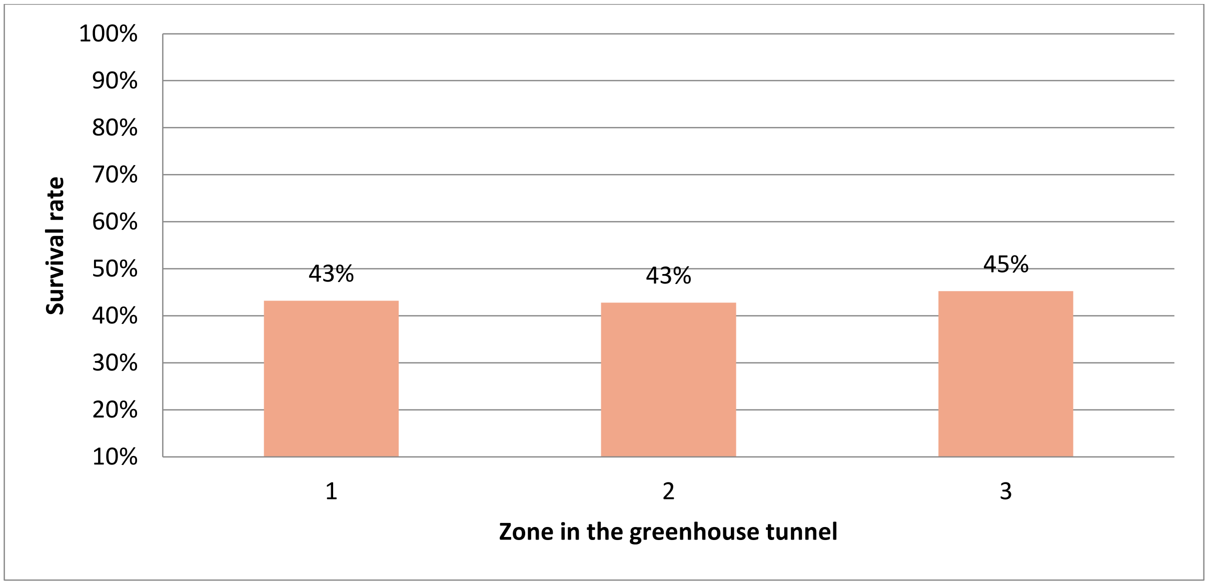

Figure 2.8: Comparative grafting success of the grafted plants kept in three distinct three zones in the greenhouse tunnel, where 1 is near the wet wall, 2 is in the middle and 3 is near the door

Table 2.4: Mean Temperature and Humidity for the wet-wall, middle, and door zones in the tunnel

Zone	**1 (Wet wall)**		**2 (Middle)**		**3 (Door)**	
Data	Temperature (°C)	RH (%)	Temperature (°C)	RH (%)	Temperature (°C)	RH (%)
Mean	20.21	84.20	20.08	86.38	20.39	84.40

2.4. Discussion

The process of grafting involves attaching scions from appropriate trees onto rootstocks of either the same or different species (Biondi and Thorpe, 1982; Gardner, 1996). Due to the nature of the technique, care was taken when selecting suitable scions and rootstocks for grafting, in an attempt to avoid incompatibility (McComb and Bennett, 1986). It has been previously reported that rootstock size may play an important role in the grafting success of eucalypts because underdeveloped rootstocks may reduce success in the grafting procedure because it affects the level of contact of the vascular cambium of the rootstock and the scion (Gardner, 1995; Gardner, 1996). The comparative grafting success of the grafts may be affected by the physiological size of the rootstock types and scion types.

The grafting success of 44% is relatively high compared to the success of grafting of Eucalypts conducted in the past. Prior levels of successful grafts had been in the region of 10% to 20 % (Gardner, 1996). This may be attributed to the grafted plants previously being kept in an open air shade house after grafting. This is in marked contrast to the conditions of this research, where the grafted plants were kept in a controlled environment in a greenhouse tunnel, with the cooling system set to 24°C, and a high level of humidity being maintained by an 80 bar fogging system which came on for 3 minutes and went off for 2 minutes on hot days, and stayed off on cool days. Maintaining a high humidity after grafting is essential to ensuring that the scion tissue does not dehydrate before the scion and rootstock cambial tissues merge, and water transfer can take place into the scion tissue (Durner, 2013).

Scion 62-T had the highest grafting success (66%). In contrast, Scion 47-BT had the lowest grafting success (30%). This poor graft take by Scion 47-B was hardly surprising as most of the collected scions were in a reproductively active state at the time of collection (see figure 2.1).

According to Gardner (1995) poor take of scions could be attributed to its physiological reproductive state as opposed to its inherent ability. Furthermore, Scion 136-BM had moderate grafting success (47%) compared to the other two scions – less compared to Scion 62-T which had the least flowra abundance during grafting.

Grafting success was not considered to be a significant factor in flowering potential; neither was grafting periods, or temperature and humidity variations in different zones within the greenhouse tunnel (wet wall, middle and door).

Rootstock R1 (*E. grandis*) had the lowest grafting success out of all rootstocks. The species *E. grandis* is considered to be a vigorous grower (Myers et al., 1996) with good rooting properties, and a precocious flowerer that produces buds in roughly 15 to 20 months, hence this outcome was disappointing. Interspecies compatibility may have been the problem.

The rootstock R2, a natural hybrid of *E. nitens* x *E. grandis,* was chosen for this study owing to its excellent rooting properties and early flowering abilities. However, its grafting success was only 37%. It was hoped that the compatible genetics and host provided by one *E. nitens* parent would improve the grafting compatibility with *E. nitens*. However, this did not appear to be the case.

Rootstock R3 (*E. globulus*) performed poorly, and few grafts took, and the resultant plants were weak and did not perform in the field (Chapter 3). A lack of compatibility of the two species was probably the basis for its failure as a rootstock.

Rootstock R4 (*E. nitens*) performed extremely well as a host rootstock, and provided the second best host for the scions, which makes sense given that they scion and rootstock were genetically compatible.

The R5 rootstock (*E. grandis x E. nitens)* had the highest grafting success at 67%. This results was what had been planned, but is in contrast to the poor performance of R2, which has similar genetics to R5, both of the being G x N hybrids.

The R6 (*E. nitens*) rootstock had a 40% grafting success. This rootstock was chosen for this study based on its early flowering results in previous trials by the ICFR compared to other *E.*

nitens selections. It can be categorized as a strong rootstock, having developed the greatest height (>4m) and the third widest collar diameter (<80mm).

2.5. Conclusions

The controlled environment greenhouse tunnel provided excellent conditions for grafting. The mean take of grafts of 44% was substantially better than the 14% grafting take (Gardner, 1996) being achieved in a nearby shade house with highly variable environmental conditions.

Under these particular environmental conditions, grafting success was between 36% and 67%, depending upon the scion x rootstock combinations.

Rootstocks need to be the same species or a related hybrid as the scions to provide for physiological compatibility, leading to high levels of grafting take.

Future research in achieving better grafting success can be done on the use of fertilizer, combined with these particular environmental conditions. Providing optimum physiological and environmental conditions during grafting will yield even higher levels of grafting take.

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CHAPTER 3: FACTORS AFFECTING EARLY FLOWERING IN *E.NITENS*, USING DWARFING AND PROCOCIOUS ROOTSTOCKS

Abstract

For flower initiation, environmental factors such as day length (light and dark periods), temperature, and topography may affect the flowering characteristics of *E. nitens*, factors which are often geographically specific. Research was conducted into the use of interspecies and sub-species rootstocks for early flowering in *E. nitens*, and to monitor the impact of light, cold temperatures (via chilling units) and site location on floral induction. Six rootstocks were selected from *Eucalyptus* taxa and provenances, including three species and two hybrids. Three scions were selected from three *Eucalyptus* provenances: Tallaganda, Barren Mountain, and Barrington Tops. Exposed sites with good air drainage and low winter day/night temperature amplitudes are considered 'good flowering sites'. Of the six rootstocks selected, amongst the ungrafted trees, the hybrid with a strong *E. nitens* appearance (R5) had the highest budding percentage while amongst the grafted trees, the hybrid rootstock with a strong *E. grandis* appearance (R2) induced the highest budding percentage on the *E. nitens* scions. This study provides further insight into the selection of rootstocks and site conditions that yielded good flowering results within the subtropical climatic conditions found in South Africa.

3.1 Introduction

3.1.1 Grafting for stimulating seed production

Eldgridge and Griffin (1983) noted that *E. nitens is* slow to flower, even under natural conditions in Australia. It is even slower to flower in South Africa, taking 10 to 12 years with most seeded parents. This impacts on any attempt to breed the species, with the cycle times being so slow. Furthermore, cross-pollination is poor in South Africa because flowering occurs over the winter period when pollinating insects are at their lowest level of activity.

The flowering characteristics of *E. nitens* and the environmental factors that affect flower initiation have been previously described (Barrington, 2005; Gardner and Bertling, 2005;

Moncur and Hasan, 1994; Moncur et al., 1994). Flower initiation is affected by environmental factors such as day length (light and dark periods), temperature, and rainfall (Gardner, 2003). These factors are generally geographically specific. Light, along with cold temperatures (chilling units) affect flower initiation by diverting nutrients such as sucrose within the plant (Gardner and Bertling, 2005; Salisbury and Ross, 1992). Furthermore, changes in the levels of growth substances (abscisic acid, auxins, cytokinins, ethylene, gibberellins, etc.) as well as changes in the levels of metabolites and cofactors, lead to synthesis of substances that invoke the initiation of flower primordia.

Optimum geographical site location along with grafting and the application of plant growth hormone (Paclobutrazol) should yield ideal flowering results (Gardner and Bertling, 2005; Moncur and Hasan, 1994). Within the context of this project, the effects of some interspecies and sub-species rootstocks on *E. nitens* were investigated. The tree growth of rootstock scions combinations and the effects of rootstocks on the budding time on the scions varieties were also examined.

The aim of this chapter was to investigate the use of interspecies and sub-species rootstocks for early flowering in *E. nitens*, and to select rootstocks that yield good results within South African climatic conditions.

3.2 Materials and Methods

3.2.1. Rootstock Selection

As discussed in Chapter 2, three scions were grafted onto six rootstocks.

Scions

1. Barrington Tops (47): This provenance is renowned as a precocious and strong flowerer.
2. Barren Mountain (136): this provenance has a medium flowering level compared to the other two provenances.
3. Tallaganda (62): This is the least precocious provenance and weakest flowerer of the three scions.

Rootstocks

1. *E. grandis* (R1): This species is reported to be a vigorous grower (Myers et al., 1996) with good rooting properties, and is a precocious flowerer (15 to 20 months) that flowers well in tropical regions.

2. *E. nitens* x *E. grandis* (R2): This has an *E. nitens* mother and an *E. grandis*-like appearance, and was selected for its excellent rooting properties, early flowering abilities (+2 years), and its probable compatibility with an *E. nitens* scion.

E. globulus (R3): This is an unimproved provenance grown from Australian seed, with slow vegetative growth (dwarfed) and it flowers in only 15 to 20 months.

E. nitens (R4): This *E. nitens* is a commercial seedling. It is a vigorous grower and time to first flower is between ten and twelve years, and was selected as a Control.

E. grandis x *E. nitens* (R5): The female parent is an *E. grandis* parent but looks phenotypically similar to *E. nitens*. It is a precocious flowerer (+3 years).

E. nitens (R6): This E. *nitens* seedling (probably selfed), is highly precocious under South African conditions.

3.2.2. Field site for an *E. nitens* orchard for optimal flowering

The chosen site was situated on Shandon farm in Mount West, near Mooi River, in KwaZulu-Natal, South Africa at latitude -29° 13' 6.93”, longitude 30° 7' 49.19”, and an altitude of 1622m. It is a site with a low mean temperature (MAT: 15.0°C) and low rainfall (MAP: 781mm). It has a slope that faces southwest to east, and receives shade early in the afternoon, summer and winter. According to Gardner and Bertling (2005), certain high altitude sites with similar altitude, latitude, longitude, MAP, and MAT as the chosen site are either ‘good flowering’ or’ bad flowering’ sites. It appears that the only major environmental difference between the two site types pertain to the degree of topographical relief and associated winter day/night temperature amplitudes. The ‘good flowering’ sites are always in exposed positions on crests, or are on cool, shady slopes that face southwest to east, where air drainage is good and winter day/night temperature amplitudes are low. In contrast, the ‘poor flowering’ sites occur on flatter terrain where winter day/night temperature amplitudes are relatively high, and cold air settles at night. This phenomenon suggests that uniform cool conditions in winter promote flowering in *E. nitens* more than extreme daily variances in temperature.

3.2.3. Graft combinations in the field trial

The grafted plants chosen for the field trials were plants that survived the final stages of grafting. Due to the low survival rate of grafted plants for some rootstocks, the numbers of the grafted combinations in the field were unequal.

A randomized blocks design was employed for the field trial. The trial initially contained six replicates of two tree plots, representing each graft combination, providing sufficient plants were available.

The planting site was prepared by brush cutting and ground clearing. Planting holes were dug with spades, 0.5 m deep, and 300 mm wide. A 10 L bucket of water was emptied into each planting hole directly prior to the trees being planted. Subsequent to planting, the hole was covered and a bracket was created around each planted tree to hold water. Tree combinations were planted from the 23rd February until the 25th February 2010. The tree combinations were planted simultaneously with a surrounding border, which consisted of two rows of commercially grown *E. nitens* seedling plants. The trees were watered once weekly for two weeks after planting, due to a lack of rainfall during the initial reestablishment of the trees for the field trial.

3.2.4. Field parameter measured

Flowering

Flower bud scoring was assessed in August 2010 and August to December 2011. The presence or absence of buds was scored, denoted by “0” for “no flower bud” and “1” for “flower bud present”.

Bud abundance was scored from 0 to 4.

0 – No buds,

1 – Very light bud load,

2 – Light bud load,

3– Medium bud load,

4– Heavy bud load.

A Kruskal–Wallis test was used for analysis of variance of bud abundance on the different rootstocks in the field trial because the measurement variables did not meet the normality assumption of an anova. The Kruskal-Wallis Statistic: H was calculated using statistical software, Genstat 14.1.

3.3. Results

3.3.1. Flowering characteristics

3.3.1.1. Budding percentage of trees on different rootstocks

Amongst the ungrafted trees, R5 had the highest budding percentage, followed by R3 and R2, respectively (See Figure 3.1). Amongst the grafted trees, R2 had the highest budding percentage followed by R4, R6, R5, R3 and R1. The budding result for grafted R3 trees was not substantial due to the large number of R3 grafts that died during the grafting process, which resulted in a low R3 combination count in the field trial. For this reason, R3 will not be considered in the discussion of the budding result.

The data collected was not sufficient for a comprehensive statistical analysis for the presence of buds in the rafted combinations to the corresponding rootstocks. This led to the result being analysed by simply looking at the means to see which rootstock performed better on average to induce buds. Based on the results, R2 performed best overall with regards to bud presence.

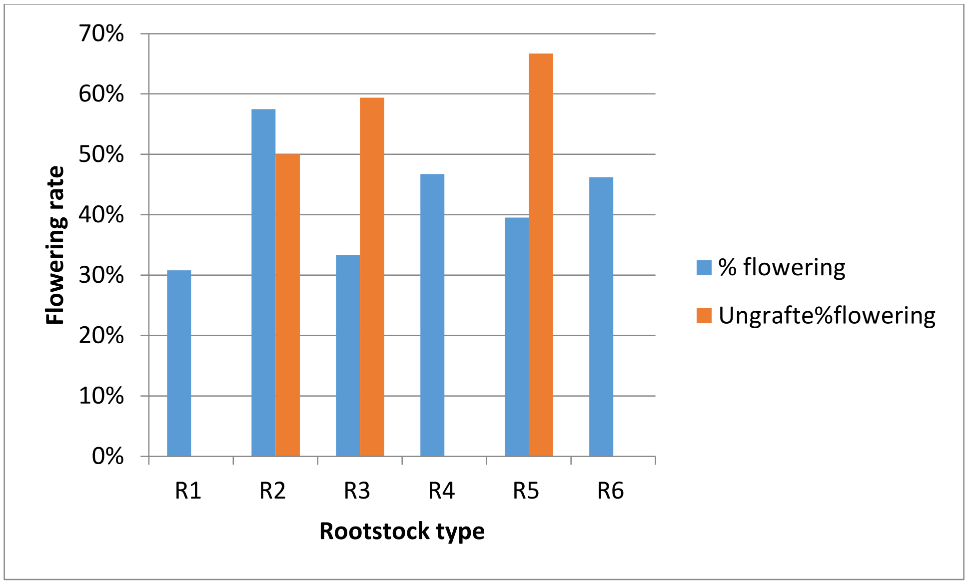

Figure 3.1: Comparative rootstock effect on the formation of flower buds of grafted trees versus un-grafted rootstocks

Figures 3.2-3.4 display the performance of the different rootstocks for each scion type. For Scion 47 on different rootstocks, Rootstock R2 performed the best with 80% budding, followed by R4 and R6 at 60%, then R5, and R1at 50% and >30%, respectively. For Scion 62-T grafted on different rootstocks, R5 performed best (<30%), followed by R4 (<20%), R2 (>10%), and R1 (<10%). For Scion 136-BM, R2 was the best rootstock (80%) followed by R6 (<80%), R4 (>60%), R1 (50%), and R5 (40%).

When Scions 47 and 136-BM were grafted onto R2, the plants attained an astonishing 80% incidence of flower buds. In contrast, Scion 62-T formed few flower buds with any of the rootstocks. Scion 62-T is notorious for being a shy flowerer in South Africa when compared to the other two scions.

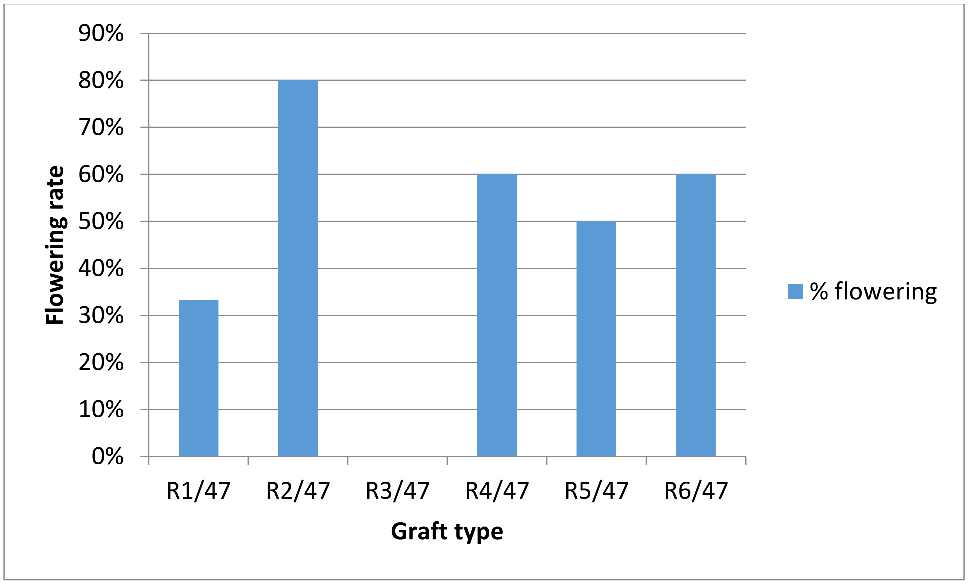

Figure 3.2: Rootstock effect on the budding of the Scion 47-BT trees.

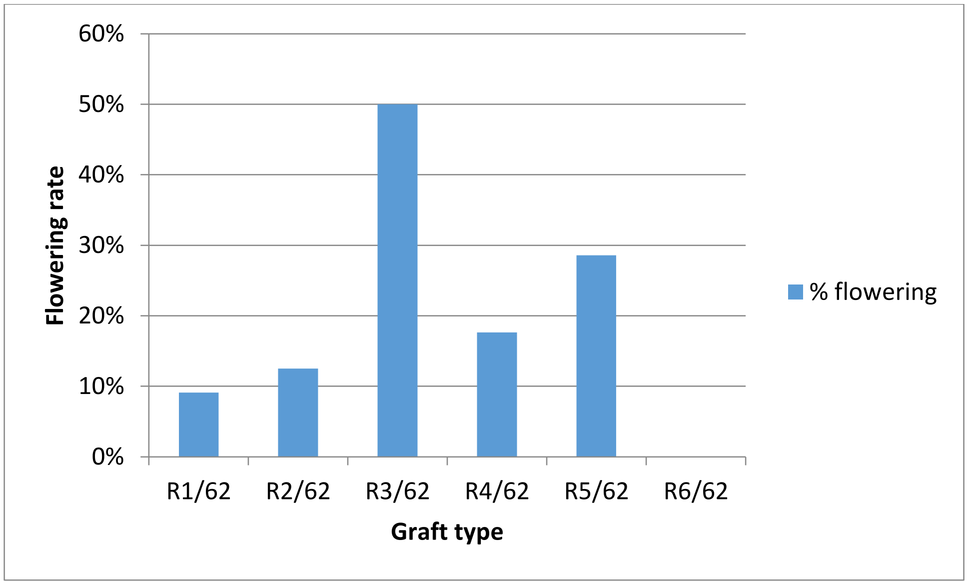

Figure 3.3: Rootstock effect on the budding of the Scion 62-T trees.

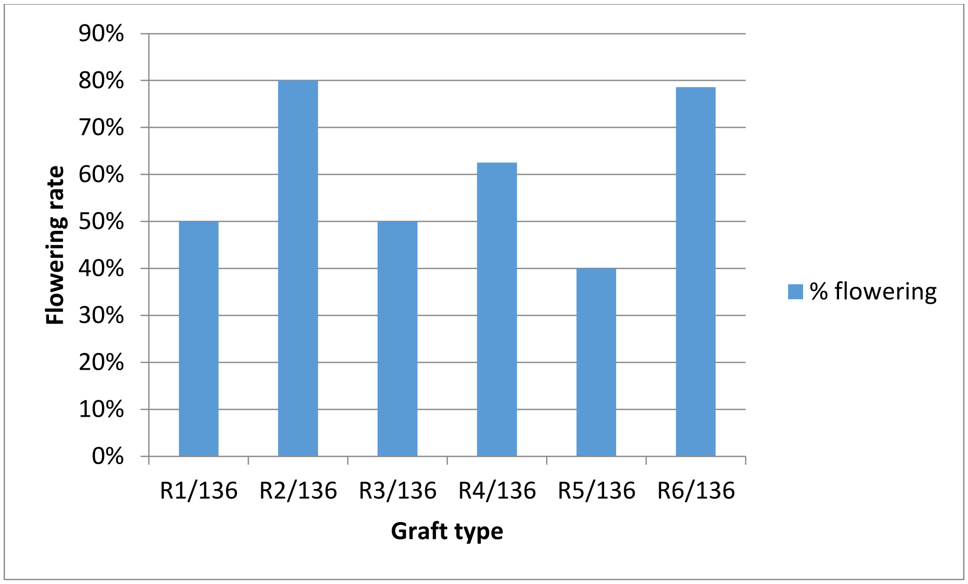

Figure 3.4: Rootstock effect on the budding of the Scion 136-BM trees

3.3.3.2. Abundance

Group factor: Treatment (TMT)

Value of H = 16.92

Adjusted for ties = 20.40

Table 3.1: Kruskal-Wallis one-way analysis of variance of bud abundance on the different rootstocks in the field trial.

Sample	Size	Mean rank
R1	9	18.44
R2	13	39.38
R3	4	38.38
R4	11	16.55
R5	6	32.5
R6	15	33.5

Variate: Flower
Group factor: TMT
Value of H = 1.808
Adjusted for ties = 2.534

Sample Size Mean rank
Group 11 22 63.64
Group 22 30 73.73
Group 33 5 82.80
Group 44 30 64.67
Group 55 13 68.15
Group 66 36 68.44

Degrees of freedom = 5
Chi-square probability = 0.771

The chi-square test was used to test for the "goodness to fit" between observed and expected data obtained from flowering results. The probability suggests that the effect of the different rootstocks on the rate of precocious flowering in the *E. nitens* scion was due to chance. This suggests the presence of additional factors which may play a role in the early flowering of *E. nitens* grafts.

3.4. Discussion

In this study, the R1 species displayed the lowest budding rates with Scion 47-BT (>30%), and Scion 62-T (<10%), which were classified as the strongest and weakest flowerers respectively (See Figures 3.2 and 3.3). Among the other rootstocks, it had the second lowest budding rate (approx. 50%) with Scion 136-BM, the medium flowerer (See Figure 3.4). The R1 species had the lowest grafting success out of all rootstocks. The species (*E. grandis*) was reported to be a vigorous grower (Myers et al., 1996) with good rooting properties, and a precocious flowerer that produces buds in roughly 15 to 20 months. However, it was also reported that R1 flowers at its optimum rate in tropical regions, hence the temperatures used in this study may have been too low for the rootstock to achieve its maximum flowering potential. It will be interesting to test the effect of R1 rootstocks on *E. nitens* in tropical regions

The R2 species had the highest budding rates (80%) with Scions 47-BT and 136-BM, the strongest and medium flowerers respectively (See Figures 3.2 and 3.4). It had the second lowest budding rate (>10%) with Scion 62-T, the weakest flowerer (See Figure 3.3). The R2 natural hybrid clone (*E. nitens* x *E. Grandis)* was chosen for this study owing to its excellent rooting properties and early flowering abilities. Although grafting success was only 37% (See Chapter 2), R2 had the highest bud presence out of all rootstocks. This budding success may be attributed to it being a strong rootstock, as strong rootstocks are linked to a reduction in the number of days to flowering (Notaguchi et. al 2008). The physiology of one *E. nitens* parent was assumed to improve the grafting compatibility of the rootstock. This is supported by the observation that R2 was the only rootstock where budding presence in the grafted species was higher than in the ungrafted species (See Figure 3.1).

The R3 species (*E. globulus*) has a slow vegetative growth and flowers in approximately 15 to 20 months. As a result, the *E. globulus* maintained its high precocity and slow rate of growth. However, during the setting up of the field trial, the R3 rootstock performed poorly and very few plants were actually established and remained in the trial.

Rootstock R4 (a selection of *E. nitens*) is a commercial seedling that was used as a control in this study. It is classified as strong rootstock and a vigorous grower, and slow to flower (10 to 12 years). Scions 47-BT and 136-BM performed surprisingly well on R4, whereas Scion 62-T performed poorly on R4.

R5 was classified as a miscellaneous rootstock because several of the grafted plants dies in the field not allowing enough data to be collected. Despite this, R5 was still considered a precocious flowerer, and displayed modest budding (50%) with Scion 47-BT, the strongest flowerer. The second highest budding percentage was observed in Scion 62-T grafted onto R5, despite this scion being the weakest flowerer. The lowest budding percentage (40%) was observed when R5 was grafted with Scion 136-BM, the medium flowerer.

Rootstock R6 (a selection of *E. nitens*) was chosen for this study based on its positive flowering results in previous trials by the ICFR compared to other *E. nitens* species. It was categorized as a strong rootstock due to performances in commercial forestry. It displayed 60% budding percentage with Scion 47-BT, the strong flowerer. No budding was recorded with Scion 62-T, the weakest flowerer, although budding with Scion 136-BM, the medium flowerer was among the highest recorded (<80%).

Scion 62-T displayed the lowest flowering potential of all three scions as predicted, despite having the highest grafting success (66%). Budding was highest in this scion with semi-dwarfed rootstock R3 (50%) and lowest with R1 (<10%), which was also semi-dwarfed. In contrast, Scion 47-BT had the lowest grafting success (30%) and the highest flowering potential. Scion 47-BT was documented as the most precocious *E. nitens* species in all ICFR flowering trials. Its highest budding percentage was recorded with strong rootstock R2 (80%), and its lowest was with semi-dwarfed rootstock R1 (>30%). Scion 136-BM had both medium flowering potential and medium grafting success (47%) compared to the other two scions. Its highest budding percentage was also with the strong rootstock R2 (80%) and it lowest was with miscellaneous rootstock R5 (40%).

It is largely accepted that the mechanism behind plant flowering involves the transfer of the flowering hormone florigen from the leaves to the shoot apical meristem. In several plant species, this mechanism is regulated by photoperiods, which is the relative exposure of leaves to periods of light, also referred to as long days (LD) and short days (SD). Although the identity and nature of the leaf-derived hormone florigen (or flowering stimulus) remains to be elucidated, numerous graft experiments have provided evidence for the transmission of signals, genetically transcribed flowering-related proteins (e.g. flowering locus T (FT) gene found in *Arabidopsis thaliana*), and other nutrients via the vascular system to induce flowering (Suarez López, 2005; Corbesier et al., 2007).

In spite of the rootstock and scion performance, overall success of this study is attributed to the location and environmental conditions of the chosen study site. The level of topographical relief and winter day/night temperature amplitudes are key factors to consider when distinguishing between 'good flowering' and 'bad flowering' sites (Moncur and Hasan, 1994; Gardner, 2003; Barrington, 2005; Gardner and Bertling, 2005; He et al., 2007) .

3.5. Conclusion

Several reports are available that present promising results for *E. nitens* breeders who wish to increase their rate of generation turnover. Eucalypts, like other plants, contain a vascular system that functions as a long-distance signalling network and transport pathway, and previous grafting studies have provided evidence for the role of this system in processes such as floral induction. This study utilised grafting techniques to investigate flower regulation via photoperiods, which is proposed as a classic example of long-distance signalling. Results indicate that higher budding rates and quicker time to first flowering were achieved with strong rootstocks compared to semi-dwarfed rootstocks, when grafted to strong and medium flowering scions. In addition, the possibility of grafting material to various distantly related species was also demonstrated. It was shown here that the size and girth of rootstocks play a key functional role in budding success, but not necessarily grafting success. Rootstocks classified as semi-dwarfed, with the shortest height and collar diameter, displayed less flowering in contrast to rootstocks classified as strong, although a similar pattern was not observed for grafting success. The study also showed that the location site and temperature amplitudes chosen were sufficient to induce flowering, which was noted as the time to first budding was observed at approximately 20 months after field planting. This adds evidence and provides rationale for continued research into growing conditions for optimum floral production in eucalypt species. The natural hybrid clone R2 (*E. nitens* x *E. grandis*- NH58), a strong rootstock, produced the highest overall budding percentage when grafted to Scion 47-BT, which is documented by ICFR as the most precocious flowerer. Based on results obtained from this study, further investigations into the use of various precocious *E. nitens* hybrids are warranted, such as the study commenced in 2011 involving the use of a very precocious *E. grandis* x *E. nitens* hybrid (PP2348) from NCT as a rootstock. Preliminary results already show floral induction from the ungrafted rootstocks of PP2348 after just 8 months of being established in a sub-tropical field trial.

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4: DISSERTATION OVERVIEW

4.1. Introduction

Rootstock age and size, nutrients and environmental conditions have been noted as significant elements for consideration in the grafting success of in eucalypts in South Africa. Identification of the specific conditions for facilitating the ease of grafting in eucalypts provides an important knowledge base for orchard management as well as manipulated flowering research. This study focused on investigating the success of grafting of interspecies and sub-species rootstocks as well as assessing rootstocks for early flowering in *E. nitens*. The study outcomes have the potential of facilitating breeding programs of *E. nitens.*

4.2. The specific objectives were:

In order to achieve this project's aim, the following objectives were addressed:

1. Grafting interspecies and subspecies rootstocks and scions of *Eucalyptus* taxa and provenances,
2. Monitoring the agro-meteorological conditions in an optimized environment tunnel and measuring grafting success, and
3. Investigating the factors affecting early flowering in *E. nitens*¸ specifically using dwarfing and precocious rootstocks.

3.3. Summary of research findings

4.3.1. Literature review

In orchard management, cultural techniques, involving both physical and chemical manipulation are important for increasing flowering, seed production and improving harvesting. Physical manipulation such as pruning, girdling and grafting have been well document on their ability to facilitate floral induction in the eucalyptus trees (Gardner, 1996; Pinkard and Beadle, 1998; Williams *et al.*, 2003; Therios, 2008). Chemical manipulation with paclobutrazol was also found to enhance flowering in *E. nitens* (Moncur *et al.*, 1994; Moncur and Boland, 2000).

To further improve the efficiency of orchard management, understanding the mechanism controlling floral induction is considered essential for the development of flowering management practices in South Africa (Barrington, 2005). The timing of cultural practices to coincide with the appropriate cultural conditions such as suitable photoperiods and low temperatures (Chiaperro and Swain, 2003; Gardner and Bertling, 2005). Other important cultural conditions include moisture stress and mineral nutrition (Chambers *et al.*, 1997; Campion *et al.,* 2006). It is important to understand the interaction among all the cultural conditions to effectively manage the orchard. According to Gardner (2003) in order to successfully induce a flowering response in *E. nitens* in South Africa, a selection of a planting site above 1400 meters, deep rich soils, with a record of exceptionally cold winters to achieve the level of cumulative cold requirement implicated for floral induction, plus good rainfall (>950mm).

Furthermore, dwarfing rootstocks has been shown to enhance flowering (Atkinson and Else, 2001). Dwarfing rootstocks induces precocious scion flowering. Not only does it reduce time taken to flower but also a large numbers of flowers are produced per unit tree compared to a normal seedling tree (Long and Kaiser, 2010). The effect of dwarfing rootstocks has been documented to improve crop production and it should be considered essential as a technique for enhance seed production in *E. nitens.*

4.3.2. Optimizing conditions for the grafting of *E. nitens* using various scions and rootstock

Based on the literature, grafting success in *E. nitens* is a function of the rootstocks and environmental conditions during the grafting process (Fan et al., 2006; Bomfim et al. 2011; Cookson et al. 2013; Trinchera et al. 2013). This further emphasizes the importance of empirically investigating the optimum conditions for grafting success in *E. nitens.*

This section of the study involved assessing the grafting success of interspecies and sub-species rootstocks of *Eucalyptus* taxa and provenance, considering three scions chosen for their differential flowering ability, combined with six different rootstocks. The environmental conditions in the greenhouse tunnel were controlled and optimized.

The controlled (optimized) environment greenhouse tunnel provided excellent conditions for grafting. The mean take of grafts of 44% was substantially better than the 14% grafting take achieved in a nearby shade house with highly variable environmental conditions. Under the controlled environmental conditions, grafting success was between 36% and 67%, depending upon the scion x rootstock combinations. It was also noted that the position of the plants in the greenhouse tunnel did not significantly affect grafting success. Grafting in early and late spring 2009 also did not show a significant influence on the grafting success.

This study shows that grafting rootstocks of the same species or a related hybrid as the scions provides for better physiological compatibility, leading to high levels of grafting take.

4.3.3. Factors affecting early flowering in *E. nitens*, using dwarfing and precocious rootstock

The flowering characteristics of *E. nitens* are affected by geographical characteristics such as day length (photoperiods), topography as well as temperature (Moncur et al., 1994; Barrington, 2005; Gardner and Bertling, 2005). The selection of an optimum geographical location combined with grafting and chemical manipulation, for example using paclobutrazol, should therefore yield ideal flowering results.

This research involved the use of grafting techniques to investigate flower regulation via photoperiods. Temperature and site location were also considered, and monitored, as factors influencing floral initiation. The recorded results suggest that higher budding rates and faster flowering were achieved with strong rootstocks compared to semi-dwarfed rootstocks, when grafted to strong and medium flowering scions.

This study noted that the selected location site and temperature amplitudes were sufficient to induce flowering, which was recorded as the time to first budding and was observed at approximately 20 months after field planting.

The natural hybrid clone R2 (*E. nitens* x *E. grandis*- NH58), a strong rootstock, produced the highest overall budding percentage when grafted to Scion 47-BT, which is documented by ICFR as the most precocious flowerer. However, without disregarding rootstock and scion performance, an additional attribute pertaining to attaining floral induction is attributed to the location and environmental condition of the selected study site.

4.3.4. The way forward

The information unearthed during this study prompts that future research be carried out to:

1. Assess the use of fertilizers combined with optimum physiological and environmental conditions during grafting, to investigate the impact of these conditions on grafting take,
2. further investigate the use of various precocious *E. nitens* hybrids in enhancing floral induction, and
3. further investigation into various growing conditions (and optimization of these conditions) for desired floral production in eucalypt species.

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