Vegetative propagation of *Corymbia henryi* and its hybrids in South Africa through cuttings and mini-grafting techniques

By

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DISSERTATION ABSTRACT

Eucalyptus species are the most commonly planted hardwood species in South Africa, composing 42% of the total plantation area in the country but as the forestry planation areas in South Africa are not expanding, the only way to increase timber supply is to use the available land productively. This also increases the demand to efficiently propagate eucalyptus species, in an easy and economically viable way. This can be achieved through optimising vegetative propagation techniques which ensures mass multiplication of superior genotypes/phenotypes and the maintenance of individual characteristics in order to ensure that a plant is genetically identical to the original/donor plant. Spotted gum (*Corymbia* species) and their inter-specific hybrids have been identified as promising taxa for commercial forestry in South Africa due to their superior survival and growth across a broad range of edaphic and climatic conditions. The major benefits of these hybrids include superior growth characteristics, disease and insect resistance and frost tolerance, making them desirable for propagation. The hybrids of *Corymbia torelliana* and *C. citriodora* are some of the extensively used *Corymbia* hybrids since they combine frost tolerance (*C. citriodora*) and disease resistance (*C. torelliana*). The widely used vegetative propagation techniques include propagation through rooted cuttings and grafting.

The aim of the study was to investigate the vegetative propagation of *Corymbia henryi* (*C. henryi)* hybrids through the rooting cuttings, and the grafting of *C. henryi*.

The propagation of the hybrids through cuttings was carried out in three experiments, comparing cutting material collected from coppice stumps and ramets. The cuttings were collected from 14 *C. henryi* hybrid genotypes grown in Zululand. The cuttings were maintained in a rooting tunnel for four weeks before being placed in greenhouse conditions to acclimatize to the natural environment. The use of ascorbic acid (AA) (40 mg L^{-1}) with Seradix® 2 (IBA 3g kg^{-1}) yielded the highest rooting incidence, ranging from 37.5-55.5%, with the lowest rooting resulting from the application of propiconazole with Seradix®2 (IBA 3g kg^{-1}) at >2%. The combination of AA with IBA has shown to be synergistic, since AA acts by protecting the rooting hormone from oxidation therefore allowing it to enhance tolerance of the plants to greenhouse conditions and alleviate stress. The cuttings selected from coppice had a higher rooting survival than those collected from hedge material. The genotype had an effect on the rooting success of cuttings, with the highest rooting percentage occurring with C. *torelliana* x C. *citriodora* subsp. *variegata* hybrids, with rooting ranging from 25-70%, while a *C. torelliana* x C. *henryi* hybrid had the least rooting success (%). The *Corymbia* species are considered difficult-to-root therefore the results have shown that these species can be propagated through rooting if the better rooting hybrid genotypes are selected. There is still a need to perform more

trials to test the genotypes that have been found to root better in order to reach the commercial requirements of rooting rate of 70% and above.

The propagation through grafting allows for the union of more than one genotype, whether belonging from the same species or different species and offers propagation of species which may be hard-to-root therefore cannot be produced through cuttings. The grafting experiment was carried out to optimize grafting techniques by comparing mini-grafting and conventional grafting techniques for *Corymbia henryi*. The rootstocks were grown and maintained at the ICFR nursery until time of grating. The scion material was collected from the Zululand region from *C. henryi* provenance mix. The grafting and mini-grafting was carried out in the grafting tunnel at the Institute for Commercial Forestry Research (ICFR) nursery, which had continuous mist for the duration of the experiments. The methods applied on the grafts were cleft and splice grafting. The evaluations made were based on the grafting method applied, comparing age of the grafts (mini- grafting and conventional grafting) and the effect of different treatments applied onto the grafts which included the control treatment, use of Parafilm® to tie the graft union, covering the grafts with polyethylene plastic (for one week) and use of an antitranspirant, Vapor Guard®. The control treatment, where grafts were tied with Parafilm® had the highest graft survival (33.3%), with the lowest graft survival on grafts covered with polyethylene plastic (%).Grafting onto four- month old seedlings(mini-grafting) had the highest survival at 55.6%, compared to grafting onto 10-month old seedlings (conventional grafting), with a survival of 22.2%.

Mini-grafting has been tested over some species due to its ease of handling and is being evaluated for its potential in the propagation of *Corymbia henryi*. This type of grafting offers advantages of efficient management of plants that are grown in the nursery, allowing ease in irrigation, nutrition and pest disease control. The use of younger seedlings allows for grafting to be a commercially viable technique due the reduced time to grow the plant, flexible and pliable cambium layers of younger rootstocks and rapidness of grafting these younger seedlings. Mini-grafting was evaluated with the use of commercially available anti-transpirants and antioxidants, to assess effect of these treatments on graft success. The different ancillary treatments applied were the control, use of Parafilm® to tie the graft union, the use of anti-transpirants such as Nu-Film 17®, Vapor Gard® and Greenstim®, and the use of an antioxidant, ascorbic acid. Foliar application of ascorbic acid had the highest graft survival (60%) compared to the other treatments applied. The use of anti-transpirants in grafting of *C. henryi* was not successful therefore more research needs to be done on the commercially available anti-tranpirants in alleviating water stress in *Eucalyptus* species, as each crop may have different requirements for anti-transpirant application and doses may differ from one crop to another.

Vegetative propagation of *Corymbia henryi* and its hybrids shows some potential for future use, but more research needs to be done to optimise these techniques to be able to have an impact on the commercial scale and in research outputs.

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DEDICATION

To Hlelolenkosi and Ndalwenhle, may you always remember:

“The beautiful thing about learning is that no one can take it away from you” B.B. King

Love always, Mummy.

Contents

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THESIS INTRODUCTION

Eucalyptus species are the most commonly planted hardwood species in South Africa and there is a need to ensure a reliable supply of superior clones of these tree species to provide a sustainable source of timber products. In South Africa, commercial forestry contributes ZAR 70 billion per annum to the gross domestic product (GDP). Most of the *Eucalyptus* plantations are grown for the exportation of wood chips to Japan to manufacture paper. The timber products play an important role in the wood and pulp industry, in gardening and for ornamental purposes. Eucalypt species can adapt to various environmental conditions and are known for their rapid growth and their ability to re-sprout after harvesting.

Due to the growing demand for wood products, there is a need to increase plantation forests, and vegetative propagation methods allow for the rapid production of elite genotypes with desirable characteristics. Propagation of *Eucalyptus* species through seeds has been used but there have been limitations because seedlings need to be established in wet soil (Hettasch *et al*., 2005), under conditions that are also favourable to the growth of parasitic fungi, resulting in damping-off, and due to the limited amount of seed available. The use of *Eucalypt* hybrids developed through vegetative propagation techniques allows for the development of genotypes with special combinations of properties, such as adaptation to a wide variety of environments, disease resistance, and specific timber properties (Hettasch *et al*., 2005).

The *Corymbia* species and their F1 hybrids are gaining importance in the forestry industry due to their wide adaptability to marginal and sub-tropical regions (Lee, 2007). The hybrids offer advantages over their parentals as their properties include superior growth, disease, insect resistance, and frost tolerance (Lee 2007, Dickinson *et al*., 2010). The hybrids of *Corymbia torelliana* and *Corymbia citriodora* are some of the extensively used *Corymbia* hybrids since they combine frost tolerance (C. *citriodora*) and disease resistance (*C. torelliana*).

High performing *Eucalyptus* clones have rooting difficulties in (recalcitrance) (do Prado *et al*., 2019). There is therefore a need to find ways to enhance the production of these elite genotypes, by promoting adventitious roots systems and their development (do Prado *et al.,* 2019).

There is a need to investigate methods to ensure the elite genotype of the *Corymbia* hybrids are propagated effectively to meet global demand for species with properties essential for the establishment of plantation forests.

Project Objectives

The two major areas of interest were to improve the rooting the *Corymbia* hybrids from cuttings, and to establish an effective mini-grafting technique, which would allow for grafting all-year round. Therefore, this study set the following specific goals:

Rooting of *Corymbia* hybrids:

1. To assess the effect of antioxidants and rooting agents applied to cuttings of *Corymbia* hybrids to identify the best of the 6 treatments used;
2. To determine the best rooting genotypes among the *Corymbia* hybrid selections used.
3. To determine best rooting material to use between coppice vs ramets.

Mini- grafting of *Corymbia henryi*:

1. To compare conventional grafting (using 10-month old seedlings) with mini- grafting (4-month old seedlings);
2. To determine the best grafting method to use between splice and cleft grafting;
3. To determine the most effective treatment to apply on the grafts to alleviate stress and promote successful graft-take.

The referencing system used in the chapters of this thesis is based on the Harvard system of referencing (De Montfort University), and follows the specific style used in the Biological Control Journal.

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is the dominant thesis format adopted by the University of KwaZulu-Natal because it facilitates the publishing of research from a thesis far more easily than the older monograph form of a thesis. As such, there is some unavoidable repetition of references and some introductory information between chapters.

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CHAPTER ONE: LITERATURE REVIEW

1.1 Eucalyptus history and importance

There are over 800 eucalypt species which belong to the myrtle family (Myrtaceae), sub-family *Leptospermoideae*, which have been recently grouped in the Eucalypteae. *Eucalyptus* species are classified into 3 genera: *Eucalyptus* (>740 sp.), *Corymbia* (113 sp.) and *Angophora* (14 sp.), closely related genera within *Eucalypteae* (Vilasboa *et al.,* 2019), which are native to Australia and a few Pacific Islands. One of the first eucalypt species to be cultivated from seed outside its native range in the United Kingdom was *Eucalyptus obliqua*, with *Eucalyptus globulus* being the most cultivated of the eucalypts (Trueman and Richardson, 2011). *Eucalyptus globulus* is the primary source of global eucalyptus oil production, China being the largest commercial producer (Trueman and Richardson, 2011). The major Eucalypt growing countries are China (170 million ha), India (2.5 mil. ha), and Brazil (3.7 mil ha) (Oballa *et al*., 2010). South Africa has the largest area under Eucalypt plantations in Africa, covering almost 1.2 million hectares (1.1% of S.A. land area) (Albaugh *et al*., 2013). These plantations were first established in 1875 after recognizing that the demand for timber was increasing and exceeding supply from the indigenous forests (Albaugh *et al*., 2013). The five commercially grown species which contribute to most of the eucalyptus plantations in S.A are *Eucalyptus grandis, E. nitens, E. smithii, E. macarthurii*, and *E. dunnii*, with *E. grandis* and its hybrids being the most cultivated of the eucalypts (Melesse and Zewotir, 2017). Commercial forestry contributes ZAR 70 billion per annum to the gross domestic product (GDP) in South Africa (Dept. of Agriculture, Forestry and Fisheries [DAFF], 2011; van der Berg, 2017). Forestry contribution to GDP varies per province in South Africa: Mpumalanga (6.0%), KwaZulu-Natal (5.3%), Eastern Cape (0.7%) and Limpopo (0.4%), with the Western Cape contributing the least (0.3%) (Forestry SA, 2017).

In South Africa, eucalypts are grown for industrial purposes, for the exportation of wood chip to Japan and to manufacture paper (Kilomo Trust, 2011). In the eastern and southern regions of Africa, *Eucalyptus* is the main supplier of firewood poles which are used in construction, for timber and as charcoal. The eucalypt species are a valuable sink for carbon dioxide, with the hybrids in Brazil showing a high carbon capturing capacity, able to sequester close to 80 tons of carbon dioxide per hectare (FAO, 2014; Vilasboa *et al.,* 2019). These species also play an important role in the wood and pulp industry, in forestry, gardening and for ornamental purposes (Di Battista *et al*., 2019). The eucalyptus oil and its ingredients are included in many commercial treatments for coughs and the common cold, as a mouthwash, for treatment of gum disease and as an ingredient in insect repellent (Sappi FAQ). Eucalypts act as shelter for livestock, as windbreaks, and contribute to the honey bee forage and pollination services (Allen *et al*., 2017; du Toit *et al*., 2017). Beyond these multiple uses, the eucalypt species can adapt to various environmental conditions, are known for their rapid growth and their ability to re-sprout for additional harvests (Melesse and Zewotir, 2017), all of which make them important species to plant.

1.2 Eucalyptus hybrids and significance

The hybridisation of eucalyptus species began in South Africa in 1984 and has become an important part of the commercial forestry sector (Denison and Kietzka, 1993a, 1993b; Lambrechts, 2018). The biggest gains in forestry are due to clonal distribution of eucalypt hybrid genetic material. Hybridisation of species is the cross-breeding of different parental species, to ensure high production of the 2nd generation genetic material. *Eucalyptus* hybrids have developed significantly because of potential genotypes with special combinations of properties contributing to the increased genetic resources, such as specific timber properties, disease resistance and greater vigour on specific sites compared to the combinations of the same properties in the pure species (Hettasch *et al*., 2005; van der Berg, 2017). Some examples of interspecific hybridisation occur between *Eucalyptus grandis x E. urophylla (GU),* where *E. urophylla* is resistant to more pests and diseases than *E. grandis*, while *E. grandis* grows much faster and has excellent stem form. Therefore, this cross produces a hybrid with a combination of the best traits from each parent, the hybrid possessing good stem form, fast growth and improved disease resistance (Melesse and Zewotir, 2017). This hybrid combination is planted in the subtropical coastal region of South Africa and plays an important role in pulp production in the forestry industry (van der Berg, 2017). The *Corymbia* hybrids and their F1 hybrids are increasing in importance due to their wide adaptability to tropical and sub-tropical environments and high-quality timber (Lee, 2007; Dickinson *et al*., 2012). The *Corymbia* hybrids used in forestry are primarily derived using *C. torelliana* as the maternal parent and either *C. citriodora* subsp. *variegata*, *C. citriodora* subsp. *citriodora* or *C. henryi* as the pollen parents. These hybrids offer advantages over their parental species such as good tolerance to *Quambalaria pitireka* (Ramularia shoot blight), moderate to high tolerance of erinose mite (*Rhambacuss* spp.), longicorn beetles (*Phoracanta* spp.), red-shouldered beetles (*Monolepta australis*), good frost resistance and superior growth over a wide range of environments (Dickinson *et al.,* 2012).

1.3 Vegetative propagation

There is a growing need to find better and more efficient ways to enhance productivity of *Eucalyptus* species. The supply of seed is limited by sparse, irregular flowering and expenses associated with pollination and seed collection in the canopy (Kilkenny *et al*., 2012). Sexual propagation through seeds is also limited by the risk of losing vital genetic properties from the donor plant (Henrique *et al.,* 2006). These limitations can be resolved using vegetative propagation, where elite clones from the diverse species available, can be reproduced.

Vegetative propagation ensures that the elite clones are genotypically and phenotypically uniform to the original or donor plant and that this uniformity is maintained (Vilasboa *et al*., 2019). Maintaining genetic uniformity is important, ensuring the production of high-value hybrid clones from the best performing species (FAO, 2014). Vegetative propagation enables the production of improvements in yield and quality, more rapidly as compared to the use of seed provenances or breeding (Jannat *et al*., 2017).

1.4 Vegetative propagation through cuttings

Root quality is an important factor to investigate since it governs the soil exploitation capacity of the plant and ensures that the trees are anchored in the soil protecting them from wind damage (Saha *et al*., 2019). The ability of a cutting to produce a quality root system is vital and ensures that the best clones are chosen during out-planting of the trees. Vegetative propagation by cuttings is widely used because of its low cost and ease of handling plant material (Titon *et al*., 2006; Saha *et al*., 2019). Propagation through cuttings is an important step because it allows for the rapid ramping up of selected material such as elite clones or genotypes (Gehlot *et al*., 2014). In vegetative propagation, healthy, vigorous, mature and young shoots with viable buds are usually chosen as a source of cuttings (Gehlot *et al*., 2014). The formation of adventitious roots is a response that is elicited by many plant species to biotic or abiotic stresses (Bellini *et al*., 2014) and is an essential process in the propagation of economically important woody species (Fett-Neto *et al*., 2001). This process is dependent on the ability of plant cells to dedifferentiate and give rise to a new plantlet provided the cells have an intact nucleus and cell membrane (totipotency) (Fett-Neto *et al*., 2001). Roots usually emerge through the formation of callus tissue, which is an irregular mass of parenchyma cells in various stages of lignification that develop at the end of a cutting, under favourable environmental conditions. (Taiz and Zeiger, 2002). In species that root easily, callus tissue and root formation are separate events that have no effect on one another, even though they both involve cell division. The formation of callus tissue may be a signal for adventitious root formation in some species, while it may delay the rooting process in others (Hartman *et al*., 2002). In *Eucalyptus* species, a negative relationship has been observed between the species' ability to form adventitious roots and the accumulation of secondary metabolites such as phenolic acids, flavonoids, terpenoids and steroids, that have a rooting inhibitory effect (Paton *et al*., 1981; Di Batista *et al*., 2019). The rooting failure in cuttings is also attributed to aging in certain species due to the accumulation of rooting inhibitors, as is found in certain eucalypt species such as *Eucalyptus grandis* (Di Batista *et al*., 2019). The inability to form roots is a problem in most Eucalypt species (including *Corymbia citriodora*, C. *torelliana*, *E. cloenziana*, *E. dunnii, E. globulus and E. nitens*) (Assis *et al*., 2004; Trueman and Richardson, 2008; Kilkenny *et al*., 2012) and these are characterised as "hard-to-root" since they lack the essential rooting substances or cell-sensitivity that should respond to endogenous rooting stimuli (Hartman *et al*., 2002). When the level of root inhibitors does not completely stop the root formation, eucalypt species may respond positively to the exogenous addition of rooting hormones , the most commonly used being indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA), which enhance rooting in a manner that may differ from one species to another, and among clones of the same species (Bennet *et al*., 1994; Di Battista *et al*., 2019).

1.4.1 Factors affecting the rooting of cuttings

Successful rooting is a process that is affected by several different factors, which are discussed briefly below:

1.4.1.1 Age of the tissues and mother plant

Stem cuttings are characterised either as hardwood, semi-hardwood, soft-wood or herbaceous based on the age of the mother plant from which they are obtained (Evans, 1999; Rice *et al*.,1990; Waman *et al*, 2019).It is important to ensure that parental trees receive adequate levels of water, light, warmth and fertilizer because the ability of a species to form roots may be maintained throughout its whole-life cycle or may decline significantly with age (Diaz-Sala, 2014; Di Battista *et al*., 2019). The observed decrease in rooting ability of cuttings from mature parent plants may be due to the loss of rooting competence which is associated with ageing (Naidu and Jones, 2016). This rooting failure could be associated with accumulation of rooting inhibitors (endoperoxides, phloroglucinol metabolites) (Gavrilan *et al*., 2001), lower sensitivity of tissues to exogenous application of rooting hormones and reduced endogenous concentration of rooting hormone promoters (Hartman *et al*., 1997). For high rooting success, the parent plants need to be maintained at the juvenile phase because in this physiological state, the plant material is known to have a greater capacity for the formation of shoots and adventitious roots rather than mature plant material (Naghmouchi *et al*., 2008).

1.4.1.2 Effect of nutrients

The mineral nutrition of cuttings is one of the key factors that limits adventitious rooting (Blazich, 1988). Although all nutrients influence the rooting process, some play a more influential role than others (de Almeida *et al.,* 2017).

Macro-nutrients

Nitrate plays a role in stimulating root branching, acting as a signal in modulating root growth and architecture (O'Brien *et al*., 2016; de Almeida *et al*., 2017). A high carbohydrate or high nitrogen level is recommended for optimal rooting of cuttings under mist while the stock plants are being maintained. A high carbohydrate to a low or moderate nitrogen ratio is optimal for rooting dormant hardwood cuttings (Hartman *et al*., 2002). The use of nitrogen (N) in rooting is dependent upon the mineral source and environmental conditions. A high ratio of C/N has a positive effect on rooting of eucalypts (Da Cunha, 2009; de Almeida *et al*., 2017). Nitrogen can be accessed from fertilisers in the form of nitrate and ammonium. The rooting of *E. globulus* showed that an increase in the concentration of nitrogen in the mother plant that resulted in improved rooting of mini-cuttings (Vasconcelos *et al.*, 2007).

Calcium (Ca) is required for cell division, membrane integrity and the transport of auxin (Bellamine *et al*., 1998; Maathuis, 2009; de Almeida *et al*., 2017). This macronutrient is considered a secondary messenger, responsible for sending signals for cell recruitment to form new root meristem tissues (Maathuis, 2009).

Potassium (K) is known to play a role in ensuring that cuttings do not lose water by preserving stomatal regulation and turgor pressure. In order to avoid water loss, which can result in desiccation of the microcutting, a correct concentration of potassium is needed (de Almeida *et al*., 2017). In studies of rooting *E. dunnii* mini-cuttings, Oberschelp and Goncalves (2016) found that high doses of K stimulated rooting, although reports by Vasconcelos *et al.* (2007) showed that the supply of K was not a limiting factor in rooting of *E. globulus* mini-cuttings.

Micro-nutrients

Iron (Fe) plays an essential role in the initial root formation phase where it supports respiratory metabolism and modulates auxin content, which aids in root growth and differentiation (de Almeida *et al*., 2017). In cuttings of *E. globulus*, the presence of Fe was found to promote the root induction phase, its activity decreasing the activity of certain peroxidases (heme enzymes), improving auxin availability, an important factor during this rooting phase. (Schwarmbach *et al*, 2005; de Almeida *et al.,* 2017). This activity is also linked to availability of manganese (Mn), which promotes enzymes (peroxidases) that break-down IAA and polymerize lignin (Requesens *et al*., 2014; de Almeida *et al*., 2017).

Cell wall synthesis, lignification and structure are associated with the activity of boron (B), which acts in the metabolism of the plant (Hansch and Mendel, 2009; de Almeida *et al*., 2017).

Zinc is required in rooting for the biosynthesis of tryptophan, an important auxin precursor (Blazich, 1988; de Almeida *et al*., 2017). The availability of Zn in higher concentrations has been found to favour rooting responses as it has an influence on auxin availability, this being seen in the microcuttings of *E. globulus*, where more roots were formed when the concentration of Zn was increased from 9 to 60 µm (Alscher *et al*., 2002; Schwambach *et al*., 2005; de Almeida *et al*., 2017).

Nutrients that are known to also play a vital role in the rooting process include calcium, which has been found to act as a peroxidase activator, manganese which is only needed in small concentrations, and other nutrients such as boron and zinc are essential for the rooting process. Monitoring the nutritional concentration in mother plant leaves is important to ensure there are adequate levels of macro and micro nutrients to improve the rooting success of cuttings (de Almeida et al., 2017).

1.4.1.3 Environmental factors

The amount of light and temperature received by the mother plant is important to ensure that maximum rooting is achieved (Rosa *et al*., 2018) because these two factors influence the uptake and metabolism of nutrients and hormones, which act as rooting promoters or inhibitors (Hartmann *et al*., 1997, Assis *et al*., 2004). The quality and amount of light received by the mother plant influences photosynthetic activity, and this has an impact on the production of substances important for the development of roots in cuttings (Taiz and Zeiger, 2003). Light and dark conditions influence the rate of entry of exogenous IAA, endogenous auxins, the breakdown of IAA and carbohydrate availability and distribution throughout the plant (Tam and Normanly, 1998; de Almeida *et al*., 2017). The effect of light may vary during the rooting phase. In rooting of *Populus tremula* x *tremuloides*, light had a negative impact on rooting success, only positively accelerating the sprouting of the cuttings (Stenvall *et al*., 2005; de Almeida *et al*., 2017).

Temperature influences several factors during the rooting process, from growth of the donor plant to development of roots (Kristiansen *et al*., 2005). The ability of auxins to moderate the effect of temperatures have been found, when *Eucalyptus saligna* (easy-to-root) and *E. globulus* (hard-to-root) were subjected to auxin treatments, both species were found to tolerate extreme temperatures, showing the ability of auxins to control stress caused by temperature (de Almeida *et al*., 2017).The stock plants of *Corymbia citriodora* and *E. dunni* cultivated at 28°C and 33°C respectively, resulted in a high percentage of rooted cuttings. The results from mother plants of cultivated *E. cloeziana* at 33°C showed an increase in the percentage of cuttings with adventitious roots, and each stock plant produced a high number of rooted cuttings (Trueman *et al*., 2013; de Almeida *et al*., 2017). In greenhouse experiments, daytime air temperatures ranging from 21°C-27°C and 15°C at night are recommended for rooting of species grown in mild climates (Hartmann *et al.* 2002; de Almeida *et al*., 2017) but higher day or night temperatures can be used in the cultivation of other species, e.g. tropical and sub-tropical species. Extremely high temperatures are not recommended during the rooting process because these may stimulate lateral buds before root development, which can trigger transpiration, causing leaves to lose too much water causing tissue necrosis (Hartmann *et al*., 2002; de Almeida *et al*., 2017). The rooting percentage of cuttings may reduce significantly during winter months, and this may be solved by increasing the environmental temperature of both mother plants and supplying additional light in the nursery and within rooting facilities.

1.4.1.4 Water and relative humidity

An adequate water balance is a key factor to ensure successful rooting. Uncontrolled water levels may result in poor aeration negatively affecting growth, whereas a lack of water may stop growth, leading to desiccation (Zalesny *et al*., 2004; Franco *et al*., 2011; de Almeida *et al*., 2017). The emergence of adventitious roots depends on the development stage of the plant, water temperature, flooding depth and species (Argus *et al*. 2015; Steffens and Rasmussen 2016; de Almeida *et al*., 2017). An adequate supply of water is required during the initial phases of root development, because the young cuttings are vulnerable when roots are not available. The high supply of water needs to be monitored and decreased over time because the new plant is formed to ensure that it is hardened and able to adapt in field conditions which may have lower access to water (de Almeida *et al*., 2017). Water is therefore critical to monitor since it needs to be accessible for optimum growth of the shoots but needs to be regulated to avoid disease (de Almeida *et al*., 2017).

Temporarily flooding the cuttings may save them from drought stress and helps ensuring adventitious root formation. When cuttings are soaked in water, the development of root primordia is stimulated, ensuring that the cuttings can absorb water (de Almeida *et al*., 2017). In greenhouses, the use of an intermittent mist system may lower the rate of transpiration, leaf temperature and physiological stress (Hartmann *et al*., 2002; Mateja *et al*., 2007; de Almeida *et al*., 2017). It is important to ensure phytosanitary control under intermittent mist systems since the high humidity can favour disease development (Assis *et al*., 2004).

Relative humidity (RH) has a significant effect in rooting because excess humidity may impair gaseous exchange in the cuttings and may cause an increase in disease development. In most cases, high RH levels decrease transpiration and water loss, which results in a high turgor pressure, which is beneficial in the rooting process because it allows for cell expansion and growth of the roots and prevents desiccation (Loach 1988; de Almeida *et al*., 2017).

1.4.1.5 Type of hormones required by the cuttings

Plant growth regulators have been successfully used in several plant species and have been found to improve the rooting ability of stem cuttings (Soundy *et al*., 2008). There are several plant growth regulators produced such as abscisic acid (ABA), gibberellins (GA), ethylene (ET), salicylic acid (SA), jasmonic acid (JA), brassinosteroids (BR), auxins, cytokinins (CK) and peptide hormones (Bari and Jones, 2009). Ethylene may be indirectly responsible for promoting rooting by stimulating cytokinin catabolism and may enhance sensitivity of the roots to auxin (Visser *et al*., 1996). Cytokinins are a group of phytohormones active throughout the plant's life cycle, involved in cell division, control of bud development and differentiation, shoot initiation and growth (Roitsch and Ehneß, 2000). The cytokinins may interact with auxins, ethylene and abscisic acid (Crowe *et al*., 1990, Izhaki *et al*., 1996), negatively affecting rooting. The combination of high auxin/low cytokinin ratio may allow for adventitious root formation, whereas a lower auxin/ high cytokinin ratio favours bud formation (Bonusa *et al*., 1994). Auxins, which dominate the rooting process of cuttings, are known to stimulate rooting, while cytokinins and gibberellins inhibit it. Auxins play an important role in the process of adventitious root formation because they are responsible for a few developmental responses such as cell extension and division on a cellular level, vascular differentiation, and root and shoot initiation, and tropisms (Hagen and Guilfoyle, 2002). Adventitious root numbers in *Eucalyptus* species can be promoted by auxins, where the most effective treatments induce one to four adventitious roots per eucalypt shoot. In the case of the application of auxins, great variation has been observed among the concentrations, formulations and forms of application (Wendling *et al.*, 2000; Fogaça and Fett-Neto, 2005; Wendling and Xavier, 2005; de Almeida *et al*., 2007; Schwambarch *et al*., 2008; Wendling *et al.*, 2010; Brondani *et al*., 2012). The most common endogenous auxin in plants is indole-3-acetic acid (IAA), although indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are more effective than IAA in promoting rooting (Pop *et al.,* 2011). This has been attributed to IAA having longer persistence in tissues as well as in differences in metabolism and transport (Li *et al*., 2009). The type, concentration, forms and time of auxin application varies with the species involved, and the type and age of cuttings. Indole-3-butyric acid is the standard active ingredient in most commercial rooting stimulation products. The optimum concentration of each auxin is dependent on the species and type of cutting (Titon *et al*., 2006 Lana *et al*., 2008). The performance of IBA as opposed to IAA can be explained by its higher stability, differences in metabolism and in transport, and because IBA is a slow release source of IAA. In younger cuttings, there is a substantial supply of endogenous auxins, which help initiate the rooting process, therefore the application of exogenous auxins to young shoots may induce excessive and toxic concentrations of that auxin and may result in inhibition of the rooting process, also reducing the survival of cuttings (Blythe *et al*., 2007). The endogenous levels of auxins vary with the different phases of rooting (Pop *et al*., 2011). Exogenous application of auxins increases the rooting percentage due to their ability to act in plant tissues, which are found near the region of contact with the plant growth regulator (Fogaça and Fett-Neto, 2005; Husen and Pal, 2007) and can also be related to the timing of applications (Luckman and Menary, 2002; Brondani *et al*., 2012). According to Luckman and Menary (2002), delaying the application of IBA for 6 weeks after planting allowed for good rooting on *Eucalyptus nitens*.

1.4.1.6 Media and its management

Rooting media plays an important role in the success of rooting of stem cuttings. The rooting medium used in horticulture and forestry consists of organic and inorganic components such as soil, coir, perlite, softwood or hardwood bark, or vermiculite (Hartman *et al*., 1997). A mixture composed of peat:sand:vermiculite (1:1:1) was found to work better than each substrate on its own, with regards to rooting success (Maile and Nieuwenhuis, 1996). The aeroponic rooting of cuttings allows dissolved oxygen to move easily at the stem surface-to-water interface as compared to traditional rooting media (Soffer and Burger, 1988). This results in oxygen-intensive respiration which is needed for root initiation and growth. Porous substrates allow for better aeration and water drainage but the medium needs to be dense and firm to be able to hold the cuttings in place during rooting or germination (Hartman *et al*., 2002; Shi *et al*., 2007). Substrates which are aerated and have been used in the propagation of cuttings include carbonised rice husk and vermiculite (1:1) (Teixeira *et al*., 2007) and sawdust (Agbo and Omaliko, 2006).

1.5. Propagation through grafting

Grafting is most commonly used in agriculture and forestry in asexual propagation to produce clonal orchard trees. In most cases, one plant is selected for its roots 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). A successful graft is determined by a contact of the vascular cambium tissues of the rootstock and the scion plants, while keeping both tissues alive. Grafting has become an important method to quickly enhance the adaptation and resistance of different cultivars to stress conditions (Pardo-Alonso *et al*., 2019). This process allows to produce scion and bud material that is genetically identical to the mother plant. To ensure optimum graft success, it is important to know which factors are crucial and to ensure they are maintained properly. The most crucial factors involved in grafting are reviewed below.

1.5.1 Factors affecting the success of grafting

1.5.1.1 Time of the year

It is generally best to graft during spring to late summer, when the environmental conditions are conducive for active growth of the plants. During this time, the cambial activity is restored and there is a flow of sap, which allows easy removal of slippery bark to aid the grafting process (Sadhu, 2005). The success of grafting during summer is due to the fact that there are longer, warmer days (reaching temperatures of 28.6 °C) as opposed to the days in autumn-winter, where temperatures are cooler (reaching maximum of 27.5 °C (Sadhu, 2005).

1.5.1.2 Compatibility between the scion and rootstock

For the graft to grow successfully, the scion and stock need to be compatible with each other. Plants that are closely related tend to have a better chance of forming a union as opposed to those that are distantly related (Mudge *et al*., 2009). The union between the scion and rootstock is established with the formation of callus tissue, which is produced either from the plants' cambium or from neighbouring wood and bark cells (Mudge et al., 2009). Once this union has been formed, it should be followed by suppression of any shoot growth from the rootstock, in order to allow the scion to become the new aerial part of the plant (Lewis and Alexander, 2009).

1.5.1.3 Temperature and Humidity

Temperature and humidity are two important factors that are monitored during the grafting process as they have an impact on the formation of callus in the graft union (Nguyen and Yen, 2017). Grafting is usually completed during the dormant season when temperatures are cool. The graft union will be slower at temperatures below 4°C or lower. The temperature should be maintained at 15°C or less for 2-3 weeks post grafting, since higher temperatures may result in the growth of shoot buds which give rise to leaves, these leaves may then deplete any moisture stored in the scion before a graft union is formed. Temperatures should be maintained for this period unless the scion buds are still in their rest period. The union after summer grafting occurs when temperatures reach about 21°C when callus formation is rapid. Temperatures that are above 32°C slow/stop the callus formation. Maintaining a high humidity is important to ensure that the scion does not dry out (Sadhu, 2005).

1.5.1.4 Age of plants

The scions are usually suitable at 1-2 years old, whereas rootstocks should be 2 years or younger.

1.5.4.5 Scion collection and rootstock maintenance

For a successful graft union, the stock and scion should be oriented the way they normally grow) (Hartmann, 2002). Scion material is best collected and used on the same day unless it can be stored in a cool, moist area, possibly in a sealed plastic bag.

During the grafting process, the rootstock cutting is prepared first, followed by the scion with a minimum time between cutting the scion and sealing it. This is done to ensure that there are no delays during the grafting process, not allowing the surfaces of the cut scion and stock to dry out or oxidize, which would reduce any chances of success (Lewis and Alexander, 2009).

1.5.4.6 Care of grafted plants

The open surfaces of the stock and scion at the union need to be protected from drying out. This may be done by covering the surface after the scion and stock are fitted together with grafting wax or other protective materials. The removal of all shoot growth from the scion as soon as it appears is encouraged to prevent the scion from being overgrown by the rootstock cutting. Also, any grafting tape should be removed before any restriction in growth occurs (Lewis and Alexander, 2009).

1.5.4.7 Contact of the stock and scion

Graft unions are promoted by a good fit between the root stock and the scion. In some methods, such as cleft grafting, the fit occurs naturally. In other methods, tying the union with materials to secure proper contact between the stock and scion aids the union. Clean and smooth cuts on the rootstock and scion are required to promote maximum contact (Hartmann, 2002).

1.5.4.8 Common grafting methods

1.5.4.8.1 Splice/whip grafting

Splice grafting is the simplest method to join a rootstock and scion. It is best used in herbaceous plants in a protected environment. The rootstock and scion should be less than 25mm in diameter and of equal length and sealing tape/other tying materials hold the parts together. Additional protection with wax or similar materials is usually advisable for younger plant materials (De, 2019).

1.5.4.8.2 Whip and tongue grafting

This method is a variation of splice grafting and is one of the most common and useful methods for grafting woody plants. The best results are found when working with a rootstock and scion of equal diameter that is less than 25mm. Once the tongues are cut, the scion section is inserted into the rootstock section, until they are interlocked. The union is then secured by wrapping tightly with tape/other tying materials (De, 2019).

1.5.4.8.3 Cleft grafting

This method is usually used before the stock shows any signs of active growth. The scions are usually 6 to 7mm in diameter and have 2-3-buds. The rootstocks should be about 25 to 100mm in diameter and be straight. The cambium layers of the stock and scion should be aligned without regard of the outside surfaces, and the cut surfaces should be covered with wax, including the splits down the side of the stock and the tip of the scion (De, 2019).

1.5.4.8.4 Bark grafting

Bark grafting can also be used for top working large trees (up to 300mm in diameter) than is used for cleft grafting, but the scions should be similar in size (6 to 7mm in diameter and have 2-3-buds). Several scions can be inserted around the stock. The union for this type of graft is usually weak for a period of 1-2 years, thus the scions may need to be tied up for support after growth begins (De, 2019).

1.5.4.8.5 Side grafting

This type of grafting can be used for production of new plants such as evergreen plants grafted onto smaller seedling stocks. The scion is grafted into an oblique cut on the side of the stem of the rootstock. After the graft union heals, the rootstock shoot is cut off just above the union.

1.5.4.9 Hormones involved in the rootstock-scion interaction

1.5.4.9.1 Formation of the rootstock-scion union

Successful grafting depends on the compatibility between the vascular connections of the scion and the rootstock (Cohen *et al*., 2017). During the grafting process, a vital hormone, IAA (an auxin), is involved in the development of a successful union. Indole-3-acetic acid (IAA) is released from the vascular strands of the rootstock and scion and is responsible for bringing about differentiation of the vascular tissues (Mattson *et al*., 2003). Treatment with plant growth regulators were found to increase apical grafting success.

Indole-3-acetic acid is produced in the shoot apexes and is then translocated to the roots, where it ensures root development, morphology and functioning. The production and activity of cytokinins has also been linked with auxin. Cytokinins are produced in the roots, move up to the shoots and they control shoot development, i.e., shoot growth and productivity (Albacete *et al*., 2009). According to Sorce *et al*., (2002), non-grafted plants have been shown to have a balanced auxin/cytokinin ratio, as opposed to non-grafted plants, where the auxin/cytokinin balance is upset.

1.5.4.9.2 Rootstock-scion communication

The breakdown of rootstock-scion connections in incompatible grafts is due to a hormonal imbalance, which is mainly due to auxins and ethylene in the root system, following establishment of the root grafting connections (Aloni *et al*., 2008). The incompatibility was also associated with exposure of the grafted plants to high temperatures following grafting, resulting in inhibition of root and shoot growth, and collapse of the incompatible grafted plants as compared to the development of the compatible grafts. In normal conditions, reactive oxygen species (ROS), such as superoxide radical (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) are produced (Miller, 2002; Foyer and Noctor, 2003). During degradation processes in the rootstock, oxidative stress activities are linked to cell death symptoms, which may occur in the plant tissue. In incompatible grafts, the auxins may reach high levels in the rootstock, resulting in higher levels of ethylene production and inhibiting the graft union.

1.5.2 Mini- Grafting

Mini-grafting is a non-destructive vegetative propagation method that is used to graft apical segments onto adult donor plants derived from rootstocks (Oliari *et al*., 2016). The mini-grafting union is initially formed by the division of cells of the callus tissue, originally from the graft and rootstock, which further differentiate to form the vascular cambium. For successful grafting to occur, there are 3 distinct stages that need to take place:

i. The adhesion between graft and rootstock cells;
ii. Callus cell proliferation;
iii. Vascular differentiation in the grafting interphase (Hartman *et al*., 2011).

Parenchyma cells have been shown to play an important role in healing injuries such as grafting (Oliari *et al*., 2016). This is due to their ability to be re-programmed to the meristematic cytological status, which is responsible for reprogramming the cells from other cellular types. The new parenchymatous cells grow both in the graft and the rootstock, filling the space between the slot and the graft (Oliari *et al*., 2016). The cells found in the actively growing parts of the graft are usually younger and respond better to injury as opposed to those of the rootstock. Usually there is a high auxin content in these tissues (Taiz and Zeiger, 2013), which has a positive impact on healing of the graft region. The presence of starch in the reverse parenchyma is seen as a biochemical marker of the compatibility in the mini-grafting process (Oliari *et al*., 2016).

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CHAPTER TWO: ROOTING OF VARIOUS *CORYMBIA* HYBRIDS

2.1. ABSTRACT

Vegetative propagation of cuttings is a commonly used technique in forestry and horticulture to ensure mass multiplication of superior genotypes/phenotypes and to maintain individual characteristics in order to ensure that a plant is genetically identical to the original/donor plant.

The aim of this study was to vegetatively propagate selected clones of *Corymbia* hybrids through cuttings. It specifically assessed the effects of growth regulators and different rooting chemicals applied to the cuttings to enhance rooting, assessing their effect on the survival of rooted cuttings, the best rooting chemical combinations, the effect of environmental factors on the success of the rooting process and the best hybrid genotypes amongst those tested.

The study was separated into three different experiments, comparing cutting material collected from coppice stumps and ramets. The material was collected from 14 genotypes to select for the best rooting genotypes. The cuttings were prepared at Sunshine Seedling's Nursery and were placed in Rooting Tunnel 5 until the cuttings were rooted. The results showed that using ascorbic acid (40 mg L^{-1}) + Seradix® 2 (IBA 3g kg^{-1}) resulted in the highest rooting success across all three experiments, with rooting ranging from 37.5 - 55.5%. The lowest rooting resulted from the use of propiconazole + Seradix® 2 (IBA 3g kg^{-1}) in the 1st experiment (1.19%), while the control treatment where no chemical was applied, yielded the lowest survival for rooted cuttings in the third experiment, with 17.86 %.

Ascorbic acid is an important antioxidant, which when combined with rooting hormones, works synergistically to alleviate plant stress. The antioxidant protects the rooting hormone from oxidation, therefore allowing them to enhance rooting and increase the tolerance of the plants to conditions in the greenhouse.

2.2. INTRODUCTION

Propagation of cuttings is commonly used to ensure rapid and large-scale growth, uniformity, and product quality in forestry and horticulture (Leakey 2004; Snedden *et al*. 2010; Navarrete-Campos *et al.* 2012). The eucalypt species that rely on the use of cutting production include species of two eucalypt genera, *Corymbia* and *Eucalyptus*, which are the world's most widely planted hardwood trees (Teulie`res *et al*. 2007; Nichols *et al*. 2010; Madhibha *et al*. 2012). Many species that were initially considered difficult-to-propagate have proven amenable to vegetative propagation once the physiological state of the mother plant, the propagation environment, and the post-severance treatments, such as auxin application, have been optimised (Leakey 2004; Atangana *et al*. 2006; Trueman *et al*. 2007; Wendling *et al*. 2014; Majada *et al*. 2011).

The aim of this experiment was to vegetatively propagate selected clones of *Corymbia* hybrids by cuttings. It specifically assessed the effects of growth regulators applied to enhance the production of rooted cuttings, assessing their effect on root length and biomass.

2.3. MATERIALS AND METHODS

2.3.1. Collection of cutting material

The collection of *Corymbia* hybrid material from coppice produced on stumps, took place on the 1st November 2016 at the Mfezi site in Zululand. The collection was done before sunrise, from 28 stumps with coppice material to choose from. Only 12 were selected from the 28 stumps for this trial.

The collection was undertaken by having a bucket for each stump number. The coppice material was cut from the chosen stumps and then bundled using an elastic band and a ticket with the selection number assigned to it, this was placed within each bundle. Buckets were half-filled with water to ensure the cuttings were kept moist. Once all the coppice material had been collected from all 14 stumps, the buckets were then placed in a cooler box, to ensure the cuttings were moist and cool during transportation. The material was transported back to Pietermaritzburg to the Sunshine Seedlings Services, to be prepared and struck.

2.3.2. Preparation of cuttings

The cuttings were prepared by firstly working with one selection at a time to ensure that the material was not mixed up. This was done by firstly cutting the material to about 8cm in length, leaving 2-3 nodes. Thereafter, leaf lamina area was reduced to a third of the original area to reduce transpiration. These cuttings were placed in buckets and Spore kill (a.i. didecyldimethylammonium chloride (Hygrotech Sustainable Solution, Mkondeni, Pietermaritzburg) was added for surface sterilization. This process was done for all 14 genotypes collected, each genotype having been allocated a selection number (MF number) and placed in its designated bucket, with its ticket. These were then taken to the rooting tunnel for treatment and placing into the propagation medium.

2.3.3. Treatment preparation

In the tunnel, the cuttings were separated into the specific chemical groups they would be treated with, treated and planted. For each of the 12 genotypes collected, four cuttings per genotype received this treatment, this was replicated three times, having a total of 12 cuttings per genotype.

Table 2. 1 : Treatments that were applied on the cuttings for the 1st experiment with coppice material

Treatment and dose	Application method
Standard IBA (Seradix® 2) (3g kg^{-1}) **- *root hormone***	Roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (40mg 1L^{-1}) **+ Standard IBA (Seradix® 2) (3g kg^{-1})**	Dip cuttings for 5 seconds in ascorbic acid, then roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (40mg L^{-1}) ***-anti-oxidant vs stress oxygen***	Dip cuttings for 5 seconds
Eco-T® (Trichoderma *harzianum*) **0.25g in 1L** ***-biocontrol agent + root hormone* (IAA)**	Eco-T® suspension, Dip cuttings for 5 seconds
Propiconazole (Tilt®) 0.2ml L^{-1} + standard IBA (Seradix® 2) (3g kg^{-1}) ***-Fungicide + anti-gibberellin***	Dip cuttings for 5 seconds
Control	No treatment

The experiment was repeated twice

Table 2.2: Treatments that were applied on the cuttings for the 2nd experiment with hedge material

Treatment and dose	**Application method**
Standard IBA (Seradix® 2) (3g kg^{-1}) **- *root hormone***	Roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (40mg in 1L) **+ Standard IBA (Seradix® 2) (3g kg^{-1})**	Dip cuttings for 5 seconds in ascorbic acid, then roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (80mg L^{-1}) **+ Standard IBA (Seradix® 2) (3g kg^{-1})** ***-anti-oxidant vs stress oxygen***	Dip cuttings for 5 seconds
L-arginine + Standard IBA (Seradix® 2)	Eco-T® suspension, Dip cuttings for 5 seconds
Glycine betaine + Standard IBA (Seradix® 2) **(3g kg^{-1})** ***-Fungicide + anti-gibberellin***	Dip cuttings for 5 seconds
Control	No treatment

This experiment was repeated twice.

Table 2. 3 The treatments that were used for rooting cuttings for the 3rd experiment with coppice material

Treatment and dose	Application method
Standard IBA (Seradix® 2) (3g kg^{-1}) ***- root hormone***	Roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (40mg L^{-1}) **+ Standard IBA (Seradix® 2) (3g kg^{-1})**	Dip cuttings for 5 seconds in ascorbic acid, then roll 2cm of tip of the cutting in Seradix® 2 powder
Ascorbic acid (80mg L^{-1}) **+ Standard IBA (Seradix® 2) (3g kg^{-1})** ***-anti-oxidant vs stress oxygen***	Dip cuttings for 5 seconds
L-arginine + Standard IBA (Seradix® 2)	Eco-T® suspension, Dip cuttings for 5 seconds
Glycine betaine + Standard IBA (Seradix® 2) **(3g kg^{-1})** ***-Fungicide + anti-gibberellin***	Dip cuttings for 5 seconds
Control	No treatment

Once in the tunnel, the cuttings were monitored weekly for root emergence, and any dead cuttings were removed. After four weeks, the cuttings were removed from the rooting tunnel and placed in a greenhouse at Sunshine Seedlings where the root length was measured and recorded. After measuring, they were assessed for rooting or callusing, and were moved to a greenhouse with natural environmental conditions.

2.3.4. Installation of data loggers

Two I-buttons were installed three days after the cuttings were set, to measure temperature (℃) and relative humidity (%) in the rooting tunnel for the duration of the experiment. The I-buttons were placed in plastic containers. One was installed at the height of the cuttings and the second one was placed at the height of the sprinklers.

2.3.5 Data analysis

An Analysis of variance (ANOVA) was performed using the results obtained using Genstat, 18th edition.

2.4. RESULTS

a. The environmental conditions measured at Sunshine Seedlings, Tunnel 5 during the rooting experiment

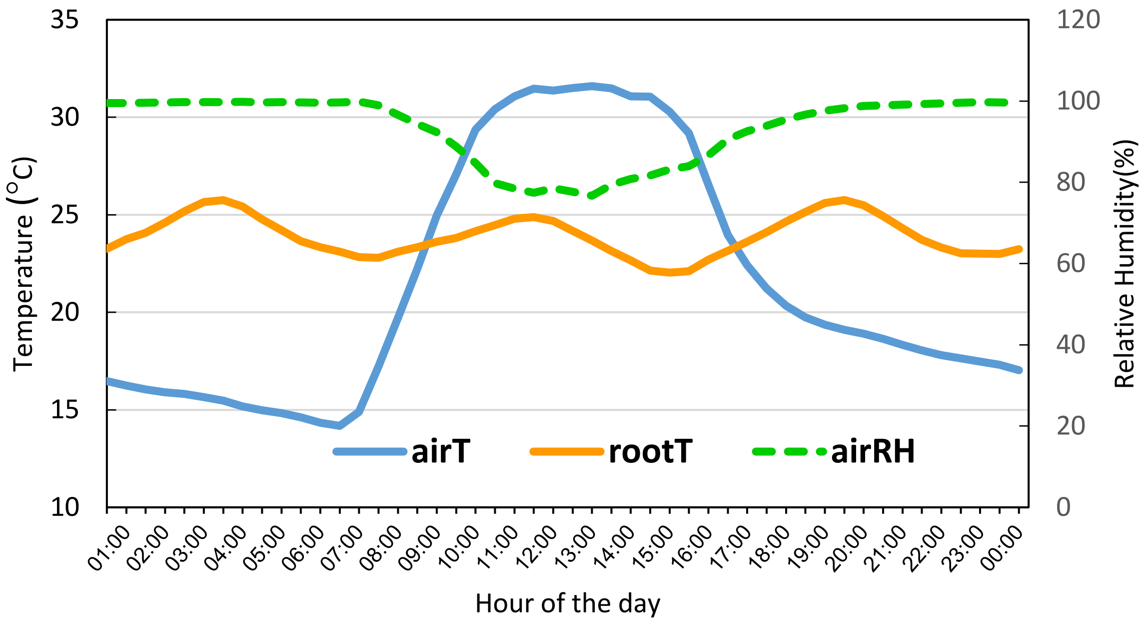

Figure 2.1: The fluctuation of environmental conditions monitored in Tunnel 5 at Sunshine Seedlings over the period of the rooting experiment.

b. An analysis of variance (ANOVA) was performed to determine the effect of different chemical treatments on rooting.

Experiment 1

Table 2. 4 The mean rooting percentage for each chemical treatment applied on the *Corymbia* hybrids in the 1st experiment from coppice

Chemical treatment	Cuttings rooted (%)
Seradix® 2	36.31^{cd}
Ascorbic acid (40 mg L^{-1})	27.38^{bc}
Ascorbic acid (40 mg L^{-1}) + Seradix® 2	47.02^{d}
***Trichoderma harzianum* + Seradix® 2**	26.19^{bc}
Propiconazole + Seradix® 2	1.19^{a}
Control	17.86^{b}

Table 2.4 shows the percentage of rooted cuttings and the use of ascorbic acid (40 mg L^{-1}) + Seradi ® 2 gave the highest results of 47%, showing a clear need for auxin and antioxidant. The lowest results from the use of propiconazole + Seradix® 2, with a rooting of 1.19%. The use of rooting powder, Seradix® 2, doubles the rooting of the control.

Table 2. 5: The ANOVA table of results for the 1st experiment in rooting of *Corymbia* hybrids using different chemical treatments

Source of Variation	df	F-value (vr)	P (F. pr)
Block stratum	2	0.49	0.627
Treatment	5	16.38	<0.001

CV %= 7.4 %, LSD= 12.22%

Significant differences ($p < 0.05$) were observed in the rooting as a result of the different treatments that were used, with the mean rooting success ranging from 26-46%.

Experiment 2

Table 2. 6 : The mean rooting percentages for each chemical rooting treatment applied on the *Corymbia* hybrids in the 2nd experiment from ramets

Chemical Treatment	Cuttings rooted (%)
Seradix ® 2	44.6^{c}
Ascorbic acid (40mg L^{-1}) + Seradix® 2	55.4^{d}
Ascorbic acid (80mg L^{-1}) + Seradix® 2	43.8^{bc}
L-arginine + Seradix® 2	33.0^{a}
Glycine betaine + Seradix® 2	36.6^{abc}
Control	33.9^{ab}

Table 2.6 shows the rooting of cuttings for the 2nd experiment, with the highest rooting percentage at 55% resulting from the use of ascorbic acid (40mg L^{-1}) + Seradix® 2. The lowest rooting resulted from the use of L-arginine + Seradix® 2, at 33%. The control treatment (33.9%)

and glycine betaine + Seradix® 2 (36.6%) all of which were not significantly different each other, ($p > 0.05$).

Table 2. 7: The ANOVA table of results for the 2nd experiment in rooting of *Corymbia* hybrids using different chemical treatments

Source of Variation	df	F-value (vr)	P (F. pr)
Block stratum	3	6.64	0.005
Treatment	5	5.78	0.004

CV % = 18.0%; LSD= 10.64%.

There were significant differences in the rooting results for the 2nd experiment.

Experiment 3

Table 2. 8: The mean rooting percentages for each chemical rooting treatment applied on the *Corymbia* hybrid in the 3rd experiment, collected from ramet seedlings

Chemical Treatment	Cuttings rooted (%)
Seradix ® 2	23.21^{ab}
Ascorbic acid (40mg L^{-1}) + Seradix® 2	37.50^{c}
Ascorbic acid (80mg L^{-1}) + Seradix® 2	37.50^{c}
L-arginine + Seradix® 2	32.14^{bc}
Glycine betaine + Seradix® 2	34.82^{bc}
Control	17.86^{a}

Table 2.8 shows the rooting for the 3rd experiment, where ascorbic acid at 40 mg L^{-1} and at 80 mg L^{-1} + Seradix® 2, had the same effect and produced the same rooting percentage of 37%, which was the highest for all the treatments tested. The lowest percent rooting was with the control treatment at 17% and Seradix® 2 (23.%) which were not significantly different from each

other (p>0.05), but not significantly higher than L- arginine + Seradix® 2 and Glycine betaine + Seradix® 2 (p>0.05).

Table 2. 9 The ANOVA table of results for the 3rd experiment in rooting of *Corymbia* hybrids using different chemical treatments

Source of Variation	df	F-value (vr)	P (F-pr)
Block stratum	3	1.80	0.19
Treatment	5	3.61	0.024

CV%=15.4, LSD=12.94

In the 3rd experiment, there was a significant difference in the chemical treatments that were used for rooting, p<0.05.

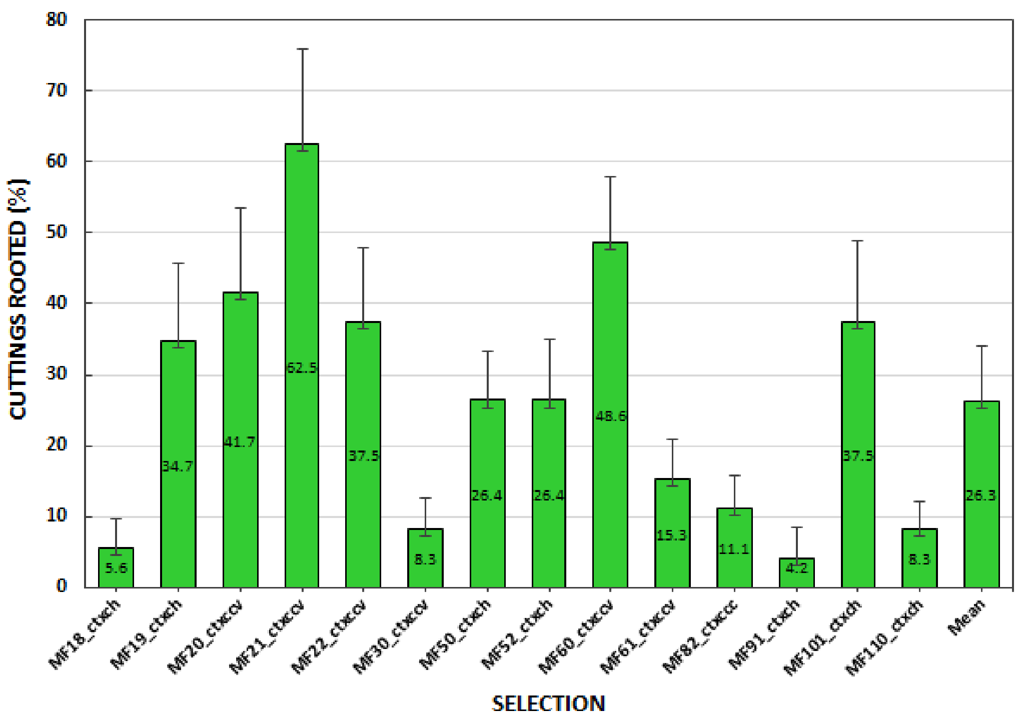

Figure 2.2: The average rooting percentage of cuttings based on the 14 different selections that were tested across the experiments.

2.5 DISCUSSION

Effect of environmental conditions on the rooting of cuttings

High temperatures in the greenhouse may have negative effects on the rooting of cuttings because of water deficiency. Low levels of water availability may lead to numerous cell function failures, resulting in impaired cell cycles and division (Costa *et al*., 2013; Brondani *et al*., 2018). Figure 2.1 shows the average environmental conditions in Tunnel 5 at Sunshine Seedlings which were measured for the duration of the rooting experiment. The data shows that root temperature ranged from 24 to 26 ºC, which was stable throughout the experiment, and showed a good impact on the rooting ability of some of the tested genotypes. The air temperature measured in rooting Tunnel 5 ranged from 15 to 30 ºC, which is a good temperature to allow for rooting as it closely relates to the temperature range to which cuttings of woody species are usually submitted to, (Alfenas *et al*., 2004; Brondani *et al*., 2018), this was also reported in studies by Hartman *et al* (2002), who found that day time air temperatures ranging from 21 -27 ºC and 15 ºC at night are good for the rooting of cuttings of temperate species in greenhouse conditions.

The relative humidity (RH) levels that are presented in figure 1 range from 25-30 %, which are very low RH for rooting cuttings. In most studies looking at rooting cuttings, a high RH level has been used, propagating cuttings with RH near 100% in controlled environments which allows for maximum root initiation and establishment (Elgimabi 2009; Rusnak and Braun, 2017). Higher RH levels are known to reduce transpiration and water loss which allows for turgor pressure which allows for cell expansion and growth of adventitious roots, preventing desiccation (Loach, 1988).

The role of plant regulating hormones in rooting of cuttings

Plant regulating hormones are known to enhance the rate of success of rooting and to increase the final root percentage and number of roots (Gehlot *et al.,* 2014). For this reason, a series of experiments were conducted to measure the effect of plant growth regulators (PGRs), some antioxidants and other stress-relieving substances that are known to affect the rooting process.

In the experiments conducted, the use of ascorbic acid at 40 mg L^{-1} produced the highest rooting percentage when it was combined with the rooting hormone, Seradix ® 2 (IBA 3g kg^{-1}), with percentages ranging from 37 to 55% (Tables 2.4, 2.6 & 2.8). This was an excellent result for this genus, especially for some of the hybrids used, which are known to be difficult-to-root. The success of using the antioxidant together with the rooting hormone was in accordance with studies by Vasar (2004) who^{-1} demonstrated that a mixture of two antioxidants synergistically increased the rooting of cuttings of *Pinus ovum* L. Ascorbic acid is an important antioxidant, found mainly in the chloroplasts of leaf tissues and other plant cells, and is known to play a crucial role in cellular defence against oxidative stress (Noctor and Foyer, 1998). The exogenous application of ascorbic acid alleviates abiotic stresses in plants, such as water deficiency, helping the plant to achieve improved survival, increased nutrient uptake and improved leaf and root growth. Its main mode of action appears to be via a reduction in lipid peroxidation and reduced oxidative stress (Khan *et al*., 2011). The use of ascorbic acid as an antioxidant stems from its history of ensuring longevity in plants and for its known action of reducing oxidative stress (Mazid *et al*., 2011). The antioxidant also protects the rooting hormone (IBA) from oxidation, thus allowing the rooting hormones to enhance rooting and to increase the tolerance of the plants to greenhouse conditions (Vasar, 2004). In this experiment, ascorbic acid was applied at 80mg/ L^{-1} with Seradix® 2 (IBA 3g kg^{-1}), but the rooting rates were lower or the same as those of the lower dose of 40mg/ L^{-1} (Tables 2.6 & 2.8). This effect has also been noted by Katay *et al* (2011), who found that the effectiveness of the ascorbigen was dependent on the dosage of the antioxidant and the time interval between inoculations and pre-treatment. In Table 2.4, significant differences ($p< 0.05$) were observed between the different chemicals used, with the mean rooting ranging from 26-46%.

The use of auxins in clonal propagation is primarily to improve the rooting percentage and to improve the number of roots, root system symmetry and for tree growth (Bryant and Trueman, 2015). Indole-3-butyric acid (IBA) was tested as a rooting hormone because of its recognized ability to stimulate adventitious roots in apical cuttings of some plants (Arya *et al*., 2007). In the experiments performed, the application of a rooting powder, Seradix® 2 (3g kg^{-1} IBA), produced good rooting percentages, which ranged from 23-45% whereas the rooting percentages of the untreated control ranged from 18-34%. Trueman and Richardson (2011) also found that the application of an auxin powder to cuttings can increase the percentage of rooted cuttings and the number of adventitious roots that are produced per cutting.

Indole-3-butyric acid (IBA) is known to increase rooting rates in eucalypt cuttings (Correa and Fett-Netto, 2004; Fogaca and Fett-Neto, 2005). Treatment with IBA was effective when compared to the untreated control, which yielded a rooting percentage from 17-33%. Trueman and Adkins (2013) found that IBA (3g kg^{-1}), increased the rooting cutting production, root number and root weight of *Corymbia* and *Eucalyptus* cuttings. An excess of auxins causes senescence, leaf abscission and death of eucalypt cuttings (Kilkenny *et al*., 2012; Trueman and Adkins, 2013), therefore choosing a low dose for rooting eucalypt cuttings is usually recommended (Trueman and Adkins, 2013).

There are reports on the use of *Trichoderma* species to enhance the growth of flowers, contributing to the shoot and root fresh & dry weights, height, number of flower buds of

Chrysanthemum & Petunia (Windham *et al*., 1986). The mechanism by which *Trichoderma harzianum* works may be due to the production of growth regulating substances, including indole acetic acid (IAA), which usually stimulates root production. However, in some cases, there may be negative effects because the IAA may accumulate and may become phytotoxic if extreme concentrations of this fungus are applied (Hartman *et al*., 1990). The results in Table 2.4, show that the rooting percentage for the cuttings treated with *Trichoderma harzianum* + Seradix® 2 , had a rooting percentage of 26%, which was low when compared to the use of Seradix® 2 (36.3 %) but similar to the use of ascorbic acid alone (27.38%), and higher than the control (17.86),showing that it may have potential for use in rooting cuttings.

The triazole fungicides offer both broad spectrum control of many fungal diseases, but many of them are also plant growth regulators related to anti-gibberellins(Magnitskiy *et al*.,2006). Propiconazole is a systemic fungicide that is also known for its plant growth regulating properties and for stimulating root growth indirectly by slow stem elongation (Yan, 1993). However, in the current study, the propiconazole treatment yielded the lowest rooting percentage, of less than 5% (Table 2.4). The fungicide had deleterious effects on the cuttings, with some cuttings dying or callusing, a result which was not consistent with previous research on the use of this fungicide on cuttings. Rademacher (2000) found the exogenous application of propiconazole and other fungicide treatments may result in an endogenous shift in the balance of hormones during heat and moisture stress, increasing the level of cytokinin's and IAA, whilst decreasing the levels of gibberellins. They also decrease the toxic effects of abscisic acid (ABA), and ethylene, thus affecting the growth of the plants positively. However, the negative effect of propiconazole on the cuttings in this study supports the findings of Dickens (1990) who reported that the fungicide is known to have a growth retardant effect on some species. However, this may have been a dose effect because most plant hormones are growth stimulators at once concentration and a growth retardant at another concentration.

Potential for *Corymbia* hybrids to propagate vegetatively

The *Corymbia* hybrids have shown great potential for establishment in plantation forests, this is due to their wide adaptability to marginal tropical and sub-tropical environments and high-quality timber (Lee, 2007). These hybrids are obtained through the controlled pollination of *Corymbia torelliana* (section *Torelliannae*) as the maternal taxon and species from the spotted gums (section *Maculatae*): *C. citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegate* or *Corymbia henryi* as the paternal taxon (Lee 2007, Dickinson *et al*., 2010). The hybrid *C. torelliana* x *C. citriodora* is desirable because of the combination of fast growth, disease resistance and frost tolerance (*C. torelliana*) and good quality wood with desirable stem form (*C. citriodora*). The spotted gums, *Corymbia citriodora*, *C. henryi* and *C. maculata* are important plantation eucalypts but are considered difficult to propagate vegetatively. The results of the effect of the genotype on rooting success, represented in Figure 2.2, show that the hybrids *Corymbia torelliana* x *C. citriodora* subsp. *variegata* (ct x ccv) had the highest rooted cuttings (%) as compared to rooting from *C. torelliana* x *C. henryi* (ctx ch) (%) and *C. torelliana* x *C. citriodora* subsp*. citriodora* (ctxccc) (%). The rooting outcomes from *C. torelliana* x *C. citriodora* cuttings varied from studies, where Shepherd *et al*., (2007) reported a rooting of 17%, 11-67% (Trueman and Richardson, 2008), 30-42% (Kilkenny *et al*., 2012) and 46% (Trueman and Adkins, 2013). From the current studies, Figure 2.2 shows that the hybrids show some amenability through cuttings if the good rooting selections are chosen, such as selection MF 21 (ctxccv) which yielded 62.5% rooting, in agreement with the findings of Trueman and Richardson (2008), which is very close to the commercial level required by nurseries (Wendling *et al*. 2014) with a rooting requirement of >70% (Trueman, 2006).

The cuttings harvested from the coppice material in experiment 2 showed higher rooting (55.4%) than the cuttings collected from ramet seedling plants in experiment 3 (38%) for the best treatment of ascorbic acid (40mg /L^{-1}) with Seradix® 2 (IBA 3 g/ kg^{-1}).

2.6 CONCLUSION

The use of ascorbic acid (40mg L^{-1}) with Seradix® 2 (IBA 3 g /kg^{-1}) is important since the antioxidant protects the rooting hormone from oxidation, allowing IBA to increase stress tolerance of the plants to the greenhouse conditions, which enable rooting to occur. The selections from the hybrid *Corymbia toreliana* x *C. citriodora* subsp. *variegate* (ct x ccv) have shown higher rooting success compared to those of the *C. torelliana* x *C. henryi* (ct x ch) and *C. torelliana* x *C. citriodora* subsp. *citriodora* (ct x ccc,) therefore vegetative propagation by cuttings can be established for plantations using this hybrid genotype. The hybrids showed good rooting under the stable root temperature of 24-26 ºC in the rooting tunnel, but a higher relative humidity (RH) would increase rooting success since it is known to reduce transpiration therefore allowing for the promotion of turgor pressure, cell expansion, which results in the growth of adventitious roots. There is a need to optimise the vegetative propagation of cuttings using the *Corymbia* hybrids to reach the commercial nursery requirements of rooting rates of 70% and above and to identify if these hybrids can survive in the field once they have been out planted.

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CHAPTER THREE: OPTIMIZING GRAFTING BY COMPARING CONVENTIONAL AND MINI-GRAFTING TECHNIQUES FOR *CORYMBIA HENRYI*

3.1 ABSTRACT

Grafting refers to the uniting of a shoot or bud (scion) with a growing plant by insertion or by placing in close contact, which requires a matching of the cambial layer of the scion with the cambial layer of the rootstock. This technique is commonly used in agriculture and forestry for asexual propagation to produce clonal orchard trees. The aim of this chapter was to optimize grafting techniques by comparing mini-grafting with conventional grafting techniques for *Corymbia henryi* scions grafted onto rootstocks of the same species from various clones, and to evaluate the effect of different treatments on grafting success.

The rootstock material used was obtained from ten-month old seedlings of *Corymbia henryi* and four-month old seedlings grown at the ICFR nursery. These were to be grafted onto, using different provenances of scion collected from the Zululand region. The grafting process took place at the grafting tunnel at the ICFR, with intermittent fogging to keep the plants moist. The technicians performed splice and cleft grafting on the seedlings in order to compare which method would yield a higher graft take. The comparisons made were between grafts grown onto four-month old rootstocks (mini-grafting), in comparison with grafts onto older, 10-month old rootstocks (conventional grafting). The different ancillary treatments applied were the control, where the graft union was tied with Parafilm®, covering the grafts with polythene plastic (one week) and dipping the grafts in $25ml^{-1}$ Vapor Gard® (VG), thereafter securing the graft union with Parafilm®.

The most successful grafting treatment was tying the control graft unions with Parafilm® (33.3%). The lowest graft success was from covering the grafts with polythene plastic (11.11%). When comparing the age of rootstocks, the four-month old rootstocks, which were splice grafted, yielded better graft survival (55.6%) than the 10-month rootstocks, which were cleft grafted (22.2%) in the control.

3.2 INTRODUCTION

Grafting refers to the uniting of a shoot or bud (scion) with a growing plant by insertion or by placing in close contact the two tissues, which requires a matching of the cambial layer of the scion with the cambial layer of the rootstock. Grafting is most commonly used in agriculture and forestry for asexual propagation to produce clonal orchard trees. 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). For a graft to be successful, the rootstocks need to well fertilized and watered (Nisar *et al*., 2012) and the grafting environment needs to be stable, with a high humidity and a moderate temperature. It is important to select suitable scion and rootstock parent plants for grafting, to avoid incompatibility (McComb & Bennet, 1986). Cambium contact of the rootstock and scion is an important step for callus formation and enhancing re-union (Bala *et al*., 2017).

The aim of this chapter was to optimize grafting for *Corymbia henryi* scions onto rootstocks of the same species from different clones by evaluating the effect age of rootstock, graft method and treatments applied to the grafts, on grafting success. This experiment was carried out in an environmentally controlled glasshouse, where the conditions (temperature and RH) were monitored to determine their effect on the grafting success.

3.3. MATERIALS AND METHODS

3.3.1. Rootstock planting and selection

In the experiment rootstock plants were grown in 5L bags to an age of 10 months, with a stem diameter of 4 to 5mm at 30cm above the soil medium level at the time of grafting. Younger rootstock plants were grown in 230ml seedling tray inserts (Speedling 98 Forestry trays, www.automa.co.za), and were used when four-months old, with stem diameters of 2 to 3mm at 15cm above the soil medium level at the time of grafting. The rootstocks were grown from South African commercial seed (mixed provenances). These were monitored, received foliar fertiliser applications and were watered daily with the use of sprinklers in the ICFR nursery. The rootstock plants were chosen by physical properties they showed which were based on their diameter, height, health and if they had leaves along the stem.

3.3.2. Scion collection and selection

The scions were collected by two people from the ICFR with different skills. One was an expert tree climber and the other was a supervisor. The chosen scion collections were from a provenance mix of good scion materials of *C. henryi*, which were sourced from the Zululand region. The scion parents were chosen – to evaluate a diverse array of scion material for their compatibility with the chosen rootstock plants. Branches of the scion selections were placed in buckets filled with water to ensure that the material was kept moist. These buckets were then placed in a cooler box inside a pickup truck and driven from Zululand to the ICFR nursery in Pietermaritzburg. On arrival at the ICFR nursery, the assigned grafting team were given these selections and they chose suitable material to use, based on the age and shoot diameters of the rootstock plants.

3.3.3. Grafting experiment

The grafting experiment was carried out in the ICFR grafting tunnel, where two skilled technicians from the ICFR were responsible for the grafting process. Cleft and splice grafts were chosen as two grafting methods, in order to determine which grafting method worked better on the two rootstock sizes. Cleft grafting (also known as wedge grafting) was chosen for the 10-month rootstocks because of the diameter of the main stem of the rootstock plants. With the four-month rootstocks, half were splice grafted and the other half were cleft grafted. One technician was responsible for grafting the four-month old rootstocks in the 230ml inserts, and the other technician grafted the 10- month rootstocks in 5L bags.

The cleft graft method was performed by trimming the base of the scion with a clean, sharp knife. The leaves were trimmed to reduce transpiration loss. The seedling rootstock received a 2-3cm slit in the middle of the stem. The prepared scion was then inserted into the rootstock to ensure that both cambia were aligned, thereafter, the entire graft was then wrapped firmly with sealing Parafilm® to ensure good contact.

The splice graft was performed by cutting the scion and rootstock at an approximate angle of 45° to provide a wide surface are for the cambia of the rootstock and scion to align. The two cut surfaces were then placed together, and the union was sealed by wrapping Parafilm® around the union.

Table 3.1: Factors evaluated in grafting applied to *Corymbia henryi*

Scion material (used for 10-month and four-month rootstocks)	C1: Montigny (ICFR 1st generation *C. henryi* breeding selection no.14)	C2: BSO (ICFR 1st generation *C. henryi* breeding selection no.91)	C3: Kwambo (ICFR 1st generation *C. henryi* breeding selection no. 42)
Rootstock Age	10-month	Four-month	
Graft method	Cleft	Cleft	Splice
Treatments applied to grafts	T1: Control (Parafilm®)	T1: Control (Parafilm®)	T1: Control (Parafilm®)
	T2: Polyethylene Plastic Cover (one week)	T2: Polyethylene Plastic Cover (one week)	T2: Polyethylene Plastic Cover (one week)
	T3: Vapour Gard® Dip (25 ml L^{-1})	T3: Vapour Gard® Dip (25 ml L^{-1})	T3: Vapour Gard® Dip (25 ml L^{-1})

Table 3.1 shows the factors that were evaluated during the grafting of *C. henryi* seedlings. Three scion materials were used, shown as C1, C2 and C3. The scion materials were all used in grafting with the four and 10- month old rootstocks. The graft methods applied on the four-month old rootstocks were cleft and splice grafting, whereas only cleft grafting was applied on the 10-month old rootstocks. A control and two treatments were applied on the grafts, which are shown as T1, T2 and T3, respectively.

3.3.4. Experimental design

A randomised complete blocks design was adopted for the grafting experiment, replicated three times. Each plot consisted of four grafted plants. The treatments were a factorial arrangement of two plant ages, two scion genotypes and three anti-stress treatments and graft method.

3.3.5. Glasshouse

Once the grafting experiment had been completed, all the grafts were taken to glasshouse no. 4. The temperature and humidity were set at 25°C with 80% RH. The misting frequency was set to occur every eight minutes for 10 seconds, keeping the plants moist as they require humid conditions. The misting frequency was adjusted depending on the weather conditions; i.e., on hotter days, misting had to be on for a longer period than on cooler days, when misting frequency was lowered. Two hobo temperature and humidity loggers were placed in the middle of the tables where the experiments were taking place, to measure temperature and humidity in the glasshouse. Heating mats were installed on the tables on which the grafts were placed to warm the base of the trays or pots.

3.3.6. Monitoring and maintenance of the grafted plants

The grafted plants were monitored every day to ensure that they received an adequate amount of water and monitored for any plant diseases. The grafts were assessed after two weeks for bud development. Any excess buds on the rootstock were removed to ensure that they did not inhibit the flow of water and nutrients from the rootstock to the scion bud. This was done using secateurs. Any weeds growing in the medium were removed. The grafted plants were fertilised with Osmocote Mini® at the recommended rate (http://osmocote.co.za/) once they were in the glasshouse.

3.3.7 Assessments and measurements

One parameter was measured, graft survival %.

3.3.8 Statistical Analysis

An analysis of variance (ANOVA) was performed to evaluate the effect of the different treatments used on the graft success using Genstat, 18th edition.

3.4 RESULTS

Table 3. 2 The ANOVA results for the grafting experiment using Fischer's unprotected test

Source of variation	df	F-value	P(F.pr)
Block	2	0.31	1.91
Treatment	2	2.15	0.124

CV%= 20.4, l.s.d. = 21.75

Table 3. 3: The mean percentage of survival for the grafts for each treatment that was applied in the grafting experiment

Treatment applied	Graft survival (%)
T1: Control (Parafilm®)	33.33^{b}
T2: Polyethylene Plastic Cover (one week)	11.11^{a}
T3: Vapour Gard® Dip (25 ml L^{-1})	18.52^{ab}

*Treatments with the same letter are not significantly different (p>0.05).

Table 3. 4: The mean percentage of graft survival with regards to the treatment applied versus age of rootstock and graft methods applied

	Age of rootstock and graft method applied		
Treatment Applied	**10-month,cleft graft**	**four-month, cleft graft**	**four-month, splice graft**
T1: Control (Parafilm®)	22.2%	22.2%	55.6%
T2: Polythylene Plastic Cover (one week)	11.1%	0	22.2%
T3: Vapour Gard® Dip (25 ml L^{-1})	33.3%	0	22.2%

Table 3.4 shows that the control treatment had highest survival of grafted plants for the four-month old rootstocks, which were splice grafted.

The four-month old rootstocks which were splice grafted and covered with polythene plastic cover (T2) showed the highest graft survival (55.6%), which was twice the survival of the 10-month rootstocks (22.2%). The use of Vapor Gard® on the 10-month rootstocks which were cleft-grafted, yielded a graft survival of 33.3%, whereas the four-month old rootstocks, which were cleft grafted and treated with Vapor Gard®, did not survive.

3.4 DISCUSSION

Tables 3.2 and 3.3 show the ($p>0.05$) results from the grafting experiment and that there were significant differences between the treatments applied to the grafts. Use of Vapor Gard® (VG) ,showed no significant difference ($p>0.05$), when compared with the other treatments, although it had a higher graft survival percentage than the grafts covered with polythene plastic. Vapor Gard® is an antitranspirant which has been found useful in reducing transpiration from plants. This anti-transpirant is known to form a thin film on the surface of leaves, closing stomata, thereby reducing transpiration water loss (Aldosoro *et al*., 2019). Anti-transpirants have been used to reduce abiotic stress related issues in plants such as ozone damage and have been used to reduce physical damage and fungal infections (Aldosoro et al., 2019). Aldoroso *et al*. (2019) found that drought stressed plants were treated with VG were able to maintain their water status parameters close to those of well-watered plants in the short term. In this study, the use of VG did yield some successful grafts, for the 10-month rootstocks but not for the four-month rootstocks. In this experiment, VG was applied at 25%(v/v), which was a higher concentration than treatments used in another study where the VG concentration was at 6% (v/v) and was effective for 30-40 days when applied on sweetcorn during the dry season. Similarly, when VG was applied to oilseed rape at a concentration of 1% (v/v) it was effective for 20-25 days (Aldosoro *et al*., 2019).

In Table 3.3, the control treatment had the highest percentage of graft survival as compared to application of the other treatments. The control treatment, where the graft union was wrapped with 2mm strip of Parafilm ® (33.3% graft survival) was the best treatment as opposed to covering the grafts with polythene plastic for 1 week, which yielded a graft survival of 11.11%. The low survival rate of the grafts covered with polythene agrees with the results found by Ajamgard *et al* (2016), where the use polyethylene cover film reported the lowest success rate of the grafts and this may have been because the callus quality within the four-week period is still low. When comparing with the results in this experiment, it suggests that the polythene plastic cover may have been removed too early, before the union between the scion and rootstock had developed, thus causing a weak callus formation. The use of Parafilm®

in the control treatment is a standard treatment in most grafting trials performed because of its stretching property and that it is easy to remove once the graft union has been established (Rodriguez *et al*., 2015; Nguyen & Yen, 2017). The other advantages of using Parafilm® are its ability to evenly distribute physical forces to the grafting region, sealing the union and ensuring that there is no water accumulation in this region (Oliari *et al*., 2016).

Table 3.4 shows the outcome of the interaction between the age of the rootstock and the grafting method applied when compared to the treatments applied. The highest graft survival was 55.6% was on the four-month old rootstock, after it was wrapped with Parafilm® and splice grafted.

When comparing the age of rootstocks, the four- month old rootstocks (with splice grafting) yielded the best survival as compared to the 10-month rootstocks (cleft grafted), except with the Vapor Gard® treatments, where the 10-month old rootstock had a graft survival of 33.3%,higher than the four-month old rootstocks. According to Rodriguez *et al* (2015), the advantages of grafting small plants include the reduced growth time of the plant, flexible and pliable cambium layers and the presence of smaller stem areas that are cut. The younger rootstocks performing better than older rootstocks is also because the young seedlings store more carbohydrates and other vital food substances allowing better growth and healing of the graft union (Nguyen & Yen, 2017).

3.5 CONCLUSION

The use of younger rather than older rootstocks is desirable because younger seedlings can be grafted all-year round. Younger seedlings have flexible and pliable cambial layers, making them easier to graft, which results in better growth and quicker healing of the graft union. The use of polyethylene grafting tape instead of Parafilm® was not successful. With the use of Vapor Gard®, the 10-month old rootstocks yielded higher graft survival (33.3%) as compared to the younger, four-month old rootstocks (22.2%). Splice grafting yielded higher graft survival (%) for the four-month old rootstocks, compared to cleft grafting. The 10-month old rootstocks showed higher graft survival (%) with respect to the cleft grafting method as opposed to the four month old rootstocks which were also cleft-grafted.

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CHAPTER FOUR: EFFECTIVENESS OF DIFFERENT TREATMENTS APPLIED TO MINI-GRAFTS OF *CORYMBIA HENRYI*

4.1 ABSTRACT

Mini-grafting allows for the grafting of smaller, more flexible and pliable cambium layers of younger rootstocks, reduced time of growth of the plant and smaller stem areas to cut and shape as compared to the conventional grafting of larger, older rootstocks. One of the limiting success in grafting is due to the loss of water through transpiration, it is therefore important to find suitable anti-transpirants that will lower loss of water. The aim of this chapter was to investigate the effect of some commercial anti-transpirants and antioxidants on increasing graft survival of *Corymbia henryi* mini-grafts compared to a standard control, Parafilm®. The different ancillary treatments applied were the control, which were tied with Parafilm®, the use of anti-transpirants such as Nu-Film 17®, Vapor Gard® and Greenstim®, and the use of an antioxidant, ascorbic acid.

Ascorbic acid was used as a foliar spray after the grafting process and yielded the highest graft survival (60%), compared to the other treatments. The treatments with the lowest graft survival were from the control treatment, 14.81%,whereas the use of Greenstim® and Nu-film 17®, resulted in no graft survival, respectively. The results suggest that mini-grafting could be adopted in the forestry industry as a standard practice for *C. henryi*. The use of anti-transpirants to reduce water loss can have positive effects on the grafts if applied at the required concentration for that crop, and if the number of applications is in accordance with the crop type. The use of antioxidants such as ascorbic acid has shown positive effects on stress alleviation and is encouraged to promote graft-take. For further applications, it is necessary to investigate the number of doses of anti-transpirants required by *C. henryi* and *Eucalytpus* species as a collective in order to determine the specific requirements for the crop and to test more commercially available anti-transpirants to find the most effective treatments.

4.2 INTRODUCTION

The use of grafting as a propagation technique is helpful as it allows for the rescue of genetic genotypes of interest and for propagation on a commercial scale (Perreira and Leal., 2002; Wendling *et al*.,2017; Stuepp *et al*, 2018). Grafting offers the advantage of allowing the union of more than one genotype, whether it belongs to the same species (Hartmann *et al*., 2015). The use of mini-grafting over the conventional grafting technique is supported as mini-grafting offers efficient management in nursery grown seedlings for activities such as irrigation, pest disease control and nutritional feed, coupled with a higher quality of seedlings (Xavier *et al.*, 2003; Schmidt *et al*. 2018). The grafting of small plants is encouraged due to the advantages if offers which include ease of handling of smaller seedlings compared to older, larger seedlings, the reduced time of plant to grow, and the flexible and pliable cambium layers of smaller seedlings (Ewens and Felker, 2003). One of the limiting factors in obtaining graft success is through the loss of water by the grafts during the hardening and healing phases (Shirai and Hagimori, 2004), resulting in desiccation at the graft union. It is therefore important for the plant to minimise transpiration after grafting. This can be achieved through the application of anti transpirant products, which are commercially available (Tambussi and Bort., 2007; Dabirian and Miles, 2017). The anti-transpirants decrease water loss from the leaves, through the size and number of stomata (Thorat *et al*., 2018) and by keeping the grafted plants in an environment with very high humidity.

The aim of this chapter was to evaluate the use of commercial anti-transpirant products on the survival of *Corymbia henryi* mini-grafts.

4.3 MATERIALS AND METHODS

4.3.1 Rootstock planting and selection

In this experiment, 360 rootstocks were planted and grown in 230ml seedling tray inserts (Speedling 98 Forestry trays, www.automa.co.za), until they were four- months - old and had stem diameters of 2-3mm at 15cm above medium level at time of grafting. The rootstocks were grown from South African commercial seed (mixed provenances). These were monitored, received foliar fertiliser applications and were watered daily with the use of sprinklers in the ICFR nursery. The rootstock plants were chosen by physical properties they showed which were based on their diameter, height, health and if they had leaves along the stem.

4.3.2. Scion collection and selection

The scions were collected by two people from the ICFR with different skills. One was an expert tree climber and the other was a supervisor. The scion wood was collected from Montigny *C. henryi* CSO (a provenance mix of good scion material) at sunrise, in the Zululand region. The scion parents were chosen to evaluate a diverse array of scion material for their compatibility with the chosen rootstock plants. Branches of the scion selections were placed in buckets filled with water to ensure that the material was kept moist. These buckets were then placed in a cooler box inside a pickup truck and driven from Zululand to the ICFR nursery in Pietermaritzburg. On arrival at the ICFR nursery, the assigned grafting team were given these selections and they chose suitable material to use, based on the age and shoot diameters of the rootstock plants.

4.3.3. Grafting experiment

Table 4. 1 Mini-Grafting of *Corymbia henryi* scion material onto rootstocks of four genotypes and then treated with four ancillary treatments

Scion material	C1	C2	C3	C4
Rootstock Age	10-month		Four-month	
Graft method	Splice		Splice	
Treatments applied to grafts	T1: Control (Parafilm®)		T1: Control (Parafilm®)	
	T2: Dip scion in Greenstim® (0.1mol)		T2: Polythylene Plastic Cover (one week)	
	T3: Dip scion in Nu-Film 17®		T3: Dip scion in Nu-Film 17®	
	T4: Spray with Ascorbic acid (100 mg L^{-1})		T4: Spray with Ascorbic acid (100 mg L^{-1})	
	T5: Vapor Gard® Dip (25 ml L^{-1})		T5: Vapor Gard® Dip (25 ml L^{-1})	

Table 4.1 shows the factors that were evaluated during the grafting of *C. henryi* seedlings. Four scion materials were used, shown as C1, C2 C3 and C4. The scion materials were all used in grafting with the four and 10- month old rootstocks. The graft method applied on the four-month and 10- month old rootstocks was splice grafting. A control and four treatments were applied on the grafts, which are shown as T1(scion laminae reduced to ¼ of original length at grafting, graft union wrapped with Parafilm) (control), T2 (Greenstim (a.i. glycine betaine) (dip scions into 0.1mol Greenstim®(Hygrotech Sustainable Solution, Mkondeni, Pietermaritzburg) solution (dilute 11.7g in 1L water), T3 (Nu-film 17® (a.i. 904 g/L di-1-p-menthene) (Hygrotech Sustainable Solution, Mkondeni, Pietermaritzburg), T4 (Ascorbic acid (100 mg L-1), T5 (Vapor Gard®(Miller Chemical, Pretoria.)

4.3.4. Experimental design

A randomised complete blocks design was adopted for the grafting experiment, replicated three times. Each plot consisted of four grafted plants. The treatments were a factorial arrangement of two rootstock ages, four scion genotypes and four anti-stress treatments and a control.

4.3.5. Glasshouse

Once the grafting experiment had been completed, all the grafts were taken to glasshouse no. 4. The temperature and humidity were set at 25°C with 80% RH. The misting frequency was set to occur every eight minutes for 10 seconds, keeping the plants moist as they require humid conditions. The misting frequency was adjusted depending on the weather conditions; i.e., on hotter days, misting had to be on for a longer period than on cooler days, when misting frequency was lowered. Two hobo temperature (℃) and humidity (%) loggers were placed in the middle of the tables where the experiments were taking place, to measure temperature and humidity in the glasshouse. Heating mats were installed on the tables on which the grafts were placed to warm the base of the trays or pots.

4.3.6. Monitoring and maintenance of the grafted plants

The grafted plants were monitored every day to ensure that they received an adequate amount of water and monitored for any plant diseases. The grafts were assessed after two weeks for bud development above the graft union. Any excess buds on the rootstock were removed to ensure that they did not inhibit the flow of water and nutrients from the rootstock to the scion bud. This was done using secateurs. Any weeds were growing in the medium were removed. The grafted plants were fertilised with Osmocote Mini® at the recommended rate (http://osmocote.co.za) once they were in the glasshouse.

4.3.7 Assessments and measurements

Parameters measured were graft survival (%)

4.3.8 Statistical Analysis

An analysis of variance (ANOVA) was performed to evaluate the effect of the different treatments used on the graft success using Genstat, 18th edition.

4.4 RESULTS

Table 4. 2. The ANOVA output for the mini-grafting experiment

Source of variation	d.f.	F-value	P(F.pr)
Block	2	0.37	0.692
Treatment	4	110.67	< 0.001

CV % = 5.4

Table 4. 3 The mean percentage of graft survival for each treatment that was applied in the mini-grafting

Treatment applied	Graft survival mean (%)
Control (Parafilm®)	14.81^{b}
Greenstim®	0.00^{a}
Nu-film 17®	0.00^{a}
Ascorbic acid Solution	60.00^{d}
Vapor Gard®	28.15^{c}

*Treatments with the same letter are not significantly different.

Table 4.3 showed that spraying the grafts with ascorbic acid solution gave the best results, with 60% grafts surviving, followed by graft treatment with Vapor Gard®. The lowest graft survival was found with the use of Greenstim® and Nu-Film®.

4.5 DISCUSSION

The use of antioxidants has been widely encouraged in stress alleviation for plants. In studies by Alzate *et al*. (2002) and Abbasifar and Valizadehkaji (2017), various antioxidants enhanced graft success. These included polyvinylpyrrolidone (PVP), ascorbic acid and citric acid to lower the secretion of phenolic compounds in the site of the graft (Alzate et al. 2002). Thomas (2008) also reported that antioxidant materials can be used to reduce the secretion of substances with high phenolic content in micro-propagation and to prevent the browning of the medium and explants in tissue culture conditions. The secretion of these substances with high phenol content in the graft site may prevent graft-take, reducing the success of the process. Ascorbic acid is an important antioxidant that has been used broadly in propagation of plants and is also present in various cell organelles and in the protoplast (Ergin *et al*., 2014). Previous studies reported that the exogenous application of ascorbic acid decreased negative effects of stress conditions in plants such as heat stress in rice, sunflower, bean, mung bean and wheat. The endogenous content of ascorbic acid has also been found to increase through exogenous application through the rooting medium, as a foliar spray and as a seed priming (Ergin *et al*., 2014). In this study, ascorbic acid was used as a foliar spray after the grafting process and yielded significantly ($p > 0.05$) high survival of grafts as compared to the other treatments applied, with a graft survival of 60%. The positive effect of ascorbic acid in grafting is supported through various studies. Abbasifar & Valizadehkaji (2017) reported that ascorbic acid (AA) and polyvinylpyrrolidone (PVP) stimulated the growth of the scions in the budding of Persian walnut. The use of antioxidants such as AA is encouraged since they have been found to lower the activity of compounds with high phenolic content, which interfere with the differentiation of vascular tissues, and limit the transfer of auxins to the rest of the plant, causing negative effects on graft success (Farsi *et al*., 2018). Ascorbic acid has also been reported to eliminate the reactive oxygen species (ROS) that are released when the graft injury occurs. The elimination of ROS allows for successful grafting through maintaining high cell activity at the graft site (Johkan *et al*., 2008). The positive effects of AA have also been found on grafting of sweet pepper plants (Johkan *et al*., 2008), where various concentrations of AA were applied as foliar sprays to the leaf surface of old-stage scions and rootstocks. This yielded an increase in the thickness of the callus that formed at the cut surface of the scion stems. The role of antioxidant substances is positive in grafting because these substances can decrease or eliminate the activity of phenols, which have negative impacts when secreted at the wound site of the graft. These prevent movement of auxins and cytokinins, which are essential for the growth and differentiation of cells in the plants (Abbasifar & Valizadehkaji, 2017). Ascorbic acid has been reported to affect scion formation from callus in tissue culture of tobacco in, and is now thought to have an effect also on the differentiation of vascular bundles and is thus its use is encouraged for use in grafting, either through immersing the scions in an AA solution before grafting occurs, or as a foliar spray on the leaf surface of the scions and rootstocks (Dabirian *et al*.,2017)

The treatments with the lowest survival rates were the use of Greenstim® and Nu-Film®, which had 0 survival. Greenstim® is based on the activity of glycine betaine (GB), which occurs naturally in several organisms and in some plants. In plants, GB works as an osmoprotectant by adjusting the osmotic balance inside plant cells and tissues (ref). This compound accumulates naturally in certain plants (e.g., sugar beet, spinach), in response to stress issues such as drought, cold and salinity (ref). There are several reports recording success in the application of exogenous GB on the growth of plants and yield increase under drought stress in plants such as barley, wheat, soybean, tobacco, maize, sunflower and common beans (Fariduddin *et al*., 2003). The exogenous application of GB has been found to rapidly penetrate through leaf surfaces and be easily transported to other plant organs, aiding the improvement of stress tolerance in the plants. In a study conducted by Tisarum *et al*. (2019), it was found that a foliar application of 100mM GB had an influence on growth performances, yield and physio-biochemical responses in *Indica* rice that was under extreme water deficit conditions at the booting stage. The application of Greenstim® (11.7g L^{-1}) did not yield good results, since none of the treated plants survived. There are contradictory findings on the application of GB for alleviation of abiotic stress in crops, where some crops have been found to benefit from GB application in order to induce tolerance to drought and salt tolerance, especially in crops such as maize, tomato, rice and wheat, whereas in cotton, the use of GB has been found to not show any significant effect (Meek and Oosterhuis, 2003; Ibrahim *et al.,* 2006). The latter findings in the use of GB on cotton agree with the results in this study, where no positive effect was found from use of this osmoprotectant. The report by Arfan *et al*. (2007) suggested that the effectiveness of foliar application of GB is dependent on factors such as the species, plant development stage at which GB is applied, the concentration of GB used and the number of applications. The ineffectiveness of GB has also been reported by various researchers in different crops, such as the report by Lopez *et al*(2002), who identified an increase in the leaf area after a GB application of 10mM in kidney beans, but no increase was observed at an application of 30mM GB. A report by Mickelbart *et al* (2006) also showed that an exogenous application of GB at 50mM reduced the leaf area and growth rate. In a study by Wilson (2001), 25mM GB application in field-grown grapevines resulted in phytotoxic effects. The negative effects of the exogenous application of GB in this study may be due to the concentration used (may be too high/too low for absorption by the plants), the limited number of applications, since the plants only received one application after grafting and the inability for the *Corymbia henryi* to be able to absorb GB when applied exogenously, since there are no reports of the use of this compound in these plants. According to Mickelbart *et al*. (2006), before exogenous application of GB, it is vital to find out the endogenous levels of GB for that certain plant/species, the ability of the leaves to absorb GB, its effects on growth for that plant and the concentrations at which it might be toxic to a certain species. In this study, a negative effect of GB was found, and thus more studies need to be carried out on the use of such compounds in grafting of *Corymbia* species and other eucalyptus plants.

The use of anti-transpirants to reduce water loss in plants under stressful conditions has been widely used in several different crops (Aldasoro *et al*.,2019). These substances are used to reduce transpiration rates, reduce water loss from the soil and aid water use efficiency in plants suffering from water stress conditions such as drought (Khalel, 2015). Nu-film 17® (a.i. poly-1-menthene, low viscosity, Hygrotech, KZN, South Africa) is an anti-transpirant which is hydrophobic, thus creates a low water potential on the surface of the leaves. In this experiment, the use of Nu-film 17® did not have a positive effect on the graft takes and survival, yielding no successful grafts as compared to other treatments. The potential value of an anti-transpirant during the grafting process is because the cambia of the scion and the rootstock must merge tissues, and until this happens, the graft tissue may dehydrate and die. Therefore, it is essential to reduce transpiration to avoid the scion tissue drying out and dying. (Trueman *et al*., 2018).

One way to do so is to use anti-transpirants, the other being able to keep the grafted plants in an environment with very high humidity. However, in this study, the application of Nu-Film® 17 on the grafted plant was not effective, and Vapor Gard® was partially successful. The use of the film-forming polymers has been found to be a temporary solution to water related stress and is dependent on the experimental conditions under which it is applied.

3.5 CONCLUSION

The use of an anti-oxidant ascorbic acid was successful in reducing graft stress and increased grafting success substantially. In contrast, the use of anti-transpirants in grafting of *Corymbia* was not successful with the doses and frequencies used but may hold potential for future research. The evaluation of more anti-transpirant products on grafting needs to be investigated. The success of the anti-transpirants may also be due to the number of doses applied, which needs to be further investigated in *Corymbia henryi* and *Eucalyptus* species since most of this information is limited.

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CHAPTER FIVE: THESIS OVERVIEW

5.1 INTRODUCTION

In South Africa, of the 1.2 million ha of forests, 512 225 ha are devoted to plantations of *Eucalyptus* species and their hybrid combinations (FSA, 2015; Dlamini *et al*., 2018). With the increasing demand for timber and pulpwood, it is important to ensure the availability of fast - growing trees that will ensure gains in productivity (Mellesse and Zewotir, 2017). Natural hybridization in *Eucalyptus* species is a common practice and has contributed to genetic improvement programmes to develop hybrids with desirable traits such as disease resistance, increased yield, increased uniformity, and rapid multiplication (Mellesse and Zewotir, 2017). The efficiency of eucalyptus species in the forestry sector is largely dependent on vegetative propagation techniques, which ensure the mass multiplication of superior genotypes (Babu *et al*., 2018), both as pure and interspecific hybrids (Vilasboa *et al*, 2019) to maximise the productivity of high - value hybrid clones (FAO, 2014). *Corymbia* species and their hybrids are timber eucalypts of increasing importance, due to their unique properties. However, they are hard to propagate from seed. Therefore, it is essential to do more research on the propagation of these hybrids. *Corymbia* hybrids were chosen for more research because of the advantages they have over their paternal parents, which include superior growth, disease, insect and frost tolerance and adaptability to marginal of subtropical and tropical regions (Lee, 2007). With the limitations of propagation of *Corymbia* hybrids from seeds, propagation by cuttings and grafting seems to be a better alternative.

In this context, this study was performed to investigate the vegetative propagation of *Corymbia* hybrids through the rooting of cuttings and the grafting of *Corymbia henryi*.

5.2 SUMMARY OF RESEARCH FINDINGS

5.2.1 Rooting of various *Corymbia* hybrids

The conclusion in Chapter 2 was that the use of ascorbic acid (40 mg L^{-1}) together with the rooting hormone, Seradix 2 (IBA 3 g^{-1} kg^{-1}) resulted in the highest root percentage across all the experiments. This combination appeared to work synergistically because ascorbic acid is known to act by protecting IBA from oxidation, therefore allowing it to act on the plants by enhancing their tolerance to stress and greenhouse conditions. It is advised to use a lower dose of ascorbic acid (40 mg L^{-1}) because the higher dose (80 mg L^{-1}) tested in the experiments, resulted in lower root survival. The use of IBA as a rooting hormone is a standard and results in good rooting, but combined synergistically with ascorbic acid, resulted in greater rooting success.

Cuttings material harvested from coppice plants showed a higher rooting of cuttings than material harvested from hedge plants. Three hybrid genotypes were tested in the experiments (ct x ccv; ct x ch; ct x ccc) among 14 different selections. The *Corymbia torelliana* x C. *citriodora* subsp. *variegata* (ct x ccv) hybrid had the highest rooting survival, with the selections showing rooting percentages ranging from 25-70%. Among the selections, the others identified with good rooting properties were MF 20, MF 22, MF 60(ct x ccv) and MF 100 (ct x ch). Therefore, clones can be selected according to their rooting ability, a measurable phenotypic trait.

This chapter highlighted the positive outcome that high percentages of rooting could be achieved in difficult-to-root species if rooting hormones were applied with antioxidants.

5.2.2. Optimizing Grafting by Comparing Conventional and Mini-Grafting Techniques for *Corymbia henryi*

The main conclusion made in Chapter 3 was that the use of mini-grafting (use of four-month old seedlings) can be adopted as an alternative to conventional grafting (use of 10-month old seedlings) because there was a higher survival rate when grafting onto the younger plants. This allows for grafting to be performed more times in the year than with conventional grafting, where more time is taken waiting for plants to grow older.

The use of Parafilm resulted in the highest graft survival across the treatments, in the younger scion.

When comparing grafting methods in the 4-month rootstocks, splice grafting resulted in the highest survival, when compared to cleft grafting.

5.2.3 Effectiveness of Different Treatments Applied to Mini-Grafts of *Corymbia henryi*

The conclusion made in Chapter 4 was that ascorbic acid can be used successfully in alleviating stress in grafts and increase graft success significantly. The use of anti-transpirants in grafting of *Corymbia* was not successful but, further investigations may need to be made for each crop requirement as different crops require different doses of anti-transpirant treatments.

5.3 RECOMMENDATIONS AND FUTURE RESEARCH

More research is required to optimize rooting, especially on hybrids that possess good characteristics and those which are difficult-to-root because the study showed that it is still not possible to achieve rooting rates of greater than 70%, which is the level that commercial nurseries need to achieve.

Some specific recommendations include:

a. An increase in the number of hybrid genotypes that are tested is essential to make a better overall recommendation on the hybrids that need to be selected for outplanting in the field.
b. Increase rooting trial tests for *C. torelliana* x *C. citriodora* subsp. *variegata* selections

c. More mini-grafting studies for *Corymbia henryi* and to familiarise grafters with the use of younger, more fragile rootstocks.
d. Review the use of anti-transpirants in combination with anti-oxidants such as ascorbic acid to alleviate stress on the plants, to optimize grafting.

Overall, the study has provided an insight into the use of *Corymbia* hybrids in advancing vegetative propagation and has given a platform for further studies into these important species and has shown good potential for the commercialisation of these hybrids through vegetative propagation techniques such as cuttings and mini-grafting.

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