THE PERFORMANCE AND ROOTING OF *Eucalyptus grandis* x *nitens* CUTTINGS

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PREFACE

The experimental work described in this thesis was carried out at the University of KwaZulu-Natal, Durban and the Trahar Technology Centre, Mondi Business Paper from February 2005 to October 2007, under the supervision of Professor M.P. Watt, Professor D.J. Mycock (University of Witwatersrand) and Dr. Oscar Mokotedi (Co-supervisor).

These studies represent work by the author and have not been submitted in any form to another tertiary institution. Where use was made by work of others, it has been duly acknowledged in the text.

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Nelisha Murugan

I certify the above statements to be true:

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Prof. M.P. Watt (Supervisor)

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Date

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ABSTRACT

Hybrid clones of *Eucalyptus grandis* and *E. nitens* (GN) have consistently been shown to be suitable for planting in cold, dry, marginal plantation sites, where they exhibit high yields and superior pulp properties. However, their clonal propagation is hindered by the very poor rooting success of cuttings. The present study aimed at assessing the effect of cutting type, time of year of setting cuttings and Seradix 2 application on rooting and development of cuttings of a commercially important *Eucalyptus grandis* x *Eucalyptus nitens* clone (GN107).

Cuttings were prepared from clonal hedge coppice at the Mondi Business Paper, Trahar Technology Centre, Hilton. Three cutting types were used (cut at different distances from the node) for each terminal (situated below the apical bud) and non-terminal cuttings. The leaves were trimmed and, for half the cuttings, the base of the stem of cuttings were dipped in Seradix 2 rooting powder (3 g kg^{-1} 4-(indole-3-yl)-butyric acid (IBA). They were then placed into rooting trays (128 inserts/ tray arranged as 8 rows x 16 columns). Seradix 2-treated and Seradix 2-untreated terminal and non-terminal cuttings, cut at, above and below the node (twelve treatments in total) were set in trays with one treatment per column of eight replicates, per tray. There were nineteen trays overall. The trays were filled with peat, perlite and vermiculite (3:3:1) and were maintained in a Mondi greenhouse, with air temperature at 25°C to 27°C (thermostatically activated fans), root zone temperature at 28°C (bed heaters) and 20 second misting at 10 minute intervals (automatic misters). The study was carried out in November 2005, April 2006 and June 2006. In the first experiment, both terminal and non-terminal cuttings were used; thereafter only non-terminal cuttings were used.

The plantlet yield was very low, regardless of cutting type, Seradix 2 treatment and the time of year the cuttings were set. The highest plantlet production (12.5%) and rooting frequencies (13.8%) were achieved with non-terminal cuttings treated with Seradix 2. Although not statistically significant, Seradix 2 inhibited shoot production (31.4% for Seradix 2-untreated and 24.2% for treated cuttings). The position at which inserts were cut in relation to the node did not significantly affect the number of plantlets produced

and non-terminal cuttings appeared hardier and performed better than terminal cuttings. The time of year of setting cuttings did not have any significant effect on plantlet yield, nonetheless, plantlet yield was highest in cuttings set in November (9.2%) and lowest in April (0.4%). In addition, cuttings set in November (spring), had superior shoot development in terms of the number of cuttings that produced shoots (regardless of root production), shoot length and the mass of shoots relative to root mass. The highest percentages of cuttings that produced roots (regardless of shoot growth) (10%) and the highest number of roots per cutting (2) were part of the June trial. Therefore, cuttings set in June (winter) had superior root development as compared with cuttings set in November (spring) or April (autumn).

In all of the studies, three rooting patterns were observed in cuttings: roots produced only from the cut area only (type 1), only from the sides of the stem (type 2) and from both sites (type 3). Non-terminal cuttings treated with Seradix 2 showed a higher incidence of types 2 and 3 rooting patterns than the terminal cuttings. Seradix 2 application increased the prevalence of types 2 and 3 rooting patterns. Although not statistically different, cuttings dipped 2.5 cm into Seradix 2 produced more types 2 and 3 rooting patterns than cuttings dipped at the abaxial end only. Light microscopy of stem sections of cuttings indicated that roots appeared to originate from the xylem archs as well as from the cambium.

The collected data indicate that it is necessary to continue research towards improving the efficiency of plantlet production of GN107 via cuttings. It appears that cuttings of this clone may be set throughout the year and that terminal cuttings should be avoided. In addition, the present practice at the Mondi Hilton nursery of treating cuttings with Seradix 2 needs to be reconsidered as although it increases rooting, it does not increase plantlet production due to its apparent inhibitory effect on shoot development.

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LIST OF ABBREVIATIONS

Anon	anonymous
BCE	before common era
©	copyright
Ca	calcium
CE	common era
cm^2	centimetre
°C	degrees Celsius
°	degrees
FAA	formalin acetic acid alcohol
GN	*E. grandis* x *nitens*
GN107	*E. grandis* x *nitens* clone 107
$g\ kg^{-1}$	grams per kilogram
IAA	indole-3-acetic-acid
IBA	indole-3-butyric-acid
K	potassium
L	litre
m^3/Ton	metres cube per ton
Max	maximum
Min	minimum
Mg	Magnesium
ml^2	milliletres square
mm	millimetre
NAA	naphthalene-acetic-acid
N	nitrogen
P	phosphorous
%	percentage
v/v	volume by volume
w/v	weight by volume
≤	less than or equal to
≥	greater than or equal to

SPSS	statistical package for social science
ANOVA	analysis of variance
HSD	honestly significant difference

1. INTRODUCTION & LITERATURE REVIEW

1.1 Brief history and importance of *Eucalyptus*

Eucalyptus (family Myrtaceae), with more than 450 species, is the most widely propagated tree genus throughout the world (Zacharin, 1978; Turnbull, 1991). According to a variety of authors (Zacharin, 1978; McComb and Bennett, 1986; Eldridge *et al.*, 1994; Campinhos, 1999; Turnbull, 1999; Smit and Pitcher, 2003), since it was first discovered in Australia over 200 years ago, seed dispersal by travellers, traders, gold miners, soldiers, priests and botanists have spread various species to many parts of the world. Although indigenous to Australia and its northern neighbours, such as the Philippines, West Timor and New Guinea, many eucalypt species and its hybrids are extensively planted in temperate and subtropical regions in countries that include Argentina, Brazil, India, Morocco, Portugal, South Africa and Spain. At the time of their discovery, the potential utilisation of eucalypts as a major source of commercial forestry products was not recognised, and they were mainly used as a source of firewood. Since then, eucalypts have proved to be very versatile and are now utilised to produce industrial charcoal, sawn timber, mine props, railroad sleepers, fibreboard, furniture, firewood, essential oils, honey, tannins, pulp and paper.

The genus *Eucalyptus* contains a wide range of species with respect to adaptation to sites, types of management systems and variety of uses, both as natural forests and plantation forests (Eldridge *et al.*, 1994). According to Eldridge *et al*. (1994), the top ranking *Eucalyptu*s species around the world in terms of mean annual increment of wood are *E. grandis*, *E. camaldulensis*, *E. tereticornis*, *E. globulus*, *E. urophylla*, *E. saligna*, *E. viminalis*, *E. deglugpta*, *E. exserta*, *E. citriodora*, *E. paniculata* and *E. robusta*.

Eucalypts were first introduced into South Africa as early as 1803. Since then, critical timber shortages as a result of the discovery of gold (and the subsequent establishment of the mining industry) and the First World War, prompted the government to promote

and expand the forestry industry (King, 1951; Zacharin, 1978). As the over-utilised indigenous forest trees were unable to recover quickly enough to meet timber demands, exotic species, such as *Eucalyptus* were imported to South Africa and propagated via seeds and cuttings (King, 1951; Luckhoff, 1973; Smith, 1996).

According to numerous authors (Zacharin, 1978; Blake, 1983; Gupta and Mascarenhas, 1987; Turnbull, 1991; Bouillet *et al*., 2004; Pallett and Sale, 2004), eucalypts display a number of features that make them popular exotic plantation species. They are fast-growing trees, with a short rotation period and they produce better quality wood and more uniform stands than most indigenous trees. Part of their global success is due to their ability to adapt to a range of soil types and climatic conditions, including nutritionally poor soil and even acidic soil. Eucalypt seeds are classified as orthodox and can therefore be stored for long periods and are easily transported/distributed. In addition, eucalypt trees produce coppice readily, many are relatively easy to clone and pure species can be crossed to produce hybrids with desirable characteristics. Furthermore, a host of valuable end products can be produced from these trees, perhaps the most important of these in South Africa, are pulp and paper (Denison, 1999).

In 1999, it was estimated that there were approximately 12 – 14 million hectares of eucalypt plantations around the world, of which the plantation area of eucalypts in South Africa comprised approximately 600 000 hectares (Turnbull, 1999). In the last three years, many plantations in countries around the Mediterranean coast, such as Spain, France, Portugal and most recently in Greece, have been destroyed by forest fires. In 2005, 1.1% of the total area of South Africa was reported to be used for forestry, which at the time comprised of 37.2% eucalypts, 54.1% pine, 8.1% wattle and 0.5% other species (Godsmark, 2006). The majority of eucalypt plantations are made up of five commercial species and their hybrid clones, viz. *Eucalyptus grandis*, *E. nitens*, *E. smithii*, *E. macarthurii* and *E. dunni* (Pallett and Sale, 2004). Eucalypts are grown primarily along the east coast of South Africa in Kwazulu-Natal and Mpumalanga, but plantations have also been established in the Eastern and Western Cape and the Northern Province (Denison and Quaile, 1987; Godsmark, 2003; Smit and Pitcher, 2003) (Figure 1.1).

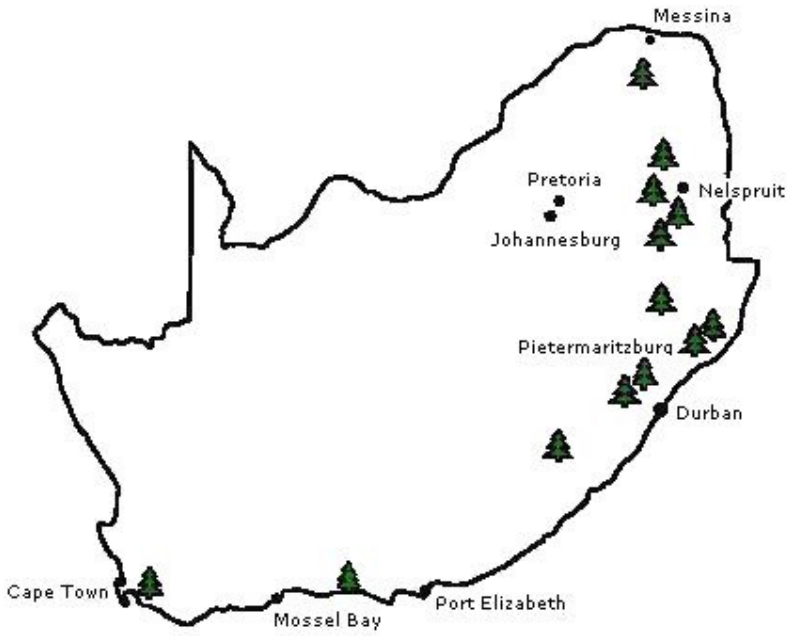

Figure 1.1: Geographic locations of commercial forestry plantations in South Africa (Adapted from Anon, 2004).

1.2 The importance of the Forestry Industry to the South African economy

Since its establishment in this country, the Forestry Industry has expanded rapidly and has become one of the fastest growing sectors of the South African economy (Denison and Kietzka, 1993a; Anon, 2004; Louw, 2004, Chamberlain *et al*., 2005). It supplies wood products both locally and internationally and this contributes to valuable foreign exchange (Cellier, 1993; Edwards, 2000; Smit and Pitcher, 2003). In 2001, the industry was valued at over R12 billion (Harvett, 2001). In addition, the forestry industry and related forestry products sector represents an important employer in South Africa; employing over 150 000 people in 2003 (Smit and Pitcher, 2003).

Although eucalypt plantations were first established in this country predominantly for the production of mining timber, the common end products now also include pulp for

the paper industry and industrial cellulose (Le Roux and van Staden, 1991; Denison and Kietzka, 1993a; Smit and Pitcher, 2003; Pallett and Sale, 2004; Anon, 2006). However, the pulpwood has emerged as being most prominent and profitable among the common end-products of eucalypts (Table 1.1). *Eucalyptus* makes excellent pulp suitable for printing, writing and tissue paper; this is attributable to its wood that produces uniform material with high brightness, good density and bulk (Turnbull, 1991). In 2002, the South African pulp and paper industry produced over R15 billion worth of pulp, and exported over R6 billion worth of product (Hunt, 2003). There are currently over 27 pulp and paper mills in South Africa of which 13 are in KwaZulu-Natal. These represent 75% of the national pulp making capacity and 56% of the national paper making capacity (Hunt, 2003). South Africa manufactures around 2.2 million tons of pulp and 2.6 million tons of paper a year, making it the 14th largest pulp producing nation in the world and the 24th largest producer of paper in the world (Anon, 2004).

Table 1.1: Sales of roundwood harvested from plantations in South Africa for the year 2004/2005 by quantity and value (Anon, 2006).

Product	Sales by quantity	Sales by value (Rand million)
Sawlogs & veneer logs	5,475,441 m^3	1,131.9
Pulpwood	11,757,666 Tons	3,568.2
Mining timber	652,789 Tons	137.0
Poles	504,611 m^3	85.7
Charcoal & Firewood	218,923 Tons	21.9

In 1968, Mondi Forests (Mondi Business Paper since 2005) was established and since then it has been involved in tree research and the propagation of trees with improved quality. Over the years, the emphasis of the company has shifted from the production of softwood sawlogs (pine) hardwood fibre and pulp production, which consequently, resulted in the formation of a clonal eucalypt programme (Denison and Quaile, 1987; Denison and Kietzka, 1993a). The research collaboration between Mondi and the

University of KwaZulu-Natal has been in place for over 15 years and the present study was undertaken within that research programme.

1.3 Hybrid forestry

As mentioned previously, the Forestry Industry is one of the fastest growing industries of the South African economy, and afforestation, land purchases and expansion have exploded in recent years. However, as prime areas of good climate, rainfall and soil are also in great demand for agricultural crops and livestock, forestry expansions have occurred largely on marginal sites (cold, dry and often nutritionally-poor sites) (Denison and Kietzka, 1993a; Bouillet *et al*., 2004). In addition, the government's Department of Water Affairs and Forestry has imposed strict policies controlling the use of land and water and minimising competition of productive land with the agricultural sector (Anon, 2006). In order for the forestry industry to remain productive and to meet the ever-increasing demands for forestry products, productivity on existing plantations and marginal sites needs to be maximised (Denison and Kietzka, 1993a; February *et al*., 1995; Dye, 2000; Bouillet *et al*., 2004). This has lead to an increased focus on hybrid forestry.

Hybridisation enables foresters to combine desired characteristics of two (or more) pure species in the hybrid individual. Hybrid forestry has provided the forestry industry with a means to match clones to sites and, in this manner, increase productivity on existing plantations sites. Often hybrid individuals exhibit greater vigour than the parents, known as hybrid vigour or heterosis. On marginal sites, hybrids can surpass the pure species in terms of growth and survival and are consistently more resistant to diseases, pests, cold, heat and drought (Denison and Quaile, 1987; Denison and Kietzka, 1993a; Jones and van Staden, 1994; Denison, 1999). However, several authors advise that care should be practised in the assessment of hybrid vigour, as it is affected by time and location (Zobel and Talbert, 1984; Martin, 1988; Denison and Kietzka, 1993a). In South Africa, hybrid forestry has made it possible to extend tree planting to marginal areas previously considered unsuitable or "off-site" for plantation forestry (Denison and Kietzka, 1993a; Jones and van Staden, 1994; Wex and Denison, 1997). In this country,

E. grandis is prominent as a pure species or as a hybrid (Denison and Quaile, 1987; Van Wyk, 1990) and its climatic range and location has been extended significantly as a result of hybridisation (Aimers-Halliday *et al*., 1999). In subtropical areas, the most commonly planted hybrids are *E. grandis* crossed with *E. urophylla, E. camaldulensis* or *E. tereticornis* and in temperate areas, are *E. grandis* crossed with either *E. nitens* or *E. macarthurii* (both cold-tolerant eucalypts) (Denison and Kietzka, 1993a). Darrow (1996) stated that *E. nitens* (pure species) is probably the most popular cold-tolerant eucalypt in South Africa as it is extremely frost-hardy and exhibits excellent qualities in terms of tree growth, height and survival.

The benefits of hybrid forestry, as discussed by Denison and Quaile (1987) and Denison and Kietzka (1993a; 1993b), include hybrid vigour, resistance to diseases, higher wood density and adaptability of hybrids to marginal sites. Another benefit is increased nursery efficiency given that hybrids root more rapidly (and require less time in the nursery), are less sensitive to handling, heat and drought than pure species (Denison and Kietzka, 1993a). In the early 1990's it was envisaged that the use of cold-tolerant hybrids, such as *E. grandis* x *E. nitens* (GN), would increase in South Africa as vegetative propagation techniques improved (Denison and Kietzka, 1993a). Studies have shown that clones of *E. grandis* x *E. nitens* hybrid adapt to sites more readily than pure species and may even require less water and use water more efficiently for production than pure species (Denison and Kietzka, 1993a; February *et al*., 1995). In addition, this hybrid has been found to have good wood qualities ideal for the pulp and paper industry. For these reasons, this clone has been widely propagated in KwaZulu-Natal and other parts of South Africa. In the 1990s clones of *E. grandis* x *E. nitens* gained status as replacement planting stock for sites that were previously planted with *E. grandis* (Denison and Kietzka, 1993a).

1.4 Propagation of *Eucalyptus*

Traditional methods of propagation of forest trees, including eucalypts, have depended upon the growth of bulked seed collected in nature or from seeds collected from randomly pollinated superior trees (Ahuja, 1993). However, those forests are

characterised by a large variation in growth, form and vigour. In terms of commercial forestry, these traits are considered undesirable where a uniform stand of superior trees ideally needs to be established (Lakshmi Sita, 1986; Schuch, 1991; Ahuja, 1993; Bell *et al*., 1993). In addition, seeds may be susceptible to genetic damage and rapid loss of viability (Ahuja, 1993). In the case of *Eucalyptus*, certain species are characterised by irregular seed set, as is the case for *E. dunnii* (Lakshmi Sita, 1986) and *E. nitens* (Eldridge *et al*., 1994; Maile and Nieuwenhuis, 1996) while other species such as *E. dives*, *E. nipophila* and *E. pauciflora* require stratification for improved germination (Hartmann *et al*., 1997). Jones *et al*. (2000) reported that irregular flowering and high abortion rates are characteristics of many eucalypts, which also contribute to limited seed supply. However, despite these setbacks, sexual reproduction of forest tree species is vital in breeding programmes, as it provides a diverse genetic base from which superior trees can be selected (Harvett, 2001). In the case of eucalypts, superior trees are pollinated and the resulting seed is collected and planted out in provenance trials (Denison and Kietzka, 1993a).

The breeding of *Eucalyptus* requires maximising genetic gain while minimising genetic erosion (Burley, 1989). In light of their long maturation times (years or decades) and changes with time in growth rate and morphology (Hartney, 1980), the ideal approach to maximise genetic gain of eucalypts is through selection followed by asexual propagation (Watt *et al*., 2003). Asexual (vegetative) propagation enables the production of individuals that are genetically identical (clones) to the parent plant. Such clones can be derived from individual cells, calli, tissues, *in vitro* cultures, cuttings (conventional vegetative propagation) and specialised plant structures (e.g. bulbs and rhizomes) (George, 1993; Hartmann *et al*., 1997). Every cell of a plant has the ability to divide and regenerate into an entire new plant. This inherent ability of a plant cell is called totipotency and it has been manipulated to bring about various vegetative propagation techniques, which will be discussed in this section, with emphasis being placed on cutting propagation. Vegetative propagation of forest trees or clonal forestry, results in the production of genetically identical individuals thus overcoming the problem of variation observed in material derived from seed orchards (Ahuja, 1993). In addition, vegetative propagation allows superior material to be commercially

propagated more rapidly than through seed orchards and can be used as a tool to maintain hybrid vigour (Ahuja, 1993).

There are various methods of vegetative propagation such as grafting, layering, micropropagation and propagation through cuttings (Hartmann *et al*., 1997). Grafting involves the attachment of scions from superior trees onto seedling stocks of the same species or different species (Konar and Nagmani, 1973; Biondi and Thorpe, 1981; Gardner, 1998). This technique is regarded as labour intensive and expensive and care must be taken to select the appropriate graft to prevent graft incompatibility (McComb and Bennett, 1986). In the case of eucalypts, Gardner (1995; 1996; 1998) reported that the size of the rootstock has an effect on the success of the graft procedure and rootstocks that are not fully developed may delay grafting. Graft incompatibility between the scion and the rootstock is a common problem in *E. macurthii* in the field and in the nursery (Gardner, 1996). Late grafting (later than August and September) of *E. nitens* may result in low survival rates (9.5%), as was the case for *E. smithii* (2.5%) (Gardner, 1998). Air layering is also considered expensive and is associated with a high failure rate and is therefore implemented in special purpose plantations only (Cresswell and de Fossard, 1974; Hartney, 1980; McComb and Bennett, 1986).

In vitro techniques have been applied successfully to a number of plant species, including ornamentals, crops, horticultural plants and commercially important forest tree species (see George, 1993), including eucalypts (Watt *et al*., 2003). Biotechnological methods encompass a number of techniques that enhance existing tree improvement programmes (Cheliak and Rogers, 1990; Dvorak, 2001). This type of propagation has the major advantage over the other methods of enabling the mass production of genotypes (and clones) in a short period and it yields high multiplication rates (Bonga, 1977; Mascarenhas *et al*., 1981; Lakshmi Sita and Shoba Rani, 1985; Nel, 1985; Lakshmi Sita, 1986; Ahuja, 1993; Zobel, 1993; Haines, 1994; Yang *et al*., 1995; Watt *et al*., 1999). While micropropagation techniques offer several advantages over conventional methods of propagation, there are drawbacks: a highly specialised and expensive facility is needed and the technique is labour-intensive and highly technical. Perhaps the most pertinent limitation to *in vitro* plant propagation is clonal specificity,

which means that fairly specific methods are required for optimum results with each species, variety and explant (George, 1993; Watt *et al*., 2003). For eucalypts, the variability of rooting among clones and the gradual decrease in rooting ability in aging parent plants creates drawbacks in its vegetative propagation. With some clones such as the cold-tolerant *E. grandis* x *E. nitens*, the problem of poor rooting persists *in vitro* and is often accompanied by large amounts of callus production, which may hinder the development of roots (Mokotedi *et al*., 2000).

Micropropagation and particularly axillary bud proliferation is now used extensively to support *Eucalyptus* breeding and clonal programmes at Mondi Business Paper and by other international forestry industry companies (Watt *et al*., 2003). However, vegetative propagation by stem cuttings remains a prominent method for the production of material for plantations. As cutting propagation is the focus of the study reported here, it is discussed in detail in the ensuing section.

1.5 Propagation by cuttings

Vegetative propagation by the rooting of cuttings may be the most ancient form of asexual propagation (Hartmann, 1988). According to Haissig and Davis (1994), for some species evidence of cutting propagation exists in antiquity, as supported by the writings of Aristotle (384 – 322 BCE), Theophrastus (371- 287 BCE) and Pliny the Elder (23 – 79 CE). Considerable research efforts have since led to the discovery and use of auxins, mist and sterile tissue culture techniques in cutting propagation, among others, and the variety of plants that can be rooted and rooted more rapidly is remarkable (Hartmann, 1988).

As previously mentioned, stem cuttings are the preferred method of commercial vegetative propagation of forest trees (including *Eucalyptus*) because a large number of cuttings can be obtained from a single tree, cuttings are generally cheaper to obtain than material for other methods of vegetative propagation such as grafting and air-layering, and the problem of graft incompatibility can be avoided (Hartney, 1980; McComb and Bennett, 1986, Van Wyk, 1997). Propagation through cuttings occurs when a portion of

a stem, root or leaf is cut from the parent plant, maintained under favourable environmental conditions and induced to form roots and shoots, thus producing a new independent and genetically identical plant (George, 1993; Hartmann *et al*., 1997). The propagation of elite genotypes through propagation by cuttings has become a major part of plantation forestry around the world (Zobel, 1993). Countries such as Brazil (Zobel, 1993), France (Eldridge *et al*., 1994), Portugal (Cotterill and Brindbergs, 1997), Congo (Eldridge *et al*., 1994) and South Africa (Denison and Quaile, 1987; Denison and Kietzka, 1993a) have adopted successful cutting propagation programmes for eucalypt species and their hybrids.

The main regenerative process required in most cutting propagation methods, including those for *Eucalyptus*, is adventitious root formation. Adventitious roots develop naturally in various plant species and arise form parts of the plant body other than the apex of the embryo and the pericycle of relatively mature roots. They can of two types, namely, pre-formed roots and (wound-) induced roots (Fahn, 1974; Haissig, 1974; Nemeth, 1986; Hartmann *et al*, 1997, Dickison, 2000). Pre-formed or latent roots lie dormant until the stems are made into cuttings and placed under favourable environmental conditions and then emerge as adventitious roots. Wound-induced roots develop *de-novo* after the cutting is made at the wound site (or cut end of the stem) and is preceded by callus production (Hartmann *et al*, 1997; Anon, 2005). Many economically important woody plants (species or specific genotypes) have a low genetic and physiological capacity for adventitious root formation (Hartmann *et al*., 1997). Consequently, as demonstrated by the present study, it is necessary to continue the efforts to improve rooting of the many genotypes (in forestry usually referred to as clones) deemed of commercial value. This, as discussed below, may involve research into the various parameters that affect successful cutting establishment.

1.5.1 Factors affecting and/or influencing the regeneration of plants from cuttings

1.5.1.1 Stock plant treatments and harvesting material

Maintenance of parent (stock) plants is the first step to successful cutting propagation. Parent plants for cuttings may be maintained as hedges, in pots, as inserts in the nursery, as mini-gardens and in hydroponic systems (Hartney, 1980; Hartmann *et al*., 1997; Wilson, 1999a; McNabb *et al*., 2000). Clonal hedges form the source of material for most cutting propagation programmes (Hartmann *et al*., 1997; Williams, 2000). Hedge plants can be produced from improved seed or in the case of clonal programmes, from cuttings of coppice obtained from superior trees (Langman, 1993). Coppice is harvested from these hedges/clone banks to produce cuttings. To ensure healthy and productive ramets, the hedges are routinely de-weeded, irrigated and surveyed and controlled for pests and diseases (Hartmann *et al*., 1997; Pierce, 1997; Williams, 2000).

The ease of adventitious root formation declines with age of the parent plant or hedge plant (Kester, 1976; Hackett, 1988; Hartmann *et al*., 1997; Mitchell, *et al*., 2004; Dick and Leakey, 2006). As the hedge plant ages, a natural loss of juvenility occurs, this is called maturation or meristem aging, and it has a significant effect on the rooting ability, root strike and root quality of the coppice material in the nursery (Adendorff and Schon, 1991) and is associated with a reduction in tree survival, growth and form in the field (Mitchell *et al*., 2004). For eucalypts, the ontogenetic loss of cuttings to form adventitious roots varies with species (Hackett, 1988). Paton *et al.* (1970) found that the highest rooting (80%) in *E. grandis* stem cuttings were obtained when cuttings were derived from the epicotyl or second internode of the parent plant and poor rooting (10%) was obtained when cuttings were taken from the tenth internode. Furthermore, Paton (1984) reported that the rooting ability in cuttings of *E. viminalis* and *E. paciflora* seedlings was completely lost by the 4^{th} node, and by the 15^{th} node in *E. grandis* seedlings. However, 50% rooting was observed at the 100^{th} node in *E.camaldulensis*, and 100% rooting was obtained in cuttings taken from the 100^{th} node of *E. deglupta* (Paton, 1984).

Maile and Nieuwenhuis (1996) found that cuttings taken from 3 year old *E. nitens* stock plants rooted better than cuttings taken from 11 year old plants (56% and 7% rooting, respectively). In South Africa, eucalypts coppice on average every 2 months, but this varies between clones, between species and with season, fertigation and age of parent plant (Wallis, *pers. comm.*).

There are various pre-conditioning techniques that may be used to treat stockplants and thereby improve rooting. These include light exclusion (etiolation, blanching or banding), winter pruning, girdling, raising stockplants through micropropagation, chemical treatment of stockplants and modifying light quality, photoperiod, CO_2, water and mineral nutrition (Hartney, 1980; Blazich, 1988a; Blazich, 1988b; Hackett, 1988; Maynard and Bassuk, 1988; Schmidt, 1989; Howard, 1994; Hartmann *et al*., 1997). Rejuvenation of hedges to the juvenile phase can occur through the grafting of adult scions onto seedling rootstocks, from which basal epicormic shoots are then induced to form on the scion and these may be used as cuttings or to form a clone bank of hedges (Kester, 1976; Hartney, 1980; Hartmann *et al*., 1997). Rejuvenation can also occur through micropropagation (George, 1993; Hartmann *et al*., 1997).

At the Mondi Business Paper production and research facilities, eucalypt clonal hedges are established in close proximity to the nursery; this ensures that the hedges are under close surveillance and cuttings arriving at the nursery are fresh and not in a condition of stress (personal observations and discussions with nursery staff). In addition, the eucalypt clonal hedges at are kept healthy through techniques such as drip irrigation, controlled fertilization, selective harvesting and the use of anti-oxidants during collection of the cuttings. These techniques minimise stress on the ramets and increase rooting of the cuttings harvested (Denison and Kietzka, 1993a, McAllister, *pers. comm.*; Wallis, *pers. comm.*).

The general rule in harvesting coppice material is to take cuttings early in the morning when the plant material is in a turgid condition as water-stressed cuttings are more prone to diseases and pests (Hartmann *et al*., 1997). In the case of eucalypts, Langman (1993) suggested that coppice should be collected early in the morning, in misty

weather or following rain to minimise drying out. Bayley and Nixon (1998) recommended that once the eucalypt coppice material is harvested, it should immediately be placed in a bucket of water containing sugar or boric acid to keep the material supple, and boric acid minimises the exudation of phenolics from the cut surfaces. The latter is the general approach used at the Mondi Business Paper nurseries.

The type of material harvested is also important to consider. In the literature and within the Forestry Industry, terms such as macrocuttings, microcuttings and minicuttings are used. For the purpose of this study, the terms terminal cuttings and non-terminal cuttings will be used. Terminal cuttings refer to cuttings taken from the stem just below the apical meristem, due to its location on the stem, they were termed terminal. Non-terminal cuttings refer to cuttings taken from the stem approximately two to three nodes below the apical meristem and below terminal cuttings. Various reports indicate that there are many differences in the rooting responses of these two cutting types in different species. For example, Day and Loveys (1998) found that terminal and stem cuttings of *Boronia megastigma* were not significantly different in their rooting responses. However, stem cuttings of *Hypcalymma angustifolium* propagated better and had longer roots than terminal cuttings (Day and Loveys, 1998). *Osyris lanceolata* cuttings taken from the basal portion of the stem rooted better than cuttings originating form the terminal part of the stem (Teklehaimanot *et al*., 2004). Fillmore (1965) stated that the position from which the cutting is taken from the adult plant affects the ability to root and the stature of the resultant plant. For eucalypts, specific data are scarce as most studies are done 'in-house' and are regarded as confidential.

1.5.1.2 Season/ timing of collection

The season of harvesting coppice material from hedge plants is another factor to consider when propagating through stem cuttings (Fordham, 1965). Many plant species have an optimum rooting period in the year, although easy-to-root species may be harvested throughout the year (Hartmann *et al*, 1997). In a review by Barnes and Lewandowski (1991), the importance of identifying the appropriate stage of plant growth for cutting collection was highlighted and it was suggested that "the key to cuttings success is not only in knowing *how* to do it, but *when*". For *Eucalyptus*,

coppice cuttings of *E. resinifera* displayed highest rooting between February and August, with the highest recorded in February (mean < 25%) (McComb and Wroth, 1986). However, earlier studies on this species indicate rooting as high as 43% in the Congo with optimum rooting period between October and February (Chaperon and Quillet, 1977). Maile and Nieuwenhuis (1996) found that, cuttings taken from three-year old *E. nitens* stock plants showed a 56% and 30% rooting in September and March, respectively.

1.5.1.3 Placing of cuttings and propagation substrate

Propagating cuttings in containers (inserts) is now a widely accepted commercial practice and under variable planting conditions in South Africa, plants in containers may yield better survival rates than open-rooted plants (Barnett and Brissette, 1986; Mitchell, *et al.*, 2005). In a study by Mitchell *et al*. (2005) on pine cuttings, five container types were tested, each differing in root volume (as listed below within parenthesis): BCC 81© (60ml^2), Sappi 49 standard (80ml^2), Sappi 49 deep (130ml^2), Unigro 98© (90ml^2) and Unigro 72© (125ml^2). Root volume and the volume of growing media within the containers were found to significantly influence the growth of cuttings (height, diameter and volume) seven years after planting with the best results obtained with the Unigro 72© tray (Mitchell *et al*., 2005). This is the same container type used for eucalypts at the Mondi Business Paper nursery and consequently, in the present study. As discussed in section 1.6, container volume also influences growth in eucalypt cuttings.

The propagation mixture or rooting medium used in horticulture and forestry consists of organic and inorganic components; these include soil, peat, moss, softwood or hardwood bark, sand, coir, perlite and vermiculite, among others (Hartmann *et al*., 1997). For *E. nitens*, Maile and Nieuwenhuis (1996) reported that a mixture of peat: sand: vermiculite (1:1:1) was found to be superior to each substrate on its own in terms of rooting success. Cuttings set in the mixture produced a 67% rooting success, while cuttings set in pure peat, sand and vermiculite showed 20%, 33% and 50% rooting respectively. For the present study, cuttings were set in peat: perlite: vermiculite (3:3:1), as this is the standard practice at Mondi for eucalypts.

The size of the cutting may also influence rooting and Langman (1993), Pierce (1997) and Williams (2000) have several recommendations for eucalypts. According to those authors, the thickness of cuttings should be between 2 and 8 mm and the length between 2.5 and 12 cm with leaves halved. Langman (1993) states that the number of nodes per cutting is dependent on the growth of the tree, stock plant management and amount of material available.

1.5.1.4 Application of rooting enhancers to cuttings

Before synthetic enhancers were used to improve rooting of cuttings, other chemicals were tried with limited success (Kefford, 1973; Blazich, 1988b). Synthetic auxins such as indole-butyric-acid (IBA), indole-3-acetic-acid (IAA) and naphthalene-acetic acid (NAA) are now regularly used in promoting the production of adventitious roots in cuttings of many species, including *Eucalyptus*. The response, however, is not universal, as cuttings of some difficult-to-root species show no rooting improvement with auxin application as demonstrated in a study on *E. nitens* by Maile and Nieuwenhuis, (1996) in which IBA application had no significant influence on adventitious root formation as compared with cuttings not treated with IBA. Fogaca and Fett-Neto (2005) showed that microcuttings of *E. saligna* rooted best with IBA and IAA as opposed to treatment with NAA or IAA with an aspartate conjugate. *E.globulus* microcuttings rooted best with exposure to IBA and showed an intermediate rooting response with exposure to IAA (Fogaca and Fett-Neto, 2005). To determine the best auxin and optimum concentration to use for a particular species under a given set of environmental conditions, a number of trials must be undertaken (Hartmann *et al*., 1997). Further, the method of auxin application also has an affect on rooting. There are several such methods of auxin application including foliar application or a basal dip into an auxin-containing solution or powder.

As early as 1939, Hitchcock and Zimmerman (1939) investigated three methods of auxin application (various concentrations) in over sixty plant species. Basal ends of cuttings were immersed in an auxin-containing solution for 24 hours, dipped into a solution containing the auxin, or dipped into a powder incorporating the auxin. It was found that all three methods produced essentially the same rooting response and that the

powder preparation was more effective, if the basal end of the cutting was moistened before being dipped. In addition, it was shown that the concentration requirements for optimum rooting depended on the kind and form of rooting agent, the plant species, age of the shoot, time of year and the method of applying the rooting agent to the cutting (Hitchcock and Zimmerman, 1939). Several authors recommend dipping eucalypt cuttings in an IBA hormone powder to promote rooting (Shepherd, 1986; Pierce, 1997; Bayley and Nixon, 1998).

1.5.1.5 Intermittent mist application (and leaching of nutrients)

In the 1940s and 1950s, it was discovered that mist application could prolong the life of cuttings; this knowledge has since revolutionised commercial and experimental propagation of cuttings and mist application became standard practice (Hartmann, 1988). Since then, studies have shown that misting can severely leach nutrients such as N, P, K, Ca and Mg and this can further delay root initiation of cuttings of difficult-to-root species (Good and Tukey, 1966; Blazich and Wright, 1979; Blazich, *et al*., 1983; Hartman, *et al*., 1997). Good and Tukey (1966) demonstrated that hardwood cuttings of several ornamental species grew less and lost greater quantities of metabolites through leaching than softwood cuttings. Loach (1992) suggested that to enhance the rooting of cuttings it is necessary to minimise water loss through transpiration and maintain a favourable tissue temperature. Transpiration can be minimised through shading of leaves or wetting the leaves (misting) (Loach, 1992). At the Mondi nurseries, mist is applied to cuttings for 20 seconds at 10 minute intervals (Wallis, *pers. comm.*).

1.5.1.6 Environmental conditions

It is widely believed that there is no other factor more critical than optimum temperature control for propagation (Loach, 1992; Eldridge *et al*., 1994; Hartmann *et* al., 1997). Seed germination, rooting of cuttings, growth of tissue culture plantlets, graft union development and specialised structure development are all temperature-driven plant responses (Hartmann *et al*., 1997). It is more cost effective to manipulate temperature at the propagation bench level or at the root zone, rather than at manipulating the temperature for the entire greenhouse. The most common way to achieve this is to heat the propagation bench itself on which the cutting trays are placed or through heated

solutions beneath the propagation bench. Landhauser (2003) reported that Balsam poplar cuttings grown in soil temperatures of 5°C did not produce roots after 6 weeks, but cuttings grown in soil with temperatures maintained at 15°C and 25°C showed 100% rooting. In cuttings of *Pinus patula* and the hybrid *Pinus elliotii x Pinus caribaea*, the addition of bottom heat during the rooting period was shown to improve rooting; in addition, root dry mass was positively influenced by bottom heat, irrespective of season (Mitchell, 2002). Fett-Neto *et al.* (2001) found that the adventitious rooting of *E. globulus* microcuttings was delayed if cuttings were not exposed to light during the root initiation phase. The consensus with regard to the optimum temperature for propagation is between 18°C and 25°C for temperate species and 7°C higher for warm climate species (Hartmann *et al*., 1997). At Mondi Business Paper, the root zone temperature of *Eucalyptus* cuttings is maintained at 28°C and controlled by bed heaters.

1.6 Studies on the propagation of *E. grandis* x *E. nitens* hybrid

Vegetative propagation through stem cuttings has become commonplace in the forestry industry and clonal plantations of selected genotypes of *Eucalyptus* are routinely established this way (Yang *et al*., 1995). As mentioned previously, cold-tolerant clones of *E. grandis* x *E. nitens* (GN) have superior wood qualities and they can be matched to marginal sites and thereby increase productivity on existing plantations sites (Denison and Kietzka, 1993a). This is due to the natural qualities of both true species involved. Pure species of *E. grandis* and *E. nitens* are known for their rapid growth and superior wood qualities; however, *E. grandis* is inherently frost-sensitive and restricted to planting in areas with warmer climates while *E. nitens* is inherently cold tolerant and suitable for planting in temperate areas that are subject to frost attack, and in South Africa, is currently grown in the Mpumalanga Highveld region, where frost conditions are frequent (Purnell and Lundquist, 1986; Denison and Kietzka, 1993a; Eldridge *et al*., 1994; Bandyopadhyay *et al*., 1999; Denison, 1999).

Aimers-Halliday *et al*., (1999) found that cuttings from *E. grandis* x *nitens* clones behaved more like *E. grandis* with respect to its ability to coppice and produce rooted cuttings. In addition, those authors found that gradually cutting back and starving the

hybrid stock plants N, P, K (delivered via fertilizers), gave poorer coppicing results although this was necessary to obtain coppice in *E. nitens* plants.

Observations from our laboratory on macropropagated plants indicate that the roots of these plants are often seen aboveground in close proximity to the stem, and this is thought to be as a result of abnormalities in root growth and development (Mokotedi, 2006). In addition, eucalypt clones have been shown to produce roots nearly 180^{o} apart, and this may prove to be inadequate anchorage as entire eucalypt plantations have been known to collapse due to strong winds (McComb and Bennett 1986).

In studies by Mokotedi *et al*. (2003) and Mokotedi (2006), micropropagated and macropropagated GN clones were compared. The results showed that there was no significant difference in terms of growth and physiological responses (such as water relations and photosynthesis) between both types of plants when grown in containers (1L and 25L pots) and in the field. However, the root and shoot dry masses increased with pot size. The most significant differences between young micro- and macropropagated trees were found belowground in their root anchorage and architecture. It was established that macropropagated plants produced better quality roots (in terms of anchorage) earlier during plant development than micropropagated plants. Furthermore, macropropagated plants developed what the author termed 'tap sinkers' (adventitious equivalent of a tap root) by 16 months of field growth, which notably improved their anchorage efficiency. In micropropagated plants the absence of 'tap-sinkers' consequently contributed to the asymmetrical distribution of roots at the root-shoot junction.

The anatomy and histology of eucalypt roots has received little attention. In anatomical studies on *E. obliqua* and *E. st. johnii*, roots were observed to be tetrarch and differences were noted in the cortex and the rate of polyphenol accumulation and these differences were thought to be related to the age and growth rates of the roots sampled (Tippett and O' Brien, 1976). Although it is not clear how adventitious roots develop in eucalypt cuttings, or how cells respond to stimuli from the externally applied rooting hormones, observations from our laboratory on GN clones indicate that adventitious

roots of cold tolerant eucalypt hybrids develop from the archs of the xylem (Mokotedi, 1999). Mokotedi (2006) suggests that it may be possible that fewer cells respond to the root promoting substance and that roots produced nearly 180^{o} apart may be a result of roots being produced from two archs on opposite sides of the stem.

1.7 Aim

Clones of the *E. grandis* x *E. nitens* hybrid that have been produced and selected through breeding programmes at the Mondi nurseries have consistently been shown to be suitable for planting in marginal plantation sites. These clones exhibit high yields and superior pulp properties, which makes them extremely valuable for commercial plantation propagation. However, many such clones are very poor rooters, in particular GN107. Apart from the above-mentioned studies and those done 'in-house' in the forestry industry, little is known about the basis for the rooting ability and performance of GN107 clones. Therefore, the aim of this work was to investigate parameters that may influence rooting of GN107 clones. Toward this end, the effects of cutting type, rooting powder application and the time of year of setting cuttings were assessed. Specifically, the growth and performance of terminal cuttings and non-terminal cuttings, the position at which cuttings were cut in the region of the node (at, above or below) and the effect of the application Seradix 2 powder on the performance of cuttings were assessed. Seradix 2 (containing IBA) is the rooting powder currently used for the cutting propagation of GN clones at Mondi Business Paper's Hilton nursery. In addition, the effect of season or times of year cuttings were set on cuttings performance was assessed. Finally, the root ontogeny of GN107 cuttings was investigated.

2. MATERIALS & METHODS

2.1 Plant material, growth conditions and cutting preparation

Plant material of the hybrid clone *E. grandis* x *E. nitens* (GN107) used in this study was obtained from the Trahar Technology Centre, Mondi Business Paper, Hilton (KwaZulu-Natal, South Africa). Cuttings of GN107 were prepared from the clonal hedges at the Trahar Technology Centre. Coppice was collected from these hedges and placed in buckets of water (no additives) and made into cuttings of approximately 5 to 10 cm in length. All cuttings contained two buds. The number of nodes in each cutting was dependent on the type of cutting made (see 2.2 and Figure 2.1 below). The leaves at the apex of the cutting were trimmed to about one third their original length and the cuttings were placed into black trays (65 cm x 33 cm x 10.5 cm) with 128 inserts per tray containing a mixture of peat: perlite: vermiculite, (3:3:1). Seradix 2 is the commercial rooting powder currently used at the Trahar Technology Centre nursery for the cutting propagation of GN clones. To test the effect of Seradix 2 (3 g kg^{-1} 4-(indole-3-yl)-butyric acid (IBA) (Bayer Crop Science, Germany) on the root development of cuttings, the cut ends (base) of cuttings were dipped into the powder. The trays were placed in the rooting greenhouse at the Trahar Technology Centre, Hilton. The air temperature within the greenhouse was maintained between 25°C and 27°C by thermostatically activated fans and the root zone temperature was maintained at 28°C by bed heaters. The cuttings were misted for 20 seconds at 10 minute intervals by automatic misters; no artificial light was provided in the greenhouse.

Plant material for the root ontogeny studies was supplied by Mondi Business Paper's Hilton nursery. Cuttings of GN107 were prepared at the Hilton nursery (128 inserts per tray containing peat: perlite: vermiculite, 3:3:1) and were placed in the greenhouse at the University of KwaZulu-Natal, Durban. The cuttings were placed in a mist tent at approximately 85% humidity.

2.2 The effect of cutting type on the performance of cuttings

2.2.1 Experimental design

This experiment was aimed at investigating the effects of cutting type, the position at which the cuttings were cut at the abaxial cut end in relation to the node, and Seradix 2 treatment on cutting performance. At the Hilton nursery, non-terminal cuttings (cuttings that are located below the apical meristem) are the primary cutting type utilised. In the initial experiment, two types of cuttings were employed. Cuttings were made from the stem directly below the apical shoot (due to their position on the stem, they were termed terminal cuttings), and cuttings were made from the parent stem approximately 3 nodes below the apical shoot of that stem and below terminal cuttings (termed non-terminal cuttings) (Figure 2.1). Once the plant material was harvested from the clonal hedges at the Trahar Technology Centre, the cuttings were cut at various points on the stem: at the node, approximately 1 cm above the node and approximately 1 cm below the node, resulting in three cutting types each for terminal and non-terminal cuttings; designated as types one to six (Figure 2.1). The length and number of nodes per cutting ranged from 5 cm to 11 cm and 1 to 3 nodes, respectively, depending on the cutting type made. Half the cuttings were dipped into Seradix 2 rooting powder, while half remained untreated.

Seradix 2-treated and -untreated terminal and non-terminal cuttings, cut at, above and below the node (twelve treatments in total) were set in trays with one treatment per column per tray, containing eight replicates (Figure 2.2). There were nineteen trays overall. Based on those results, thereafter, only non-terminal cuttings were used in subsequent studies.

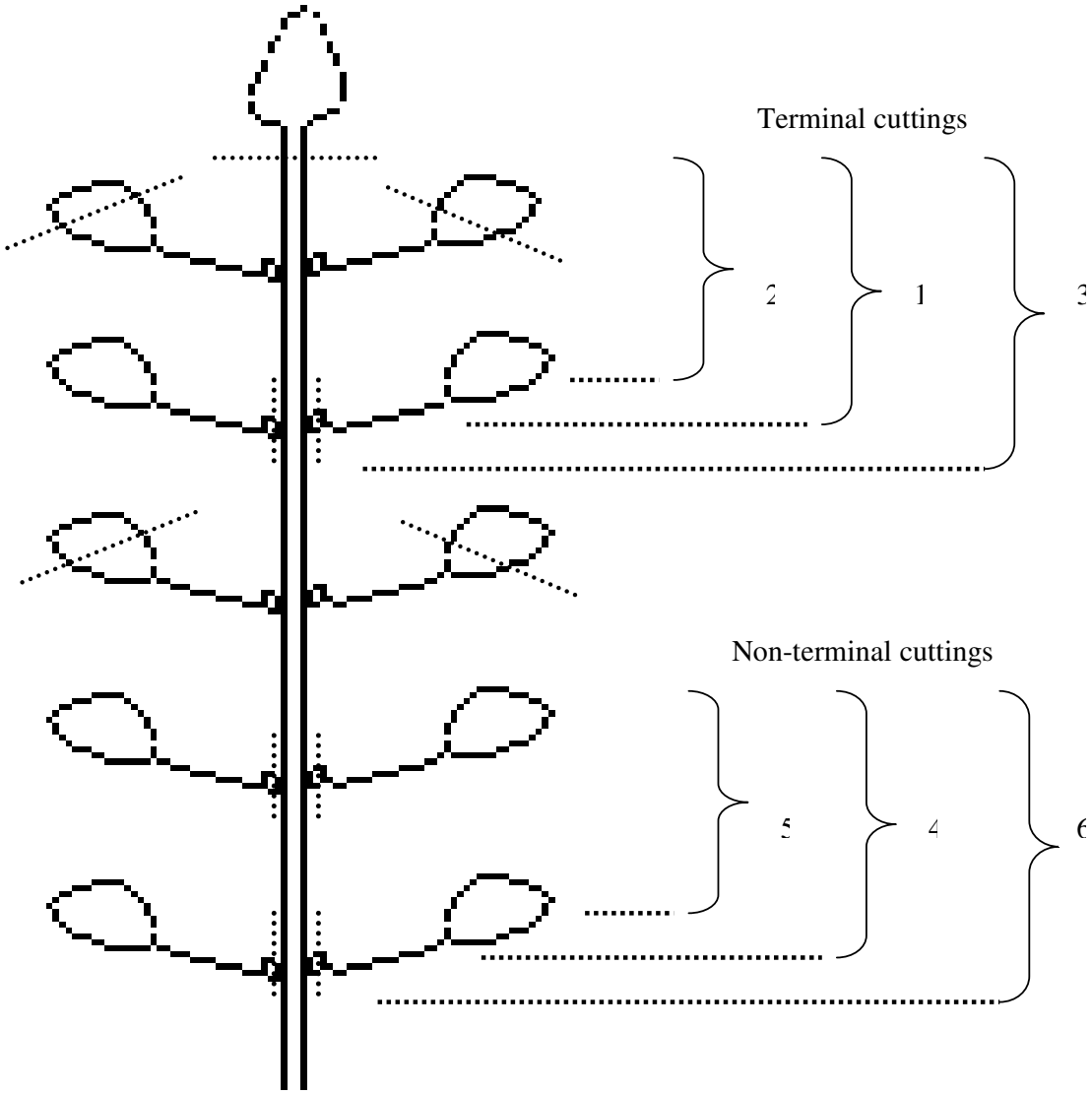

Figure 2.1: Diagrammatic representation of the positions of terminal and non-terminal cutting types (1 to 6) and the points at which they were cut in the region of the node. Terminal cuttings (1, 2 and 3) and non-terminal cuttings (4, 5 and 6) were cut at the node, approximately 1 cm above the node or approximately 1 cm below the node, respectively. Each cutting contained two buds, the leaves on either side of the buds were trimmed to about one third their original size, and all other leaves and buds below the topmost buds were removed. The apical meristem was discarded.

NB. Non-terminal cuttings are the standard cutting type utilised at Mondi Business Paper for GN107 and other clones.

16 inserts/ row

8 inserts/ column

X	X	1T	1U	2T	2U	3T	3U	4T	4U	5T	5U	6T	6U	X	X
		↓													

Figure 2.2: Illustration of a cutting tray (8 x 16 cells) showing an example of a layout of the different treatments. Cutting types 1, 2, 3 = terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node, respectively; cutting types 4, 5, 6 = non terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node, respectively, T = treated with Seradix 2, U = untreated. X = cuttings not used. The twelve treatments were arranged in nineteen trays.

2.3 The effect of the time of year of setting cuttings on cutting performance

2.3.1 Experimental design

To assess the influence of season and environmental temperature on GN107 hedge plants and the effect on the performance of cuttings, the experiment was repeated at different times of the year. As in the initial study, it was observed that non-terminal cuttings resulted in a greater plantlet yield than terminal cuttings, and that there were no significant differences amongst non-terminal cutting types 4, 5 and 6, subsequent studies employed only non-terminal cuttings (type 5).

Plant materials from the GN107 hedges at the Mondi Business Paper nursery were harvested in October 2005, March 2006 and May 2006, and cutting results were recorded 4 weeks later in each study, in November 2005, April 2006 and June 2006,

respectively. Cuttings were prepared with and without Seradix 2 treatment and set in trays, as previously described.

Table 2.1 illustrates the average daily temperature, amount of rain and average daily humidity by month experienced by hedge plants for the period of the study.

Table 2.1: Average daily temperature, amount of rain and average daily humidity by month at the Trahar Technology Centre, Hilton during the period of the study.

	Temperature (^{o}C)		Rain (mm)	Humidity (%)
	Max.	Min.		
October 2005	26.7	14.3	2.2	68.6
November 2005	26.6	16.1	2.4	71.9
December 2005	26.7	15.4	3.4	70.1
January 2006	28.4	18.8	5.9	78.2
February 2006	28.3	19.4	2.3	80.5
March 2006	25.9	15.1	3.3	74.9
April 2006	25.2	13.5	3.6	73.4
May 2006	21.5	7.2	2.3	68.9
June 2006	21.6	4.8	0.1	62.6

2.4 The effect of Seradix 2 on rooting patterns

2.4.1 Experimental design and cutting preparation

Dipping the abaxial cut ends of cuttings into the rooting powder is not a precise practise as the extent to which the cutting is dipped varies with cutting length and amongst nursery workers. To investigate any potential effect of this, an experiment was conducted in which only non-terminal cuttings were used. This study aimed at investigating if the depth the stem was placed into the rooting powder had any effect on rooting, callusing or the type of rooting pattern produced. The abaxial cut ends of cuttings were dipped into Seradix 2 approximately 0.5 cm and compared with cuttings that were dipped approximately 2.5 cm into the rooting powder. Cuttings were prepared at the Hilton nursery and all cuttings were dipped into Seradix 2 rooting powder and set in trays as described above. Non-terminal cuttings (type 5) were used in this study. The

trays were placed in the greenhouse at the Trahar Technology Centre, Hilton and environmental conditions maintained as previously described. Results were recorded 4 weeks after cuttings were set.

2.5 Root ontogeny of GN107 cuttings

For all treatments and cutting types, the positions of root emergence (at the abaxial cut end or above the abaxial end) were recorded. In addition, after 4 weeks of setting cuttings, the root-shoot junctions (regions of root emergence) were excised trimmed to approximately 1.5 cm and fixed in 5 ml formalin/ acetic acid/ alcohol (FAA) for 24 hours at room temperature. The mixture contained 10 ml formalin (37 - 40% v/v formaldehyde), 5 ml glacial acetic acid, 50 ml ethanol (95% v/v) and 35 ml distilled water. The samples were then dehydrated through a series of butanol/ ethanol/ water solutions after which they infiltrated with Paraplast paraffin wax (Lancer, Ireland) through a series of wax/ butanol solutions. The root-shoot junction samples were then incubated in pure Paraplast paraffin wax overnight and thereafter embedded in plastic peel away moulds (2.2 x 2.2 x 2.2 cm) (Polyscience, USA) using fresh paraffin wax and allowed to set overnight. Wax embedded samples were sectioned with a rotary microtome (AO 820, American Optical, Buffalo, New York, USA). Sections were adhered to slides pre-treated with Haupt's Adhesive, which comprised of 1 g gelatin, 15 ml glycerol, 2 g phenol crystals and 100 ml water. Sections were stained with 0.1% (w/v) Toluidene Blue and DPX mountant (Unilab Saarchem, South Africa) was used to mount coverslips to the slides. Slides were viewed using a Carl Zeiss light microscope and images were captured using a Nikon DXM 1200C digital camera.

2.6 Data collection, statistical analysis & photography

For the first two studies, the following parameters were measured: survival, percentage of plantlets produced, percentage of rooted and unrooted cuttings, percentage of cuttings that did and did not produce new shoots and the percentage of cuttings that produced callus. In addition, the number of roots per cutting, the length of longest root per cutting, average shoot length per cutting, site of root emergence and rooting pattern per

cutting were recorded. Sampling was performed 4 weeks after treatment as this is the standard practice at the Mondi nurseries, since roots are well established at this stage while not being overgrown in insert containers.

To determine the fresh and dry mass, roots, shoots and callus were harvested and placed in brown paper bags to minimise water loss. These were promptly weighed using an electronic scale at the Mondi laboratory which is situated adjacent to the rooting greenhouse in which the trays were placed. The bags were then placed in an oven at 80°C for 48 hours and the contents thereafter re-weighed to determine the dry weight. The shoot: root ratio based on the fresh mass and dry mass were also determined.

Cuttings that produced roots were categorised by their site of root emergence, i.e. cuttings with roots from the abaxial end (1), cuttings with roots from above the abaxial end (2) and cuttings with roots from both (3) (see also section 3.3). The incidences (percentage) of these rooting patterns were assessed 4 weeks after cuttings were set.

In the first study (November), nineteen trays were used, each with eight cuttings per each of the twelve treatments. In the April and June trials, eight trays and five trays were used, respectively, with 128 replicates in each tray. For the studies on the extent of dipping cuttings into Seradix 2, one tray was used with 128 replicates.

Statistical analyses were carried out with the Statistical Package for Social Science (SPSS) software package version 13.0. All data were subjected to the Kolmogorov-Smirnov's test for normal distribution. Data that were normally distributed were analysed by a one-way analysis of variance test (ANOVA) and Tukey's Honestly Significant Difference (HSD) test ($p \leq 0.05$). Data that were not normally distributed even after log-transformation, were analysed by the Kruskal-Wallis and Mann-Whitney U tests ($p \leq 0.05$).

Images of root emergence and ontogeny were captured using a Nikon DXM 1200C digital camera.

3. RESULTS

3.1 The effect of cutting type on the performance and outcome of cuttings

In the initial investigation of this study, the effect of cutting type and rooting powder were investigated. Terminal cuttings and non-terminal cuttings were harvested from clonal hedges and their base was cut at different places along the internodes and at the node (Figure 2.1). Half of these were treated with Seradix 2 rooting powder, while the other half remained untreated.

3.1.1 Survival of cuttings

At the time results were taken (4 weeks after cuttings were set), it was found that a large number of cuttings did not survive. The percentage of cuttings that did not survive and the summary of the comparisons made between the different cutting types and cuttings treated and untreated with Seradix 2 are presented in Tables 3.1 and 3.2. Wilted or desiccated cuttings were recorded as dead. Overall, mortality was very high with 65.2% of Seradix 2-untreated and 69.9% of Seradix 2-treated cuttings assessed as dead (Table 3.1). Amongst Seradix 2-untreated cutting types 1 to 6, differences in mortality were not significantly different (Table 3.1). Further, differences were not significant between terminal and non-terminal cuttings ($p = 0.061$), within terminal cuttings ($p = 0.330$) and within non-terminal cuttings ($p = 0.782$) (Table 3.2).

Amongst Seradix 2-treated cutting types, the highest mortality was observed in cutting type 2 (86.3%) (Table 3.1). Moreover, when the collective data for Seradix 2-treated terminal and non-terminal cuttings were compared (Table 3.2), terminal cuttings were observed to have a higher mortality than non-terminal cuttings ($p = 0.029$). Within terminal cuttings, type 2 was different from types 1 and 3 ($p = 0.032$), however no differences were found with respect to mortality amongst non-terminal cuttings ($p = 0.656$) (Table 3.2). Furthermore, overall no significant differences were found between Seradix 2-untreated cuttings and -treated cuttings at the 0.05% level of significance ($p = 0.218$).

Table 3.1: The effect of cutting type on % mortality of Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1, 2, 3 = terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node, respectively; cutting types 4, 5, 6 = non-terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node, respectively. Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2. Results were recorded after 4 weeks.

Cutting type	% Mortality	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	71.7 ± 0.37 [a]	71.1 ± 0.41 [a]
2	75.0 ± 0.46 [a]	86.3 ± 0.30 [b]
3	66.4 ± 0.43 [a]	70.4 ± 0.40 [a]
4	64.5 ± 0.46 [a]	64.5 ± 0.56 [a]
5	57.2 ± 0.70 [a]	67.8 ± 0.51 [a]
6	56.6 ± 0.60 [a]	59.2 ± 0.55 [a]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152 for each cutting type).

Table 3.2: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for morality of cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents the collective mean. Data from Table 3.1.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.061
Terminal cuttings 1 vs. 2 vs. 3	0.330
Non-terminal cuttings 4 vs. 5 vs. 6	0.782
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.029
Terminal cuttings 1 vs. 2 vs. 3	0.032
Non-terminal cuttings 4 vs. 5 vs. 6	0.656
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.218
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.964
Cutting type 2	0.191
Cutting type 3	0.654
Cutting type 4	0.813
Cutting type 5	0.311
Cutting type 6	0.848

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 152 for each cutting type).

3.1.1.1 Summary

Overall, regardless of Seradix application, cutting mortality was high and non-terminal cuttings were more resilient and survived longer than terminal cuttings.

3.1.2 Plantlet yield

The plantlet production of cutting types 1 to 6 as a percentage of cuttings that were set is presented in Table 3.3, and a summary of the comparisons made between the different cutting types and cuttings treated and untreated with Seradix 2 is presented in Table 3.4. As discussed later, some cuttings produced new shoots and roots (plantlets), while some cuttings produced roots only or shoots only. Therefore, plantlet production (Table 3.3) was scored as those cuttings that produced new shoots and roots for each cutting type.

In Seradix 2-untreated cuttings, the plantlet yield was generally low (< 5%), and no distinction in the frequency of plantlet yield was observed amongst the cutting types (1 to 6). Cutting type did not have any effect on plantlet yield in Seradix 2-untreated cuttings (terminal vs. non-terminal cuttings, amongst terminal or amongst non-terminal cuttings) (Table 3.4).

Table 3.3: The effect of cutting type on % plantlet production from Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. Results were recorded after 4 weeks.

Cutting type	% Plantlet production	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	1.3 ± 0.07 [a]	5.3 ± 0.14 [ab]
2	2.0 ± 0.09 [a]	2.0 ± 0.09 [a]
3	4.6 ± 0.14 [a]	7.2 ± 0.16 [bc]
4	4.0 ± 0.13 [a]	9.9 ± 0.28 [bc]
5	4.0 ± 0.11 [a]	9.2 ± 0.21 [bc]
6	2.0 ± 0.09 [a]	12.5 ± 0.20 [c]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152 for each cutting type).

For Seradix 2-treated cuttings (Table 3.3), a significant difference in plantlet yield was observed between cutting types 2 and 6, with type 6 producing the highest plantlet yield. Further, non-terminal cuttings (types 4, 5, 6) were higher yielding than terminal cuttings

(types 1, 2, 3) (p = 0.008). However, the improved yield from non-terminal cuttings was not affected by the position at which the cutting was cut, i.e. no difference was observed within non-terminal cutting types (4, 5, 6) (Table 3.4). There were also no significant differences observed in plantlet yield amongst terminal cuttings.

Table 3.4: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for plantlets produced. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents the collective mean. Data from Table 3.3.

Parameter	p value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.508
Terminal cuttings 1 vs. 2 vs. 3	0.216
Non-terminal cuttings 4 vs. 5 vs. 6	0.520
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.008
Terminal cuttings 1 vs. 2 vs. 3	0.094
Non-terminal cuttings 4 vs. 5 vs. 6	0.405
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.000
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.055
Cutting type 2	1.000
Cutting type 3	0.311
Cutting type 4	0.156
Cutting type 5	0.173
Cutting type 6	0.001

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 152 for each cutting type).

Seradix 2 treatment significantly improved plantlet yield, irrespective of cutting type (terminal/ non-terminal and the layout of the cutting) (p = 0.000, Table 3.4). This was due to the marked difference in plantlet yield between Seradix 2-untreated and Seradix

2-treated cutting type 6 (1.97% and 12.5% respectively (Table 3.3) (p = 0.001, Table 3.4).

3.1.2.1 Summary

In summary, Seradix 2 application was found to improve plantlet yield, with the highest plantlet production observed in non-terminal cuttings.

3.1.3 Root development

Root development was assessed as the percentage of set cuttings that developed roots, the number of roots and the length of the longest root per cutting (Tables 3.5 to 3.10). Percentage rooting (Table 3.5) was scored after four weeks as the percentage of cuttings that produced roots of the total number of cuttings set for each cutting type, including plantlets (cuttings with new shoots and roots, Table 3.3) and cuttings that produced roots and no shoots (data presented later).

Table 3.5: The effect of cutting type on % rooting in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1 Results were recorded after 4 weeks.

Cutting type	% Rooting	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	1.3 ± 0.07 [a]	7.2 ± 0.19 [ab]
2	2.6 ± 0.09 [a]	3.3 ± 0.10 [a]
3	4.6 ± 0.14 [a]	9.2 ± 0.18 [ab]
4	3.9 ± 0.13 [a]	11.2 ± 0.29 [ab]
5	4.0 ± 0.11 [a]	9.9 ± 0.22 [ab]
6	2.0 ± 0.09 [a]	13.8 ± 0.23 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152 for each cutting type).

The percentage rooting of cuttings (regardless of cutting type or Seradix 2 treatment) was generally low (< 14%, Table 3.5). The cutting type used (terminal or non-terminal and the position at which the cutting was cut) did not have any effects on rooting in Seradix 2-untreated cuttings (Table 3.5 and Tables 3.6). That is, cutting types 1, 2, 3 were not significantly different from each other (p = 0.265, Table 3.6), as was the case for non-terminal cuttings 4, 5, 6 (p = 0.520, Table 3.6).

Table 3.6: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for rooted cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents the collective mean. Data from Table 3.5.

Parameter	p value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.663
Terminal cuttings 1 vs. 2 vs. 3	0.265
Non-terminal cuttings 4 vs. 5 vs. 6	0.520
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.035
Terminal cuttings 1 vs. 2 vs. 3	0.170
Non-terminal cuttings 4 vs. 5 vs. 6	0.480
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.000
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.043
Cutting type 2	0.707
Cutting type 3	0.137
Cutting type 4	0.076
Cutting type 5	0.145
Cutting type 6	0.001

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 152 for each cutting type).

For Seradix 2-treated cuttings (Table 3.6), there was a significant difference in percentage rooting between terminal cuttings and non-terminal cuttings (particularly between types 2 and 6) (p = 0.035, Table 3.6). However, there were no differences in percentage rooting amongst terminal cuttings (p = 0.170) or amongst non-terminal cuttings (p = 0.480) (Table 3.6).

In addition to percentage rooting, the effects of the treatments on the number of roots produced per cutting (Tables 3.7 and 3.8) and root length (Tables 3.9 and 3.10) were investigated. As can be seen from the data in Tables 3.7 and 3.8, there were significant differences in the average number of roots produced per cutting in Seradix 2-untreated cuttings. Overall, amongst those cuttings not treated with Seradix 2, the highest average number of roots was produced in cutting type 1 and the lowest in types 4 and 5. However, terminal cuttings were not significantly different from non-terminal cuttings (p = 0.142), and there were also no differences amongst terminal cuttings (p = 0.395) and amongst non-terminal cuttings (p = 0.135) (Table 3.8).

Table 3.7: The effect of cutting type on number of roots per cutting in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. Results were recorded after 4 weeks.

Cutting type	Number of roots	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	2 ± 0.00 [a]	2 ± 0.30 [a]
2	2 ± 0.50 [ab]	2 ± 0.77 [a]
3	1 ± 0.29 [ab]	2 ± 0.27 [a]
4	1 ± 0.00 [b]	2 ± 0.17 [a]
5	1 ± 0.00 [b]	2 ± 0.19 [a]
6	1 ± 0.33 [ab]	2 ± 0.20 [a]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 2 - 21).

For Seradix 2-treated cuttings (Table 3.8), there was no significant difference in the number of roots per cutting between all terminal cuttings and all non-terminal cuttings (p = 0.864). Further, there were no significant differences amongst cutting types 1, 2 and 3 (p = 0.376), as well as amongst cutting types 4, 5 and 6 (p = 0.123). Therefore the position at which the base of the cutting was cut, did not affect the number of roots produced per cutting. Overall, Seradix 2-treated and -untreated cuttings were found to be significantly different with respect to the number of roots per cutting (p = 0.024, Table 3.8).

Table 3.8: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for number of roots per cutting. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.7.

Parameter	p value
Comparing Cuttings Preparation:	
Seradix 2-untreated:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.142
Terminal cuttings 1 vs. 2 vs. 3	0.395
Non-terminal cuttings 4 vs. 5 vs. 6	0.135
Seradix 2-treated:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.864
Terminal cuttings 1 vs. 2 vs. 3	0.376
Non-terminal cuttings 4 vs. 5 vs. 6	0.123
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.024
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.923
Cutting type 2	0.730
Cutting type 3	0.938
Cutting type 4	0.227
Cutting type 5	0.267
Cutting type 6	0.310

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 2 - 21).

The mean lengths of the longest root for Seradix 2-untreated cuttings were not significantly different (Table 3.9). Further analyses (Table 3.10), showed that there was no significant difference within Seradix 2-untreated terminal types (p = 0.644) and within non-terminal types (p = 0.509) and between these two groups (p = 0.576). Therefore the position at which the cutting was cut, and the point at which it was taken from the stem (terminal vs. non-terminal) had no effect on the length of the longest root in Seradix 2-untreated cuttings.

Table 3.9: The effect of cutting- type on the length of the longest root in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. Results were recorded after 4 weeks.

Cutting type	Length of the longest root (mm)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	113.0 ± 92.0 [a]	111.9 ± 15.72 [a]
2	61.5 ± 21.83 [a]	93.6 ± 26.02 [a]
3	92.3 ± 23.05 [a]	98.8 ± 17.45 [a]
4	58.7 ± 25.72 [a]	79.9 ± 9.31 [a]
5	75.5 ± 18.45 [a]	76.1 ± 8.19 [a]
6	101.0 ± 6.03 [a]	89.1 ± 7.24 [a]

a - b = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 2 - 21).

In the case of Seradix 2-treated cuttings (Table 3.10), no significant differences were detected between all terminal and non-terminal cuttings (p = 0.085), amongst terminal cuttings (p = 0.798) or amongst non-terminal cuttings (p = 0.508). Further, no significant difference was observed between Seradix 2-untreated (1 to 6, collectively) and Seradix 2-treated cuttings (1 to 6, collectively) (p = 0.386, Table 3.10).

Table 3.10: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the length of the longest root per cutting. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.9.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.576
Terminal cuttings 1 vs. 2 vs. 3	0.644
Non-terminal cuttings 4 vs. 5 vs. 6	0.509
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.085
Terminal cuttings 1 vs. 2 vs. 3	0.798
Non-terminal cuttings 4 vs. 5 vs. 6	0.508
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.386
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.983
Cutting type 2	0.392
Cutting type 3	0.827
Cutting type 4	0.337
Cutting type 5	0.971
Cutting type 6	0.550

Analyses were performed using ANOVA and T-test where applicable ($p \leq 0.05$, n = 2 - 21).

3.1.3.1 Summary

In summary, Seradix 2 application significantly improved rooting (p = 0.000, Table 3.6), and non-terminal Seradix 2-treated cuttings (types 4, 5 and 6) rooted better than terminal cuttings (types 1, 2 and 3) (Table 3.6). Regardless of the cutting type used, treatment of cuttings with Seradix 2, increased the number of roots produced as compared with cuttings not treated with Seradix 2 (Tables 3.7 and 3.8). Cutting type and Seradix 2 treatment had no effect on the length of the longest root in cuttings.

3.1.4 Shoot development

Shoot development was assessed as the percentage of cuttings that developed new shoots and length of new shoots (Tables 3.11 to 3.14). New shoot growth (Table 3.11) was scored as the percentage of cuttings that produced new shoots of the total number of cuttings set for each cutting type, including plantlets (cuttings with new shoots and roots, Table 3.3), cuttings that only produced shoots and cuttings that produced new shoots with callus at the base of the stem (data presented later).

Of the number of cuttings that were set, 31.4% in Seradix 2-untreated cuttings and 24.3% in Seradix 2-treated cuttings produced new shoots. For Seradix 2-untreated cuttings, a significant difference was observed amongst cutting types 1 to 6, with cutting type 2 yielding the lowest new shoot production, and types 5 and 6 the highest (Table 3.11). In addition, terminal cuttings were different from non-terminal cuttings (p = 0.013, Table 3.12). However, there were no differences in shoot yield amongst terminal cuttings (p = 0.210) or amongst non-terminal cuttings (p = 0.752) (Table 3.12).

Table 3.11: The effect of cutting type on % new shoot growth in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1 Results were recorded after 4 weeks.

Cutting type	% New shoot growth	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	23.0 ± 0.40 ^{ab}	15.8 ± 0.31 ^{b}
2	17.8 ± 0.40 ^{a}	4.6 ± 0.17 ^{a}
3	29.6 ± 0.44 ^{ab}	23.7 ± 0.41 ^{bc}
4	34.2 ± 0.49 ^{ab}	32.2 ± 0.44 ^{c}
5	42.1 ± 0.69 ^{b}	30.9 ± 0.51 ^{bc}
6	41.4 ± 0.61 ^{b}	38.2 ± 0.55 ^{c}

a - c = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152 for each cutting type).

With regards to Seradix 2-treated cuttings a significant difference was observed amongst cutting types 1 to 6 for new shoot growth, with type 2 producing the lowest shoot yield and types 4 and 6 producing the highest yield (Table 3.11). Non-terminal cuttings were significantly better than terminal cuttings in terms of shoot yield (p = 0.000, Table 3.12). However, there were no significant differences in percentage new shoot growth amongst non-terminal types 4, 5 and 6 (p = 0.745, Table 3.12).

Table 3.12: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for cuttings with new shoot growth. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.11.

Parameter	p value
Comparing Cutting Preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.013
Terminal cuttings 1 vs. 2 vs. 3	0.210
Non-terminal cuttings 4 vs. 5 vs. 6	0.752
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.000
Terminal cuttings 1 vs. 2 vs. 3	0.003
Non-terminal cuttings 4 vs. 5 vs. 6	0.745
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.075
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.289
Cutting type 2	0.073
Cutting type 3	0.466
Cutting type 4	0.870
Cutting type 5	0.326
Cutting type 6	0.825

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 152 for each cutting type).

Although Seradix 2-untreated cuttings had a higher frequency of new shoot production than cuttings treated with Seradix 2 (31.4% vs. 24.3%), Seradix application did not influence shoot yield overall ($p = 0.075$, Table 3.12).

Shoot development was also assessed as length of new shoots (Table 3.13), which amongst Seradix 2-untreated cutting types 1 to 6 was significantly different, with the longest shoots produced by cutting type 3. There were no significant differences amongst types 4, 5 and 6 (non-terminal) ($p = 0.595$). However, amongst the terminal cuttings, type 3 produced significantly longer shoots than type 1 ($p = 0.012$) (Table 3.14). Nevertheless, terminal cuttings were not different from non-terminal cuttings with respect to shoot length ($p = 0.621$, Table 3.14)

Table 3.13: The effect of cutting type on length of new shoots in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. Results were recorded after 4 weeks.

Cutting type	Shoot length (mm)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	14.4 ± 1.42 [a]	15.7 ± 2.11 [a]
2	15.9 ± 1.34 [ab]	16.5 ± 1.48 [abc]
3	19.4 ± 1.24 [b]	20.5 ± 1.36 [c]
4	17.3 ± 1.12 [ab]	16.9 ± 1.12 [ab]
5	18.2 ± 1.18 [ab]	14.2 ± 1.13 [ab]
6	18.5 ± 1.02 [b]	16.6 ± 1.04 [ab]

a - c = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 7 -100).

Amongst Seradix 2-treated cutting types 1 to 6, a significant difference was observed in the length of new shoots, with type 3 (terminal cutting, cut below the node) producing the longest shoots (20.5 mm) (Table 3.13). Terminal cuttings (types 1, 2 and 3) produced shoots that were significantly longer than non-terminal cuttings (types 4, 5

and 6) ($p = 0.017$). There were no significant differences amongst terminal cuttings ($p = 0.095$) or amongst non-terminal cuttings ($p = 0.195$) (Table 3.12). Seradix 2 treatment did not have any effect on the shoot length of cuttings ($p = 0.292$, Table 3.14).

Table 3.14: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for length of new shoots of cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.13.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.621
Terminal cuttings 1 vs. 2 vs. 3	0.012
Non-terminal cuttings 4 vs. 5 vs. 6	0.595
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.017
Terminal cuttings 1 vs. 2 vs. 3	0.095
Non-terminal cuttings 4 vs. 5 vs. 6	0.195
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.292
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.771
Cutting type 2	0.665
Cutting type 3	0.435
Cutting type 4	0.992
Cutting type 5	0.026
Cutting type 6	0.231

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 7 - 100).

3.1.4.1 Summary

As was the case for percentage plantlet production and percentage rooting, non-terminal treated cuttings were superior to terminal cuttings for new shoot growth. Although not statistically different, cuttings not treated with Seradix 2 produced more shoots than

those cuttings treated with Seradix 2 (Table 3.11 and 3.12). From the data in Tables 3.13 and 3.14, it was observed that in Seradix 2-treated cuttings the point at which the cuttings were cut did not have any effect on shoot length but the choice of material did. Terminal cuttings produced longer shoots than non-terminal cuttings. Overall, Seradix 2 treatment did not have any effect on the shoot length of cuttings (Table 3.14).

3.1.5 Other parameters and overview

As previously mentioned, not all cuttings that rooted, produced shoots and not all cuttings in which shoot growth occurred, rooted. In addition, some cuttings (with and without new shoots) produced callus, while a number of cuttings were alive but unresponsive to the treatments, and the majority of cuttings did not survive. A summary of the above data and the comparisons made between the treatments are presented in Tables 3.15 and 3.16.

Less than 1% of all Seradix 2-untreated cuttings produced roots exclusively (i.e. cuttings with roots and without new shoots) (Table 3.15), and in this regard no significant differences were observed amongst cutting types 1 to 6. Terminal cuttings were not significantly different from non-terminal cuttings with respect to the frequency of cuttings that produced roots only ($p = 0.317$) and there were no significant differences in the frequency of cuttings that produced roots exclusively amongst terminal cuttings ($p = 0.368$) and amongst non-terminal cuttings ($p = 1.000$) (Table 3.16).

Of those cuttings treated with Seradix 2, 1.4% produced roots only (Table 3.15), however, this result was not found to be significantly different amongst cutting types 1 to 6. Similarly, there were no significant differences between terminal and non-terminal cuttings ($p = 0.241$), amongst terminal cuttings ($p = 0.867$) and amongst non-terminal cuttings ($p = 0.788$) (Table 3.16).

Table 3.15: Summary of the outcome of Seradix 2-untreated and Seradix 2-treated cuttings. The outcome of cuttings after 4 weeks were as follows: plantlets, cuttings that formed roots only, cuttings that formed basal callus only, cuttings that formed new shoots only, cuttings that formed new shoots with basal callus and cuttings that were unresponsive to the treatments and dead cuttings. Cutting types 1, 2, 3 = terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node respectively; cutting types 4, 5, 6 = non-terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node respectively. Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2.

Outcome of cuttings	%					
	Cutting type					
	1	2	3	4	5	6
	Seradix 2-untreated cuttings					
Plantlets*	1.3 ^{a}	1.9 ^{a}	4.6 ^{a}	4.0 ^{a}	4.0 ^{a}	1.9 ^{a}
Roots only	0 ^{a}	0.7 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
Callus only	0 ^{a}	0 ^{a}	0.7 ^{a}	0 ^{a}	0.7 ^{a}	1.3 ^{a}
New shoots only	17.7 ^{ab}	14.5 ^{a}	21.7 ^{abc}	28.9 ^{abc}	36.2 ^{bc}	39.5 ^{c}
New shoots and callus	4.0 ^{a}	1.3 ^{ab}	3.3 ^{a}	1.3 ^{ab}	1.9 ^{ab}	0 ^{b}
Unresponsive	5.3 ^{ab}	6.6 ^{a}	3.3 ^{ab}	1.3 ^{ab}	0.0 ^{c}	0.7 ^{bc}
Dead*	71.7 ^{a}	75.0 ^{a}	66.4 ^{a}	64.5 ^{a}	57.2 ^{a}	56.6 ^{a}
	Seradix 2-treated cuttings					
Plantlets*	5.3 ^{ab}	1.9 ^{a}	7.2 ^{bc}	9.9 ^{bc}	9.2 ^{bc}	12.5 ^{c}
Roots only	1.9 ^{a}	1.3 ^{a}	1.9 ^{a}	1.3 ^{a}	0.7 ^{a}	1.3 ^{a}
Callus only	4.6 ^{b}	1.3 ^{ab}	0 ^{b}	0.7 ^{ab}	0 ^{b}	0 ^{b}
New shoots only	7.2 ^{ab}	1.9 ^{a}	11.2 ^{b}	19.0 ^{b}	19.0 ^{b}	25.0 ^{b}
New shoots and callus	3.3 ^{ab}	0.7 ^{a}	5.3 ^{b}	3.3 ^{ab}	2.6 ^{ab}	1.3 ^{a}
Unresponsive	6.6 ^{a}	6.6 ^{ab}	4.0 ^{ab}	1.3 ^{bc}	0.7 ^{c}	0.7 ^{bc}
Dead*	71.1 ^{a}	86.3 ^{b}	70.4 ^{a}	64.5 ^{a}	67.8 ^{a}	59.2 ^{a}

*Data for plantlets and dead cuttings as in Tables 3.3 and 3.1, respectively.

a - c = mean separation across columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152 for each cutting type).

Table 3.16: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the outcome of cuttings. The outcome of cuttings, cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.15. ∑ represents collective mean. Data from Table 3.15.

Parameter	*p* value					
	Outcome of cuttings					
	Roots only	Callus only	New shoots only	New shoots and callus	Unresponsive	Dead
Seradix 2-untreated cuttings:						
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.317	0.311	0.003	0.056	0.002	0.061
Terminal cuttings 1 vs. 2 vs. 3	0.368	0.368	0.348	0.439	0.692	0.330
Non-terminal cuttings 4 vs. 5 vs. 6	1.000	0.355	0.609	0.171	0.355	0.782
Seradix 2-treated cuttings:						
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.241	0.028	0.000	0.743	0.001	0.029
Terminal cuttings 1 vs. 2 vs. 3	0.867	0.046	0.041	0.102	0.826	0.032
Non-terminal cuttings 4 vs. 5 vs. 6	0.788	0.368	0.565	0.223	0.806	0.656

Table 3.16 (continued):

Parameter	*p* value					
	Outcome of cuttings					
	Roots only	Callus only	New shoots only	New shoots and callus	Unresponsive	Dead
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.002	0.232	0.000	0.453	0.352	0.218
Seradix 2-untreated vs. Seradix 2-treated:						
Cutting type 1	0.075	0.018	0.038	0.472	0.569	0.964
Cutting type 2	0.553	0.152	0.034	0.553	1.000	0.191
Cutting type 3	0.075	0.317	0.064	0.449	0.542	0.654
Cutting type 4	0.152	0.317	0.218	0.102	1.000	0.813
Cutting type 5	0.317	0.317	0.151	0.945	0.317	0.311
Cutting type 6	0.317	0.152	0.154	0.317	0.553	0.848

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 152 for each cutting type).

Treatment of cuttings with Seradix 2 increased the tendency of cuttings to produce roots only, as indicated by the significant difference between all Seradix 2-untreated and all Seradix 2-treated cuttings (p = 0.002, Table 3.16). Thus, although the type of cutting used (terminal vs. non-terminal and the position at which the cutting was cut) had no effect on the frequency of cuttings producing roots only for both Seradix 2-treated and Seradix 2-untreated cuttings, overall, Seradix 2 application increased the outcome of cuttings that produced roots only.

As previously mentioned, some cuttings (with and without new shoots), produced callus. As seen from the data in Table 3.15, a small percentage of cuttings produced callus exclusively. Cuttings not treated with Seradix 2 showed a low prevalence for this type of outcome (≤ 1.3%) and no significant difference was observed amongst cutting types 1 to 6 (Table 3.15). Furthermore, there were no distinctions between terminal and non-terminal cuttings (p = 0.311), amongst terminal cuttings (p = 0.368) and amongst non-terminal one (p = 0.355) for this response (Table 3.16).

With regard to callus production, a significant difference was observed in Seradix 2-treated cutting types 1 to 6 for cuttings that produced callus only (Table 3.15). Type 1 exhibited the highest incidence of callus production (4.6%), while types 3, 5 and 6 had no incidence of this response. Therefore, collectively, terminal cuttings had a higher occurrence of callus production than non-terminal cuttings (p = 0.028, Table 3.16). However, there were no significant differences observed amongst terminal types (p = 0.046) or amongst non-terminal types (p = 0.368) with respect to the frequency of cuttings that produced callus only.

The production of callus only in cutting type 1 (terminal cutting, cut at the node) was found to be affected by Seradix 2 application (0% vs. 4.6%, Table 3.15) (p = 0.018, Table 3.16). Nevertheless, when all Seradix 2-untreated were compared with all Seradix 2-treated cuttings, they were found to be statistically similar in the prevalence of cuttings that formed callus only (p = 0.232, Table 3.16).

As previously stated, 31.4% of Seradix 2-untreated cuttings and 24.3% of Seradix 2-treated cuttings produced new shoots (Table 3.11). Of these, a considerable amount comprised of cuttings that produced shoots only (and no roots) (Table 3.15). There were significant differences amongst the cutting types that were not treated with Seradix 2. Cutting type 2 (terminal shoot, cut above the node) had the lowest frequency of cuttings that produced new shoots only (14.5%) and type 6 resulted in the highest incidence of this response (39.5%). A comparison of the different treatments (Table 3.16), revealed that terminal cuttings were different from non-terminal cuttings with respect to this response (p = 0.003). However, the frequency of cuttings that produced shoots only were not significantly different amongst terminal types 1, 2 and 3, (p = 0.348) and amongst non-terminal types 4, 5 and 6 (p = 0.609).

Amongst Seradix 2-treated cutting types, again types 2 and 6 resulted in the lowest and highest incidence of cuttings with new shoots only (2% and 25%, respectively). A comparison of the different types of cuttings (Table 3.16) revealed that there was a significant difference between Seradix 2-treated terminal and non-terminal cuttings in the production shoots only (p = 0.000). While no significant difference was observed amongst types 4, 5 and 6 (p = 0.565), types 1, 2 and 3 did differ in this response (p = 0.041) (Table 3.16).

When the percentage of cuttings with new shoots only in all Seradix 2-treated cuttings was compared with all Seradix 2-untreated cuttings (i.e. regardless of cutting type), a significant difference was observed at the 95% level of confidence (p = 0.000, Table 3.16). Therefore, Seradix 2-treated cuttings resulted in fewer cuttings with new shoots only than cuttings not treated with Seradix 2, which suggests that Seradix 2 treatment inhibits shoot growth in cuttings.

A small percentage of cuttings produced shoots and basal callus (without roots) but this was less than 2% of all Seradix 2-untreated cuttings and less than 3% of all Seradix 2-treated cuttings (Table 3.15). Amongst Seradix 2-untreated cutting types, although types

1 and 3 were significantly different from type 6 (Table 3.15), further statistical analysis (Table 3.16) revealed no differences for Seradix 2-untreated cuttings; this was probably a consequence of the large variation of the data. Similarly, in Seradix 2-treated cuttings, cutting types 2 and 6 were significantly different from type 3 for this response (Table 3.15). However, statistically, there were no differences amongst Seradix 2-treated cutting types. Moreover, there was no significant difference observed when Seradix 2-treated and -untreated cuttings were compared. Therefore, both cutting type and Seradix 2 treatment did not influence the prevalence of cuttings that produced shoots and callus.

As previously discussed, a large proportion of cuttings that were set were did not survive (Table 3.1). In addition, it was noticed that some cuttings were alive but completely unresponsive to the treatments (cuttings that survived but did not produce roots, shoots or callus). The proportions of cuttings that were unresponsive were 2.9% in Seradix 2-untreated and 3.4% in Seradix 2-treated cuttings. Amongst Seradix 2-untreated cutting types, type 2 had the highest frequency of cuttings that were totally unresponsive to the treatments and type 5 had the lowest frequency of this response (Table 3.15). Terminal cuttings had a higher response of this outcome than non-terminal cuttings (p = 0.002), but there were no differences amongst terminal or amongst non-terminal cutting types (Table 3.16).

Amongst the cuttings that were treated with Seradix 2, type 5 had the lowest prevalence of cuttings that were unresponsive (Table 3.15). Furthermore, a significant difference was observed when terminal cuttings were compared with non-terminal cuttings, with a higher response of unresponsive cuttings observed amongst terminal cuttings (p = 0.001, Table 3.16). However, cutting types 1, 2, 3 were not different from each other, as was the case for types 4, 5, and 6 (Table 3.16).). Additionally, when all cuttings treated with Seradix 2 (regardless of type) were compared with all untreated cuttings, no significant difference was observed at the 95% level of confidence. Therefore, with respect to the frequency of unresponsive cuttings and the mortality of cuttings (Table 3.15), non-terminal shoots were more resilient and survived longer than terminal cuttings, regardless of Seradix 2 treatment.

3.1.5.1 Summary

In summary, non-terminal cuttings treated with Seradix 2 produced the most plantlets and there were no differences amongst types 4, 5 and 6. Root growth was enhanced by Seradix 2 application, however, shoot growth appeared to be inhibited in cuttings treated with Seradix 2. Terminal cuttings produced more cuttings that were unresponsive to the treatments than non-terminal cuttings. Regardless of Seradix application, the mortality of cuttings was very high.

3.1.6 Fresh mass & dry mass

In addition to recording number of roots, root length and shoot length per cutting as growth measurements, the fresh and dry mass of roots, new shoots and callus were also determined (Tables 3.17 to 3.28). Although precautions were taken when measuring and recording fresh mass of roots, shoots and callus, inevitable water loss from the plant material may have occurred after excision of the plant material in the greenhouse and before weighing in the laboratory a few metres away.

3.1.6.1 Roots

The fresh mass of roots was not significantly different in Seradix 2-untreated cuttings (Table 3.17). Additionally, there were no significant differences in the root fresh mass between terminal and non-terminal types ($p = 0.773$), amongst terminal cuttings (types 1, 2 and 3) ($p = 0.764$), and amongst non-terminal cuttings (types 4, 5 and 6) ($p = 0.707$) (Table 3.18).

With regards to Seradix 2-treated cuttings, although cutting types 1 to 6 were not significantly different with respect to their root fresh masses (Table 3.17), terminal cuttings were found to have a significantly higher root fresh mass than non-terminal cuttings ($p = 0.018$, Table 3.18). However, there were no differences in the fresh mass of roots amongst terminal types 1, 2 and 3 ($p = 0.665$) and amongst non-terminal type 4, 5 and 6 ($p = 0.275$).

Treatment with Seradix 2 increased the root fresh mass (p = 0.014, Table 3.18). Therefore, while the cutting type and the position at which the cuttings were cut did not have any effect on root fresh mass, the application of Seradix 2 increased the root fresh mass of cuttings.

Table 3.17: The effect of cutting type on the fresh mass of roots in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Root fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.17 ± 0.17 [a]	0.18 ± 0.07 [a]
2	0.04 ± 0.03 [a]	0.20 ± 0.10 [a]
3	0.05 ± 0.03 [a]	0.15 ± 0.06 [a]
4	0.03 ± 0.01 [a]	0.03 ± 0.01 [a]
5	0.02 ± 0.02 [a]	0.05 ± 0.02 [a]
6	0.02 ± 0.01 [a]	0.07 ± 0.02 [a]

a = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 2 - 21).

Table 3.18: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the fresh mass of roots. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1 Results were recorded after 4 weeks. ∑ represents collective mean. Data from Table 3.17.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.773
Terminal cuttings 1 vs. 2 vs. 3	0.764
Non-terminal cuttings 4 vs. 5 vs. 6	0.707
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.018
Terminal cuttings 1 vs. 2 vs. 3	0.665
Non-terminal cuttings 4 vs. 5 vs. 6	0.275
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.014
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.739
Cutting type 2	0.121
Cutting type 3	0.157
Cutting type 4	0.102
Cutting type 5	0.306
Cutting type 6	0.255

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 2 - 21).

There were no differences in the root dry mass amongst cutting types 1 to 6 in Seradix 2-untreated cuttings (Table 3.19). Similarly, as depicted in Table 3.20, there were no differences in root dry mass between terminal (types 1, 2 and 3) and non-terminal cuttings (types 4, 5 and 6) (p = 0.467), amongst terminal cuttings (p = 0.673) and amongst non-terminal cuttings (p = 0.854).

In Seradix 2-treated cuttings, the root dry mass of cutting type 1 was found to be significantly different from types 4, 5 and 6 (Table 3.19). This is supported by the data in Table 3.20, wherein a comparison between the root dry mass of terminal cuttings (1, 2 and 3) and non-terminal cuttings (4, 5 and 6) showed a statistical difference (p = 0.003) but no differences were observed amongst terminal cuttings (p = 0.420) or amongst non-terminal cuttings (p = 0.329).

When the root dry mass of all Seradix 2-untreated cuttings were compared with Seradix 2-treated cuttings, a statistical difference was observed (p = 0.032, Table 3.20). Therefore, as was the case for root fresh mass, the root dry mass of terminal cuttings appeared to be higher than non-terminal cuttings, and Seradix 2 application increased the dry mass of roots.

Table 3.19: The effect of cutting type on the dry mass of roots in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Root dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.025 ± 0.02 [a]	0.032 ± 0.01 [a]
2	0.010 ± 0.01 [a]	0.016 ± 0.01 [ab]
3	0.009 ± 0.00 [a]	0.025 ± 0.01 [ab]
4	0.007 ± 0.00 [a]	0.007 ± 0.00 [b]
5	0.004 ± 0.00 [a]	0.004 ± 0.00 [b]
6	0.005 ± 0.00 [a]	0.012 ± 0.00 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 2 - 21).

Table 3.20: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the dry mass of roots. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.19.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.467
Terminal cuttings 1 vs. 2 vs. 3	0.673
Non-terminal cuttings 4 vs. 5 vs. 6	0.854
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.003
Terminal cuttings 1 vs. 2 vs. 3	0.420
Non-terminal cuttings 4 vs. 5 vs. 6	0.329
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.032
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.737
Cutting type 2	0.439
Cutting type 3	0.257
Cutting type 4	0.390
Cutting type 5	0.307
Cutting type 6	0.340

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 2 - 21).

3.1.6.2 Shoots

The fresh mass of shoots were significantly different in Seradix 2-untreated and -treated cuttings (Table 3.21). For Seradix 2-untreated cuttings, types 2 and 5 had significantly lower shoot fresh masses than type 4. However, non-terminal cuttings were not significantly different from terminal cuttings (p = 0.248, Table 3.22). There were significant distinctions in the fresh mass of shoots amongst Seradix 2-treated cuttings 1 to 6 (Table 3.21). However, terminal and non-terminal cuttings were not significantly different in this regard (p = 0.973, Table 3.22). Furthermore, when the fresh mass of

shoots of all Seradix 2-treated cuttings were compared with the Seradix 2-untreated cuttings, no significant difference was observed at the 95% level of confidence (p = 0.221, Table 3.22); therefore, Seradix 2 application did not affect shoot fresh mass.

Table 3.21: The effect of cutting type on the fresh mass of shoots in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Shoot fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{abc}
2	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{bc}
3	0.04 ± 0.00 ^{ab}	0.05 ± 0.01 ^{a}
4	0.06 ± 0.02 ^{b}	0.04 ± 0.00 ^{b}
5	0.03 ± 0.00 ^{a}	0.02 ± 0.00 ^{c}
6	0.05 ± 0.01 ^{ab}	0.05 ± 0.02 ^{bc}

a - c = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 7 - 64).

Table 3.22: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the fresh mass of shoots. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.21.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.248
Terminal cuttings 1 vs. 2 vs. 3	0.293
Non-terminal cuttings 4 vs. 5 vs. 6	0.028
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.973
Terminal cuttings 1 vs. 2 vs. 3	0.449
Non-terminal cuttings 4 vs. 5 vs. 6	0.001
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.221
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.555
Cutting type 2	0.485
Cutting type 3	0.885
Cutting type 4	0.846
Cutting type 5	0.167
Cutting type 6	0.263

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 7 - 64).

Shoot dry mass was significantly different amongst Seradix 2-untreated cutting types 1 to 6 (Table 3.23). Terminal cuttings (types 1, 2 and 3) were significantly different from non-terminal cuttings (types 4, 5 and 6), with non-terminal cuttings producing shoots with a higher dry mass ($p = 0.004$, Table 3.24). There were no significant differences amongst terminal cutting type 1, 2 and 3 ($p = 0.254$), as was the case amongst non-terminal cuttings ($p = 0.050$) (Table 3.24).

For Seradix 2-treated cuttings, no significant difference was observed when the shoot dry mass data of terminal cuttings were compared with the non-terminal cuttings (p = 0.823, Table 3.24). However, significant differences in the shoot dry mass were observed amongst terminal cuttings and amongst non-terminal cuttings (Table 3.24). Therefore, in Seradix 2-treated cuttings, terminal cuttings cut below the node (type 3), exhibited a higher shoot dry mass than terminal cuttings cut at or above the node (type 1 and 2, respectively). In addition, non-terminal cuttings cut at or below the node (types 4 and 6) were observed to have a higher shoot dry mass than non-terminal cuttings cut above the node (type 5). Furthermore, a significant difference in shoot dry mass was observed when Seradix 2-treated cuttings were compared with Seradix 2-untreated cuttings, irrespective of cutting type (p = 0.002, Table 3.24).

Table 3.23: The effect of cutting type on the dry mass of shoots in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Shoot dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.011 ± 0.00 [a]	0.007 ± 0.00 [a]
2	0.010 ± 0.00 [a]	0.006 ± 0.00 [a]
3	0.015 ± 0.00 [abc]	0.013 ± 0.00 [c]
4	0.016 ± 0.00 [b]	0.014 ± 0.00 [c]
5	0.013 ± 0.00 [ab]	0.008 ± 0.00 [abc]
6	0.018 ± 0.00 [c]	0.012 ± 0.00 [c]

a - d = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 7 - 64).

Therefore cutting type had an effect on the dry mass of shoots and cuttings not treated with Seradix 2 had higher shoot dry masses than those cuttings that were treated with Seradix 2 (Table 3.23 and 3.24).

Table 3.24: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for shoot dry mass of cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.23.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.004
Terminal cuttings 1 vs. 2 vs. 3	0.254
Non-terminal cuttings 4 vs. 5 vs. 6	0.050
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.823
Terminal cuttings 1 vs. 2 vs. 3	0.028
Non-terminal cuttings 4 vs. 5 vs. 6	0.000
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.002
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.202
Cutting type 2	0.371
Cutting type 3	0.975
Cutting type 4	0.697
Cutting type 5	0.001
Cutting type 6	0.003

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 7 - 64).

3.1.6.3 Shoot: root mass ratios

The data presented in Tables 3.17, 3.19, 3.21 and 3.23 were calculated as ratios of shoot mass to root mass and are presented in Table 3.25. For Seradix 2-untreated cuttings, shoot: root fresh mass and dry mass were higher in non-terminal cuttings than in terminal cuttings ($p = 0.011$ and $p = 0.019$, respectively). However, amongst Seradix 2-treated cuttings, the ratios for fresh and dry mass were not significantly different ($p = 0.095$ and $p = 0.052$ for the ratio of fresh mass and dry mass, respectively). Overall,

Seradix 2 application did not influence shoot: root fresh mass or dry mass ratios (p = 0.105 and p = 0.122, respectively). Although not proven statistically, the results for shoot: root mass ratios indicate that Seradix 2 application enhanced rooting and inhibited shoot development.

Table 3.25: The effect of cutting type on shoot: root fresh mass and dry mass ratios in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. Significant differences are highlighted in the text.

Cutting type	Shoot: root ratio			
	Seradix 2-untreated cuttings		Seradix 2-treated cuttings	
	Fresh mass	Dry mass	Fresh mass	Dry mass
1	0.2	0.4	0.2	0.2
2	0.5	1.0	0.1	0.4
3	0.8	1.7	0.3	0.5
4	2.0	2.3	1.3	2.0
5	1.5	3.3	0.4	2.0
6	2.5	3.6	0.7	1.0

3.1.6.4 Callus

As illustrated by the data presented in Table 3.26, no significant differences were observed in callus fresh mass in Seradix 2-untreated and Seradix 2-treated cuttings. In Seradix 2-untreated cuttings (Table 3.27), the fresh mass of callus was statistically similar. There were no significant differences observed for callus fresh mass amongst terminal cuttings (types 1, 2 and 3) (p = 0.393), amongst non-terminal cutting types 4, 5 and 6) (p = 0.917) and when terminal cuttings were compared with non-terminal cuttings (p = 0.695). Similarly, for Seradix 2-treated cuttings (Table 3.27), no significant differences were observed for callus mass amongst terminal cuttings (p = 0.965), amongst non-terminal cuttings (p = 0.487) and when terminal cuttings were compared with non-terminal cuttings (p = 0.406). Overall, the callus fresh mass of

Seradix 2-treated cuttings was higher than untreated cuttings (p = 0.006, Table 3.27). Therefore, Seradix 2 increased callus fresh mass.

Table 3.26: The effect of cutting type on the fresh mass of callus in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Callus fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.01 ± 0.00 [a]	0.12 ± 0.05 [a]
2	NR	NR
3	0.03 ± 0.02 [a]	0.12 ± 0.09 [a]
4	0.03 ± 0.02 [a]	0.10 ± 0.04 [a]
5	NR	0.08 ± 0.01 [a]
6	0.03 ± 0.00 [a]	0.01± 0.00 [a]

a = mean separation within columns, ANOVA, ± standard error ($p \leq 0.05$, n = 2 - 12).
NR = results not recorded.

Table 3.27: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the fresh mass of callus. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.26.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.695
Terminal cuttings 1 vs. 2 vs. 3	0.393
Non-terminal cuttings 4 vs. 5 vs. 6	0.917
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.406
Terminal cuttings 1 vs. 2 vs. 3	0.965
Non-terminal cuttings 4 vs. 5 vs. 6	0.487
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.006
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.155
Cutting type 2	NR
Cutting type 3	0.485
Cutting type 4	0.303
Cutting type 5	NR
Cutting type 6	0.001

Analyses were performed using ANOVA and T-tests, where applicable ($p \leq 0.05$, n = 2 - 12). NR = results not recorded.

The dry mass of callus was not affected by cutting type amongst cuttings not treated with Seradix 2 (p = 0.859) or amongst cuttings treated with Seradix 2 (p = 0.775) (Table 3.28). For Seradix 2-untreated cuttings, there were no significant differences in callus dry mass amongst terminal cuttings (p = 0.617) or amongst non-terminal cuttings (p = 0.454) (Table 3.29). Similarly, in Seradix 2-treated cuttings, no significant differences were observed for callus dry mass amongst terminal cuttings (p = 0.559) or amongst non-terminal cuttings (p = 0.575) (Table 3.29). In addition, terminal cuttings

were not significantly different in callus dry mass to non-terminal cuttings irrespective of Seradix 2 treatment (Table 3.29). However, when all Seradix 2-treated cuttings were compared with all Seradix 2-untreated cuttings, a significant difference was found at the 95% level of confidence (p = 0.010, Table 3.29). Therefore the effect of Seradix 2 application recorded for callus fresh mass is reflected also in the dry mass data.

Table 3.28: The effect of cutting type on the dry mass of callus in Seradix 2-untreated and Seradix 2-treated cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1.

Cutting type	Callus dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
1	0.005 ± 0.00 [a]	0.015 ± 0.00 [a]
2	NR	NR
3	0.007 ± 0.01 [a]	0.022 ± 0.02 [a]
4	0.004 ± 0.00 [a]	0.019 ± 0.01 [a]
5	NR	0.011 ± 0.00 [a]
6	0.002 ± 0.00 [a]	0.003 ± 0.00 [a]

a = mean separation within columns, ANOVA, ± standard error ($p \leq 0.05$, n = 2 - 12).
NR = results not recorded.

Table 3.29: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the dry mass of callus. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.1. ∑ represents collective mean. Data from Table 3.28.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.539
Terminal cuttings 1 vs. 2 vs. 3	0.617
Non-terminal cuttings 4 vs. 5 vs. 6	0.454
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.774
Terminal cuttings 1 vs. 2 vs. 3	0.559
Non-terminal cuttings 4 vs. 5 vs. 6	0.575
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.010
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	0.139
Cutting type 2	NR
Cutting type 3	0.515
Cutting type 4	0.334
Cutting type 5	NR
Cutting type 6	0.005

Analyses were performed using ANOVA and T-tests, where applicable ($p \leq 0.05$, n = 2 - 12). NR = results not recorded.

3.1.6.5. Summary

In summary, the fresh and dry masses of roots in Seradix 2-treated terminal cuttings were higher than those of non-terminal cuttings. The fresh and dry masses of shoots in Seradix 2-untreated non-terminal cuttings were higher than terminal cuttings. In addition, when all Seradix 2-untreated and -treated cuttings were compared, shoot dry mass was higher in the former. The fresh mass and dry mass of callus was observed to be significantly higher in Seradix 2-treated cuttings. In addition, when considering

shoot: root fresh and dry mass ratios, Seradix 2 application appears inhibit shoot development. Therefore, the results for root, shoot and callus fresh mass and dry mass, suggest that Seradix 2 application may increases root and callus growth but inhibits shoot growth.

3.2 The effect of season and time of year of setting cuttings on cutting performance and outcome

Many plant species have an optimum rooting period in the year and seasonal effects on rooting have been reported in eucalypt cuttings. Knowing the optimum rooting period in the year of a plant species is of great value to the forestry industry so that propagation of that species can be exploited at that time. To test the seasonal effects on rooting in the commercially important clone GN107, cuttings from hedge plants were harvested and rooted at the end of November 2005, April 2006 and June 2006. Seradix 2 application was performed as before (see sections 2.3 and 3.1). In the initial study undertaken in November 2005, different cutting types (1 - 6) were used. From the data obtained from that study (section 3.1), it was observed that cutting types 4, 5 and 6 were statistically similar to each other and superior to types 1, 2 and 3. Therefore, only type 5 was subsequently used in the April and June trials and this was compared with type 5 of the November trial.

3.2.1 Survival of cuttings

Regardless of the time of year at which the cuttings were set, a substantial amount of cuttings did not survive (Table 3.30). For cuttings not treated with Seradix 2, the mortality of cuttings was significantly different in cuttings set in November, April and June. For Seradix 2-untreated and -treated cuttings, the highest losses occurred in April (87.7% and 92.3%, respectively) and the lowest mortality in June (47.8% and 50%, respectively). In total, 64.3% and 70.1% of Seradix 2-untreated and Seradix 2-treated cuttings did not survive and these figures were not found to be significantly different from each other (p = 0.300, Table 3.31). Therefore, Seradix application did not affect the survival of cuttings.

Table 3.30: The effect of the time of year of setting cuttings on % mortality in Seradix 2-untreated and Seradix 2-treated cuttings. Results were recorded after 4 weeks in each study in November 2005, April 2006 and June 2006, respectively**.** Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2.

Time of year	% Mortality	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	57.2 ± 8.67 [ab]	67.8 ± 6.43 [ab]
April 2006	87.7 ± 2.87 [b]	92.3 ± 3.17 [b]
June 2006	47.8 ± 4.4 [a]	50.0 ± 5.95 [a]

a - b = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

Table 3.31: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for mortality of cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.30.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.300
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.311
April 2006	0.292
June 2006	0.776

Analyses were performed using T-tests ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

3.2.1.1 Summary

The mortality of cuttings was influenced by the time of year of cuttings were set, with the best time to set cuttings (in terms survival) observed in June.

3.2.2 Plantlet yield

Regardless of Seradix 2 treatment, plantlet production was exceedingly low (Table 3.32). For Seradix 2-untreated and -treated cuttings, the highest percentage of plantlets produced was in November 2005 (4% and 9.2%, respectively) and the lowest in April 2006 (0.2% and 0.4%, respectively). However, for both Seradix 2-untreated and -treated cuttings there were no significant differences with respect to plantlet production and the time of year cuttings.

There were no significant differences in plantlet yield between Seradix 2-untreated and Seradix 2-treated cuttings (p = 0.099, Table 3.33). Likewise, there were no significant differences in the percentage plantlet production for Seradix 2-untreated and treated cuttings within the November, April and June trials (p = 0.173, p = 0.535 and p = 0.119, respectively).

Table 3.32: The effect of the time of year of setting cuttings on % plantlet production from Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	% Plantlet production	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	4.0 ± 0.11 [a]	9.2 ± 0.21 [ab]
April 2006	0.2 ± 0.13 [a]	0.4 ± 0.16 [a]
June 2006	0.6 ± 0.25 [a]	3.1 ± 1.05 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

Table 3.33: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for plantlet production. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents the collective mean. Data from Table 3.32.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.099
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.173
April 2006	0.535
June 2006	0.119

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

3.2.2.1 Summary

Although not shown statistically, there is an indication that plantlet yield in GN107 cuttings is highest in November. The application of Seradix 2 to cuttings had no affect on plantlet yield.

3.2.3 Root development

Root development was evaluated as the percentage of set cuttings that developed roots, the number of roots and the length of the longest root per cutting (Tables 3.34 to 3.39). As indicated by the data in Table 3.34, for Seradix 2-untreated cuttings, there were no significant differences in the percentage of cuttings that rooted amongst the three study periods. In Seradix 2-treated cuttings, the highest percentage of cuttings that developed roots were set in November (9.9%) and June (10%) and the lowest in April (1.4%). Overall, Seradix 2 application influenced the number of cuttings that produced roots ($p = 0.046$), with the most notable difference occurring in June ($p = 0.015$) (Table 3.35).

Table 3.34: The effect of the time of year of setting cuttings on % rooting of Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	% Rooting	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	4.0 ± 0.11 [a]	9.9 ± 0.22 [ab]
April 2006	0.8 ± 0.38 [a]	1.4 ± 0.39 [a]
June 2006	1.9 ± 0.37 [a]	10.0 ± 1.81 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

Table 3.35: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for rooted cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.34.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.046
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.145
April 2006	0.506
June 2006	0.015

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

The average number of roots produced per cutting in Seradix 2-untreated cuttings was not significantly different for the three times, with 1 root per cutting being produced (Table 3.36). In Seradix 2-treated cuttings, statistically, cuttings set in June produced more roots per cutting than those set in November or April (Table 3.36). When all Seradix 2-untreated cuttings were compared to all Seradix 2-treated cuttings (Table 3.37), a significant difference was observed ($p = 0.007$), with the most notable difference occurring in cuttings set in June ($p = 0.034$).

Table 3.36: The effect of the time of year of setting cuttings on the number of roots per cutting in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Number of roots	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	1 ± 0.00 [a]	2 ± 0.19 [a]
April 2006	1 ± 0.00 [a]	1 ± 0.17 [a]
June 2006	1 ± 0.17 [a]	2 ± 0.22 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.37: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the number of roots per cutting. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.36.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.007
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.267
April 2006	0.414
June 2006	0.034

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 4 - 32).

For Seradix 2-untreated cuttings, the average length of the longest root per cutting was highest in cuttings set in November (75.5 mm) and lowest in April (21.8 mm) (Table 3.38). For Seradix 2-treated cuttings, the longest roots were produced in cuttings set in November 2005 and April (Table 3.38), however, these results were not found to be significantly different (Table 3.38). Furthermore, when all Seradix 2-untreated cuttings were compared with all the -treated cuttings, no difference was observed ($p = 0.766$, Table 3.39). Therefore, Seradix 2 treatment did not have any affect on the length of the longest roots in cuttings.

Table 3.38: The effect of the time of year of setting cuttings on the length of the longest root per cutting in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Length of the longest root (mm)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	75.5 ± 18.45 [a]	76.1 ± 8.19 [a]
April 2006	21.8 ± 7.89 [b]	68.0 ± 20.73 [a]
June 2006	67.2 ± 8.57 [ab]	46.4 ± 5.64 [a]

a - b = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.39: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the length of the longest root per cutting. The time of year of settings cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.38.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.766
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.971
April 2006	0.080
June 2006	0.115

Analyses were performed using T-tests ($p \leq 0.05$, n = 4 - 32).

3.2.3.1 Summary

To summarize, the data (Tables 3.34 to 3.39) indicated that cuttings set in June 2006 (winter) and treated with Seradix 2 had the highest percentage rooting and the highest number of roots per cutting but the shortest roots per cutting.

3.2.4 Shoot development

Shoot development was measured after four weeks as the percentage of set cuttings with new shoot growth and shoot length (Tables 3.40 to 3.43). The percentage of cuttings with new shoot growth (Table 3.40) refers to those cuttings that produced new shoots, regardless of root production. Although not statistically different from the values obtained for the other times of the year, the highest incidence of new shoot production in Seradix 2-untreated cuttings was observed in November (42.1%) (Table 3.40). For Seradix 2-treated cuttings (Table 3.40), the percentage of cuttings that produced shoots in November (30.9%) was found to be higher than that for cuttings set in April (2.9%).

Overall, when Seradix 2-untreated and -treated cuttings were compared, no significant difference in new shoot growth was observed (p = 0.654, Table 3.41).

Table 3.40: The effect of the time of year of setting cuttings on % new shoot growth in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year and Seradix 2 treatment as explained in Table 3.30.

Time of year	% New shoot growth	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	42.1 ± 0.69 [a]	30.9 ± 0.51 [b]
April 2006	8.6 ± 1.38 [a]	2.9 ± 0.95 [a]
June 2006	6.9 ± 1.50 [a]	10.0 ± 1.81 [b]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

Table 3.41: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for cuttings with new shoot growth. Time of year and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.40.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.654
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.326
April 2006	0.061
June 2006	0.338

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June, respectively).

The length of shoots of cuttings set in November, April and June were significantly different amongst Seradix 2-untreated cuttings (Table 3.42) and the longest shoots were produced in November (18.2 mm). In Seradix 2-treated cuttings, there was no effect of the time of year at which cuttings were set on shoot length (Table 3.42). With regard to shoot length of cuttings set in November, Seradix 2-untreated cuttings were significantly different from Seradix 2-treated cuttings ($p = 0.026$, Table 3.43). However, when the shoot length of all Seradix 2-treated cuttings was compared with those of Seradix 2-treated cuttings, no effect of Seradix 2 application on shoot length was found ($p = 0.596$, Table 3.43).

Table 3.42: The effect of the time of year of setting cuttings on shoot length in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Shoot length (mm)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	18.2 ± 1.18 [b]	14.2 ± 1.13 [a]
April 2006	12.2 ± 0.92 [a]	12.6 ± 1.58 [a]
June 2006	12.8 ± 1.13 [a]	14.6 ± 1.14 [a]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 22 - 78).

Table 3.43: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for length of new shoots of cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.42.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.596
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.026
April 2006	0.749
June 2006	0.332

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 22 - 78).

3.2.4.1 Summary

In summary, the highest new shoot growth (36.2%) and shoot length (18.2 mm) was observed in cuttings not treated with Seradix 2 set in November (Tables 3.40 and 3.42).

3.2.5 Other parameters and overview

As reported earlier (section 3.1), a range of responses in cuttings was recorded. A summary of the outcome of cuttings for the studies conducted in November 2005, April 2006 and June 2006 and the comparisons made between the treatments and the trials are presented in Tables 3.44 and 3.45, respectively. The outcomes of cuttings for the three studies were recorded four weeks after cuttings were set.

To reiterate the previously presented results, the incidence of plantlet production (Table 3.32) was relatively low (1.6% in Seradix 2-untreated cuttings and 4.2% in Seradix 2-treated cuttings) and the highest frequency of plantlet production was observed in Seradix 2-treated cuttings set in November (9.2%).

A small percentage of cuttings that were set produced roots only (without shoots or callus). This response was found to be significantly higher in cuttings in June in Seradix 2-untreated cuttings (1.3%) and in Seradix 2-treated cuttings (6.9%) compared with cuttings set in November and April (Table 3.44). As seen in Table 3.45, there were no significant differences in the percent cuttings that produced roots only between Seradix 2-untreated and Seradix 2-treated cuttings in November or April. However, there was a significant difference between Seradix 2-untreated and Seradix 2-treated cuttings in June (p = 0.011, Table 3.45). Despite this, when all Seradix 2-untreated cuttings were compared with Seradix 2-treated cuttings, no significant difference was observed (p = 0.145, Table 3.45). Therefore, cuttings set in June showed the highest production of roots only (without shoots).

Table 3.44: Summary of the outcome of Seradix 2-untreated and Seradix 2-treated cuttings at different times of the year. The outcome of cuttings after 4 weeks were as follows: plantlets, cuttings that formed roots only, cuttings that formed basal callus only, cuttings that formed new shoots only, cuttings that formed new shoots with basal callus and cuttings that were unresponsive to the treatment or dead. Results were recorded after 4 weeks in each study in November 2005, April 2006 and June 2006, respectively**.** Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2.

Outcome of cuttings	%		
	Nov 2005**	Apr 2006	June 2006
	Seradix 2-untreated cuttings		
Plantlets*	4.0 [a]	0.2 [a]	0.6 [a]
Roots only	0 [a]	0.6 [b]	1.3 [b]
Callus only	0.7 [a]	0.6 [b]	3.5 [ab]
New shoots only	36.2 [a]	7.4[a]	5.6 [a]
New shoots and callus	1.9 [a]	1.0 [a]	0.6 [a]
Unresponsive	0.0[a]	2.5[b]	40.6[c]
Dead*	57.2[b]	87.7[b]	47.8[a]
	Seradix 2-treated cuttings		
Plantlets*	9.2 [ab]	0.4 [a]	3.1 [b]
Roots only	0.7 [a]	1.0 [b]	6.9 [c]
Callus only	0 [a]	3.3 [b]	8.4 [b]
New shoots only	19.0 [a]	1.6 [a]	5.3 [a]
New shoots and callus	2.6 [a]	1.0 [a]	1.6 [a]
Unresponsive	0.7[ab]	0.4[a]	24.7[b]
Dead*	67.8[ab]	92.3[b]	50.0[a]

*Data for plantlets and dead cuttings as in Tables 3.32 and 3.30, respectively.

** Data for November 2005 as for cutting type 5 in section 3.1.

a - c = mean separation across columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June respectively).

Table 3.45: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings at different times of the year for the outcome of cuttings. Time of year and Seradix 2 treatment as explained in Table 3.44. ∑ represents collective mean. Data from Table 3.44.

Parameter	*p* value					
	Outcome of Cuttings					
	Roots only	Callus only	New shoots only	New shoots and callus	Unresponsive	Dead
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.145	0.220	0.148	0.863	0.509	0.321
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:						
November 2005	0.317	0.317	0.151	0.945	0.317	0.311
April 2006	0.608	0.113	0.061	0.644	0.063	0.205
June 2006	0.011	0.113	0.822	0.637	0.028	0.832

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 152, 512 and 320 for November, April and June respectively).

A fraction of all cuttings set in all three trials produced basal callus only after four weeks (2.8%) (cuttings with callus and without roots or shoots). For Seradix 2-untreated cuttings, the highest occurrence of this response was observed in cuttings set in June, however, this was not statistically different from cuttings set in November or April (Table 3.44). Amongst Seradix 2-treated cuttings, the percent cuttings with callus only was significantly higher in cuttings set in April (3.3%) and June (8.4%) than those set in November (0%) (Table 3.44). Treatment of cuttings with Seradix 2 did not have an effect on the percentage cuttings that produced callus only for each trial and overall (all cuttings compared) (Table3.45). Therefore, the percentage of cuttings that produced callus exclusively was significantly different amongst the three trials, and the highest incidence of this response was observed in cuttings set in June (Table 3.44).

There were no significant distinctions in the percentage cuttings that produced new shoots only amongst the three trials for both Seradix 2-untreated and Seradix 2-treated cuttings (Table 3.44). Furthermore, although 16.4% of Seradix 2-untreated cuttings and 8.6% of Seradix 2-treated cuttings produced shoots exclusively (Table 3.44), the use of Seradix 2 did not significantly increase the incidence of this response ($p = 0.148$, Table 3.45). This could be attributed to the high accompanying standard errors (data not shown).

Less than 2% of all cuttings that were set (regardless of Seradix 2 treatment) produced new shoots and callus at the basal cut end (Table 3.44). However, as depicted by the data in Tables 3.44 and 3.47, there were no significant differences in terms of the effect of season and Seradix 2 application on the percentage of cuttings that produced new shoots and callus.

Regardless of the time of year at which the cuttings were set and Seradix 2 treatment, the majority of cuttings did not survive. As observed in the initial study (section 3.1), some cuttings set in November, April and June were unresponsive to the treatments and others were dead (Table 3.44).

For those cuttings not treated with Seradix 2, the percentages of cuttings that were unresponsive were significantly different in November, April and June, with the highest percentage of this response occurring in June (40.6%). Similarly, for Seradix 2-treated cuttings, the incidences of unresponsive cuttings were significantly different amongst the three times of the year, with the highest occurrence of this outcome observed in June (24.7%). Overall, Seradix 2 application did not influence the outcome of unresponsive cuttings ($p = 0.509$, Table 3.45).

As discussed previously (Table 3.30), the mortality of cuttings was lowest in June. However, cuttings set in June had the highest prevalence of unresponsive cuttings. Therefore, it appears that the best time to set cuttings with the lowest mortality as well as the lowest number of unresponsive cuttings is in November.

3.2.5.1 Summary

In summary, although the highest plantlet yield was produced in Seradix 2-treated cuttings set in November, Seradix 2 application had no effect on the percentage plantlet production. It appears that root and callus growth were enhanced when cuttings were set in June (winter) and shoot growth was enhanced when cuttings were set in November (spring). As indicated by the data in Table 3.45, Seradix 2 application did not influence any of the abovementioned responses; however, it appears as though cuttings treated with Seradix 2 show differences with respect to these responses and the time of year at which they were set (Table 3.44).

3.2.6 Fresh mass & dry mass

The fresh mass and dry mass data of roots, shoots and callus of Seradix 2-untreated and Seradix 2-treated cuttings set in November, April and June, and the comparisons made between the treatments are presented below in Tables 3.46 to 3.49.

3.2.6.1 Roots

The fresh mass of roots of cuttings set in November, April and June were not significantly different for Seradix 2-untreated and Seradix 2-treated cuttings (Table 3.46). Although the highest root fresh mass was observed in Seradix 2-treated cuttings set in April (0.08 g), no statistical differences were found, probably as a consequence of the high standard error for this value. Furthermore, there was no significant difference in root fresh mass between Seradix 2-treated and Seradix 2-untreated cuttings set in November, April and June (Table 3.47). Therefore, the time of year at which cuttings were set and Seradix 2 application did not affect root fresh mass.

Table 3.46: The effect of the time of year of setting cuttings on the fresh mass of roots in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Root fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	0.02 ± 0.02 [a]	0.05 ± 0.02 [a]
April 2006	0.02 ± 0.02 [a]	0.08 ± 0.04 [a]
June 2006	0.04 ± 0.01 [a]	0.05 ± 0.01 [a]

a = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.47: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the fresh mass of roots. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.46.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.262
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.306
April 2006	0.493
June 2006	0.837

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 4 - 32).

The dry mass of roots for cuttings not treated with Seradix 2 was not statistically different as was the case for Seradix 2-treated cuttings (Table 3.48). Furthermore, when all Seradix 2-treated and all -untreated cuttings were compared, no difference was found in root dry mass ($p = 0.063$, Table 3.49). Thus, the time of year at which cuttings were set and Seradix 2 treatment did not have an effect on root dry mass.

Table 3.48: The effect of the time of year of setting cuttings on the dry mass of roots in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Root dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	0.004 ± 0.002 [a]	0.01 ± 0.00 [a]
April 2006	0.003 ± 0.002 [a]	0.02 ± 0.01 [a]
June 2006	0.01 ± 0.002 [a]	0.01 ± 0.00 [a]

a = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.49: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the dry mass of roots. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.48.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.063
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.307
April 2006	0.244
June 2006	0.421

Analyses were performed using T-tests ($p \leq 0.05$, n = 4 - 32).

3.2.6.2 Shoots

Shoot fresh mass in Seradix 2-untreated cuttings was higher in cuttings set in November and June than those set in April (Table 3.50). For Seradix 2-treated cuttings, the shoot fresh mass of was highest in cuttings set in November (Table 3.50). Seradix 2-untreated shoots had a higher average shoot fresh mass than Seradix 2-treated cuttings (0.023 g vs. 0.017 g), and this difference was found to be statistically significant ($p = 0.040$, Table 3.51). Therefore, cuttings not treated with Seradix 2, performed better in terms of shoot growth and yielded a higher average shoot fresh mass than Seradix 2-treated cuttings.

Table 3.50: The effect of the time of year of setting cuttings on the fresh mass of shoots in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Shoot fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	0.03 ± 0.00 [a]	0.02 ± 0.00 [a]
April 2006	0.01 ± 0.00 [b]	0.01 ± 0.01 [b]
June 2006	0.03 ± 0.01 [a]	0.02 ± 0.00 [b]

a = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 15 - 53).

Table 3.51: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the fresh mass of shoots. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.50.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.040
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.167
April 2006	0.053
June 2006	0.056

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 15 - 53).

The average shoot dry mass for cuttings not treated with Seradix 2 was 0.01g (Table 3.25). Statistically, the shoot dry mass of cutting set in November was highest as compared with April or June (Table 3.52). For Seradix 2-treated cuttings, the time of year at which cuttings were set did not affect shoot dry mass (Table 3.52). Overall, the application of Seradix 2 to cuttings reduced the shoot dry mass ($p = 0.000$, Table 3.53). This is corroborated by the significant difference between Seradix 2-treated and -untreated cuttings set in November ($p = 0.001$, Table 3.53). Therefore, GN107 cuttings not treated with Seradix 2 had higher shoot dry masses than cuttings treated with

Seradix 2 as was the case for shoot fresh mass; supporting the idea that Seradix 2 application may inhibit shoot growth.

Table 3.52: The effect of the time of year of setting cuttings on the dry mass of shoots in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Shoot dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]
April 2006	0.01 ± 0.00 [b]	0.01 ± 0.00 [a]
June 2006	0.01 ± 0.00 [b]	0.01 ± 0.00 [a]

a - b = mean separation within columns, Mann-Whitney U test, ± standard error ($p \leq 0.05$, n = 15 - 53).

Table 3.53: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the dry mass of shoots. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.52.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.000
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	0.001
April 2006	0.441
June 2006	0.178

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 15 - 53).

3.2.6.3 Shoot: root mass ratios

The shoot: root ratios for both fresh mass and dry mass were lower in Seradix 2-treated cuttings (Table 3.54). When the shoot: root ratios based on fresh mass of Seradix 2-untreated and -treated cuttings were compared, no significant difference was found ($p = 0.072$). Similarly, no difference was apparent in the shoot: root ratio based on dry mass between Seradix 2-treated and untreated cuttings ($p = 0.244$). Therefore, although

there is an indication that Seradix 2 application increased root fresh and dry mass, statistically no difference was found between Seradix 2-untreated and -treated cuttings for shoot: root mass ratios.

Table 3.54: The effect of the time of year of setting cuttings on shoot: root fresh mass and dry mass in Seradix 2-untreated and Seradix 2-treated cuttings. Time of year and Seradix 2 treatment as explained in Table 3.30. Significant differences are highlighted in the text.

Time of year	Shoot: root			
	Seradix 2-untreated cuttings		Seradix 2-treated cuttings	
	Fresh mass	Dry mass	Fresh mass	Dry mass
November 2005	1.5	3.3	0.4	2.0
April 2006	0.6	2.6	0.1	0.4
June 2006	0.8	0.7	0.4	0.4

3.2.6.4 Callus

Of those cuttings that were set in November, 2.6% of Seradix 2-untreated cuttings and 2.6% of Seradix 2-treated cuttings produced callus (Table 3.44), however the mass of this callus was not recorded. There was no significant difference in callus fresh mass in cuttings not treated with Seradix 2 set in November, April or June (Table 3.55). Seradix 2-treated cuttings set in April produced more callus per cutting (0.10 g) than those set in November (0.08 g) or June (0.05 g), however these figures were not statistically different from each other (Table 3.55). The fresh mass of callus was significantly higher in cuttings treated with Seradix 2 than untreated cuttings, regardless of the time of year at which the cuttings were set ($p = 0.007$, Table 3.56). Therefore, Seradix 2 application significantly increased the amount of callus per cutting, with the most callus produced per cutting in Seradix 2-treated cuttings set in April.

Table 3.55: The effect of the time of year of setting cuttings on the fresh mass of callus in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Callus fresh mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	NR	0.08 ± 0.01 [a]
April 2006	0.04 ± 0.02 [a]	0.10 ± 0.01 [a]
June 2006	0.04 ± 0.01 [a]	0.05 ± 0.01 [a]

a = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.56: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the fresh mass of callus. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.55.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.007
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	NR
April 2006	0.016
June 2006	0.174

Analyses were performed using T-tests ($p \leq 0.05$, n = 4 - 32). NR = no result.

With respect to callus dry mass, cuttings not treated with Seradix 2 set in November, April and June were not significantly different (Table 3.57). Similarly, the dry mass of callus for Seradix 2-treated cuttings was similar (Table 3.57). As was the case for callus fresh mass, callus dry mass was higher in Seradix 2-treated cuttings as compared with cuttings not treated with Seradix 2 ($p = 0.016$, Table 3.58). Hence, Seradix 2 application to the base of cuttings increased the dry mass of callus as compared with the callus produced in cuttings not treated with Seradix 2.

Table 3.57: The effect of the time of year of setting cuttings on the dry mass of callus in Seradix 2-untreated and Seradix 2-treated cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30.

Time of year	Callus dry mass (g)	
	Seradix 2-untreated cuttings	Seradix 2-treated cuttings
November 2005	NR	0.01 ± 0.00 [a]
April 2006	0.01 ± 0.00 [a]	0.02 ± 0.00 [a]
June 2006	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]

a = mean separation within columns, Tukey's HSD test, ± standard error ($p \leq 0.05$, n = 4 - 32).

Table 3.58: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings for the dry mass of callus. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.30. ∑ represents collective mean. Data from Table 3.57.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.016
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings in:	
November 2005	NR
April 2006	0.006
June 2006	0.355

Analyses were performed using T-tests ($p \leq 0.05$, n = 4 - 32). NR = no result.

3.2.6.5 Summary

In summary, the fresh mass and dry mass of roots in Seradix 2-treated and Seradix 2-untreated cuttings were not significantly affected by the time of year at which the cuttings were set and Seradix 2 application. However, both callus fresh and dry masses were highest in cuttings set in April (autumn) and Seradix 2 was found to increase callus mass. In contrast, shoot fresh mass and dry mass was highest in cuttings set in November (spring), and Seradix 2 was found to negatively affect shoot mass and possibly inhibit shoot growth.

3.3 Root development in GN107 cuttings

From all the primary studies, it was apparent that roots emerged from different points of the cutting. Some roots emerged from the abaxial cut end of the cutting (base) and some emerged from above the abaxial end (sides of the stem just above the base) (Figure 3.1). These root emergence patterns were recorded as three categories of cuttings, i.e. cuttings with roots from the abaxial cut end only (1), cuttings with roots from above the abaxial end only (2) and cuttings with roots from both (3). As the significance, of these root emergence patterns is not known, the incidences of the different rooting categories in the six cutting types (Table 3.59), and throughout the year were investigated in this study (Table 3.61). In addition, a study was undertaken to investigate if the site of root emergence was affected by the extent of which cuttings were dipped into the rooting powder. Two application methods were utilised and the percentage rooting, percentage callusing and the rooting pattern of both methods were then compared (Tables 3.63 and 3.64).

Figure 3.1: Root emergence patterns in GN107 cuttings. (A) Cutting with root emerging from the abaxial cut end, bar = 1.8 cm and (B) cutting with root emerging from above the abaxial cut end, bar = 2.1 cm.

3.3.1 The effect of Seradix 2 on rooting patterns

In Seradix 2-untreated cuttings, regardless of cutting type, cuttings that produced roots showed a greater tendency to develop from the abaxial cut end of the cutting (category 1) (Table 3.59). However, there were no significant differences among the six cutting types in the percentages of cuttings in each rooting category (Table 3.59). There were also no differences between terminal and non-terminal cuttings, amongst terminal cuttings (types 1, 2 and 3) or amongst non-terminal cuttings (types 4, 5 and 6) (Table 3.60).

For Seradix 2-treated cuttings, there was a significant difference between terminal and non-terminal cuttings in the percentage of cuttings in each category, with non-terminal cuttings producing a higher incidence of roots emerging from the side or emerging from both the base and side of the cutting stem (categories 2 and 3, respectively) ($p = 0.000$, Table 3.60). In addition, Seradix 2 application influenced the percentages of cuttings in each category, as cuttings treated with Seradix 2 showed a significantly diverse response compared with cuttings not treated with Seradix 2 ($p = 0.005$, Table 3.60). Therefore, non-terminal cuttings showed a greater tendency to develop roots emerging from the side of the stem of the cutting, and this was further enhanced by Seradix 2 application.

Table 3.59: Summary of the rooting categories in Seradix 2-untreated and Seradix 2-treated cuttings. Rooting category 1 = roots emerging from the bottom only, rooting category 2 = roots emerging from the side only, and rooting category 3 = roots emerging from the bottom and the side. Cutting types 1, 2, 3 = terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node respectively; cutting types 4, 5, 6 = non terminal cuttings, cut at the node, cut 1 cm above the node and cut 1 cm below the node respectively. Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2. Results were recorded after 4 weeks of setting in November 2005.

Rooting category	%					
	Cutting type					
	1 [A, a]	2 [A, ab]	3 [A, ab]	4 [A, bc]	5 [A, c]	6 [A, c]
	Seradix 2-untreated cuttings					
1	100	100	100	83.3	83.3	100
2	0	0	0	16.7	16.7	0
3	0	0	0	0	0	0
	Seradix 2-treated cuttings					
1	100	100	84.6	64.7	46.6	42.8
2	0	0	7.7	29.4	26.7	28.6
3	0	0	7.7	5.9	26.7	28.6

A = mean separation across columns for Seradix 2-untreated cuttings, a - c = mean separation across columns for Seradix 2-treated cuttings, Mann-Whitney U test ($p \leq 0.05$, n = 2 - 21).

Table 3.60: Levels of significance in Seradix 2-untreated, Seradix 2-treated cuttings, terminal (cutting types 1, 2, 3) and non-terminal cuttings (cutting types 4, 5, 6) for the rooting categories cuttings. Cutting types 1 - 6 and Seradix 2 treatment as explained in Table 3.59. ∑ represents collective mean. Data from Table 3.59.

Parameter	*p* value
Comparing Cutting preparation:	
Seradix 2-untreated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.180
Terminal cuttings 1 vs. 2 vs. 3	1.000
Non-terminal cuttings 4 vs. 5 vs. 6	0.764
Seradix 2-treated cuttings:	
∑ Terminal cuttings (1, 2, 3) vs. ∑ Non-terminal (4, 5, 6)	0.000
Terminal cuttings 1 vs. 2 vs. 3	0.280
Non-terminal cuttings 4 vs. 5 vs. 6	0.227
Comparing Seradix 2 treatment:	
∑ Seradix 2-untreated vs. ∑ Seradix 2-treated cuttings	0.005
Seradix 2-untreated vs. Seradix 2-treated:	
Cutting type 1	1.000
Cutting type 2	1.000
Cutting type 3	0.287
Cutting type 4	0.384
Cutting type 5	0.106
Cutting type 6	0.087

Analyses were performed using Kruskal-Wallis and Mann-Whitney U tests, where applicable ($p \leq 0.05$, n = 2 - 21).

The incidences of cuttings in the three rooting categories in November, April and June are presented in Table 3.61. There was no effect of the time of year cuttings were set on the percentage cuttings in each category in Seradix 2-untreated cuttings (Table 3.61). Cuttings treated with Seradix 2 showed a significant difference in the percentage cuttings in each category in November, April and June (Table 3.53). Cuttings set in November showed a more diverse response in terms of the type of root emergence pattern than cuttings set in April or June (Table 3.61). Overall, however, Seradix 2 application did not significantly influence the prevalence of each rooting category ($p = 0.075$, Table 3.62). Therefore, the highest incidence of type 2 and type 3 categories was observed in cuttings set in November.

Table 3.61: Summary of the rooting categories produced in Seradix 2-untreated and Seradix 2-treated cuttings at different times of the year. Seradix 2-untreated cuttings = cuttings not treated with Seradix 2, Seradix 2-treated cuttings = cutting base dipped into Seradix 2. Results were recorded after 4 weeks of setting in November 2005, April 2006 and June 2006. Rooting category as explained in Table 3.59.

Rooting category	Nov 2005 [A, a]	Apr 2006 [A, b]	June 2006 [A, b]
		%	
		Seradix 2-untreated cuttings	
1	83.3	100	100
2	16.7	0	0
3	0	0	0
		Seradix 2-treated cuttings	
1	46.6	100	90.3
2	26.7	0	3.2
3	26.7	0	6.5

Data for November 2005 as for cutting type 5 in section 3.1.

A = mean separation across columns for Seradix 2-untreated cuttings, a - b = mean separation across columns for Seradix 2-treated cuttings, Mann-Whitney U test ($p \leq 0.05$, n = 4 - 32).

Table 3.62: Levels of significance in Seradix 2-untreated and Seradix 2-treated cuttings at different times of the year for the rooting categories of cuttings. The time of year of setting cuttings and Seradix 2 treatment as explained in Table 3.61. ∑ represents collective mean. Data from Table 3.61.

Parameter	*p* value
∑ Seradix 2-untreated cuttings vs. ∑ Seradix 2-treated cuttings	0.164
Seradix 2-untreated cuttings vs. Seradix 2-treated cuttings for:	
November 2005	0.106
April 2006	1.000
June 2006	0.441

Analyses were performed using Mann-Whitney U tests ($p \leq 0.05$, n = 4 - 32).

3.3.2 The effect of the method of Seradix 2 application on cuttings

In the commercial propagation of cuttings, the practice of dipping cuttings into Seradix powder is not precise and the depth of Seradix application is not identical in each cutting. To investigate the effect of the application method of Seradix 2, cuttings were dipped into Seradix 2 powder at the surface of the cut abaxial end or cuttings were dipped into Seradix 2 powder to an approximate depth of 2.5 cm. The percentage rooting, percentage callusing and the rooting categories were recorded (Tables 3.63 and 3.64).

As illustrated by the data in Table 3.63, the percentage rooting was 25% for cuttings dipped at the abaxial end and 24.2% for cuttings dipped 2.5 cm into Seradix 2. There was no significant difference in the rooting frequency between the two methods of Seradix 2 application ($p = 0.885$). Cuttings dipped 2.5 cm into the rooting powder had a higher incidence of callus production (63.3%) than cuttings dipped at the base (46.9%) ($p = 0.008$, Table 3.63). Therefore the extent at which cuttings were dipped into Seradix 2 did not affect the percentage rooting of cuttings but did influence the percentage callusing.

The percentage cuttings within each rooting category between cuttings dipped at the abaxial end and those dipped 2.5 cm are presented in Table 3.64. Although the percentage of cuttings with category 2 and 3 rooting was numerically highest in cuttings dipped 2.5 cm into Seradix 2, statistically, no significant difference was observed between the treatments (p = 0.348, Table 3.64). Therefore, the extent at which cuttings were dipped into Seradix 2 did not affect the percentage of cuttings in each rooting category (1, 2 or 3).

Table 3.63: The effect of the extent at which cuttings were dipped into Seradix 2 on % rooting and % callusing. Cuttings were dipped into Seradix 2 powder at the abaxial (base) of the cutting only or cuttings were dipped into Seradix 2 powder up to approximately 2.5 cm above the abaxial end. Results were recorded after 4 weeks.

Parameter	%	
	Cuttings dipped at abaxial end	Cuttings dipped 2.5 cm
Rooted cuttings	25.0 ± 0.38 [a]	24.2 ± 0.38 [a]
Cuttings with Callus	46.9 ± 0.44 [a]	63.3 ± 0.43 [b]

a - b = mean separation across columns, Mann-Whitney U tests, ± standard error ($p \leq 0.05$, n = 128 each, for cuttings dipped at the abaxial end and cuttings dipped 2.5 cm).

Table 3.64: The effect of the extent at which cuttings were dipped into Seradix 2 on % rooted cuttings in each rooting category. Cuttings were dipped into Seradix 2 powder at the base of the cutting only or cuttings were dipped into Seradix 2 powder up to approximately 2.5 cm above the base. Rooting category as explained in Table 3.59. Results were recorded after 4 weeks.

Rooting category	% Rooted cuttings	
	Cuttings dipped at abaxial end [a]	Cuttings dipped 2.5 cm [a]
1	90.7	83.9
2	9.4	3.2
3	0	12.9

a = mean separation across columns between treatments ($p \leq 0.05$, n = 128 each, for cuttings dipped at the abaxial end and cuttings dipped 2.5 cm.

In summary, non-terminal cuttings were found to generate more diverse root emergence patterns than terminal cuttings and Seradix 2 treatment influenced the incidence of roots emerging from the bottom and sides of the stem. Furthermore, a higher incidence of roots emerging from the sides occurred in cuttings set in November and this was enhanced by Seradix 2 application. However, the method of Seradix 2 application, in particular the extent at which the bases of cuttings were dipped into the powder, did not affect the rooting pattern but influenced callus production.

3.3.3 The anatomy and ontogeny of roots of GN107 cuttings

As previously mentioned, 4 week old cuttings developed roots that emerged from the cutting stem at the abaxial cut end (base) or from above the abaxial end (sides of the stem). Anatomical studies were performed to investigate if roots that emerged from the abaxial end and from above the abaxial end of the cutting differed in their point of origin.

Figure 3.2 illustrates the stems of GN107 cuttings prior to root development, showing tetrarch xylem. In cuttings in which roots emerged from the abaxial cut end of the stem, appear to have root primordia in the xylem arch as well as the cambium (Figure 3.3). Similarly, cuttings in which roots emerged from above the abaxial end of the stem also appear to have root primordia in the xylem arch as well as the cambium (Figure 3.4). In those cuttings in which roots were believed to develop from the xylem arch, a connection existed between the vascular bundle in the centre of the stem and the developing root (Figures 3.3 and 3.4 A and B). However, in those cuttings in which roots appeared to originate from the cambium, no such connection was present in the sections investigated (Figures 3.3 and 3.4 C and D).

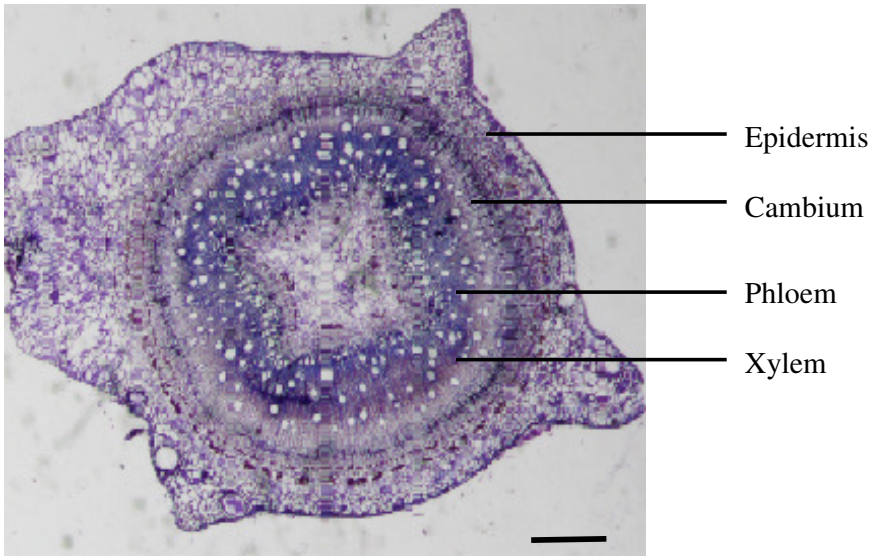

Figure 3.2: Cross-section of a stem of a GN107 cutting. Cutting at 2 weeks, before root development. The vascular bundle is seen in the centre of the figure with tetrarch xylem. Samples were embedded with paraffin wax, sectioned and then stained with Toluidene Blue (0.1%). Bar = 0.44 mm.

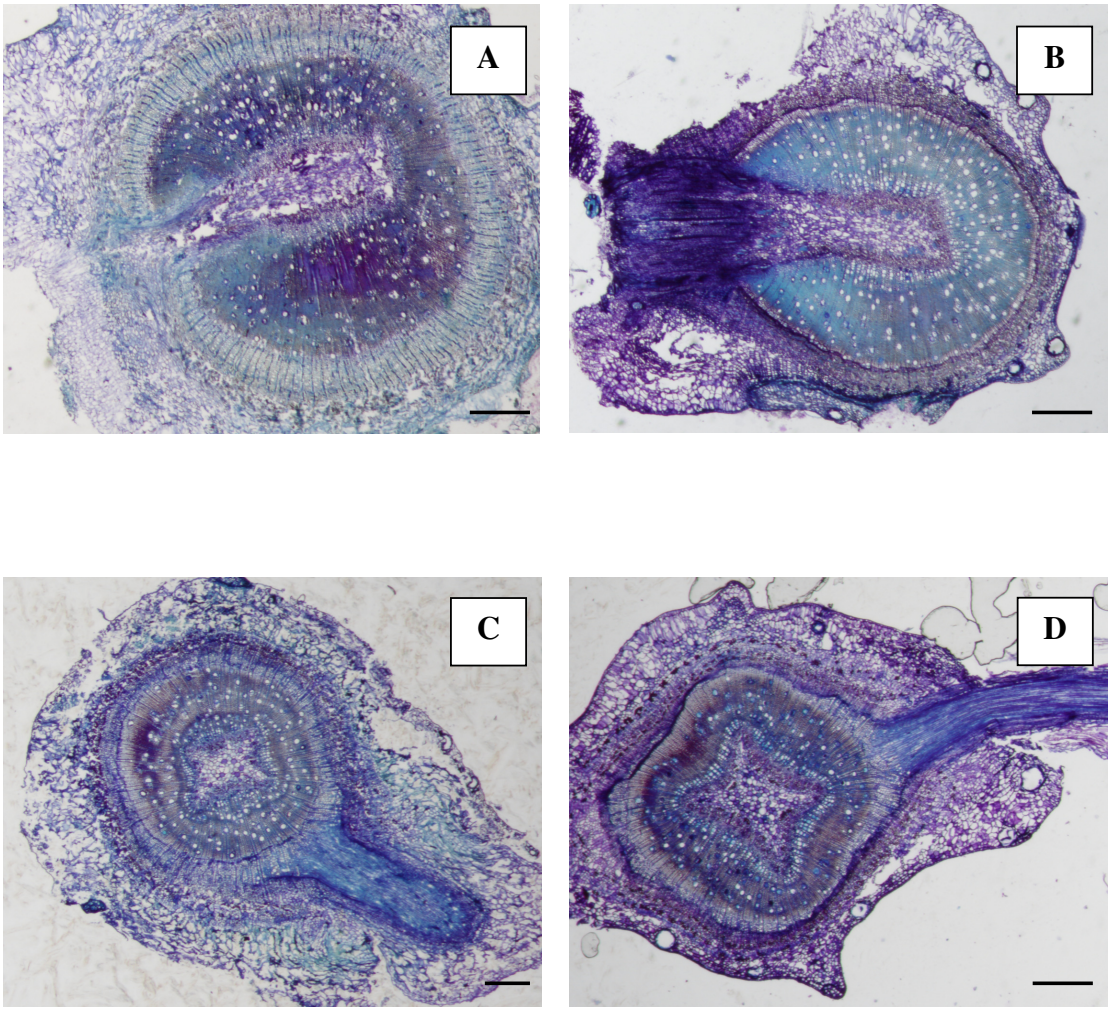

Figure 3.3: Cross sections of stems of GN107 cuttings from which root emergence occurred at the abaxial cut end (Figure 3.1 A). (A) and (B) root primordia appear to originate in the xylem archs, bar = 0.40 mm and 0.39 mm, respectively; (C) and (D) root primordia appear to originate in the cambium, bar = 0.32 mm and 0.48 mm, respectively. Samples were embedded with paraffin wax, sectioned and then stained with Toluidene Blue (0.1%).

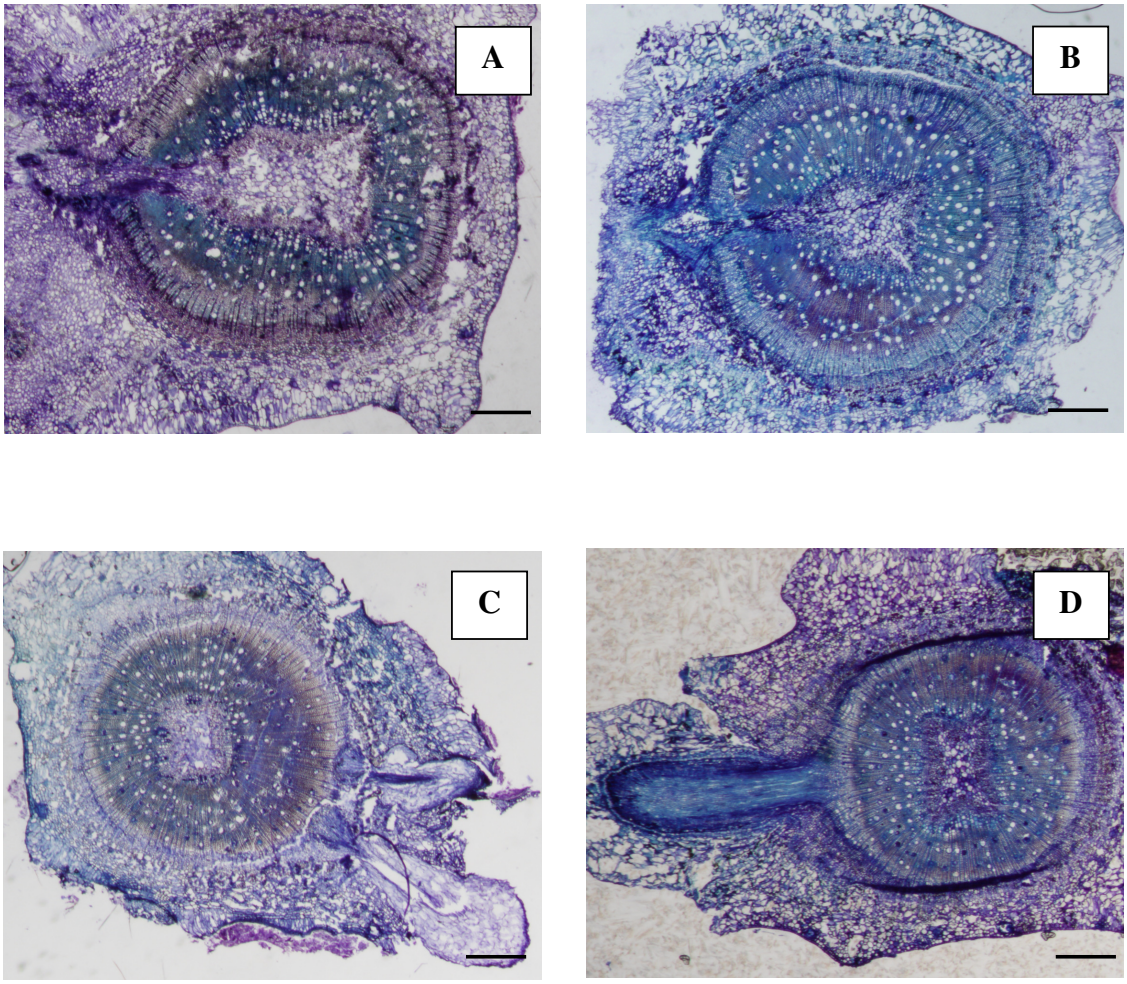

Figure 3.4: Cross sections of stems of GN107 cuttings from which root emergence occurred above the abaxial cut end (Figure 3.1 B). (A) and (B) root primordia appear to originate in the xylem archs, bar = 0.38 mm and 0.32 mm, respectively; (C) and (D) root primordia appear to originate in the cambium, bar = 0.45 mm and 0.40 mm, respectively. Samples were embedded with paraffin wax, sectioned and then stained with Toluidene Blue (0.1%).

From the observations made in this study, there were no patterns to indicate that the different root emergence patterns (roots emerging from the abaxial cut end or from above the cut end) had different points of origin. Roots were observed to develop from the xylem archs as well as from the cambium. However, further investigation is necessary in order to eradicate the possibility that the root primordia and point of origin in the stem sections were somehow overlooked during the sectioning process. Furthermore, studies should be conducted on cuttings at different stages in their

development, and stem sections should be made at those different stages of growth so as to locate correctly the root primordia at the time that roots first develop.

4. DISCUSSION

4.1 Overview

In order for the South African forestry industry to remain productive and to meet the escalating demands for forestry goods, productivity on existing plantations and marginal sites needs to be maximised (Denison and Kietzka, 1993a; Dye, 2000). This has been achieved, in part, through hybrid forestry. One of the hybrids used is the *E. grandis* x *E. nitens* (GN), as many of its clones adapt to sites more readily and use water more efficiently than pure species (Denison and Kietzka 1993a; February *et al*., 1995). In addition, clones of *E. grandis* x *E. nitens* have been found to have superior wood qualities that make it ideal for use by the pulp and paper industry (Denison and Kietzka, 1993a).

As previously discussed, successful cutting propagation programmes are dependent on the root-ability of cuttings, and the capability of cuttings to perform as well as, or superior to seedlings (Sasse and Sands, 1997). Not only is successful cutting propagation species- and clone-dependent, but yield depends also on the type of material used, the position from which the material originates form the parent plant, the age of the parent plant, the time of year of harvesting plant material, the propagation substrate, the type of rooting enhancers used, container type, and the soil temperature, to name a few (Paton *et al*., 1970; Hartney, 1980; Paton, 1984; McComb and Wroth, 1986; Shepherd, 1986; Wilson, 1993; Maile and Nieuwenhuis, 1996; Tibbits *et al*., 1997; Bayley and Nixon, 1998; Wilson, 1999a).

Studies have shown varying rooting percentages in eucalypt cuttings, with rooting percentages ranging from 0% to 100%, depending on the species and clone (McComb and Wroth, 1986; Wilson, 1994; Maile and Nieuwenhuis, 1996; Tibbits *et al*., 1997; Aimers-Halliday *et al*., 1999). The Hilton nursery reports an average root strike of 30 - 40% for cold-tolerant clones (McAllister, *pers. comm.*; Wallis, *pers. comm*.). However there are many cold-tolerant species (notably *E. grandis* x *nitens*, including the clone GN107) with a much lower root strike, and for which little or no research into

the factors that affect rooting has been undertaken. At the Hilton nursery, propagation of *E. grandis* x *nitens* cuttings involves the utilisation of stem cuttings (below the apical shoot) and the application of the IBA-containing commercial rooting powder Seradix 2 (3 g kg^{-1} IBA) to the base of the cutting. However, reports in the literature on eucalypt cuttings indicate that cuttings taken from different positions from the parent plant root differently (Paton *et al*., 1970; Wilson, 1993) and IBA treatment and concentration, as well as its method of application to cuttings (Carter and Slee, 1993; Maile and Nieuwenhuis, 1996) also affect root strike. Furthermore, a seasonal effect on cutting performance among eucalypt species has been documented (Hartney, 1980; McComb and Wroth, 1986; Maile and Nieuwenhuis, 1996; Tibbits *et al*., 1997). As such effects on rooting of cuttings of GN107 have not been reported, they were investigated in this study.

Many plant species have an optimum rooting period in the year (Fordham, 1965; Hartmann *et al*, 1997). McComb and Wroth (1986) found that optimum rooting in *E. resinifera* cuttings was observed in February (< 25% rooting). Maile and Nieuwenhuis (1996) found that cuttings taken from three-year old *E. nitens* plants showed a 30% and 56% rooting in March (spring) and September (autumn), respectively. Research has shown that the rate of rooting and root development of cuttings are improved when the root zone temperature and bottom heat are increased (Hartmann *et al*., 1997). Mitchell (2002) established that without the use of rooting hormones, root strike in *Pinus patula* and *P. elliotii* x *P. caribaea* cuttings could be improved by the addition of bottom heat (25 - 28ºC), and although pines (gymnosperms) are not directly comparable to eucalypts, that study indicates the importance of the relationship of the heat requirement during the rooting of cuttings.

Throughout the present study, as rooting did not necessarily occur in conjunction with shoot growth, the percentage rooting was not equivalent to the plantlet yield. The highest percentages of cuttings that produced roots (regardless of shoot growth) were part of the June (10%) and November trials (9.9%) (Table 3.34). In addition, cuttings set in June and November produced the highest numbers of roots per cutting (Table 3.36). The proportion of cuttings that produced only roots and the proportion of cuttings that

produced only callus were highest amongst cuttings set in June as compared with those set in November or April (Table 3.44). Therefore, cuttings set in June (winter) had improved root development as compared with cuttings set in November (spring) or April (autumn). Similarly, Tibbits *et al.* (1997) found that *E. nitens* cuttings set in winter had a higher percentage rooting (average 27%) than cuttings set at other times of the year. On the other hand, in the present study, cuttings set in November (spring), had superior shoot development in terms of the number of cuttings that produced shoots, shoot length and the mass of shoots relative to root mass. The results indicate that high-yielding shoot development in GN107 cuttings may have a seasonal influence and may depend on the quality of the hedge plant material at the time of setting cuttings. Furthermore, while shoot development appears to be enhanced in November (spring), root development was greater in June (winter). In terms of cutting success, although not shown statistically, there was an indication that plantlet yield was highest in cuttings set in November and lowest in April (Table 3.32).

Reports in the literature indicate that the rooting ability of cuttings is affected by the position at which the cuttings are taken from the parent plant. Different species exhibit varying rooting success for cuttings taken from the apical, sub-apical, mid-position and basal region of the parent stem (Wilson, 1993). Bachelard and Stowe (1963) reported that mid-position and basal cuttings of *E. camaldulensis* rooted better than tip cuttings. Wilson (1993) demonstrated that the survival and rooting ability of *E. globulus* stem cuttings and the relation to the origin of the cuttings on the parent plant were clonally influenced. Survival percentages tended to be higher in apical and sub-apical cuttings in one clone but not in the other clone tested and rooting percentages decreased with increasing distance form the shoot apex (Wilson, 1993). Similarly, the rooting ability of conifers has been found to decrease as the node position from the apical bud increases (Mitchell, 2005). However, in species such as *Cordia allidora* and *Osyris lanceolata*, the relationship between rooting success and cutting origin was shown to be variable and influenced by season (Mesén *et al*., 1997; Teklehaimanot *et al*., 2004). Cuttings of *O. lanceolata* set in winter and spring showed basal cuttings rooting better than terminal cuttings and in cuttings set in summer the opposite effect was observed (Teklehaimanot *et al*., 2004). In the case of eucalypts, an increase in ontogenetic age of the cutting tissue

is associated with a decline in rooting ability and has been found to be related to an increase in rooting inhibitor at the base of the cutting (Paton *et al*., 1970).

In the initial investigation of this study, terminal cuttings (situated below the apical bud), and non-terminal cuttings were employed (for description see section 2.2). The abaxial ends of terminal and non-terminal cuttings were cut at different points on, above or below the lowest node, to examine the effect of this on rooting. Of the two types, terminal cuttings had the highest mortality of cuttings (Table 3.1), the lowest plantlet yield (Table 3.3), the lowest percentage of cuttings that produced roots (Table 3.5) and the lowest percentage of cuttings that produced new shoots (Table 3.11). This suggests that the juvenility of the cutting affects survival, rooting and plantlet yield negatively. Better results were obtained with the more mature and hardier non-terminal cuttings, in particular type 6 (non-terminal cutting, cut below the abaxial node). With regard to the point at which the abaxial end of the cuttings was cut in relation to the node, there were no significant differences amongst terminal cuttings types 1, 2 and 3 or amongst non-terminal cutting types 4, 5 and 6.

Although terminal cuttings had a higher mortality, they produced longer roots, slightly more roots per cutting and a higher root fresh mass and dry mass than non-terminal cuttings. However, the root strike and survival of non-terminal cuttings was superior to terminal cuttings. These differences could be related to the differences in the endogenous auxin content within terminal cuttings and non-terminal GN107 cuttings may differ.

As discussed in section 1.5, the main regenerative process required in cutting propagation is adventitious root formation; however, the capacity to produce adventitious roots is low in many woody plant species and may be influenced by juvenility and season (Hartmann *et al*., 1997). Root development is characterized by the manufacture and change of levels of physiologically important substances such as rooting co-factors. However the ability to synthesize such substances may be lost or gained with ageing (juvenility) and over time (season) (Paton *et al*., 1970). In the present study, cuttings success (plantlet production) may have been influenced by the

presence or absence of rooting compounds in the plant tissue and the subsequent effects of this on adventitious root formation.

It is well known that high concentrations of auxins are necessary for adventitious root formation, and endogenous auxins originate in the shoot apex and shoot axillary region (Haissig, 1986; Gaspar and Hofinger, 1988; Hartmann *et al*., 1997; De Klerk *et al*., 1999). In cutting propagation, exogenous hormones applied to cuttings to promote rooting are commonly used. However their concentration and method of application influence the rooting of cuttings (Carter and Slee, 1993; Maile and Nieuwenhuis, 1996). Although Seradix 2 (3 g kg^{-1} IBA) is the commercial rooting powder most commonly used at the Mondi Hilton nursery for GN107 and other cold-tolerant clones, the effects of Seradix 2 application, cutting type and season on GN107 cuttings have not previously been reported.

Regardless of the cutting type used or the time of year at which cuttings were set, cuttings treated with Seradix 2 had a higher plantlet yield (Table 3.3) and a higher percentage of cuttings that produced roots (Tables 3.5 and 3.34), as compared with untreated cuttings. In addition, the former had higher root and callus fresh and dry masses (Tables 3.17, 3.19, 3.55 - 3.58) than the latter.

In contrast to the results obtained in this study, Aimers-Halliday *et al*. (1999) reported that the percentage rooting of *E. grandis* x *nitens* cuttings treated with a 3% (w/v) IBA in a gel base was not significantly different from that of cuttings not treated with the hormone (36.5% and 34.5%, respectively). Two points can be inferred from the findings of those authors in comparison with the results presented in this study. Firstly, a higher concentration of externally applied IBA (3% vs. 0.3% IBA in the present study) enhanced rooting. However, even without the IBA application, their reported rooting percentage was still above 30%, implying that the *E. grandis* x *nitens* clone used in that study may have been less difficult-to-root than the GN107 genotype.

Reports in the literature on the pure species *E. grandis* and *E. nitens* indicate that each have their own auxin requirements for rooting. For example, the overall percentage

rooting in *E. nitens* cuttings increased from 30% to 56.3% when the IBA concentration was lowered from 0.8% to 0.2% (Maile and Nieuwenhuis, 1996). On the contrary, *E. grandis* cuttings exhibited similar rooting responses when treated with IBA concentrations ranging from 0.2% to 1%, and the magnitude of the response varied with season and the highest mean rooting percentage was observed in January (summer) (Carter and Slee, 1993).

In the present study, although Seradix 2 application increased plantlet yield, it also increased the tendency of cuttings to form roots exclusively. Furthermore, although not statistically different from Seradix 2-untreated cuttings, cuttings treated with Seradix 2 had the highest prevalence of callus formation at the bases of the cuttings (Table 3.15). The percentage new shoot growth amongst cuttings not treated with Seradix 2 was found to be higher than in treated cuttings (31.4% vs. 24.3%) (Table 3.11). Overall, when all three trials were compared, the shoot fresh and dry masses among Seradix 2-untreated cuttings were found to be greater than in cuttings to which Seradix 2 was applied (Tables 3.50 to 3.54). The shoot: root fresh and dry mass ratios (Tables 3.25 and 3.54) indicated that cuttings not treated with Seradix 2 had higher shoot fresh and dry masses. This suggests that Seradix 2 may have inhibited shoot development, but statistically there was no difference between Seradix 2-treated and -untreated cuttings.

Therefore Seradix 2 promoted root growth and development but appears to have inhibited or delayed shoot growth in GN107 cuttings. This indicates that GN107 requires auxin application for successful setting of cuttings but at a concentration other than that found in Seradix 2 (as IBA); such 'optimal' concentration still needs to be determined for GN107. In addition, it is suggested that other hormones (alone or in combination) be tested on the clone GN107, such as, IAA and NAA, which have also been shown to improve rooting of cuttings (Blazich, 1988b; Hartmann *et al.*, 1997; Blythe and Sibley, 2003). Mixtures of rooting hormones have also been shown to be effective in rooting cuttings than either hormone alone (Fazio, 1964; Hartmann *et al.*, 1997; Blythe and Sibley, 2003).

Furthermore, Maile and Nieuwenhuis (1996) demonstrated that although Seradix (0.8% IBA) on its own did not significantly influence rooting in *E. nitens* cuttings, it improved rooting when used in combination with specific time of year of harvesting cuttings and propagation medium (Maile and Nieuwenhuis, 1996). Those authors also demonstrated that the percentage rooting in juvenile cuttings set in late summer was influenced by the time of year of harvesting cuttings, the propagation medium and Seradix application. They found significant differences in the percentage rooting between Seradix-untreated and Seradix-treated cuttings propagated in vermiculite (50% vs. 16.7%, respectively). When cuttings were set in a mixture of peat, sand and vermiculite (1:1:1), Seradix-untreated cuttings yielded a higher percentage rooting (66.7%) than Seradix-treated cuttings (23.3%). However, they did not observe any differences in the percentage rooting between Seradix-treated and untreated woody cuttings set in mid-winter for the different propagation media that were tested in that study. The results obtained by those authors indicated that the propagation media utilised affected the rooting of cuttings of the pure species *E. nitens*. It is possible, therefore, that the propagation substrate used in the present study had an inhibitory effect on rooting of the hybrid clone, but this was not investigated.

Tibbits *et al*. (1997) reported that the rooting ability in *E. nitens* cuttings appeared to be highly inherited and suggested that a selection program for *E. nitens* could identify superior rooting plants. Several years ago, it was determined that the *E. grandis* x *nitens* hybrid behaved more like the *E. grandis* parent than the *E. nitens* parent in its ability to coppice and produce rooted cuttings (Aimers-Halliday *et al*., 1999). Perhaps the low incidence of rooting obtained with the GN107 genotype in the present study may be a consequence of inherent, poor rooting traits and the poor plantlet yield for GN107 (< 13%) may be an extreme and rare case.

As stated by Sasse and Sands (1997) for cuttings to be considered successful in tree improvement programmes, their root systems must function as well as or better than those of seedlings. From personal observation, and those of researchers and forestry workers, abnormal root growth is often observed in cuttings, and this aberrant growth usually persists after cuttings have been established in the field (Lindström and Rune,

2000; Mokotedi, 2007). Eucalypt cuttings have been known to produce roots that grow 180^{o} apart, which, are often seen above-ground in close proximity to the stem (Mokotedi, 2006). Furthermore, GN107 cuttings have been reported to produce 'tap sinkers' by 16 months of field growth that serve to improve the anchorage of the plants (Mokotedi, 2007). In the present study, as early as four weeks after cuttings were set, it was apparent that two types of root emergence patterns were evident amongst GN107 cuttings. Roots emerged from the abaxial cut end of the cutting (base) while others emerged from the sides of the stem, just above the base. As the significance of these root emergence patterns on the development of the roots of macropropagated plants is not known, the incidence the rooting patterns and the origin of the roots in cuttings were investigated.

Three rooting patterns amongst cuttings of GN107 were distinguished: cuttings that gave rise to roots emerging from the bottom only (abaxial end) (type 1), cuttings with roots emerging from the side (above the abaxial end) (type 2), and cuttings with both roots (type 3). A higher incidence of type 2 and 3 rooting was observed in non-terminal cuttings as compared with terminal cuttings, and the highest incidence of this response was observed amongst cuttings set in November.

Blythe and Sibley (2003) highlighted that it is not only the rooting hormone used and its concentration, but also the duration of hormone application that affect the rooting ability of cuttings. However, *E. grandis* cuttings dipped into and IBA solution for 1 second was found to be equally effective in root formation than cuttings dipped for 5 or 10 seconds (Carter and Slee, 1993). In the commercial nursery, the practise of dipping cuttings into Seradix powder is not precise. As labourers set thousands of cuttings a day, the possibility exists that the depth of Seradix application is not identical in each cutting; the implications of this on subsequent root growth, rooting patterns and possible aberrant root growth in the nursery and in the field is not known. Therefore, the depth of Seradix 2 application was investigated in the present study. Although not statistically different, cuttings dipped 2.5 cm into Seradix 2 produced more roots that emerged from the side (type 2) and from both the bottom and side (type 3) of the stem than cuttings dipped at the abaxial end only (Table 3.64).

Anatomical investigations of roots that emerged from the abaxial end and roots that emerged from above the abaxial end, revealed that they appeared to develop from both the cambium as well as the xylem archs. However, although not statistically compared, there was no apparent relationship between the site of root emergence (root ontogeny) and the type of root emergence pattern (type 1, 2 or 3). Therefore, the type of cutting used and the time of year at which cuttings were set had an effect on the rooting pattern of GN107 cuttings, however, the depth of Seradix 2 application did not. The implications of the rooting patterns described in the present study on the long term development of roots and anchorage of cuttings in the field still need to be determined.

4.2 Conclusions

The plantlet yield for GN107 cuttings obtained in this study was poor (< 13%). Despite this, the results suggested that the rooting and plantlet yield were influenced by Seradix 2 application, cutting type and season. Seradix 2 appeared to have an adverse affect on shoot growth as cuttings treated with Seradix 2 gave poorer results for shoot development than untreated cuttings. In addition, non-terminal cuttings outperform terminal cuttings when Seradix 2 is used, regardless of how they were cut (at, above or below the node). There appears to be a seasonal effect on root, callus and shoot growth in GN107 cuttings, with the best period for rooting observed in June (winter), while the best shoot development was exhibited in November (spring). Overall, the high mortality rate (regardless of the time of the year cuttings were set) and low plantlet yield emerges as the major concern. Treatments with the best rooting performance (highest percentage rooting and highest number of roots) were set in June. However, cuttings set in June had the lowest shoot: root ratio. These points emerge as another concern as the growth and competitiveness of plants in the field may be impeded in plants with shorter roots and a reduced shoot: root ratio.

Based on the work of other researchers and as discussed, there are numerous parameters that influence cutting development. It is suggested that for the clone GN107, other concentrations of IBA, perhaps lower than that found in Seradix 2 powder (0.3% IBA), be tested. Since Seradix 2 appeared to have inhibited shoot growth in GN107, lower

concentrations of IBA should reduce callus production and increase shoot production, and thereby increase plantlet production. In addition, the effect of combinations of hormones (such as IBA and NAA) and the effect of a dipping the cutting into a solution containing the hormone (rather than the powder itself) on the rooting of GN107 should be investigated.

Seeing that a substrate of peat, sand and vermiculite increased the rooting results obtained in *E. nitens* cuttings (Maile and Nieuwenhuis, 1996), it is suggested that other combinations of propagation substrates, such as those that include sand, be investigated for GN107. Moreover, increasing the temperature of propagation medium by the application of bottom heat may also improve the rooting percentages of GN107; however, further research into the optimum root zone temperature is necessary.

Wilson (1999b) demonstrated that heavy and light pruning of mother plants, both weekly and fortnightly gave varying rooting percentages (35 - 46%) in *E. globulus* cuttings. Therefore, it is suggested that pruning and rejuvenation of hedge plants should be carried out at the Hilton nursery.

This study established that cuttings of GN107 have an optimum rooting period and a separate optimum shoot development period in the year. This, together with the relationship with auxin treatment, needs to be investigated further. Since overall plantlet yield was very low (< 13%) and the mortality of GN107 was very high, it is suggested that the study be repeated with a bigger sample size. In addition, other statistical analyses should be performed (such as multivariate tests) so as to determine the relationships between the three parameters tested (i.e. cutting type, Seradix 2 treatment and season). Other parameters that may affect shoot development, such as, the use of other auxins (e.g. NAA) and different concentrations of auxins should be investigated for GN107. In addition, the effects of the pruning regime for hedge plants on shoot development should be investigated. As it appears that specific parameters and conditions are required for cutting propagation of the commercially important clone GN107, future research should concentrate on identifying and exploiting those parameters to improve plantlet yield.

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