Optimising mini-plug transplanting of *Eucalyptus dunnii* seedlings

by

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“Success is not final, failure is not fatal:

it is the courage to continue that counts”

-Winston Churchill

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Optimising mini-plug transplanting of *Eucalyptus dunnii* seedlings

I, Tracy Yvonne Newmarch, student number: 205524985 declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is the result of my own endeavours at the Mountain Home Nursery, Tree Improvement Department, Mondi Limited, Hilton and in the Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg;

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Student's Full Name: Tracy Yvonne Newmarch

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Thesis Title: Optimising mini-plug transplanting of *Eucalyptus dunnii* seedlings

Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the Faculty of Science and Agriculture Higher Degrees Office for examination by the University appointed Examiners.

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DETAILS OF CONTRIBUTIONS TO PUBLICATIONS AND CONFERENCES that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication)

CONFERENCES ATTENDED:

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Contributions: Trial work and analysis was co-ordinated and conducted by the first author under the supervision and guidance of the last three authors.

Signed_____

ABSTRACT

Mini-plug transplanting has only recently been described as a useful propagation method for use in forestry nurseries. Mini-plug seedlings are seedlings that have been pre-cultivated in containers with volumes less than 33-ml. These seedlings are re-planted into containers of a larger volume at a later stage in the growing season. The many advantages of mini-plug transplanting include decreased seedling production costs, improved space use efficacy and the flexibility of producing larger seedlings for harsh field conditions. However, the effect of mini-plug transplanting on seedling quality and root architecture is unknown and may influence seedling survival after planting in the field. This research aimed to optimise a mini-plug transplanting protocol for use in a commercial forestry nursery to limit the development of root deformities and enhance seedling quality in *Eucalyptus dunnii* seedlings.

The effects of mini-plug container type and mini-plug cultivation time prior to transplanting on seedling quality were evaluated. Seedlings were grown in two mini-plug containers, MP288 and MP544, for one, two, three and four months before transplanting the mini-plug seedlings into the final container. In this experiment it was confirmed that the mini-plug container design had an effect on mini-plug seedling quality. Seedlings grown in MP288 containers had lower sturdiness and shoot-to-root ratios compared to MP544-reared seedlings. This was attributed to the lower planting density and higher cavity volume of the MP288 trays. However, MP288 trays increased the frequency of root deformities in the *E. dunnii* mini-plug seedlings as these trays were constructed out of plastic which encouraged root coiling. The MP544 trays produced seedlings with actively growing, fibrous roots which were attributed to the root pruning properties of the copper oxychloride that the polystyrene mini-plug containers were initially treated with. It is therefore recommended that the MP544 tray should be used for the cultivation of mini-plug seedlings. The age of the mini-plug seedlings also affected transplanting success. Based on the results it was concluded that the optimal age to transplant mini-plug seedlings was between two and three months after sowing. Transplanting one-month-old seedlings tended to increase the frequency of J-roots after transplanting and four-month-old seedlings delayed seedling growth.

Once the optimal seedling age and mini-plug tray type to be used for container-to-container transplanting was confirmed, the effects of the propagation method (directly-sown and transplanting) on seedling quality were compared using various containers. Transplanted seedling quality was inferior compared to directly-sown seedlings. This was caused by delayed growth directly after transplanting. The substrate composition that the mini-plug seedlings had been grown in had not been optimised for mini-plug cultivation and may have reduced mini-plug seedling quality which resulted in delayed seedling growth after transplanting. In addition, transplanting increased the incidence of root deformities and using polystyrene containers as the final container for transplanting did not reduce root deformation. The presence of J-roots was believed to be caused by the transplanting process itself. The negative effects of container design observed in the mini-plug containers were also observed in the final container after transplanting. Low plug volumes decreased seedling growth and increased seedling competition due to the higher planting densities.

Following the results of the second study, the optimal substrate composition to be used for mini-plug seedling cultivation and in the final container after transplanting was investigated. This investigation confirmed that the substrate composition affected seedling development and growth. In the mini-plug trays, the physical properties of the substrate were shown to be important factors affecting both root development and seedling growth. Substrates with low air-filled porosity values reduced root growth and the negative effects of low air-filled porosity were enhanced when seedlings were cultivated in mini-plug containers. The results also indicated that different substrate compositions were required for the different seedling propagation methods tested and for the different physiological stages in seedling growth. It was recommended that *E. dunnii* mini-plug seedlings are cultivated using an 80:20 (v/v) mixture of 6-mm pine bark and perlite. For the final container, the recommended substrate was a 90:10 (v/v) mixture of 6-mm pine bark and perlite.

The effects of vermicompost leachate, seaweed extract and smoke-water on seedling growth after transplanting were evaluated as many beneficial effects of these supplements have been reported. The use of vermicompost leachate, seaweed extract and smoke-water were not effective in improving *E. dunnii* seedling growth after transplanting in this study. However, these supplements may have needed to be applied to the seedlings more

regularly and at higher concentrations. Further research is required to optimise the concentration and frequency of application to ensure improved seedling growth after transplanting.

Although, during the course of this study some improvements to the container to-container transplanting procedure were made, the seedling quality of directly-sown seedlings was still superior. By transplanting mini-plug seedlings a delay in seedling growth was observed and the incidence of J-rooting was increased. Neither, tray type, cultivation time nor substrate composition was able to limit the incidence of J-roots in transplanted seedlings or prevent the delayed seedling growth. For a mini-plug transplanting method to be successful in a commercial forestry nursery, further research is required to overcome the limitations of this method.

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LIST OF ABREVIATIONS

MP:	Mini-plug
S.D.:	Standard deviation
ANOVA:	Analysis of Variance
VCL:	Vermicompost leachate
SW:	Smoke-water solution
K:	Kelpak®
SVI:	Seedling vigour index
SET:	Seedling emergence time
EC:	Electrical conductivity
SAR:	Sodium absorption ratio
AMP:	Adenosine monophosphate
ADP:	Adenosine diphosphate
ATP:	Adenosine triphosphate
GA:	Gibberellic acid
ABA:	Abscisic acid
LSD:	Least significant difference

CHAPTER 1

Introduction

Commercial forestry plantations are important in South Africa because they supply raw materials for the pulp and paper industry, timber, mine supports, particle board and telephone poles, to name a few (**FORESTRY SOUTH AFRICA, 2013**). Planting commercial forestry plantations ensures a continuous supply of these raw materials whilst conserving our natural forests (**NAMBAIR, 1999**). Exotic tree species are used for the establishment of these plantations as they are fast growing and able to meet the high demand for raw materials (**ELDRIDGE *et al*., 1993**)

Successful establishment of a forestry plantation is largely dependent on seedling survival after planting (**DAVIS & JACOBS, 2005; HAASE, 2008; BINOTTO *et al*., 2010; PINTO *et al.*, 2011**). Forest seedlings are exposed to variable environmental conditions after planting in the field. These conditions may be detrimental to seedling survival if the seedlings are unequipped to endure the initial establishment phase. Good quality seedlings are essential to limit mortality immediately after planting. These seedlings need to be able to initiate new root growth quickly in order to access available water and nutrients for further growth and development (**DAVIS & JACOBS, 2005; HAASE, 2008; BINOTTO *et al*., 2010; PINTO *et al.*, 2011**). Poor quality seedlings lack the ability to develop new roots quickly, often due to ineffective root systems; the result is decreased water and nutrient up-take. In an effort to minimise the loss of water through transpiration, the stomata close, limiting photosynthesis and the production of carbohydrates which may lead to eventual death (**GROSSNICKLE, 2005**).

In a forestry seedling nursery, seedling quality is assessed prior to sending the plants out to the field for planting. Seedling quality provides an indication of seedling viability and is assessed by evaluating both morphological and physiological characteristics. These include shoot height, root collar diameter, shoot and root mass, root growth potential, root electrolyte leakage, stomata conductance, and chlorophyll content, to name a few. These characteristics give the nurseryman an insight into the seedlings ability to survive in the field (**HAASE, 2008; RICHIE & LANDIS, 2010**). Various nursery cultural practices will have an

impact on seedling quality and care needs to be taken to ensure good practices are employed in the nursery to prevent the production of inferior seedlings (**DAVIS & JACOBS, 2005**). Producing seedlings with active root systems is one of the most important criteria to ensure seedling survival and growth after establishment in the field (**GROSSNICKLE, 2005**).

Mini-plug seedling production is not novel to the horticultural industry but has only recently been described as a useful technique to be employed in the production of forestry seedlings (**LANDIS, 2007**). Mini-plug seedlings offers various advantages to seedling production in forestry nurseries including decreased seedling productions costs, improved space usage and flexibility of producing larger seedlings without losing valuable production space (**STEINFELD, 2004; LANDIS, 2007**).

Seedlings are initially cultivated in small mini-plug trays and are re-planted at a later stage into trays with a bigger volume (**STEINFELD, 2004**). The transplanting process may have a detrimental effect on the root system (**RICHIE, 2003**). Transplanting may cause the development of root deformities, decrease the substrate-to-root contact after transplanting and result in physical damage to the roots **(NESMITH, 1999; GROSSNICKLE, 2005; JOHANSSON *et al*., 2012**). The resulting damage to root system function will impact on above-ground seedling growth (**DAVIS & JACOBS, 2005; GROSSNICKLE, 2005**).

It is clear that the use of mini-plugs in a forestry seedling nursery, offers various advantages. However, the influence of mini-plugs on final plant quality should be investigated as it would have a substantial effect on field survival. The aim of this research study was to optimise the mini-plug transplanting procedure to (1) ensure that the effects of transplant shock was limited, (2) reduce the development of poor quality mini-plug seedlings and 3) reduce the development of poor root architecture. This study was carried out at Mountain Home Nursery in Hilton, Kwa-Zulu Natal using *Eucalyptus dunnii* seed sourced from Mondi's seed orchards. *E. dunnii* was selected for this study because it represented the highest proportion of Mountain Home Nursery's seedling production. A series of experiments testing various aspects of nursery cultural practises and their influences on seedling growth, development and root architecture were implemented.

CHAPTER 2

Literature review

2.1. Forestry and the importance of *Eucalyptus* species in South Africa

The Forestry, Pulp and Paper Industry in South Africa manufactures and supplies a wide range of products including raw materials such as timber, mine supports, particle board, paper pulp (both bleached and unbleached) and paper products such as kraft packaging papers, newsprint and graphic paper. The South African forestry industry sold forestry products to the value of R19 586 million (TABLE 2.1) during 2010/11 (**FORESTRY SOUTH AFRICA, 2013**). A proportion of these products are exported and the industry is therefore reliant on fast growing, low cost raw material to compete on international markets (**PALLET *et al*., 2000**).

TABLE 2.1. The sale of forestry products extracted from South African Forestry facts for 2010/2011 (**FORESTRY SOUTH AFRICA, 2013**).

PRODUCT	**UNITS**	**TOTAL VOLUME ('000m^3/tons)**	**RAND VALUE (MILLIONS)**	**% TOTAL**
Sawn Timber	m^3	1568	4 035	18.9
Pulp	Tons	2379	12 862	60.1
Mining Timber		386	345	1.6
Poles	m^3	397	520	2.4
Charcoal	Tons	52	218	1.0
Chips/ Mill Residues	Tons	1606	1 606	7.5

South Africa's vegetation consists mainly of non-woody species due to the low average rainfall patterns in South Africa (**OWEN & VAN DER ZEL, 2000**). Natural forests make up approximately 0.3% of the total surface area in South Africa. In an effort to conserve these forests and to meet growing public demand, commercial plantations were planted (**NAMBAIR, 1999**). One of the first plantations established in 1670 was a stand of oaks planted at Newlands, but it was only in 1875 that exotic species were established to supply fuel wood for the railways (**OWEN & VAN DER ZEL, 2000**).

The Industry is supplied by commercial plantations consisting mainly of exotic species including *Pinus*, *Acacia* and *Eucalyptus* trees. In 2005/6 51% of the plantations were planted to *Pinus,* 40% to *Eucalyptus* and 8% to *Acacia* tree species (**FORESTRY SOUTH AFRICA, 2013**).

The *Eucalyptus* genus was first described in 1788 by the French botanist Charles-Louis L. Heriter de Brutelle (**BOLAND *et al*., 1984**). This economically important genus is native to Australia and its surrounding islands and comprises of approximately 700 species, many of which are planted in both tropical and temperate regions around the world. Throughout the world including South Africa, the *Eucalyptus* genus is chosen for its fast growth habit and its ability to grow and survive in harsh conditions (**ELDRIDGE *et al*., 1993**). *Eucalyptus* is utilized in the South African forestry industry for a wide range of applications including paper pulp, charcoal, fuelwood, poles, mining timber, fibreboard, timber and volatile oils (**POYNTON, 1979**).

Eucalyptus is important to the pulp and paper industry because it produces superior fibre in a short rotation time (**LAINE & DAVID, 1994**). The ten most commonly grown species throughout the world for their fibre properties are *E. grandis, E. camaldulensis, E. tereticornis, E. globulus, E. urophylla, E. viminalis, E. saligna, E. deglupta, E. exserta* and *E. citriodora* (**ELDRIDGE *et al*., 1993**). In South Africa several different *Eucalyptus* species and their hybrids are established in plantations across a variety of sites for the pulp and paper industry (**POYNTON, 1979**). *Eucalyptus grandis* is by far the most commercially superior species within the *Eucalyptus* genus planted in South Africa. This species is favoured for its excellent fibre qualities, fast growth rate, good form and excellent rooting potential (**WEX & DENISON, 1997; ARBUTHNOT, 2000**). It is however not suited for growth in cold dry regions and is limited by its susceptibility to disease (TABLE 2.2) (**WEX & DENISON, 1997**). Other important species grown in South Africa include *E. nitens, E. macarthurii, E. dunnii, E. smithii, E. camaldulensis* and *E. urophylla* (**SWAIN & GARDNER, 2004**). *Eucalyptus nitens* and *E. macarthurii* are favoured in South Africa for their good form and ability to grow in extreme cold and frost (TABLE 2.2). *Eucalyptus dunnii* and *E. smithii* however, are less adapted to extreme growing conditions (TABLE 2.2) (**SWAIN & GARDNER, 2004**). *Eucalyptus urophylla* is used in the forestry industry for its superior growth, good stem form and ability

to propagate easily (TABLE 2.2) and *E. camaldulensis* is able to tolerate drought and is relatively free from pests and diseases (TABLE 2.2, **VAN WYK & VERRYN, 2000**). *Eucalyptus grandis* is especially useful as a hybrid partner with *E. nitens, E. camaldulensis, E. macarthurii*, or *E. urophylla* thus producing progeny containing both good fibre qualities, increased resistance to disease and adaptation to extreme growing conditions which will allow for greater productivity off marginal land (**DENISON & KIETZKA, 1993; VAN WYK & VERRYN, 2000; SWAIN & GARDNER**, **2004**).

TABLE 2.2. The growth conditions, properties and potential uses of important *Eucalyptus* species utilized in the South African forestry industry.

Species	Natural growing conditions	Properties	Uses	Reference
E. smithii	Temperate to cool climates, humid. Rainfall: 750mm-1700mm annually	Reasonably tolerant to frost or cold	Pulpwood, essential oils, fencing, fuel, poles	**PRYOR & JOHNSON, 1971; BOLAND *et al*., 1984**
E. nitens	Temperate to cool climates, humid. Rainfall: 750mm-1750mm	Frost tolerant, good form	Mining timber, pulpwood	**POYNTON**, **1979**, **BOLAND *et al*.**, **1984**
E. macarthurii	Temperate, fairly humid. Rainfall: 750mm-1150mm per annum	Frost tolerant, reasonably free from attack by pests and diseases, Good form,	Essential oils, mining timber, fuel, pulpwood	**POYNTON**, **1979**; **BOLAND *et al*.**, **1984**
E. grandis	Sub-tropical, warmer climates, humid. Rainfall: 1000mm-3500mm per annum	Good wood properties, rapid growth	Construction work furniture, telephone poles, mining, pulp	**PRYOR & JOHNSON**, **1971**; **BOLAND *et al*.**, **1984**; **ELDRIDGE *et al*.**, **1993**
E. globulus	Cool climate, humid. Rainfall: 600mm-1400mm	Rapid growth, large flowers and prolific coppicing	Heavy and light construction, sleepers, poles, paper pulp, fibre and particle board, furniture and essential oils	**PRYOR & JOHNSON, 1971**

Species	Natural growing conditions	Properties	Uses	Reference
E. camaldulensis	Temperate to tropical climates. Rainfall: 200mm-1100mm per annum	Moderately frost tolerant, withstands high temperatures and drought, coppices readily, deep root penetration, tolerant to water logging, not generally attacked by insects or fungi	Construction, flooring, sleepers, poles, charcoal	**PRYOR & JOHNSON, 1971; ELDRIDGE *et al*., 1993**
E. tereticornis	Warmer or sub-tropical climates, humid to sub-humid. Rainfall: 3000mm per annum	Moderately drought resistance, free from insect and fungal damage, coppices easily	Mining timber, pulpwood, fuel building poles	**POYNTON, 1979**; **BOLAND *et al*., 1984**
E. fastigata	Cooler climates, humid. Rainfall: 750-1150mm	Frost tolerant, not attacked by pests and disease, good form	General carpentry, flooring, fibreboard, pulping, fuel, mining timber	**POYNTON, 1979; ELDRIDGE *et al*., 1993**
E. urophylla	Tropical-warmer climates, humid. Rainfall: 1000-1500mm per annum	Rapid growth, good stem form, easily propagated	Construction, furniture, firewood, fuel	**POYNTON, 1979; ELDRIDGE *et al*., 1993**

Species	Natural growing conditions	Properties	Uses	Reference
E. dunnii	Warm climate, humid. Rainfall: 1000-1750mm per annum	Grows in a wide range of soil types, Fast growth, good pulping properties	Saw logs, pulp production	**BOLAND *et al*., 1984; ARNOLD *et al*., 2004; SMITH & HENSON, 2007**
E. saligna	Warmer to temperate or sub-tropical climates, humid. Rainfall: 900-1800mm per annum	Good stem form, dense timber, no end splitting, coppices readily	Light construction, fibre and hard board, railway sleepers	**POYNTON, 1979; ELDRIDGE *et al*., 1993**

2.2. Forest seedling nurseries

Plantation forestry relies on the production of exotic tree species in forestry nurseries (**VILJOEN *et al*., 1992**). A forestry nursery is a managed area where high quality forestry seedlings are grown in optimal conditions to ensure the mass production of quality seedlings for plantation establishment. Nurseries ensure that seedlings are grown with care during the juvenile growth phase in order to guarantee the production of vigorously growing plants. The seed propagated in the nurseries is often sourced from the seed orchards which contain trees that were selected through vigorous tree breeding programmes (**ROSHETKO *et al*., 2010**).

Forestry seedling nurseries may either propagate seedlings using bare root methods or containers (**ROSE & HAASE, 2005**). Bare root nurseries propagate the seedlings directly in the soil and the seedlings are lifted prior to sale. The container nurseries propagate seedlings in containers filled with artificial growing media where the seedlings will remain until planting out (**O'REILLY *et al*., 2002**). Bare root nurseries require large areas for plant propagation, a high labour contingent and seedlings are more prone to physical damage, particularly during lifting prior to sale (**ROSE & HAASE, 2005**). However, these seedlings are easy to pack and transport without concern for the return of containers. As the seedlings are planted directly in the soil, growth media and nutrient supply concerns are limited. The use of containers for seedlings offers many advantages. These include extended planting seasons, quicker seedling production and uniform seedlings. In container nurseries, containers make the transport of seedlings to the field difficult and often these containers are not returned which incurs a cost to the nursery (**BRISSETTE *et al*., 1991**). Container-reared seedlings require more intensive management in terms of pests, diseases, irrigation and nutrition (**BRISSETTE *et al*., 1991**). However, container-reared seedlings are more space efficient, can be maintained in a controlled environment and suffer less transplant shock at planting (**BRISSETTE *et al*., 1991**). In South Africa, Mondi's seedling nurseries are container nurseries and for this reason further topics discussed will refer to container-grown seedlings.

2.3. Benefits of mini-plug transplanting in the commercial nursery

Mini-plug transplanting is a nursery practice which was introduced into nursery systems to speed up nursery production and reduce production costs (**LANDIS, 2007**). Mini-plug transplanting has been common practice in the vegetable industry for several years, in the forestry industry however, mini-plug transplanting is not a common practice (**LANDIS, 2007**). Seeds are germinated in small seedling trays (generally with a plug volume of less than 33-ml), these seedlings are allowed to grow until the roots have consolidated the plug and then transplanted into either a bareroot bed or in the case of a container nursery, a larger container, where the seedlings will remain until the time of sale (**STEINFELD, 2004**).

Mini-plug transplants optimise production through improved space use efficiency, decreased media usage and less labour requirements (**STEINFELD, 2004; LANDIS, 2007**). A standard nursery tray such as a Unigro® 128 tray with a volume of 65-ml has a growing density of 590 plants per m^2 whereas a 288 tray with a volume of 6-ml has a growing density of 2090 plants per square metre, increasing the nursery capacity three fold (**LANDIS, 2007**). The increased space is available to the nursery manager either to increase plant production capacity or to transplant seedlings into trays with a larger plug size. Larger seedlings tend to produce a more robust plant which improves overall field survival.

The mini-plug system also offers the advantage of decreased growth media requirements. The total media required for initial germination stages is reduced by almost 90% of the total substrate if the seeds are germinated in a 288 tray (TABLE 2.3). In addition, since only germinated seedlings are transplanted to larger containers, the media in cells containing un-germinated seeds is significantly less than what would be expected if seeds were sown directly into larger containers.

TABLE 2.3. Estimated volume of growth media required on a daily, monthly and annual basis for five different tray types (Unigro® 128, Unigro® 98, mini-plug (MP) 200, MP288 and MP406). These figures are based on a nursery producing eight million seedlings per year with an average germination rate of 67%.

Tray type	No cavities	Volume/ cavity (ml)	Volume/ Tray (l)	Daily (l)	Monthly (l)	Annually (l)
U128	128	66	8.45	3 573.50	71 470.08	857 640.96
U98	98	80	7.84	4 335.52	86 710.40	1 040 524.80
MP200	200	11	2.20	594.00	11 880.00	142 560.00
MP288	288	5	1.73	324.86	6 497.28	77 967.36
MP406	406	4	1.62	217.62	4 352.32	52 227.84

In mini-plug transplanting, the current processes of pricking and patching, sizing and consolidating will all take place at the transplanting stage thus reducing labour requirements. The seedlings are sized earlier in the crop cycle thus minimising the plant mortality due to variations in size of seedlings in one tray (**LANDIS, 2007**)

While mini-plug transplanting offers many advantages to nursery operations, the final plant quality needs to be considered as this has a substantial effect on field survival.

2.4. **The importance of seedling quality**

The successful establishment of forest plantations is dependent on the quality of seedlings produced in the nursery. Cultural practises in the nursery influence initial growth and survival in the field after planting, particularly in harsh variable growing conditions (**DAVIS & JACOBS, 2005; HAASE, 2008**; **BINOTTO *et al.*, 2010; PINTO *et al.*, 2011**). When seedlings are established in the field they are often exposed to both biotic and abiotic stresses which may be detrimental to seedling health and may decrease or inhibit initial growth and subsequent field survival. Water and nutrients are not always readily available; the seedlings may be exposed to extreme temperature conditions and may also be physically damaged due to handling (**DAVIS & JACOBS, 2005; HAASE, 2008; JOHANSSON *et al.*, 2012**).

Good quality seedlings are plants that are able to survive these stresses and result in successful plantation establishment (**DAVIS & JACOBS, 2005; HAASE, 2008**).

Seedling quality can be determined by measuring morphological and physiological parameters of the seedlings prior to despatch. The morphological parameters that can be measured are shoot height, root collar diameter, sturdiness ratio, shoot-to-root ratio, fresh and dry weight of shoots and roots and colour. The physiological parameters that can be measured are cold hardiness, root growth potential, stomatal conductance, chlorophyll fluorescence, and plant moisture stress (**HAASE, 2008; RICHIE & LANDIS, 2010**) (TABLE 2.4).

TABLE 2.4. Morphological and physiological measurements used to interpret seedling quality in the nursery.

Characteristic	Description
Morphological indicators of seedling quality	
Shoot height	Shoot height gives an indication of photosynthetic and transpiration area. Tall seedlings compete easily against weeds but will be prone to water loss due to greater transpiration area. Taller seedlings will also be difficult to plant (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Root collar diameter	Root collar diameter is a good indicator of potential survival in the field. The greater the root collar diameter the greater the potential survival in the field. Root collar diameter has also been correlated positively with root volume. A greater root volume will improve field survival due to increased root-to-soil contact and increased nutrients and water uptake (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Sturdiness ratio	The sturdiness ratio is a ratio of shoot height and root collar diameter. A high value indicates a tall spindly seedling and a low value indicates a shorter, robust seedling. The potential survival in the field is less for tall spindly seedlings. These seedlings tend to have smaller root systems which are unable to support the above-ground plant material (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Shoot mass (fresh and dry)	A greater shoot mass is an indication of greater photosynthetic potential. However, seedlings with a disproportionate shoot and root mass may be detrimental to seedling survival. A seedling with a shoot mass greater than the root mass indicates that the seedling will transpire faster than it can absorb water causing plant water stress, particularly on drier sites (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).

Characteristic	**Description**
Shoot-to-root ratio	The shoot-to-root ratio gives an indication of the seedling balance between the area of transpiration (shoots) and water uptake. In container grown seedlings the ratio should be less than 2:1 (**HAASE, 2007**; **HAASE, 2008; RICHIE & LANDIS, 2010**)
Colour	Seedling colour is an indicator of plant stress such as poor nutrient status or diseases (**HAASE, 2008; RICHIE & LANDIS, 2010**).
Physiological indicators of seedling quality	
Cold hardiness	Is an indication of stress resistance and is influenced by seed source, environment and nursery practices (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Root growth potential	Indicates seedling vigour and determines the seedlings ability to initiate new root growth which is essential for survival after planting in the field (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Plant moisture stress	Plant moisture stress increases with increasing water stress. When seedlings become water stressed the stomata close, decreasing photosynthesis which decreases plant growth and in severe cases results in plant mortality (**HAASE, 2007; HAASE, 2008; RICHIE & LANDIS, 2010**).
Chlorophyll fluorescence	Chlorophyll fluorescence gives an indication of photosynthetic activity. Stressed or poor quality seedlings exhibit lower levels of fluorescence indicating decreased photosynthetic activity (**HAASE, 2008; RICHIE & LANDIS, 2010**).

Root system quality is possibly one of the most important characteristics that should be measured to determine potential field survival after transplanting. Unfortunately it is one of the least measured characteristics because it requires destructive sampling and takes time. A good quality root system is essential as it may limit transplant shock after transplanting. Transplanting shock often occurs because the seedling is unable to initiate new root growth quickly and the root to soil contact is then limited (**DAVIS & JACOBS, 2005**). The main

function of the root system is to absorb nutrients and water, anchor and support the above-ground plant parts and act as a storage organ (**JANICK, 1986**). In order to minimise transplant shock, the root system must initiate new roots quickly to ensure a good root-to-soil or -substrate contact for optimal uptake of nutrients. The ability of the roots to absorb water and nutrients from the substrate may be inhibited by root volume, soil temperature and root development. Root systems with a greater root volume will increase the surface area available to absorb water and nutrients and increase the potential root to soil or substrate contact (**RICHIE, 2003**).

Root function is affected by soil temperature. Under low soil or substrate temperatures water viscosity increases, hindering the hydraulic conductance of the root system. New root growth depends on photosynthetic activity and transport of carbohydrates to the roots. However, under lower temperatures, stomatal conductance in the leaves decreases thereby decreasing photosynthetic activity and subsequently decreasing root growth. Since new root growth is suspended, water uptake is limited (**RICHIE, 2003**).

Shoot-to-root balance is critical, particularly in container grown plants where root growth is restricted. Roots depend on shoots for photosynthetic products and growth regulators and in turn shoots require water and nutrients from the roots. High shoot-to-root ratios may increase transplanting shock after transplanting because the root volume is too small and therefore, unable to supply sufficient water and nutrients for important metabolic processes such as photosynthesis (**KRIZEK & DUBIK, 1987; RICHIE, 2003**).

Seedling quality is determined by the genetic potential of the seedling, the seedling size and seedling vigour. As previously discussed, the quality of the seedlings is largely dependent on cultural practises in the nursery which include growth media, container shape, size and density, irrigation, and nutrition to name but a few (**DAVIS & JACOBS, 2005**).

2.5. Factors affecting seedling quality

2.5.1. Container types

Nursery container type is one of the most influential factors determining seedling quality and general seedling management (**KOSTOPOULOU *et al*., 2011**). Comparisons between bare root grown seedlings and container-reared seedlings have shown that containers improve seedling quality and field survival (**ANNAPURNA *et al*., 2004; SOUTH *et al*., 2004**). **SOUTH *et al.*** (**2004**) showed that field survival of container-grown seedlings was 22 points higher than bare root grown seedlings. The increased field survival rate was attributed to more actively growing fibrous root systems in container-grown seedlings. In a root growth potential test, container-grown seedlings produced an average of 5.5 new roots compared to an average of 1.7 new roots produced by bare root seedlings (**SOUTH *et al*., 2004**). In addition, bare root-produced seedlings tended to develop deformed root systems (L-shaped roots and J-rooting) and under-developed tap roots with the effects of these deformities being evident up to seven years after planting (**SLOAN *et al*., 1987**).

Nursery containers contain the substrate for individual seedlings which provide seedlings with nutrients, water, oxygen, plant support and root protection prior to planting in the field. There are many different container options, shapes, sizes and configurations. The characteristics of the container will influence the morphological and physiological aspects of seedlings. These characteristics include volume, shape, espacement (density), drainage and manufacturing materials (plastic or polystyrene), (**HANDRECK & BLACK, 1994; KOSTOPOULOU *et al*., 2011**). The ideal container type for seedling production depends on the species, planting site, planting ease and cost (**LANDIS, 1990; O'REILLY *et al*., 2002**). The container is designed to promote the development of an actively growing fibrous root system (**SLOAN *et al*., 1987; LANDIS, 1990**). A balanced shoot-to-root relationship should be promoted during seedling growth (**LANDIS, 1990**). The container should allow for easy extraction of the seedling with the root "plug" intact (**MULLAN & WHITE, 2001**).

Root development and morphology are the most critical factors influenced by container design with the in-field survival of seedlings being dependant on the seedling's ability to send out new roots and become quickly established in the soil (**LANDIS, 1990)**. Common

problems caused by growing seedlings in containers are root deformities (J-rooting and spiralling/coiling) and restriction of root growth (**MULLAN & WHITE, 2001**). Various methods are used by manufacturing companies to limit these problems (**LANDIS**, **1990**). Lateral root development is ideal while seedlings are maintained in their containers because they increase the number of active root tips. This is important for nutrient uptake and encourages substrate consolidation making exaction of seedlings from containers easier. To encourage branching, the roots are pruned, either through physical, chemical or desiccation methods (**RUNE, 2003; SAYER *et al*., 2009**). Containers are designed with a hole at the base allowing drainage and root pruning. Some containers have side slits which increases air pruning of the roots on the sides. Root pruning prevents spiralling within the root plug. Un-pruned roots retain their apical dominance and continue to grow and spiral in the container, eventually causing strangulation of the seedling (**RUNE, 2003; HAYWOOD *et al*., 2012**). The use of ribs in plastic containers is designed to direct root growth downward towards the drainage hole, preventing root spiralling (**JAENICKE, 1999**). However, roots of seedlings grown in containers with ribs often develop a cage-like appearance which may cause swelling of the root collar, constricting movement of nutrients between the roots and stem (**BURDETT, 1978**).

Density, spacing or number of plants per square meter is largely influenced by the volume of the plug which impacts the sturdiness of the seedling. The closer the seedlings are to each other the taller they become as they compete for light to photosynthesise. Decreasing the space between cells reduces air movement through the plants thus increasing the incidence of disease, decreasing the number of leaves on the stem and inhibiting root collar diameter expansion (**LANDIS, 1990; RADOGLOU *et al*., 2011; DINI-PAPANASTASI *et al*., 2012**). **RADOGLOU *et al***. (**2011**) found that mini-plug seedlings grown in containers with lower planting densities increased the production of new roots because there was less competition for light to photosynthesise and therefore exhibited higher carbohydrate content for root development. Similar results were observed in a study conducted by **DINI-PAPANASTASI *et al*.** (**2012**) where the authors showed that planting density was especially important in seedling survival and the longer the cultivation period the more important planting density became. However, increasing the spacing between cells takes up valuable

space in the nursery. Therefore, the nursery manager will need to weigh up the need for space versus that of plant quality when choosing container design (**LANDIS, 1990**).

Containers are made in various shapes and sizes. Shape will have an impact on plant density; square containers increase the number of plants per square meter compared to round containers. Round containers allow for easier extraction of the seedling from the container but tend to promote root spiralling. Tapered containers are useful for ease of extraction but extreme tapering may cause a decrease in the root growth potential as the majority of the roots will be produced at the bottom of the plug (**LANDIS, 1990; JAENICKE, 1999**).

Root plug volume affects total plant biomass, root collar diameter, time in the nursery, plant density and ultimately plant cost. Increased volume increases the root biomass, which increases the root plug surface area in contact with the soil **(LANDIS, 1990)**. A larger container will produce a more robust plant with a greater root collar diameter and root plug, which is considered to be good for field survival (**LANDIS, 1990; HAASE 2008**). However, a large volume container is not always ideal as it increases the bulk weight of the tray, decreases plant density, decreasing nursery space efficiency and increases plant cost. Most nurseries typically only use larger volume containers for plants meant for harsh field sites since plants produced in large volume containers will have a higher buffering capacity (**LANDIS**, **1990**).

Container depth affects the physical properties of the substrate which affect seedling growth. A shallow container increases the moisture content of the substrate and substrate requirements should be adjusted accordingly (**HANDRECK & BLACK, 1994**). Container depth will influence the growth and development of the root system because container depth affects container volume. Restriction of root volume decreases shoot growth due to the inability to absorb nutrients and water, decreases oxygen supply and causes irregular distribution of growth substances between the roots and shoots (**NISHIZAWA & SAITO, 1998**). Research has shown that seedling development is influenced by container depth where deeper containers produced seedlings with longer roots, and a greater shoot and root biomass (**KOSTOPOULOU *et al*., 2010b**). Smaller containers increase the severity of the symptoms of restricted roots and by extending the time in the container, these symptoms will be more pronounced (**NESMITH & DUVAL, 1998**).

It is clear that many design parameters of containers may impact on the growth of the seedling in the nursery and after planting in the field.

2.5.2. Substrate composition

A good quality substrate allows for better gaseous exchange, retains sufficient water for plant growth, which creates a reservoir of minerals and nutrients, provides better plant support. It should not be too heavy (**REID, 2005; BANACH *et al*., 2013**). The type of media used has an effect on the resulting plant growth and quality. Physical properties of a growing media refer to several different criteria including total porosity, air-filled porosity, water holding capacity and bulk density. All of which are relatively easy to test and will indicate to the grower how plant quality and growth will be influenced by the substrate (**HANDRECK & BLACK, 1994; REID, 2005**).

Total porosity refers to the total space in the medium that is not filled with growing media (**HANDRECK & BLACK, 1994**). Total porosity includes the total space available for oxygen diffusion and water. The pore space and size of pores is important because it gives an indication of the substrate's ability to satisfy the seedling requirements for optimum growth. Water is retained in the small pores of the substrate and air is diffused into the larger pores. The volume of water that is left in the substrate after draining is referred to as the water holding capacity and the air-filled porosity of the substrate is volume of air in the substrate after draining (**HANDRECK & BLACK, 1994**).

Air-filled porosity gives an indication of the potential volume of gaseous exchange that may occur. High air-filled porosity means that there are many large pores in the medium which increases the gaseous exchange but decreases the substrate's water retaining capacity. A medium with a high air-filled porosity needs to be irrigated frequently to prevent drying out. Low air-filled porosity reflects a substrate with many small pores which decrease gaseous exchange and increase water holding capacity of the substrate. An air-filled porosity that is too low may cause the waterlogging if irrigated to frequently (**HANDRECK & BLACK, 1994; MATHERS *et al*., 2007**). As a result, decreased oxygen will decrease in the growth rate of the seedlings due to:

- an inability to repair and produce root cells;
- decreased ability to absorb water and nutrients;
- changes in hormone production;
- increase attack by pathogenic microbes;
- decreases the activity of beneficial microbes;
- and production of toxic substances (**HANDRECK & BLACK, 1994; MATHERS *et al*., 2007**).

When the air-filled porosity is low, the irrigation frequency needs must be considered. Sufficient time between irrigation scheduling must be allowed to ensure that enough water has drained from the substrate to encourage oxygen diffusion into the substrate (**HANDRECK & BLACK, 1994**).

The water holding capacity of a substrate indicates the volume of water available to the plants between irrigation events. A high water holding capacity indicates that there are many small pores for water to be adhered to. For a substrate with a high water holding capacity, less irrigation periods are required. Care must be taken when irrigating substrates with high water holding capacities to prevent over watering. In some cases however, the water holding capacity may be high but the pores within the substrate are very small. This decreases water availability in the substrate. Water is inaccessible to the root system because it is difficult for root tips to penetrate the small pores (**HANDRECK & BLACK, 1994**).

Bulk density is the weight per volume of growing media and is inversely proportional to porosity (**HANDRECK & BLACK, 1994; JANENICKE, 1999; JACOBS *et al*., 2009**). Bulk density gives an indication of the weight of the substrate. It is recommended that a lighter medium is used for container propagation. Generally substrates with a higher bulk density increases compaction, particularly when it has been over watered. Compaction results when the particles of the substrate are pressed together reducing the pore spaces. Compacted substrates affect seedling growth by decreased infiltration of water and air resulting in a decreased nutrient, water and oxygen supply. Coupled to this, the increased soil strength restricts root growth due to decreased pore spaces and increased physical resistance to the root tip. More energy is thus directed away from above-ground plant structures resulting in

stunted plants with small root systems (**HANDRECK & BLACK, 1994; HARRISON *et al*., 1994; THOMAS *et al*., 2008; DINI-PAPANASTASI *et al*., 2012**).

Cation exchange capacity (CEC) refers to the ability of the substrate to store nutrients. Cation exchange capacity is measured in milliequivalents and is the sum of the ions that can be exchanged with the root system. A high cation exchange capacity means that the substrate can retain more nutrients. Substrates with high cation exchange capacities are recommended in container nurseries because it decreases leaching due to high irrigation requirements and acts as a buffer against sudden changes in pH. Inorganic materials tend to have low cation exchange capacities whilst organic materials such as bark have high cation exchange capacities (**LANDIS**, **2007**; **JACOBS *et al*., 2009**).

The pH of the medium is the measure of relative acidity or alkalinity and should be between 5.5 and 6.5. The pH has an effect on the solubility of nutrients in the substrate and extreme pH may result in nutrient deficiencies. The type and abundance of microorganisms in the susbtrate are also affected by the pH. Very alkaline media encourage the growth of fungal pathogens such as *Fusarium* (**LANDIS, 2007; JACOBS *et al*., 2009**).

2.5.3. Irrigation

2.5.3.1. Water quality

Knowledge of irrigation water quality is an important aspect of seedling cultivation (**HARTMANN *et al*., 1997; JAENICKE, 1999**). Water quality can have an influence on seedling quality (**JAENICKE, 1999)** and it is recommended that the irrigation water is tested on a regular basis since water quality can change depending on season and weather conditions (**HARTMANN *et al*., 1997**).

A standard water quality test measures pH, electrical conductivity (EC), macro- and micro-elements, and microbial activity. The pH and conductivity of the irrigation water should be monitored at the nursery on a daily basis and adjusted as required. As mentioned previously the pH of the water affects the availability of nutrients and microbial activity in the substrate. High pH levels may indicate high levels of sodium or calcium which may be harmful to seedling growth. A lower pH level will prevent scale deposits in the irrigation

pipes (**HARTMANN *et al*., 1997**). EC measures the total dissolved salts (TDS) in solution but will not indicate which salts are present in solution (**HANDRECK & BLACK, 1994; ROSHETKO *et al*., 2010**). Less fertilizer should be added to water with high conductivity measurements and the irrigation frequency should be increased to prevent salt build-up. Dissolved carbonates, bicarbonates and hydroxides are measured by alkalinity (**HARTMANN *et al*., 1997)**. High alkalinity may cause nutrient imbalances and high salinity levels (**ROSHETKO *et al*., 2010**). Sodium absorption ratio (SAR) is used to determine the sodium-to-calcium-to-magnesium ratio and gives an indication of sodium content. Water with a high ratio of sodium-to-calcium-to-magnesium is not suitable for irrigation purposes. The high levels of sodium disrupt nutrient absorption. As plants dry out after irrigation the salt content builds up around the roots. This increases the osmotic pressure and may induce calcium and potassium deficiencies (**LANTZKE, 2004**). Plants irrigated with highly saline water are stunted, have burnt leaf edges and tips and often shows signs of wilting even when the plants are moist (**HANDRECK & BLACK, 1994; LANTZKE, 2004**). High concentrations of chlorides and sodium ions may also result in toxicities. The chloride ions accumulate in the leaves causing premature leaf yellowing and tip burn (**HARTMANN *et al*., 1997; LANTZKE, 2004; ROSHETKO *et al*., 2010**) and in severe cases leaf abscission which in turn disrupts photosynthesis (**EL-JUHANY *et al*., 2008**).

2.5.3.2. Irrigation of seedlings in the nursery

The time and frequency of irrigation is critical in seedling cultivation, correct irrigation regimes improves plant growth, quality and conserves water (**MATHERS *et al*., 2005**). To manage watering effectively, plants with similar watering requirements should be grouped together. The amount of water required is usually determined by the amount of water lost since the last irrigation. Determining when and how much to irrigate depends on the climate, plant species and seedling age (**LANDIS, 1989; HANDRECK & BLACK, 1994; MATHERS *et al*., 2005**). It is recommended to irrigate pre-dawn to decrease water loss due to evaporation and improve irrigation uniformity since generally there are fewer winds. Leaves will also have an opportunity to dry out after irrigation, preventing the development of foliar diseases (**MATHERS *et al*., 2005**).

Newly germinated seedlings require less water since water is mostly lost through evaporation and to replenish this, shorter more frequent irrigations are necessary. However, these seedlings need to be monitored closely as short irrigation periods may cause saturation at the top of the container and result in the development of dry areas at the bottom of the container. Consequently, roots only develop in the top of the container and will not fully consolidate the root plug (**LANDIS, 1989; HANDRECK & BLACK, 1994**). The irrigation schedule should be adjusted as the plants grow. Older seedlings begin to lose water through transpiration and not evaporation, increasing the water requirements. Older seedlings are usually irrigated less frequently but for longer periods. Over-irrigating seedlings may flood or wash out the media and/or seeds and may cause the development of anaerobic conditions in the root plug which are ideal for root borne diseases and poor quality root development (**LANDIS, 1989; HANDRECK & BLACK, 1994; HARTMANN *et al*., 1997**).

2.5.4. Nutrient supply

Seedling growth, development and seedling quality is influenced by nutrient availability. To ensure production of high quality seedlings with optimal nutrient levels guaranteeing field survival and growth, mineral nutrients should be supplied in balanced proportions at optimal concentrations (**LANDIS *et al*., 2005; HAWKINS, 2011**). There are 16 essential nutrients that the plant requires for growth. An essential element has been defined as an element that is either part of a molecule important to the plant structure and thus its metabolic activity and/or if the element is deficient in the plant it will impact normal plant growth and development (**LANDIS *et al*., 2005; HAWKINS, 2011**). These 16 elements are divided into micro- and macro-nutrients, of these 16 elements, carbon, hydrogen and oxygen are found in water and carbon dioxide. The remaining 13 are referred to as mineral elements found in the soil (**LANDIS *et al*., 2005**). Macro-nutrients are elements that are required by the plant in relatively high concentrations and consist of nitrogen, potassium, calcium, magnesium, phosphorous and sulphur. Macro-nutrients are often part of cellular constituents, with the exception of phosphorous, and are involved in physiological processes (**LANDIS *et al*., 2005**) (TABLE 5). A micro-nutrient refers to an element that is only required by the plant in small (trace) concentrations. The micro-nutrients are chlorine, iron, manganese, boron, zinc, copper, molybdenum, nickel, colbalt and sodium. These nutrients

are generally involved in metabolic processes such as redox reactions and physiological processes including respiration and photosynthesis (**LANDIS *et al*., 2005; FAROOQ *et al*., 2012**) (TABLE 2.5).

Seedling growth is influenced by nutrient concentration, a low nutrient content decreases plant growth and when the nutrient content increases plant growth increases. At optimal or critical nutrient concentration plant growth is at its maximum and increasing the nutrient concentration at levels greater than the optimal or critical levels will have no impact on seedling growth. The excess nutrients will begin to accumulate in the plant tissues and further accumulation of nutrients will become toxic to the plant and result in decreased plant growth (**DELL *et al*., 1982; LANDIS *et al*., 2005; HAWKINS, 2011**).

Plant tissues with low levels of nutrients will show signs of nutrient deficiencies. Symptoms of nutrient deficiencies for different elements are observed in different plant organs which is dependent on element mobility. Mobile elements are elements that are translocated within the xylem and phloem easily. Symptoms of nutrient deficiencies caused by these elements will be observed in older leaves because these elements are concentrated in actively growing younger leaves. Elements not easily translocated within the xylem and phloem are referred to as immobile elements. Symptoms of nutrient deficiencies are often observed in younger leaf tissues as these elements are concentrated in older foliage (**DELL *et al*., 1982; LANDIS *et al*., 2005; HAWKINS, 2011**). Variable immobile elements are elements that only move from the leaves under certain conditions (**DELL *et al*., 1982**).

Nutrient deficiencies are observed in various forms but the five most common signs of plant nutrient deficiencies are leaf chlorosis (yellowing of the leaf tissues), leaf necrosis, leaf reddening, deformation of leaves (such as irregularly shaped leaf margins and leaf curling) abnormally small leaves and shoot die back (**DELL *et al*., 1982**). As the severity of the nutrient deficiency increases the symptoms will spread to other parts of the plant depending on element mobility within the xylem and phloem (**DELL *et al*., 1982**).

TABLE 2.5. Macro- and micro-elements required by plants for optimal seedling growth.

Element	**Concentration range**	**Mobility**	**Function**	**Symptoms of deficiency**	**Reference**
Nitrogen (N)	0.5-6%	Mobile	Found in all amino acids, proteins, nucleic acids, nucleotides and polyamines.	Pale green to yellow in the interveinal areas. Initially observed in mature leaves. Severe cases necrotic spot appear (purple to white in the center).	**DELL *et al*., 1982; HAWKINS**, **2011**
Potassium (K)	0.8-8%	Mobile	Activates enzymes and cellular osmoticum.	Necrosis and scorching seen in the interveinal areas midway between the midrib and leaf margin, leaf tip curls.	**DELL *et al*., 1982; HAWKINS**, **2011**
Calcium (Ca)	0.51-6%	Immobile	Cell wall support, signalling and regulating enzymes.	Scorching of the young expanding leaf tips and margins. Young leaves buckle or become sickle shaped.	**DELL *et al*., 1982; HAWKINS**, **2011**

Element	Concentration range	Mobility	Function	Symptoms of deficiency	Reference
Magnesium (Mg)	0.05-1%	Variable mobility	Forms part of chlorophyll and activates enzymes.	Green tongue form along the main vein of young expanding leaves flanked by yellow and may become purple. Development of beaked leaves.	**DELL *et al*., 1982; HAWKINS, 2011**
Phosphorous (P)	0.15-5%	Highly mobile	Plays a key role in nucleotides and nucleic acid, energy metabolism, adenosine phosphate (AMP, ADP and ATP).	Purple blotches develop in the interveinal areas of mature leaves. Stunted growth and purple foliage occurs.	**DELL *et al*., 1982; HAWKINS, 2011**
Sulphur (S)	0.1-1.5%	Variable mobility	Forms part of amino acids, proteins and co-enzymes.	Pale green to yellow in the interveinal areas of expanding leaves.	**DELL *et al*., 1982; HAWKINS, 2011**
Chlorine (Cl)	10-80 000ppm		Essential in photosynthesis because it is part of the water splitting complex in photosystem II.		**HAWKINS, 2011**

Element	**Concentration range**	**Mobility**	**Function**	**Symptoms of deficiency**	**Reference**
Iron (Fe)	20-600ppm	Immobile	Enzyme activation, forms part of haemoproteins and Fe-S proteins. Essential for chlorophyll synthesis.	Pale green chlorosis develops in the interveinal areas of expanding leaves.	**DELL *et al*., 1982; HAWKINS, 2011**
Manganese (Mn)	10-600ppm	Immobile	Nitrogen metabolism and part of the water splitting complex in photosystem II in photosynthesis, activates enzymes and found in superoxide dismutase.	Margins of both expanding and mature leaves are pale green and eventually turn yellow. Terminal buds may die.	**HAWKINS, 2011; FAROOQ *et al.,* 2012**
Boron (B)	0.2-800ppm	Immobile	Metabolism of nucleic acid, carbohydrates, proteins, auxins, cell division, cell growth, cell wall synthesis, membrane integrity and metabolism, associated with Ca use.	Limits shoot growth, purple pigments or chlorosis accumulates along the margins. Leaf rolling and shoot die back.	**DELL *et al*., 1982; HAWKINS, 2011; FAROOQ *et al.,* 2012**

Element	**Concentration range**	**Mobility**	**Function**	**Symptoms of deficiency**	**Reference**
Zinc (Zn)	10-250ppm	Immobile	Activates enzymes and is found in metalloenzymes.	Poor growth, small brown spots on leaves, profuse growth at shoot tips and interveinal leaf chlorosis, purple areas may appear.	**DELL *et al*., 1982; HAWKINS, 2011; FAROOQ *et al.,* 2012**
Copper (Cu)	2-50ppm	Immobile	Essential for photosynthesis, carbon assimilation, nitrogen metabolism, lignin biosynthesis increasing cell strength, found in metalloenzymes.	Growth retardation, leaf chlorosis, stunted growth and stem die-back. Development of cupped, twisted leaves and irregular margins.	**DELL *et al*., 1982; HAWKINS, 2011; FAROOQ *et al.,* 2012**
Molybdenum (Mo)	0.1-10ppm	Immobile	Nitrogen assimilation, found in nitrogenase and nitrate reductase.	Similar to nitrogen deficiency but appear in expanding leaves.	**DELL *et al*., 1982; HAWKINS, 2011; FAROOQ *et al.,* 2012**
Nickel (Ni)	0.05-5ppm		Found in urease which is associated in the conversion of urea to ammonium.		**HAWKINS, 2011**

Element	Concentration range	Mobility	Function	Symptoms of deficiency	Reference
Cobalt (Co)	0.01ppm		Component of co-enzymes and enzymes, metabolism and growth, inhibits ethylene synthesis, slows down leaf senescence, stimulates alkaloid biosynthesis.		**HAWKINS, 2011**

2.5.5. Supplements

2.5.5.1. Smoke-water

Burning has traditionally been used to remove excess slash from forestry land and agricultural fields in preparation for planting (**MANDAL *et al*., 2004; KULKARNI *et al*., 2008; LONG *et al*., 2011**). Burning is an inexpensive and quick method of removing waste from the land and the remaining ash improves soil nutrient content. Burning acts as a seed germination stimulant and improves seedling vigour which improves crop yields (**KULKARNI *et al*., 2008**). However, burning agricultural land destroys beneficial soil microbes, insects and causes air pollution (**MANDAL *et al*., 2004**).

The potential benefits of smoke have been captured through the use of smoke-extracts (**KULKARNI *et al*., 2008**). Smoke is generated by burning wood, straw or other plant materials and bubbling the smoke through water (**KULKARNI *et al*., 2011**)**. CHUMPOOKAM *et al*.** (**2012**) was able to generate smoke-water by burning dry rice straw. Compounds found in smoke dissolve easily in water, resulting in a smoke-water solution. The smoke-water solution is diluted and can be applied to seedlings as a drench or foliar spray. Alternatively, the active compounds in smoke-water can be isolated and dissolved in solution. This solution may then be applied to the seedlings as a drench or foliar spray. **AREMU *et al*.** (**2012**) showed that smoke-water applied as a soil drench at a dilution of 1:500 (v/v) was more effective on seedling growth than using a foliar spray. These results were supported by research conducted by **KULKARNI *et al*.** (**2007**) which showed that all growth parameters, except the root weight, increased in plants treated with smoke-water.

Extensive research has been conducted on smoke-water properties and its use. Smoke-water is reported to improve seed germination, stimulate seedling vigour, increase seedling growth, increase root biomass and improve shoot-to-root ratios (**KULKARNI *et al*., 2007; KULKARNI *et al*., 2011; AREMU *et al*., 2012; ZHOU *et al*., 2013**). Increases in germination, seedling growth and seedling vigour have been observed in more than 1200 species (**BROWN & BOTHA, 2004**). A butenolide compound called karrikinolide and a family of plant growth regulators (termed karrikins) (**FLEMATTI *et al*., 2004**) were isolated from smoke-water. These compounds are thought to be responsible for the improved seed germination

and seedling growth (**VAN STADEN *et al*., 2004**). **CHIWOCHA *et al*.** (**2009**) suggested that karrikins increases gibberellic acid (GA) and decreases abscisic acid (ABA) levels in the seed. GA releases seed dormancy whilst ABA prolongs it. Furthermore, smoke-water has been reported to exhibit antimicrobial properties which reduce endophytic fungi (**KULKARNI *et al*., 2011**). Other compounds such as ethylene, nitrogen oxides and glyceronitrile have also been isolated from smoke-water and contribute to improved seedling germination (**KULKARNI *et al*., 2011**).

Many benefits of smoke-water application in the horticultural industry have been reported. It has potential uses in the agricultural industry as an organic fertiliser or supplement. Smoke-water can be used to scarify or prime seeds before sowing. The antibacterial properties make smoke-water an attractive option in enhancing plant resistance to disease. Smoke-water may be used to enhance root development and seedling growth. However, much research is still required to appreciate its possible application in the agricultural and horticultural sectors (**KULKARNI *et al*., 2011**).

2.5.5.2. Seaweed extract

Seaweed concentrates have become increasingly popular in the agricultural and horticultural industries for their growth promoting properties (**ALDWORTH & VAN STADEN, 1987; PISE & SABALE; 2010; AHMEND & SHALABY, 2012**). They can be used as a substitute for inorganic fertilisers which pollute the environment (**SRIDHAR & RENGASAMY, 2010; AHMED & SHALABY, 2012**). The macro- and micro-nutrients, auxins, cytokinins and amino acids present in these extracts have been reported to promote seedling growth, stimulate root initiation and enhance seed germination (**CHALLEN & HEMINGWAY, 1965; ALDWORTH & VAN STADEN, 1987; PISE & SABALE; 2010; AHMED & SHALABY, 2012; EL KAOAUA *et al*., 2013; PAPENFUS *et al*., 2013**). **ALDWORTH & VAN STADEN** (**1987**) showed improved root development and decreased transplant shock in cabbage seedlings that were dipped in a 1:500 (v/v) concentration of Kelpak® (a seaweed extract). These authors also demonstrated that marigold seedlings treated with Kelpak® were healthier and more robust than the control treatments. Other research has shown that the use of seaweed concentrates increase chlorophyll, carbohydrate, protein and lipid content in crop seedlings (**SRIDHAR & RENGASAMY, 2010).** Application of seaweed extracts result in healthier plants resistant to

environmental stresses and disease (**SRIDHAR & RENGASAMY, 2010; EL KAOAUA *et al*., 2013**). This was demonstrated when drought stress symptoms were reduced in *Salvia officinalis* seedlings treated with a seaweed extract (**EL KAOAUA *et al*., 2013**).

2.5.5.3. Vermicompost

Vermicompost is produced through the biological degradation of organic waste products using the beneficial interactive relationship between microorganisms and earthworms (**BACHMAN & METZGER, 2007; KANDARI *et al*., 2011b; RESÉNDEZ *et al*., 2012; ABDULI *et al*., 2013; POOR & RAFIEI, 2013; POUR *et al*., 2013**). The resulting earthworm castings and leachate are rich in nutrients, humic acid, fulvic acid, microbes and plant hormone-like compounds (**NAGAVALLEMMA *et al*., 2006; BACHMAN & METZGER, 2007; RESÉNDEZ *et al*., 2012; ABDULI *et al*., 2013; POOR & RAFIEI, 2013; POUR *et al*., 2013**). Vermicompost may be produced using different waste products including animal manure, paper waste, sugar cane waste, cotton residue, kitchen and municipal wastes (**THEUNISSEN *et al*., 2010; POOR & RAFIEI, 2013**). However, the biological and chemical composition of vermicompost may differ depending on the origin of the composted organic waste (**BACHMAN & METZGER, 2007; THEUNISSEN *et al*., 2010)**.

Vermicompost is beneficial to the horticultural industry as it has been reported to improve seed germination, increase plant growth and stimulate root development (**BACHMAN & METZGER, 2007; RESÉNDEZ *et al*., 2012; POOR & RAFIEI, 2013**). Vermicompost has a high nutrient content which is easily accessible to plants for absorption (**BACHMAN & METZGER, 2007; POOR & RAFIEI, 2013)**. Humic and fulvic acid present in vermicompost stimulates root growth which improves nutrient uptake (**ALVAREZ & GRIGERA, 2005; PANT *et al*., 2009; THEUNISSEN *et al*., 2010**). **POUR *et al*.** (**2013**) demonstrated that by incorporating vermicompost into potting soil the zinc and auxin concentrations in cabbage seedlings increased. The authors found that soil mixtures with lower concentrations of vermicompost increased the mean number of leaves which was positively correlated with auxin and zinc concentration. These authors postulated that the improved nutrient status was due to the humic acid content in the vermicompost. Microbial activity in vermicompost has also been reported to increase plant resistance to pathogens and enhance nutrient uptake though microbe interactions (**ALVAREZ & GRIGERA, 2005**).

The high nutrient content of vermicompost makes the castings and leachate useful as an organic fertiliser (**KANDARI *et al*., 2011b**). The castings may be incorporated into the substrate as a substrate component. Vermicompost has been reported to improve substrate drainage, water holding capacity, air-filled porosity, CEC and increase microbial activity (**BACHMAN & METZGER, 2007; RESÉNDEZ *et al*., 2012**). When the leachate is used as an organic fertiliser it can be applied to the plants as a foliar spray or soil drench (**AREMU *et al*., 2012**). **AREMU *et al*.** (**2012**) showed that a vermicompost leachate drench was more effective than a foliar spray. Banana plants were able to continuously absorb the leachate solution over a longer period compared to foliar spraying which contributed to improved plant growth. In a study on different *Eucalypt* species, it was observed that vermicompost leachate can be used as an alternative fertiliser for tree seedling propagation. However, different species responded differently to different concentrations of vermicompost leachate suggesting that vermicompost application needs to be optimised for each species (**KANDARI *et al*., 2011b**).

2.5.5.4. Mycorrhizae

Mycorrhiza refers to the mutually beneficial relationship between soil borne fungi and higher plants. These fungi are split into two groups characterised by their structure (**CASTELLANO & MOLINA, 1989; TURK *et al*., 2006**). Ectomycorrhizae form a sheath around the outside of the plant's root system. A Hartig net is developed within the epidermal and cortex cells where nutrients and water are exchanged. The vesicular-arbuscular mycorrhizae are classified as endomycorrhizae which are invisible to the naked eye (**CASTELLANO & MOLINA, 1989; MISBAHUZZAMAN & INGLEBY, 2005; TURK *et al*., 2006**). The vesicular-arbuscular mycorrhizae consist of vesicles and arbuscules. The arbuscules are fine branches that are found inside the cells and facilitate nutrient exchange between the plant host and the fungi. Balloon shaped structures called vesicles, often found outside of the root cells, are utilised for energy storage and reproduction (**TURK *et al*., 2006**). *Eucalyptus* species have been reported to form both ecto- and endomycorrhizae associations (**MISBAHUZZAMAN & NEWTON, 2006**).

Mycorrhizae associations can form in container-grown seedlings through artificial inoculation as soilless substrates do not naturally contain mycorrhizae. Ectomycorrhizae can also be inoculated naturally by wind dispersion. Vesicular-arbuscular mycorrhizae however, are not dispersed by wind, rather by small animals and insects and therefore, container-grown seedlings cannot form natural vesicular-arbuscular mycorrhizae associations (**CASTELLANO & MOLINA, 1989**).

Mycorrhizae have many known benefits to plants. Mycorrhizae provide protection against pathogens, reduce root respiration, increase root longevity, decreases transplant stock, increase salt and drought tolerance and improve water and nutrient absorption. Water and nutrient uptake is enhanced by increasing the surface area of the root system. Fungal hyphae extend the exploratory capabilities of the plant's root system and stimulate the production of plant growth regulators which promote root growth. The plants are protected against pathogens because the mycorrhizae form a protective barrier around the root system thereby preventing entry of harmful microorganisms. In addition, antibiotics produced by mycorrhizae eliminate pathogens and improve disease resistance (**CASTELLANO & MOLINA, 1989**). **DIXON** (**1986**) showed that mycorrhizae increased shoot height, root collar diameter and plant survival and in wheat, plant growth increased when they were treated with mycorrhizae (**AL-KARAKI, 1998**).

CHAPTER 3

Consequences of the mini-plug container design and seedling age at transplanting

3.1. INTRODUCTION

Seedling growth, development and cultivation time are often influenced by container design, shape, volume and planting density (**HANDRECK & BLACK, 1994; AMOROSO *et al*., 2010; KOSTOPOULOU *et al*., 2011;**). Cultivation of seedlings in containers is often the cause of poor root structure and poorly proportioned seedlings which may be detrimental to seedling growth after transplanting (**TSAKALDIMI *et al*., 2009**). Usually, the smaller the container, the greater the negative effects on plant growth (**NESMITH & DUVAL, 1998**). The aims of this study were: (1) to evaluate the influence that plastic and polystyrene mini-plug trays have on root growth, root physiology and the development of the mini-plug transplants (seedlings), and (2) to determine the optimal transplanting age of the seedlings to prevent any root deformation as a result of extended cultivation periods in the mini-plug container and/or the transplanting process itself. The mini-plug tray which shows the least detrimental effect on survival, growth and root plug quality of seedlings in the nursery will be recommended as the mini-plug container for use in the subsequent transplanting process.

3.2. MATERIALS AND METHODS

3.2.1. Seed germination

Eucalyptus dunnii seed, stock number M9671, supplied by Mountain Home Nursery was sown into two types of mini-plug containers: (1) a mini-plug 288 (MP288) cavity plastic container with a plug volume of 5-mL and (2) a mini-plug 544 (MP544) cavity polystyrene tray with a volume of 4.5-mL filled with 6-mm pine bark (TABLE 3.1) supplied by Organics for Africa, Pietermaritzburg, South Africa. The polystyrene MP544 tray was coated in copper oxychloride (according to the manufacturer's instructions) to prevent the roots from growing into the side walls of the container. The MP288 containers were not dipped in copper oxychloride because the copper oxychloride solution did not adhere to the walls of the plastic containers. In order to evaluate seedling age when the mini-plugs were

transplanted to the final container, the seed was sown at one month intervals over a period of four months. These seedling containers were placed into a germination chamber with a temperature of 24 °C and a relative humidity of 100 % for two days in the dark to initiate germination. Thereafter, the seedling containers were moved into a semi-controlled plastic-covered tunnel where the temperature was maintained between 15 and 25 °C. The seedling trays were irrigated twice daily for 5-minutes and fertilised twice weekly with a solution of Natgro (Omnia®) at an EC of 1.2 mS, and once every second week with Xtracal (Omnia®). Fertilisation only commenced when the seedlings had four leaves and two cotyledons. The germinated seedlings from each of the four age classes (one-month-old, two-month-old, three-month-old and four-month-old) were transplanted at the same time by gently pulling the mini-plug seedlings out of their containers and transferring them to either Unigro 128® trays to determine the effect of the mini-plug container type and seedling age on seedling quality and growth or to polyethylene bags for the root growth potential test to evaluate the treatment effects on the initiation of new roots.

TABLE 3.1. The specifications of the two mini-plug containers tested.

Mini-plug container	Manufacturing material	Number of cavities per tray	Plant density per m^2	Cavity volume (mL)	Cavity depth (mm)
MP288	Plastic	288	2090	5.0	25
MP544	Polystyrene	544	2220	4.5	30

3.2.2. Transplanting seedlings from the mini-plug container to Unigro® 128 trays

Eucalyptus dunnii seedlings from each treatment were transplanted to Unigro® 128 cavity containers (65 mL) containing a coir (imported from Sri Lanka, supplied by CPS Nursery in Greytown, South Africa) and 6-mm pine bark mix in a ratio of 1:3 (v/v). All treatments were repeated four times where one container was considered one replicate with 32 seedlings transplanted per treatment per replication. After transplanting, the seedlings remained in the plastic-covered tunnel where they were irrigated on a daily basis for ten minutes and fertilised using a solution of Natgro with an EC of 1.2 mS twice weekly.

3.2.3. Influence of the mini-plug container and seedling age on new root initiation

The effect of mini-plug containers and seedling age on the ability to initiate new root growth was evaluated using the root growth potential test. Twenty randomly selected, healthy seedlings from the MP288 and MP544 containers were transplanted into 5-L polyethylene bags containing a mixture of perlite and vermiculite in a ratio of 1:1 (v/v) (FIGURE 3.1) and placed in a semi-controlled plastic-covered tunnel for 28 days where the temperature was regulated to between 15 °C and 28 °C. The seedlings were watered daily with 200-mL tap water and not fertilised. After 28 days, number of new roots and the new root dry mass were measured. The new roots were defined as all roots longer than 1-mm protruding from the original root plug (FIGURE 3.1). These roots were excised from the original mini-plug, dried at 70 °C for two days and weighed using an analytical balance to determine the new root dry mass.

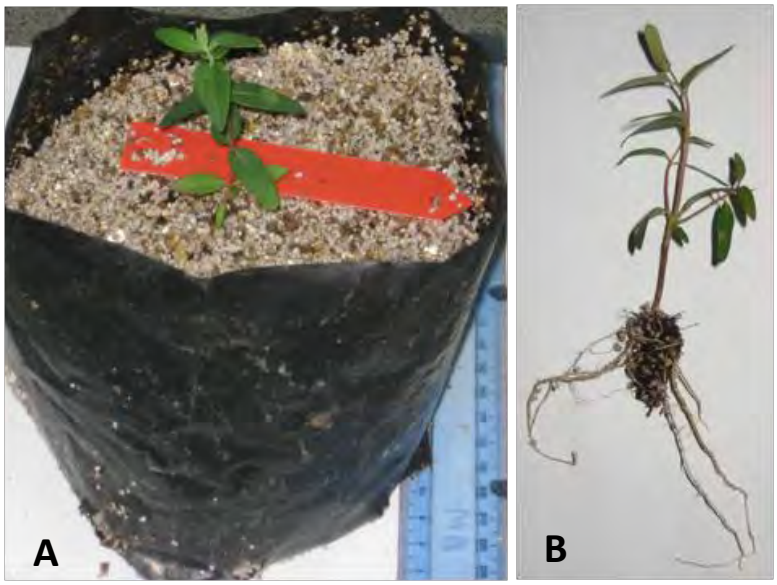

FIGURE 3.1. Mini-plug seedlings transplanted to polyethylene bags (A) for the root growth potential test to evaluate the number of new roots initiated (B) 28 days after transplanting.

3.2.4. The effects of seedling age and mini-plug container on the seedling characteristics, quality and growth

To determine the effect of the mini-plug container type and mini-plug seedling age; shoot height, root collar diameter, root length, dry shoot and root mass of 20 randomly selected seedlings per treatment were recorded prior to transplanting and once a month for 5-months thereafter. Shoot height was determined by measuring the distance between the base of the stem and the bottom of the auxiliary bud using a stainless steel ruler (FIGURE 3.2). Root collar diameter was measured using a digital vernier calliper (FIGURE 3.2). In order to assess the root system, growth media was removed from the roots and rinsed gently in tap water. The root length was determined by measuring the length of the roots from the base of the shoot to the root tip. The dry shoot and root mass measured by separating the shoots from the roots and drying the plant material for two days at 70 °C. The sturdiness ratio of each seedling was determined by dividing the shoot height (mm) by the root collar diameter (mm) and the shoot-to-root ratios calculated by dividing dry shoot mass (mg) by dry root mass (mg). The total dry mass of the seedlings was calculated once a month after transplanting and used to plot a growth curve to determine how the mini-plug and seedling age influenced growth.

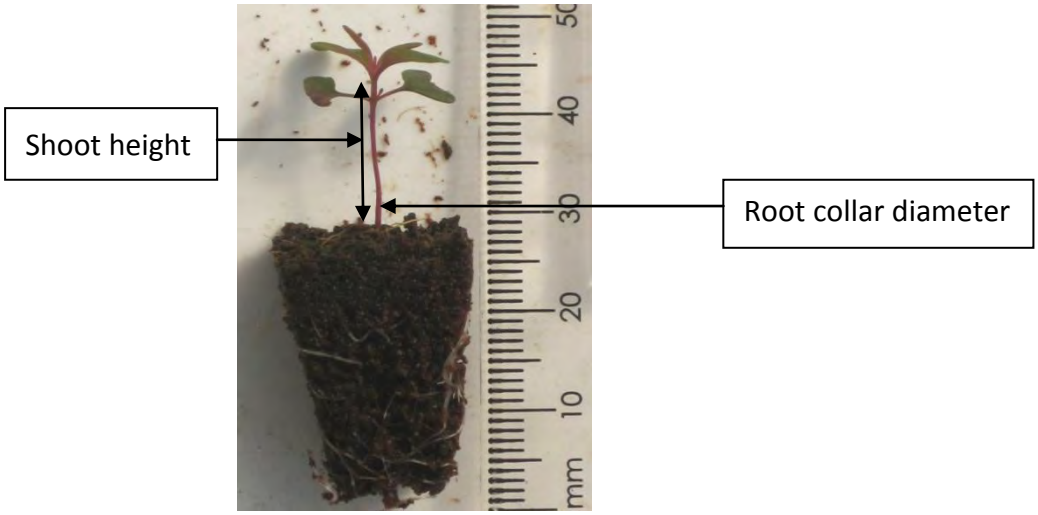

FIGURE 3.2. Shoot height and root collar diameter was measured on 20 randomly selected seedlings from each treatment.

3.2.5. Influence of mini-plug container and seedling age on root architecture

The presence of J-roots and root coiling (FIGURE 3.3) of 20 randomly selected seedlings per treatment (five per replicate) was assessed using a modified J-rooting assessment procedure outlined by **THOMAS *et al.*** (**2008**)**.** The severity of J-rooting was rated on a scale of zero to five, where zero referred to no J-roots, and five to very severe J-rooting (TABLE 3.2). A similar assessment was used to evaluate the incidence of root coiling in the root plug. The root architecture was assessed at the time of transplanting (0 months) and then once a month for five months following transplanting to the final container.

FIGURE 3.3. An example of root coiling (A) and J-rooting (B) in container-grown seedlings.

TABLE 3.2. The J-root and root coiling scoring system used to evaluate the root architecture (**THOMAS *et al*., 2008**).

Criteria for root coiling	Criteria for J-roots	Score
No coiling present	No J-roots present	0
Just beginning to coil	Deflected less than 30° from vertical	1
Half a coil	Bent 30° and 70° from the vertical	2
A full coil	Bent 70° and 90° from the vertical	3
One-and-a-half to two full coils	Greater than 90°	4
More than two coils	Greater than 90° with a U-shape	5

3.3. RESULTS

3.3.1. The effects of seedling age and mini-plug container on seedling quality

Prior to transplanting, seedlings grown in MP544 containers exhibited greater sturdiness ratios than the MP288 seedlings within the same age class, although not significant ($p<0.05$), with the exception of three-month-old seedlings. The sturdiness ratio of four-month-old seedlings prior to transplanting for MP288 and MP544 seedlings was 52.79 and 53.13 respectively, greater than all treatments evaluated (TABLE 3.3). One month after transplanting MP288-grown seedlings transplanted one and three months after sowing exhibited higher ($p<0.05$) sturdiness ratios compared the remaining treatments. The reason for this increase in sturdiness is unclear. Seedlings transplanted from MP288 containers were found to have higher sturdiness ratios two months after transplanting, compared to MP544-reared seedlings with the exception of the two-month-old treatments. Three and four months after transplanting, the opposite was seen, where the sturdiness ratios were higher in MP544-reared seedlings. However, no significant differences were observed three months after transplanting and four months after transplanting only the two-month-old seedlings grown in MP288 containers exhibited significantly a lower sturdiness ratio. Five months after transplanting, the sturdiness ratios of four-month-old seedlings transplanted four months after sowing, was higher than one-, two- and three-month-old seedlings five months after transplanting (TABLE 3.3).

TABLE 3.3. Sturdiness ratios of seedlings cultivated in MP544 and MP288 mini-plug containers for one-, two-, three and four-months after sowing. Different letters represent significantly different means (±S.D.) at ($p<0.05$).

Seedling age		One-month-old		Two-months-old		Three-months-old		Four-months-old		LSD
Mini-plug		288	544	288	544	288	544	288	544	
Time after transplanting (months)	0	38.66± 4.40^{c}	40.25± 14.19^{bc}	36.91± 6.33^{c}	40.19± 5.75^{bc}	35.58± 6.62^{c}	31.67± 6.52^{c}	52.79± 17.57^{ab}	53.13± 12.48^{a}	54.40
	1	117.64± 16.93^{a}	68.26± 27.05^{b}	93.79± 15.45^{ab}	72.53± 13.88^{b}	109.41± 21.32^{a}	69.06± 36.86^{b}	89.10± 16.50^{ab}	77.26± 29.51^{b}	77.26
	2	49.34± 15.84^{ab}	34.95± 13.51^{b}	44.61± 8.83^{ab}	50.24± 8.13^{ab}	63.29± 12.75^{a}	50.80± 15.68^{ab}	59.25± 11.33^{a}	57.41± 16.13^{a}	57.41
	3	67.14± 25.71^{a}	76.10± 13.87^{a}	74.39± 13.62^{a}	78.57± 9.79^{a}	72.95± 20.56^{a}	83.90± 24.39^{a}	73.54± 20.55^{a}	84.43± 26.49^{a}	84.43
	4	82.51± 17.43^{ab}	92.03± 20.81^{a}	62.86± 17.61^{b}	92.03± 20.95^{a}	67.94± 25.32^{ab}	84.32± 15.53^{ab}	87.57± 14.41^{ab}	92.37± 22.06^{a}	92.37
	5	60.09± 10.87^{bc}	52.54± 5.28^{cd}	54.53± 10.59^{bcd}	46.87± 13.90^{d}	59.36± 8.94^{bc}	57.53± 7.72^{bcd}	64.98± 6.96^{ab}	72.45± 10.18^{a}	72.45

Prior to transplanting (0 months), seedlings cultivated from MP288 mini-plug containers resulted in seedlings with lower shoot-to-root ratios ($p>0.05$) than MP544-reared seedlings within each age class. Seedlings grown in MP288 mini-plug containers for two months exhibited the lowest overall shoot-to-root proportions prior to transplanting ($p>0.05$). In contrast, two-month-old seedlings grown in MP544 containers exhibited the highest overall shoot-to-root ratio prior to transplanting ($p>0.05$). The differences in shoot-to-root ratios, however, were not significant and differences were only significant three months after transplanting. Three months after transplanting, the shoot-to-root ratio of one-month-old MP544-cultivated seedlings was significantly higher ($p<0.05$) than both three- and four-month-old MP288-grown seedlings (TABLE 3.4). Further to this, seedlings initially grown in MP544 containers exhibited higher ($p>0.05$) shoot-to-root ratios than seedlings grown in the MP288 containers three months after transplanting. After four months, an interaction

between tray type and seedling age was found, where one- and two-month-old seedlings transplanted from MP288 mini-plug containers had better (i.e. lower) shoot-to-root ratios than seedlings transplanted at three- and four-months-old. In MP544 containers, three- and four-month-old seedlings exhibited significantly better shoot-to-root ratios (i.e. close to a ratio of one thus indicating a more balanced seedling (**RICHIE AND LANDIS, 2010**) than one-month-old seedlings. The effects of container type and seedling age on shoot-to-root ratios, was not observed five months after transplanting (TABLE 3.4).

TABLE 3.4. Shoot-to-root ratios (w/w) of seedlings grown in MP544 and MP288 mini-plug containers for one-, two-, three- and four-months. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Seedling age		**One-month-old**		**Two-months-old**		**Three-months-old**		**Four-months-old**		**LSD**
Mini-plug		**288**	**544**	**288**	**544**	**288**	**544**	**288**	**544**	
Time after transplanting (months)	0	2.54± 1.00^{a}	3.43± 1.08^{a}	1.98± 0.65^{a}	3.47± 1.89^{a}	2.58± 1.29^{a}	2.67± 0.77^{a}	2.95± 1.04^{a}	3.13± 0.52^{a}	3.13
	1	2.60± 0.69^{a}	2.12± 0.67^{a}	2.79± 0.89^{a}	2.47± 1.37^{a}	3.08± 1.20^{a}	2.50± 0.69^{a}	3.08± 1.02^{a}	2.88± 1.16^{a}	2.88
	2	1.64± 0.25^{a}	1.35± 0.35^{a}	1.28± 0.28^{a}	1.52± 0.33^{a}	1.39± 0.42^{a}	1.36± 0.29^{a}	1.98± 2.54^{a}	1.54± 0.58^{a}	1.54
	3	2.15± 0.52^{abc}	2.56± 0.44^{a}	1.82± 0.61^{abc}	2.49± 0.98^{ab}	1.60± 0.44^{bc}	2.30± 0.53^{abc}	1.56± 0.36^{c}	2.32± 0.73^{abc}	2.32
	4	1.55± 0.43^{b}	2.28± 0.45^{a}	1.43± 0.32^{b}	1.71± 0.50^{ab}	1.82± 0.48^{ab}	1.57± 0.34^{b}	1.61± 0.38^{b}	1.62± 0.40^{b}	1.62
	5	1.81± 0.46^{a}	1.95± 0.84^{a}	1.95± 0.49^{a}	1.80± 0.48^{a}	1.78± 0.46^{a}	1.67± 0.20^{a}	1.63± 0.49^{a}	1.62± 0.25^{a}	1.62

3.3.2. The effects of mini-plug containers and seedling age on growth after transplanting

Mini-plug container type and seedling age both had an influence on seedling growth after transplanting. However, these effects were only observed in seedlings transplanted three and four months after sowing (FIGURE 3.4A). One-month-old seedlings grew exponentially for one month after transplanting, reached a lag in growth for two months and thereafter seedling growth increased for a further two months. Seedlings transplanted at two-months-old grew steadily for three months after transplanting, after which seedling growth began to slow down. Seedlings transplanted after three months initially grew rapidly but one month after transplanting, seedling growth slowed down. Four-month-old seedlings exhibited a rapid increase in plant mass in the first month after transplanting, thereafter seedling growth reached a lag phase. No significant differences in plant growth were observed between the two containers tested when the seedlings were transplanted one and two months after sowing. However, seedlings transplanted three and four months after sowing had a greater total plant mass when they were initially cultivated in MP544 mini-plug containers (FIGURE 3.4).

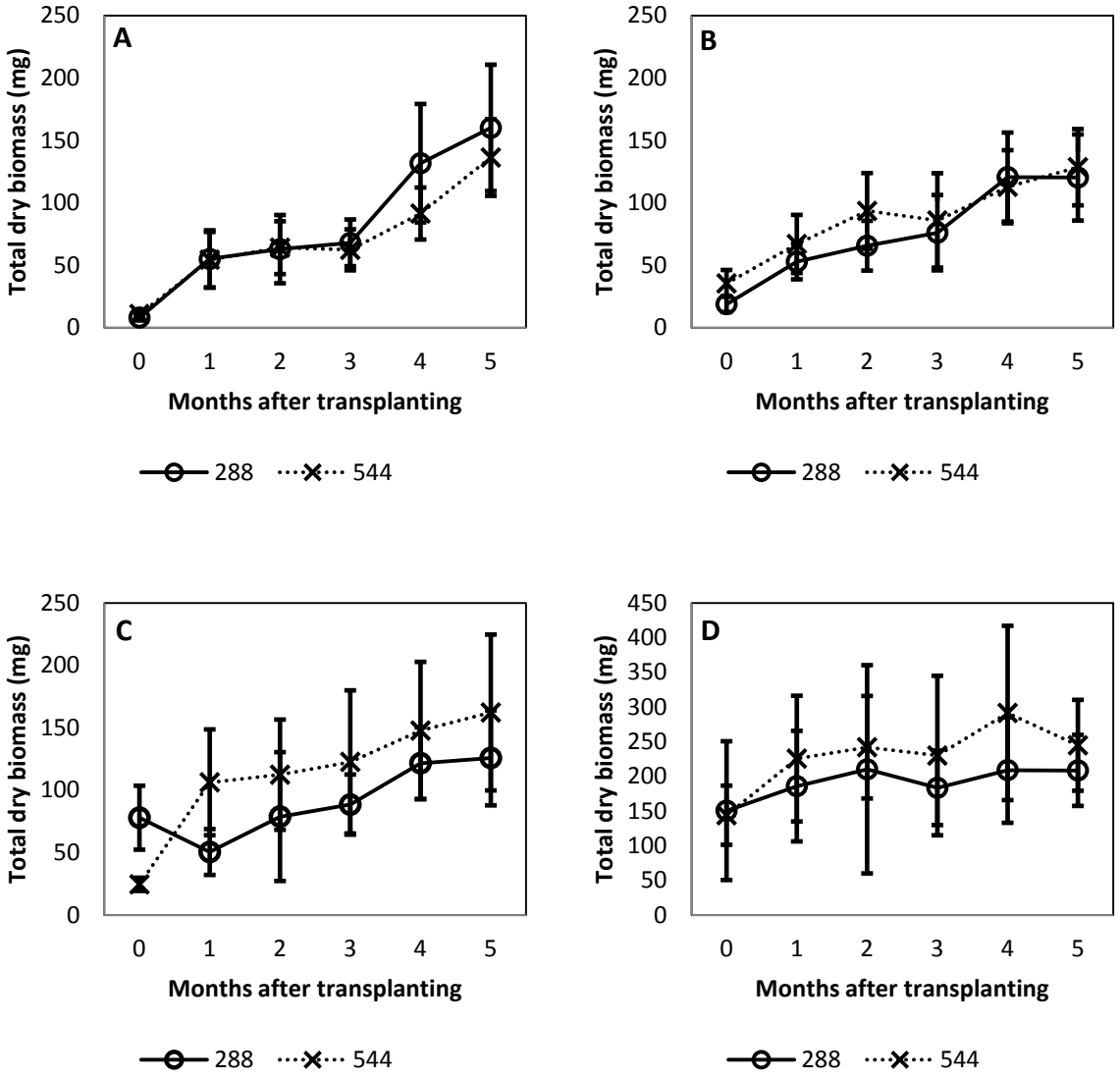

FIGURE 3.4. Total dry seedling mass (mg) over five months, after transplanting one- (A),two- (B), three- (C) and four-month-old (D) seedlings from MP288 and MP544 containers. Error bars represent S.D.

3.3.3. The effect of mini-plug container and seedling age on root development and growth

The effects of the mini-plug container and seedling age at transplanting on root growth and development were determined by measuring the longest root length and dry root biomass of these seedlings prior to transplanting and every month for five months thereafter. Root length appeared to be influenced by container type rather than seedling age. The seedlings

initially grown in polystyrene MP544 trays exhibited significantly shorter roots than seedlings grown in MP288 plastic trays prior to transplanting. The roots from seedlings grown in MP288 containers had begun to circle around the bottom of the MP288 mini-plugs within two months after sowing. No significant differences in root length were observed between age classes of seedlings within container types after transplanting (FIGURE 3.5).

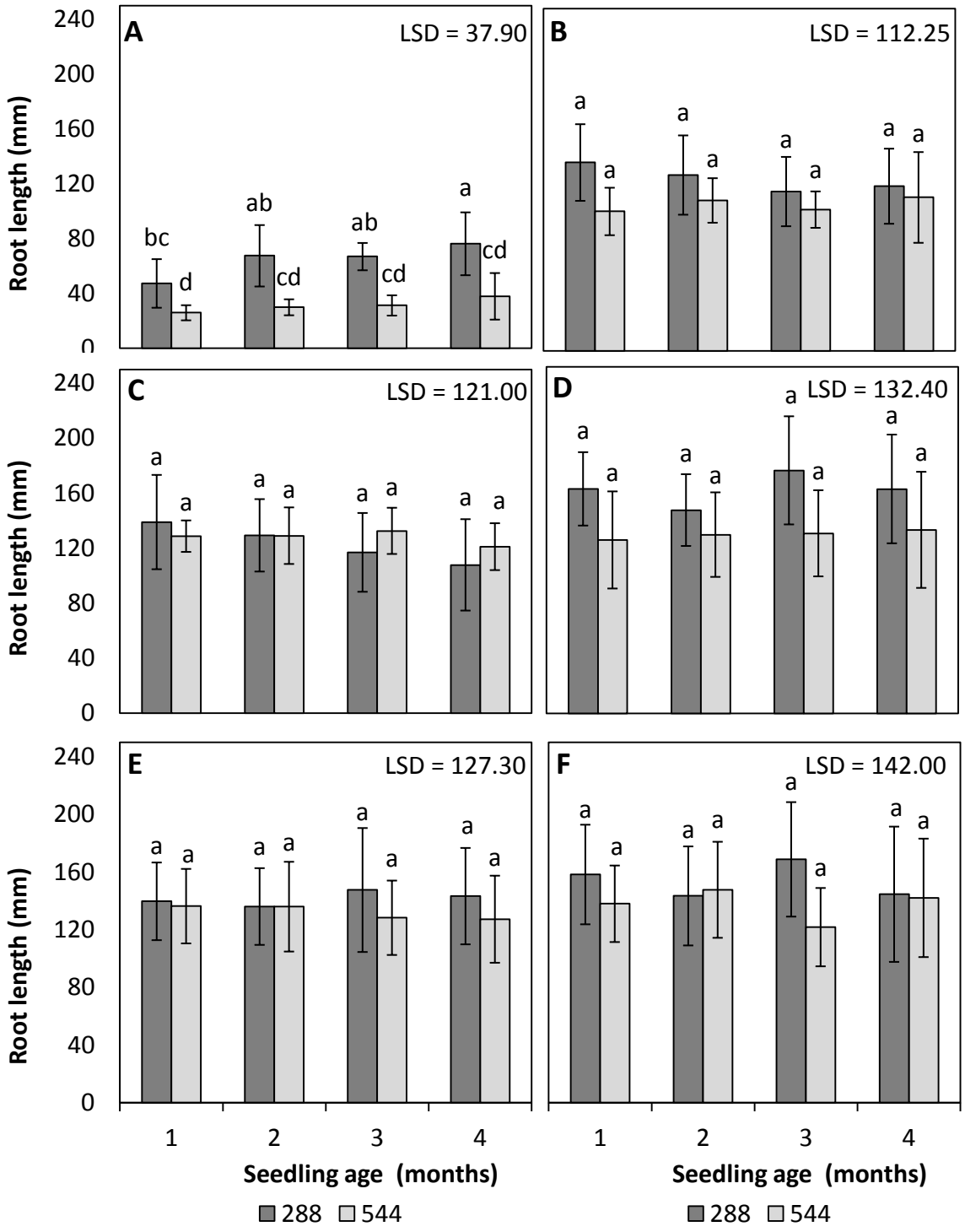

FIGURE 3.5. Root length (mm) of transplanted MP544 and MP288-grown *E. dunnii* seedlings before transplanting (A) and one (B), two (C), three (D), four (E) and five (F) months thereafter. Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

Dry root mass was affected by container type and seedling age prior to transplanting. The mean dry root mass of the four-month-old MP288-reared seedlings was significantly ($p<0.05$) higher than MP288-reared seedlings in all other age classes (FIGURE 3.6A). The dry root mass of three- and four-month-old seedlings cultivated in MP544 containers was lower than the dry root mass of three-and four-month-old seedlings cultivated in MP288 containers but only the three-month-old MP544-reared seedlings were significantly ($p<0.05$) lower (FIGURE 3.6A).

After transplanting it appeared that initial cultivation container did not affect root growth. The difference in dry root mass observed after transplanting was a result of the initial dry root mass when the seedlings were transplanted to the final container (FIGURE 3.6B-F).

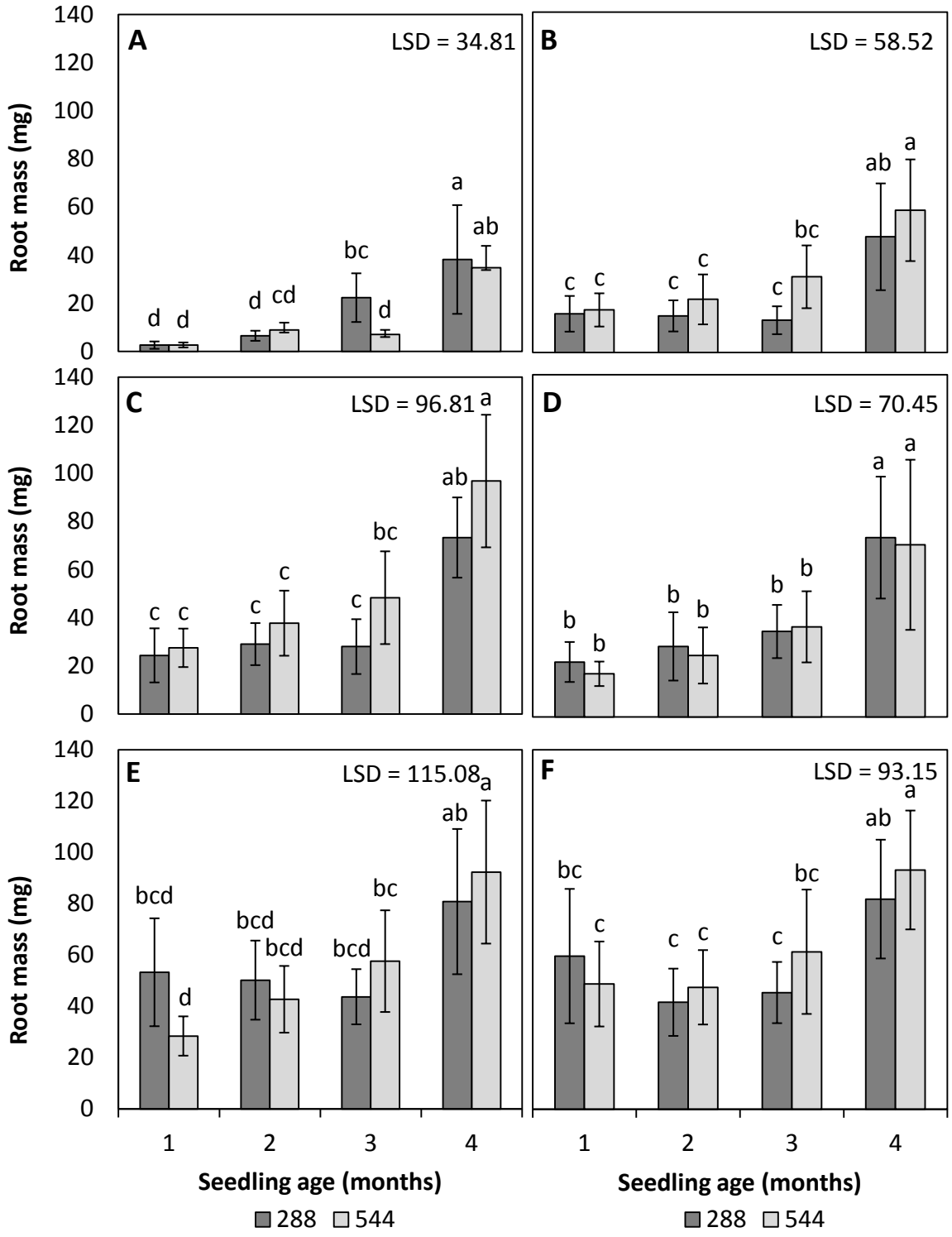

FIGURE 3.6. Dry root mass measured prior to transplanting (A) and for five months after transplanting for all treatments (one month (B), two months (C), three months (D), four months (E) and five months (F)). Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

One-, two-, three- and four-month-old seedlings grown in plastic MP288 and polystyrene MP544 containers were transplanted from mini-plug containers into polyethylene bags containing vermiculite and perlite in order to test the root growth potential of these seedlings. Seedlings grown in MP288 trays generally produced more roots than the seedlings grown in MP544 trays, with the exception of one-month-old MP544-grown seedlings, which produced the highest number of new roots during the root growth potential test when compared to all other treatments (TABLE 3.5). Three-month-old MP544 seedlings exhibited the lowest number of new roots produced. Although the highest number of new roots was observed in MP544 one-month-old seedlings the greatest dry new root mass was found in MP288 two-month-old seedlings with the lowest observed in MP544 three-month-old seedlings (TABLE 3.5).

TABLE 3.5. Effects of container type and seedling age prior to transplanting on the root growth potential. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Seedling age	**One-month-old**		**Two-months-old**		**Three-months-old**		**Four-months-old**		**LSD**
Container	**288**	**544**	**288**	**544**	**288**	**544**	**288**	**544**	
Number of new roots	6.65± 2.15^{bc}	11.25± 3.19^{a}	10.55± 2.01^{a}	10.50± 2.50^{ab}	7.20± 2.38^{abc}	5.90± 1.17^{c}	9.70± 2.96^{abc}	9.45± 4.70^{abc}	9.45
New root mass (mg)	4.80± 2.67^{b}	6.14± 3.74^{b}	19.63± 2.01^{a}	4.91± 2.29^{b}	9.40± 5.79^{ab}	3.88± 2.34^{b}	13.07± 3.03^{ab}	13.78± 8.09^{ab}	13.78

3.3.4. The effects of mini-plug containers and seedling age on root architecture

The incidence of J-rooting and J-rooting severity was low prior to transplanting in seedlings transplanted one, two and three months after sowing, regardless of the container type in which the seedlings were grown. For the four-months-old seedlings, the frequency of J-roots was the highest at 40% and 60% in MP288 and MP544 containers, respectively. The J-roots that were observed were, however, rated acceptable since they only exhibited J-rooting scores of one (FIGURE 3.7A) (**THOMAS *et al.*, 2008**).

By one month after transplanting all treatments exhibited J-roots but the severity of J-rooting was low and J-root scores ranged between one and two (FIGURE 3.7B). After two months, J-rooting frequency in one-month-old seedlings grown in MP544 trays increased to 80% but the severity of the J-roots remained low. Unacceptable levels of J-roots were observed two months after transplanting in three- and four-month-old seedlings originally grown in MP288 trays where J-root scores of four and five were observed (**THOMAS *et al*., 2008**). Seedlings transplanted from MP544 containers only exhibited unacceptable J-roots in seedlings that had been transplanted at four-months-old (FIGURE 3.7C). The frequency of J-rooting increased dramatically five months after transplanting, where J-rooting ranged from 90 to 100% in MP288-reared seedlings and 70 to 100% in MP544-reared seedlings. In addition, the number of treatments that exhibited undesirable J-roots increased where J-root scores of three to four were recorded in one-, two-, three- and four-month-old MP544-reared seedlings and in three- and four-month-old MP288-reared seedlings (FIGURE 3.7F).

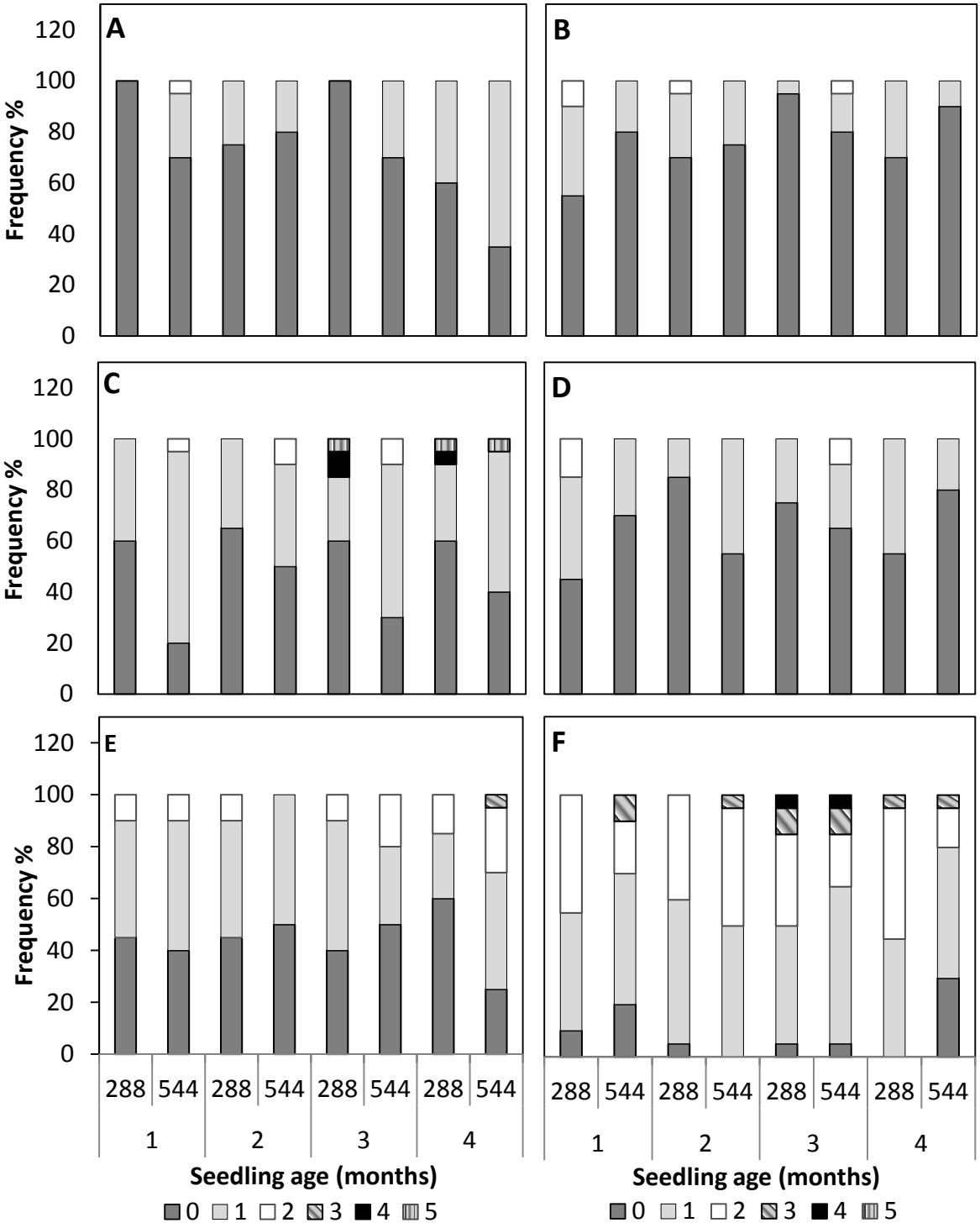

FIGURE 3.7. J-root frequency of one-, two-, three- and four-month-old *E. dunnii* seedlings transplanted from MP288 and MP544 containers at transplanting (A) and one (B), two (C), three (D), four (E) and five (F) months thereafter. Different shading within bars (outlined by the graph key) represents the degree of J-rooting according to the scoring system outlined in TABLE 3.2.

As with J-rooting, the frequency and severity of root coiling increased with the age of the seedlings when they were transplanted to the final container. The highest number of seedlings that developed root coils was observed in four-month-old seedlings at transplanting. Furthermore, the frequency of root coiling was higher in two- and four-month-old seedlings grown in MP288 containers at transplanting (FIGURE 3.8A). Seedlings transplanted three-months after sowing from the MP288 mini-plug containers exhibited root coiling scores of five, two months after transplanting (FIGURE 3.8C), and seedlings transplanted four-months after sowing, had coiling scores of five only one month after transplanting (FIGURE 3.8B). Although root coiling was recorded in seedlings grown in the MP544 mini-plug containers and the incidence of root coiling increased with seedling age at transplanting, severe coiling (scores of four and five) was not observed. One month after transplanting, only 5% of the seedlings transplanted one-and two-months after sowing exhibited root coiling scores of two, compared to 20% and 15% of seedlings transplanted at three- and four-months-old, respectively (FIGURE 3.8B). In addition, 15% of seedlings transplanted at four-months-old exhibited root coiling scores of three, one month after transplanting (FIGURE 3.6 B). The levels of root coiling increased substantially five months after transplanting. The frequency of coiling ranged from 90 to 100% in seedlings grown in MP288 trays and from 60 to 100% in MP544-reared seedlings (FIGURE 3.8F).

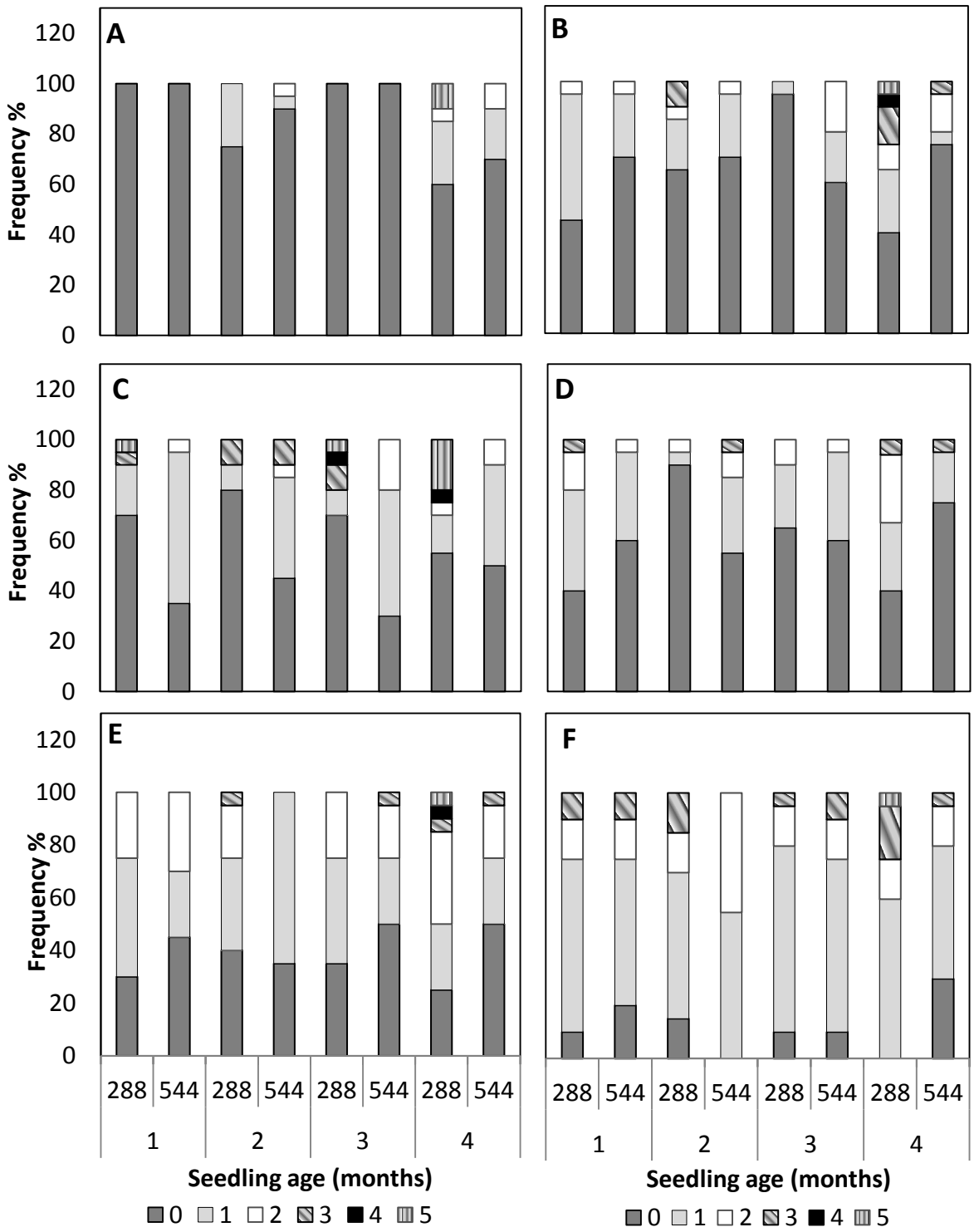

FIGURE 3.8. Root coiling frequency of one-, two-, three- and four-month-old *E. dunnii* seedlings transplanted from MP288 and MP544 containers at transplanting (A) and one (B), two (C), three (D), four (E) and five (F) months thereafter. Different shading within bars (outlined by the graph key) represents the degree of J-rooting according to the scoring system outlined in TABLE 3.2.

3.4. DISCUSSION

3.4.1. The impact of container characteristics on seedling quality

In this study, it was shown that container characteristics impacted seedling quality. Seedlings grown in MP288 containers developed better proportions than in MP544 containers before transplanting. They exhibited lower shoot-to-root ratios, sturdiness ratios and greater dry root mass which may have been due to the lower plant density and greater volume of MP288 mini-plug containers. Container volume tends to limit the seedling's access to water, nutrients, air and space in order to grow. This restricted volume available for root growth and development often results in increased shoot-to-root ratios (**NESMITH & DUVAL, 1998; MATHERS *et al*., 2007**). Distortion of shoot-to-root balance is often observed in container-grown plants and is not conducive to relieving plant stress after transplanting (**MATHERS *et al*., 2007**). In cucumber seedlings it was shown that seedlings restrict the growth of both the shoots and roots when root volume was restricted (**ROBBINS & PHARR, 1988**). In MP544 trays, the root mass was greater, although not significantly, than MP288 trays in the first two months after sowing, but after three months the root mass of MP288-grown seedlings were significantly greater than MP544-grown seedlings. Suggesting, that root growth of seedlings grown in MP544 trays only became restricted three months after sowing. In general, the smaller the container, the greater the negative effects of container volume on the morphological characteristics of seedlings (**NESMITH & DUVAL, 1998**).

An increased plant density in container-grown plants increases competition between plants for light in order to photosynthesise. Taller, thinner seedlings are a consequence of this, where energy is directed to the shoots to increase their exposure to light for photosynthesis (**BARNETT, 1980; ANNAPURNA *et al*., 2004; DINI-PAPANASTASI *et al*., 2012**). **SCARRAT** (**1989**) found that seedlings grown in trays with high plant density had significantly higher sturdiness ratios than seedlings cultivated in trays with a lower plant density. These seedlings were smaller, thinner and did not develop side shoots. The effects of higher planting densities were also shown in a study conducted on Italian cypress where the higher the planting density, the shorter the root length, which according to the authors was as a result of the decrease in carbohydrates stored in the roots due to decreased photosynthetic

activity (**KOSTOPOULOU *et al*., 2010a**). Similarly, the root length and the root dry mass of black locust (*Robinia pseudoacacia*) seedlings grown in mini-plugs were reported to decrease with increasing plant densities (**DINI-PAPANASTASI *et al*., 2012**)**.**

The negative effect of container planting density was enhanced by the length of time that the seedlings remained in the designated container (**BARNETT & BRISSETTE, 1986**). Seedlings cultivated for four months in mini-plug containers became tall and spindly. This was illustrated by the higher sturdiness ratios recorded prior to transplanting when compared to the seedlings transplanted earlier. Thus, indicating that more resources had been allocated to shoot development to ensure sufficient light energy was captured for photosynthesis. Contrary to this, planting density, in the study on Italian cypress (*Cupressus sempervirens L*.), did not influence the shoot height or shoot dry weight of the mini-plug seedling, this was attributed to the short cultivation times of the study (**KOSTOPOULOU *et al*., 2010**). Similarly, the negative effects of planting density in the mini-plug-grown seedlings was not observed by **RADOGLOU *et al*.** (**2011**) as the *Pinus brutia* seedlings evaluated were transplanted to the destination container four weeks after sowing.

The initial effects of plant density and container volume in mini-plug trays were initially extended after transplanting into the final container. The sturdiness and shoot-to-root ratios, one month after transplanting, were greater in seedlings from MP288 containers compared to MP544 containers within each age class. The dry root mass one month after transplanting, was only slightly greater ($p>0.05$) in MP544-grown seedlings than MP288-grown seedlings that were one- and two-months-old at transplanting. However, the root mass of three- and four-month-old MP544-grown seedlings were greater ($p>0.05$) than MP288 seedling root mass one month after transplanting. A well-balanced seedling allows for immediate shoot growth after transplanting since there are sufficient roots for water and nutrient absorption (**JOHANSSON *et al*., 2012**). Seedlings originating from MP288 containers were able to invest more resources into shoot growth rather than root growth after transplanting due to their better shoot-to-root balance. Higher shoot-to-root ratios as a result of the restricted root volume and competition between MP544 seedlings for light meant more investment in root mass accumulation immediately after transplanting (**BARNETT, 1980; DINI-PAPANASTASI *et al*., 2012**).

3.4.2. The impact of container characteristics on root development and architecture

Seedlings cultivated in MP544 containers produced seedlings with superior root architecture. Prior to transplanting, the seedlings grown in MP544 trays developed root systems with significantly shorter roots and a lower incidence of root coiling than MP288-reared seedlings, regardless of seedling age at transplanting. Coiling was observed in MP288-grown seedlings within two months of sowing. Since the MP288 trays were not dipped in copper oxychloride and these trays only had one hole at the bottom of the container for air pruning, the roots continued to grow unchecked thereby increasing the incidence of root coiling (**MATHERS *et al*., 2007**). In this study, the MP544 polystyrene containers were dipped in copper oxychloride to prevent the roots from growing into the walls of the polystyrene container which would make removal of seedlings from the mini-plugs difficult. Copper is often used to prevent the roots from circling in containers. The copper burns or kills the root tips when they come into contact with the walls of the container causing secondary branching of the roots which results in a fibrous root system (**DUMROESE & WENNY, 1997; MATHERS *et al*., 2007**). The shorter roots of MP544-reared seedlings were due to chemical root pruning in MP544 containers which contributed to the decreased incidence of root coiling after transplanting. Similar results were reported by **SVENSON & BROSCHAT** (**1992**) where West Indies Mahogany container grown seedlings showed decreased frequencies of root circling from 18% to 1% when the containers were treated with copper oxychloride. Three-month-old seedlings grown in MP544 trays produced a significantly ($p<0.05$) lower root mass compared to the MP288-grown seedlings (FIGURE 3.6A). Furthermore, the two- and three-month-old MP544-cultivated seedlings exhibited a lower new dry root mass compared to MP288-cultivated seedlings, although significantly differences were only observed within the two-month-old seedlings ($p<0.05$) (TABLE 3.5). In a study conducted on Littleleaf Linden the seedlings that were grown in air pruning containers also exhibited a lower root mass than the solid plastic container which the authors contributed to the constant need for regeneration of new roots in seedlings grown in air pruning containers (**AMOROSO *et al*., 2010**).

Whilst the seedlings grown in MP288 trays generally exhibited more new roots than those grown in MP544 trays, the number of new roots developed within each of the age classes

tested was not significantly higher. The slight increase in the number of new roots formed in the root growth potential test by seedlings initially grown MP288 containers was associated with the lower planting density and higher container volumes of these trays. New root development is dependent on photosynthesis and in the event of reduced photosynthetic activity, as would be the case in higher density containers, the number of new roots produced is limited (**RICHIE, 2003; ANNAPURNA *et al*., 2004**). Similar results were observed in black pine, black locust, brutia pine and long leaf pine where the number of new roots developed in the root growth potential test increased with decreased planting densities (**SOUTH *et al*., 2004; KOSTOPOULOU *et al*., 2010b**). However, seedlings from MP544 trays produced significantly more new roots in the one-month-old seedlings than the MP288 seedlings of the same age. This suggests that the effects of density and container volume on root growth potential were dependant on cultivation time in the trays and the effects of higher planting densities in the trays had not yet limited photosynthetic activity (**RICHIE, 2003)**. The copper treatment further enhanced the root growth potential of one-month-old MP544 mini-plug seedlings. In a comparative study on the effects of various container types on seedling production of Longleaf pine, it was shown that seedlings grown in copper treated Stryoblock® containers had resulted in a higher root growth potential than seedlings grown in trays without copper (**SOUTH *et al.*, 2004**). This improved root growth potential may have been due to an higher total root surface area as a result of the more fibrous root system which increased water absorption and decreased plant stress after transplanting (**RICHIE, 2003; DEVINE *et al*., 2009**). Further to this, increased root growth potential of these seedlings may also be due to the fact that the roots of the seedlings grown in MP544 trays were more actively growing unsuberized roots than the roots of seedlings grown in MP288 trays as a result of the constant regeneration of new roots. Unsuberized roots take up water more effectively thereby, decreasing water stress, increasing photosynthesis and initiating new root growth quickly (**GROSSNICKLE, 2005**).

Prior to transplanting, the incidence of J-rooting, with the exception of the four-month-old seedlings, was negligible suggesting that J-root formation was a result of the transplanting procedure. This theory was proposed in a study done by **THOMAS *et al.*** (**2008**) which showed similar results. It was suggested that J-rooting was a result of the transplanting

process which was further complicated by human error and the growth medium used and was not an effect of the mini-plug container (**THOMAS *et al*., 2008**).

Poor root architecture was enhanced with the age of the seedlings at transplanting. Seedlings grown in MP544 and MP288 containers needed to be transplanted within three months after sowing to reduce the incidence and severity of J-rooting and root coiling. However, transplanting seedlings too early, particularly MP544-reared seedlings, increased J-root development. Prolonging growing time in the containers increased the severity of root deformities (**MATHERS *et al*., 2007**), as was observed in the seedlings transplanted four months after sowing. The negative effects of extended growing times in the containers was further demonstrated by the dramatic increase in frequency of J-roots five months after transplanting, particularly in seedlings transplanted after four months, where five months after transplanting the seedlings were nine months old.

The prolonged growing time of the four-month-old seedlings also had a negative impact on seedling growth after transplanting as it delayed or slowed down subsequent growth. The higher incidence of root deformities in the four-month-old seedlings, particularly coiling of the roots indicated that these seedlings had become root bound (**MATHERS *et al*., 2007**). Root bound seedlings take longer to establish after transplanting and cause delays in subsequent seedling growth (**SCHRADER, 2000**).

3.5. CONCLUSIONS

- Seedlings cultivated in MP288 trays produced superior plant quality specifications as a result of the increased container volume and decreased plant density.
- This superior plant quality of the MP288-grown seedlings, however, was not extended after transplanting.
- The root architecture of the seedlings was better in MP544-reared seedlings as a result of the copper pruning which prevented root coiling and encouraged more actively growing roots.
- J-rooting occurred in the seedlings after transplanting regardless of the container type and transplanting was found to be the main cause of J-root formation.

- Seedling age at transplanting was critical in reducing delayed growth and root deformation due to extended periods in containers.
- Based on these results, MP544 containers were selected for further experimentation.

CHAPTER 4

Mini-plug transplanting and its effect on the growth and development of Eucalyptus dunnii *seedlings*

4.1. INTRODUCTION

Transplanting shock or growth check refers to the delayed growth after seedlings are transplanted to new growing conditions (**CLOSE *et al*., 2005; GROSSNICKLE, 2005**). Fast establishment in the new environment is essential to ensure seedling survival (**JOHANSSON *et al*., 2012**). Transplanting seedlings disturbs the shoot and root symmetry (**NESMITH, 1999**). Damage to the root systems may occur when seedlings are pulled from the mini-plug containers during transplanting and in mini-plug systems this stress is enhanced because the seedlings are still very small (**SCHRADER, 2000**). Delayed growth is often due to decreased absorption of nutrients and water (**JACOBS *et al*., 2004**). Since seedlings continue to transpire after transplanting, rapid initiation of new roots is essential to avoid plant stress and in extreme incidences, mortality (**HATHAWAY & WHITCOMB, 1977; NESMITH, 1999**). The aims of this experiment were to compare the performance of directly-sown seedlings with transplanted seedlings and to evaluate the impact of different container types on both directly-sown and transplanted seedling quality.

4.2. MATERIALS AND METHODS

4.2.1. Seedling cultivation

In order to compare propagation techniques and their influence on seedling growth, development and quality two propagation methods were used to propagate *Eucalyptus dunnii* seedlings; (1) sowing directly into the final propagation container and (2) transplanting previously germinated seedlings from mini-plug containers to the final propagation container. For the purposes of clarity, seedlings that were cultivated directly in final containers are referred to as 'directly-sown' seedlings and transplanted mini-plug seedlings are referred to as 'transplanted' seedlings.

For the directly-sown seedling treatment, *Eucalyptus dunnii* seeds, stock number M9671, supplied by Mondi's Mountain Home Nursery were sown into three different sized plastic containers (Unigro® 128-, Unigro® 98- and Unigro® 72-cavity) and three different sized

polystyrene containers (Polystyrene 128-, 98- and 72-cavity) (TABLE 4.1) previously dipped in copper oxychloride. The seedlings sown directly into the Unigro® and polystyrene 128-, 98-, and 72-cavity containers were not transplanted and served as experimental controls.

In transplanted seedling treatments, *E. dunnii* seeds were initially sown into MP544 (mini-plug 544-cavity polystyrene) containers. After two months, seedlings germinated in the MP544 containers were transplanted into three different sized plastic containers (Unigro® 128-, Unigro® 98- and Unigro® 72-cavity) and three different sized polystyrene containers (Polystyrene 128-, 98- and 72-cavity) (TABLE 4.1 & FIGURE 4.1). The same cultivation techniques described in Chapter 3, section 3.2.1 and 3.2.2 were used for the cultivation of both the directly-sown and transplanted treatments.

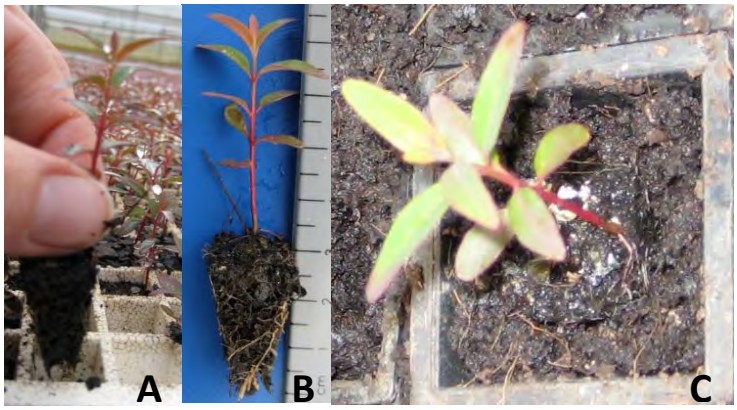

FIGURE 4.1. Sequence of transplanting. Pulling the mini-plug seedling (A) from the 544-cavity tray, the mini-plug seedling (B) and the mini-plug seedling transplanted into the Unigro 128®-cavity container (C).

TABLE 4. 1. Final container specifications.

Container	Manufacturing material	Number of cavities per container	Plant density per m^3	Cavity volume (ml)	Cavity depth (mm)
Unigro® 128	Plastic	128	522	65	100
Unigro® 98	Plastic	98	400	88	100
Unigro® 72	Plastic	72	288	104	100
Polystyrene 128	Polystyrene	128	522	65	100
Polystyrene 98	Polystyrene	98	400	88	100
Polystyrene 72	Polystyrene	72	288	104	100

4.2.2. Evaluation of seedling growth, quality and root architecture

Shoot height, root collar diameter, dry shoot mass and dry root mass of 20 seedlings per treatment were measured. The seedlings were measured when the root plugs had fully consolidated in the containers as this is a specification required for planting in the field. Therefore at assessment, the seedlings were four-, five- and six-months-old for the Unigro® and polystyrene 128-, 98- and 72-cavity trays, respectively, as it took longer for the larger cavity containers to develop consolidated root plugs. The root architecture of the same seedlings was assessed using the methods described in Chapter 3, section 3.2.5. A root growth potential test was carried out on 20 randomly selected seedlings per treatment using the same procedures described in Chapter 3, section 3.2.3 to determine the treatment effects on new root initiation.

4.3. RESULTS

4.3.1. The effects of transplanting and final container type on seedling quality

The overall mean sturdiness ratio for transplanted seedling treatments was higher than for directly-sown seedlings ($p>0.05$). In contrast, the overall shoot-to-root ratio of transplanted seedlings was lower compared to the directly-sown seedlings ($p>0.05$) (TABLE 4.2).

TABLE 4.2. Overall mean sturdiness and shoot-to-root ratios of directly-sown and transplanted seedlings. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Propagation method	Sturdiness ratio	Shoot-to-root ratio
Directly-sown	84.23±18.48[a]	2.69±0.97[a]
Transplanted	86.79±20.93[a]	2.22±0.75[a]
LSD	5.02	0.22

Seedlings transplanted to Unigro® 128 and 98-cavity containers exhibited lower sturdiness ratios than seedlings that were directly-sown into the Unigro® 128 and 98-cavity containers. The shoot-to-root ratios recorded for transplanted seedlings were all lower than their directly-sown counterparts with the exception of the Unigro® 98- and polystyrene 98-cavity containers ($p>0.05$) (TABLE 4.3).

TABLE 4.3. Sturdiness and shoot-to-root ratios of seedlings directly-sown and transplanted into different containers. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Propagation method	Container type	Sturdiness ratio	Shoot-to-root ratio
Directly-sown	Polystyrene 128	74.03 ± 14.36^{de}	3.91 ± 0.62^{a}
	Polystyrene 98	67.53 ± 10.52^{e}	2.38 ± 0.77^{bc}
	Polystyrene72	94.13 ± 11.70^{abcd}	3.13 ± 0.86^{ab}
	Unigro® 128	83.55 ± 14.39^{cde}	2.50 ± 0.89^{abc}
	Unigro® 98	79.68 ± 12.87^{cde}	1.97 ± 0.47^{c}
	Unigro® 72	106.48 ± 16.28^{ab}	2.30 ± 0.53^{bc}
Transplanted	Polystyrene 128	85.17 ± 15.16^{bcde}	2.43 ± 0.74^{bc}
	Polystyrene 98	81.77 ± 9.80^{cde}	3.01 ± 0.85^{ab}
	Polystyrene72	99.13 ± 10.53^{abc}	1.96 ± 0.59^{c}
	Unigro® 128	73.47 ± 13.27^{de}	2.22 ± 0.48^{bc}
	Unigro® 98	68.90 ± 6.60^{e}	2.19 ± 0.56^{bc}
	Unigro® 72	112.31 ± 26.24^{a}	1.57 ± 0.36^{c}
LSD		68.90	2.18

Generally the transplanted seedling treatments displayed inferior seedling characteristics (shoot height, root collar diameter, dry shoot and root mass) compared to the directly-sown seedlings. Mean shoot height of directly-sown seedlings was slightly, but not significantly ($p>0.05$) higher than transplanted seedlings when these seedlings were propagated in all containers (FIGURE 4.4A). No significant ($p>0.05$) differences in root collar diameter between directly-sown and transplanted seedlings grown in 128- and 72-cavity containers were observed. However, directly-sown seedlings grown in polystyrene 98-cavity containers exhibited a significantly ($p<0.05$) higher mean root collar diameter compared to seedlings transplanted to polystyrene 98-cavity containers (FIGURE 4.4B). The mean shoot dry mass and the mean root dry mass were lower in all transplanted seedlings, within the different

container types, compared to directly-sown treatments, but only the dry shoot mass was significantly ($p<0.05$) lower in seedlings cultivated in both the 72-cavity container types (FIGURE 4.2; 4.3 and 4.4 C and D).

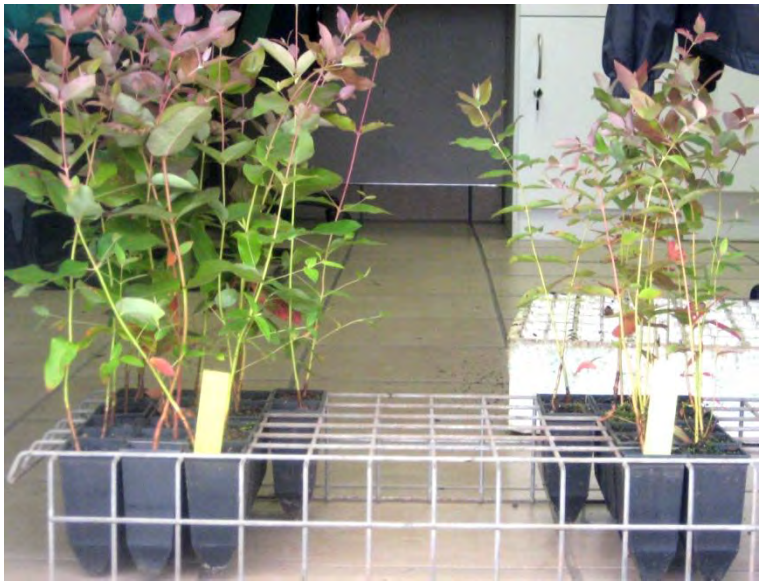

FIGURE 4.2. Directly-sown seedlings (left) compared to transplanted seedlings (right) propagated in Unigro® 72-cavity containers.

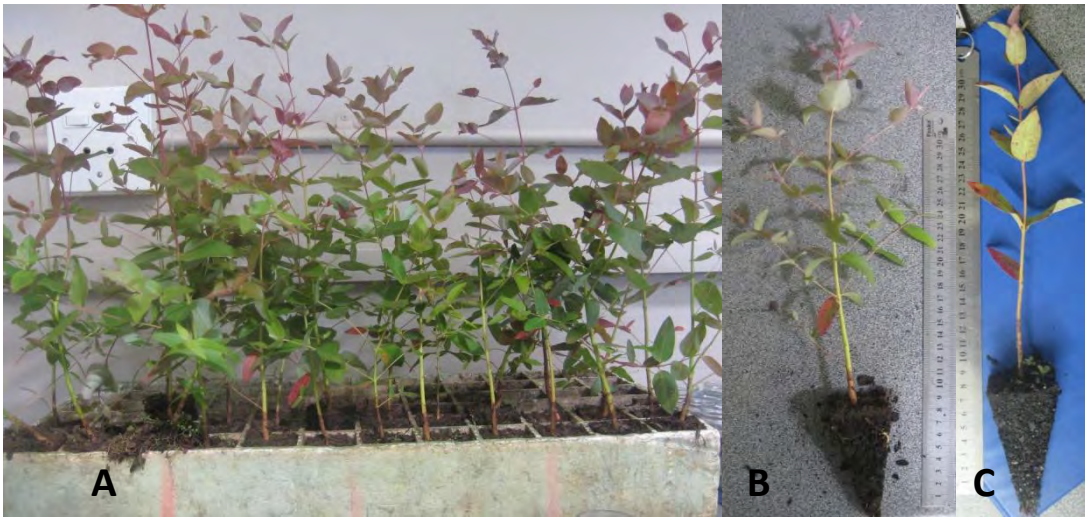

FIGURE 4.3. Directly-sown seedlings (A, B) compared to transplanted seedlings (C) propagated in polystyrene 72-cavity containers.

The 72-cavity containers produced the largest seedlings regardless of the propagation method employed. The 72-cavity containers exhibited the highest mean shoot height, dry root mass and dry shoot mass of all seedling treatments. However, significant differences were only found in shoot height. The mean dry shoot mass of directly-sown seedlings grown in 72-cavity polystyrene containers were significantly ($p<0.05$) greater than for seedlings sown directly into Unigro® 72-cavity containers. This was followed by the 98-cavity containers, and the smallest plants were produced in 128-cavity containers. No significant ($p>0.05$) differences in shoot height and dry root mass were observed between polystyrene and plastic containers within different sized containers (FIGURE 4.4). However, the root collar diameter of seedlings cultivated in 98-cavity polystyrene containers were significantly ($p<0.05$) larger than seedlings grown in Unigro® 98-cavity containers (FIGURE 4.4B).

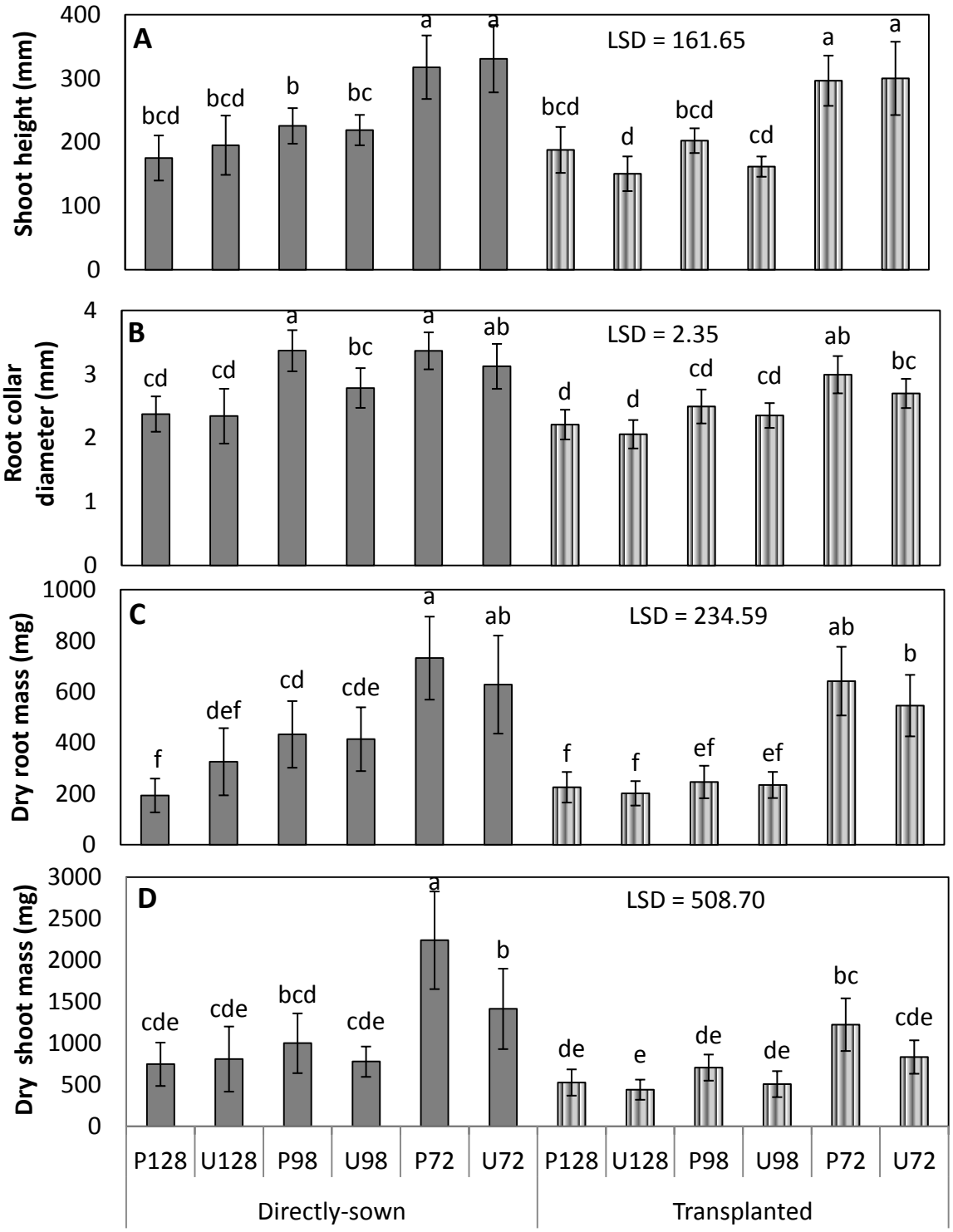

FIGURE 4.4. Mean shoot height (A), root collar diameter (B) , dry root mass (C) and dry shoot mass (D) of *E. dunnii* seedlings directly-sown and transplanted into 128-, 98-, 72-cavity polystyrene (P) and Unigro® (U) trays. Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

4.3.2. The effects of transplanting and the final container on root architecture

The frequency and severity of root coiling and J-rooting was higher when seedlings had been transplanted. Of all directly-sown seedlings, 47% exhibited J-rooting, with the highest J-root score of two being recorded for Unigro® 98-cavity containers. For transplanted seedlings, however, 67% developed J-roots and the highest J-rooting score of five was observed (FIGURE 4.5A) for Unigro® 128- and 98-cavity containers

J-root formation and severity were generally more frequent in seedlings transplanted to plastic Unigro® trays, except for seedlings transplanted into 98-cavity trays. Seedlings transplanted to Unigro® 128- and Unigro® 72-cavity trays had the highest frequency of J-rooting (90% and 95% respectively). It was observed that 20% and 10% of seedlings transplanted to Unigro® 128-cavity trays developed J-roots with scores of four and five respectively and 35% of seedlings transplanted to polystyrene 98-cavity trays exhibited a J-root score of five (FIGURE 4.5A).

Seedlings transplanted into 128-cavity trays exhibited a greater incidence of root coiling, with 60% of the roots coiling in polystyrene 128-cavity trays and 65% in Unigro® 128-cavity trays. However, severity of root coiling was higher in polystyrene 98-cavity and in both 72-cavity containers (FIGURE 4.5B).

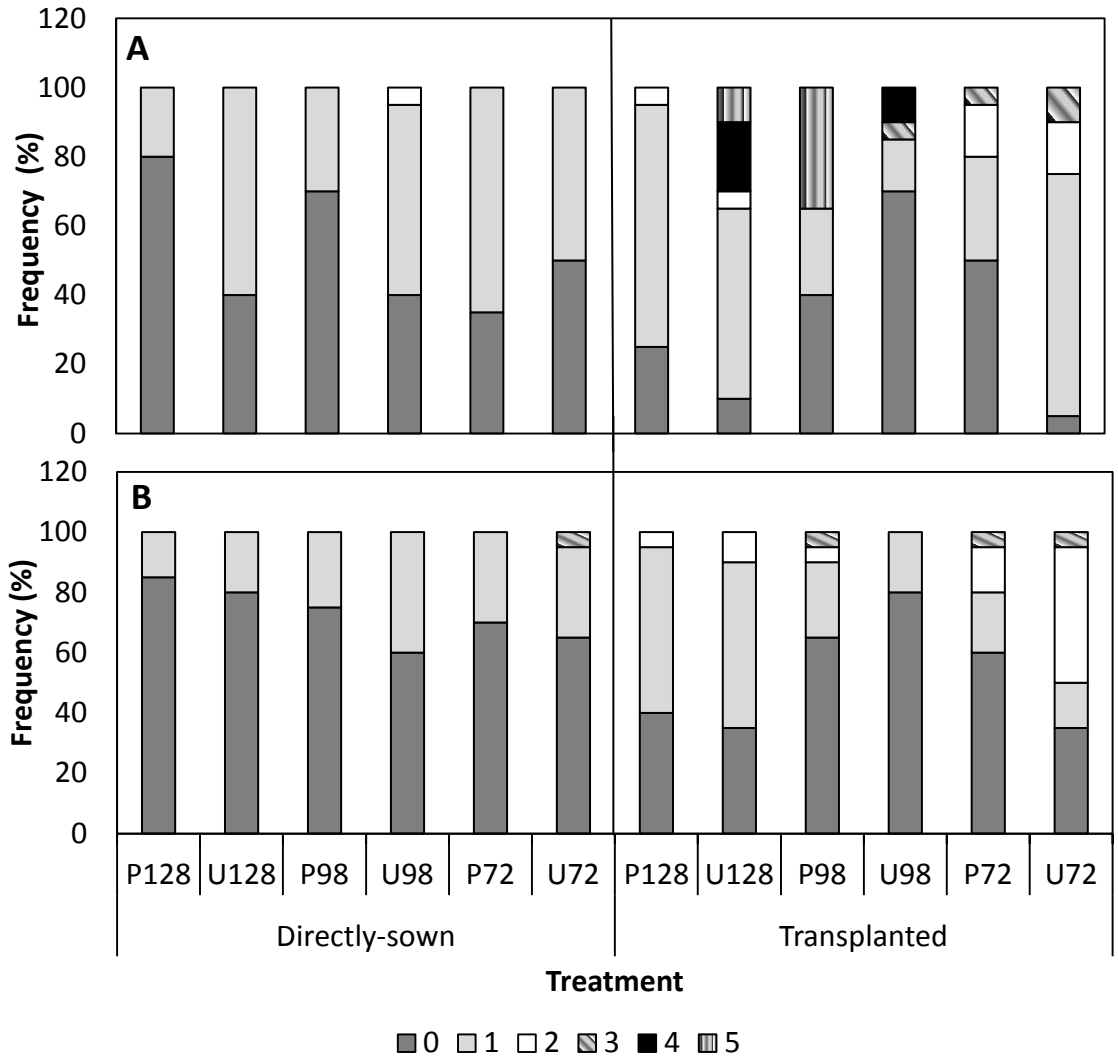

FIGURE 4.5. The frequency of J-roots (A) and root coiling (B) as a result of the final container. Different shading within bars (outlined by the graph key) represents the degree of J-roots and root coils according to the scoring system outlined in TABLE 3.2.

4.3.3. The effect of transplanting and the final container on root growth potential

The directly-sown seedlings produced significantly ($p<0.05$) more new roots during the root growth potential test than transplanted seedlings. In addition, these seedlings exhibited greater new dry root mass than transplanted seedlings ($p<0.05$). The overall mean new root dry mass was 108.32 mg for the directly-sown treatments compared to 87.30 mg of the transplanted treatments. Root length did not appear to be effected by transplanting or container type in this experiment ($p>0.05$) (TABLE 4.4 and FIGURE 4.6A).

TABLE 4.4. Overall mean number of new roots, root length and new root dry mass that developed during the root growth potential test of directly-sown and transplanted *E. dunnii* seedlings. Different letters represent significantly different means (±S.D.) at p<0.05.

Propagation method	Number of new roots	Root length (mm)	New root dry mass (mg)
Directly-sown	17.62±9.26[a]	291.24±67.71[a]	108.32±65.31[a]
Transplanted	13.37±8.28[b]	260.80±74.05[a]	87.30±43.76[b]
LSD	2.23	18.05	18.81

In directly-sown treatments, the mean dry mass of new roots was consistently greater (p>0.05) in seedlings that had been directly-sown into the 128- and 72-cavity polystyrene trays and only slightly higher in polystyrene 98-cavity trays (FIGURE 4.6C). The number of new roots in directly-sown treatments was greatest in the polystyrene 128-cavity and 72-cavity containers (FIGURE 4.6B). In transplanted seedling treatments, the mean number of new roots was greatest in seedlings transplanted to polystyrene 128-cavity, 98-cavity and Unigro®-98 cavity containers. No significant differences (p>0.05) in the number of new roots produced between polystyrene and plastic containers were observed within 98-cavity and 72-cavity treatments. However, for seedlings transplanted into polystyrene 128-cavity trays, the number of new roots produced was significantly more than seedlings transplanted into Unigro® 128-cavity containers (p<0.05) (FIGURE 4.6B). Similarly, the new root dry mass showed no significant differences between polystyrene and plastic containers within different tray sizes tested (FIGURE 4.6C).

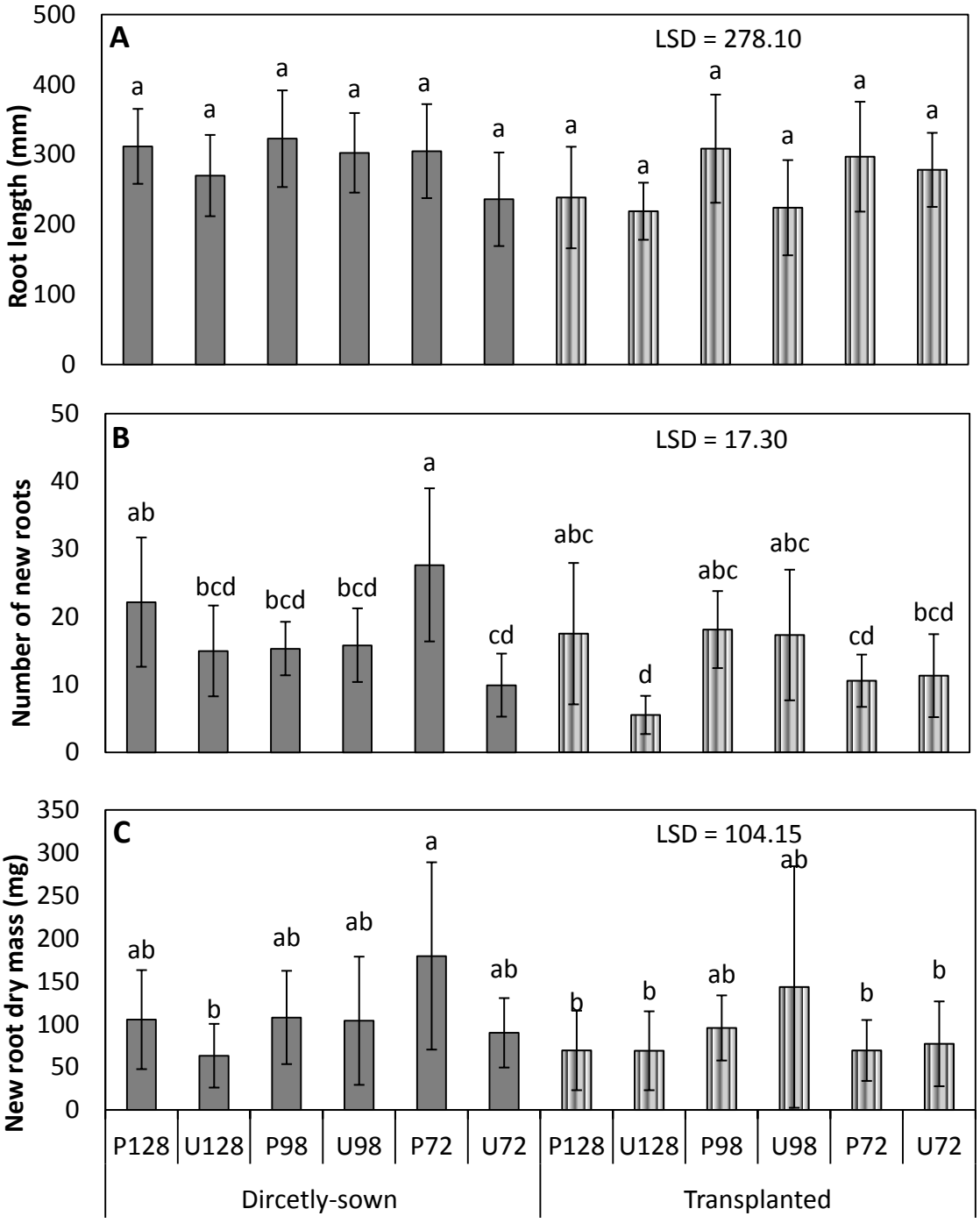

FIGURE 4.6. Mean root length (A), number of new roots (B) and new dry root mass (C) after the root growth potential test of *E. dunnii* seedlings directly-sown and transplanted into 128-, 98-, 72-cavity polystyrene (P) and Unigro® (U) trays. Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

4.4. DISCUSSION

4.4.1. Transplant shock

Transferring a seedling to a new environment causes delayed growth while the seedling adapts to new conditions. This delay may be caused by many factors which affect various metabolic processes within the plant. Mechanical damage, poor seedling quality, low nutrient status, limited water uptake and poor growing conditions are just some of these factors that impede seedling growth (**SCHRADER, 2000, CLOSE *et al*., 2005; GROSSNICKLE, 2005**). Root initiation is important to ensure fast growth after transplanting (**BURDETT, 1990; RICHIE, 2003; CLOSE *et al*., 2005**). The faster new roots are initiated after transplanting the less the effects of transplant shock (**SANDS, 1984; VAN IERSEL, 1998**).

In this study, container-to-container transplanting affected seedling growth and development. Although, nursery conditions were the same for all treatments, shoot height, root collar diameter, root mass and shoot mass were lower in transplanted seedlings compared to directly-sown seedlings. Transplanting also reduced new root initiation during the RGP test. Similar results were reported by **SCARATT** (**1989**) when Black spruce seedlings were transplanted from mini-plug containers into larger paper pots. The results of this study supported by the study conducted by **SCARATT** (**1989**) suggest that transplanted seedlings experienced transplant shock caused by a variety of cultural factors (**CLOSE *et al*., 2005**).

Container-reared seedlings are frequently subject to root damage which is not always immediately detectable (**BURDETT, 1990**). When mini-plug seedlings were pulled from the MP544 trays during transplanting some seedlings were damaged during this process. This often occurred when the seedlings had not fully consolidated the root plug. The shoots of these seedlings detached from the roots, with half the seedling remaining in the MP544 tray. These seedlings were discarded without transplanting. However, it is possible that the seedlings that were successfully transplanted were damaged when they were extracted from the mini-plug tray. Mechanical damage to the root system reduces root viability and root growth (**BURDETT, 1990**).

Root growth is affected by both the previous cultural practices and the new growing conditions that seedlings are transplanted into. Growing conditions that have an impact on

root growth include substrate temperature, moisture availability and oxygen content (**RICHIE, 2003**). It is well documented that water stress is one of the main factors that cause transplant shock. However, these reports refer to seedlings that were transplanted into the field where environmental conditions are not easily controlled (**ORLANDER, 1984; SANDS, 1984; BURDETT, 1990; ROSE *et al*., 1991; GROSSNICKLE, 2005**). In this study, MP544 seedlings were transplanted under controlled conditions in the nursery, where both directly-sown and transplanted seedlings were irrigated at regular intervals throughout the day. It is therefore unlikely that the decreased plant growth observed in the transplanted seedlings was due to water stress.

Root growth increases with increasing substrate temperatures but growth is limited when the substrate temperature drops below 8˚C (**RICHIE, 2003**). Reduced root growth affects physiological process within seedlings and subsequently affects overall seedling growth. This may result in mortality after transplanting. Root system viability and hydraulic conductivity are important in limiting seedling growth (**GROSSNICKLE, 2005**). It was concluded, however, that substrate temperature did not delay seedling growth in transplanted seedlings since both directly-sown and transplanted seedlings were exposed to the same temperature conditions.

In container systems, water logging as a result of poorly draining substrates can have serious consequences on root growth and development (**HANDRECK & BLACK, 1994; SCHRADER, 2000; MATHERS *et al*., 2007**)**.** Excess water cannot be absorbed by the seedlings and creates unfavourable growing conditions. Waterlogged substrates are a consequence of a low AFP which decreases the available oxygen required for growth and increases the incidence of root borne diseases (**HANDRECK & BLACK, 1994; SCHRADER, 2000; MATHERS *et al*., 2007**)**.** In a study on Black spruce, **SCARRAT** (**1989**) found that in mini-plug seedlings, the substrate remained saturated long after irrigation. The negative effects of the waterlogged substrate delayed subsequent growth of the mini-plug seedlings after they had been transplanted to larger paper pots. **SCARRAT** (**1989**) suggested that initial growing conditions may have affected the seedlings ability to grow optimally after transplanting. In this study similar observations were made, the substrate in the MP544 tray did not dry out completely between irrigation events. The substrate used in this study to produce mini-plug seedlings

was not optimal and influenced the root system viability of the seedlings. Therefore, a separate study was conducted to optimise the substrate composition for container-to-container transplanting (Chapter 5).

The delayed growth of transplanted seedlings observed by the results of this study was likely caused by a combination of contributing factors. However, the most obvious are the possible negative effects caused by mechanical damage and the inappropriate substrate used in the mini-plug containers. By optimising the environmental conditions the negative effects of transplant shock may be reduced. Studies have shown that optimising environmental conditions improves seedling growth thereby reducing plant stress (**SOUTH & MITCHELL, 1999; JOHANSSON *et al*., 2012**). Seedling growth of Norway spruce was increased when environmental conditions were optimised (**JOHANSSON *et al*., 2012**). **SOUTH & MITCHELL** (**1999**) illustrated that both seedling quality and optimum growing environment increased growth and survival after planting *Pinus elliotii* seedlings. Therefore, more focus on the production of high quality seedlings was required to improve root growth and development after transplanting.

4.4.2. Final container size and seedling quality

The larger the container volume the greater the seedling size (**ANNAPURNA *et al*., 2004; CLOSE *et al*., 2006; MATHERS *et al*., 2007**) The 72-cavity containers produced the largest seedlings, followed by the 98-cavity containers with the smallest seedlings grown in 128-cavity containers. Similar results were observed for *E. globulus* seedlings grown in three different size containers where the seedling size increased with an increase in container volume (**CLOSE *et al*., 2006**). Larger seedlings are reported to perform better after planting because they have more resources than smaller seedlings. Smaller seedlings reportedly have less developed root systems compared to larger seedlings, which affects their ability to absorb nutrients and water (**CLOSE *et al*., 2006**). In this study, although, larger container volume did produce larger seedlings, the cultivation time in the containers began to affect overall seedling quality. In the 128- and 72-cavity trays the sturdiness ratios were higher than in the 98-cavity trays. The 72-cavity seedlings were grown in these trays longer than those seedlings grown in 128- and 98-cavity containers as they needed more time for root plug consolidation prior to despatch to the field. Consolidated root plugs make it easier to

remove the seedlings from containers and decrease possible root deformities when seedlings are planted in the field. However, over consolidation of the root plugs should be avoided since root bound seedlings reduce root system vigour once they are planted in the field. The increased volume in the container allowed seedlings in 72-cavity containers to grow continuously without restricting the root system. The seedlings grew quickly and began to develop side shoots. Over time however, the seedlings become overcrowded in the trays, limiting air flow and light. The sturdiness ratio increased due to the increased competition for light to photosynthesise (**SHARMA, 1996; POORTER *et al*., 2012**). In *Santalum album* (Indian sandalwood) seedlings, similar symptoms were observed where the sturdiness ratio was higher in seedlings grown in containers with a high container volume compared to seedling grown in containers with a smaller volume but with a lower planting density (**ANNAPURNA *et al*., 2004**). The seedlings from 128-cavity trays had the highest planting density and the lowest container volume. This meant that the seedlings began to compete for photosynthetic radiation faster than seedlings in 98- and 72-cavity trays (**POORTER *et al*., 2012**). The results of this experiment indicate that spacing seedlings within trays to improve light accessibility and air flow between seedlings will need to be investigated to reduce the negative effects of seedling competition thereby improving final seedling quality.

4.4.3. Root architecture

Transplanting seedlings increased the severity of root deformities in the seedlings. These results concur with the research conducted by **THOMAS *et al*.,** (**2008**) and similar results were reported in Chapter 3. **THOMAS *et al*.,** (**2008**) postulated that dibbling holes in the media for seedling placement during transplanting compacted the substrate, restricting root-tip penetration. To avoid compaction, substrates with less fine particles and lower bulk densities should be used. Substrates with many fine particles decrease the pore space through which the root tips may penetrate. Bulk density increases soil strength and more energy is required by the root tips to penetrate the substrate.

Root pruning in container-grown plants - whether pruned using chemical or air-pruning methods is an efficient way of reducing root deformities. In *Pinus radiata* air pruning containers produced seedlings with roots having a ‘fish-bone’ appearance where no L-

shaped roots or spiral roots were found compared to smooth plastic walled containers where 50% of the seedlings had L-shaped roots (**ORTEGA *et al*., 2006**). In this study, the use of polystyrene containers did not reduce the severity or frequency of root deformities, particularly in seedlings that had been transplanted. The use of polystyrene containers improved root architecture slightly in seedlings transplanted to 128- and 72-cavity containers as well as for all the directly-sown seedlings. This was due to the root pruning properties of the copper oxychloride **(MATHERS *et al*., 2007)** with which the polystyrene trays had previously been treated prior to transplanting and/or sowing (FIGURE 4.7).

FIGURE 4.7. The fibrous root systems of *E. dunnii* seedlings grown in polystyrene 72-cavity containers.

4.5. CONCLUSIONS

- Transplant shock was observed in transplanted seedlings and was caused by a variety of different factors that all contributed to decreased seedling growth.
- Further optimisation of the transplanting protocol is required to ensure the production of high quality seedlings to limit delayed growth after transplanting.
- While root deformation was observed in all treatments, transplanting increased the frequency and severity of root deformities.
- Although, an increase in container volume increased seedling size, seedling quality was influenced by planting density restrictions over time.

- There is a need to investigate the effects of spacing seedlings in the nursery, to decrease light competition for the production of robust seedlings suitable for field conditions.

CHAPTER 5

Substrate composition for container-to-container transplanting

5.1. INTRODUCTION

Substrate composition should be optimised according to the propagation techniques and container design used for seedling production (**SALIFU *et al*., 2006; MATHERS *et al*., 2007; CHOI *et al*., 2012; BANACH *et al*., 2013**). Chemical and physical properties of the substrate affect seedling growth and development. These properties affect the quantity of water that is accessible to the plants, the volume of oxygen available for respiration and the amount of nutrients available for seedling growth (**HANDRECK & BLACK, 1994; MATHERS *et al*., 2007; SURRAGE *et al*., 2010; CHOI *et al*., 2012; BANACH *et al*., 2013**). These properties can be altered using varying amounts of individual substrate components to produce optimal growing conditions for seedling production (**HANDRECK & BLACK, 1994; MATHERS *et al*., 2007; CHOI *et al*., 2012**).

The aims of this experiment were (1) to determine how the physical properties of various substrates influenced the growth and development of directly sown and transplanted seedlings; and (2) to determine the optimal substrate for the production of high quality seedlings in MP544 (mini-plug 544-cavity) and Unigro® 128 trays.

5.2. MATERIALS AND METHODS

5.2.1. Seed germination and seedling cultivation in mini-plug containers

An experiment was conducted to assess the effects of various substrates on the growth and development of mini-plug seedlings. The MP544 trays previously dipped in copper oxychloride, and Unigro®128 trays were filled with twelve different substrate combinations that were mixed using the ratios outlined in TABLE 5.1. Pine bark was sourced from Organics for Africa in Pietermaritzburg, South Africa. The coir imported from Sri Lanka was supplied by CPS Nursery in Greytown, South Africa and the perlite was supplied by Pratley's in Johannesburg, South Africa.

Eucalyptus dunnii seed, stock number M9728, supplied by Mondi's Mountain Home Nursery in Hilton was hand sown into the prepared MP544 trays, with one seed per cavity. The germination and propagation conditions used in Chapter 3, section 3.2.1, with the exception of the substrate composition, remained the same. Eight weeks after sowing, the seedlings were transplanted into Unigro®128-trays containing the same substrate from which they had originally been grown (S1 to S1, S2 to S2, ect.).

At the same time as the seed was sown into the MP544 trays, *Eucalyptus dunnii* seed, stock number M9728 was sown into previously prepared Unigro®128 trays. As with the mini-plug treatment the germination and propagation conditions remained the same as those described in Chapter 3, section 3.2.1, with the exception of the substrate composition. The resulting seedlings were not transplanted after eight weeks and were referred to as the directly-sown treatment for purposes of clarity.

TABLE 5.1. The twelve substrate combinations evaluated for transplanting mini-plug seedlings.

Substrate #	**Substrate composition (v/v or v/v/v)**
S1	10-mm pine bark
S2	6-mm pine bark
S3	90% 10-mm pine bark: 10% perlite
S4	90% 6-mm pine bark: 10% perlite
S5	80% 10-mm pine bark: 20% perlite
S6	80% 6-mm pine bark: 20% perlite
S7	80% 10-mm pine bark: 10% perlite: 10% coir
S8	80% 6-mm pine bark: 10% perlite: 10% coir
S9	70% 10-mm pine bark: 20% perlite: 10% coir
S10	70% 6-mm pine bark: 20% perlite: 10% coir
S11	80% 10-mm pine bark: 10% perlite: 10% vermiculite
S12	80% 6-mm pine bark: 10% perlite: 10% vermiculite

5.2.2. Experimental design

All treatments were repeated four times in a randomised complete block design with two MP544 trays and two Unigro®128 trays per replicate, each tray contained six substrates. In total 160 seeds per substrate were sown into MP544 trays and 32 seeds per substrate were sown into the Unigro®128 trays.

5.2.3. Evaluation of substrate properties

The total porosity, air-filled porosity and water holding capacity of all the substrate compositions were determined using a modified method described by **HANDRECK & BLACK** (**1994**). A 65-ml container was filled with substrate and was tapped lightly to ensure that the substrate had settled in the container. The substrate was drenched in water until saturation point which was observed when the surface of the substrate began to glisten. The volume of water used to saturate the substrate was recorded. The substrate was allowed to drain for one hour and the water that had drained from the substrate was collected. The volume of water drained was recorded and the total porosity, water holding capacity and air-filled porosity calculated using the following formulae:

Total Porosity was calculated as: $\frac{S \times 100}{C}$

Air-filled porosity was calculated as: $\frac{D \times 100}{C}$

Water holding capacity was calculated as:

Total porosity – Air-filled porosity

Where:

S - volume of water required to saturate the substrate, D - volume of water collected, C - total container volume.

These measurements were repeated ten times and the mean air-filled porosity, water holding capacity and total porosity were determined.

5.2.4. Seedling growth, quality and root development

Two weeks after sowing, germination and shoot height of the seedlings cultivated in MP544 cavity trays were recorded. Thereafter, shoot height of ten samples per rep per treatment was measured every two weeks up until eight weeks to determine the influence of growth media on shoot growth in the mini-plug containers. After eight weeks 20 seedlings were destructively harvested to measure shoot height, root collar diameter, root length, dry shoot and dry root mass. These characteristics were measured using the same procedures described in Chapter 3, section 3.2.4. The incidence and severity of J-rooting and root coiling was determined using the method previously described in Chapter 3, section 3.2.5. A root growth potential test, as described in Chapter 3, section 3.2.3 was carried out on seedlings grown in MP544 trays to assess the effects of substrate composition on new root initiation.

After transplanting, shoot height of the same ten samples per rep per treatment was measured once every two weeks until the seedlings were 20 weeks old. In the directly-sown treatments, the seedling height of ten samples per rep per treatment was initially measured two weeks after sowing, and thereafter every two weeks until the seedlings were 20 weeks old.

At 20 weeks, both directly-sown and transplanted seedlings of eight seedlings per treatment (two seedlings per replicate) were destructively harvested to measure shoot height, root collar diameter, root length, dry shoot and dry root mass to evaluate the effects of growth media on the final seedling quality using the same procedures described in Chapter 3, section 3.2.4.

In order to determine the differences in the ability of the seedlings to initiate roots, both directly-sown and transplanted seedlings cultivated in the 12 different growth media were subjected to a root growth potential test described in Chapter 3, section 3.2.3. The influence of transplanting versus directly-sowing in the 12 growth media tested on root structure was determined by measuring the incidence and severity of J-rooting and root coiling using the methods described in Chapter 3, section 3.2.5.

5.2.5. Data analysis

Mean values for seedling parameters measured were compared using an analysis of variance (ANOVA) and significant means were separated using Tukey's test at a 95% confidence interval using SAS Enterprise Guide 5.1, 2012.

5.3. RESULTS

5.3.1. Substrate properties

The total porosity of the substrates tested ranged from 44% to 67%, with the highest air-filled porosity measured in S5 and the lowest in S8. The highest water holding capacity was recorded for S4 and the lowest in S12 (TABLE 5.2).

TABLE 5.2. Physical properties of the twelve substrate combinations. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Substrate	Total porosity (%)	Air-filled porosity (%)	Water holding capacity (%)
S1	60.3 ± 2.54^{cd}	41.6 ± 2.90^{ab}	18.7±2.10
S2	58.0 ± 3.02^{de}	22.5 ± 5.08^{ef}	35.4 ± 4.13^{b}
S3	62.7 ± 2.14^{bc}	40.9 ± 3.11^{ab}	21.8 ± 3.63^{cd}
S4	67.0 ± 4.15^{a}	24.5 ± 2.30^{de}	42.5 ± 3.34^{a}
S5	64.3 ± 1.53^{ab}	45.0 ± 4.43^{a}	19.2 ± 4.38^{de}
S6	62.5 ± 3.22^{bc}	29.6 ± 3.21^{c}	32.9 ± 3.36^{b}
S7	48.5 ± 1.57^{f}	31.3 ± 3.60^{c}	17.2 ± 2.65^{e}
S8	44.4 ± 2.89^{g}	18.5 ± 2.28^{f}	25.8 ± 1.91^{c}
S9	47.4 ± 1.68^{fg}	28.5 ± 2.28^{cd}	18.8±1.95
S10	49.9 ± 1.94^{f}	31.0 ± 1.52^{c}	18.8 ± 2.10^{de}
S11	56.2 ± 2.29^{e}	38.6 ± 1.88^{b}	17.5 ± 1.93^{de}
S12	57.5 ± 2.38^{de}	42.5 ± 5.34^{ab}	15.0 ± 3.84^{e}
LSD	3.82	5.05	4.61

5.3.2. Effect of substrate composition on germination

The highest germination results were found in substrate S1 for seeds sown in both MP544 and Unigro® 128 trays. However, the germination percentage was only significantly higher than substrate S8 when the seeds were sown into the MP544 trays and substrate S2 when sown in Unigro® 128 trays (FIGURE 5.1). No interaction between container type and substrate composition were observed.

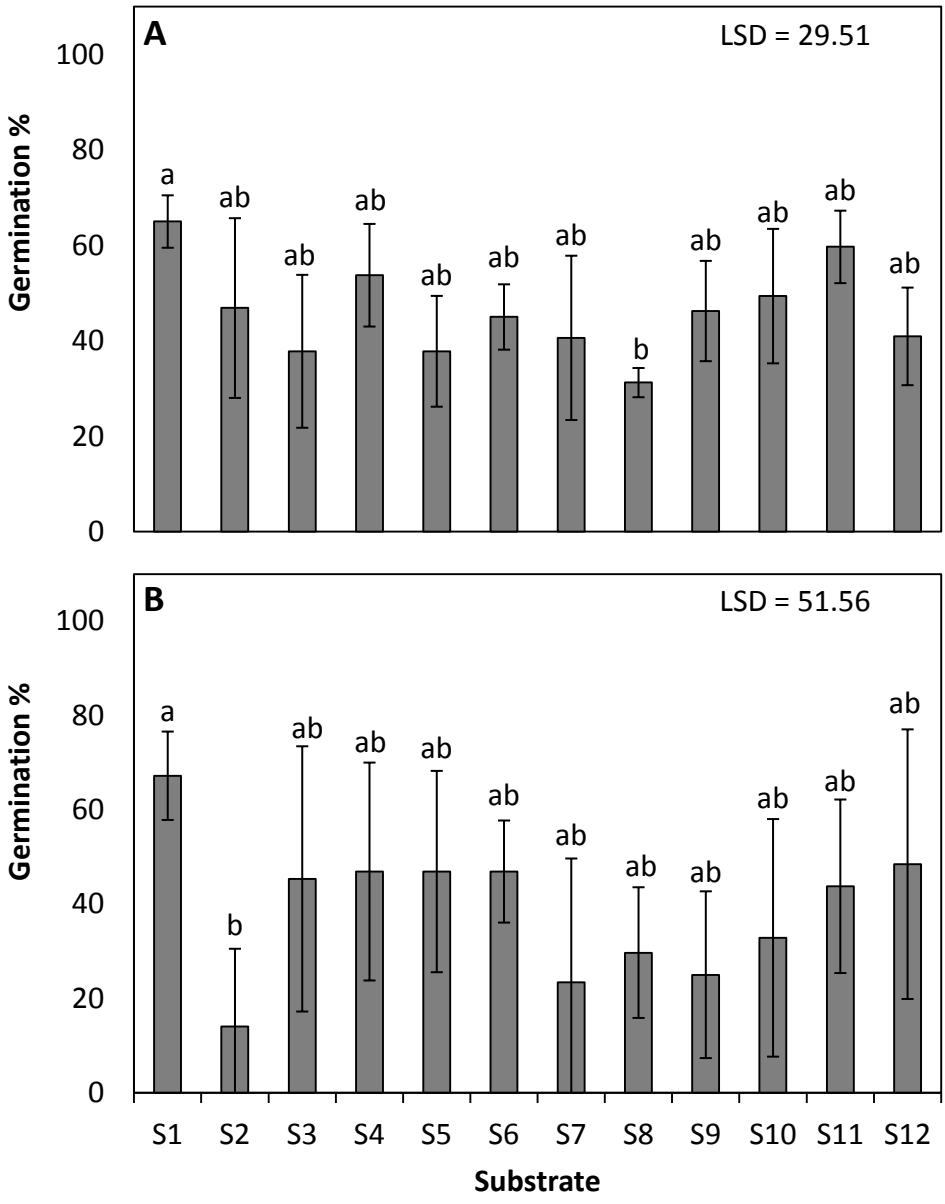

FIGURE 5.1. Germination of *E. dunnii* seedlings cultivated in MP544 (A) and Unigro® 128 trays (B) with twelve substrate combinations. Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

5.3.3. Seedling growth

Growth of seedlings in Unigro® 128 trays (directly-sown) was slower than seedlings grown in MP544 trays (transplanted) in all substrates except S3, S4, S5, S6, and S8 during the first eight weeks after sowing. In substrates S3, S4, S5 and S6 seedling growth in Unigro® 128 trays (directly-sown) began to increase after six weeks and at eight weeks, these seedlings were taller compared to MP544-cultivated seedlings (FIGURE 5.2). Seedlings directly sown into substrate S12 grew at approximately the same rate as those that had been sown into MP544 trays with the same substrate.

After transplanting (MP544) at eight weeks, seedlings originally grown in MP544 trays (transplanted seedlings) in substrates S9 and S10 continued to grow faster than the directly-sown (Unigro® 128 trays) seedlings. However, after eight weeks, seedlings grown in Unigro® 128-cavity trays (directly-sown) in substrates S1, S3, S4, S5, S6, S8 and S12 grew more rapidly than transplanted seedlings that had originally been cultivated in the MP544 trays with the same substrates. The growth rate for directly-sown (Unigro® 128 trays) and transplanted (MP544) seedlings appeared to be similar when seedlings were grown in substrates S2, S7 and S11 from eight to 20 weeks (FIGURE 5.2).

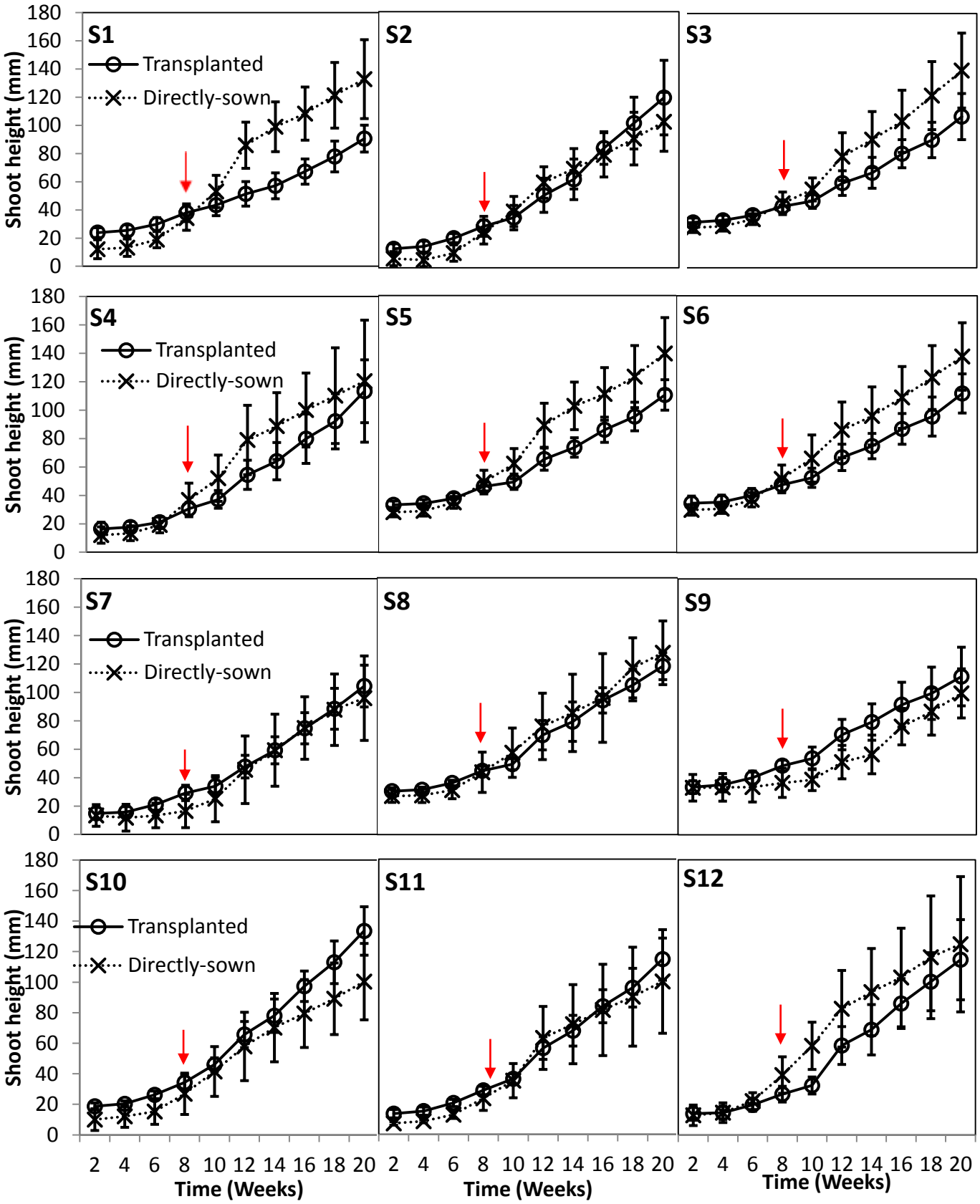

FIGURE 5.2. Growth of *E. dunnii* seedlings before and after transplanting into twelve different substrates (listed in TABLE 5.1). Seedlings were transplanted at week eight, as indicated by the arrows. Error bars indicate S.D.

5.3.4. Mini-plug seedling characteristics

The morphological parameters measured (shoot height, root collar diameter, dry root mass and dry shoot mass) were varied between substrates. The tallest seedlings were grown in S10 and were only significantly ($p<0.05$) taller than substrates S3, S4, S5, S6 and S12 with the shortest seedlings cultivated in S4 which were only significantly ($p<0.05$) shorter than S9, S10 and S11. Root collar diameter was greatest in S11 but was only significantly different to seedlings grown in S4 which exhibited the lowest mean root collar diameter. No significant ($p>0.05$) differences were found between dry shoot mass and dry root mass between treatments. The greatest dry root mass was observed in seedlings grown in S1, whilst S2 seedlings exhibited the lowest dry root mass (TABLE 5.3).

TABLE 5.3. Morphological characteristics of *E. dunnii* seedlings grown in MP544 trays for two months. Different letter notations represent significant differences between means (±S.D.) at $p<0.05$.

Substrate	Shoot height (mm)	Root collar diameter (mm)	Root dry mass (mg)	Shoot dry mass (mg)	Sturdiness ratio	Shoot-to-root ratio
S1	30.65± 5.30^{abcd}	0.45± 0.07^{a}	4.16± 1.32^{a}	15.98± 4.74^{a}	67.81± 15.11^{ab}	3.85± 1.43^{a}
S2	31.45± 5.93^{abcd}	0.44± 0.07^{ab}	2.99± 1.48^{a}	13.61± 6.02^{a}	71.56± 9.96^{ab}	4.56± 3.12^{a}
S3	30.40± 5.49^{bcd}	0.47± 0.05^{a}	3.90± 1.79^{a}	15.06± 4.67^{a}	65.03± 8.39^{ab}	6.65± 5.73^{a}
S4	27.95± 3.99^{d}	0.36± 0.07^{b}	4.14± 1.80^{a}	12.08± 3.60^{a}	77.00± 17.54^{ab}	2.92± 0.98^{a}
S5	28.90± 3.85^{cd}	0.44± 0.08^{ab}	3.58± 1.46^{a}	13.97± 4.85^{a}	65.31± 17.52^{b}	3.91± 1.15^{a}
S6	29.45± 5.53^{bcd}	0.42± 0.05^{ab}	4.08± 1.62^{a}	13.03± 4.25^{a}	69.70± 11.52^{ab}	3.20± 1.50^{a}
S7	31.25± 5.73^{abcd}	0.46± 0.08^{a}	3.43± 1.43^{a}	14.30± 6.12^{a}	67.42± 12.58^{b}	4.17± 2.55^{a}
S8	30.80± 4.03^{abcd}	0.48± 0.08^{a}	3.60± 1.91^{a}	12.87± 5.39^{a}	64.71± 10.17^{ab}	3.57± 3.11^{a}
S9	33.80± 4.73^{abc}	0.46± 0.09^{a}	3.81± 1.04^{a}	16.97± 4.24^{a}	72.84± 20.69^{ab}	4.46± 1.57^{a}
S10	35.80± 5.69^{a}	0.46± 0.09^{a}	3.93± 1.41^{a}	16.03± 5.16^{a}	78.51± 18.41^{a}	4.08± 1.68^{a}
S11	34.25± 4.77^{ab}	0.49± 0.10^{a}	3.77± 1.47^{a}	18.18± 5.26^{a}	69.97± 16.53^{ab}	4.82± 4.56^{a}
S12	30.45± 3.67^{bcd}	0.44± 0.08^{ab}	3.01± 1.33^{a}	13.48± 3.94^{a}	70.00± 11.81^{ab}	4.48± 3.90^{a}
LSD	5.18	0.08	1.59	16.09	15.34	3.12

5.3.5. Root development of mini-plug seedlings

Seedlings grown in substrate S2 had the lowest overall root growth potential. These seedlings initiated the least number of new roots with the lowest new root mass. Seedlings produced from substrates S1 and S4 initiated the most new roots but not the greatest new root mass which was found in substrates S9 and S11 but not significantly different ($p>0.05$) (FIGURE 5.3).

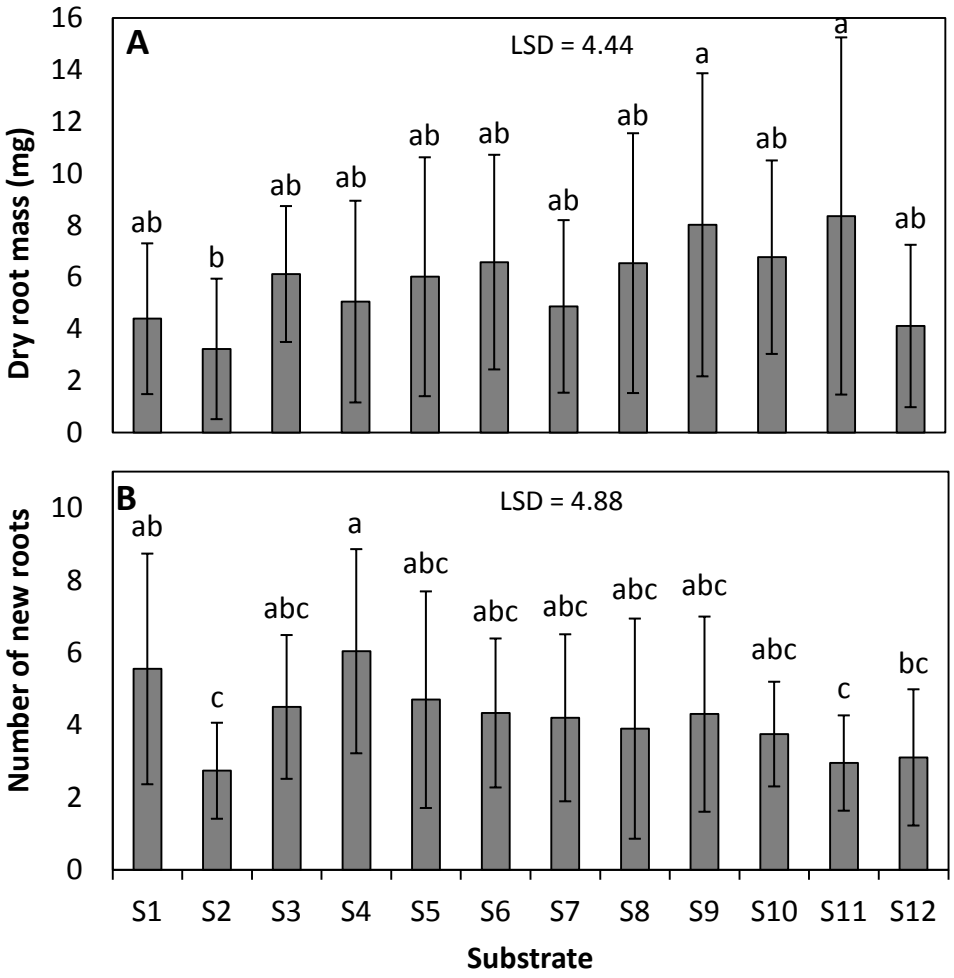

FIGURE 5.3. The dry mass (A) and number of new roots (B) developed during a root growth potential test of mini-plug grown *E. dunnii* seedlings. Different letters represent significant differences between means at $p<0.05$. Error bars represent S.D.

Although J-rooting and root coiling were observed for all treatments, the severity of the J-rooting and coiling ranged between scores of one to three. J-root and root coiling scores of one and two have been defined as acceptable and three as undesirable, but acceptable (Chapter 3). The lowest frequency of J-roots was seen in substrate S1 and the highest in S11.

The frequency of root coiling was highest when seedlings were grown in S11 and S12 with the lowest observed in S5. Seedlings that were cultivated in S2 exhibited J-rooting and root coiling scores of 3 (FIGURE 5.4).

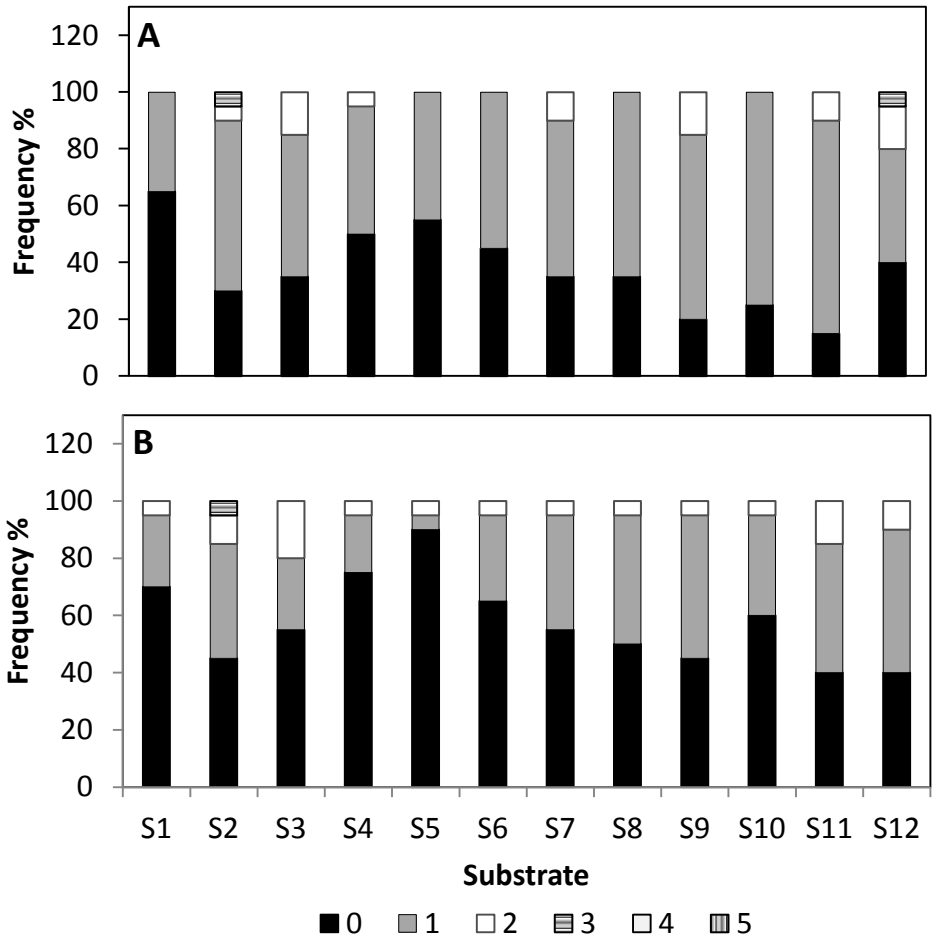

FIGURE 5.4. Frequency of J-rooting (A) and root coiling (B) in *E. dunnii* seedlings grown in MP544 trays filled with the different substrates detailed in TABLE 5.1 two months after sowing. Different shading within bars (outlined by the graph key) represents the frequency of J-rooting and root coiling severity according to the scoring system outlined in TABLE 3.2.

5.3.6. Seedling characteristics

Differences in the morphological characteristics were observed between treatments; however, no one substrate appeared to produce consistently better morphological characteristics between the substrates tested for both directly-sown and transplanted seedlings (TABLE 5.4 and TABLE 5.5). Shoot height of directly-sown seedlings was highest in S3 and was only significantly ($p<0.05$) higher than S2, S9 and S11. Root collar diameter of

directly-sown seedlings was found to be greatest when seedlings were grown in S12 and was only significantly ($p<0.05$) greater than S2 and S9. In directly-sown seedlings, substrate S7 produced seedlings with significantly more leaves than substrate S1, S4, S9, and S11. Substrate S7 cultivated seedlings also exhibited a greater dry root mass in directly-sown seedlings which was significantly ($p<0.05$) greater than S1, S2, S3, S4, S5, S9, S10 and S11. The dry shoot mass was highest when seedlings were grown in S8 and was significantly greater than S2, S3, S9, S10 and S11 (TABLE 5.4.)

TABLE 5.4. Morphological characteristics of directly-sown *E. dunnii* seedlings 20 weeks after sowing. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Substrate	Shoot height (mm)	Root collar diameter (mm)	Number of leaves	Dry root mass (mg)	Dry shoot mass (mg)	Shoot-to-root ratio	Sturdiness ratio
S1	153.38± 14.99^{abc}	1.93± 0.25^{abcd}	21± 4.2^{b}	154.38± 38.63^{bc}	410.66± 143.27^{abcd}	2.66± 0.48^{a}	79.47± 9.54^{a}
S2	112.25± 31.29^{c}	1.67± 0.44^{cd}	28± 17.11^{ab}	159.45± 85.75^{bc}	296.04± 187.42^{bcd}	1.86± 0.28^{bc}	67.27± 12.34^{a}
S3	169.13± 26.32^{a}	2.12± 0.32^{abcd}	23± 10.15^{ab}	112.84± 40.17^{c}	227.64± 90.92^{c}	2.02± 0.43^{cd}	79.73± 10.96^{a}
S4	160.13± 18.03^{ab}	1.90± 0.15^{abcd}	18± 4.96^{b}	133.73± 30.21^{bc}	337.94± 63.88^{abcd}	2.53± 0.49^{ab}	84.17± 12.49^{a}
S5	154.63± 29.02^{abc}	1.93± 0.21^{abcd}	23± 15.73^{ab}	149.10± 50.21^{bc}	318.45± 96.47^{abcd}	2.14± 0.41^{abc}	80.27± 16.47^{a}
S6	158.25± 24.48^{ab}	2.22± 0.17^{ab}	31± 20.06^{ab}	218.69± 48.65^{ab}	420.94± 115.98^{abcd}	1.92± 0.38^{abc}	71.20± 8.11^{a}
S7	157.13± 30.19^{ab}	2.20± 0.36^{abc}	42± 15.28^{a}	279.34± 53.09^{a}	459.64± 199.14^{abc}	1.65± 0.60^{c}	71.42± 12.94^{a}
S8	153.38± 21.12^{abc}	2.23± 0.29^{ab}	27± 9.05^{ab}	260.25± 64.81^{a}	546.65± 248.39^{a}	2.10± 0.76^{abc}	68.78± 11.13^{a}
S9	123.00± 33.89^{bc}	1.60± 0.45^{d}	17± 5.42^{b}	155.95± 80.26^{bc}	244.95± 139.08^{bcd}	1.57± 0.55^{c}	76.81± 15.72^{a}
S10	138.25± 15.06^{abc}	1.92± 0.29^{abcd}	27± 9.38^{ab}	144.20± 45.93^{bc}	295.83± 102.18^{bcd}	2.05± 0.34^{abc}	71.91± 13.11^{a}
S11	123.38± 33.61^{bc}	1.72± 0.36^{abcd}	15± 3.60^{b}	123.60± 38.45^{bc}	209.71± 65.84^{d}	1.70± 0.28^{c}	71.83± 12.93^{a}
S12	164.25± 24.63^{ab}	2.37± 0.38^{a}	31± 16.39^{ab}	276.31± 74.97^{a}	479.35± 151.51^{ab}	1.73± 0.38^{c}	69.41± 9.70^{a}
LSD	43.76	0.54	20.61	95.75	242.24	0.79	20.75

In the transplanted seedlings (TABLE 5.5), S12 produced the tallest seedlings and were significantly ($p<0.05$) taller than S1, S2, S3, and S5. Root collar diameter was significantly ($p<0.05$) greater with S6 compared to S1, S2, S3, S5, S9 and S11. No significant ($p>0.05$) differences were observed between substrate treatments with respect to leaf number, although seedlings from S12 had the highest number of leaves. Root and shoot mass was found to be higher in S3 and lowest in S1. Root mass of the S3-grown seedlings was significantly ($p<0.05$) greater compared to S1, S2, S4, S5, S9 and S11. Shoot mass of the S3 seedlings were significantly ($p<0.05$) higher than S1, S2, S5, S7, S8, S9 and S11 (TABLE 5.5).

In all criteria assessed, the directly-sown seedlings (TABLE 5.4) performed better than transplanted seedlings, with a few exceptions (TABLE 5.5). Transplanted seedlings cultivated with substrate S2 exhibited taller seedlings; S4 and S9 exhibited greater root collar diameters. Greater dry root mass, dry shoot mass and leaf number were observed when seedlings were propagated in S11 and seedlings grown in substrate S3 exhibited a greater dry root and shoot mass when compared to their directly-sown counterparts. Generally, transplanted seedlings (TABLE 5.5) had superior proportions compared to the directly-sown seedlings (TABLE 5.4). Sturdiness ratios were lower in transplanted seedlings in all substrates except S2, S7 and S12 (TABLE 5.5). The shoot-to-root ratios of directly-sown seedlings (TABLE 5.4) were only found to be less than the transplanted seedlings (TABLE 5.5) when they had been cultivated in S6, S7 and S12.

TABLE 5.5. Morphological characteristics of transplanted *E. dunnii* seedlings, 20 weeks after sowing and 12 weeks after transplanting. Different letters represent significant different means (±S.D.) at $p<0.05$.

Substrate	Shoot height (mm)	Root collar diameter (mm)	Number of leaves	Dry root mass (mg)	Dry shoot mass (mg)	Shoot-to-root ratio	Sturdiness ratio
S1	96.38± 8.73^{c}	1.42± 0.05^{d}	15± 4.24^{a}	92.45± 15.93^{c}	148.65± 21.34^{d}	1.61± 0.31^{ab}	67.75± 6.64^{a}
S2	113.00± 12.72^{bc}	1.59± 0.12^{cd}	14± 4.03^{a}	131.19± 30.21^{bc}	174.80± 39.94^{cd}	1.33± 0.28^{b}	70.96± 7.32^{a}
S3	115.25± 13.05^{bc}	1.73± 0.23^{bcd}	14± 4.16^{a}	205.79± 85.86^{a}	415.20± 135.19^{a}	2.02± 0.45^{a}	66.52± 11.54^{a}
S4	127.13± 17.77^{ab}	1.91± 0.25^{abc}	14± 4.47^{a}	133.81± 33.27^{bc}	286.13± 97.36^{abc}	2.14± 0.52^{a}	66.47± 7.22^{a}
S5	116.50± 16.46^{bc}	1.70± 0.16^{bcd}	17± 8.92^{a}	122.05± 17.26^{bc}	205.11± 50.34^{cd}	1.68± 0.38^{ab}	68.38± 11.84^{a}
S6	146.88± 22.34^{a}	2.08± 0.16^{a}	15± 7.85^{a}	156.61± 14.97^{abc}	349.13± 83.31^{ab}	2.23± 0.62^{a}	70.61± 10.10^{a}
S7	141.50± 18.71^{ab}	1.79± 0.19^{abc}	12± 2.27^{a}	147.31± 37.47^{abc}	264.24± 39.47^{bcd}	1.79± 0.29^{ab}	79.16± 10.31^{a}
S8	123.63± 15.58^{abc}	1.92± 0.26^{abc}	15± 5.44^{a}	153.60± 87.70^{abc}	260.00± 87.71^{bcd}	1.69± 0.47^{ab}	64.35± 11.47^{a}
S9	119.88± 11.16^{abc}	1.64± 0.26±^{cd}	15± 7.56^{a}	128.28± 34.05^{bc}	199.13± 43.56^{cd}	1.55± 0.39^{ab}	73.04± 13.16^{a}
S10	125.50± 20.95^{ab}	1.77± 0.19^{abc}	14± 4.92^{a}	151.88± 28.19^{abc}	285.43± 91.59^{abc}	1.88± 0.45^{ab}	71.00± 12.24^{a}
S11	121.38± 20.74^{abc}	1.70± 0.19^{bcd}	16± 6.25^{a}	132.28± 34.37^{bc}	211.45± 68.94^{cd}	1.60±0. 23^{ab}	71.55± 10.05^{a}
S12	148.38± 22.41^{a}	2.02± 0.23^{ab}	20± 6.54^{a}	183.91± 42.71^{ab}	345.71± 111.67^{ab}	1.88± 0.34	73.50± 16.99^{a}
LSD	29.05	0.34	9.83	65.08	134.13	0.69	18.97

5.3.7. Root development after transplanting

Although differences in root initiation were noticed between treatments, they were not significant ($p>0.05$) (FIGURE 5.5). The mean number of new roots initiated was only greater in directly-sown seedlings when the seedlings had been grown in substrates S1, S2, S6, S7 and S12 (FIGURE 5.5A). Seedlings directly-sown in substrate S4 initiated the same number of new roots during the root growth potential test as those seedlings that had been transplanted into the same substrate. Seedlings raised in substrates S3, S5, S8, S9, S10 and S11 initiated more new roots during the root growth potential test when the seedlings had been transplanted compared to when the seedlings where directly-sown (FIGURE 5.5A). In nine out of the twelve substrates tested, transplanted seedlings had longer roots than seedlings that had been directly sown (FIGURE 5.5B). Of the directly-sown treatments, the substrates that exhibited a greater dry root mass than the transplanted seedlings were S1, S6, S7, S10 S11 and S12 (FIGURE 5.5C). When the seedlings had been transplanted, S3 initiated the highest number of new roots, S11 the longest roots and S4 the greatest new root mass compared to the other substrates tested. In contrast, S2 initiated the least number of new roots, S10 had the shortest root length and S1 had the lowest dry root mass. Seedlings cultivated directly in S12 exhibited the highest number of new roots, root length and dry new root weight within the substrate tested. Seedlings that were directly grown in S6 and S12 exhibited more new roots, longer root length and greater new dry root mass than their transplanted counterparts. Transplanted seedlings grown in S5, S8 and S9 produced greatest number of new roots, the longest root length and highest dry new root mass compared to their directly sown counterparts (FIGURE 5.5).

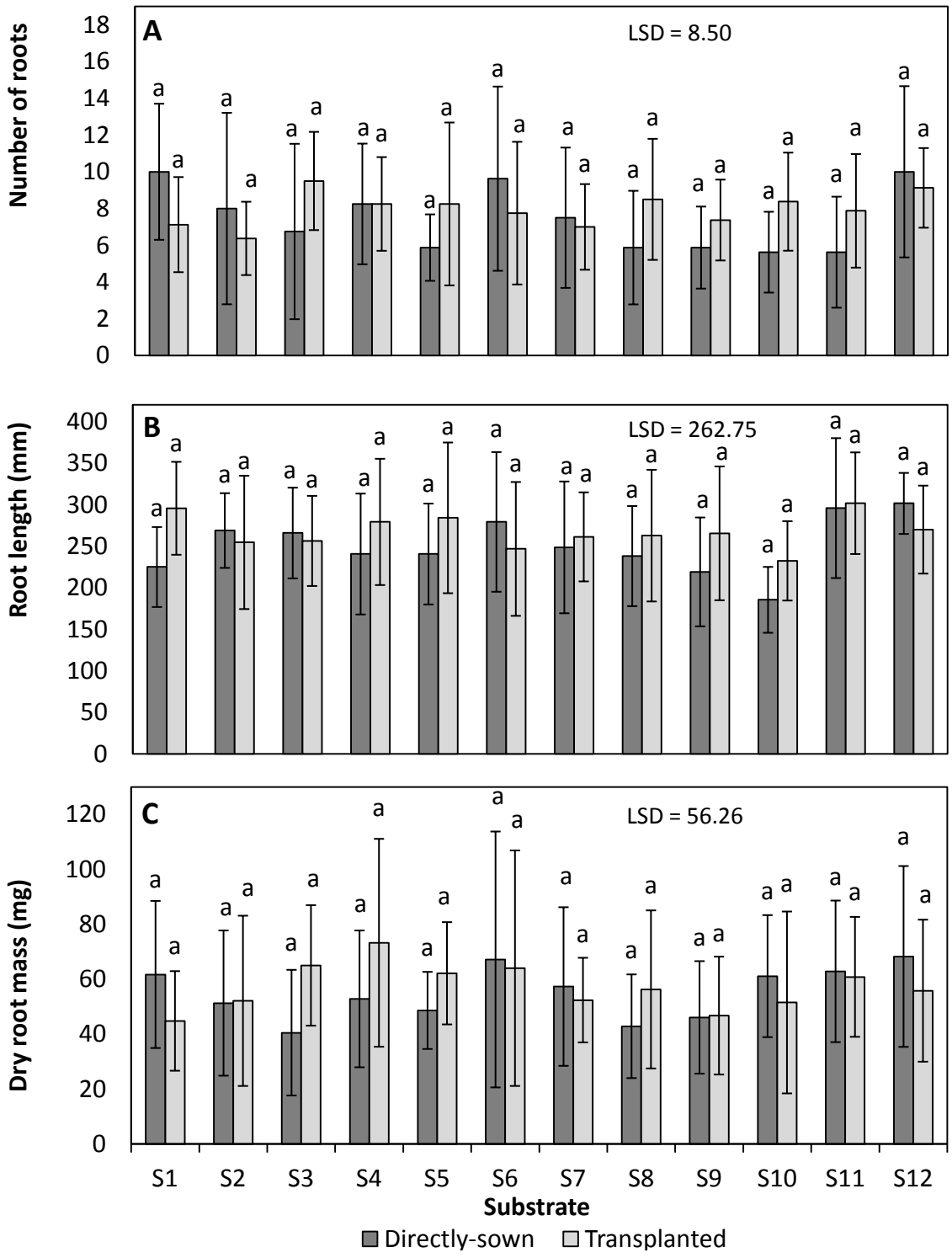

FIGURE 5.5. Number of new roots (A), root length (B) and the new root dry mass (C) of *E. dunnii* seedlings as determined after a root growth potential test on directly-sown and transplanted seedlings subjected to different substrates (TABLE 5.1). Different letters represent significantly different means at $p<0.05$. Error bars indicate S.D.

All treatments exhibited varying degrees of root deformities (FIGURE 5.6). The frequency of J-rooting and root coiling in the directly-sown treatments ranged from 75% to 100%, where three was recorded as the highest J-rooting (S9) and root coiling (S8) score (FIGURES 5.6A and C). All the directly-sown seedlings that were assessed from substrates S6, S8, S9 and S10 exhibited both J-roots and root coils (FIGURES 5.6A and C). The lowest incidence of J-roots was recorded in S12-cultivated seedlings (FIGURE 5.6A). Treatments S5, S11 and S12 exhibited the least number of seedlings that had root coiling within all substrates tested when the seedlings had been propagated directly (FIGURE 5.6C).

The incidence and severity of J-rooting and root coiling was greater in the seedlings that had been transplanted (FIGURES 5.6B and D). The incidence of J-rooting and coiling ranged from 75% to 100% with the highest J-rooting score observed being five and the highest root coiling score being four (FIGURES 5.6B and D). All the seedlings assessed from S3, S4 S5, S11 and S12 had both J-roots and root coiling (FIGURES 5.6B and D). All the transplanted seedlings assessed in treatments S7 and S8 had J-rooting. Seedlings that had been transplanted to S2, S8, S9 and S10 exhibited J-rooting scores of one to two, which is considered acceptable. Seedlings transplanted to S1, S4, S6, and S7 had J-root scores of three, seedlings transplanted to S3, S11 and S12 had J-rooting scores of four, and seedlings transplanted to S5 exhibited J-rooting scores of three and five (FIGURE 5.6B). Seedlings that were transplanted to S2, S5, S7, S9 and S11 only exhibited coiling scores of one and two. Root coiling scores of three and/or four where observed in S1, S3, S4 S6, S8, S10 and S12 (FIGURE 5.6D).

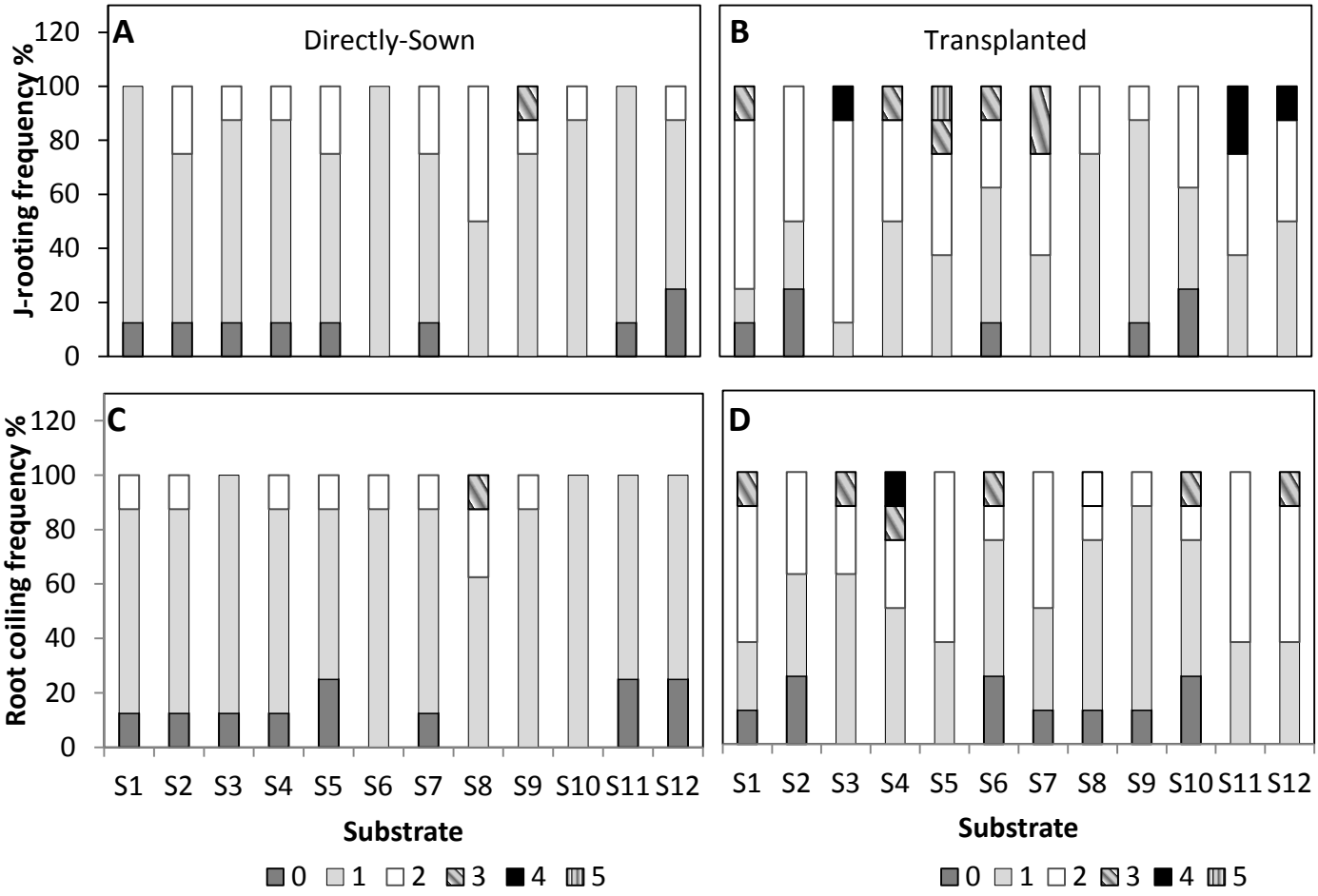

FIGURE 5.6. Effect of different substrate treatments on J-roots (directly-sown (A) and transplanted (B) *E. dunnii* seedlings) and root coiling (directly-sown (C) and transplanted (D) *E. dunnii* seedlings) frequency after five months. Different shading within bars (outlined by the graph key) represents the degree of J-roots and root coils according to the scoring system outlined in TABLE 3.2.

5.4. DISCUSSION

The morphological differences observed between seedlings from the different treatments were caused by the physical and chemical properties of the substrates tested. Physical properties of a substrate can have an impact on root system function, particularly with regards to water and nutrient uptake (**HANDRECK & BLACK, 1994; SIRIN *et al*., 2010**). Water holding capacity and air-filled porosity are important as these properties influence water availability, aeration and drainage, which in turn impacts on root growth, development and vitality thus affecting overall seedling growth and development (**NESMITH, 1999; SIRIN *et al*., 2010**).

Substrate S2 (6mm-pine bark) negatively affected the growth and development of roots when the seedlings had been cultivated in MP544 trays. These seedlings produced the lowest dry root weight and influenced the initiation of new roots during the root growth potential test. Substrate S2 did not drain well and was able to retain a large volume of water as was illustrated by the high water holding capacity and low air-filled porosity results. The poor root growth and low root viability suggested the development of anaerobic conditions due to insufficient drainage in S2 (**HANDRECK & BLACK, 1994; RICHIE, 2003; MATHERS *et al*., 2007**). In addition, in this study, the high water content of S2 in MP544 containers, coupled with insufficient drainage may have caused compaction of the substrate over time affecting root growth as suggested by a review of the literature (**HANDRECK & BLACK, 1994; RICHIE, 2003; MATHERS *et al*., 2007**). Compacted substrates have increased soil strength and small pores making it difficult for roots tips to penetrate the medium (**HANDRECK & BLACK, 1994; MATHERS *et al*., 2007**). The resulting effect is the development of a less extensive root system and smaller above-ground seedling mass since more energy is required for root growth (**ALAMEDA & VILLAR, 2012**). This was illustrated by the low dry shoot mass of the MP544 seedlings grown in S2. In contrast, seedlings grown in S1 (10-mm pine bark), S4 (90:10 6-mm pine bark: perlite (v/v) and S6 (80:20 6-mm pine bark: perlite (v/v)) exhibited the highest root mass of MP544-cultivated seedlings.

Whilst the water holding capacity of S4 was higher than for S2, the air-filled porosity was also higher, which indicated that S2 did not drain well and resulted in anaerobic conditions. Substrate S4 was mixed with perlite which is commonly used in substrate combinations to encourage aeration and drainage. Perlite increases water availability because water adheres to the perlite particle surface with a low surface tension making water easily accessible to the roots (**HARTMANN *et al*., 1997; AWANG *et al*., 2009; SIRIN *et al*., 2010**). Similarly, seedlings grown in S3 (90:10 10-mm pine bark: perlite (v/v)) after transplanting into Unigro® 128 trays from MP544 trays exhibited high dry root and shoot mass compared to the other transplanted treatments. The substrate had a high total porosity and air-filled porosity thus increasing drainage and oxygen exchange. In a study conducted on *Celosia cristata* seedlings, **AWANG *et al*.** (**2009**) demonstrated that differences in seedling morphology were caused by the physical properties of the substrates they had tested.

Particle size also increases drainage and aeration of a medium (**HANDRECK & BLACK, 1994; RICHIE, 2003; MATHERS *et al*., 2007**). In contrast to S2, S1 exhibited the highest air-filled porosity with a low water holding capacity although both substrates were 100% pine bark, the difference related to particle size, where substrate S2 was a 6-mm pine bark compared to the 10-mm particle size of S1. Seedlings from MP544 trays grown in S1 promoted root growth, development and root viability. These seedlings produced actively growing roots as they initiated the highest number of new roots during the root growth potential test and had the greatest root mass compared to all MP544 substrate treatments. Red Oak seedlings grown in three different substrate combinations of peat, coir, pine bark and vermiculite grew better when pine bark was present in greater proportion. The improved morphological characteristics were attributed to an increase in air-filled porosity. The increased air-filled porosity of the pine bark used in that study indicated that it had a greater average particle size than 6-mm pine bark (S2) (**SALIFU *et al*., 2006**).

In nursery substrates, it is important that a balance between air-filled porosity and water holding capacity should exist; a good substrate should have a high water holding capacity with enough large pores to allow for sufficient oxygen exchange (**REID, 2005; JACOBS *et al.,* 2009**). Root development and new root initiation in S12 (80:10:10 6-mm pine bark: perlite: coir) was lower than in S1 despite the higher air-filled porosity recorded in S12. Too high an air-filled porosity, with a low water holding capacity (as seen in S12), meant that the seedlings needed to be watered more frequently to prevent wilting. A higher water holding capacity will offer a buffer in the event of irrigation problems (**MATHERS *et al*., 2007**). Substrate S6 exhibited the best substrate balance between water holding capacity and air-filled porosity which was translated to overall seedling quality. Seedlings grown in this substrate produced seedlings with actively growing roots, a low sturdiness ratio and a lower shoot-to-root ratio.

The substrate requirements for development of good quality seedlings appeared to change after transplanting. This was observed in seedlings grown in substrate S1 which developed good root system whilst they were cultivated in the MP544 trays. However, after transplanting these seedlings into the same substrate, root growth declined as the dry root mass at 20 weeks was the lowest and affected the above-ground seedling development.

This may have been due to the increased irrigation requirements as the seedlings grew (**HANDRECK AND BLACK, 1994; HARTMANN *et al*., 1997**). Since the air-filled porosity of this substrate was high and the water holding capacity low, the substrate did not hold sufficient water to sustain increased growth. In contrast, seedlings transplanted to substrate S12 grew well after transplanting, despite the low water holding capacity. The reason for the improved growth of seedlings transplanted to S12 is, however, unclear. It may have been attributed to other chemical or physical properties of the substrate that were not evaluated during the course of this study.

Container depth affected substrate properties, which influenced seedling development and growth. The effects of the high water holding capacity of S2 was not as severe when the seedlings were grown directly into Unigro® 128 containers. Seedlings grown in S2 from Unigro® 128 containers were able to initiate an average of eight new roots, and the average dry root mass developed was not the lowest. Similar results were observed in Crimean juniper seedlings which were cultivated in 20-, 25- and 30-cm long containers in various substrates (**GÜLCÜ *et al*., 2010**). It was shown that seedlings from the 30-cm long containers produced superior morphological characteristics than both 20-and 25-cm deep containers (**GÜLCÜ *et al*., 2010**). Substrate water content decreases with increasing container depth (**HANDRECK & BLACK, 1994**) and in this study Unigro® 128 trays were deeper (100 mm) when compared to the MP544 cavities (30 mm).

Substrate type did not eliminate the negative effects of transplanting. In most substrates tested, directly-sown seedlings had taller, thicker, heavier seedlings with more leaves and were, therefore, more metabolically active compared to transplanted seedlings. This indicates that transplanted seedlings experienced some form of stress after transplanting. Transplanting stress is often caused by mechanical damage to the root system and the disturbance of the root-to-substrate contact (**GUEHL *et al.*, 1989**). Root growth potential tests provides an indication of the root system viability of a seedling (**GROSSNICKLE, 2005**), and since transplanted treatments generally exhibited lower root growth potential results than directly-sown seedlings, this suggests that the root systems of the transplanted seedlings were damaged during transplanting. This may have negative effects when seedlings are planted in the field, where the conditions are harsher than in the nursery

environment. Seedlings produced in the nursery should have actively growing roots systems that are able to initiate new roots quickly to reduce water stress, thereby improving seedling survival in the field (**NESMITH, 1999; GROSSNICKLE, 2005; JOHANSSON *et al*., 2012**).

Whilst all seedlings exhibited root deformities, transplanted seedlings exhibited more severe incidences of J-rooting and coiling. Since all treatments exhibited root deformities; indicates that these were due to the cultivation of the seedlings in the containers rather than the transplanting process. However, transplanting did enhance the development of more severe root deformities. Substrate composition did not eliminate the development of J-roots and root coiling.

5.5. CONCLUSIONS

- Air-filled porosity and water holding capacity were critical to root development which influenced above-ground seedling development.
- Propagation method influenced the selection of suitable substrate composition.
- Transplanting of seedlings increased the development of root deformities, regardless of the substrate composition.
- All transplanted seedlings were subject to transplanting stress regardless of the substrate composition used.
- Substrate S6, the 80:20 6-mm pine bark: perlite (v/v) substrate combination was chosen as the optimal substrate for the cultivation of seedlings in MP544 trays. Seedlings grown in this substrate produced seedlings with actively growing roots, low sturdiness and shoot-to-root ratios. This substrate also exhibited a good balance between air-filled porosity and water holding capacity with a total porosity above 60% as recommended by **HANDRECK & BLACK** (**1994**).
- Substrate S3, the 90:10 6-mm pine bark: perlite (v/v) substrate combination was selected as the optimal final substrate for transplanting seedlings into Unigro® 128 trays. This substrate exhibited a total porosity of 67%, a high air-filled porosity and

moderate water holding capacity. Seedlings cultivated in this medium produced higher dry shoot mass, low sturdiness ratios and actively growing roots.

CHAPTER 6

The use of supplements to enhance seedling growth

6.1. INTRODUCTION

Supplements have been used to stimulate improved seed germination, root development, seedling growth and development (**JANEICKE, 1999**). Various types of supplements are available on the market today, however, only the application of vermicompost, seaweed extract and smoke-water were investigated in this study.

Vermicompost is produced by the degradation of waste products using earthworms (**BACHMAN & METZGER, 2007; KANDARI *et al*., 2011b**). Vermicompost improves seed germination, increases plant growth and stimulates root development (**BACHMAN & METZGER, 2007; RESÉNDEZ *et al*., 2012; POOR & RAFIEI, 2013**). Root growth is stimulated by the presence of humic and fulvic acids that occur in vermicompost (**ALVAREZ & GRIERA, 2005; PANT *et al*., 2009; THEUNISSEN *et al*., 2010**). Naturally occurring micro-organisms in the vermicompost improve seedling quality by forming beneficial associations with the plant, thereby increasing resistance to disease and enhancing nutrient uptake (**ALVAREZ & GRIERA, 2005**).

Seaweed extracts such as Kelpak® have been used as organic fertilisers (**SRIDHAR & RENGASAMY, 2010; AHMED & SHALABY, 2012**). The macro- and micro nutrients present in the seaweed extract are responsible for improved seedling growth and development (**ALDWORTH & VAN STADEN, 1987; PISE & SABALE; 2010; AHMED & SHALABY, 2012; EL KAOAUA *et al*., 2013; PAPENFUS *et al*., 2013**). Furthermore, high concentrations of auxins, cytokinins and amino acids are thought to stimulate root initiation and enhance seed germination. The resulting high quality seedlings have increased resistance to plant stresses and pathogenic micro-organisms (**SRIDHAR & RENGASAMY, 2010; EL KAOAUA *et al*., 2013**).

Smoke-water has potential to improve seedling propagation in commercial nurseries (**KULKARNI *et al*., 2011**). A group of plant growth regulators called karrikinolides have recently been isolated from smoke-water (**FLEMATTI *et al*., 2004**) and are thought to be responsible for improved seed germination, seedling vigour, root development and overall

plant growth (**KULKARNI *et al*., 2007; KULKARNI *et al*., 2011**; **AREMU *et al*., 2012; ZHOU *et al*., 2013**). The improved seed germination has also been attributed to the presence of ethylene, nitrogen oxides and glycernitrile in the smoke-water. In addition, smoke-water has antimicrobial properties which have been shown to reduce the presence of endophytic fungi (**KULKARNI *et al*., 2011**).

The aims of this study were two-fold (1) to evaluate the effects of vermicompost leachate and smoke-water on seed germination and seedling vigour and (2) to evaluate the effects of smoke-water, vermicompost leachate and Kelpak on seedling growth after transplanting.

6.2. MATERIALS AND METHODS

6.2.1. Enhancing germination using smoke-water and vermicompost leachate

Eucalyptus dunnii seed, stock number M9753, supplied by Mondi Mountain Home Nursery was sown into mini-plug 544-cavity (MP544) trays that had been treated with copper oxychloride and filled with a 80:20 6-mm pine bark: perlite (v/v) mixture. Germination was initiated using the same procedures described in Chapter 3 and cultivation conditions remained the same. The experiment consisted of five treatments; a control (no additives), seeds soaked in smoke-water solution (supplied by the Research Centre for Plant Growth and Development) for one hour prior to sowing, seeds soaked in a solution of vermicompost leachate (produced by Wizzard Worms) for one hour prior to sowing, a smoke-water and a vermicompost leachate drench directly after sowing. The smoke-water was prepared using a 1:500 (v/v) dilution and the vermicompost leachate solution was prepared using a 1:10 (v/v) dilution for both soak and drench applications. All five treatments were represented in one MP544 tray, with a total of 96 seeds sown per treatment per replicate and arranged in a complete block design. Each tray was considered to be one replicate and treatments were repeated six times.

6.2.2. Data collection

The number of germinated seedlings was counted every day for 32 days after sowing. The seedlings were not removed from the tray after germination and to differentiate the germinated and ungerminated seedlings, the germinated seedlings were marked with a

plastic tag after germination had been recorded. After 32 days, the seedling length of ten seedlings per replicate per treatment was measured from the base of the growing point to the tip of the root. The data collected was used to calculate the total number of germinated seeds, the seedling vigour index (SVI) and the seedling emergence (SET) time.

The seedling vigour index was estimated by:

SVI = mean germination (%) x mean seedling length (mm).

The SET was calculated by:

$$SET = \sum(n/d),$$

where d - day of germination and n - number of germinated seeds on the day of counting.

6.2.3. Enhancing root growth after transplanting using supplements

In a second experiment, *Eucalyptus dunnii* seed was sown into MP544 trays filled with a 80:20 6-mm pine bark: perlite (v/v) mixture. The germination and seedling cultivation procedures were the same as described in Chapter 3. When the mini-plug seedlings had consolidated the root plug in the MP544 trays, the seedlings were transplanted to previously prepared Unigro®128 trays. The Unigro®128 trays were filled with the 90:10-mm pine bark: perlite mixture (v/v) which was found to be the most suitable substrate for use in the final container after transplanting (see Chapter 5). This experiment consisted of four treatments with each replicate consisting of two Unigro®128 trays. Each tray consisted of two treatments per tray and all treatments were replicated six times and arranged in a randomised complete block design. Solutions of smoke-water (1:500, v/v); vermicompost leachate (1:10, v/v) and Kelpak® (1:500, v/v) were prepared and applied as a drench to the transplanted seedlings directly after transplanting and once every three weeks thereafter. On the day of application, the seedlings were only irrigated once in the morning and the afternoon irrigation was replaced with the treatment application. These treatments were applied three times during the experiment.

6.2.4. Data collection and analysis

Treatment effects on seedling growth were determined by measuring the shoot height and root collar diameter after transplanting and every two weeks thereafter to establish a growth curve. The treatment effects on seedling morphology were evaluated three months after transplanting. The shoot height, number of leaves, root collar diameter, dry shoot and

dry root mass, the number of lateral roots and the length of the longest roots were determined using the same procedures described in Chapter 3. Mean values were compared using an ANOVA and significant means were separated using Tukey's test at a 95% confidence interval. Analysis was done using SAS Enterprise Guide 5.1, 2012.

6.3. RESULTS

6.3.1. Germination

No significant differences between treatments were found and the mean germination for the different treatments ranged from 73 % for seeds drenched in smoke-water to 82 % for seed soaked in vermicompost leachate. The germination percentages of the control, smoke-water soaked seed and seed drenched in vermicompost leachate were 80 %, 78 % and 76 % respectively (FIGURE 6.1A). The SET ranged from 4.15 days in the smoke-water drenched seeds to 4.82 days in the control seeds although these differences were not significant (FIGURE 6.1).

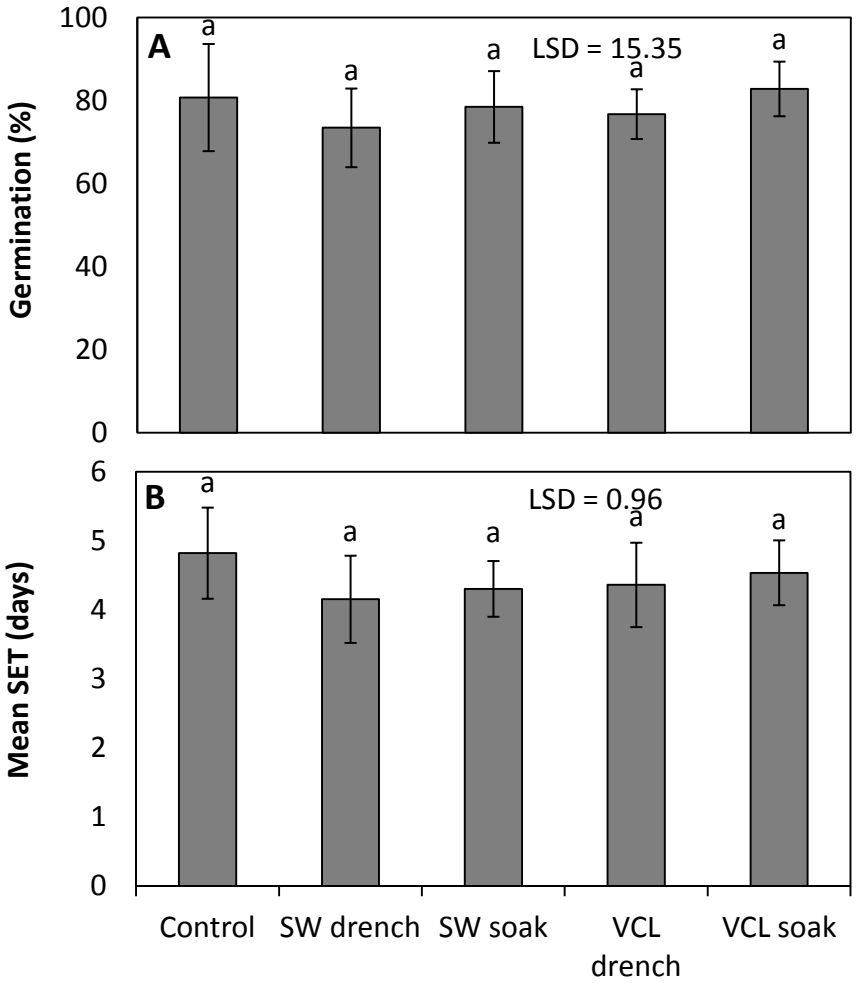

FIGURE 6.1. Mean germination percentage (A) and emergence time (B) of the *E. dunnii* seeds treated with smoke-water (SW) and vermicompost leachate (VCL). Different letters represent significantly different means at $p<0.05$. Error bars represent S.D.

6.3.2. Seedling vigour

The control treatment produced the tallest and heaviest seedlings. However, these seedlings were not significantly ($p>0.05$) taller than seedlings resulting from smoke-water soaked and vermicompost leachate drenched treatments. Significant differences ($p>0.05$) between treatments were not observed for seedling dry mass (TABLE 6.1).

TABLE 6.1. Effect of smoke-water (SW) and vermicompost leachate (VCL) on *E. dunnii* seedling length and dry mass 32 days after sowing. Different letters refer to significantly different means (± SD) at $p<0.05$.

Treatment	Seedling length (mm)	Seedling dry mass (mg)
Control	48.27±7.26[a]	6.27±1.21[a]
SW drench	44.28±8.00[b]	5.68±1.66[a]
SW soak	47.22±7.26[ab]	5.81±1.12[a]
VCL drench	44.78±6.97[ab]	6.12±1.78[a]
VCL soak	44.32±8.58[b]	5.67±1.04[a]
LSD	3.83	1.61

The SVI was highest for the control seedlings and lowest for the smoke-water drenched seedlings although significant differences were not recorded (FIGURE 6.2).

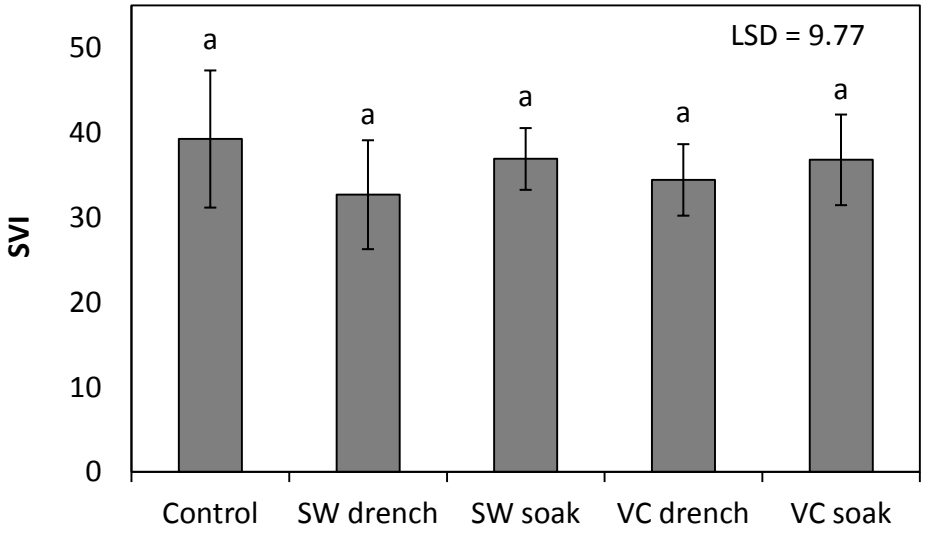

FIGURE 6.2. Mean SVI of treated *E. dunnii* seedlings. Different letters refer to significantly different means at $p<0.05$. Error bars represent S.D.

6.3.3. Seedling growth

The resulting growth curves for all treatments displayed similar patterns and no significant differences ($p>0.05$) in growth were observed. The increase in the root collar diameter of treated seedlings appeared to be rapid during the eight weeks after transplanting. The

increase in the root collar diameter of Kelpak®-treated seedlings was lower in the first two weeks after transplanting compared to the other seedling treatments. A slight difference between the control seedlings and the Kelpak®-, smoke-water- and vermicompost leachate-treated seedlings occurred at week four, where the increase in root collar diameter between weeks two and four was slightly lower in the control seedlings (FIGURE 6.3).

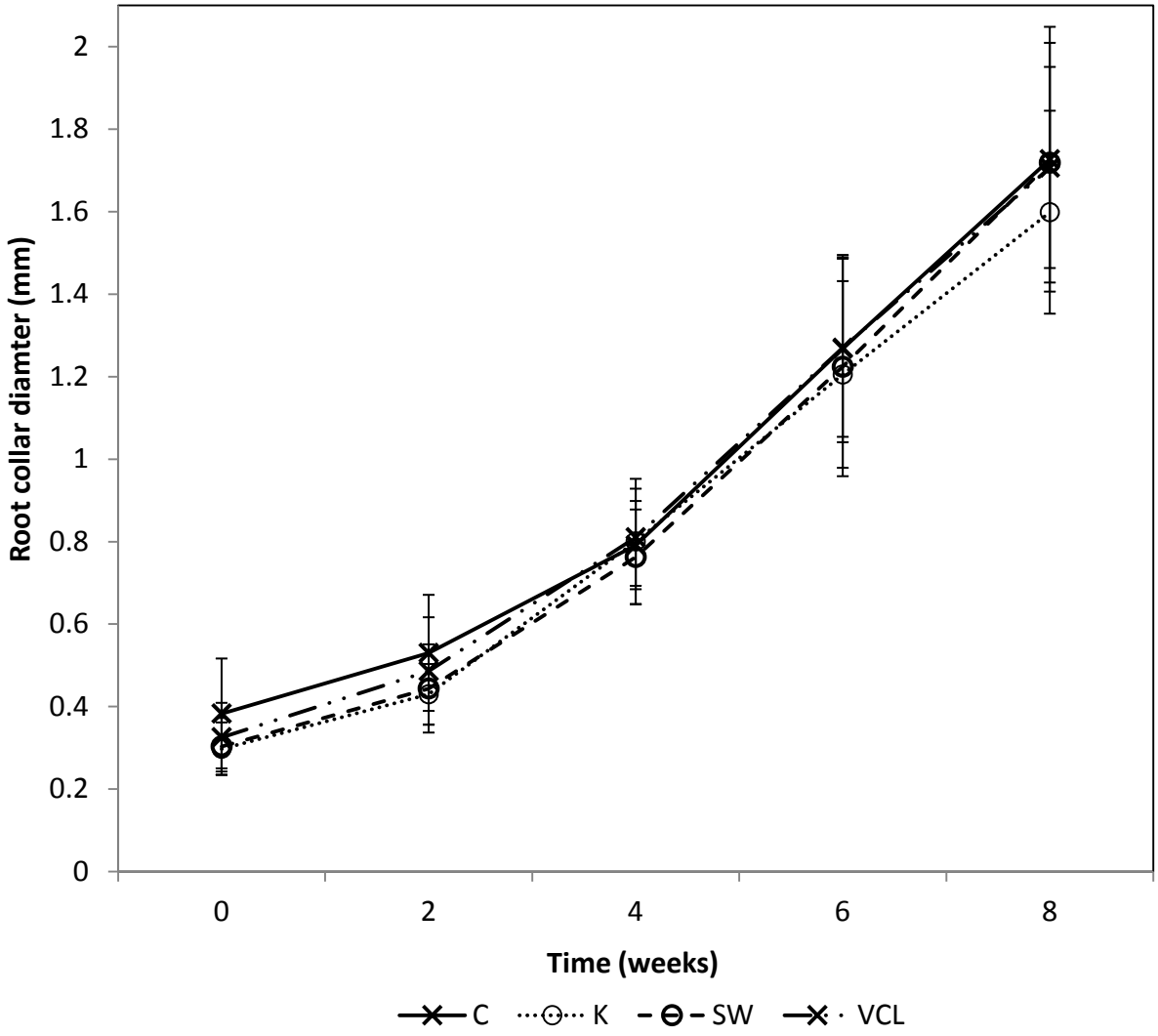

FIGURE 6.3. Comparison of the effect of the control, smoke-water, Kelpak® and vermicompost leachate treatments on root collar diameter of *E. dunnii* seedlings over eight weeks after transplanting. Error bars represent S.D.

As seen in the resulting root collar diameter growth curves, shoot height of the seedlings displayed similar growth patterns. In the first four weeks after transplanting, a slow but steady increase in shoot height was observed. Thereafter, a greater increase in the seedling height was observed between weeks six and eight (FIGURE 6.4).

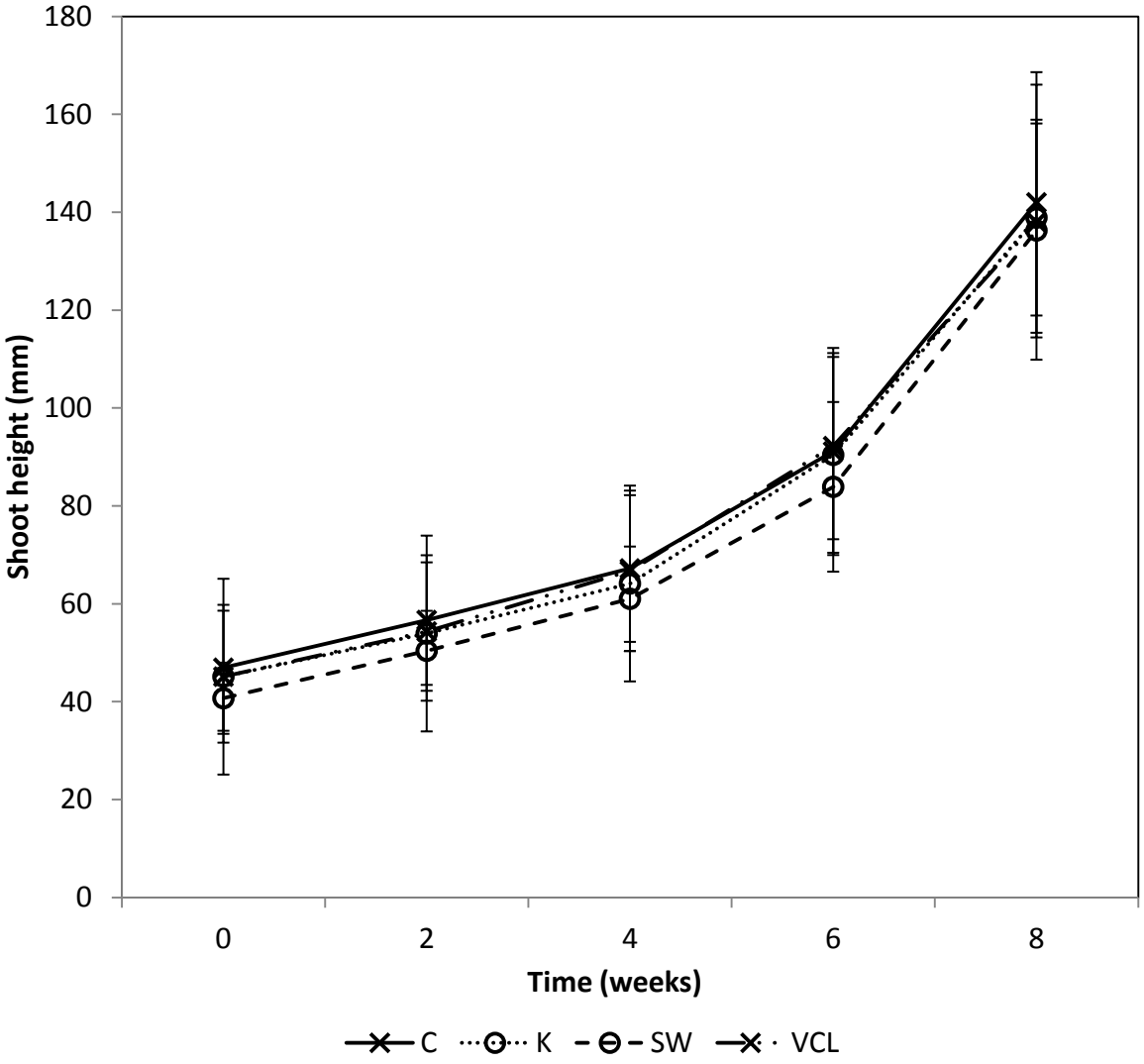

FIGURE 6.4. Comparison of the effects of the control, smoke-water, Kelpak®, and vermicompost leachate treatments on the shoot height of *E. dunnii* seedlings, eight weeks after transplanting. Error bars represent S.D.

6.3.4. Seedling quality

No significant differences ($p>0.05$) in seedling quality were observed when *E. dunnii* seedlings were treated with Kelpak®, vermicompost leachate or smoke-water, although seedlings treated with Kelpak® had the lowest shoot-to-root ratios (TABLE 6.2).

TABLE 6.2. Effect of Kelpak®, smoke-water and vermicompost leachate on the shoot-to-root and sturdiness ratios of *E. dunnii* seedlings. Different letters represent significantly different means (±S.D.) at $p<0.05$.

Treatment	Shoot-to-root ratio	Sturdiness ratio
Control	3.26±0.86[a]	86.93±11.01[a]
Smoke-water	3.26±0.71[a]	80.16±11.35[a]
Kelpak®	3.14±0.80[a]	85.26±11.05[a]
Vermicompost leachate	3.25±0.90[a]	83.60±23.49[a]
LSD	0.60	9.11

6.3.5. Morphological characteristics

The effects of treating *E. dunnii* MP544-seedlings with smoke-water, vermicompost leachate and Kelpak® after transplanting are shown in FIGURE 6.5. No significant differences ($p>0.05$) in shoot height (FIGURE 6.5A), root collar diameter (FIGURE 6.5B), number of lateral roots (FIGURE 6.5E), dry root (FIGURE 6.5F) and dry shoot mass (FIGURE 6.5G) of the seedlings between treatments were observed. Smoke-water treated seedlings developed the longest roots, and were only significantly longer than vermicompost leachate-treated seedlings (FIGURE 6.5D). The mean number of leaves produced by the test seedlings ranged from 16 to 22 leaves. However, significant differences ($p<0.05$) in leaf number where only observed when the *E. dunnii* seedlings had been treated with vermicompost leachate (FIGURE 6.5C).

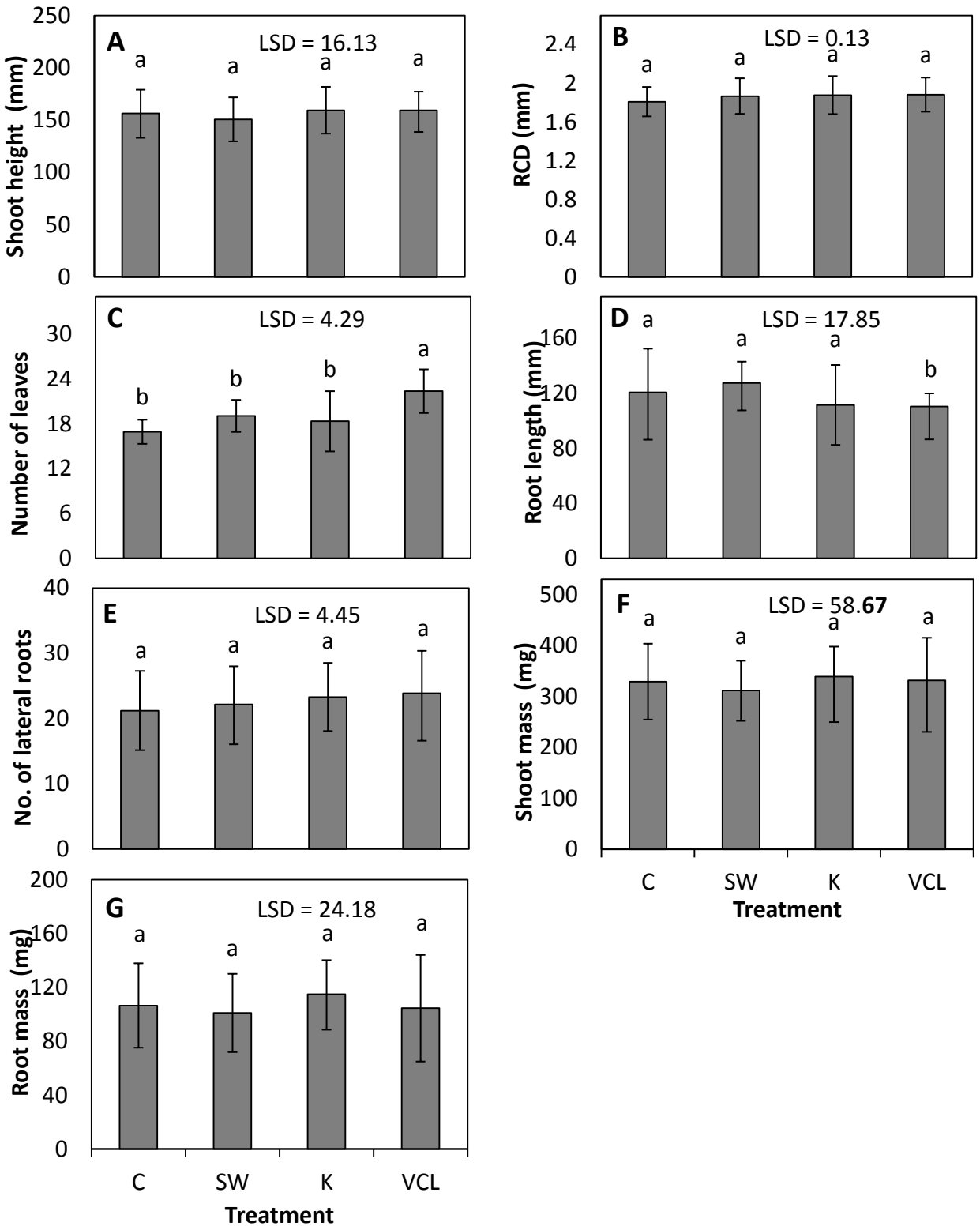

FIGURE 6.5. Mean shoot height (A), root collar diameter (B), number of leaves (C), root length (D), number of lateral roots (E), dry shoot mass (F) and dry root mass (G) of transplanted *E. dunnii* control seedlings (C) compared to those seedlings treated with smoke-water (SW), Kelpak® (K) and vermicompost leachate (VCL). Different letters represent significant differences at $p<0.05$. Error bars represent ±S.D.

6.4. DISCUSSION

6.4.1. Effect of smoke-water and vermicompost leachate on germination and seedling vigour

Neither smoke-water nor vermicompost application improved overall germination or seedling vigour of the *E. dunnii* seed. Germination of the control seeds was higher than the smoke-water and vermicompost leachate treated seeds. However, although not significant, smoke-water and vermicompost treatments decreased SET. The seedlings from the control treatment exhibited a higher SVI compared to the smoke-water and vermicompost leachate treatments. The seedlings produced from the control treatment were heavier and taller than smoke-water and vermicompost leachate treatments.

KANDARI *et al*. (**2011b**) conducted a similar study on *E. dunnii, E. nitens and E. smithii*. The influence of vermicompost leachate on seed germination, seedling vigour and growth were investigated. Vermicompost leachate had a positive influence on all three species tested. Improvements in seedling emergence, rate of emergence and seedling growth were improved with the addition of vermicompost leachate. Other studies on the effects of vermicompost have been conducted on tomatoes (**ARTHUR *et al*., 2012**) and bananas (**AREMU *et al*., 2012**) both of which showed positive results. Similarly, smoke-water has been reported to enhance seedling germination, vigour and growth (**KANDARI *et al*., 2011a; AREMU *et al*., 2012**).

The application method in this study had an influence on seed germination and seed vigour. In the experiments conducted by **KANDARI *et al*.** (**2011a**), **KANDARI *et al*.** (**2011b**), **AREMU *et al*.** (**2012**) and **ARTHUR *et al*.** (**2012**) the vermicompost leachate and smoke-water solutions were applied more regularly, than in this study, where they were applied only once. This was possibly not sufficient to generate the positive effects observed by **KANDARI *et al*.** (**2011b**). Further to this, although not significant, the results of this experiment demonstrated that seeds soaked in smoke-water and vermicompost leachate exhibited improved germination and SVI compared to the drenched seedlings. Prolonging the exposure of the plant material to vermicompost leachate and smoke-water solutions ensured that the active ingredients of smoke-water and vermicompost leachate were

absorbed. This evidence is supported by **BROWN & VAN STADEN** (**1997**) who suggested that smoke-water is absorbed through the surface of the seed coat and its stimulatory effects are retained after drying the seed.

6.4.2. Effects of supplements on seedling growth and development after transplanting

Several research studies have shown that smoke-water, vermicompost leachate and Kelpak® have shown positive contributions towards the growth and development of plants. These studies have shown increases in plant growth, shoot and root mass, shoot height and improved photosynthesis due to the increased chlorophyll concentrations. Smoke-water has been reported to improve seedling growth and enhance root development (**VAN STADEN *et al*., 2006; KULKARNI *et al*., 2008; AREMU *et al*., 2012; CHUMPOOKAM *et al*., 2012**).

In this study, while some positive effects of supplement application to transplanted *E. dunnii* seedlings were observed, they were not consistently better than the control. Vermicompost leachate application significantly ($p<0.05$) increased the number of leaves compared to the control which was similar to the results reported by **KANDARI *et al.*** (**2011b**). Smoke-water treated seedling produced the longest roots and exhibited more robust seedlings. In a similar study, smoke-water increased the root length of banana seedlings (**AREMU *et al*., 2012**). Kelpak® produced seedlings with the best shoot-to-root ratios.

In banana plants, **AREMU *et al*.** (**2012**) demonstrated that the method of application was important. When the test seedlings were treated with a foliar spray of smoke-water, the effect was less obvious compared to drenched seedlings. Similar results were observed for vermicompost leachate treatments in the same study. According to **PARKUNAN *et al*.** (**2011**), soil drenching allows for the additive solution to be available to the seedlings for a longer time, thus ensuring that the active components can be taken up by the plants. In this study, the additives were applied to the seedlings as a drench, but were only applied three times during the experiment. In contrast, **KULKARNI *et al*.** (**2008**) and **AREMU *et al*.** (**2012**) both applied the solutions to the seedlings twice a week. Therefore, as with the method of application, the frequency of the application is essential in ensuring the active ingredients of the smoke-water, vermicompost leachate and Kelpak® are absorbed by the plants.

KANDARI *et al*. (**2011b**) found that vermicompost leachate increased seedling development of *E. dunnii* seedlings significantly. However, this was dependant on the concentration of the solution applied to the seedlings. When a 1:10 (v/v) concentration of vermicompost leachate was applied to *E. dunnii* seed a positive effect in seed germination was observed but increases in seedling growth parameters were only observed in seedling dry weight. By increasing the concentration of the vermicompost solution to 1:5 (v/v) significant improvements in seedling length, seedling mass, number of leaves and number of lateral roots were found (**KANDARI *et al*., 2011b**). In this study, the concentration of vermicompost leachate was 1:10 (v/v), and the results were similar to those reported by **KANDARI *et al*.** (**2011b**). Vermicompost leachate solution concentration was important to generate the advantageous effects of the additive for transplanting seedlings. Furthermore, different concentrations of the vermicompost leachate solutions may be required at different physiological stages.

Kelpak® has been reported to improve seedling growth. **SRIDHAR & RENGASAMY** (**2010**) showed that seedling growth improved and increased photosynthetic pigment concentrations when a 1% (v/v) seaweed extract solution was applied to *Tagetes erecta* seedlings. In other research, a positive effect of Kelpak® on seedling growth was observed when a Kelpak® solution was applied at a concentration of 1.5% (v/v) (**NEDUMARAN & PERUMAL, 2009**). However, in this study the effects of Kelpak on seedling growth after transplanting were not remarkable.

Kelpak® concentration and frequency of application may be important to ensure beneficial effects of Kelpak® on seedling growth. **ALDWORTH & VAN STADEN** (**1987**) reported an increased vigour of cabbage seedlings when they had been immersed in a 1:500 (v/v) solution of Kelpak® before transplanting. However, when a 1:250 (v/v) solution was used, no differences in growth were observed compared to the control seedlings. Too high concentrations may become inhibitory, whilst too low concentrations may have no beneficial effects (**ALDWORTH & VAN STADEN, 1987; NEDUMARAN & PERUMAL, 2009**). In this study, a Kelpak® solution of 1:500 (v/v) was applied to the transplanted seedlings three times, which was much lower than the concentrations reported by **NEDUMARAN & PERUMAL** (**2009**) and **SRIDHAR & RENGASAMY** (**2010**)**.** The positive effects on seedling

growth in the study carried out by **NEDUMARAN & PERUMAL (2009**) were only achieved when the frequency of application increased. In addition, **ALDWORTH & VAN STADEN** (**1987**) immersed the cabbage seedlings in Kelpak® for a full five minutes. However, in this experiment, the Kelpak® solution was applied as a drench to the *E. dunnii* seedlings after transplanting which limited the exposure time of the seedlings to Kelpak®. The concentration and application frequency of Kelpak® will need to be investigated further to evaluate the benefits of the Kelpak®.

6.5. CONCLUSIONS

- Some positive effects of the additives tested in this study were observed.
    - Vermicompost leachate showed slight improvements in seedling vigour
    - Vermicompost leachate-treated seedlings showed an increase in leaf number
    - Smoke-water increased root length
    - Kelpak® improved the shoot-to-root ratios slightly
- Further experiments are needed to optimise concentration and application methods of smoke-water, vermicompost and Kelpak® on transplanted *E. dunnii* seedlings

CHAPTER 7

Concluding remarks

7.1. SUMMARY OF THE RESEARCH FINDINGS

Seedling quality gives an indication of health and vigour of seedlings cultivated in a nursery environment which affects their ability to grow after transplanting (**HAASE, 2008**). The importance of mini-plug seedling quality was highlighted during this study. Poor mini-plug seedling quality delayed seedling growth after transplanting. The factors that affected quality of mini-plug seedlings included mini-plug container design, mini-plug seedling cultivation time prior to transplanting and substrate composition.

Mini-plug container design impacted mini-plug seedling development. Larger seedlings were produced when seedlings were sown into MP288 trays. These seedlings had better shoot-to-root and sturdiness ratios when compared to seedlings grown in MP544 trays. This was attributed to the lower planting density and the larger container volume of the MP288 seedling tray (**BARNETT, 1980; ANNAPURNA *et al*., 2004; DINI-PAPANASTASI *et al*., 2012**). The higher planting density of MP544 trays increased competition between seedlings and limited space available for growth. This resulted in tall, spindly seedlings since most of the plant energy was directed towards shoot growth to increase light exposure for photosynthesis (**BARNETT, 1980; ANNAPURNA *et al*., 2004; DINI-PAPANASTASI *et al*., 2012**). Coupled to this, the small container volume of MP544 trays restricted root growth and resulted in high shoot-to-root ratios. This phenomenon is often observed in container-grown seedlings and may cause plant stress after transplanting (**NESMITH & DUVAL, 1998; MATHERS *et al*., 2007**).

Although, MP288 trays produced seedlings with lower shoot-to-root and sturdiness ratios, MP288 containers affected root architecture. Seedlings grown in plastic MP288 trays developed root coils which became more pronounced the longer the seedlings remained in the mini-plug container. Although, some root coils were observed in MP544-reared seedlings, the severity of coiling remained low compared to the MP288-reared seedlings regardless of seedling age. Seedlings cultivated in MP544 trays had shorter more fibrous, actively growing roots than seedlings grown in MP288 containers. This improved root

growth was caused by chemical pruning of the seedling roots as they were exposed to copper oxychloride in which the MP544 trays had been treated prior to sowing (**DUMROESE & WENNY, 1997; MATHERS *et al*., 2007**). Even though, the smaller, more densely populated MP544 trays resulted in seedlings with high shoot-to-root and sturdiness ratios, the active root systems of these seedlings meant that they were able to initiate new roots quickly after transplanting.

The cultivation time of mini-plug seedlings affected mini-plug seedling quality. Seedlings grown in mini-plug containers for too long, delayed seedling growth after transplanting. Furthermore, the longer the seedlings remained in the mini-plug containers the worse the negative effects of high planting densities and restricted root volumes became. In four-month-old transplanted seedlings, the sturdiness and shoot-to-root ratios were higher than one-, two- and three-month-old seedlings within tray types tested. However, when seedlings were transplanted too early, the root plugs were damaged when the seedlings were extracted from the mini-plug tray. In addition, since the root plug had not yet become consolidated at transplanting, the incidence of J-root formation increased. This was because the roots tended to "bend" when the seedlings were transferred into the final container as the root plug was not firm.

Substrate composition affected mini-plug quality, and it was therefore important that substrate selection was optimised for container-to-container transplanting (**SALIFU *et al*., 2006; MATHERS, 2007; CHOI *et al*., 2012; BANACH *et al*., 2013**). Seedlings sown into 6–mm pine bark produced a lower root mass and initiated fewer roots during the root growth potential test than other substrates tested. This poor performance was attributed to the high water holding capacity of this substrate which retained a large amount of water and did not drain well. The small volumes of mini-plug container enhanced the negative effects of the poorly draining substrate, creating an anaerobic environment which was not conducive to the development of healthy roots (**HANDRECK & BLACK, 1994; RICHIE, 2003; MATHERS, 2007**). Based on the results it was concluded that the best substrate for mini-plug cultivation was a 80:20 (v/v) mixture of 6-mm-pine bark and perlite. The perlite increased the drainage and oxygen content at the root zone and promoted the development of healthier, more vigorous roots (**HARTMANN *et al*., 1997; AWANG *et al*., 2009; SIRIN *et al*., 2010**). The

substrate composition used for seedling propagation in mini-plug containers could not be used in the final container after transplanting. This was because the seedling requirements and container dimensions after transplanting were different (**HANDREK AND BLACK, 1994**). The results of this study indicated the optimal substrate used in the final container after transplanting was a 90:10 (v/v) mixture of 6-mm-pine bark and perlite.

To ensure optimal growth after transplanting, the development of healthy, vigorously growing roots was essential (**BURDETT, 1990; RICHIE, 2003; CLOSE *et al*., 2005**). When seedlings were grown in mini-plug trays for extended periods it resulted in delayed growth after transplanting and enhanced J-root development. Unhealthy roots developed when seedlings were grown in poorly draining substrates which effected mini-plug seedling growth and root initiation. Root initiation is important to ensure fast growth after transplanting (**BURDETT, 1990; RICHIE, 2003; CLOSE *et al*., 2005**). The faster new roots are initiated after transplanting, the less the effects of transplant shock (**SANDS, 1984; VAN IERSEL, 1998**). Increased root growth increases the volume of substrate that is accessible to the plants for nutrients and water (**BURDETT, 1990**).

The use of supplements to enhance root growth after transplanting seedlings was investigated to determine if such supplements could minimise delayed growth after transplanting. The use of smoke-water, vermicompost leachate and Kelpak® were tested since these additives have all shown beneficial effects on seedling growth and stimulation of root growth (**BACHMAN & METZGER, 2007; RESÉNDEZ *et al*., 2012; POOR & RAFIEI, 2013**). Unfortunately, no effects on seedling development were found during this study. This was attributed to the low concentration and application frequency of these supplements to the seedlings. Further investigations to optimise the concentration and application of the supplements are recommended as other research studies conducted into the use of smoke-water, vermicompost leachate and Kelpak® have reported beneficial effects (**BACHMAN & METZGER, 2007; KULKARNI *et al*., 2007; KULKARNI *et al*., 2011; AHMED & SHALABY, 2012; AREMU *et al*., 2012; RESÉNDEZ *et al*., 2012; POOR & RAFIEI, 2013; SRIDHAR & RENGASAMY, 2013**; **ZHOU *et al*., 2013**).

7.2. CONCLUSIONS AND RECOMMENDATIONS

To determine the best container-to-container transplanting method the main research questions asked during the course of this study were:

- How did the mini-plug tray design influence mini-plug seedling quality, growth and root architecture;
- What was the best age at which the mini-plug seedlings could be transplanted to ensure continued seedling growth after transplanting and to minimise root deformities;
- How did substrate composition effect root growth and development of mini-plug seedling before and after transplanting;
- Is it possible to limit the development of root deformities and
- Can the addition of supplements enhance seedling growth after transplanting?

It was concluded that the optimal seedling age to transplant mini-plug seedlings was between two and three months after sowing. Transplanting early increased root deformities, and late delayed subsequent seedling growth. The polystyrene MP544-cavity tray dipped in copper oxychloride was selected as the preferred tray for mini-plug seedling production as the resulting root structure was fibrous and exhibited fewer root deformities before transplanting. Substrate composition had an impact on root vigour, a poorly draining substrate decreased seedling growth and overall quality, particularly in mini-plug containers. In addition, substrates used for cultivation of mini-plug seedlings should not be used in the final container after transplanting since the container characteristics were shown to influence the substrate properties.

Whilst some conclusions regarding the questions asked at the beginning of this research study could be drawn, several questions still remain. Transplanted seedlings exhibited higher levels of root deformities than directly-sown seedlings. Furthermore, directly-sown seedling quality was far superior when compared to transplanted seedlings. The main concern of container-to-container transplanting in seedling nurseries was the development of root deformities which may affect seedling growth in the field. Various methods to limit the development of J-roots and root coiling in transplanted seedlings were evaluated during the course of this research. These included: (1) determining the optimal time of

transplanting; (2) investigating the use of polystyrene trays dipped in copper oxychloride to minimise root coiling; and (3) evaluating the effects of substrate composition on the development of J-roots and root coiling. Despite this, however, transplanted seedlings continued to develop J-roots and root coiling. In addition, the small volume of the mini-plugs enhanced the negative effects of poor horticultural techniques. Therefore, nursery cultivation practices are intensively managed in order to minimise the negative effects associated with poor quality seedlings. Currently the negative effects on seedling quality and root structure caused by transplanting mini-plug seedlings outweighs the reported benefits and despite our efforts, further research is still required to ensure the production of vigorously growing seedlings using a container-to-container transplanting protocol which could be used in a commercial nursery.

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