

**Effect of coloured shade nets and plastics on *Eucalyptus* hybrid
mini-hedge stock plant morphology and subsequent cuttings
rooting potential**

Donna Louise Gilbert

**Submitted in fulfilment of the academic requirements for the degree of
Master of Science in Agriculture
Discipline of Horticultural Science
School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal, Pietermaritzburg, South Africa**

2014

ABSTRACT

Eucalyptus grandis (W. Hill) × *E. nitens* (Maiden) (G × N) clonal hybrids are bred to produce trees more cold-tolerant than *E. grandis* alone due to the cold-resistant *E. nitens* parent. Some of these selected hybrid clones have superior wood and pulp properties but are considered “difficult-to-root”; thus, any technique that improves their rooting percentage is valuable to the industry. As the radiation spectrum can affect growth and development of plants, manipulation of the spectrum received by mini-hedge stock plants through cultivating them under certain shade nets and plastics could alter the rooting potential of the subsequent mini-cuttings. Therefore, the effects of the covering factor at eight levels (shade nets: black 30 %, green 40 %, Apple blue 20 %, Photo red 30 % and silver Aluminet® 40 % and plastics: Clarix E Blue® as well as Patilite®, plus the control (no covering)) on morphology and subsequent rooting potential on the G x N clone factor at two levels (GN018B: difficult-to-root or PP2107: easy-to-root) were evaluated. A further factor, fertilizer, at two levels (inorganic or organic) was also evaluated. Statistical analysis was carried out using GenStat®. Data were analysed using analysis of variance (ANOVA) where data were orthogonally distributed. Where data were not orthogonally distributed the algorithm restricted maximum likelihood (REML) was used to estimate variance parameters in the multivariate linear mixed model.

To pinpoint the effect of the covering employed, firstly, the alteration of environmental parameters (temperature, relative humidity (RH) and radiation spectrum) were analysed as well as the seasonal effect on stock plants and rooting of mini-cuttings and root system quality of mini-cuttings were analysed. One greenhouse was used for stock plants and one for mini-cuttings. In the stock plant tunnel the shade nets and plastic were draped over wire trellising over bricked beds where the top and two sides were covered but the ends left open to allow sufficient ventilation. Thermometers and HOBOS® were hung above the plants and a spectroradiometer placed centrally under a specific covering to measure environmental parameters. It was found that Aluminet® and black nets and Patilite® plastic act as neutral covers with regard to radiation transmission, while the blue, red and green shade nets as well as Clarix E Blue® plastic covers altered the transmission spectrum; thus, they can be considered photoselective. Similarly, the red to near infrared ratios (R:NIR) were altered significantly by the coverings, however, this did not significantly affect the shoot internode length or leaf area (LA) of stock plants. Leaf area was determined with a leaf area meter

using ten of the first fully expanded leaves collected per treatment and averaging them to run analyses based on LA per leaf. The irradiance levels in the stock plant tunnel were lowest under Aluminet® 40 % (PPFD of 204.6 $\mu\text{mol s}^{-1} \text{m}^2$) during winter, resulting in low rooting percentages, while higher irradiance under blue 20 % shade net and control (no cover) (PPFD 604.4 and 931.5 $\mu\text{mol s}^{-1} \text{m}^2$, respectively) during spring 2011, achieved average rooting percentages. There was no clear trend of an optimal radiation intensity to expose G \times N stock plants to, in order to achieve high rooting percentages; however, a tendency was documented, whereby black 30 % and green 40 % at 200 to 450 $\mu\text{mol s}^{-1} \text{m}^2$ PPFD gave good rooting percentages.

Shade nets as well as plastic coverings increased minimum and decreased maximum temperatures, as well as increasing RH under all coverings with the exception of black 30 %. Maximum temperatures varied significantly under the various nets in the stock plant tunnel, but minimum temperatures did not. The control had a tendency towards producing the highest number of mini-cuttings per stock plant (production) and green 40 % and Aluminet® 40 % the lowest.

Three replications of 16 mini-cuttings from each of the 32 treatments (2 clones \times 2 fertilizers \times 8 coverings), where each stock plant treatment consisted of one clone, one fertilizer and one shade net factor. The cuttings were placed in three different plastic rooting trays, which were then placed on the floor in a grid pattern in the rooting tunnel with misting sprayers. All mini-cuttings were left to root for six weeks before evaluation. Final rooting percentage was calculated as the percentage of mini-cuttings that formed roots and were still alive at the time of assessment from the original 16 mini-cuttings placed. Application of organic fertilizer tended to result in higher rooting percentages than inorganic fertilizer during autumn 2011, however, organic and inorganic fertilizer applications did not significantly alter rooting percentage of mini-cuttings during winter 2011, spring 2011 and summer 2011/12, while in autumn 2012 the inorganic fertilizer gave significantly better rooting than the organic fertilizer. In line with industry experience, Clone PP2107 rooted consistently better than GN018B, over all seasons and fertilizer regimes. The coloured shade nets and plastic coverings that supported the highest rooting percentages, over all seasons, were black 30 %, green 40 %, Clarix E Blue® and Patilite®, the lowest rooting percentages were found to be from mini-cuttings collected under Aluminet® 40 %. However, the shade nets or plastics that supported the highest average rooting percentages changed with each season,

making it difficult to recommend the best shade net or plastic for use in G × N vegetative propagation. The highest overall rooting percentage of both G × N clones was achieved during spring 2011 and the lowest during autumn 2011 and 2012. To determine root quality the rooted mini-cuttings for each treatment were rated on a four-point scale. The seasons that produced the best root quality and highest root numbers for rooted mini-cuttings were autumn and spring 2011. The season with the worst root quality for rooted mini-cuttings and the highest percentage of callus was winter 2011. This may indicate that rooting conditions were more suited to root than callus formation during spring 2011, but during winter 2011 conditions may have been more suited to callus formation.

In South Africa, extremely high temperatures may be a greater limiting factor for G × N root formation than extremely low temperatures. Seasons when temperatures are more moderate, such as spring and autumn, seem best to root G × N mini-cuttings. It is recommended to use black 30 % or green 40 % for stock plants and Clarix E Blue® or Patilite® for the rooting tunnel, possibly with a removable shade net of 30 to 40 % shading factor.

DECLARATION

The experimental work described in this thesis was carried out at the University of KwaZulu-Natal, Pietermaritzburg from 2010 to 2012, under the supervision of Ass. Professor Isa Bertling (University of KwaZulu-Natal)

I, **Donna Louise Gilbert**, **204506391** declare that these studies are my own original work and have not been submitted in any form to another tertiary institution. Where the work of others has been used, this is appropriately and duly acknowledged in the text.

Signed: _____ **Date:** _____

D. L. Gilbert

I hereby certify that this statement is correct.

Signed: _____ **Date:** _____

Ass. Prof I. Bertling (Supervisor)

CONFERENCE CONTRIBUTION

Gilbert, D.L., Bertling, I. and Savage M.J. 2013. Radiation transmission through coloured shade netting and plastics and its effect on *Eucalyptus grandis* × *E. nitens* hybrid mini-hedge shoot internode length, stem diameter and leaf area. Proceedings of 2nd All Africa Horticulture Congress. Eds K. Hannweg and M. Penter. *Acta Horticulturae* 1007, 773-780. (see Chapter 2)

ACKNOWLEDGEMENTS

I am grateful to the following people and institutions:

- My supervisor, Ass. Prof Isa Bertling for always making time for me in her busy schedule, helping me find structure and improve my writing skills. Thank you for always encouraging and believing in me and helping me find the end of the tunnel.
- Dr Oscar Mokotedi (formerly CSIR) for his academic insight and helping me to think like a scientist.
- Ms Flic Blakeway (CSIR) for offering me the opportunity to study further and giving me insight on how strong women can succeed in business. Her help was invaluable in the initial stages of planning and establishing tunnels and introducing me to many people in the forestry industry.
- Prof. John Bower (formerly UKZN) for his advice and help in planning experiments and initial establishment of tunnels.
- Prof. Michael Savage (UKZN) for his advice and expertise on the subject of radiation and transmission and allowing me to use his equipment and laboratory.
- Prof. Annabel Fossey (formerly CSIR) for her help and advice in planning experiments and helpful suggestions with literature research and scientific writing.
- Mr Bryn Pollard (Sunshine Seedling Services) for all of his help and advice on running a commercial nursery, explaining what challenges need to be overcome in the forestry nursery industry. His advice on which clones to choose, fertilizer and fungicides to use, irrigation scheduling, as well as providing growing medium, plants and rooting trays was invaluable.
- Mr Matt Erasmus (Senior field technician UKZN) for all of his help and advice with setting up my stock plant and rooting tunnel and providing labourers and transport when needed. Particularly his expertise in irrigation systems and setting timers and application of pesticides and fungicides.
- Mr Arthur Philip (Talborne Organics KZN) for guidance in selecting organic fertilizer and advice for application of fertilizer and beneficial fungi/ bacteria mix.
- Mr Keith Hartley and Ms Denise Foster (FilmFlex Plastics Natal CC) and Ms Debbie Herbst (Knittex® (Multiknit® (Pty) Ltd.)) for advice on shade nets and plastics.

- Mr Craig Hay (Tunnel Quip CC) for advice on Weedstop weed matting and instructions for programming the Irritrol Junior Max® irrigation controller in the rooting tunnel.
- Ms Karen Eatwell (CSIR) for her information regarding the selection of the $G \times N$ clones used in these experiments.
- Ms Rochelle Parsons (formerly CSIR) for all her help and advice on experimental design and statistical analyses.
- Ms Jacqui Wallis (Mondi, nursery manager at Mountain home) for showing us how a forestry nursery is run and advising on what thesis topics would be useful in the “real world” of forestry nursery management.
- Dr Samson Tesfay (UKZN) for advice on statistics and research in general and help with laboratory work.
- Mr Xolani Sibozza (UKZN) for help in the laboratory and purchasing laboratory consumables.
- Mrs Jessica Chetty, Mrs Gloria Andrews (CSIR), Mrs Celeste Clarke and Mr Kamenthren Govender (UKZN) for help with ordering and purchasing key components and consumables for my tunnel and laboratory experiments.
- Mr Matabaro Ziganira (UKZN) and Mrs Noma Nene (CSIR) as well as Bongani and Philani (work experience) and Florence, Thembi, Anna, Thandi and Nosipho (“Ukalinga” farm, UKZN) for help in harvesting and setting one or more rooting experiments.
- Leonie Berjak and Digby Gold for proofreading and advice on scientific writing.
- Last but not least my family and parents-in-law for all their support, encouragement and interest as well as use of office space, computers and printers.
- To my loving husband Nigel Berjak, for his financial and emotional support during this long road to MSc. Thank you for all of your patience, encouragement, help in the greenhouse, refining my spreadsheet capturing system, proofreading and always believing in me. You are my rock as well as my spreadsheet guru.

Many thanks to the Council for Scientific and Industrial Research (CSIR), National Research Foundation (NRF) Scarce skills scholarship 2011 and Donald Moor Agricultural Bursary for financial support.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
DECLARATION	v
CONFERENCE CONTRIBUTION	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xv
Chapter 1: Introduction and literature review	1
1.1. <i>Eucalyptus</i> propagation in South Africa	1
1.2. Clones commonly propagated in South Africa	2
1.3. Propagation techniques	3
1.4. Internal factors of rooting success	6
1.5. Radiation quantity	9
1.6. Radiation quality	12
1.6.1. Altering radiation quality	13
1.6.1.1. Effects of specific coloured shade nets	13
Aluminet®	13
Black	14
Blue	15
Green	16
Red	16
1.6.1.2. Specific plastic covering	16
Clarix E Blue®	16
Patilite®	17
1.6.2. Effect of radiation quality on elongation	17
1.6.3. Effect of radiation quality on leaf area	20
1.7. Other environmental parameters	21
1.7.1. Temperature	21
1.7.2. Relative humidity	23
1.7.3. Season	24

1.8. Problem statement	25
REFERENCES	26

Chapter 2: Radiation transmission through coloured shade netting and plastics and its effect on *Eucalyptus grandis* × *E. nitens* hybrid mini-hedge shoot internode length, stem diameter and leaf area

Keywords	32
ABSTRACT	32
2.1. Introduction	33
2.2. Materials and methods	35
2.2.1. Plant material	35
2.2.2. Spectral irradiance	35
2.2.3. Morphology	36
2.2.4. Statistical analysis	36
2.3. Results and discussion	37
2.3.1. Spectral irradiance	37
2.3.2. Morphology	38
2.4. Conclusion	39
ACKNOWLEDGEMENTS	39
REFERENCES	40
TABLES	41
FIGURES	43

Chapter 3: Effect of environmental parameters on *Eucalyptus grandis* × *E. nitens* hybrid stock plants

3.1. Introduction	44
3.1.1. Temperature	45
3.1.2. Relative humidity	48
3.1.3. Production	48
3.1.4. Leaf area and stem diameter	49
3.1.5. Pests and diseases	51
3.2. Materials and methods	52
3.2.1. Environmental parameters	52
3.2.1.1. Temperature and relative humidity (RH)	52

3.2.2. Morphological parameters of stock plants	53
3.2.2.1. Production	53
3.2.2.2. Dry mass	53
3.2.2.3. Leaf area and stem diameter	53
3.2.3. Pests and diseases	53
3.2.4. Statistical analysis	54
3.3. Results	55
3.3.1. Environmental parameters	55
3.3.1.1. Temperature and relative humidity (RH)	55
3.3.2. Morphology parameters of stock plants	64
3.3.2.1. Production	64
3.3.2.2. Dry mass	66
3.3.2.3. Leaf area and stem diameter	68
3.4. Discussion	69
3.5. Conclusion	74
REFERENCES	75

Chapter 4: Effect of growing *Eucalyptus grandis* × *E. nitens* mini-hedge stock plants under coloured shade nets on rooting of mini-cuttings

4.1. Introduction	79
4.1.1. Seasonal effects on rooting	82
4.1.2. Radiation quantity and quality effects on rooting	83
4.1.3. Effect of cutting type on root system quality	85
4.1.4. Callus formation	86
4.2. Materials and methods	87
4.2.1. Plant material	88
4.2.2. Harvest procedure	89
4.2.3. Rooting conditions	90
4.2.4. Rooting assessments	91
4.2.5. Statistical analysis	92
4.3. Results	94
4.3.1. Effect of fertilizer and clones on rooting	94
4.3.2. Effect of coloured shade netting on rooting	96
4.3.3. Seasonal effects on rooting	98

4.3.4. Human effect on rooting	102
4.3.5. Quality of rooted mini-cuttings	104
4.3.5.1. Root types	104
4.3.5.2. Root number	109
4.3.5.3. Callus	114
4.3.5.4. Basal stem diameter	119
4.3.6. General observations	121
4.4. Discussion	121
4.5. Conclusion	131
REFERENCES	132
Chapter 5: Final outlook, conclusions and recommendations	136
5.1. Outlook	136
5.2. Conclusion	140
5.3. Recommendations for future research	141
Appendices	143
Appendix A – Layouts	143
Appendix B – Additional statistical evaluations, tables, graphs and photographs regarding chapter 2	145
Appendix C – Statistical tables	152

LIST OF TABLES

	Page
Chapter 2	
Table 2.1. Parameters determined from the spectroradiometer data for clear-sky measurements in autumn, winter and spring (2011). Photosynthetic irradiance is the irradiance in the PAR region.	41
Table 2.2. Ratios determined from red (R), blue (B) and near infrared (NIR) radiation; nb = narrow band (10 nm); wb = wide band (100 nm).	41
Table 2.3. Average transmissivity for the PAR region (and PAR plus NIR region) in comparison with manufacturer supplied shading percentage %.	42
Table 2.4. Average internode length (mm), leaf area (LA) (cm ²) and stem diameter (mm) during the evaluation period.	42
 Chapter 3	
Table 3.1. Mean temperature and relative humidity (% RH) in the stock plant tunnel and rooting tunnel recorded by HOBO® data loggers and displayed as month or season.	56
Table 3.2. Mean maximum and minimum temperatures in the stock plant tunnel measured with thermometers suspended above the plants in the centre of the shade net treatment over the time of the experiment.	58
Table 3.3. Mean maximum and minimum temperature in the stock plant tunnel measured with thermometers for the experimental seasons.	59
Table 3.4. Mean temperature and RH recorded hourly by HOBO® data loggers under each shade net during spring 2011.	61
Table 3.5. Comparison of the minimum and maximum temperatures recorded with thermometers in 2011 under shade nets and after removal of the nets in 2012 at the same time of year.	62
Table 3.6. Mean production of mini-cuttings per stock plant under shade nets divided into their respective clone and fertilizer treatments in spring 2011.	64
Table 3.7. Mean production of mini-cuttings per stock plant under shade nets over seasons.	65

Table 3.8. Mean dry mass in grams and percentage calculated from five mini-cuttings per three replications from various shade nets and clones.	67
Table 3.9. Mean leaf area and stem diameter of five mini-cuttings as affected by shade nets and clones.	69

Chapter 4

Table 4.1. Mean rooting percentage of mini-cuttings from stock plants grown under two clones vs. two fertilizer regimes based upon predicted means of the analysis of variance.	96
Table 4.2. Mean rooting percentage, root number per rooted mini-cutting, callus percentage, root plus callus percentage and basal stem diameter (BSD) for all five seasons analysed.	100
Table 4.3. Mean rooting percentages of mini-cuttings from stock plants grown under shade net (data displayed as a difference from the control (no shade) treatment for each season).	101
Table 4.4. Mean rooting percentage of mini-cuttings from stock plants over time according to specific labourer placing mini-cuttings.	104
Table 4.5. Basal stem diameter (BSD) (mm) of rooted mini-cuttings taken from plants grown under each shade net, clone and fertilizer factor over five seasons.	120

LIST OF FIGURES

	Page
Chapter 2	
Figure 2.1. Transmissivity of the eight curves shade nets and plastics used, during spring, where the difference between neutral and photosensitive nets are displayed.	43
Chapter 3	
Figure 3.1. Diurnal temperature fluctuations recorded over two days, while mini-cuttings were harvested in the stock plant tunnel.	57
Figure 3.2. Diurnal relative humidity (RH) recorded over two days, while mini-cuttings were harvested in the stock plant tunnel.	57
Figure 3.3. Temperature under shade netting during spring 2011, data recorded hourly by HOBO® data loggers.	63
Figure 3.4. Relative humidity under shade netting during spring 2011, data recorded hourly by HOBO® data loggers.	63
Figure 3.5. Mean production of mini-cuttings per stock plant for all shade nets over seasons divided into the clone and fertilizer treatments. Where the LSD = 1.1352 for clone × fertilizer × season interaction at 1 % level of significance.	66
Figure 3.6. Mean dry mass in grams (A) and as a percentage of the fresh mass (B) for five mini-cuttings per three replications divided into shade nets and clones. Where LSD = 0.07901 for dry mass (g) for shade net × plant part interaction at 1 % level of significance and LSD = 3.922 for dry mass (%) for shade net × plant part interaction at 1 % level of significance.	68
Chapter 4	
Figure 4.1. Examples of rated rooted mini-cuttings, where (1) displays root type 1 – a weak rooted mini-cutting, (2) root type 2 – an average rooted mini-cutting, (3) root type 3 – a good rooted mini-cutting and (4) root type 4 – a very strong rooted mini-cutting. Where the white square next to the ruler is 2 × 2 cm.	92
Figure 4.2. Seasonal rooting percentage of mini-cuttings from stock plants grown under inorganic or organic fertilizer treatments; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with least significant difference (LSD) for rooting percentage = 6.073 for the fertilizer × season interaction.	94

- Figure 4.3. Seasonal rooting percentage of mini-cuttings from PP2107 vs. GN018B clone stock plants; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 6.073 for the clone \times season interaction.95
- Figure 4.4. Rooting percentage of mini-cuttings from stock plants grown under various shade nets over time to indicate the relationship between clone and fertilizer; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 8.589 for the clone \times fertilizer \times season interaction.96
- Figure 4.5. Rooting percentage of mini-cuttings from stock plants grown under various shade nets; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 5.432 for the factor shade net.97
- Figure 4.6. Rooting percentage of mini-cuttings from stock plants grown under various shade nets and plastic; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 12.146 for the shade net \times season interaction.98
- Figure 4.7. Rooting percentage of mini-cuttings from stock plants grown under control (no shade) treatment over time; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 8.53 for the clone \times fertilizer \times season interaction.99
- Figure 4.8. Comparison of rooting percentage of mini-cuttings from stock plants grown under various shade nets in autumn 2011 and 2012; letters I = Inorganic and O = Organic fertilizer treatments; PP = PP2107 and GN = GN018B clones; error bars represent the standard errors of differences of predicted means (s.e.d.) = 9.464 for the shade net \times clone \times fertilizer \times season interaction. LSD and s.e. could not be determined for REML analyses.102
- Figure 4.9. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in autumn 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong.105
- Figure 4.10. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in winter 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.106

- Figure 4.11. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in spring 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.107
- Figure 4.12. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in summer 2011/12, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.108
- Figure 4.13. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in autumn 2012, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.109
- Figure 4.14. Mean number of roots per rooted mini-cutting and total rooting percentage in autumn 2011; error bars are based on the root number s.e. = 0.944 for the shade net \times clone \times fertilizer interaction. No s.e. could be calculated for rooting percentage for this season as only one replication of each treatment was set.110
- Figure 4.15. Mean number of roots per rooted mini-cutting and total rooting percentage in winter 2011; error bars are based on the root number s.e. = 0.2342 and rooting percentage s.e. = 5.409 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.111
- Figure 4.16. Mean number of roots per rooted mini-cutting and total rooting percentage in spring 2011; error bars are based on the root number s.e. = 0.3912 and rooting percentage s.e. = 6.49 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.112
- Figure 4.17. Mean number of roots per rooted mini-cutting and total rooting percentage in summer 2011/12; error bars are based on the root number s.e. = 0.401 and rooting percentage s.e. = 7.04 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.113
- Figure 4.18. Mean number of roots per rooted mini-cutting and total rooting percentage in autumn 2012; error bars are based on the root number s.e. = 0.4307 and rooting percentage s.e. = 4.805 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.114
- Figure 4.19. Total callus percentage and percentage of root plus callus of mini-cuttings during autumn 2011 compared with the rooting percentage of mini-cuttings; the

- autumn 2011 rooting experiment consisted of only one replication per treatment; hence, it could not be analysed due to lack of variation.115
- Figure 4.20. Total callus percentage and percentage of root plus callus of mini-cuttings during winter 2011 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 6.88, callus with root percentage s.e. = 4.983 and total rooting percentage s.e. = 5.409 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.116
- Figure 4.21. Total callus percentage and percentage of root plus callus of mini-cuttings during spring 2011 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 10.07, callus with root percentage s.e. = 6.35 and total rooting percentage s.e. = 6.49 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.117
- Figure 4.22. Total callus percentage and percentage of root plus callus of mini-cuttings during summer 2011/12 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 8.47, callus with root percentage s.e. = 6.41 and total rooting percentage s.e. = 7.04 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.118
- Figure 4.23. Total callus percentage and percentage of root plus callus of mini-cuttings during autumn 2012 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 8.24, callus with root percentage s.e. = 4.448 and total rooting percentage s.e. = 4.805 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.119
- Figure 4.24. Variety of rooted mini-cuttings; (A) root emergence from the mini-cutting stem at the abaxial cut end (base), (B) left: mini-cutting with root emergence from the abaxial end together with callus formation, right: mini-cutting with root emergence from above the abaxial end (sides of the stem), (C) mini-cutting that failed to root with excessive callus production at the base.121

Chapter 1

Introduction and literature review

1.1 *Eucalyptus* propagation in South Africa

Eucalyptus is the most cultivated tree genus worldwide due to fast growth rates and economic viability making it a common choice for commercial forest plantations. Different *Eucalyptus* species can be used in diverse climates for many purposes such as timber for furniture or poles as well as a major source of pulp and cellulose for the paper industry (Rocha Corrêa and Fett-Neto, 2004). In South Africa *Eucalyptus* wood is predominantly used for pulpwood for paper (Zwolinski and Bayley, 2001).

The first plantations of exotic trees, *Eucalyptus globulus*, were established in South Africa in 1876, in order to preserve natural woodlands by supplying intensive timber production for the growing needs of the country at the time (Zwolinski and Bayley, 2001). Since then the forestry industry has grown tremendously in importance as a viable industry as well as in the variety of trees grown. In South Africa, the area under forestry is 1,257 million hectares (ha) or approximately one percent of the total land area of 122,3 million ha (DAFF, 2011b). In South Africa the export value of forestry products amounted to R2.4 billion in the first quarter of 2011 and forestry imports amounted to R1.8 billion in the first quarter of 2011 (DAFF, 2011a). Contrary to global trends, South African timber is predominantly used as pulpwood, although not a significant producer on the global scale, it produces pulp and paper of world-class quality (Zwolinski and Bayley, 2001). The forestry sector contributes about one percent to the GDP and in terms of regional GDP, forestry in KwaZulu-Natal contributes 4.7 %, in Mpumalanga 5.5 % and 0.8 % in Limpopo (DAFF, 2011b). The forestry sector employed approximately 165 900 people in 2011, the bulk of which were employed in direct or indirect forestry as well as in the pulp and paper sub-sector, the sawmilling and timber board sub-sector and other smaller sectors (Godsmark, 2013). South Africa has a relatively low rainfall, where only 7 % of the total land area receives sufficient rainfall for intensive forest production (≥ 800 mm/ annum), situated in a narrow belt along the south and east coasts, and in the mountains on the eastern side of South Africa. Therefore, industrial

forestry is dependent on efficiency and innovation in order to limit the exploitation of natural resources (Zwolinski and Bayley, 2001). Due to limited land and resources the development of timber plantations in South Africa is highly regulated and the South African timber grower has to apply for planting permits issued depending on the outcome of impact studies, in particular the impact of tree planting on surface water resources (Zwolinski and Bayley, 2001).

1.2 Clones commonly propagated in South Africa

The objective of commercial plantation forestry is to make a profit. Integration of breeding and nursery technology, site selection, protection against pests and diseases and enhanced tolerance of trees to frost and drought is important in order to optimise product output (Zwolinski and Bayley, 2001). Tree breeders have made use of inter-specific hybridisation and establishment of clonal plantations to improve productivity, product quality and production costs (Assis *et al.*, 2004; Titon *et al.*, 2006). *Eucalyptus* species hybridise readily with one another and different hybrids are best suited for different purposes and locations. Hybridisation between species can combine superior wood characteristics with tolerance to biotic and abiotic stress to meet industrial requirements (Assis *et al.*, 2004). Various *Eucalyptus* species make up 39 % of the area planted to commercial trees in South Africa; the rest is planted to *Pinus* species (52 %) and *Acacia mearnsii* (black wattle 8 %) (Foelkel, 2008). *Eucalyptus grandis* was the most popular species, but the importance of using hybrids and clones has increased in recent years (Zwolinski and Bayley, 2001). Vegetative propagation has been vigorously implemented in South Africa to speed up tree breeding programmes and has become common practice in many nurseries for commercial production (Zwolinski and Bayley, 2001) to multiply clones of pure species and hybrids. Five commercial species make up the majority in *Eucalyptus* plantations in South Africa, these being: *E. grandis*, *E. nitens*, *E. smithii*, *E. macarthurii* and *E. dunnii* (Pallet and Sale, 2004). Tree species and hybrids commonly propagated vegetatively in South Africa are: *Eucalyptus grandis*, *E. grandis* × *E. urophylla*, *E. grandis* × *E. camaldulensis*, *E. grandis* × *E. nitens*, *E. grandis* × *E. tereticornis*, *Pinus elliottii* × *P. caribaea* and *P. patula* (Zwolinski and Bayley, 2001). Other tree species that can be vegetatively propagated are: *Pinus taeda*, *Acacia mearnsii*, *Ilex paraguariensis* (Assis *et al.*, 2004), hybrid aspen (*Populus tremula* × *P. tremuloides*) (Haapala *et al.*, 2004), neem (*Azadirachta indica*) (Palanisamy and Kumar,

1997), *E. saligna*, *E. globulus* (Rocha Corrêa and Fett-Neto, 2004) and Sweetgum (*Liquidambar styraciflua*) (Wendling *et al.*, 2010).

Two hybrid clones of *Eucalyptus grandis* (W. Hill) × *E. nitens* (Maiden) (G × N), one easy-to-root genetically improved clone (PP2107) and one difficult-to-root unimproved clone (GN018B) were used in these experiments. *Eucalyptus grandis* × *E. nitens* clonal hybrids are bred to be more cold-tolerant than *E. grandis* alone due to the *E. nitens* that can survive cold temperatures at high altitudes (Pallett and Sale, 2004; McMahon *et al.*, 2010), where other crops are not suitable. *Eucalyptus grandis* grows rapidly and has a tall, straight form suitable to many purposes such as solid timber, wood panelling and processed wood such as pulp, paper and woodchips (McMahon *et al.*, 2010). The optimal altitude and corresponding temperature ranges for *E. grandis* and *E. nitens* are 1000 to 1250 m (16 to 20 °C) and 1250 to 1900 m (13.5 to 16 °C), respectively (Gardner, 2007). The capital investment in trees for South African plantations in 2008, stood at R10.5 billion (DAFF, 2011b). Therefore, it is important that rooted cuttings that are transplanted in the field are healthy and of good quality to grow and thrive.

1.3 Propagation techniques

There are various methods of plant propagation such as micropropagation (i.e., *in vitro* tissue culture), rooting cuttings from woody or herbaceous shoots (i.e., macropropagation) and seed propagation (Haapala *et al.*, 2004; Titon *et al.*, 2006). Seed propagation is the traditional propagation method and is still a vital method in forestry and forestry breeding programmes worldwide. Superior genotypes capable of producing better wood quality and higher volumetric growth are propagated commercially for use in establishing plantation forests. The development of large-scale vegetative propagation methods has become important to increase the competitiveness of the plantation forestry industry (Palanisamy and Kumar, 1997; Fett-Neto *et al.*, 2001; Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004; Schwambach *et al.*, 2008). The cutting technique is the most widely used for propagating *Eucalyptus* around the world due to its ease of handling compared with micropropagation methods (Titon *et al.*, 2006). In recent years, *Eucalyptus* cuttings have increased to 28 % of the total stock production (Zwolinski and Bayley, 2001) compared with seed production in South Africa. In vegetative propagation terms a clone is the resulting offspring plant propagated by means of grafting, rooting of cuttings or tissue culture. Clones are duplicates

of the stock plant (mother plant) with the same genotype (Hettasch and Lunt, 2002; Wendling *et al.*, 2010); therefore, all observed variation is attributed to the environment (Sasse and Sands, 1997). The original single-tree-ancestor of a vegetatively propagated clone is called an ortet and the vegetative offspring of an ortet, or an individual member of a clone is called a ramet (Hettasch and Lunt, 2002).

Micropropagation (tissue culture) is the production of plants from very small plant parts, tissues, or cells (often only the apical meristem) grown in a laboratory, under sterile conditions, in a test tube or other container where the environment and nutrition are controlled (Hettasch and Lunt, 2002). Macropropagation using macro-cuttings is a widely used method, whereby a fairly long portion of a stem, root, or leaf is cut from the stock plant and is subsequently placed under favourable environmental conditions and often induced with rooting hormones, to form adventitious roots and shoots (Hettasch and Lunt, 2002). A rooted cutting should be healthy looking with good fibrous roots and a single stem when ready to be planted out in the field (Zwolinski and Bayley, 2001). In many *Eucalyptus* nurseries the macro-cutting system has been replaced by a mini-cutting system, which delays maturation, improves rooting rates, lowers production costs and improves root system formation (Stape *et al.*, 2001; Assis *et al.*, 2004; Romero, 2004; Wendling *et al.*, 2010). Conventional outdoor clonal hedges are replaced with mini-hedge stock plants grown intensively under protection in plastic growth tunnels where environmental conditions such as temperature and radiation can be more easily controlled (Stape *et al.*, 2001; Assis *et al.*, 2004). New ramets for clonal hedges are established by rooting macro-cuttings from coppice re-growth of selected ortets in the field. Clonal hedges are planted at 0.5 × 0.5 m spacing and are between 0.2 and 0.7 m in height (Hettasch and Lunt, 2002), sometimes larger. Whereas mini-hedge plants are much smaller and are grown at densities of 25 to 50 plants per square meter (about 100 mm spacing between plants). Mini-hedge stock plants are allowed to grow between 70 and 100 mm in height and are kept short by frequent harvesting every two to six weeks (Hoad and Leakey, 1996; Stape *et al.*, 2001; Wendling *et al.*, 2010; Pollard, *pers. comm.*¹). Mini- and micro-cutting techniques are very similar in both concept and operational procedures, differing mainly in the origin of the initial propagules (Assis *et al.*, 2004; Titon *et al.*, 2006). Micro-cuttings are obtained from shoot apices originating from

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

micro-propagated plants (tissue culture) and mini-cuttings are derived from shoots, retaining their apical buds, from mini-hedge stock plants (Assis *et al.*, 2004; Titon *et al.*, 2006).

Some disadvantages of the various methods of propagation are that micropropagation is very costly and requires sophisticated facilities and skilled staff, seed propagation can result in highly variable phenotypes in the progeny, and rooting of macro-cuttings can be difficult and/or slow (Zwolinski and Bayley, 2001; Hettasch and Lunt, 2002; Haapala *et al.*, 2004). Similarly, macro-cuttings can cost two to three times more than seedlings because of a higher initial investment in infrastructure (tunnels, cost of heating or cooling etc.) and labour (Stape *et al.*, 2001). Using macro-cuttings for propagation can have a loss of rooting competence due to ontogenetic aging of the clonal hedges and poor root system quality (Assis *et al.*, 2004; Titon *et al.*, 2006). Sasse and Sands (1997) compared root architecture of *Eucalyptus globulus* macro-cuttings with apex removed and normal seedlings. Seedlings had more primary roots and the number of roots increased with time, whereas the number of primary (adventitious) roots of macro-cuttings remained constant (Sasse and Sands, 1997). The quality of macro-cutting root systems was more variable than in seedlings due to variability in a range of root system parameters (Sasse and Sands, 1997). Plantations grown from clones have a much higher degree of uniformity than those grown from seedlings (Stape *et al.*, 2001; Hettasch and Lunt, 2002), to allow trees to grow as a uniform stand. Vegetative propagation is an effective method for propagating plants that produce little or no seed or if hybrids are sterile (Hettasch and Lunt, 2002). This method can be used for establishment of plantations on a commercial scale of trees such as neem (*Azadirachta indica*), which are inhibited by rapid loss of seed viability (Kamaluddin and Ali, 1996). Advantages of using mini-cuttings and mini-hedges over macro-cuttings and outdoor clonal hedges include increased productivity as more cuttings can be produced per year per unit area (Assis *et al.*, 2004; Romero, 2004). Costs of labour intensive activities can be reduced due to limiting the area of field operations such as soil preparation, the amount of fertilizer, irrigation water, and pesticides and fungicides used, weeds are easier to control and distances are shorter to transport harvested material to rooting areas (Assis *et al.*, 2004). Although the rooting potential of cuttings varies between species and clones, generally those species or clones that are “difficult-to-root” as macro-cuttings perform better as mini-cuttings (Assis *et al.*, 2004). Increases in rooting ability and speed of root initiation of mini-cuttings compared with macro-cuttings have been attributed to higher levels of juvenility and optimal nutritional content of the stock plants (Assis *et al.*, 2004; Titon *et al.*, 2006). By increasing the rooting

rate of mini-cuttings, the time that plants are held indoors to root is frequently reduced to half compared with macro-cuttings, allowing more efficient use of facilities and reducing the exposure period of cutting tissue to pathogenic fungi (Assis *et al.*, 2004). The mini-cutting technique is more widely used than micropropagation due to being less costly by not being dependent on tissue culture to supply stock plants (Assis *et al.*, 2004). However, the micro-cutting technique can be used as an efficient and cost effective method to multiply clones that are difficult-to-root (Titon *et al.*, 2006), if facilities are available. The improved rooting of mini- and micro-cuttings compared with macro-cuttings may be due to lower lignification of the more juvenile tissue (Assis *et al.*, 2004).

1.4 Internal factors of rooting success

Adventitious rooting is a complex process, which is affected by numerous factors such as phytohormones (plant hormones), phenolic compounds, plant nutritional status and genetic characteristics as well as how a plant responds to stress such as wounding, water and temperature stress (Hartmann *et al.*, 1990; Fett-Neto *et al.*, 2001).

All plant hormones regulate growth but not all plant growth regulators are hormones (Hartmann *et al.*, 1990). Plant hormones are organic compounds produced by plants, which naturally regulate plant physiological processes by moving within the plant from the production site to the action site (Hartmann *et al.*, 1990). Plant growth regulators are often synthetic compounds but can be plant hormones, which regulate growth by mimicking hormones, influencing hormone synthesis, destruction or translocation, or by modifying hormonal action sites. Various types of growth regulators such as auxins, cytokinins, gibberellins and ethylene, influence root initiation (Hartmann *et al.*, 1990). Of these growth regulators, auxins have the greatest effect on root formation in cuttings (Hartmann *et al.*, 1990; Fett-Neto *et al.*, 2001; Rocha Corrêa and Fett-Neto, 2004; Trobec *et al.*, 2005). Using exogenous auxin increases the percentage of cuttings that form roots, hastens root initiation, increases the number and quality of roots produced per cutting and increases rooting uniformity of cuttings (Hartmann *et al.*, 1990; De Klerk *et al.*, 1999; Fett-Neto *et al.*, 2001). Cuttings from species that root easily may not need additional auxin to form adventitious roots; however, auxin is beneficial to difficult-to-root species, although there are exceptions where difficult-to-root species root poorly after auxin application if auxin is not the limiting factor (Hartmann *et al.*, 1990). Naturally occurring indole-3-acetic acid (IAA) is an auxin

and a rooting hormone. The most reliable synthetically manufactured auxins for stimulating adventitious rooting in cuttings are indole-butyric acid (IBA) and naphthalene-acetic acid (NAA) (Hartmann *et al.*, 1990; De Klerk *et al.*, 1999). Indole-butyric acid is often used as it is generally non-toxic to plants over a wide concentration range and is effective in promoting rooting of a large number of plant species (Hartmann *et al.*, 1990). Osterc *et al.* (2009) found that endogenous basal IAA levels of ornamental cherry (*Prunus subhirtella*) cuttings were high directly after severance and at the beginning of the root developing phase, but decreased during the induction phase of rooting and as the roots start developing (Osterc *et al.*, 2009). The first four days after severance, including the induction phase, are auxin active requiring a continuous supply of auxin, either from a terminal bud or from applied auxin, in order to form roots (Hartmann *et al.*, 1990). Juvenile cuttings contained, nearly twice as much IAA in their bases at the time of severance as the cuttings from mature stock plants (Osterc *et al.*, 2009). Similarly, endogenous IAA levels were greater in leaves and softwood cuttings than in hardwood cuttings (Yoo and Kim, 1996). Endogenous IAA levels of white forsythia (*Abeliophyllum distichum*) hardwood cuttings were low during winter, but increased after dormancy in late winter and decreased during the flowering season in the middle of spring, increasing again in summer, which corresponded with the rooting ability of the cuttings during these seasons (Yoo and Kim, 1996). Rooting potentials in *Eucalyptus* historically improved when vegetative propagation procedures changed from using macro-cuttings harvested from large stumps to mini-cuttings harvested from outdoor mini-hedges and then further improved with using mini-cuttings harvested from indoor intensively managed mini-hedges (Assis *et al.*, 2004). These rooting improvements were partially related to the better nutritional status and to current and stored carbohydrates in the stock plants and mini-cuttings (Assis *et al.*, 2004). Vegetative propagation procedures such as using tissue culture, mini-cuttings or coppicing of a mature tree stump leads to physiological rejuvenation, whereby the newly propagated tissue is considered juvenile, which improves the rooting potential of difficult-to-root species (Hartmann *et al.*, 1990; Haapala *et al.*, 2004; Kibbler *et al.*, 2004; Osterc *et al.*, 2009).

The juvenile phase begins with the germination of seed, although the end of the juvenile stage and beginning of the mature stage is often not clearly defined, it is usually regarded as when the plant is first able to form flowers (Haapala *et al.*, 2004). The juvenile phase is often characterised by specific morphological and physiological traits that differ from the mature plant such as leaf shape, thorniness, vigour and disease resistance as well as having a greater

ability to form adventitious roots (Hartmann *et al.*, 1990). Many *Eucalyptus* species have rounded sessile leaves (no petiole) during the juvenile phase and narrow leaves with petioles and form flowers and seedpods when mature (Hartmann *et al.*, 1990). The ability to form adventitious roots from cuttings often declines with maturation as reported for, *Eucalyptus*, Douglas fir (*Pseudotsuga menziesii*) (Hartmann *et al.*, 1990); hybrid aspen (*Populus tremula* × *P. tremuloides*) (Haapala *et al.*, 2004); *Backhousia citriodora* (Kibbler *et al.*, 2004) and ornamental cherry (*Prunus subhirtella*) (Osterc *et al.*, 2009). Adventitious root formation of cuttings is strongly influenced by genotype (Kibbler *et al.*, 2004). This decrease in rooting ability with advancing ontogeny may possibly be explained by the increasing production of rooting inhibitors as plants age (Hartmann *et al.*, 1990). Therefore, the biological or ontogenetic age, and not the chronological age, of propagules is the more important factor for rooting success (Hartmann *et al.*, 1990; Osterc *et al.*, 2009). Mature woody species and basal ends of shoots may contain more lignin, which according to Trobec *et al.* (2005) may inhibit rooting of cuttings due to higher IAA oxidase activity in more lignified tissue leading to lower endogenous IAA levels. Higher lignification of tissue can additionally present a mechanical barrier, whereby emergence of the adventitious roots becomes difficult (Trobec *et al.*, 2005). Loss of rooting capacity with stock plant age was more pronounced in *E. globulus* than in *E. saligna*, but the application of exogenous auxin to *E. globulus* reversed this effect to some extent (Fett-Neto *et al.*, 2001). Leafy macro-cuttings of ornamental cherry *Prunus subhirtella* ‘Autumnalis’ were harvested from juvenile three-year-old stock plants and mature 40-year-old trees (Osterc *et al.*, 2009). Cuttings from the more juvenile stock plants developed a significantly higher number of primary roots and higher rooting percentage (76.7 %) than cuttings from mature stock plants (32.3 %) and due to the higher-quality root system of young cuttings, stronger sprout growth was achieved (Osterc *et al.*, 2009). Rooting potential can be preserved at the base of plants (usually below node 15 from the base) where tissue remains biologically juvenile and can be used to vegetatively propagate difficult-to-root mature plants (Kibbler *et al.*, 2004). Continuous pruning is, however, not a permanent solution as over time rooting success and quality and survival of stock plants declines. Although this time period varies for different species, *Backhousia citriodora* hedge plants declined over a five-year period (Kibbler *et al.*, 2004) and in hybrid aspen (*Populus tremula* × *P. tremuloides*) stock plants, where cuttings were taken every four weeks, displayed a decline over eight months (Haapala *et al.*, 2004).

1.5 Radiation quantity

Radiation quantity (intensity), duration (photoperiod or daylength) and spectral quality (wavelength) influence the condition of stock plants with regard to growth and development and subsequent rooting of cuttings (Hartmann *et al.*, 1990). The level of irradiance that stock plants or cuttings are exposed to is important for adventitious rooting as radiant energy influences the level, translocation of and cell sensitivity to endogenous hormones such as auxin as well as photosynthates (Palanisamy and Kumar, 1997; Fett-Neto *et al.*, 2001; Assis *et al.*, 2004). Potential stock plant effects include changes in nutrient uptake due to radiation quantity effects on stomatal opening and transpiration (Assis *et al.*, 2004). Radiation contributes to the seasonal variation of rooting ability of cuttings. Differing irradiance intensities applied to stock plants can effect rooting by inhibiting, promoting, or not affecting root initiation and development (Hartmann *et al.*, 1990; Pellicer *et al.*, 1998). Possible explanations for light inhibition of rooting include; inadequate IAA synthesis, inhibition of rooting cofactor synthesis, increased destruction of factors promoting root formation, increased peroxidase activity, and formation of histological barriers (Hartmann *et al.*, 1990).

Mung bean (*Phaseolus aureus*) cuttings had enhanced rooting when treated with IBA under high continuous irradiance (40 W m^{-2} (400 to 750 nm)), compared with those not treated with auxin, although for this species, rooting is generally enhanced when stock plants are grown under high irradiance (Jarvis and Ali, 1985). Some woody plants react to short photoperiods, such as during winter, with a dormancy response, whereby vegetative growth is slowed leading to reduced rooting, in this case stock plants can be manipulated to continue to grow by extending the photoperiod with low irradiance artificial lighting (Hartmann *et al.*, 1990). Supplemental lighting in the greenhouse can be used to improve stock plant growth in order to improve vigour and enhance production of cuttings (Pellicer *et al.*, 1998). Although when supplementary lighting of $50 \mu\text{mol s}^{-1} \text{ m}^{-2}$ was provided to larch (*Larix × eurolepis*) stock plants, for an 18 hour photoperiod, when natural radiation intensity fell below 40 W m^{-2} , it promoted the growth of stock plants and the number of cuttings collected, but it adversely affected the rooting percentage in the subsequent rooting of cuttings (Pellicer *et al.*, 1998). Cuttings from Pine (*Pinus sylvestris*) stock plants grown at a low irradiance of 8 W m^{-2} rooted faster and with a greater rooting percentage than those from stock plants grown at 40 W m^{-2} (Hansen *et al.*, 1978). When these two irradiance levels were used during the rooting period, only minor effects on the rooting rate were observed (Hansen *et al.*,

1978). The level of irradiance during the stock plant stage affected the rooting process more than the irradiance level during root formation (Hansen *et al.*, 1978).

Due to their physical properties, additives to the plastics and the knitting design, shade nets may differ in their efficiency to transmit diffused or scattered radiation as well as their ability to scatter direct radiation passing through such nets (Shahak, 2002). In addition to the protection of crops from excessive irradiation, nets can be used as a mechanical barrier against birds, bats and insects as well as hail and heavy rain (Oren-Shamir *et al.*, 2001; Anon, 2006; Stamps, 2009). Using shade nets can partially restrict air movement, thus reducing wind damage to the crop and soil moisture evaporation, leading to higher relative humidity (RH) beneath shade nets (Anon, 2006; Stamps, 2009). Crop protection using hail and shade nets expanded from being mainly used in ornamental nurseries to being used in fruit tree orchards has led to the improved quality of harvested products (Oren-Shamir *et al.*, 2001). Some heavy black netting or black opaque plastic can be used to eliminate natural light in the greenhouse to induce short-day conditions, but if used for extended periods this may cause etiolation. Etiolation is the development of plants or plant parts in complete darkness. This leads to plants with small, unexpanded leaves, elongated shoots and lack of chlorophyll resulting in plant parts tending towards a yellow or white colour. However, plant propagators often use this term when forcing stock plants to grow new shoots under heavy shading conditions (Hartmann *et al.*, 1990), which may lead to confusion. The term shade-avoidance response may be more fitting when discussing heavy shading instead of total elimination of light. The phenomenon of shade-avoidance response is usually attributed to reduction of the red to near infrared (R:NIR) from shading, or to the deficiency in blue radiation and is characterised by elongated plant shoots that tend to be thin and weak (Oren-Shamir *et al.*, 2001; Franklin and Whitelam, 2005). Shade-avoidance response can lead to reduced chlorophyll content, reduction in leaf thickness as well as elevated leaf angles and increased apical dominance, leading to reduced branching (Franklin and Whitelam, 2005). Auxin is often applied in varying concentrations to stimulate rooting of hardwood or softwood cuttings that are usually more difficult-to-root than herbaceous cuttings. Etiolation can be used to increase rooting of selected difficult-to-root species in conjunction with applied auxin, where auxin alone is not sufficiently effective (Hartmann *et al.*, 1990). This may be because etiolation greatly enhances a stem's sensitivity to absorb and utilise exogenous auxin and stem tissue consists of more undifferentiated parenchyma cells, which may increase the potential for initiation of root primordia (Hartmann *et al.*, 1990). Furthermore,

there are reduced mechanical barriers due to reduced production of lignin (for structural support in cells), which may alter the availability of phenolic metabolites, which are channelled to enhance root initiation instead of forming lignin (Hartmann *et al.*, 1990).

James and Bell (2000) grew *Eucalyptus globulus* ssp. *globulus* from two areas in Australia under full sunlight as well as 50 and 90 % shading in order to determine the effect of radiation intensity on plant growth and leaf morphology. Saplings grown under full sunlight or 50 % shading were significantly greater in height, number of leaves and basal diameter than saplings grown in 90 % shade. Saplings under 90 % shading had the smallest total leaf area (LA), 50 % shading had an intermediate LA and saplings in full sunlight had the greatest LA (James and Bell, 2000). However, when calculating specific LA with respect to cm² per gram dry mass the radiation treatments reversed in rank order whereby saplings under 90 % shading had the largest specific LA, 50 % had an intermediate specific LA and full sunlight had the smallest specific LA (James and Bell, 2000). The time it took for juvenile leaves to start transitioning to mature leaves was four months for plants under full sunlight and 50 % shading and nine months under 90 % shading; the cause of this delay in maturation under low radiation may be due to reduced growth rates and carbohydrate concentrations (James and Bell, 2000). When biomass allocation increases in leaves at the expense of non-photosynthetic stems and roots under low radiation like in 90 % shade, it is in order to maximise radiation interception and growth rates (James and Bell, 2000). Saplings in full sunlight seemed to have enough photosynthates in order to sustain large leaves and to allocate carbohydrates to structural components, such as basal diameter and number of branches, whereas saplings under 50 % shading seemed to preferentially allocate available carbohydrates into leaves for increased sunlight capture (James and Bell, 2000) as structural components had smaller biomass than under full sunlight. The energy cost of producing a larger leaf in 90 % shading may outweigh the benefit of increased radiation interception and carbohydrate gain (James and Bell, 2000).

In the management of stock plants for vegetative propagation, the importance of radiation is well established, although the effects on subsequent rooting are less clear because of the difficulty of separating differences in radiation quantity and quality (Hoad and Leakey, 1996). Grinberger *et al.* (2000) maintains that the quality of the radiation is the more relevant factor than its absolute quantity for plant growth and development.

1.6 Radiation quality

The quality of radiation can be manipulated by using different coloured shade nets to cover the area where stock plants are grown. Plants respond to quantity and quality of radiation, but requirements differ according to species (Shahak, 2002). In order to improve rooting frequency of difficult-to-root *Eucalyptus grandis* × *E. nitens* (G × N) clones the ideal radiation conditions must be identified in order to produce the best quality mini-cuttings with regard to health, carbohydrate status and endogenous hormone concentrations. The growth form or morphology of leafy plants can be altered for better yield and quality, depending on desired parameters, by placing them under different coloured shade nets (Oren-Shamir *et al.*, 2001), thus decreasing the need for growth regulator applications or pruning. Shade netting can, however, decrease yield of some crops due to the decreased photosynthetic activity (Grinberger *et al.*, 2000). The quality of a cutting cannot be improved after severance from the stock plant, but only maintained until the cutting forms roots with optimal radiation and high relative humidity (RH). For *Eucalyptus* hybrids that are known to be difficult-to-root, any improvement in rooting percentage could increase the profit margins of commercial nurseries as space and labour can be better utilised and more cuttings can be produced in the same time period.

Green leafy plants usually have a high absorption of radiation in the blue (B), between 400 and 500 nm, and in the red (R) region, between 600 and 700 nm of the radiation spectrum. The photosynthetically active radiation (PAR) region lies between 400 and 700 nm and plants exhibit high reflectance in the green (G) region, between 500 and 600 nm and far red (FR) region beyond 700 nm (Grant, 1997). What is commonly referred to as far red (FR) radiation is more accurately near infrared (NIR) radiation (Savage, *pers. comm.*²) and will be referred to as such from this point on. Radiation in the orange to red end of the radiation spectrum seems to favour rooting of cuttings more than that in the blue spectrum, but there are conflicting reports on the effect of spectral radiation on stock plants (Hartmann *et al.*, 1990). In order to understand the effect of specific wavelengths on stock plants, the specific spectral irradiance reaching the plants and transmissivity (ratio of spectral irradiance under the net divided by that above the net at the same specific wavelengths) of the material plants are grown under must be known.

² Prof. Michael Savage. University of KwaZulu-Natal, Discipline of Agrometeorology, Savage@ukzn.ac.za, Pietermaritzburg, South Africa.

Eucalyptus research has not focused on the effects of coloured shade nets and plastics. Most nurseries propagating woody species grow stock plants under direct sunlight, under clear plastic in tunnels or under a black or green shade net of 30 to 40 % shading intensity, in order to reduce total solar irradiance, and often use different shade percentages for different plant types. Different nurseries use different shade net percentages according to preference, but (Wallis, *pers. comm.*³) recommended using 30 or 40 % as the greenhouse becomes too dark when using any higher shading percentage. After rooting, many nurseries allow rooted cuttings to harden under thin hail netting that gives about 18 to 20 % shading intensity (Pollard, *pers. comm.*¹). Many authors describe the conditions that *Eucalyptus* species of different types of cuttings are rooted under, in a greenhouse, shade house or other protected structure (Stape *et al.*, 2001) or conditions after rooting, where *E. grandis* micro- and mini-cuttings were transferred to a shade house (50 % shade), for acclimation and then allowed to harden outdoors (Titon *et al.*, 2006), but not many describe any shading conditions for the stock plants themselves. Depending on geographical location and local climatic conditions as well as the specific crop grown, shade netting of different shading percentages can be used. It is suggested that 30 to 40 % shade netting is ideal for germination of seeds and development of seedlings or other young, vulnerable plants and 50 to 55 % shade netting is recommended for flower cultivation and for general nursery stock (Anon, 2006). Nets that are made of clear or black plastic transmit radiation evenly throughout the spectrum, without modifying PAR; act as neutral density filters (Oren-Shamir *et al.*, 2001). Coloured shade nets that modify the spectrum of transmitted radiation (in the UV, visible or NIR regions) are referred to as photosensitive netting (Shahak, 2002).

1.6.1 Altering radiation quality

1.6.1.1 *Effects of specific coloured shade nets*

Aluminet®

Aluminet® is a high quality aluminised (silver) woven shade net that is often called a thermal net as it can be used to moderate day and night temperatures to assist with microclimate control in greenhouses and nurseries (Anon, 2005, Anon, 2008). This net comes in various

³ Ms Jacqui Wallis. Mondi – Mountain home, jacqui.wallis@mondigroup.co.za, Hilton, South Africa.

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

shading percentages and can be installed as a thermal blanket outside or as a shade net inside a greenhouse. Aluminet® is more expensive than black shade net, but provides many advantages due to the highly reflective nature of the net (Anon, 2008). Such as, reflecting radiation away from the net, thus reducing the ambient temperature in the greenhouse (Anon, 2008). Similarly at night, when the plants and floor of the greenhouse cool down by giving off infrared radiation, Aluminet® reflects this radiation back to the plants maintaining warmer leaf and air temperatures (Anon, 2008). This decreases condensation on leaves in warmer months (Anon, 2008) and protects against frost damage and saves energy in heated structures (Anon, 2005) in colder months and climates. Aluminet® provides for more diffused radiation in the plant canopy compared with black shade netting (Oren-Shamir *et al.*, 2001; Anon, 2005; Anon, 2008). Direct radiation onto plants results in the upper leaves receiving excessive radiation and the lower leaves being shaded, whereas diffused radiation is spread more evenly throughout the canopy (Anon, 2008). Aluminet® transmits radiation neutrally in terms of radiation quality at specific wavelengths. *Pittosporum variegatum*, a species whose decorative branches are used as additives or fillers in bouquets or as pot plants, were grown under 50 % shading green, red, blue, grey, black, and reflective Aluminet® shade nets (Oren-Shamir *et al.*, 2001). Under Aluminet® the elongation rates were not significantly different to green, grey or black nets, but the Aluminet® and grey dramatically enhanced side branching. Commercial yield of *Pittosporum* plants is calculated as a combination of number of branches per plant, number of branches of commercial length (longer than 30 cm) and mass per branch. The number of commercial branches per plant for the grey net treatment was the highest of all nets followed by Aluminet®, red and green. Leaf photosynthesis rates measured showed no significant differences among the *Pittosporum* plants grown under the Aluminet® compared with green, red and black (Oren-Shamir *et al.*, 2001). Ityel *et al.* (2000) used black shade netting at 30, 40 and 50 % and Aluminet® 40 % shading intensity for a trial on four cultivars of red peppers (*Capsicum annuum*). The Aluminet® 40 % had the highest yield, in terms of mass in kg m⁻² and number of fruit per m², for all cultivars tested.

Black

Black knitted shade nets are frequently used in nurseries and garden centres to shade plants and keep them cooler than under direct sunlight. In general, black shade nets absorb radiation resulting in higher temperatures under the net (Leite *et al.*, 2002; Anon, 2008). Treatment with 50 % black net resulted in a lowest yield of red peppers (*Capsicum annuum*), in terms

of mass in kg m^{-2} and number of fruit per m^2 , for all the four varieties tested. The 30 and 40 % black netting had an interaction with the variety (Itzel *et al.*, 2000). In the ‘Slika’ and ‘Mazurka’ varieties 30 % black gave higher yield; in the ‘Lorka’ variety no difference was found, and in the ‘Torkal’ variety the 40 % black shade netting gave higher yield (Itzel *et al.*, 2000). The second lowest total yield of *Pittosporum* commercial branches per plant was obtained under the black net (Oren-Shamir *et al.*, 2001).

Blue

Blue copper sulphate (CuSO_4) filters can be used to reduce plant height and internode length and reduce stem dry mass of Chrysanthemum (*Dendranthema × grandiflorum*) and miniature roses (*Rosa × hybrida*) where the number of lateral shoots were increased compared with control filters (Rajapakse *et al.*, 1993; Rajapakse and Kelly, 1994; Rajapakse *et al.*, 1999). Additionally to dwarfing, the copper sulphate filters induced darker green leaves containing more chlorophyll (Rajapakse *et al.*, 1999). Blue shade nets have been used to try replicate the results achieved using copper sulphate filters. Anon (2010) claim that plants grown under blue shade net are more compact with dark green foliage than plants grown under other shade nets. However, Oren-Shamir *et al.* (2001) observed no significant differences between the chlorophyll content of the green parts of the *Pittosporum* plants grown under the blue net compared with any of the other nets studied. *Pittosporum* plants grown under blue netting were dwarfed with shorter branches and internodes (Oren-Shamir *et al.*, 2001). The rate of photosynthesis of plants grown beneath blue shade netting is decreased and the time to flower and fruit ripening is delayed (Anon, 2010). Blue nets change the radiation spectrum transmitted to plants, by reducing the red and NIR radiation transmitted and by increasing the blue radiation transmitted. Flowering can be delayed and the grower benefits by the ability to direct the flowering time. The net is most suitable for nurseries that produce pot plants who wish to reduce transport volume and want to delay flowering or fruiting according to market requirements (Anon, 2010). Contrary to Rajapakse and Kelly (1994), Oren-Shamir *et al.* (2001) found that under the blue shade net the plants developed the least number of side branches leading to a decreased yield of commercial branches. The apparent photosynthesis rates measured under the blue net were consistently lower than all other nets, but had a greater percentage of variegation (Oren-Shamir *et al.*, 2001).

Green

Green knitted shade nets are frequently used in nurseries and garden centres along with or in place of black nets. These nets are often used for decorative purposes, as wind screens at building sites or golf courses and as shade netting at private residences as it blends in well with the natural surroundings (Anon, 2006). Green filters increased plant height but inhibited lateral buds (Rajapakse *et al.*, 1999; Oren-Shamir *et al.*, 2001). The only net that markedly reduced the red to near infrared (R:NIR) was the green (Oren-Shamir *et al.*, 2001).

Red

Photosensitive red shade nets reduce the amount of blue, green and yellow radiation and increase the red and NIR radiation transmitted to plants grown beneath them (Anon, 2010). Red netting accelerates photosynthesis of plants beneath it and promotes early ripening of fruit and early flowering without decreasing flower quality. Red netting promotes larger leaf area, longer and thicker stems and total foliage volume or yield is higher (Anon, 2010). The net is most suitable for growers who wish to accelerate growth and increase foliage volume of plants, such as house plants, decorative branches, non-flowering herbs (Anon, 2010) and who want to accelerate flowering or fruiting to take advantage of early market prices. Under red netting there was a pronounced stimulation of branch and internode elongation (Oren-Shamir *et al.*, 2001) resulting in taller plants. The average *Pittosporum* branch growing under the red netting was 45 % longer than under the blue netting (Oren-Shamir *et al.*, 2001). The red netting induced more branching in *Pittosporum* than the standard black netting and produced less branching than grey or Aluminet®, but this branching was slower to develop than other nets (Oren-Shamir *et al.*, 2001).

1.6.1.2 Specific plastic covering

Clarix E Blue®

Clarix E Blue® is a specific photo-selective Ethylene Vinyl Acetate copolymer (EVA) screening film. Clarix E Blue® allows transmission of 82 to 88 % of total PAR and is 200µm thick (Hartley, *pers. comm.*⁴). Clarix E Blue® can be used to grow more compact plants as the blue radiation transmitted will reduce leaf and overall plant size and slow down plant development. It is suggested that growers increase the plant density by 10 % as Clarix E Blue® film transmits between 10 and 15 % more radiation than standard greenhouse films

⁴ Mr Keith Hartley. Film Flex Plastics Natal CC, Keithhartley@filmflex.co.za, New Germany, Durban, South Africa.

(Hartley, *pers. comm.*⁴). Clarix E Blue® is most suitable for fruiting crops grown in a trellised manner, such as tomatoes (*Solanum lycopersicum*), cucumbers (*Cucumis sativus*), aubergines (*Solanum melongena*) as well as strawberries (*Fragaria × ananassa*) and other bedding plants and seedling production in nurseries (Hartley, *pers. comm.*⁴). Clarix E Blue® films come standard with anti-drip additives (AG), which reduces the surface tension of condensed water vapour, thus avoiding fogging and droplets forming on the film (Hartley, *pers. comm.*⁴).

Patilite®

Patilite® is imported "thermal" EVA greenhouse sheeting. This film is created in three layers with micro-bubbles of carbon dioxide gas in the central layer that enhances the thermal ability as the micro-bubbles create an insulating effect and the radiation diffusing capabilities of the film in both hot and cold climates and is, therefore, cooler in summer and warmer in winter (Hartley, *pers. comm.*⁴). Patilite® allows transmission of more than 80 % of total PAR and is 210µm thick (Hartley, *pers. comm.*⁴). Patilite® transmits a high degree of diffused radiation as well as good direct radiation in the PAR spectrum which is necessary for the plants growth (Hartley, *pers. comm.*⁴). Patilite® enhances the growth and fruit-bearing properties of plants due to the diffused radiation combined with the reduced temperatures leading to well-balanced plant growth and an extension of the production period and avoiding conditions of sunburn. Water supplied to plants can be reduced as evaporation is reduced due to the combination of diffused radiation and reduced temperatures, particularly in hydroponics systems. Patilite® films do not contain anti-drip additives as the micro-bubble structure and highly insulating effect of the film reduces the water vapour condensation onto the film surface naturally (Hartley, *pers. comm.*⁴).

Unlike greenhouses, shade houses cause only minor changes to the plant microclimate, but are able to modify both the quantity and the quality of the radiation transmitted (Oren-Shamir et al., 2001). Coloured shade netting can help to control different plant properties such as flowering date, leaf and fruit size, colour, root development, yield, branching and plant height (Anon, 2010).

1.6.2 Effect of radiation quality on elongation

Growing stock plants at very low radiation intensities leads to a shade-avoidance response, which can result in improved rooting and increased number of roots per cutting in some

species, while decreasing these parameters in others (Assis *et al.*, 2004; Haapala *et al.*, 2004). This technique can be used on stock plants of difficult-to-root species whereby the resultant cuttings are easier to root and the cuttings are handled as softwood or herbaceous cuttings under mist propagation (Hartmann *et al.*, 1990). Due to the negative characteristics of etiolated or shade-avoidance plants, such as weak and pale plants, it would be preferable to get the same effects with good rooting using a lower shading percentage. Radiation quality affects plant growth and development and is controlled by the combined action of several photoreceptor systems such as the phytochrome and cryptochrome systems. Phytochromes are responsible for detecting NIR and red radiation (Hartmann *et al.*, 1990; Hoad and Leakey, 1996; Franklin and Whitelam, 2005) and to a lesser extent blue and UV radiation (Rajapakse *et al.*, 1993; Grant, 1997; Oren-Shamir *et al.*, 2001; Runkle and Heins, 2001). Cryptochromes are responsible for detecting blue and UV-A radiation (Grant, 1997; Oren-Shamir *et al.*, 2001; Runkle and Heins, 2001; Franklin and Whitelam, 2005; Stamps, 2009). Stem elongation due to shade-avoidance response is specifically influenced by the amount of red radiation relative to NIR radiation (R:NIR) reaching the plants (Rajapakse *et al.*, 1993; Hoad and Leakey, 1996). Growing plants under a low R:NIR, any ratio less than that of daylight (approximately 1.2 all year round) but usually less than 1.0 (Hoad and Leakey, 1996; Franklin and Whitelam, 2005), such as where smaller plants are shaded by the upper canopy and receive more NIR radiation than red radiation resulting in a low R:NIR, tends to promote stem elongation. Underneath canopies of vegetation, R:NIR are typically in the range between 0.05 and 0.7 (Franklin and Whitelam, 2005). A high R:NIR, more than 1.2, inhibits stem elongation and can cause dwarfing (Appelgren, 1991; Oren-Shamir *et al.*, 2001). The dwarfing effect by means of inhibition of stem elongation due to blue radiation exposure is controlled by cryptochromes (Runkle and Heins, 2001). Furthermore, the ratios of blue to red (B:R) and blue to near infrared (B:NIR) can be measured (Runkle and Heins, 2001). A high ratio of B:R is known to inhibit stem elongation in many plants especially at higher irradiance levels as the inhibitory effect is less pronounced at lower irradiance levels (Appelgren, 1991). Appelgren (1991) found that low ratios of B:R and B:NIR seem to be more indicative of stem elongation than low ratio of R:NIR. Runkle and Heins (2001) found that the expected stem elongation response related to R:NIR is invalid when blue radiation levels differ significantly from that in the natural environment. Blue radiation seems to play a role equal to or greater than that of R or NIR radiation in controlling stem elongation in long-day plants (Runkle and Heins, 2001). Apart from long-day and short-day plants that are sensitive to changing radiation conditions, shade-avoiding species such as *Campanula*

carpatica and *Coreopsis* × *grandiflora* are sensitive to R:NIR and shade-tolerant species such as *Lobelia* × *speciosa* do not respond strongly to a low R:NIR with regard to stem elongation (Runkle and Heins, 2001).

Hoad and Leakey (1996) grew *E. grandis* mini-hedges under filters to induce radiation conditions with differing R:NIR ranging from 0.4 to 6.5 in order to take cuttings. Rooting success of *E. grandis* was significantly greater in longer cuttings harvested from mini-hedges grown under low R:NIR conditions compared with shorter cuttings from high R:NIR. Better rooting ability may be associated with longer cuttings as they tend to have the most favourable leaf and stem morphology, such as, greatest leaf and stem dry weights and larger leaf area (Hoad and Leakey, 1996). The cuttings from mini-hedges grown under low R:NIR were longer due to longer internodes as all cuttings consisted of two nodes. Cutting size, determined by internode lengths, was closely correlated with percentage cuttings rooted, where the longer cuttings at the apical end rooted best, although when all cuttings were cut to the same length, basal cuttings rooted best (Leakey, 1985). Hoad and Leakey (1996) found that poor rooting of shorter *E. grandis* cuttings were, however, confounded by greater susceptibility of short cuttings to rotting and subsequent death due to the close proximity of their leaves to the surface of the medium. Long cuttings maintained their original leaves and produced significantly more new leaves than short cuttings, implying that longer cuttings were more photosynthetically active and perhaps were better at mobilising stored and using current photosynthates during propagation (Hoad and Leakey, 1996). Red netting stimulates stem elongation resulting in taller plants and blue netting exhibited dwarfing with shorter plants and stems such as *Pittosporum variegatum* (Oren-Shamir *et al.*, 2001), Leather-leaf ferns (*Rumohra adiantiformis*) and *Ruscus hypoglossum* (Shahak, 2002), *Dracaena marginata* ‘Colorama’ (Kawabata *et al.*, 2007) and Pea (*Pisum sativum*) (Cummings *et al.*, 2008). Alternatively, Costa *et al.* (2010) found no significant difference in plant height *Ocimum selloi* medicinal perennial shrubs between red and blue shade nets at 50 % shading, although shaded plants were taller than the control plants, which were grown in full sunlight. *Dracaena marginata* ‘Colorama’ plants under the 70 % red shade net produced the longest new cane growth compared with 70 % black, blue and grey netting; however, other cultivars such as *Dracaena deremensis* ‘Janet Craig’ were not affected by radiation quality as greatly (Kawabata *et al.*, 2007). An important parameter determining the commercial value of cut flowers is the length and weight of the flowering stems. When grown under 50 % red netting *Lisianthus* stems were 100 mm longer than under 50 % black netting (Shahak, 2002).

Lupines had a similar vegetative response to *Lisianthus* (Shahak, 2002). *Eucalyptus globulus* grown under 90 % shading did not display a shade-avoidance response as saplings were shorter overall and had the shortest internodes compared with 50 % shading and full sunlight, although stem diameter was narrower under 90 % shade compared with 50 % shade and full sunlight (James and Bell, 2000).

1.6.3 Effect of radiation quality on leaf area

Oren-Shamir *et al.* (2001) growing *Pittosporum variegatum* found that red and green shade nets resulted in larger average leaf area (LA) compared with the blue, grey, black, and Aluminet® shade nets. Plants under blue net had a smaller LA than under red and green but not smaller than under grey or black netting (Oren-Shamir *et al.*, 2001). Blue net at 60 % shading resulted in dwarfing of *Aralia* and *Philodendron monstera* plants. *Philodendron monstera* leaves are often too large for export when grown under commercial netting, consequently, it was suggested to use blue netting when growing for export (Shahak, 2002) as such netting reduces the size of the leaves. There is, however, a local market in Israel for large *Philodendron* leaves; consequently, the red netting at 60 % shading was suggested to be used to produce extremely large *Aralia* and *Philodendron monstera* leaves aimed at that market (Shahak, 2002). In contrast, Kawabata *et al.* (2007) found that *Dracaena marginata* ‘Colorama’ plants under the 70 % red shade net produced the highest number of new leaves with the smallest LA compared with 70 % black, blue and grey netting. In the leafy herb basil (*Ocimum basilicum*), a greatly increased yield in terms of kilograms harvested per unit area was realised with 50 % red shade over an existing plastic tunnel (Reshef, 2001). Red nets at 30 % shading also gave excellent yields in terms of average mass in grams per lettuce head compared with the control of no net and a blue net (Grinberger *et al.*, 2000). Leaf area and leaf dry mass of *E. grandis* at severance were significantly greater in cuttings originating from lower R:NIR of 0.4 or 0.7 than in cuttings from higher R:NIR of 3.5 and 6.5 (Hoad and Leakey, 1996). Rooting and new leaf growth was significantly greater in cuttings originating from low R:NIR than those from high R:NIR (Hoad and Leakey, 1996).

The presence of leaves on cuttings is known to stimulate root initiation (Hartmann *et al.*, 1990). It has been well documented that leafless summer softwood and herbaceous cuttings rarely root, although leafless winter hardwood cuttings root well, particularly toward the end of winter (Leakey, 1985; Leakey and Coutts, 1989; Hartmann *et al.*, 1990). Since softwood and herbaceous cuttings, particularly in summer, are dependent on current photosynthesis of

leaves present to form roots (Leahey, 1985; Leahey and Coutts, 1989; Hartmann *et al.*, 1990). Leahey and Coutts (1989) suggested that root formation and development of cuttings with small LA depend more on reserves than cuttings with large LA as larger leaves can produce more photosynthates during photosynthesis. Apart from translocation of carbohydrates from leaves, a more direct root-promoting effect of leaves and buds is that they are strong auxin producers, where the auxin is transported from the apex to the base of the cutting where the roots form (Hartmann *et al.*, 1990). Wendling *et al.* (2010) observed that the sweetgum (*Liquidambar styraciflua*) clone with the largest mini-cutting root systems had the highest number of leaves; therefore, a good quality root system may improve the number of new leaves formed and conversely a higher number of leaves on cuttings may also improve the quality of roots formed. The optimal LA of cuttings can influence rooting ability particularly in difficult-to-root species (Leahey, 1985; Leahey and Coutts, 1989; Kamaluddin and Ali, 1996). *Triplochiton scleroxylon* cuttings had their leaves trimmed to 10, 50 or 100 cm² before being rooted. Cuttings with LA of 10 and 50 cm² exceeded 80 % rooting, although the 10 cm² cuttings produced fewer roots per rooted cutting than those with larger leaves and the largest leaves of 100 cm² only reached 65 % rooting (Leahey and Coutts, 1989). Similarly, Leahey (1985) found that leaf areas greater than 50 cm² per cutting were detrimental in *Triplochiton scleroxylon* and *Cleistopholis glauca*, but not in *Terminalia ivorensis* and *Nauclea diderrichii*. Kamaluddin and Ali (1996) found that rooting percentage of Neem (*Azadirachta indica*) macro-cuttings was not significantly affected by the LA as all treatments (100 % or decreased to 50 or 30 % LA per cutting) rooted well, between 90 and 100 %. Water loss from leaves through the process of transpiration, can lead to wilting of cuttings that can lead to mortality before the cutting has an opportunity to form roots. Water losses from leaves are minimised naturally by stomatal closure, but this limits photosynthesis by reducing carbon dioxide intake (Leahey and Coutts, 1989). Leaf area reduction or removal of some leaves from cuttings is a common practice to balance the positive effect of photosynthesis and the negative effect of transpiration (Leahey, 1985; Leahey and Coutts, 1989; Kamaluddin and Ali, 1996).

1.7 Other environmental parameters

1.7.1 Temperature

Shade netting can affect thermal components of the immediate plant environment due to its transmittance or reflectance of infrared wavelengths (Shahak, 2002). Shade nets are of great

importance in warm, sunny climates, where they serve to reduce both radiation intensity and air temperature during the day (Grinberger *et al.*, 2000; Oren-Shamir *et al.*, 2001). Various citrus seedlings under 50 % Aluminet® had a 4 to 5 °C lower leaf temperature than the ambient Brazilian summer temperatures (Leite, 2001). Grinberger *et al.* (2000) found that air temperatures under 30 % Aluminet® were higher than under pearl (white), blue and red coloured netting of the same shading percentage, although the control of no net, had the highest overall temperature. In general, black shade nets reduce the radiation that plants receive but increase the air temperature underneath (Leite *et al.*, 2002). Good ventilation is essential to negate differences in air and leaf temperature under different shade nets (Oren-Shamir *et al.*, 2001). Temperature extremes can influence root initiation of cuttings by interfering with water and nutrient uptake as well as rate of metabolism and the promoting or inhibiting enzyme action (Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004). Rocha Corrêa and Fett-Neto (2004) investigated the effects of different temperature treatments (15, 20, 25 and 30 °C) applied to stock plants and micro-cuttings on adventitious rooting of *Eucalyptus saligna*, an easy-to-root species and the difficult-to-root *Eucalyptus globulus*. Micro-cuttings of *E. saligna* kept at the lower temperature treatment of 15 °C developed a greater number of roots and rooted more quickly than the controls, particularly when exogenous IBA auxin was applied and had good rooting percentages at all temperatures (Rocha Corrêa and Fett-Neto, 2004). *Eucalyptus globulus* was more affected by temperature than *E. saligna*, rooting more consistently at intermediate temperatures (20 and 25 °C). Maintenance of micro-cuttings at 40 °C was lethal for both *E. globulus* and *E. saligna* species (Rocha Corrêa and Fett-Neto, 2004). The method of heating rooting benches from the bottom, while the upper portion of cuttings are exposed to cooler air temperatures, has been successful for rooting difficult-to-root cuttings of many species; this can be more cost effective than heating the whole greenhouse (Hartmann *et al.*, 1990). The optimal temperature to propagate many temperate climate species is between 18 to 25 °C at the base of cuttings, with daytime air temperatures of 21 to 27 °C and night temperatures of 15 °C, although some root better at lower temperatures (Hartmann *et al.*, 1990). *Eucalyptus globulus* macro-cuttings were rooted in a greenhouse with a bench temperature maintained at 24 °C and an air temperature of 24 to 26 °C during the day and 20 to 22 °C at night (Sasse and Sands, 1997). Similarly, mini-cuttings of *E. grandis* were rooted with a controlled bench temperature of 27 ± 2 °C (Hoad and Leakey, 1996). Leaf temperatures of *Eucalyptus* cuttings should be kept between 18 and 20 °C during rooting, consequently, misters are used to wet the cuttings and cool the leaves down through evaporation (Hettasch and Lunt, 2002).

1.7.2 Relative humidity

High humidity and temperatures can lead to the ideal breeding conditions for fungal diseases. Several fungal diseases can be responsible for severe losses of *Pinus* and *Eucalyptus* propagules in South African nurseries (Zwolinski and Bayley, 2001). Stock plants should be kept disease-free to ensure that cuttings are disease-free (Pollard, *pers. comm.*¹) and all plant material leaving the nursery should be uninfected and healthy to ensure good establishment in the field after transplanting, and to avoid poor growth or timber properties after establishment (Zwolinski and Bayley, 2001). Relative humidity (RH) is often higher under netting than outside as a result of transpiration by the plants beneath and reduced integration with drier air outside the netted area, even under higher temperatures (Stamps, 2009). Relative humidity is affected by the different properties of nets, such as the knitting or weaving pattern and the size of holes in the pattern, which controls the shading percentage and can affect the movement of air and moisture through the netting (Anon, 2005; Anon, 2008). Shahak *et al.* (2004) noted that there was a 3 to 10 % increase in the minimal daily relative humidity under nets at 30 % shading. Relative humidity under 50 % Aluminet® netting increased between 10 and 25 % compared with greenhouses without shade nets (Leite, 2001). The RH under 50 % Aluminet® was significantly higher than under 50 % black net, which is likely due to the decreased air temperature under Aluminet® (Leite *et al.*, 2002). The recommended RH for growing miniature rose plants is between 70 and 75 %. Any RH higher than this increases the probability of the occurrence of pathogens such as *Botrytis* (Leite *et al.*, 2002). However, in the environment where cuttings are rooted, it is advantageous to increase the RH by means of misters as a high RH of 80 to 90 % assists in the rooting process by decreasing transpiration from leaves (Hansen *et al.*, 1978; Hettasch and Lunt, 2002; Haapala *et al.*, 2004; Schwambach *et al.*, 2008; Osterc *et al.*, 2009). If the medium is too dry, the cutting experiences water stress, which retards rooting. Alternatively, if the medium becomes saturated due to excessive misting, the oxygen available for the development of roots becomes limited, which encourages pathogenic fungi growth, thus RH balance must be maintained (Hettasch and Lunt, 2002).

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

1.7.3 Season

Many species are limited by the season during the year when cuttings can be taken or the time period when rooting is the most successful (Leahey, 1985; Hartmann *et al.*, 1990). Deciduous cuttings from hardwood species are usually taken during the dormant season from late autumn until spring bud development and softwood leafy cuttings can be prepared during the growing spring and summer seasons (Leahey, 1985; Hartmann *et al.*, 1990). When mature plants are rejuvenated by means of coppicing or softwood cuttings are taken from hardwood plants, the subsequent cuttings tend to root more easily; furthermore, if cuttings are treated with IBA, often the seasonal rooting effects are negated (Leahey, 1985; Hartmann *et al.*, 1990; Yoo and Kim, 1996). Therefore, by using the mini-hedge method under controlled conditions, mini-cuttings should be able to be harvested and rooted all year round. The rooting percentage of sub-tropical *Eucalyptus* mini-cuttings decreases in cold winter months, a problem that may be resolved by supplying additional light and increasing the temperature of the stock plant environment to greater than 20 °C (Assis *et al.*, 2004). Cuttings can be rooted all year round from *Eucalyptus* species, but a decline in rooting is experienced during the winter months in colder areas (Hettasch and Lunt, 2002). However, the commercial forestry nursery Sunshine Seedling Services found that the rooting of cuttings from the two cold-tolerant *Eucalyptus grandis* × *E. nitens* clones used in this research, had the highest rooting percentages during winter (Pollard, *pers. comm.*¹). This may be because temperate climate species can survive and form roots in climates approximately 7 °C colder than warm climate species (Hartmann *et al.*, 1990). The effects of seasons are often a reflection of the response of cuttings to environmental conditions at different times during the year. Newly expanding buds and shoots are competing sinks for metabolites and phytohormones, which can be detrimental to rooting (Hartmann *et al.*, 1990). Therefore, active vegetative growth can lead to poor rooting such as with junipers (*Juniperus* spp.) where rooting was much higher during the dormant period (Hartmann *et al.*, 1990). Softer leafy cuttings are often affected by seasonal variations in rooting as they are more sensitive to high summer levels of irradiance and temperature, water stress and flowering than hardwood cuttings, which may contribute to decreased rooting ability (Leahey, 1985).

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

1.8 Problem statement

The *Eucalyptus* forestry nursery industry is moving toward intensively grown mini-hedge stock plants under protection. The process of rejuvenating stock plant material has improved rooting for some clones, but there are still clones that are difficult-to-root with low rooting percentages. Any significant improvement in rooting percentages will increase profits and efficiency in nurseries. This investigation aims to deepen the understanding of how the radiation spectrum under certain shade nets and plastics can be manipulated and to observe the effect this has on the morphology of mini-hedge stock plants grown under these conditions for production of cutting and subsequent rooting potential.

References

- ANON. 2010. Polynet Aust (Pty) Ltd. Colored Shade Cloth and Nets <http://www.polynet.com.au/> Accessed 25/02/2010.
- ANON. 2008. The benefits of Aluminet are multiple. <http://www.igcusa.com/greenhouse-shade-cloth-aluminet.html> Accessed 26/05/2010.
- ANON. 2006. Alnet (Pty) Ltd. Agricultural Shade Cloth. <http://www.protect-o-net.com/> Accessed 25/02/2010.
- ANON. 2005. Polysack – Better solutions for better crops. ChromatiNet® Spectrum Management Solutions. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- APPELGREN, M. 1991. Effects of light quality on stem elongation of *Pelargonium* in vitro. *Scientia Horticulturae* 45, 345-351.
- ASSIS, T.F., FETT-NETO, A.G. and ALFENAS, A.C. 2004. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: C. Walter and M. Carson (eds.), *Plantation Forest Biotechnology for the 21st Century*, 303-333. Research Signpost, Kerala, India.
- COSTA, L.C.B., PINTO, J.E.B.P., CASTRO, E.M., ALVES, E., BERTOLUCCI, S.K.V. and ROSAL, L.F. 2010. Effects of coloured shade netting on the vegetative development and leaf structure of *Ocimum selloi*. *Bragantia: Revista de Ciências Agronômicas* 69(2), 349-359.
- CUMMINGS, I.G., FOO, E., WELLER, J.L., REID, J.B. and KOUTOULIS, A. 2008. Blue and red photosensitive shade cloths modify pea height through altered blue irradiance perceived by the cry1 photoreceptor. *Journal of Horticultural Science and Biotechnology* 83(5), 663-667.
- DAFF (DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES), Republic of South Africa. 2011a. Quarterly Economic Overview of the Agriculture, Forestry and Fisheries sector: January 2011 to March 2011. 9(1), pp 9-10.
- DAFF (DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES). 2011b. Pocket Guide to South Africa 2010/11. pp 117-119.
- DE KLERK, G.J., VAN DER KRIEKEN, W. and DE JONG, J.C. 1999. Review: The formation of adventitious roots: new concepts, new possibilities. *In Vitro Cellular and Developmental Biology - Plant* 35, 189-199.

- FETT-NETO, A.G., FETT, J.P., VIEIRA GOULART, L.W., PASQUALI, G., TERMIGNONI, R.R. and FERREIRA, A.G. 2001. Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. *Tree Physiology* 21, 457-464.
- FOELKEL, C. 2008. The world of *Eucalyptus*: South Africa. *Eucalyptus* Online Book and Newsletter. August, volume 16. http://www.eucalyptus.com.br/newseng_ag08.html Accessed 11/04/2013.
- FRANKLIN, K.A. and WHITELAM, G.C. 2005. Botanical briefing: Phytochromes and shade-avoidance responses in plants. *Annals of Botany* 96, 169-175.
- GARDNER, R.A.W. 2007. Investigating the environmental adaptability of promising subtropical and cold-tolerant eucalypt species in the warm temperate climate zone of KwaZulu-Natal, South Africa. *Southern Hemisphere Forestry Journal* 69(1), 27-38.
- GODSMARK, R. 2013. Employment in the South African Forestry and Forest Products Industry 2011. *Forestry South Africa*. Accessed 11/04/2013.
- GRANT, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *International Journal of Biometeorology* 40, 26-40.
- GRINBERGER, A., SHOMRON, M. and GANELEVIN, R. 2000. Shading nets testing. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- HAAPALA, T., PAKKANEN, A. and PULKKINEN, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* × *P. tremuloides*). *Forest Ecology and Management* 193, 345-354.
- HANSEN, J., STROMQUIST, L.H. and ERICSSON, A. 1978. Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Plant Physiology* 61, 975-979.
- HARTMANN, H.T., KESTER, D.E. and DAVIES, F.T., Jr. 1990. Plant propagation – Principles and practices. Fifth edition. Prentice Hall Career & Technology, Englewood Cliffs, New Jersey, USA.
- HETTASCH, M.H. and LUNT, K.A. (eds). 2002. Chapter 4: Vegetative propagation – cuttings. In: An introduction to forest nursery practices: with emphasis on select *Eucalyptus*, *Pinus*, *Podocarpus* and *Acacia* species grown in South Africa. CSIR Environmentek, pp 53-69.

- HOAD, S.P. and LEAKEY, R.R.B. 1996. Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden cutting morphology, gas exchange and carbohydrate status during rooting. *Trees* 10, 317-324.
- ITYEL, E., OFFENBACH, R., MADDOEL, A. and GANELEVIN, R. 2000. Reshetplast – Agrotechnique for growing peppers for export at Kikar Sedom. Ministry of Agriculture, Jewish National Fund, Arava R&D and Kfar Development Training Bureau, Negev, Israel. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- JAMES, S.A. and BELL, D.T. 2000. Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. *Tree Physiology* 20, 1007-1018.
- JARVIS, B.C. and ALI, A.H.N. 1985. The influence of irradiance on root regeneration in stem cuttings of mung bean. *New Phytologist* 101, 233-239.
- KAMALUDDIN, M. and ALI, M. 1996. Effects of leaf area and auxin on rooting and growth of rooted stem cuttings of neem. *New Forests* 12, 11-18.
- KAWABATA, A.F., LICHTY, J.S., KOBAYASHI, K.D. and SAKAI, W.S. 2007. Effects of photoselective shade cloths on potted *Dracaena deremensis* ‘Janet Craig’ and *Dracaena marginata* ‘Colorama’. *Pacific Agriculture and Natural Resources* 14, 49-54.
- KIBBLER, H., JOHNSTON, M.E. and WILLIAMS, R.R. 2004. Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell. 1. Plant genotype, juvenility and characteristics of cuttings. *Scientia Horticulturae* 102, 133-143.
- LEAKEY, R.R.B. 1985. Chapter 9: The capacity for vegetative propagation in trees. In: Cannell, M.G.R.; Jackson, J.E., (eds.) Attributes of trees as crop plants. Abbots Ripton, Institute of Terrestrial Ecology, pp 110-133.
- LEAKEY, R.R.B. and COUTTS, M.P. 1989. The dynamics of rooting in *Triplochiton scleroxylon* cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. *Tree Physiology* 5, 135-146.
- LEITE, C. 2001. The Aluminet I 50% Effect on Photosynthesis for Growing Citrus in Greenhouses. *International Agriculture* (August). http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.

- LEITE, C.A., FAGNANI, M.A. and OLIVEIRA DA SILVA, I.J. 2002. Comparison between thermal reflect net and black net in mini-roses crop in Holambra-sp, Brazil. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- MCMAHON, M.J. and KELLY, J.W. 1995. Anatomy and pigments of chrysanthemum leaves developed under spectrally selective filters. Short Communication. *Scientia Horticulturae* 64, 203-209.
- MCMAHON, L., GEORGE, B. and HEAN, R. 2010. Primefacts for profitable, adaptive and sustainable primary industries. Primefact 1055, A Treesmart factsheet, September. <Http://www.industry.nsw.gov.au> Accessed 22/05/2012.
- OREN-SHAMIR, M., GUSSAKOVSKY, E.E., SHPIEGEL, E., NISSIM-LEVI, A., RATNER, K., OVADIA, R., GILLER, Y.E. and SHAHAK, Y. 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum*. *Journal of Horticultural Science and Biotechnology* 76, 353-361.
- OSTERC, G., ŠTEFANČIČ, M. and ŠTAMPAR, F. 2009. Juvenile stockplant material enhances root development through higher endogenous auxin level. *Acta Physiologiae Plantarum* 31, 899-903.
- PALANISAMY, K. and KUMAR, P. 1997. Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (*Azadirachta indica* A. Juss). *Forest Ecology and Management* 98, 277-280.
- PALLET, R.N. and SALE, G. 2004. The relative contributions of tree improvement and cultural practice toward productivity gains in *Eucalyptus* pulpwood stands. *Forest Ecology and Management* 193, 33-43.
- PELLICER, V., CAZET, M., VERGER, M. and RIVIÈRE, L.M. 1998. Effect of stock plant lighting on bulk vegetative propagation of hybrid larch (*Larix × eurolepis* Henry). *Forest Ecology and Management* 102, 323-332.
- RAJAPAKSE, N.C., MCMAHON, M.J. and KELLY, J.W. 1993. End of day far-red light reverses height reduction of chrysanthemum induced by Copper sulphate spectral filters. *Scientia Horticulturae* 53, 249-259.
- RAJAPAKSE, N.C. and KELLY, J.W. 1994. Influence of spectral filters on growth and postharvest quality of potted miniature roses. *Scientia Horticulturae* 56, 245-255.

- RAJAPAKSE, N.C., YOUNG, R.E., MCMAHON, M.J. and OI, R. 1999. Reviews: Plant height control by photoselective filters: current status and future prospects. *HortTechnology* 9(4), 618-624.
- RESHEF, G. 2001. Basil culture under different shading nets, Summer 2001. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- ROCHA CORRÊA, L.D. and FETT-NETO, A.G. 2004. Effects of temperature on adventitious root development in microcuttings of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill. *Journal of Thermal Biology* 29, 315-324.
- ROMERO, J.L. 2004. A review of propagation programs for *Gmelina arborea*. *New Forests* 28, 245-254.
- RUNKLE, E.S. and HEINS, R.D. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. *Journal of the American Society for Horticultural Science* 126(3), 275-282.
- SASSE, J. and SANDS, R. 1997. Configuration and development of root systems of cuttings and seedlings of *Eucalyptus globulus*. *New Forests* 14, 85-105.
- SCHWAMBACH, J., RUEDELL, C.M., RODRIGUES de ALMEIDA, M., PENCHEL, R.M., de ARAÚJO, E.F. and FETT-NETO, A.G. 2008. Adventitious rooting of *Eucalyptus globulus* × *maidenii* mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. *New Forests* 36, 261-271.
- SHAHAK, Y. 2002. Current research in ornamental: Colored shade nets a new agro-technology. <http://www.polysack.com/files/cf8ed7312fe796d76fb634a6fb0899ed.pdf> or <http://wendell-trading.com/files/Colour%20Shade%20Net.pdf> Accessed 14/05/2010.
- SHAHAK, Y., GUSSAKOVSKY, E.E., GAL, E. and GANELEVIN, R. 2004. Colornets: crop protection and light-quality manipulation in one technology. *Acta Horticulturae* 659, 143-151. <http://www.polysack.com/files/0840ec45a16ef4de16f28cf3a66006d6.pdf> Accessed 14/05/2010.
- STAMPS, R.H. 2009. Use of colored shade netting in horticulture. *Hortscience* 44(2), 239-241.
- STAPE, J.L., GONÇALVES, J.L.M. and GONÇALVES, A.N. 2001. Relationships between nursery practices and field performance for *Eucalyptus* plantations in Brazil: A historical overview and its increasing importance. *New Forests* 22, 19-41.

- TITON, M., Xavier, A. and Otoni, W.C. 2006. Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting techniques. *Scientia Forestalis* 71, 109-117.
- TROBEC, M., ŠTAMPAR, F., VEBERIČ, R. and OSTERC, G. 2005. Fluctuations of different endogenous phenolic compounds and cinnamic acid in the first days of the rooting process of cherry rootstock 'GiSelA 5' leafy cuttings. *Journal of Plant Physiology* 162, 589-597.
- WENDLING, I., BRONDANI, G.E., DUTRA, L.F. and HANSEL, F.A. 2010. Mini-cuttings technique: a new ex vitro method for clonal propagation of sweetgum. *New Forests* 39(3), 343-353.
- YOO, Y.K. and KIM, K.S. 1996. Seasonal variation in rooting ability, plant hormones, carbohydrate, nitrogen, starch, and soluble sugar contents in cuttings of white forsythia (*Abeliophyllum distichum* Nakai). *Journal Korean Society for Horticultural Science* 37(4), 554-560.
- ZWOLINSKI, J. and BAYLEY, A.D. 2001. Research on planting stock and forest regeneration in South Africa. *New Forests* 22, 59-74.

Chapter 2

Radiation transmission through coloured shade netting and plastics and its effect on *Eucalyptus grandis* × *E. nitens* hybrid mini-hedge shoot internode length, stem diameter and leaf area

D.L. Gilbert¹, I. Bertling¹ and M.J. Savage²

¹ Horticultural Science

² Agrometeorology

School of Agricultural, Earth and Environmental Sciences

University of KwaZulu-Natal

Pietermaritzburg

South Africa

Keywords: blue: red, propagation, red: near infrared, spectral irradiance, transmissivity

Abstract

Quantity and spectral quality of radiation is important when growing plants under protection (tunnels) and particularly for vegetative propagation due to the varying effect of the radiation spectrum on plant morphology. Five coloured shade nets and two plastics were evaluated with regard to their transmission of radiation and alteration of spectral irradiance (300 to 1100 nm waveband) in autumn, winter and spring using a spectroradiometer. Transmissivity, total spectral irradiance, photosynthetically active radiation (PAR), photosynthetic photon flux density (PPFD), red to near infrared ratio (R:NIR) and blue to red ratios (B:R) were determined. Using an incandescent lamp in the laboratory, transmissivity for each shade net and plastic was compared with that for the propagation tunnel. The Aluminet® (silver), black shade nets and Patilite® clear plastic transmitted radiation neutrally over the entire spectrum, while the blue, red and green shade net as well as Clarix E Blue® plastic altered the transmission of certain wavelengths. The R:NIR values for shade nets and plastics were significantly different over the seasons, but did not vary enough to affect the shoot internode length ranging from 1.13 to 1.22 in autumn to 1.05 to 1.16 in winter. Plants under the blue and green nets had the shortest internode length, while those under the Clarix E Blue® and red net had the longest, corresponding with results that Clarix E Blue® had the smallest R:NIR values and the blue shade net the largest. The average transmission percentage of PAR, using an incandescent lamp, in a laboratory gave more accurate measurements compared with the shading percentage given by the manufacturers. When the NIR was added to the PAR, the average transmission percentage increased for all shade nets and plastics, particularly those that exhibited photoselective spectra. Laboratory transmission measurements were generally much lower than those under field tunnel conditions. Manufacturers seem to base the shading factor percentages of nets and plastics on PAR, excluding NIR and wavelengths

beyond. For photoselective materials these excluded wavelengths may skew the expected absolute transmission of radiation.

2.1 Introduction

Hardwood tree species can be propagated vegetatively more easily from juvenile stock plant material (Assis *et al.*, 2004; Haapala *et al.*, 2004). The trend in recent years is to grow *Eucalyptus* stock plants intensively in a mini-hedge style, which maintains juvenility, under protection in plastic growth tunnels. When stock plants are grown at low short-wave irradiance, inducing etiolation, the rooting of hybrid aspen and other ligneous plants is improved (Haapala *et al.*, 2004), but can decrease rooting in some species (Assis *et al.*, 2004). In order to improve rooting of difficult-to-root *Eucalyptus grandis* (W. Hill) \times *E. nitens* (Maiden) hybrids (G \times N) stock plants, the ideal short-wave radiative conditions must be quantified in order to produce the best quality cuttings with regard to health, carbohydrate content and endogenous hormones to optimise rooting potential. Stock plants morphology should be short and produce shoots for cuttings all year round. The mini-cutting shoots should be hardy enough to survive and not too soft to prevent fungal infection (Assis *et al.*, 2004) to be able to form adventitious roots easily. Mini-cuttings consisting of at least two nodes and an internode are taken from side shoots of the stock plants between 40 to 85 mm in length and should not have a shoot diameter less than 0.5 mm according to local nursery standards. Therefore, longer internodes may improve rooting.

The quantity and spectral quality of radiation, reaching plants is important when growing plants in tunnels and particularly for vegetative propagation due to the varying effects of the radiation spectrum on plant morphology. The morphology of leafy plants (stem elongation, side branching, leaf area) as well as leaf colour can be altered by placing them under different coloured shade nets, thereby affecting yield and quality depending on parameters important for commercial value (Oren-Shamir *et al.*, 2001).

In order to understand the effect of radiation on stock plants, the irradiance and transmissivity of shade nets and plastics covering the plants must be known. Due to their physical properties, shade nets may differ in their efficiency in transmitting diffuse or scattered radiation as well as their ability to scatter direct radiation passing through them (Oren-Shamir *et al.*, 2001). The most commonly used nets are made of black plastic and transmit evenly throughout the spectrum, without modifying photosynthetically active

radiation (PAR), thus acting as neutral density filters (Oren-Shamir *et al.*, 2001). Radiation transmitted through coloured shade nets exhibit peaks and troughs over the spectrum. Oren-Shamir *et al.* (2001) found that blue nets transmitted a broad peak at 470 nm as well as into the NIR radiation beyond 750 nm. The red net had major transmittance beyond 590 nm and a minor peak around 400 nm.

Plant growth and development is influenced specifically by the amount of red (R) radiation relative to NIR radiation (R:NIR ratio) (Rajapakse *et al.*, 1993; Hoad and Leakey, 1996). Phytochrome photoreceptors are responsible for the detection of NIR and red radiation and can sense blue and ultraviolet (UV) radiation (Oren-Shamir *et al.*, 2001). It has been established that growing plants under a low R:NIR ratio promotes stem elongation, whereas a high R:NIR inhibits stem elongation and can cause a dwarfing effect (Appelgren, 1991; Oren-Shamir *et al.*, 2001). Generally, red netting stimulates branch elongation resulting in taller plants and blue netting resulting in dwarfing with shorter internodes and branches with plants under red and green shade nets having the longest internodes and a larger average leaf area (LA). Blue netting resulted in plants with smaller LA than red and green but not compared with grey or black (Oren-Shamir *et al.*, 2001). A decrease in the ratio of R:NIR, from that of daylight (approximately 1.2) to values less than 1, may increase stem elongation and reduce shoot production and lateral branching by strengthening apical dominance (Hoad and Leakey, 1996). The same authors found that radiation quality did not significantly affect shoot diameter. The majority of research on radiation quality and stem elongation has focussed on R:NIR; however, it has been shown that phytochrome undergoes radiation-induced changes in the blue (B) region of the spectrum and phytochrome phototransformation is sensitive to B radiation (Rajapakse *et al.*, 1993). Cryptochromes are photoreceptors absorbing B radiation, thereby participating in inhibition of stem elongation (Runkle and Heins, 2001). The same authors found that stem elongation under low R:NIR was invalidated when B radiation alters greatly from the natural environment. Appelgren (1991) reported that a high B:R ratio inhibits stem elongation with low B:R and B:NIR seemingly more important for stem elongation than a low R:NIR alone as a radiation source with a high R:NIR may have a low B:R and negate the expected effects.

Most nurseries propagating woody species grow stock plants in direct sunlight, under clear plastic in tunnels or under a black or green shade net to reduce total spectral irradiance. Five shade nets and two plastics were placed in a plastic tunnel and compared with a control. This

investigation aims to deepen the understanding of how the radiation spectrum can be manipulated under certain shade nets and plastics and to observe the effect on the morphology of mini-hedge stock plants grown under these conditions.

2.2 Materials and methods

The experiment was carried out in a multi-span tunnel in Pietermaritzburg, South Africa. Five shade nets (Black 30 %, Green 40 %, Apple Blue 20 %, Photo Red 30 %), were obtained from Knittex® (Multiknit® (Pty) Ltd.) as well as Aluminet® (silver) 40 %, were compared with a blue plastic (Clarix E Blue®) and a clear plastic containing CO₂ bubbles (Patilite®) obtained from FilmFlex Plastics Natal CC (percentage shading as shading factor determined by the manufacturer). Shade nets with identical shading factors were not available locally. The control treatment consisted of no shading other than that from the tunnel structure. Shade netting and plastic were draped over wire trellising over bricked beds where the top and two sides were covered but the ends left open for ventilation. The trellising was standardised to 1.32 m above the soil level of each bed. The nets were replicated once each in three blocks. Due to logistical reasons, only one block was measured three times and averaged.

2.2.1 Plant material

Two G × N clones were used as stock plants. Stock plants were collected as eight-week-old rooted cuttings from a local supplier and were transplanted singularly into 2.5 L black plastic nursery bags filled with a Perlite®, vermiculite and coir mixture. Mini-hedge plants were kept short at a height of 100 mm above the base and cuttings were harvested from side shoots.

2.2.2 Spectral irradiance

A LI-COR® portable research spectroradiometer (LI-1800, Lincoln, Nebraska, USA) was used to measure the incoming spectral irradiance (SI) ($\text{Wm}^{-2}\mu\text{m}^{-1}$) from 300 to 1100 nm in autumn, winter and spring. Readings were taken around midday on cloudless, sunny days. The instrument was placed on a stand to elevate the sensor above the mini-hedge plants to reduce false readings from unwanted reflected colours. The data were used to determine transmissivity, total spectral irradiance, PAR and photosynthetic photon flux density (PPFD). In order to determine R:NIR a narrow band of 10 nm (R = 656-666 nm and NIR = 726-736 nm) was used according to the manufacturer's instruction. Similar bands were used by Newton *et al.* (1996), Grant (1997), Oren-Shamir *et al.* (2001) and Runkle and Heins

(2001). A wide band of 100 nm was used to be able to compare the R:NIR and B:R (R = 600-700 nm, NIR = 700-800 nm and B = 400-500 nm) according to Rajapakse *et al.* (1993), Grant (1997) and Runkle and Heins (2001). These authors found the wide band to be more reliable for predicting elongation. When using the spectroradiometer, the control treatment of no shade netting was used between every two shade netting readings to be able to negate any change in radiation conditions over the time period the readings were taken.

A laboratory experiment was conducted using small pieces of the shade nets and plastics to cover the spectroradiometer sensor. To test the accuracy of transmission data recorded with time delays between measurements, a 60 W incandescent bulb was used as a static radiation source, secured in a lamp on a stand 150 mm above the spectroradiometer. Transmissivity is the ratio of spectral irradiance under the net divided by that above the net at a specific wavelength. The ratio should be equivalent when using natural or artificial radiation sources as the netting material is constant.

2.2.3 Morphology

Morphological effects on stock plants, caused by the coloured shade net treatments, were determined by measuring internode length, LA and shoot diameter of new growth. Internode length was calculated by measuring the length of a new shoot and dividing it by the number of nodes on that shoot minus one. Three plants per treatment were randomly selected from the centre of the group of stock plants and three shoots chosen per plant to determine internode length. Leaf area was measured using a Li-Cor® LI-3000L leaf area meter. Ten of the first fully expanded leaves were collected per treatment and averaged to determine the LA per leaf. Shoot diameter was measured using digital callipers.

2.2.4 Statistical analysis

The experiment was a factorial design with three levels. Statistical analyses were carried out using GenStat® 14th edition (VSN International, 2011). Shoot diameter and LA data were analysed by ANOVA and the internode data were analysed by REML as the data were not orthogonal.

2.3 Results and discussion

2.3.1 Spectral irradiance

The parameters of SI, photosynthetic irradiance and PPFD changed over the seasons, with higher values in spring than in autumn or winter (Table 2.1). The PPFD was generally consistent with the percentage shading when comparing the shade nets and plastics to the control (outside clear sky measurements) except for red 30 % and Aluminet® 40 %. The R:NIR values in the narrow band (nb) and wide band (wb) for shade nets and plastics varied significantly over the seasons (Table 2.2), with a $P_{0.05} < 0.001$ at a 5 % level of significance. The R:NIR (nb) ranged from 1.13 to 1.22 in autumn, 1.05 to 1.16 in winter and 1.10 to 1.19 in spring. However, these variations did not affect internode length as reported by Appelgren (1991) and Rajapakse *et al.* (1993) who, using artificial lighting and spectral filters, respectively, observed a larger range of R:NIR (0.72 to 80.44 and 1.1 to 5.8, respectively). Clarix E Blue® consistently had the smallest R:NIR (nb) values and blue 20 % the greatest. Values for all shade nets and plastics were consistently smaller than those for all R:NIR and B:R ratios for outside measurements, with the R:NIR ratio close to the value of 1.2 stated by Hoad and Leakey (1996). The B:R values were all less than 1.0 and did vary significantly from each other with a $P_{0.05} < 0.001$ at a 5 % level of significance. Of particular interest is that the rank of Clarix E Blue® which changed from the smallest R:NIR to one of the treatments with the greatest B:R values. The greatest B:R observed in autumn were green 40 % and Clarix E Blue® at 0.66, while in winter blue 20 % and Clarix E Blue® at 0.65 and in spring green 40 % and blue 20 % were 0.70 and 0.67, respectively. The smallest B:R was consistently for red 30 % at 0.51, 0.48 and 0.54 in autumn, winter and spring, respectively. Appelgren (1991) and Rajapakse *et al.* (1993) reported B:R ranging from 0.0001 to 216.26 and 0.6 to 1.6, respectively. This may explain the low variation in internode length and LA observed.

The average transmission percentage of PAR using an incandescent lamp gave a more accurate measurement when compared with the shading percentage given by the manufacturers (Table 2.3). Only the blue 20 % was close to the transmission of 80.1 % PAR recorded for the incandescent lamp. When the NIR was added to the PAR, the average transmission percentage increased for all shade nets and plastics, particularly for those that exhibited photosensitive spectra. Transmission was generally much lower than expected under tunnel conditions. This may be due to the time differences between measurements or

due to the separation distance between the shade net to the instrument and the distance from the nets to the tunnel roof. It seems, therefore, that manufacturers base the shading factor percentages of nets and plastics on PAR, excluding NIR and beyond. For photosensitive materials these excluded wavelengths may skew the expected absolute transmission of radiation.

The transmission of radiation during spring verifies that black 30 % is a neutral net as it displays a fairly neutral line over the entire spectrum crossing at 50 % (Fig. 2.1), although it was expected to be closer to 70 % transmissivity. Similarly, the Patilite® clear plastic and Aluminet® showed fairly constant transmissivity as they too are neutral nets. The blue 20 % net had an increase in spectral irradiance transmitted, between 450 to 500 nm, within the blue radiation range, then decreasing and flattening towards 750 nm after which it increases steadily beyond the NIR. The opposite was observed for the red 30 % transmissivity, a clear decrease in SI transmission from 410 to 590 nm, in the violet, blue, green and yellow areas of the radiation spectrum. The red 30 % net increased transmission from 600 nm into the NIR and then flattened out. Clarix E Blue® displayed similar qualities to the blue 20 % net, with an increase from 420 to 520 nm in the blue and extending into the green area. A distinct decrease from 530 to 740 nm, only increasing again in the NIR wavelengths, was found. The green shade net showed an increase from 460 to 540 nm, partially in the blue and green areas of the spectrum, then decreased and flattened off until 800 nm where it increased again.

2.3.2 Morphology

The shade net treatments were not significantly different from each other with respect to internode lengths at 5 % level for any of the seasons, with a $P_{0.05} = 0.054$ in autumn, 0.35 in winter and 0.097 for spring, all greater than 0.05. Season, clone and fertilizer treatments affected internode length significantly. The relatively small variation in internode length between treatments may be due to R:NIR and B:R values of the nets smaller than the outside ratios. Plants under blue 20 % and green 40 % tended towards shorter internode lengths, while internodes of plants under the Clarix E Blue® and red 30 % were longer, which corresponds with the results that Clarix E Blue® had the smallest R:NIR value and the blue shade net the greatest for winter (Table 2.4).

Shade net treatments did not significantly affect LA ($P_{0.05} = 0.16$), but clone, fertilizer and shade net by fertilizer interaction were highly significant with $P_{0.05} < 0.001$ at 5 % level. Leaf

area did not correlate strongly with the internode data with a Spearman's rank correlation coefficient of 0.379. The Clarix E Blue® that had the largest LA, Aluminet® 40 % the second largest and red 30 % the third largest LA; these treatments also had the longest internodes, an observation supporting the findings by Oren-Shamir *et al.* (2001) with regard to red and blue nets. The control treatment had the smallest LA and black 40 % the second smallest LA coupled with the shortest internodes (Table 2.4).

2.4 Conclusion

In conclusion, it is important to understand the optical properties of the various shade netting and plastics used and their effect on plant growth and morphology. Although the Clarix E Blue® looks blue to the eye, with respect to internode length and LA, it is more similar to the red 30 % than the blue 20 % net. This is due to the R:NIR and B:R ratios of each product compared with the control and outside conditions. It is clear that shade nets and plastics can be used to manipulate the radiation spectrum and, therefore, plant morphology, but with the particular products used effects were not significant enough to replace labour intensive tasks such as pruning; however, blue 20 % could be used to assist in these tasks. In future, a larger experimental area using the most influential treatments such as Clarix E Blue®, blue and red shade nets should be evaluated also incorporating other *Eucalyptus* clones.

ACKNOWLEDGEMENTS

Mr B. Pollard and Sunshine Seedlings Services are thanked for providing plant material. CSIR, NRF and the Donald Moor Scholarship provided funding.

References

- APPELGREN, M. 1991. Effects of light quality on stem elongation of *Pelargonium* in vitro. *Scientia Horticulturae* 45, 345-351.
- ASSIS, T.F., FETT-NETO, A.G. and ALFENAS, A.C. 2004. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: C. Walter and M. Carson (eds), Plantation Forest Biotechnology for the 21st Century, 303–333. Research Signpost, Kerala, India.
- GRANT, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *International Journal of Biometeorology* 40, 26-40.
- HAAPALA, T., PAKKANEN, A. and PULKKINEN, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* × *P. tremuloides*). *Forest Ecology and Management* 193, 345-354.
- HOAD, S.P. and LEAKEY, R.R.B. 1996. Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden Cutting morphology, gas exchange and carbohydrate status during rooting. *Trees* 10, 317-324.
- NEWTON, A.C., DICK, J. McP., MCBEATH, C. and LEAKEY, R.R.B. 1996. The influence of R:FR ratio on the growth, photosynthesis and rooting ability of *Terminalia spinosa* Engl. and *Triplochiton scleroxylon* K. Schum. *Annals of Applied Biology* 128, 541-556.
- OREN-SHAMIR, M., GUSSAKOVSKY, E.E., SHPIEGEL, E., NISSIM-LEVI, A., RATNER, K., OVADIA, R., GILLER, Y.E. and SHAHAK, Y. 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum*. *Journal of Horticultural Science and Biotechnology* 76, 353-361.
- RAJAPAKSE, N.C., MCMAHON, M.J. and KELLY, J.W. 1993. End of day far-red light reverses height reduction of chrysanthemum induced by CuSO₄ spectral filters. *Scientia Horticulturae* 53, 249-259.
- RUNKLE, E.S. and HEINS, R.D. 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. *Journal of the American Society for Horticultural Science* 126(3), 275-282.
- VSN INTERNATIONAL. 2011. GenStat for Windows, 14th Edition. VSN International, Hemel Hempstead, UK.

Tables

Table 2.1. Parameters determined from the spectroradiometer data for clear-sky measurements in autumn, winter and spring (2011). Photosynthetic irradiance is the irradiance in the PAR region.

		Shade net or plastic								
		Clarix							Patilite	
Clear-sky measurements	Units	Aluminet 40%	Blue 20%	Red 30%	Black 30%	Green 40%	E Blue plastic	clear plastic	Control Ave	Outside Ave
Autumn										
Spectral irradiance (SI)	W m ⁻²	117.4	203.2	137.1	146.0	139.5	162.3	161.3	275.4	533.5
Photosynthetic irradiance	W m ⁻²	59.5	98.1	64.3	74.0	67.9	74.3	78.4	138.2	284.5
Photosynthetic photon flux density (PPFD)	μmol s ⁻¹ m ⁻²	282.7	464.9	308.3	351.5	320.7	351.4	373.3	657.9	1334.4
Winter										
Spectral irradiance (SI)	W m ⁻²	89.5	120.4	100.3	100.1	100.8	112.7	126.4	190.1	361.0
Photosynthetic irradiance	W m ⁻²	42.9	56.6	44.6	47.7	46.6	49.7	60.0	93.2	184.9
PPFD	μmol s ⁻¹ m ⁻²	204.6	267.8	214.5	227.4	221.3	235.4	286.4	444.3	871.2
Spring										
Spectral irradiance (SI)	W m ⁻²	217.0	260.4	230.9	184.8	170.5	270.7	236.6	387.5	687.3
Photosynthetic irradiance	W m ⁻²	108.9	127.9	109.8	93.0	80.4	123.9	115.5	196.0	365.6
PPFD	μmol s ⁻¹ m ⁻²	517.5	604.4	524.9	441.6	378.1	586.2	550.4	931.5	1713.4

Table 2.2. Ratios determined from red (R), blue (B) and near infrared (NIR) radiation; nb = narrow band (10 nm); wb = wide band (100 nm).

		Shade net or plastic								
		Clarix							Patilite	
Clear-sky measurements		Aluminet 40%	Blue 20%	Red 30%	Black 30%	Green 40%	E Blue plastic	clear plastic	Control Ave	Outside Ave
Autumn										
R:NIR (nb)		1.22	1.22	1.20	1.21	1.20	1.13	1.17	1.21	1.28
R:NIR (wb)		1.18	1.15	1.13	1.17	1.14	1.01	1.13	1.18	1.22
B:R (wb)		0.60	0.63	0.51	0.60	0.66	0.66	0.57	0.58	0.78
Winter										
R:NIR (nb)		1.14	1.16	1.11	1.13	1.12	1.05	1.11	1.16	1.19
R:NIR (wb)		1.13	1.12	1.07	1.12	1.10	0.99	1.11	1.14	1.18
B:R (wb)		0.55	0.65	0.48	0.56	0.60	0.65	0.56	0.57	0.69
Spring										
R:NIR (nb)		1.17	1.19	1.15	1.18	1.15	1.10	1.16	1.19	1.22
R:NIR (wb)		1.16	1.15	1.12	1.17	1.11	1.02	1.14	1.17	1.22
B:R (wb)		0.59	0.67	0.54	0.61	0.70	0.65	0.57	0.60	0.75

Table 2.3. Average transmissivity for the PAR region (and PAR plus NIR region) in comparison with manufacturer supplied shading percentage %.

Transmissivity	Shade net or plastic							
	Aluminet 40%	Black 30%	Blue 20%	Green 40%	Clarix E Blue plastic	Patilite clear plastic	Red 30%	Tunnel plastic
	Incandescent lamp (laboratory)							
PAR+NIR	52.4	67.9	84.9	68.3	86.4	89.3	82.7	
PAR	53.4	67.8	80.1	63.1	81.5	88.7	75.9	
	Autumn							
PAR+NIR	42.4	53.5	74.1	48.7	58.7	55.7	49.5	54.4
PAR	41.5	53.7	71.7	46.7	53.9	53.5	46.3	48.1
	Winter							
PAR+NIR	52.5	50.3	61.9	51.3	58.4	63.5	51.1	56.7
PAR	51.7	48.0	58.5	46.8	51.5	60.3	45.4	52.3
	Spring							
PAR+NIR	51.4	51.0	62.5	48.3	76.4	63.4	64.2	58.1
PAR	50.8	50.6	60.3	43.4	67.3	60.7	59.2	52.2

Table 2.4. Average internode length (mm), leaf area (LA) (cm²) and stem diameter (mm) during the evaluation period.

	Internode length (mm)			Leaf area (cm ²)	Stem Diameter (mm)
	Autumn	Winter	Spring		
Aluminet 40%	15.97 a ¹	13.34 a	14.54 ab	9.213 a	1.256 c
Black 30%	14.85 a	13.59 a	12.54 c	8.751 b	1.293 c
Blue 20%	15.38 a	12.13 a	13.12 abc	9.005 ab	1.253 c
Green 40%	14.01 a	12.81 a	14.70 a	8.903 ab	1.357 bc
Red 30%	17.99 a	14.23 a	14.31 abc	9.045 ab	1.606 a
Clarix E Blue	15.96 a	15.22 a	14.28 abc	9.262 a	1.312 c
Patilite	14.74 a	14.60 a	13.60 abc	8.729 b	1.392 abc
Control	16.14 a	12.88 a	12.80 bc	8.878 ab	1.540 ab

¹Treatment means sharing the same symbol (abc) are not significantly different at 5% level.

Figures

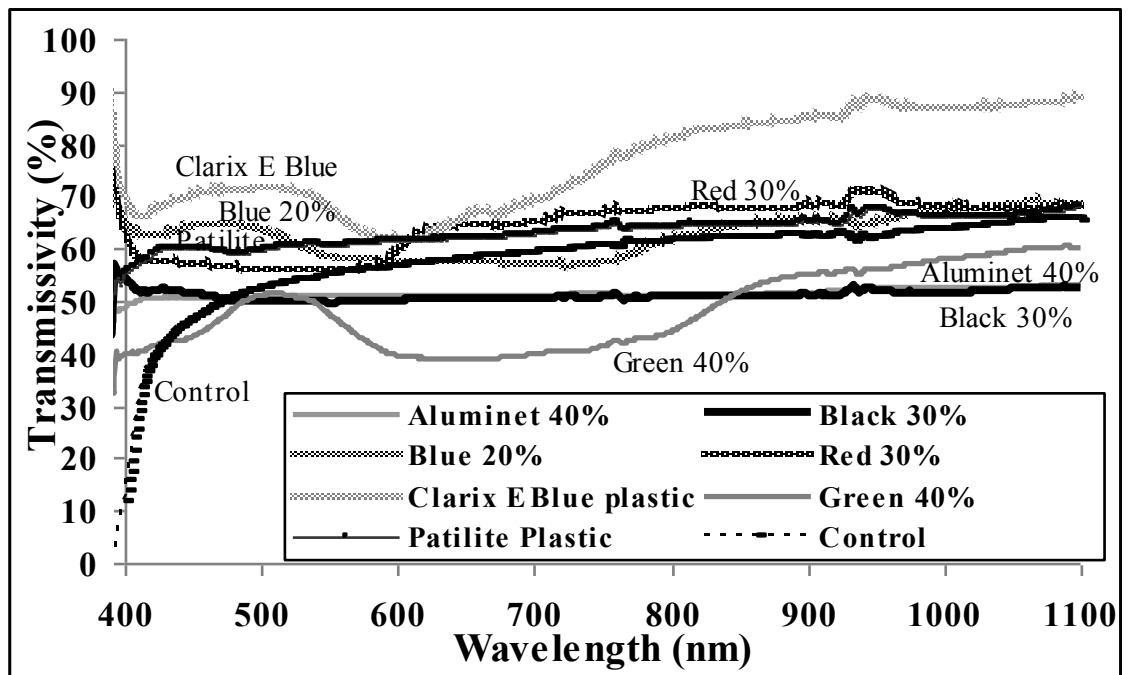


Figure 2.1. Transmissivity of the eight curves shade nets and plastics used, during spring, where the difference between neutral and photosensitive nets are displayed.

Chapter 3

Effect of environmental parameters on *Eucalyptus grandis* × *E. nitens* hybrid stock plants

3.1 Introduction

The ambient environment of an area is altered when protective coverings such as shade netting or plastic tunnels are used. The weave pattern, shading percentage and colour of the net can affect the specific temperature experienced underneath a certain shade net (Shahak, 2002). Shade netting can affect thermal components of the immediate plant environment due to its transmittance or reflectance of infrared wavelengths (Shahak, 2002). Ambient temperature and relative humidity (RH) affect the growth rate of stock plants, impacting on nursery management through the quantity and quality of mini-cuttings produced. The temperature and RH can create ideal conditions for the spreading of pests and diseases throughout the nursery. Therefore, the ambient environment should be controlled and adapted to specific species, as much as possible, to ensure the maximum multiplication of plant material without the loss of rooting ability of cuttings.

Hybrid clones of *Eucalyptus grandis* (W. Hill) × *E. nitens* (Maiden) (G × N), such as those used in these experiments, have been selected to be more cold-tolerant than the *E. grandis* parent due to the high cold tolerance of the *E. nitens* parent. Naturally occurring in New South Wales and Queensland at altitudes from sea level to 600 m, *E. grandis* can also be found in northern Queensland, at up to 1100 m altitude. The optimal altitude and corresponding temperature ranges for *E. grandis* and *E. nitens* are 1000 to 1250 m (16 to 20 °C) and 1250 to 1900 m (13.5 to 16 °C), respectively (Gardner, 2007). Temperature limits of *E. grandis* production lie between 0 and 34 °C; the species can tolerate light, but not heavy frosts (McMahon *et al.*, 2010), resulting in *E. nitens* being the preferred species for planting at high altitudes in south-eastern Australia as mean annual temperatures of this area lie below 10 °C (Battaglia *et al.*, 1996; Close and Beadle, 2003).

3.1.1 Temperature

Shade netting is of great importance for vegetable and decorative branch production in warm, sunny climates, where it serves to reduce both radiation intensity and air temperature during the day (Grinberger *et al.*, 2000; Oren-Shamir *et al.*, 2001). Shade netting with 50 % Aluminet® has been reported to decrease leaf temperature by 4 to 5 °C below ambient summer temperatures (Leite, 2001). Grinberger *et al.* (2000) found that air temperatures under Aluminet® (30 % shading) were higher than under pearl (white), blue and red coloured netting of the same shading percentage, although the control of no net had the highest mean maximum temperature. Temperatures under shade netting can be higher than outside temperatures such as in enclosed shade houses (Stamps, 2009), without fans or other forms of ventilation. Good ventilation, by leaving one side of the shade netting open, was found important to negate differences in air and leaf temperature under different shade nets (Oren-Shamir *et al.*, 2001). At night infrared radiation is reflected off plants and the ground up toward the sky and is reflected back toward the plants by nets such as Aluminet®, maintaining a warmer temperature than would occur with no covering, thus together with good ventilation, condensation on leaves is decreased (Anon, 2008) and disease incidence is reduced. Shade nets can also be used to reduce the risk of frost damage at night during winter as the net amount of infrared radiation off the ground during the night is reduced, thus keeping the plant temperature under the net higher than ambient (Teitel *et al.*, 1996). Generally black shade nets absorb radiation resulting in higher temperatures under the net (Leite *et al.*, 2002; Anon, 2008), resulting in the air and leaf temperature under 50 % Aluminet® to be about 15 and 20 % lower, respectively than under 50 % black net (Leite *et al.*, 2002). The most important difference in leaf temperature under Aluminet® was found to be the ability to restrict leaf temperature to below 30 °C, while under the black net leaf temperature often rose above 30 °C (Leite *et al.*, 2002), causing damage to roses and negatively affecting photosynthesis. When deviations from optimal photosynthetic temperatures occur in *Eucalyptus* for long periods, photosynthesis and respiration have to acclimate to a new optimum (Battaglia *et al.*, 1996). During this acclimation period the rate of carbon uptake is affected and, consequently, the synthesis of photosynthates as well as the carbohydrate stores (Battaglia *et al.*, 1996). Leaves exhibit different photosynthetic responses to temperature at different ages. When tested in summer, the youngest *E. globulus* plant materials, apical shoots, had an optimal photosynthetic temperature of 10 to 15 °C. In contrast, expanding leaves, fully expanded leaves from current year's growth, and fully expanded leaves from the previous year's growth had an optimal photosynthetic temperature

of 15, 20 and 20 to 25 °C, respectively (Battaglia *et al.*, 1996). The optimum temperature for photosynthesis of *E. nitens* increased from 14 to 20 °C when the mean daily temperature increased from 7 to 19 °C. The photosynthetic rate of *E. nitens* was similar across a range of 20 to 25 °C, indicating that *E. nitens* has a broader optimal photosynthetic temperature than *E. globulus* (Battaglia *et al.*, 1996). In *E. nitens*, photosynthesis was only slightly diminished at 10 °C, even in midsummer, whereas *E. globulus* showed a marked decline in photosynthetic performance at temperatures below 15 °C.

Due to the large temperature variations between macro- and micro- climates in South Africa, as well as the variations between seasons, the success of growing stock plants under protection in a greenhouse or plastic tunnel can be affected by the prevailing conditions. Temperature extremes can be decreased under protected cultivation, however, temperature variations remain. There have been reports of differences in rooting percentage of *Eucalyptus* cuttings between seasons, such that mini- and micro-cuttings of *Eucalyptus grandis* × *E. urophylla*, *E. saligna* and *E. globulus* show reduced rooting percentage during cold winter months (Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004) compared with warm summer months. To this end, Assis *et al.* (2004) found that rooting improved when temperatures of stock plants were maintained above 20 °C. Rocha Corrêa and Fett-Neto (2004) found that both easy-to-root *E. saligna* and difficult-to-root *E. globulus* reacted more strongly to the effect of different temperatures (15 to 30 °C) on micro-cuttings with regard to rooting than when the same temperatures were applied to stock plants. The *E. saligna* had high rooting percentages of 90 to 100 % for all stock plant temperatures, but the highest number of roots and fastest rate of rooting was under 15 °C using exogenous auxin (Rocha Corrêa and Fett-Neto, 2004). *Eucalyptus globulus* was more affected by stock plant temperatures than *E. saligna*. The highest rooting percentages of about 88 % as well as the highest root numbers per cutting were in the intermediate stock plant temperatures between 20 and 25 °C for *E. globulus*. *Eucalyptus saligna*, maintained a fairly high root number for all stock plant temperatures, but the *E. globulus* was reduced at the lowest and highest temperatures (15 and 30 °C) (Rocha Corrêa and Fett-Neto, 2004). The *E. globulus* micro-cuttings rooted best under a daily thermoperiod of 30 °C during the day and 20 °C at night. Tsipouridis *et al.* (2006) rooted macro-cuttings with bottom heating where the bases of macro-cuttings were maintained between 18 and 20 °C, however, the ambient temperature experienced by the stock plants in the experimental orchards in Naoussa, Greece were not discussed, therefore, the following temperatures are based on monthly temperature means

for the area compiled by Bolstad *et al.* (2013). Tsipouridis *et al.* (2006) produced contradictory results to previous investigations, whereby rooting percentages of macro-cuttings were higher in mid-to-late autumn (16 to 20 °C) and late winter (12 °C) and lower in spring and summer (13 to 25 °C) and early to mid-winter (12 to 13.5 °C) (Bolstad *et al.*, 2013).

Temperature extremes can influence root initiation by interfering with water and nutrient uptake as well as the metabolic rate and the promotion or inhibition of enzymes due to the prevailing temperature (Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004). Cold storage has been shown to promote the rooting of chrysanthemum (*Dendranthema × grandiflorum*) (Druege *et al.*, 2000) and carnation (*Dianthus caryophyllus*) (Garrido *et al.*, 1996) herbaceous cuttings. Druege *et al.* (2000) grew stock plants at approximately 20 °C air temperature and held cuttings in cold-storage under dark conditions for two, three or four weeks at either 0.5 or 5 °C. Comparing two chrysanthemum cultivars, ‘Cassa’ and ‘Puma’, ‘Cassa’ had the higher rooting capacity when unstored, using number of roots formed and root length as rooting parameters. After cold-storage ‘Puma’ experienced a promotional effect on root length, whereas ‘Cassa’ experienced only a minor increase in root length (Druege *et al.*, 2000). However, the number of roots formed by either cultivar was not significantly affected by cold storage. Garrido *et al.* (1996) using the carnation cultivars ‘Oriana’, ‘Elsy’ and ‘Virginie’ stored cuttings at 4 ± 2 °C with a 12-hour photoperiod using fluorescent light for two to twelve weeks. Carnation cuttings were rooted with or without auxins (15.3 µM indole-3-butyric acid (IBA) and 9.88 µM naphthalene acetic acid (NAA)). The optimal rooting period was defined as the minimum time required for 95 % of plants to show commercial rooting quality (Garrido *et al.*, 1996). This optimal rooting period, for cuttings not treated with any exogenous auxin, was shortened from 26 to 22 days when the storage period increased from two to ten weeks in ‘Oriana’, and from 22 to 18 days when the storage period increased from two to eight weeks in ‘Elsy’ cuttings; however, storage did not affect the optimal rooting period of untreated ‘Virginie’ cuttings, which remained at 22 days for all storage periods. Rocha Corrêa and Fett-Neto (2004) investigated the effects of heat-shock on rooting of *E. saligna* and *E. globulus* stock plants, by exposing vials to 40, 50 or 60 °C for one hour in the dark, one day before taking the mini-cuttings. Mini-cuttings were then rooted in the dark at 25 °C. Long-term exposure to 40 °C was lethal for both, *E. globulus* and *E. saligna* micro-cuttings. A short-term exposure to 40 °C for one hour had a positive effect on *E. saligna* root numbers and length in the absence of auxin, but such an

effect was not observed in *E. globulus* (Rocha Corrêa and Fett-Neto, 2004). Exposure to even higher temperatures of 50 °C and 60 °C of one hour strongly diminished rooting percentage (Rocha Corrêa and Fett-Neto, 2004).

3.1.2 Relative humidity

Relative humidity is often higher under netting than outside as a result of transpiration by the plants beneath and reduced integration with drier air outside the netted area, even under higher temperatures (Stamps, 2009). Relative humidity under 50 % Aluminet® was significantly higher (10 to 25 %) than under greenhouse conditions without netting (Leite, 2001). There may be a gradient where the highest RH is between the stock plants and decreases toward the netting of a shade house. Good ventilation is important for the regulation of RH and to decrease the incidence of fungal diseases that are prone to occur under high humidity. Anon (2008) reports better movement of hot air through Aluminet® due to the open weave pattern compared with other nets of the same shading percentage; additionally, Aluminet® allows moisture to pass through the netting, maintaining a lower RH at plant level. However, Leite *et al.* (2002) in their study on roses found that Aluminet® 50 % had approximately 7 % higher RH than black 50 % netting, explaining that this may be due to the higher temperatures experienced under the black 50 %, which increases the water holding capacity for the air. Druege *et al.* (2000) grew chrysanthemum stock plants at an average RH of 60.3 %. Best results for the rooting of mini-cuttings have been obtained at a high RH of 80 to 90 % as such conditions decrease transpiration (Hansen *et al.*, 1978; Haapala *et al.*, 2004).

3.1.3 Production

Successful production of mini-cuttings is dependent on temperature and RH as the plant metabolism and health of the plant will determine how many new shoots the plant can produce over time. The more mini-cuttings produced per stock plant, the more profitable that plant is to the nursery. Thus if a particular clone has a higher production potential coupled with good rooting rate, it is a profitable clone to invest in. The average production rate of mini-cuttings per mini-hedge plant of sweetgum (*Liquidambar styraciflua*) was 2.5 every 30 days (Wendling *et al.*, 2010); although *Eucalyptus* usually has a higher production rate of about six mini-cuttings per mini-hedge plant every four to six weeks (Pollard, *pers.*

*comm.*¹). Annual production of *Liquidambar styraciflua* mini-cuttings can be estimated around 2,953 sprouts per m². Using *Liquidambar styraciflua* mini-cuttings from mini-hedge systems compared with macro-cuttings from field-grown clonal hedges can increase production of *Liquidambar styraciflua* up to 3,076 % per m² per year (Wendling *et al.*, 2010). Saya *et al.* (2008) converted from *E. urophylla* × *E. grandis* macro-cuttings, collected from coppicing stock plant stumps grown over a large area, to mini-cuttings, collected from intensively grown nursery mini-hedge stock plants and reported a higher quantity of rooted cuttings produced in the mini-cutting system, averaging 400 cuttings per annum per mini-hedge stock plant compared with 35 for field grown stock plant stumps (Saya *et al.*, 2008). In addition to increased production per plant and per unit area, rooted cuttings can be produced all-year-round, whereas field cuttings are more dependent on the rainy season (Saya *et al.*, 2008).

3.1.4 Leaf area and stem diameter

Radiation quality can affect stem elongation, whereby radiation transmitted through red netting stimulates stem elongation resulting in taller plants, while blue netting results in dwarfed plants with shorter internodes. Oren-Shamir *et al.* (2001) found that *Pittosporum variegatum* plants grown under red and green shade netting produced the longest internodes, and had a larger average leaf area (LA) compared with blue, grey, black, and Aluminet® shade netting. Plants under blue netting had a smaller LA than those under red and green netting, while no differences were observed under grey or black netting (Oren-Shamir *et al.*, 2001). When grown for export under commercial netting, *Philodendron monstera* leaves are often too large, consequently, using blue netting to reduce leaf size can be beneficial in the opinion of growers consulted (Shahak, 2002). Furthermore, red netting at 60 % shading can be employed to produce large *Aralia* and *Philodendron monstera* leaves supplying the local market in Israel with large leaves of these species (Shahak, 2002). In contrast, Kawabata *et al.* (2007) found that *Dracaena marginata* ‘Colorama’ plants grown under 70 % red shade net produced a higher number of new leaves with the smallest LA than plants under 70 % black, blue or grey netting. Leaf area and leaf dry mass of *E. grandis* cuttings at severance were significantly greater in explants originating from treatments with a lower R:NIR (red to near infrared ratio) of 0.4 or 0.7. This lower R:NIR was associated with stem elongation,

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

while stock plants maintained under a higher R:NIR of 3.5 and 6.5 were dwarfed (Hoad and Leakey, 1996).

Wendling *et al.* (2010) observed that the *Liquidambar styraciflua* clone with the largest mini-cutting root systems had the highest number of leaves. The authors went on to hypothesise that a good quality root system may improve the number of new leaves formed and conversely a higher number of leaves on cuttings may improve the quality of roots formed. Water loss from leaves through the process of transpiration can lead to wilting of cuttings and ultimately mortality before the cutting is able to form roots. Water loss from leaves can be minimised by stomatal closure, however, this limits photosynthesis by reducing carbon dioxide intake (Leakey and Coutts, 1989). Therefore, in order to minimise transpiration and water stress, softwood cuttings should have a smaller LA and relative humidity increased in the rooting environment (Leakey and Coutts, 1989; Hartmann *et al.*, 1990). Leaf area reduction or removal of some leaves from cuttings is a common practice to balance the positive effect of photosynthesis and the negative effect of transpiration (Leakey, 1985; Leakey and Coutts, 1989; Kamaluddin and Ali, 1996). The optimal LA of cuttings can influence rooting ability, particularly in difficult-to-root species (Leakey, 1985; Leakey and Coutts, 1989; Kamaluddin and Ali, 1996). Difficult-to-root cuttings, such as certain avocado (*Persea americana*) cultivars, are prone to leaf abscission under misting and subsequently die, whereas other avocado cultivars that root more easily, can retain their leaves for up to nine months (Hartmann *et al.*, 1990). Therefore, the environmental conditions that cuttings are rooted under are important to retain leaves. Apart from translocation of carbohydrates from leaves to areas of root initiation, a more direct root-promoting effect of leaves and buds is their strong ability to produce auxins; these plant hormones are transported from the apex to the base of the cutting assisting in root formation (Hartmann *et al.*, 1990). Softwood and herbaceous cuttings depend on the current leaf photosynthesis to produce carbohydrates to form roots (Leakey, 1985; Leakey and Coutts, 1989; Hartmann *et al.*, 1990). *Triplochiton scleroxylon* cuttings respond with increased rooting when the LA of cuttings as well as temperature and relative humidity of the rooting environment are controlled (Leakey and Coutts, 1989). When leaves of such cuttings were trimmed to 10, 50 or 100 cm², cuttings with a LA of 10 and 50 cm² displayed a rooting percentage which exceeded 80 %, whereas larger leaves of 100 cm² only reached 65 % rooting, although the 10 cm² cuttings produced fewer roots per rooted cutting than those with larger leaves (Leakey and Coutts, 1989). The same authors found that in cuttings with a smaller LA (10 cm²) carbohydrate levels in the

stem declined over the six week rooting period, suggesting that rooting of cuttings with smaller LA depends more on the reserves in the stem than rooting of cuttings with a larger LA (Leahey and Coutts, 1989). Leahey (1985) found that a LA greater than 50 cm² per cutting was detrimental to root development in *Triplochiton scleroxylon* and *Cleistopholis glauca*, but not in *Terminalia ivorensis* and *Nauclea diderrichii*. Difficult-to-root species may be more sensitive to the deleterious effects of a too large or too small LA (Leahey, 1985). Kamaluddin and Ali (1996) studied *Azadirachta indica* macro-cuttings, whereby an IBA solution was applied at 0, 0.2, 0.4 or 0.8 % and LA was kept at 100 % or decreased to 50 or 30 % per cutting. All treatments rooted well between 90 and 100 %, thus the authors concluded that rooting percentage was not significantly affected by the LA or auxin treatment, although in general, root number per cutting increased with increasing IBA concentrations from 0 to 0.8 % and with an increase in LA. Root dry mass increased significantly with an increased LA but was not affected by IBA treatment (Kamaluddin and Ali, 1996). Cuttings rooted best at 100 % LA and when treated with 0.2 or 0.4 % IBA solution (Kamaluddin and Ali, 1996). Chrysanthemum (*Dendranthema × grandiflorum*) leaves grown under light filtered through a blue copper sulphate solution had an increased dry mass and a reduced LA compared with those illuminated with light filtered through a blue dye solution or through water (control) (McMahon and Kelly, 1995).

Radiation quality in terms of specific wavelengths that reach the plants has not been reported on prolifically with regard to its effect on shoot diameter. Hoad and Leahey (1996) found that radiation quality, over a wide range of R:NIR ratio (0.4 to 6.5) affected elongation of *Eucalyptus grandis* cuttings, with no significant effect on shoot diameter. Growing the leather-leaf fern *Rumohra adiantiformis* and *Ruscus hypoglossum* under 50 % red netting resulted in longer branches with increased branch diameter (Shahak, 2002). The flower crops *Lisianthus* and *Lupinus luteus* grown under the 50 % yellow net had heavier flowering stems (Shahak, 2002) leading to better quality and prices for these cut flowers.

3.1.5 Pests and diseases

Reflected infrared radiation at night, together with good ventilation, decreases condensation on leaves (Anon, 2008) consequently, reducing disease incidence. This decreased leaf wetness, with a reduced incidence of leaf diseases increases the chance of producing pathogen-free mini-cuttings (Assis *et al.*, 2004).

The environmental parameters, particularly temperature and RH can greatly affect the ability of a stock plant to produce good quality cutting material that subsequently has good rooting potential. If a particular shade net or plastic keeps the stock plants cooler, then rooting may be improved in a cold-tolerant hybrid such as *E. grandis* × *E. nitens* and if the temperature is excessive the plants may have experienced some heat stress. Relative humidity should be higher in the rooting tunnel than in the stock plant tunnel to decrease the incidence and assist in pest and disease control. This experiment aims to quantify the effect shade netting has on environmental parameters such as temperature and relative humidity as well as to monitor the effect of these environmental parameters on *Eucalyptus grandis* × *E. nitens* hybrid stock plants.

3.2 Materials and methods

3.2.1 Environmental parameters

3.2.1.1 Temperature and relative humidity (RH)

During all rooting experiments from summer 2010 to autumn 2012 temperature and RH were recorded by Onset® HOBO® U12-012 series data loggers placed in the stock plant tunnel as well as in the rooting tunnel. Hourly Temperature and RH were recorded. Stock plants were grown with a dripper system to keep the moisture at soil level in order to not wet the foliage and reduce disease incidence.

Due to limited equipment, temperature and RH were only recorded under one of each of the three replicated coloured shade net treatments. Eight minimum/maximum thermometers were placed in the middle of each coloured shade netting treatment and data were recorded daily. Additionally, eight HOBO® data loggers were placed under the same coloured shade nets as the thermometers, in order to measure hourly temperature and RH. These eight HOBO®s were downloaded weekly for six weeks in spring 2011 in order to determine differences in temperature and RH between the five shade nets, the two plastics and control treatment. When the netting was removed in autumn 2012 eight HOBO® were installed in the same positions in order to determine any gradient in temperature and RH in the tunnel.

3.2.2 Morphological parameters of stock plants

3.2.2.1 Production

The experimental design for the stock plant layout was a factorial design with three factors, where the covering (shade net) factor had eight levels (black 30 %, green 40 %, blue 20 %, red 30 %, Aluminet® 40 %, Clarix E Blue®, Patilite® and control), the clone factor had two levels (PP2107 and GN018B) and the fertilizer factor had two levels (organic and inorganic). The shade nets with clone and fertilizer treatments beneath them were replicated three times within the greenhouse. The stock plant production – as the number of new shoots per stock plant was noted when taking mini-cuttings. This was repeated over seasons to determine the change in production over seasons. Production was determined from three plants in each of the 32 stock plant treatments (8 covering × 2 clone × 2 fertilizer) over three replications; essentially nine replications that are representative of the entire greenhouse.

3.2.2.2 Dry mass

The factors used to assess differences in dry mass of mini-cuttings were covering (shade net) at eight levels, clone factor at two levels and plant part at two levels (leaves and stalks) as only inorganically fertilized plants were assessed for this parameter. Fresh mass of five mini-cuttings (experimental unit) from each of the 32 stock plant treatments (8 covering × 2 clone × 2 plant part), replicated three times, were recorded in spring and summer 2011. Samples were then freeze-dried and weighed again to determine dry mass and percentage dry mass.

3.2.2.3 Leaf area and stem diameter

The factors used for LA and stem diameter were covering (shade net) at eight levels and clone at two levels, replicated five times with a single mini-cutting as one experimental unit. Leaf area was measured using a Li-Cor® leaf area meter (LI-3000L). Mini-cuttings were collected and prepared as for the rooting experiments, whereby bottom leaves were removed and the remaining leaves were reduced by one half to a third depending on the size of the leaf. Stem diameter was measured at the base of each cutting using digital callipers.

3.2.3 Pests and diseases

Pests and diseases were monitored on the stock plants weekly over the entire experimental period. In the second week after planting, some powdery mildew was noticed on some of the stock plants. Powdery mildew is caused by several species of the genera *Erysiphe* and *Sphaerotheca* belonging to the family Erysiphaceae (Old *et al.*, 2003), thus periodically,

after identifying fungal diseases, all plants were sprayed with various fungicides in an attempt to curb disease spread and to decrease the chance of developing fungicidal resistance. For powdery mildew mostly Funginex®, Bravo®, Folicur®, Benlate®, and Dithane® were used curatively. Alliette® and Avigard® are used as preventative fungicides. Mikal® with 20 % milk solution and lime sulphur with 10 % milk was used as a suggestion as a more environmentally friendly control method for powdery mildew (Pollard, *pers. comm.*¹). For fungal disease control Sunshine Seedlings Services apply chemicals only when necessary, using Agrisil® and Agricure® when powdery mildew is identified on plants (Pollard, *pers. comm.*¹). To prevent disease outbreak, Sporekill®, an agricultural disinfectant, and Nitrosol®, an organic liquid fertilizer, were sprayed on a weekly basis using a backpack sprayer. Slug bait was laid down infrequently, only when slugs or snails were noticed on the stock plants.

3.2.4 Statistical analysis

The experimental design for the stock plant layout was a factorial design with three factors at various levels. The first factor, covering (shade nets), consisted of eight levels (five shade nets, two plastics and a control). The second factor was plant material with two levels of G × N clones, one easy-to-root (PP2107) and one difficult-to-root (GN018B). The third factor was fertilizer regimes at two levels, one inorganic and one organic-based. The shade nets with clone and fertilizer treatments beneath them were replicated three times within the greenhouse. For the statistical analysis of morphological parameters a factorial design was used with all three main factors or variation of them; however, data of the environmental parameters were collected only with regard to the shade net factor over different seasons, where season was regarded as a factor at times; it was therefore considered a completely randomised design. Statistical analysis was carried out using GenStat® 14th edition (VSN International, 2011). All data were orthogonally distributed, thus was analysed using analysis of variance (ANOVA). Blocking was used where appropriate to negate the effect of a temperature gradient from cooler to warmer in the stock plant tunnel from the wet wall end to the fan end. Where treatments were highly significant at 5 % level of significance, a least significant difference (LSD) of 1 % level of significance was used in line with Fisher's LSD (protected LSD) in order to limit experimental error and make the LSD range narrower (protected), as the test does not correct for multiple comparisons.

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

3.3 Results

3.3.1 Environmental parameters

3.3.1.1 Temperature and relative humidity (RH)

As expected in an area such as Pietermaritzburg, which is situated between the sub-tropical coastal and temperate interior climatic region according to the South African national standard (SANS 204-2) with a summer rainfall (Conradie, 2012), the temperature and RH analysed with ANOVA varied significantly (5 % level of significance, $P_{0.05} < 0.001$) over both seasons and months (see table 3.1 for means and see ANOVA table C. 10 to C. 17 in appendix C), allowing differences to be displayed with a LSD at 1 % level of significance.

The temperatures experienced in the two tunnels used for stock plant production and rooting of mini-cuttings were in the expected range. Summer temperatures in the stock plant tunnel were higher than the other seasons and summer 2010/11 was not significantly different from summer 2011/12 (Table 3.1). Autumn and spring 2011 temperatures were not significantly different from one another; winter 2011 had the coldest mean temperature and was significantly different from the other seasons (Table 3.1). In the rooting tunnel, overall temperatures were slightly higher than in the stock plant tunnel; this could be explained by the fact that the tunnel used was relatively small with a lower roof making it more difficult to dissipate built-up heat. Temperatures in the rooting tunnel lagged behind the stock plant tunnel. Autumn 2011 was on average hotter than the previous season, summer 2010/11, as February and March 2011 had higher temperatures than what was recorded over the previous November to January period (Table 3.1).

The RH over seasons varied more in the stock plant tunnel than in the rooting tunnel (Table 3.1), with the two summer seasons similar to each other displaying the highest RH, while winter 2011 had the lowest RH; since stock plants were irrigated by drippers four times a day for five minutes in summer, while in winter the irrigation frequency was decreased to twice a day due to lower ambient temperatures. No significant differences in RH were observed in the rooting tunnel between seasons and months, possibly due to mini-cuttings being irrigated with misters, keeping the tunnel RH at a high level (mostly above 70 % RH (Table 3.1) and as high as 90 % RH (Fig. 3.2)). The intervals between two minute misting

cycles varied from 15 to 45 minutes depending on the stage of the mini-cuttings and were not run at night in order to avoid disease outbreak.

Table 3.1. Mean temperature and relative humidity (% RH) in the stock plant tunnel and rooting tunnel recorded by HOBO® data loggers and displayed as month or season.

	<i>Season</i>			
	Stock Plant Tunnel		Rooting Tunnel	
	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)
Summer 2010/11	24.378 a ¹	74.99 ab	25.099 a	75.71 a
Autumn 2011	21.407 b	73.97 b	26.121 a	70.59 a
Winter 2011	15.073 c	69.77 d	16.880 c	73.11 a
Spring 2011	21.460 b	72.13 c	22.163 b	71.96 a
Summer 2011/12	24.792 a	75.76 a	25.577 a	74.91 a
	<i>Month</i>			
	Stock Plant Tunnel		Rooting Tunnel	
	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)
Nov-10	25.333 a	70.83 a	25.513 ab	67.88 a
Dec-10	22.919 ab	77.54 a	23.927 ab	75.62 a
Jan-11	24.839 a	75.41 a	25.736 ab	74.73 a
Feb-11	25.467 a	71.76 a	26.418 a	76.54 a
Mar-11	25.299 a	72.35 a	26.121 ab	70.59 a
Apr-11	20.793 bc	75.72 a		
May-11	18.111 cd	73.91 a		
Jun-11	14.524 d	71.79 a	14.515 c	79.47 a
Jul-11	13.948 d	69.09 a		
Aug-11	16.730 cd	68.49 a	20.381 b	63.69 a
Sep-11	20.593 bc	68.79 a	21.238 ab	68.75 a
Oct-11	21.573 b	72.99 a	22.251 ab	74.12 a
Nov-11	22.211 ab	74.59 a	22.97 ab	72.83 a
Dec-11	23.662 a	78.21 a	24.904 ab	75.08 a
Jan-12	25.384 a	75.33 a	26.282 a	74.72 a
Feb-12	26.541 a	69.19 a		

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where each column of temperature and RH are calculated separately. In the stock plant tunnel for seasons, LSD for temperature = 0.4925 and RH = 1.55. For months, LSD for temperature = 4.6861 and RH = 15.245. In the rooting tunnel for seasons, LSD for temperature = 1.6655 and RH = 5.374. For months, LSD for temperature = 5.7996 and RH = 18.852. Missing data in the rooting tunnel during April, May and July 2011 and February 2012 were due to a faulty HOBO® data logger.

Diurnal temperatures in the stock plant tunnel over five seasons over the period when mini-cuttings were taken (Fig. 3.1) indicate, as expected, highest temperatures for all seasons are between 12:00 and 14:00 and the lowest temperatures in the early mornings. Winter temperatures were lowest, nonetheless still reaching 25 °C during the day. The highest temperature (38 °C) was recorded in autumn 2011, indicating an unusually warm autumn, with summer 2010/11 and 2011/12 being fairly cool due to overcast or rainy days.

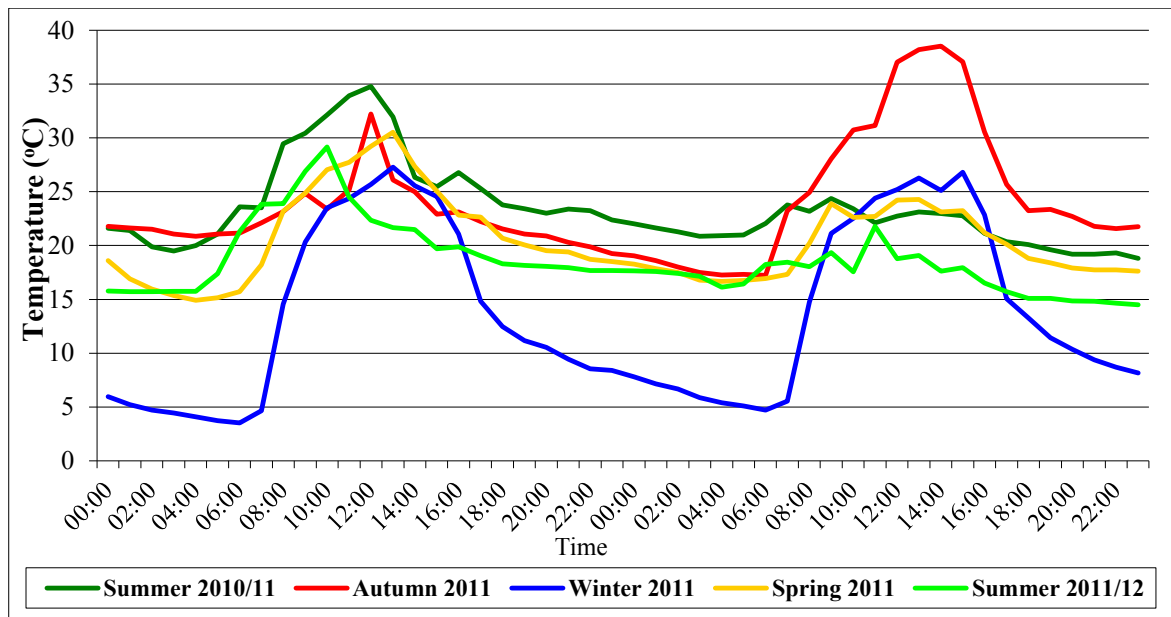


Figure 3.1. Diurnal temperature fluctuations recorded over two days, while mini-cuttings were harvested in the stock plant tunnel.

The diurnal RH in the stock plant tunnel (Fig. 3.2) displays the biggest variation in winter and the second day of the autumn rooting experiment where the RH dropped to between 30 and 40 % during the hottest part of the day between 12:00 and 14:00, which may be due to the reduced irrigation frequency. All seasons had similar maximum RH of between 84 and 92 % humidity.

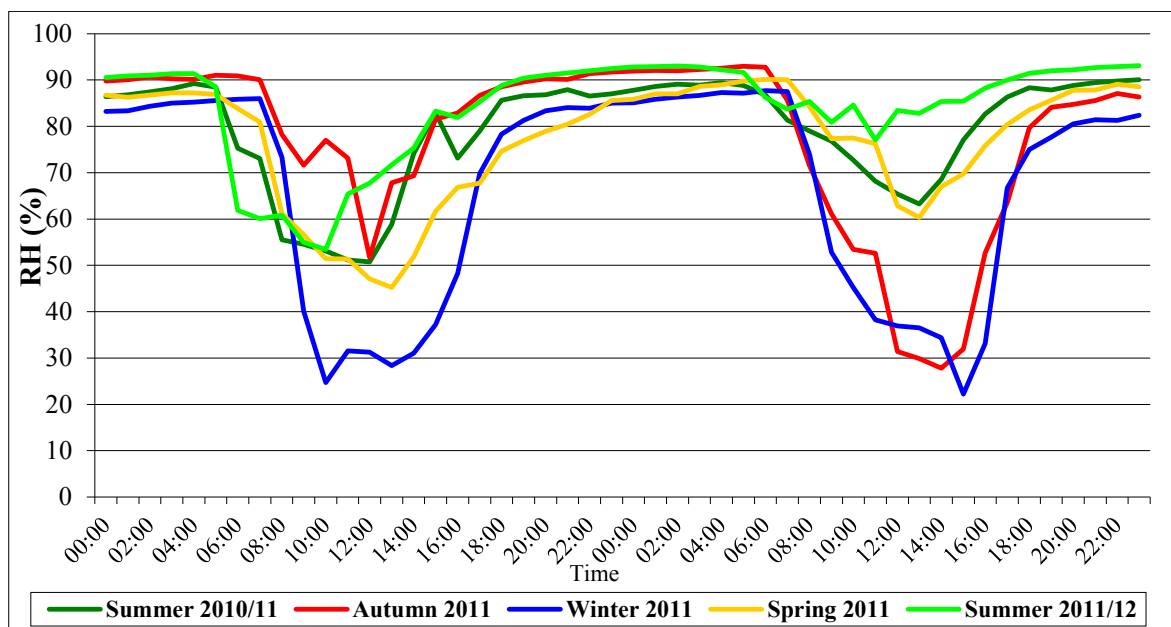


Figure 3.2. Diurnal relative humidity (RH) recorded over two days, while mini-cuttings were harvested in the stock plant tunnel.

The micro-environmental conditions under shade nets were compared over the entire experimental period using maximum and minimum temperatures. Minimum temperatures

analysed by ANOVA did not reveal the shade net factor ($P_{0.05} = 0.503$) to be significant. The maximum temperature under the various nets differed significantly ($P_{0.05} = 0.003$) as well as at 1 % level of significance revealed by the LSD (Table 3.2). Although minimum temperatures were not significantly different, the temperatures under the control and Clarix E Blue® were coolest and temperatures under green 40 % and red 30 % warmest. The maximum temperatures under shade nets differed significantly, where Clarix E Blue® plastic as a covering had the coolest maximum temperatures, but was not significantly different from the green 40 %, Aluminet® 40 %, black 30 % and blue 20 % nets. Temperatures under the red 30 % had a tendency towards being the warmest, but were not significantly different from the control, Patilite®, blue 20 % and black 30 % nets.

Table 3.2. Mean maximum and minimum temperatures in the stock plant tunnel measured with thermometers suspended above the plants in the centre of the shade net treatment over the time of the experiment.

	Temperature (°C)	
	Maximum	Minimum
Aluminet® 40 %	31.91 bc ¹	15.18 a
Black 30 %	32.30 abc	15.37 a
Blue 20 %	32.32 abc	15.05 a
Clarix E Blue® plastic	31.34 c	14.89 a
Control	32.95 ab	14.84 a
Green 40 %	31.68 bc	15.58 a
Patilite® clear plastic	32.71 ab	15.24 a
Red 30 %	33.28 a	15.53 a

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where columns for minimum and maximum temperatures are calculated separately. The maximum temperature LSD for shade net = 1.360 and minimum temperature LSD for shade net = 1.061.

Temperatures under the shade nets varied significantly with regard to seasons ($P < 0.001$) for both minimum and maximum temperatures at both 5 and 1 % level of significance. Similarly, at 5 and 1 % level of significance, for both, minimum and maximum temperature, the interaction between shade net and season was not significantly different ($P = 1.00$). When using LSD at 1 % level of significance, the minimum and maximum temperatures for seasons and shade nets varied (Table 3.3). Maximum temperatures displayed more variation than minimum temperatures over shade nets and seasons. Although shade nets moderate temperatures to some extent, the maximum temperatures in summer 2011/12 reached 40 °C, a range potentially dangerous to plant metabolism as it can cause physiological damage. The minimum temperature in winter 2011 did not fall below 6 °C; therefore, frost damage was avoided. The hottest maximum temperatures were measured under all shade nets in Summer 2011/12, but temperatures were not significantly different compared with most shade nets

during summer 2010/11 and spring 2011 for maximum. As expected, the coolest maximum temperatures were measured during winter 2011, but were not significantly different compared with a few shade nets during autumn 2011 and 2012 (Table 3.3). The warmest minimum temperatures were measured during summer 2010/11, but were not significantly different from summer 2011/12 and autumn 2011. The coldest minimum temperatures were measured during winter 2011.

Table 3.3. Mean maximum and minimum temperature in the stock plant tunnel measured with thermometers for the experimental seasons.

	Temperature (°C)					
	Summer 2010/11		Autumn 2011		Winter 2011	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Aluminet® 40 %	34.60 ab ¹	20.81 a	31.67 bc	16.17 ab	26.82 c	6.97 d
Black 30 %	34.92 ab	20.79 a	32.10 b	16.35 ab	26.96 c	7.43 d
Blue 20 %	34.64 ab	20.37 a	32.23 b	16.07 ab	27.18 c	7.18 d
Clarix E Blue® plastic	33.99 ab	20.14 a	30.98 bc	15.83 ab	26.22 c	7.18 d
Control	35.13 a	20.22 a	32.96 b	15.85 ab	27.91 c	6.83 d
Green 40 %	34.59 ab	20.78 a	31.61 bc	16.52 ab	25.80 c	7.76 cd
Patilite® clear plastic	35.05 a	20.74 a	32.57 b	16.25 ab	27.84 c	7.13 d
Red 30 %	35.90 a	20.73 a	33.13 b	16.56 ab	27.96 c	7.71 cd
Mean	34.85	20.57	32.16	16.2	27.08	7.27
	Temperature (°C)					
	Spring 2011		Summer 2011/12		Autumn 2012	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Aluminet® 40 %	34.85 ab	12.66 bc	39.50 a	15.89 ab	32.16 b	13.17 b
Black 30 %	35.68 a	13.45 b	40.73 a	15.86 ab	32.84 b	13.48 b
Blue 20 %	35.43 a	12.75 bc	39.23 a	15.53 ab	33.34 b	12.86 bc
Clarix E Blue® plastic	34.94 ab	12.78 bc	39.79 a	15.18 b	31.57 bc	12.95 bc
Control	35.98 a	12.47 bc	39.98 a	15.73 ab	33.64 b	12.79 bc
Green 40 %	34.83 ab	13.22 b	39.98 a	16.07 ab	31.48 bc	13.79 b
Patilite® clear plastic	35.75 a	12.71 bc	40.83 a	15.72 ab	32.83 b	13.39 b
Red 30 %	36.14 a	13.23 b	40.46 a	16.51 ab	34.24 ab	13.51 b
Mean	35.45	12.91	40.06	15.81	32.76	13.24

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where minimum and maximum temperatures are calculated separately. The interaction between shade and seasons has a LSD for maximum temperature = 6.881 and minimum temperature = 5.369.

Mean temperatures and RH under shade nets were analysed by ANOVA with blocking to correct for any temperature gradient from wet wall to fans during six weeks in spring 2011. Maximum and minimum temperatures and % RH were recorded for the same spring 2011 time period (Table 3.4). Temperatures were significantly affected by the shade net factor ($P_{0.05} = 0.030$). The % RH for shade net was highly significant ($P_{0.05} < 0.001$); recalculation at 1 % level of significance revealed significant differences in % RH in spring 2011 where Clarix E Blue® had a tendency towards being the coolest shade covering, but was not significantly different at 1 % level of significance from all nets, except for red 30 %.

Mean temperatures calculated from HOBO® data loggers over six weeks during spring 2011, tended towards being the warmest under the red 30 % shade net; however, these temperatures were not significantly different from other nets, except for Clarix E Blue®, which tended to be coolest (Table 3.4). Although maximum and minimum temperatures recorded on HOBO® data loggers (Table 3.4) were not the same as the maximum and minimum temperatures measured with thermometers (Table 3.3, spring 2011) there were similar trends. For example in table 3.3, spring 2011, measured with thermometers, the control (no covering) had the coolest minimum (12.47 °C) but one of the warmest maximum temperatures (35.98 °C), and red 30 % had the warmest maximum (36.14 °C); whereas in table 3.4, spring 2011, measured with HOBO® data loggers, the coolest minimum was Aluminet® (8.63 °C) and the warmest maximum temperatures were control and red 30 % (40.14 and 40.06 °C, respectively). The maximum and minimum temperatures in table 3.4 are more extreme than in table 3.2 or 3.3 as they were the absolute maximum and minimum values recorded with a HOBO® over six weeks in spring 2011 and the values in table 3.2 were means of minimum and maximum data collected over more than a year and table 3.3 were means of minimum and maximum data collected over three months (one season) recorded with a thermometer. Thus the minimum and maximum temperature and RH values in table 3.4 could not be statistically analysed as they were single values. Mean RH values were significantly different for shade nets; the highest RH was recorded under blue 20 %, being significantly different from all other shade nets and plastics. Similarly, black 30 % had the lowest mean RH (Table 3.4), being significantly lower than other shade nets and plastics.

Table 3.4. Mean temperature and RH recorded hourly by HOBO® data loggers under each shade net during spring 2011.

	Temperature (°C)			Relative humidity (RH %)		
	Mean	Max	Min	Mean	Max	Min
Aluminet® 40 %	19.95 ab ¹	37.44	8.63	67.72 c	91.80	23.50
Black 30 %	19.99 ab	39.22	10.99	61.30 d	89.30	23.50
Blue 20 %	20.37 ab	38.77	10.60	75.65 a	96.70	23.50
Clarix E Blue® plastic	19.74 b	39.46	10.71	70.25 b	93.61	10.52
Control	20.27 ab	40.14	10.25	67.86 bc	92.08	9.07
Green 40 %	20.10 ab	39.40	10.49	69.10 bc	92.26	10.95
Patilite® clear plastic	20.41 ab	38.32	10.21	69.25 b	99.30	14.00
Red 30 %	20.63 a	40.06	10.66	66.70 c	93.44	10.46

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where mean temperature and RH for shade nets are calculated separately. Where LSD for mean temperature under shade nets = 0.7131 and LSD for mean RH under shade nets = 2.427.

Minimum and maximum temperatures were compared for part of May and June 2011 with shade nets covering the stock plants and for the same time period in 2012 when the shade nets and plastics had been removed. The temperatures were analysed by ANOVA using a blocking factor based on the theory that there is a temperature gradient in greenhouses and tunnels from the wet wall to the fans, where the first block (theoretically warmest) was closest to the fans. The ANOVA minimum temperatures indicated that the variation between presence/absence of shade nets was highly significant ($P_{0.05} < 0.001$), but the shade net factor ($P_{0.05} = 0.993$) and the interaction between shade net and presence/absence of shade nets ($P_{0.05} = 1.000$) were both not significantly different. The maximum temperatures between presence/absence of shade net was highly significant ($P_{0.05} < 0.001$), but the shade net factor ($P_{0.05} = 0.712$) and the interaction between shade net and presence/absence of shade nets ($P_{0.05} = 0.720$) were both not significantly different. Therefore, as it is difficult to separate the effects of the presence/absence of shade nets and the type of shade net, the LSD at 1 % level of significance was used in table 3.5 for the interaction between these two factors as the LSD can indicate apparent differences even when the ANOVA indicates that there is no significant difference. The minimum temperatures for the shade net \times presence/absence interaction were not significantly different at 1 % level of significance (Table 3.5 all under letter 'a'), where the red 30 % during the shaded period in 2011 was the warmest (10.3 °C) and Clarix E Blue® was the coolest (6.69 °C) minimum temperature with no shading in 2012. Although the maximum temperatures for the shade net \times presence/absence interaction were not significantly different at 1 % level of significance, the difference between presence in 2011 and absence of shade nets in 2012 was significant at this level, which gave an

apparent significant difference when viewing LSD in table 3.5. Where black 30 % during the no shade 2012 period had the warmest maximum temperature (32.69 °C), but was not significantly different to any of the nets and plastics in no shade 2012 and red 30 % in the shaded 2011 period. The coolest maximum temperature was the Aluminet® 40 % during the shaded period 2011 (26.47 °C) but was not significantly different from any of the nets and plastics in the shaded 2011 period (Table 3.5). More variation was evident for maximum temperature than minimum temperature using LSD, although not significantly.

Table 3.5. Comparison of the minimum and maximum temperatures recorded with thermometers in 2011 under shade nets and after removal of the nets in 2012 at the same time of year.

	Minimum temperature (°C)		Maximum temperature (°C)	
	Shade 2011	No shade 2012	Shade 2011	No shade 2012
Aluminet® 40 %	9.88 a ¹	6.94 a	26.47 b	32.03 a
Black 30 %	9.98 a	7.03 a	27.19 b	32.69 a
Blue 20 %	9.76 a	6.92 a	27.42 b	31.08 a
Clarix E Blue® plastic	9.41 a	6.69 a	26.56 b	30.67 a
Control	9.28 a	6.73 a	28.33 b	30.94 a
Green 40 %	10.17 a	7.51 a	26.78 b	32.33 a
Patilite® clear plastic	9.83 a	7.05 a	27.58 b	32.31 a
Red 30 %	10.30 a	7.30 a	29.06 ab	32.11 a

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where minimum and maximum temperatures are calculated separately. Minimum temperature LSD for shade net × presence/absence interaction = 4.021 and maximum temperature LSD for shade net × presence/absence interaction = 3.819.

In spring 2011 the Aluminet® 40 % shade net was generally cooler than all other nets and plastics at the coolest and hottest part of the day with a minimum of 9 °C and a maximum of about 28 °C, except for the last day shown where all nets reached about 36 to 38 °C (Fig. 3.3). In general, temperatures under the red 30 % and blue 20 % were hottest, reaching a maximum of 32 to 34 °C most days and a minimum of 11 to 12 °C. Other shade nets and plastics were similar to each other and the control with regard to temperature ranges.

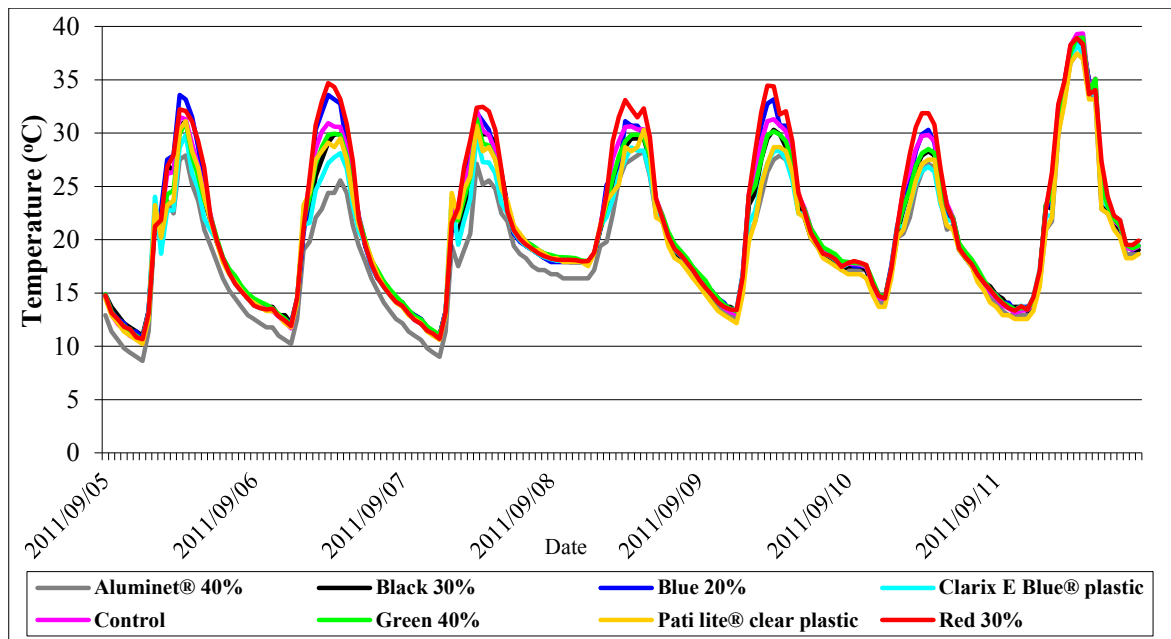


Figure 3.3. Temperature under shade netting during spring 2011, data recorded hourly by HOBO® data loggers.

The diurnal fluctuations of RH (Fig. 3.4) display slightly more variation than temperatures (Fig. 3.3). Typically RH was highest during the night and lowest at the hottest time of the day between 12:00 and 14:00. In general, blue 20 % shade net (Fig. 3.4) had the highest RH. Maximum % RH was similar under all other shade nets with RH variations during the day. Conditions under the blue 20 %, Clarix E Blue® and green 40 % seem to remain at a higher RH level during the heat of the day. The lowest RH levels were recorded under red 30 %, Patilite® and black 30 % ranging between 22 to 42 % during the heat of the day.

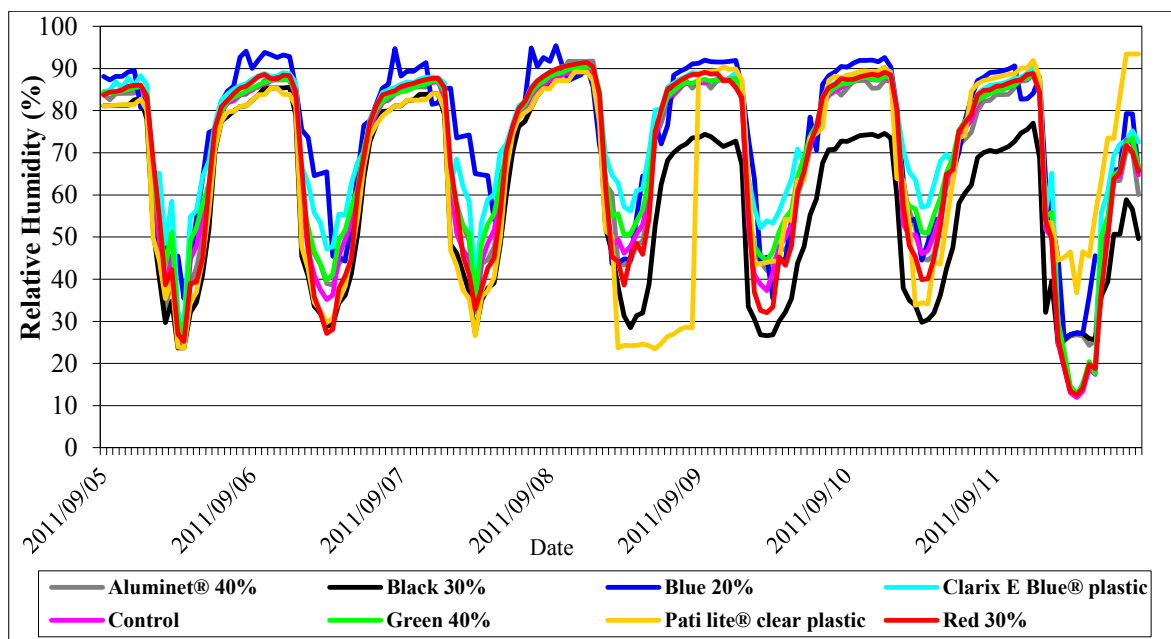


Figure 3.4. Relative humidity under shade netting during spring 2011, data recorded hourly by HOBO® data loggers.

3.3.2 Morphology parameters of stock plants

3.3.2.1 Production

Production in spring 2011 was analysed by ANOVA using replication for blocking. The shade net factor was found to be highly significant ($P_{0.05} < 0.001$). The interaction between clone and fertilizer was significant ($P_{0.05} = 0.015$). The interaction between shade net, clone and fertilizer was not significantly different ($P_{0.05} = 0.194$) at 5 % level of significance. The control PP2107 organic treatment had the highest production of mini-cuttings per stock plant, but this treatment was not significantly different from the other treatments under control, black 30 %, Clarix E Blue® plastic or red 30 % and most treatments under blue 20 %, green 40 % or Patilite® (Table 3.6). The Aluminet® GN018B organic treatment had the lowest production of mini-cuttings per stock plant, but this treatment was not significantly different from the other treatments under Aluminet® or blue 20 % and most treatments under black 30 % or green 40 % (Table 3.6). In general, PP2107 organic treatments had the highest production over all nets followed by GN018B inorganic; GN018B organic had the lowest production and PP2107 inorganic the second lowest (means not shown). The overall means of the shade nets were calculated with their own LSD at 1 % level of significance (Table 3.6). Control had the highest production of mini-cuttings, but was not significantly different to black 30 %, blue 20 %, Clarix E Blue®, green 40 %, Patilite® or red 30 %. Aluminet® had the lowest mini-cutting production, but was not significantly different from blue 20 %.

Table 3.6. Mean production of mini-cuttings per stock plant under shade nets divided into their respective clone and fertilizer treatments in spring 2011

		Aluminet® 40 %	Black 30 %	Blue 20 %	Clarix E Blue® plastic	Control	Green 40 %	Patilite® clear plastic	Red 30 %
GN018B	Inorganic	2.716 ab ¹	2.810 ab	2.033 b	3.370 a	3.518 a	3.402 a	3.210 a	3.158 a
	Organic	1.960 b	3.137 ab	3.210 ab	2.902 ab	3.053 ab	2.552 b	2.497 b	3.188 ab
PP2107	Inorganic	2.022 b	3.225 ab	2.865 ab	3.013 ab	3.111 ab	3.009 ab	3.021 ab	2.819 ab
	Organic	2.100 b	3.496 a	2.858 ab	3.872 a	3.972 a	3.195 ab	3.510 a	3.425 a
Mean		2.199 b	3.167 a	2.742 ab	3.289 a	3.413 a	3.04 a	3.059 a	3.148 a

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where the shade net × clone × fertilizer interaction and the shade net means are calculated separately. Production of shade net × clone × fertilizer interaction LSD = 1.3443 and shade net means LSD = 0.6721.

The shade net and fertilizer factors were found to be highly significant (both $P_{0.05} < 0.001$), but the clone factor was not significantly different ($P_{0.05} = 0.395$) with regard to production

of mini-cuttings over the experimental period. The season factor was found to significantly affect ($P_{0.05} < 0.001$) cutting production. The interaction between shade net and season was significant ($P_{0.05} = 0.001$). In general, winter and autumn 2011 had the highest production of mini-cuttings per stock plant (Table 3.7). Summer 2010/11 had the third highest production and spring 2011 the lowest. The mini-cutting production was not specifically documented during harvests in summer 2011/12 and autumn 2012 as just enough mini-cuttings were taken to fill the rooting experiment requirements. Over time there was some stock plant mortality, so during rooting experiments toward the end of the study some of the treatments produced mini-cuttings unevenly. The mean production of each shade net over all seasons (Table 3.7), similarly to that of the two clones and fertilizer treatments (Table 3.6), indicates that the control (no shade net) had a tendency towards the highest number of mini-cuttings per stock plant; however, this was not significantly different from production of mini-cuttings under blue 20 %, Clarix E Blue® or red 30 %. The shade net with the tendency towards the lowest production of mini-cuttings was green 40 %, but this treatment was not significantly different from production under Patilite®, Aluminet® or black 30 %.

Table 3.7. Mean production of mini-cuttings per stock plant under shade nets over seasons.

	Summer 2010/11	Autumn 2011	Winter 2011	Spring 2011	Mean
Aluminet® 40 %	4.276 c ¹	6.900 ab	6.917 a	2.199 d	5.073 bc
Black 30 %	4.431 c	5.450 b	7.250 a	3.167 d	5.074 bc
Blue 20 %	5.987 b	8.100 a	6.083 b	2.742 d	5.728 ab
Clarix E Blue® plastic	5.314 bc	5.650 b	8.333 a	3.289 d	5.647 ab
Control	7.086 a	7.800 a	7.083 a	3.413 d	6.346 a
Green 40 %	4.417 c	5.400 b	6.000 b	3.040 d	4.714 c
Patilite® clear plastic	4.624 c	5.450 b	6.000 b	3.059 d	4.783 c
Red 30 %	6.545 b	7.100 a	6.667 b	3.148 d	5.865 ab
Mean	5.335 b	6.481 a	6.792 a	3.007 c	

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where the season × shade net interaction, mean season and mean shade net LSD are calculated separately. Mean production of season × shade net interaction LSD = 1.6055, the mean season LSD = 0.5676 and the mean shade net LSD = 0.8027.

The interaction between season, clone and fertilizer was significant ($P_{0.05} = 0.003$). Over time the production of mini-cuttings increased from summer 2010/11 to winter 2011 with a 50 % decrease in spring 2011. During spring 2011, none of the clone × fertilizer treatments were significantly different (Fig. 3.5), similar to production during summer 2010/11 and autumn 2011, which were not significantly different for GN018B organic and inorganic and PP2107 inorganic, but the PP2107 organic summer 2010/11 production was significantly

lower and the autumn 2011 production was significantly higher. Interestingly, in winter 2011 the inorganic treatment for both clones was statistically similar and low; the organic treatments for both clones were statistically similar and much higher.

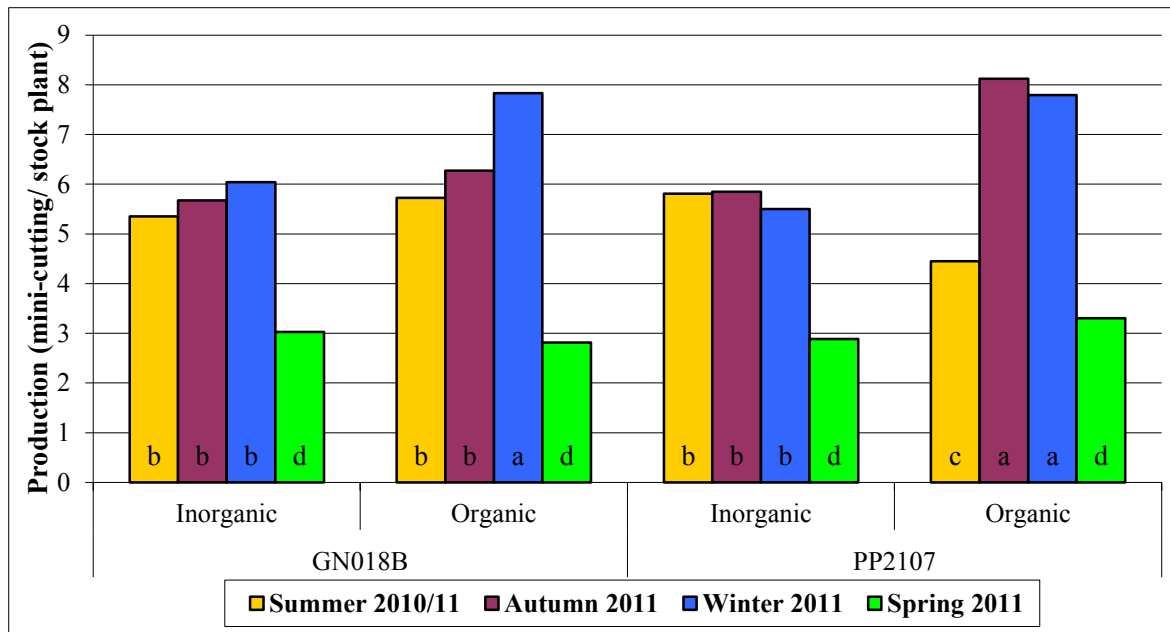


Fig 3.5. Mean production of mini-cuttings per stock plant for all shade nets over seasons divided into the clone and fertilizer treatments. Where the LSD = 1.1352 for clone \times fertilizer \times season interaction at 1 % level of significance.

3.3.2.2 Dry mass

Using dry mass data (grams (g)), the shade net factor was not significant ($P_{0.05} = 0.917$), but the clone and plant part (leaves and stalks) factors were significantly different ($P_{0.05} = 0.035$ and $P_{0.05} < 0.001$, respectively). All of the interactions of these factors were not significant. Using percentage dry mass, shade net and clone factors were found to be significant ($P_{0.05} = 0.049$ and $P_{0.05} = 0.001$, respectively), however, the plant part factor was not significantly different ($P_{0.05} = 0.693$), neither were the interactions between these factors. The dry mass (g) of leaves for all shade nets and clones was significantly larger than that of stalks (Table 3.8); however, no differences were found between the various shade nets, which all displayed a dry mass of approximately 30 % (Table 3.8; Figure 3.6). Within plant part such as leaf, the dry mass (g) of mini-cuttings with regard to clone did not differ significantly; clone PP2107 under Aluminet® 40 % had a tendency to produce the greatest leaf dry mass, while clone GN018B under green 40 %, had a tendency to produce the lowest leaf dry mass. Similarly, no significant differences were found between shade nets with regard to dry mass of leaves or stalks. Stalks of clone PP2107 under red 30 % had a tendency towards the largest dry mass, while those of clone GN018B under black 30 % had a tendency towards the lowest

dry mass per cutting. In general, clone PP2107 had bigger leaves and stalks than GN018B by observation (see Table 3.9); possibly explaining why PP2107 had slightly larger dry mass (g) than GN018B, particularly for stalks (Table 3.8).

Table 3.8. Mean dry mass in grams and percentage calculated from five mini-cuttings per three replications from various shade nets and clones.

Shade net	Clone	Dry Mass (g)		Dry Mass (%)	
		Leaves	Stalks	Leaves	Stalks
Aluminet® 40 %	PP2107	0.3818 a ¹	0.2055 b	30.22 a	29.88 a
	GN018B	0.3484 a	0.1790 b	32.38 a	32.84 a
Black 30 %	PP2107	0.3672 a	0.2228 b	31.61 a	30.94 a
	GN018B	0.3394 a	0.1688 b	30.99 a	31.65 a
Blue 20 %	PP2107	0.3669 a	0.2175 b	31.14 a	31.19 a
	GN018B	0.3643 a	0.1929 b	32.34 a	32.57 a
Clarix E	PP2107	0.3372 a	0.2166 b	28.54 a	28.62 a
Blue® plastic	GN018B	0.3306 a	0.1778 b	31.47 a	30.68 a
	PP2107	0.3389 a	0.2115 b	28.36 a	27.04 a
Control	GN018B	0.3746 a	0.2208 b	30.25 a	30.07 a
	PP2107	0.3283 a	0.2470 b	28.84 a	28.75 a
Green 40 %	GN018B	0.3038 a	0.1755 b	29.56 a	29.51 a
	PP2107	0.3616 a	0.2276 b	31.15 a	30.48 a
Patilite® plastic	GN018B	0.3671 a	0.1895 b	31.98 a	31.23 a
	PP2107	0.3331 a	0.2499 b	28.87 a	28.14 a
Red 30 %	GN018B	0.3401 a	0.1777 b	31.65 a	32.45 a

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where the two columns under dry mass (g) and the two columns under dry mass (%) are calculated separately. dry mass (g) of clone × shade net × plant part interaction LSD = 0.11174 and the mean dry mass (%) of clone × shade net × plant part interaction LSD = 5.547.

The data displayed in figure 3.6 is the simplified representation of table 3.8 where the mean data of both clones is used in order to simplify the figure to display only the dry mass of leaves and stalks in terms of the shade nets, thus ignoring the effect of the clone on the dry mass. Figure 3.6 clearly displays the dry mass (g) variation between leaves and stalks compared with little variation in dry mass percentage. The tendency was similar in figure 3.6 to table 3.8 where the treatment with the greatest leaf dry mass (g) was clone PP2107 under Aluminet® 40 % and the lowest leaf dry mass (g) was clone GN018B under green 40 %; in figure 3.6 the treatment with the greatest leaf dry mass (g) was blue 20 %, Aluminet® 40 % and Patilite® and the lowest was green 40 %. However, where in table 3.8 the PP2107 clone under red 30 % had the greatest dry mass (g) for stalks, and clone GN018B under black 30 % had the lowest dry mass (g) for stalks per cutting; in figure 3.6 the treatment with the greatest stalk dry mass (g) was the control and the lowest stalk dry mass (g) was Aluminet® 40 %.

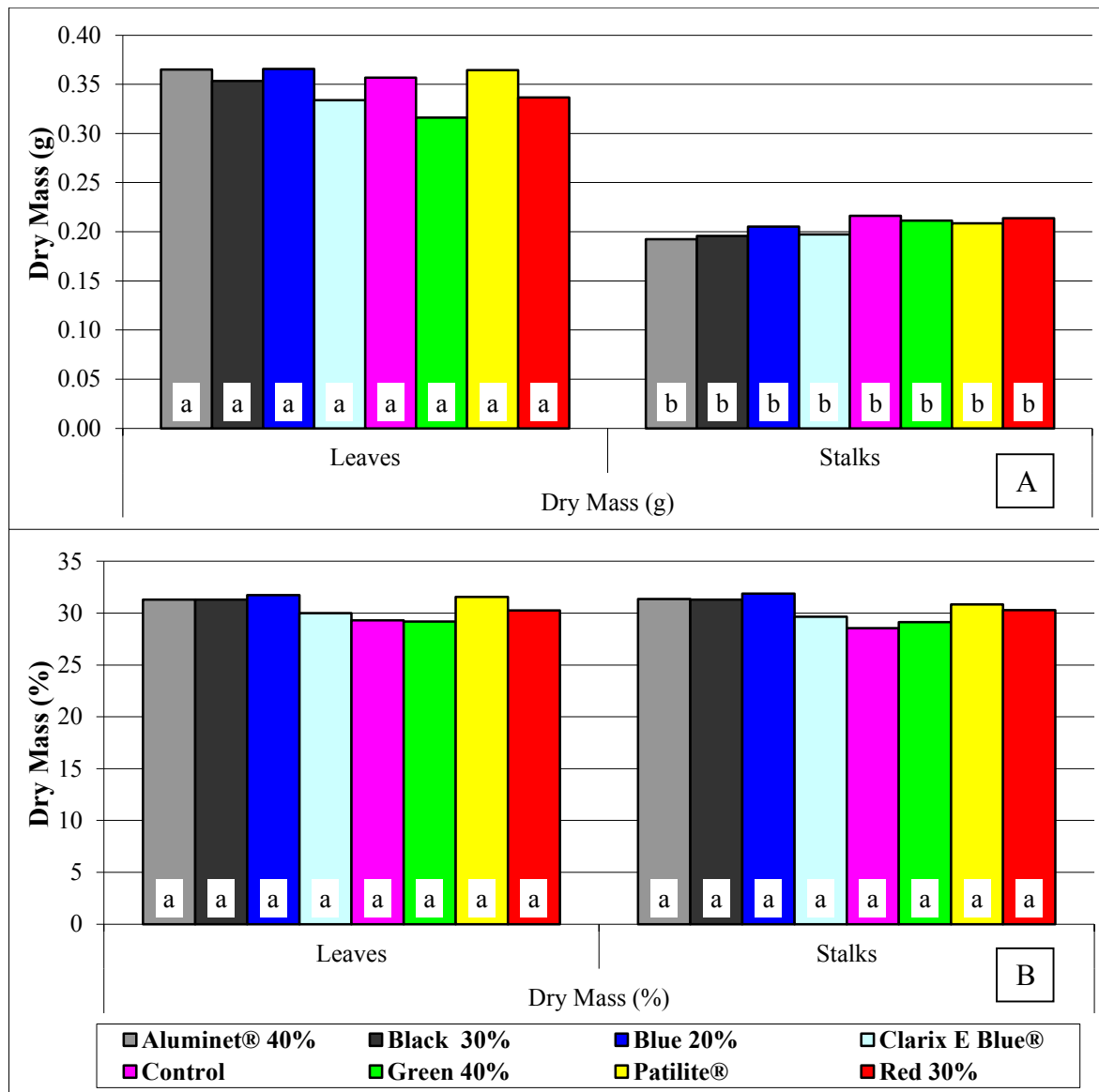


Figure 3.6. Mean dry mass in grams (A) and as a percentage of the fresh mass (B) for five mini-cuttings per three replications divided into shade nets and clones. Where $LSD = 0.07901$ for dry mass (g) for shade net \times plant part interaction at 1 % level of significance and $LSD = 3.922$ for dry mass (%) for shade net \times plant part interaction at 1 % level of significance.

3.3.2.3 Leaf area and stem diameter

The shade net factor significantly affected LA per prepared mini-cutting ($P_{0.05} < 0.001$), but the clone factor and shade net by clone interaction were not significant ($P_{0.05} = 0.263$ and 0.339 , respectively) with regard to LA per mini-cutting. Shade net and clone factors significantly affected stem diameter ($P_{0.05} = 0.010$ and 0.008 , respectively). The shade net by clone interaction was highly significant ($P_{0.05} = 0.003$). Leaf area appeared larger for the clone PP2107, but once the mini-cuttings leaves were reduced in preparation for rooting, the clones did not vary significantly with regard to LA; however, using LSD at 1 % level of

significance revealed some variation. Mini-cuttings from the PP2107 clone had a tendency towards producing the largest LA under the black 30 % net, while a tendency towards producing the smallest LA was determined for Patilite®, PP2107 mini-cuttings (Table 3.9). Similarly, mini-cuttings stem diameter (mm) of PP2107 had a tendency to be greatest under red 30 %, but was not significantly different to the stem diameter of control, GN018B. The mini-cuttings stem diameter of GN018B had a tendency to be the smallest under Aluminet® 40 %, but was not significantly different from many other stem diameters (see Table 3.9). In general, clone PP2107 had bigger leaves than GN018B in six out of eight shade net treatments, the exceptions being green 40 % and Patilite®. Similarly, clone PP2107 also had a greater stem diameter than GN018B except for blue 20 % and the control.

Table 3.9. Mean leaf area and stem diameter of five mini-cuttings as affected by shade nets and clones.

	Leaf area (cm ²)		Stem diameter (mm)	
	GN018B	PP2107	GN018B	PP2107
Aluminet® 40 %	10.08 b ¹	12.24 a	1.132 c	1.38 bc
Black 30 %	12.22 a	14.37 a	1.192 c	1.394 bc
Blue 20 %	8.99 b	9.49 b	1.33 bc	1.176 c
Clarix E Blue® Plastic	9.32 b	10.01 b	1.256 bc	1.368 bc
Control	12.65 a	13.59 a	1.656 ab	1.424 bc
Green 40 %	11.93 ab	11.29 ab	1.278 bc	1.436 bc
Patilite® plastic	10.65 b	8.63 b	1.324 bc	1.460 bc
Red 30 %	12.66 a	12.98 a	1.244 c	1.968 a

¹Treatment means sharing the same symbol (abc) are not significantly different at 1 % level where the two columns under leaf area and the two columns under stem diameter are calculated separately. Mean leaf area of shade net × clone interaction LSD = 3.417 and the mean stem diameter of shade net × clone interaction LSD = 0.4062.

3.4 Discussion

The evaluation of environmental conditions is often complicated by the multitude of parameters that contribute to the overall effect. Newer tunnels and greenhouses provide a more controlled environment due to the presence of sensors that activate misting or fogging devices and fans at a frequency dependent on the temperature and RH. The tunnels used for growing stock plants and rooting mini-cuttings in this experiment were not perfectly environmentally controlled but did regulate temperature and RH to a certain extent.

Season plays a vital part in management aspects of any nursery and, although temperatures reached 40 °C in summer months in the stock plant tunnel, these periods were not extended. On the opposite side of the scale, temperatures did not drop below 5 °C that could cause

permanent damage to plant organs (Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004). If minimum and maximum temperatures fall below or above a certain threshold value for a certain species/clone temperature stress occurs. Rocha Corrêa and Fett-Neto (2004) reported that short-term exposure to 40 °C for one hour had a positive effect on *E. saligna* root density and length in the absence of auxin, but such an effect was not observed in *E. globulus*. In this experiment stock plants were only exposed to such high temperatures for a maximum of one to three hours (Fig. 3.1 and 3.3), before temperatures decreased to normal levels. According to Leite *et al.* (2002) the most important difference in leaf temperature of miniature rose plants was recorded between Aluminet®, where temperatures did not rise above 30 °C, and black shade net where leaf temperature often rose above 30 °C, disrupting photosynthesis and damaging plants. Although leaf temperature is correlated with air temperatures, other factors such as stem water potential, relative humidity, photosynthetically active radiation (PAR) (400 - 700 nm) and wind speed also dictate leaf temperature (Day, 2000). Day (2000) found that red spruce (*Picea rubens*), a forestry tree that is adapted to cool temperate climates, exhibited a broad net photosynthesis optimum for leaf temperature (16 to 32 °C), which then decreased as leaf temperature increased from 32 to 36 °C. The same author used leaf temperature since measuring air temperature rather than leaf temperature may have biased the resulting photosynthetic response curve. At high irradiances, leaf temperature may vary considerably from ambient air temperature, in natural and artificial environments (Day, 2000). Using detached shoots of red spruce (*Picea rubens*), it was observed that leaf temperatures often exceeded air temperatures by more than 5 °C under both natural and artificial lighting (Day, 2000). However, by using shade netting, the variation between leaf and air temperatures may be decreased. Contrary to Day (2000), 50 % Aluminet® shade netting has been reported to decrease *Citrus* leaf temperature by 4 to 5 °C below ambient summer temperatures without covering (Leite, 2001). Leite *et al.* (2002) reported that under 50 % Aluminet® thermal reflective net, the leaf temperature of miniature rose plants was 1 to 2 °C lower than the air temperature under Aluminet®. Under 50 % black net the leaf temperature was lower than that of the air temperature, but at the hottest part of the day the difference was insignificant and both temperatures were above 30 °C for most of the day. The air and leaf temperatures under 50 % Aluminet® were significantly lower than air and leaf temperatures under 50 % black net (Leite *et al.*, 2002). It is not known what the deviation between leaf and air temperatures under other coloured nets may be. Air temperatures under Aluminet® 40 % did not exceed 30 °C during the week measured in spring 2011, while under black 30 % and green 40 % often reached temperatures of 30 °C

and above (Fig. 3.3); air temperatures under other nets such as blue 20 % and red 30 % as well as the control (no netting) often exceeded 30 °C. The G × N clones used in this experiment are bred to be cold tolerant in order to be able to grow in high mountainous areas; this might explain why stock plants thrived and produced more clones in the cooler autumn and winter months than under summer conditions (Table 3.7 and Fig. 3.5).

To some extent the temperature and RH are dependent on one another (Day, 2000). The RH is dependent on the saturated vapour pressure, which increases as the air temperature increases, that is to say warmer air can hold more water vapour than cold air (Schulze, 2006). It was anticipated that Clarix E Blue® and Patilite® would result in the highest RH due to no ventilation through the plastic, but blue 20 % resulted in the environment with the highest RH with Clarix E Blue® and Patilite® creating a slightly less humid environment (Table 3.4). Under the red 30 % shade net air temperatures were highest (Table 3.4), more so than under the black 30 %, although it was expected that the black colour would absorb more radiation (Leite *et al.*, 2002; Anon, 2008); however, the micro-environment under black 30 % had the lowest RH (Table 3.4 and Fig. 3.4). As plants receive the same amount of water through the drip irrigation system under all nets, the heat absorbed by the black 30 % net might have resulted in evaporative cooling. Considering the temperature gradient from wet wall (cooler) to fan (warmer) and the position of the experimental units in the tunnel, red 30 % and Clarix E Blue® were in the first and hottest block, blue 20 % and black 30 % in the second block, control and green 40 % in the third block and Aluminet® 40 % and Patilite® in the fourth and coolest block (see appendix A, Fig. A.2). This gradient was, however, not greatly evident, especially in the minimum temperatures and the effect of the positioning of the nets in the tunnel was seemingly reduced by using the blocking structure in the statistical analysis when comparing the mean temperature and RH under different shade nets (Table 3.4) and minimum and maximum temperatures under different shade nets comparing with (2011) or without shade net (2012) cover in the same position (Table 3.5). The coolest and second coolest minimum temperatures with shade nets in 2011 were the control and Clarix E Blue®, respectively; whereas with no shading in 2012 the coolest and second coolest were Clarix E Blue® and control respectively (Table 3.5), thus trading places. Similarly, the warmest and second warmest minimum temperatures with shade nets in 2011 were red 30 % and green 40 %, respectively; whereas with no shading in 2012 the warmest and second warmest minimum temperatures were green 40 % and red 30 %, respectively. More variation was noted in the maximum temperatures, where the coolest maximum temperatures with

shade nets in 2011 were Aluminet® 40 % and Clarix E Blue® and with no shading in 2012 the coolest maximum temperatures were Clarix E Blue® and control. The warmest maximum temperatures with shade nets in 2011 were red 30 % and control and with no shading in 2012 the warmest maximum temperatures were black 30 % and green 40 % (Table 3.5). In general, minimum temperatures were colder and the maximum temperatures were hotter when the plants had no covering by shade nets or plastic compared with being covered with shade net or plastic covering. As previously stated, 50 % Aluminet® shade netting can decrease *Citrus* leaf temperature by 4 to 5 °C below ambient summer temperatures without covering (Leite, 2001); however, in this experiment minimum temperatures were 2.94 °C higher and the maximum temperatures were 5.56 °C lower under 40 % Aluminet® than the same spot with no shade net (Table 3.5), but was not significantly different from the control during the same year. Grinberger *et al.* (2000) found that air temperatures under 30 % Aluminet® were higher than under blue and red coloured netting of the same shading percentage, although the control (no net), had the highest overall temperature; however, in this experiment maximum temperatures under Aluminet® 40 % had a lower temperature than blue 20 % and red 30 %, although they were of lower shading percentages, which may let more radiation through resulting in higher temperatures under these nets (Table 3.5). The control had a higher temperature than Aluminet® 40 %. Seemingly, a high shading percentage as afforded by the Aluminet® 40 % and the green 40 % resulted in similar temperature under these nets, indicating that shading percentage might be a more important factor than the colour of the shade net. Diurnal and seasonal fluctuations in temperature and RH are natural, although greenhouses and tunnels can regulate environmental conditions to some extent, there is still variation within (Table 3.1 and Fig. 3.1 and 3.2). Shade netting can further regulate or accentuate temperature and RH fluctuations in comparison to the control (Fig. 3.3 and 3.4).

Production of mini-cuttings depends on many factors; season played a certain role (compare seasonal production in Table 3.7 and Fig. 3.5). With regard to this experiment the gradual increase in production from summer 2010/11 to winter 2011 may have been due to the stock plants being very young in summer 2010/11 as it was their first harvest to produce shoots for mini-cuttings (Fig. 3.5). The high level of production in winter 2011 may have been due to the stock plants now being fully established as well as the fact that the G × N clones are bred to be cold tolerant and grow well in winter. The reduction in production in spring 2011 (Fig. 3.5) may have been due to the plants recovering in terms of nutrition stores after the

good winter production and may have been experiencing some physiological shock due to the high temperatures experienced over that time period (Table 3.3 and Fig. 3.3). The organic fertilizer is applied as solid granules that need a beneficial fungi and bacteria mixture in order to become available for absorption by plants when using an inert medium (Philip, *pers. comm.*²), whereas the inorganic fertilizer can be applied directly as a diluted solution. Production in winter 2011 for the inorganic treatment for both clones was lower than the organic treatment (Fig. 3.5); this may be due to the beneficial fungi and bacteria mixture increasing the uptake of nutrients from the organic fertilizer in the cold soil during winter compared with the inorganic fertilizer so that the stock plant receives a beneficial effect or it simply took that long for the optimal amount of organic fertilizer to become available to the stock plants. For more conclusive results on the condition of stock plants under the organic versus inorganic fertilization regime of mini-hedges over time, stock plant morphology should be assessed for longer than a year. With the organic fertilizer regime it was difficult to keep the plant nutrition even over time as there was a lag phase every time fertilizer was applied as the beneficial fungi and bacteria was needed to make the nutrients available to the plants. Dry mass (g) was not significantly different with regard to shade net treatments, but was significantly different with regard to plant part (leaves and stalks), which is as should be expected as stalks contain more dense tissue. In chapter 2, LA was based on the first fully expanded leaf on a cutting, but in this chapter LA was based on the total LA per average mini-cutting, based on one person trimming mini-cuttings to an industry standard discussed in chapter 4. In chapter 2, Clarix E Blue® had the largest LA, Aluminet® 40 % the second largest and red 30 % the third largest LA; two of the treatments also had the longest internodes (Aluminet® 40 %, green 40 % and red 30 %), an observation supporting the findings by Oren-Shamir *et al.* (2001) with regard to red and blue nets. In this chapter the cuttings with the largest LA were grown under black 40 %, control and red 30 %, in which only red 30 % is the same top three ranking as chapter 2, which may be due to human error in reducing the leaf size of cuttings. In chapter 2 the Patilite® treatment had the smallest LA and black 40 % the second smallest and control the third smallest LA coupled with the shortest internodes being under black 40 % and the control. In this chapter the smallest LA was in cuttings grown under blue 20 %, Clarix E Blue® and Patilite®, in which only Patilite® is the same bottom three ranking as in chapter 2. The LA of an average cutting ranged from 8.63 to 14.37 cm², which is a significant difference in LA. It is also much closer

² Mr Arthur Philip. Talborne Organics KZN, talbornekzn@vodamail.co.za, Tongaat, South Africa.

to the poorer rooting *Triplochiton scleroxylon* cuttings that had a LA of 10 cm² than to the optimal 50 cm² of Leakey and Coutts (1989), although the G × N mini-cuttings were much smaller and more delicate and would not need as large a LA as 50 cm².

3.5 Conclusion

It is vital to know what environmental conditions to grow stock plants of a particular species under and if possible to control those conditions. When growing a crop that is sensitive to fungal disease, under shade netting, it may be prudent to use a net such as black 30 % that had the lowest RH and raise the net high enough for good air flow around the plants. A high RH is required for root formation of cuttings, so a net such as blue 20 % or green 40 % or a plastic like Clarix E Blue® or Patilite® should be used; alternatively, the shade nets could be hung lower to increase the RH near the cuttings set for rooting. Recording the variation between *E. grandis* × *E. nitens* leaf and air temperatures under shade netting may be useful. Further investigation into the particular optimal LA needed for *E. grandis* × *E. nitens* mini-cuttings in conjunction with the photosynthetic balance of the cuttings would be helpful and subsequent training of staff in nurseries would need to be carried out to ensure as little human variation as possible.

References

- ANON. 2008. The benefits of Aluminet are multiple. <http://www.igcusa.com/greenhouse-shade-cloth-aluminet.html> Accessed 26/05/2010.
- ASSIS, T.F., FETT-NETO, A.G. and ALFENAS, A.C. 2004. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: C. Walter and M. Carson (eds.), Plantation Forest Biotechnology for the 21st Century, 303-333. Research Signpost, Kerala, India.
- BATTAGLIA, M., BEADLE, C., and LOUGHHEAD, S. 1996. Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens*. *Tree Physiology* 16, 81-89.
- BOLSTAD, E., ERIKSEN, T.G. and ELIASSEN, A. 2013. Norwegian Meteorological Institute and Norwegian Broadcasting Corporation: Weather statistics for Naoussa, South Aegean (Greece). http://www.yr.no/place/Greece/South_Aegean/Naoussa/statistics.html Accessed 18/12/2013.
- CLOSE, D.C., and BEADLE, C.L. 2003. Chilling-dependent photoinhibition, nutrition and growth analysis of *Eucalyptus nitens* seedlings during establishment. *Tree Physiology* 23, 217-226.
- CONRADIE, D.C.U. 2012. South Africa's climatic zones: Today, tomorrow. International green building conference and exhibition future trends and issues impacting on the built environment. Sandton, South Africa. http://researchspace.csir.co.za/dspace/bitstream/10204/6064/1/Conradie2_2012.pdf Accessed 19/12/2013.
- DAY, M.E. 2000. Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). *Tree Physiology* 20, 57-63.
- DRUEGE, U., ZERCHE, S., KADNER, R. and ERNST, M. 2000. Relation between nitrogen status, carbohydrate distribution and subsequent rooting of chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. *Annals of Botany* 85, 687-701.
- GARDNER, R.A.W. 2007. Investigating the environmental adaptability of promising subtropical and cold-tolerant eucalypt species in the warm temperate climate zone of KwaZulu-Natal, South Africa. *Southern Hemisphere Forestry Journal* 69(1), 27-38.
- GARRIDO, G., CANO, E.A., ARNAO, M.B., ACOSTA, M., and SÁNCHEZ-BRAVO, J. 1996. Influence of cold storage period and auxin treatment on the subsequent rooting of carnation cuttings. *Scientia Horticulturae* 65, 73-84.

- GRINBERGER, A., SHOMRON, M. and GANELEVIN, R. 2000. Shading nets testing. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- HAAPALA, T., PAKKANEN, A. and PULKKINEN, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* × *P. tremuloides*). *Forest Ecology and Management* 193, 345-354.
- HANSEN, J., STROMQUIST, L.H., and ERICSSON, A. 1978. Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Plant Physiology* 61, 975-979.
- HARTMANN, H.T., KESTER, D.E. and DAVIES, F.T., Jr. 1990. Plant propagation – Principles and practices. Fifth edition. Prentice Hall Career & Technology, Englewood Cliffs, New Jersey, USA.
- HOAD, S.P. and LEAKEY, R.R.B. 1996. Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden cutting morphology, gas exchange and carbohydrate status during rooting. *Trees* 10, 317-324.
- KAMALUDDIN, M., and ALI, M. 1996. Effects of leaf area and auxin on rooting and growth of rooted stem cuttings of neem. *New Forests* 12, 11-18.
- KAWABATA, A.F., LICHTY, J.S., KOBAYASHI, K.D. and SAKAI, W.S. 2007. Effects of photoselective shade cloths on potted *Dracaena deremensis* ‘Janet Craig’ and *Dracaena marginata* ‘Colorama’. *Pacific Agriculture and Natural Resources* 14, 49-54.
- LEAKEY, R.R.B. 1985. Chapter 9: The capacity for vegetative propagation in trees. In: Cannell, M.G.R.; Jackson, J.E., (eds.) Attributes of trees as crop plants. Abbotts Ripton, Institute of Terrestrial Ecology, pp 110-133.
- LEAKEY, R.R.B. and COUTTS, M.P. 1989. The dynamics of rooting in *Triplochiton scleroxylon* cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. *Tree Physiology* 5, 135-146.
- LEITE, C. 2001. The Aluminet I 50% Effect on Photosynthesis for Growing Citrus in Greenhouses. *International Agriculture* (August). http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.

- LEITE, C.A., FAGNANI, M.A. and OLIVEIRA DA SILVA, I.J. 2002. Comparison between thermal reflect net and black net in mini-roses crop in Holambra-sp, Brazil. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lan=en&geo_action=done Accessed 14/05/2010.
- MCMAHON, M.J and KELLY, J.W. 1995. Anatomy and pigments of chrysanthemum leaves developed under spectrally selective filters. Short Communication. *Scientia Horticulturae* 64, 203-209.
- MCMAHON, L., GEORGE, B. and HEAN, R. 2010. Primefacts for profitable, adaptive and sustainable primary industries. Primefact 1055, A Treesmart factsheet, September. <Http://www.industry.nsw.gov.au> Accessed 22/05/2012.
- OLD, K.M., WINGFIELD, M.J. and YUAN, Z.Q. 2003. A manual of diseases of eucalypts in South-East Asia. Center for International Forestry Research. Jakarta, Indonesia.
- OREN-SHAMIR, M., GUSSAKOVSKY, E.E., SHPIEGEL, E., NISSIM-LEVI, A., RATNER, K., OVADIA, R., GILLER, Y.E. and SHAHAK, Y. 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum*. *Journal of Horticultural Science and Biotechnology* 76, 353-361.
- ROCHA CORRÊA, L.D. and FETT-NETO, A.G. 2004. Effects of temperature on adventitious root development in microcuttings of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill. *Journal of Thermal Biology* 29, 315-324.
- SAYA, R.A., MANKESSI, F., TOTO, M., MARIEN, J.N. and MONTEUUIS, O. 2008. Advances in mass clonal propagation of *Eucalyptus urophylla* × *E. grandis* in Congo. *Bois et Forêts des Tropiques* 297(3), 15-25.
- SCHULZE, R. E. 2006. Relative humidity: General background. In: Schulze, R. E. (Ed). 2006. South African atlas of climatology and agrohydrology. Water Research Commission, Pretoria, RSA, WRC Report 1489/1/06, Section 12.1.
- SHAHAK, Y. 2002. Current research in ornamental: Colored shade nets a new agrotechnology. <http://wendell-trading.com/files/Colour%20Shade%20Net.pdf> Accessed 14/05/2010.
- STAMPS, R.H. 2009. Use of colored shade netting in horticulture. *Hortscience* 44(2), 239-241.
- TEITEL, M., PEIPER, U.M. and ZVIELI, Y. 1996. Shading screens for frost protection. *Agricultural and Forest Meteorology* 81, 273-286.

- TSIPOURIDIS, C., THOMIDIS, T. and BLADENOPOULOU, S. 2006. Rhizogenesis of GF677, Early Crest, May Crest and Arm King stem cuttings during the year in relation to carbohydrate and natural hormone content. *Scientia Horticulturae* 108, 200-204.
- VSN INTERNATIONAL. 2011. GenStat for Windows, 14th Edition. VSN International, Hemel Hempstead, UK.
- WENDLING, I., BRONDANI, G.E., DUTRA, L.F., HANSEL, F.A. 2010. Mini-cuttings technique: a new ex vitro method for clonal propagation of sweetgum. *New Forests* 39(3), 343-353.

Chapter 4

Effect of growing *Eucalyptus grandis* × *E. nitens* mini-hedge stock plants under coloured shade nets on rooting of mini-cuttings

4.1 Introduction

Large-scale vegetative propagation has become important to multiply superior genotypes in order to establish competitive commercial plantation forests of economically important woody species (Palanisamy and Kumar, 1997; Fett-Neto *et al.*, 2001; Negash, 2002; Assis *et al.*, 2004; Rocha Corrêa and Fett-Neto, 2004; Schwambach *et al.*, 2008), including *Eucalyptus* species. Various methods of propagation used in forestry include micropropagation (i.e., *in vitro* tissue culture), rooting of cuttings from woody or herbaceous shoots (i.e., macropropagation) and seed propagation (Haapala *et al.*, 2004; Titon *et al.*, 2006). Seed propagation is the traditional propagation method and is still a vital method in forestry worldwide as well as in breeding programmes. Micropropagation is the production of plants from very small plant parts, tissues or cells grown in a laboratory under sterile conditions, in a test tube or other container under controlled conditions (Hettasch and Lunt, 2002). Macropropagation using macro-cuttings is a common method, whereby a portion of a stem, root, or leaf is cut from the stock plant and placed under favourable environmental conditions to form adventitious roots (Hettasch and Lunt, 2002). *Eucalyptus* macro-cuttings can be prepared between 60 and 100 mm in length, with basal diameters of 2.0 to 5.0 mm (Stape *et al.*, 2001; Hettasch and Lunt, 2002). However, traditional macro-cuttings are often much larger depending on the type such as hardwood (100 to 760 mm and diameter 6 to 25 mm) or softwood (75 to 125 mm) (Hartmann *et al.*, 1990). Mini- and micro-cuttings are both smaller than macro-cuttings and the techniques used to produce them are very similar in both concept and operational procedures, differing mainly in the origin of the initial propagules (Assis *et al.*, 2004; Titon *et al.*, 2006). Micro-cuttings are obtained from shoot apices originating from micro-propagated plants (tissue culture) and mini-cuttings are derived from shoots, retaining their apical buds, from mini-hedge stock plants (Assis *et al.*, 2004; Titon *et*

al., 2006). Plantations grown from vegetatively propagated clones have a much higher degree of uniformity than those grown from seedlings (Stape *et al.*, 2001; Hettasch and Lunt, 2002), to allow trees to grow as a uniform stand. Adventitious rooting is a complex process, affected by numerous aspects such as plant hormones, the plant's nutritional status and its genetic characteristics as well as its response to stress such as wounding, water and temperature stress (Hartmann *et al.*, 1990; Fett-Neto *et al.*, 2001). The cutting technique has been adopted by many commercial nurseries around the world for the propagation of *Eucalyptus* species due to its ease of handling compared with micropropagation methods (Titon *et al.*, 2006). In many *Eucalyptus* nurseries the macro-cutting system has been replaced by a mini-cutting system, in order to maintain juvenility of the stock plants, to improve rooting rates of cuttings, lower production costs and produce a healthy root system (Stape *et al.*, 2001; Assis *et al.*, 2004; Romero, 2004; Wendling *et al.*, 2010). Increases in rooting ability and speed of root initiation of mini-cuttings compared with macro-cuttings have been reported, particularly for difficult-to-root species or clones; this has been attributed to higher levels of juvenility and optimal nutritional content of the stock plants (Assis *et al.*, 2004; Titon *et al.*, 2006). Conventional outdoor clonal hedges are replaced with mini-hedge stock plants grown intensively under protection in plastic tunnels (Stape *et al.*, 2001; Assis *et al.*, 2004). Advantages of using mini-cuttings and mini-hedges over macro-cuttings and outdoor clonal hedges include increased productivity as more cuttings can be produced per unit area per year (Assis *et al.*, 2004; Romero, 2004). Costs of labour intensive activities can be reduced due to limiting the area of field operations, the amount of fertilizer, irrigation water, and pesticides and fungicides used, easier weed control and shorter transport distances of harvested material to rooting areas (Assis *et al.*, 2004). Clones of sub-tropical *Eucalyptus grandis* × *E. urophylla* (G × U or GU) hybrids are much more productive as mini-cuttings than are those of other species (Assis *et al.*, 2004). Generally, better rooting and productivity result from mini-hedges and mini-cuttings of G × U clones compared with macro-hedges and macro-cuttings (Pollard, *pers. comm.*¹).

Wendling *et al.* (2010) found that mini-cuttings of Sweetgum (*Liquidambar styraciflua*) can be successfully harvested from mini-hedge plants all-year-round and have much better survival and rooting percentages than macro-cuttings. Schwambach *et al.* (2008) vegetatively propagated an *E. globulus* × *E. maidenii* hybrid clone 19 (Aracruz Celulose

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

S.A) using mini-cuttings. This clone is known to be difficult-to-root with relatively high initial mini-cutting losses; however, 20 days after being placed for rooting the majority (up to 89 %) of the mini-cuttings had formed roots (Schwambach *et al.*, 2008), giving acceptable rooting percentages as rooting greater than 60 % in a commercial nursery is considered to be a high rooting percentage (Pollard, *pers. comm.*¹). Titon *et al.* (2006) investigated differences in *E. grandis* rooting and survival comparing mini- and micro-hedges and cuttings of four clones (CC1, CC8, CC11 and CC12). Most micro- and mini-cuttings had rooting percentages greater than 87.5 % after 28 days; however, one clone (CC11) had a significantly lower rooting percentage for the mini-cutting technique of 68.8 % after 28 days (Titon *et al.*, 2006). In general, clones root better using the micro-cutting technique with mean rooting percentages of 94 and 88 % for micro- and mini-cuttings, respectively (Titon *et al.*, 2006). In testing difficult-to-root *Eucalyptus* species, such as *E. citriodora*, *E. maculata* and *E. paniculata*, Assis *et al.* (2004) found best rooting when using juvenile stock plants and the micro- or mini-cutting method compared with the macro-cutting method. Species such as *Backhousia citriodora* cuttings take many weeks to form roots, thus making it difficult to distinguish between the characteristics that increase rooting and those that enhance survival (Kibbler *et al.*, 2004). The ability to form adventitious roots from cuttings often declines with maturation as reported for *Eucalyptus*, Douglas fir (*Pseudotsuga menziesii*) (Hartmann *et al.*, 1990), apple (*Malus × domestica*) (Pawlicki and Welander, 1995), hybrid aspen (*Populus tremula × P. tremuloides*) (Haapala *et al.*, 2004), *Backhousia citriodora* (Kibbler *et al.*, 2004), ornamental cherry (*Prunus subhirtella*) (Osterc *et al.*, 2009) and *Pinus radiata* (Menzies *et al.*, 2001). This decrease in rooting ability with advancing ontogeny may possibly be explained by the increasing production of rooting inhibitors as plants age (Hartmann *et al.*, 1990). Cuttings from more mature trees tend to develop fewer roots, which may lead to poor field survival (Menzies *et al.*, 2001). Mature woody species and basal ends of shoots may contain more lignin, which according to Trobec *et al.* (2005) may inhibit rooting of cuttings due to higher IAA oxidase activity in more lignified tissue leading to lower endogenous indole-3-acetic acid (IAA) levels. Therefore, the biological or ontogenetic age, and not the chronological age, of propagules is of major importance for rooting success (Hartmann *et al.*, 1990; Osterc *et al.*, 2009). Higher lignification of tissue can, furthermore, present a mechanical barrier, whereby emergence of adventitious roots becomes physically difficult (Trobec *et al.*, 2005). *Eucalyptus globulus* is grown in many

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

areas of the world due to its frost resistance and lower lignin levels compared with *E. saligna*; this is particularly important for the pulp and paper industry in southern Brazil as a high lignin content of the wood interferes with the chemical extraction of cellulose (Fett-Neto *et al.*, 2001). In contrast to the hypothesis of Trobec *et al.* (2005), Fett-Neto *et al.* (2001) found that the cuttings of *E. globulus*, which contained less lignin than those of *E. saligna*, were more difficult-to-root. Easy-to-root *E. saligna* micro-cuttings achieved a relatively high rooting percentage of 75 % after eight days even when rooted in the absence of indole-3-butyric acid (IBA), whereas difficult-to-root *E. globulus* micro-cuttings only reached 60 % rooting (Fett-Neto *et al.*, 2001). *Eucalyptus saligna* micro-cuttings responded better to lower auxin concentrations than *E. globulus* as rooting of *E. globulus* micro-cuttings was only promoted by higher concentrations of IBA (Fett-Neto *et al.*, 2001). Loss of rooting capacity with seedling age was more pronounced in *E. globulus* than in *E. saligna*, but the application of exogenous auxin to *E. globulus* reversed this effect to some extent (Fett-Neto *et al.*, 2001). Leafy macro-cuttings of the ornamental cherry *Prunus subhirtella* ‘Autumnalis’ were harvested from juvenile three-year-old stock plants and mature 40-year-old trees; the rooting success of cuttings from more juvenile stock plants was 76.7 %, whereas cuttings from mature stock plants only achieved 32.3 % rooting (Osterc *et al.*, 2009). A higher rooting potential of more juvenile material can be preserved at the stem base as this tissue remains biologically juvenile and, therefore, can be used to vegetatively propagate difficult-to-root mature plants (Kibbler *et al.*, 2004). Continuous pruning is, however, not a permanent solution as over time rooting success, quality and survival of stock plants decline such as observed in *Backhousia citriodora* hedge plants where cutting production declined over a five-year period (Kibbler *et al.*, 2004) and hybrid aspen (*Populus tremula* × *P. tremuloides*), where cutting production and stock plant survival declined over eight months (Haapala *et al.*, 2004).

4.1.1 Seasonal effects on rooting

There are seasonal variations of rooting ability of cuttings, which are attributed to variations in radiation received by stock plants (Hartmann *et al.*, 1990). *Eucalyptus* mini-hedges that are grown outdoors are susceptible to adverse climatic conditions and are susceptible to poor nutritional status and leaf diseases, particularly during winter under wet conditions. Under plastic growth tunnels, however, the environmental conditions such as temperature and radiation, can be more easily controlled (Assis *et al.*, 2004). In winter the main problems of outdoor mini-hedge productivity are reduced photosynthetic rate, reduced nutrient uptake

and high levels of nutrient losses by leaching during periods of excessive rainfall or irrigation. In softer, leafy cuttings seasonal variations in rooting are often more pronounced as these are more susceptible to higher summer irradiance levels, temperature and water stress (Leakey, 1985). All stresses, typically lead to flower initiation and these conditions may contribute to decreased rooting ability (Leakey, 1985). *Eucalyptus* cuttings can be rooted all year round, but a decline in rooting is often experienced during the winter months in colder areas (Hettasch and Lunt, 2002). The rooting percentage of subtropical *Eucalyptus* mini-cuttings decreases in cold winter months, however, rooting improved when temperatures of stock plants were maintained above 20 °C (Assis *et al.*, 2004). The highest rooting percentages and root numbers per cutting were when stock plant temperatures were between 20 and 25 °C for *E. globulus* (Rocha Corrêa and Fett-Neto, 2004). Temperate climate species can survive and root in climates about 7 °C colder than warm climate species and some can root at lower than average temperatures (Hartmann *et al.*, 1990). *Eucalyptus saligna* had the highest number of roots and fastest rate of rooting when stock plants were held at 15 °C and exogenous auxin was used on micro-cuttings (Rocha Corrêa and Fett-Neto, 2004). This explains why the commercial forestry nursery Sunshine Seedling Services found that the rooting of cuttings from ramets of the two cold-tolerant *E. grandis* × *E. nitens* (G × N or GN) clones generally rooted best during winter 2010 (July and August for PP2107 at 61.5 % and August for GN018B at 51.4 %), although rooting varied over time and seemed unpredictable (Pollard, *pers. comm.*¹). The effect of seasons is often a reflection of the response of cuttings to environmental conditions prevailing at different times during the year. In spring, newly expanding buds and shoots are competing sinks for metabolites and plant hormones, resulting in conditions that can be detrimental to rooting (Hartmann *et al.*, 1990).

4.1.2 Radiation quantity and quality effects on rooting

Radiation quantity (intensity), duration (photoperiod or daylength) and spectral quality (wavelength) influence the growth and development of stock plants and subsequent rooting of cuttings (Hartmann *et al.*, 1990). Irradiance levels applied to stock plants are important for adventitious rooting as radiant energy influences the level, translocation of and cell sensitivity to endogenous hormones such as auxins, as well as of photosynthates and nutrient uptake (Palanisamy and Kumar, 1997; Fett-Neto *et al.*, 2001; Assis *et al.*, 2004).

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

No detailed information on the effect of coloured shade nets and plastics on *Eucalyptus* cutting performance is available. Most nurseries propagating woody species grow stock plants under direct sunlight, under clear plastic in tunnels or outdoors under a black or green shade net (30 to 40 % shading), in order to reduce total solar irradiance, often using different shading percentages for different plant types. Different nurseries prefer to use different shade net percentages; Wallis (*pers. comm.*²) recommended using 30 or 40 % shading as the greenhouse becomes too dark when using higher shading percentages. The SAPPI forests (Pty) Ltd research nursery uses 40 % shade netting to cover the area where rooting trays are held for rooting (Naidu and Jones, 2009). Rieckermann *et al.* (1999) observed best rooting when sweetgum (*Liquidambar styraciflua*) cuttings were kept in a greenhouse covered with 55 % shade netting for ten weeks. After rooting, many nurseries allow rooted cuttings to harden under thin hail netting with 18 to 20 % shading intensity (Pollard, *pers. comm.*¹). Many authors describe conditions used to successfully root *Eucalyptus* cuttings (under greenhouse or shade house conditions as well as other protected structures) (Stape *et al.*, 2001). Titon *et al.* (2006) elaborated on conditions after rooting, where *E. grandis* micro- and mini-cuttings were transferred to a shade house (50 % shading) for acclimation and were allowed to harden outdoors; however, not many authors describe shading conditions for the stock plants themselves. Sasse and Sands (1997) grew *E. globulus* stock plants in a glasshouse; after harvesting, trays of macro-cuttings were placed in an automated glasshouse to root under 50 % shading. *Eucalyptus globulus* × *E. maidenii* stock plants were cultivated as clonal hedges inside a greenhouse, using intermittent flooding trays or drip fertigated sand bed systems; from this material mini-cuttings were harvested and rooted in the greenhouse and transferred to a hardening area once rooted (Schwambach *et al.*, 2008). Cuttings from pine (*Pinus sylvestris*) stock plants grown at a low irradiance of 8 W m⁻² rooted faster and with a greater rooting percentage than those from stock plants grown at 40 W m⁻²; however, when these two irradiance levels were used on cuttings during the rooting period only minor effects on the rooting rate were observed (Hansen *et al.*, 1978). Therefore, the level of irradiance during the stock plant stage affects the rooting process more than the irradiance level during root formation (Hansen *et al.*, 1978). Growing stock plants at low radiation intensity can be a useful tool for difficult-to-root species whereby the resultant cuttings are

² Ms Jacqui Wallis. Mondi – Mountain home, jacqui.wallis@mondigroup.co.za, Hilton, South Africa.

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

easier to root (Hartmann *et al.*, 1990). The effects of radiation on stock plants and on subsequent rooting can be difficult to separate into radiation quantity and quality effects (Hoad and Leakey, 1996). Grinberger *et al.* (2000) maintain that the quality of the radiation is a more relevant factor than its absolute quantity for plant growth and development. As radiation intensity and temperature were increased from 37 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 23 °C day/ 21 °C night to 66 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and 27 °C day/ 21 °C night the rooting percentage of *E. grandis* \times *E. nitens* increased up to 75 % with well-formed roots free from callusing (Mokotedi *et al.*, 2000). The effects of spectral radiation on the morphology and vegetative growth of stock plants has been investigated (chapter 2); however, information on the effects of placing stock plants under shade netting on the subsequent rooting of cuttings is lacking. Hartmann *et al.* (1990) explained that some difficult-to-root plants had an enhanced rooting potential when stock plants were grown and rooted under reduced irradiance levels. These conditions often cause a shade avoidance response of stem elongation (Hartmann *et al.*, 1990); therefore, as red shade netting promotes stem elongation (Appelgren, 1991; Hoad and Leakey, 1996; Oren-Shamir *et al.*, 2001; Cummings *et al.*, 2008), red netting could have a positive effect on rooting potential of cuttings. There is little information on the effect of radiation quality on shoot diameter. Hoad and Leakey (1996) found that radiation quality, over a wide range of red to near infrared ratios (R:NIR) (0.4 to 6.5) affected elongation of *E. grandis* stock plant shoots, but did not significantly affect shoot diameter. A reduced shoot diameter may be advantageous as the slender shoot is less sclerified and may, therefore, root more easily due to a lower mechanical barrier.

4.1.3 Effect of cutting type on root system quality

The number of adventitious roots initiated is an important propagation feature as it determines the size of the structural and functional root system (Sasse and Sands, 1997). A higher number of adventitious roots leads to a larger surface area of fine roots in order to absorb water and nutrients and form a strong young tree to be planted out in the field. Increasing the root number of cuttings is beneficial as this increases the ability to exploit soil water and nutrients and in turn increases overall plant growth (Negash, 2002). To enhance root anchorage it is preferable that more roots emerge from different quadrants of the cutting base (Menziez *et al.*, 2001); a cutting with a good root system consists of many fibrous roots. Compared with macro-cuttings, micro-cuttings typically have finer, more prolific and more fibrous roots that are more effectively connected to the base of the cutting (Hettasch and Lunt, 2002). In order to score the efficiency of root plugs, a scoring system has been

developed by SAPPI forests (Pty) Ltd research for G × U cuttings. In this system rooted cuttings are scored as zero if a root plug is not colonised by roots or is pot-bound, a score of 15 is given if partially colonised by roots, and a score of 30 is given, if the root plug is firm and fully colonised by roots (Naidu and Jones, 2009). In another assessment system, root systems of *Liquidambar styraciflua* rooted cuttings were evaluated and considered to be acceptable for planting if the cutting was alive and the roots together with medium maintained the container shape when the cutting was removed from the container (Rieckermann *et al.*, 1999). Poor root morphology can decrease tree growth and stability in the field and often trees derived from macro-cuttings lack a typical taproot, unlike seedlings, thus compensating by forming multiple primary adventitious roots, which are essential for anchorage (Sasse and Sands, 1997; Stape *et al.*, 2001). The propagation method can affect root density whereby in general, rooted macro-cuttings have fewer primary and secondary roots than seedlings (Sasse and Sands, 1997; Stape *et al.*, 2001). Once established, the fine root system of cutting-propagated trees is as effective as seedling-propagated ones with regard to water and nutrient uptake (Stape *et al.*, 2001). The same authors explain that as macro-cutting systems are replaced by mini-cutting systems, rooting rates and root system formation are improved; therefore, initial growth of mini-cuttings in the field is equal or better than that of macro-cuttings or seedlings. Osterc *et al.* (2009) found that cuttings from more juvenile stock plants produced a significantly higher number of primary roots and a higher-quality root system than cuttings from mature stock plants, thus more juvenile stock plants are characterised by stronger shoot growth.

4.1.4 Callus formation

Poor rooting of *Eucalyptus* macro-cuttings and micropropagated tissue has been attributed to the trend whereby the shoots form excess callus tissue at the base, limiting efficient absorption of water and nutrients (Mokotedi *et al.*, 2000). Such callus usually develops at the basal end of a cutting when it is placed under favourable rooting conditions (Hartmann *et al.*, 1990). As the strength of the nutrient solution in which micropropagated cuttings were cultured was reduced from full-strength to one quarter strength, the percentage of rooting increased from 0 to 52 % and callus formation decreased from 100 to 48 %. Roots obtained in one third and one quarter strength micropropagation nutrient solution were long and thick with well-developed lateral roots, while those produced in half-strength nutrient solution were stunted (Mokotedi *et al.*, 2000). Frequently, roots appear through the callus, leading to the belief that callus formation is essential for rooting; however, the formation of callus and

the formation of roots are independent processes, although both involve cell division. Callus and root formation often occur simultaneously due to their dependence on similar internal and environmental conditions (Hartmann *et al.*, 1990). In some species, callus formation is a precursor of adventitious root formation. Difficult-to-root species such as *Pinus radiata*, *Sedum* spp. and English ivy (*Hedera helix*) (adult phase) have adventitious roots that emerge through the callus tissue formed at the base of the cutting (Hartmann *et al.*, 1990). At times, when basal temperatures exceed 27 °C, excessive callusing can be observed and poor rooting and field survival results (Hartmann *et al.*, 1990). Trobec *et al.* (2005) found that callus formation of the cherry rootstock (*Prunus avium*) ‘GiSelA 5’ was strongest for terminal macro-cuttings, when IBA was not applied (6.9 % callusing), whereas IBA-treated terminal cuttings had only 1.2 % callusing. Callus formation may indicate that internal or environmental conditions at the time of root initiation were not optimal, but acceptable for cell division (Trobec *et al.*, 2005). Similarly, callus formation of cuttings derived from five-month-old stock plants declined as the IBA concentration increased from zero to 3.2 % IBA (Negash, 2002), indicating that IBA concentrations exceeding a certain threshold value for a certain species reduce callusing and this reduced callusing enhances root formation.

Regarding difficult-to-root *Eucalyptus* hybrids, any improvement in rooting percentage could increase the profit margins of commercial nurseries as space and labour can be better utilised and more cuttings can be produced over the same time period. The aim of the experiment was to assess if the shade net or plastic coverings under which stock plants are grown affect rooting in terms of percentage cuttings rooted and quality of roots formed and if different responses are found in different seasons.

4.2 Materials and methods

Rooting experiments were carried out at the University of KwaZulu-Natal (UKZN) in Pietermaritzburg in South Africa (Lat (S): -29.626388° and Lon (E): 30.403917°). Stock plants were grown in a multi-span greenhouse and mini-cuttings were rooted in a single span clear polyethylene tunnel nearby. Stock plants were grown with a dripper irrigation system. The experimental design for the stock plant layout was a factorial design with three factors, where the covering (shade net) factor had eight levels, the clone factor had two levels (PP2107 and GN018B) and the fertilizer factor had two levels (organic and inorganic). The shade nets with clone and fertilizer treatments beneath them were replicated three times

within the greenhouse. The eight levels of the covering factor consisted of five shade nets (Black 30 %, Green 40 %, Apple Blue 20 %, Photo Red 30 %, Aluminet® (silver) 40 %) that were compared with a blue plastic (Clarix E Blue®) and a clear plastic covering, the latter containing CO₂ bubbles (Patilite®) as well as a control that consisted of no shading other than that from the tunnel structure. All covering material was purchased from either Knittex® (Multiknit® (Pty) Ltd.) or FilmFlex Plastics Natal CC. The percentage following the shade net name indicates the shading factor determined by the manufacturer. Shade nets with identical shading factors were not available locally. Radiation levels were compared and discussed previously (chapter 2).

4.2.1 Plant material

Two inter-specific hybrid G × N clones, one easy-to-root genetically improved clone (PP2107) and one difficult-to-root unimproved clone (GN018B), were provided by Sunshine Seedling Services as eight-week-old rooted mini-cuttings. Clone PP2107 is relatively new to the market, developed by Project Pulp, an improvement programme for *Eucalyptus* hybrid material, initiated by the Council for Scientific and Industrial Research (CSIR) and Natal Co-operative Timber, (NCT Forestry Co-operative Limited) funded by the Department of Science and Technology (DST) Innovation fund, while GN018B is an established clone that is popular with growers. Each stock plant was planted into a 2.5 L black plastic nursery bag filled with a Sunshine Seedlings Services medium mixture of Perlite®, vermiculite and palm coir (2:3:5) and allowed to establish for eight weeks before being cut back to a standard height of 100 mm above the medium in each bag. This method is in alignment with Hoad and Leakey (1996), Stape *et al.* (2001), Titon *et al.* (2006) and Wendling *et al.* (2010) who cut back mini-hedge stock plants to between 70 and 100 mm in height and kept stock plants short with frequent pruning in order to achieve acceptable mini-cutting yields. Similarly, in this experiment, plants were kept short with only side shoots allowed to grow in order to become a mini-hedge from which to harvest cuttings. Shade netting or plastic were draped over wire trellising over bricked beds where the top and two sides were covered but the ends left open for ventilation. The trellising was standardised to 1.32 m above the soil level of each bed with the plants placed on top of black polywoven Weedstop weed matting (Tunnel Quip CC) at soil level, which deters the growth of weeds but allows excess water to pass through. The inorganic fertilizer was applied on a bi-weekly basis, alternating Hortichem 3:1:3 (38) or Hortichem 3:1:5 (45), both with a small amount of Microplex trace element mixture (Ocean Agriculture (Pty) Ltd) and the organic fertilizer was applied every six weeks,

alternating Vita Green 5:1:5 (16) or Vita-fruit and Flower 3:1:5 (18), both with Opticrop fungi and bacteria mix from Argaz Organic Soil Nutrients CC, trading as Talborne Organics KZN. A weekly foliar application of Nitrosol® fertilizer was also provided to all stock plants. The organic fertilizer concentrations were matched as closely as possible to the inorganic fertilizer. Stock plants were consistently monitored for pests and diseases. At times between harvests, the stock plants had to be pruned back as the side shoots had become too long for use as cutting material; this practice also ensured that all cuttings were at the same physiological age when harvested for the next rooting experiment.

4.2.2 Harvest procedure

In order to harvest mini-cuttings, young, disease-free side shoots, 40 to 85 mm in length, were removed from the stock plants with sharp, clean scissors or secateurs. Mini-cuttings were immediately submerged in a bucket half-filled with tap water to avoid dehydration and taken to the processing area. To maintain uniformity, mini-cuttings were cut smaller with sharp, clean scissors in the processing area; each mini-cutting consisted of two nodes and one internode, at least 25 mm in length with a diameter of more than 0.5 mm (Pollard, *pers. comm.*¹). This is in line with Stape *et al.* (2001) and Titon *et al.* (2006) who used mini-cuttings between 20 and 30 mm in length with diameter between 0.4 and 1.0 mm and mini- and micro-cuttings between 40 and 60 mm in length, respectively. Mini-cuttings were cut below the bottom node as rooting ability of such cuttings is better than when the cut is made in the middle of the internode (Hettasch and Lunt, 2002). Leaves from the basal node were removed and the pair of leaves on the upper node were reduced by half to a third of the original size in order to reduce transpiration and water stress, while allowing photosynthesis of the recently severed cuttings. Such procedure is in accordance with Hartmann *et al.* (1990), Hettasch and Lunt (2002) as well as Naidu and Jones (2009). Furthermore, by reducing leaf area and removing bottom leaves, overlapping of leaves is prevented in the rooting trays and leaves do not touch the rooting medium, thereby reducing the risk of fungal infection in the nursery (Hettasch and Lunt, 2002). When trimming mini-cuttings the shoot apex was retained as well as any small leaves growing at the shoot apex. Presence of the shoot apex *in vitro* is important to achieve a good quality root system, because apex presence induces a taproot-like system (Assis *et al.*, 2004). Black plastic Unigro® 128 rooting trays were used for rooting cuttings (128 individual removable cells of eight rows by 16 columns

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

(680 mm × 350 mm)), typically used by forestry nurseries. Mini-cuttings were drenched in a solution of non-ionic sticker-spreader NU-FILM P® (registered and distributed by Hygrotech Sustainable Solutions (Properties (Pty) Ltd.) for a short time, to further limit transpiration from leaves, before dipping in approximately 10 mm of the base of the mini-cutting into Seradix® B No. 2 (IBA at 3 g/kg) powder and placing the mini-cuttings into the centre of an Unigro® 128 rooting tray cell, approximately 10 mm deep into the medium. Tray columns were labelled and trays subsequently placed into the rooting tunnel. Mini-cuttings were harvested, cut to size and placed into the Unigro® 128 rooting trays to root by several individuals (labourer 1, 2, 3, 4, 5, 6 and 7) over the five seasons rooting experiments were conducted. The harvesting period of mini-cuttings for any rooting experiment were kept as short as possible to limit potential physiological changes in the stock plants. Between rooting experiments trays were emptied of old medium, sterilised and filled with new medium.

4.2.3 Rooting conditions

All of the mini-cuttings were rooted under the same temperature, relative humidity and light intensity to be able to accurately observe whether there were any significant differences between the stock plant treatments on rooting. Mini-cuttings from each stock plant treatment, consisting of one clone, one fertilizer treatment and one shade net were placed over two labelled columns (16 mini-cuttings) with spaces between treatments in plastic rooting trays. As the mini-cuttings were harvested from three replications the mini-cuttings were also placed as three replications into three separate rooting trays (16 × 3 replications = 48 mini-cuttings per rooting experiment). Trays were placed on the floor, in a grid pattern, in the rooting tunnel with misting sprayers arranged so that all trays received misting. The Irritrol Junior Max® JRMAX-4-220 irrigation controller was set to run for one minute every ten or 15 minutes, from 07h00 to 18h00 at the beginning of the experiment. All mini-cuttings were left to root for six weeks before evaluation. The misting interval was lengthened to 30, 45 and a maximum of 60 minutes to start hardening off mini-cuttings between four to six weeks and on cooler days when not as much relative humidity was needed. Misting was not applied at night to avoid pathogenic fungal and bacterial growth. During winter the scheduling of misters was changed to run from 07h30 to 17h30 depending on the time of sunrise and sunset.

4.2.4 Rooting assessments

Rooting assessments were carried out in the propagation tunnel six weeks after mini-cuttings were placed. Parameters to evaluate the success of rooting of mini-cuttings were: final rooting percentage; number of primary roots formed; and callus presence or absence. Final rooting percentage was calculated as the percentage of mini-cuttings that form roots and are still alive during the assessment after six weeks. In addition the basal stem diameter (BSD) of rooted mini-cuttings above the root emergence zone was determined with a pair of digital callipers. To determine root quality the rooted mini-cuttings for each treatment were rated on a four-point scale (Fig. 4.1): root type 1 – weak rooted mini-cutting, broken or missing root or apical meristem; 2 – average rooted mini-cutting, poor top and average root or average top and poor root; 3 – good rooted mini-cutting, strong top and root needs more time to form, has root hairs but roots do not fully colonise rooting cell; 4 – very strong rooted mini-cutting, strong root and growing tip, has root hairs and fully colonises rooting cell and holds medium strongly when removed from the cell.



Figure 4.1. Examples of rated rooted mini-cuttings, where (1) displays root type 1 – a weak rooted mini-cutting, (2) root type 2 – an average rooted mini-cutting, (3) root type 3 – a good rooted mini-cutting and (4) root type 4 – a very strong rooted mini-cutting. Where the white square next to the ruler is 2×2 cm.

4.2.5 Statistical analysis

The stock plants were laid out as a factorial design with three factors (shade, clone and fertilizer factors). The first factor, shade nets, consisted of eight levels (five shade nets, two plastics and a control). The second factor had two levels of $G \times N$ clones consisting of one easy-to-root (PP2107) and one difficult-to-root (GN018B). The third factor had two fertilizer levels, one being inorganic and the other organic based. Therefore, the $8 \times 2 \times 2$ factorial treatment structure consisted of 32 stock plant treatments per replication. There were three replications of the shade net factor with the stock plant clone and fertilizer factors below each covering, whereby one replication fitted into two benches in the greenhouse. Therefore, 3 replications \times 32 treatments = 96 individual treatments over six benches were used. Due

to being a biological study, the stock plant growth and rooting of mini-cuttings was found to be affected by season and so in the statistical analyses season was often used as a factor with five levels to help separate the effects of the other factors. When assessing specific parameters such as the skill of the labourer placing cuttings or root quality the factor “labourer” was used at varying levels within seasons and the factor “root type” was used with four levels over all seasons and within seasons. Statistical analysis was carried out using GenStat® 14th edition (VSN International, 2011). Data were analysed using analysis of variance (ANOVA) where data were orthogonally distributed. Where data were not orthogonally distributed the algorithm restricted maximum likelihood (REML) was used to estimate variance parameters in the multivariate linear mixed model. All statistical analyses were conducted at a 5 % level of significance. Blocking was used according to placement in the propagation tunnel for the rooting treatments winter 2011, spring 2011, summer 2011/12 and autumn 2012 in order to minimise the effect of the temperature gradient present in the propagation tunnel. Correlation was used to compare parameters of quality and quantity such as rooting percentage, callus percentage, callus plus root, root number, basal stem diameter. For the autumn 2011 rooting experiment 56 mini-cuttings were placed per treatment filling about half a rooting tray per treatment. This rooting experiment had missing data and only one replication of the data represented each treatment due to an event on the 28th March 2011 when the stock plants were sprayed for an infestation of mealybugs (family Pseudococcidae, various species) with the pesticide Avigard (active ingredient Mercaptothion) and the plants experienced phytotoxicity whereby many leaves shrivelled up and appeared burnt; therefore, further cuttings could not be taken at that time. The next rooting experiment conducted in winter 2011 was redesigned to improve the statistical analysis by using 16 mini-cuttings per treatment × three stock plant replications × by three replications, blocking was also introduced; however, using three stock plant replications as well as three replications proved to be very time consuming and labour intensive, therefore, for the remaining rooting experiments conducted in spring 2011, summer 2011/12 and autumn 2012, the harvested shoots from all three stock plant replications were bulked and then placed as 16 mini-cuttings per treatment × three replications in different rooting trays.

4.3 Results

4.3.1 Effect of fertilizer and clones on rooting

The rooting percentage over time was analysed using ANOVA as the data were orthogonal. Rooting percentage was analysed according to the factors shade net, clone, fertilizer and season and their interactions without blocking. Some variation was noted in the fertilizer factor, although this variation was not significant at the 5 % level ($P_{0.05} = 0.731$). The season factor was significant ($P_{0.05} = 0.001$) as well as the interactions between shade net \times fertilizer, fertilizer \times season, shade net \times fertilizer \times season and clone \times fertilizer \times season ($P_{0.05} = 0.005, 0.009, 0.008$ and 0.012 , respectively). A trend was noted, whereby the organic fertilizer had a higher rooting percentage than inorganic fertilizer during autumn 2011, winter 2011 and spring 2011, while in summer 2011/12 the rooting percentage was almost equal and, finally, in autumn 2012 the inorganic fertilizer gave significantly better rooting than the organic fertilizer. The organic treatment had a tendency towards the highest rooting percentage during spring 2011, but this value was not significantly different from the spring 2011, winter 2011 or summer 2011/12 inorganic and autumn 2011 or winter 2011 organic treatments. A tendency towards a lower rooting percentage was found in the autumn 2012 organic treatment, which was not significantly different from autumn 2011 inorganic (Fig. 4.2).

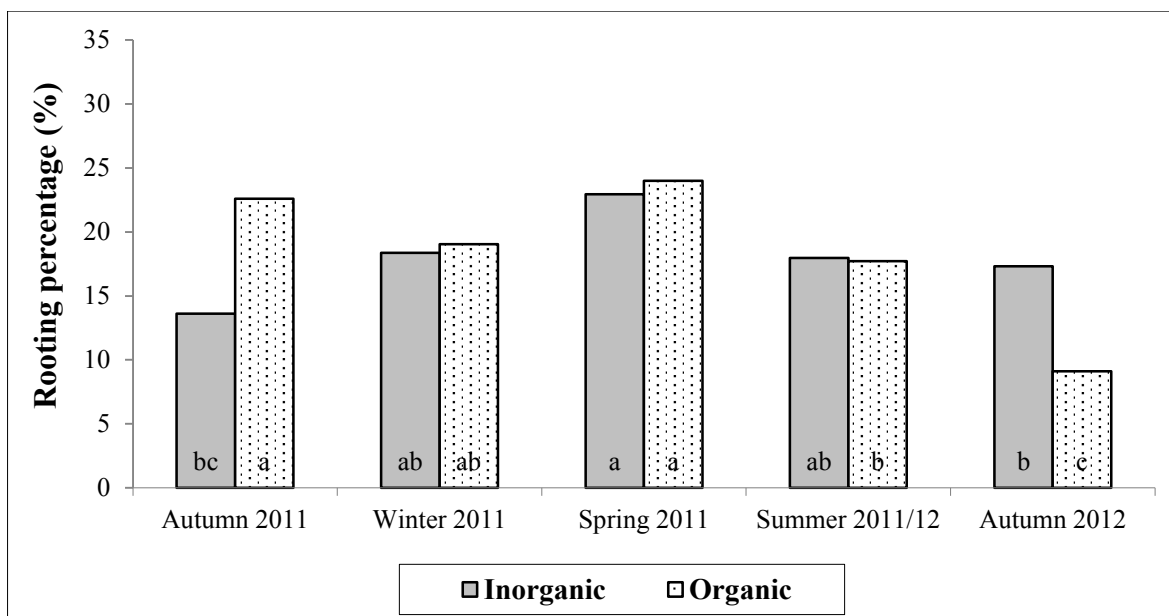


Figure 4.2. Seasonal rooting percentage of mini-cuttings from stock plants grown under inorganic or organic fertilizer treatments; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with least significant difference (LSD) for rooting percentage = 6.073 for the fertilizer \times season interaction.

The factor clone was highly significant ($P_{0.05} < 0.001$), while the interactions between clone \times season, shade net \times clone \times season and clone \times fertilizer \times season were merely significant ($P_{0.05} = 0.007, 0.036$ and 0.012 , respectively). The interactions between shade net \times clone, fertilizer \times clone and shade net \times clone \times fertilizer were not significant ($P_{0.05} = 0.076, 0.699$ and 0.335 , respectively). As expected, the easy-to-root clone PP2107 rooted consistently higher over all seasons compared with clone GN018B. The highest rooting for PP2107 was during summer 2011/12, but this value was not significantly different from autumn 2011, winter 2011 or spring 2011. Conversely, the highest rooting percentage for GN018B was during spring 2011. The lowest overall rooting percentage was during autumn 2012 for both clones (Fig. 4.3).

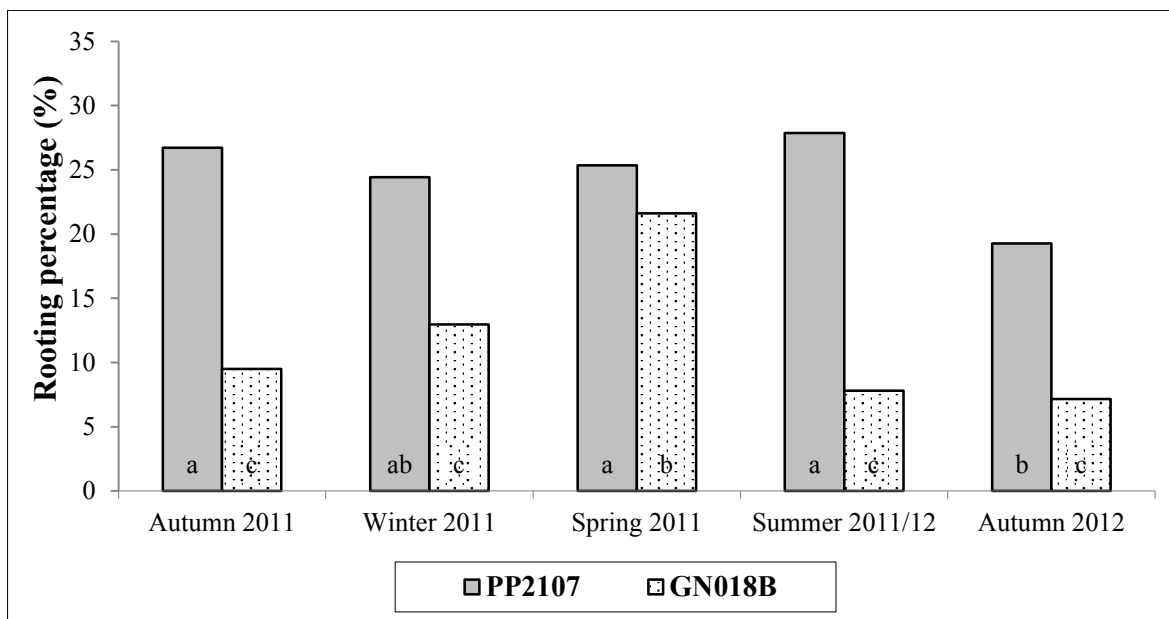


Figure 4.3. Seasonal rooting percentage of mini-cuttings from PP2107 vs. GN018B clone stock plants; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 6.073 for the clone \times season interaction.

Using the predicted means over all seasons, the interactions of clone \times fertilizer using least significant difference (LSD) further indicates that clone was a significant factor but fertilizer and the clone \times fertilizer interaction were not significantly different (Table 4.1). As expected, the easy-to-root clone PP2107 had a significantly higher rooting percentage than GN018B (Table 4.1 and Fig. 4.3). The inorganic and organic fertilizers had very little influence on the mean rooting percentage of all seasons (Table 4.1), although a significant variation was calculated during autumn 2011 and 2012 (Fig. 4.2) due to the season factor being significantly different ($P_{0.05} = 0.001$) when all data were analysed. The mean rooting

percentage over all five seasons minimises the variation, hence, for both PP2107 and GN018B the inorganic and organic fertilizers did not significantly affect rooting (Table 4.1).

Table 4.1. Mean rooting percentage of mini-cuttings from stock plants grown under two clones vs. two fertilizer regimes based upon predicted means of the analysis of variance.

	PP2107	GN018B
Inorganic	24.76 a ¹	11.33 b
Organic	24.70 a	12.30 b

¹Treatment means sharing the same symbol (ab) are not significantly different at 5 % level with LSD for mean rooting percentage for clone × fertilizer interaction 3.841.

Rooting percentage over seasons displayed variation between the interactions of clone and fertilizer. In general, PP2107 had a higher rooting percentage than clone GN018B during most seasons (Fig. 4.4). The PP2107 organic treatment had a tendency towards the highest rooting percentage during autumn 2011, winter 2011 and spring 2011; however, PP2107 inorganic tended towards the highest rooting percentage during summer 2011/12 and autumn 2012 (Fig. 4.4).

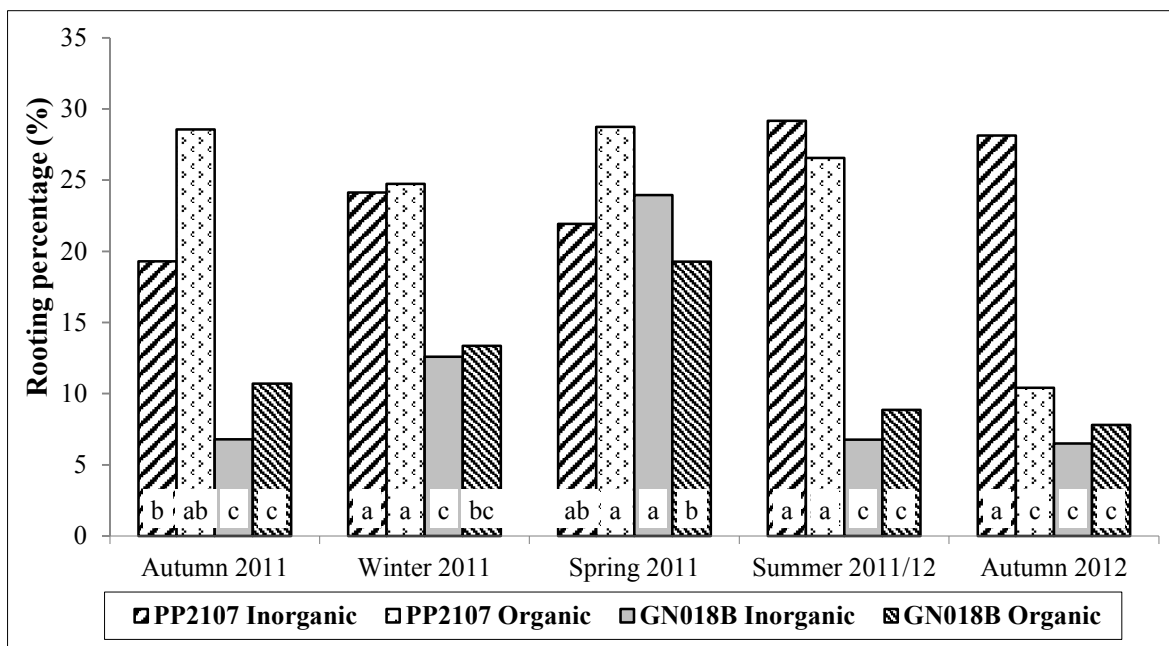


Figure 4.4. Rooting percentage of mini-cuttings from stock plants grown under various shade nets over time to indicate the relationship between clone and fertilizer; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 8.589 for the clone × fertilizer × season interaction.

4.3.2 Effect of coloured shade netting on rooting

The mean rooting percentage of mini-cuttings obtained from plants grown under coloured shade nets over all seasons were analysed using ANOVA. The shade net factor was found

to be highly significant ($P_{0.05} = 0.006$). Over all seasons Aluminet® 40 % and control had the lowest rooting percentages (13.07 and 13.61 %, respectively), while black 30 %, green 40 % and Clarix E Blue® plastic had the highest rooting percentages (24.00, 21.22 and 20.05 %, respectively; Fig. 4.5).

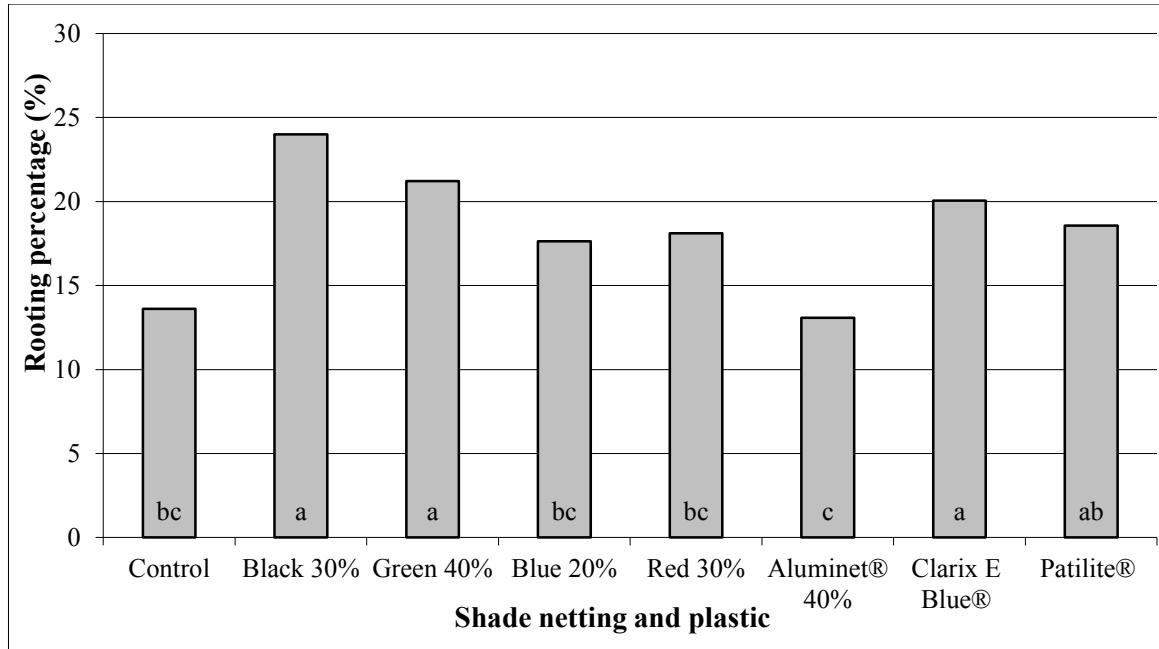


Figure 4.5. Rooting percentage of mini-cuttings from stock plants grown under various shade nets; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 5.432 for the factor shade net.

The interactions between shade net \times fertilizer, shade net \times clone \times season, and shade net \times fertilizer \times season were statistically significant ($P_{0.05} = 0.005$, 0.036 and 0.008, respectively). The shade net \times clone, shade net \times season, and shade net \times clone \times fertilizer interactions were not significant ($P_{0.05} = 0.076$, 0.065 and 0.335, respectively). Generally coloured shade nets had the highest rooting percentage during spring 2011 except for blue 20 %, red 30 % and Patilite®, which had higher rooting in autumn 2011, winter 2011 and summer 2011/12, respectively (Fig. 4.6). The type of shade netting did not consistently affect rooting over each season. Control mini-cuttings had the highest rooting percentages during spring 2011 and lowest rooting during winter 2011; black 30 % and green 40 % both had the highest rooting during spring 2011 and the lowest during summer 2011/12. There was no clear trend over the five seasons of which shade net or plastic treatments had the highest or lowest rooting percentages. The shade net or plastic with the highest rooting percentage changed with each season (Fig. 4.6). During autumn 2011 the highest rooting percentage was from mini-cuttings grown under blue 20 %. While winter 2011 and autumn 2012 the highest rooting percentage was observed in mini-cuttings grown under black 30 %. During spring

2011 highest rooting was observed under Clarix E Blue® and during summer 2011/12 under Patilite®; however, the shade nets with the lowest rooting percentages for some seasons displayed the highest in other seasons (Fig. 4.6).

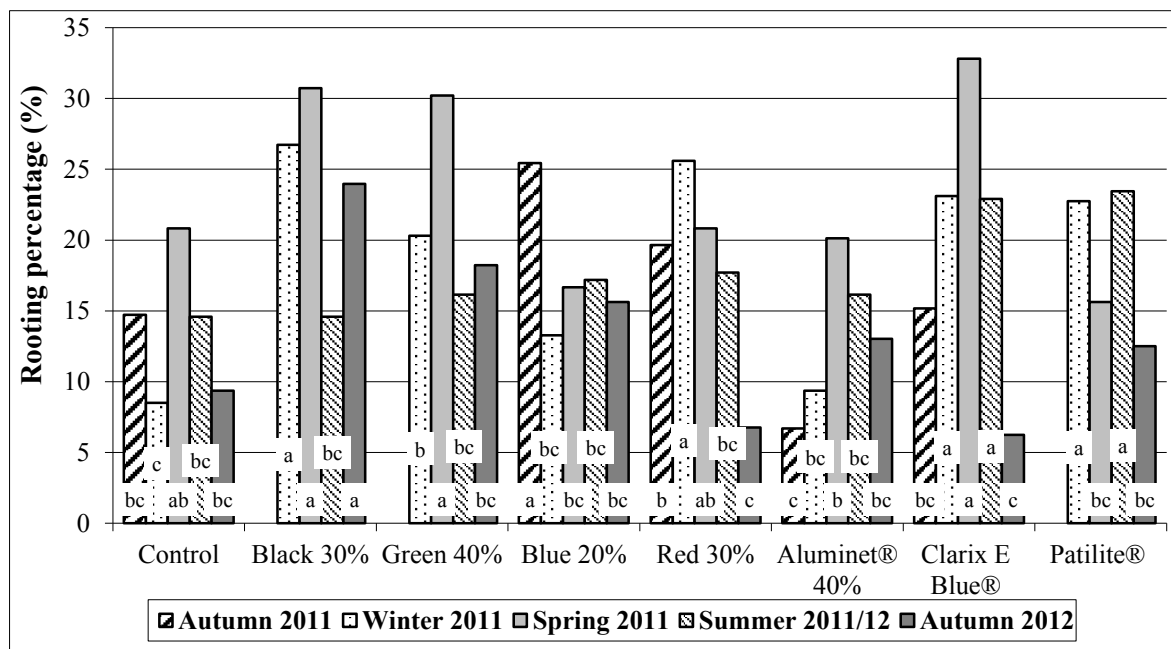


Figure 4.6. Rooting percentage of mini-cuttings from stock plants grown under various shade nets and plastic; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 12.146 for the shade net × season interaction.

4.3.3 Seasonal effects on rooting

Rooting potential of mini-cuttings can be affected by season as well as by the clone and fertilizer factors. By observing the rooting percentage of the control over time it was evident that unshaded mini-cuttings rooted best during spring 2011, except for the control PP2107 organic treatment that had a low rooting percentage (4.17 %). The PP2107 clone, which generally had good rooting, had poorer rooting during winter 2011, autumn 2012 and summer 2011/12 for PP2107 inorganic. The control GN018B generally had low rooting with the exception of spring 2011 inorganic and organic that achieved more than 20 % rooting (Fig. 4.7).

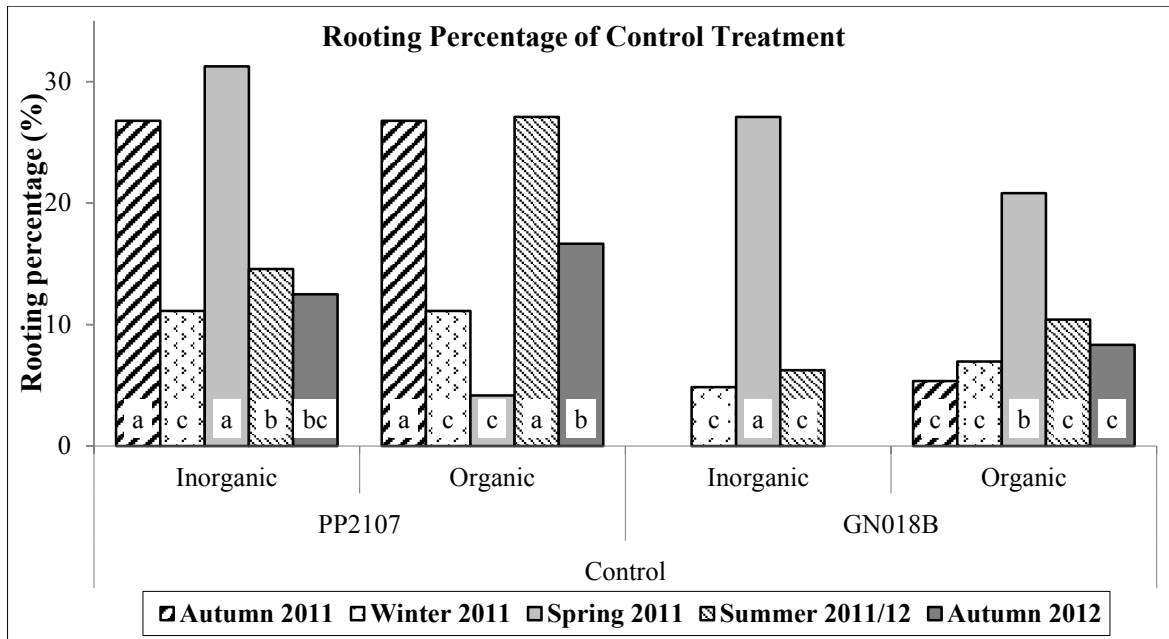


Figure 4.7. Rooting percentage of mini-cuttings from stock plants grown under control (no shade) treatment over time; treatment means sharing the same symbol (abc) are not significantly different at 5 % level with LSD for rooting percentage = 8.53 for the clone \times fertilizer \times season interaction.

Various parameters were analysed over five seasons in order to clearly display the significance that seasons play in rooting experiments. The parameters of rooting percentage, root number per rooted mini-cutting and callus percentage with roots all had highest means during spring 2011. The lowest rooting was during autumn 2012, but was not significantly different from autumn 2011 at 5 % level of significance. Total callus percentage and BSD had the greatest values during winter 2011. The lowest total callus percentage was during spring 2011; this was, however, not significantly different from autumn 2011 and 2012, or summer 2011/12. The BSD were significantly greater during winter 2011, spring 2011 and autumn 2012 and smaller during summer 2011/12 and autumn 2011 (Table 4.2).

Table 4.2. Mean rooting percentage, root number per rooted mini-cutting, callus percentage, root plus callus percentage and basal stem diameter (BSD) for all five seasons analysed.

	Rooting (%)	Root number per mini-cutting	Total callus (%)	Root plus callus (%)	Cutting basal stem diameter (mm)
Autumn 2011	16.34 bc ¹	2.086 b	28.66 b	8.66 c	2.222 c
Winter 2011	18.71 b	1.820 c	45.23 a	15.69 b	2.693 a
Spring 2011	23.48 a	2.409 a	27.21 b	20.38 a	2.645 a
Summer 2011/12	17.84 b	1.860 c	30.86 b	14.65 b	2.366 b
Autumn 2012	13.22 c	1.804 c	28.71 b	10.74 c	2.562 a
LSD	4.294	0.2194	4.361	3.036	0.1421

¹Treatment means sharing the same symbol (abc) are not significantly different at 5 % level; LSD was calculated separately for each parameter in a specific column for season factor, indicated in the row marked LSD.

The seasons were further analysed in comparison with the control (Table 4.3), where black numbers in the left hand column under each shade net indicate the rooting percentage of each treatment and red and blue numbers in the right hand column under each shade net indicate the difference in rooting percentage, whether positive or negative, between shade net treatments and control for each season. The best rooting was achieved by PP2107 mini-cuttings collected under black 30 % inorganic in autumn 2012, green 40 % inorganic in spring 2011, Clarix E Blue® inorganic in summer 2011/12, blue 20 % organic in autumn 2011 and red organic in autumn 2011, with rooting percentages of 68.75, 52.08, 54.17, 57.14 and 44.64 %, respectively (Table 4.3). During autumn 2011, all shade nets had improved rooting compared with the control for GN018B. Clone PP2107 only showed low rooting in the Aluminet® 40 % and Clarix E Blue® treatments. During winter 2011 most treatments had improved rooting compared with the control, except for Aluminet® 40 % and blue 20 %. During spring 2011, mini-cuttings from plants grown under several shade nets rooted consistently better than the control (black 30 %, green 40 %, red 30 % and Clarix E Blue®), while other shade nets produced mini-cuttings that consistently rooted more poorly than the control (blue 20 % and Patilite®). During summer 2011/12 green 40 % and Patilite® rooted consistently better than the control, while blue 20 %, Aluminet® 40 % and Clarix E Blue® rooted consistently poorer. During autumn 2012 green 40 % rooted better than the control. Therefore, during most seasons mini-cuttings from stock plants grown under green 40 %, black 30 % and red 30 % shade nets rooted better than those under no shade net (control) (Table 4.3). Clone GN018B generally had lower rooting percentages than clone PP2107.

Table 4.3. Mean rooting percentages of mini-cuttings from stock plants grown under shade net (data displayed as a difference from the control (no shade) treatment for each season).

		<i>Autumn 2011</i>														
		Control	Black 30%		Green 40%		Blue 20%		Red 30%		Aluminet® 40%		Clarix E Blue®		Patilite®	
PP2107	Inorganic	26.79	missing	missing	19.64	-7.14	16.07	-10.71	10.71	-16.07	23.21	-3.57	missing			
	Organic	26.79	missing	missing	57.14	30.36	44.64	17.86	3.57	-23.21	10.71	-16.07	missing			
GN018B	Inorganic	0.00	missing	missing	10.71	10.71	7.14	7.14	3.57	3.57	12.50	12.50	missing			
	Organic	5.36	missing	missing	14.29	8.93	10.71	5.36	8.93	3.57	14.29	8.93	missing			
		<i>Winter 2011</i>														
		Control	Black 30%		Green 40%		Blue 20%		Red 30%		Aluminet® 40%		Clarix E Blue®		Patilite®	
PP2107	Inorganic	11.11	34.72	23.61	23.61	12.50	21.15	10.04	31.57	20.46	8.33	-2.78	34.13	23.02	28.47	17.36
	Organic	11.11	38.89	27.78	33.33	22.22	14.58	3.47	31.94	20.83	10.42	-0.69	23.61	12.50	34.03	22.92
GN018B	Inorganic	4.86	15.97	11.11	15.28	10.42	13.19	8.33	19.44	14.58	3.47	-1.39	18.06	13.19	10.42	5.56
	Organic	6.94	17.36	10.42	9.03	2.08	4.17	-2.78	19.44	12.50	15.28	8.33	16.67	9.72	18.06	11.11
		<i>Spring 2011</i>														
		Control	Black 30%		Green 40%		Blue 20%		Red 30%		Aluminet® 40%		Clarix E Blue®		Patilite®	
PP2107	Inorganic	31.25	6.25	-25.00	52.08	20.83	14.58	-16.67	6.25	-25.00	12.98	-18.27	45.83	14.58	6.25	-25.00
	Organic	4.17	39.58	35.42	27.08	22.92	20.83	16.67	18.75	14.58	44.56	40.40	41.67	37.50	33.33	29.17
GN018B	Inorganic	27.08	27.08	0.00	35.42	8.33	14.58	-12.50	37.50	10.42	2.08	-25.00	29.17	2.08	18.75	-8.33
	Organic	20.83	50.00	29.17	6.25	-14.58	16.67	-4.17	20.83	0.00	20.83	0.00	14.58	-6.25	4.17	-16.67
		<i>Summer 2011/12</i>														
		Control	Black 30%		Green 40%		Blue 20%		Red 30%		Aluminet® 40%		Clarix E Blue®		Patilite®	
PP2107	Inorganic	14.58	27.08	12.50	22.92	8.33	35.42	20.83	22.92	8.33	39.58	25.00	54.17	39.58	16.67	2.08
	Organic	27.08	29.17	2.08	33.33	6.25	22.92	-4.17	16.67	-10.42	20.83	-6.25	20.83	-6.25	41.67	14.58
GN018B	Inorganic	6.25	0.00	-6.25	6.25	0.00	4.17	-2.08	22.92	16.67	4.17	-2.08	2.08	-4.17	8.33	2.08
	Organic	10.42	2.08	-8.33	2.08	-8.33	6.25	-4.17	8.33	-2.08	0.00	-10.42	14.58	4.17	27.08	16.67
		<i>Autumn 2012</i>														
		Control	Black 30%		Green 40%		Blue 20%		Red 30%		Aluminet® 40%		Clarix E Blue®		Patilite®	
PP2107	Inorganic	12.50	68.75	56.25	43.75	31.25	39.58	27.08	18.75	6.25	27.08	14.58	12.50	0.00	2.08	-10.42
	Organic	16.67	12.50	-4.17	22.92	6.25	0.00	-16.67	6.25	-10.42	10.42	-6.25	0.00	-16.67	14.58	-2.08
GN018B	Inorganic	0.00	10.42	10.42	2.08	2.08	18.75	18.75	0.00	0.00	12.50	12.50	8.33	8.33	0.00	0.00
	Organic	8.33	4.17	-4.17	4.17	-4.17	4.17	-4.17	2.08	-6.25	2.08	-6.25	4.17	-4.17	33.33	25.00

Note: Numbers in black lettering, in the left hand column under each shade net, represent the recorded rooting percentage of mini-cuttings from each treatment, regarding the clone and fertilizer factors in the same row as the particular value. The numbers in red and blue lettering, in the right hand column under each shade net, represent the difference (shade net treatment – control treatment), whether positive (blue) or negative (red), between the particular shade net treatment and control treatment for each season.

In order to demonstrate the rooting percentage variation over two years during the same season, autumn 2011 and autumn 2012 data were compared. These data were analysed using the linear mixed models REML procedure to estimate variance parameters due to the unbalanced nature of the data. As expected, the Wald test for fixed effects indicated that the two years were not significantly different from each other ($P_{0.05} = 0.188$) at 5 % level of significance; however, the factors shade net, clone and fertilizer were highly significant ($P_{0.05} = 0.002, < 0.001$ and < 0.001 , respectively). Therefore, the differences displayed were due to these interactions (Fig. 4.8). The highest rooting during autumn 2011 was from PP2107 mini-cuttings harvested under blue 20 % and red 30 % organic treatments; however, missing data from the black 30 %, green 40 % and Patilite® treatments made proper comparison impossible. The highest rooting percentage during autumn 2012 was in PP2107 mini-cuttings from the black 30 % and green 40 % inorganic treatments (Fig. 4.8).

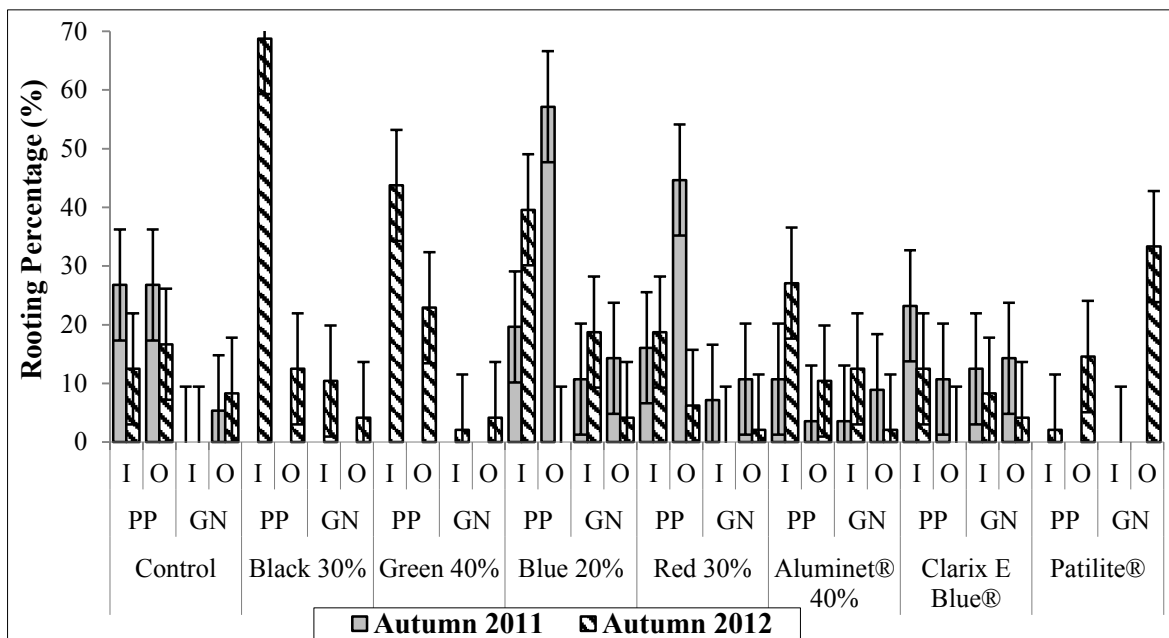


Figure 4.8. Comparison of rooting percentage of mini-cuttings from stock plants grown under various shade nets in autumn 2011 and 2012; letters I = Inorganic and O = Organic fertilizer treatments; PP = PP2107 and GN = GN018B clones; error bars represent the standard errors of differences of predicted means (s.e.d.) = 9.464 for the shade net \times clone \times fertilizer \times season interaction. LSD and s.e. could not be determined for REML analyses.

4.3.4 Human effect on rooting

As several individuals carried out cutting preparation, the effect of a particular labourer preparing mini-cuttings on final rooting percentage was analysed. The rooting data were analysed for the specific labourer preparing mini-cuttings for each season using ANOVA; LSD was used to differentiate rooting averages for each labourer. In all seasons labourer

significantly affected rooting of cuttings in winter 2011, spring 2011, summer 2011/12 and autumn 2012 ($P_{0.05} < 0.001$, = 0.004, = 0.017 and = 0.007, respectively). There were variations in the time it took each labourer to cut and place a treatment and, hence, some labourers were able to place more mini-cuttings than others per day. During winter 2011, labourer 4 prepared cuttings for three days averaging 496 mini-cuttings placed per day, while labourer 3 worked for two days averaging 480 mini-cuttings placed per day and labourer 1 for one day placed 288 mini-cuttings and labourer 2 placed 1872 mini-cuttings over six days (Table 4.4). The winter 2011 rooting experiment was three times as large as the following three rooting experiments and due to fewer labourers available cutting preparation took six days. In contrast, during spring 2011 the rooting experiment was completed in one day due to a smaller sample size and four labourers working full day. During this rooting experiment labourer 1 was able to place 432 mini-cuttings. The highest rooting percentage of all seasons was of mini-cuttings prepared by labourer 2 during spring 2011 (38.54 %) and the lowest rooting percentage was of mini-cuttings prepared by labourer 1 during autumn 2012 (6.60 %). Treatments were randomly assigned to each labourer to prepare and place, therefore, if a labourer placed more GN018B than PP2107 clones the rooting percentage is skewed towards lower rooting percentages due to the difficult-to-root tendency of the GN018B clone (Table 4.1). During winter 2011 labourer 1 and 3 had the highest rooting percentages for that season (27.81 and 23.61 %, respectively); both prepared a higher percentage of PP2107 to GN018B mini-cuttings (75.00 to 25.00 % and 83.33 to 16.67 %, respectively). However, this is not always the case, during spring 2011 labourer 1 had a fairly low rooting percentage (14.12 %), even though the percentage of PP2107 clone to GN018B clone prepared was higher (66.67 to 33.33 %, respectively), while labourer 5 had a good rooting percentage (28.13 %), where the percentage of PP2107 clone to GN018B clone prepared was lower (16.67 to 83.33 %, respectively; Table 4.4).

Table 4.4. Mean rooting percentage of mini-cuttings from stock plants over time according to specific labourer placing mini-cuttings.

<i>Winter 2011</i>						
Number of reps placed	Number of mini-cuttings placed	Rooting %	PP2107 % of reps	GN018B % of reps	Labourer	LSD
18	288	23.61 a ¹	83.33	16.67	Labourer 1	8.54
117	1872	11.22 b	46.15	53.85	Labourer 2	8.54
60	960	27.81 a	75	25	Labourer 3	8.54
93	1488	21.10 a	32.26	67.74	Labourer 4	8.54
<i>Spring 2011</i>						
Number of reps placed	Number of mini-cuttings placed	Rooting %	PP2107 % of reps	GN018B % of reps	Labourer	LSD
27	432	14.12 c	66.67	33.33	Labourer 1	14.42
6	96	38.54 a	50	50	Labourer 2	14.42
18	288	28.13 ab	16.67	83.33	Labourer 5	14.42
24	384	26.04 abc	62.5	37.5	Labourer 6	14.42
21	336	23.81 bc	42.86	57.14	Labourer 7	14.42
<i>Summer 2011/12</i>						
Number of reps placed	Number of mini-cuttings placed	Rooting %	PP2107 % of reps	GN018B % of reps	Labourer	LSD
42	672	22.47 a	57.14	42.86	Labourer 2	6.75
54	864	14.24 b	44.44	55.56	Labourer 5	6.75
<i>Autumn 2012</i>						
Number of reps placed	Number of mini-cuttings placed	Rooting %	PP2107 % of reps	GN018B % of reps	Labourer	LSD
18	288	6.60 b	66.67	33.33	Labourer 1	9.45
30	480	10.42 b	30	70	Labourer 2	9.45
27	432	12.73 b	55.56	44.44	Labourer 5	9.45
21	336	23.51 a	57.14	42.86	Labourer 3	9.45

¹ Mean rooting percentages of each labourer sharing the same symbol (abc) were not significantly different at 5 % level, where each rooting percentage LSD for labourer mini-cutting was calculated separately for each season indicated in the LSD column.

4.3.5 Quality of rooted mini-cuttings

4.3.5.1 Root types

Each season of rooting experiments were assessed and the four root types were displayed as a percentage of the total mini-cuttings rooted for each treatment. Interestingly, when all root quality data were analysed by ANOVA using season as a blocking tool, the root type factor and root type \times shade net interaction were found to be significant ($P_{0.05} < 0.001$ and 0.035 , respectively), but the shade net, clone and fertilizer factors were not significant; however, when seasons were analysed separately using ANOVA such as for autumn 2011, only the shade net factor was highly significant ($P_{0.05} < 0.001$) and factor root type was not significant ($P_{0.05} = 0.053$). The autumn 2011 rooting experiment indicates that the highest percentage of

mini-cuttings that rooted were type 3 or 4 (good or strong), particularly those harvested under blue 20 % (Fig. 4.9). At least 50 % of rooted mini-cuttings obtained from red 30 % PP2107 inorganic and Aluminet® 40 % PP2107 organic were rated as type 1 (weak), while control GN018B inorganic had no mini-cuttings that rooted (Fig. 4.9). There were missing treatments in autumn 2011, whereby no cuttings could be taken of black 30 %, green 40 % or Patilite® treatments.

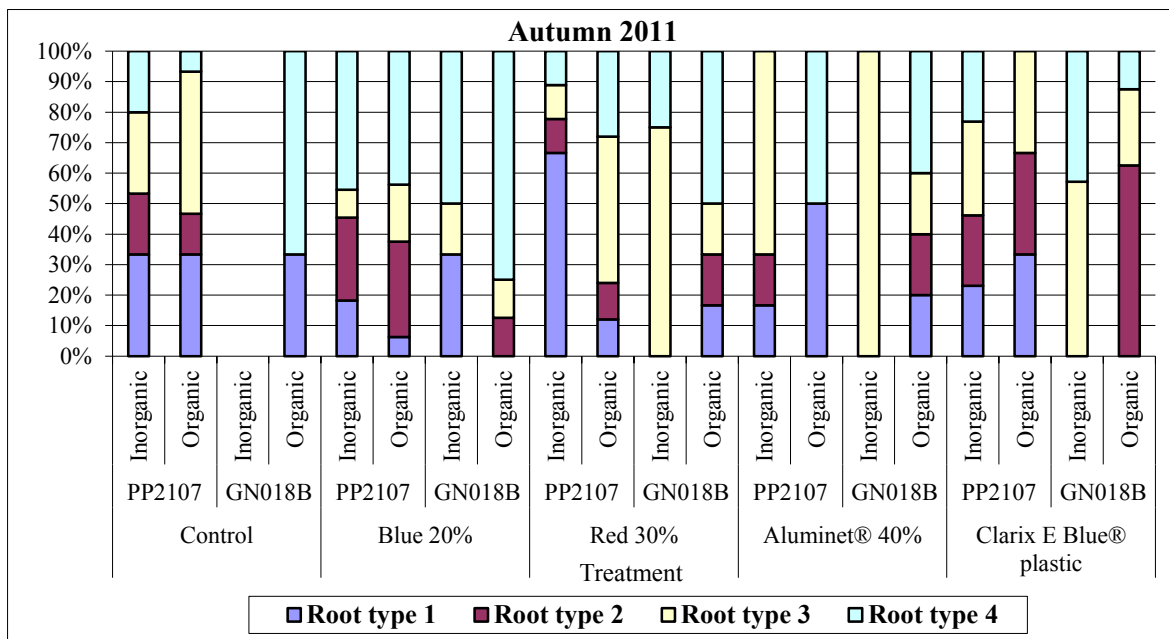


Figure 4.9. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in autumn 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong.

In the winter 2011 rooting experiment, the root type factor and root type \times clone interaction were both highly significant ($P_{0.05} < 0.001$) and the root type \times shade net \times clone interaction was significant ($P_{0.05} = 0.05$). In the same rooting experiment, treatments with the highest percentage, reaching up to 80 % of type 3 and 4 (good as well as strong) rooted mini-cuttings were black 30 %, green 40 %, red 30 %, Aluminet® 40 % and Patilite® GN018B organic and inorganic as well as Clarix E Blue® GN018B organic (Fig. 4.10). Although GN018B mini-cuttings did not have high rooting percentages (Table 4.3), the rooted GN018B mini-cuttings were of superior quality compared with most PP2107 treatments. The treatments with the highest percentage of type 1 (weak) rooted mini-cuttings were red 30 % PP2107 organic, Clarix E Blue® PP2107 inorganic and blue 20 % GN018B organic (Fig. 4.10).

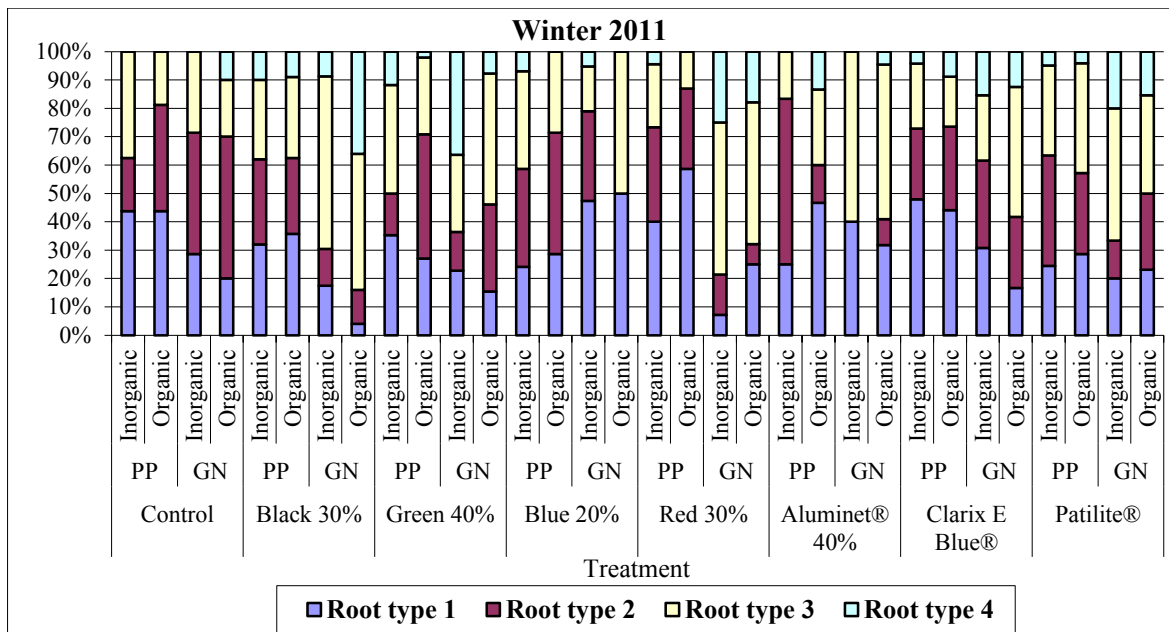


Figure 4.10. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in winter 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.

In the spring 2011 rooting experiment, root type was the only highly significant factor ($P_{0.05} < 0.001$). For most treatments the spring 2011 rooting experiment had higher rooting percentages than the other four seasons, reaching up to 52 % (Table 4.2 and 4.3); this experiment also had improved percentages, reaching up to 75 % of type 4 (strong) rooted mini-cuttings compared with previous seasons, where in many of the treatments more than 50 % of the total rooted mini-cuttings fell into the category type 3 or 4 (good or strong) rooted mini-cuttings (Fig. 4.11). Treatments with the highest percentage of such type 4 rooted mini-cuttings were control GN018B inorganic, black 30 % GN018B inorganic, Patilite® PP2107 organic and inorganic, Clarix E Blue® PP2107 organic and blue 20 % GN018B organic. The only treatment with 50 % type 1 (weak) rooted mini-cuttings was Patilite® GN018B organic (Fig. 4.11).

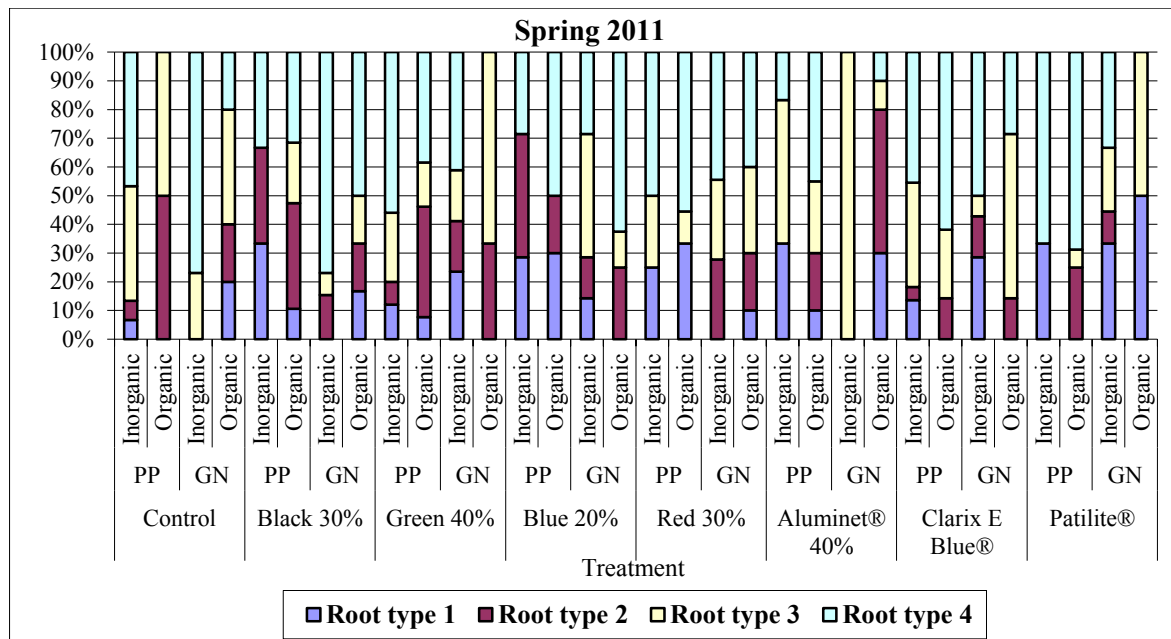


Figure 4.11. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in spring 2011, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.

In the summer 2011/12 rooting experiment, root type was the only highly significant factor ($P_{0.05} < 0.001$). The summer 2011/12 rooting experiment had a lower percentage of type 4 (strong) rooted mini-cuttings than the spring 2011 rooting experiment; however, there was a higher percentage of type 2 and 3 rooted mini-cuttings (Fig. 4.12). The only treatment that had 50 % type 4 (strong) rooted mini-cuttings was Clarix E Blue® GN018B organic. The treatments that had the highest percentage of type 3 and 4, (good and strong) rooted mini-cuttings combined were red 30 % GN018B organic, control GN018B inorganic, Patilite® PP2107 organic and inorganic, green 40 % PP2107 inorganic, blue 20 % and Clarix E Blue® PP2107 organic. The treatments that failed to root in summer 2011/12 were black 30 % GN018B inorganic and Aluminet® 40 % GN018B organic. The treatments that had more than 50 % type 1 (weak) rooted mini-cuttings were green 40 % GN018B organic, blue 20 % GN018B inorganic, Aluminet® 40 % and Patilite® GN018B inorganic (Fig. 4.12). In summer 2011/12 the rooting percentage was generally low (Table 4.3); in general, clone PP2107 had better rooting quality than clone GN018B, except for the control and red 30 % (Fig. 4.12).

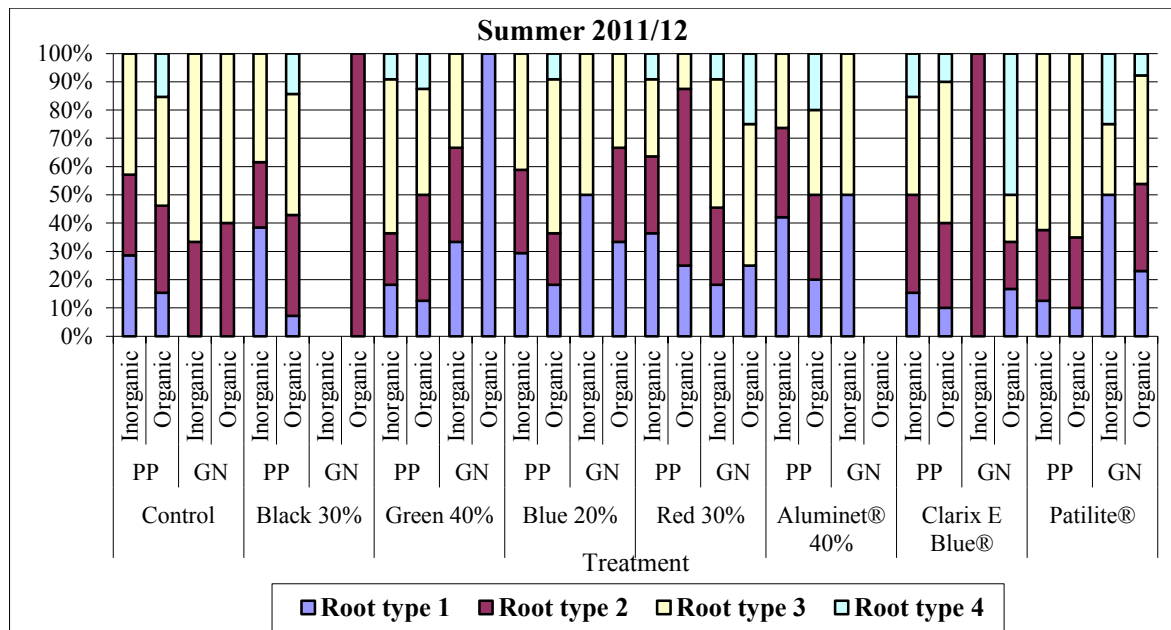


Figure 4.12. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in summer 2011/12, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.

In the autumn 2012 rooting experiment, root type was the only highly significant factor ($P_{0.05} < 0.001$); other factors were probably not significant as rooting percentages were very erratic (Table 4.3). In autumn 2012 the percentages of type 4 (strong) rooted mini-cuttings were low, reaching up to 30%. Treatments with the highest percentage of type 3 and 4 (good and strong) rooted mini-cuttings were red 30%, green 40% and Patilite® PP2107 organic and control and Aluminet® 40% PP2107 inorganic as well as Patilite® and green 40% GN018B organic and Clarix E Blue® and Aluminet® 40% GN018B inorganic (Fig. 4.13). There were more treatments (blue 20% and Clarix E Blue® PP2107 organic and control, red 30% and Patilite® GN018B inorganic) that had no rooting in autumn 2012 than in other seasons. The treatments that had 50% or more type 1 (weak) mini-cuttings were Aluminet® 40% and control PP2107 organic, Clarix E Blue® PP2107 inorganic and red 30%, Aluminet® 40%, blue 20% and control GN018B organic (Fig. 4.13).

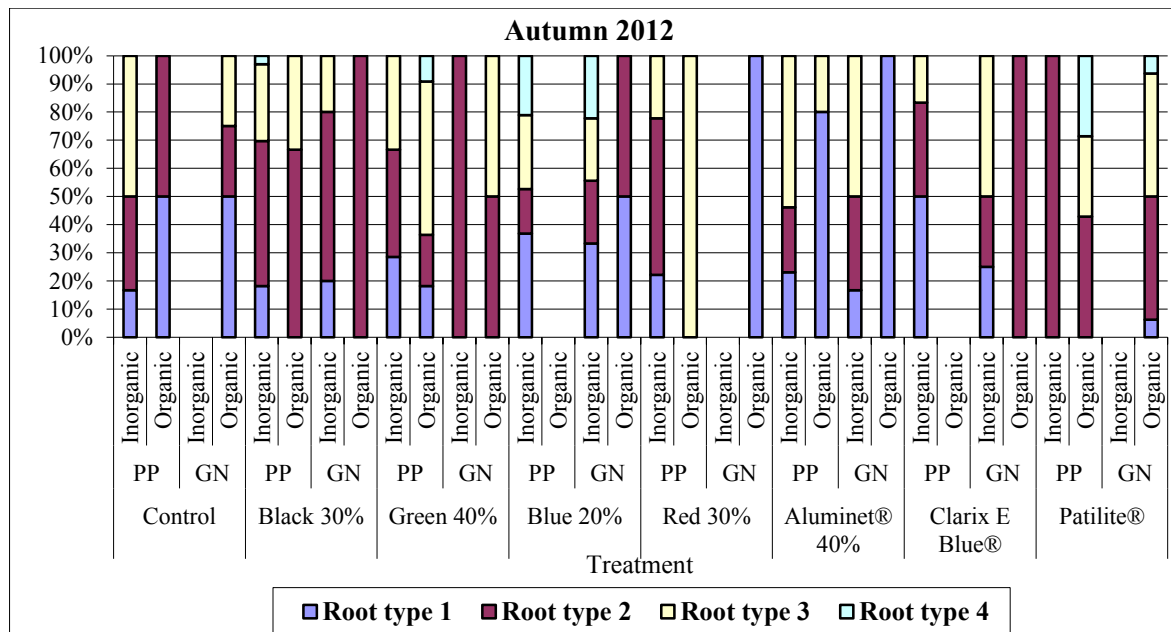


Figure 4.13. Root type quality of rooted mini-cuttings as a percentage of total rooted mini-cuttings of the stock plant treatment in autumn 2012, where root type 1 is weak, 2 is average, 3 is good and 4 is strong; PP and GN stand for PP2107 and GN018B clones, respectively.

4.3.5.2 Root number

The quality of rooted mini-cuttings was further evaluated as root number per mini-cutting. Root number data were analysed by ANOVA using replications \times season for blocking. Root number data indicated that the clone factor, shade net \times fertilizer and clone \times fertilizer interactions were significant ($P_{0.05} < 0.001$, = 0.015 and = 0.003, respectively), but the shade net as well as the fertilizer factors were not significant ($P_{0.05} = 0.081$ and 0.239, respectively). When the root number data were analysed separately for each season the root number ANOVA for the autumn 2011 season indicated that the clone factor, shade net \times fertilizer and shade net \times clone \times fertilizer interactions were significant ($P_{0.05} < 0.001$, = 0.022 and = 0.015, respectively). When all seasons were analysed there was a positive correlation between root number and rooting percentage ($r = 0.431$); however, during autumn 2011 there was a strong positive correlation between root number and rooting percentage ($r = 0.7889$). Root number was generally between one and three roots per rooted mini-cutting except for Aluminet® 40 % PP2107 inorganic, which had nearly eight roots per rooted mini-cutting, significantly higher than other treatments. The treatments that displayed the highest rooting percentages (blue 20 % and red 30 % PP2107 organic), both had root numbers of three roots per rooted mini-cutting, although the Aluminet® 40 % PP2107 inorganic treatment had a low rooting percentage with the highest root number per rooted mini-cutting (Fig. 4.14),

indicating that, although there was some correlation between root number and rooting percentage, there were exceptions.

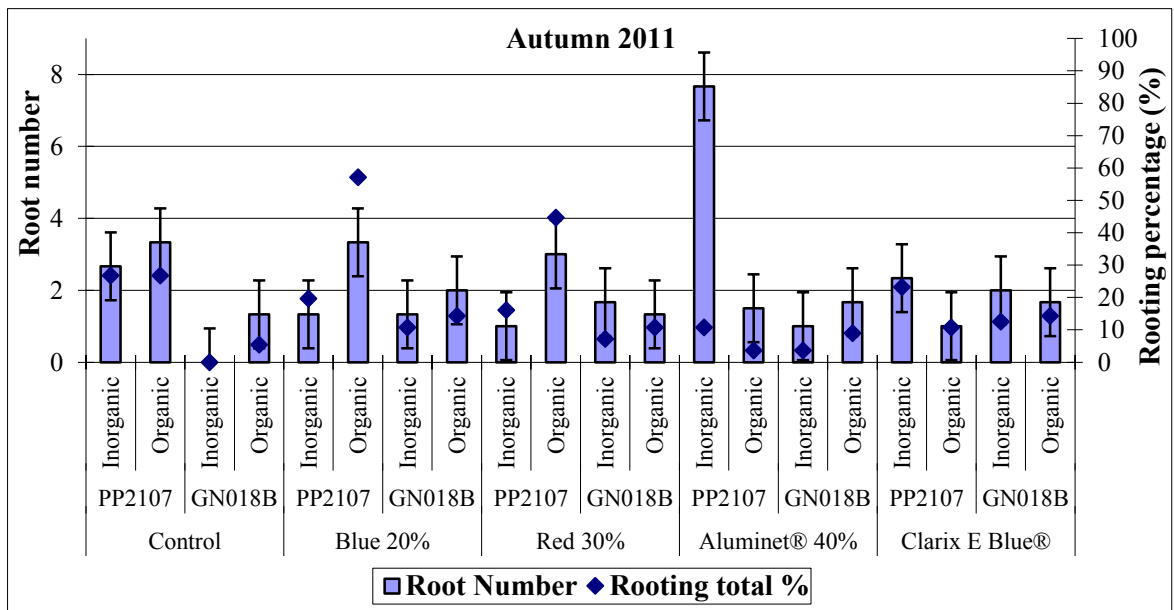


Figure 4.14. Mean number of roots per rooted mini-cutting and total rooting percentage in autumn 2011; error bars are based on the root number s.e. = 0.944 for the shade net \times clone \times fertilizer interaction. No s.e. could be calculated for rooting percentage for this season as only one replication of each treatment was set.

The shade net as well as the clone factors affected root number in winter 2011 significantly ($P_{0.05} = 0.008$ and < 0.001 , respectively), but the fertilizer factor was not significant ($P_{0.05} = 0.222$), leading to variation within interactions. There was a moderately positive correlation between root number and rooting percentage ($r = 0.5194$). During winter 2011 the root numbers per rooted mini-cutting were lower than in autumn 2011 or spring 2011, but were not significantly different from summer 2011/12 or autumn 2012 (Table 4.2), averaging between one and two and a half roots per rooted mini-cutting during winter 2011 (Fig. 4.15). However, the mean rooting percentages were higher during winter 2011 than during autumn 2011 (Table 4.2). The highest root number was recorded for green 40 % PP2107 inorganic. Some of the higher root numbers per rooted mini-cutting had higher rooting percentages (Fig. 4.15).

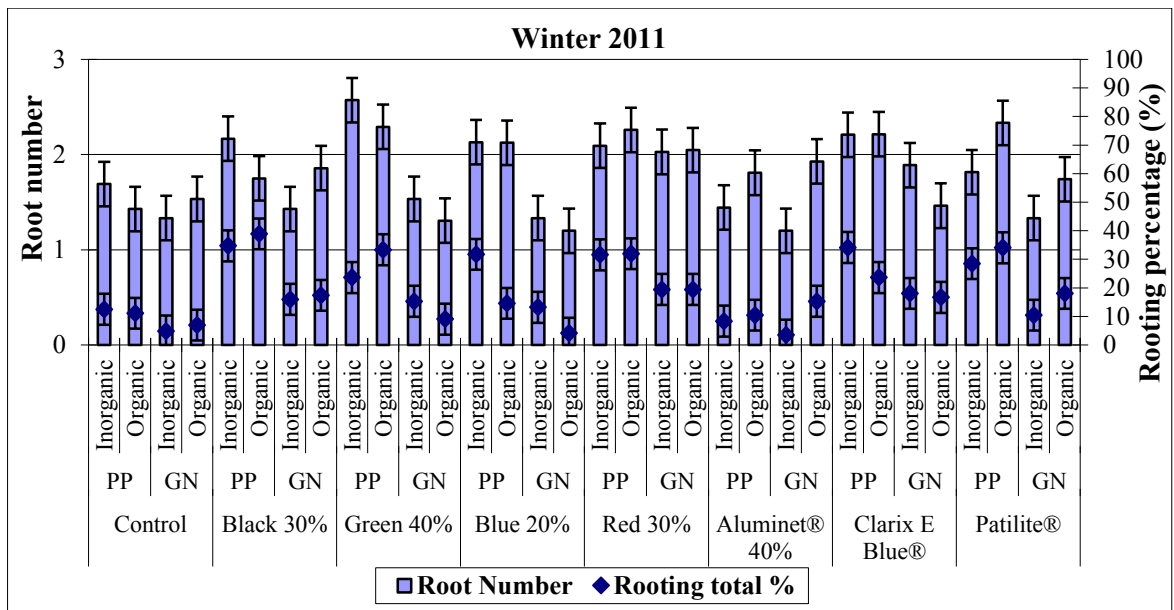


Figure 4.15. Mean number of roots per rooted mini-cutting and total rooting percentage in winter 2011; error bars are based on the root number s.e. = 0.2342 and rooting percentage s.e. = 5.409 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

Root number data for spring 2011 indicated that all factors, shade net, clone and fertilizer ($P_{0.05} = 0.035$, < 0.001 and $= 0.035$, respectively) as well as shade net \times clone, shade net \times fertilizer and shade net \times clone \times fertilizer interactions were significant ($P_{0.05} = 0.004$, < 0.001 and $= 0.002$, respectively). During spring 2011 there was a weak positive correlation between root number and rooting percentage ($r = 0.2888$). Although the spring 2011 season had the highest overall mean rooting percentage and root number per rooted mini-cutting (Table 4.2), the rooting percentage and root numbers were erratic during this period (Table 4.3 and Fig. 4.16). The highest root numbers were found in the green 40 % PP2107 inorganic and Clarix E Blue® PP2107 organic treatments, which both had more than four roots per rooted mini-cutting, while cuttings from control PP2107 organic had the lowest root number (Fig. 4.16).

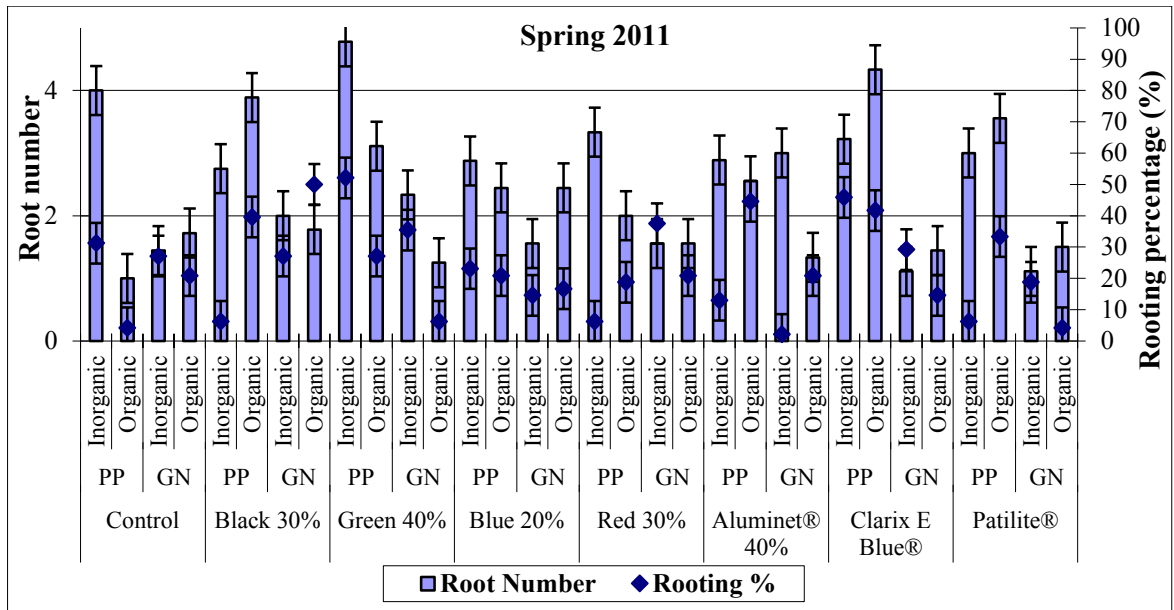


Figure 4.16. Mean number of roots per rooted mini-cutting and total rooting percentage in spring 2011; error bars are based on the root number s.e. = 0.3912 and rooting percentage s.e. = 6.49 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

Root number data for summer 2011/12 indicated that shade net and clone factors were significant (both $P_{0.05} = 0.019$) as well as the shade net \times clone, shade net \times fertilizer and clone \times fertilizer interactions ($P_{0.05} = 0.025, 0.006$ and 0.031 , respectively). There was a weak positive correlation between root number and rooting percentage ($r = 0.3298$). The summer 2011/12 season had lower rooting percentages than spring 2011, but not significantly different from winter 2011 and low root number per mini-cutting during this season (Table 4.2). The rooting percentages (Table 4.3) and the root numbers were fairly erratic, ranging from one to three roots per mini-cutting (Fig. 4.17). Highest root numbers were recorded for treatment Clarix E Blue® GN018B organic and control PP2107 organic, with more than three roots per rooted mini-cutting (Fig. 4.17).

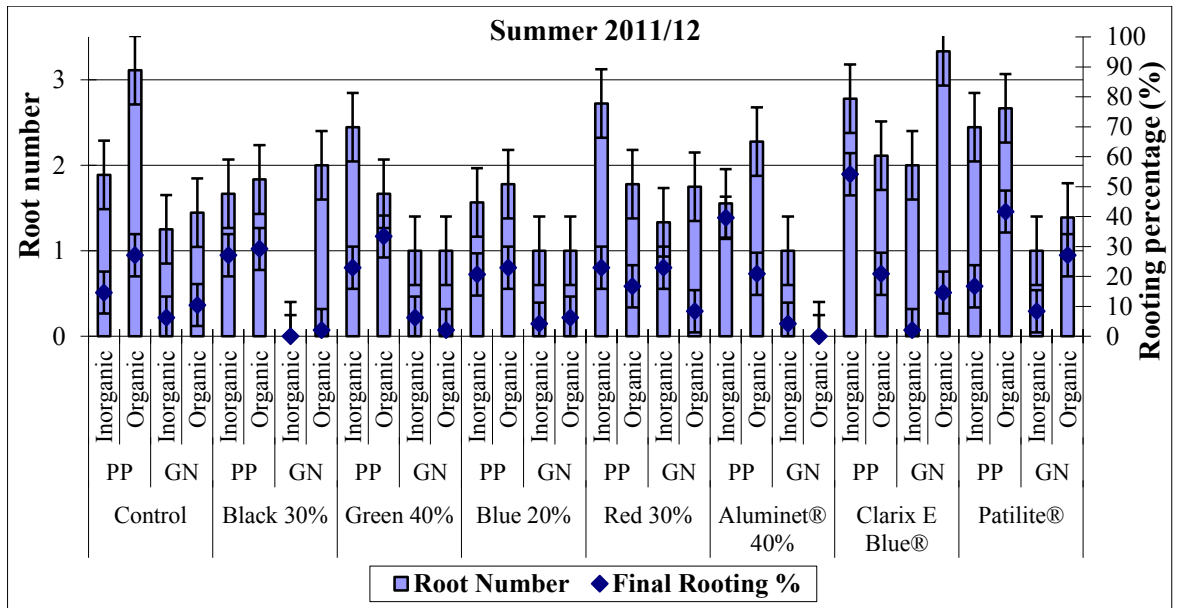


Figure 4.17. Mean number of roots per rooted mini-cutting and total rooting percentage in summer 2011/12; error bars are based on the root number s.e. = 0.401 and rooting percentage s.e. = 7.04 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

Root number data for autumn 2012 indicated that shade net and clone factors were significant ($P_{0.05} = 0.005$ and < 0.001 , respectively). There was a moderately positive correlation between root number per mini-cutting and rooting percentage ($r = 0.4178$). Rooting percentage and root number per mini-cutting were both low during this season (Table 4.2). The autumn 2012 root numbers did not vary greatly ranging from one to two roots per rooted mini-cutting. The highest root number per rooted mini-cutting was reported for black 30 % PP2107 inorganic (Fig. 4.18).

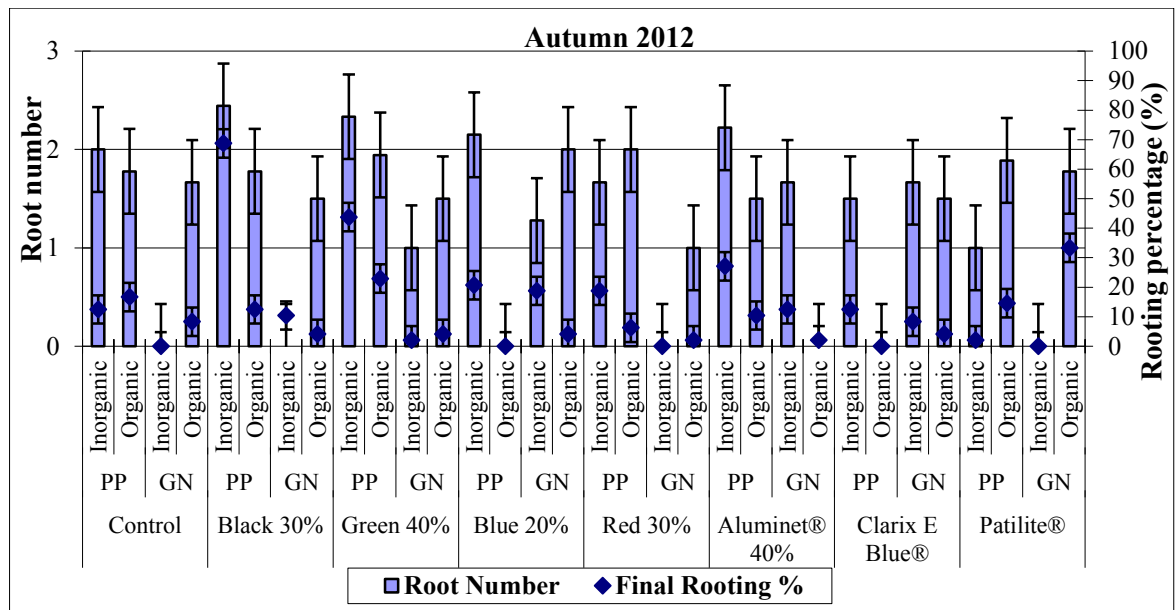


Figure 4.18. Mean number of roots per rooted mini-cutting and total rooting percentage in autumn 2012; error bars are based on the root number s.e. = 0.4307 and rooting percentage s.e. = 4.805 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

4.3.5.3 Callus

Callus data were analysed for all five seasons using REML, as the data was unbalanced, where the shade net and clone factors were found to be highly significant ($P_{0.05} < 0.001$) as well as the shade \times fertilizer interaction ($P_{0.05} = 0.006$). Callus data were subsequently analysed separately for each season by ANOVA as data was orthogonal within seasons. As the autumn 2011 rooting experiment only consisted of one replication per treatment, it could not be analysed, but correlation could be analysed. There was a strong positive correlation between total callus and rooted mini-cuttings with callus present (root plus callus), total callus and total rooting percentage, and total rooting percentage and root plus callus ($r = 0.8683, 0.7901$ and 0.9434 , respectively). Autumn 2011 had the second lowest rooting percentage, total callus percentage and the lowest percentage of root plus callus of all seasons (Table 4.2). In general, total callus percentage was higher than total rooting percentage except for blue 20 % PP2107 organic, which had the highest total rooting percentage overall (57 %) and total callus percentage (51 %), although the percentage of root plus callus was high (40 %). The second highest total rooting percentage was recorded for red 30 % PP2107 organic (45 %), which had the same percentage of total callus (45 %), although the root plus callus were nearly half (21 %) of the total rooted mini-cuttings. Some of the treatments with poor rooting such as the Aluminet® 40 % treatments and control GN018B organic had no root plus callus, indicating that there were more mini-cuttings that survived without

developing roots, than those that formed roots. The easy-to-root clone PP2107 had the highest percentages of total callus and mini-cuttings with root plus callus (Fig. 4.19).

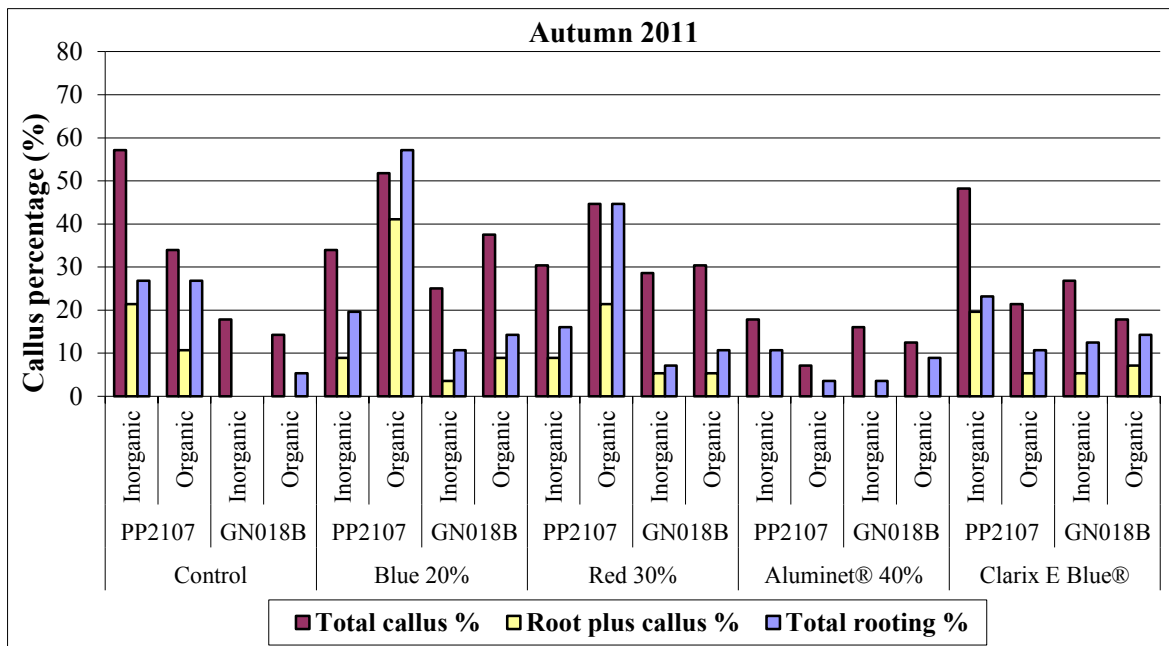


Figure 4.19. Total callus percentage and percentage of root plus callus of mini-cuttings during autumn 2011 compared with the rooting percentage of mini-cuttings; the autumn 2011 rooting experiment consisted of only one replication per treatment; hence, it could not be analysed due to lack of variation.

The winter 2011 total callus percentage data indicated significant interactions between shade net \times clone, shade net \times fertilizer and clone \times fertilizer ($P_{0.05} = 0.015, 0.034$ and 0.040 , respectively). Root plus callus as well as total rooting percentage data of mini-cuttings were both significantly affected by shade net and clone factors ($P_{0.05} < 0.001$). There was moderately positive correlation between total callus and root plus callus, total callus and total rooting percentage ($r = 0.6614$ and 0.6256 , respectively), and a strong positive correlation between total rooting percentage and root plus callus ($r = 0.9473$). During winter 2011 the total callus percentage was the highest of all seasons; however, the total rooting percentage and the percentage of root plus callus were lower than spring 2011 values, but not significantly different from summer 2011/12 (Table 4.2). In general, total callus percentage was high (between 40 and 60 %), while total rooting percentage (5 to 35 %) and mini-cuttings with root plus callus (0 to 30 %) were not very high (Fig. 4.20); this indicates that there were many mini-cuttings not developing roots but surviving and developing callus. The treatment with the highest total callus percentage (68 %) was black 30 % PP2107 organic, which had a fairly good total rooting percentage (38 %) and most of the black 30 % PP2107 organic mini-cuttings that rooted also developed callus (35 % root plus callus). The total callus percentage was high for both clones, mostly above 45 % (Fig. 4.20).

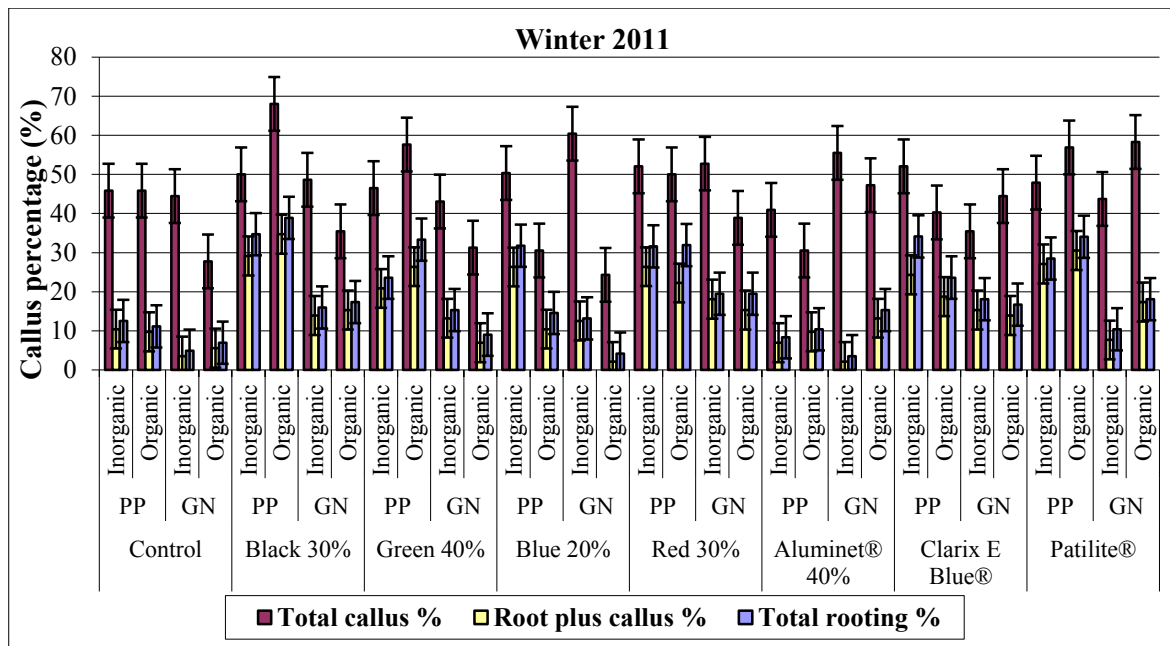


Figure 4.20. Total callus percentage and percentage of root plus callus of mini-cuttings during winter 2011 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 6.88, callus with root percentage s.e. = 4.983 and total rooting percentage s.e. = 5.409 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

The total callus percentage was significantly affected by the factors clone and fertilizer as well as the shade net \times fertilizer, clone \times fertilizer and shade net \times clone \times fertilizer interactions ($P_{0.05} < 0.001$, = 0.022, < 0.001 , = 0.011 and = 0.035, respectively). Data for mini-cuttings with root plus callus indicated that all factors and factor interactions were significant, except for fertilizer. The ANOVA for total rooting percentage indicated that shade net, shade net \times clone, shade net \times fertilizer and clone \times fertilizer were significant ($P_{0.05} < 0.001$, < 0.001 , < 0.001 and = 0.015, respectively). There was a strong positive correlation between total rooting percentage and root plus callus ($r = 0.9505$); there was a moderate positive correlation between total callus percentage and root plus callus as well as between total callus and total rooting percentage ($r = 0.4118$ and 0.3591 , respectively). In general, rooting percentage and root plus callus were highest in spring 2011, but the total callus percentage in spring 2011 was lower than in winter 2011, but not significantly different from summer 2011/12 or autumn 2011 or autumn 2012 (Table 4.2). The highest rooting percentages for spring 2011 were reported of mini-cuttings from the black 30 %, green 40 % and Clarix E Blue® shade net treatments; in addition these treatments had a high percentage of mini-cuttings with root plus callus. The highest total callus percentages were reported for blue 20 % GN018B organic, green 40 % and red 30 % GN018B inorganic (Fig. 4.21).

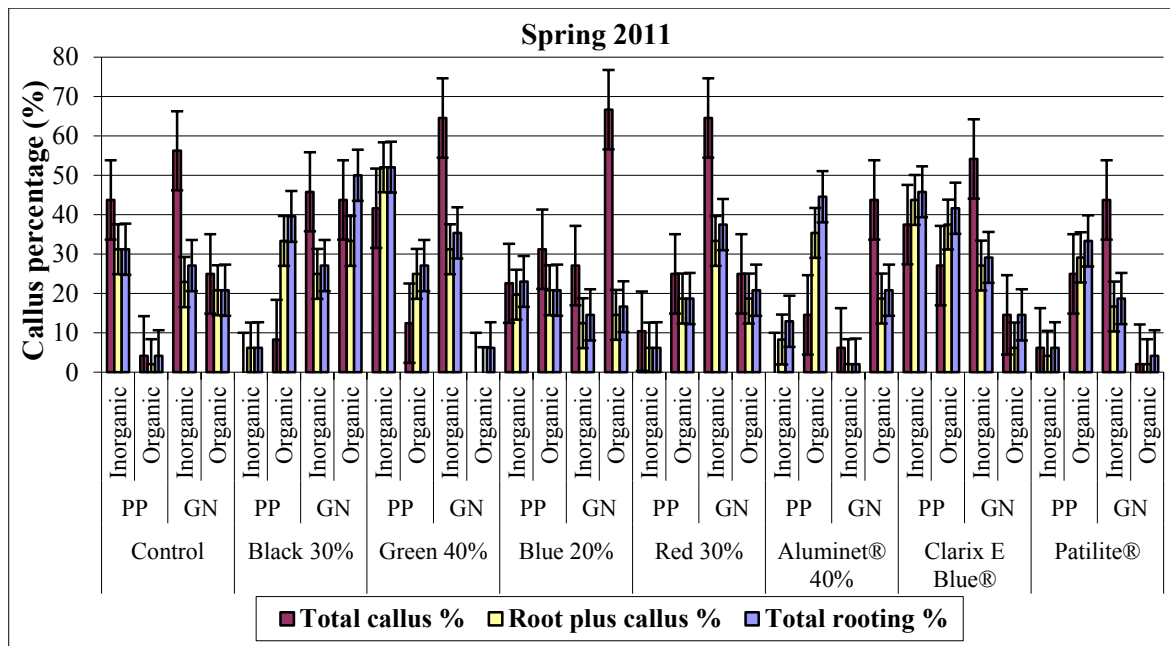


Figure 4.21. Total callus percentage and percentage of root plus callus of mini-cuttings during spring 2011 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 10.07, callus with root percentage s.e. = 6.35 and total rooting percentage s.e. = 6.49 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

In summer 2011/12, total callus percentage was significantly affected by shade net and clone factors as well as shade net \times fertilizer and shade net \times clone interactions ($P_{0.05} = 0.018$, < 0.001 , < 0.001 and $= 0.004$, respectively). The root plus callus data were significantly affected by the factor clone and the shade net \times fertilizer interaction ($P_{0.05} < 0.001$ and $= 0.021$, respectively). The total rooting was significantly affected by the clone factor and the shade net \times fertilizer interaction ($P_{0.05} < 0.001$ and $= 0.017$, respectively). There was a strong positive correlation between total callus percentage and root plus callus, between total callus percentage and total rooting percentage, and between total rooting percentage and root plus callus ($r = 0.7761$, 0.7276 and 0.9217 , respectively). The highest rooting percentage (54 %) in summer 2011/12 was determined in cuttings from the Clarix E Blue® PP2107 inorganic treatment, which also had the highest total callus percentage (75 %) and root plus callus percentage (49 %) (Fig. 4.22).

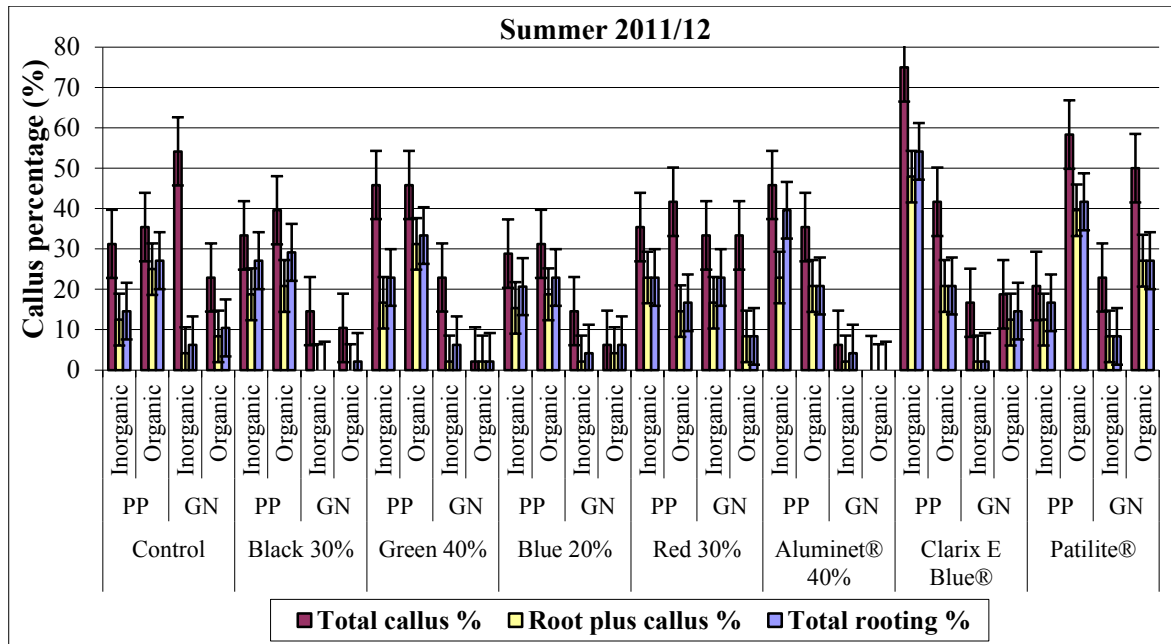


Figure 4.22. Total callus percentage and percentage of root plus callus of mini-cuttings during summer 2011/12 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 8.47, callus with root percentage s.e. = 6.41 and total rooting percentage s.e. = 7.04 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

The autumn 2012 total callus percentage data indicated that in this season, the factors shade net, clone and fertilizer as well as the shade net \times fertilizer and the shade net \times clone \times fertilizer interactions were significant ($P_{0.05} < 0.001$, = 0.040, < 0.001, < 0.001 and = 0.012, respectively). The root plus callus data were significantly affected by all factors ($P_{0.05} < 0.001$) as well as the shade net \times clone \times fertilizer interaction ($P_{0.05} = 0.019$). All factors had a highly significant ($P_{0.05} < 0.001$) effect on total rooting percentage, so did the shade net \times clone \times fertilizer interaction ($P_{0.05} = 0.033$). There was a moderate to strong positive correlation between total callus percentage and root plus callus, between total callus percentage and total rooting percentage, and between total rooting percentage and root plus callus ($r = 0.7247$, 0.6429 and 0.9587, respectively). The highest total callus percentage (71 %) for autumn 2012 was recorded for black 30 % PP2107 inorganic; this treatment also had the highest total rooting percentage (69 %) and root plus callus (59 %). High total callus percentages were also determined for blue 20 % GN018B inorganic (58 %) and green 40 % PP2107 organic (54 %), which both had low rooting percentages of about 20 % (Fig. 4.23). In general, rooting percentages were low during autumn 2012, while total callus percentages in this season were lower than in winter 2011 but not significantly different from autumn 2011, spring 2011 or summer 2011/12 (Table 4.2). The percentage of mini-cuttings with root plus callus were low compared with other seasons, but not significantly different from

autumn 2011. Rooting percentage and root plus callus were similar in this season, indicating that the majority of rooted mini-cuttings developed callus (Table 4.2 and Fig. 4.23).

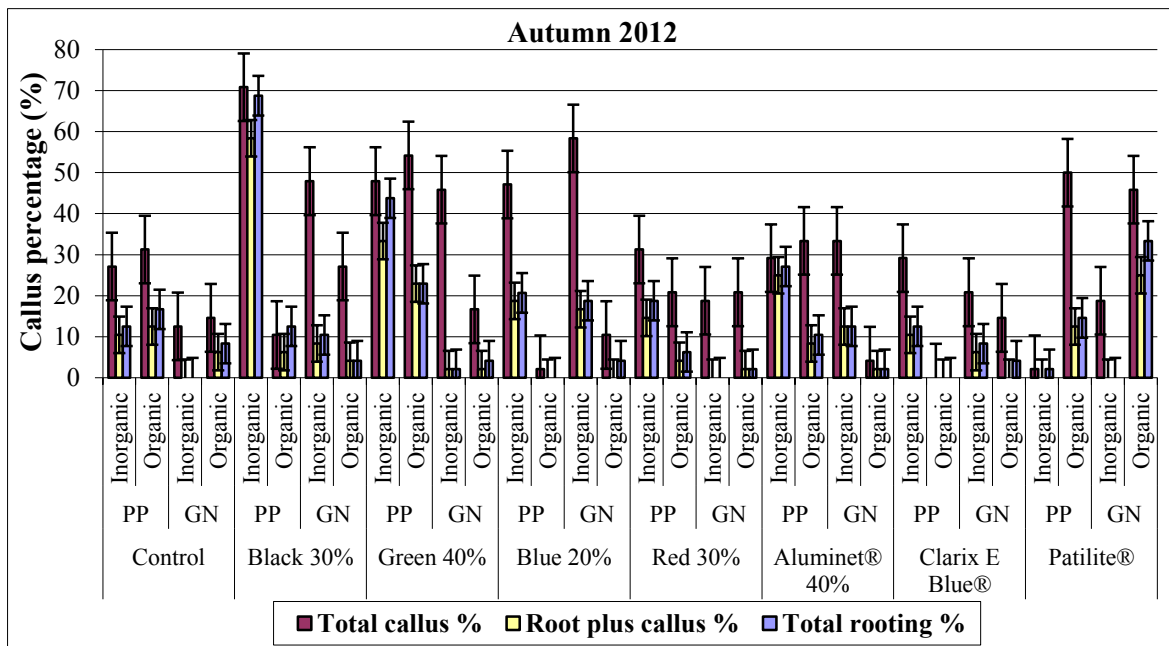


Figure 4.23. Total callus percentage and percentage of root plus callus of mini-cuttings during autumn 2012 compared with the rooting percentage of mini-cuttings; where the error bars are based on the total callus percentage s.e. = 8.24, callus with root percentage s.e. = 4.448 and total rooting percentage s.e. = 4.805 for the shade net \times clone \times fertilizer interaction; PP = clone PP2107, GN = clone GN108B.

4.3.5.4 Basal stem diameter

Basal stem diameter (BSD) was measured as an indicator of callus growth at the stem base for rooted mini-cuttings. Data were analysed using ANOVA over all five seasons where the factors clone, fertilizer and season were found to be significant ($P_{0.05} < 0.001$, = 0.015 and < 0.001 , respectively); however, BSD was not significantly affected by the factor shade net ($P_{0.05} = 0.105$); therefore, data were analysed separately for each season. Autumn 2011 BSD indicated that the shade net \times clone interaction was the only significant factor ($P_{0.05} = 0.002$). During autumn 2011, Aluminet® 40 % GN018B inorganic had the greatest BSD but was not significantly different from control and red 30 % GN018B organic; cuttings from Clarix E Blue® PP2107 organic had the smallest BSD, which was not significantly different from most treatments in the same season (Table 4.5). The BSD was weakly correlated with rooting percentage, total callus percentage, root plus callus, and root number for all five seasons; however, there was a moderate positive correlation between BSD and root plus callus during summer 2011/12 ($r = 0.5178$). In all seasons BSD varied between treatments; there was a tendency towards smaller BSD in autumn 2011 and summer 2011/12, while winter 2011, spring 2011 and autumn 2012 tended to have greater BSDs (Table 4.2 and 4.5). In all seasons

clone PP2107 tended to have greater BSDs than clone GN018B. In general, BSD ranged from 1.5 to 4.1 mm, more than the required 0.5 mm (Pollard, *pers. comm.*¹); however, some of the greater diameters may have been exaggerated due to excessive callus formation (see Fig. 4.24 (C)).

Table 4.5. Basal stem diameter (BSD) (mm) of rooted mini-cuttings taken from plants grown under each shade net, clone and fertilizer factor over five seasons.

		<i>Autumn 2011</i>							
		Control	Black 30%	Green 40%	Blue 20%	Red 30%	Aluminet® 40%	Clarix E Blue®	Patilite®
PP2107	Inorganic	1.92 bc ¹	missing	missing	2.30 bc	3.02 a	1.69 c	2.37 bc	missing
	Organic	2.29 bc	missing	missing	2.64 bc	2.65 bc	1.97 bc	1.59 c	missing
GN018B	Inorganic	1.94 bc	missing	missing	2.46 bc	1.72 bc	3.85 a	1.75 bc	missing
	Organic	2.78 ab	missing	missing	2.23 bc	1.53 c	2.43 bc	1.87 bc	missing
		<i>Winter 2011</i>							
		Control	Black 30%	Green 40%	Blue 20%	Red 30%	Aluminet® 40%	Clarix E Blue®	Patilite®
PP2107	Inorganic	3.18 a	2.89 a	2.90 a	2.87 a	2.89 a	2.82 a	2.76 b	2.68 b
	Organic	3.01 a	2.85 a	3.01 a	2.30 c	2.62 b	2.60 b	2.80 ab	2.98 a
GN018B	Inorganic	3.12 a	2.70 b	2.30 c	2.62 b	2.88 a	2.12 c	2.76 b	2.14 c
	Organic	2.68 b	2.27 c	2.77 ab	1.99 c	2.60 b	2.67 b	2.46 b	2.27 c
		<i>Spring 2011</i>							
		Control	Black 30%	Green 40%	Blue 20%	Red 30%	Aluminet® 40%	Clarix E Blue®	Patilite®
PP2107	Inorganic	3.34 a	2.64 b	3.22 a	2.71 ab	2.60 b	2.06 b	3.01 a	2.87 a
	Organic	2.52 b	2.54 b	3.16 a	2.77 a	2.90 a	2.58 b	2.77 a	3.12 a
GN018B	Inorganic	2.53 b	2.89 a	2.53 b	2.20 b	2.52 b	2.38 b	2.49 b	2.37 b
	Organic	2.58 b	2.76 a	1.23 c	3.15 a	2.43 b	2.25 b	2.24 b	1.68 c
		<i>Summer 2011/12</i>							
		Control	Black 30%	Green 40%	Blue 20%	Red 30%	Aluminet® 40%	Clarix E Blue®	Patilite®
PP2107	Inorganic	2.21 c	2.11 cd	2.10 cd	2.27 c	2.38 bc	1.99 cd	3.64 a	2.85 b
	Organic	3.03 b	2.50 b	2.96 b	2.35 c	2.66 b	2.31 c	2.29 c	2.81 b
GN018B	Inorganic	1.98 cd	0.00	1.69 d	1.94 cd	2.04 cd	2.10 cd	4.07 a	2.17 c
	Organic	1.61 d	2.07 cd	1.48 d	1.99 cd	2.27 c	0.00	2.84 b	2.41 b
		<i>Autumn 2012</i>							
		Control	Black 30%	Green 40%	Blue 20%	Red 30%	Aluminet® 40%	Clarix E Blue®	Patilite®
PP2107	Inorganic	2.35 c	2.77 b	2.49 b	2.83 b	2.35 c	2.43 bc	2.60 b	1.70 cd
	Organic	2.18 cd	3.26 a	2.74 b	0.00	1.71 c	2.07 cd	0.00	2.77 b
GN018B	Inorganic	0.00	0.00	3.51 a	3.45 a	0.00	3.14 ab	1.81 cd	0.00
	Organic	2.06 cd	3.82 a	2.80 b	1.53 d	1.51 d	0.00	1.72 cd	2.47 b

¹ Basal stem diameter treatments sharing the same symbol (abc) are not significantly different at 5 % level; LSD was calculated separately for each season with the LSD for BSD autumn 2011 = 1.1252, winter 2011 = 0.4105, spring 2011 = 0.6898, summer 2011/12 = 0.6449 and autumn 2012 = 0.7472 for the shade net × clone × fertilizer interaction. 'Missing' data in autumn 2011 refers to plant material that could not be used in the experiment due to phytotoxicity; however, any zero values are due to treatments that failed to root during the rooting experiment.

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

4.3.6 General observations

While some rooted mini-cuttings developed roots that emerged from the mini-cutting stem at the abaxial cut end (base) with minimal callus formation (Fig. 4.24 A), other mini-cuttings showed adventitious root formation from the abaxial end together with callus formation (Fig. 4.24 B). Some mini-cuttings that failed to root displayed excessive callus production at the base of the mini-cutting (Fig. 4.24 C).

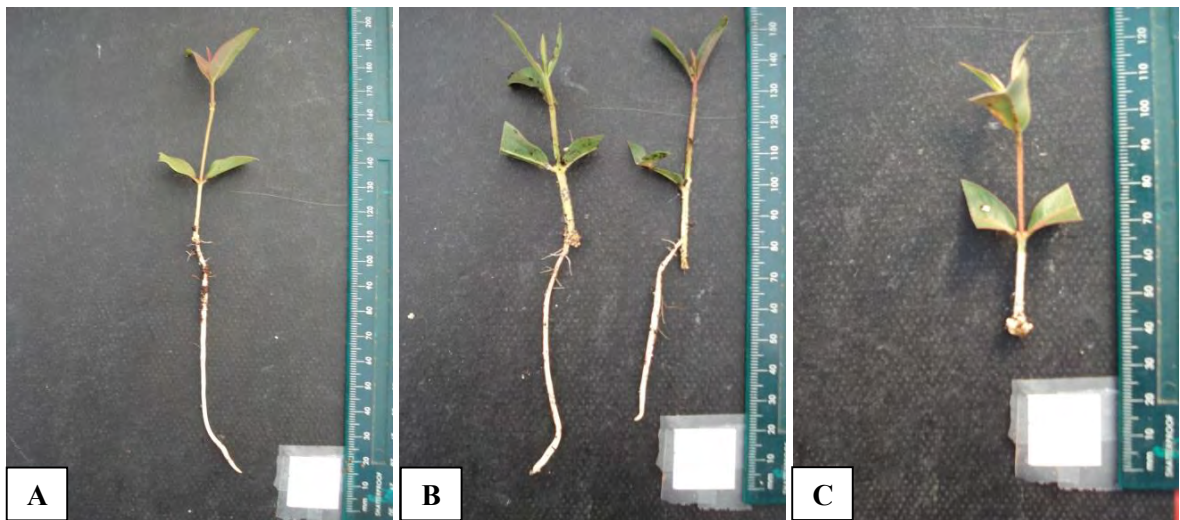


Figure 4.24. Variety of rooted mini-cuttings; (A) root emergence from the mini-cutting stem at the abaxial cut end (base), (B) left: mini-cutting with root emergence from the abaxial end together with callus formation, right: mini-cutting with root emergence from above the abaxial end (sides of the stem), (C) mini-cutting that failed to root with excessive callus production at the base.

4.4 Discussion

Applying either organic or inorganic fertilizer to stock plants does not significantly affect rooting ($P_{0.05} = 0.731$) (Fig. 4.2). Similarly, the factor fertilizer had no significant effect on rooting during winter 2011, spring 2011 and summer 2011/12 ($P_{0.05} = 0.718$, 0.645 and 0.917, respectively); however, during autumn 2012 the factor fertilizer was significant ($P_{0.05} < 0.001$). Possibly, fertilizer was also highly significant in autumn 2011, but this could not be statistically analysed due to lack of replication. Organic fertilizers commonly take longer (four to six months) to be metabolised into a form usable by the stock plants (Philip, *pers. comm.*³). A population of beneficial fungi and bacteria needs to be established to convert the organic fertilizer into a plant-available form, allowing vigorous plant growth. Under greenhouse conditions the organic fertilizer takes much longer to be broken down naturally due to the stock plant medium mixture being partially made of materials that contain minimal

³ Mr Arthur Philip. Talborne Organics KZN, talbornekzn@vodamail.co.za, Tongaat, South Africa.

plant-available nutrients (Perlite® and vermiculite) and not natural soil (Philip, *pers. comm.*³) as well as the lack of temperature fluctuations, earthworms or other microorganisms in the medium. In the first few months, some of the stock plants in the organic treatment displayed chlorosis (personal observation); however, by the time the autumn 2011 rooting experiment was undertaken, the green colour had returned to the leaves of stock plants in the organic fertilizer treatment, indicating sufficient presence of plant available nutrients. There was a trend, whereby the organic fertilizer had a higher mean rooting percentage than the inorganic fertilizer during autumn, winter and spring 2011, while in summer 2011/12 the rooting percentage was almost equal and, finally, in autumn 2012 the inorganic fertilizer gave significantly better rooting than the organic fertilizer. Furthermore, from the time of planting until March 2012 (20 months), stock plants treated with organic fertilizer had higher mortality than those treated with inorganic fertilizer (73 vs. 61 %) and particularly the clone PP2107 treated with organic fertilizer (82 % mortality) (means derived from Appendix B, Table B.2). The higher mortality of clone PP2107 compared with the unimproved clone GN018B, when treated with organic fertilizer over nearly two years, may possibly be due to the fast growing nature of the clone as PP2107 was partially selected for its promising growth parameters and rooting ability (Eatwell, 2008). The PP2107 organic treatment had a high production of mini-cuttings per stock plant during autumn and winter 2011; similarly the GN018B organic had high production during winter 2011 and all clone and fertilizer treatments had a great decrease in production during spring 2011 (Chapter 3, Fig. 3.5), when the mortality of stock plants increased rapidly (Appendix B, Table B.2). Wendling *et al.* (2010) found that periods of plant mortality and poor shoot production were related to high salt build-up in the medium, fed by a semi-hydroponic fertigation system, or possibly due to depleted internal nutrient reserves of mini-hedge stock plants. The inorganic fertilizer treatment did not display symptoms of nutrient deficiency, during these experiments, although there were some algae and moss growth on the medium surface of many stock plants, it was not excessive and there was no notable salt residue. The inorganic fertilizer was delivered manually as a biweekly measured allotment in solution and stock plants were irrigated with tap water on a daily basis, thus minimising the chance of salt build-up. The organic fertilizer was applied in granular form and may have caused some inconsistencies in the internal nutrient levels initially, due to slow release of nutrients, but these fertilizers should not have caused a salt build-up as such natural organic fertilizers are not bound to

³ Mr Arthur Philip. Talborne Organics KZN, talbornekzn@vodamail.co.za, Tongaat, South Africa.

any synthetically produced salts (Talborne organics, 2014). In general, clone PP2107 rooted consistently better than clone GN018B over all seasons (Fig. 4.3) and fertilizer regimes (Table 4.1); however, there was variation of rooting percentages within shade nets and plastics such as black 30 % PP2107 inorganic and organic (6.25 and 39.58 %, respectively) being significantly lower than GN018B inorganic and organic (27.08 and 50.00 %, respectively) during spring 2011 (Table 4.3). Clone GN018B had much improved rooting percentages for most shade nets and plastics during the spring 2011 season (Fig. 4.3, 4.4 and Table 4.3).

Coloured shade nets and plastics significantly altered the radiation quality measured as R:NIR over seasons and compared with outside values, but not sufficiently to alter the morphology of the stock plants with regard to internode length and leaf area (Chapter 2, Table 2.2). There were significant differences in rooting percentages, where black 30 %, green 40 %, Clarix E Blue® and Patilite® had the highest rooting and Aluminet® 40 % the lowest. The blue 20 % and red 30 % shade nets were expected to have contrasting effects on the radiation quality (Oren-Shamir *et al.*, 2001; Cummings *et al.*, 2008), consequently, affecting plant growth and rooting potential differently; however, these two shade nets had average rooting percentages that were not statistically different from one another (Fig. 4.5). The transmission (direct radiation allowed through the net) should be 60 % under a shade net with a 40 % shading factor and 70 % under a 30 % shading factor. However, average transmission for the photosynthetically active radiation (PAR) region of the radiation spectrum, under controlled laboratory conditions using an incandescent lamp, for these shade nets were 53.4, 67.8 and 63.1 % for Aluminet® 40 %, black 30 % and green 40 %, respectively (Table 2.3); which converts to shading factors of 46.6, 32.2 and 36.9 for Aluminet® 40 %, black 30 % and green 40 %, respectively. Therefore, the Aluminet® and black had higher shading properties and the green lower shading properties than expected. During spring 2011, the shade nets and plastics that had the highest rooting percentages that were not significantly different from one another, were black 30 %, red 30 %, green 40 % and Clarix E Blue® (Fig. 4.6). The mean PAR transmission for these shade nets were 50.6, 59.2, 43.4 and 67.3 %, respectively (Table 2.3), which converts to shading factors of 49.4, 40.8, 56.6 and 32.7 % for black 30 %, red 30 %, green 40 % and Clarix E Blue®, respectively, which are all higher shading properties than expected. This indicates that the optimal transmission for a shade net used to grow stock plants under should be between 40 to 70 % (equivalent to a shading factor of 30 to 60 %, respectively). However, there were

exceptions whereby the blue 20 % and Patilite® had the lowest rooting percentages during spring 2011, but had transmission of 60.3 and 60.7 %, which converts to shading factors of 39.7 and 39.3 %, which were similar to the measured shading factor of the red 30 % net (40.8 %). Therefore, it may be useful to investigate the effect of a particular shade net at varying shading factors between 30 and 60 % on rooting potential. There was no clear trend whereby a particular shade net or plastic covering had the highest or lowest rooting percentage as these changed with each season (Fig. 4.6), highlighting the difficulty of selecting the best shade net or plastic for use in vegetative propagation. Seasonal variations of rooting ability of cuttings are common, particularly for softer leafy cuttings as these are more susceptible to variations in radiation as well as temperature and water stress inflicted on stock plants during the year (Leakey, 1985; Hartmann *et al.*, 1990). Shade nets and plastic coverings do regulate radiation received, temperature and RH to some degree, but there are still seasonal variations. Mini-cuttings harvested from stock plants grown under control conditions had the highest rooting percentages during spring 2011 and lowest rooting during winter 2011; black 30 % and green 40 % both had the highest rooting during spring 2011 and the lowest during summer 2011/12. However, the shade nets with the most consistently high rooting were black 30 % and green 40 %.

In general, the highest rooting percentages were obtained during spring 2011 and the lowest during autumn 2012 (Table 4.2). The lowest photosynthetic photon flux density (PPFD) measured over three seasons was under Aluminet® 40 % during winter 2011 ($204.6 \mu\text{mol s}^{-1} \text{m}^{-2}$; Table 2.1) that had a low rooting percentage (9.38 %; Fig. 4.6) and the highest PPFD under shade nets was measured under blue 20 % during spring 2011 ($604.4 \mu\text{mol s}^{-1} \text{m}^{-2}$; Table 2.1) that had an average rooting percentage (16.67 %; Fig. 4.6). The PPFD measured under the control treatment (no shade nets) was higher than under any other shade net or plastic treatments in the same season, where the highest PPFD was during spring 2011 and the lowest during winter 2011 (931.5 and $444.3 \mu\text{mol s}^{-1} \text{m}^{-2}$, respectively); the highest outside PPFD was during spring 2011 ($1713.4 \mu\text{mol s}^{-1} \text{m}^{-2}$; Table 2.1). There was no clear trend to indicate the optimal radiation intensity at which to grow $G \times N$ stock plants to achieve high rooting percentages of mini-cuttings. Mean maximum irradiance over three days, during the rooting period, in the rooting tunnel was 475.1 , 128.8 and $172.3 \mu\text{mol s}^{-1} \text{m}^{-2}$ PPFD during autumn 2011, winter 2011 and spring 2011, respectively (unpublished data). The PPFD values measured in the rooting tunnel were lower than those measured in the stock plant tunnel control (no shade covering) for all three seasons; however, during winter

and spring 2011 the rooting tunnel PPFDs were both much lower than the PPFD values in the stock plant tunnel. This data was calculated from readings recorded by Onset® HOBO® U12-012 series data loggers and not the LI-COR® portable research spectroradiometer (LI-1800, Lincoln, Nebraska, USA) used in chapter 2, thus may not be as accurate in measuring irradiance levels.

Many plant species result in poor rooting of cuttings when stock plants are grown at very low irradiances, however, rooting increases with increasing irradiance until the optimal PPFD is reached; above this optimum, rooting ability again declines (Mesén *et al.*, 2001). Supplemental lighting can be used in greenhouses in regions with low radiation and/or short daylengths during winter, to improve stock plant growth and thereby to enhance production of cuttings (Pellicer *et al.*, 1998). *Albizia guachapele* (Leguminosae; Mimosaceae) is a fast growing, drought-tolerant timber species that is native to the dry tropical forest of Central America, where it is used as wood for fuel, for posts, live fences and timber as well as a nitrogen fixer (Mesén *et al.*, 2001); where *Eucalyptus* species are also fast growing and are used for timber, large poles and pulpwood for paper (Rocha Corrêa and Fett-Neto, 2004); although these species are not related, they are used for similar purposes and so can be compared. Stock plants of *Albizia guachapele* were maintained at 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$ PPFD at canopy level as a low irradiance treatment and at 500 $\mu\text{mol s}^{-1} \text{m}^{-2}$ as a high irradiance treatment in separate controlled environment chambers (Mesén *et al.*, 2001). Although *E. grandis* stock plants were grown under various R:NIR, the PPFD was maintained at a constant 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (Hoad and Leakey, 1996). Mesén *et al.* (2001) found that rooting percentages of *Albizia guachapele* were significantly higher (53.8 %) under low irradiance and low nutrient levels (200 $\mu\text{mol s}^{-1} \text{m}^{-2}$ and 0.25 % v/v of NPK) than under high irradiance and high nutrient levels (500 $\mu\text{mol s}^{-1} \text{m}^{-2}$ and 1.25 % v/v of NPK), which had lower rooting percentages (11.2 %). These *Albizia guachapele* stock plants grown under low irradiance and low nutrient levels produced elongated cuttings that possessed a higher rooting ability (Mesén *et al.*, 2001), which may have been due to a shade avoidance effect. The optimal PPFD of G \times N hybrids may be the reason that as radiation intensity and temperature were increased from 37 $\mu\text{mol s}^{-1} \text{m}^{-2}$ PPFD and 23 °C day/ 21 °C night to 66 $\mu\text{mol s}^{-1} \text{m}^{-2}$ PPFD and 27 °C day/ 21 °C night with a 16-hour photoperiod, under controlled laboratory conditions, the rooting percentage of micropropagated G \times N clones increased up to 75 % with well-formed roots free from callusing (Mokotedi *et al.*, 2000). However, cuttings from Pine (*Pinus sylvestris*) stock plants grown in a controlled environment chamber at a low

irradiance (8 W m^{-2}) rooted faster and with a greater rooting percentage (95 %) than those from stock plants grown under higher irradiance (40 W m^{-2}), which produced cuttings with a good but significantly lower rooting percentage (80 %) (Hansen *et al.*, 1978). A conversion from spectral irradiance (SI) to PPFD can only be performed if the wavelengths of measured irradiance are known, therefore, a calculation of $\text{PPFD} = 0.00835945 \times \sum (\text{all irradiance} \times \lambda)$ was performed according to the wavelengths (λ) 400 to 715 nm as used by Hansen *et al.* (1978) on *Pinus sylvestris* stock plants. From this conversion Hansen *et al.* (1978) irradiance of 8 W m^{-2} is equivalent to $37.3 \mu\text{mol s}^{-1} \text{ m}^{-2}$ PPFD and 40 W m^{-2} to $186.4 \mu\text{mol s}^{-1} \text{ m}^{-2}$ PPFD, where the former is extremely low and the latter is similar to the low irradiance treatment ($200 \mu\text{mol s}^{-1} \text{ m}^{-2}$) used by Mesén *et al.* (2001) that gave good rooting results for *Albizia guachapele*. The irradiance levels described in chapter 2 measured under shade nets and plastics in the stock plant tunnel had a minimum PPFD of $204.6 \mu\text{mol s}^{-1} \text{ m}^{-2}$, photosynthetic irradiance (PAR) of 42.9 W m^{-2} and SI of 89.5 W m^{-2} for Aluminet® 40 % during winter 2011 (Table 2.1), which was similar to the higher irradiance used by Hansen *et al.* (1978) and the low irradiance used by Mesén *et al.* (2001). Although the aforementioned authors reported that these light regimes of approximately $200 \mu\text{mol s}^{-1} \text{ m}^{-2}$ PPFD resulted in good rooting (53.8 and 80 % (Mesén *et al.*, 2001 and Hansen *et al.*, 1978), respectively), this lower PPFD recorded under Aluminet® 40 % during winter 2011 in this G × N hybrid study had a fairly low rooting percentage of 9.38 %; however, the maximum rooting percentage of 32.81 % under Clarix E Blue® during spring 2011 (Fig. 4.6) had a PPFD of $586.2 \mu\text{mol s}^{-1} \text{ m}^{-2}$ (Table 2.1), indicating that these G × N hybrids may have a higher optimal PPFD for stock plants than *Albizia guachapele* or *Pinus sylvestris*. In all subsequent experiments the PPFD and SI under various shade nets were higher than those used by Hansen *et al.* (1978) and Mesén *et al.* (2001), particularly during spring 2011. Therefore, in South Africa supplemental lighting is usually not necessary for G × N cutting production and was not a limiting factor to the rooting percentage during these experiments; however, PPFDs on the higher end of the spectrum ($> 600 \mu\text{mol s}^{-1} \text{ m}^{-2}$) may have been limiting to root formation such as under blue 20 % and control during spring 2011; therefore, shade nets such as black 30 % and green 40 % that give a range of 200 to $450 \mu\text{mol s}^{-1} \text{ m}^{-2}$ PPFD over three or more seasons should be recommended.

A certain photoperiod, particularly when combined with a specific temperature, can influence the rooting predisposition of shoots (Assis *et al.*, 2004). Photoperiod and nutrition are two important factors that control the growth rate and duration of the growing season in

trees (Rieckermann *et al.*, 1999). In order to avoid retarded growth of seedlings and/or rooted cuttings of woody species during short day periods, the natural photoperiod can be extended by using supplemental lighting. In sweetgum (*Liquidambar styraciflua*), seedlings grown under the 14- or 16-hour days grew more quickly and continued to grow longer than seedlings under 8- or 12-hour days. A comparison of cuttings receiving ambient light with those receiving the 3-hour night interruption as studied by Rieckermann *et al.* (1999), revealed no significant differences in surviving percentages (75 % ambient, 77 % night interruption) and the percentages of cuttings deemed plantable (59 % ambient, 58 % night interruption). In Pietermaritzburg, where the experiments were carried out, maximum daylength (summer solstice) provides 13-hours and 51 minutes daylight, while at the winter solstice only 10-hours and four minutes (Savage, *pers. comm.*⁴); since *E. nitens* is not strongly influenced by photoperiod as a stimulus for flowering (Moncur and Hasan, 1994), the adventitious rooting of *Eucalyptus* hybrids with *E. nitens* as one of the parents should not be strongly influenced by photoperiod. Production of cuttings was still high during winter 2011 (Table 3.7), therefore, the photoperiod experienced by G × N clones did not have a strong effect on stock plant growth and production of mini-cuttings.

The highest overall rooting percentage of both G × N clones together was achieved during the spring 2011 season; the lowest during autumn 2011 and 2012, while winter 2011 and summer 2011/12 had intermediate rooting percentages (Table 4.2). Although mean temperatures in the stock plant tunnel were not significantly different during spring and autumn 2011, the mean temperatures in the rooting tunnel during spring 2011 were significantly cooler than during autumn 2011 and summer 2011/12 (Table 3.1), which may have been the reason for the improved rooting percentages during spring 2011. Winter 2011 had significantly colder temperatures in both the stock plant and rooting tunnels. Total callus percentage and BSD were greatest, together with intermediate rooting percentages, during winter 2011 (Table 4.2), which may indicate that, although G × N clones are cold-tolerant, they still have an optimal temperature range for vegetative propagation of about 12 to 30 °C (spring 2011 minimum and maximum temperatures, Table 3.3). The optimal temperature ranges for growing *E. grandis* and *E. nitens* are 16 to 20 °C and 13.5 to 16 °C, respectively (Gardner, 2007). Temperature limits of *E. grandis* production lie between 0 and 34 °C and can tolerate light, but not heavy frosts (McMahon *et al.*, 2010), therefore, as *E. nitens* can

⁴ Prof. Michael Savage. University of KwaZulu-Natal, Discipline of Agrometeorology, Savage@ukzn.ac.za, Pietermaritzburg, South Africa.

tolerate colder temperatures it is the preferred species for planting at high altitudes in south-eastern Australia, where mean annual temperatures lie below 10 °C (Battaglia *et al.*, 1996; Close and Beadle, 2003). The minimum temperatures under shade nets and plastics in the stock plant tunnel were mostly within a range optimal for G × N production from summer 2010/11 to autumn 2012 (12 to 21 °C) except during winter 2011, when temperatures ranged from 6.8 to 7.8 °C; however, during autumn 2011, spring 2011, summer 2011/12 and autumn 2012, maximum temperatures exceeded 30 °C in the stock plant tunnel (Table 3.3). In general, mean temperatures for seasons and months were slightly higher in the rooting tunnel than in the stock plant tunnel. Poor rooting may be due to very high temperatures, particularly in the rooting tunnel during summer, which could be curbed by using more efficient temperature control such as using overhead misters controlled by sensors measuring temperature and relative humidity (RH) (not just a timer) to make use of evaporative cooling as well as by using tunnels with a higher roof, stronger extraction fans and by using approximately 20 to 30 % shade netting in existing tunnels. Alternatively, to counter very cold temperatures, bottom heating in the rooting tunnel can encourage root formation at the base of cuttings.

Hoad and Leakey (1996) and Mesén *et al.* (2001) found that poor rooting of less elongated *E. grandis* and *Albizia guachapele* macro-cuttings were confounded by greater susceptibility of these shorter macro-cuttings to rotting and subsequent death due to the close proximity of leaves to the medium. Similarly, in this experiment some algal growth and rotting at the base of mini-cuttings was observed in some rooting trays during all seasons; however, this was worst during winter 2011 and summer 2011/12 (personal notes - thicker algae, as well as algae and rotted cuttings reaching up to 80 % of some rooting trays), which may be due to extreme temperatures and extreme variations in RH. During these seasons mini-cuttings did not root well. The winter 2011 and summer 2011/12 rooting experiments were prepared by four and two labourers, respectively. The labourers were taught to remove bottom leaves in order to avoid this rotting problem, but during assessments it was noted that some small leaf portions had occasionally been left behind (personal observation). During autumn 2012 an irrigation malfunction damaged some of the mini-cuttings and some medium was washed out of a few rooting trays, which may have contributed to the resultant low rooting percentage of that season.

Autumn 2011 had fairly poor rooting percentages (above 16 %) and spring 2011 had the highest rooting percentages (above 23 %) (Table 4.2), but those mini-cuttings that did root had a high percentage of type 3 and 4 (good and strong) root quality (Fig. 4.9 and 4.11). Autumn 2012 had a high percentage of type 1 and 2 (weak and average), some type 3 (good), but very few type 4 (strong) roots (Fig. 4.13). Although the autumn seasons had poor rooting percentages (above 13 %), there were good rooting percentages of blue 20 % PP2107 organic during autumn 2011 (57.14 %) and black 30 % PP2107 inorganic during autumn 2012 (68.75 %) (Fig. 4.8 and Table 4.3); together with the good root quality during autumn 2011, that may suggest that spring and autumn may be the best seasons to root G × N mini-cuttings, when temperatures are not extreme.

The number of roots per mini-cutting was weakly or moderately positively correlated with rooting percentage for all seasons, except for autumn 2011 when it was strongly correlated, possibly due to missing treatments in autumn 2011 and no replication in autumn. Spring 2011 had significantly higher number of roots per mini-cutting compared with other seasons; the lowest number of roots per mini-cutting were observed in winter 2011, summer 2011/12 and autumn 2012 (Table 4.2). Most mini-cuttings during all seasons had between one and three roots per rooted mini-cutting. Rocha Corrêa and Fett-Neto (2004) found that the highest rooting percentages (87 %) and root numbers (four to six roots per cutting) were when stock plant temperatures were between 20 and 25 °C for *E. globulus*. *Eucalyptus saligna* had the highest number of roots (nine roots per cutting) and fastest rate of rooting (four days) when stock plants were held at 15 °C and exogenous auxin (10 mg/L) was used on micro-cuttings (Rocha Corrêa and Fett-Neto, 2004). This may be why the spring 2011 season had improved rooting percentages, root quality and number of roots per mini-cutting as mean temperature in the stock plant tunnel was 21.5 °C and in the rooting tunnel was 22.2 °C, while temperatures in the rooting tunnel either exceeded 25 °C or were below 20 °C (Table 3.1). The commercial forestry nursery Sunshine Seedling Services, found that the rooting of cuttings from ramets of the two cold-tolerant G × N clones generally had the highest rooting during winter 2010 (July and August for PP2107 at 61.5 % and August for GN018B at 51.4 %), although rooting varied over years (Pollard, *pers. comm.*¹). Rapaka *et al.* (2005) found that *Pelargonium × hortorum* cv. ‘Isabell’ cuttings had the highest root number (ten roots per cutting) overall when low-light-adapted stock plants grown during

¹ Mr Bryn Pollard. Sunshine Seedlings Services, bryn@sunshinseedlings.co.za, Pietermaritzburg, South Africa.

winter were rooted at a controlled PPFD of $100 \mu\text{mol s}^{-1} \text{m}^{-2}$; however, below $100 \mu\text{mol s}^{-1} \text{m}^{-2}$ these authors found rooting to be poor. Similarly, high-light-adapted cuttings grown in summer had a higher root number (more than eight roots per cutting) when rooted in the greenhouse at a PPFD of $154 \mu\text{mol s}^{-1} \text{m}^{-2}$ compared with those rooted in the control chamber at $100 \mu\text{mol s}^{-1} \text{m}^{-2}$ (seven roots per cutting) (Rapaka *et al.*, 2005). The PPFD measurements in this experiment ranged between 378.1 and $604.4 \mu\text{mol s}^{-1} \text{m}^{-2}$ under shade nets and plastics in the stock plant tunnel during spring 2011, and the PPFD was calculated at $172.3 \mu\text{mol s}^{-1} \text{m}^{-2}$ in the rooting tunnel during the same season; however, the calculated PPFD in the rooting tunnel was much higher at $475.1 \mu\text{mol s}^{-1} \text{m}^{-2}$ during autumn 2011; therefore, installing removable shade netting in the structure used for rooting in this experiment, could improve rooting in that particular tunnel.

Callus and root formation often occur simultaneously due to their dependence on similar internal and environmental conditions (Hartmann *et al.*, 1990); however, excessive callus formation can hinder root formation and may indicate that the environmental conditions were not ideal for root initiation or that the plant species or clone is difficult-to-root (Hartmann *et al.*, 1990). The winter 2011 season had significantly higher total callus percentage than other seasons, while callusing was lowest in spring (Table 4.2). Within seasons, the total callus percentage was strongly positively correlated with rooting percentage for autumn 2011, winter 2011, summer 2011/12 and autumn 2012; however, in spring 2011 total callus percentage and rooting percentage were only weakly correlated. This may indicate that rooting conditions were more suited to root than to callus formation during spring 2011, but during the other seasons may have been more suited to callus formation. Within seasons (autumn 2011, winter 2011, spring 2011, summer 2011/12 and autumn 2012), rooting percentage was strongly positively correlated with the percentage of mini-cuttings with root plus callus, indicating that for all seasons, a high percentage of the rooted mini-cuttings developed callus. When basal temperatures exceed $27 \text{ }^{\circ}\text{C}$, excessive callusing can be observed and poor rooting and field survival results (Hartmann *et al.*, 1990). This may have been the case during summer 2011/12 and autumn 2011 and 2012, which were warmer than anticipated for autumn (Table 3.3). The BSD was significantly greater during winter 2011, spring 2011 and autumn 2012, although winter 2011 had a significantly higher total callus percentage and lower rooting percentage than spring 2011 (Table 4.2). The BSD was weakly correlated with rooting percentage, total callus percentage, and root number for

all of the five seasons. In general, clone PP2107 had a greater BSD than clone GN018B (Table 4.5).

4.5 Conclusion

Black or green woven or knitted plastic shade nets, of varying shade factors, are commonly used in nurseries as the covering material for shade houses. Alternatively, nurseries use tunnels or greenhouses covered in clear plastic sheeting or a combination of shade nets and plastics can be used. The coloured shade nets and plastic coverings that supported the highest rooting percentages in these experiments were black 30 %, green 40 %, Clarix E Blue® and Patilite®, thus validating the use of these coloured shade nets and plastic coverings by commercial nurseries. If nursery facilities for the stock plants are established it may be beneficial to add black or green shade netting on the inside or outside of the tunnel or greenhouse structure. Nurseries should be prepared to replace between 50 to 80 % of originally planted stock plants due to mortality over a two year cycle. If new tunnels are raised it may be beneficial to use the Clarix E Blue® or Patilite® plastics over the tunnels. It is important to be able to control temperatures and RH, particularly in the rooting tunnel and possibly to add shade netting above rooting mini-cuttings in order to decrease temperature and irradiance. Further studies could ascertain the beneficial effects on rooting of cuttings using these plastics in combination with different shading factors.

References

- APPELGREN, M. 1991. Effects of light quality on stem elongation of *Pelargonium* in vitro. *Scientia Horticulturae* 45, 345-351.
- ASSIS, T.F., FETT-NETO, A.G. and ALFENAS, A.C. 2004. Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. In: C. Walter and M. Carson (eds.), *Plantation Forest Biotechnology for the 21st Century*, 303-333. Research Signpost, Kerala, India.
- BATTAGLIA, M., BEADLE, C., and LOUGHHEAD, S. 1996. Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens*. *Tree Physiology* 16, 81-89.
- CLOSE, D.C., and BEADLE, C.L. 2003. Chilling-dependent photoinhibition, nutrition and growth analysis of *Eucalyptus nitens* seedlings during establishment. *Tree Physiology* 23, 217-226.
- CUMMINGS, I.G., FOO, E., WELLER, J.L., REID, J.B. and KOUTOULIS, A. 2008. Blue and red photoselective shade cloths modify pea height through altered blue irradiance perceived by the cry1 photoreceptor. *Journal of Horticultural Science and Biotechnology* 83(5), 663-667.
- EATWELL, K.A. 2008. Annual Technical Report 2 Innovation Fund Project T50028 Project Pulp. CSIR/NRE/FOR/ER/2008/0402/B, NRE, CSIR
- FETT-NETO, A.G., FETT, J.P., VIEIRA GOULART, L.W., PASQUALI, G., TERMIGNONI, R.R. and FERREIRA, A.G. 2001. Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. *Tree Physiology* 21, 457-464.
- GARDNER, R.A.W. 2007. Investigating the environmental adaptability of promising subtropical and cold-tolerant eucalypt species in the warm temperate climate zone of KwaZulu-Natal, South Africa. *Southern Hemisphere Forestry Journal* 69(1), 27-38.
- GRINBERGER, A., SHOMRON, M. and GANELEVIN, R. 2000. Shading nets testing. http://www.polysack.com/index.php?page_id=1&lang_action=change_lang&to_lang=en&geo_action=done Accessed 14/05/2010.
- HAAPALA, T., PAKKANEN, A. and PULKKINEN, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* × *P. tremuloides*). *Forest Ecology and Management* 193, 345-354.

- HANSEN, J., STROMQUIST, L.H. and ERICSSON, A. 1978. Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). *Plant Physiology* 61, 975-979.
- HARTMANN, H.T., KESTER, D.E. and DAVIES, F.T., Jr. 1990. Plant propagation – Principles and practices. Fifth edition. Prentice Hall Career and Technology, Englewood Cliffs, New Jersey, USA.
- HETTASCH, M.H. and LUNT, K.A. (eds). 2002. Chapter 4: Vegetative propagation – cuttings. In: An introduction to forest nursery practices: with emphasis on select *Eucalyptus*, *Pinus*, *Podocarpus* and *Acacia* species grown in South Africa. CSIR Environmentek, pp 53-69.
- HOAD, S.P. and LEAKEY, R.R.B. 1996. Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden cutting morphology, gas exchange and carbohydrate status during rooting. *Trees* 10, 317-324.
- KIBBLER, H., JOHNSTON, M.E. and WILLIAMS, R.R. 2004. Adventitious root formation in cuttings of *Backhousia citriodora* F. Muell. 1. Plant genotype, juvenility and characteristics of cuttings. *Scientia Horticulturae* 102, 133-143.
- LEAKEY, R.R.B. 1985. Chapter 9: The capacity for vegetative propagation in trees. In: Cannell, M.G.R.; Jackson, J.E., (eds.) Attributes of trees as crop plants. Abbots Ripton, Institute of Terrestrial Ecology, pp 110-133.
- MCMAHON, L., GEORGE, B. and HEAN, R. 2010. Primefacts for profitable, adaptive and sustainable primary industries. Primefact 1055, A Tre SMART factsheet, September. <http://www.industry.nsw.gov.au> Accessed 22/05/2012.
- MENZIES, M.I., HOLDEN, D.G. and KLOMP, B.K. 2001. Recent trends in nursery practice in New Zealand. *New Forests* 22, 3-17.
- MESÉN, F., LEAKEY, R.R.B. and NEWTON, A.C. 2001. The influence of stockplant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele*. *New Forests* 22, 213-227.
- MOKOTEDI, M.E.O., WATT, M.P., PAMMENTER, N.W. and BLAKEWAY, F.C. 2000. *In vitro* rooting and subsequent survival of two clones of a cold-tolerant *Eucalyptus grandis* × *E. nitens* hybrid. *Hortscience* 35(6), 1163-1165.
- MONCUR, M.W. and HASAN, O. 1994. Floral induction in *Eucalyptus nitens*. *Tree Physiology* 14, 1303-1312.

- NAIDU, R.D. and JONES, N.B. 2009. The effect of cutting length on the rooting and growth of subtropical *Eucalyptus* hybrid clones in South Africa. *Southern Forests* 71(4), 297-301.
- NEGASH, L. 2002. Successful vegetative propagation techniques for the threatened African pencil cedar (*Juniperus procera* Hoechst. Ex Endl.). *Forest Ecology and Management* 161, 53-64.
- OREN-SHAMIR, M., GUSSAKOVSKY, E.E., SHPIEGEL, E., NISSIM-LEVI, A., RATNER, K., OVADIA, R., GILLER, Y.E. and SHAHAK, Y. 2001. Coloured shade nets can improve the yield and quality of green decorative branches of *Pittosporum variegatum*. *Journal of Horticultural Science and Biotechnology* 76, 353-361.
- OSTERC, G., ŠTEFANČIČ, M. and ŠTAMPAR, F. 2009. Juvenile stockplant material enhances root development through higher endogenous auxin level. *Acta Physiologiae Plantarum* 31, 899-903.
- PALANISAMY, K. and KUMAR, P. 1997. Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (*Azadirachta indica* A. Juss). *Forest Ecology and Management* 98, 277-280.
- PAWLICKI, N. and WELANDER, M. 1995. Influence of carbohydrate source, auxin concentration and time of exposure on adventitious rooting of the apple rootstock Jork 9. *Plant Science* 106, 167-176.
- PELLICER, V., CAZET, M., VERGER, M. and RIVIÈRE, L.M. 1998. Effect of stock plant lighting on bulk vegetative propagation of hybrid larch (*Larix × eurolepis* Henry). *Forest Ecology and Management* 102, 323-332.
- RAPAKA, V.K., BESSLER, B., SCHREINER, M. and DRUEGE, U. 2005. Interplay between initial carbohydrate availability, current photosynthesis, and adventitious root formation in *Pelargonium* cuttings. *Plant Science* 168, 1547-1560.
- RIECKERMANN, H., GOLDFARB, B., CUNNINGHAM, M.W. and KELLISON, R.C. 1999. Influence of nitrogen, photoperiod, cutting type, and clone on root and shoot development of rooted stem cuttings of Sweetgum. *New Forests* 18, 231-244.
- ROCHA CORRÊA, L.D. and FETT-NETO, A.G. 2004. Effects of temperature on adventitious root development in microcuttings of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill. *Journal of Thermal Biology* 29, 315-324.
- ROMERO, J.L. 2004. A review of propagation programs for *Gmelina arborea*. *New Forests* 28, 245-254.

- SASSE, J. and SANDS, R. 1997. Configuration and development of root systems of cuttings and seedlings of *Eucalyptus globulus*. *New Forests* 14, 85-105.
- SCHWAMBACH, J., RUEDELL, C.M., RODRIGUES de ALMEIDA, M., PENCHEL, R.M., de ARAÚJO, E.F. and FETT-NETO, A.G. 2008. Adventitious rooting of *Eucalyptus globulus* × *maidenii* mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. *New Forests* 36, 261-271.
- STAPE, J.L., GONÇALVES, J.L.M. and GONÇALVES, A.N. 2001. Relationships between nursery practices and field performance for *Eucalyptus* plantations in Brazil: A historical overview and its increasing importance. *New Forests* 22, 19-41.
- TALBORNE ORGANICS. 2014. Fertilisers and Edu-info. <http://www.talborne.co.za/> Accessed 14/04/2014.
- TITON, M., XAVIER, A. and OTONI, W.C. 2006. Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting techniques. *Scientia Forestalis* 71, 109-117.
- TROBEC, M., ŠTAMPAR, F., VEBERIČ, R. and OSTERC, G. 2005. Fluctuations of different endogenous phenolic compounds and cinnamic acid in the first days of the rooting process of cherry rootstock ‘GiSelA 5’ leafy cuttings. *Journal of Plant Physiology* 162, 589-597.
- VSN INTERNATIONAL. 2011. GenStat for Windows, 14th Edition. VSN International, Hemel Hempstead, UK.
- WENDLING, I., BRONDANI, G.E., DUTRA, L.F. and HANSEL, F.A. 2010. Mini-cuttings technique: a new ex vitro method for clonal propagation of sweetgum. *New Forests* 39(3), 343-353.

Chapter 5

Final outlook, conclusions and recommendations

The aim of this study was to deepen the understanding of possible alterations to the radiation spectrum occurring under certain shade nets and plastics and how these alterations affect the morphology of mini-hedge stock plants. The subsequent rooting potential of mini-cuttings from stock plants grown under these conditions was also assessed. Of particular interest were the specific transmission of radiation quantity and quality through the particular shade nets and plastics, the effect of temperature, relative humidity (RH) and season on stock plants and rooting of mini-cuttings, root system quality of mini-cuttings and the interactions of clone, fertilizer regime and shade netting on rooting percentages.

5.1 Outlook

Most nurseries propagating woody species grow stock plants under direct sunlight, under clear plastic in tunnels or outdoors under a black or green shade net (30 to 40 % shading), in order to reduce total solar irradiance, often using different shading percentages for different plant types. Such practice alters the radiation spectrum perceived by the stock plant and, consequently, the morphology as well as possibly the physiology of mini-hedge stock plants grown under these conditions are likely to be affected. Aluminet®, black shade nets and Patilite® plastic act as neutral covers with regard to radiation transmission, while the blue, red and green shade nets as well as Clarix E Blue® plastic covers alter the transmission of certain wavelengths and can, thus, be considered photoselective. It was found that shade nets do not eliminate specific wavelengths altogether, unlike filters, nor do they provide only specific wavelengths like artificial lighting, since shade nets allow natural radiation through the holes between the weaves, no waveband is entirely eliminated.

Stock plants grown at low red to near infrared ratios (R:NIR), display stem elongation, with resultant improved rooting percentages; however, although the R:NIR under shade nets and plastics in these described experiments were significantly different over three seasons, R:NIR did not vary to such an extent as to affect shoot internode length or leaf area (LA) of

stock plants. It is, therefore, most likely that the R:NIR under the shade nets and plastics used in these experiments did not affect rooting percentage of mini-cuttings significantly. It was expected that the shade nets that tended to enhance internode elongation, such as Clarix E Blue® and red 30 %, would have the highest rooting percentages; however, this was not the case as red 30 % often had average rooting, although Clarix E Blue® had good rooting in most seasons.

Radiation intensity was not a limiting factor with regard to root formation for *Eucalyptus grandis* × *E. nitens* (G × N) during these rooting experiments; however, high irradiance levels, such as PPFD > 600 $\mu\text{mol s}^{-1} \text{m}^{-2}$, may have been limiting root formation under blue 20 % and Patilite® during spring 2011. There was no clear trend to indicate the optimal radiation intensity at which to grow G × N stock plants to achieve high rooting percentages of mini-cuttings; however, shade nets such as black 30 % and green 40 % with a range of 200 to 450 $\mu\text{mol s}^{-1} \text{m}^{-2}$ PPFD over three or more seasons gave good rooting percentages. Therefore, although coloured shade nets and plastics do not alter the radiation spectrum sufficiently to greatly affect stock plant morphology, the presence of shade netting does benefit plant growth and rooting, particularly with regard to decreasing irradiance levels to a range optimal for root formation. Although manufacturers of coloured shade nets claim that the rate of photosynthesis of plants grown beneath red shade netting is increased and decreased beneath blue shade netting and that the time to flower and fruit ripening is accelerated under red shade netting and delayed under blue shade netting, in the case of G × N mini-hedges the effects were not significant. The common black 30 % and green 40 % shade nets are perfectly acceptable for use in vegetative propagation.

Eucalyptus grandis × *E. nitens* clonal hybrids have been bred to produce a tree more cold-tolerant than *E. grandis* due to the *E. nitens* parent that can survive cold temperatures at high altitudes. Environmental parameters, particularly temperature and RH, can greatly affect the ability of stock plants to produce good quality cutting material with good rooting potential. It was found that the presence of shade net or plastic covering increases minimum, while decreasing maximum temperatures (Table 3.5); additionally, RH increased under all shade nets and plastics, with the exception of black 30 % (Table 3.4). Temperatures in the rooting tunnel during spring 2011 were significantly cooler than during autumn 2011 and summer 2011/12 (Table 3.1), possibly providing conditions for the improved rooting percentages during spring 2011. In the hotter months of the year it is important to keep temperatures

below 30 °C; therefore, in future rooting experiments misters should be activated more frequently or be attached to a sensor that measures temperature and RH.

Maximum temperatures varied significantly under the various nets in the stock plant tunnel, but minimum temperatures did not. In general, the control often had the highest temperature fluctuations (coolest minimum and warmest maximum temperatures) during all six seasons recorded (Table 3.3), while temperatures under Clarix E Blue® were as cool as the control, with temperatures under red 30 %, Patilite® were as warm as the control (Table 3.2); therefore, the presence of any shade net provides a moderating effect on temperature fluctuations and decreases irradiance levels. Thus, the presence of shade nets decreases stock plant temperature stress and reduces the occurrence of photoinhibition. The highest mean RH was recorded under blue 20 % (75.7 %) and the lowest under black 30 % (61.3 %) (Table 3.4); this should be considered when growing a crop or clone prone to fungal diseases as high humidity is likely to exacerbate disease outbreaks.

The high production of mini-cuttings from the control was likely due to high irradiance levels; similarly, the lower number of mini-cuttings produced by green 40 % were likely due to lower irradiance levels (Table 2.1); however, in most seasons as well as clone and fertilizer treatments the green 40 % shade net produced cuttings with a higher rooting percentage than the control (Table 4.3), suggesting that, although fewer mini-cuttings were produced under the green 40 % shade net, they were more efficient as fewer resources are needed to produce the same number of successfully rooted mini-cuttings compared with mini-cuttings produced under the control.

In many *Eucalyptus* nurseries the macro-cutting system has been replaced by a mini-cutting system, in order to maintain juvenility of the stock plants, to improve rooting rates of cuttings, lower production costs and produce a healthy root system. The assessments of the rooting experiments revealed that the fertilizer factor did not significantly alter rooting percentage of mini-cuttings during winter 2011, spring 2011 and summer 2011/12; however, during autumn 2011 mini-cuttings from plants treated with organic fertilizer had better rooting than those treated with inorganic fertilizer. During autumn 2012 the mini-cuttings from plants treated with inorganic fertilizer had better rooting than those treated with organic fertilizer (Fig. 4.2), indicating that the inorganic fertilizer gave more predictable rooting over time. Considering the ease of application and the consistency of results it is recommended

to use an inorganic fertilizer solution rather than organic fertilizer granules until a more efficient application method can be developed.

As expected, clone PP2107 (easy-to-root) rooted consistently higher than clone GN018B (difficult-to-root) over all seasons (Fig. 4.3) and fertilizer regimes (Table 4.1). Although clone GN018B had significantly lower rooting percentages than clone PP2107 for most seasons, clone GN018B had much improved rooting percentages for most shade nets and plastics during spring 2011 (Fig. 4.3, 4.4 and Table 4.3). The coloured shade nets and plastic coverings generally producing the highest rooting percentages, over all seasons, were black 30 %, green 40 %, Clarix E Blue® and Patilite®, while the lowest rooting percentages were found of mini-cuttings collected under Aluminet® 40 % (Fig. 4.5); however, shade nets and plastic coverings that resulted in the highest average rooting percentages changed seasonally (Fig. 4.6), emphasising the difficulty of recommending ‘the best’ shade net or plastic for use in $G \times N$ vegetative propagation.

The highest overall rooting percentage of both $G \times N$ clones together was achieved during spring 2011, while autumn 2011 and 2012 gave the lowest rooting, with winter 2011 and summer 2011/12 displaying intermediate rooting percentages (Table 4.2). In general, the seasons that produced the best root quality of rooted mini-cuttings were autumn and spring 2011, which both produced a high percentage of type 3 and 4 (good and strong) root systems (Fig. 4.9 and 4.11). On the other hand, the season with the worst root quality of rooted mini-cuttings was winter 2011, which had a high percentage of type 1 and 2 (weak and average) roots (Fig. 4.10). The summer 2011/12 and autumn 2012 also had a high percentage of type 1 and 2 (weak and average) root systems (Fig. 4.13). Increased rooting percentages, root quality and number of roots per mini-cutting during spring 2011 may be related to the average temperatures experienced during this season and could be aligned with the low callus percentage of this season (Table 4.2). During all seasons, a high percentage of the rooted mini-cuttings developed callus. Total callus percentage and basal stem diameter (BSD) were greatest, together with intermediate rooting percentages during winter 2011 (Table 4.2); this may indicate that although $G \times N$ clones are cold-tolerant, the optimal temperature range for vegetative propagation lies between 12 to 30 °C (spring 2011 minimum and maximum temperatures, Table 3.3) and mini-cuttings are more sensitive to temperature fluctuations than larger plants. The root quality and callus results seem to indicate that temperatures optimal for rooting (moderate) are different from those optimal

for callus formation, possibly less than 15 °C and greater than 27 °C, for the latter; however, this should be investigated further. The optimal temperature ranges for growing *E. grandis* and *E. nitens* are 16 to 20 °C and 13.5 to 16 °C, respectively. The minimum temperatures under shade nets and plastics in the stock plant tunnel were mostly within this optimal range for G × N plant growth from summer 2010/11 to autumn 2012 (12 to 21 °C) except during winter 2011, when minimum temperatures ranged from 6.8 to 7.8 °C; however, during autumn 2011, spring 2011, summer 2011/12 and autumn 2012 maximum temperatures often exceeded 30 °C in the stock plant tunnel (Table 3.3).

5.2 Conclusion

The replacement of the macro-hedge and macro-cutting system with the mini-hedge and mini-cutting system shows much promise for *Eucalyptus* nurseries in South Africa; however, these systems need careful management and the correct use of infrastructure to maintain stock plants that produce mini-cuttings with a high rooting potential all year round. The common practice in South African nurseries of using clear plastic tunnels and black or green shade netting for growing or hardening-off stock plants and rooted cuttings is suitable, but can be improved upon. When managing a G × N production nursery, extremely high temperatures are a greater limiting factor to root formation than extremely low temperatures, particularly in the rooting tunnel. Spring and autumn, when temperatures are more moderate, seem to be the best seasons to root G × N mini-cuttings. Therefore, if possible, temperatures should be controlled from 12 to 30 °C; choosing a tunnel with a high roof and natural ventilation for stock plant growth and rooting cuttings, together with extraction fans and a wet wall can achieve temperature control; additionally, more efficient temperature control tools, such as overhead misters controlled by sensors measuring temperature and RH (not just a timer) to make use of evaporative cooling should be employed; further, by installing permanent or removable shade netting in the stock plant or rooting tunnel, such as green 40 % or permanently covering the entire outside of the tunnel with plastic, such as Clarix E Blue®, should keep temperatures lower than other coverings due to reduced irradiance levels and temperatures. Alternatively, to counter very cold temperatures, bottom heating in the rooting tunnel can encourage root formation.

At the time of setting up these experiments in 2010, black 30 % was the least costly shade net available followed by blue 20 %, green 40 % and red 30 % (R20.70, R22.14, R22.40 and

R24.15 per m, respectively) and Aluminet® 40 % was the most costly followed by Clarix E Blue® and Patilite® (R28.50, R9.50 and R9.35 per m², respectively). All shade nets were available in rolls of 3 m width, but Clarix E Blue® and Patilite® plastics were only available in rolls of 6.5 and 3.5 m width, respectively. The relative humidity should be higher in the rooting tunnel than in the stock plant tunnel to decrease pest and disease outbreaks on stock plants; thus, it is recommended to use black 30 % or green 40 % for stock plants and Clarix E Blue® or Patilite® for the rooting tunnel, possibly with a removable shade net of 30 to 40 % shading factor.

5.3 Recommendations for future research

Further studies should be carried out on G × N stock plants to investigate the effect of black and green, as well as possibly red and blue, shade nets at varying shading factors, between 30 and 60 %, on rooting.

Further studies should be carried out on other G × N clones as well as other *Eucalyptus* species where a larger experimental area is under one shade net (Clarix E Blue®, black 30 %, green 40 % and red 30 %) to eliminate overlapping effects from adjacent shade nets. This could be done separately or in conjunction with a study to ascertain the beneficial effects on rooting potential of G × N mini-cuttings using Clarix E Blue® and Patilite® plastics in combination with different shade nets such as black 30 % and green 40 %.

A possible oversight of this study was the recording of air temperatures rather than leaf temperature; therefore, the variation between various G × N stock plant leaf temperature and air temperatures near plants under Clarix E Blue® plastic, black 30 % and green 40 % shade nets together with a control should be recorded. Furthermore, in order to investigate the reason for excessive callus production, root zone temperatures of the medium in the cutting trays as well as leaf and air temperatures should be measured in the rooting tunnel or in a controlled environment room where low (5 to 15 °C), medium (15 to 25 °C) and high temperatures (25 to 35 °C) can be consistently controlled.

In order to improve uniformity in *Eucalyptus* nurseries, further investigations into the particular optimal leaf area needed for G × N and other *Eucalyptus* species and hybrid clone mini-cuttings to achieve optimal photosynthesis of the mini-cuttings and to achieve

maximum rooting percentages would be of interest. Labourers should be trained in nurseries to adjust harvesting and cutting procedures to produce mini-cuttings of a uniform optimal LA for photosynthesis.

Lastly, due to the stock plant mortality experienced in this study, it is recommended that commercial nurseries as well as studies involving mini-hedges take stock plant mortality into account at the beginning of the study and build the cost of replacing at least 50 to 80 % of originally planted stock plants over two years into their budgets.

Appendix A – Layouts

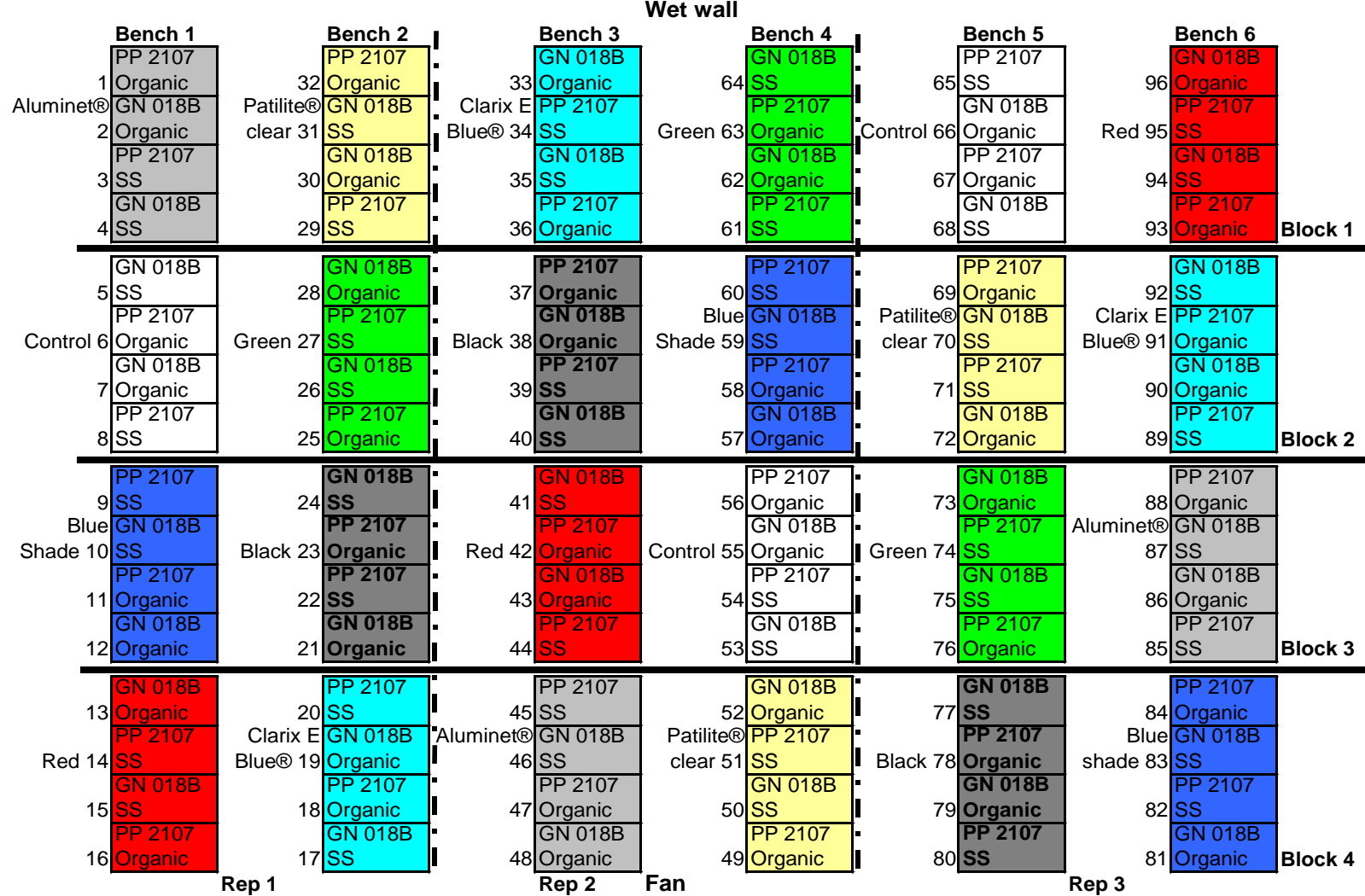


Figure A.1. Tunnel layout of stock plants under shade netting.

Wet wall end of tunnel

Aluminet® 40 %	Patilite® clear plastic
Control	Green 40 %
Blue 20 %	Black 40 %
Red 30 %	Clarix E blue® plastic

Fan end of tunnel

Figure A.2. Layout of the first replication of shade nets in the stock plant tunnel under which the environmental parameters of temperature and relative humidity were measured with thermometers and HOBO®s.

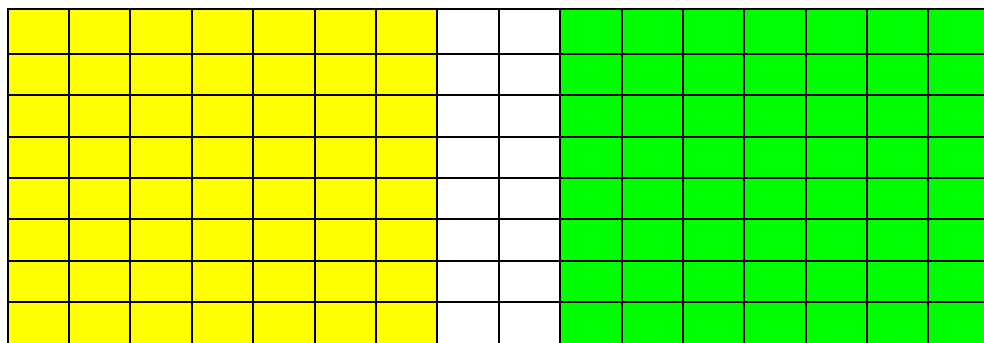


Figure A.3. Layout of a 128 tray for the rooting experiment in autumn 2011 where half a tray (8 rows × 7 columns = 56 mini-cuttings and 2 spaces between) were used for a specific treatment i.e. PP2107, inorganic under red 30 % shade net.

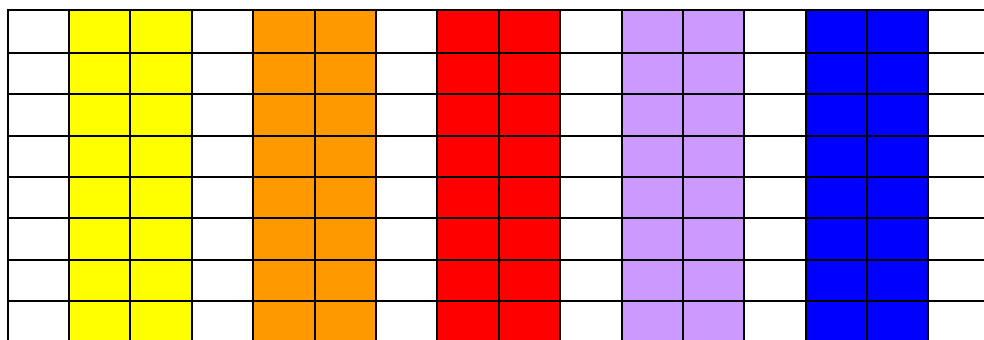


Figure A.4. Layout of a 128 tray for the rooting experiments in winter 2011, spring 2011, summer 2011/12 and autumn 2012 where each specific treatment i.e. PP2107, organic under blue 20 % shade net is split into 3 replications of 8 rows × 2 column = 16 cuttings (each replication would be in a different tray). Giving a total of 16 × 3 = 48 mini-cuttings per treatment.

Appendix B – Additional statistical evaluations, tables, graphs and photographs regarding chapter 2

The paper “Radiation transmission through coloured shade netting and plastics and its effect on *Eucalyptus grandis* × *E. nitens* hybrid mini-hedge shoot internode length, stem diameter and leaf area” was published in: Proceedings of 2nd All Africa Horticulture Congress Eds.: K. Hannweg and M. Penter *Acta Horticulturae* 1007, 773 – 780. ISHS 2013. Due to the paper being limited in length some graphs and detail were omitted. Due to the paper having already gone to print, no further changes could be made to the chapter, therefore, the following comments are to add value or clarify statements that have already been made.

Statistical evaluations:

A factorial design with three factors, where the factor shade net had eight levels (black 30 %, green 40 %, blue 20 %, red 30 %, Aluminet® 40 %, Clarix E Blue®, Patilite® and control), the factor clone had two levels (PP2107 and GN018B) and the factor fertilizer had two levels (organic and inorganic) was used. The shade nets with clone and fertilizer treatments beneath them were replicated three times within the greenhouse. The morphological measurements such as leaf area (LA), internode length and stem diameter were statistically analysed using shade net, clone and fertilizer as factors, but replications varied according to the morphological observational unit measured (e.g. nine shoots chosen per treatment to determine internode length), which are described in the materials and methods section of chapter 2.

The experimental design of the spectral radiation data, however, was collected only with regard to the shade net factor and was, therefore, a completely randomised design. Statistical analyses were carried out using GenStat® 14th edition (VSN International, 2011). All spectral radiation data as well as stem diameter and LA data were analysed by ANOVA and the internode data were analysed by REML as the data were not orthogonal (see tables in Appendix C.1 – Chapter 2).

The term ‘blocking’ used in the published Chapter 2 should rather be called ‘replication’, as the stock plant treatments were replicated three times over six brick beds over the width of the greenhouse, therefore, one complete replication covered two brick beds. Although there may have been some variation caused by shadows over the width of the greenhouse in the

early morning and late afternoon, blocking for this was not necessary as on a clear cloudless day between 12:00 and 14:00 PM readings taken with a handheld Hansatech® quantitherm lightmeter indicated a similar range of light intensity ($\mu\text{mol s}^{-1} \text{m}^{-2}$) under each replication of a specific shade net. When using the more sophisticated and accurate spectroradiometer, it was difficult to move this larger instrument to each shade net treatment; therefore, the spectroradiometer was programmed to take three sets of measurements under each shade net, over one replication of shade nets over a shorter time period. In order to ensure accurate R:NIR calculations, repeated readings of the control (no covering) were used, as the ratios of a specific shade net were calculated using the control reading closest to the time the specific shade net reading was measured; this method was likely to introduce less bias and error than measuring in all three replications as by then the sun was no longer at its zenith.

Tables, graphs and photographs:

Table B.1. Range of Irradiance wavelengths in the visible spectrum.

Colour	Irradiance (nm)	Typical Irradiance (nm)
Violet	390 to 455	430
Dark blue	455 to 485	470
Light blue	485 to 505	495
Green	505 to 550	530
Yellow-green	550 to 575	560
Yellow	575 to 585	580
Orange	585 to 620	600
Red	620 to 760	640

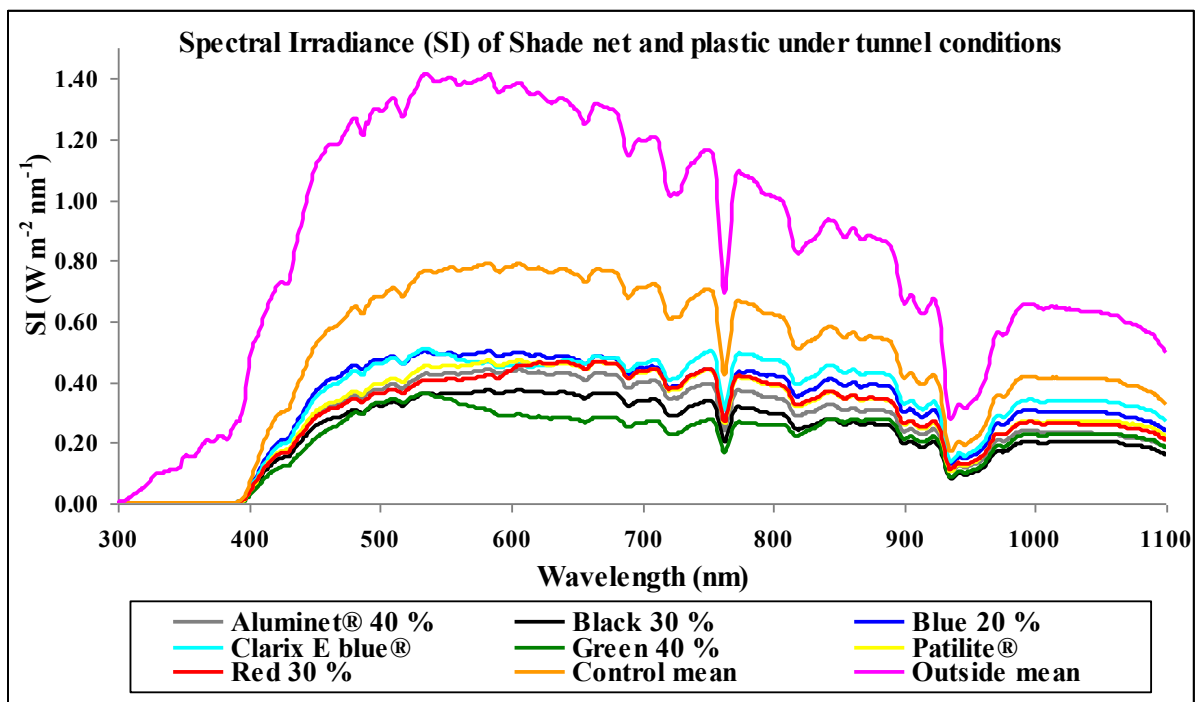


Figure B.1. Spectral irradiance perceived under each of the shade nets and plastics as well as the control, which has no covering besides the hard plastic of the stock plant tunnel and the outside irradiance averaged over the two days readings were taken during spring 2011.

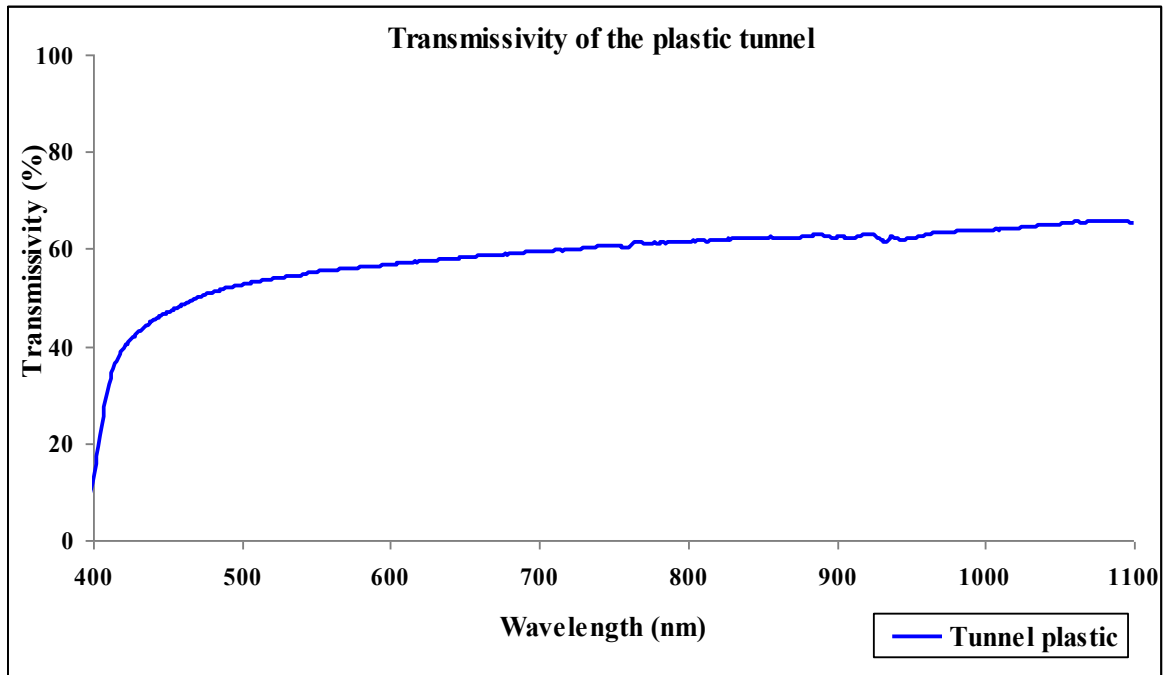


Figure B.2. The transmission of irradiation of the hard plastic of the stock plant tunnel was calculated from the control irradiance divided by the outside irradiance during spring 2011 and appears to let through between 50 and 60% of the natural irradiance.

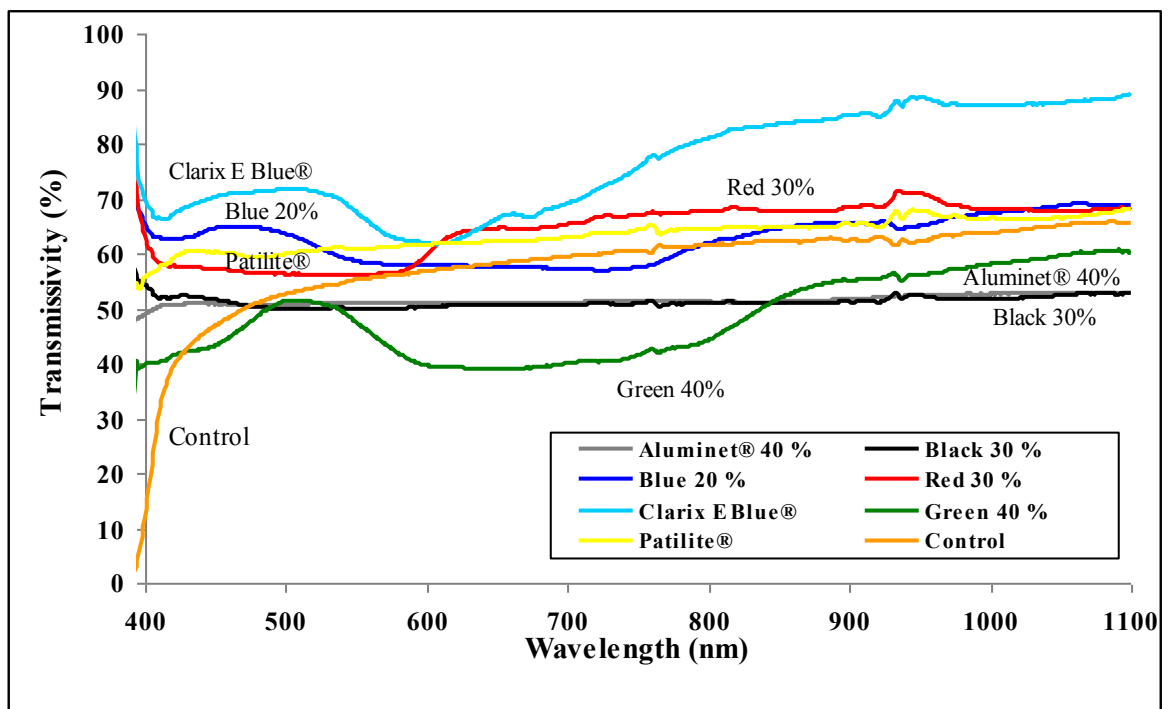


Figure B.3. The transmission of irradiation of each shade net or plastic, calculated from the control irradiance divided by the outside irradiance during spring 2011. Colour representation of figure 2.1 in chapter 2.

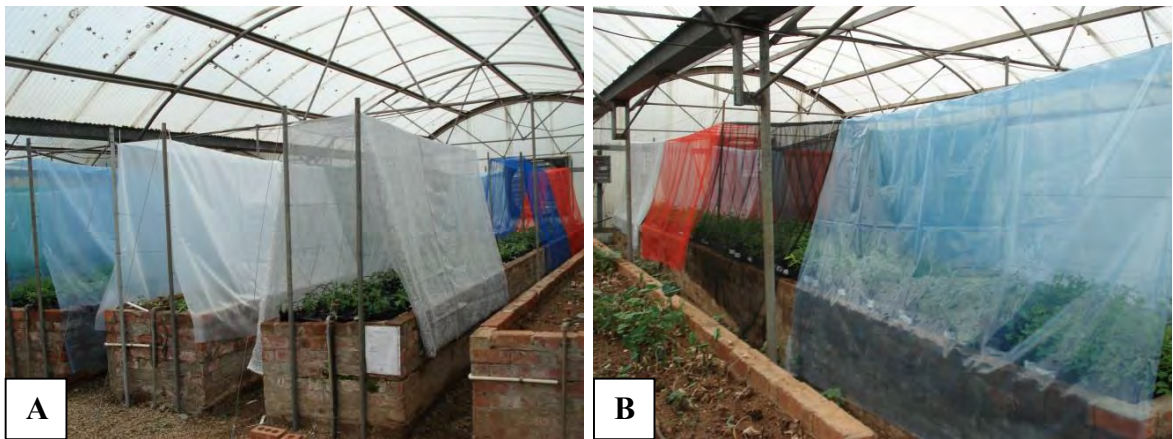


Figure B.4. Arrangement of shade nets and plastic coverings over bricked beds in the stock plant tunnel, looking from the wet wall end toward the fan end of the tunnel.

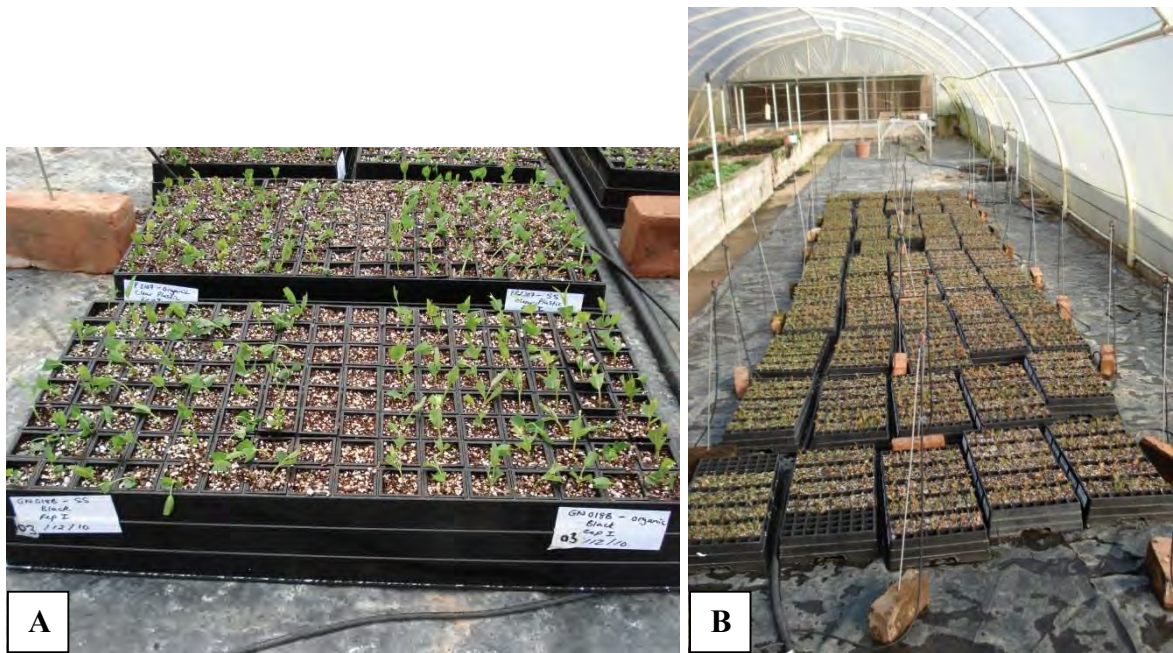


Figure B.5. (A) Mini-cuttings placed in a tray during autumn 2011 and (B) all rooting trays laid out in the rooting tunnel with mist sprayers placed at regular intervals.



Figure B.6. Using the spectroradiometer (A) in the stock plant tunnel showing the sensor on top, (B) outside away from buildings and plants. (C) Setting the parameters on the computer and (D) the spectroradiometer was placed above the stock plants to avoid reflection off leaves that can lead to false readings.

Table B.2. Mean number of stock plants per treatment over time, where final survival and mortality were calculated from the last stock plant count in March 2012 as a percentage of the original number of stock plants at the end of 2010.

			Number of stock plants per treatment						
			Dec 2010	Mar 2011	May 2011	Sep 2011	Mar 2012	Final survival %	Final mortality %
Control	PP2107	Inorganic	24	18	18	10.3	8.3	34.7	65.3
		Organic	24	20	20	10.3	4.3	18.1	81.9
	GN018B	Inorganic	24	20	20	11.7	8.0	33.3	66.7
		Organic	24	21	20	13.0	9.7	40.3	59.7
Black 30%	PP2107	Inorganic	24	18	18	11.0	8.0	33.3	66.7
		Organic	24	20	20	11.7	4.3	18.1	81.9
	GN018B	Inorganic	24	18	18	12.3	10.3	43.1	56.9
		Organic	24	22	22	13.7	11.0	45.8	54.2
Green 40%	PP2107	Inorganic	24	18	16	11.3	10.7	44.4	55.6
		Organic	24	20	18	11.7	2.0	8.3	91.7
	GN018B	Inorganic	24	22	21	11.0	8.3	34.7	65.3
		Organic	24	22	21	13.7	10.0	41.7	58.3
Blue 20%	PP2107	Inorganic	24	17	15	11.3	8.0	33.3	66.7
		Organic	24	20	19	12.0	1.7	6.9	93.1
	GN018B	Inorganic	24	19	19	12.0	10.0	41.7	58.3
		Organic	24	23	23	13.7	8.3	34.7	65.3
Red 30%	PP2107	Inorganic	24	18	14	11.7	9.7	40.3	59.7
		Organic	24	20	20	12.0	4.7	19.4	80.6
	GN018B	Inorganic	24	22	21	12.0	10.3	43.1	56.9
		Organic	24	21	21	13.7	8.7	36.1	63.9
Aluminet® 40%	PP2107	Inorganic	24	17	17	11.0	9.7	40.3	59.7
		Organic	24	20	20	12.0	8.7	36.1	63.9
	GN018B	Inorganic	24	21	22	12.7	11.3	47.2	52.8
		Organic	24	16	16	13.7	10.3	43.1	56.9
Clarix E Blue®	PP2107	Inorganic	24	18	19	11.0	8.7	36.1	63.9
		Organic	24	20	20	12.0	4.7	19.4	80.6
	GN018B	Inorganic	24	20	20	11.3	9.7	40.3	59.7
		Organic	24	24	22	13.7	6.7	27.8	72.2
Patilite®	PP2107	Inorganic	24	18	18	10.7	9.0	37.5	62.5
		Organic	24	20	20	11.0	4.0	16.7	83.3
	GN018B	Inorganic	24	21	20	12.3	8.3	34.7	65.3
		Organic	24	19	19	13.7	5.3	22.2	77.8

Appendix C – Statistical tables

C.1 Chapter 2

Table C.1. Analysis of variance of R:NIR ratio (wide band), at 5 % level of significance, relevant to section 2.3.1 and Table 2.2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Shade	8	0.07254572	0.00906821	128.98	<.001
Season	2	0.00880292	0.00440146	62.60	<.001
Residual	16	0.00112489	0.00007031		
Total	26	0.08247353			

Table C.2. Analysis of variance of R:NIR ratio (narrow band), at 5 % level of significance, relevant to section 2.3.1 and Table 2.2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Shade	8	0.03369125	0.00421141	46.61	<.001
Season	2	0.02349436	0.01174718	130.01	<.001
Residual	16	0.00144567	0.00009035		
Total	26	0.05863128			

Table C.3. Analysis of variance of B:R ratio, at 5 % level of significance, relevant to section 2.3.1 and Table 2.2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Shade	8	0.1096023	0.0137003	33.93	<.001
Season	2	0.0087550	0.0043775	10.84	0.001
Residual	16	0.0064605	0.0004038		
Total	26	0.1248179			

Table C.4. Analysis of variance of PPF, at 5 % level of significance, relevant to section 2.3.1 and Table 2.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Shade	8	2455646.	306956.	28.23	<.001
Season	2	597845.	298922.	27.50	<.001
Residual	16	173944.	10871.		
Total	26	3227434.			

Table C.5. Stem internode lengths during autumn 2011 analysed using REML tests for fixed effects, at 5 % level of significance, relevant to section 2.3.2 and Table 2.4.

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade	14.61	7	2.09	84.9	0.054
Clone	122.63	3	40.37	37.6	<0.001
Fert	6.26	1	6.26	83.8	0.014
Shade.Clone	24.53	21	1.15	74.7	0.319
Shade.Fert	7.50	7	1.07	83.8	0.389
Clone.Fert	4.73	3	1.58	83.8	0.202
Shade.Clone.Fert	37.92	21	1.81	83.8	0.031
Dropping individual terms from full fixed model					
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade.Clone.Fert	37.92	21	1.81	83.8	0.031

Table C.6. Stem internode lengths during winter 2011 analysed using REML tests for fixed effects, at 5 % level of significance, relevant to section 2.3.2 and Table 2.4.

Sequentially adding terms to fixed model					
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade	7.99	7	1.14	57.7	0.350
Clone	92.69	1	92.69	56.0	<0.001
Fert	72.20	1	72.20	56.0	<0.001
Shade.Clone	15.08	7	2.15	56.0	0.052
Shade.Fert	15.19	7	2.17	56.0	0.051
Clone.Fert	27.56	1	27.56	56.0	<0.001
Shade.Clone.Fert	34.33	7	4.90	56.0	<0.001
Dropping individual terms from full fixed model					
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade.Clone.Fert	34.33	7	4.90	56.0	<0.001

Table C.7. Stem internode lengths during spring 2011 analysed using REML tests for fixed effects, at 5 % level of significance, relevant to section 2.3.2 and Table 2.4.

Sequentially adding terms to fixed model					
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade	13.55	7	1.88	41.4	0.097
Clone	6.14	1	6.14	54.6	0.016
Fert	34.43	1	34.43	54.6	<0.001
Shade.Clone	8.20	7	1.17	54.6	0.335
Shade.Fert	4.47	7	0.64	54.6	0.723
Clone.Fert	0.03	1	0.03	54.6	0.869
Shade.Clone.Fert	2.85	7	0.41	54.6	0.893
Dropping individual terms from full fixed model					
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Shade.Clone.Fert	2.85	7	0.41	54.6	0.893

Table C.8. Analysis of variance of leaf area (cm²), at 5 % level of significance, relevant to section 2.3.2 and Table 2.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0805	0.5402	1.84	
Rep.*Units* stratum					
Shade_Net	7	3.2410	0.4630	1.57	0.160
Clone	1	51.6413	51.6413	175.49	<.001
Fertiliser_Treatment	1	28.7657	28.7657	97.75	<.001
Shade_Net.Clone	7	3.5615	0.5088	1.73	0.119
Shade_Net.Fertiliser_Treatment	7	12.4228	1.7747	6.03	<.001
Clone.Fertiliser_Treatment	1	0.2233	0.2233	0.76	0.387
Shade_Net.Clone.Fertiliser_Treatment	7	2.2591	0.3227	1.10	0.377
Residual	62	18.2448	0.2943		
Total	95	121.4399			

Table C.9. Correlation between internode (cm) and leaf area (cm²) using Spearman's rank coefficient, relevant to section 2.3.2.

Internode	1	1.000	-
LA	2	0.379	1.000
		1	2
Sample size:	96		

C.2 Chapter 3

Table C.10. Analysis of variance of temperature over seasons recorded in the stock plant tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Season	4	127201.04	31800.26	900.69	<.001
Residual	10479	369975.77	35.31		
Total	10483	497176.82			

Table C.11. Analysis of variance of temperature over months recorded in the stock plant tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Month	15	155441.23	10362.75	317.43	<.001
Residual	10468	341735.59	32.65		
Total	10483	497176.82			

Table C.12. Analysis of variance of relative humidity (RH) over seasons recorded in the stock plant tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Season	4	47955.3	11988.8	34.28	<.001
Residual	10479	3665114.3	349.8		
Total	10483	3713069.6			

Table C.13. Analysis of variance of relative humidity (RH) over months recorded in the stock plant tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Month	15	96196.7	6413.1	18.56	<.001
Residual	10468	3616873.0	345.5		
Total	10483	3713069.6			

Table C.14. Analysis of variance of temperature over seasons recorded in the rooting tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Season	4	37078.46	9269.61	199.88	<.001
Residual	5797	268834.40	46.37		
Total	5801	305912.85			

Table C.15. Analysis of variance of temperature over months recorded in the rooting tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Month	11	45067.58	4097.05	90.94	<.001
Residual	5790	260845.27	45.05		
Total	5801	305912.85			

Table C.16. Analysis of variance of relative humidity (RH) over seasons recorded in the rooting tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Season	4		16270.5	4067.6	8.43	<.001
Residual	5794	(3)	2797341.0	482.8		
Total	5798	(3)	2813600.4			

Table C.17. Analysis of variance of relative humidity (RH) over months recorded in the rooting tunnel, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.1.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Month	11		59030.0	5366.4	11.27	<.001
Residual	5787	(3)	2754580.5	476.0		
Total	5798	(3)	2813600.4			

Table C.18. Analysis of variance of minimum temperatures recorded with min/max thermometers under various shade nets over seasons in the stock plant tunnel, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.2 and 3.3.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade	7	37.84	5.41		
Residual	-4	0.00			
Block.*Units* stratum					
Shade	7	78.07	11.15	0.90	0.503
Season	5	24014.86	4802.97	388.89	<.001
Shade.Season	35	17.05	0.49	0.04	1.000
Residual	1117	13795.33	12.35		
Total	1167	37943.15			

Table C.19. Analysis of variance of maximum temperatures recorded with min/max thermometers under various shade nets over seasons in the stock plant tunnel, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.2 and 3.3.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade	7	268.38	38.34		
Residual	-4	0.00			
Block.*Units* stratum					
Shade	7	441.24	63.03	3.11	0.003
Season	5	10338.06	2067.61	101.92	<.001
Shade.Season	35	63.16	1.80	0.09	1.000
Residual	1117	22659.18	20.29		
Total	1167	33770.03			

Table C. 20. Analysis of variance of mean temperatures recorded with HOBO® data loggers under various shade nets during spring 2011, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade	7	1679.31	239.90		
Residual	-4	0.00			
Block.*Units* stratum					
Shade	7	528.08	75.44	2.21	0.030
Week	5	4102.96	820.59	24.07	<.001
Shade.Week	35	454.63	12.99	0.38	1.000
Residual	7069	240994.48	34.09		
Total	7119	247760.50			

Table C. 21. Analysis of variance of mean relative humidity (RH) recorded with HOBO® data loggers under various shade nets during spring 2011, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade	7	32730.2	4675.7		
Residual	-4	0.0			
Block.*Units* stratum					
Shade	7	98991.2	14141.6	35.83	<.001
Week	5	107079.2	21415.8	54.26	<.001
Shade.Week	35	19199.4	548.6	1.39	0.063
Residual	7069	2790129.0	394.7		
Total	7119	3048136.5			

Table C. 22. Analysis of variance of minimum temperatures recorded with min/max thermometers under various shade nets in 2011 under shade nets and after removal of the nets in 2012 at the same time of year, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.5.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade net	7	3.81	0.54		
Residual	-4	0.00			
Block.*Units* stratum					
Shade net	7	11.57	1.65	0.16	0.993
Cover	1	283.36	283.36	26.65	<.001
Shade net.Cover	7	0.75	0.11	0.01	1.000
Residual	125	1329.17	10.63		
Total	143	1628.66			

Table C. 23. Analysis of variance of maximum temperatures recorded with min/max thermometers under various shade nets in 2011 under shade nets and after removal of the nets in 2012 at the same time of year, with blocking, at 5 % level of significance, relevant to section 3.3.1.1 and Table 3.5.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum					
Shade net	7	24.056	3.437		
Residual	-4	0.000			
Block.*Units* stratum					
Shade net	7	43.750	6.250	0.65	0.712
Cover	1	680.340	680.340	70.94	<.001
Shade net.Cover	7	43.160	6.166	0.64	0.720
Residual	125	1198.833	9.591		
Total	143	1990.139			

Table C. 24. Analysis of variance of production of mini-cuttings per stock plant in spring 2011 under shade nets, clone and fertilizer factors, at 5 % level of significance, relevant to section 3.3.2.1 and Table 3.6.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	12.9316	6.4658	16.85	
Rep.*Units* stratum					
Clone	1	0.7344	0.7344	1.91	0.172
Fert	1	0.2468	0.2468	0.64	0.426
Shade	7	12.1971	1.7424	4.54	<.001
Clone.Fert	1	2.4023	2.4023	6.26	0.015
Clone.Shade	7	1.1615	0.1659	0.43	0.878
Fert.Shade	7	2.2965	0.3281	0.85	0.547
Clone.Fert.Shade	7	3.9507	0.5644	1.47	0.194
Residual	62	23.7967	0.3838		
Total	95	59.7176			

Table C. 25. Analysis of variance of production of mini-cuttings per stock plant over four seasons under various shade nets, at 5 % level of significance, relevant to section 3.3.2.1 and Table 3.7 and Fig. 3.5.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Shade	7	37.4749	5.3536	8.33	<.001
Clone	1	0.4853	0.4853	0.75	0.395
Fert	1	19.0679	19.0679	29.65	<.001
Season	3	282.7459	94.2486	146.57	<.001
Shade.Clone	7	5.8703	0.8386	1.30	0.296
Shade.Fert	7	14.3906	2.0558	3.20	0.018
Clone.Fert	1	0.5793	0.5793	0.90	0.353
Shade.Season	21	53.0085	2.5242	3.93	0.001
Clone.Season	3	9.9418	3.3139	5.15	0.008
Fert.Season	3	32.8361	10.9454	17.02	<.001
Shade.Clone.Fert	7	19.9532	2.8505	4.43	0.004
Shade.Clone.Season	21	19.0075	0.9051	1.41	0.220
Shade.Fert.Season	21	23.4522	1.1168	1.74	0.107
Clone.Fert.Season	3	12.3281	4.1094	6.39	0.003
Residual	21	13.5039	0.6430		
Total	127	544.6455			

Table C. 26. Analysis of variance of dry mass (g) of mini-cuttings from various shade nets and clones, at 5 % level of significance, relevant to section 3.3.2.2 and Table 3.8 and Fig. 3.6.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.079105	0.039552	14.91	
Rep.*Units* stratum					
Shade	7	0.006854	0.000979	0.37	0.917
Clone	1	0.012353	0.012353	4.66	0.035
Plant_part	1	0.497261	0.497261	187.51	<.001
Shade.Clone	7	0.009874	0.001411	0.53	0.807
Shade.Plant_part	7	0.010422	0.001489	0.56	0.784
Clone.Plant_part	1	0.006814	0.006814	2.57	0.114
Shade.Clone.Plant_part	7	0.003184	0.000455	0.17	0.990
Residual	62	0.164422	0.002652		
Total	95	0.790289			

Table C. 27. Analysis of variance of dry mass (%) of mini-cuttings from various shade nets and clones, at 5 % level of significance, relevant to section 3.3.2.2 and Table 3.8 and Fig. 3.6.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	127.851	63.926	9.78	
Rep.*Units* stratum					
Shade	7	99.485	14.212	2.18	0.049
Clone	1	72.774	72.774	11.14	0.001
Plant_part	1	1.030	1.030	0.16	0.693
Shade.Clone	7	30.004	4.286	0.66	0.708
Shade.Plant_part	7	2.632	0.376	0.06	1.000
Clone.Plant_part	1	1.520	1.520	0.23	0.631
Shade.Clone.Plant_part	7	3.604	0.515	0.08	0.999
Residual	62	405.123	6.534		
Total	95	744.023			

Table C. 28. Analysis of variance of leaf area (LA) (cm²) of mini-cuttings from various shade nets and clones, at 5 % level of significance, relevant to section 3.3.2.3 and Table 3.9.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	4	55.024	13.756	3.34	
Rep.*Units* stratum					
Shade	7	193.920	27.703	6.72	<.001
Clone	1	5.269	5.269	1.28	0.263
Shade.Clone	7	33.483	4.783	1.16	0.339
Residual	60	247.459	4.124		
Total	79	535.154			

Table C. 29. Analysis of variance of stem diameter (mm) of mini-cuttings from various shade nets and clones, at 5 % level of significance, relevant to section 3.3.2.3 and Table 3.9.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	4	0.62914	0.15729	2.70	
Rep.*Units* stratum					
Shade	7	1.20927	0.17275	2.96	0.010
Clone	1	0.44551	0.44551	7.64	0.008
Shade.Clone	7	1.45456	0.20779	3.56	0.003
Residual	60	3.49802	0.05830		
Total	79	7.23650			

C.3 Chapter 4

Table C. 30. Analysis of variance of rooting percentage of mini-cuttings grown under various shade nets, clones and fertilizer factors over five seasons, at 5 % level of significance, relevant to section 4.3.1, 4.3.2 and 4.3.3 and Table 4.1, 4.2, Fig. 4.2, 4.3, 4.4, 4.5 and 4.6.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		1867.22	266.75	3.83	0.006
Clone	1		6668.34	6668.34	95.86	<.001
Fert	1		8.41	8.41	0.12	0.731
Season	4		1698.14	424.53	6.10	0.001
Shade.Clone	7		1045.05	149.29	2.15	0.076
Shade.Fert	7		1931.84	275.98	3.97	0.005
Clone.Fert	1		10.67	10.67	0.15	0.699
Shade.Season	25	(3)	3224.78	128.99	1.85	0.065
Clone.Season	4		1252.43	313.11	4.50	0.007
Fert.Season	4		1191.68	297.92	4.28	0.009
Shade.Clone.Fert	7		588.45	84.06	1.21	0.335
Shade.Clone.Season	25	(3)	3633.18	145.33	2.09	0.036
Shade.Fert.Season	25	(3)	4714.56	188.58	2.71	0.008
Clone.Fert.Season	4		1124.71	281.18	4.04	0.012
Residual	25	(3)	1739.04	69.56		
Total	147	(12)	28267.21			

Table C. 31. Analysis of variance of rooting percentage of mini-cuttings grown under control (no shade) treatment over five seasons, at 5 % level of significance, relevant to section 4.3.3 and Fig. 4.7.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Clone	1	770.35	770.35	9.45	0.003
Fert	1	1.77	1.77	0.02	0.883
Season	4	1567.07	391.77	4.80	0.002
Clone.Fert	1	72.26	72.26	0.89	0.351
Clone.Season	4	966.40	241.60	2.96	0.027
Fert.Season	4	1174.02	293.51	3.60	0.011
Clone.Fert.Season	4	335.30	83.83	1.03	0.401
Residual	56	4565.97	81.54		
Total	75	9453.16			

Table C. 32. Analysis of variance of root number per mini-cutting grown under various shade nets, clones and fertilizer factors over five seasons, at 5 % level of significance, relevant to section 4.3.3 and Table 4.2.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		5.7789	0.8256	0.92	0.490
Clone	1		103.5727	103.5727	115.48	<.001
Fert	1		0.3586	0.3586	0.40	0.528
Season	4		35.1056	8.7764	9.79	<.001
Shade.Clone	7		10.2136	1.4591	1.63	0.126
Shade.Fert	7		11.5840	1.6549	1.85	0.077
Clone.Fert	1		4.6206	4.6206	5.15	0.024
Shade.Season	25	(3)	54.9323	2.1973	2.45	<.001
Clone.Season	4		32.8620	8.2155	9.16	<.001
Fert.Season	4		4.5422	1.1355	1.27	0.283
Shade.Clone.Fert	7		9.2805	1.3258	1.48	0.173
Shade.Clone.Season	25	(3)	47.9099	1.9164	2.14	0.001
Shade.Fert.Season	25	(3)	55.9164	2.2367	2.49	<.001
Clone.Fert.Season	4		2.8845	0.7211	0.80	0.523
Residual	388	(152)	347.9814	0.8969		
Total	510	(161)	632.0950			

Table C. 33. Analysis of variance of total callus percentage of mini-cuttings grown under various shade nets, clones and fertilizer factors over five seasons, at 5 % level of significance, relevant to section 4.3.3 and Table 4.2.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		3419.2	488.5	1.38	0.213
Clone	1		2615.5	2615.5	7.37	0.007
Fert	1		5283.8	5283.8	14.90	<.001
Season	4		68806.9	17201.7	48.50	<.001
Shade.Clone	7		4976.8	711.0	2.00	0.053
Shade.Fert	7		10579.7	1511.4	4.26	<.001
Clone.Fert	1		2192.5	2192.5	6.18	0.013
Shade.Season	28		38341.2	1369.3	3.86	<.001
Clone.Season	4		19121.6	4780.4	13.48	<.001
Fert.Season	4		2086.2	521.5	1.47	0.210
Shade.Clone.Fert	7		5214.0	744.9	2.10	0.042
Shade.Clone.Season	28		16867.7	602.4	1.70	0.015
Shade.Fert.Season	28		37660.3	1345.0	3.79	<.001
Clone.Fert.Season	4		1971.7	492.9	1.39	0.236
Residual	476	(64)	168838.9	354.7		
Total	607	(64)	349846.5			

Table C. 34. Analysis of variance of percentage of mini-cuttings grown under various shade nets, clones and fertilizer factors over five seasons developed callus and roots, at 5 % level of significance, relevant to section 4.3.3 and Table 4.2.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		5904.5	843.5	4.91	<.001
Clone	1		15393.0	15393.0	89.57	<.001
Fert	1		72.1	72.1	0.42	0.518
Season	4		12780.1	3195.0	18.59	<.001
Shade.Clone	7		2018.7	288.4	1.68	0.112
Shade.Fert	7		4028.8	575.5	3.35	0.002
Clone.Fert	1		18.9	18.9	0.11	0.740
Shade.Season	28		13510.5	482.5	2.81	<.001
Clone.Season	4		2014.6	503.7	2.93	0.021
Fert.Season	4		1689.6	422.4	2.46	0.045
Shade.Clone.Fert	7		792.2	113.2	0.66	0.707
Shade.Clone.Season	28		9094.5	324.8	1.89	0.004
Shade.Fert.Season	28		12846.4	458.8	2.67	<.001
Clone.Fert.Season	4		2158.4	539.6	3.14	0.014
Residual	476	(64)	81804.6	171.9		
Total	607	(64)	154390.9			

Table C. 35. Analysis of variance of basal stem diameter (BSD) of mini-cuttings grown under various shade nets, clones and fertilizer factors over five seasons developed callus and roots, at 5 % level of significance, relevant to section 4.3.3 and Table 4.2.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		4.5037	0.6434	1.71	0.105
Clone	1		11.1566	11.1566	29.65	<.001
Fert	1		2.2418	2.2418	5.96	0.015
Season	4		19.7449	4.9362	13.12	<.001
Shade.Clone	7		4.9589	0.7084	1.88	0.071
Shade.Fert	7		9.0578	1.2940	3.44	0.001
Clone.Fert	1		0.6934	0.6934	1.84	0.175
Shade.Season	25	(3)	31.7239	1.2690	3.37	<.001
Clone.Season	4		4.8347	1.2087	3.21	0.013
Fert.Season	4		1.8293	0.4573	1.22	0.304
Shade.Clone.Fert	7		1.4555	0.2079	0.55	0.794
Shade.Clone.Season	25	(3)	20.4913	0.8197	2.18	0.001
Shade.Fert.Season	25	(3)	26.1257	1.0450	2.78	<.001
Clone.Fert.Season	4		1.0629	0.2657	0.71	0.588
Residual	389	(151)	146.3882	0.3763		
Total	511	(160)	239.1236			

Table C. 36. Rooting percentage of mini-cuttings grown under various shade nets, clones and fertilizer factors over two autumn seasons 2011 and 2012, analysed using REML and Wald tests for fixed effects, at 5 % level of significance, relevant to section 4.3.3 and Fig. 4.8.

Sequentially adding terms to fixed model				
Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Shade	22.86	7	3.27	0.002
Clone	60.62	1	60.62	<0.001
Fert	12.11	1	12.11	<0.001
Season	1.74	1	1.74	0.188
Shade.Clone	43.58	7	6.23	<0.001
Shade.Fert	64.05	7	9.15	<0.001
Clone.Fert	20.81	1	20.81	<0.001
Shade.Season	60.69	7	8.67	<0.001
Clone.Season	0.60	1	0.60	0.440
Fert.Season	13.18	1	13.18	<0.001
Shade.Clone.Fert	11.69	7	1.67	0.111
Shade.Clone.Season	29.87	7	4.27	<0.001
Shade.Fert.Season	35.47	7	5.07	<0.001
Clone.Fert.Season	10.83	1	10.83	<0.001
Shade.Clone.Fert.Season	12.39	7	1.77	0.089
Dropping individual terms from full fixed model				
Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Shade.Clone.Fert.Season	12.39	7	1.77	0.089

Table C. 37. Analysis of variance of rooting percentage of mini-cuttings according to the labourer who placed the mini-cutting during winter 2011, at 5 % level of significance, relevant to section 4.3.4 and Table 4.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	60.0	30.0	0.10	
Rep.*Units* stratum					
Labourer	3	12473.7	4157.9	14.16	<.001
Residual	282	82789.5	293.6		
Total	287	95323.2			

Table C. 38. Analysis of variance of rooting percentage of mini-cuttings according to the labourer who placed the mini-cutting during spring 2011, at 5 % level of significance, relevant to section 4.3.4 and Table 4.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1658.8	829.4	3.21	
Rep.*Units* stratum					
Labourer	4	4256.9	1064.2	4.12	0.004
Residual	89	23017.4	258.6		
Total	95	28933.2			

Table C. 39. Analysis of variance of rooting percentage of mini-cuttings according to the labourer who placed the mini-cutting during summer 2011/12, at 5 % level of significance, relevant to section 4.3.4 and Table 4.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	74.1	37.0	0.14	
Rep.*Units* stratum					
Labourer	1	1601.8	1601.8	5.87	0.017
Residual	92	25119.4	273.0		
Total	95	26795.2			

Table C. 40. Analysis of variance of rooting percentage of mini-cuttings according to the labourer who placed the mini-cutting during autumn 2012, at 5 % level of significance, relevant to section 4.3.4 and Table 4.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	237.6	118.8	0.47	
Rep.*Units* stratum					
Labourer	3	3256.1	1085.4	4.27	0.007
Residual	90	22902.4	254.5		
Total	95	26396.1			

Table C. 41. Analysis of variance of root quality of mini-cuttings assessed as four root types over five seasons, at 5 % level of significance, relevant to section 4.3.5.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Season stratum	4	9218.7	2304.7	5.16	
Season.*Units* stratum					
Root_type	3	12532.4	4177.5	9.35	<.001
Shade	7	3000.0	428.6	0.96	0.461
Clone	1	250.0	250.0	0.56	0.455
Fert	1	62.5	62.5	0.14	0.709
Root_type.Shade	21	15482.3	737.3	1.65	0.035
Root_type.Clone	3	911.6	303.9	0.68	0.565
Shade.Clone	7	1000.0	142.9	0.32	0.945
Root_type.Fert	3	1038.8	346.3	0.77	0.509
Shade.Fert	7	1187.5	169.6	0.38	0.914
Clone.Fert	1	562.5	562.5	1.26	0.262
Root_type.Shade.Clone	21	9797.4	466.5	1.04	0.408
Root_type.Shade.Fert	21	14357.6	683.7	1.53	0.062
Root_type.Clone.Fert	3	1875.1	625.0	1.40	0.242
Shade.Clone.Fert	7	687.5	98.2	0.22	0.981
Residual	529	236477.3	447.0		
Total	639	308441.1			

Table C. 42. Analysis of variance of root quality of mini-cuttings assessed as four root types during autumn 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.9.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Root_type	3	2172.3	724.1	3.01	0.053
Shade	7	17421.9	2488.8	10.34	<.001
Clone	1	78.1	78.1	0.32	0.575
Fert	1	78.1	78.1	0.32	0.575
Root_type.Shade	21	8653.2	412.1	1.71	0.113
Root_type.Clone	3	1819.1	606.4	2.52	0.086
Shade.Clone	7	546.9	78.1	0.32	0.934
Root_type.Fert	3	1940.4	646.8	2.69	0.073
Shade.Fert	7	546.9	78.1	0.32	0.934
Clone.Fert	1	78.1	78.1	0.32	0.575
Root_type.Shade.Clone	21	4714.3	224.5	0.93	0.563
Root_type.Shade.Fert	21	10648.5	507.1	2.11	0.048
Root_type.Clone.Fert	3	1759.5	586.5	2.44	0.093
Shade.Clone.Fert	7	546.9	78.1	0.32	0.934
Residual	21	5053.7	240.7		
Total	127	56057.9			

Table C. 43. Analysis of variance of root quality of mini-cuttings assessed as four root types during winter 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.10.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Root_type	3	11827.8	3942.6	29.14	<.001
Shade	7	0.0	0.0	0.00	1.000
Clone	1	0.0	0.0	0.00	1.000
Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade	21	3494.4	166.4	1.23	0.320
Root_type.Clone	3	4417.3	1472.4	10.88	<.001
Shade.Clone	7	0.0	0.0	0.00	1.000
Root_type.Fert	3	9.3	3.1	0.02	0.995
Shade.Fert	7	0.0	0.0	0.00	1.000
Clone.Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade.Clone	21	5921.3	282.0	2.08	0.050
Root_type.Shade.Fert	21	3071.9	146.3	1.08	0.430
Root_type.Clone.Fert	3	284.2	94.7	0.70	0.562
Shade.Clone.Fert	7	0.0	0.0	0.00	1.000
Residual	21	2840.9	135.3		
Total	127	31867.1			

Table C. 44. Analysis of variance of root quality of mini-cuttings assessed as four root types during spring 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.11.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Root_type	3	10895.3	3631.8	11.97	<.001
Shade	7	0.0	0.0	0.00	1.000
Clone	1	0.0	0.0	0.00	1.000
Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade	21	9456.5	450.3	1.48	0.186
Root_type.Clone	3	1933.2	644.4	2.12	0.128
Shade.Clone	7	0.0	0.0	0.00	1.000
Root_type.Fert	3	1798.4	599.5	1.98	0.148
Shade.Fert	7	0.0	0.0	0.00	1.000
Clone.Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade.Clone	21	10713.9	510.2	1.68	0.121
Root_type.Shade.Fert	21	12150.1	578.6	1.91	0.074
Root_type.Clone.Fert	3	1277.4	425.8	1.40	0.270
Shade.Clone.Fert	7	0.0	0.0	0.00	1.000
Residual	21	6369.4	303.3		
Total	127	54594.2			

Table C. 45. Analysis of variance of root quality of mini-cuttings assessed as four root types during summer 2011/12, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.12.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Root_type	3	13578.4	4526.1	11.00	<.001
Shade	7	937.5	133.9	0.33	0.934
Clone	1	312.5	312.5	0.76	0.393
Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade	21	10320.3	491.4	1.19	0.344
Root_type.Clone	3	1091.1	363.7	0.88	0.465
Shade.Clone	7	937.5	133.9	0.33	0.934
Root_type.Fert	3	984.5	328.2	0.80	0.509
Shade.Fert	7	1250.0	178.6	0.43	0.870
Clone.Fert	1	0.0	0.0	0.00	1.000
Root_type.Shade.Clone	21	12691.2	604.3	1.47	0.193
Root_type.Shade.Fert	21	8654.8	412.1	1.00	0.498
Root_type.Clone.Fert	3	504.4	168.1	0.41	0.748
Shade.Clone.Fert	7	1250.0	178.6	0.43	0.870
Residual	21	8637.7	411.3		
Total	127	61149.8			

Table C. 46. Analysis of variance of root quality of mini-cuttings assessed as four root types during autumn 2012, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.13.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Root_type	3	17615.1	5871.7	12.67	<.001
Shade	7	1171.9	167.4	0.36	0.915
Clone	1	78.1	78.1	0.17	0.686
Fert	1	78.1	78.1	0.17	0.686
Root_type.Shade	21	19955.2	950.2	2.05	0.054
Root_type.Clone	3	1579.4	526.5	1.14	0.357
Shade.Clone	7	3046.9	435.3	0.94	0.498
Root_type.Fert	3	832.6	277.5	0.60	0.623
Shade.Fert	7	3046.9	435.3	0.94	0.498
Clone.Fert	1	1953.1	1953.1	4.21	0.053
Root_type.Shade.Clone	21	13102.8	623.9	1.35	0.251
Root_type.Shade.Fert	21	19160.8	912.4	1.97	0.064
Root_type.Clone.Fert	3	3027.9	1009.3	2.18	0.121
Shade.Clone.Fert	7	1171.9	167.4	0.36	0.915
Residual	21	9732.7	463.5		
Total	127	95553.3			

Table C.47. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) over five seasons, relevant to section 4.3.5 and 4.3.6.

	1	2	3	4	5
Rooting %	1	-			
Callus_total	2	0.5392	-		
Callus_with_root	3	0.9446	0.5815	-	
Root_number	4	0.4310	0.1091	0.4370	-
BSD	5	0.2040	0.2655	0.3113	0.2015

Number of observations: 473

Table C.48. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) during autumn 2011, relevant to section 4.3.5 and 4.3.6 and Fig. 4.14, 4.19 and Table 4.5.

Rooting %	1	-				
Callus_total	2	0.7901	-			
Callus_with_root	3	0.9434	0.8683	-		
Root_number	4	0.7889	0.7215	0.7281	-	
BSD	5	0.1089	-0.0425	0.0653	0.1519	-
		1	2	3	4	5

Number of observations: 20

Table C.49. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) during winter 2011, relevant to section 4.3.5 and 4.3.6 and Fig. 4.15, 4.20 and Table 4.5.

Rooting %	1	-				
Callus_total	2	0.6256	-			
Callus_with_root	3	0.9473	0.6614	-		
Root_number	4	0.5194	0.2606	0.5000	-	
BSD	5	0.1660	0.2898	0.2893	0.1535	-
		1	2	3	4	5

Number of observations: 230

Table C.50. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) during spring 2011, relevant to section 4.3.5 and 4.3.6 and Fig. 4.16, 4.21 and Table 4.5.

Rooting %	1	-				
Callus_total	2	0.3591	-			
Callus_with_root	3	0.9505	0.4118	-		
Root_number	4	0.2888	-0.0770	0.3186	-	
BSD	5	0.2238	0.0871	0.3056	0.3654	-
		1	2	3	4	5

Number of observations: 86

Table C.51. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) during summer 2011/12, relevant to section 4.3.5 and 4.3.6 and Fig. 4.17, 4.22 and Table 4.5.

Rooting %	1	-				
Callus_total	2	0.7276	-			
Callus_with_root	3	0.9217	0.7761	-		
Root_number	4	0.3298	0.2525	0.4244	-	
BSD	5	0.4084	0.3686	0.5178	0.4252	-
		1	2	3	4	5

Number of observations: 75

Table C.52. Correlations between rooting percentage, total callus percentage, callus with roots, root number per mini-cutting and basal stem diameter (BSD) during autumn 2012, relevant to section 4.3.5 and 4.3.6 and Fig. 4.18, 4.23 and Table 4.5.

Rooting %	1	-				
Callus_total	2	0.6429	-			
Callus_with_root	3	0.9587	0.7247	-		
Root_number	4	0.4178	0.3662	0.4571	-	
BSD	5	0.0822	0.2576	0.1772	-0.0133	-
		1	2	3	4	5

Number of observations: 62

Table C. 53. Analysis of variance of number of roots per rooted mini-cutting over five seasons, at 5 % level of significance, relevant to section 4.3.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		131.242	65.621	16.13	
Season stratum	4		105.573	26.393	6.49	
Rep.Season stratum	8		32.537	4.067	2.43	
Rep.Season.*Units* stratum						
Shade	7		21.278	3.040	1.82	0.081
Clone	1		248.530	248.530	148.48	<.001
Fert	1		2.323	2.323	1.39	0.239
Shade.Clone	7		11.130	1.590	0.95	0.467
Shade.Fert	7		29.395	4.199	2.51	0.015
Clone.Fert	1		14.750	14.750	8.81	0.003
Shade.Clone.Fert	7		7.289	1.041	0.62	0.738
Residual	1063	(715)	1779.235	1.674		
Total	1108	(715)	2145.190			

Table C. 54. Analysis of variance of number of roots per rooted mini-cutting during autumn 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.14.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.425	0.213	0.08	
Rep.*Units* stratum						
Shade	4	(3)	12.456	3.114	1.16	0.342
Clone	1		41.572	41.572	15.55	<.001
Fert	1		0.166	0.166	0.06	0.804
Shade.Clone	4	(3)	24.183	6.046	2.26	0.082
Shade.Fert	4	(3)	35.083	8.771	3.28	0.022
Clone.Fert	1		5.691	5.691	2.13	0.153
Shade.Clone.Fert	4	(3)	38.334	9.584	3.58	0.015
Residual	36	(26)	96.238	2.673		
Total	57	(38)	234.569			

Table C. 55. Analysis of variance of number of roots per rooted mini-cutting during winter 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.15.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		23.535	11.768	7.95	
Rep.*Units* stratum						
Shade	7		28.479	4.068	2.75	0.008
Clone	1		32.478	32.478	21.94	<.001
Fert	1		2.213	2.213	1.49	0.222
Shade.Clone	7		18.702	2.672	1.80	0.084
Shade.Fert	7		13.760	1.966	1.33	0.235
Clone.Fert	1		0.355	0.355	0.24	0.625
Shade.Clone.Fert	7		7.875	1.125	0.76	0.621
Residual	493	(337)	729.927	1.481		
Total	526	(337)	812.402			

Table C. 56. Analysis of variance of number of roots per rooted mini-cutting during spring 2011, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.16.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		40.223	20.111	14.60	
Rep.*Units* stratum						
Shade	7		21.364	3.052	2.22	0.035
Clone	1		145.148	145.148	105.37	<.001
Fert	1		6.210	6.210	4.51	0.035
Shade.Clone	7		30.395	4.342	3.15	0.004
Shade.Fert	7		39.954	5.708	4.14	<.001
Clone.Fert	1		5.264	5.264	3.82	0.052
Shade.Clone.Fert	7		32.601	4.657	3.38	0.002
Residual	183	(71)	252.089	1.378		
Total	216	(71)	524.249			

Table C. 57. Analysis of variance of number of roots per rooted mini-cutting during summer 2011/12, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.17.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		69.059	34.529	23.86	
Rep.*Units* stratum						
Shade	7		25.230	3.604	2.49	0.019
Clone	1		8.150	8.150	5.63	0.019
Fert	1		4.643	4.643	3.21	0.075
Shade.Clone	7		24.125	3.446	2.38	0.025
Shade.Fert	7		30.092	4.299	2.97	0.006
Clone.Fert	1		6.886	6.886	4.76	0.031
Shade.Clone.Fert	5	(2)	12.645	2.529	1.75	0.128
Residual	141	(113)	204.011	1.447		
Total	172	(115)	300.855			

Table C. 58. Analysis of variance of number of roots per rooted mini-cutting during autumn 2012, at 5 % level of significance, relevant to section 4.3.5 and Fig. 4.18.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		34.057	17.029	10.20	
Rep.*Units* stratum						
Shade	7		35.987	5.141	3.08	0.005
Clone	1		27.055	27.055	16.20	<.001
Fert	1		6.059	6.059	3.63	0.060
Shade.Clone	7		24.115	3.445	2.06	0.054
Shade.Fert	7		19.482	2.783	1.67	0.125
Clone.Fert	1		4.756	4.756	2.85	0.094
Shade.Clone.Fert	2	(5)	0.549	0.275	0.16	0.849
Residual	105	(149)	175.302	1.670		
Total	133	(154)	219.731			

Table C. 59. Total callus percentage of mini-cuttings grown under various shade nets, clones and fertilizer factors over five seasons, analysed using REML and Wald tests for fixed effects, at 5 % level of significance, relevant to section 4.3.6.

Sequentially adding terms to fixed model						
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr	
%_variable	60.45	1	60.45	3.8	0.002	
%_variable.%_Shade	38.36	7	5.48	571.8	<0.001	
%_variable.%_Clone	81.31	1	81.31	571.8	<0.001	
%_variable.%_Fert	0.24	1	0.24	571.8	0.628	
%_variable.%_Shade.%_Clone	11.20	7	1.60	571.8	0.132	
%_variable.%_Shade.%_Fert	19.95	7	2.85	571.8	0.006	
%_variable.%_Clone.%_Fert	0.51	1	0.51	571.8	0.476	
%_variable.%_Shade.%_Clone.%_Fert	5.49	7	0.78	571.8	0.601	
Dropping individual terms from full fixed model						
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr	
%_variable.%_Shade.%_Clone.%_Fert	5.49	7	0.78	571.8	0.601	

Table C. 60. Analysis of variance of total callus percentage of mini-cuttings during winter 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.20.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1151.0	575.5	1.35	
Rep.*Units* stratum					
Shade	7	5284.3	754.9	1.77	0.093
Clone	1	1148.0	1148.0	2.69	0.102
Fert	1	1467.0	1467.0	3.44	0.065
Shade.Clone	7	7558.6	1079.8	2.53	0.015
Shade.Fert	7	6575.5	939.4	2.20	0.034
Clone.Fert	1	1825.1	1825.1	4.28	0.040
Shade.Clone.Fert	7	5115.0	730.7	1.71	0.106
Residual	254	108232.7	426.1		
Total	287	138357.2			

Table C. 61. Analysis of variance of mini-cuttings with callus and roots during winter 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.20.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	35.0	17.5	0.08	
Rep.*Units* stratum					
Shade	7	9520.3	1360.0	6.09	<.001
Clone	1	6386.9	6386.9	28.58	<.001
Fert	1	1.2	1.2	0.01	0.941
Shade.Clone	7	2093.0	299.0	1.34	0.233
Shade.Fert	7	1946.5	278.1	1.24	0.279
Clone.Fert	1	6.6	6.6	0.03	0.863
Shade.Clone.Fert	7	660.7	94.4	0.42	0.888
Residual	254	56770.6	223.5		
Total	287	77420.7			

Table C. 62. Analysis of variance of rooting percentage of mini-cuttings during winter 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.20.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	60.0	30.0	0.11	
Rep.*Units* stratum					
Shade	7	13355.0	1907.9	7.25	<.001
Clone	1	9458.5	9458.5	35.93	<.001
Fert	1	34.4	34.4	0.13	0.718
Shade.Clone	7	2754.9	393.6	1.49	0.169
Shade.Fert	7	1764.4	252.1	0.96	0.463
Clone.Fert	1	0.6	0.6	0.00	0.962
Shade.Clone.Fert	7	1024.7	146.4	0.56	0.791
Residual	254	66870.7	263.3		
Total	287	95323.2			

Table C. 63. Analysis of variance of total callus percentage of mini-cuttings during spring 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.21.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2212.7	1106.4	3.64	
Rep.*Units* stratum					
Shade	7	3540.0	505.7	1.66	0.135
Clone	1	8204.8	8204.8	26.98	<.001
Fert	1	1666.7	1666.7	5.48	0.022
Shade.Clone	7	3904.6	557.8	1.83	0.096
Shade.Fert	7	17252.6	2464.7	8.10	<.001
Clone.Fert	1	2109.4	2109.4	6.94	0.011
Shade.Clone.Fert	7	4987.0	712.4	2.34	0.035
Residual	62	18855.0	304.1		
Total	95	62732.7			

Table C. 64. Analysis of variance of mini-cuttings with callus and roots during spring 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.21.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1233.7	616.9	5.11	
Rep.*Units* stratum					
Shade	7	2789.3	398.5	3.30	0.005
Clone	1	618.9	618.9	5.12	0.027
Fert	1	33.0	33.0	0.27	0.603
Shade.Clone	7	4156.5	593.8	4.92	<.001
Shade.Fert	7	6344.0	906.3	7.50	<.001
Clone.Fert	1	824.0	824.0	6.82	0.011
Shade.Clone.Fert	7	1998.3	285.5	2.36	0.033
Residual	62	7490.2	120.8		
Total	95	25487.9			

Table C. 65. Analysis of variance of rooting percentage of mini-cuttings during spring 2011, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.21.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1658.8	829.4	6.57	
Rep.*Units* stratum					
Shade	7	3820.4	545.8	4.32	<.001
Clone	1	333.3	333.3	2.64	0.109
Fert	1	27.1	27.1	0.21	0.645
Shade.Clone	7	4960.5	708.6	5.61	<.001
Shade.Fert	7	7725.8	1103.7	8.74	<.001
Clone.Fert	1	793.5	793.5	6.28	0.015
Shade.Clone.Fert	7	1784.4	254.9	2.02	0.067
Residual	62	7829.2	126.3		
Total	95	28933.2			

Table C. 66. Analysis of variance of total callus percentage of mini-cuttings during summer 2011/12, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.22.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	185.5	92.8	0.43	
Rep.*Units* stratum					
Shade	7	4002.3	571.8	2.66	0.018
Clone	1	10157.9	10157.9	47.25	<.001
Fert	1	162.8	162.8	0.76	0.388
Shade.Clone	7	6293.9	899.1	4.18	<.001
Shade.Fert	7	5078.1	725.4	3.37	0.004
Clone.Fert	1	162.8	162.8	0.76	0.388
Shade.Clone.Fert	7	2252.6	321.8	1.50	0.185
Residual	62	13330.1	215.0		
Total	95	41626.0			

Table C. 67. Analysis of variance of mini-cuttings with callus and roots during summer 2011/12, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.22.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	119.6	59.8	0.49	
Rep.*Units* stratum					
Shade	7	1663.0	237.6	1.93	0.080
Clone	1	6771.2	6771.2	54.94	<.001
Fert	1	146.9	146.9	1.19	0.279
Shade.Clone	7	1077.1	153.9	1.25	0.291
Shade.Fert	7	2232.7	319.0	2.59	0.021
Clone.Fert	1	10.2	10.2	0.08	0.775
Shade.Clone.Fert	7	1340.7	191.5	1.55	0.166
Residual	62	7640.8	123.2		
Total	95	21002.2			

Table C. 68. Analysis of variance of rooting percentage of mini-cuttings during summer 2011/12, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.22.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	74.1	37.0	0.25	
Rep.*Units* stratum					
Shade	7	1014.0	144.9	0.97	0.458
Clone	1	9650.1	9650.1	64.87	<.001
Fert	1	1.6	1.6	0.01	0.917
Shade.Clone	7	1834.3	262.0	1.76	0.111
Shade.Fert	7	2810.9	401.6	2.70	0.017
Clone.Fert	1	131.8	131.8	0.89	0.350
Shade.Clone.Fert	7	2055.7	293.7	1.97	0.073
Residual	62	9222.8	148.8		
Total	95	26795.2			

Table C. 69. Analysis of variance of total callus percentage of mini-cuttings during autumn 2012, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.23.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	351.6	175.8	0.86	
Rep.*Units* stratum					
Shade	7	6715.1	959.3	4.71	<.001
Clone	1	898.8	898.8	4.42	0.040
Fert	1	3988.0	3988.0	19.60	<.001
Shade.Clone	7	1773.7	253.4	1.25	0.292
Shade.Fert	7	16913.7	2416.2	11.87	<.001
Clone.Fert	1	0.4	0.4	0.00	0.964
Shade.Clone.Fert	7	4052.3	578.9	2.84	0.012
Residual	62	12617.2	203.5		
Total	95	47310.8			

Table C. 70. Analysis of variance of mini-cuttings with callus and roots during autumn 2012, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.23.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	148.93	74.46	1.25	
Rep.*Units* stratum					
Shade	7	2265.22	323.60	5.45	<.001
Clone	1	2669.68	2669.68	44.99	<.001
Fert	1	1514.08	1514.08	25.51	<.001
Shade.Clone	7	2535.40	362.20	6.10	<.001
Shade.Fert	7	5058.19	722.60	12.18	<.001
Clone.Fert	1	1322.02	1322.02	22.28	<.001
Shade.Clone.Fert	7	1096.60	156.66	2.64	0.019
Residual	62	3679.20	59.34		
Total	95	20289.31			

Table C. 71. Analysis of variance of rooting percentage of mini-cuttings during autumn 2012, at 5 % level of significance, relevant to section 4.3.6 and Fig. 4.23.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	237.63	118.82	1.72	
Rep.*Units* stratum					
Shade	7	3020.43	431.49	6.23	<.001
Clone	1	3519.29	3519.29	50.82	<.001
Fert	1	1614.99	1614.99	23.32	<.001
Shade.Clone	7	4081.62	583.09	8.42	<.001
Shade.Fert	7	6311.44	901.63	13.02	<.001
Clone.Fert	1	2168.38	2168.38	31.31	<.001
Shade.Clone.Fert	7	1148.68	164.10	2.37	0.033
Residual	62	4293.62	69.25		
Total	95	26396.08			

Table C. 72. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings over five seasons, at 5 % level of significance, relevant to section 4.3.6.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Shade	7		4.5037	0.6434	1.71	0.105
Clone	1		11.1566	11.1566	29.65	<.001
Fert	1		2.2418	2.2418	5.96	0.015
Season	4		19.7449	4.9362	13.12	<.001
Shade.Clone	7		4.9589	0.7084	1.88	0.071
Shade.Fert	7		9.0578	1.2940	3.44	0.001
Clone.Fert	1		0.6934	0.6934	1.84	0.175
Shade.Season	25	(3)	31.7239	1.2690	3.37	<.001
Clone.Season	4		4.8347	1.2087	3.21	0.013
Fert.Season	4		1.8293	0.4573	1.22	0.304
Shade.Clone.Fert	7		1.4555	0.2079	0.55	0.794
Shade.Clone.Season	25	(3)	20.4913	0.8197	2.18	0.001
Shade.Fert.Season	25	(3)	26.1257	1.0450	2.78	<.001
Clone.Fert.Season	4		1.0629	0.2657	0.71	0.588
Residual	389	(151)	146.3882	0.3763		
Total	511	(160)	239.1236			

Table C. 73. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings during autumn 2011, at 5 % level of significance, relevant to section 4.3.6 and Table 4.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		1.8799	0.9400	2.03	
Rep.*Units* stratum						
Shade	4	(3)	2.5176	0.6294	1.36	0.266
Clone	1		0.0009	0.0009	0.00	0.965
Fert	1		0.2599	0.2599	0.56	0.458
Shade.Clone	4	(3)	9.7670	2.4417	5.28	0.002
Shade.Fert	4	(3)	2.4757	0.6189	1.34	0.274
Clone.Fert	1		0.1270	0.1270	0.27	0.603
Shade.Clone.Fert	4	(3)	2.9677	0.7419	1.60	0.194
Residual	37	(25)	17.1151	0.4626		
Total	58	(37)	34.0066			

Table C. 74. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings during winter 2011, at 5 % level of significance, relevant to section 4.3.6 and Table 4.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		1.4954	0.7477	1.27	
Rep.*Units* stratum						
Shade	7		12.8745	1.8392	3.12	0.003
Clone	1		22.2976	22.2976	37.84	<.001
Fert	1		3.5616	3.5616	6.04	0.014
Shade.Clone	7		10.6750	1.5250	2.59	0.013
Shade.Fert	7		23.3190	3.3313	5.65	<.001
Clone.Fert	1		0.0346	0.0346	0.06	0.809
Shade.Clone.Fert	7		3.2777	0.4682	0.79	0.592
Residual	493	(337)	290.5428	0.5893		
Total	526	(337)	325.5599			

Table C. 75. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings during spring 2011, at 5 % level of significance, relevant to section 4.3.6 and Table 4.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		1.8003	0.9002	1.64	
Rep.*Units* stratum						
Shade	7		3.3903	0.4843	0.88	0.523
Clone	1		13.9074	13.9074	25.29	<.001
Fert	1		1.0353	1.0353	1.88	0.172
Shade.Clone	7		14.6356	2.0908	3.80	<.001
Shade.Fert	7		4.7780	0.6826	1.24	0.282
Clone.Fert	1		0.2281	0.2281	0.41	0.520
Shade.Clone.Fert	7		10.3792	1.4827	2.70	0.011
Residual	183	(71)	100.6538	0.5500		
Total	216	(71)	131.4248			

Table C. 76. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings during summer 2011/12, at 5 % level of significance, relevant to section 4.3.6 and Table 4.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		1.4487	0.7243	1.51	
Rep.*Units* stratum						
Shade	7		32.5939	4.6563	9.72	<.001
Clone	1		7.9800	7.9800	16.67	<.001
Fert	1		0.0157	0.0157	0.03	0.857
Shade.Clone	7		12.0786	1.7255	3.60	0.001
Shade.Fert	7		17.7745	2.5392	5.30	<.001
Clone.Fert	1		2.5328	2.5328	5.29	0.023
Shade.Clone.Fert	5	(2)	4.4775	0.8955	1.87	0.103
Residual	141	(113)	67.5152	0.4788		
Total	172	(115)	105.3827			

Table C. 77. Analysis of variance of basal stem diameter (BSD) of rooted mini-cuttings during autumn 2012, at 5 % level of significance, relevant to section 4.3.6 and Table 4.5.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		2.8330	1.4165	2.22	
Rep.*Units* stratum						
Shade	7		32.4784	4.6398	7.26	<.001
Clone	1		0.0000	0.0000	0.00	0.996
Fert	1		3.2060	3.2060	5.02	0.027
Shade.Clone	7		17.9643	2.5663	4.02	<.001
Shade.Fert	7		40.4224	5.7746	9.04	<.001
Clone.Fert	1		4.8482	4.8482	7.59	0.007
Shade.Clone.Fert	2	(5)	5.2687	2.6344	4.12	0.019
Residual	105	(149)	67.1046	0.6391		
Total	133	(154)	94.6921			