RECIRCULATING HYDROPONIC SYSTEMS: EVALUATING CUTTINGS YIELD AND ROOTING ABILITY OF COLD TOLERANT *Eucalyptus* HYBRIDS

by

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" ...soilless culture does not make any sense; nobody would seriously be interested."

International Society for Horticultural Science, 1959

DECLARATION

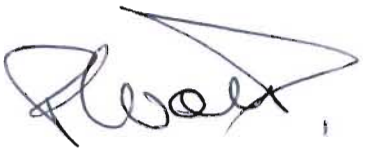
I hereby certify that the research work reported in this dissertation is the result of my own original investigation except where acknowledged. The experimental work described in this thesis was carried out at the Trahar Technology Center, Mondi Forests from January 2001 to January 2004, under the supervision of Profs. M.P. Watt and N.W. Pammenter. These studies have not been submitted in any form to another university.

Signed: 

Jacqueline Tanya Wallis

January 2004

We certify that the above statement is correct

Signed: 

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ABSTRACT

In South Africa, clonal forestry of *Eucalyptus* and its hybrids has been implemented to increase the productivity on existing forestry lands and marginal sites and to facilitate the production of desired fibre types for timber processing operations. The cold-tolerant *Eucalyptus grandis* x *E. nitens* hybrids have produced consistently high yields, and are propagated clonally with limited success via a macro-cutting system currently in use for other hybrid species. The heart of vegetative propagation operations is the clonal hedge and its management, and nutrition in particular, is an important element of any vegetative propagation programme. However, achieving and sustaining an optimum nutritional balance in macrohedges is difficult in practice and, in order to accurately predetermine the optimum plant nutrition required all year round and to ensure optimal levels of rooting, a more controllable nutrient environment is essential. Hydroponics may facilitate this control of nutrition. At the same time it may be possible to manipulate the system to determine accurately what levels of each nutrient may contribute to the highest rooting and more importantly allow forest nursery managers to maintain those levels in a practical manner. The main aims of the present work were to obtain and compare cuttings and rooting yields from hydro-ramets in different hydroponic substrates and systems and to investigate the possible roles of essential nutrients on those parameters. Modified Nutrient Film Technique (NFT), ebb-and-flow and aeroponic tables were used in this study. The former consisted of eight individual gutters, allowing for eight different substrates to be tested simultaneously. One gutter was set up as an unmodified NFT table and the other seven gutters had gravel, Leca, peat, perlite, perlite: vermiculite mix, Rockwool® and sand as substrates; all were supplied with the same nutrient solution. Three commercial clones were used throughout these trials: GN107, GN156 and NH00. Rooting results and data from plant elemental analyses indicated that certain elements (Ca, Cu, Zn, Mn and B) appeared to play a more important role in rooting than others (N, P, K, Mg, Na and Fe). It was also found that when comparing the hydroponic systems, the substrate and / or method of irrigation affected the availability and uptake of different nutrients, which in turn affected the rooting of coppice collected from those ramets. The rooting performance of coppice from the eight different substrates tested in the NFT

system was compared. Within each of the four harvests undertaken, both clone and substrate had a significant effect on the rooting performance. However, when the four harvests were compared, only harvest number /time had a significant effect on the rooting performance of the cuttings derived from the hydro-hedges. For both the ebb-and-flow and aeroponics systems (where there was no substrate), only the clone had a significant effect on the rooting performance. In addition to this, the plants from the ebb-and-flow system produced the highest number of cuttings to be placed overall (7.9 cuttings per mother plant per harvest) while those from the gravel substrate had the highest rooting percentage overall (26.9 %). When combining these two factors into a success rate, the perlite substrate rated highest (1.7 rooted cuttings per mother plant per harvest). From a cost efficiency perspective, perlite was the most cost effective substrate, as it required the least initial capital outlay to produce one million rooted clones per year from a hydroponics system (R6 533 655). The plants in the perlite substrate also produced the highest number (6 700) of rooted cuttings per year from 1 000 mother plants with a low cost per plant (R2.33 per rooted plant).

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LIST OF ABBREVIATIONS

®	Registered name or trademark
ANOVA	Analysis of variance
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
BC	Before Christ
BER	Blossom end rot
C.D.M	Consolidated Diamond Mines of South West Africa Limited
CBA	Cost-benefit analysis
CEC	Cation exchange capacity
CG	*Eucalyptus camaldulensis* x *E. grandis* natural hybrid
CTE	Cold tolerant eucalypts
DFT	Deep flow technique
EC	Electrical conductivity
FPPWG	Forestry Plant Propagators Working Group
FRP	Fibre re-inforced plastic
FSC	Forestry Stewardship Certification
GM	*Eucalyptus grandis* x *E. macarthurii*
GN	*Eucalyptus grandis* x *E. nitens* hybrid
GN107	*Eucalyptus grandis* (female) x *E. nitens* (male) clone
GN156	*Eucalyptus grandis* (female) x *E. nitens* (male) clone
GU	*Eucalyptus grandis* x *E. urophylla* hybrid
IAA	Indole-3-acetic acid
IBA	Indolebutyric acid
IP	International Paper
ISOSC	International Society of Soilless Culture
NASA	National Aeronautical and Space Administration
NFT	Nutrient Film Technique
NH	*Eucalyptus nitens* x *E. grandis* natural hybrid
NH00	*Eucalyptus nitens* (female) x *E. grandis* (male) clone
ns	not significant
P:V	Perlite – vermiculite mix
PGR	Plant growth regulator
QNM	Quantitative Nutrient Management
R	Rands
TDS	Total dissolved salts
UK	United Kingdom
USA (US)	United States of America
USSR	United Soviet Socialist Republic

Chapter 1

INTRODUCTION

In South Africa, excellent agricultural and forestry growth potential exists in the prime areas of good rainfall, climate and soils. However, in South Africa as in many other countries, this type of land is limited and the forestry industry has to compete with agricultural crops and livestock. Forestry expansions have therefore occurred largely on more marginal sites (Denison & Kietzka, 1993a; Harvett, 2000).

South Africa comprises 122 103 000 ha (Anon, 1995) of which forestry plantation areas cover slightly less than 1.4 % (Owen & van der Zel, 2000). As illustrated in Figure 1, 29.0 % of this is planted to *Eucalyptus grandis* and 10.3 % to other eucalypt species and hybrids (Owen & van der Zel, 2000). During the past two decades a large number of additional commercial eucalypt species and clonal hybrids have been introduced because of the great diversity of site conditions and the above-mentioned expansion of forestry into marginal areas (Herbert, 2000).

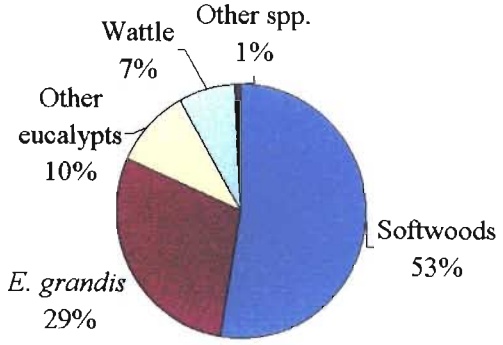

Figure 1: **South Africa's plantation area by species**

Eucalyptus is one of the most important genera in the timber, pulp and paper industry and is a major contributor to the worlds hardwood pulp market plantations (Durand-Cresswell, Boulay & Franclet, 1982). With the global demand for forestry products increasing, and particularly that for the eucalypts' high quality pulp, it is no surprise that the importance of *Eucalyptus* has grown dramatically over the years and is still increasing. *Eucalyptus* rates as

one of the most productive forest crops in plantations (Durand-Cresswell *et al.*, 1982), which is partially due to its fast growth (Turnbull & Boland, 1984). The relative ease with which most economically important eucalypts can be cloned has further extended and increased the global interest in the genus, particularly in the warmer regions of the globe where most of the forestry expansion is occurring (Denison & Quaile, 1987). This has also provided opportunities for improving the productivity and quality of eucalypt species (Denison & Quaile, 1987).

Eucalyptus grandis is prominent as either a pure species or as a hybrid. The climatic range of *E. grandis* has been considerably extended by hybridization (Aimers-Halliday, Dibley, Hong & Menzies, 1999) which has added considerable flexibility to the species in terms of growing areas (Denison & Quaile, 1987). Historically, *E. grandis* is the most important hardwood for the South African forestry industry (Swain, 1997), favored for its excellent growth potential, good form, desirable pulp and timber properties and the rooting ability of cuttings (Wex & Denison, 1997). Furthermore, the increasing demand for pulp and paper has resulted in hardwood plantations expanding into colder areas previously unsuitable for *E. grandis* (Swain, 1997). The susceptibility to cold temperatures poses serious limitations on its success as a pure species in these regions (Zobel, 1988; Wex & Denison, 1997).

Eucalyptus nitens is an economically important *Eucalyptus* species but unlike most eucalypts, it is a shy seed bearer (Maile & Nieuwenhuis, 1996). It is cold resistant and can be planted at higher altitudes than *E. grandis*. It is also a desirable species for pulpwood production and is currently grown in the Mpumalanga Highveld of South Africa, where frosts are common and rainfall is moderate (Purnell & Lundquist, 1986).

A clonal approach to commercial forestry is becoming increasingly popular, especially with *Eucalyptus* species, because of the higher yields, better quality and greater stand uniformity of clones (Hoad & Leakey, 1994; Denison, 1998). On marginal sites, the growth and survival of eucalypt hybrids are higher than those of pure, parent species and hybrids are consistently more resistant to diseases, pests, cold, heat and drought (Denison & Kietzka, 1993a). According to these authors, the wood and growth properties of the hybrids are normally intermediate between the parent species, but superior growth, compared to both

parent species, is common. Hybrid vigor, or heterosis, is always correlated with the environment in which the hybrid is grown (Strickberger, 1985; Martin, 1988) and it is more prevalent in zones that are considered marginal for the pure parent species. This environmentally-dependent hybrid vigor is the basis for the success of the eucalypt hybrid programmes of Aracruz in Brazil and more recently Mondi's hybrid programmes in South Africa (Denison & Kietzka, 1993b).

The relationship between hybrid vigor and adaption to new environments is of significance in developing economically viable forests in marginal areas of South Africa (Denison & Kietzka, 1993a). The potential for hybrid forestry in South Africa was recognized for many years but progress was slow, owing to the difficulties related to obtaining hybrid seed in sufficient quantities and problems multiplying the selected material through vegetative propagation (Denison & Kietzka, 1993a). Clonal forestry has been implemented by various forestry companies in South Africa and overseas (van Wyk & Verryn, 2000) to increase the productivity on existing forestry lands and also to extend forestry to areas initially considered off-site for plantations, i.e. marginal sites (Denison & Quaile, 1987; Denison & Kietzka, 1993a). Those authors predicted that hybrids would play an important role on marginal sites and it is in this way that the use of hybrids and clonal material has revolutionized *Eucalyptus* forestry in South Africa (Denison & Kietzka, 1993a). Vegetative propagation and clonal forestry are practiced successfully with eucalypts in several parts of the world (Denison & Kietzka, 1993a).

A decade ago, Denison & Kietzka (1993a) postulated that the continued successful progress of Mondi's commercial *Eucalyptus* programme would be dependent on a strong clonal programme and the vegetative multiplication of selected clones. For this reason, in the last ten years alternative propagation methods have been developed at Mondi. These propagation methods involve macrocuttings, microcuttings, tissue culture and hydroponics.

Hydroponics and its application to cutting production in eucalypt nurseries had had very little attention outside South America until 1998 when Mondi Forests investigated the feasibility of hydroponically sustained parent plants to replace field hedges as discussed below (da Costa Alpoim, 2002). Mondi has focussed on the evaluation of recirculating

hydroponic systems for the cutting production of various *Eucalyptus* species and their hybrids.

After a visit to various forestry companies in Brazil, Janse (2001) acknowledged the merits of changing to hydroponically-derived ramets (hydro-ramets or microgardens). Based on this, Mondi Forests decided to develop both a pilot and commercial scale ebb-and-flow hydroponic system, because at least 80 million eucalypt plantlets are produced via hydroponically-fed microgardens in South America. Janse (2001) reported that Aracruz Cellulose obtains the same number of cuttings off 0.5 ha of microgardens, compared to seven hectares of macrohedges, and they intend to convert fully to microgardens. The pilot scale production unit at Mountain Home Nursery was built to produce between 800 000 and 1.2 million plants of *E. grandis* x *E. nitens* (GN) clones per annum.

In South Africa, the current system of extensive clonal hedges is costly and difficult to maintain. For example, Mondi Forest's KwaMbonambi nursery has to maintain clonal hedges of 25 ha in order to produce the required eight million eucalypt plants annually. This system has massive logistical implications and is extremely labour intensive. The nursery managers are also reliant on prevailing weather conditions and other seasonal changes. Due to extra numbers of subtropical clones that have been ordered, Mondi was forced to increase production at KwaMbonambi nursery by close to 50 % in the short term. It was proposed that this was to be accomplished using microgardens with an aim to phase out the macrohedges and be fully converted to microgardens after five years.

Da Costa Alpoim (2002) conducted several trials to investigate different systems, substrates, fertilizers and the responses of different clones to these treatments. He also investigated the influence of light on growth and other factors such as disease-incidence in the hydroponic systems and the severity of cut-back on ramets maintained in hydroponic systems. Da Costa Alpoim (2002) established that with the correct nutrient and substrate combination the hydro-hedges resulted in cuttings with a higher rooting percentage than those from field hedges. He concluded that a gravel substrate in combination with the Hydroponica® fertiliser gave the best results.

Following on from the preliminary work started by da Costa Alpoim (2002) in 1998 it was decided to investigate methods to improve the productivity of hydroponics systems. In this regard the main aims of the present work were to:

(a) improve the overall efficiency of the hydro-ramets in a recirculating hydroponic system and to provide possible protocols from which nurserymen can plan their hydroponic strategies for cold-tolerant eucalypt vegetative propagation;

(b) compare the cuttings' yield and rooting data from hydro-ramets in different hydroponic systems; and

(c) investigate the essential nutrients within the *Eucalyptus* clones as well as how they were affected by system and over time, and in addition take note any possible influences particular nutrients may have on cutting yield or rooting in hydroponics systems.

Chapter 2

LITERATURE REVIEW

2.1 Vegetative propagation of *Eucalyptus* species and hybrids

Vegetative propagation is a form of asexual reproduction whereby new daughter plants develop from individual cells, tissues, calli or specialised multicellular plant structures (e.g. tubers, bulbs) (Hartmann, Kester & Davies, 1990; Martin & Hine, 2000). This is possible because of the totipotency of cells, which is the property of every living cell of a plant to regenerate an entire new plant (Hartmann, Kester, Davies & Geneve, 1997; Pierce, 1997). Artificial methods of vegetative propagation include grafting, cuttings and tissue culture (Hartmann *et al.*, 1997; Pierce, 1997).

The terms used throughout this thesis are employed by the forestry industry and consequently some definitions may assist the reader. In forestry, the donor tree, from which the vegetative propagules are originally taken, is called the ortet (Zobel & Talbert, 1984). The vegetative offspring of an ortet, or an individual member of a clone, is called a ramet (Zobel & Talbert, 1984; Pierce, 1997; Williams, 2000). The sum of propagules (cuttings) arising from one ortet is referred to, as a group, as a clone (Zobel & Talbert, 1984). In turn, a clone is defined as a group of cells, organism, or a population of organisms arising from a single cell (Martin & Hine, 2000). All offspring of a particular clone are genetically identical (Pierce, 1997; Martin & Hine, 2000; Williams, 2000).

Many countries with large forestry companies are implementing vegetative propagation as a strategy for the deployment of improved *Eucalyptus* species and hybrids. These include Portugal (Cotteril & Brindbergs, 1997), Brazil (Bertolucci & Penchel, 1993; Zobel, 1993; Ikemori, Penchel & Bertolucci, 1994), South Africa (Denison & Quaile, 1987; Denison & Kietzka, 1993b), Southern France (Eldridge, Davidson, Harwood & van Wyk, 1994), and Congo (Eldridge *et al.*, 1994). Such an approach is used as a tool to capture specific clonal traits that would not be possible, or too time-consuming, through conventional tree breeding. Vegetative propagation can be used to maintain hybrid vigour and to speed up the

implementation of results obtained from eucalypt tree breeding trials to the operational phase of tree improvement raising productivity substantially (Harvett, 2000; White, 2000; Williams, 2000). For example, in 1991, Turnbull reported that Aracruz Florestal increased its average yield of *Eucalyptus* species and hybrids by 112 % by these means.

The use of vegetative propagation makes it possible to capture and transfer the full genetic potential (additive and non-additive variation) of a selected tree (the donor or ortet) to its asexually reproduced progeny (Monteuuis, 1988; Denison & Kietzka, 1993a; Pierce, 1997). Furthermore, the superior tree may be multiplied to make large numbers of exact superior copies, whereas sexual crosses yield variable offspring, some of which may be inferior (van Wyk & Verryn, 2000). In addition to the long life cycle of trees, many woody species are highly heterozygous and this is a major limitation to sexual breeding. This limits the use of inbreeding and controlled genetic gain because inbreeding of heterozygous plants leads to severe growth depression in the offspring (van Wyk & Verryn, 2000). Nevertheless, with continuous breeding and selection over several generations, some tree improvement can be expected but the gain is likely to be less than if selected trees are propagated vegetatively (Bonga, 1982).

However, as Wilson (1998a) and Williams (2000) have pointed out, vegetative propagation is more expensive, labour-intensive and time consuming than seedling production. Denison and Kietzka (1993a) also noted the high costs attached to clonal plant production and that they are even higher in cooler and less humid areas where cuttings need to be kept in greenhouses equipped with elaborate heating and cooling systems. The success rate is influenced by various factors such as clone (genotype), time of year, physiological condition of the stock plants, fluctuating climatic conditions and propagation environment (Hartmann *et al.*, 1990; Denison & Kietzka, 1993b).

In addition to plantation productivity, vegetative propagation of selected eucalypt clones can also be used to improve the uniformity of the timber produced (Wignall, Brown & Purse, 1991; Zobel, 1993). A greater uniformity of raw material is becoming increasingly important as manufacturing processes become more sophisticated (Zobel, 1993). This can

offset the production difficulties and the high cost of clonal plants (Denison & Kietzka, 1993a).

2.1.1 Vegetative propagation methods

The most important vegetative propagation techniques for tree species are propagation by grafting, various methods of layering, stem or root cuttings, and various techniques of micropropagation (Wiessman & Jaenicke, 2000).

Grafting is the connecting of two pieces (a stem or a bud, the scion, to the rootstock) of plant together in such a manner that they will unite and subsequently grow and develop as one plant (Langman, 1993). It is the technique of choice when a single genotype does not possess all required characteristics (Wiessman & Jaenicke, 2000). Grafting is possible for many important timber species but is expensive and so has limited use in routine plantation forestry. It can be used to establish seed orchards and to 'rejuvenate' shoots for use either as cuttings or as explants for tissue culture. However, there has been a high incidence of graft incompatibility leading to graft failure (McComb & Bennett, 1986; Shepherd, 1986). Grafting is used in eucalypt species because grafted plants bear seed earlier compared to plants originating from seed, thus resulting in an earlier supply of improved seed (van Wyk & Verryn, 2000).

Layering is a propagation method by which adventitious roots are caused to form on a stem while the stem is still attached to the parent plant (Hartmann *et al.*, 1990; Wiessman & Jaenicke, 2000). This technique of propagation is similar to cuttings production, with the advantage that the propagules are detached from the mother plant only after roots have formed. It is therefore a method that can provide rooting success with difficult-to-root species. Although the multiplication rate is lower than with cuttings, it can yield larger individual plants (Wiessman & Jaenicke, 2000). Air layering is a labour-intensive process and eucalypts are very slow to root when layered and the technique is seldom used (McComb & Bennett, 1986).

In the 1950s, a French forester, Bouvier, experimented for the first time, with vegetative propagation of juvenile eucalypts (Eldridge *et al.*, 1994). It has been subsequently found that some eucalypt species and hybrids are easy to propagate via cuttings if the leafy cuttings are taken from seedlings, juvenile trees or epicormic shoots (Durand-Cresswell *et al.*, 1982; McComb & Bennett 1986). Also, the manipulation of the state of juvenility of the shoots has allowed cuttings to be used with great success (Durand-Cresswell *et al.*, 1982; McComb & Bennett 1986).

Since the late 1970s when clonal forestry of eucalypts started, the potential of stem cutting propagation to improve yield and timber quality became apparent. By the early 1980s clonal forestry cuttings became an integral part of forestry in many companies in a number of countries across the world (Denison & Quaile, 1987; Adendorff & Schön, 1991; Bertolucci & Penchel, 1993; Hoad & Leakey, 1994; Ikemori *et al.*, 1994; Lambeth, Endo & Wright, 1994).

Much progress has been made in the use of rooted cuttings, and a number of large operational programmes are in use. Many organisations in a number of countries use rooted cuttings, particularly of eucalypt species, on a large scale. Companies in South Africa, Venezuela, Brazil, Uruguay, New Zealand, Australia, Colombia, Congo and Portugal have implemented the intense use of rooted cuttings of eucalypts or are using them exclusively (Aimers-Halliday *et al.*, 1999; Denison & Quaile, 1987; Bertolucci & Penchel, 1993; Denison & Kietzka, 1993b; Zobel, 1993; Eldridge *et al.*, 1994; Ikemori *et al.*, 1994; Cotterill & Brindbergs, 1997). The commercial production of rooted cuttings has become increasingly important in South African forestry because the technology of producing cuttings successfully and economically has developed rapidly, and because of growing awareness of the potential for increased production on limited forestry land (van Wyk & Verryn, 2000).

When propagating using cuttings, a portion of a stem, root, or leaf is cut from the parent plant, after which this plant part is placed under favourable environmental conditions and induced to form roots and shoots, thus producing a new independent plant which is genetically identical to the parent plant (Pierce, 1997). Taking stem cuttings is considered

the most common way to vegetatively propagate trees (Van Wyk, 1997; Wiessman & Tchoundjeu, 1997) because the process is relatively simple and a single mother or stockplant can yield many cuttings.

Cuttings must perform as well as, or better than seedlings in order for cuttings programmes to be commercially successful. The root systems of cuttings are fundamentally different from those of seedlings (Sasse & Sands, 1997); if the differences influence growth, their consequences must be identified and the propagation system manipulated to improve performance of propagules. Cuttings are a viable alternative to seedlings as planting stock only if the method of propagation does not affect their growth and development adversely (Sasse & Sands, 1997).

There are three main advantages to using stem cuttings as a means of vegetative propagation: (a) a large number of cuttings can be obtained from a single tree thus conserving the superior traits of the individual which has been selected; (b) the problem of grafting incompatibility is avoided; and (c) the rooting of the cuttings is cheaper than some other vegetative propagation methods as this approach does not require the special techniques necessary in grafting, layering or micropropagation (Durand-Cresswell *et al.*, 1982; Spanos, Pirrie & Woodward, 1999). Zobel (1993) explained that the major benefits of rooted cuttings in an operational program are increased growth, improved tree quality, better wood and tree uniformity, greater pest tolerance and better adaptability to differing environments – all qualities which have been selected or bred for in the breeding programme. However, stem cuttings of some species of genotypes are very fragile before they develop roots and mortality can be high (Wilson, 1998a).

In spite of some eucalypt species not sprouting readily or at all, or that sprout readily but root with difficulty, stem cuttings have been shown to be more operationally useful in clonal eucalypt forestry than other methods of vegetative propagation (Yang, Chung & Chen, 1995). The disadvantages of using cuttings, especially macrocuttings, are that only one plant is obtained from each cutting and the success of the process is often genotype-dependent. As mentioned, it can also be very difficult to get the cuttings to root. According

to Geneve (1995), root formation in cuttings is a complex, integrated phenomenon that responds to environmental, nutritional and physiological factors.

Until recently, in both South Africa and Brazil, the clonal production of *Eucalyptus*, via cuttings, had largely remained unchanged since its inception. The Brazilians, however, have made some changes in the details of their systems in the last 20 years, resulting in the reduction in the size of the area utilised (Figures 2A & 2B) and decreased cutting size. In South Africa, Mondi has also recently made some similar changes (Figures 2C & 2D).

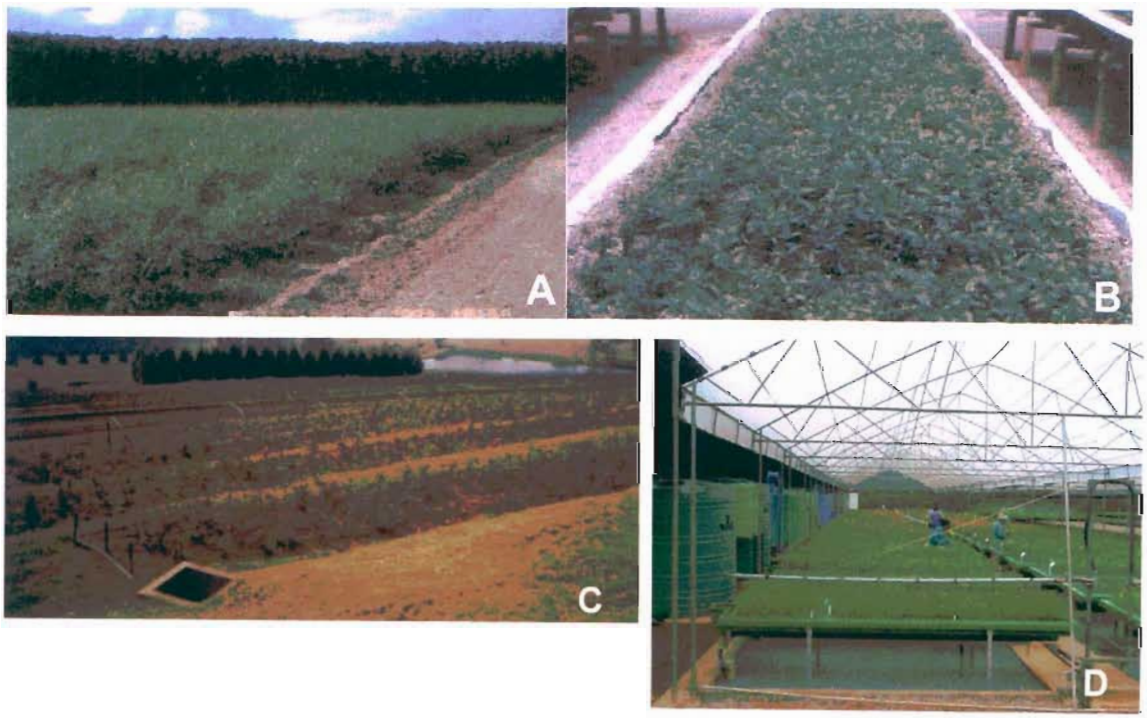

Figure 2: **(A) A Brazilian field of clonal hedges with 40 000 plants per ha (B) Brazilian mini-garden: cement gutters with sand housing 100 plants per m^2** (From: Higashi, Silveira & Gonçalves, 2002) **(C) Extensive field hedges (22 500 ramets per ha) in South Africa (D) South African hydroponic (ebb-and-flow) hedges (95 ramets per m^2)**

According to da Costa Alpoim (2002) the essential difference between micro- and minicuttings is the source of the material: microcuttings are from ramets that were produced *in vitro* whereas minicuttings are from ramets that were produced from stem cuttings. De Assis (2001) explains that microcuttings are shoot apices and mini-cuttings are axillary bud shoots. He also notes that the two techniques are very similar and the designated terms are by convention only. At Mondi, a minicutting has always been

considered as a smaller macrocutting while a microcutting is also smaller than a conventional macrocutting, has a growing point and is not necessarily produced *in vitro*. A macrocutting is considered as a cutting that is five to 10 cm in length and has no growing point.

The development of this intensive cloning system has set the stage for a new phase of mass vegetative propagation of eucalypts. Compared to stem cuttings from existing hedge systems, the rooting of micro- or minicuttings improves rooting potential, rooting speed, root system quality and reduces production and maintenance costs (De Assis, Rosa & Gonçalves, 1992, Xavier & Comério, 1996). In this new system ramets are managed more intensively than field hedges. The conventional field hedges could thus be replaced by 'minihedges' or hydroponic hedges, which provide a higher level of juvenility. Although the success of these techniques has been attributed to this maintenance of juvenility, new findings indicate that their high rooting potential is also related to the better nutritional status of the minicuttings (De Assis *et al.*, 1992, Xavier & Comério, 1996). In general, mini- or microcuttings root better than stem cuttings of the same clone harvested from field hedges, as a result of the hedge plant's improved nutritional status (De Assis *et al.*, 1992, Xavier & Comério, 1996).

Micropropagation involves the mass production of plants from very small plant parts, tissues, or cells grown aseptically in a test tube or other container where the environment and nutrition are accurately controlled (McAteer & Davis, 1994). Micropropagation can allow a very high multiplication rate as from a single plant thousands of new daughter plants can be produced. These techniques need high initial investment in equipment and training and are usually done for commercially important agricultural and horticultural crops (Shepherd, 1986; Ahuja, 1993; Libby & Ahuja, 1993). Many tree species for plantation forestry have proved difficult to propagate using conventional tissue culture techniques but with a great deal of research have proven to be very successful (Shepherd, 1986; Lakshmi sita, 1993; van Wyk & Verryn, 2000).

One major application of micropropagation is to establish virus / pathogen-free stock plants. This can then be followed by propagation *ex vitro* (macropropagation) (Libby &

Ahuja, 1993; Kubota & Kozai, 2001). In this way, tissue cultured plants are used to restock clone banks and introduce new clones (Williams, 2000). There is the potential for this interface between micro- and macropropagation to be increasingly used in eucalypt vegetative propagation systems.

2.1.2 *Eucalyptus* propagation by cuttings

Figure 3 shows a diagrammatic representation of the vegetative propagation programme of *Eucalyptus* species and hybrids in Mondi Forests' tree improvement and propagation continuum.

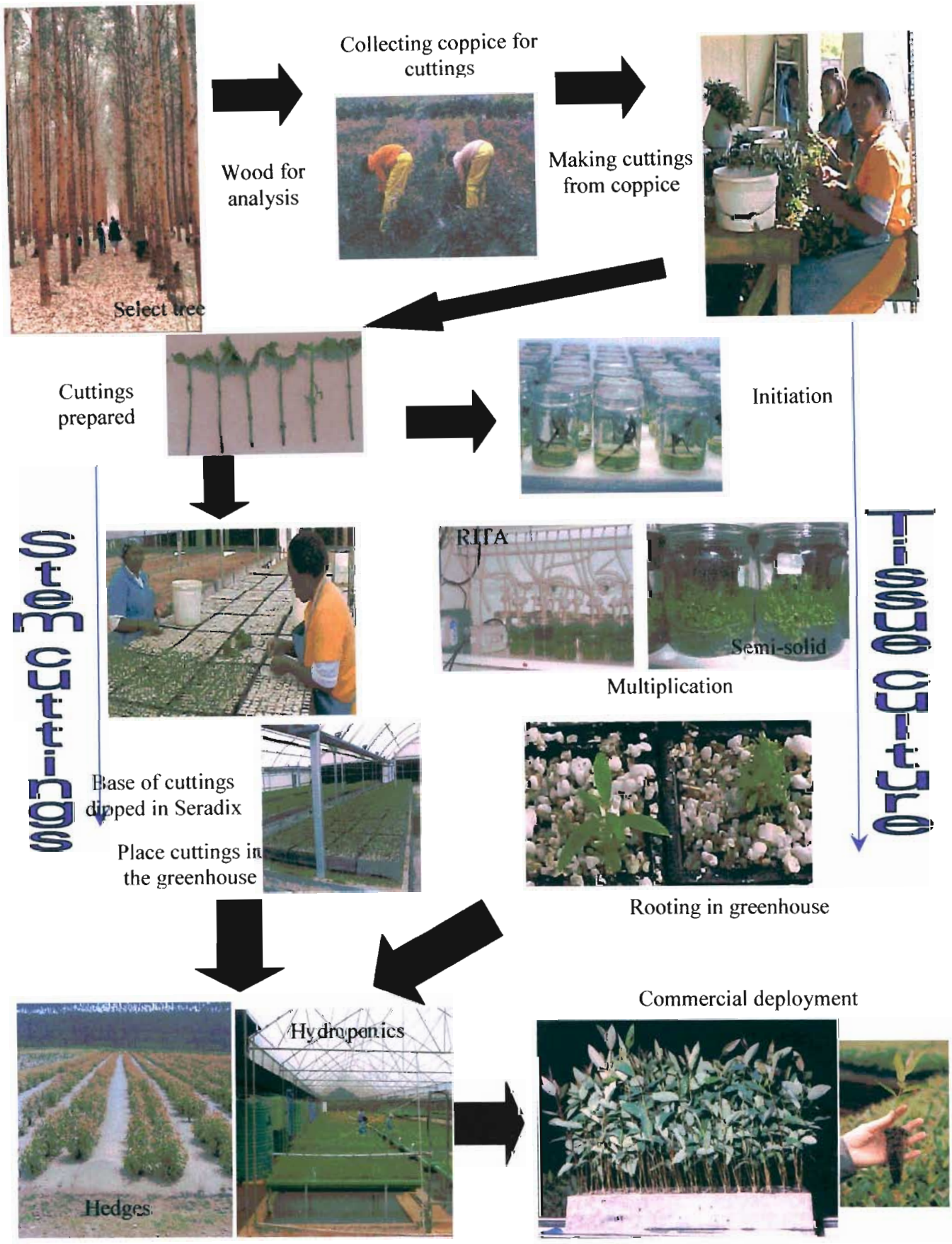

Figure 3: **Production of vegetative propagules**

2.1.2.1 Production areas: clonal hedges

The heart of most vegetative propagation operations is the clone bank or clonal hedge (Hartmann *et al.*, 1997; Williams, 2000). Clonal hedges or clonal gardens are clone banks used for production purposes and coppice is harvested from these to produce cuttings (Hartmann *et al.*, 1997; Pierce, 1997; Williams, 2000). The hedge plants can be produced from improved seed or from cuttings from coppice from superior mature trees (Langman, 1993). The eucalypt material planted in the bank ideally comes from the tree breeding programme and is selected for superior growth, disease resistance and / or fibre characteristics in trials or plantations. Good rooting potential is also a selection criterion in some forestry vegetative propagation programmes.

In the case of eucalypts, new ramets in clone banks are established by rooting cuttings from eucalypt coppice regrowth of selected ortets. Clone bank management is an important element of any vegetative propagation programme as healthy ramets are required to produce strong cuttings. General maintenance to ensure healthy, productive ramets includes weeding, pest and disease control, nutrition and irrigation (Hartmann *et al.*, 1997; Pierce, 1997; Williams, 2000).

2.1.2.2 Harvesting material

According to Adendorff and Schön (1991), obtaining good root strike and root quality depends largely on the juvenility of the coppice material. Pierce (1997) observed that in South Africa the eucalypt coppice growth period is on average two months, but it varies from clone to clone and with season, fertigation and age of hedge mother plant.

The effect of hedge plant maturation poses one of the most challenging difficulties in producing cuttings from hedge plants over an extended period (Bonga, 1982; Barnes & Lewandowski, 1991; Wignall *et al.*, 1991; Geneve, 1995; Hartmann *et al.*, 1997; Williams, 2000). Maturation is the natural loss of juvenility as trees become older or as shoots selected for propagation are selected from the upper portions of trees. Hedge maturation is

not as obvious with eucalypts and generally, a reduction in the rooting ability of cuttings indicates that the condition of the ramet has declined (Williams, 2000).

Marien (1991) found large initial differences in rooting between two different categories of mother plants, *viz.* (a) coppice from adult trees (12 %) and (b) four-month-old seedlings (45 %). The clones used were mainly *E. camaldulensis* or natural *E. camaldulensis* x *E. grandis* hybrids. The difference diminished after two years, which Marien (1991) attributed to a better rooting environment. He found through his experiments that the quality of the mother plant plays a predominant role in the quality of the next generation cuttings.

Wignall *et al.* (1991) used six *E. grandis* clones to test the rooting success (%) using cuttings from four different sources of starting material (Table 1). They determined that the rooting success reflected the vigour of the cutting material.

Table 1: **Variation in rooting success (%) of six *E. grandis* clones with source of cuttings** (Wignall *et al.*, 1991)

	Clone number						
Cutting source	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**
Primary graft: shoots grafted onto seedlings and hedged	76	48	40	65	33	43	51
Crown shoots: shoots taken from top of eight-month old rooted cuttings approx. two metres tall	10	37	20	23	13	7	18
Hedge shoots 1: Eight-week old coppice regrowth after eight-month old plants were cut at 20 cm above soil level	95	92	87	94	100	93	94
Hedge shoots 2: Second harvest of coppice regrowth eight weeks after first crop removed.	100	90	100	89	100	75	92

2.1.2.3 Placing of cuttings

Zobel (1993) suggests that there is no one method of rooting for all *Eucalyptus* species and hybrids and recommends that each organisation work out techniques suitable for clonal propagation for their specific conditions. The selection of the correct type of shoot suitable for rooting is very important. The optimal size and physiological stage of eucalypt cuttings should be determined for every system (containers and greenhouses or other structure) and species (including hybrids) (Zobel, 1993; Pierce, 1997). Bayley, Young, de Swardt & Snell (2000) concur and state that *E. grandis* hybrid clones required different rooting compounds,

media and misting regimes for optimum rooting. Some recommendations for the cutting production of eucalypts are listed in Table 2.

Table 2: **Dimensions and other recommendations for cutting production of eucalypts**

Length	Thickness	Comments	Reference
6 – 10 cm	2 – 5 mm	• Length of the cutting determined by internode length • Two leaves per cutting • Leaves halved	Pierce (1997)
2.5 – 12 cm	4 – 8 mm	• No. of nodes dependent on growth habit of tree; management of stock plants and amount of material available • Single-noded cuttings: internode length >1.5 cm • Two to three node cuttings suitable for shoots with shorter internodes, those lacking leaves or buds and for hard-to-root species	Langman (1993)
Approx. 10 cm	3 – 5 mm	• Cut between lowest node and above next node • Two leaves per cutting • Leaves halved	Williams (2000)

According to Langman (1993), eucalypt coppice should be collected in the early morning, in misty weather or just after rain to keep drying out to a minimum. Wentzel (1998) and Bayley & Nixon (1998) recommend that in the clone banks, coppice should be placed in a bucket of water and sugar or water and boric acid solution. Shepherd (1986), Wentzel (1988), Pierce (1997), Bayley & Nixon (1998), de Swardt (1998) and Kemp (1998) all suggest that once the eucalypt cutting has been made and the leaves have been halved, that the cuttings be submerged in a fungicide / water solution for three seconds, shaken dry and the bottom part of the eucalypt cutting dipped into an IBA (indolebutyric acid) hormone powder. Either Seradix 2® (IBA 3 $mg.kg^{-1}$) or Seradix 3® (IBA 8 $mg.kg^{-1}$) should be used depending on the species or genotypes being propagated (Wentzel, 1988; Bayley & Nixon, 1998; Kemp, 1998). Langman (1993) suggests that eucalypt cuttings remain in the greenhouse for 25 to 30 days to root, depending on the external environment and internal greenhouse conditions. Wentzel (1988), Bayley & Nixon (1998), de Swardt (1998) and Kemp (1998) all report that rooting takes three to seven weeks, depending on species.

Maile and Nieuwenhuis (1996) conducted a series of trials using *E. nitens* stem cuttings to determine the effects of substrate, growth hormone application and seasonal variation (cuttings were collected at various times of the year) on the rooting ability of cuttings (Table 3).

Table 3: **Combinations of treatments used by Maile and Nieuwenhuis (1996)**

Treatment	Substrate	Hormone
PO	Peat	No application
P1	Peat	Seradix® 3
S0	Sand	No application
S1	Sand	Seradix® 3
V0	Vermiculite	No application
V1	Vermiculite	Seradix® 3
PSV0	Peat: sand: vermiculite	No application
PSV1	Peat: sand: vermiculite	Seradix® 3

The results from the three experiments using cutting material from 11-year old trees showed that these cuttings root with difficulty regardless of treatment with hormones and use of different substrates. In general the peat: sand: vermiculite mixture proved superior to peat, followed by vermiculite. Positive interactions between IBA and substrate were primarily restricted to peat where percentage rooting was significantly increased. The rooting ability of the juvenile material was higher than that of the mature material (30 % and 7 %, respectively), especially in a mixture of peat, sand and vermiculite. In the one experiment with juvenile material, sand had the highest percentage rooting (85 %) followed by peat and then vermiculite. Rooting hormone had a negative effect on the rooting of juvenile material.

The nutritional status of the cutting material and the stock plant has an influence on rooting in cuttings. This is because nutritional status can have a pronounced effect on plant growth and development (Blazich, 1988). Although rooting and the nutritional status of the plant are closely related, understanding the interactions is difficult because root formation is a multi-stage process and few studies have distinguished between the nutrient effects at the various stages (Hartmann *et al.*, 1990; Carlson, Bower & Ward, 2003). One could argue that any nutrient involved in the multitude of metabolic processes associated with root formation is essential. Researchers have however been unable to identify those nutrients

essential for root initiation. The situation with respect to root growth and development is much clearer as greater knowledge of this stage exists (Blazich, 1988).

Marien (1991) used five clones of natural hybrids between *E. camaldulensis* and *E. grandis* (CGs) in a trial to show the effect mother plant nutrition has on rooting of cuttings. Three formulae were tested with two or four grams per litre (one litre per plant) (Table 4).

Table 4: **Formulation of fertiliser treatments used by Marien (1991)**

	% of nutrient		
Fertiliser	**N**	**P**	**K**
Green	20	5	10
Red	8	12	24
Blue	15	11	15

The results indicated the important enhancement of rooting ability when applying complete fertilisers to the mother plants. With these treatments, it was possible to get nearly 39 % more rooted cuttings compared with the control (Table 5).

Table 5: **Effect of different types of fertiliser on the rooting percentage of CG cuttings**

	Dose ($g.l^{-1}$)		
Fertiliser	**0**	**2**	**4**
Green	58	79	76
Red	58	78	78
Blue	58	83	91

Special attention must be paid to greenhouse conditions as environmental conditions can influence the rooting of cuttings. Temperature control is very important, with the gradient between leaf and root temperature being the most crucial factor (Pierce, 1997). In South Africa, Pierce (1997) recommends a leaf temperature between 18 °C and 20 °C, which can be controlled by misters as they wet the leaves and the evaporation of this moisture off the leaves cools them down. Further, bottom heat will usually hasten and enhance root formation (Williams, 2000). Pierce (1997) also indicates that optimum root growth occurs at temperatures between 23.8 °C and 29.4 °C, and a standard procedure in South Africa is to install electric heating cables inside the benches and cover with sand to provide an even distribution of the heat. It has been shown that root initiation and root development have different temperature optima, 30 °C and 20 °C respectively (Gaspar & Coumans, 1987).

Using this knowledge, growers can improve root development by reducing root-zone temperature after roots emerge (Preece, 1995).

Misting during the root initiation phase of *Eucalyptus* cuttings is essential not only for evaporative cooling, but also for maintaining a high relative humidity in the rooting area (Pierce, 1997). Water is lost from the stem tissue by transpiration through leaves and can be replaced by the uptake of moisture through the stem base if moisture is available (Pierce, 1997). The atmosphere must be controlled so that it is conducive to low water loss and can maintain turgor in the leaves (Hartmann *et al.*, 1990). In South Africa, Pierce (1997) reports that with *Eucalyptus* cuttings, an effort should be made to keep at least 80 % of the leaf area moist at all times. This necessitates watering every few minutes with fine misting nozzles.

Light also plays an important role in the growth of the eucalypt cuttings, as sufficient light must be present for photosynthesis to take place (Hartmann *et al.*, 1997; Pierce, 1997). Hartmann *et al.* (1990) recommends 1.5 to 3.0 $MJ.m^{-2}.d^{-1}$. Light that is above this recommendation causes overheating of the eucalypt cuttings resulting in partial or whole dessication and / or photoinhibition. On the otherhand, light less than the recommended lower limit is below the light compensation point for photosynthesis (Langman, 1993).

2.1.2.4 Rooting process

Rooting is influenced by various endogenous and exogenous factors including age of the stock plant, season, type and size of cuttings, presence of leaves and / or buds, light, air and soil temperatures, harvesting conditions and nutrient status of stockplant and resultant eucalypt cuttings (Nemeth, 1986; Hartmann *et al.*, 1990). The primary internal controls on the initiation and development of adventitious roots include the carbon, water and nutrient budgets of the cutting, as well as endogenous plant growth regulators (Hartmann *et al.*, 1990). Light intensity, air and soil temperatures, vapour pressure deficit and nutrient supply, both when the eucalypt cutting is a part of the stockplant and after it has been detached and placed in a propagation bed, all influence root initiation regulators (Hartmann *et al.*, 1990).

While much experimental work has been done to identify factors which influence the ability of eucalypt cuttings to root, the mechanism of root formation remains unclear (Hartmann *et al.*, 1990). The initiation and development of adventitious roots involves many interactive processes, but experiments on rooting usually consider only a few of the most easily assessed factors known to influence rooting (Hartmann *et al.*, 1990). For example, Carter and Slee (1991) studied propagation media and rooting; Marien (1991) studied, in separate trials, the influence of the position of the cutting from the mother plant and the nutrition of the mother plant; Wignall *et al.* (1991) looked at the influence of stockplant management on rooting and there have been various in-house, unpublished studies by forestry companies (e.g. work done by Kopp (1997) and a study on the influence of plant growth regulators by McAlister, Wallis and Alborough (2002)) and research institutes on rooting and factors that influence rooting. Rooting is generally accepted as being a multi-step process (Nemeth, 1986; Hartmann *et al.*, 1990), involving totipotent cells which dedifferentiate, produce undifferentiated meristematic cells which then differentiate as root initials, elongate and emerge as visible roots from the stem tissues (Hartmann *et al.*, 1990).

2.1.2.5 Hardening off

Hardening off is the acclimatization of plants from the greenhouse to the field by the gradual decrease of shade and water (Langman, 1993). This weaning process forces the rooted eucalypt cutting to become more 'self-reliant' in taking up nutrients and water, in photosynthesizing and hardening-off leaves and stems to tolerate the shock of lower relative humidity, higher temperature and higher light intensity outside than compared to in a greenhouse.

Eucalypt cuttings deteriorate if left under mist after having rooted (Hartmann *et al.*, 1990). The decrease in root quality causes premature leaf drop, and slows down growth, which not only delays the production period, but also produces a poorer quality plant (Hartmann *et al.*, 1990). The hardening-off process also encourages more efficient secondary root development.

Eucalypt cuttings are moved to a shade-net area after being in the greenhouse for about a month (this depends on species, season and where production is located). They remain in the shade-net area for approximately 35 days where the cuttings are hardened off. Fungicide preventative treatment is frequently advisable at this stage because cuttings have grown and the overlapping of leaves in the tray provides an ideal environment for fungus development. Shading is then removed and in areas where hail occurs, the only protection afforded is the erection of a hail net (Pierce, 1997). In South African forestry nurseries eucalypt cuttings take three to six months from initial set before they are ready to be deployed, depending on the season of the year and the genotype being produced (Denison & Quaile, 1990; Bayley & Nixon, 1998; de Swardt, 1998; Kemp, 1998 and Wentzel, 1998).

2.1.3 Advantages of vegetative propagation

The main advantages of vegetative propagation in forestry and, in particular for eucalypt species and hybrids are:

a) Maintenance of genotypes (Biondi & Thorpe; 1981; Pierce, 1997; Wiessman & Jaenicke, 2000). Cloning is particularly important for the reproduction of a highly heterozygous genotype (Zobel & Talbert, 1984; Pierce, 1997; White, 2000). If an individual is reproduced by seed, the recombination of genes during the process leads to segregation with loss of heterozygosity and many important characteristics might disappear. If a superior individual tree has been identified, its genetic information can be fixed by vegetative propagation, thus allowing reproduction of the same superior individual in subsequent generations (Wiessman & Jaenicke, 2000).

b) Propagation of seedless plants (Langman, 1993; Pierce, 1997; Wiessman & Jaenicke, 2000). Vegetative propagation can offer a sound solution in the following instances: (a) if plants produce little or no seed (e.g. *E. nitens*); (b) for hybrids which are sterile; (c) if pests or diseases destroy the seed crop; (d) when it is difficult to collect seed and (e) when material is young, before trees reach flowering stage (Langman, 1993; Yang *et*

al., 1995). In those cases, vegetative propagation might be a suitable and cheaper alternative to seedling production (Langman, 1993; Wiessman & Jaenicke, 2000).

c) Rapid introduction of improved trees into progressive genetic improvement and deployment programmes (Biondi & Thorpe, 1981; Langman, 1993; Pierce, 1997; Wiessman & Jaenicke, 2000). Many tree species have not undergone domestication by man and breeding is much slower than with conventional agricultural crops. Vegetative propagation allows more rapid genetic improvement, as trees with desirable, inherited characters can be used directly to produce improved planting stock (Langman, 1993). In this way, new, improved clones can be deployed faster because one does not have to wait for the tree to produce seed.

d) Controlling phases of development (Wiessman & Jaenicke, 2000). A plant undergoes several age phases that can be distinguished by its growth vigour and flowering. Juvenile plants are vigourous, have a strong apical dominance and regenerate easily by vegetative propagation. Mature plants are not as vigorous and they do not regenerate easily by vegetative propagation. Through vegetative propagation techniques, the non-flowering juvenile phase can be shortened resulting in a reducing of the reproduction cycle of tree (Wiessman & Jaenicke, 2000). This is particularly useful when a seed orchard is being established. The plants will mature faster and the improved seed will be available sooner.

e) Uniformity of plantations (Biondi & Thorpe, 1981; Wiessman & Jaenicke, 2000). A decrease in product variability is a first step in improving an undomesticated tree species (Wiessman & Jaenicke, 2000). Through cloning, it is possible to obtain greater uniformity in size, quality and wood properties of the tree crop than is possible through regeneration by seed, as all the members of the clone are genetically identical (Pierce, 1997; Martin & Hine, 2000; Williams, 2000). For many commercial species, uniformity of growth form is economically very important.

2.2 Hydroponics

According to Harris (1982) and Resh (1998a) hydroponics is "the science of growing plants in a medium, other than soil, using mixtures of the essential plant nutrient elements dissolved in water." The word hydroponics was originally used in the United States as a synonym for soilless culture (Schwarz, 1995). This covers all methods and systems of growing plants without soil. There is no physiological difference between plants grown hydroponically and those grown in the soil (Carruthers, 1998). The process of nutrient and water uptake by plants is the same for both environments. The fundamental difference is the way in which the nutrients are delivered to the plant and the level of control of environmental conditions that can be exercised.

Hydroponic systems offer a highly efficient way of providing the necessary nutrients and water to the plants (Carruthers, 1998). In soil, water and nutrients are heterogeneously distributed and the production of roots to access these resources constitutes an energy and carbon cost. In hydroponic systems, the nutrients are homogenously dispersed and are in readily assimilable forms (Harris, 1982). This means that they are immediately available. Both water and nutrients are transported directly to the plants' roots. The plants can therefore grow faster and can be harvested sooner because they are putting their energy into aboveground biomass rather than root biomass.

Hydroponic systems in greenhouses have long been used in research because this is perceived to be a 'ideal' environment, where a plant is never lacking in water, nutrients and light (Soffer, 1986). These factors can also be easily controlled and manipulated.

Before continuing with this review a note needs to be made that there is extremely little work on *Eucalyptus* species in hydroponics and no published works were found although research has been done by da Costa Alpoim (2002).

2.2.1 Historical review of hydroponics and current applications

The development of hydroponics has not been rapid and its recorded history is quite vague. Various authors suggest different origins and place varying degrees of importance on specific breakthroughs. The summary in Table 6 has attempted to include all significant findings mentioned by the referenced authors.

Resh (1998a) described how some researchers believe that hydroponics was developed from the findings of experiments carried out to determine what substances make plants grow and develop. Those works date back as early as the 1600s. However, both Carruthers (1998) and Resh (1998a), indicate that hydroponic growing techniques have in fact been used since the Hanging Walls of Babylon.

Table 6: **Summary of the history of hydroponics**

Date	Person / Place	Event	Reference
Ancient times		• Hanging gardens of Babylon • Floating gardens of Kashmir • Aztec Indian of Mexico: Plants grown on rafts in shallow lakes • Egyptian hieroglyphic records, dating back to several hundred years BC, describe growing plants in water	Carruthers (1998); Resh (1998a)
1600	Van Helmont, Belgium	• Showed plants obtain substances from water	Schwarz (1995); Resh (1998a)
1699	Woodward, England	• Cultivated plants in water to which was added various types of soil showing that substances, derived from soil, are responsible for plant growth	Harris (1982); Schwarz (1995); Resh (1998a)
1804	De Saussure, France	• Proposed that plants are composed of chemical elements obtained from water, soil and air	Harris (1982); Schwarz (1995); Resh (1998a)
1851	Boussingault, France	• Verified De Saussure's proposal • Grew plants in sand, quartz and charcoal to which were added solutions of known chemical composition • Concluded that water was essential for plants growth in providing hydrogen and that plant dry matter consisted of hydrogen, carbon and oxygen • Also found that plants contain nitrogen and other mineral elements	Harris (1982); Schwarz (1995); Resh (1998a)
1850s	Various research workers	• Demonstrated that plants could be grown in an inert medium that was moistened with a water and nutrient solution.	Schwarz (1995); Resh (1998a)

Date	Person / Place	Event	Reference
1860/1	Sachs and Knop, Germany	• Eliminated the medium entirely and grew the plants in a water and nutrient solution. • Showed that normal plant growth could be achieved by immersing the roots of a plant in a water solution containing minerals • Made synthetic solution of essential plant nutrients in water. These did not include trace elements	Harris (1982); Harris (1987); Schwarz (1995); Resh (1998a)
1925	Research workers in U.S. Agricultural Experiment Stations	• Showed an interest in the possible use of nutrient solutions for large-scale crop production.	Resh (1998a); Jensen (1999)
1925 to 1935		• Extensive development took place in modifying the lab techniques to large-scale crop production.	Resh (1998a); Jensen, (1999).
1930s	Gericke (U.S.A)	• Grew tomatoes in solutions of plant nutrient elements and put early laboratory experiments on to a commercial basis. • The term 'hydroponics' was first used.	Harris, (1982); Schwarz (1995); Resh (1998a);
	New Jersey Agricultural Experiment Station	• Improved the sand culture method	Jensen, (1999).
	Californian Agricultural Experimental Station	• Water and sand culture methods were used for large-scale production	Jensen, (1999).
1934	New Jersey and Indiana Agricultural Experiment station	• Introduction of the sub-irrigation system	Jensen, (1999).
World War II		• Plants were grown in solid media rather than soil	Harris (1982); Carruthers (1998); Resh (1998a)
1945	U.S. Air Force stationed on Pacific islands	• Problem of providing its personnel with fresh vegetables • To solve this problem, hydroponics was successfully practiced on a large scale	Harris (1982); Schwarz (1995); Carruthers (1998); Resh (1998a)
After the war	U.S. military	• Continued to use hydroponics. • For example, the U.S. Army established a 22 hectare project at Chofu, Japan.	Harris (1982); Schwarz (1995); Carruthers (1998); Resh (1998a)
1950s		• Commercial use of hydroponics expanded throughout the world to other countries such as Italy, Spain, France, England, Germany, Sweden, the USSR and Israel	Harris (1982); Schwarz (1995); Resh, (1998a).

Date	Person / Place	Event	Reference
		• Development of plastics resulted in a renewed interest in hydroponics. • Plastics are used in the glazing of greenhouses, lining the growing beds, also important in the introduction of drip irrigation	Resh (1998a); Jensen (1999)
		• With technological progress of pumps, timers, plastic plumbing, solenoid valves and other equipment, the entire hydroponic system could be automated, reducing both capital and operational costs	Jensen (1999)
Early 1970s	Allen Cooper, U.K.	• The ebb-and-flow growing system, in various configurations, was the traditional hydroponic growing technique • Nutrient Film Technique (NFT): Growing plants without a root support medium and within a simply designed system	Cooper (1996)
	Massantini, Italy	• Another growing system, aeroponics, was designed. Plants roots were suspended in a mist of nutrient solution	Benton-Jones, Jr (1999)

Hydroponics has become a reality for greenhouse growers in virtually all climatic areas, and large hydroponic installations exist throughout the world (Resh, 1998a). Greenhouses in combination with hydroponics are becoming increasingly popular, particularly for horticultural crops (Jensen, 1999).

Today, hydroponics is playing an increasingly important role in the world's agricultural development. Population pressures, climatic changes, soil erosion, inequitable water distribution and polluted groundwater are all factors which are influencing alternative horticultural methods (Carruthers, 1998). Carruthers (1998) reports how hydroponics is being adapted to many situations, from outdoor field culture and indoor greenhouse culture to a highly specialized application in nuclear submarines, which provides fresh vegetables for the crews. Harris (1986) describes the hydroponic gardens in Namibia which was installed by the Consolidated Diamond Mines of South West Africa Limited (C.D.M). This facility had a combination of indoor greenhouse and outdoor field hydroponic beds. The reasoning behind the installation was that food crops such as tomatoes were being transported from Cape Town some 800 km away. Harris (1986) refers to the C.D.M's hydroponic installation as an oasis in the desert providing the surrounding mining community with fresh fruit and vegetables such as tomato, rhubarb, spinach and lettuce.

Hydroponics is a 'space-age' science, but at the same time is being used in developing countries of the Third World to provide intensive food production in resource-limited areas, such as in the rural areas of Southern Africa where children suffer from vitamin A and C deficiencies (Combrink & Harms, 2001). Combrink & Harms (2001) developed a system that can be used in rural areas that have no electricity to produce hydroponically grown vegetables at any time of the year. Its only constraints are sources of fresh water and nutrients. And with plans to establish a permanent space-station colony and a colony on the moon within our lifetime, the future of hydroponics is assured. Research by National Aeronautical and Space Administration is bringing us closer to achieving that, and is also revealing a great deal about plants (Carruthers, 1998; Resh, 1998a).

In South Africa, it was estimated in 1986 that there was about 300 ha under soilless culture (Harris, 1986). Harris (1986) states that hydroponics was little more than a curiosity until after the 1950s when some individuals and companies set up installations of various sizes which were generally not more than a quarter of a hectare. Some of these installations were initiated by mining companies to supply their personnel with fresh vegetables in areas where these are either unable to be cultivated or too distant from normal areas of supply (Harris, 1986). However, for South Africa, there is very little information recording the progress of hydroponics although research work has been conducted at various universities and research institutions and many hydroponic installations do exist for fruit, vegetable and flower production.

At a recent symposium, Schwarz (2001) indicated that, worldwide, in 1955 there was a mere one hectare of hydroponics compared to over 30 000 ha in the year 2000. Data on crops, substrates and systems in many countries are not readily available but a summary has been attempted (Appendix 1).

2.2.2 The benefits of hydroponics

As already mentioned, the main advantages of hydroponics over conventional soil culture are the more efficient use of water and fertilisers and the higher density planting that leads

to an increased yield per area planted (Schwarz, 1995; Resh, 1998a). Other advantages include the following:

a) Less water is used, particularly in recirculating systems (Carruthers, 1998; Resh, 1998a). The quantity of water used per unit weight of crop produced is less than that required under conventional agriculture (Schwarz, 1995). This considerable reduction in the amount of water required to produce a given crop is often the determining factor for the adoption of hydroponic systems in arid regions (Carruthers, 1998; Ikeda, 2001).

b) pH can be easily controlled. Inert growing media are almost exclusively used in hydroponics which means that pH can be altered, almost precisely, by simply adjusting the pH of the nutrient solution (Harris, 1982; Resh, 1998a). It is also easier to provide more or less nitrogen, phosphorous, potassium and other nutrients should the plants require special treatment (Harris 1982; Ikeda, 2001). One can therefore accurately control the nutritional supply to the plant and consequently growth control can also be achieved.

c) Larger yields may be expected and the crop is usually of a better quality compared to a field-grown crop (Harris, 1982; Carruthers, 1998). Harris (1982) defines yield as "the return per unit growing area per unit time". According to this definition the hydroponics method is superior to a field-grown crop. The reasons for this are that plants mature faster, and in addition, because there is no competition by plants for nutrients, denser plantings of crops is possible (Carruthers, 1998; Resh, 1998a). This at least results in a higher yield per unit area (Schwarz, 1995; Resh, 1998a).

In work by Silveira, Camargo, Luca & Luz (1995a,b) and Higashi, Silveira, Paula, Zanardo & Gon◊alves (1998) it was found that in a comparison of soil and hydroponic systems the levels of all nutrients in the new shoots were higher in the hydroponics system after 28 days. These authors concluded that the higher concentrations of nutrients available to the plants per square metre in the hydroponics was the result of greater shooting productivity, in a shorter time period, than in the clonal macrogarden (soil system).

Janse (2001) reported that International Paper (IP) has three clonal nurseries which each produce 10 million rooted cuttings. The nursery at Mogi Guacu is the largest and is based exclusively on hydroponics. Whilst IP has been working on hydroponic feeding of hedge plants for a number of years, it was due to their inability to ensure maximum productivity in each of their nurseries using traditional macrocuttings, that they were forced to fast track the hydroponics system. Currently, IP achieves a rooting percentage of 90 – 95 % across all *E. grandis* x *E. urophylla* (GU) clones. While IP is converting to hydro-hedges, the rooting system is the same as for the field hedge system.

At Aracruz Cellulose, Janse (2001) recorded 160 macrocuttings per square metre per month being obtained from conventional hedges compared to 1600 cuttings per square metre per month from microgardens. When taking the increased survival into account they are achieving almost a 300 times increase in productivity. Current survival rate after 55 days is 60 % from macrohedges, whilst the cuttings from microhedges attain a 90 - 95 % survival at 55 days. Aracruz is converting their hedge systems for propagation from field to hydro-hedges but the rooting systems will remain identical.

d) A uniform substrate is used and if the system is properly set-up and monitored the nutrient availability is homogenous and constant over the whole system resulting in a uniform crop. Certain climatic factors, such as temperature and humidity, can also be controlled within limits, depending on the degree of automation (Harris, 1982).

e) Soil pests and diseases are reduced because the sterilisation of the aggregate and containers is simple and inexpensive (Carruthers, 1998; Resh, 1998a).

f) Many operations in soil cultivation can be eliminated (e.g. hoeing, ploughing, tending) (Harris, 1982; Resh, 1998a; Ikeda, 2001).

g) Less maintenance is required because many of the substrates used in hydroponics are sterile in nature and therefore contain no weed seeds (Harris, 1982; Carruthers, 1998;

Resh, 1998a; Ikeda, 2001). This means there is little to no chance of the occurrence of weeds except those that are wind-borne which are easy enough to eradicate by hand (Harris, 1982).

The main disadvantages of hydroponics are the high initial capital cost, the fact that diseases can spread rapidly through the system, and nutritional imbalances can occur. Although most of these disadvantages can be overcome, the following must be considered:

a) Costs can be high depending on scale of operation, degree of automation, equipment used etc (Harris, 1982; Carruthers, 1998; Resh, 1998a). The more elaborate and automatic the system employed, the greater will be its efficiency, and the higher the costs. However, simple, low-cost hydroponic systems can be improvised (Carruthers, 1998). Such simple methods may often be satisfactory but will not necessarily provide all the possible advantages to be gained from hydroponics (Schwarz, 1995).

b) Diseases can spread rapidly in a hydroponics system and nutritional deficiencies and toxicities can also be a problem (Carruthers, 1998; Resh, 1998a). These can be avoided by implementing a good management programme which involves periodically taking measurements of pH and EC of the nutrient solution and implementing good hygiene protocols (Carruthers, 1998).

   Aracruz has experienced many problems since the nursery has included hydroponically fed microgardens. One of these was disease management. It was clear that the plants were really tightly packed against one another and that this microclimate could cause problems. Outbreaks of both aphids and *Pythium* have already occurred in the gardens within the first six months of operation (Janse, 2001).

c) Some chemistry, plant physiology and plant nutrient metabolism knowledge is required. On a large scale it is useful to have a basic knowledge of agricultural chemistry and plant physiology, but on a small-scale commercially available plant nutrient mixtures can be purchased to provide the necessary nutrients to the plants (Harris, 1982). One

must also have sufficient knowledge of the particular crop to be grown in order to utilise hydroponic methods successfully (Schwarz, 1995).

Another problem Aracruz experienced related to their understanding of the best hydroponic solution for the individual clones and also to the fact that they had problems with precipitation of microelements in their irrigation lines. Furthermore, there have been problems with bad nutrition on the gardens as indicated by the fact that the hedges do not show shoot elongation (Janse, 2001).

Higashi, Silveira & Gonçalves (2002) reported that for a clonal hydroponic mini-garden, problems encountered are those relating to nutrient toxicity rather than deficiencies. Toxicity of manganese is often observed with concentrations often reaching 1000 $mg.kg^{-1}$ in the leaves and another common toxicity is that of boron. It was found that the main cause of such problems is usually the lack of care in the preparation of the nutrient solution by non-technical personnel that often make errors in weighing. It is often observed that the leaves exhibit chlorosis of the veins, similar to the symptoms of Fe and Mg deficiencies, even though the foliar concentration of these elements are deemed adequate. In most cases this is found when the plants are sprayed with fungicides which contain Mn and Zn, and certain types of insecticides. Another common deficiency is that of Ca, particularly when N and water are supplied in large quantities. The main symptom is the rotting of the base of the stem near the root. In these cases the Ca concentration at the apical parts of the shoot is lower than 4 $g.kg^{-1}$. The recommendation is to decrease the supply of N and water and increase Ca with a foliar spray.

d) There are environmental problems associated with hydroponics. For example, the eutrophication of groundwater, dams and lakes caused by nutrient solution spills, or of used growing media, such as Rockwool® (which is not biodegradable) when it is not properly disposed of (Schwarz, 1995).

e) There is only a small buffer capacity for plants roots, especially in a nutrient film technique (NFT), in event of a breakdown or malfunction of equipment (Schwarz, 1995). A breakdown may quickly result in irreparable damage to plants.

2.2.3 Examples of hydroponic systems

Authors here classified hydroponic systems differently from one another based on different factors. For example, Harris (1982) differentiated between two distinct types of hydroponics, *viz.* water culture and aggregate culture, where the latter employs some solid medium in which to grow plants. He further divided aggregate culture into sand or vermiculite culture and gravel culture. In contrast, Carruthers (1998) distinguished between hydroponic systems based on the type of media used. These are water culture, aggregate culture, Rockwool® culture, sawdust culture and aeroponics. Another author, Schwarz (1995) grouped systems according to their type and size of support medium, and the installation of the system. He recognised four main groups, namely water culture, sand culture, gravel culture and aeroponics.

Ikeda (2001) divided hydroponic systems into three main groups: water culture; aeroponics and media culture. The latter, is then further separated into organic or inorganic media (Figure 4).

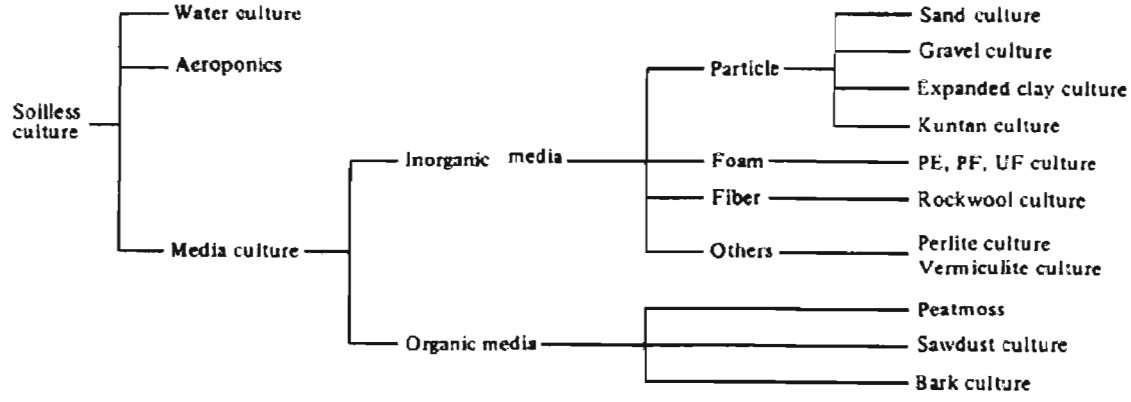

Figure 4: **Diagrammatic representation of different hydroponic systems** (PE = poly-ethylene; PF = polyurethane foam ; UF =ureaformaldehyde foam)

Systems have also been categorised as either 'open' or 'closed' (Harris, 1982; Carruthers, 1998). An open or non-recirculatory system is a run-to-waste system, which means that the nutrient solution passes the root zone only once before being discarded. Over 90 % of commercial hydroponic production uses these types of systems (Carruthers, 1998). On the other hand, in closed systems the nutrient solution is continuously recirculated. Different systems can be either run continuously (e.g. NFT) or intermittently (e.g. flood-and-drain). Carruthers (1998) reported that less than ten percent of commercial hydroponic production used these types of systems.

There is a great deal of interest in the closed system for growing protected horticultural crops to prevent the seepage of fertilisers and pesticides to the groundwater (O'Shea & Prasad, 1999). Thus there has been a swing from growing in soil *in situ*, to artificial media with recirculation of the feed solution, thereby preventing any seepage of nutrients into the ground water (O'Shea & Prasad, 1999). In countries where hydroponic techniques are more developed, the wastes of depleted nutrient solutions may pollute the environment and therefore nutrient solution recycling methods have been promoted (Chagvardieff, Pean, Ravel & Vidal, 1999).

2.2.3.1 The Nutrient Film Technique (NFT)

The work leading to the NFT was started in the late 1960s and the system was originally designed and developed by A. J. Cooper (Harris, 1982). It is officially described by the International Society for Soilless Culture (ISOSC) as a system in which "a very shallow stream of water containing all the dissolved nutrients required for growth is recirculated past the bare roots of the crop plants in a watertight gully". This society makes the following recommendations: "Ideally, the depth of the recirculatory stream should be very shallow, little more than a film of water – hence the name nutrient film. This ensures that the thick root mat, which develops in the bottom of the gully, has an upper surface which, although moist, is in the air. Consequently, there is an abundant supply of oxygen to the roots of the plants" (Carruthers, 1998). This supply of oxygen is one of the many advantages of the NFT system.

A typical NFT system is shown in Figure 5. The plants are grown in channels so that the roots are bathed to a depth of about one to two millimetres in a thin film of continuously flowing nutrient solution in order to ensure a maximum oxygenation of the root system (Harris, 1982; Carruthers, 1998; Resh, 1998a). There is no substrate, as it is represented by the nutrient film itself (Harris, 1982; Resh, 1998a). Irrigation or circulation of the nutrient is a continuous process and NFT systems are generally recirculated or closed systems (Harris, 1982). Trough covers provide plant support and help decrease algal growth in recirculating nutrient solutions (Albright, Both, Langhans & Scholl, 1999).

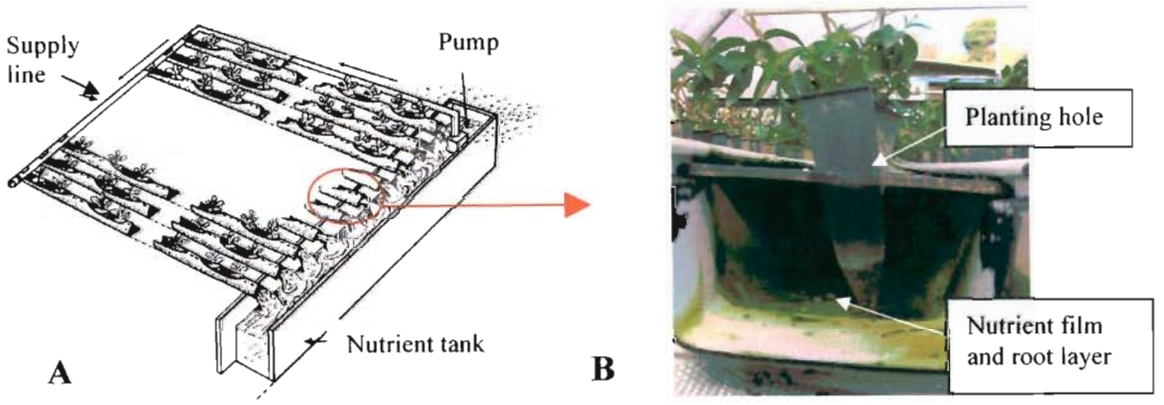

Figure 5: **(A) Layout of NFT gutters and nutrient solution tank showing recirculation of nutrient solution** (Adapted from Resh, 1998a and Carruthers, 1998). **(B) A photograph showing the film of water and root layer in an NFT system**

The main advantage of the NFT system is that there is no support medium (Harris, 1982), which reduces both running costs and waste. NFT systems can produce high yields and high quality crops from high-density plantings because, due to their design, they can meet the three basic plant requirements (i.e. nutrients, water and oxygen) (Carruthers, 1998). Other advantages include the precise control of the nutrients and easy adjustment of nutrient solution to control plant growth under changing conditions. It is also simple to administer systemic pesticides in the nutrient solution to control insects and diseases (Resh, 1998a). The technique of NFT is more difficult than other hydroponic methods and experience in raising plants is necessary particularly in a commercial project (Harris, 1982).

A major concern with NFT is the risk of flooding, waterlogged roots, drying out of roots and other problems due to poor design, construction and / or operation. The NFT system is

also entirely dependent on reliable sources of water and electricity. If a breakdown does occur and no suitable back-ups exist the grower can suffer serious losses as there is no degree of buffering (Carruthers, 1998).

While the size, design and construction can vary between large commercial units, research laboratory units and units designed for the small home gardener, the basic principles of NFT remain the same (Carruthers, 1998).

2.2.3.2 Ebb-and-flow systems

Ebb-and-flow systems are also referred to as flood-and-drain or sub-irrigation and are a type of closed hydroponic system (Carruthers, 1998; Resh, 1998a). They consist of shallow tables with inlets and outlet valves situated at the bottom of the tables. Periodically, according to the climatic and environmental conditions, the tables are flooded and then drained to allow plant roots to aerate. The movement of the nutrient solution can be seen in Figure 6. Once the water level has reached a set depth during the flood cycle, overflow valves allow the nutrient solutions to drain back into reservoirs where they are recirculated by pumps, thereby providing a continuous flow of fresh nutrient solution to the plants (Carruthers, 1998).

The ebb-and-flow system displaces the stale air out of the root zone during the flood cycle and replenishes the root zone with fresh air during the drain cycle. This movement of nutrient solution provides roots with the necessary water, nutrients and oxygen.

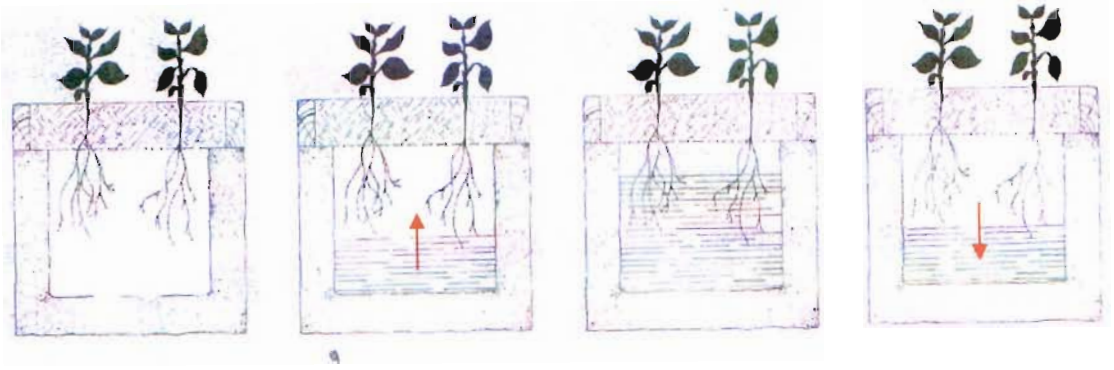

Figure 6: **Cross section of a simplistic ebb-and-flow system** (adapted from Resh, 1998a) (⟶ Movement of nutrient solution)

Ebb-and-flow methods are widely used in pot and bedding plant production (Treder, Matysiak, Nowak & Nowak, 1999). In such production systems a nutrient solution is very uniformly distributed in potting medium, and less variation is found in plant growth compared to top irrigation (Treder *et al.*, 1999; Resh, 1998a). The advantage is that there is less chance of crop loss during a breakdown, when compared to the NFT system. The substrate continues to provide water and nutrients to the plant until the malfunction is repaired (Carruthers, 1998). The system is easily automated and because it is a recirculating system there is a decrease in runoff which means a decrease in water and fertiliser cost and a decrease in environmental damage. Like all hydroponic systems, ebb-and-flow requires reliable water and electricity (Treder *et al.*, 1999). With ebb-and-flow systems, several media types can be used.

2.2.3.3 Aeroponics systems

The most recent development in hydroponics is aeroponics, defined by the ISOSC as "a system in which the plants' roots are continuously or discontinuously in an environment saturated with fine drops (a mist or aerosol) of nutrient solution" (Carruthers, 1998; Ikeda, 2001). The system requires no substrate, the plants are grown with their roots suspended in a growth chamber, with the roots periodically moistened with a fine mist of atomised nutrients. The recirculating nutrient solution is sprayed onto the bare root system and an optimum supply of nutrients, water and oxygen can be achieved (Molitar & Fischer, 1999). The system requires one hundred percent relative humidity to prevent drying (Resh, 1998a).

The chamber can be of any size and design, as long as it is moisture-proof and dark (Carruthers, 1998; Resh, 1998a). A generalised aeroponic system (Figure 7) requires a means to support the aerial portion of the plant while its root system is exposed to a source of nutrient mist and protected from direct sunlight. A distribution system of nozzles generates the mist from water pumped from a nutrient solution storage tank. The frequency and duration of a mist application is controlled by an electrical timing device connected to the pump which ensures that precise and timely applications will be provided (Giacomelli & Smith, 1989).

Since its development some thirty years ago, the aeroponic technique has been very successful for propagation on a research scale, but has yet to prove itself on a larger commercial scale (Carruthers, 1998). Giacomelli & Smith (1989) and Resh (1998a) both record exceptions found in Italy and Israel (e.g. the Ein-Gedi system) where research has led to development of commercially available systems, and grower installations. Carruthers (1998) and Resh (1998a) both report that aeroponics is widely used in laboratory studies of plant physiology, and is also being used in the USA to research controlled environment life support systems to be used in space stations of the future, and to support visitors to Mars. The aeroponic technique has primarily been utilised by the researcher because of its unique capabilities and characteristics. In studies for plant growth and development, various parameters can be easily controlled for observation of the effects of nutrition, growth regulators, root zone aeration and water stress analysis (Giacomelli & Smith, 1989).

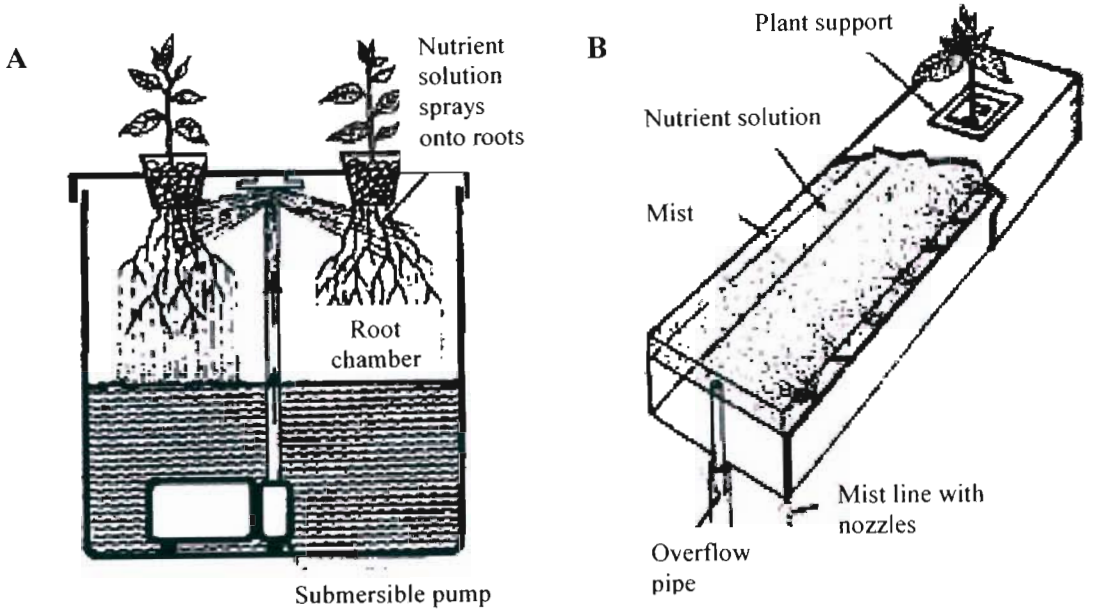

Figure 7: **(A) A generalised aeroponic system showing roots getting sprayed with nutrient solution** (adapted from Carruthers, 1998) **(B) The Ein-Gedi system which was developed by the Israelis is an example of an aeroponics system** (adapted from Resh, 1998a)

According to Carruthers (1998), the main advantage of aeroponics is the optimum supply of nutrients, water and oxygen. Another advantage is the closed irrigation system which prevents groundwater pollution and the media-free cultivation also lowers the running costs and waste problem (Molitar & Fischer, 1999). However, as there is no substrate, there exists little or no buffer for nutrients or moisture uptake for plants' roots in event of a breakdown e.g. no electricity.

In addition to the relatively high set-up costs, the aeroponics technique is mechanically quite elaborate, susceptible to malfunction, requires regulation and control of water and nutrients, and has no buffer capacity to sustain even slight malfunctions (Carruthers, 1998). The requirement for reliable equipment and permanent automatic control is high, and the consumption of electrical energy is high (Molitar & Fischer, 1999). The complexity and maintenance requirements of the system have been the primary factors which have precluded major acceptance within industry (Giacomelli & Smith, 1989).

2.2.4 Examples of commercial substrates used in hydroponic systems

The medium or substrate used in hydroponics is more than simply a means of support for plants (Carruthers, 1998). For optimum results, it must also hold oxygen and water effectively and offer good drainage (Harris, 1982; Carruthers, 1998). The following must be considered when deciding on an appropriate substrate:

a) Toxic substances and salts must be absent (Harris, 1982; Carruthers, 1998; Resh, 1998a). Many substrates consist of cinders, which may be acidic or alkaline in nature or contain fine dust or silt, and must be pre-treated or washed before use (Harris, 1982).

b) Good moisture retention is essential (Harris, 1982; Carruthers, 1998). The moisture retention of a medium is one of the most important characteristics and is influenced by the size, shape and porosity of the particles and pore spaces (Harris, 1982; Carruthers, 1998; Resh, 1998a). For certain given shapes a packing of smaller size particles produces smaller voids, hence greater capillary attraction compared to larger size particles. Chippings with irregular facets pack better and have greater moisture-holding capacity than rounded particles of the same size, and voids between the chips are smaller, hence more water is held within the substrate by virtue of its stronger capillary attraction (Harris, 1982; Resh, 1998a). The more porous the material, the greater the quantity of water that can be stored within the particles themselves and thus the higher the water-retention of the medium (Carruthers, 1998; Resh, 1998a). Smaller particles are more water retentive than large ones because they have proportionately greater surface area and pore space and they pack together more tightly (Carruthers, 1998).

c) Good drainage of the rooting substrate is essential. Particle size and drainage must be considered together (Harris, 1982; Carruthers, 1998). All free liquid should be removed from the voids within the substrate after gravitation back into the reservoir. The object is to leave a film of nutrient moisture around each particle for nourishing the plant. The voids should be filled with air-saturated water vapour to allow normal respiration of root systems and healthy metabolic activity. Therefore, a compromise must be brought

about between moisture retention on the one hand and good drainage on the other (Harris, 1982).

d) Substrates must be durable (Carruthers, 1998; Resh, 1998a), so that they do not disintegrate or lose their structure. This would lead to compaction which interferes with free drainage and hence poor root aeration and anaerobic conditions may result.

e) Sharpness in a substrate can prove injurious, particularly to the crowns of plants. Sharp particles may cut roots and stems of plants (Harris, 1982; Resh, 1998a). Resulting injuries make it possible for disease-producing organisms to enter the plant tissues (Harris, 1982).

f) Cation exchange capacity (CEC) is important. Cations are particles that have positive charges and many important plant nutrients occur in the nutrient solution as cations. These ions attach themselves to medium particles that have a negative charge, thereby staying in the medium and not leaching away. As a result, they are available to the plant roots for longer. Substrates that attract a large number of cations are described as having a high CEC, which is a desirable characteristic. Clearly, hydroponic substrates with a high CEC require less frequent applications of nutrients than those with a lower CEC (Carruthers, 1998).

g) Essentially, hydroponic substrates need to be inert i.e. neither contributing nor altering nutrients, and thereby giving the grower complete nutritional control. Hydroponic support substrate should not influence the pH of the nutrient solution (Carruthers, 1998).

Martinez & Barbosa (1999) explain that the appropriate physical characteristics can be obtained by mixing a variety of substrates. Before purchasing substrates, it is important to know their characteristics, so that the mix appropriate for any particular hydroponic system can be achieved (Carruthers, 1998). The choice between substrates will be dictated to an extent, by factors such as availability, cost, climate and system type.

Higashi *et al.* (2002) recommend that a substrate should be inert (in terms of its composition) and able to maintain its physical characteristics (such as particle size) through-out the production duration. Those authors found that substrates such as sand or bark possess the required physical and chemical characteristics (Table 7).

Table 7: **Physical and chemical characteristics of substrates tested by Higashi *et al.* (2002)**

	Fine sand	**Bark**	**Expanded clay**	**Mineral wool**	**Vermiculite**
Water retention capacity	High	Low	Low	High	High
Porosity	Low	Moderate	High	High	Moderate
Particle size	Small	large	large	Fibres	
Overall density (apparent)	High	High	Moderate	Low	Low
Capillary action	Moderate	Low	Low	High	High
Water loss at the surface	Moderate	Moderate	Moderate	High	High
Loss of structure with use	Low	None	Low	Moderate	Moderate
Possibility of recycling	Good	Good	Good	Poor	Good
pH	7.2 variable	6.9	6.6	7.1	7.3 5.5-9.0
Possibility of cation exchange (mg.l-1)	Low 10-40	Low	Low 0-1	High 0-1	High 50-150
Na concentration (mg.kg-1)	20	-	16	-	-

2.2.4.1 Organic substrates

Peat has long been used as a major component of horticultural substrates due to its highly suitable chemical and physical properties (Table 8) for propagating and growing plants (Handreck & Black, 1994). Peat is formed as a result of partial decomposition of plants under anaerobic or semi-anaerobic conditions. Peat bogs are formed under climatic conditions of high precipitation, low evaporation, low solar radiation and low temperatures. There are several types of peat currently used such as palm peat, moss peat and various genera of *Sphagnum* peat (Schwarz, 1995). The composition of different peat deposits varies depending upon the vegetation from which it originated, the degree of decomposition, the mineral content and the degree of acidity (Resh, 1998a). Peat is still the preferred medium of professional growers in many countries on grounds of reliability, uniformity and continuity of supply (Carlile, 1999). Peat has a low pH and low levels of nutritional elements (Schwarz, 1995). There have however been some serious allegations from environmental groups about the destruction of peat bogs (Carlile, 1999).

2.2.4.2 Inert substrates

a) Sand is probably the oldest hydroponic substrate known (Carruthers, 1998), and can vary in size, shape, composition and colour. Any particle from 0.25 to 2.5 mm may be described as sand (Harris, 1982; Carruthers, 1998; Ikeda, 2001). However, not all sands are alike and not all are suitable for use in a hydroponic system (Table 8) (Harris, 1982; Carruthers, 1998). Calcareous (lime-containing) minerals, such as shell, may be present or the sand may be contaminated with silt or organic matter (Harris, 1982). Granitic or silica-type sands should be used and not calcareous sands, which are too alkaline (Carruthers, 1998; Resh, 1998a). Beach sand is also unsuitable because of its high levels of NaCl (Carruthers, 1998). The ideal sand aggregate is river sand, washed free of all fine silt and clay (Harris, 1982). The particle size should be between 0.6 and two millimetres in diameter, which allows the aggregate to drain freely (Harris, 1982; Carruthers, 1998). Builders' sand is often suitable and almost universally obtainable (Harris, 1982).

b) Gravel is one of the oldest and most widely used hydroponic substrates. It was one of the first substrates used by Gericke (1930s), who pioneered the modern revival of hydroponics using sub-irrigation techniques. After gravel has been irrigated, enough water and nutrients cling to the gravel and roots to supply the plant until the next irrigation cycle (Carruthers, 1998).

Gravel is described as a material belonging to a size group rather than conforming to a type. Aggregates between 1.5 and nine millimetres are recognised as gravel (Harris, 1982; Ikeda, 2001). Gravel differs enormously in size, porosity, shape, hardness and composition. All these factors aid in determining whether or not a gravel is suitable for hydroponics. The characteristics (Table 8) of gravel are similar to those of sand but the particles are larger (Harris, 1982). As with sands, gravels of calcareous origin, such as limestone and coral, should be avoided, since they increase alkalinity of the nutrient solution (Harris, 1982; Carruthers, 1998; Resh, 1998a).

According to Carruthers (1998) the best choice of gravel is crushed granite of irregular shape, free of fine particles less than two millimetres in diameter and coarse particles more than 15 mm in diameter. At least half the total volume of gravel should be about 10 mm in diameter. Gravel is suitable for many irrigation techniques.

c) Perlite, essentially a mined mineral of volcanic origin (a potassium sodium aluminium silicate) has extensive horticultural use in the United States (Harris, 1982; Resh, 1998a).

For horticultural purposes the natural mineral is screened and heated to a temperature of 910 °C – 1000 °C (Harris, 1982). On being heated these crystals explode like popcorn and form soft white or grey granules which have a foam-like texture and countless tiny cavities on a glass-like surface (Carruthers, 1998; Resh, 1998a). Perlite expands to four times its original volume. In so doing the crude rock has an amazingly low density (Harris, 1982). The surface area of each particle is greatly increased from the original mineral (Harris, 1982). In South Africa, the grade most commonly used is two to 30 mm in diameter and weighs 80 to 110 $kg.m^{-3}$ (Harris, 1982). Perlite is an excellent substrate (Table 8) for germinating seeds or striking cuttings in hydroponics (Harris, 1982; Resh, 1998a). It can be used solely as a growing medium or, in situations where additional water retention is required, can be used in conjunction with vermiculite (Carruthers, 1998).

d) Vermiculite is a mica mineral – a complex hydrated magnesium aluminium iron silicate in chemical composition (Harris, 1982; Carruthers, 1998; Resh, 1998a). It exists naturally in plates of lamellae, which are thin. To produce horticultural vermiculite it is necessary to obtain a deposit which, when suspended in water, will give a nearly neutral pH value. A suitable size is a fine vermiculite whose average particle size is about 1.25 mm. The larger the particles, the more easily they flake apart along their cleavage planes.

The mined mineral is first milled and screened to a size suitable for growing purposes (Harris, 1982). It is then placed in a furnace at approximately 1000 °C where a change known as exfoliation takes place. In this process the water of hydration instantaneously

turned into steam which forces the hundreds of lamellae outwards like a concertina. This splits the layers apart and creates light, spongy particles that are excellent for hydroponic cultures (Harris, 1982; Carruthers, 1998; Resh, 1998a). The original particle finally expands to between twelve and twenty times its normal thickness (Harris, 1982). The very light, soft particles so formed will retain water up to 50 % by volume (Table 8) (Carruthers, 1998).

e) The term Leca is derived from the initial letters of the words Light Expanded Clay Aggregate. As the name suggests the pellets are made from clay and have a very low bulk density (300 to 600 $kg.m^{-3}$). The clay pellets, about the size of a marble, are prepared by heating in a furnace at about 1100 °C (Harris, 1997; Ikeda, 2001). Leca or expanded clay, is formed by blending and firing clay in rotary kilns (Carruthers, 1998). Furnaces for the production of Leca are to be found in most countries of Europe and Scandinavia (Harris, 1997). It has proved to be a popular and successful substrate for more sophisticated systems, particularly for flood-and-drain tables (Carruthers, 1998) (Table 8).

f) Rockwool® was developed in Denmark in 1969 and is now the most popular substrate in Scandinavia (Carruthers, 1998). Worldwide, Rockwool® is used in over 80 % of hydroponic systems and it is also widely used as a propagation medium (Carruthers, 1998). In Holland there are over 2000 ha of crops grown in Rockwool® under drip irrigation (Harris, 1982). The properties of this substrate make it extremely suitable (Table 8). In recent years, however, some growers have been moving away from Rockwool® as it is not biodegradable, and thus poses a problem of disposal (Carruthers, 1998).

Rockwool® is an inert fibrous material manufactured from three natural raw materials. A mixture of volcanic rock (diabase), limestone and coke are melted at a temperature of 1500 °C to 2000 °C (Harris, 1982; Carruthers, 1998; Ikeda, 2001). The resultant molten mass is spun into fibres of 0.005 mm diameter which, together with certain additives (phenol resin to reduce surface tension) are pressed into sheets and then cubes (Carruthers, 1998).

Table 8: **Characteristics/ properties of the substrates discussed** (Harris, 1982; Handreck & Black, 1994; Schwarz, 1995; Harris, 1997; Carruthers, 1998; Resh, 1998a; Ikeda, 2001)

Property/ Characteristic	Peat	Sand	Gravel	Perlite	Vermiculite	Leca	Rockwool®
Water-holding capacity	Good	Good	Poor	Fair	Good	Fair	Good
pH buffering capacity	Yes	None	None	None	Yes	Yes	None, except first time which can be compensated for
Drainage/ Aeration	Poor	Fair	Good	Good	Fair	Good	Good
Sterility on purchase	Yes, relatively	No	No	Yes	Yes	Yes	Yes
Requires sterilization after initial use	Yes	Yes	Yes	Yes	Yes but not easily done	Yes	Yes
Cation Exchange Capacity	Some	None	None	None	High	Some	None
Inert: materials contribute/ alter the solution	Low levels of nutritional elements	None, if sterilised	None, if sterilised	None	Mg and K released to plants	None	None
Consistency of material	Variable in composition	Variable in size and shape of particles	Variable in size and shape of particles	Consistent	Consistent	Consistent	Consistent
Problems with handling	Has a low bulk density.		Has sharp edges which can injure plants; heavy material.	Soft media causes powdering; white colour encourages algal growth; Lightweight and easy to handle but easily blown away by wind.	Breaks down over time; lightweight and easy to handle but easily blown away by wind.	Low bulk density; small pore sizes a blockage problem with algae.	Easy to handle and lightweight but user needs to wear protective gloves, mask and goggles.
Durable	Yes	Yes	Yes	Yes	No	Yes	Yes

2.3. The nutrient environment

Nutrients, or mineral elements, are the common ground between conventional agriculture and hydroponics. These elements are absorbed by the plant from the soil (conventional agriculture) or nutrient solution (hydroponics) (Harris, 1982). As was previously mentioned, modern hydroponics had its origins in plant physiology experiments where plant constituents were being studied, and this led to the discovery of essential plant elements (Carruthers, 1998). It can be thus said that the science of hydroponics is based on an understanding of plant nutrition. The hydroponic method enables growers to monitor, control and modify the availability of nutrients through the nutrient solution, which can result in larger yields of better quality crops (Barry, 1996; Carruthers, 1998). Simply, the optimisation of plant nutrition is more easily achieved in hydroponics than in conventional agriculture (Janick, 1986; Landis, Tinus, McDonald & Barnett, 1989; Carruthers, 1998).

Of the 92 natural elements that exist only 16 are considered essential for plant growth (Barry, 1996; Carruthers, 1998; Resh, 1998b). To be considered essential, elements must fulfil the following three criteria: (1) the element must be necessary for the plant to complete its life cycle; (2) its action must be specific (i.e. it cannot be replaced by any other element) and (3) it must be directly involved in the growth or metabolism of the plant (i.e. it must be required for the action of an enzyme) and not some indirect effect such as by antagonising another element present at a toxic level (Arnon & Stout, 1939; Benton-Jones, Jr, 1998; Carruthers, 1998; Resh, 1998b). The essential elements are divided into macro- and microelements with the former being required at concentrations greater ($g.l^{-1}$) than the latter ($mg.l^{-1}$).

Although most plants require only the 16 essential elements, some species do absorb and use other elements (Resh, 1998b). Table 9 shows a summary of these 16 elements and their functions in the plant. For extensive details on nutrients and their roles in plants refer to George, Puttock & George (1988), Dell, Malajczuk & Grove (1995) and Hartmann *et al.*, (1997).

Table 9: **Summary of the elements and their functions within the plant** (Harris (1982), George, *et al.,* (1988), Edward-Muckle, (1993), Barry (1996), Benton-Jones, Jr, (1998), Carruthers (1998), Resh (1998b))

Element	Function	Form taken up
	MACROELEMENTS	
Carbon (C)	• main constituent of all organic compounds • part of virtually every metabolic process and structure within the plant	CO_2
Hydrogen (H)	• important for the cation exchange in plant-medium relations • one of the keys in the process of releasing energy • major constituent of plant structure as component of water	H_2O
Oxygen (O)	• essential in the anion exchange between roots and the supporting medium • appears to be important in every metabolic process in the plant • key factor in fuelling the transport system of the plant • component of virtually every molecular structure in the plant	O_2, H_2O
Nitrogen (N)	• combines with C, H, O and sometimes S to form amino acids, enzymes, nucleic acids, chlorophyll, alkaloids and purine bases • used in various forms to promote rapid vegetative growth, leaf, flower, fruit and seed development • ammonium required for lignin/ cellulose creation	NO_3^-/ NH_4^+
Phosphorus (P)	• component of certain enzymes and proteins, adenosine triphosphate (ATP), ribonucleic acids (RNA), deoxyribonucleic acids (DNA), phospholipids and certain co-enzymes • promotes and stimulates early growth, blooming and root growth • influences hydrolysis and the synthesis of starch • appears to be a regulator of the uptake and utilisation of nitrogen • acts as a buffering agent on the acidity of cell sap	HPO_4^{2-}/ $H_2PO_4^-$ / PO_4^{3-}
Potassium (K)	• acts as coenzyme or activator for many enzymes • used by the plant to regulate cellular osmotic pressure • required for the accumulation and translocation of newly formed carbohydrates • essential for promoting strong growth and is found in areas of high physiological activity • promotes disease resistance	K^+
Calcium (Ca)	• enhances pollen germination and growth • may be important for protein synthesis and carbohydrate transfer, and its presence may serve to detoxify the presence of heavy metals in the plant • involved in cell-wall building, permeability of cell membranes, cell division and elongation • neutraliser of organic acids • enhances uptake of some nitrogen forms, as well as transport and retention of other nutrients	Ca^{2+}
Magnesium (Mg)	• component of the chlorophyll molecule • essential to maintain ribosome structure • promotes the absorption and translocation of phosphorus • activates many enzymes and it appears to aid in the formation of oils and fats • plays a role in carbon dioxide assimilation • serves in non-specific functions establishing osmotic potentials	Mg^{2+}

Element	Function	Form taken up
Sulphur (S)	• required for protein synthesis and is part of amino acids, cystine and thiamine • reduces the incidence of disease in many plants • needed in the synthesis of important metabolites such as, coenzyme A, (oxidation and synthesis of fatty and amino acids) • found in vitamins, thiamine and biotin	SO_4^{2-}
	MICROELEMENTS	
Iron (Fe)	• acts as a catalyst in the photosynthesis and respiration process, and it is essential for the formation of sugars and starches • acts an oxygen carrier and as an enzyme catalyst • required for chlorophyll synthesis • essential part of cytochromes (electron carriers)	Fe^{2+} / Fe^{3+}
Chlorine (Cl)	• essential for photosynthesis where it acts as an enzyme activator during the production of oxygen from water. • Raises the cell osmotic pressure, affects stomatal regulation and increases the hydration of plant tissue. • Functions include activity as a counter ion (Cl^- balances the positive electrical charge of Ca^{2+}, K^+, Mg^{2+}, etc.)	Cl^-
Boron (B)	• has catalytic agents for various physiological functions • believed to be important in the synthesis of one of the bases for RNA (uracil) formation and in cellular activities (i.e. division, differentiation, maturation, respiration growth etc.) • associated with pollen germination and growth and it improves the stability of pollen tubes • influences and may even control the ratio in which anions and cations are taken in by the plant • facilitates the uptake of calcium	BO_3^{3-}/ $B_4O_7^{2-}$
Manganese (Mn)	• has general function of catalyst • activates one or more enzymes in fatty acid synthesis and the enzymes responsible for DNA and RNA formation • involved in carbohydrate metabolism and chlorophyll synthesis • controlling catalyst for nitrate reduction • key factor in the energy storage metabolism of plants in the form of high-energy phosphate bonding • oxidises excess iron in the plant	Mn^{2+}
Copper (Cu)	• internal catalyst and acts as an electron carrier • participates in protein and carbohydrates metabolism and nitrogen fixation. • Involved in the desaturation and hydroxylation of fatty acids • Influences the disease resistance of the plant	Cu^{2+} / Cu^+
Zinc (Zn)	• Linked to chlorophyll synthesis • Essential for auxin (IBA) metabolism. • best described as a regulating catalyst • aids in the removal of CO_2 from the plant so it may be a specific regulator in plant transport metabolism	Zn^{2+}
Molybdenum (Mo)	• acts as an electron carrier in the conversion of nitrate to ammonium • appears to be a factor in both nitrogen and carbohydrate processes as well as an enzyme co-ordinating catalyst	MoO_4^{2-} / MoO^-

Grove, Thomson, and Malajczuk (1996) and Cromer, Cameron, Rance, Ryan & Brown (1993a) highlighted that there are a number of gaps in our knowledge of the nutritional physiology of eucalypts, despite the vast literature on nutrient responses in *E. grandis* and a large number of other eucalypts. For eucalypts, much work has been done on forest soils, fire and nutrition, nutrient cycling (organic matter / debris), silviculture and nutrients, plantation fertilisation, deficiencies in plantations and macroelements, particularly N and P for growth (Table 10). Higashi *et al.* (2002) have recently published a paper on the nutrition and fertilisation of eucalypts in a clonal mini-garden. A better knowledge of eucalypt nutrition will help formulate management options aimed at sustaining and improving the productivity of plantation forests and the increased rooting in propagation of the genus. In fact as far back as 1984, Cromer (1984) had already recognised the need for further research on nutrient status of eucalyptus.

Table 10: **Examples of literature on studies of *Eucalyptus* and nutrition**

Topic	**References**
Forest soils	Erasmus & Levin (1991); McLaughlin (1996); Specht (1996); Adams (1996)
Fire and nutrition	Adams (1996)
Nutrient cycling (organic matter / debris)	O'Connell & Grove (1996); Attiwill, Polglase, Weston & Adams (1996); Turner & Lambert (1996)
Silviculture and nutrients	Cromer (1996); Herbert (1996); Dala-Tea & Marcó (1996); de Barros & de Novais (1996)
Plantation fertilisation	Dicks, Jackson & Kirk (1965); Bhimaya & Kaul (1966); Ranwell (1975); Lamb (1976); Lamb (1977); Schönau (1981); Schönau & Herbert (1982); Mattos & Maciel (1984); Schönau (1983); Herbert (1990); Cromer, *et al.* (1993a); Dell *et al.* (1995); Herbert (1996); Dala-Tea & Marcó (1996); de Barros & de Novais (1996); Prado & Toro (1996); Pereira, Tomé, Madeira, Oliveira, Tomé, Almeida (1996); Huoran & Wenlong (1996); Negi & Sharma (1996)
Deficiencies in plantations	Dell *et al.* (1995); Huoran & Wenlong (1996); Dell (1996)
Macroelements, particularly N and P for growth	Lamb (1977); Halsall, Forrester & Moss (1983); Schönau & Herbert (1982); Cromer (1984); Mattos & Maciel (1984); Herbert (1990); Cromer, Cameron, Rance, Ryan & Brown (1993b); Kriedemann & Cromer (1996); Knight & Nicholas (1996); Negi & Sharma (1996)
Hydroponics	Higashi *et al.*, (2002)

2.3.1 Nutrient solutions

Resh (1998b) defines a nutrient solution as pH-adjusted water and adequate (balanced) fertilisers to attain a solution of the required pH and electrical conductivity (EC). Since hydroponics involves growing plants in an inert substrate, all essential elements must be

combined with irrigation (fertigation) in the form of nutrient solution, which consists of fertiliser salts dissolved in water (Janick, 1986; Carruthers, 1998; Resh, 1998a; Robbertse, 2002).

Each plant species and its varieties have their own particular nutritional requirements which change during the life of a plant but many species can grow and develop with the same general purpose nutrient solution. This is because the individual nutrient concentrations need to be within a certain range of concentrations rather than a specific concentration (Janick, 1986; Barry, 1996). Carruthers (1998) noted that while optimum nutrition is easy to achieve in hydroponics, so is damage to plants due to errors in making up the nutrient solution and / or failure to adjust it regularly. Therefore, the success or failure of a hydroponic system depends primarily on a strict nutrient management programme, and this is achieved by carefully monitoring and managing the pH, temperature and electrical conductivity (EC) of the nutrient solution (Barry, 1996; Carruthers, 1998).

Whether dealing with conventional agriculture or hydroponics, 'pH' is an extremely important factor, as each type of plant functions best within a definite pH range. Landis *et al.* (1989) stated that in soil, most plants prefer a pH which is slightly acidic whereas hydroponically grown plants prefer a more acidic solution, with the optimum pH being between 5.8 and 6.5. If the pH drifts outside this range, then nutritional problems can occur as some nutrients have either precipitated out of solution or are unavailable for absorption (Harris, 1982; Barry, 1996; Carruthers, 1998; Resh, 1998a; Ross, 1998). Higashi *et al.* (2002) regard a pH range of 5.5. to 6.0 as the best for *Eucalyptus* species.

Not only does pH affect the nutrient availability in general but it also affects the nutrient availability differently for different culture conditions. One can see an example of this in Figure 8, where hydroponic culture is being compared to conventional agriculture (soil) (Edward-Muckle, 1993).

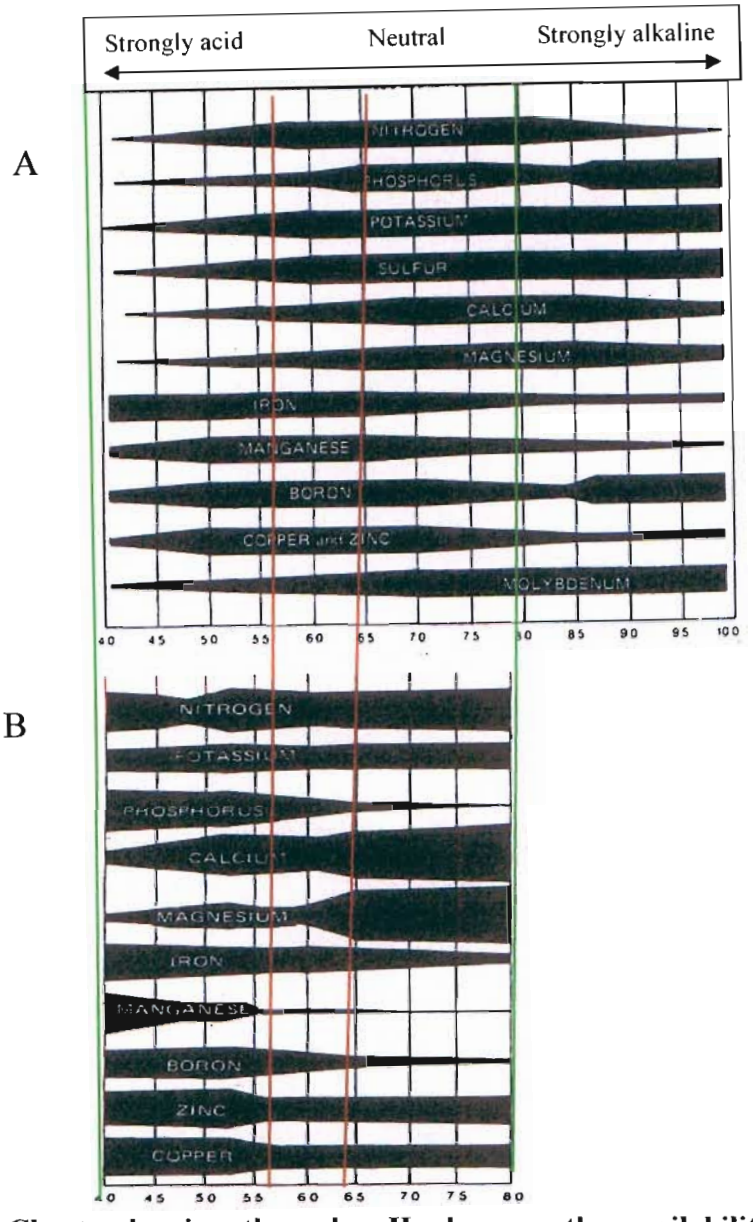

Figure 8: **Charts showing the role pH plays on the availability of elements in conventional agriculture (A) and hydroponic culture (B). The width of the band indicates the relative availability of each element at various pH levels** (Adapted from Edward-Muckel, 1993; Ross, 1998). Green lines indicate absolute maximum and minimum range. Red lines indicate optimal range

According to Harris (1982) a pH value of 4.0 is usually accepted as the lowest tolerated by plants in hydroponic culture as root growth is usually retarded or roots are injured under such acid conditions. When plants are grown in a pH below 5.0, rather high levels of Ca are required for satisfactory growth (Harris, 1982). Below 5.5, the availability of P, K, Ca, Mg and Mo declines rapidly and below pH 6.0, the solubility of phosphoric acid,

Ca and Mn decreases (Carruthers, 1998; Resh, 1998a; Ross, 1998). Alkaline solutions above 7.0 can cause complications in the hydroponic system as a high pH causes the precipitation of Fe, Mn, P, Ca and Mg (Harris, 1982; Ross, 1998), resulting in deficiency symptoms and, if prolonged, death (Harris, 1982). For pH values above 7.5, Cu, Zn and B become less available to plants (Carruthers, 1998; Resh, 1998a).

When nutrient salts are dissolved in water they dissociate to a greater or lesser degree into positively and negatively charged particles called ions which conduct electricity (Harris, 1997). Electrical conductivity is a measure of the ability of a nutrient solution to conduct electricity, which is dependent upon the ion concentration and nature of the elements present and is of critical importance in hydroponics (Barry, 1996; Resh, 1998b). It is possible to measure the conducting power of these ions by means of a conductivity meter. This reading effectively gives us a means of measuring the concentrations of salts (nutrients) in solution but it gives no information relating to the composition of the salts in solution (Harris, 1997; Resh, 1998a).

As plants remove elements, the EC will decrease and if a recirculating system is being used, a close watch should be kept, by regular measurements, to ensure that the strength does not fall below the prescribed level (Ross, 1998). Harris (1997) explains that during growth, plants take up the elements they require, thereby altering the balance of the remaining nutrient solution. If the EC is high, the plants are taking up water faster than they are taking up elements. It follows that, as water is removed by plants, the volume of the solution decreases, with subsequent increase in nutrient concentration.

Higashi *et al.* (2002) explain that a nutrient solution should, at the very least, possess the following characteristics: (a) all essential nutrients; (b) be suitable for the culture type; (c) have a pH of 5.8 – 6.2; and (d) have an EC of between 1.5 – 4 $mS.cm^{-1}$ dependent on the culture. For nutrient solution replenishment in closed systems, Higashi *et al.* (2002) recommend that the pH and EC be regularly measured and further recommend that once the EC drops below 1 $mS.cm^{-1}$ that the solution be replenished. These authors also

suggest that as *Eucalyptus* species can tolerate acidic conditions a pH range of 5.5. – 6.0 is regarded as best.

Silveira, Higashi, Gonçalves, Bonini & Vale (1999) used an *E. grandis x E. urophylla* clone to determine a nutrient regime for microcuttings in an hydroponic garden, under greenhouse and full sunlight conditions. The productivity of cutting production per square metre was measured, with the maximum productivity (shooting, new shoots / sprouting) obtained with 80 % of standard solution and an EC of 1.25 – 2.3 $mS.cm^{-1}$. It was also found that the productivity of the garden under greenhouse conditions was 9.6 % greater than that under full sunlight. With both environmental conditions, solutions with greater than 80 % of the standard solution resulted in high mortality and the highest survival was found with 25 % of the standard. Silveira *et al.* (1999) also assessed the frequency of nutrient application to the mini-garden, and found that the content of N, P, B, Fe and Mn in the new shoots increased with increasing nutrient concentration and supply frequency but this also resulted in increased mortality.

Resh (1998a) reported that EC varies not only with the concentration of salts present, but also in accordance with the chemical composition of the nutrient solution. Some fertiliser salts conduct electricity better than others e.g. ammonium sulphate conducts twice as much as calcium nitrate and more than three times that of magnesium sulphate, whereas urea does not conduct electricity at all. Although this statement may not be entirely true as both ammonium sulphate and calcium nitrate dissociate into three ions.

Another major advantage of hydroponics is that it provides the facility to control the root environment more precisely than is possible with conventional agriculture, particularly the root-zone temperature. This can be controlled simply by controlling the temperature of the recirculating water. Cooper (1996) points out that the cost of control will be the main determinant of the degree and precision. Temperature fluctuations in a hydroponic solution can affect not only the pH but also the solubility of nutrients (Carruthers, 1998). According to that author studies have shown that the ideal water temperature for total solubility, and therefore plant health, is between 20 °C and 22 °C. It is however, difficult

and expensive to cool the hydroponic nutrient solution during summer. Therefore, the grower should run the solution at a lower EC as the plants require more water than nutrients at high temperatures as the rate of transpiration is greater. Barry (1996) suggested that a nutrient solution should not drop below 15 °C as cold roots do not absorb water or minerals well with the result that plant growth slows. Carmelo (1997) found that the best solution temperatures for eucalypts are 24 °C (day) and 15 °C (night) in the warmer seasons and 16 °C (day) and 10 °C (night) in the cooler seasons.

Nutrient elements are not taken up from a nutrient solution in the same proportion in which they are present (Schwarz, 1995; Carruthers, 1998). The rate of absorption depends upon the plant, climatic and environmental conditions (light intensity and duration, temperature and humidity) as well as the type of culture and the plants' stage of development (Schwarz, 1995). Uptake of water and nutrients by the plant will constantly change the nutrient solution, disrupting the balance of the elements (Schwarz, 1995; Carruthers, 1998). As a result, some elements become in short supply before others. The composition of the solution can be determined by atomic absorption analyses. Both Carruthers (1998) and Resh (1998a) indicated that such analyses can be done only in costly laboratory facilities. Those authors recommend that in order to safeguard against nutrient disorders, the nutrient solution should be replaced regularly (Carruthers, 1998; Resh, 1998a).

As previously mentioned, there is very little information in the literature concerning pH, EC and temperature of nutrient solutions for *Eucalyptus* propagation in hydroponic systems except where mentioned. There is, however, vast information for other crops (Table 11).

Table 11: **Literature found for crops other than *Eucalyptus* on pH, EC and temperature of the nutrient solution**

Crop	Findings	Reference
Tomato	To optimise the absorption of Ca by the roots: (a) Ca concentration of 120 ppm is suitable, (b) root zone temperature should exceed 25 °C and (c) low oxygen levels (<3 $mg.l^{-1}$) should be avoided. To improve the distribution of Ca to rapidly expanding fruit: (a) high airflow or low humidity should increase canopy transpiration and (b) vapour pressure deficit of greater than 0.5 kPa should be avoided. Maintenance of the balance between Ca and other nutrients by apply low but sufficient N (180 ppm), sufficient but not too high K (400 ppm) and avoiding P depletion (>5 ppm) in the feed. This should reduce the incidence of BER and goldspot.	Ho, Hand & Fussell (1999)
	A pH of 4 was more effective at reducing the incidence of the bacteria *Clavibacter michiganensis* subsp. *michiganensis* in tomato plants than a pH of 6 with no effect on the fresh weight of the plant.	Huang & Tu (1999)
	Reduced nutrient strength decreased soluble solids, titratable acidity, and vitamin C, but did not affect the flavour volatiles. Reduced macronutrient to quarter strength for 8 months and terminating top up of nutrient supply for 8 days did not affect levels of sugars, acidity, pH and EC of tomato fruit.	Lin & Glass (1999)
	The yield of first class fruits increased significantly with increasing K concentration. Early yield also increased with increasing K concentration.	Economakis, Daskalaki & Bitsaki (2001)
	Plants grown in higher nutrient solution with additional NaCl showed increased plant height and darker leaf colour at harvest of the first cluster although total yields were lower. The disadvantage in yield was slightly overcome by adding NaCl throughout the growing period. The incidence of BER increased with increasing nutrient concentrations associated with additional NaCl at later stage of plant growth. Fruits sugar contents were considerably higher in the plants grown with the enriched NaCl to the nutrient solutions, the values showed no difference among the plants of high nutrient level. Water uptake was greatly restricted by high nutrient concentration and NaCl application. The uptakes of some cations, such as K, Ca and Mg decreased markedly by NaCl application.	Hohjo, Ganda, Maruo, Shinohara & Ito (2001)
	Tomato plants were treated with nutrient solution of various K:N ratios and EC. It was concluded that EC and K:N ratio are tools that can be used to control growth without detrimental carryover effects into the production season. For good quality transplants it was found that the EC should be maintained between 3000 – 6000 $\mu S.cm^{-1}$ and the K:N ratio between 2:1 –4:1.	Khosla & Papadopoulos (2001)

	At the same water potential gradients, the rate of water flow through the stem was increased by 250 % when the root temperatures changed from 12 to 20 °C at constant light radiation and air humidity and temperature of the canopy. When roots were cooled from 20 to 12 °C the water flux dropped immediately to the same value observed in plants that their roots were kept constantly at 12 °C. The presence of ammonium in the root zone solution is beneficial when the root zone temperatures are low. Ammonium becomes detrimental at high root temperatures. The differences in sensitivity between plants to ammonium toxicity are due to the differences in the sugar concentration in the root. The upward flux of nitrate, phosphate and potassium is very much reduced with low root temperatures.*	Kafkafi (2001)
Maize	*	Kafkafi (2001)
Strawberry	With an increase in temperature from 20 °C to 25 °C there was increased plant growth and root / shoot ratio was the highest for this treatment. Yield and earliness were significantly improved by nutrient solution warming and the highest yield / plant was obtained from the higher solution temperature regime. Although the average fruit was not influenced the number of harvested fruits per plant increased with increasing solution temperature.	Economakis & Krulj (2001)
	*	Kafkafi (2001)
Pepper	The amounts of water and N added in treatments with EC threshold values of 7 and 2.5 $dS.m^{-1}$ were 300 $l.m^{-2}$ and 36 N $g.m^{-2}$, and 770 $l.m^{-2}$ and 110 N $g.m^{-2}$, respectively. The marketable fruit yield and incidence of BER in fruits in those treatments were 5.9 and 6.7 $kg.m^{-2}$ and 16 and 13 %, respectively.	Bar-Yosef, Markovich & Levkovich (2001)
	Container size became the predominant growth-limiting factor when nutrient supply was high. The restriction of nutrient supply and root growth volume significantly increased partition of assimilates to the fruits. There was no significant effect of container size and nutrient supply on the water contents of all plant parts. Increasing nutrient and water supply could not sufficiently compensate the influences of extreme restriction of root volume imposed by container size. Relative large size of container combined with ample nutrition and water is needed for high yield production of pepper plant.	Xu & Kafkafi (2001)
Gerbera	Gerbera plants were maintained for nine months in a recirculating system with a solution of a target EC and nutrient ratios. After this period nutrient solution samples were collected and analysed and the results indicated that the K:Ca, Mg:Ca and P: (SO_4^{2-} + NO_3^-) ratios in the reference nutrient solution should be higher than the values for the supply solution.	Savvas (2001)
Rose	Management of recycling using EC measurement proved to be reliable. There are good relationships between EC and ion concentrations for supplied solutions and leachate solutions recycled and not recycled.	Brun, Settembrino & Couve (2001)
Melon	In general, CO^2 enrichment up to 800 ppm increased the surface area and the chlorophyll density of the measured leaves. In concentration higher than 800 ppm, CO^2 increased the leaf surface area and chlorophyll density to a lesser degree. Addition of NaCl in the nutrient solution caused significant reduction in total yield and total fresh shoot weight of the plants, in all cases. Measurements of gas exchange showed that exposure to 25 mM and 50 mM NaCl inhibited significantly net carbon exchange rates. Stomatal conductance was affected most by NaCl at 50 mM NaCl. Substomatal concentration of CO^2 was affected most by NaCl at 1200 ppm CO^2.	Mavrogianopoulos, Spanakis & Tsikalas (1999)

	Salinization increased only slightly the concentration of sugars in the fruits and, in summer, promoted the occurrence of flesh vitrescence. Fruit yield and quality was not affected by root cooling, which instead increased the plant's susceptibility to root death.	Pardossi, Malorgio, Incrocci, Tagnoni & Campiotti (2001)
Spinach	A quantitative nutrient management (QNM) method, which has an initial low concentration with subsequent daily additions of individual nutrients. The daily nutrient uptake was estimated and each nutrient was supplied in a quantity equivalent to the daily uptake. It was found that there was no difference in the marketable weight of the spinach crop from this system and the conventional system where a constant EC (2.7 $dS.m^{-1}$) was maintained.	Maruo, Hoshi, Hohjo, Shinohara & Ito (2001)
Paprika	Blossom end rot (BER) in paprika fruits is induced at maximum temperatures above 30 °C and especially at minimal relative air humidity levels below 60 %. In the non-cooled media temperatures fluctuate between 23 and 33 °C whereas the fluctuations in the cooled media is between 17 and 22 °C. BER was reduced in the cooled media. There was also more calcium in the basal and distal fruits (11 and 43 % respectively) from the cooled media. These favourable results were ascribed to the stimulating effects of higher oxygen content in the cooler root environment.	Benoit & Ceustermans (2001)
Lettuce	With CO_2 enrichment and optimal light intensity, nutrient levels did not affect growth.	Park & Lee (2001)
Potato	Each nutrients concentration in upper leaves necessary to induce deficiency symptoms appeared to 1.1 – 2.2 % K and 0.1 – 0.3 % Ca.	Chang, Kim, Jeong, Shin & Lee (2001)
	Results obtained confirmed that the optimum range of solution temperatures for the growth and mineral uptake of potato foliage is considered 20 –25 °C.	Chang, Kim, Jeong, Shin & Lee (2001)
Osteospermum	The maximum fresh weight was obtained at 2.24 – 3.35 mM P, the maximum plant height and diameter at 1.61 - 3.35 mM P. The plants grown at 1.61 – 3.25 mM P had the greatest leaf size and the number of flower buds and flowers. The average flower diameter increased with increasing P level. The number of days from planting to flowering decreased with increasing concentration of in the medium. Overall, good plant appearance and root growth was achieved with P levels from 1.61 – 3.25 mM. Although P stress did not produce any visual deficiency symptoms on the leaves, the use of P below 1.61 mM cannot be recommended to the delay in flowering time.	Nowak (2001)
Impatiens	The P nutrition had a pronounced effect on growth. P stress reduced plant height and diameter, shoot number, leaf number and leaf dimensions. The leaves and flowers of P deficient plants had darker colour, increasing its ornamental value. No dark spots on leaves, or any other detrimental effects of P deficiency were observed on plants at all treatment levels. P deficient plants flowered about 7 days earlier.	Nowak & Stroka (2001)
Sweet potato	At three different concentration of Hoaglands solution, there was a similar biomass production for all three treatments. The main response was the significant decrease in foliage dry weight of plants replenished with a higher concentration (but not the highest), although storage root yields were similar. These results indicate that sweetpotato can be grown successfully in NFT with either of the protocols from this study.	Mortley, Bonsi, Hill & Hill (2001)

2.3.2 Plant analysis

Plant, or leaf analysis, is a technique used to determine the elemental content of the whole plant or of its parts. It usually refers to a laboratory analysis and interpretation is based on the assumption that there is a significant biological relationship between the elemental content of part of the plant assayed and its physical appearance, growth rate, yield or quality of harvested product (Benton-Jones, Jr, 1998). Plant analysis methods used in diagnosing nutrient disorders and predicting nutrient requirements of plants are based on these relationships (Dell, Malajczuk, Xu, & Grove, 2001).

There are a number of ways of dealing with foliar data, the most common of which is the use of critical levels. Using established critical or standard values, or sufficiency ranges, a comparison is made between the laboratory analysis result with one or more of these known values or ranges in order to access the plants' nutritional status (Figure 9). This is generally defined as the concentration, or narrow range, above which the plant is sufficient and below which the plant is deficient (Smith, 1986).

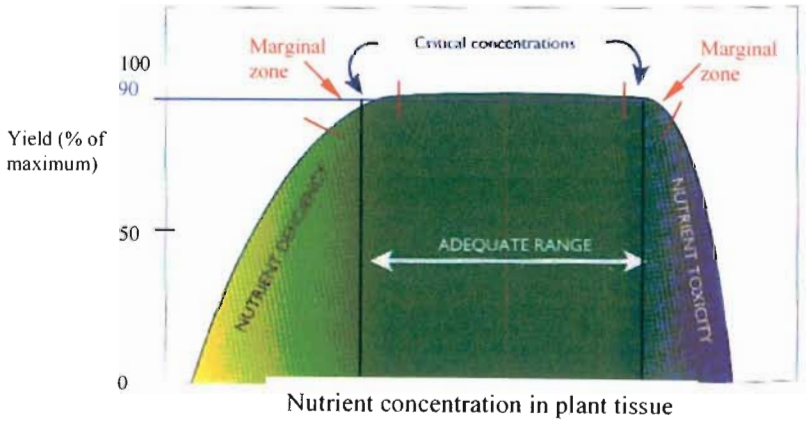

Figure 9: **Relationship between nutrient concentration in plant tissue and plant growth** (Adapted from Smith & Loneragan, 1997 and Dell *et al.*, 2001)

Dell *et al.* (2001) explains that these relationships are established by experimentation with a number of rates of application of a single nutrient that are used to determine the 'critical nutrient concentration', the concentration in the plant tissue above which there is no significant increase in growth. The critical concentration is often set at 90 % of maximum yield.

A major drawback to this method is that each element is examined in isolation – with the assumption that the other elements are at optimal concentrations. This is in fact how critical levels are established and factors, nutritional or otherwise, that limit growth can alter the concentration deemed to be critical (Smith, 1986). To overcome this problem, most nutritionists examine the ratios of each element with other elements.

Plant analysis as a diagnostic technique has a considerable history of application and dates back to the early 1800s (Smith & Loneragan, 1997). It has been developed primarily to provide information on the nutrient status of plants as a guide to nutrient management for optimal plant production (Benton-Jones, 1985). More recently, Smith & Loneragan (1997) explain that plant analysis results are being used to determine the combined soil and crop nutrient element status from which a prescribed fertiliser recommendation can be made. A number of objectives in utilising plant analysis results have been proposed (Smith & Loneragan, 1997):

a) verification / diagnosis of deficiency symptoms;
b) evaluation of the nutritional status of the soil / crop environment; and
c) determination of the nutrient element needs for optimal crop production.

By conducting both plant-tissue and nutrient-solution analyses, plant physiological upsets and imbalances of various mineral elements in the nutrient solution can be compared and related (Resh, 1998b). It is possible to control the changes in the nutrient solution once precise relationships are established between fluctuations in mineral elements in the plant tissue and those in the nutrient solution (Resh, 1998b). The nutrient solution can then be adjusted before visual symptoms appear in the plant tissue, which would prevent any mineral stress from occurring within the plant and thus increase yields by allowing the plant to grow under optimum mineral nutrition conditions (Janick, 1986).

An advantage of tissue analysis over nutrient analysis is that the former indicates what has been absorbed from the nutrient solution by the plant. The actual uptake by the plant

from the nutrient solution may be restricted by conditions of the medium, solution, environmental factors or the plant itself (Janick, 1986; Hartmann *et al.*, 1997).

Resh (1998b) discusses that to relate tissue analysis results accurately to nutritional requirements of plants, considerable data must be available on optimum levels of nutrients in the specific plant species and tissues to be compared. A table which summarizes the results of foliar analyses on *Eucalyptus* species is provided in Appendix 2. The nutrient concentrations considered optimal for vegetative growth, as provided by a number of authorities, mainly for plantations, are given. In constructing this table, emphasis was placed on the foliar concentrations of *Eucalyptus* species that are considered to be optimal for growth of established trees.

2.3.3 Nutrients and *Eucalyptus* propagation

The development of adventitious roots is an essential step in the vegetative propagation of economically important woody species. Adventitious rooting is a complex process which is affected by multiple factors (Hand, 1994), as well as associated stress responses (Fett-Neto, Fett, Vieira Goulart, Pasquali, Termignoni, & Ferreira, 2001). These influence both the initiation and development of roots which in turn impacts on both the success of the rooting of the cuttings and the quality of the root systems produced (Fett-Neto *et al.*, 2001). These factors include environmental conditions and physiological status of the cutting hedges, the treatment of the cuttings, and the environmental conditions during rooting. Many authors including Janick (1986), Hartmann *et al.* (1997) and Carlson *et al.* (2003) suggested that such variables can be controlled and manipulated so as to optimise the rooting of cuttings.

Blazich (1988) suggested that the nutritional status of the hedges is one of the primary factors that determine the rooting success of cuttings. Investigations into the role of nutrition in the rooting of cutting material tends to be confounded by the fact that root formation is a multi-stage process and few studies distinguish between mineral effects at each stage (Blazich, 1988; Hartmann *et al.*, 1997) i.e. between root initiation and root

growth / development. Research has shown that the effect of the stock plant nutrition can override any effect of the environment (Welander, 1994). Furthermore, optimal nutrient concentrations are known to be specific for species, genotype and age of the hedge plant (Hartmann *et al.*, 1997; Blazich, 1998).

However, despite the importance of the hedge plant nutrition on the rooting processes, suprisingly little work has been conducted in this field. Although, there exists considerable amounts of information regarding nutrient concentrations that are required for optimal vegetative growth of plants (Ericsson, Ryter & Linder, 1992; Linder, 1995; Marshner, 1995), it is not known whether they are the same as those that enhance adventitious root formation in cuttings. In fact, Carlson *et al.* (2003) hypothesized that cuttings taken from hedges that are grown rapidly are not likely to root successfully. The basis for this reasoning is that tissue that is strongly favouring shoot growth is likely to have distinctly different levels and forms of carbohydrates, as well as differences in concentrations of plant growth regulators when compared to plant material that favours adventitious root formation.

Erasmus & Levin (1991) explained that the most important consideration of any clonal nursery is the improvement of the rooting of cuttings and hence the overall productivity of the nursery. To achieve this, consideration must be given to the fertilisation of the clonal hedges but, as Erasmus & Levin (1991) point out, without any guidelines on the optimal foliar nutrient levels, this task is extremely difficult.

In their studies, Erasmus & Levin (1991) found significant differences in foliar nutrient levels between clones. They also discovered various factors affecting the yield (the number of shoots harvested from each stump) and rooting percentage. For example, the yield of all clones increased with increasing Cu, decreasing S and an earlier date of harvest. Results also showed that better rooting appeared to be achieved with increased Ca and Mn levels, reduced S and Cu levels and an earlier date of harvest. Based on these results, Erasmus & Levin (1991) suggest that rooting percentage is related to foliar nutrient levels and the date on which harvest takes place. Erasmus & Levin (1991) also

highlighted that the apparent trade-off, where increased foliar Cu levels appear to increase coppice yield yet reduce the rooting percentage, is contrary to the common belief that the 'healthier' the coppice the 'better' the rooting and conclude that maximizing coppice yield may not be the answer to increasing nursery production. Erasmus & Levin (1991) propose that there is a critical foliar nutrient level that will optimize both coppice yield and rooting percentage and that it is also possible that these optimum levels could be different for different clones. According to these authors, the most important conclusion from their study is that more detailed research is required to elucidate the essential information regarding mineral nutrition and vegetative propagation and that such research would be highly beneficial to the forestry industry in terms of increased nursery production.

Novais, Barros, Teixeira, Neves, Goulart & Macedo (1991), showed the necessity of a distinct fertiliser recommendation for a variety of *Eucalyptus* species by testing the differential nutrient responses among a few of them. The results of their studies stress the differences in nutritional requirements of the species. They also advise that in the production of two or more species the nurseryman must be conscious that one fertiliser regime may cause nutritional disorders and unsatisfactory stock growth.

From their studies, Barnes & Lewandowski (1991) proposed methods for manipulating stock plants to improve the quality and rooting of *Eucalyptus* cuttings. These were: (a) feeding specific fertilisers to avoid high N concentrations in the plant and (b) supplying B and Zn as essential trace elements that are known to influence rooting.

More recently, in 2003, the Forestry Plant Propagation Working Group (FPPWG) (made up of representatives from Mondi, Sappi, Institute of Commercial Forestry Research, University of Natal and some private growers and is based in Pietermaritzburg) evaluated nutritional data collected from *Eucalyptus* hybrid hedges of Sappi and Mondi. The results indicated significant differences in the nutrient concentrations on N, P, Ca, Mg, Fe, Mn, Cu and B depending on whether foliar samples or whole cuttings were analyzed for the nutrient content. Carlson *et al.* (2003) reported that there were large differences in the

rooting percentages of the different clones, but stated however, that the trend is for the rooting to be slightly improved with time. Despite variability, this data has provided indications of optimal foliar nutrient concentrations which can be examined more closely in further trials. The 20 samples for which all the conditions below occurred, showed a 24.9 % improvement in rooting, compared to the 108 samples which failed to meet these criteria. Carlson *et al.* (2003) recommended that these broad nutrient concentration ranges need to be further investigated, refined and narrowed in further studies under more controlled conditions. These criteria are:

a) N concentration of between 1.75 and 4.25 % (m/m);
b) P:N nutrient ratio of between 0.6 and 1.4;
c) K:N nutrient ratio of between 3.0 and 5.0;
d) Ca:N nutrient ratio of between 1.5 and 3.5; and
e) Zn:N nutrient ratio of between 0.006 and 0.008.

Paula, Silveira, Higashi & Gonçalves (2000) investigated the effect of different levels of K on the productivity and rooting of plants growing in 8 l containers of sand. The authors observed that the level of K supplied affected the productivity, length of the new shoots, root number and root dry mass significantly. In contrast, rooting percentage, number of new shoots per stem and root length were all unaffected.

Higashi, Silveira & Gonçalves (2000a) established adequate ranges for macro- and micronutrients for clonal mini-gardens of *Eucalyptus* through various assays. Higashi *et al,* (2000a) recommended that the concentrations need to be adapted for every clone and time of the year. These authors also suggested that the relationship between foliar nutrient content and productivity, and the relationship between foliar nutrient content and rooting, be taken into account. The ranges of macro- and micronutrients concentrations deemed adequate and deficient for the production of microcuttings are presented in Table 12 (Higashi *et al.*, 2000a).

Table 12: **Values for macro- and micronutrients of new *Eucalyptus* shoots aged 7-14 days**

Nutrient	Higher	Adequate	Lower	Deficient
		Macronutrient g.kg^{-1}		
N	>40	28-40	20-28	<20
P	>4	2.5-4	1.5-4	<1.5
K	>30	15-30	10-15	<10
Ca	>7	5-7	3-5	<3
Mg	>4	2-3	1-2	<1
S	>2.5	2-2.5	1.3-2	<1.3
		Micronutrients mg.kg^{-1}		
B	>70	35-70	20-35	<20
Cu	>15	8-15	5-8	<5
Fe	>220	101-220	75-100	<75
Mn	>700	250-500	150-250	<150
Zn	>80	30-60	20-30	<20

The influence of the nutritional status of the cutting on rooting of 14 clones of *Eucalyptus* (nine of *E. grandis* and five of Rio Claro hybrids) was evaluated by Higashi, Silveira, Firme, Leite & Gonçalves (2000b). According to those authors, the most important conclusions were:

(a) P concentrations higher than 3.5 g.kg^{-1} in the new shoots decrease rooting;

(b) the critical level of Ca (in the new shoots) for rooting is 5.5 g.kg^{-1};

(c) Mg in the new shoots > 2.5 g.kg^{-1} to obtain rooting of 70 % or higher;

(d) the adequate range for Ca:P in the new shoots of *E. grandis* is 1.3 – 2 and 1.1 – 2.1 in the clones;

(e) Ca:N > 0.12;

(f) N:P > 9; and

(g) Ca:Mg > 2.

Authors do not indicate whether the values mentioned above are for dry or fresh plant mass.

Higashi, Silveira, Valle, Bonine, Bouchardet & Gonçalves (2000c) also observed the effect of N on nutrient levels, shoot production and rooting of microcuttings of *Eucalyptus* grown in a gutter system of clonal mini-garden. The results indicate that the effect of N concentration and the clones on rooting (Table 13). Clone A showed higher rooting in the 40 to 320 N mg.l^{-1} range than clone B. There was a significant difference between clones regarding their rooting response to N supply; only clone B showed a

linear increase in rooting with increased N. Levels of P, Ca and S were unaffected by N but increase N supply resulted in decreased foliar content of B, Cu, Fe and Mn in both clones, and Zn in clone A.

Table 13: **Rooting % as a function of N concentration** ($p < 0.05$)

	Rooting (%)		
N mg.l^{-1}	**Clone A**	**Clone B**	**Average**
40	77.1 a x	60.7 b y	68.9 b
160	76.2 a x	69.4 a x	72.8 b
320	82.8 a x	77.0 a y	79.9 a
Average	78.7 x	69.0 y	

Letters in red denote differences (if any) between N concentrations and letters in blue denote differences (if any) between the two clones

There was no significant effect of N concentration on shoot productivity for either clone although there were isolated effects on the number of cuttings produced. Clone A produced 27 % more cuttings than B. Using regression, it was established that the maximum productivity (cuttings m^{-2}) was obtained with 261 and 299 N mg.l^{-1} for clones A and B, respectively, which results in a foliar N concentration of 37.3 and 42.6 N g.kg^{-1}, respectively. The N concentration that yields 90 % of maximum new shoot production was found to be 36 and 32.5 N g.kg^{-1} for clones A and B. In terms of foliar content these ranges were 36 – 42.6 g.kg^{-1} for clone A and 32.5 – 37.3 g.kg^{-1} for clone B. The level at which deficiency occurs was identified as below 32 g.kg^{-1} for A and 29 g.kg^{-1} for B. Again, authors do not indicate whether the values mentioned above are for dry or fresh plant weight.

da Costa Alpoim (2002) reported that most forestry nurserymen strongly believe that the rooting potential of genetically improved eucalypt stock plants is strongly linked to their nutrient status. However, achieving and sustaining an optimum nutritional balance is difficult and complex in practice. In order to predetermine as accurately as possible the optimum plant nutrition required all year round, to ensure economic levels of rooting, a more controllable environment is essential and this is where hydroponics can play a big role. Hydroponic systems allow for the precise control of nutrient applications. At the same time it may be possible to manipulate the system to accurately determine what level

of each nutrient will provide the highest rooting and more importantly allow us to practically maintain that level.

Chapter 3

MATERIALS AND METHODS

3.1 Source of material

Three clones from Mondi's *Eucalyptus* clonal programme were selected on the basis of mean rooting performance (Table 14) for testing in the hydroponic tables. Da Costa Alpoim (2002) used these three clones in his work and so it was decided to continue using these to make the work comparable.

Table 14: **Rooting of selected clones from field hedges, 8 / 1997 to 4 / 1999** (da Costa Alpoim, 2002)

Clone	**NH00**	**GN156**	**GN107**
Mean rooting (%)	54.6	60.1	54.3

Cuttings of clones *E. grandis* x *E. nitens* (GN156 and GN107) and *E. nitens* x *E. grandis* (NH00), were obtained from the Trahar Technology Centre, Mondi Forests, Hilton (KwaZulu-Natal, South Africa). The GNs are a product of controlled crosses with a maternal *E. grandis* and a paternal *E. nitens* while the NHs are a natural hybrid produced by open pollination between a maternal *E. nitens* and a paternal *E. grandis.*

These rooted plants were transferred into the hydroponic systems to serve as stock plants. The cuttings where originally from ramets in Mountain Home Nursery's clonal hedges.

3.2 Coppice collection, cuttings preparation and placing

The methods used to collect coppice from the clonal hedges, make cuttings and place cuttings in the greenhouse was based on what was described in Section 2.1.2. Briefly, coppice was collected from the hydroponic ramets and placed into buckets of water. The coppice was then cut up into cuttings that were one to two nodes in length (approximately three to eight centimetres) with two half leaves attached. The cuttings were placed into

trays in the rooting greenhouse, both of which are discussed later. Before being placed, the base of the cuttings was dipped into Seradix 2® rooting hormone.

3.3 Trays and rooting substrate

All cuttings were rooted in Unigro® 128 trays (conventional method). These were then transplanted into the hydroponic tables.

All cuttings from the hedges were placed in Mondi's conventional mix for rooting cuttings. This was a mix of perlite, coir and vermiculite in a ratio of three parts perlite, one part coir and six parts vermiculite. As these are all manufactured products they are sterile upon initial use. The substrate was thoroughly mixed and wetted before being transferred into trays.

3.4 Rooting greenhouse

Cuttings from clonal hedge plants were placed in trays in a rooting greenhouse (Figure 10, Table 15) where they were left to root for a 50-day period. After this period, the trays were moved to a plastic covered tunnel (same conditions as hydroponics tunnel discussed in Section 3.5) to be hardened off by increasing light intensity and decreasing watering intervals (Figure 11, Table 16). The environmental parameters in the rooting greenhouse were controlled as listed in Table 15.

Figure 10: **Trays of cuttings placed in the rooting greenhouse where watering, humidity and temperature are controlled**

Table 15: **Environmental conditions in the rooting greenhouse used in this study**

	Greenhouse (cuttings)
Made from	Fibreglass reinforced plastic (FRP)
Size	16 m x 30 m
Air temperature control	Thermostatically activated fans 28 °C
Root zone temperature control	Bed heaters set to 28 °C
Artificial light	None
Humidification	Automatic misters set to 20 s spray time every 10 min.
Watering and nutrients	No fertilisers in water
Disease and insect control	Attention to hygiene. Spray upon detection i.e. curative programme

3.5 Hydroponic systems

The NFT, ebb-and-flow and aeroponic tables (described in Sections 3.5.1. to 3.5.3) were designed and constructed by either Mondi staff or contractors. The hydroponic tables were placed in the same tunnel, at Mondi's Trahar Technology Centre (Figure 11 and Table 16).

Figure 11: **Simple plastic-covered tunnel at Mondi's Trahar Technology Centre with hydroponic tables in background**

Table 16: **Environmental conditions in the hydroponics tunnel**

	Tunnel (hydroponic hedge plants)
Made from	180 micron plastic with 80 % shade net sides
Size	8 m x 30 m
Air temperature control	Two small extraction fans to ensure air movement. Sides open to allow natural ventilation
Root zone temperature control	None
Artificial light	None
Humidification	None
Watering and nutrients	Both water and nutrients were supplied to the plants via nozzles from the appropriate hydroponic tanks.
Disease and insect control	Attention to hygiene. Spray upon detection i.e. curative programme

The nutrient solution was identical for all hydroponic systems. The commercially available fertilisers Hydroponica® and Agrisol®: Plant Calcium (Table 17) were used. Based on work by da Costa Alpoim (2002) a nutrient solution protocol was devised (Appendix 3). The solution pH was maintained at a range of 5.5 to 6.5, whilst EC levels were maintained at 1.2-1.5 $mS.cm^{-1}$. Each hydroponic table had its own tank which held the nutrient solution supplied to the plants (Table 18). Solutions were replaced every 10 - 14 days and reservoirs were well rinsed prior to being refilled. The nutrient solutions were replaced to avoid a build-up of salts and pathogens and to ensure the availability of a balanced solution. At each refill, one millilitre of Sporekill® (quartenary ammonium chloride) or Purogene FB®

(chlorine dioxide) disinfectant was added to the solution to curb the proliferation of pathogens.

Once the tanks had been emptied, cleaned and refilled with water, the pH of the water was measured and then remediated with nitric acid (90 %). Once this was done the fertilisers were added and the EC measured. Both pH and EC were measured on a daily basis using waterproof, portable hand-held pH and EC meters (Eutech Instruments - pHScan 1 and TDScan 4). pH and EC were corrected on a daily basis. Electrical conductivity was corrected by adding the above mentioned fertilisers to the relevant tank.

Table 17: **Elemental composition of the two fertiliser formulations used**

Element		**Hydroponica®**	**Agrisol®: Plant calcium**
N	$g.kg^{-1}$	56	140
P	$g.kg^{-1}$	46	0
K	$g.kg^{-1}$	274	16
S	$g.kg^{-1}$	112	0
Mg	$g.kg^{-1}$	30	26
Ca	$g.kg^{-1}$	0	129
B	$mg.kg^{-1}$	440	2000
Fe	$mg.kg^{-1}$	690	0
Mn	$mg.kg^{-1}$	300	0
Zn	$mg.kg^{-1}$	270	0
Mo	$mg.kg^{-1}$	90	50
Cu	$mg.kg^{-1}$	140	0

Table 18: **Volume of hydroponic tanks and the amounts of fertiliser added to each tank of water to obtain a refreshed nutrient solution every 10 – 14 days**

Tank	**Volume of tanks (litres)**	**Amount of Hydroponica® fertiliser to be added (ml)**	**Amount of Agrisol®: Plant Calcium fertiliser to be added (ml)**
Modified NFT	45	50	34
Ebb-and-flow	500	570	340
Aeroponics	300	340	200

Three different insert types were used throughout these trials. The Unigro® 128 (Figure 12), are the conventional inserts for rooting cuttings and these were used throughout. The cuttings used as stockplants in the NFT substrate in the NFT table (see Section 3.5.1) were

transferred from Unigro® 128 to Unigro® 98 (Figure 12) before being placed into the NFT table. The latter are slightly larger than the Unigro® 128. The cuttings used as stockplants in the ebb-and-flow table were left in the Unigro® 128 inserts throughout the study period. The cuttings used for the aeroponic table were transferred from the standard Unigro® 128 to a modified Unigro® 128 (Figure 12) insert before being placed in the aeroponic table to allow for an increased exposure of the root system to the nutrient solution.

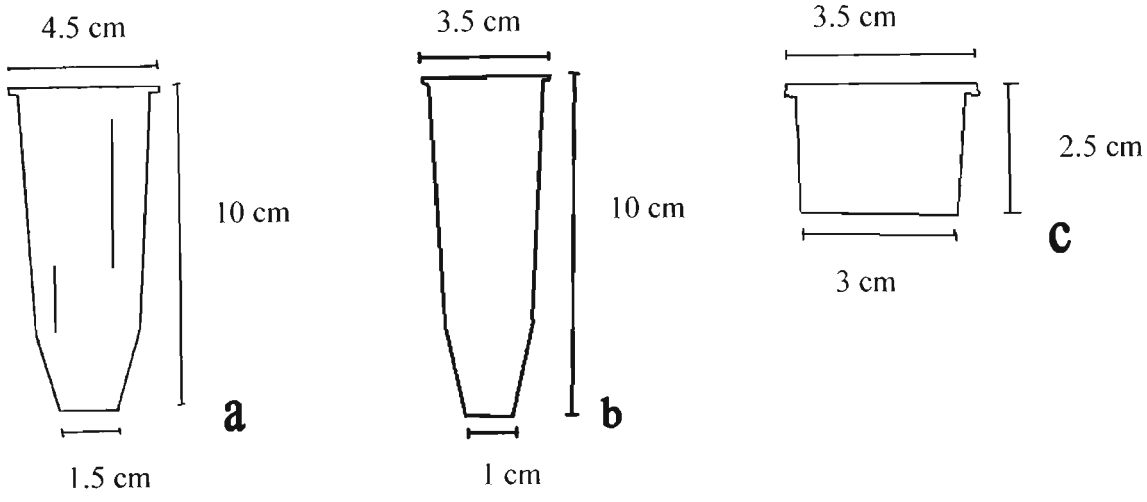

Figure 12: **Different inserts used in the hydroponic tables (a) Unigro® 98; (b) Unigro® 128 and (c) Modified Unigro® 128**

3.5.1 NFT

The system used was a modified NFT design and not a true one as different substrates were tested. The NFT recirculating tables comprised 4 x 110 mm polypipe sections split longitudinally and braced onto a frame (Figure 13). The polypipe sections were painted black to darken the root growth zone and were lined with capillary matting to ensure the even distribution of liquid feed. Each unit was irrigated via a submersible pump (maximum volume 1400 $l.hr^{-1}$ to a maximum head of 1.8 m) installed in a 45 l plastic tank. Unequal cyclic timers were fitted to the individual units. The timer for the unit was set to run for 25-minutes with an off-time interval of three hours. Both timers were linked to an electronic clock that disrupted power supply at 18h00 and reconnected at 06h00. This flow of the nutrient solution is also shown in Figure 13.

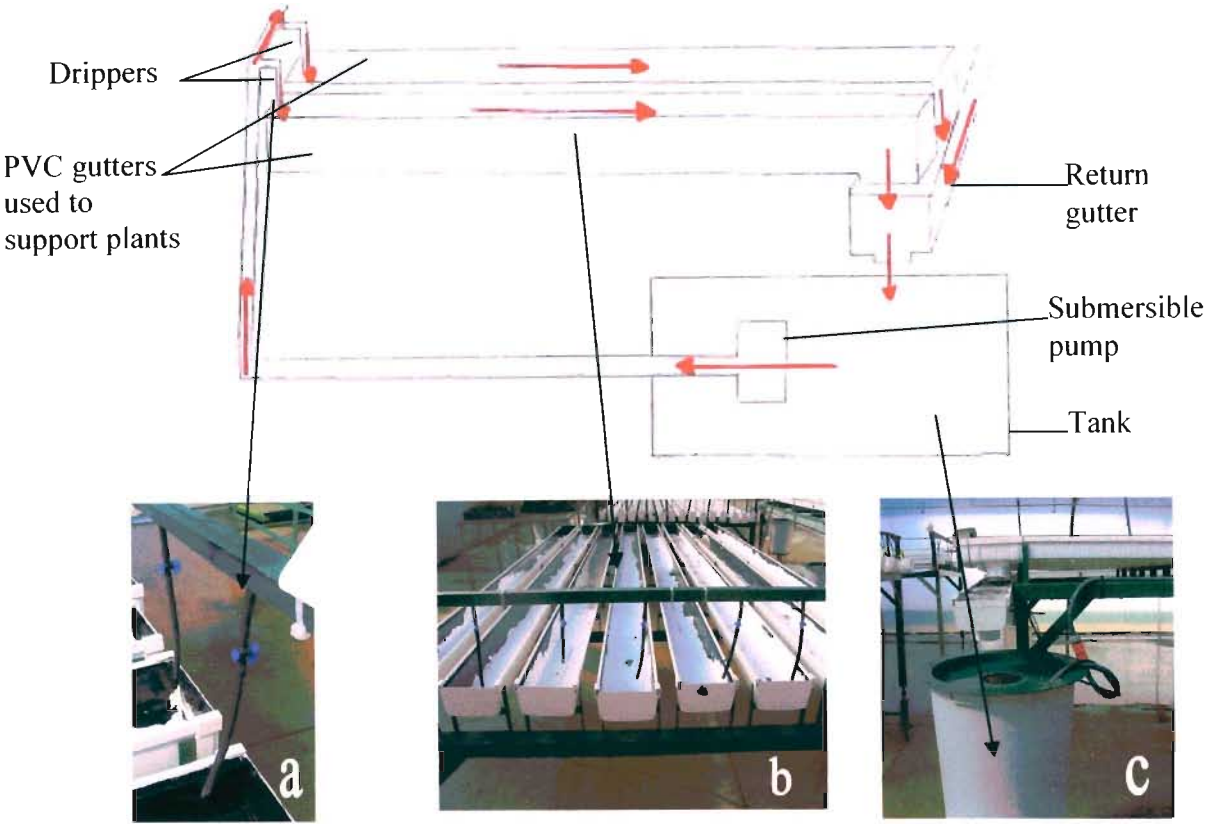

Figure 13: **Drawing and photographs of modified NFT table (→ Flow of water) (a) drippers; (b) gutters and (c) tank**

The modified NFT table had eight individual gutters, allowing for eight different substrates to be tested simultaneously. One gutter was set up as an unmodified NFT table (Figure 14a & b). The plants were held in inserts and roots were allowed to grow out through the bottom to the root zone to form a root mat.

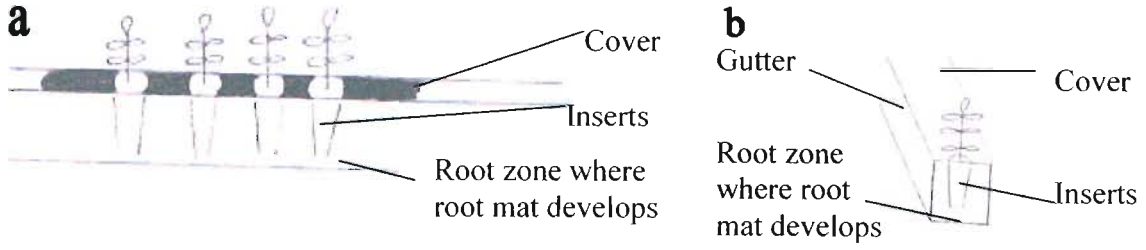

Figure 14: **NFT substrate in the unmodified NFT table showing inserts in gutter (a) cross section; (b) longitudinal section**

The other seven gutters had gravel (6.2 mm), Leca, peat with polystyrene cover, perlite (horticultural grade), a perlite: vermiculite (P:V) mix (3:1), Rockwool® or sand as substrates (Figures 15). Properties of all substrates used are listed in Chapter 2 (Table 8). Plants to be used as hedge material were removed from the inserts and planted into each substrate type except for the true NFT gutter which held the plants in the inserts with no support substrate. The drippers were adjusted for each substrate to avoid problems such as flooding or drying out of the substrate.

The stock plants were planted in June 2001 and the first harvest was conducted on the 14/09/01. After this initial harvest, the next harvests were conducted at three-month intervals over a period of a year.

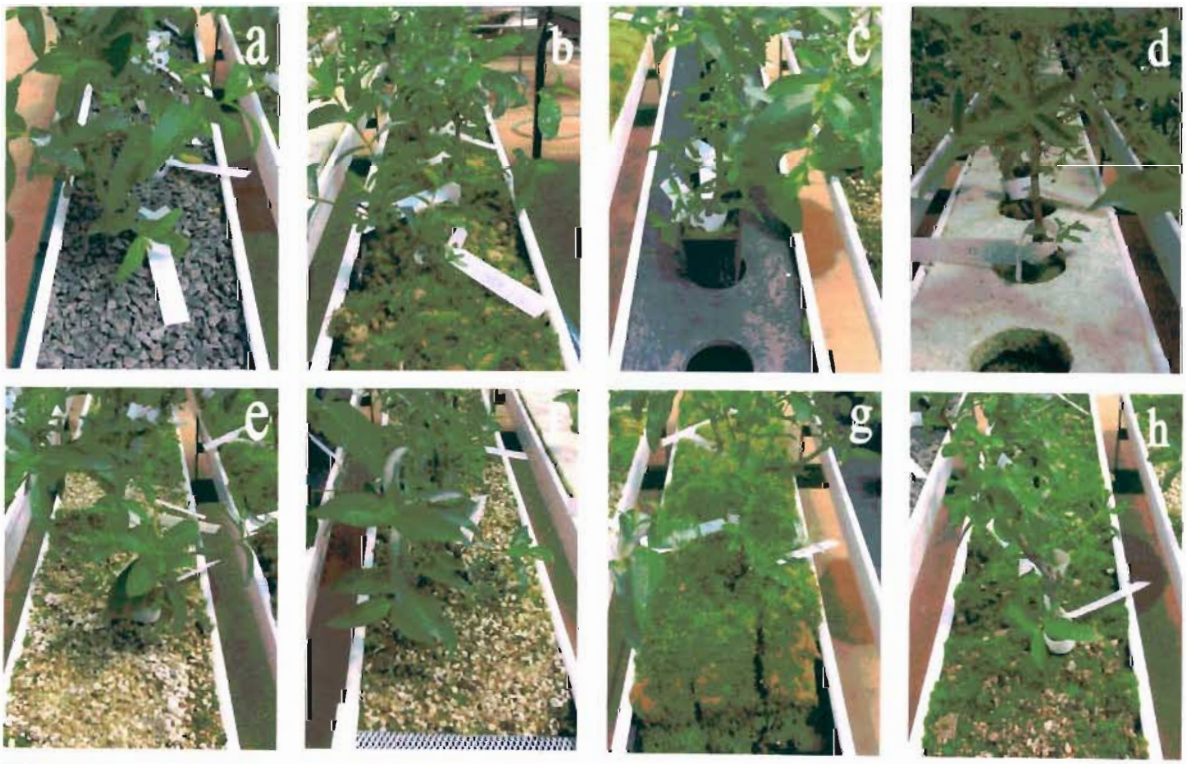

Figure 15: **Cold tolerant eucalypts growing in gutters containing (a) gravel; (b)Leca; (c) NFT (Unigro® 128 inserts); (d) peat with polystyrene cover; (e) perlite; (f) perlite: vermiculite; (g) Rockwool® and (h) sand**

3.5.2 Ebb-and-flow

The ebb-and-flow recirculating table comprised a 2.46 m x 1.245 m fibreglass basin with a depth of 90 mm on the outer edge and a depth of 160 mm at the centre (Figure 16). The table was irrigated via two submersible pumps (maximum volume 1400 $l.hr^{-1}$ to a maximum head of 1.8 m) both of which were installed in a 500 l horizontal plastic tank. These pumps therefore operated at the same time and pumped the same nutrient solution from the same tank. An unequal cyclic timer was fitted to control irrigation times. The timer for the table was set to run for 25-minutes with an off-time interval of four hours. The timer was linked to an electronic clock that disrupted power supply at 18h00 and reconnected at 06h00.

The ebb-and-flow table consisted of a flood and a drain cycle. During the flood cycle the bed was flooded with the nutrient solution pumped from the tank (Figure 16a). The flooding process took 20-minutes to complete. It was imperative that the fibreglass trough was completely full to ensure the activation of the siphon system. After 25-minutes the pump switched off and no water was pumped into the bed. The drain cycle commenced as soon as the siphon unit fed back to the tank (Figure 16b). A four-hour interval between irrigation cycles was introduced to ensure that roots obtained an adequate oxygen supply. Automatic timers were used to ensure proper cycling of nutrient solution.

By definition, there was no actual substrate used in this system. Unigro® 128 inserts held the plants in position in the bed. Therefore the only substrate present was that which was originally in the insert from when the cutting was rooted in the greenhouse.

The stock plants were planted in June 2002 and the first harvest was conducted in September 2002. It was decided that the first harvest would be a sculpting harvest where the ramets are pruned to obtain a maximum number of side shoots (coppice). This decision was based on the results of harvest 1 for the NFT system as poor yield and rooting were obtained. After this initial harvest, the next harvest was conducted in December 2002.

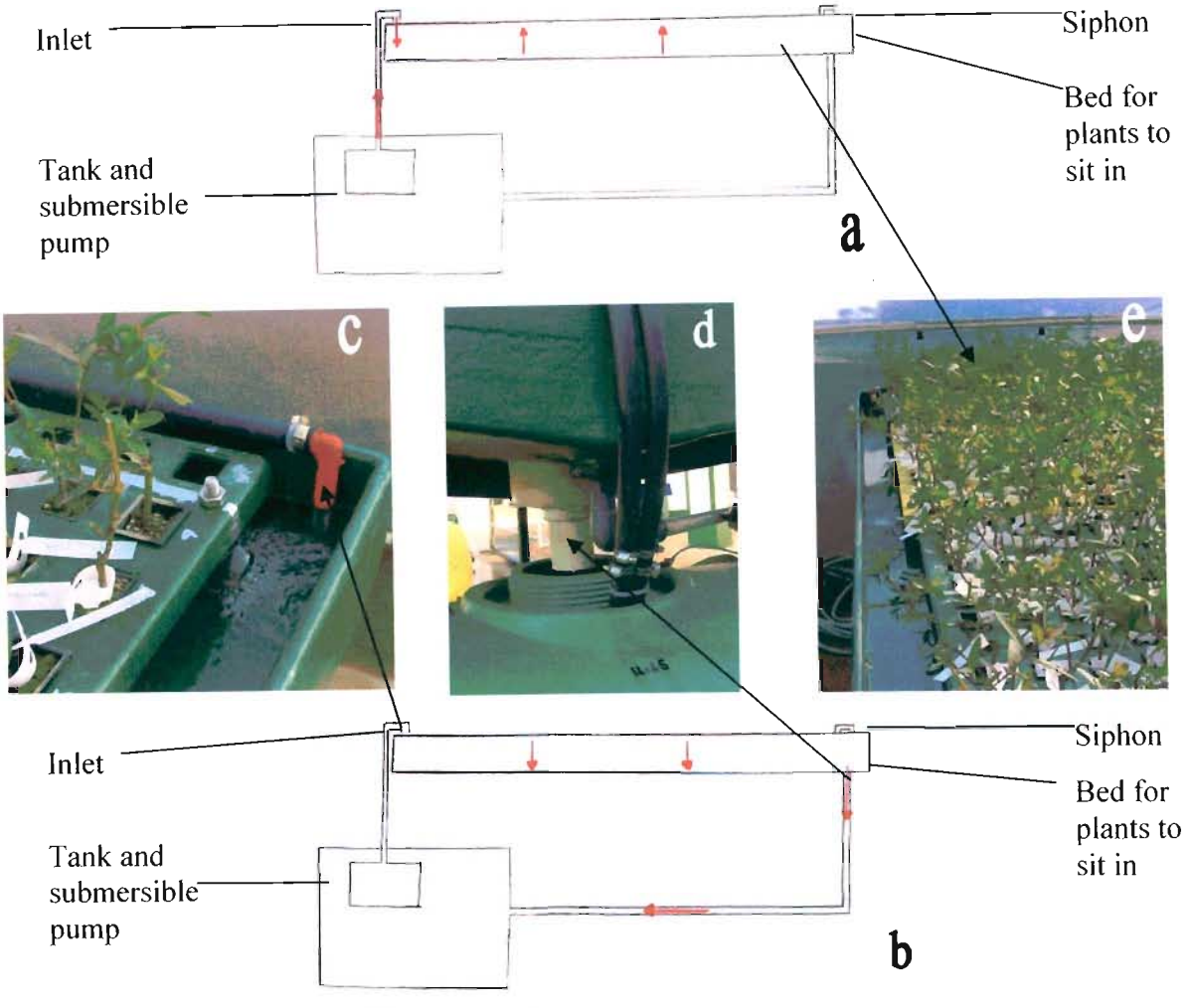

Figure 16: **Ebb-and-flow table (→ Flow of water) in (a) flood and (b) drain cycle showing (c) inlet for nutrient solution; (d) bottom of bed where siphoned water is sent back to tank and (e) plants in ebb-and-flow bed**

3.5.3 Aeroponics

The aeroponic recirculating tables (Figure 17) comprised a 2.085 m x 1.05 m basin constructed from 1.5 mm galvanised sheet metal, with a depth of 80 mm. The table had a wall made from five millimetres thick rubber skirting which was joined to the fibreglass frame which held the plants and overlapped the galvanised basin (Figure 17c). The table was irrigated via a CPM-100 centrifugal pump (displacing 300 $l.hr^{-1}$) installed in line after a 300 l horizontal plastic tank (Figure 17b). An unequal cyclic timer was fitted to control

irrigation times. The timer for the table was set to run for 60 seconds with an off-time interval of five minutes. The timer was linked to an electronic clock that disrupted power supply at 18h00 and reconnected at 06h00.

The ramets in the aeroponics table were placed above a network of misters (Figure 17a / d). The chamber was approximately 195 mm in height and was kept dark (rubber skirting). The humidity in the chamber was kept high. To ensure this, an automatic timer was used to switch the misters on for one minute and off for five minutes to allow the roots to obtain an optimum supply of nutrient solution and oxygen.

In this system there was no substrate. The plants roots were exposed to the nutrient rich mist. Inserts were modified to hold the plants in position and ensure optimum darkness and humidity in the chamber (Figure 17c).

The stock plants were planted in June 2002 and the first harvest was undertaken in September 2002. It was decided that the first harvest would be a sculpting harvest where the ramets are pruned to obtain a maximum number of side shoots (coppice). This decision was based on the results of harvest 1 for the NFT system as poor yield and rooting were obtained. After this initial harvest, the next harvest was conducted in December 2002.

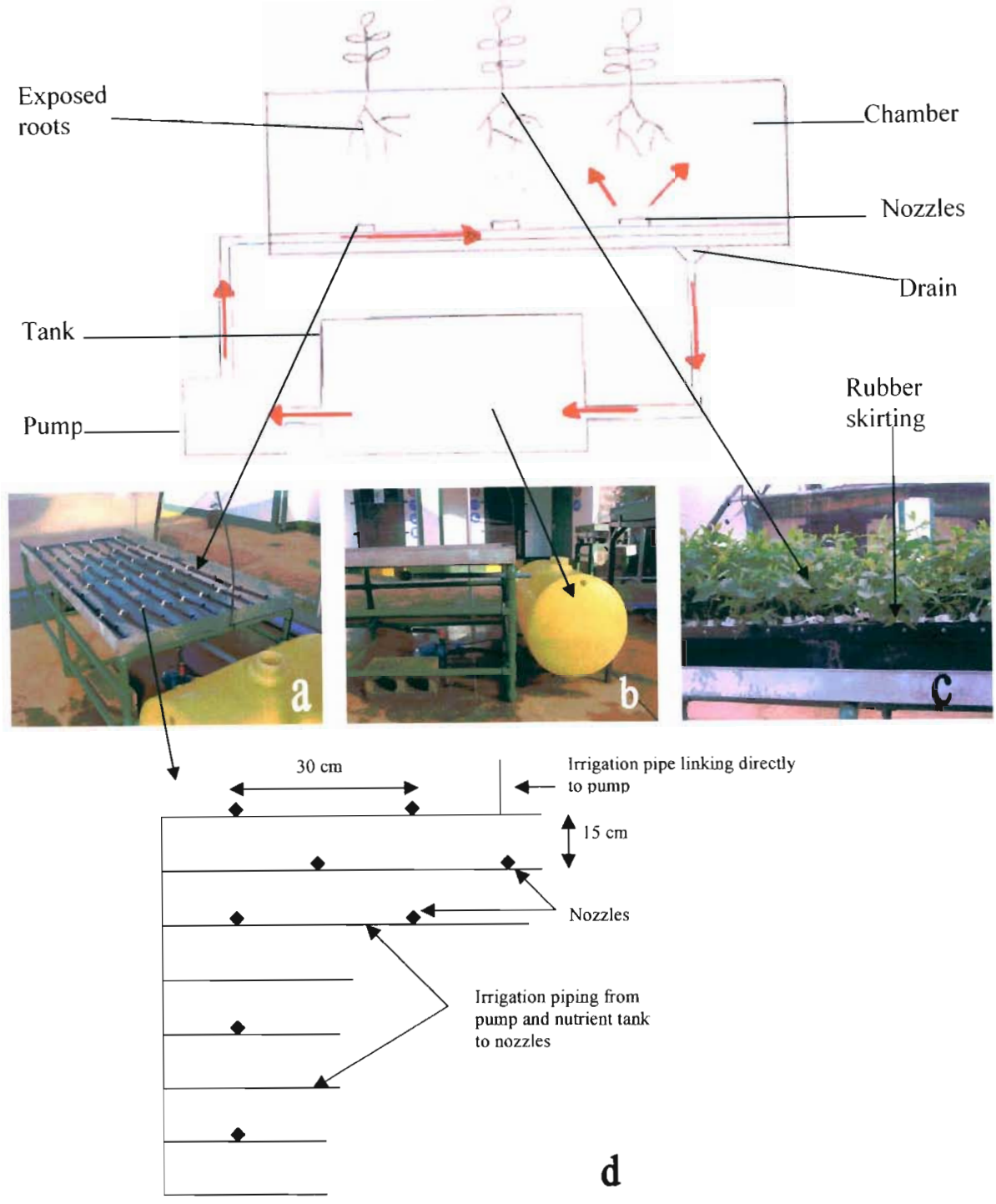

Figure 17: **Drawing and photographs of aeroponics system (⟶ flow of water) (a) nozzles; (b) tank; (c) plants in the system and (d) set-up of nozzles**

3.6 Experimental design and layout

The NFT trial consisted of one NFT table that had eight gutters and two nutrient solution tanks. Four gutters were fed from a single tank. The eight substrates were randomly allocated to the gutters i.e. there was one substrate per gutter. Each gutter could accommodate 30 hedge plants. As there were three clones, it was decided that nine plants of each clone would be randomly allocated a position in each gutter. This made a total of 27 plants per gutter.

Due of the cost of the beds and the lack of space to house them, it was not possible to have more than one bed in the tunnel at the time of the NFT experiment and therefore no true replications of substrate type exist for the NFT trials. It was assumed that because all the substrate types used (except peat) are inert, this should have little or no influence in altering the nutrient solution composition. In addition, because of the frequency of the solution being discarded and replenished, it was assumed that the trial design would not affect the results of the trial in any significant manner. This must be kept in mind though when looking at the results.

In reality, all of the above are pseudo replicates but because of the reasons and rationale explained above, normal ANOVA and statistical tests were performed on the data obtained from the trials. In commercial concerns these types of trials are acceptable as they are more practical to conduct and the results can easily establish the optimal substrate / system to be used under these conditions. Therefore, the outcome of the statistical analysis of the results must be treated with caution.

In the ebb-and-flow and aeroponics trial there was one of each table. The ebb-and flow table can hold 576 plants and the aeroponic table can hold 378 plants. In each case the total number was divided by three (192 and 126 respectively) to determine how many plants of each clone the tables would hold and then plants were randomly assigned positions on the table.

All data were analysed statistically using multiple analysis of variance (ANOVA) on GENSTAT. Where necessary differences were compared using Duncan's multiple range test.

3.7 Collecting of samples and recording data

The number of cuttings placed for each system, harvest, clone and substrate was recorded. After cuttings had been in the hardening off tunnel for 25 days (75 days since being placed) the plants were destructively harvested. The number of cuttings that rooted was recorded and a percentage of rooted cuttings were obtained for each system, harvest, clone and substrate. For the purposes of this investigation, the presence of even a single root was considered a rooted cutting, in the destructive harvest.

For all systems, harvests, clones and substrates the excess coppice material after cuttings were made was sent to the laboratory at the Cedara Agricultural Development Institute, KwaZulu-Natal for plant analysis. In the NFT system, due to the weight constraints (there was insufficient material), clones from each substrate type were combined for analyses and therefore no clonal differences have been taken into account.

Nutrient solution samples (500 ml) were taken from individual tanks that held the nutrient solution used to feed the stock plants in the NFT system. The samples were collected directly after the tanks had been thoroughly cleaned and a new solution prepared (day 0). Fourteen days later when the tanks where due to be emptied and replenished, samples where again taken. The samples were kept in glass Schott bottles and were sent by courier to the ARC (Agricultural Research Council) laboratory in Nelspruit to determine specific nutrient levels. A tap water sample (control) was also sent for analysis.

3.8 Analyses of plant samples

The laboratory at Cedara Agricultural Institute required plant samples with a fresh weight of between 50 and 500 g (weight constraint mentioned above). These were dried at 75 °C for 24 hours and milled. Dry samples were ground to pass through a 40-mesh screen fitted

to a rotary mill. Results were reported on a percent dry-matter basis. A schematic representation of the approach is summarised in Figure 18.

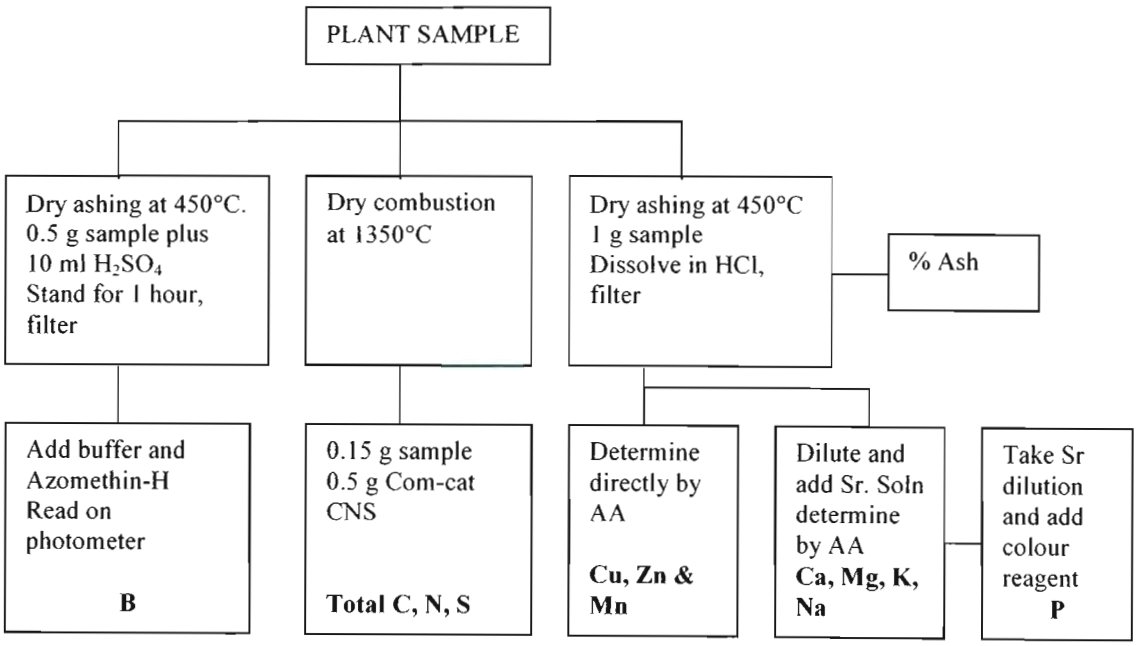

Figure 18: **Summary of nutrient analysis technique performed by Cedara Plant Laboratory** (Riekert and Bainbridge, 1998)

Chapter 4

RESULTS AND DISCUSSION

4.1 Nutrient film technique

4.1.1 Rooting results for four harvests

In an unpublished report, on the average rooting percentages for the cold tolerant eucalypts (CTEs) at Mountain Home Nursery (Table 19), Kopp (1997) highlighted that at times the rooting percentages for individual batches dropped as low as 20 %. More recently, for CTEs at Mondi's Mountain Home Nursery, Bonner (2003) explained that it was difficult to summarise the trends that occurred in rooting, as they varied dramatically between clones and at different times of the year and depended on the climatic influences on the hedges as well as hedge age. In addition, at Mt. Home Nursery it was necessary to budget for an average of 38 % rooting, although this was probably only true for the better rooting clones (e.g. GN108) whereas some of the poorer clones (e.g. NH69) average between 8 and 10 % rooting. A clonal rooting variation between Mountain Home and Fountains nurseries exists due to different soil types, climatic conditions, greenhouses etc. De Haas (2003) concurred with Bonner (2003) by indicating that each clone had a different success rate and added that CTEs are difficult to root. De Haas (2003) also observed that the best rooting results were achieved in the winter months (May – September) and the worst rooting in the summer months (December – February). De Haas (2003) at Fountains Nursery budgets for a rooting percentage of 35 % across all CTE clones and notes that during the season of 1998 - 1999 the average rooting for Fountains nursery was 27 % (Table 19).

Table 19: **Average rooting percentages for CTE clones from actual production figures at Mondi's nurseries**

Clone No.	Rooting (%) As per Kopp (1997)	Clone No.	Rooting (%) As per de Haas (2003)
GN0055	45	GN0016	60
GN0107	50	GN0107	34
GN0156	50	GN0108	36
GN0188	45	GN0121	36
NH0000	55	GN0065	40
NH0058	50	NH0058	21
NH0081	50	NH0069	25
NH0064	50		

With the aim of improving rooting percentages and / or decrease the variability of rooting between clones and throughout the year, a series of trials were established in hydroponic systems.

Hedge plants of three cold tolerant clones (GN107, GN156 and NH00) were maintained in a NFT hydroponic system in which eight different substrate types were tested. Plant material (coppice) was collected from these hedge / mother plants and cuttings were made to be placed in the greenhouse to be rooted using conventional methods (see Section 3.2). The number of cuttings placed and rooted from each clone from each substrate at each of the four harvests (one year) was recorded. From these data the rooting percentage of plants from all substrates and clones were calculated.

For each of the four harvests data the following questions were posed:
(a) Were there any differences between clones within a harvest?
(b) Were there any differences between substrates within a harvest?
(c) Were there any interactions between clone and substrate within a harvest?
(d) Were there any differences between harvests?

The total number of cuttings placed in the greenhouse at harvest 1 was very low (Figure 19) for plants from all substrate types. The total number of cuttings increased at harvest 2 (Figure 19). For plants in Rockwool® (all clones), leca (all clones), sand (all clones), perlite (all clones), NFT (GN156 only) and P:V (GN156 and NH00) substrates the total number of cuttings increased. There was a decrease in the total number of cuttings placed at harvest 3 (Figure 19) except for NH00 and GN107 plants in the NFT substrate. The total number of cuttings placed decreased again at harvest 4 (Figure 19) except for clone GN156 grown in the NFT, gravel, P:V and perlite; GN107 plants grown in gravel and P:V and NH00 plants in gravel and leca. The total number of cuttings placed at harvest 4 was only lower than that of harvest 1 for the clones grown in the peat substrate.

All clones grown in Rockwool®, gravel, sand and peat (Figure 19) showed similar results across the four harvests. Clones grown in the other four substrates had different results. Overall, harvest 2 resulted in the most cuttings placed.

As mentioned, the cuttings from all harvests were set to root. As total numbers of rooted cuttings are important to nurserymen, these are presented in Figure 20. The total number of cuttings rooted at harvest 1 was very low (Figure 20) for all clones grown in all eight substrates. At harvest 2 the total number of cuttings that rooted increased except for GN107 cuttings taken from plants in the NFT system (Figure 20). Harvest 3 showed an increase in the total number of rooted cuttings except for all the clones grown in Rockwool®; NH00 cuttings from plants in NFT, leca and P:V and GN156 cuttings from plants in P:V (Figure 20). The total number of cuttings rooted at harvest 4 was very low with no cuttings rooted from mother plants maintained in NFT and GN156 mother plants in peat (Figure 20).

The total number of cuttings that rooted (Figure 20) was dependent on the total number of cuttings that were placed (Figure 19). These data were used to determine the rooting percentages for each clone and substrate treatment (Figure 21).

The rooting percentage of cuttings (Figure 21) at harvest 1 was low (below 10 %) except for GN107 plants maintained in sand and NFT, GN156 cuttings from plants in gravel and peat and NH00 plants in gravel, leca and P:V. The rooting percentage of cuttings generally increased from harvest 1 to harvest 2 (Figure 21) except for those of clone GN107 grown in the NFT substrate and those from GN156 maintained in peat. The rooting percentage increased from harvest 2 to harvest 3 (Figure 21) except for GN156 mother plants maintained in peat and all clones in Rockwool®, as expected based on rooted numbers (Figure 20). There was a decline in the rooting percentage (Figure 21) from harvest 3 to harvest 4 for all clones and substrates.

For most substrates, a similar pattern emerged (Figure 21) with the rooting percentage of cuttings increasing from harvest 1 to harvest 3 and then declining from harvest 3 to harvest 4. This effect may be caused by the change of the seasons or by the ageing of the mother plants.

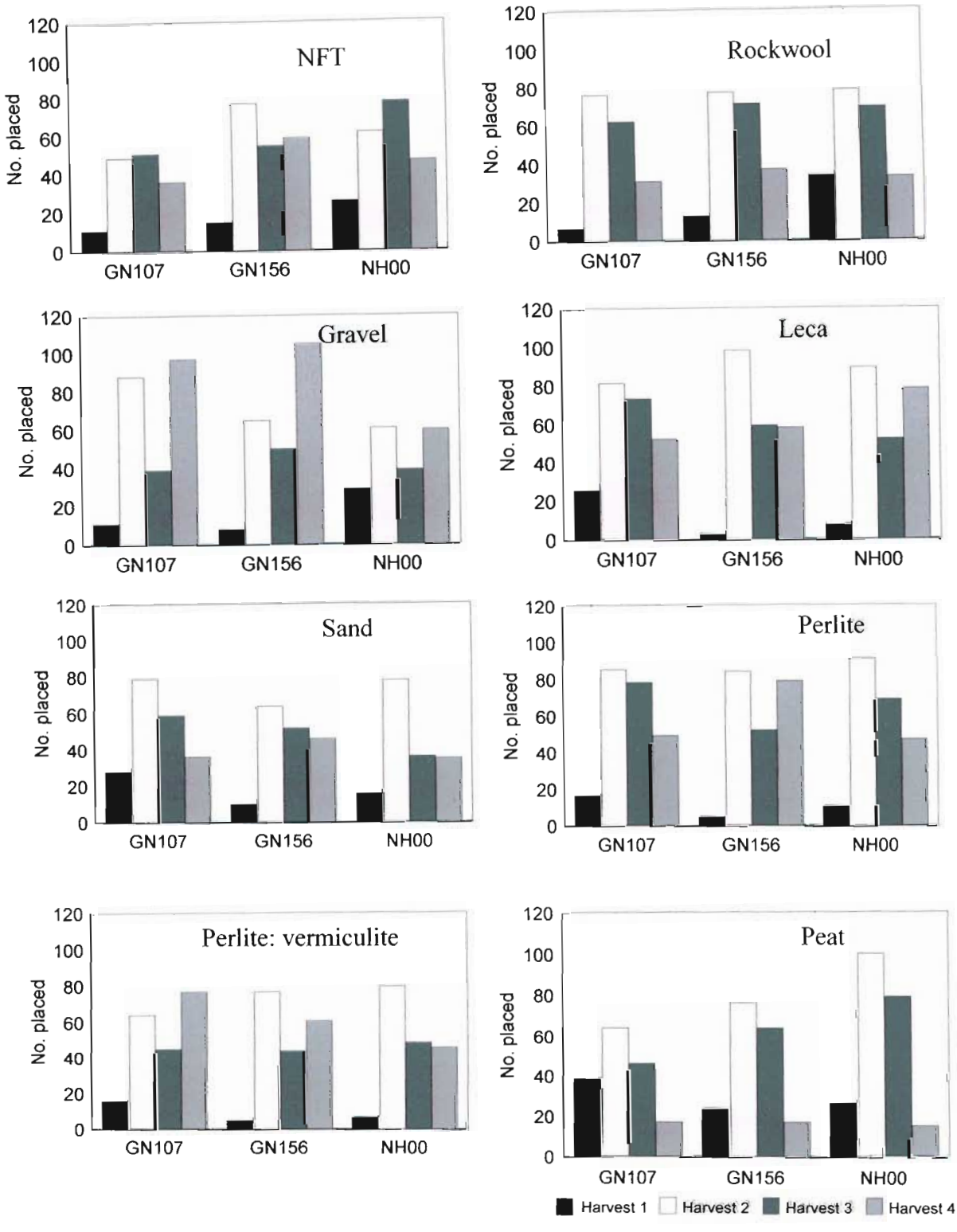

Figure 19: **Effect of substrate type on total number of cuttings placed for three clones (GN107, GN156 & NH00) at four harvests (1- Sept 2001; 2- Dec 2001; 3- Mar 2002 & 4- Jun 2002) from parent plants in the NFT system**

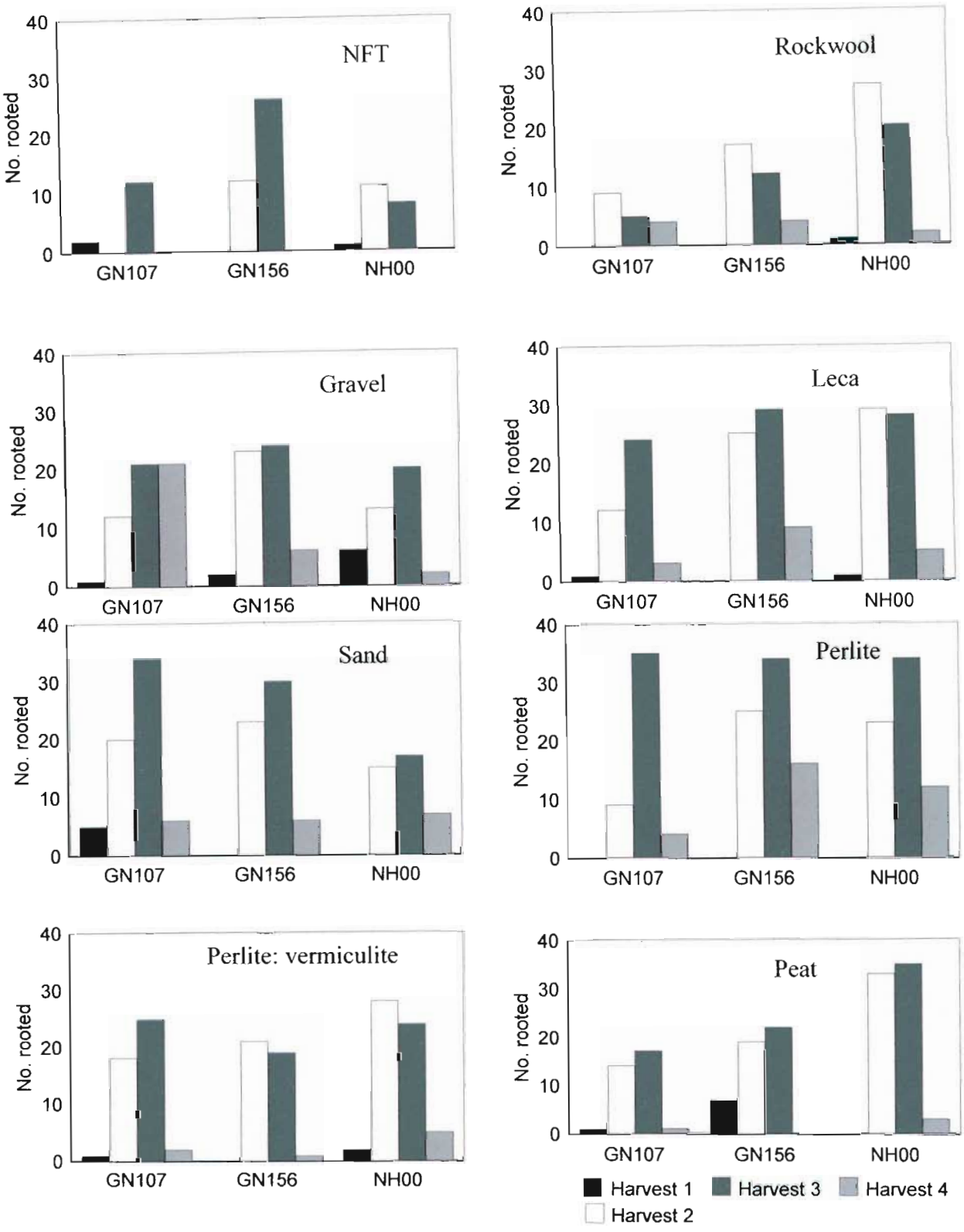

Figure 20: **Effect of substrate type on total number of cuttings rooted for three clones (GN107, GN156 & NH00) at four harvests (1- Sept 2001; 2- Dec 2001; 3- Mar 2002 & 4- Jun 2002) from parent plants in the NFT system**

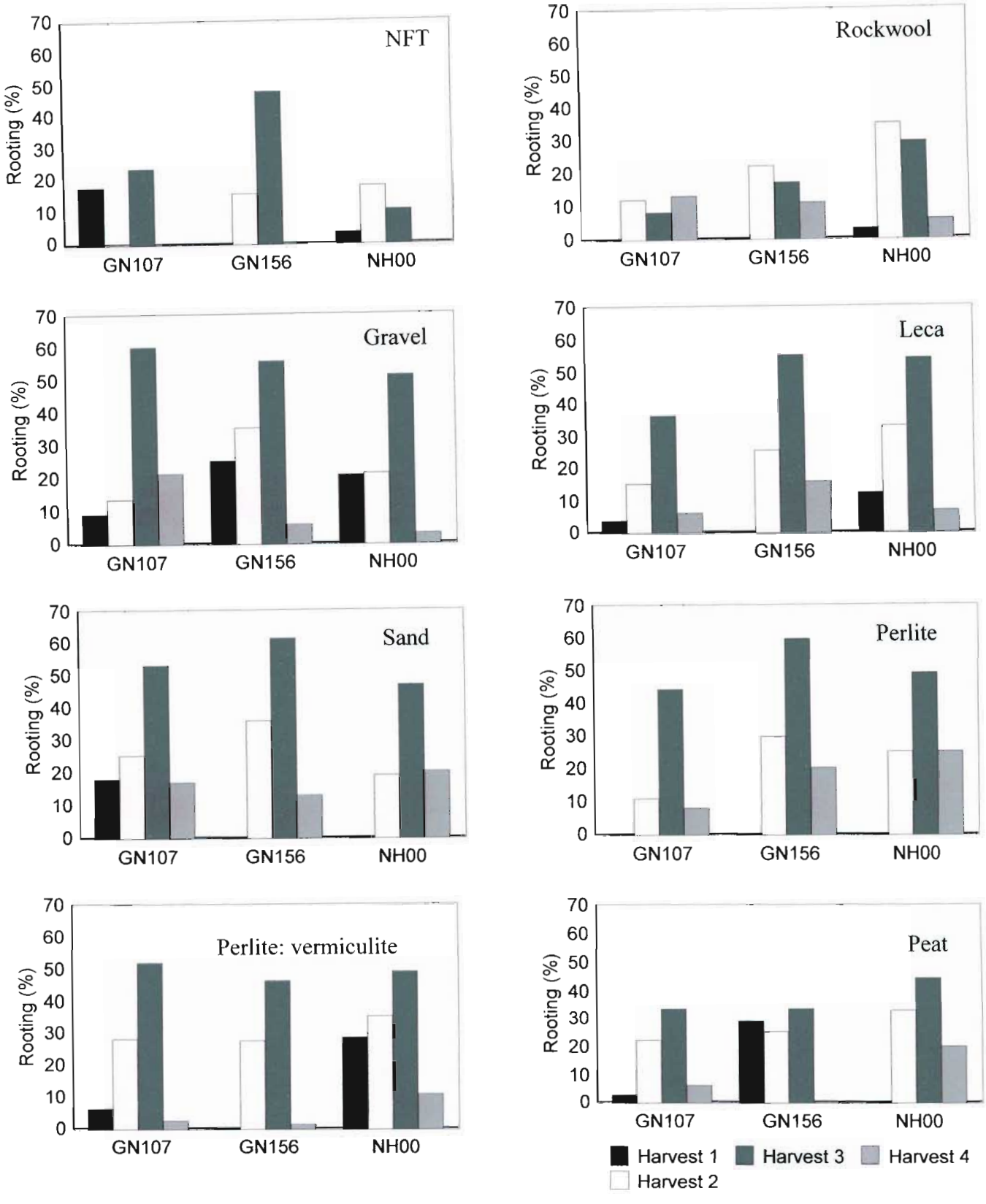

Figure 21: **Effect of substrate type on rooting percentages of three clones (GN107, GN156 & NH00) at four harvests (1- Sept 2001; 2- Dec 2001; 3- Mar 2002 & 4- Jun 2002) from parent plants in the NFT system (Data from figures 19 & 20)**

In these trials, cuttings from harvest 3 had a rooting percentage of 40 % or higher which is above the figures budgeted for from field hedges in the commercial nurseries (Bonner, 2003; de Haas, 2003). The exceptions were clones GN107 and NH00 maintained in NFT, clones GN107 and GN156 maintained in peat and all clones in the Rockwool®.

Da Costa Alpoim (2002) found that for three different trials on CTE clones, clone (genotype) played a significant role in rooting percentages but neither fertiliser, substrate nor, any interactions of these factors played a significant role in rooting. From his first trial (troughs vs. pots), that researcher concluded that rooting variances were due to other horticultural practices (e.g. disease, light etc), which had not been taken into account. In his second trial (comparison of three different nutrient solutions over four substrates), analysis of variance showed that any significant differences were due to clone and not fertiliser, substrate or any interactions of these factors. The data from his last trial (comparison of seven different nutrient solutions) indicated that clone effect was significant for all three harvests at the 1 % level of significance, while nutrient solution type was significant for harvest 1 only at the 5 % level. Substrate, interactions (fertiliser by substrate) and nutrient solution were otherwise found not to be significant. For all three harvests, the order of rooting for the clones did not change i.e. NH00 > GN156 > GN107. From those studies, da Costa Alpoim (2002) concluded that the best fertiliser type for *E. grandis* x *E. nitens* hybrids in hydroponics was Hydroponica® used in combination with the gravel substrate type.

In accordance with the results of da Costa Alpoim (2002), gravel (Table 20) was the best substrate and overall the rooting response of the clones was similar to what he reported.

Table 20: **Mean rooting percentages for harvest, clone, substrate and overall mean** (data from Figure 21 – all data was grouped and averaged)

Harvest	Mean rooting (%)	Substrate	Mean rooting (%)
1	7.5	NFT	11.4
2	23.4	Rockwool®	13.0
3	42.6	Gravel	26.9
4	9.7	Leca	21.8
		Sand	25.8
Clone		Perlite	22.7
GN107	17.8	P:V	24.0
GN156	22.3	Peat	20.7
NH00	22.3		
		Overall mean (%)	20.8

Eleni, Sabri & Dimitra (2001) grew two rose cultivars on the following substrates: cocopeat, perlite-cocopeat (3:1) and perlite-zeolite (3:1). They found that although there were differences in flower quality and yield these were not significant and were cultivar dependent. Similarly, in a study on tomatoes, Angelis, Papdantonakis, Spano & Petrakis (2001) found that fruit quality was not affected by substrate but by the variety. Siomos, Beis, Papadopoulou, Nasi & Kaberidou (2001) investigated the effect of three substrates (perlite and pumice: 0-8 mm and 5-8 mm grades) on the aerial and root biomass as well as on the visual quality of four lettuce cultivars. They found that the most significant effect on productivity and visual quality of the lettuce was found to be that of the cultivar and not that of the substrate.

From the above examples one can summarise that when different culivars of a species have been compared there were significant differences between the cultivars and not the substrates for the measured parameters. In contrast, as discussed below in other trials, substrate type showed significant difference and the authors attributed these differences to the different properties of each substrate.

Schroeder & Foerster (2001) compared polyester fleece with various commonly used substrates (e.g. Rockwool®, perlite and expanded clay). Those authors found that there were no significant differences in the early yield of cucumbers. The total yield was lower for Rockwool® but fruit quality was not influenced. Schroeder & Foerster (2001) attributed these differences to the different water holding capacity, porosity and the consequent varying root growth of the substrates. Similarly, Tüzel, Tüzel, Gül, Meriç, Yavuz & Eltez (2001) compared tomato yields on different substrates. When comparing perlite, volcanic tuff, perlite + peat (4:1) and volcanic tuff + peat (4:1) they obtained the

highest yield with the perlite: peat mix. This mix also had the highest water and nutrient consumption rates recorded. Blom (1999) grew cut roses for two years in a recirculating system in either coco-coir or granulated Rockwool®. In the first year coir produced about 15.6 % more marketable stems and 18 % more fresh weight compared to Rockwool®; however there were no significant difference between the substrates in the second year. El-Behairy, Abou-Hadid, Medany & Awad (2001) found that a sponge medium (ureaformaldehyde foam) gave higher early and total yields when compared to the NFT system for growing strawberries. Scarascia-Mugnozza, Vox & Mancini (2001) compared lettuce yield in blast furnace and recycled plastic residues with the traditional substrates peat, perlite and expanded clay. Lettuce had similar and significantly more marketable yield when grown with peat, blast furnace residue and perlite compared with lettuce grown in expanded clay and recycled plastic (Scarascia-Mugnozza *et al.*, 2001). Scarascia-Mugnozza *et al.*, (2001) attributed these differences to substrate temperatures and temperature fluctuations within a substrate, heat capacities, water retention and other substrate-properties.

Hahn, Jeon & Paek (2001) grew two cultivars of *Gerbera* (Ensophy and Estel) in substrate (Rockwool®, cocopeat, perlite and vermiculite) and soil cultures. They found that the number of flowers per plant, flower height, flower weight and flower diameter were far better in the substrate culture than in soil culture for both cultivars. In addition Rockwool® led to better results in number of flowers per plant, plant height, plant weight and flower diameter with Ensophy, while no significant differences were observed among growing media in Estel. Relatively smaller number of flowers per plant were produced in cocopeat for both cultivars. Hahn *et al.*, (2001) found significant differences due to substrate and cultivar.

In the present study (all four harvests), clone, substrate and their interaction had a significant effect on (Table 21) the number of cuttings placed and percent rooted. For the number of cuttings rooted, the above was true for all but harvest 1, where clone was not significant (Table 21). The number rooted was very low for all clones in each substrate. This verifies the clonal differences established by da Costa Alpoim (2002). However, as per the findings of Hahn *et al.*, (2001), there were also significant differences due to substrate type and its interaction with clone type. This could have been due to the fact that four additional substrate types were used in addition to the four

which da Costa Alpoim (2002) had originally used in his trials. It may also have been due, as da Costa Alpoim (2002) suggested, to other horticultural practices or as Schroeder & Foerster (2001) and Scarascia-Mugnozza *et al.*, (2001) suggested, one or more of the substrates properties, which were not recorded or taken into account. However, the effect of substrate type found in the present study must be assessed with caution because of the feeding method of the eight substrates.

Table 21: **Summary of analyses of variance for harvest 1 to 4 for rooting results** (Data from Figures 19, 20 & 21)

	Clone	**Substrate**	**Clone x substrate interaction**
	Number of cuttings placed		
Harvest 1	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 2	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 3	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 4	$p < 0.001$	$p < 0.001$	$p < 0.001$
	Number of cuttings rooted		
Harvest 1	$p = 0.404$	$p < 0.001$	$p < 0.001$
Harvest 2	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 3	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 4	$p = 0.014$	$p < 0.001$	$p < 0.001$
	Rooting percentages		
Harvest 1	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 2	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 3	$p < 0.001$	$p < 0.001$	$p < 0.001$
Harvest 4	$p < 0.001$	$p < 0.001$	$p < 0.001$

After conducting a three-way ANOVA with clone, substrate and harvest number as factors, it was found that only harvest number had a significant effect ($p < 0.0001$) for percentage of cuttings rooted. As mentioned earlier, this trial was conducted over a year and therefore one cannot conclude whether or not this significance may be due to maturity of the mother plants, to a seasonal effect or perhaps both. The trial would have to be conducted again over a longer period to determine whether age, maturity or both played any role in the varying rooting percentages.

When investigating the differences within the harvests, the rooting percent was affected by clone, substrate and their interaction significantly but when examining the differences between the harvests, there was no significant difference on the rooting percent for clones, substrate type or their interaction but only for harvest number.

Each substrate type had particular advantages and disadvantages, as outlined in Table 8 and it was imperative that the practicality, availability and the cost of the substrate had to be taken into account when considering a commercial installation. In Chapter 5, a cost-benefit analysis has been presented, comparing the eight substrate types tested with the ebb-and-flow and aeroponics systems.

4.1.2 Plant analyses: harvest 1 vs. harvest 3

A plant analysis was conducted on the excess coppice material after placing cuttings from harvests 1 and 3 of the NFT system. As discussed in Section 3.7, clones (GN107, GN156 and NH00) from each substrate type had to be combined due to the sample weight constraints and therefore no clonal differences have been taken into account. The data was compared for differences between substrates but because the same three clones were bulked for each substrate type this must be interpreted with some caution. The results were compared to determine if there was a change in nutrient content between harvests in the hedge plants.

For each nutrient the following questions were posed:

(a) Are there any differences between the two harvests?

(b) Are there any differences among the substrates?

(c) Is there any interaction between the harvests and substrates?

Plant macronutrient concentrations were significantly different between the two harvests (Table 22). Substrate and the harvest by substrate interactions were not significant for all macroelement concentrations except P, where both had a significant effect (Table 22). Plant N, P and K (Figure 22) decreased from harvest 1 to harvest 3. The decrease in N and K concentrations was not significant for plants grown in all substrates. However, the decrease in P concentration was significant for plants in all substrates. Plant Ca and Mg levels (Figure 22) increased from the first to the third harvest in plants in all substrates. For Ca the increase was significant in plants in all substrate types but the increase in Mg concentrations was not significant. Plant Na (Figure 22) increased from the first to the third harvest in plants grown in all substrates except P:V. The only significant change was for perlite where Na concentrations increased from harvest 1 to harvest 3.

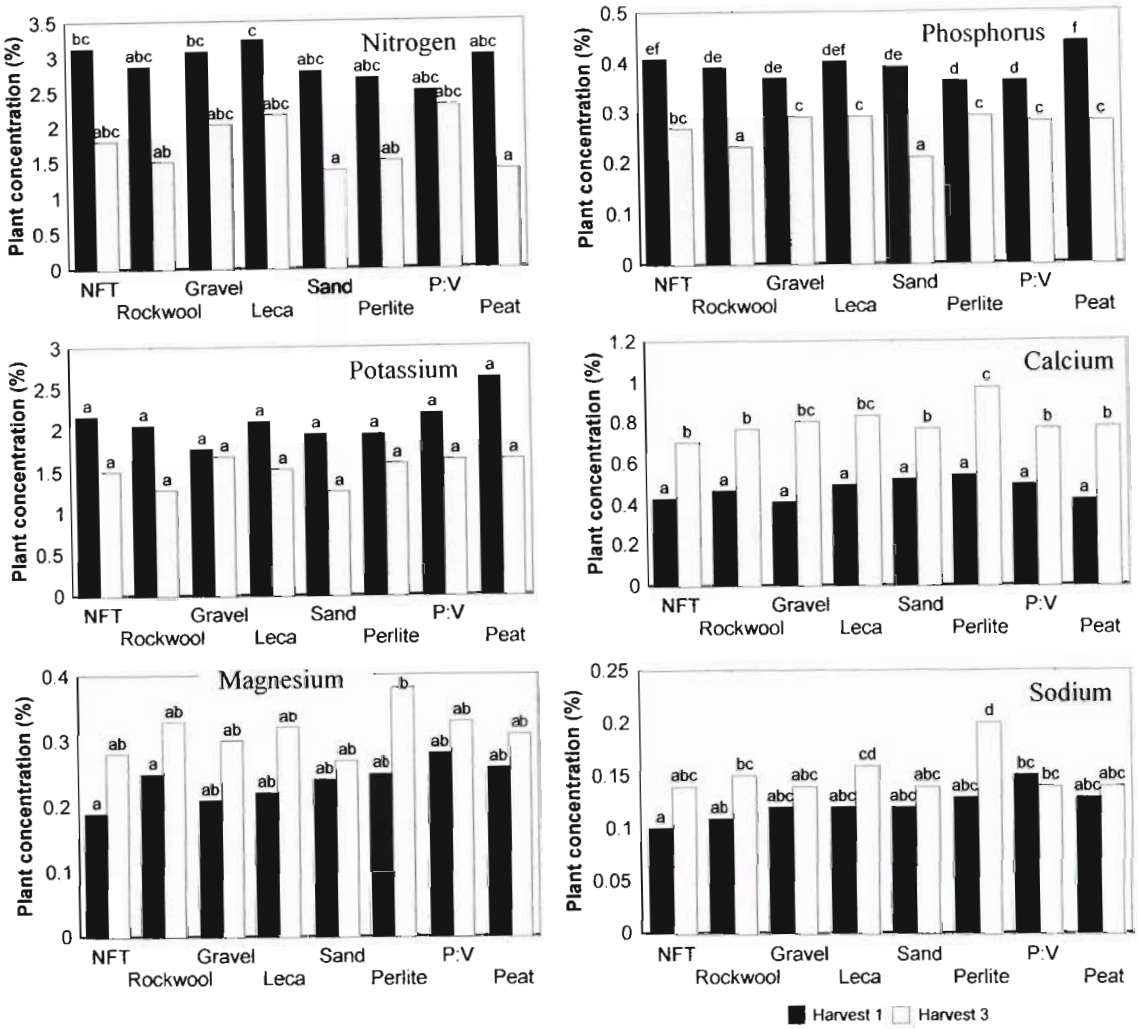

Figure 22: **Macroelement concentrations of plants grown in the NFT system at harvests 1 and 3 ($p < 0.05$)**

Barnes & Lewandowski (1991) proposed that avoiding high N levels in *Eucalyptus* mother plants improved the quality and rooting of cuttings. Nitrogen was used to promote vegetative growth (Table 9). Carlson *et al.*, (2003) suggested that the optimum N concentration in plants for the rooting of *Eucalyptus* was 1.75 – 4.25 % N while Higashi *et al.* (2000a) proposed the narrower range of 2.8 – 4 % N. From the results in this trial (Figure 22) one would expect better rooting from harvest 1 compared to that of harvest 3 for clones in all substrates except possibly P:V mix. This was because the N concentration for harvest one was between 2.5 – 3.5 % N (Figure 22) whereas the N concentration for harvest three was between 1 – 2.5 % N. According to Carlson *et al.* (2003) and Higashi *et al.* (2000a), the N content at harvest three are too low for the optimal rooting of eucalypts. From the rooting results (Figure 21), the rooting percentage for all three clones from every substrate type was higher at harvest 3 than at harvest 1. This indicated that a lower plant N concentration might be related to better

rooting. One must bear in mind that Carlson *et al.* (2003) worked with GCs (*E. grandis* x *E. camaldulensis*), GUs (*E. grandis* x *E. urophylla*) and GNs (*E. grandis* x *E. nitens*) and while there was no information on which species of *Eucalyptus* Higashi *et al.* (2000a) used it was likely that these authors used GUs and possibly GCs. Carlson *et al.* (2003) had a lowest optimum value which was lower than that of Higashi *et al.* (2000a) and therefore it was possible that GNs require lower concentrations of N compared to GU or GC clones.

Phosphorus promotes and stimulates root growth (Table 9). Higashi *et al.* (2000a) recommended a foliar range of 0.25 – 0.4 % P in the hedge plants. In later work, Higashi *et al.* (2000c) found that the P levels higher than 0.35 % decrease rooting. This might explain why cuttings from harvest 1, with high P levels did not show high rooting percentages (Figure 21). All results from these analyses fall into the 0.11 – 0.6 % range recommended by Carlson *et al.* (2003) (Figure 22).

Potassium is used by the plant to regulate osmotic pressure and is found in parts of the plant with high physiological activity (Table 9). Paula *et al.* (2000) observed that K levels affected productivity, length of new shoots, root number and root dry mass but not rooting percentage, number of new shoots per stem or root length of eucalypts. Higashi *et al.* (2000a) recommends 1.5 – 3.0 % K for the production of micro-cuttings. Again, harvest 1 should have had the better rooting results as these concentrations of K were all above 1.5 % whereas those for harvest three were either less than or equal to 1.5 % K. Paula *et al.* (2000) suggested that K does not influence the rooting percentage. According to Carlson *et al.* (2003) the required K range was 0.53 – 2.13 %. Although there was no significant differences between the actual K levels at the two harvests (Figure 22), there was a significant difference between the rooting at the two harvests (Figure 21) and therefore the lower K may have positively influenced the rooting.

Calcium is important for cell division, elongation and in building cell walls (Table 9). As previously mentioned (Table 11) Ho *et al.* (1999) recommended a Ca level of 0.012 % to ensure optimal uptake by the roots of tomato plants. Ho *et al.* (1999) also stressed how important it was to maintain a balance between Ca and other nutrients, especially N (0.018 %), K (0.04 %) and P (> 0.0005). Higashi *et al.* (2000a) recommended 0.5 – 0.7 % Ca for hedge plants to get optimum rooting but later Higashi *et al.* (2000d) specified

that the critical level was 0.55 %. In this case, Carlson *et al.* (2003) results were higher than those of Higashi *et al.* (2000a). This and the corresponding rooting results suggest that a Ca level higher than those of Higashi *et al.* (2000d) and Carlson *et al.* (2003) was required for optimal rooting of GN hybrids (Figures 21 & 22).

Magnesium is a component of the chlorophyll molecule and promotes the absorption and translocation of P (Table 9). Higashi *et al.* (2000a) recommended a Mg level of 0.2 – 0.3 % and later (Higashi *et al.*, 2000d) observed that to obtain a rooting percentage of 70 % or higher the level should be 0.25 %. In the present study, although there was no significant difference in plant Mg levels for plants in most substrates (except NFT and Rockwool®) (Figure 22) the rooting (Figure 21) was higher at the higher levels of Mg plant concentrations.

Harvest number, substrate type and their interaction had a significant effect (Table 22) on all microelement concentrations except plant Cu for which harvest number was not significant. Plant Fe concentration (Figure 23) decreased significantly from harvest 1 to harvest 3 in plants grown in the NFT, Rockwool®, leca, sand, P:V and peat substrates but increased significantly in plants in gravel and perlite. Copper concentrations (Figure 23) decreased significantly in plants in Rockwool® and decreased non-significantly in plants in the NFT and sand substrates. There were no significant changes in plants grown in leca and P:V, while there was a significant increase in Cu concentration in those growing in peat, but Cu increased non-significantly in plants in gravel and perlite. Plant Zn (Figure 23) decreased significantly in plants in all substrates from harvest 1 to harvest 3. Manganese levels (Figure 23) increased significantly in those grown in gravel and perlite but decreased significantly in plants in the other substrates. Plant B (Figure 23) decreased significantly in plants grown in NFT, Rockwool® and gravel from the first to the third harvest but increased significantly in those in the other four substrates.

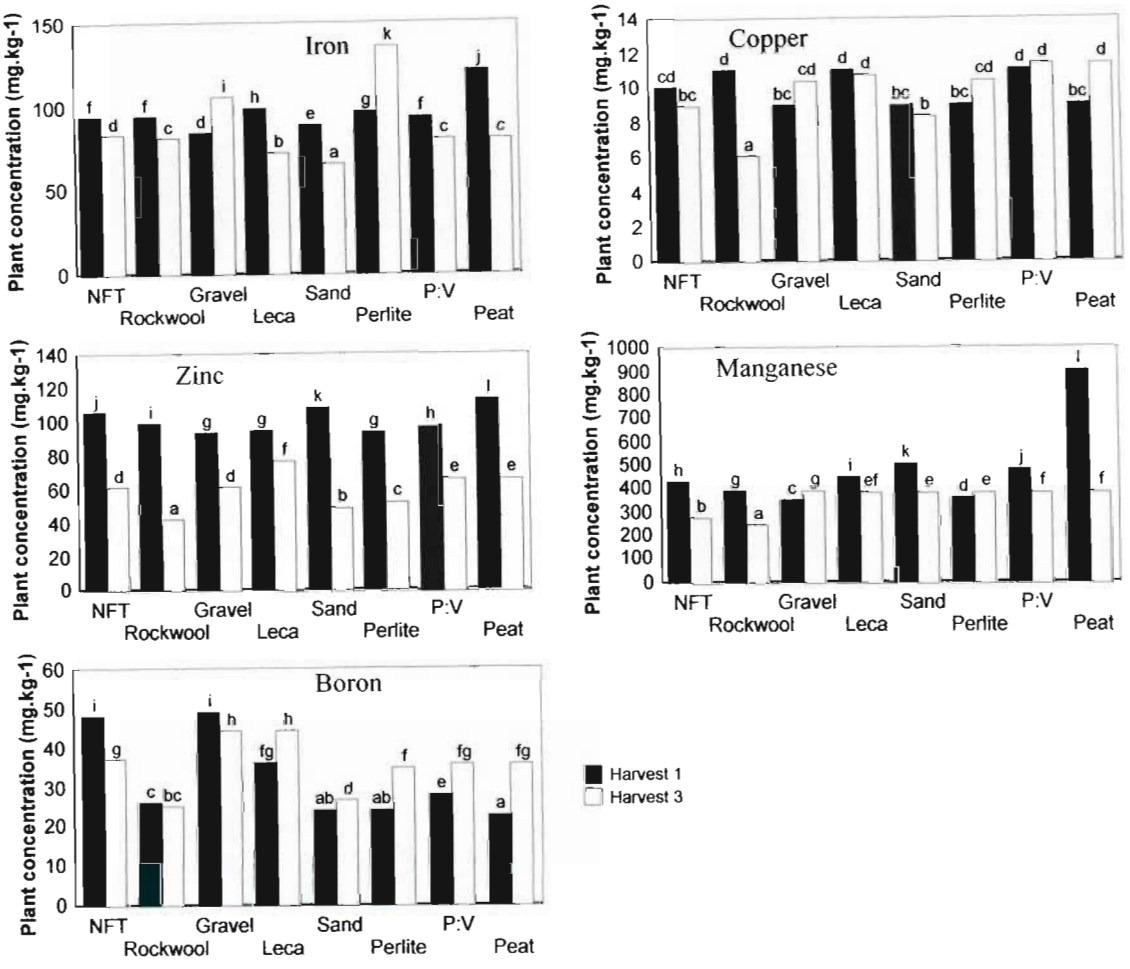

Figure 23: **Microelement concentration of plants grown in the NFT system at harvests 1 and 3 ($p < 0.05$)**

In 1976, Lamb noted that seasonal changes in the distribution of plant nutrients in *E. deglupta* were most noticeable with microelements, particularly Mn, B and Fe. Iron is essential as various catalysts and enzymes in plant metabolism (Table 9). Higashi *et al.*, (2000a) recommended a level of 101 – 220 $mg.kg^{-1}$ for Fe, and in the present study, the only results obtained which fell into this range were from plants kept in perlite and gravel (both harvest 3) and peat (harvest 1) (Figure 23). The rooting of cuttings from plants in perlite and gravel substrates was higher than 40 % at harvest 3 (Figure 21). This was however not the case for plants in peat at harvest 1 (Figure 21).

Copper is another important catalyst in plant metabolism and also acts as an electron carrier (Table 9). As previously mentioned, Erasmus & Levin (1991) found that Cu levels influenced rooting percentages. They observed that high plant Cu levels increased the coppice yield but was detrimental to the rooting percentage. From this information,

they proposed that there is a optimal plant Cu level for both yield and rooting. Higashi *et al.* (2000a) recommended a range of 8 – 15 $mg.kg^{-1}$ Cu in the hedges for micro-cutting production. All results from this trial fell into this range except for those from plants in Rockwool® at harvest 3 which were the lowest (Figure 23). In addition, the rooting percentage for plants grown in the Rockwool at harvest three were lower than for those from harvest 2, which had the highest rooting percent (Figure 21).

Zinc is essential for auxin synthesis and is best described as a regulating catalyst (Table 9). Barnes & Lewandowski (1991) suggested that adding Zn to a feed solution improved the rooting of *Eucalyptus* species. Reichman, Asher, Mulligan & Menzies (2001) showed that Zn concentration in *E. camaldulensis* root and shoot tissue increased as Zn in solution increased. Root tissue concentrations were higher than those of shoot at all Zn solution concentrations. The critical tissue Zn concentrations were approximately 0.415 and 0.370 $mg.g^{-1}$ for *E. camaldulensis* for the youngest fully expanded leaf and total shoot, respectively. On the other hand, Higashi *et al.*, (2000a) reported that a level of 30 – 60 $mg.kg^{-1}$ was optimal. Coppice from harvest 1 had levels of Zn that were too high for cutting production when comparing against Higashi *et al.* (2000a) results (Figure 23). As previously mentioned, the rooting percentage at harvest 3 was greater than those at harvest 1 for all treatments and therefore results from this trial concurred with the findings of Higashi *et al.* (2000a) (Figure 21).

Manganese has a general function of a catalyst but is involved in carbohydrate and energy storage metabolism and chlorophyll synthesis (Table 9). Erasmus & Levin (1991) also mention that it has an effect on rooting percentages. Higashi *et al.* (2000a) recommended 250 –500 mg. kg^{-1} Mn for eucalypt cuttings production. All results fell into this range except those grown in perlite (harvest 1) (Figure 23). Coppice from Rockwool® and NFT had the lowest Mn levels at harvest 3 and this corresponded with low rooting levels (Figure 21). This was especially true for coppice from Rockwool® where Mn levels and rooting was lowest (Figures 21 & 23).

Boron has catalytic functions for many physiological activities. It is known to influence and may even control the ratio of anions to cations taken up by the plant (Table 9). Barnes & Lewandowski (1991) recommended that adding B to the feed solution for hedge plants improved the quality and rooting of cuttings taken from the hedges.

Higashi *et al,* (2000a) found that the optimum B level in eucalypt hedges was 35 – 70 mg. kg^{-1}. Only plants that had been grown in NFT, gravel and leca fell into this range (both harvests) (Figure 23). In the present study coppice from the plants in the gravel had the best rooting overall (Figure 21), there seemed to be no correlation between plant B concentration and rooting.

Table 22: **Summary of analyses of variance for plant analyses: harvest 1 vs. harvest 3** (data from Figures 22 & 23)

	Harvest	**Substrate**	**Harvest x substrate interaction**
N (%)	$p < 0.001$	$p = 0.941$	$p = 0.960$
P	$p < 0.001$	$p = 0.017$	$p = 0.004$
K	$p = 0.049$	$p = 0.991$	$p = 0.997$
Ca	$p < 0.001$	$p = 0.108$	$p = 0.742$
Mg	$p = 0.11$	$p = 0.868$	$p = 0.993$
Na	$p < 0.001$	$p = 0.188$	$p = 0.302$
Fe ($mg.kg^{-1}$)	$p < 0.001$	$p < 0.001$	$p < 0.001$
Cu	$p = 0.473$	$p < 0.001$	$p < 0.001$
Zn	$p < 0.001$	$p < 0.001$	$p < 0.001$
Mn	$p < 0.001$	$p < 0.001$	$p < 0.001$
B	$p < 0.001$	$p < 0.001$	$p < 0.001$

In three separate trials, da Costa Alpoim (2002) found that there was no significant differences for both macro- and microelements due to substrate. He did, however, find that certain elements changed significantly over time. Nitrogen, P, Ca, B, Zn, and Mn concentrations had all increased significantly over a 103-day period while plant Mg had decreased significantly over that period. Higashi *et al.* (1998) results' concurred with these. They found that N, P, Ca, Mg, S, Zn, Cu, Fe and Mn concentrations all increased linearly over a 28-day period. The results discussed for the plant analysis of harvests 1 and 3 were for a longer period (approximately 180 days) compared to Higashi *et al.* (1998) findings and this may explain the differences in results but the time of year and fertiliser type may also have played a role.

In summary:

N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn	B
↓	↓	↓	↑	↑	↑	*	*	↓	*	*
✓	✓	X	✓	X	✓	*	*	✓	*	*

↑- increase over time; ↓ - decrease over time; ✓ - significant; X – not significant

* Fe, Cu, Mn and B act differently for each substrate over time and can not be generalised to fit in the above summary table.

Results from this trial and others mentioned indicated that no one single nutrient was responsible for rooting or growth and instead each played a part in the process, some to a lesser degree than others. While it is possible to suggest that individual elemental levels may influence rooting, it is more likely that element ratios are what are important and these should be looked at in depth in future studies.

4.1.3 Plant analyses: mother material vs. cutting material

Wignall *et al.,* (1991) suggested that many of the limitations associated with attempts to intensify the management of operational clonal nurseries could be linked to the way in which the stock plants (mother material) were managed. Those authors indicated that poor rooting and mother plant death were typical symptoms of inappropriate mother plant management. They suggested that attention to irrigation, nutrition, site preparation and the use of feeder branches could all improve the vigour of mother plants and in turn improve the rooting of cuttings taken from those plants. Williams (2000) supported this and noted that one of the advantages of a more intensive system was the better control over the nutritional status of the mother plants and the consequent superior quality material which was produced by them. Barnes & Lewandowski (1991) recommended feeding specific fertilisers to avoid high N levels and supplying B and Zn as methods for manipulating mother plants to improve the quality and rooting of cuttings.

According to Welander (1994) the opportunity to manipulate the rooting potential of cuttings are generally much greater when favourable conditions are given to the mother plants rather than the cuttings taken from them, and he gives the mineral status of the mother plant as one example of how the manipulation of the mother plants can affect rooting of cuttings. In a similar fashion, Marien (1991) reported that it was possible to obtain nearly 39 % more rooted cuttings when the mother plants were treated with a complete fertiliser. Marien (1991) found that mother plants play a predominant role in the quality of the next generation of cuttings and special attention must be given to the fertilisation of these mother plants.

On the other hand, Aimer-Halliday *et al.* (1999) found that for 135 *E. grandis* x *E. nitens* hybrids, a clonal influence was the only critical factor in the rooting of cuttings.

Coppice treatments which included various topping methods and dates, fertilisation and various combinations of these produced no significant differences in the rooting of the hybrids.

With this in mind, a plant analysis was conducted on hedge material (mother material) from harvest 3 and then on rooted material (cutting material). A destructive harvest was conducted 75 days after the cuttings were placed to root in the greenhouse. Both sets of samples that were collected contained leaf and stem parts. The cutting material also contained any roots that had developed. These were then compared to see whether there were differences in nutrients before and after the plant material had rooted, i.e. what nutrients were used and how they changed in the rooting process.

For each nutrient the following questions were posed:

(a) Were there any differences between mother and cutting material?

(b) Were there any differences between substrates?

(c) Was there any interaction between material types and substrates?

No significant differences were found for substrate type or the interaction (substrate by material) for any of the macroelements. There were however significant differences between mother and cutting material for all macroelements except Na (Table 23).

For N, P, K and Ca within plants, the concentration decreased from the mother material to the cutting material (Figure 24). For N concentrations there was a significant difference between mother and cutting material for plants grown in gravel, leca and P:V substrates. For P and K, these differences were not significant. Plant Ca only decreased significantly in the coppice from plants grown in perlite. Magnesium and Na concentrations, on the other hand, increased in the coppice from plants grown in all substrates (Figure 24), except the Na levels in the plants grown in perlite (Figure 24). For Mg the increase was significant in plants grown in all substrates except Rockwool, P:V and peat.

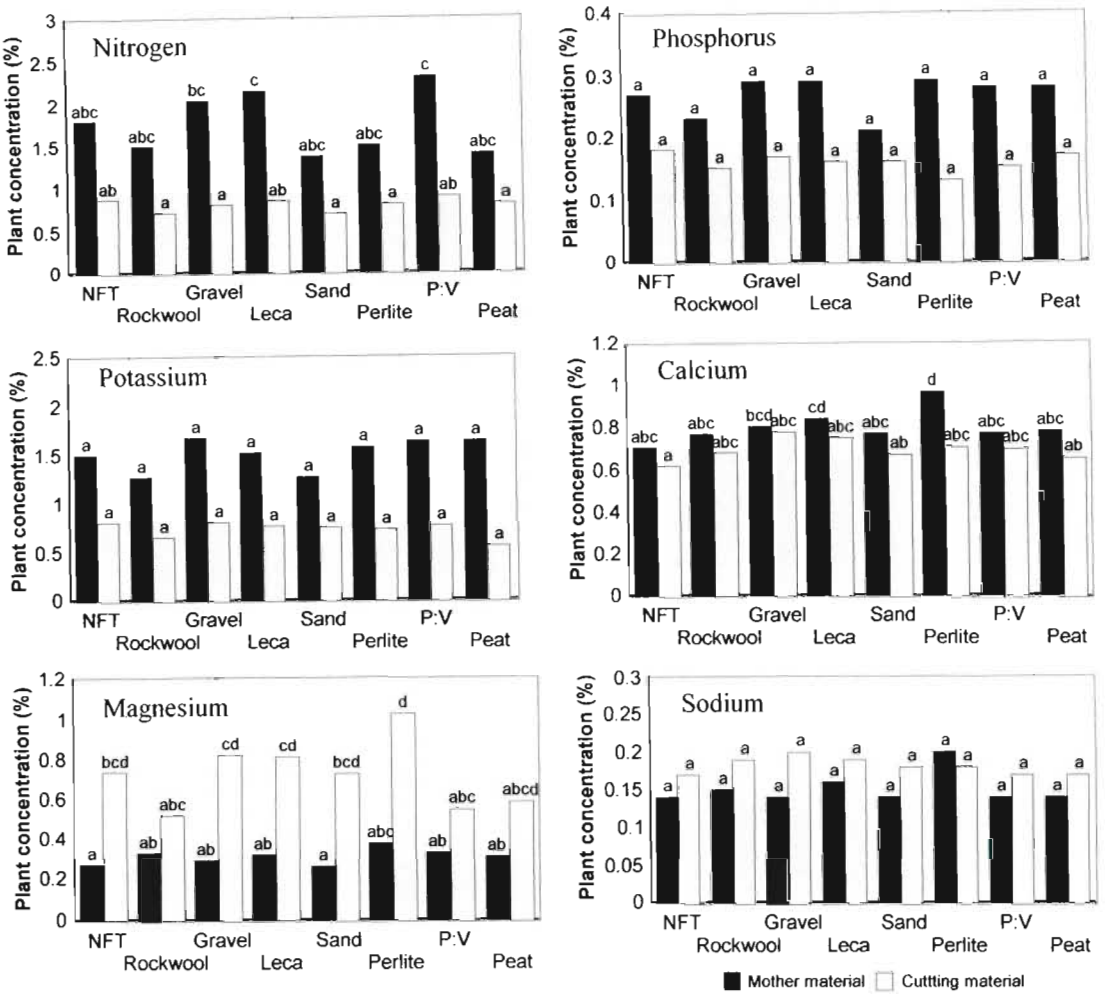

Figure 24: **Macroelement concentrations of mother material at harvest 3 and the cutting material from these plants subsequent to rooting (cutting material) ($p < 0.05$)**

Of the microelements, only Cu, Zn and B concentrations showed significant differences between mother and cutting material for substrate type. It is again emphasized that, the substrate results must be treated with caution due to the experimental design of the study. However, Fe, Zn, Mn and B concentrations showed significant differences for material and for the substrate by material interaction, Cu, Zn and B concentrations were significant (Table 23).

The Fe values (Figure 25) for cutting material were unrealistically high. An error in analysis is suspected, and therefore the data should be disregarded.

Plant Cu decreased significantly in plants grown in gravel and P:V while there was no significant change in those grown in NFT, leca, perlite and peat (Figure 25). Plant Cu levels increased significantly in coppice from plants in Rockwool® and sand. Plant Zn

levels decreased significantly with rooting in plants maintained in all substrate types except Rockwool® (Figure 25). Plant Mn and B both decreased significantly from mother material to cutting material in mother plants in all substrate types (Figure 25).

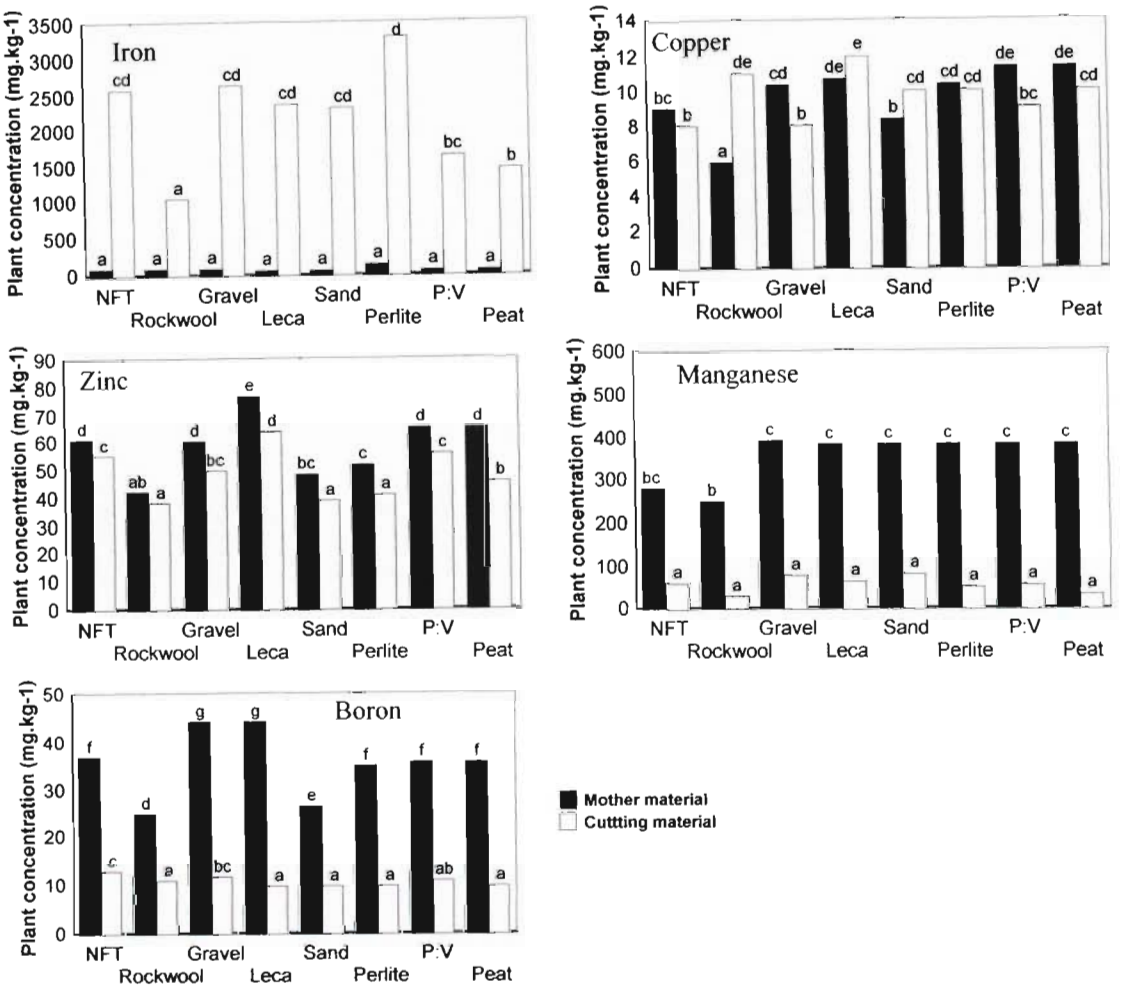

Figure 25: **Microelement concentrations of mother material at harvest 3 and the cutting material from these plants subsequent to rooting (cutting material) ($p < 0.05$)**

Many elements, such as N, P, Ca, Mg, Mn, B and Zn, are thought to be active in root initiation (Anderson, 1986; Blazich, 1988). Of these, Zn, Mn and B are known to influence the metabolic status of indole-3-acetic acid (IAA) (Jarvis, Ali & Hunt, 1984; Salami & Kenefick, 1970; Takaki & Kushizaki, 1970; Thomaszewski & Thimann, 1966). Zinc also promotes the formation of the auxin precursor, tryptophan and the formation of auxin from tryptophan (Salami & Kenefick, 1970; Takaki & Kushizaki, 1970). Conversly, Mn acts as an activator of the IAA-oxidase enzyme system (Thomaszewski & Thimann, 1966) and B enhances IAA-oxidase activity (Jarvis et al., 1984). Mengel & Kirby (1982) reported that the Fe status of plants would be regulated

partially by auxins. Despite the importance of these micronutrients, their role in adventitious root formation has received little attention (Hartmann *et al.*, 1990; Jarvis *et al.*, 1983; Reuveni & Raviv, 1980).

Table 23: **Summary of analyses of variance for the plant analyses: mother material vs. cutting material** (data from figures 24 & 25)

	Substrate	**Material**	**Substrate x material interaction**
N (%)	$p = 0.810$	$p < 0.001$	$p = 0.942$
P	$p = 0.989$	$p < 0.001$	$p = 0.988$
K	$p = 0.998$	$p < 0.001$	$p = 0.998$
Ca	$p = 0.099$	$p < 0.001$	$p = 0.675$
Mg	$p = 0.675$	$p < 0.001$	$p = 0.793$
Na	$p = 0.998$	$p = 0.307$	$p = 0.999$
Fe ($mg.kg^{-1}$)	$p = 0.161$	$p < 0.001$	$p = 0.196$
Cu	$p < 0.001$	$p = 0.772$	$p < 0.001$
Zn	$p < 0.001$	$p < 0.001$	$p = 0.002$
Mn	$p = 0.281$	$p < 0.001$	$p = 0.623$
B	$p < 0.001$	$p < 0.001$	$p < 0.001$

Svenson & Davies, Jr (1995) recorded the variation in tissue elemental concentration in apical stem cuttings of two poinsettia cultivars during the initiation and development of adventitious roots over a 23-day period. Analyses were conducted on day 1 (cuttings placed), day 13 (root primordia were viewed microscopically) and on day 23 (cuttings rooted). After day 13 cuttings were irrigated daily with a nutrient solution which contained no microelements. After 23 days, Svenson & Davies, Jr (1995) found that the concentration of N, P and K all decreased over time while Ca appeared to decline with initiation only. Conversely, they found that Mg increased with rooting (Note: Ca and Mg were supplied in nutrient solution). Although no nutrient solution or fertilisers were available to the *Eucalyptus* cuttings in this trial, after 75 days, the response in the macroelements was similar to that of Svenson & Davies, Jr (1995). As Svenson & Davies, Jr (1995) explain this decline in macronutrients probably resulted from a concentration dilution caused by an increase in plant mass.

Svenson & Davies, Jr (1995) found that for both Cu and Zn there was an increase in concentration in the basal portion of the poinsettia stem throughout the rooting process. As there were no microelements available in the nutrient solution, they must have

moved from one less physiologically active area in the poinsettia plant to this area of high physiological activity. Svenson & Davies, Jr (1995) explained that an increase in dry mass concurrent with an increase in elemental concentration for a particular section of a cutting provided evidence for the redistribution of mineral elements within the cutting. For the *Eucalyptus* cuttings in this trial, Cu concentration was affected significantly by substrate type while material, substrate and their interaction affected plant Zn. For plant Cu, there were different results depending on the substrate type that the plants were grown in. From this, one would suspect that the substrate in which the plants were grown influenced the uptake of Cu by the mother plant and the change in Cu concentration in the *Eucalyptus* cutting was influenced by the original concentration within the cutting.

In the *Eucalyptus* cuttings, plant Zn decreased in plants in all substrate types, as did Mn and B levels (Figure 25). This was probably due to the 'dilution' effect discussed earlier. Svenson & Davies, Jr (1995) suggested that as there was no difference in the conductivity of the nutrient solution applied and the leachate (from the bottom of the rooting tray) that the substrate did not contribute to any significant increase in element concentrations of the poinsettia cuttings. Svenson & Davies, Jr (1995) suggested that if root initiation were related to the relative activity of IAA and IAA-oxidase, then rooting should be correlated with the relative Zn, Mn and B concentrations at the site of root initiation. These correlations will be discussed later in this chapter (Section 4.5).

In summary:

N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn	B
↓	↓	↓	↓	↑	↑	^	*	↓	↓	↓
✓	X	X	✓	✓	X	^	*	✓	✓	✓

↑- increase over time; ↓ - decrease over time; ✓ - significant; X – not significant;
^ Fe result not used; * Cu result varies for each substrate and cannot be generalised.

As Marien (1991), Wignall *et al.*, (1991), Welander (1994) and Williams (2000) proposed, the rooting process was a complicated one and trying to narrow down the key elements to hasten or improve rooting was a difficult task. Many of the results in the trial reported here concur with the findings of Svenson & Davies, Jr (1995) particularly those for the macroelements. This leads to a suggestion that the results for

macroelements would be similar for any species but the microelements would behave slightly differently because of the small quantity of each micronutrient available within the plant and the number of intricate functions these micronutrients serve.

4.1.4 Nutrient solution analyses

Nutrient solution samples from the NFT tanks were analysed to determine differences in the nutrient concentration of the nutrient solution. To do this, samples were taken immediately after the tanks were cleaned and recharged with fertilisers and again 14 days later, just before the tanks were cleaned and recharged. These results were then compared with a two-way ANOVA.

Throughout the trials two tanks fed eight gutters, each containing a different substrate. Tank A fed the gravel, sand, NFT and Rockwool® substrates and tank B fed the perlite, P:V, peat and leca substrates. The different properties (Table 8) of these substrates caused the start and end concentration of nutrients in the tanks to be slightly different. The substrates may have precipitated or absorbed some of the nutrients out of the solution affecting the availability of nutrients to the plants maintained within the substrate. Samples taken on day one were collected after recirculation to ensure the fertilisers and water were thoroughly mixed.

Tank, time and their interaction had no significant effect on the pH of the nutrient solution (Table 24). The pH decreased slightly between samples but not significantly (Figure 26). Tank and time both had a significant effect on the EC of the nutrient solution but their interaction did not (Table 24). The EC in tank A decreased more than in tank B (Figure 26), which meant that more nutrients were being taken up by plants fed from tank A than those from tank B. This was also apparent in separate elemental analysis (see later) and may have been due to the properties of the different substrates that each tank supplied. The TDS (total dissolved salts) of the solution did not differ between tanks nor was the interaction significant, but the effect of time on TDS was (Table 24). The TDS of a solution was a similar measurement to EC and in this case showed the same decline as the EC results (Figure 26).

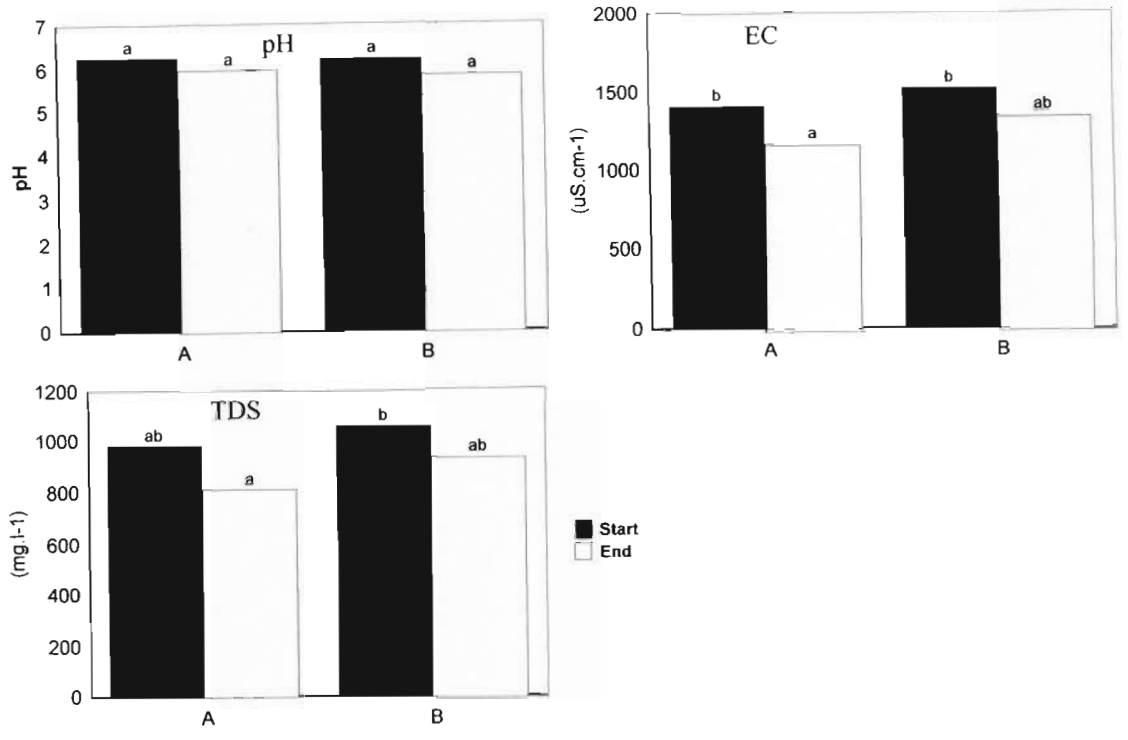

Figure 26: **Change in pH, EC and TDS of nutrient solution in the two NFT tanks (A and B) after a 14 day cycle ($p < 0.05$)**

Time and the tank by time interaction significantly affected the solution P concentrations (Table 24). Figure 27 shows that there was no significant change in P in tank A but that in tank B showed a significant and considerable decrease in P concentration over time. There was also a significant difference between the tanks to start and end with. This difference could have been due to the different substrate types that each tank fed.

For K levels, the effect of both tank number and time were significant but not the interaction between them (Table 24). From Figure 27 one can see there was a significant decrease in concentration over time. As with P there was a significant difference between the nutrient solution of the tanks at both the beginning and end of the time period.

The Mg concentration decreased over time (Figure 27); in tank A this was significant but in tank B it was not. Again this was probably due to the different substrate types that each tank fed and one would assume that the plants in substrates fed by tank A took up more Mg.

Sodium levels decreased significantly between samples in both tanks. Calcium and Cl concentration both increased but the change in Ca was not significant.

With respect to the microelements in the solution, B and Cu concentrations were significantly different between the tanks, while time had a significant effect on Fe levels only. The interaction was not significant for any microelements (Table 24).

Iron levels decreased significantly between samples in both tanks but more so in tank A than in tank B. The Cu concentration decreased in tank A and increased in tank B (Figure 28). Zinc and Mn concentrations increased between samples in both tanks but not significantly (Figure 28). Boron levels decreased between samples in both tanks but the original solutions contained different B concentrations (Figure 28).

Silberbush & Ben-Asher (2001) found that the depletion of different nutrients is in accordance to their initial concentration and plant uptake rate.

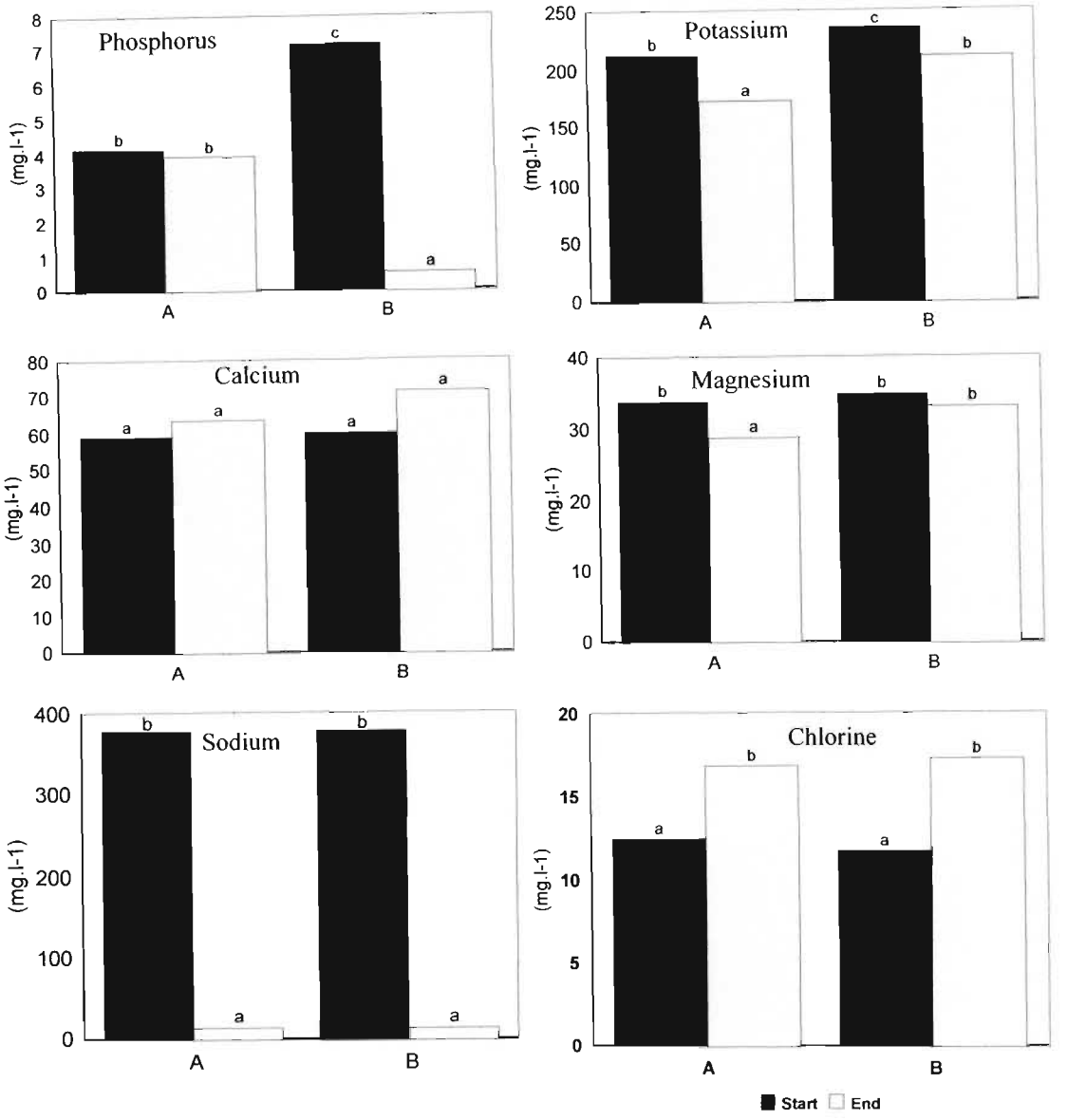

Figure 27: **Change in macroelement (mg.l^{-1}) concentration of nutrient solution in two NFT tanks (A and B) after a 14 day cycle ($p < 0.05$)**

As illustrated earlier (Figure 8) the pH of a nutrient solution affected the availability of all the elements within the solution and Higashi *et al.* (2002) recommended a pH of 5.5 – 6.0, based on their work on *Eucalyptus* species in hydroponics. The pH that was recorded for the nutrient solution in the present study was slightly above this recommended range but it was doubtful if this made any difference to the availability of the nutrients.

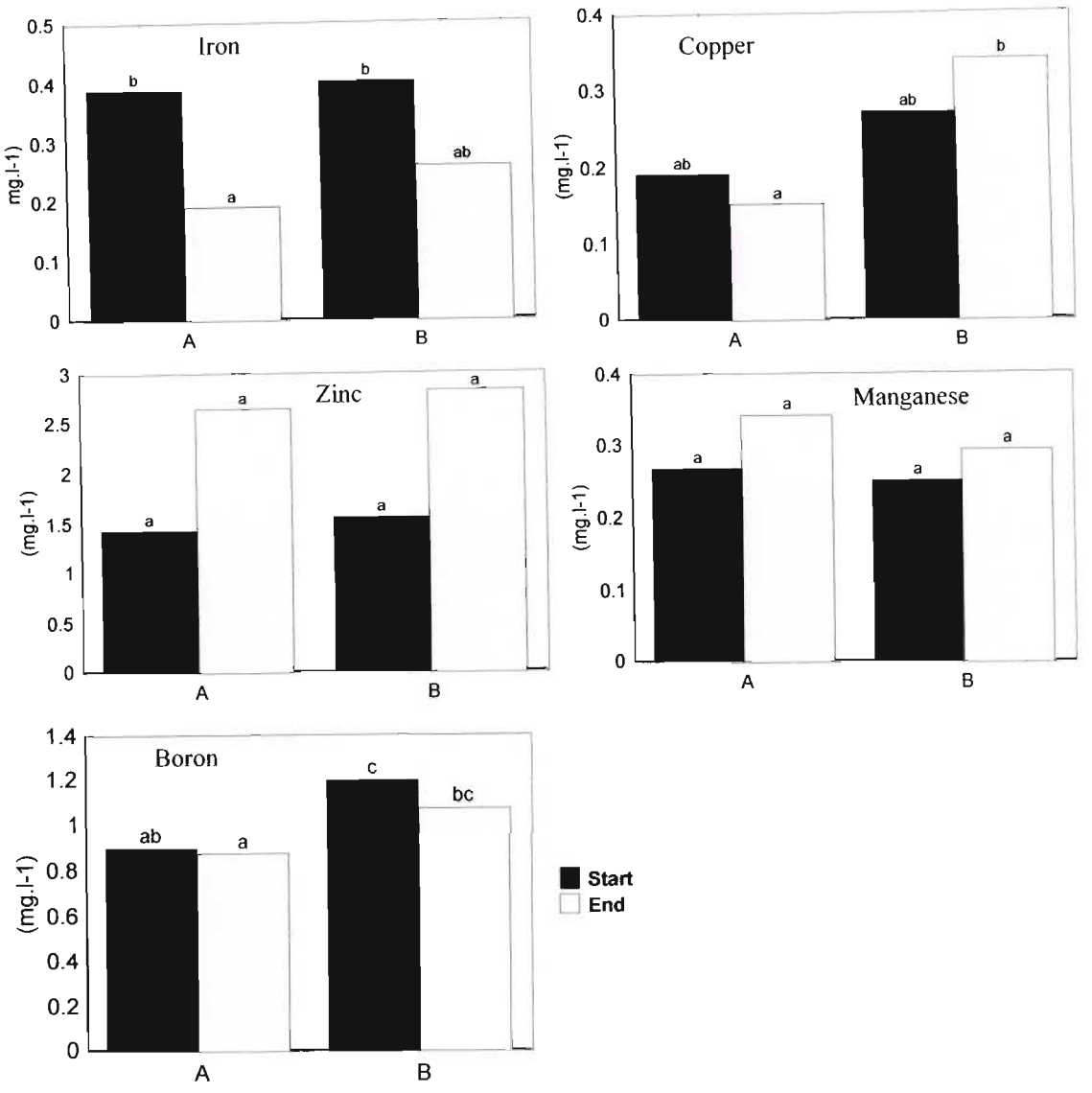

Figure 28: **Change in microelement (mg.l^{-1}) concentrations of nutrient solution in two NFT tanks (A and B) after a 14 day cycle ($p < 0.05$)**

Silveira *et al.* (1999) recommended an EC range of 1250 – 2300 μS.cm^{-1} for hydroponic *Eucalyptus* production and also recommended that once the EC dropped to 1000 μS.cm^{-1} the solution should be replenished. The EC for this nutrient solution was recorded between 1000 – 1500 μS.cm^{-1}. Over time the EC decreased (Figure 26) and as Harris (1997) suggested, this indicates that the plants were taking nutrients up. This appeared to be more so in tank A than tank B (Figure 26). Total dissolved salts (TDS) was a measurement similar to EC and therefore shows the same decline over time.

Table 24: **Summary of analyses of variance for nutrient analyses measured at the start and end of a nutrient supply cycle**

	Tank	Time	Tank x time
pH	$p = 0.887$	$p = 0.567$	$p = 0.967$
EC	$p = 0.047$	$p = 0.006$	$p = 0.545$
TDS	$p = 0.139$	$p = 0.032$	$p = 0.670$
P ($mg.l^{-1}$)	$p = 0.772$	$p < 0.001$	$p < 0.001$
K	$p < 0.001$	$p < 0.001$	$p = 0.230$
Ca	$p = 0.483$	$p = 0.211$	$p = 0.567$
Mg	$p = 0.002$	$p < 0.001$	$p = 0.019$
Na	$p = 0.933$	$p < 0.001$	$p = 0.933$
Cl	$p = 0.802$	$p < 0.001$	$p = 0.369$
Fe	$p = 0.508$	$p = 0.019$	$p = 0.617$
Cu	$p = 0.048$	$p = 0.802$	$p = 0.369$
Mn	$p = 0.583$	$p = 0.352$	$p = 0.789$
Zn	$p = 0.782$	$p = 0.063$	$p = 0.967$
B	$p = 0.002$	$p = 0.248$	$p = 0.421$

As mentioned earlier, the substrate properties may have played a role in altering the availability of certain nutrients in the solution and this appeared particularly to be the case with P and Cu concentrations. Blom (1999) recorded differences in uptake and availability that was caused by the properties of coir and Rockwool®. Blom (1999) found that solution analysis indicated that phosphate (especially in the Rockwool® substrate) and microelement concentrations decreased while Ca, Mg and chloride increased with recirculation of both substrates. This was primarily due to the addition of fresh water (contained Ca, Mg and chloride) to the tanks to reduce the increasing EC levels of the solution over time. The average K concentration did not change significantly. There were higher levels of microelements in the rose stems grown in coir than those grown in Rockwool®. This was probably due to the precipitation and / or absorption by the substrates and the indirect effect of the lower pH in the coir compared to that in the Rockwool® influencing the availability.

Premuzic, Bargiela, Garcia, Rendina & Iorio (1998) observed different effects upon levels of Ca, Fe, K, P of two organic (vermicompost and vermicompost-soil mix) and two inorganic (sand and peat-perlite mix) substrates. Those effects were also evident in the tomato fruit grown in each substrate type. Those authors also observed that a higher Ca level in the organic media was associated with reduced levels of other cations such as Na, Mg and K.

In a report, Adams (1999) explained that many factors influenced the rate of uptake and the distribution of nutrients within a plant. He explained that the nutrient solution should be specifically formulated for the crop, frequency of irrigation, substrate type, rooting volume and plant growth. That author divided the substrates up into two categories, organic and inorganic. He explained that it was not advisable to feed both types of substrates with an identical nutrient solution. Organic materials usually required extra N as they immobilised considerable amounts of N. This could in turn affect other nutrient such as Ca which in turn would affect the availability of others resulting in a snowball effect.

The Hydroponica® – Agrisol®: plant calcium combination of fertilisers used throughout these trials was formulated specifically for hydroponics but is a general fertiliser. This means that it does not take into account any special formulations propagators may require to optimise the cutting production of *Eucalyptus* species. While it was adequate for the growth of the *Eucalyptus* hedge plants there may be room for improvement for cuttings production by fine-tuning the individual elemental requirements and ratios particularly for microelements such as Zn, B and Mn which are involved in auxin metabolism and its actions. As a result one may find that rooting initiation may not only be hastened but that root quality may be improved and rooting percentages could be increased.

4.2 Ebb-and-flow technique

Similarly to the NFT system, cuttings of the same three cold-tolerant clones were planted in the ebb-and-flow system, as hedge plants. From these plants, coppice shoots was collected and these cuttings placed in the greenhouse to be rooted, as described in Section 3.2. The number of cuttings placed from each clone and the number of cuttings that rooted in the greenhouse was recorded.

The following questions were posed:

(a) Were there any differences among clones?

(b) Were there any differences between the ebb-and-flow and NFT systems? (Section 4.4)

The clone type had a significant effect on the total number of cuttings placed ($p < 0.001$) (Figure 29) and the total number rooted ($p < 0.001$) (Figure 29). Clone NH00 produced significantly higher number of cuttings to be placed and the number rooted (Figure 29). Clone GN156 produced significantly more cuttings to be placed than clone GN107 but in terms of the number rooted there were no significant differences for clones GN107 and GN156 (Figure 29).

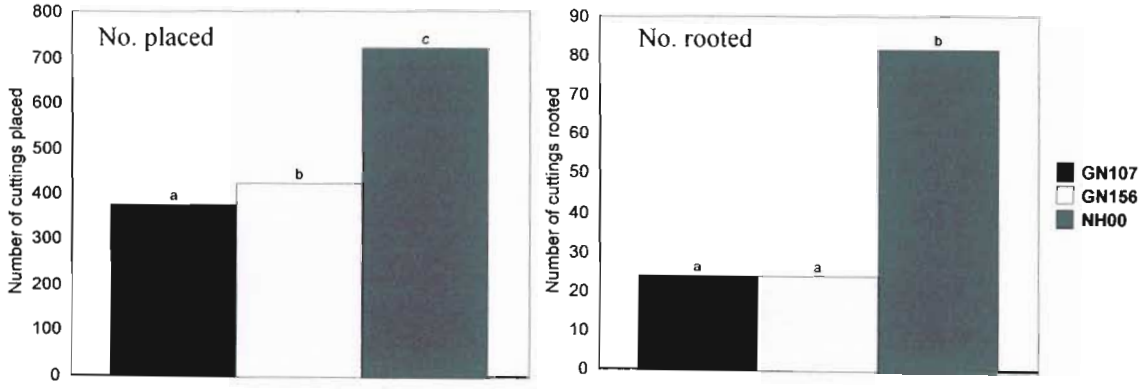

Figure 29: **Total number of cuttings placed and rooted from hedge plants grown in the ebb-and-flow system ($p < 0.05$)**

The clone type had a significant effect on the percentage of cuttings that rooted from hedges in the ebb-and-flow system ($p < 0.001$) (Figure 30). Clone NH00 had the highest rooting percentage followed by clones GN107 and GN156, respectively (Figure 30).

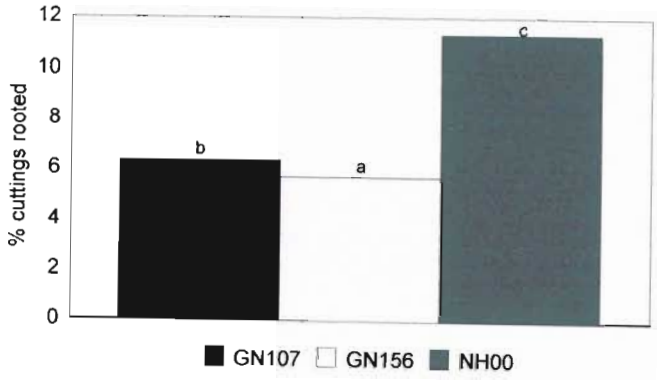

Figure 30: **Percentage of cuttings rooted from parent plants grown in the ebb-and-flow system ($p < 0.05$)**

No significant differences could be attributed to substrate type in plants from the ebb-and-flow system as all hedge plants resided in the same substrate (three parts perlite,

one part coir and six parts vermiculite) unlike in the NFT system (Figure 19, 20 & 21). Therefore, one could only attribute the differences in rooting to clonal differences. This concurred with what da Costa Alpoim (2002) found in his trials and with what Bonner (2003) and de Haas (2003) have experienced in commercial nurseries.

4.3 Aeroponics

Similarly to the NFT and ebb-and-flow systems, cuttings of the same three cold tolerant clones were planted in the aeroponic system, as hedge plants. From these planting material (coppice shoots) was also collected and cuttings placed in the greenhouse to be rooted as described in Section 3.2. A record was kept of the number of cuttings placed from each clone and the number of cuttings that rooted in the greenhouse.

The following questions were posed:
(a) Were there any differences among clones?
(b) Were there any differences between the aeroponic and NFT systems? (Section 4.4)

The clone type had a significant effect on the total number of cuttings placed ($p < 0.001$) (Figure 31) and the total number rooted ($p < 0.001$) (Figure 31). For both number placed and number rooted, clone NH00 had the highest followed by clones GN156 and GN107, respectively (Figure 31).

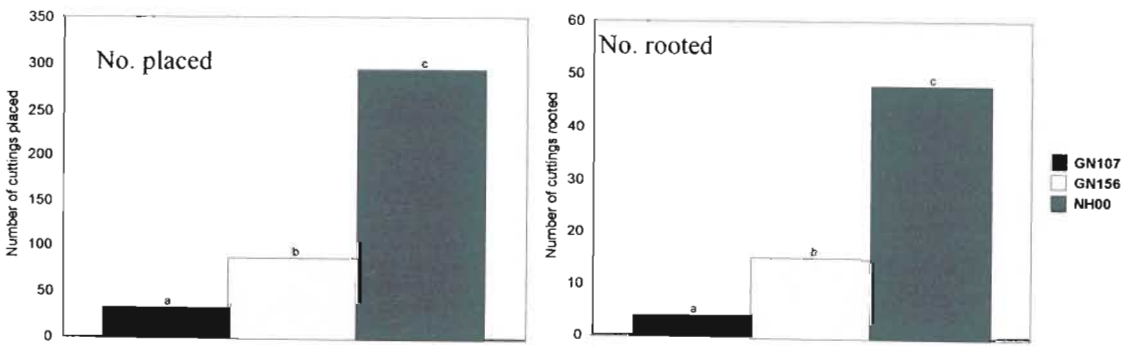

Figure 31: **Total number of cuttings placed and rooted from hedge plants grown in the aeroponic system ($p < 0.05$)**

For the aeroponics system, the percentage rooted was also significantly affected by clone ($p = 0.02$). Cuttings from clone GN156 had a significantly higher rooting

percentage (Figure 32). This was followed by clone NH00 which had a significantly higher rooting percentage than clone GN107 (Figure 32).

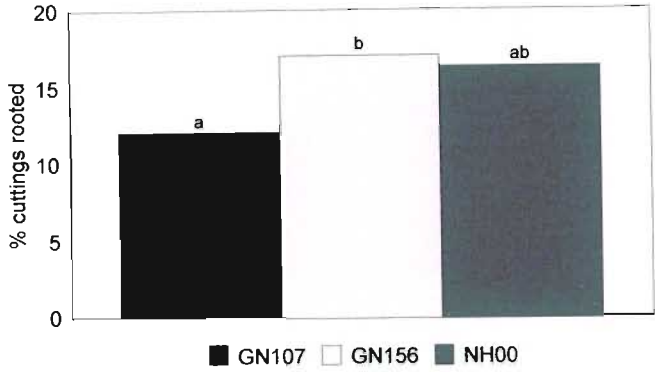

Figure 32: **Percentage of cuttings rooted from plants grown in the aeroponics system ($p < 0.05$)**

As mentioned in the Section 4.2, there was no substrate to attribute the differences in rooting percentages to and therefore these differences could have been due to clonal variation. Wilson (1998b) found that the biggest contributor to differences in rooting was the genotypic effect and not so much the environmental effects. He explained that the ease of propagation, in practice, would depend on efficient clonal selections especially for rooting potential and a good understanding of the management of the environment to favour high rooting potentials.

4.4 Comparison of the NFT, ebb-and-flow and aeroponics systems

In section 3.6, the flaws of the experimental design were explained. In this section, the results from the three systems were compared in an attempt to determine which system is best for maintaining hedge plants for cold tolerant *Eucalyptus* vegetative propagation. An ANOVA was conducted on the results obtained from the three trials. This was not the appropriate statistical test but gave results which were simple and easy to understand and this must be kept in mind when interpreting the results from the statistical analysis.

4.4.1 Rooting results

The number of cuttings placed and rooting data from harvest 2 for the three hydroponic systems was recorded and compared. For the NFT system all data from the eight substrates were pooled together to give a single value for the system.

Comparisons between a pot and a trough system by da Costa Alpoim (2002) indicated that there were no significant differences between systems for cuttings yield and rooting results. The decision by that author to continue with the trough system instead of the pot system was made for practical reasons.

There was a significant effect on both the number of cuttings placed and the number rooted due to clone, system and their interaction (Table 25).

Table 25: **Summary on analyses of variance for the three systems**

	Clone	System	Clone by system
No. placed	$p < 0.001$	$p < 0.001$	$p < 0.001$
No. rooted	$p < 0.001$	$p < 0.001$	$p < 0.001$

The plants in the NFT system produced significantly more cuttings to be placed for all clones and had significantly better rooting for all three clones (Figure 33). The plants in the ebb-and-flow system produced significantly higher numbers of cuttings to be placed for all three clones compared to those from the aeroponics system. In all three systems, clone NH00 produced significantly more cuttings to be placed than clone GN156 and clone GN156 produced significantly more cuttings to be placed than clone GN107. Clone GN156 and NH00 had no significant differences between the ebb-and-flow and aeroponics systems for the number of cuttings rooted but clone GN107 was significantly higher in the ebb-and-flow system. In the NFT system: NH00 > GN156 > GN107.

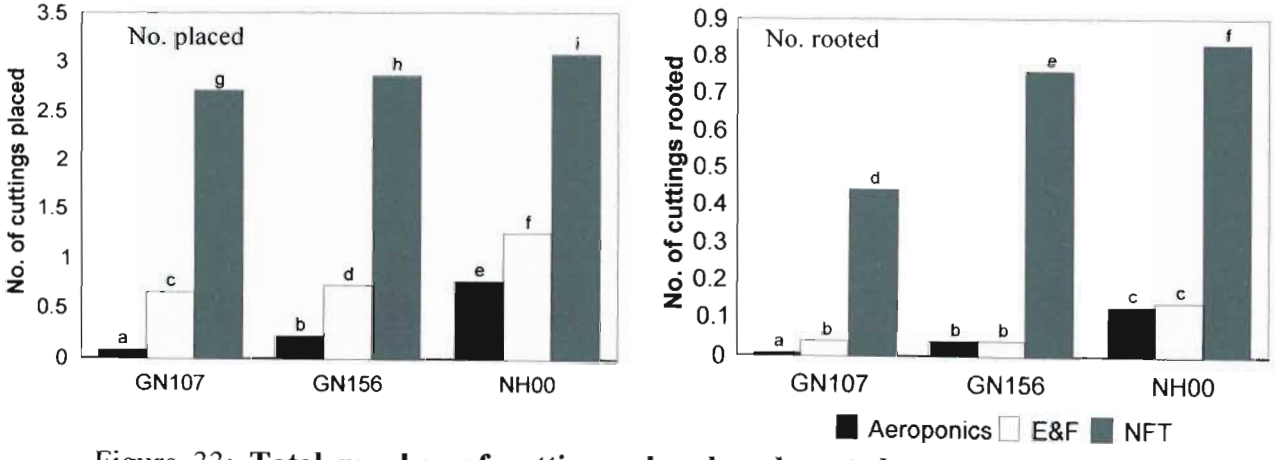

Figure 33: **Total number of cuttings placed and rooted per mother plant from hedge plants grown in the three hydroponic systems (NFT, ebb-and-flow, aeroponic) ($p < 0.05$)**

Clone ($p < 0.001$), system ($p < 0.001$) and clone by system interaction ($p < 0.001$) were all significant for rooting percentages. Data in Figure 34 show that cuttings from plants in the NFT system produced the highest rooting percentages for all clones, followed by cuttings from plants in the aeroponics and ebb-and-flow systems, respectively. Clone NH00 had the highest rooting percentage for plants grown in all three systems. In the aeroponics and NFT systems, GN156 plants had a significantly higher rooting percentage than GN107 plants but in the ebb-and-flow system GN107 plants had significantly higher rooting percentages than GN156 plants.

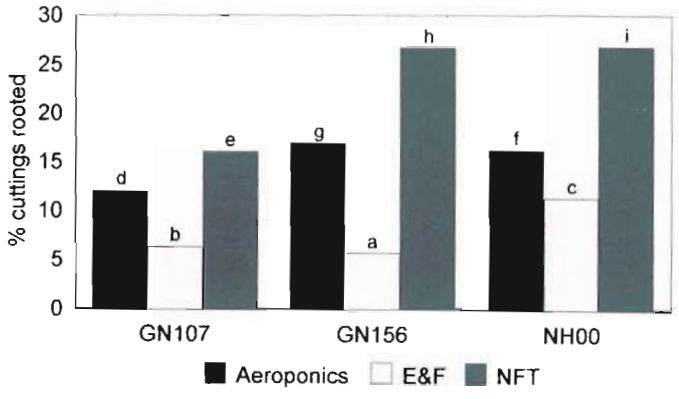

Figure 34: **Percentage of cuttings rooted per mother plant from coppice from plants in the three hydroponic systems (NFT, ebb-and-flow and aeroponics) ($p < 0.05$)**

Rodriguez-Delfin, Chang & Hoyos (2001) found with studies on different cultivars of lettuce, that the yield of a modified DFT (deep flow technique) system was similar to that of a floating raft system. They determined that certain cultivars were better suited to one system than the other.

da Costa Alpoim (2002) found that there was no significant difference between the two systems that he used in his trial but there were distinct differences which correlate more with the findings by Rodriguez-Delfin *et al.* (2001). In this case, all three clones were most successful in the NFT system. Each system has particular advantages and disadvantages, as mentioned earlier and it is imperative that the practicality, reliability and cost of each system be taken into account when considering a commercial installation. This is discussed later in Chapter 5.

4.4.2 Plant analyses: comparison of systems

A plant analysis was conducted on plants in each of the systems and the results compared. The only difference between these treatments was the hydroponic system itself; the same three clones were used and the same nutrient solution was used with identical pH and EC. For comparisons among systems, the plant nutrient content of the mother material was averaged over the eight substrates for the NFT at harvest 3.

For all macroelements, the clone and the clone by system interaction was not significant. System type was significant for all macroelements except K levels (Table 26). For all the macroelements, a pattern was evident for all clones within each element (Figure 35). Plants in the aeroponic system had the highest N and P levels (Figure 35). Plant K levels were highest, although not significantly, in plants grown in the ebb-and-flow system while Ca and Mg concentrations were the highest in plants grown in the NFT system (Figure 35). For N, K and Mg concentrations, there were no significant differences between clones in the aeroponic system (Figure 35).

For clone GN107, the P concentration of plants in the three systems were not significant (Figure 35). For clone NH00 and GN156, the P concentration from the plants in the aeroponics system was significantly higher than those in the other two systems (Figure 35).

Plants in the NFT system had significantly higher Ca concentrations compared to plants grown in the other two systems (Figure 35). Clones GN107 and GN156 had a significantly higher concentration than clone NH00 in the NFT system (Figure 35).

With regard to the microelements, clone type and the interaction (clone by system) had a significant effect on Cu, Mn and B concentrations. The system type had a significant effect on all microelements (Table 26).

Plant Fe was highest in plants in the aeroponics system except for clone GN107 where mother plants in the NFT were higher although not significantly (Figure 36). The lowest Fe levels were found (in all clones) in the ebb-and-flow system. For clones GN107 and

NH00, plants in the NFT system showed significantly higher levels of Fe compared to plants in the ebb-and-flow system.

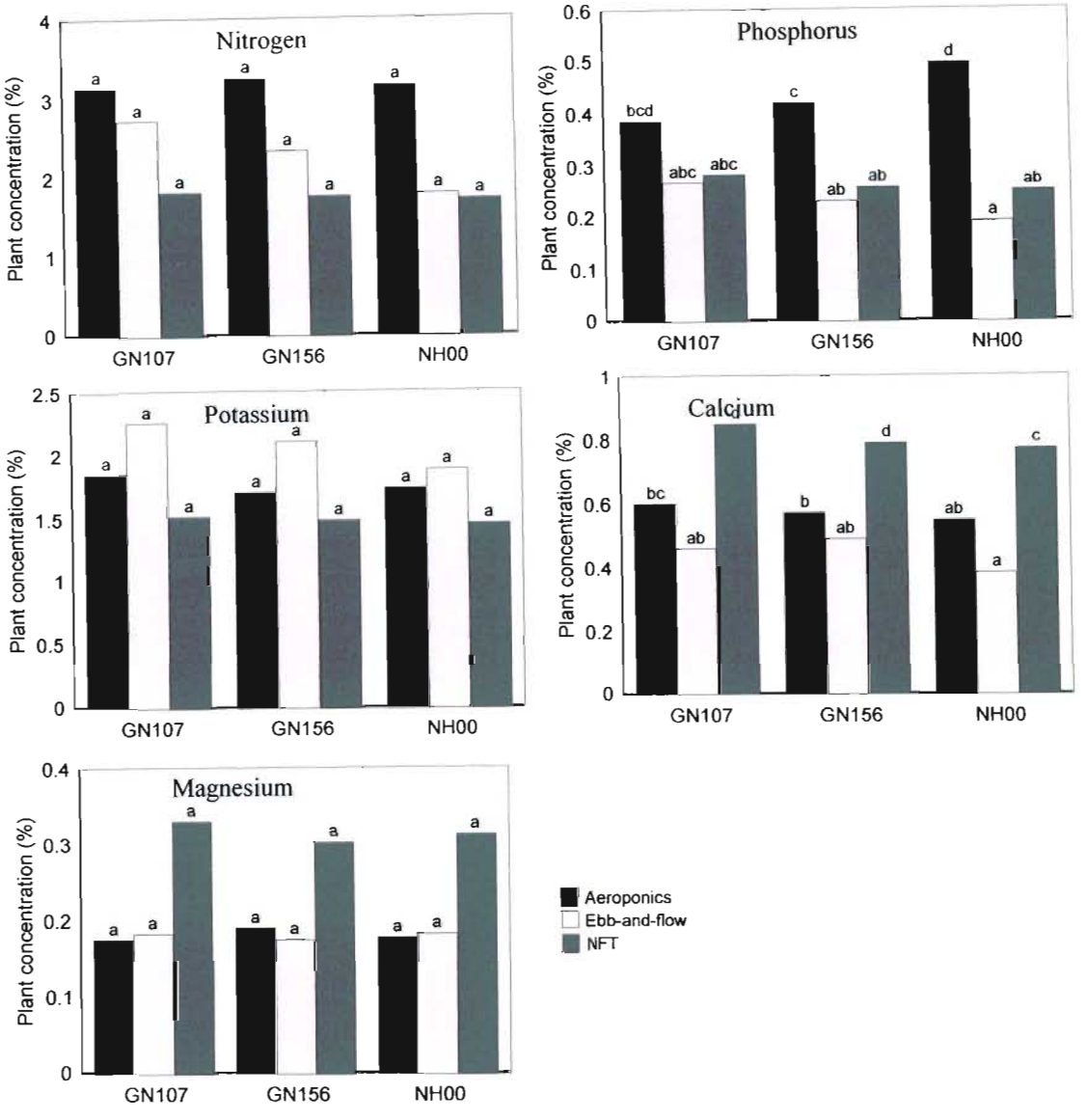

Figure 35: **Concentration of macroelements (%) in three clones (GN107, GN156 and NH00) maintained in three hydroponic systems (NFT, ebb-and-flow, aeroponics) ($p < 0.05$)**

For plant Cu, there was no particular system that produced the highest concentrations in all clones (Figure 36). For clones GN107 and NH00, plants in the aeroponic system had the highest Cu concentration followed by those in the NFT and ebb-and-flow systems, respectively (Figure 36). For clone GN156, plants in the ebb-and-flow system had the highest Cu concentration followed by plants in the aeroponics and NFT systems, respectively (Figure 36). Only in the NFT system were concentrations similar among the clones (Figure 36). For plants in the aeroponics system, clone GN107 had a

significantly higher Cu concentration than clone GN156, which was slightly higher than NH00, although not significantly (Figure 36). For the plants in the ebb-and-flow system, clone GN156 had the highest Cu concentration with clones GN107 and NH00 being significantly lower (Figure 36).

For all clones grown in the aeroponic system Zn concentration were highest (Figure 36). The mother plants in the NFT system gave the next highest concentrations, which were significantly higher than those in the ebb-and-flow system.

For Mn concentrations, NFT plants had significantly higher result than the other two systems followed by plants kept in the aeroponics, which were significantly higher than those in the ebb-and-flow system (Figure 36).

Boron concentrations also had a trend across the different systems with plants in NFT giving significantly higher values across all clones (Figure 36). For GN107 mother plants, the other two systems are not significantly different but for GN156 and NH00 mother plants, aeroponics was significantly higher than the ebb-and-flow results.

One could see there was a distinct pattern for all three clones with regards to the response to nutrients delivered via each system except for Cu concentration. This indicated that not only was the method of delivering the nutrient solution different for each system but the availability of the individual nutrients was affected by this and the presence or absence of substrates may have also influenced the nutrients' availability. As mentioned earlier, oxygen levels in the nutrient solution were not measured but this would have influenced the nutrient availability and the forms in which nutrients are taken up. Plants within the different systems produced different root systems (Figure 37, 38 & 39) and this may have influenced the availability of the nutrients.

In the aeroponics system, the root growth was rapid (Figure 37) with many white roots which are actively growing and taking up nutrients. The plants in the ebb-and-flow system produced a mass of roots (Figure 38). Many were white and therefore actively growing. The plants in the NFT system produced a mass of roots (Figure 39) although not as many were white as in the other two systems.

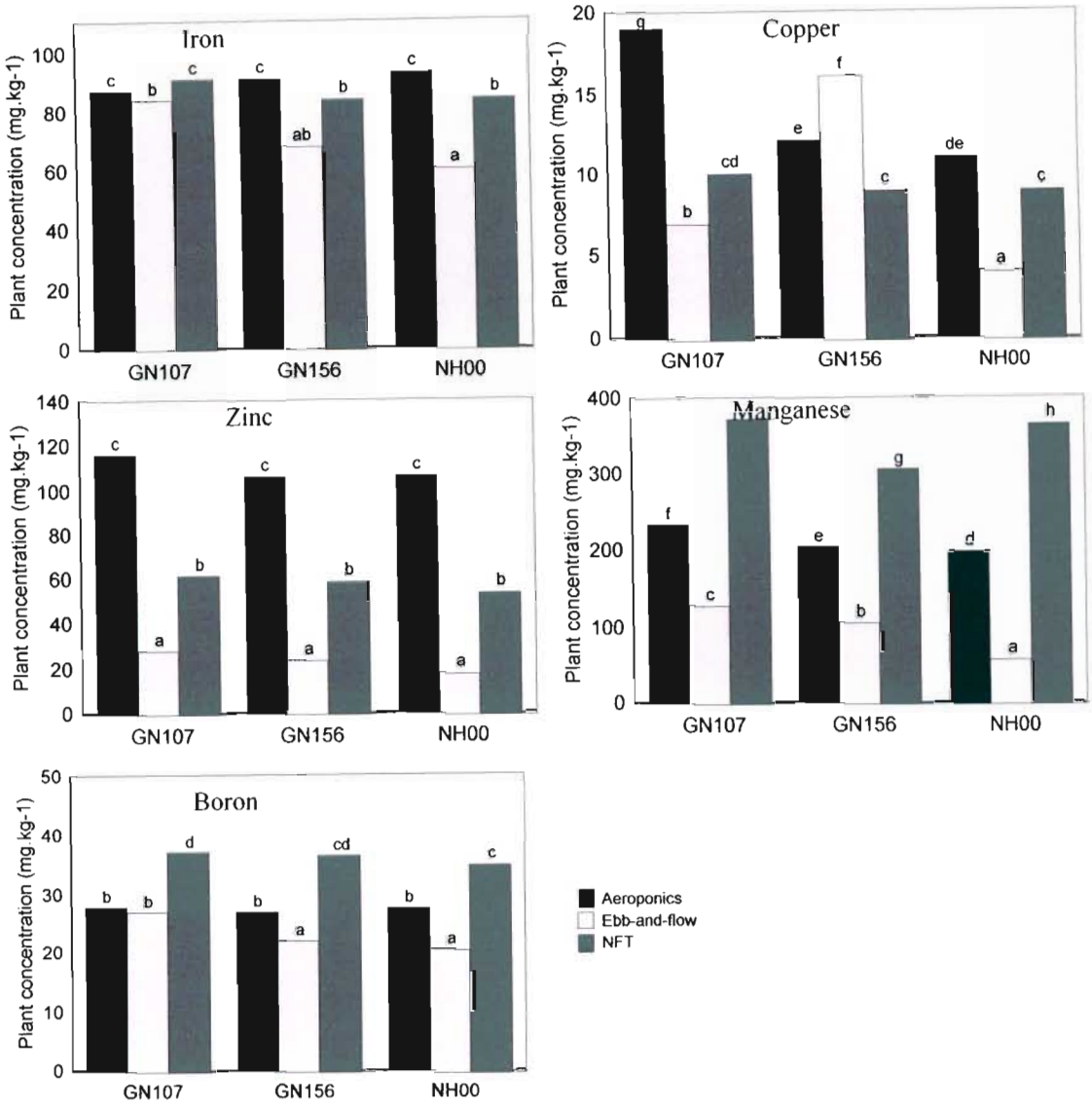

Figure 36: **Concentration of microelements (mg.kg^{-1}) in three clones (GN107, GN156 and NH00) maintained in three hydroponic systems (NFT, ebb-and-flow, aeroponics) ($p < 0.05$)**

Table 26: **Summary of analyses of variance for plant analyses measurements when comparing the three hydroponic systems**

	Clone	System	Clone x system interaction
N (%)	$p = 0.780$	$p = 0.023$	$p = 0.937$
P	$p = 0.975$	$p < 0.001$	$p = 0.591$
K	$p = 0.924$	$p = 0.464$	$p = 0.999$
Ca	$p = 0.333$	$p < 0.001$	$p = 0.903$
Mg	$p = 0.990$	$p = 0.014$	$p = 0.998$
Fe (mg.kg^{-1})	$p = 0.210$	$p = 0.001$	$p = 0.178$
Cu	$p < 0.001$	$p < 0.001$	$p < 0.001$
Zn	$p = 0.187$	$p < 0.001$	$p = 0.959$
Mn	$p < 0.001$	$p < 0.001$	$p < 0.001$
B	$p < 0.001$	$p < 0.001$	$p < 0.001$

Figure 37: **Root growth of mother plants in the aeroponics table at different stages (Five days, two weeks and approximately six months – end of trial)**

From these and previous results, it appeared that Ca concentration played a critical role in the rooting process. It has already been established (Figure 34) that plants from the hedges in the NFT system had the best rooting results. From data in Figure 35, it would appear that these plants required a minimum Ca concentration of 0.7 %. Higashi *et al.*, (2000a) recommended a Ca range of 0.5 – 0.7 %; Carlson *et al.*, (2003) recommended 0.26 – 1.49 % Ca and from Section 4.1.2 it would appear that for GN hybrids a higher level of Ca was required for optimal rooting.

Figure 38: **Root growth from mother plants established in the ebb-and-flow table**

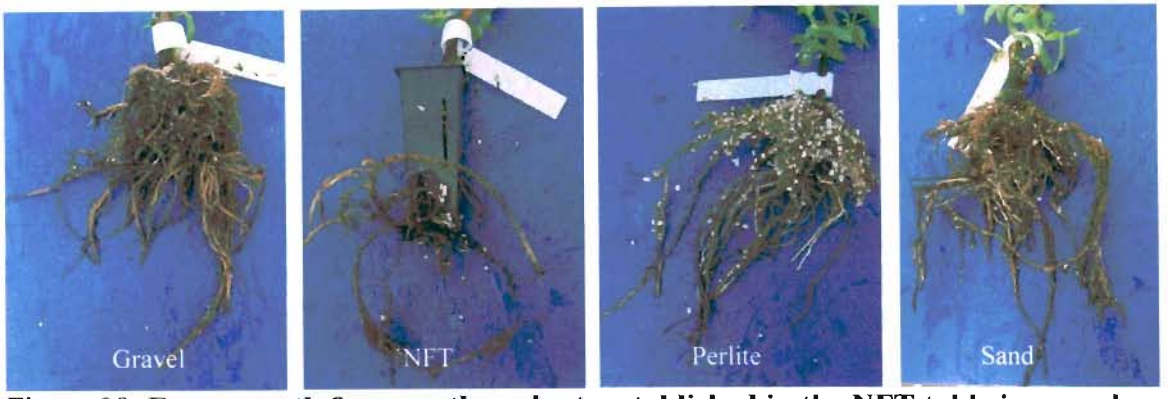

Figure 39: **Root growth from mother plants established in the NFT table in gravel, NFT, perlite and sand substrates**

If Zn, Mn and B were all as important as was previously discussed, then these results and the critical ranges determined by Higashi *et al.* (2000a) concurred. Higashi *et al.* (2000a) reported that the best ranges for the production of micro-cuttings was 30 - 60 $mg.kg^{-1}$ Zn, 250 - 500 $mg.kg^{-1}$ Mn and 35 - 70 $mg.kg^{-1}$ B. In Figure 35, the plants grown in the aeroponics system showed the highest accumulation of Zn but this was above the Higashi *et al.* (2000a) recommended limit. The plants grown in the NFT system all had a Zn level just below 60 $mg.kg^{-1}$ which was within the Highashi *et al.* (2000a) range.

From Figure 36, the plants grown in the NFT system both have levels of Mn and B, respectively, which fall into Higashi *et al.* (2000a) suggested range whereas none of the other plants in the other two systems do.

The NFT system resulted in the highest rooting percentages overall and from these plant analyses it would appear that the concentrations of Ca, Zn, Mn and B were critical.

4.5 Correlation between rooting and concentrations of plant elements

In an effort to verify the findings further in the previous section a series of correlation graphs were produced (Figures 40 & 41). These graphs compared rooting data with the concentration of essential elements within the plants. A statistical correlation was not performed as there was insufficient data.

For all three clones rooting decreases as N and K concentrations in the plants increased (Figure 40). Plant P, Mg and Na concentrations appeared to be fairly constant and therefore did not seem to play any significant role in the rooting process.
Of all the macroelements only Ca concentration changed significantly between plants in systems. In the separate graphs, one can see that as Ca concentration decreased so too did the rooting, in keeping with the suggestion that Ca plays a role in rooting. Rooting was reduced at plant Ca concentrations below 0.7 %.

The relationship between rooting and plant micronutrient concentrations are shown in Figure 41. There was considerable variation in micronutrient content in plants from different systems.

Iron concentrations did not appear to have any effect on rooting performance. If Cu and Zn concentrations were either too high or too low the rooting performance decreased. The critical range for Cu was: 9 – 11 $mg.kg^{-1}$ and for Zn: 50 – 80 $mg.kg^{-1}$. For Mn and B concentrations, as the concentration decreased so too did the rooting. The critical minimum's for Mn were: 250 $mg.kg^{-1}$ and B: 35 $mg.kg^{-1}$.

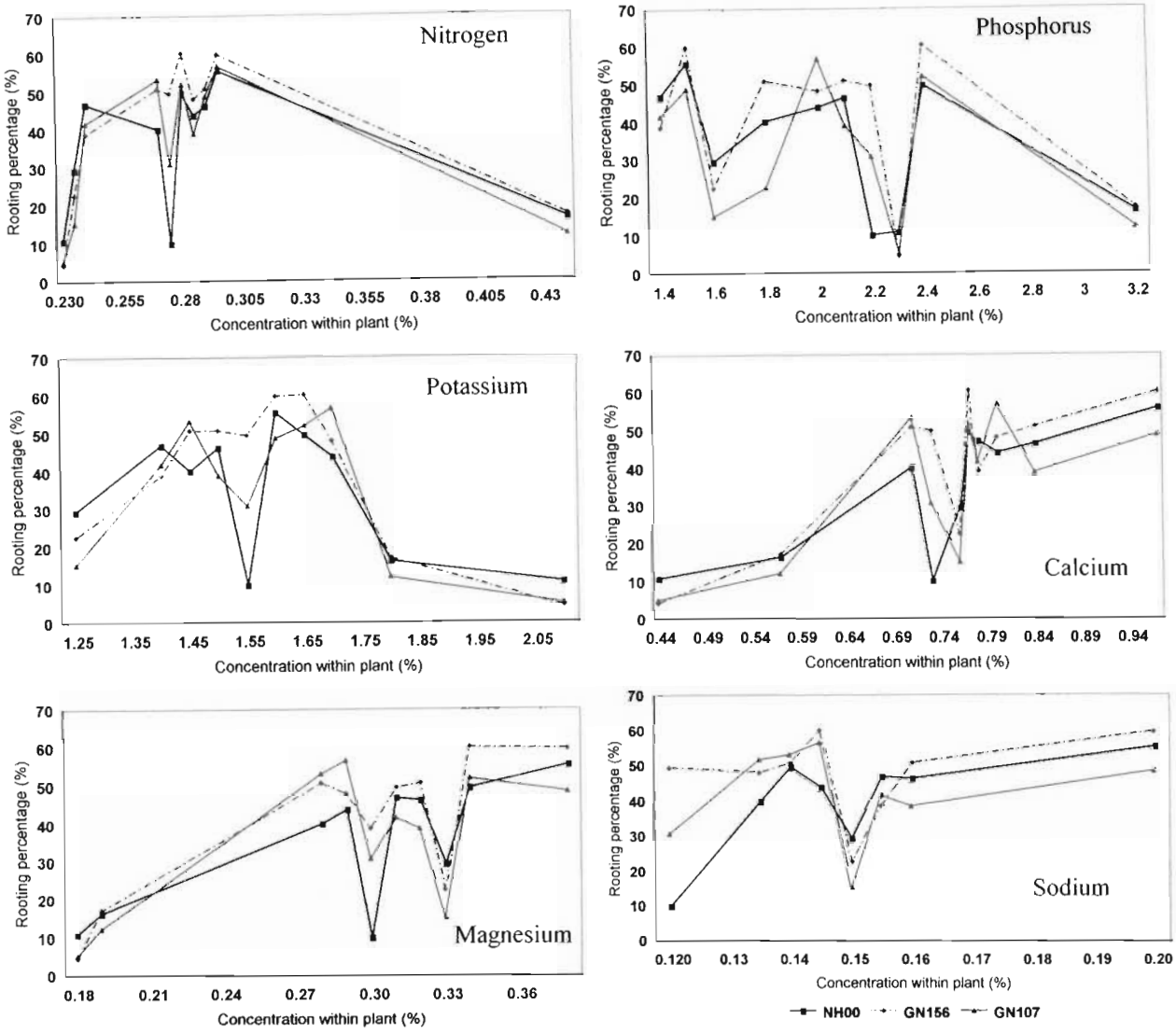

Figure 40: **Correlation of rooting and macroelements for cold-tolerant *Eucalyptus* hybrids**

From these and previous results, it would appear that Ca, Cu, Zn , Mn and B concentrations may play a role in the rooting process and that for GN hybrids, these critical values or ranges were slightly different to other *Eucalyptus* hybrids (Table 27).

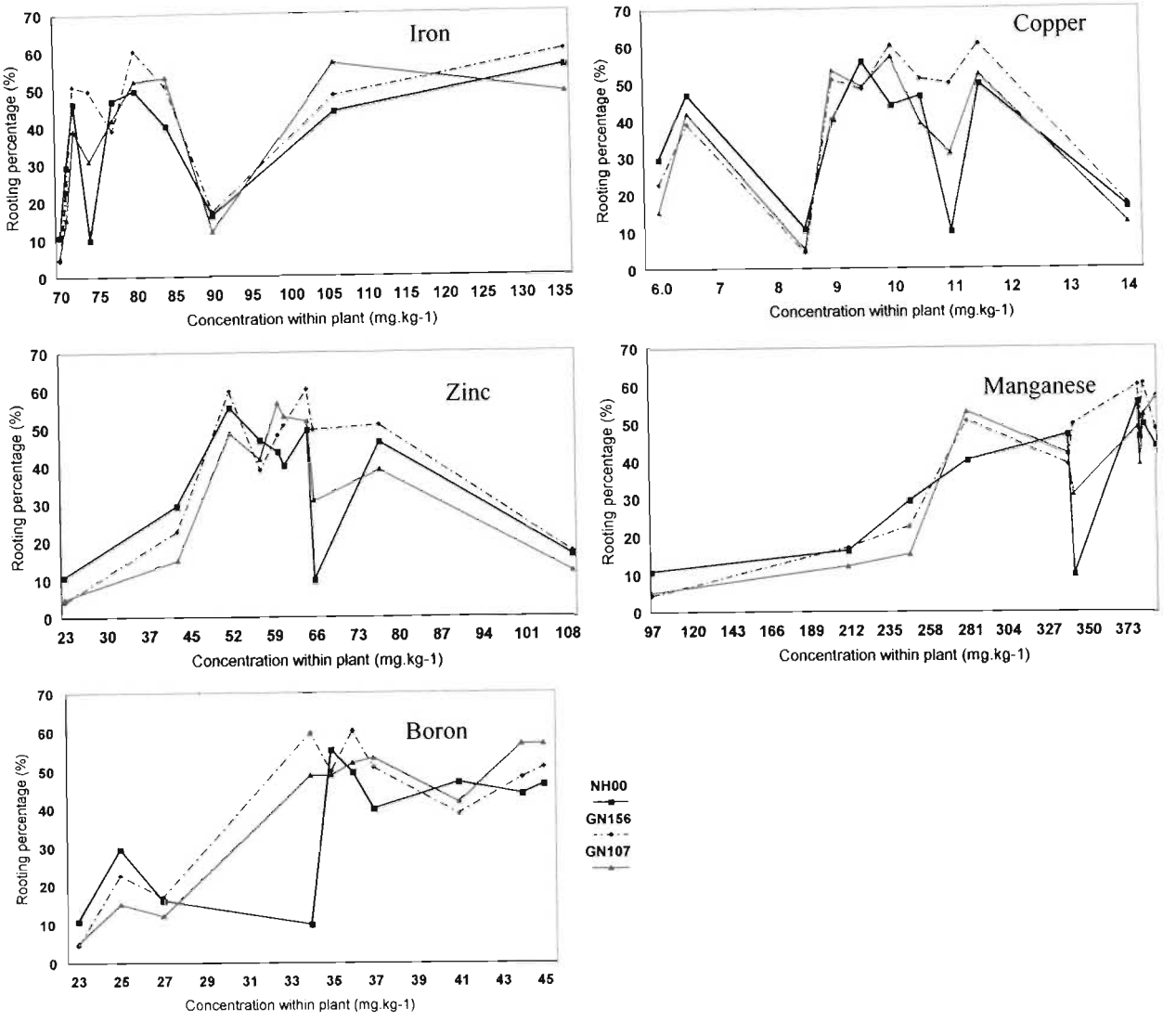

Figure 41: **Correlation of rooting and microelements for cold-tolerant *Eucalyptus* hybrids**

Table 27: **Critical concentrations of Ca, Cu, Zn, Mn and B for optimal rooting of cold tolerant *Eucalyptus grandis* x *E. nitens* hybrids**

Element	Higashi *et al.*, (2000a)	Carlson *et al.*, (2003)	Results from the trials evaluated in this chapter
Ca %	5 – 7	0.26 – 1.49	0.7 minimum
Cu $mg.kg^{-1}$	8 – 15		9 – 11
Zn $mg.kg^{-1}$	30 – 60		50 – 80
Mn $mg.kg^{-1}$	250 – 500		250 minimum
B $mg.kg^{-1}$	37 – 70		35 minimum

Chapter 5

COST-BENEFIT ANALYSIS

5.1 Introduction

Within Mondi's *Eucalyptus* tree improvement programme, the increasingly greater focus on specific clones and improved production targets prompted investigation of ways to reduce costs and increase yields in a minimum time period. The previous chapters have described how the various hydroponic systems were identified as potentially important methods of increasing propagation production yields and rooting of *Eucalyptus* clones, specifically CTEs destined for the colder marginal areas in South Africa. As discussed in Section 2.2.3, each system has different advantages and disadvantages and the type of system used affects, in various ways, the rooting of cuttings taken from mother plants grown in each system. Brazilian companies are in the process of converting from field hedges to microhedges, while continuing to research and improve the hydroponic hedge system (Janse, 2001). The value of these systems is discussed in this chapter in terms of rooting performance, costs and the application to the *Eucalyptus* plantation industry in Mondi forests.

5.2 Rooting performance

The rooting results of the three CTEs used in these trials have been discussed in the previous chapter. The rooting performance of any clone was difficult to ascertain, as one could not simply take the rooting percentage into consideration and had to consider the actual number of cuttings placed per mother plant. This gave a more reliable idea of the rooting performance for each system.

The differences due to clone type have not been taken into account in this chapter because in a commercial installation in a forestry nursery, many clones will be maintained together in the chosen substrate / system.

The average number of cuttings placed per mother plant per harvest and the average rooting percent were used to determine a success rate for each substrate system (Table 28).

Table 28: **Summary of rooting success results for each substrate / system**

System	Number of cuttings placed per mother plant	Average rooting %	Success rate * (no. placed x rooting %)
Aeroponics	1.1	15.2	0.17
Ebb-and-flow	7.9	6.8	0.54
Gravel	4.7	26.9	1.26
Leca	6.8	21.8	1.48
NFT	6.8	11.4	0.78
P:V	5.1	24.0	1.22
Peat	7.0	20.7	1.45
Perlite	7.4	22.7	1.68
Rockwool®	7.5	13.0	0.97
Sand	5.4	25.8	1.39

* Success rate = the number of rooted cuttings per mother plant

The ebb-and-flow system has the highest average number of cuttings placed (7.9) but the lowest rooting percent (6.8 %). This results in a success rate of 0.54, the lowest compared to the other substrate / systems, except the aeroponics system (0.17) (Table 28).

Ebb-and-flow, Rockwool® and perlite substrates had the highest number of cuttings placed; gravel, sand and P:V substrates had the highest rooting percent and perlite, leca and peat substrates had the highest success rate (Table 28). The plants in the last three substrates had a rooting percentage above 20 % and the number of cuttings placed was above six (Table 28). Plants in the other systems had either a high number of cuttings placed (above six) or a high rooting percentage (above 20 %) but the respective success rate was low because the corresponding rooting percentage or number placed was low (Table 28). It must be kept in mind that irrespective of their original substrate, all three clones in this trial remained in the rooting greenhouse for the same number of days.

5.3 Costs

To access the potential commercial application from this work, it was decided to do a cost-benefit analysis (CBA). This CBA looked at the 10 different systems and took the initial cost, cost of substrate (if any) and the rooting data into consideration.

The initial capital outlay for each of the experimental tables is shown in Table 29. As they maintained a different number of plants, at different planting densities (number of plants per m^2), the figures have been extrapolated to give comparable figures. The cost of the tables were extrapolated to obtain a cost for each system / substrate in which 1000 mother plants could be maintained. The CBA was based on costs for a research / trial environment and if one was to scale-up the figures (for a commercial installation) these costs would be less than those presented here but proportionally so and therefore one could still compare the figures presented. The figures do not include the cost of greenhouses or other infrastructure, only those of the hydroponic tables in which the mother plants are grown. As the aeroponic, ebb-and-flow and NFT systems had the lowest initial capital outlay, and did not require any form of a substrate, they have lower initial capital costs than the other systems / substrates.

Table 29: **Initial capital outlay for research tables of each system and initial capital outlay to maintain 1000 mother plants**

System	Research tables in Rands (R)	Capital costs per 1000 mother plants in Rands (R)
Aeroponic	1 850	7 324
Ebb-and-flow	7 600	15 244
Gravel	9 306	43 083
Leca	11 184	51 776
NFT	9 270	42 917
P:V	9 463	43 809
Peat	9 320	43 147
Perlite	9 457	43 781
Rockwool®	12 272	56 813
Sand	9 301	43 061

The other eight substrate / systems had the same initial capital cost (R 42 917), so the cost differences seen in Table 29 are due to the cost of the different substrates used. The leca

and Rockwool substrate / systems were the most expensive (R51 776 and R56 813, respectively) in terms of initial capital cost as they were imported.

A cost analysis was done (Table 30) using the average number of cuttings placed and average rooting percentages for all clones (Table 28). Calculations were based on data obtained to date which indicated that with 1 000 mother plants for each of the 10 systems, and assuming four harvests per year, 6 700 rooted plants can be obtained from mother plants in perlite and 667 rooted plants from mother plants in the aeroponics for one year.

Table 30 gives a breakdown of the number of cuttings placed, the number of rooted cuttings produced, and, from the cost of placing and maintaining a cutting, the cost of producing each rooted cutting.

The highest number of rooted plants produced were from mother plants maintained in the perlite (6 700), leca (5 943) and peat (5 798) substrates / systems while the cheapest rooted plants to propagate were from mother plants grown in gravel (R1.97 per plant), sand (R2.05 per plant) and the P:V (R2.21 per plant) substrates / systems.

In terms of initial capital outlay (Table 29) of each system, the aeroponics, ebb-and-flow and NFT systems had the lowest cost of instalment. On the other hand, the results from these three systems show that the total number of plants rooted was low and total cost per plant was high. In this instance, the low number and high cost of those rooted might negate the savings achieved in the cost of instalment.

After the aeroponics, ebb-and-flow and NFT systems, the sand, gravel and peat systems had the lowest initial capital cost (R43 061, R43 083 and R43 147 respectively). The sand and gravel systems have a low cost per plant and the peat system has a high number of rooted plants. These systems are similar in the cost of initial outlay with only the cost of the substrate making the slight difference.

Table 30: **Calculation of variable costs of 10 different hydroponic systems based on 1000 mother plants per system**

System	Number of cuttings placed per mother plant	Actual number of cuttings placed for 1000 mother plants	Rooting percent (%)	Total number of Rooted cuttings	Total cost per rooted plant in Rands (R)
	(a)	(b)	(c)	(d)	(e)
Aeroponic	1.1	4 400	15.2	667	3.50
Ebb-and-flow	7.9	31 760	6.8	2 166	7.77
Gravel	4.7	18 960	26.9	5 102	1.97
Leca	6.8	27 240	21.8	5 944	2.43
NFT	6.8	27 240	11.4	3 116	4.63
P:V	5.1	20 440	24.0	4 902	2.21
Peat	7.0	28 000	20.7	5 799	2.56
Perlite	7.4	29 480	22.7	6 701	2.33
Rockwool®	7.5	29 920	13.0	3 875	4.09
Sand	5.4	21 760	25.8	5 614	2.05

(a) Average number of cuttings placed per mother plant for each system for **each harvest** (data from trial)

(b) Number of cuttings placed from 1000 mother plants for **one year** (equivalent of four harvests)

(c) Average rooting percentage of three clones from **each system** (data from all trials)

(d) Total number of cuttings rooted from those placed at the average rooting percentage for **one year** (c). d = c x b / 100

(e) Cost of (d) rooted plants at R0.53 per plant **per year**. Cost includes media, hormone powder, labour for collecting coppice, making and placing cuttings and subsequent care, any necessary pesticides, water, electricity etc. Cost does not incl. space in the greenhouse and the cost of the tray itself as these are re-used. This figure was extrapolated from actual nursery costs. e = (b x 0.53) / d

From another point of view, if one million rooted plants from each system was required annually, i.e. that of production targets, for example, one finds that each system would need to house a different number of mother plants (Table 31). For example, the perlite system would house the least number of mother plants (149 236) and would be the most inexpensive (R 6 533 655) to install but the rockwool system would need to house 258 088 mother plants and would cost the most to install (R14 662 772)

Table 31: **Cost of initial capital outlay for each system to produce one million plants per year**

System	Total plants per year (rooted)	Number of cuttings placed per year	Number of mother plants required	Total cost of installation to house required no. of mother plants
Aeroponic	1 000 000	6 596 306	1 499 160	10 979 566
Ebb-and-flow	1 000 000	14 662 757	461 674	7 037 959
Gravel	1 000 000	3 716 091	195 996	8 444 103
Leca	1 000 000	4 582 951	168 243	8 710 960
NFT	1 000 000	8 741 259	320 898	13 771 868
P:V	1 000 000	4 170 142	204 019	8 937 832
Peat	1 000 000	4 828 585	172 449	7 440 620
Perlite	1 000 000	4 399 472	149 236	6 533 655
Rockwool	1 000 000	7 722 008	258 088	14 662 772
Sand	1 000 000	3 875 969	178 124	7 670 265

Table 31 shows the costs of initial capital outlay for housing the parent plants and does not take into account the costs that would be incurred by the additional rooting greenhouses that would be needed to complement the hydroponic systems.

Taking this analysis a step further, one can combine the production costs (Table 30) and installation costs (Table 31) to obtain a total cost (Table 32) where the capital outlay can be discounted over a number of years. For any project to be economically viable, the return on capital must be realised within a three to five year period and therefore the installation costs from Table 31 were discounted (without interest) over a five-year period in Table 32.

Over the five-year period, the rooted cuttings from plants in the sand substrate were the most inexpensive (R3.58), followed by rooted cuttings from plants in the perlite (R3. 64) and gravel systems (R3.66) (Table 32). Rooted cuttings from plants grown in the ebb-and-flow system were the least cost-effective (R9.18) (Table 32).

Table 32: **Total costs per rooted cutting from each system if producing one million cuttings per year, with capital costs discounted (without interest) over a five-year period**

System	Production cost per rooted cutting (fromTable 30)	Installation cost to produce 1 million cuttings (from Table 31)	Discount over five years (without interest)	Product cost per 1 million plants	Total cost	Total cost per rooted cutting
	A	B	C = (B/5)	D = (A * 1 000 000)	E = (C+D)	F = (E/ 1 000 000)
Aeroponic	3.50	10 979 565	2 195 913	3 500 000	5 695 913	5.70
Ebb-and-flow	7.77	7 037 959	1 407 592	7 770 000	9 177 592	9.18
Gravel	1.97	8 444 103	1 688 821	1 970 000	3 658 821	3.66
Leca	2.43	8 710 960	1 742 192	2 430 000	4 172 192	4.17
NFT	4.63	13 771 868	2 754 374	4 630 000	7 384 374	7.38
P:V	2.21	8 937 832	1 787 566	2 210 000	3 997 566	4.00
Peat	2.56	7 440 620	1 488 124	2 560 000	4 048 124	4.05
Perlite	2.33	6 533 654	1 306 731	2 330 000	3 636 731	3.64
Rockwool®	4.09	14 662 772	2 932 554	4 090 000	7 022 554	7.02
Sand	2.05	7 670 265	1 534 053	2 050 000	3 584 053	3.58

This analysis, however, did not indicate whether one particular system was more efficient or productive than the others and one would have to take the advantages and disadvantages of the individual systems into account when deciding which is most suitable to implement. For a commercial installation the practicality and availability of the substrate types are critical factors. Added to this, Mondi Forests is FSC (Forestry Stewardship Council) certified and strives to implement and improve on the relevant government requirements. Issues such as health and environmental impacts also need to be taken into consideration.

5.4 Advantages and disadvantages of substrates / systems

To make an informed decision which concerns the type of system to be installed, one would have to look at the advantages and disadvantages (Table 33) of each system tested and the practicality of each system and substrate. The designs of the systems vary as do the properties and characteristics of each substrate.

In addition to the advantages and disadvantages discussed in Section 2.2.4 the advantages and disadvantages listed in Table 33 were experienced throughout the trials:

Table 33: **Critical advantages and disadvantages of the 10 systems used in these trials**

System / Substrate	Advantage	Disadvantage
Gravel	- inexpensive - reusable - easy to clean - inert - good drainage	- heavy - difficult to plant in - sharp edges
Sand	- inexpensive - reusable - easy to clean - inert - good water-holding capacity	- heavy - prolific moss growth (habitat for problem insects e.g. fungus gnat)
Leca	- reusable - not easy to clean because of porous structure - inert - good drainage	- imported and therefore expensive - prolific moss growth (habitat for problem insects e.g. fungus gnat) - not as heavy as sand or gravel but still heavy
Perlite	- light in weight - reusable - inert - good drainage	- prolific moss growth (habitat for problem insects e.g. fungus gnat)
Peat	- good water-holding capacity	- environmental issues: loss of habitat - prolific moss growth (habitat for problem insects e.g. fungus gnat) - organic material and therefore not inert
Note: There was concern about the possibility of phenolics and organic acids being released into nutrient solution as there was a change in colour of the nutrient solution.		
P:V	- inert - light in weight	- vermiculite breaks down and therefore cannot clean or reuse
Rockwool®	- inert - good drainage	- imported and therefore expensive - health issues: tiny fibres can be inhaled causing respiratory problems - environmental issues: rockwool not biodegradable - cannot reuse - cannot clean - prolific moss growth (habitat for problem insects e.g. fungus gnat)
NFT / Aeroponics / ebb-and-flow	- no substrate therefore decreases costs - excellent drainage	- need way to anchor / hold plants

5.5 Conclusion

The costs (e.g. operational costs such as rooting media, rooting hormones, greenhouse space etc) involved in actually producing the rooted plants were identical for all systems

and the final cost per plant was dependent on the rooting percentage and number of cuttings placed from mother plants in each system. When making a decision the cost per rooted plant and the actual number of rooted plants from mother plants in each system needed to be weighed up against the cost of the initial outlay and the advantages, disadvantages and any applicable practicality concerns.

It was easy enough to identify that the three systems with no substrate (aeroponics, ebb-and-flow and to a lesser extent, NFT) were the least expensive in terms of cost of initial capital outlay; however these had the worst results with regard to cost per plant and actual number of plants rooted.

Rockwool® was not only an expensive system to install but also the rooting results were poor and cost per rooted plant was high. There are also health and environmental concerns with regard to Rockwool® that do not make further studies of this substrate feasible.

The gravel, sand, perlite, P:V, peat and leca systems / substrates had the same design but different substrates in which the mother plants resided. Of these, leca was the most expensive as it is an imported product. If a local derivation of the leca pellets could be sourced it would make it a feasible option.

The P:V, gravel, peat, perlite and sand substrates / systems were all in a similar price range. These systems all had a high number of trays of rooted plants and a low price for these plants. Mother plants grown in the sand, perlite and gravel substrates / systems produced rooted cuttings, discounted over five years without interest, which were the most cost-effective (R3.58, R3.64 and R3.66, respectively). This was where one needs to take the above-mentioned advantages and disadvantages (Table 33) into account.

Both the sand and gravel substrates, although inexpensive, easy to source and reusable, are heavy in weight and a commercial design with reinforcing of the structures would need to be considered, increasing the initial capital costs.

The P:V mix cannot be reused as the vermiculite in the mix breaks down over time. This would mean that the substrate would have to be replaced regularly to decrease the chance of blockages in the system. Another concern with the breakdown of vermiculite is that once the structure breaks down it hinders the drainage of the substrate and cause secondary problems such as rotting of the roots or harbours and encourages diseases.

Peat is an organic substrate and it is not certain what organic compounds it released into the nutrient solution and how this affects the solution's composition. As mentioned, there are environmental concerns with many environmental groups making serious allegations about the destruction of peat bogs and the consequent destruction of habitats (Section 2.2.4.1).

The perlite system is a more expensive system to outlay initially, but perlite can be easily cleaned and reused. Plants in the perlite system produced the highest number of rooted cuttings at a cost of R3.64 per rooted plant. There are various grades of perlite available and possibly a cheaper grade would not negatively influence the rooting or corresponding cost per plant and this is worth investigating in further studies.

Chapter 6

CONCLUSION AND PROSPECTS

6.1 Conclusion

According to Wilson (1998b) genotypic effect plays the biggest role in determining the rooting performance of *Eucalyptus* species, and that the efficient selection of clones, especially for rooting, and the good understanding of the management of the rooting environment would ease the propagation process.

In South Africa, Mondi Forests implemented a CTE programme to provide for operations in marginal cooler temperate areas with *Eucalyptus* trees that can survive the extremes of the higher altitudes such as cold, frost and snow (Denison & Kietzka, 1993a; Harvett, 2000). Unfortunately, the clonal propagation of these hybrids has not been easy, with budgeted rooting percentages of 35 % or less across all cold tolerant clones (de Haas, 2003). There is very little information available on the optimal growth and rooting conditions of these cold tolerant hybrid species and nurserymen have had to apply any other potentially related information, usually based on data from *E. grandis* trials.

In South Africa, until 1998, there had not been any significant research undertaken into the development and application of hydroponics to cutting production in nurseries. Since then, research in Mondi Forests has focused on the evaluation of recirculating hydroponic systems for cutting production of the genus *Eucalyptus* (Appendix 4).

Da Costa Alpoim (2002) studied hydroponics and its suitability for the propagation of exotic species that until then had been given very little attention outside of South America. The aim of his studies was to assess the feasibility of utilising recirculating hydroponic systems to improve the overall rooting efficiency of cuttings harvested from clonal *Eucalyptus* hydroponic hedge plants, and the ability of hydroponic parent plants to supply higher numbers of cuttings than can field hedges. Other major objectives were to focus on the role of nutrition and attempt to identify those nutrients that strongly correlate with the

rooting success of eucalypts and to investigate the impact of disease pathogens on mother plant production as disease is the greatest threat to the survival of stock plants in soilless culture.

The work reported in this thesis had the aim of optimising the production of GN hybrid cuttings in the hydroponic systems. Several trials were established to compare different hydroponic systems and substrates and to look at the corresponding plant elemental analyses. Three different hydroponic systems were compared, viz. variations on NFT, ebb-and-flow and an aeroponics system. The NFT systems were constructed in such a way that eight different substrate types could be tested and compared. This, in effect, meant that a total of ten systems were tested and compared.

Rooting percentage was significantly effected by clone for each of the four harvests from the NFT systems (Section 4.1.1) and for the ebb-and-flow (Section 4.2) and aeroponics (Section 4.3) systems. There was however, no clonal effect on the rooting percentage for the four NFT harvests when the differences between harvests were investigated. As discussed (Section 4.1.1), the effect of season, maturity of hedges or both, on the rooting percentage had a greater effect than clone, and this needs to be taken into account in the design of future experiments.

Rooting percentage only, of plants from a system could not be taken as an indicator of the rooting performance. The actual number of cuttings placed and the corresponding number of cuttings rooted had to be taken into consideration, to give a measure of the success of any one system.

Cuttings with the highest rooting percentage were from mother plants grown in gravel substrate but the plants that produced the highest number of cuttings to be placed were grown in the ebb-and-flow system. Both factors were used to determine a success rate for each system. It was calculated that plants in the perlite system had the best success with an average 7.4 cuttings placed per mother plant and a rooting percentage of 22.73 % giving a success rate of 1.68 successfully rooted cuttings per mother plant per harvest.

The clones may have had different nutrient requirements for optimal rooting. It was difficult to determine which elements played key roles in the rooting process but assessment of these trials provided some information. It was found that of all the macroelements, Ca showed the most notable relationship with rooting success with a minimum plant concentration of 0.7 % being required, although it was not determined what the maximum level was. With respect to the microelements analysed, only Fe concentration did not seem to have any direct relationship with rooting. Copper, Zn, Mn and B concentrations all changed as rooting increased or decreased. It was suggested that the optima for these were: 9 – 11 $mg.kg^{-1}$ for Cu; 50 – 80 $mg.kg^{-1}$ for Zn and minima of 200 $mg.kg^{-1}$ and 35 $mg.kg^{-1}$ for Mn and B, respectively (dry mass). When these values were compared with other works, it was found that they were either slightly higher or concurred.

A cost analysis was undertaken for all ten systems and the cost of the initial outlay was also determined. Most critical factors were taken into account and while the NFT, ebb-and-flow and aeroponics systems were the least expensive to install initially, the low productivity of these systems precludes them from further studies or commercial installations.

The remaining systems all housed the mother plants in a substrate and it was in fact these substrates that determined the price difference in initial outlay and the properties of these substrates that affected the rooting of plants from each system. With the current exchange rate, it was not reasonable to import substrates such as Rockwool® and leca even though the plants in the leca had a good rooting performance. It was pointed out that if a local source of leca pellets or a similar product could be found that it would be worth considering for further studies. When comparing the remaining five systems it was necessary to look at the associated advantages and disadvantages of each substrate. Gravel and sand were both heavy in weight and therefore impractical to use; peat, with its associated environmental concerns and the P:V mix, with the breakdown and subsequent problems of vermiculite were all disregarded.

Of all the substrates, only perlite seemed to be practical. Results from plants kept in perlite ranked highest in the success ratings, had a rooting percentage above 20 and produced an average of 7.4 cuttings to be placed per mother plant. The only major operational

disadvantage was the prolific moss growth that produced a habitat for unwanted insects. However, in an intensive system such as this, the control of these pests was much easier compared to a field hedge system.

Hydroponic systems offer an intensive approach to manage and produce clonal plants for the field. In keeping with experience in Brazil, the results from these trials indicate that for cold tolerant *Eucalyptus* propagation the hydroponic systems provide benefits over (and possibly problems yet not realised) relative to the current system of field hedges. This makes the future prospects of hydroponics propagation both exciting and daunting as forestry nurserymen enter into unchartered territory in the propagation of CTEs in intensive hydroponic systems.

6.2 Future recommendations

The work in this thesis was a small step into gaining more knowledge and understanding of how eucalypts, particularly CTEs, responded in hydroponic systems as hedge plants. There is still much work that can be researched in this field:

- new systems and substrates
- alternative nutrient formulations, concentrations and applications
- the addition of plant growth regulators (PGRs) to the nutrient solution
- heating / cooling of the nutrient solution to improve uptake of nutrients
- new clones and their responses
- different cutting types from the hydro-hedges
- selective vs. complete harvest of hydro-hedges
- disease control
- rooting in hydroponic systems

For much of the research mentioned above a good understanding of how eucalypts respond to various nutrients and how eucalypts root when vegetatively propagated is necessary. An understanding of what nutrients and hormones played a role in the rooting process and to what degree would also assist the researcher.

Another logical step to hydroponics culture and the commercialization of these systems is to establish the possibility of rooting eucalypt cuttings in hydroponic systems. A preliminary trial was placed at Mountain Home Nursery to compare the rooting percentages of cuttings placed in hydroponic systems with those propagated conventionally (Appendix 5).

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Appendix 1

Summary of crops and substrates used in hydroponic systems in various countries

Crop species	System / Substrate	Country	Reference
Tomato	Rockwool®, perlite.	USA	Brentlinger (1999)
	Rockwool®, NFT, peat-lite mix, sawdust and others.	Canada	Carrier (1999);Khosla (1999); Mirza & Younus (1999)
	NFT, Deep flow technique (DFT), Floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Rockwool®	Belgium	Van Os & Benoit (1999)
	Perlite, Rockwool®, pumice, volcanic cinders and stone, expanded clay, coir, peat, sand, grape marc and various mixes of the above. NFT.	Italy	Pardossi & Tognoni (1999); Nucifora, Vasquez &Giuffrida (2001)
	Rockwool®, NFT, perlite.	Greece	Mavrogianopaulos (1999)
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
	Unknown	Mexico	Steta (1999)
Cucumber	Rockwool®, perlite.	USA	Brentlinger (1999)
	Unknown	Mexico	Steta (1999)
	Rockwool®, soil, NFT, peat-lite mix sawdust and others.	Canada	Carrier (1999); Khosla (1999); Mizra & Younus (1999)
	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Rockwool®	Belgium	Van Os & Benoit (1999)
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)

Crop species	System / Substrate	Country	Reference
	NFT, leca and perlite	Antarctica	Campiotti, Balducchi, Incrocci, Pardossi, Popovski & Popovska (2001)
Lettuce	Rockwool®, perlite.	USA	Brentlinger (1999)
	NFT and floating rafts	Canada	Carrier (1999); Khosla (1999)
	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999); Rodriguez-Delfin, Chang & Hoyos (2000)
	NFT	Belgium	Van Os & Benoit (1999)
	Rockwool®, NFT, perlite.	Greece	Mavrogianopaulos (1999)
	NFT primarily	Brazil	Furlani (1999)
	DFT	Asian Countries	Rodriguez-Delfin *et al.*, (2001)
	DFT	Caribbean and other tropical regions	Rodriguez-Delfin *et al.*, (2001)
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
	NFT, leca and perlite	Antarctica	Campiotti *et al.*, (2001)
Peppers	Rockwool®, perlite.	USA	Brentlinger (1999)
	Unknown	Mexico	Steta (1999)
	Rockwool®	Canada	Carrier (1999); Khosla (1999); Mirza & Younus (1999)
	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Rockwool®	Belgium	Van Os & Benoit (1999)
	Rockwool®, NFT, perlite.	Greece	Mavrogianopaulos (1999)
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)
Herbs	Rockwool®, perlite.	USA	Brentlinger (1999)
	NFT	Belgium	Van Os & Benoit (1999)

Crop species	System / Substrate	Country	Reference
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
Celery	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
Medicinal Plants	Floating raft system	North America	Dorais, Papadopoulus, Luo, Leonhart, Gosselin, Pedneault, Angers & Gaudreau (2001)
	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
Rice, edible fungi	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
Mitsuba, Welsh onion.	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
Aubergine	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
Argula & watercress	Primarily NFT	Brazil	Furlani (1999)
Leafy vegetables	DFT	Japan and other Asian countries	Rodriguez-Delfin *et al.*, (2001)
Vegetable including Zucchini	NFT, leca and perlite	Antarctica	Campiotti *et al.*, (2001)
Leafy vegetable e.g. Swiss Chard	Ebb-and-flow	Southern Africa	Combrink & Harms (2001)
Radish	Sand, peat moss, volcanic material and surplus agricultural products e.g. rice hulls.	Colombia	Bradley & Marulanda (2001)
Potato, carrot, sweet potato, turnip and green beans	Unknown	Colombia	Bradley & Marulanda (2001)
Unknown vegetables	Unknown	Arabian Gulf, Israel, France, Italy, Netherlands, Germany, England, former USSR, USA, Japan, Canada, Singapore, India, Kuwait, Belgium and the Canary Islands	Harris (1982); Schwarz (1995); Ito (1999); Van Os & Benoit (1999); Nucifora *et al.*, (2001)

Crop species	System / Substrate	Country	Reference
Strawberry	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Perlite, Rockwool® and pumice.	Italy	Pardossi & Tognoni (1999)
Melons	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
Unknown fruit	NFT, leca and perlite	Antarctica	Campiotti *et al.*, (2001)
Carnations	Gravel	USA	Harris (1982)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)
Gladioli	Gravel	USA	Harris (1982)
Chrysanthemums	Gravel	USA	Harris (1982)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
Roses	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Perlite, Rockwool® and pumice.	Italy	Pardossi & Tognoni (1999)
Gerbera	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
	Perlite, Rockwool® and pumice.	Italy	Pardossi & Tognoni (1999)
Orchids	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
Alstroemeria	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
Antheriums	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
Freesia	Mainly Rockwool®. Alternate substrates: pumice, poly-urethane foam and coir.	Netherlands	Van Os & Benoit (1999)
Lily	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)

Crop species	System / Substrate	Country	Reference
Lisianthus	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)
Gypsofila	Volcanic cinders and stone, expanded clay, coir, peat, perlite, Rockwool®, sand, grape marc and mixes.	Italy	Nucifora *et al.*, (2001)
Unknown flowers	Gravel	USA	Harris (1982)
	Unknown	Italy, Spain, France, England, Germany, Sweden, Netherlands, former USSR, USA, Canada, Israel, Singapore, India, Kuwait and the Canary Islands	Harris (1982); Schwarz (1995)
	NFT, DFT, floating capillary hydroponics and other systems designed in China. Vermiculite, coal cinders, perlite and sand.	China	Xing & Meng (1999)
	DFT, Rockwool®, NFT and gravel.	Japan	Ito (1999)
Unknown potplants	Lava or clay granules	Netherlands	Van Os & Benoit (1999)
	85 % Rockwool®	Belgium	Van Os & Benoit (1999)

Appendix 2

Recommended nutrient concentrations published in the literature for *Eucalyptus* species. All concentrations listed are considered to be for normal for optimal growth.

Species		*E. grandis*	*E. grandis*	*E. grandis*	*E. grandis*	*E. grandis*	*E. grandis*	*E. grandis*	*E. nitens*	*E. nitens*
Age of plants		Seedlings	Young plantation	Mature trees	1 year old seedlings	3 month old seedlings	1 –2 year old	Juvenile	Juvenile	3 year old
Element										
N	%	1.25-5.3	2.51	2.8	2.38	2.5-3.8	1.8 – 3.4	1.8-3.4	2.0-3.5	1.34
P	%	0.075-0.275	0.18	0.15	0.21	1.5-2.2	0.1 – 0.22	0.1-0.3	0.1-0.2	0.08
K	%	0.75-1.75	1.12	0.75	0.80	12-14	0.9 – 1.8	0.6-1.8	0.8	0.25
Ca	%	0.55-0.95	0.61	> 1.0	0.84	5-8	0.3 – 0.6	0.3-1.0	0.3-0.5	0.65
Mg	%	0.25-0.4	0.11	0.35	0.29	1.6-2.0	0.11 – 0.21	0.1-0.35	0.09-0.15	0.27
S	%	2400-3300 ppm		0.20		1.9-3.2		0.1-0.3		
Na	%			0.32				0.30-0.42		
Cl	%							0.4		
Fe	ppm	150-190		110		65-80	63 – 128	60-130	23-75	
Mn	ppm	80-120		600		50-546	193 – 547	220-700 150-600	960-1400	
Cu	ppm	12-16		12		3.0-5.5	1.7-7.4	6-15 2-7	4.9	10
Zn	ppm	25-32		18		17-22	17-42	14-16	9-19	13
B	ppm	55-239		32		15-27	15-27	15-30	10-30	22
Mo	ppm									
Reference		Judd, Attiwill & Adams (1996)	Huoron & Wenlong (1996)	Herbert (1996)	Negi & Sharma (1996)	Dell, Malajczuk & Grove (1995)		Boardman, Cromer, Lambert & Webb (1997)		Prado & Toro (1996)

Appendix 3

Nutrient solution protocol for hydroponic systems

1. Switch off timer in pump room
2. Empty tanks and clean thoroughly with water
3. Clean pumps thoroughly with hose-pipe
4. Spray plants and media with water to remove dust
5. Refill tanks with water
6. Add Purogene FB® (see A)
7. Add fertilisers (see B)
8. Switch pumps back on
9. Once system has run, take EC ($\mu S.cm^{-1}$) and pH measurements for each tank.
10. pH should be between 5,5 and 6,5
11. The EC should be between 1,2 and 1,5 $\mu S.cm^{-1}$
12. If EC is higher than 1,5 $\mu S.cm^{-1}$: add water to tanks and take measurement again
13. If EC is lower than 1,2 $\mu S.cm^{-1}$: add fertiliser to tanks and take measurement again.
14. Fertiliser and water must be replaced every 10 days. After 10 days the tanks can be emptied and run with water only for two days before cleaning of tanks and adding Purogene FB® and fertiliser again.

A. Purogene FB®

Remember to wear gloves and a mask !!

1. In one litre of water add: 100 ml Purogene FB® (big blue bottle)
   10 ml Purogene FB® activator (small bottle)
2. Make up to two litres with water
3. Put 100 ml of solution into each of the NFT tanks
4. Put 1100 ml into ebb-and-flow tank
5. Put 660 ml into aeroponics tank

B. Fertiliser

1. Using buckets or beakers mix fertiliser with water
2. Add thoroughly mixed solution to each tank

Tank	Tank volume (l)	Amount of Hydroponica® to be added to nutrient solution (ml)	Amount of Agrisol®: Plant Calcium to be added to nutrient solution (ml)
NFT –A	45	50	34
NFT -B	45	50	34
Ebb-and-flow	500	570	340
Aeroponics	300	340	200

Appendix 4

Commercialization of hydroponic hedges to date at Mondi

Mondi Forests has installed two hydroponic systems in the last two years based on the hydroponic systems used by Brazilian companies. A small system for CTE propagation was first installed at the Mountain Home Nursery in Hilton. A second, much larger and more extensive system was installed for the propagation of subtropical eucalyptus at the KwaMbonambi nursery near Richards Bay.

The plan is to convert from a field-hedge system to a hydroponics-hedge (hydro-hedge) system. Field hedges are extensive and are vulnerable to prevailing weather conditions and / or outbreaks of pests and diseases. Hydro-hedges, particularly in a controlled greenhouse, are grown in better conditions with optimum water and nutrients. Further, the incidence of insects or diseases can quickly be controlled with the appropriate pesticides.

There have been some operational problems with both of Mondi's systems. The hydro-hedges at Mountain Home Nursery are particularly susceptible to powdery mildew (Figure 1). Fungicides have been tried and management protocols altered in order to try to combat this problem.

Figure 1: **Powdery mildew on mother plants at the Mountain Home Nursery hydroponics installation**

The system in KwaMbonambi has a totally different set of problems. The quality of the water seems to be the root of all their problems. Many of the plants are suffering from either a nutrient deficiency or toxicity (Figure 2). One clone in particular, GU178, appears to be particularly sensitive. Many changes were made rapidly in an attempt to remedy the situation, including a change in fertilisers, pH, EC and protocols with little or no effect. After conducting numerous analyses on the foliar material, nutrient solution and the source of water it appears that there was Na and Cl toxicity which aggravated the uptake of other nutrients and in particular the microelements. Subsequently, a deionising unit has since been installed at the KwaMbonambi hydroponics project with the aim of improving the water quality before adding acids and fertilisers to formulate a nutrient solution. Since this unit has been installed, the pH of the solution has been easier to manage and the plants are responding well.

Figure 2: **An unidentified nutrient deficiency or toxicity of mother plants at KwaMbonambi's hydroponics installation**

Two other possible sources of problems at KwaMbonambi's installation have also since been discovered: the frequency of irrigation and design of the beds. The frequency of irrigation may be too high especially for the cooler winter months causing the plants roots to remain wet and not receive sufficient oxygen. This was due to a slight change in the original design of that at Mountain Home Nursery which was implemented to use less water. The systems were ebb-and-flow systems and the depth of the bed was reduced in the KwaMbonambi design. This together with the high frequency of irrigation seems to be causing the rotting and subsequent death of the roots.

A new fertiliser has also been introduced at KwaMbonambi, based on the fertiliser formulations the Brazilian companies recommend and are using. This fertiliser is eucalypt-specific and although there were some changes in the composition of the nutrients and elemental ratios, further research will be necessary to determine the efficacy. The plants housed in the KwaMbonambi hydroponic system have also responded well to this change.

The decision to introduce hydroponic hedges with the aim to fully convert from field hedges has been both daunting and exciting. As this system of propagation is so intensive compared to the field hedges many suprises, some good, some bad, have been noted. Research into diseases and disease control in hydroponic systems is being investigated at the Tree Pathology Co-operative Programme at the University of Pretoria and research into

carbohydrate assimilation and its impact on rooting is underway at the University of KwaZulul-Natal, Pietermaritzburg.

If these issues and others are addressed the full benefits of the hydroponics propagation system can be realised and can become more cost effective with improved management understanding and the implementation of new techniques.

Appendix 5

Comparison of rooting systems using GM and GN hybrids

A selection of *Eucalyptus grandis* x *E. macarthurii* (GM) and GN clones were being bulked up for trial purposes. These clones were all proving to be difficult-to-root hybrids and a decision was made to attempt to root these hybrids in the hydroponic systems and to compare the results (Table 1).

Table 1: **Rooting percentages for GM and GN clones from the conventional- and hydroponic systems**

	Conventional			Hydroponics
	1999-2000	2001	Average	
Clone No.	% rooted	% rooted	% rooted	% rooted
GM001	12	2	7	0
GM008	13	1	7	0
GM010	8	4	6	0
GM013	9	1	5	2
GM015	38	2	20	19
GM016	13	1	7	3
GM018	16	3	9.5	0
GM022	14	4	9	8
GM024	8	2	5	56
GM025	7	0	3.5	0
GM027	11	0	5.5	0
GM034	58	0	29	51
GM037	25	0	12.5	0
GM041	5	1	3	2
GM047	6	4	5	0
GN002	23	1	12	14
GN003	27	0	13.5	0
GN018	3	2	2.5	6
GN037	0	1	1	0
GN063		0	0*	0
GN091	3	7	5	4
GN093	0	0	0	0
GN100	10	4	7	12
GN104	0	5	2.5	0
GN111	4	0	2	0
GN131	0	1	0.5	0
GN155		6	6*	0
GN235	1	2	1.5	7
GN311	0	9	4.5	5
GN345		1	1*	34
GN449		19	19*	31

* denotes that results available for one year only.

From Figure 1, not all clones rooted well in the hydroponic systems but in some cases, e.g. GN002, GN018, GN100, GN235 and GN311, the rooting percentage was higher than that of the conventional method. In other instances, e.g. GM024, GM034, GN345 and GN449, the rooting percentage was above 30 % in the hydroponics system.

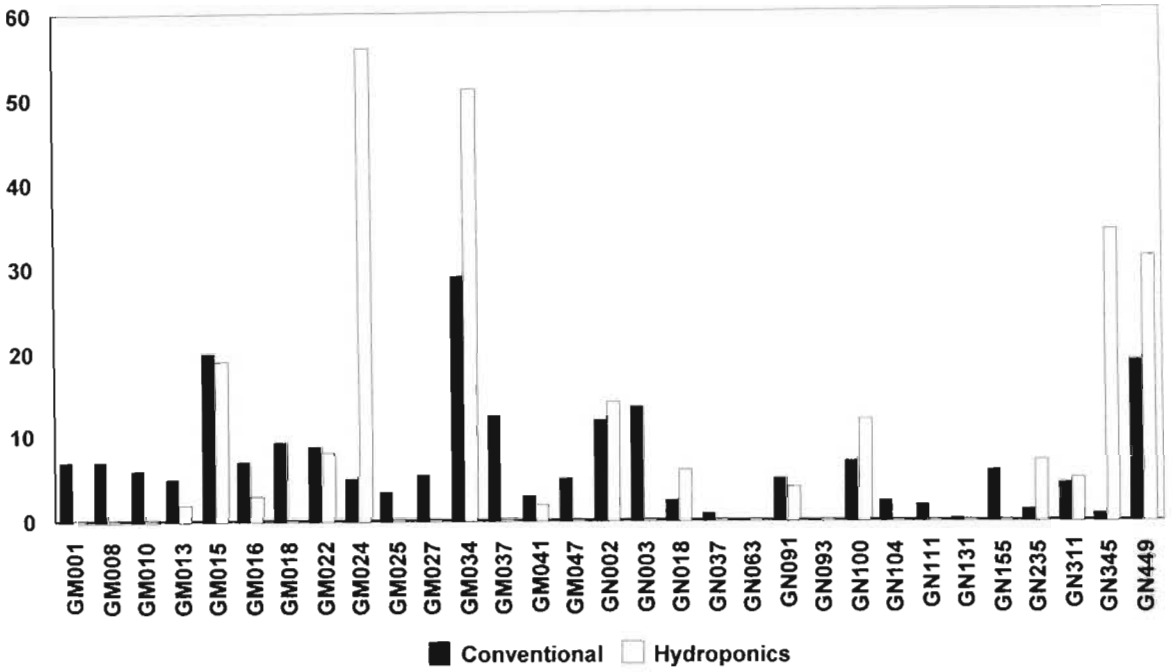

Figure 1: **Rooting percentage of GM and GN clones in conventional and hydroponics systems**

Considering the hydroponic systems have not been optimized for the rooting of cuttings, the Figure 1 shows some interesting results from the hydroponic system. The results shown are from a pilot, small-scale trial but even so it seems to show that there are clonal preferences to the hydroponic systems. A two-way ANOVA was conducted using the results available and it was found that there was no significant difference between the two systems at both the 1 % ($p = 0.3259$) and 5 % ($p = 0.2465$) level (Means: hydroponics = 8.797; conventional =7.172).

In addition to the increased rooting for some clones, the quality of the rooted cutting was superior with secondary growth and root growth occurring simultaneously (Figure 2). This was probably due to the nutrients available to the cuttings in the hydroponic system.

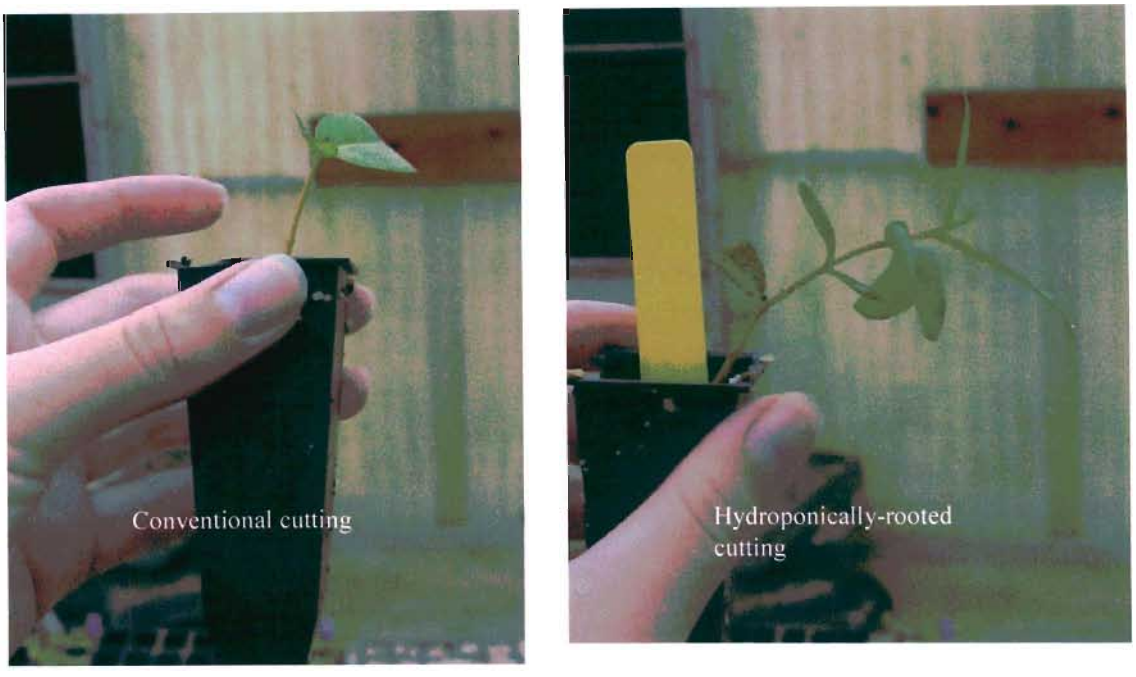

Figure 2: **Hydroponically-rooted cuttings have better growth when compared to conventional-rooted cuttings**

It would appear that no one nutrient mix or management technique would suit every clone and forestry nurserymen will have to generalize these differences in order to optimize the system whilst ensuring that the cost of the system is kept to a minimum.