NUTRITION OF CONTAINERISED PINE (*Pinus patula* Schlecht. et Cham.) SEEDLINGS GROWN IN PINE BARK

by

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ABSTRACT

NUTRITION OF CONTAINERISED PINE (*Pinus patula* Schlecht. et Cham) SEEDLINGS GROWN IN PINE BARK

Increasing demands for timber and timber products have resulted in the rapid expansion of containerised forestry seedling production. The lack of available information on pine seedling fertilization has forced nurserymen to use overseas recommendations which are based on peat and vermiculite as growing media, and not composted pine bark.

Six fertilization trials were conducted in plastic enclosed "tunnels" to provide fertilization recommendations and optimum foliar nutrient concentrations for *P. patula* seedlings grown in composted pine bark. It was concluded that pre-enrichment of the pine bark should include micronutrients, preferably in the form of Micromax®, a soluble micronutrient fertilizer, at 1000 $g.m^{-3}$. Pre-enrichment with lime was unnecessary for pine bark media since pine bark contains sufficient Ca and Mg. At an unlimed pH of 4.5, the availability of micronutrients was adequate for pine seedling growth. The addition of an N-P-K fertilizer and scheduling of nutrients, to correlate with seedling growth stage, are essential for maximum pine seedling production. Slow release forms (SRF) of N-P-K fertilizers were not beneficial, although their use may be warranted when the cost of SRF is similar to that of conventional fertilizers. Nitrogen in the form of NH_4^+-N at 80 $mg.\ell^{-1}$ was shown to produce the best quality *P. patula* seedlings. The addition of Si, in the form of pre-enriched silicate slags and Si nutrient solutions, reputed to improve growth by decreasing cuticular water loss and increasing plant rigidity, provided no added advantage to pine seedling growth under the trial conditions.

It was concluded that individual nurseries should formulate a nutritional programme for their own situation, based on these guidelines. Care should be taken when extrapolating these results to other substrates and it is recommended that the medium be analysed before a nutritional programme is decided on.

DECLARATION

I hereby certify that the research work reported in this dissertation is the result of my own original investigation, except where acknowledged.

Signed. .....

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CONTENTS

INTRODUCTION

Impending world-wide timber shortages and the growing scarcity of afforestable land are focusing the attention of researchers to ways and means of increasing forestry production per unit area. South Africa needs to double its existing yield of timber by the year 2010 without unnecessarily increasing the area under plantations (Roberts, 1990). The projected annual yield must increase almost 100% from 17 million m^3 to 30 million m^3 by 2010, while the area under plantation can only increase by 45% (Roberts, 1990). It was estimated, in 1995, that the total area of tree plantations in South Africa was 1 441 875 ha, of which 55% was afforested with *Pinus* species (Anon., 1995). Of this, 42% was *Pinus patula*, a species that was introduced into South Africa from central Mexico in 1907, and first planted at Cedara, Natal in 1914 (Wormald, 1975). Its superior rate of growth to that of other species is a great advantage, resulting in the higher percentage area planted.

As reforestation and afforestation in the world is mainly planted to conifers, the factors affecting mineral nutrition of conifer seedlings (notably *Pinus patula*) are of utmost importance, since this determines seedling quality and therefore field performance (Van den Driessche, 1992). Thus, by manipulating nutrition it is possible to influence the growth rate of seedlings and therefore govern the type of plant produced.

The need for the economical production of greater amounts of quality seedlings stimulated the change to specialised forestry nurseries, and the rapid expansion in containerised seedling production. The move to container seedling production revolutionised the forestry seedling industry. Prior to this seedlings in South Africa were grown in individual polyethylene sleeves or as bare-root seedlings, both using soil as growing media. The use of small containers resulted in the adoption of soilless media, such as pine bark, which gained wide acceptance.

In the USA and Europe peat-based media are used in most containerised seedling nurseries. However, in South Africa the

high importation costs of peat have resulted in most nurseries using composted pine bark, a cheaper and readily available waste product. This light-weight medium is favoured by growers as it is well drained, well aerated and difficult to overwater (Lea-Cox, 1989). It has also been shown to contain many essential nutrients that satisfy most seedlings' requirements (Brafield-Rolfe, 1992) and has fairly stable and predictable physical and chemical properties.

Relatively little information is available on the correct fertilization and irrigation procedures of containerised forest seedlings. That which is available, is American or European. Particular care is needed in interpreting nutrition results from other forestry areas of the world, where seedlings may require special treatments to withstand specific stress factors such as cold (Nelson, 1992). This has forced South African nurseries to use vegetable seedling recommendations, as research results are available for vegetables grown in pine bark (Van Schoor, Smith & Davis, 1990).

The production of high quality tree seedlings in containers involves the use of fertilizers (either as a pre-enrichment source of fertilization and/or as a nutrient solution), to promote growth in as short a time as possible. Maintaining optimum fertility in containers is complicated, owing to the small rooting volumes and generally low nutrient reserves of the rooting media (Brix & Van den Driessche, 1974). Nutrient levels tend to fluctuate because of frequent fertilization and irrigation which may lead to instances of accumulation, imbalance or deficiency of available plant nutrients (Timmer & Parton, 1984). Maintaining a constant, balanced supply of nutrients is therefore one of the most important criteria in the production of containerised seedlings. Thus, the nursery's aim is to utilize mineral nutrition to enhance the resources of the nursery, to produce planting stock of desired quality, to satisfy the objectives of management at minimal cost and without long-term deleterious effects on the nursery. To do so nurseries should have some understanding of the effects of mineral nutrient status on the physiological processes involved in seedling growth and

development (Van den Driessche, 1992).

The main objective of the research reported in this thesis was to provide norms from which nurseries can work when planning their nutritional programmes for containerised pine seedlings. It was hoped to highlight some of the "do's and don'ts" of pine seedling nutrition. Aspects of fertilization investigated include pre-enrichment (the pre-plant application of fertilizer to the medium) in the form of conventional and slow release fertilizers, and fertigation (the application of fertilizers in nutrient solution through the irrigation system). All trials were carried out using composted pine bark as the growing medium and *Pinus patula* as the indicator plant. This species was used as it is a major contributor to the forestry industry in South Africa and because research on its nutrition is notably lacking.

CHAPTER ONE

LITERATURE REVIEW

Most factors that affect containerised pine seedling nutrition are dealt with in this review. Aspects discussed, for a well balanced nutrition programme include: the chemical properties of pine bark media, mineral nutrient requirements of seedlings, and methods and types of fertilizer application. The physical properties of pine bark media are not discussed in this review, but can influence seedling nutrition.

1.1 CHEMICAL PROPERTIES OF PINE BARK MEDIA

Plants produced in containers have their roots confined to a limited volume of medium. To maintain adequate plant growth, frequent irrigation and fertilizer application are essential. With the importance of precise control of water and fertilizer in container-grown plants, a detailed knowledge of the growing medium's chemical properties is needed. Composted pine bark, in contrast to organic soils, represents a relatively undecomposed organic material in which the original bark structure is still apparent. Extrapolations between bark, and organic or mineral soils should be viewed with caution (Lea-Cox, 1989).

1.1.1 Nutrient Status of Pine Bark

The chemical content of pine bark should be one of the primary factors considered when using it as a growing medium, as this determines the necessary fertilizer regime. McGinnis & Parikh (1975) listed the contents of *P. taeda* bark as: neutral-soluble extractives (17.3%), alkali-soluble extractives (20.8%), lignin after alkali extraction (20.4%), hollocellulose (41.7%) and ash (0.7%). The organic constituents of bark, except for the neutral-soluble extractives such as tannins, will not be dealt with in this review. The elemental composition of bark is the fraction which contributes largely to the final quality of the pine bark medium and consequently influences fertilizer application and seedling growth. Results on the availability of

the indigenous nutrients in pine bark are often contradictory.

Porter (1973) found that the chemical composition of both softwood and hardwood barks was variable, due to chemical changes that occur after the cells have been formed. He warned that there is little point in making general comparisons between tree genera or species, as did Maggs (1985) and Bunt (1988). Because the bark nutrient content depends partly on the need of the tree which has produced the bark, surplus uptake from the soil, and the proportion of living versus dead tissue, Solbraa (1986) concluded that the concentration of elements in bark from the same tree species and geographical region is therefore fairly stable. For example, in composted Norwegian spruce bark, average concentrations of macronutrients are N 0.4%, P 0.5%, K 0.3%, Ca 1.0% and Mg 0.1%. With the exception of N and P, the concentrations of all plant nutrients in the bark are sufficient for the needs of micro-organisms and higher plants for a relatively long period of time. Magnesium, B, Cu and Mo concentrations may however be close to the minimum requirements for some plant species (Solbraa, 1986). However, Solbraa did not take into account whether these elements were available to plants.

Samples of raw bark from 15 and 25 year old trees of *Pinus patula* and *P. elliottii*, and from raw bark of 15 year old *P. taeda* trees in South Africa (Maggs, 1985), showed marked differences in mineral element concentration for different species and tree ages tested (Table 1.1). Generally, there was little difference in concentration of macronutrients between bark of the same species but of different ages. There were generally higher concentrations of micronutrients in the bark of younger trees of *P. elliottii* and *P. taeda* compared to older trees sampled, the reverse being evident for *P. patula*. Bark samples of *P. patula* contained higher concentrations of all elements, particularly Ca, Mn and Al. This may have been due to the fact that the percentage inner bark in *P. patula* is higher than for the other two species (Maggs, 1985).

An analysis by the Agricultural Development and Analysis Service

in the U.K., showed that coarsely pulverized pine bark had the following available nutrients : N - 0.3% (with 5 mg.kg^{-1} NO_3^-), P - 6 mg.kg^{-1}, K - 155 mg.kg^{-1} and Mg - 36 mg.kg^{-1}. Further grinding of the bark led to an increase in the availability of P to 18 mg.kg^{-1}, K to 210 mg.kg^{-1} and Mg to 60 mg.kg^{-1}, but had no effect on N (Aaron, 1972).

Table 1.1 Mineral element composition of different species of raw pine bark in South Africa as a percentage of total dry mass (after Maggs, 1985).

ELEMENT	*P.patula*		*P.elliottii*		*P.taeda*
	15yr	25yr	15yr	25yr	15yr
N %	0.38	0.33	0.31	0.30	0.30
Ca %	0.63	0.67	0.11	0.11	0.27
Mg %	0.16	0.15	0.05	0.04	0.08
K %	0.28	0.24	0.09	0.08	0.15
P %	0.04	0.04	0.03	0.02	0.03
Na %	0.01	0.02	0.01	0.01	0.01
Zn mg.kg^{-1}	32	32	8	9	39
Cu mg.kg^{-1}	4.6	4.7	3.4	3.1	6.4
Fe mg.kg^{-1}	2050	2275	1125	475	1700
Mn mg.kg^{-1}	166	299	60	39	137
Al mg.kg^{-1}	3633	3813	2280	1824	2106

Ashed pine bark contains only 0.2-0.3% nitrogen, with approximately 0.33 mg.ℓ^{-1} water extractable NH_4^+-N and 0.67 mg.ℓ^{-1} NO_3^--N (Pokorny, 1979). The mineral N content of bark is therefore low and remains at this level in spite of periodic fertilization (McNab, 1984).

Pokorny (1979) in the USA, found that southern ashed pine bark contained ±0.02% total elemental phosphorus and that water extracts contained 6.9-9.0 mg.ℓ^{-1} P. Having detected the presence of P in the leachate of milled bark, not fertilized with P, Yeager & Wright (1982) looked at the amount of indigenous P that was leached from pine bark. They found that after 6 weeks,

3.54mg of indigenous P had been leached from 35g of bark; this is equivalent to 16.7g of P that is leachable per m^3 of bark. The initial total ashable P was found to be 7mg per 35g, hence 50% of the indigenous P was leached. They attributed this to the fact that bark lacks Fe and Al oxides that temporarily fix P; hence the rapid leaching of P and the negligible amount bound by the bark and therefore the low P content in pine bark. Prasad (1980) contradicted this evidence by suggesting that P retention occurs mostly in the first 6 weeks after application and that it is considerable. Therefore, for all practical purposes, it can be assumed that most of the seedling's requirement for P must be met by the application of P fertilizers.

Various workers have reported that there are moderate amounts of potassium in pine bark. Ogden (1982) stated that K was found naturally in pine bark in only small amounts and that it was retained by the bark. He attributed its retention to cation exchange. Pokorny (1979) found the total K content of ashed bark to be 0.1% and the water-extractable content to be 6-28 $mg.\ell^{-1}$ K. Of interest is the following statement by Holmes, Coorts & Rosenfeld (1981), "The addition of K afforded no significant advantage to the plants grown in bark medium as indicated by the measured parameters. This may be attributed to the inherent K constituent of the bark medium". This statement was substantiated by Wright (1987) and Brafield-Rolfe (1992) who found that vegetable and forestry seedlings grown in pine bark with no supplementary K, were comparable to those on a typical K (150 $mg.\ell^{-1}$) feeding schedule. Thus, composted bark has been shown to contain relatively large quantities of inherent K, and possibly on decomposition, this K may be made available as a slow release source. This initial release rate may be sufficient to meet plant requirements, but after a period of time this could decrease and necessitate the addition of supplementary K in the liquid feed, as it is not known for how long this K would remain available.

The calcium content of ashed, aged pine bark was found to be 0.51% (Pokorny, 1979). Koch (1972) found the Ca content of *P. taeda* to be 0.214%. However, of greater importance is the

quantity of Ca available for plant growth. Pokorny (1979) working with a mixture of *P. taeda* and *P. echinata* bark reported a water-extractable concentration of 7.6 $mg.\ell^{-1}$. Starr & Wright (1984) reported a Ca concentration of 21-39 $mg.\ell^{-1}$ in pine bark and Neal & Wagner (1983) reported 40.6 $mg.\ell^{-1}$. Thus, the content of Ca in composted bark is high (McNab, 1984).

Pokorny (1979) found the total magnesium content of ashed pine bark to be 0.14%. The water-extractable Mg for bark that was not fertilized was reported as 1.6 $mg.\ell^{-1}$, which is insufficient for adequate growth. Neal & Wagner (1983) found it to be 5.9 $mg.\ell^{-1}$, therefore also reporting a low Mg status in pine bark. Starr & Wright (1984) reported that a pour-through extract of bark, receiving no fertilizer, contained 7 $mg.\ell^{-1}$ Mg and that bark alone supplies sufficient Mg for growth of holly. Pine bark has a low attraction for Mg, in the presence of large quantities of NH_4^+, K^+ and Ca^{2+} cations, because of competition for exchange sites (Ogden, 1982), and therefore the Mg content of bark can be low depending on fertilizers applied.

No references to sulphur contents were found in the literature that were specific to pine bark.

The micronutrient content of milled pine bark is given in Table 1.2. Pine bark is shown to contain most, if not all, of the micronutrients considered essential for plant growth, but their availability is uncertain (Lunt & Clarke, 1959). Ogden, Pokorny, Mills & Dunavent (1987) showed that the water-extractable content of B, Fe and Mn in bark is generally much less than the concentrations in Hoagland's solution, but not for Cu and Zn (Table 1.2). They proposed that this was due to excessive leaching of B, Fe and Mn and, that in the case of Zn, applied Zn appeared to be fixed in the bark medium. Copper has also been reported as the plant nutrient forming the strongest bonds with organic colloids and considered to be the least mobile of all the micronutrients (Ogden, 1982).

Control ash contents measured by Ogden (1982) showed that pine bark does contain sizeable quantities of Al, Fe and Mn,

indicating potential toxicity to plants grown in a pine bark medium. But this is not the case, as leachates showed that Al and Fe remained at tolerable concentrations in the water-extractable fraction. In fact, Pokorny (1979) found that Fe was not detectable (Table 1.2) and recommended soluble Fe additions to bark, as it appeared that Fe was fixed by the bark.

Table 1.2 Micronutrient analysis of milled southern USA pine bark used as a potting medium (Ogden *et al.*, 1987).

ELEMENT	TOTAL ELEMENTAL CONTENT ($mg.kg^{-1}$)	WATER - EXTRACTABLE CONTENT ($mg.\ell^{-1}$)			
		POKORNY (1979)	NEAL & WAGNER (1983)	OGDEN (1982)	HOAGLAND'S SOLUTION
B	9	0.15	1.10	0.17	0.50
Cu	77	0.17	0.33	0.12	0.02
Fe	790	*	0.61	1.10	5.00
Mn	119	0.01	0.90	1.30	0.50
Zn	112	0.06	0.36	0.30	0.05

* Not detectable

Ogden (1982) concluded that Mn at 1.30 $mg.\ell^{-1}$ was readily available from bark, in amounts comparable to those supplied by the nutrient solution (Table 1.2), as did Neal & Wagner (1983). This is in conflict with Pokorny (1979) and McNab (1984), who believed that extractable Mn is low in comparison with Hoagland's solution. Ogden (1982) also concluded that solution Zn levels in filtrates and leachates were adequate to high compared to solution culture standards. However, some supplementation of Zn would probably be required where the bark was cropped repeatedly, as the elemental bark content of Zn is not very high. Bark total elemental B content is relatively low compared to the other micronutrients. Boron is reported to be the essential element most liable to downward leaching in organic soils (Ogden, 1982),

which would explain its low extractability in pine bark. Little information exists on Mo availability in pine bark. Unlike other micronutrients, availability of Mo increases with pH.

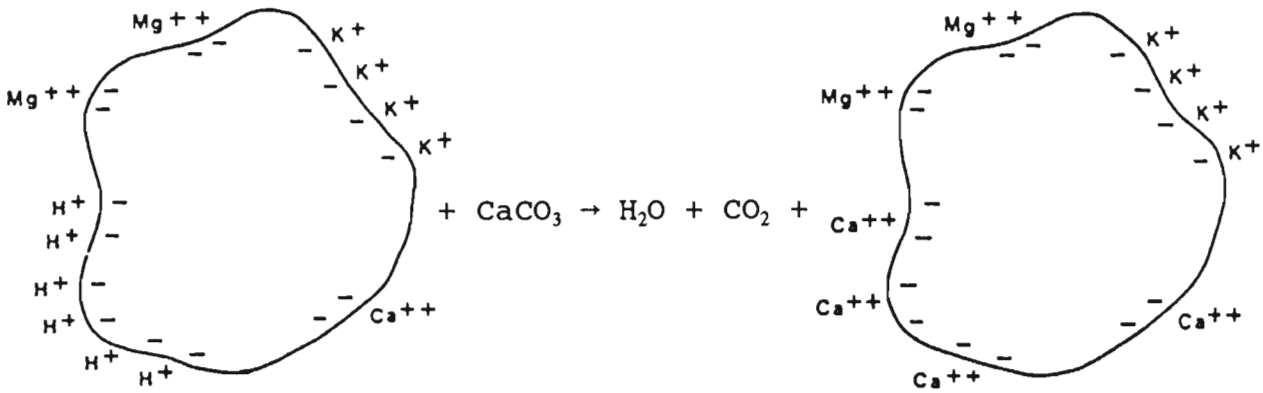
It may seem incongruous at first, but an initial low fertility level, as often occurs in composted pine bark, is actually a desirable attribute for growing media used in container nurseries (Landis, Tinus, McDonald & Barnett, 1990). An important benefit of low initial fertility in media, is that nurseries can completely control mineral nutrient concentrations in the growing medium solution through fertilization. Inherently fertile growing media or media amended with fertilizers make it impossible to completely control seedling nutrition.

1.1.2 Cation Exchange Capacity

One of the most desirable characteristics of a growing medium is the ability to store and hold essential nutrients (Handreck & Black, 1984) and to be able to maintain a supply of nutrients to the plant root zone (Joiner, 1983). This is an important factor affecting the fertility of growing media and is known as the cation exchange capacity (CEC). Cation exchange refers to the relatively weak attraction of cations in the medium solution to negatively charged sites on the organic matter complex, such as pine bark particles. The CEC can be defined as the sum of the exchangeable cations, measured in milliequivalents (meq), that a material can adsorb per unit mass or volume - the larger the number, the greater the nutrient-holding ability (Landis *et al.*, 1990).

Whilst much is known about the reactions of essential plant nutrients in mineral soils, considerably less is known about the fate of added nutrients in organic-based soilless media. The general structure of pine bark and its functional groups, and the similarities between these structures and those already investigated in the organic fractions of soils, suggests ways in which pine bark media may interact with added nutrients, in the nutrition of plants (Ogden, 1982). Each bark particle has a number of negative charges on its surface and when a fertilizer

is applied to the medium, the cations such as Ca^{2+}, Mg^{2+}, K^{+} and H^{+} are attracted to the bark particle. The cation exchange process is illustrated in Fig. 1.1 by showing the reaction which occurs when lime in the form of $CaCO_3$ is added to the medium.



acid medium + lime = neutral medium

Fig. 1.1 Diagrammatic representation of a bark particle showing the cation exchange process when $CaCO_3$ is applied to a medium (Bunt, 1988).

The number of negatively charged sites available for cation adsorption on organic colloids is pH-dependent, with elevated pH inducing greater adsorption because of the ionization of exposed hydrogen (Ogden *et al.*, 1987). Niemiera & Wright (1986) reported that changing the pH from 4 to 5 resulted in a 53% increase in the CEC of a pine bark medium. Thus, the CEC of a medium generally increases with pH. This increase in CEC, however, does not necessarily mean a greater availability of nutrients.

Cation exchange capacity has traditionally been measured on a mass basis for field soils, but when dealing with soilless container media, CEC per volume is more meaningful and is the generally accepted basis for growing media, such as pine bark. This is because of the relatively low bulk density of the media and the small volumes of the containers used (Ogden *et al.*, 1987; Bunt, 1988). Cation exchange capacity values for some typical horticultural growing media are compared in Table 1.3. Estimates

of CEC of various pine bark media are found in the literature. Bollen & Glennie (1963) reported a CEC of 39.3 meq.100 g^{-1} in Douglas fir bark and stated that bark contains phenolic acid polymers, which have neutralization equivalents ranging from 100 to 200 meq.100 g^{-1} (Ogden, 1982). Raw pine bark has a low initial fertility of ±8 meq.100 g^{-1} (Bunt, 1988), but with composting the CEC increases to +60 meq.100 g^{-1}, due to the breakdown of particles during composting and the resultant increase in surface area (Lea-Cox, 1989).

Table 1.3 Cation exchange capacities for some standard growing media (from Ogden *et al.*, 1987 & Landis *et al.*, 1990).

MEDIUM COMPONENT	CATION EXCHANGE CAPACITY (CEC)	
	MASS (meq.100 g^{-1})	VOLUME (meq.100 cm^{-3})
Sphagnum peat	180.0	16.6
Vermiculite	82.0	11.4
Perlite	3.5	0.6
Sand	1.0	1.6
Pine bark	52.6	15.3
Pine bark-sand(1:1)	8.0	7.8
Peat-vermiculite(1:1)	141.0	32.0
Peat-sand(1:1)	5.8	7.0
Peat-perlite(1:3)	11.2	1.1
Pine bark-perlite(2:1)	24.0	5.0

Hoitink & Poole (1980) proposed that small quantities of fine bark particles be retained in bark growing media, so as to increase CEC to acceptable levels without adversely affecting porosity. Thus, media containing coarse bark will have a significantly lower CEC than that containing fine bark. Brown & Pokorny (1975) reported that CEC increased from 1 to 13 meq.100

cm^{-3} as the proportion of pine bark in a bark-sand mixture increased from 0-100%. High CEC values are desirable for growing media, because they maintain a reservoir of mineral nutrients that supports plant growth between fertilizer applications and during periods of heavy rainfall. The CEC holds cations in equilibrium with the growing medium solution and this counteracts leaching and/or plant uptake, which can be very significant with the high irrigation rates used in most container tree nurseries (Wright & Niemiera, 1987). Generally speaking, the higher the CEC for a medium, the greater its resistance to nutrient leaching. Media with high CEC, which are able to selectively adsorb and release cations from the growing medium solution, are also able to buffer the seedling root system against sudden changes in pH or salinity (Landis *et al.*, 1990).

Pokorny (1987) found that bark particles have 43% internal porosity and this results in water being held and made available for plant growth. Airhart, Natarella & Pokorny (1978) confirmed that numerous sites for adsorption and absorption of water and nutrients exist within the pine bark particles, as well as evidence of small pores within internal cellular connections. The numerous small pores within the bark may, in part, account for the wettability problem encountered when bark is air and/or oven dried.

Foster, Wright, Alley & Yeager (1983), in an attempt to compare leaching of NH_4^+ and NO_3^- ions from pine bark, found that most of the leachable NH_4^+ and NO_3^- ions were removed from the pine bark after 120ml was applied, equivalent to 4 irrigations of 2.5cm (Fig. 1.2). Foster *et al.* (1983) also showed that an increase in pH increased NH_4 adsorption (pine bark has the capacity to adsorb 1.5 mg NH_4-N.g^{-1} bark). Prasad (1980) showed that the retention of N, P and K was noticeably higher for bark and wood shavings, than peats. He attributed this to a greater CEC. Brown & Pokorny (1977) have also demonstrated potassium retention in a bark-sand medium. They suggested that many exchange sites exist on the internal bark surfaces, and believed that these pores are relatively small and that the nutrient elements contained therein were not readily removed by water percolating through the pine

bark medium.

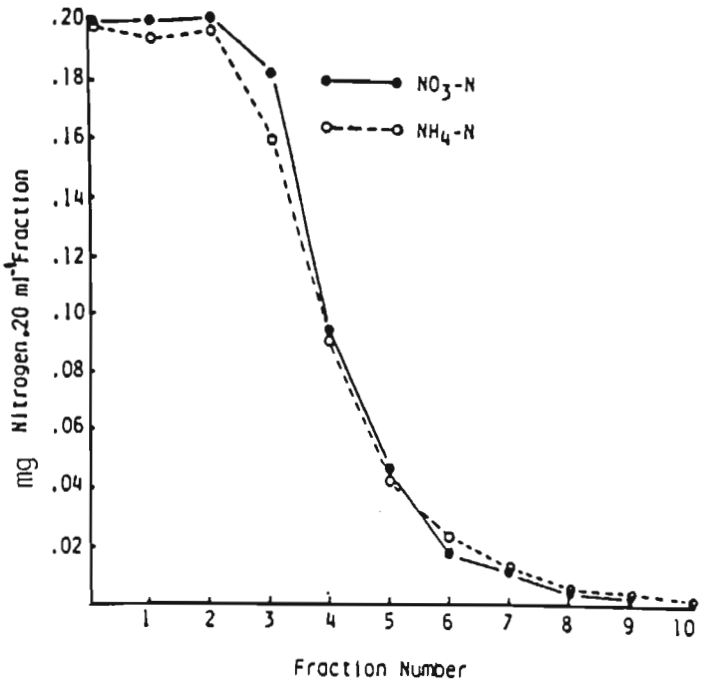


Fig. 1.2 Leaching of NH_4-N and NO_3-N ions from a column of unlimed pine bark after saturation with 200 mg N.ℓ^{-1} from NH_4NO_3 (Foster *et al.*, 1983).

Information on the retention of nutrients by pine bark media is of limited availability and certainty, but seedling nurseries would be well advised to take into consideration that bark-based mediums do definitely have a CEC, and to determine and plan fertilizer formulations and programmes accordingly (Prasad, 1980).

1.1.3 pH

The pH of the growing medium is one of the most commonly discussed, yet least understood, factors affecting mineral nutrition of container tree seedlings (Landis, Tinus, McDonald & Barnett, 1992). pH is a relative measure of the hydrogen ion (H^+) concentration expressed on a logarithmic scale and ranges from 0 (very acid) to 14 (very alkaline). In actual practice, the pH of a solution involves more than just H^+ or OH^- ions. In growing media solutions it is often a reflection of the activity of other ions, notably CO_3^{2-}, HCO_3^-, NH_4^+, NO_3^-, SO_4^{2-}, Al^{3+}, PO_4^{3-}, HPO_4^{2-}

and $H_2PO_4^-$ (Landis *et al.*, 1992). Obviously, many different ions in the soil solution have an effect on the pH reading. The final pH of a growing medium will depend on the proportion of the components, their original pH, and subsequent cultural practices, especially fertilization and irrigation.

The importance of pH on container seedling nutrition is the subject of considerable debate. Gingrich (1984) supported the widely held proposition that pH is the "most important aspect" of container seedling nutrition, whereas Whitcomb (1983) stated that pH has "little effect", as long as proper fertilization practices are followed. Tinus (1980) stated that, except at the extreme values where root injury may occur, pH does not directly affect seedling growth. One reason for this difference in opinion can be attributed to the type of growing media used. Some media, such as pine bark, are able to buffer pH changes more readily than others, and so pH measurements might not be the most important monitors of seedling nutrition. Also, in container nurseries where all essential minerals can be supplied with fertilizers, control of pH is less critical.

The principal cultural effect of pH is its influence on the availability of mineral nutrients, especially micronutrients. Several mineral nutrients can become unavailable or even toxic at extreme pH values (Landis *et al.*, 1990). Generally, as pH rises micronutrient availability declines; conversely, as pH declines micronutrients are more available to the plant (Ogden *et al.*, 1987). In a study of interactive effects of nitrogen source and liming practices on micronutrient availability in pine bark, Ogden (1982) found that liming (pH 5.5-6.5) reduced available Mn when NH_4-N and NO_3-N fertilizers were applied. Manganese may reach toxic concentrations in unlimed bark fertilized with either NH_4-N and NO_3-N and receiving micronutrient fertilizer supplying Mn (Hewitt, 1966). When urea was used as a nitrogen source, liming had no effect on Mn concentrations in water extracts.

Since the type of fertilizer used can influence the pH of the medium, it can therefore affect micronutrient availability. As a general rule, if NH_4 or urea is the dominant source of N, the

pH tends to decrease, but the use of NO_3 will lead to an increase in pH. Additions of limestone have been shown to stimulate nitrification (Niemiera & Wright, 1986), which leads to a decreased NH_4-N:NO_3-N ratio in the medium solution. Subsequent NO_3 absorption by plants results in an increased uptake of cations. High cation tissue content has been positively correlated with organic acid content, which leads to Fe immobilization (Mengel & Kirkby, 1982). Liming also increases medium solution HCO_3 levels, resulting in HCO_3 absorption, which can also lead to Fe immobilization - hence the term lime-induced iron chlorosis (Wright & Niemiera, 1987). The fact that most micronutrients are less available at high medium pH also adds to the complexity of lime-mediated growth inhibitions.

Freshly milled uncomposted South African pine bark is highly acidic, with $pH_{(H2O)}$ ranging from 3.5 to 4.5 (Maggs, 1985; Van Schoor *et al.*, 1990), as is bark in the USA (Bunt, 1988). Maggs (1985) recommended that bark be composted and limed to raise the pH. Pokorny (1979) reported pH values in untreated pine bark of 4.1-5.0, and that pine bark pH does not rise substantially with ageing, but with composting it increases to between 6.0 and 7.0. Brown & Pokorny (1975) found the pH for a pine bark mixture of *P. taeda* and *P. echinata* to be 4.1 and bark-sand mixtures to be 5.4. They recommended liming for optimum plant growth. Thus, in South Africa it is recommended that pine bark used as a growing medium be composted to raise the pH to ± 5. "Sour" bark, due to anaerobic composting, in which the pH has dropped below 4.0 is not usable since samples have been found to be extremely toxic and can kill all vegetation (McNab, 1984). As pine bark media generally have a low pH this can unfortunately result in the bacterial conversion of ammonium to nitrate being slowed, and plants suffering ammonium toxicity. This is because they take up and store ammonium-N which is often fatal, as ammonium, unlike nitrate-N, cannot be stored in excessive amounts.

Peterson (1982) has shown that the optimum pH range for nutrient availability of organic potting media is 4.0-5.2, except for Ca and Mg, which were less available below 5.2. Thus, there appears to be no advantage of liming organic container media to adjust

the pH above 5.0-5.5, if all nutrients are supplied to the medium solution in sufficient, non-toxic quantities. Experimental results often show that more vigorous growth and increased plant quality can be attained at pH values of 4-5 (Wright & Niemiera, 1987). More research is needed to investigate the interaction between species, media and lime additions.

1.1.4 Carbon to Nitrogen Ratio (C:N)

The stabilization of pine bark media by means of composting is affected by the presence of two key elements, carbon (C) and nitrogen (N). The C:N ratio represents the relative proportion of these two elements. Living organisms require available C to act as a source of energy, and both C and N for the building of protoplasm. This results in the conservation of N and the transformation of C in the bark to CO_2 and humic acids.

It has been determined that the ideal C:N ratio of fully composted bark is 10:1, as in humus, however this is hardly ever achieved (Mathur, Owen, Dinel & Schnitzer, 1993). For acceptable seedling or plant growth this ratio should be ± 30. Uncomposted bark has a C:N ratio of more than 100:1 (Wright, 1987). In the case of South African pines, this may be as high as 450:1 (Maggs, 1985). With composting this is reduced to about 40:1. If the cellulose (wood) content is high, the medium has an extremely high N demand and the final ratio drops to only 60:1 or higher (Gartner, 1981). Addition of nitrogen helps to adjust the C:N ratio. If full composting is achieved the C:N ratio falls to acceptable levels and the compost is stable and so has a low demand on added nitrogen.

Although the C:N ratio of softwood barks is high (200-100:1) in relation to some other organic materials, it can be shown that there is no simple relationship between this and the occurrence of nitrogen deficiency. Barks having similar C:N ratios do not necessarily decompose at the same rate (Bunt, 1988). In a study on the C:N ratio and pH of composting bark, Sterrett & Fretz (1977) concluded that the resultant C:N ratio was similar regardless of the initial N source used to compost. They also

concluded that it was important to add N during the composting process in order to narrow the C:N ratio prior to planting. It is also essential when fertilizing plants in bark, to allow for the often high demand created by the bark and microbial population, and still maintain sufficient N availability for healthy plant growth. Thus, when using a medium made from pine bark, it is important to match the degree to which the medium is composted with the amount of nitrogen fertilizer applied, to achieve optimal seedling growth.

1.1.5 Toxicities

Early indications of toxicity, resulting from the application of bark to croplands, were generally attributed to N deficiency due to the high C:N ratio of the bark and were found to be alleviated by the application of N fertilizers (Ogden, 1982). However, in some cases (depending on bark species used) toxicities still occurred after extra N treatments.

The degree of toxicity was and is found to depend upon several factors. These include the age of the bark, the season it was removed, the species of tree, the geographical location, and the length of time that the bark is composted. Bark from old trees (35 years old) is more toxic than bark from young trees (15 years old), with the lower bark of the tree being more toxic than the upper bark. Also, bark removed in winter is more toxic than that removed in spring (Bunt, 1988). In the Southern USA, Cobb & Keever (1984) found that fresh bark from *Pinus taeda* and *P. elliottii* had no detrimental effects on Japanese holly and euonymus. However, fresh bark from *P. taeda* grown in South Africa is toxic (Van Schoor *et al.*, 1990). Thus, in countries such as America, uncomposted bark is often used as a growing medium with no toxic effects, but in South Africa this is impossible as all bark media have to be composted in order to have no deleterious effects on plant growth.

Identification of the toxins present in softwood barks has been studied by many researchers (Bunt, 1988) and toxins in hardwood bark were identified by Still, Dirr & Gartner (1976). The toxins

present within the bark can be of organic or inorganic origin. The principal inorganic toxin is manganese and the organic toxins comprise the phenolic compounds, which include flavonoids, phenylpropanoids, tannins, melanins and lignins.

Phytotoxicity may also be due to other organic toxins, such as butyric acid, ethanol, acetic acid, lactic acid, formic acid and succinic acid, which can be produced during unfavourable anaerobic composting conditions when the polysaccharides of cellulose are broken down (Van Schoor *et al.*, 1990). All these toxins are phytotoxic to young seedlings and if present in the pine bark growing medium they can have detrimental effects on the seedlings.

1.1.5.1 Manganese

The manganese content of pine bark species used to produce growing media varies widely. Pokorny (1979) reported the total Mn content of ashed pine bark to be 119 $mg.\ell^{-1}$; Ogden (1982) 194 $mg.\ell^{-1}$; and Solbraa & Selmer-Olsen (1981) reported concentrations between 480 and 1070 $mg.\ell^{-1}$ for the bark of Norway spruce. The upper limit of exchangeable Mn for a bark compost, produced for use as a growing medium, is 200 $mg.\ell^{-1}$ according to Norwegian standards (Solbraa, 1986). The maximum concentration for Mn in plants is 500 $mg.\ell^{-1}$ (Solbraa & Selmer-Olsen, 1981). Any concentration above this may induce toxicity and reduce growth of sensitive plants. In South Africa water-extractable Mn is analysed as this is a more realistic measurement of what the plant can take up. This ranges from 0.01 to 5 $mg.\ell^{-1}$ in South African media (Jarvel, 1993). Handreck (1995) established that Australian media containing at least 1.6 $mg.\ell^{-1}$ 2 mM DTPA-extractable Mn provided an adequate amount for more than a year of plant growth. He suggested that up to ± 36 $mg.\ell^{-1}$ DTPA-extractable Mn would not be toxic to most plants growing at pH 6.0.

Pinus patula bark contains very high amounts of Mn in comparison to *P. taeda* and *P. elliottii*. Maggs (1985) found that the high concentrations of Mn in this bark were toxic to forestry

seedlings, but not to vegetable seedlings. He attributed this difference to the fact that lime was added to the bark used for vegetable seedlings. This reduced the available divalent Mn^{2+} form and he suggested that Mn toxicity can be overcome by adding lime during and/or after the composting phase. This raises the pH of the medium and precipitates out the manganese into its stable hydrated Mn dioxide ($MnO_2.H_2O$) form. Thus, correct composting procedures allow for the tie-up (i.e., it induces an oxidation to the stable hydrated $MnO_2.H_2O$); and leaching (Mn is easily leached from bark (Prasad, 1979)) and hence reduction in Mn availability, preventing a Mn toxicity from occurring. Liming also results in more Ca and Mg being available in the soil solution for plant uptake, i.e., the Mn competes with the Ca and Mg which depresses Mn uptake. Maggs (1985) also showed that by raising the Fe concentrations in the medium, Mn toxicity could be overcome. For this reason most bark media obtained from a supplier will be lime and Fe pre-enriched to combat Mn toxicity.

Manganese toxicity is a problem affecting seedling nutrition and different pine species may have varying resistances to large uptakes of Mn. Concentrations toxic to one species may therefore not be toxic to another, although growth reductions may occur.

1.1.5.2 Phenols

Phenolic compounds include a wide range of compounds which possess an aromatic ring bearing a hydroxyl component. Among the natural phenolic compounds, of which several hundred are known, the flavonoids and their relatives, the tannins, form the largest group (Moffitt, 1991). These tannins give fresh pine bark its characteristic smell and are the main phenolic compounds that cause toxicity in composted bark. They participate in the defence mechanisms in plants, i.e., they are effective in allowing plants to combat such factors as viral infection, various wounds to tissues and water deficits. They also influence seed germination, plant growth and flowering (Karolewski & Giertych, 1994).

Still *et al.* (1976) found that fresh bark of silver maple had a

high degree of inhibition on seed germination and compared the extract of this bark with 15 phenolic compounds. By using chromatography and spectral analysis they revealed the inhibitor to be similar to tannic acid at 0.45 R_F value, and both the bark extract and tannic acid at 0.45 R_F resulted in the same degree of rooting of mung bean. Bark that was aged for 30 days showed no inhibition of adventitious roots on mung bean. Yazaki & Nichols (1978) isolated the phytotoxic components of *P. radiata* bark. They found that the compounds that inhibited root and shoot growth were tannins with relatively low molecular masses, and that as they became more polymerized they showed less effect. The degree of phytotoxicity depended largely on the concentration of these compounds in the bark and whether they were soluble and appeared in the leachate. In further work, Solbraa (1979) found that while root growth could be stimulated by small concentrations of the phenol, pyrogallol, from spruce bark, at high concentrations growth was reduced and finally eliminated. The main effect was attributed to tannins, which constituted 12% of the fresh spruce bark. Aaron (1982) concluded that the growth inhibition of plants grown in bark was related to the monoterpene content of the bark. The bark from Norway spruce and Sitka spruce, each of which caused severe inhibition of growth, had 0.3% monoterpenes, whereas bark from Scots pine, Corsican pine and Japanese larch, all of which had little or no effect on growth, had less than 0.03% monoterpenes.

From the chemical analysis of fresh bark, Maggs (1985) showed that *P. elliottii* bark from 15 year old trees contained 8% tannins, which was almost double that of *P. taeda* bark (4.5%) and *P. patula* (3.7%). Perkins (1985) tested the effects of bark extracts on cucumber seed germination and found that water extracts from fresh *P. patula, P. elliottii* and *P. taeda* bark were highly phytotoxic, but extracts from composted bark were non-toxic.

Fortunately, tannins are water soluble and can be leached out of bark. It usually takes up to 12 weeks for the concentration to drop below 2% and be non-toxic (Van Schoor *et al.*, 1990). Tannins are also denatured by heat and polymerized by dilute

organic acids during active composting and this helps to reduce the soluble tannin concentration (Maggs, 1985). Thus, composting is the main means of removing or reducing this toxicity, as shown by Still *et al.* (1976) and others. However, it is not known if this is entirely due to leaching or polymerization during the composting or if both processes play a role to varying degrees.

The significance of phytotoxic compounds, present in composted bark, to the horticultural industry is considerable. Seedlings are either stunted or die. When a batch of pine seedlings grown in toxic bark is observed, they appear a blotchy yellow colour, i.e., not all seedlings in a tray appear to be affected (Jarvel, 1993).

1.2 MINERAL NUTRIENT REQUIREMENTS

The importance of mineral nutrition on both the quality and quantity of container tree seedlings cannot be overemphasized. Good mineral nutrition is fundamental to producing the target seedling which will survive outplanting in the field. The beneficial effects of adding "mineral" substances, such as lime or wood ash, to the soil to improve plant growth has been known for more than 2000 years. It was not until the 19th century, however, that Justus von Liebig proposed the "mineral element theory," which stated that certain elements are "essential" for plant growth (Landis *et al.*, 1992). It was only in the past 60 years that plant physiologists identified 14 chemical elements as being essential for the growth of higher plants. These elements are classified as six macronutrients, which plants use in relatively large amounts, and eight micronutrients, which are required in very small quantities (Welch, 1995). Among the former are nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, and among the latter are iron, manganese, zinc, copper, boron, chlorine, molybdenum and nickel.

Specific levels of nutrients for optimum forestry seedling growth can be difficult to determine, since the required concentration to be applied depends on a number of factors. Some of these are the inherent nutrient level in the growing medium and water,

fertigation frequency, seedling growth rate and the required degree of seedling hardiness (Nelson, 1992). There have been many investigations attempting to establish the nutrient requirements of container-grown tree seedlings. Direct comparisons between these experiments are difficult, due to differences in methodology and plant species used. Therefore, no attempt will be made to establish absolute nutrient levels for optimum growth, but rather guidelines for nutrient applications will be reviewed.

1.2.1 Nitrogen, Phosphorus and Potassium

The ideal nitrogen (N) level for conifer seedling growth has been the source of much discussion, with the levels prescribed in the literature showing considerable variation (Landis *et al.*, 1992). The Container Nursery Survey revealed that American nurseries used levels from 55-260 $mg.\ell^{-1}$ for the period of most rapid seedling growth (Landis *et al.*, 1992). However, the general trend has been to reduce total N levels from 200 to 100-150 $mg.\ell^{-1}$ during the rapid growth phase to control shoot growth and produce a more balanced, hardy seedling (Landis *et al.*, 1992). Scarratt (1986), Phillion & Libby (1984) and Will (1961) all found that 100 $mg.\ell^{-1}$ N produced the best seedling. Timmer & Parton (1984) found that 15-65 $mg.\ell^{-1}$ N (as NH_4^+) was optimal for red pine seedlings. However, the ideal N fertilization level will vary with the many cultural factors and between species.

Nitrogen is available in different organic (e.g., urea) and inorganic forms. The inorganic forms are applied more often. There are two different inorganic N ions that are taken up by plants: ammonium (NH_4^+) and nitrate (NO_3^-). Plants differ in their ability to acquire nitrate and ammonium from a medium, and in their tolerance of ammonium-N (Barker & Mills, 1980). The most favourable N form differs between species (Etter, 1969) and often changes with the age of the plant, nutrient availability, medium pH and with the carbohydrate content of the plant (Christersson, 1972). It is therefore difficult to say whether a plant prefers one N source or another, as this depends on many factors. Nitrate and ammonium are readily absorbed by plant roots.

However, the accumulation of NH_4 in the leaves is usually toxic, whilst plants are able to accumulate and transport high concentrations of NO_3 without major toxic effects (Van Schoor *et al.*, 1990). Theoretically, ammonium should be the preferred form. It is used more efficiently in the plant, for it need not be reduced before being incorporated into organic compounds. Ammonium is also subject to less leaching and denitrification losses than nitrate (Barker & Mills, 1980). However, toxic effects can be easily encountered when only the ammonium form is applied.

Nitrogen deficiency symptoms include chlorosis and stunting of needles, increasing with severity of deficiency. In the most severe cases, needles are short, stiff, yellow-green to yellow (Van den Driessche, 1992). Chlorosis usually appears first on the lower needles, since N is a mobile element within the plant and is transferred to the younger foliage (Lyle, 1969; Duryea & Landis, 1984). Nitrogen stunting is usually easy to diagnose, and subsequently corrected, because deficient seedlings respond rapidly to applications of N fertilizer.

Chemical analysis of tree seedling tissue shows that, in comparison to N (± 1.5%) and K (± 1.0%), the amount of P (± 0.2%) in tissue is low (Will, 1961; Landis *et al.*, 1992). Nevertheless, phosphorus (P) nutrition is an important aspect of macronutrient nutrition. Phosphorus is present in relatively large quantities in the protoplasm and nucleus of the cell. It is a component of the nucleic acids and sugar phosphates and is essential to seedling metabolism (Bunt, 1988). Phosphorus is associated with rapid root growth and concentrations of P tend to be higher in the roots (Will, 1961). Plant roots preferentially absorb P in the form of the $H_2PO_4^-$ ion, and to a lesser extent HPO_4^{2-}. This uptake is pH dependent, with the rate of P uptake decreasing with increasing pH. The $H_2PO_4^-$ ion is readily available at pH < 7 (Mengel & Kirkby, 1982).

Foliar P deficiency symptoms are extremely variable between species, with colour ranging from dull green-yellow for the younger needles, to blue-purple for the older needles (Lyle,

1969; Duryea & Landis, 1984). The entire seedling is often stunted, although leaf size may or may not be reduced (Landis *et al.*, 1992). Phosphorus toxicity can occur and symptoms include blackening of the shoot tips, the lower leaves become pale and necrotic and are then often shed (Bunt, 1988). Toxicity is aggravated under low K conditions, since K promotes the conversion of inorganic P to nucleic acids and phosphoproteins (Wright & Niemiera, 1987). Excess P results in micronutrient deficiencies, as it binds with the micronutrients making them unavailable, e.g., P-induced Zn-deficiency (Gibson, 1990) and P-Fe reactions which can lead to deficiency in the form of Fe chlorosis (Wright, 1987).

The potassium ion (K^+) is absorbed by plants at a high rate, inferring that it is a strong competitor in the uptake of other cations (Mengel & Kirkby, 1982). It has been shown to be necessary for the function of enzymes that control carbohydrate and nitrogen metabolism, and K acts as an osmotic regulator in the water relations of plants (Bunt, 1988). High concentrations of foliar K are associated with better water economy, i.e., reduced transpiration which increases drought tolerance (Duryea & Landis, 1984). Thus, plants suffering from K deficiency show a decrease in turgor, and under water stress easily become flaccid (Mengel & Kirkby, 1982). Potassium nutrition may also play a role in the development of frost hardiness and better winter survival (Ingestad, 1979). He concluded that high K concentrations and a balanced ratio of nutrients resulted in frost-free stock. Duryea & Landis (1984) showed that frost hardiness in container seedlings was more closely related to the K:N balance than to the concentration of a single nutrient. A lower K:N ratio resulted in hardier seedlings. However, Christersson (1972) proved that there is no correlation between K content and frost hardiness. According to Mengel & Kirkby (1982), plants adequately supplied with K are also more resistant to diseases. This may relate to the fact that K promotes the development of thicker cell walls, preventing disease attack.

Foliar K deficiency symptoms are variable between species, but usually short, chlorotic foliage with some green at the base are

obvious symptoms. In severe cases, purpling, tufting and spiralling occurs around the terminal bud. Browning and necrosis may also occur (Lyle, 1969; Duryea & Landis, 1984). Flaig & Mohr (1992) found that *P. sylvestris* seedlings, without additional K, approached K deficiency symptoms within 10 days after germinating when endosperm reserves were depleted. Thus, K appears to be a crucial element during the juvenile period.

Timmer & Parton (1984) concluded that 35-95 $mg.\ell^{-1}$ P and 25-115 $mg.\ell^{-1}$ K were the optimal nutrient concentrations for red pine seedling growth. Will (1961) grew *P. radiata* in water cultures and found that 1 $mg.\ell^{-1}$ P and 10 $mg.\ell^{-1}$ K were sufficient to maintain good growth in seedlings. These concentrations are low, but are similar to soil solution concentrations found in the field (Will, 1961). Tinus & McDonald (1979) recommended that 60-100 $mg.\ell^{-1}$ P and 100-150 $mg.\ell^{-1}$ K be applied for constant fertilization of container tree seedlings. In the studies of Wright (1987) and Lea-Cox (1989) good seedling growth in pine bark was achieved with 10-20 $mg.\ell^{-1}$ P and 50 $mg.\ell^{-1}$ K. Brafield-Rolfe (1992) found that eucalypt seedlings needed at least 30 $mg.\ell^{-1}$ P to achieve optimum growth.

1.2.2 Calcium, Magnesium and Sulphur

Calcium (Ca) is required by plants in regions of active cell division for the formation of cell walls, where it occurs as calcium pectate, having effects on membrane properties and thus disease control (Bunt, 1988). It also plays a role in the transport of carbohydrates and amino acids within the plant, and in the development of new roots (Bunt, 1988). It is not mobile within the plant, making up 0.5% of plant tissue (Landis *et al.*, 1992). Calcium is taken up mainly in the ionic (Ca^{2+}) form, but Ca-chelates can also be absorbed (Mengel & Kirkby, 1982). Calcium uptake is inhibited by Mg^{2+}, K^{+}, Na^{+}, Al^{3+} and NH_4^{+}, and has been shown to be stimulated by NO_3^{-} and phosphates (Mengel & Kirkby, 1982). Calcium deficiency symptoms result in chlorosis, stunting and minimal growth at all meristems. In severe cases, terminal buds may die or fail to elongate (Van den Driessche, 1992). Browning and mortality of root tips is also common, as is

resin exudation in extreme deficiencies (Lyle, 1969; Duryea & Landis, 1984).

Magnesium (Mg) is one of the elements required for the formation of chlorophyll. It is also regarded as a carrier of P within the plant and is important in metabolism (Mengel & Kirkby, 1982). Magnesium is absorbed by plants in the Mg^{2+} form and the content of dry conifer seedling tissue is 0.11-0.2% (Will, 1961; Landis *et al.*, 1992), with higher concentrations of Mg in the roots than in the shoots (Will, 1961).

Cation competitive effects during plant nutrient uptake are of particular importance for Mg, as these can lead to Mg deficiency, i.e., high NH_4^+, K^+, Ca^{2+}, Mn^{2+} and P can induce Mg deficiency (Mengel & Kirkby, 1982; Richardson, 1992). Thus, a shortage of Mg also produces a characteristic chlorosis, which is almost always strongly patterned, i.e., needle tips are yellow or orange, with a yellow middle and dark green base, occurring first in the older leaves (Lyle, 1969). This can be followed by necrosis in severe cases (Duryea & Landis, 1984), which soon leads to a decrease in photosynthesis and vegetative growth (Bunt, 1988). Lyle (1969) found that roots are more affected than shoots, when Mg is deficient.

Sulphur (S) is an important part of amino acids from which proteins are formed, and so the total S content in seedling tissue is 0.1% of the dry matter (Landis *et al.*, 1992).. Plants are unable to utilize S in the elemental form as it must be absorbed by the roots as sulphate (SO_4^{2-}) (Bunt, 1988). Sulphur deficiency is similar to that of N, and is characterised by foliage that is chlorotic to pale yellow-green, with the youngest leaves most affected. Stunting of leaves and eventual necrosis occurs in severe cases (Landis *et al.*, 1992; Van den Driessche, 1992). Another feature of S deficient tissue is the accumulation of NO_3-N (Mengel & Kirkby, 1982). However S deficiency is rare, due to fertilizers such as superphosphates and sulphates often being incorporated into the media.

In an experiment growing containerised red pine seedlings, it was

found that 30-60 mg.ℓ^{-1} Ca and 15-35 mg.ℓ^{-1} Mg produced seedlings of maximum dry matter (Timmer & Parton, 1984). Will (1961) found this to be 10 mg.ℓ^{-1} for Mg. Tinus & McDonald (1979) stated that 80 mg.ℓ^{-1} Ca, 40 mg.ℓ^{-1} Mg and 60 mg.ℓ^{-1} S were the general concentrations applied for constant liquid fertilization of tree seedlings, i.e., the application of dilute fertilizer every time the seedlings are irrigated.

1.2.3 Micronutrients

The eight elements which plants require in very small quantities are known as micro- or trace-elements (Welch, 1995). Although they are present in most plants at the following concentrations: Fe 100 mg.kg^{-1}, Mn 50 mg.kg^{-1}, Zn 20 mg.kg^{-1}, B 20 mg.kg^{-1}, Cu 6 mg.kg^{-1}, Mo 0.1 mg.kg^{-1}, Cl 100 mg.kg^{-1} and Ni 0.1 mg.kg^{-1} (Landis *et al.*, 1992; Welch, 1995), they are equally essential for plant growth. Tinus & McDonald's (1979) fertilizer recommendations for several container-grown forestry seedlings are as follows: 4 mg.ℓ^{-1} Fe, 0.5 mg.ℓ^{-1} Mn, 0.05 mg.ℓ^{-1} Zn, 0.5 mg.ℓ^{-1} B, 0.02 mg.ℓ^{-1} Cu and 0.01 mg.ℓ^{-1} Mo. No Cl is recommended because it is present in most irrigation sources. Neither is Ni because no work has been done on pine seedling nutrition. However, much remains to be learnt about the physiology of micronutrient absorption, translocation and deposition in plants, and about their functions in plant growth (Welch, 1995).

Micronutrient deficiency symptoms are extremely variable between nutrients and species, and are difficult to diagnose because symptoms are often a result of an imbalance between several micronutrients. It is recommended that tissue nutrient analysis be used to confirm visual symptoms, although foliar concentrations of some micronutrients, notably Fe, can be higher in symptomatic seedlings, as almost all the Fe in a seedling is in the roots (Will, 1961).

Iron (Fe) deficiency is a relatively common disorder in horticultural nurseries (Bunt, 1988) and is frequently referred to as Fe-chlorosis or lime-induced-chlorosis. Diffuse chlorosis (interveinal chlorosis of leaves while veins remain green) is the

first symptom of minor Fe deficiency, and this condition is usually expressed first in the newer foliage. In severe cases, the entire seedling becomes chlorotic (bright yellow to white) and stunted, and at this stage the disorder is almost impossible to correct (Landis *et al.*, 1992; Van den Driessche, 1992). Despite research, the function of Fe in plants is not clearly understood. It is not a constituent of chlorophyll but it is essential for its formation. It is a constituent of enzymes, such as peroxidases and catalases (Bunt, 1988), and has a function in biological redox systems (Welch, 1995). Iron is present in the medium as either Fe^{3+} (ferric ion), Fe^{2+} (ferrous ion) or as Fe-chelates and has to be reduced to Fe^{2+} by the plant root before it is absorbed (Bunt, 1988). The uptake of Fe is considerably influenced by other cations (Mengel & Kirkby, 1982). Thus, deficiencies can readily occur in situations where there is low aeration and soil temperature; wet conditions; low Fe supply; high Ca and bicarbonate causing high pH; high P, Zn, Mn, K, Mg and Cu and a high NO_3:NH_4 ratio (Bunt, 1988).

Copper (Cu) is involved in respiration and oxidation-reduction reactions in plants, functioning in several of the enzyme systems, and is concerned with lignification, disease resistance and pollen viability (Welch, 1995). It also accumulates in organs of active metabolism, seeds, young leaves, shoots and buds and is absorbed by the plant as Cu^{2+}. Plants deficient in Cu are low in ascorbic acid oxidase, a Cu-containing enzyme (Bunt, 1988). Deficiency is evident by needles which are twisted spirally and droop at the end of the seedling, with yellowing or bronzing of needle tips (Will, 1961; Van den Driessche, 1992). The deficiency is also compounded by high concentrations of P, Mn, Zn and NH_4. Turvey, Carlyle & Downes (1992) showed the existence of competition between Cu and Mn in *P. radiata* seedlings, illustrating that Cu deficiency can be magnified in the presence of normal concentrations of Mn in the plant. However, Cu deficiency is rarely encountered as it is found as impurities in fertilizers, fungicides and in tray dips (Ogden *et al.*, 1987). Heale & Ormrod (1982) showed that Cu toxicity in *P. resinosa* resulted in chlorotic needles and a wilted appearance of the seedling. Roots were reddish brown with few laterals but no

distinct growth defects were measured. Copper toxicity is reduced by iron chelates and Mo, as Cu is necessary for the utilization of Fe (Bunt, 1988).

Manganese (Mn) is a constituent of certain enzyme systems concerned with respiration, nitrogen metabolism and the transference of phosphate (Welch, 1995). It is only absorbed by plants in the Mn^{2+} form and is relatively immobile in the plant. Manganese also affects the form of Fe present in the leaves. When Mn is deficient, the Fe is oxidised from the ferrous to the non-available ferric form and this can be deposited in the leaf veins (Bunt, 1988). Deficiency is similar to Fe deficiency, with chlorosis of the foliage (Landis *et al.*, 1992; Van den Driessche, 1992). Deficiency in *P. radiata* has been shown to result in short needles with yellow and dead tips, with limited needle retention on the tree (Turvey *et al.*, 1992). High K, Mg, Cu and Zn concentrations cause Mn uptake to be reduced, as do the applications of iron chelates, NH_4 and P, thereby preventing Mn toxicity (Bunt, 1988).

Zinc (Zn) plays a part in metabolism, respiration, enzyme activation, and membrane integrity. It is essential for auxin and gibberellic acid production (Mengel & Kirkby, 1982), only being absorbed as Zn^{2+}. In Zn deficient plants extreme stunting of the foliage occurs, with tufting and rosetting, followed by tip dieback in extreme cases (Will, 1961; Landis *et al.*, 1992). McGrath & Robson (1984a) observed that the first symptoms of Zn deficiency were chlorosis and deformation of the apical primary leaves. The concentration of Zn in the new growth was lower than in older foliage, suggesting that Zn may be immobile in *P. radiata* seedlings. Consequently, concentrations of Zn in young tissue would provide the best indication of a seedling's Zn status. Zinc availability is reduced at high pH, by applying $MgCO_3$ and when P, N, Fe and Cu concentrations are high (Mengel & Kirkby, 1982; Bunt, 1988). McGrath & Robson (1984b) attributed this interaction to the dilution of Zn within the seedling as a result of growth, and not due to P and N impeding the absorption or utilization of Zn within the plant.

Boron (B) is one of the few elements that can be absorbed by plants in various ionic forms, viz. H_3BO_3, $H_2BO_3^-$, HBO_3^{2-} and BO_3^{3-} (Van den Driessche, 1992), with the leaves accumulating the most B (Mengel & Kirkby, 1982). It is associated with several functions in the plant, e.g., cell wall formation and stabilization, membrane integrity, stomatal regulation, carbohydrate utilization, transport of sugars, auxin activity and xylem differentiation (Bunt, 1988; Welch, 1995). Dugger (1983) proposed that the primary role of B is in the biosynthesis of lignin, and that B deficiency leads to increased levels of phenolic compounds, i.e., B has a function in phenol metabolism (Welch, 1995). Deficiency is evident by chlorosis and necrosis of the terminal bud, which leads to the conclusion that there is no re-utilization of B from older to younger plant parts (Dugger, 1983). High amounts of P and K can suppress B uptake, as can the application of organic nitrogenous fertilizers which reduce availability by temporary biological lockup, and by a rise in media pH during the first stages of mineralization (Gupta, 1979; Bunt, 1988). Boron toxicity occurs chiefly under two conditions: via the irrigation water or due to accidental application of excess B when treating B deficiency (Gupta, 1979). The ratio of toxic to adequate levels of B is smaller than that for any other nutrient element, thus correct application of B is essential (Gupta, 1979).

Molybdenum (Mo) is required by plants, in the MoO_4^{2-} form, for electron transfer reactions, nitrogen fixation, ureide metabolism, sulphate oxidation and protein synthesis (Welch, 1995). It is also required for the reduction of NO_3^- to NH_4^+ within the plant before proteins can be formed (Bunt, 1988). Thus, plants receiving NO_3^- are more likely to show Mo deficiencies, than plants receiving the NH_4^+ form. The uptake of Mo is greater in the presence of NO_3-N than NH_4-N and is enhanced by added phosphate (Mengel & Kirkby, 1982). High concentrations of sulphates, Cu, Al and Mn can reduce Mo uptake by plants (Bunt, 1988). Often liming is enough to prevent Mo deficiency, as Mo availability increases with pH. In Mo deficient plants, foliar chlorosis occurs followed by necrosis, beginning at the tip (Van den Driessche, 1992). Lyle (1969) did not observe deficiency

symptoms for Mo in *Pinus taeda*.

No chlorine (Cl) deficiency symptoms have been noted for tree seedlings, as chlorides, the ionic form in which Cl is absorbed, usually occur in sufficient quantities in the irrigation water and as impurities in fertilizers (Van den Driessche, 1992). It is thought that Cl plays a role in photosynthesis (Mengel & Kirkby, 1982), disease resistance, stomatal regulation, reactivity of enzymes, charge compensation and osmo-regulation (Welch, 1995). Chloride may replace NO_3^- or SO_4^{2-} and consequently affect seedling growth (Bunt, 1988).

Nickel (Ni) was added to the list of essential micronutrients in 1992, the first addition since Cl was adopted in 1954 (Welch, 1995). Researchers have found that Ni helps plants get maximum benefit from other nutrients, notably N and Fe. Without Ni, roots cannot absorb Fe properly and urea can build up to harmful concentrations in the leaf tips. Thus, Ni plays a role in urea and ureide metabolism, Fe absorption, seed viability, N fixation and reproductive growth (Welch, 1995). Nickel is common in most soils as Ni^{2+}, so this finding will only have relevance to plants grown in soilless media (Welch, 1995).

1.2.4 Others

Elements, other than the micronutrients, such as silicon (Si), sodium (Na) and cobalt (Co) are considered to be essential for certain plant species, but current scientific evidence does not permit the addition of these to the list of essential elements for all higher plants (Welch, 1995). Other elements such as aluminium (Al) are not required, but they can greatly influence the nutrition of pine seedlings, especially when planted out in the field (Hutchinson, Bozic & Munoz-Vega, 1986). In the near future, with advanced technology, other elements may well be discovered to be essential (Welch, 1995).

Despite the fact that silicon (Si) is the second most abundant element in the earth's crust, it is largely in an unavailable form for plant use (Tisdale, Nelson & Beaton, 1985). The form in

which Si is taken up by plants is monosilicic acid, $Si(OH)_4$ (Barber & Shone, 1966; Jones & Handreck, 1967). Plant species take up different amounts of Si (Jones & Handreck, 1967), which is not re-distributed within the plant (Wutscher, 1989). Why this occurs and what the mechanism of Si uptake is, are interesting questions about which little information is available (Mengel & Kirkby, 1982).

With the increasing use of soilless media for plant growth, the role of Si in plant nutrition needs careful re-evaluation, since its full range of effects are unknown. The evidence for Si benefits is controversial and may relate to the remedial effects of Si on nutrient imbalances or other forms of stress. As it is difficult to grow plants in the total absence of Si it is difficult to demonstrate that Si is an essential element (Okuda & Takahashi, 1964).

1.3 NUTRIENT ADDITIONS

Fertilization, probably more than any other cultural practice, with the possible exception of irrigation which results in nutrients being leached, controls both the rate and type of growth in container tree seedling nurseries, i.e., seedling growth can be manipulated during the various growth stages (Landis *et al.*, 1992). Thus, a fertilization programme for a container tree seedling nursery should be designed to maintain specific concentrations of the essential nutrients in the growing medium, keep them in balance and should allow necessary nutritional changes during the growing cycle. The medium used, the type of fertilizer applied, and the grower's management practices should be borne in mind.

1.3.1 Method and Type of Addition

There are several approaches used by nurseries to alleviate the nutrient deficiency problem inherent in container-grown tree seedlings. According to Handreck & Black (1984) there are three basic ways to apply fertilizer in a container nursery:

1. Top dressing solid fertilizers onto the surface of the

growing medium, i.e. post-plant applied.

2. Incorporating a conventional or slow release fertilizer (SRF) into the growing medium, i.e. pre-enrichment.
3. Injecting a liquid fertilizer solution into the irrigation water, i.e. fertigation.

In North American container tree seedling nurseries, liquid fertilizer is the most common fertilization technique, followed by incorporation of slow release fertilizers (Landis *et al.*, 1992). The Container Nursery Survey revealed that, although none of the nurseries used incorporated fertilizers exclusively, 26% used them in combination with liquid fertilizer injection. Top dressing generally is never done, as it is impossible due to the small top opening of containers. Fertilization in South Africa is similar, with conventional fertilizers being used more often than SRF, and nearly always being used in combination with liquid fertilizers. Thus, it is normal to use combinations of these methods.

Deciding which fertilizers to use in container tree seedling nurseries should be based on one criterion alone - seedling response to the existing cultural and environmental conditions (Landis *et al.*, 1992).

1.3.2 Pre-enrichment

The most recent ideas, regarding the best method of nutrient supply to a bark medium, involve the pre-enriching of bark prior to planting with as many of the required nutrients as possible, in the form of conventional or slow release fertilizers. The balance is then made up with a dilute nutrient feed (Wright, 1987; Lea-Cox, 1989; Brafield-Rolfe, 1992). The following is recommended for the pre-enrichment of pine bark used to grow pine seedlings: 3 $kg.m^{-3}$ of dolomitic lime, 5 $kg.m^{-3}$ of single superphosphate and 0.3 $kg.m^{-3}$ of trace elements, followed by an N-P-K liquid feed (Van Schoor *et al.*, 1990). Smith (undated) recommended: 0.45 $kg.m^{-3}$ N, 0.15 $kg.m^{-3}$ P, 0.20 $kg.m^{-3}$ K and 0.30 $kg.m^{-3}$ of fritted trace elements.

Incorporating fertilizers into the medium has certain advantages

in container nurseries. No specialized fertigation equipment is necessary. Costs involved in constantly mixing and applying liquid fertilizers are avoided, and mineral nutrient levels can be maintained during wet periods when irrigation is unnecessary and nutrient leaching is a problem (Landis *et al.*, 1992). However, there are also drawbacks to pre-enriching growing media with fertilizers. It is impossible to control the concentration and balance of nutrients in the growing medium solution. It is often difficult to obtain even distribution of fertilizer particles throughout the medium, and proper incorporation often requires extensive mixing which can result in particle size breakdown or compaction of the medium (Landis *et al.*, 1992).

One of the advantages of applying fertilizers, is that seedling growth can be precisely controlled through all phases of seedling development, but this is sacrificed by fertilizer incorporation. However, incorporation of fertilizers into the media is necessary in nurseries that lack irrigation systems or nutrient injectors.

The most common types of conventional fertilizers used for incorporation, contain nutrients that are relatively insoluble and do not leach readily, such as P, Ca and Mg. Superphosphate, calcitic and/or dolomitic lime are therefore often pre-enriched into the growing media.

Other fertilizer types that are commonly mixed into media include slow release fertilizers (SRF) or controlled release formulations. There are numerous types of SRF. Coated water-soluble fertilizers which include Osmocote®, Osmocote Mini®, Ficote®, Nutricote® and sulphur-coated urea products, such as Zipp/Nitran® and Enspan® are one group of SRF (Elston, 1992). The rate of nutrient release is controlled by irrigation frequency and medium temperatures, as well as the nutrient formulation; except for Osmocote® which is only dependent on soil temperature (Anon., undated(a)). Crowley, Maronek & Hendrix (1986) found that the best pine seedling growth and mycorrhizal formation was obtained with the (21-7-14) 8 to 9 month release Osmocote® fertilizer, at 4.5 $kg.m^{-3}$ application rate. Other types of SRF are the commercially available inorganic fertilizers of

low solubility, such as MagAmp®, for which the recommendation is 6 $kg.m^{-3}$ for seedlings (Anon., undated(b)). The organic fertilizers of low solubility are represented by the urea-formaldehyde fertilizers, such as Agriform® tablets, Nitroform® and Nitrocta®, and isobutylidene diurea (IBDU). The nutrient release from these fertilizers is controlled by the type of growing medium, medium pH, temperature and micro-organism population (Sanderson, 1987). Urea-formaldehyde has been tested under nursery conditions but was too expensive to use commercially in tree nurseries where cost considerations are of major importance (Loxton & Donald, 1987). Other slow release organic fertilizers include bone meal, hoof and horn, and dried blood. Rock phosphate (e.g., Langfos®), a naturally occurring product, and sewage sludge can also be used as SRF (Hawgood, 1988).

Micronutrients are often pre-enriched into the medium. Examples of SRF include: fritted micronutrients (such as FTE 504®), Micromax® (a soluble sulphate), Esmigran® (which consists of a mixture of micronutrients adsorbed on clay particles), and several brands of chelated micronutrient fertilizers, such as Sequestrene®, Fe-EDTA, Fe-DTPA, etc. Chelates are organically bound forms of the metal micronutrients (Fe, Mn, Zn and Cu) that prevent these ions being chemically complexed by other elements such as P, Ca, Na and HCO_3. Prasad (undated) recommended that the following amounts per m^3 be added to all bark mixes:

21.2g $CuSO_4$	14.2g $MnSO_4$	2.4g $Na(MoO_4)$	11.0g borax
35.4g $FeSO_4$	14.2g $ZnSO_4$	50.0g chelated Fe	

While there are many SRF formulations, the resin-coated materials, Osmocote® and Nutricote®, are the most popular in containerized conifer nurseries in North America (Hicklenton & Cairns, 1992). Donald (1973) concluded that the use of SRF in South African forest nurseries is unlikely to be great because of the cost and unpredictable release rates. Nutrient release rates of SRF are controlled by factors such as, temperature, moisture content and micro-organism activity, that are beyond the control of the nursery manager. Thus, further research is needed to develop a SRF that has a release rate that parallels plant

nutrient requirements and that can be controlled to a certain extent by growers.

1.3.3 Fertigation

The practice of adding soluble chemicals to the irrigation water has steadily gained in popularity since it was first used in the 1930's (Van den Driessche, 1992). Almost all container tree seedling nurseries have a fertigation programme (Landis *et al.*, 1992). The benefits of this technique include precise control of both the concentration and balance of all 14 nutrients, the ability to change the nutrient solution at any time, and a very low chance of over-fertilization and resultant salt injury. Unfortunately, fertigation is often wasteful when the areas between containers, such as aisles and roadways, receive the nutrient solution. However, fertigation is recommended whenever possible (Van den Driessche, 1992).

Fertilization frequency ranges from every day (weak solutions of 1.0-1.5 $mS.cm^{-1}$) to once or twice a week with stronger solutions of 2.0 $mS.cm^{-1}$ (Van den Driessche, 1992), with 1 or 2 applications being applied daily. Many nurseries schedule fertilizers and apply varying strengths and ratios of nutrients at different times in the growth cycle. Different nurseries use and recommend different fertilizer formulations and regimes, there being almost as many recipes as nurseries.

1.3.3.1 Timing of fertilizer application

The proper timing of fertilizer application to small seedlings conserves fertilizers and more importantly, minimizes the imposition of environmental stresses (Marshall, 1981). Traditionally growers delay the first application of fertilizer until the germinant has become established, i.e., 4-8 weeks after sowing in cold climates (Landis *et al.*, 1992) and 3-7 weeks in warmer climates (Da Costa, 1995 pers. comm.). The reasons are that damping-off fungi are stimulated by fertilizer, and concentrated fertilizer solutions may "burn" the seedlings. Another reason is that it is believed that the seed endosperm

contains enough nutrition for initial growth and establishment. Carlson (1983) stated that newly germinated seedlings take up few mineral elements until 10-14 days after germination. Marshall (1981) found that additions of nutrient solutions shortly after germination of red pine did not stimulate elongation or biomass accumulation of roots or shoots. Barnett & Brissette (1986), however, reported that a delay in initial fertilization can have a considerable effect on seedling development. A three week delay can decrease loblolly pine dry mass by nearly 20% (Fig. 1.3).

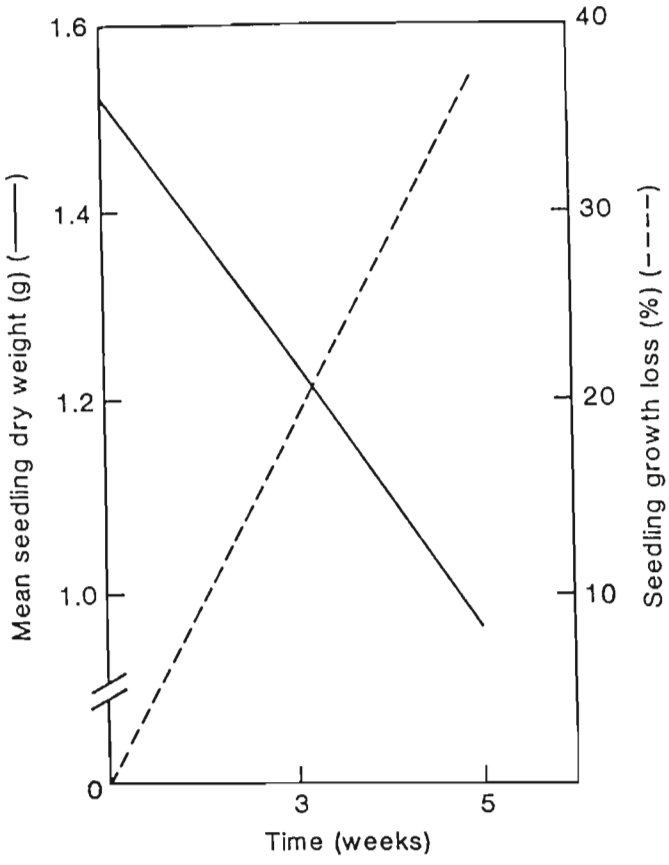


Fig. 1.3 A delay in the initial application of liquid fertilizer caused a substantial growth loss for loblolly pine seedlings (modified from Barnett & Brissette, 1986).

Edwards & Huber (1982) state that some nurseries in Canada begin liquid fertilizer applications, with high P formulations, at 1 week after sowing to stimulate root development. Many others incorporate a small amount of lime or slow release "starter" fertilizer into the growing media to support the germinant until liquid fertilizers are applied (Brix & Van den Driessche, 1974). Etter (1969) found that although high N or P concentrations did

not increase root growth of white spruce during the first 6 weeks of growth, a fertilization programme using 50-60 mg.ℓ^{-1} N produced significant increases in foliar and total seedling dry mass.

Observations made by Barnett & Brissette (1986) indicate that the timing of the initial fertilization application should be re-evaluated and that the "starter" fertilizer practice may have some merit if the crop is on a short rotation, as it may stimulate growth without harmful side effects. If a short growing period is not critical, delay until germination occurs may be beneficial from a disease management viewpoint.

1.3.3.2 Fertilizer scheduling

Nutritional schedules for tree seedlings have been an area of much research (Troeng & Ackzell, 1988). It is generally agreed that correct fertilization practises should be designed to provide the seedlings with the correct amount of nutrients according to their size and stage of development. There are however, three different ways to apply liquid fertilizers, viz. constant, periodic and exponential fertilization. The application of dilute fertilizer each time the crop is irrigated, for most of the active growth period, is known as constant fertilization (Mastalerz, 1977). Periodic fertilization consists of applying a more concentrated fertilizer solution according to some fixed schedule, such as once a week or every other irrigation, also for most of the seedling growth period. The Container Nursery Survey found that 64% of nurseries used periodic fertilization, 25% preferred constant fertilization and the remaining 11% scheduled fertilization based on the monitoring of crop development or growing medium nutrient levels (Landis *et al.*, 1992). Examples of periodic and exponential fertilization schedules are given in Table 1.4.

Mullin & Hallett (1983) compared the constant and periodic fertilization techniques and concluded the following advantages for constant fertilization: regular flushing of the growing media prevents salt build-up, nutrient levels in the growing media can be adjusted quickly, no over-fertilization since the applied

solution is the precise concentration for ideal growth, and medium nutrient concentrations are returned to target specifications with each fertilizer application. The disadvantages of constant fertilization are slightly higher fertilizer and labour costs, and problems of disposing of fertilizer solution runoff. Landis *et al.* (1992) therefore recommended that constant fertilization be applied in tree nurseries. Gingrich (1984) stated that periodic fertilization is becoming less popular in container nurseries, because of the large fluctuations in the nutrient levels and thus electrical conductivity (EC) in the growing media.

Table 1.4 Periodic and exponential liquid fertilizer application schedule for the USDA Forest Service Nursery (adapted from Myers, 1987).

Seedling growth phase	Timing (weeks)	Type of fertilizer	Fertilization frequency
germination	0-2	H_3PO_4	every other irrigation
juvenile	3-4	7-40-17	every other irrigation
exponential	5-10	20-7-19	every other irrigation

Exponential fertilization is the third way of applying liquid fertilizer. It is normally applied in conjunction with constant and/or periodic fertilization (Table 1.4), and consists of adjusting nutrient concentrations for the various growth stages during seedling development, inducing steady-state nutrition (Ingestad, 1979). Three growth stages can be recognised, the establishment phase (which is emergence and the cotyledon stage), the rapid growth phase (when seedlings grow in height at an exponential rate) and the hardening phase (which begins when seedlings have set their terminal bud and shoot growth ceases, but collar diameter and root growth increase). Therefore, Mullin & Hallett (1983) recommended a "starter" fertilizer of moderate

N (50 mg.ℓ^{-1}) during the establishment phase, a "grower" fertilizer of higher levels (100 mg.ℓ^{-1}) during the rapid growth phase and a "hardener or finisher" of low N levels (25 mg.ℓ^{-1}) during the hardening phase. Basically, the "starter" has low concentrations of N and higher concentrations of P and K and is applied ± 2 weeks after germination for approximately 4-6 weeks. The "grower" has significantly higher N concentrations and is applied throughout the major growth period, while the "hardener" provides high concentrations of P and K to maintain seedling colour and nutrient reserves (Van den Driessche, 1992). All formulations normally contain a full spectrum of the essential elements. Some growers do not apply fertilizers during the hardening period, only water. Miller & Timmer (1994) have suggested that high-dose fertilization, to build nutrient reserves before field planting, may also promote early plantation performance. The practice, termed nutrient loading, increases internal nutrient concentrations without changing total dry mass, giving rise to what is known as luxury consumption. The nutrient-loaded seedling exhibits greater growth and increased nutrient content after planting compared to conventionally fertilized seedlings. Nutrient loading must, however, be done with care to avoid nutrient toxicity. Exponentially rising fertilizer addition reduces this risk by gradually exposing plants to higher nutrient additions (Miller & Timmer, 1994).

In South Africa, however, Mondi's pine fertilizer schedule involves applying no fertilizer until 20% of the seed has germinated. This is 3 weeks in summer and 8 weeks in winter. Agrofert Blue® [3:1:2(42)] is then applied for the next week at 0.75 mS.cm^{-1}, followed by Agrofert Orange® [1:2:1(44)] at 0.75-1.2 mS.cm^{-1}, until the seedlings are ready to be hardened off. Only water is applied for the hardening period, which is done two weeks before the seedlings are dispatched. Agrofert Blue® and Orange® contain trace elements and the Orange® is applied for 5 days, followed by 2 days of watering to leach any salt build-up (Da Costa, 1995 pers. comm.).

Scarratt (1986) reported that special "starter, grower and finisher" fertilizers showed no significant improvement over

standard fertilization of jack pine seedlings in containers, as did Troeng & Ackzell (1988) using Scots pine seedlings. However, Timmer & Armstrong (1987) showed that an N rate that was gradually increased from 5 to 125 mg.ℓ^{-1} N over the fertilization period produced better red pine seedling growth, particularly root growth, compared to conventional fertilization. The results indicate that superior seedlings can be grown successfully at low concentrations of nutrient solution, applying only one-quarter of the fertilizer dose conventionally used for container seedling production. Imo & Timmer (1992) showed that seedlings had consistently higher N accumulation, matched by dry matter production, which reflected higher fertilizer-N uptake efficiency throughout the growing season, when exponential nutrient additions were applied. They observed that conventional fertility regimes caused nutrient dilution in plants with time and that nutrient uptake did not match growth in the seedlings, resulting in nutrient stress at varying stages of seedling development. Exponential fertilization has other benefits, such as less chance of salinity build-up, and it has also been found to be more effective in producing conifer seedlings with the desired characteristics for out-planting performance, than fixed fertilization regimes (Timmer & Armstrong, 1987; Troeng & Ackzell, 1988; Timmer & Miller, 1991; Miller & Timmer, 1994). Timmer & Miller (1991) found that despite smaller seedling size, the lower shoot:root ratio, higher root reserves and enhanced drought avoidance of seedlings produced by exponential fertilization, under limited irrigation, suggested better drought and nutritional pre-conditioning for out-planting performance. On the negative side, exponential fertilization schedules are more complicated to compute, and the applications are more time consuming than conventional liquid fertilization (Timmer & Armstrong, 1987). Nevertheless, the biological and economic advantages associated with exponential fertilization far outweigh the operational disadvantages (Timmer & Armstrong, 1987).

However, Nelson (1992) stressed that an exponential or relative rate of fertilizer addition could be detrimental to the seedling. He stated that common practice in South Africa is to provide seedlings with a relatively high concentration of N to obtain

maximum shoot growth. This is followed by a low N, high P programme to harden seedlings and boost root growth. Once seedlings have reached desirable sizes for planting they are held at this growth stage by totally withholding water and nutrients, or reducing the amounts of these. This can cause root die-back and lack of vigour in the seedling, resulting in unacceptable mortality rates in the field (Nelson, 1992). Therefore, a balance is needed between the forester's need for a hardy seedling and a vigorous seedling which will transplant well and grow rapidly.

1.3.4 Nutrient Balances

A healthy seedling must be well supplied with all the necessary nutrients in the correct proportions (Lavender, 1984), and so the relative proportions of the various mineral nutrients to one another in the growing medium solution is of vital importance. Will (1961) and Jones (1983) stated that the nutrient concentration ratios are more important than the absolute concentration of any one element. The balance between the various mineral nutrients is biologically important because an excess of certain ions in the medium solution may affect the availability of other nutrients, and the ionic balance affects the pH of the medium solution. Nutrient balance can be assessed in terms of ratios between nutrient concentration values. The ratios not only reflect nutrient balance, but are often less affected by growth dilution and aging processes than nutrient concentrations. Hence their use expands the usefulness and flexibility of plant diagnosis (Van den Driessche, 1992).

Steiner (1980) states that most plants will grow well in one universal nutrient solution if certain ratios of cations and anions are followed. He has designed a "universal nutrient solution" that is based on relative cation and anion ratios, total ionic concentration and pH (Jones, 1983). One of the most widely used nutrient balance theories for tree seedling culture is based on the works of Ingestad, who set the ratios of all nutrients in relation to N (Table 1.5). Ingestad (1979) proposed "nutrient ratios" for several conifer and hardwood seedlings

(Table 1.5), along with the actual nutrient concentrations for all mineral nutrients at 100 and 200 mg.ℓ^{-1} N. The nutrient proportions are fairly consistent between different species.

Table 1.5 Comparison of nutrient ratios for Douglas fir and complete nutrient level at two N concentrations (Ingestad, 1979).

Nutrient	Nutrient ratios	Fertilizer nutrient levels	
		100 mg.ℓ^{-1} N	200 mg.ℓ^{-1} N
N	1.00	100	200
P	0.30	30	60
K	0.50	50	100
Ca	0.04	4	8
Mg	0.05	5	10
S	0.09	9	18
Fe	0.007	0.7	1.4
Mn	0.004	0.4	0.8
Zn	0.0003	0.03	0.06
Cu	0.0003	0.03	0.06
Mo	0.00007	0.007	0.014
B	0.002	0.2	0.4
Cl	0.0003	0.03	0.06

The recommended ratios of the three principle macronutrients (N-P-K) also vary between the three growth phases. Barnett & Brissette (1986) stated that for the first several weeks the N:P ratio should be greater than or equal to 1, and the P:K ratio should be less than 1. Hahn (1978) recommended an N-P-K ratio of 1:5:1 during early seedling growth and a 3:1:1 ratio during the rapid growth phase. Nelson (1992) stated that as a general rule-of-thumb, N:P:K:Ca:Mg:S in the ratio of 3:1:5:3:1:1 should produce acceptable seedlings. Whitcomb (1984) suggested that an

Fe:Mn ratio of 5:1 and an Fe:Cu ratio of 10:1 are necessary to maintain maximum plant growth. Recent fertilizer experiments, however, cast some doubt on the need for special fertilizers during the different growth phases (Landis *et al.*, 1992). Starr & Wright (1984) investigated the effect of Ca and Mg ratio on growth. They concluded that as long as the applied amounts of Ca and Mg were sufficient for growth, the ratio of Ca:Mg was of no consequence.

The question of mineral nutrient balance in fertilization solutions is one of the most confusing aspects of plant nutrition. Much of the published research appears contradictory and so no hard and fast recommendations for the best nutrient balance can be made. In the meantime, nurseries should try to develop fertilization programmes that work under their own cultural regimes (Landis *et al.*, 1992).

CHAPTER TWO

GENERAL PROCEDURES

2.1 NURSERY METHODOLOGY

2.1.1 Structures

All experimentation, except for Experiment 2 (lime trial), was carried out at the University of Natal in Pietermaritzburg during 1992 and 1993. Experiments were located in 30 x 8m tunnels at the Controlled Environment Research Unit of the Agricultural Faculty, and orientated so that their length lay in a N-S direction. The tunnels used were covered in U.V. stabilised polyethylene film (180μm thickness) and their temperatures regulated by drawing air in through a wet wall for cooling, with fan heaters for heating. The one tunnel was heated with additional hot water pipes laid on the floor beneath the trays during the cold winter months. Day/night temperature set points were 16/25°C and the humistat was set at 70% relative humidity.

Experiment 2 was duplicated and put in tunnels, covered with Uvidek® plastic, at Mondi (Mountain Home, Hilton) and SAPPI (Richmond) seedling nurseries. Temperatures experienced at both nurseries were very similar, with Mondi having a range of 6°C - 32.8°C and SAPPI 4°C - 30.1°C during the duration of the experiment.

2.1.2 Containers

The two types of seedling trays used were SAPPI and Unigro® trays. SAPPI trays, which are white plastic trays, contained 49 cavities per tray. They were cleaned by means of pressure injected water before being filled. The round cavities, of 80ml volume, contain five plastic ribs on the inside to encourage roots to grow downwards and not spiral within the cavity (Fig. 2.1). The Unigro® trays, which are also plastic but black, contained 128 removable plastic plugs. These were not washed,

just shaken free of debris before being used. They were designed for Mondi, to ensure easy handling of seedlings in the nursery and to ensure that trays taken into the field are 100% full. Unigro® plugs are square-pyramidal shaped plugs with volumes of 60ml (Fig. 2.1).

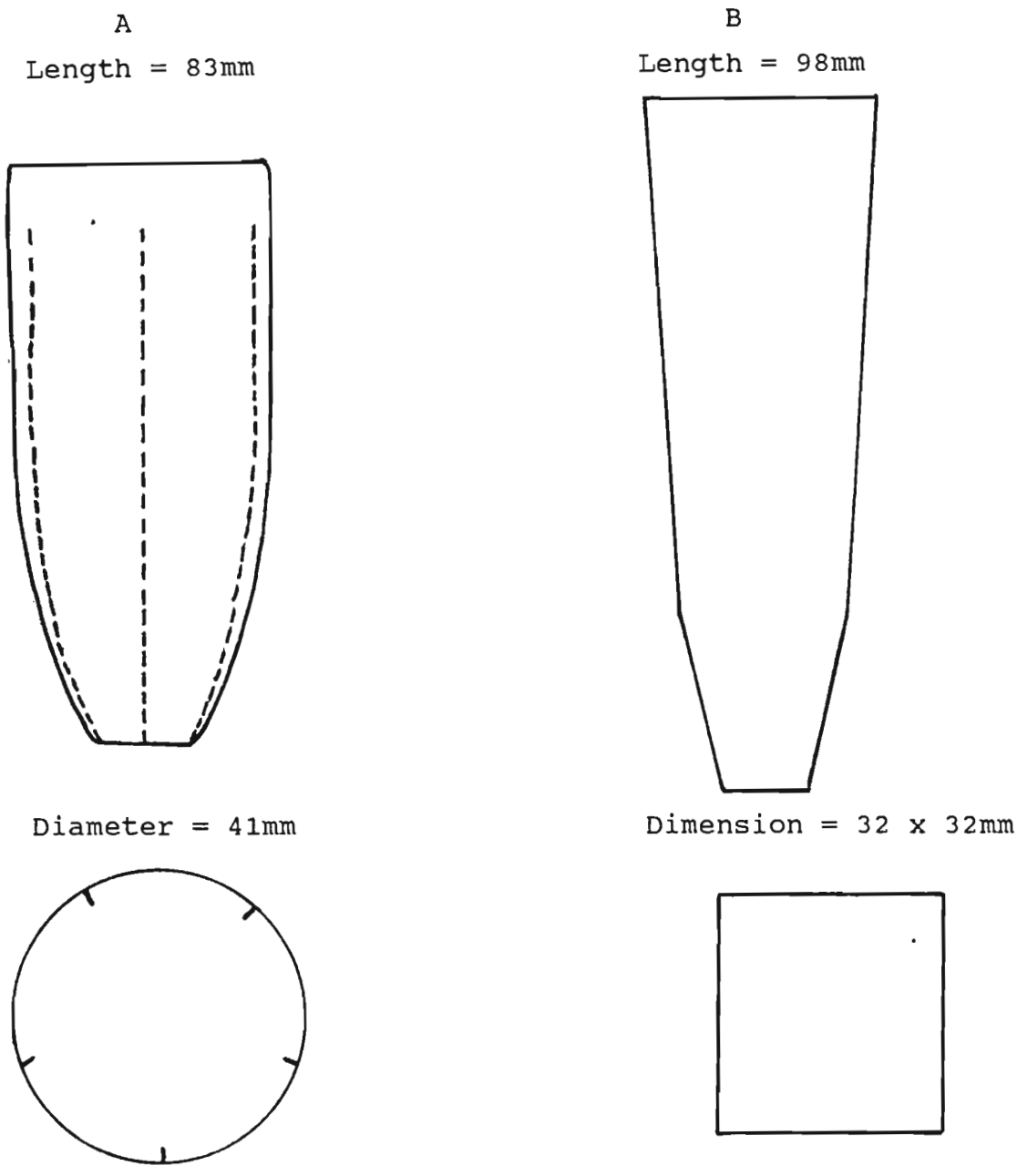


Fig. 2.1 Plan and longitudinal views of the plugs associated with the two types of trays. A = SAPPI plug, B = Unigro® plug.

Seedling trays were placed on metal racks 50-100cm above the ground to facilitate drainage and air-pruning of roots, and one

tray was used per treatment for all experiments.

2.1.3 Medium Preparation

Composted pine bark, consisting of *P. patula, P. elliottii* and *P. taeda*, was used in all experiments. Experiments 1, 2, 3 & 4 (nitrogen, lime, lime-micronutrient and slow release trials) used 8mm "fine seedling mix" bark from SAPPI (Mandini), and Experiments 5 and 6 (silicon trials) were prepared with 6mm "wattle medium" bark from Gromed (Cramond). Bark obtained from these producers was not pre-enriched with nutrients. Trials planted at Mondi (Exp.1, 4, 5 & 6) were filled with medium put through the infra-red machine, to pasteurise the bark and so hopefully kill harmful micro-organisms.

All experiments involved a certain amount of fertilizer pre-enrichment (Section 2.1.5). This was achieved by mixing predetermined amounts of fertilizer with pine bark, for 5 min in a concrete mixer to ensure even distribution throughout the medium. Trays were then filled with bark and shaken slightly to settle the medium.

2.1.4 Sowing and Germination

Experiments 1 (nitrogen), 4 (slow release), 5 (silicon pre-enrichment) & 6 (silicon nutrient feed) were planted at Mondi and Experiments 2 (lime) & 3 (lime-micronutrient) at SAPPI. Slightly different procedures were involved when planting.

In all trials, except Exp.3, seed was pre-germinated and sown mechanically. Experiment 3 was sown by hand as the planter was not in operation. Seed was obtained from either SAPPI or Mondi and pre-germinated for ±48 hr in a muslin bag, that had been placed in an aerated water bath kept at 70°C. The planting machines had automatic dibblers and sowed 2-3 seeds per plug, which were then capped with fine pine bark to cover the seed. Trays were watered with warm water. At Mondi, trays were put into a germination room at 26-27°C and stacked in a pyramid for 4-12 days. At SAPPI, trays were stacked in a block, but left in

the shed under black plastic for 11-27 days. Trays were brought to the University when transport was available. They were thoroughly watered before transporting, to ensure the least seed disturbance.

When the seedlings were approximately 2-3cm high they were thinned to one seedling per plug. If necessary seedlings were blanked into empty plugs.

2.1.5 Nutrition and Irrigation

Trials were irrigated with water only, until all seedlings had germinated. Fertilization then commenced approximately 4-8 weeks after sowing. In all experiments, except Exp.1, 2 & 6, a nutrient solution feed (conductivity 1.0 $mS.cm^{-1}$) containing 3:1:3 (38) was applied in the irrigation system, either through the boom or by microjets placed above the trays. For Exp. 1 & 6 the nutrient solutions were stored in 80ℓ black plastic drums and applied by hand with a watering can. A wire frame covered in plastic was used to prevent spray overlap into the adjacent treatments (Plate 2.1).

Plate 2.1 Application of nutrient solution to *Pinus patula* seedlings using a plastic covered frame to avoid overlap.

In Exp.1, three fertilizers (Calmag®, urea and NH_4NO_3) at five N application rates (0, 40, 80, 120, 160 $mg.\ell^{-1}$) were applied. Experiment 6 compared two Si fertilizers (Silchem-K® & Fertigro-sil®) at four rates (0, 25, 50, 100 $mg.\ell^{-1}$ Si), with a constant application of 80 $mg.\ell^{-1}$ NH_4-N. Experiment 2 (duplicated trial at Mondi and SAPPI), compared five lime fertilizers (dolomitic lime, calcitic lime, Calmag®, Langfos® & Calmafos®) at six rates (0, 2, 4, 6, 8, 10 $kg.m^{-3}$). The trial was then left to be irrigated under Mondi or SAPPI'S fertilization schedules. Mondi applied 1:2:1(44) at 0.5 $mS.cm^{-1}$ and SAPPI applied a combination of Hortichem Blue® and Orange® at 0.5 $mS.cm^{-1}$. The percentage nutrient content of all fertilizers used in the experiments is given in Appendices 1 & 2.

Applications were 1-3 times a day depending on the ambient temperatures. At each irrigation, sufficient nutrient solution was applied to ensure some leaching, to reduce salt build-up. The seedlings were not hardened before harvesting, except Exp.2, so as not to allow confounding of plant tissue analysis. Experiment 2 was hardened because one of the objectives of the trial was to compare the fertilization timing and scheduling programmes of the two nurseries.

2.1.6 Management Procedures

The conductivities of the leachates from all experiments, except Exp.2, were monitored regularly. The method implemented was a slight variation of the pour-through (PT) or leachate procedure (Wright, 1984). This entailed applying a nutrient solution to the trays, when the medium was already saturated, and collecting the displaced solution. When approximately 50ml had been collected, in a plastic bag tied to the base of the plugs, fertigation was stopped. Solutions were then analysed, and trays leached with water if the conductivities measured were too high.

The PT method was applied as it is a quick and easy way to determine a medium's nutrient availability, i.e., the medium is not handled and there is no danger of rupturing SRF particles, causing erroneously high nutrient readings. It has also been

found to be as accurate as other nutrient extraction procedures (Wright, 1984).

Weeding and scraping off of algae on plug surfaces were undertaken when necessary. Herbicides were cautiously applied twice to weeds growing beneath the racks.

2.2 SAMPLING AND STORAGE

Due to cost considerations, pine bark and seedling samples were each "pooled" for analysis. Pine bark, taken at the end of trials from 20 or 25 plugs, and milled shoots from 20 or 25 seedlings in each treatment were combined into a single sample for either pine bark or plant analysis. A subsample of the thoroughly mixed composite sample was analysed. Variations in the number of chemical analyses performed for each trial were due to cost considerations and decisions taken on the worthiness of each analysis.

2.2.1 Pine Bark

Pine bark samples, from bark left over from each treatment, were taken at the start of all experiments. Final bark samples were taken from each treatment at the termination of all trials, except Exp.5. Once the treatments had been harvested, root plugs were shaken free of medium, and the media for each treatment pooled for analysis. Samples of each treatment were kept in sealed plastic bags and put into a cold room at 5°C, until analysed for nutrient content.

2.2.2 Seedlings

Once seedlings had reached optimum transplanting size they were harvested. Samples of 4-7 month seedlings were randomly selected from the tray's centre rows, allowing 1-3 border rows around each treatment. Samples consisted of 20 or 25 seedlings. Shoots were examined for visible deficiency or toxicity symptoms and then separated from the roots, by cutting just above the top lateral root. Stem height (HT), measured from the shoot end to the

terminal bud or where the growing point is a rosette; collar diameter (CD), measured with a digital vernier calliper ±10mm from the shoot end; and shoot fresh mass (SFM) were measured. In some trials the number of lateral shoots and buds were also noted because it has been proposed that the greater the number, the better the seedling quality (Nelson, 1992). Roots were washed clean of pine bark and patted dry with paper towel, before root fresh mass (RFM) was recorded.

For research purposes, the treatments showing the best growth and maximum dry matter accumulation were considered to be the best treatments. However, these treatments will not necessarily give the best performance when planted out in the field.

The shoots and roots were placed into separate brown paper packets and dried in a Labotec forced draft oven at 65-70°C for 72 hr. This temperature was chosen as temperatures less than this may not be sufficient to remove all the moisture, and temperatures above 70°C can result in nitrogen volatilization (Donald, 1992). The shoot dry mass (SDM) and root dry mass (RDM) were then recorded. The dried shoots were then cut up and each treatment from Exp.1, 2 & 3, milled through a Kinematica mill, using a 1mm sieve. Milling ensured that plant tissue was reduced to a particle size suitable for laboratory analysis, and that composition uniformity within samples was attained. The dried, milled samples were then stored in sealed plastic bags until analysed.

High correlations between shoot fresh mass (SFM) and shoot dry mass (SDM), and between root fresh (RFM) and root dry mass (RDM) were found. Thus, only dry mass results are shown and discussed in all instances.

2.3 ANALYSES

2.3.1. Pine Bark

Many mineral soil nutrient extraction methods, based upon weak acid or organic extractants, are available and have been applied

to soilless media. However, methods based upon water extraction of nutrients may represent the actual levels of nutrients in medium solution more accurately, and thereby provide a better management tool for maintaining optimum nutrient levels in solution (Wright & Niemiera, 1987). Soilless media, such as pine bark, have also been found unsuitable for analysis by procedures prescribed for mineral soils, i.e., pine bark should not be dried out completely or sieved prior to analysis (Handreck & Black, 1984).

The procedure described by Warncke (1975), which involves using water as an extractant, is the saturated soil extract method. Variations of this method are widely used for testing organic and soilless media. Sonneveld, van den Ende & van Dijk (1974) developed an extraction method with a ratio of 1:1.5 medium to water volume. This method has been successfully used in The Netherlands for evaluating nutrient element status of organic media for 20 years. The results obtained compare favourably with other previously used procedures that are difficult to perform in the laboratory.

Pine bark extracts were obtained, from all experiments, using a modified "Quick" version of the 1:1.5 volume method of Sonneveld & van Elderen (1994) (Appendix 3). The moisture content of the bark was adjusted visibly, not using the sandbox method, to ± 35%. A volume of 100ml of bark was highly correlated to a mass of 50g (Jarvel, 1993), which was used as the standard mass for all samples, and not measured in a ring at a pressure of 10kPa. The reason for this change in technique, was that Brafield-Rolfe (1992) found the pressure procedure to yield extremely low nutrient concentrations even with increased bark quantities. The "Quick" 1:1.5 volume extraction method has been shown to give reliable results that are similar to the original 1:1.5 method, with correlation coefficients varying between 0.943 and 0.988 for nutrients extracted (Sonneveld & van Elderen, 1994).

Although moisture contents of bark were derived for all experiments, the "Quick" 1:1.5 method of Sonneveld & van Elderen (1994) shows that moisture content is not a crucial determinant

of the procedure and need not be corrected for. Only the pre-treatment of samples with a visual estimation of the water content at -3.2kPa is necessary. Sonneveld *et al*. (1974) also concluded that it was possible to use visual estimation of the moisture content of growing media in the preparation of the 1:1.5 volume method.

2.3.2 Plant

The technique of plant material analysis presents another approach in the nutrient availability determination of a growing medium. This technique is based on the concept that the content of a particular nutrient in the plant is greater the higher its availability in the medium (Mengel & Kirkby, 1982). However, plant analysis is only useful in the range of low nutrient availability in the medium, and not very sensitive in higher ranges of nutrient availability (Mengel & Kirkby, 1982). Here medium analysis is more appropriate and should be used in conjunction with plant analysis. Plant analysis is also a technique for correlating the elemental content of the plant with either its physical appearance, growth rate and yield or productivity. In most cases, the detection of nutrient deficiencies by foliar analysis complements diagnoses based on visual symptoms and medium analysis.

Unfortunately plant nutrient analysis has limitations. The amount of elements present in the shoots is known to vary with position on the plant, season, species and age of the plant (Bunt, 1988). It is therefore important to know the sources of variation in order to standardize comparisons and ensure relevant interpretations (Van den Driessche, 1992).

Dried shoot material was either ashed or digested (Appendix 4 & 5) and then analysed for nutrients (Section 2.3.3). Only Exp.1, 2 & 3 were analysed, with N and P determination done in the Department of Horticultural Science and the other nutrients by Talbot and Talbot, Pietermaritzburg.

2.3.3 Nutrients

Pine bark and plant material were analysed for nutrients once extracted, ashed or digested. Nutrient solutions from the various trials were also analysed. The NH_4^+-N, NO_3^--N, or total-N, and P contents were determined in the Department of Horticultural Science and read on a Beckman DU-65 spectrophotometer. Potassium, Ca, Mg, Fe, Cu, Mn, Zn, Na and Al were analysed by Talbot and Talbot in Pietermaritzburg, using a Varian 1275 atomic absorption spectrophotometer (AAS).

Phosphorus was determined by the method of Olsen & Sommers (1982). This procedure is based on the principle that in an acid molybdate solution containing orthophosphate ions, a phosphomolybdate complex forms that can be reduced by ascorbic acid to a molybdate blue colour, measured at 840nm. This colour is stable for up to 24 hr after formation, and the method is less susceptible to interferences than procedures using $SnCl_2$ as a reductant. It is also more sensitive and as a result is widely used for extracts containing small amounts of P, such as pine bark. The method of nitrate determination was that of Cataldo, Haroon, Schrader & Young (1975). This procedure is based on the nitration of salicylic acid under highly acidic conditions and the colorimetric determination of the resulting coloured complex, which absorbs maximally at 410nm in basic (pH > 12) solutions. The method is free of interference from other ions present in plant tissue and suitable for samples with a wide range of nitrate concentrations. Total-N was determined using the micro-Kjeldahl procedure followed by determining NH_4-N content. This determination is based on the colorimetric method of Bremner & Mulvaney (1982), in which an emerald green colour is formed by the reaction of ammonia, sodium salicylate, sodium prusside and NaOCl in a buffered, alkaline medium of pH 12.8-13.0. The complex is read at 660nm (Appendix 6).

2.3.4 Statistical

All experimental designs implemented were planned in conjunction with the Department of Biometry & Statistics at the University of

Natal, with designs being described in Steel & Torrie (1980). Data were analysed by analysis of variance, using Genstat version 5.1.

Factorial randomised block designs were used in all experiments, except for Exp.4 which was a split plot design. Due to the uniform growing conditions in the tunnels, and the small area occupied by each trial, it was calculated that two to five replications of each treatment were sufficient to contain error variance. Comparison of means was determined by least significant differences. Significant and highly significant were denoted $P \leq 0.05$ and $P \leq 0.01$, and represented by * and ** respectively. Non-significance was denoted by NS. All ANOVA tables from the data analysis have been included on the disc attached to the back cover.

Concentrations of the initial nutrient content of pine bark were not analysed using Genstat. This was because samples were collected from the treated pine bark that was used for the various replications, resulting in the initial pine bark concentrations being unreplicated. Due to cost considerations, not all the replications were analysed for final nutrient content of bark. Thus, final bark from all experiments, except Exp.1, was not analysed by Genstat. All comments relating to these data are therefore made from observations only.

2.4 ROOT GROWTH POTENTIAL TRIALS

Quality control in South African seedling nurseries has been based on morphological parameters such as height, etc. A physiological trait which is often used internationally is "root growth potential" (RGP). The object of a RGP test is to determine the ability of a seedling root system to initiate new root growth. In theory, the faster and more prolifically the seedling is able to produce new root growth, the better its chances for initial establishment and survival (Kietzka, 1993).

The most common method used is to plant representative seedlings in pots and keep them under standard favourable conditions for a

set time period. The seedlings are then extracted, and the amount of new root growth which has occurred is quantified.

Trials on RGP were carried out on Exp.3 & 4, when they were of transplantable size. A random sample, of 5 seedlings per treatment from the 2 replications of Exp.3, and 3 per treatment from the 3 replications of Exp.4, were planted out in sandbeds at the ICFR. The sandbeds were in a greenhouse, and misted every 5 min for 10 sec, to maintain a constant temperature of 25°C (Plate 2.2).

The duration of the RGP trials for Exp.3 was 17 days and for Exp.4, 28 days. This was to see if ±2 weeks, the current recommendation, or 28 days, the recommendation that has always been used, is more favourable in terms of greater significance in results (McCubbin, 1992). The seedlings were extracted, shaken free of sand, and the new roots protruding from the plug were counted, cut off and their dry mass measured.

Plate 2.2 Misting of *Pinus patula* seedlings during an RGP trial at the ICFR.

However, statistical analysis of these results was not performed as it was perceived that the trials had some limitations. It was extremely difficult in cases where plugs had lost their shape, to

clearly define which roots should be cut off and measured. Thus, excessive sampling error would have occurred. Also, seedling variability was extensive and in some cases comparisons of observed root growth simply could not be made. More often than not, seedlings from identical treatments would produce completely dissimilar root systems. McCubbin (1992), who did research on RGP also found the same problems. Should future research be done using RGP, clonal material should be used so as to reduce genetic variability.

However, nurseries can use this test to see if they are producing seedlings with the potential to do well in the field (Kietzka, 1993). Results of this test could be used to match seedling out-planting survival to the type of site and the initial handling of seedlings when planted in the field, once the test is perfected.

CHAPTER THREE

NITROGEN NUTRITION

3.1 INTRODUCTION

Nitrogen (N) is an indispensable element in plant nutrition as it is incorporated into essential organic compounds, such as amino acids, proteins and nucleic acids. Plants require N to synthesize chlorophyll - the critical determinant in photosynthesis. Nitrogen is therefore the element that elicits the most growth when applied to conifers, so most fertilizer programmes are generally established relative to the N concentration (Landis *et al.*, 1992). Overseas research on conifers has shown that higher N levels of 150-200 $mg.\ell^{-1}$ produced optimal growth, but in South Africa lower N concentrations of 75-100 $mg.\ell^{-1}$ are recommended. Thus, the N concentration required to produce the optimal *P. patula* seedling is not known.

Not only is the N concentration important, but also the different forms of N that can be applied to a seedling. There are two different inorganic N ions that are taken up by plants: ammonium (NH_4^+) and nitrate (NO_3^-). Studies on the type of N applied to coniferous seedlings have received wide attention, but have given conflicting results. The growth or total N-content of many conifer seedlings is greatest when N is supplied either as ammonium alone (McFee & Stone, 1968; Etter, 1969; Nelson & Selby, 1974; Van den Driessche & Dangerfield, 1975; Agrawal, 1986; Flaig & Mohr, 1992; Lavoie, Vézina & Margolis, 1992) or as a mixture of ammonium and nitrate in various ratios (Van den Driessche, 1971 & 1978; Christersson, 1972; Bigg & Daniel, 1978; Ingestad, 1979). There are few reports of conifer seedlings producing the best growth with only nitrate-N. Conifers are acid-loving plants, and so the preference for ammonium in solution may be ascribed to the acidification of the rhizosphere resulting from NH_4^+ absorption (Landis *et al.*, 1992). Nitrate absorption results in OH^- being released by the plant and the surrounding medium increasing in pH. As the pH of the medium is affected differently by the uptake of ammonium and nitrate, the reaction of a plant to

different N forms could partly be due to pH (Bigg & Daniel, 1978). Flaig & Mohr (1992) found that Scots pine seedlings nourished with NH_4^+ took up 3 times as much N as plants supplied with NO_3^-. Ingram & Joiner (1982) observed that chlorosis was evident on plants that received 50% or more NO_3^--N. Most of the experiments, however, have been grown in either sand, water cultures, or peat-vermiculite and not in pine bark. Little is known about the N reactions and transformations that occur in bark-based media.

Pine bark contains only 0.2-0.3% nitrogen, with approximately 0.33 mg.ℓ^{-1} water extractable NH_4^+-N and 0.67 mg.ℓ^{-1} NO_3^--N (Pokorny, 1979). This may be an effect of mineral N immobilization as well as the leaching of NO_3-N (Prasad, 1980). Foster *et al.* (1983) stated that the NO_3^--N form is readily leached from pine bark because it is a negatively charged anion, which is repelled by the negatively charged cation exchange sites. Van Schoor *et al.* (1990) stated that NH_4-N is rapidly adsorbed by pine bark and may not be available to the plant. Foster *et al.* (1983) showed that an increase in pH, increased NH_4-N adsorption (pine bark has the capacity to adsorb 1.5 mg NH_4-N g^{-1} bark), and that two types of adsorption sites for NH_4 existed. One site is effective at lower pH, the other is active as pH increases. At higher pH, the functional groups on the bark (carboxylic and phenolic groups) dissociate, exposing negatively charged sites on the bark. This accounts for the increased NH_4^+ adsorption when pH increases. They also showed that it is likely that the NH_4^+ adsorbed at low pH is a result of hydrogen bonding, rather than a typical cation exchange reaction. They concluded that incorporation of NH_4-N into a pine bark medium prior to planting may be advisable so as to prevent low N concentrations from occurring in the container solution, due to NH_4^+ binding when plants are first planted and fertilized. However, the fate and availability of NH_4^+ ions bound to bark particles is still uncertain (Ogden *et al.*, 1987). Thus, a continuous supply of N is important in pine bark media that are lacking in N and easily leached, and in restricted containers where plants cannot rely on expanding roots to provide a supply of N. The application of N in a solution feed is, thus, obviously necessary. Nitrogen nutrition promotes vegetative

growth and is therefore a major determinant of seedling stem diameter and subsequent yield based on collar diameter. Mexal & Landis (1990) found that seedling dry mass and yield were a function of the amount of N applied. Over 3 years, N accounted for 81% of the variation in dry mass and yield. However, high N fertilizer rates (> 250 $mg.\ell^{-1}$) can lead to poor seedling quality, such as an unbalanced shoot to root ratio, succulent growth, increased transpiration, excessive branching, reduction of apical dominance in the main stem, and toxicity symptoms (Will, 1971). Thus, when pines are under drought stress, high N concentrations can be detrimental. Nitrogen fertilization can also prolong seedling growth in the nursery, delay frost hardening and, later, result in frost damage (Van den Driessche, 1992). Hellergren (1981) found that there was no correlation between frost hardiness and N content in unhardened seedlings. However, the hardy seedlings with the highest N contents showed a decrease in frost hardiness of 3°C. Thus, N concentrations must be adjusted to suit environmental conditions and seedling stage of growth, as seedling hardiness is of prime concern to plantation managers who want high survival rates after transplanting.

Although the relationship between seedling growth and N nutrition has been studied for most conifer species, information is notably lacking on *P. patula* grown in pine bark. The following work, therefore, examines the growth responses of *P. patula* seedlings to different N forms and concentrations. The aim was to determine the optimum N concentration to apply to *P. patula* seedlings growing in composted pine bark, to observe which form of N produces maximum seedling growth, and which combination is the most economical to apply.

3.2 MATERIALS AND METHODS

This trial, labelled as Exp.1, was in progress from 05.06.92 (Day 0) to 04.11.92 (Day 152). *Pinus patula* (S/N 100011) were sown into Unigro® trays (Section 2.1.2) containing composted pine bark (Section 2.1.3). The bark was pre-enriched with 5 $kg.m^{-3}$ of single superphosphate, to provide a P source, and 300 $g.m^{-3}$ of FRIT 504®. No K was added, as it was assumed the bark had

sufficient inherent K. Trays were laid out in a tunnel as a 3 x 4 (+1 control) factorial random block design with three replications.

All nutrient solutions were hand-applied daily (Section 2.1.5). Three types of fertilizer (low biuret urea (NH_4^+ form), Calmag® (NO_3^- form) and NH_4NO_3 (1:1 mixture of both forms)) were applied at 5 application rates (0, 40, 80, 120 & 160 mg.ℓ^{-1} N) (Tables 3.1 & 3.2).

Table 3.1 Quantities of fertilizers used to make up the nutrient solutions for the N trial.

N CONC. (mg.ℓ^{-1})	MASS (g)		
	L. B. UREA	CALMAG®	NH_4NO_3
40	6.96	24.24	9.14
80	13.91	48.49	18.27
120	20.87	72.73	27.43
160	27.83	96.97	36.57

As can be seen from Table 3.2, the NH_4^+ concentrations in the urea treatments were very low. This was because the enzyme urease, which hydrolyses urea to NH_4, is not present in water (see Section 3.3.3 for details).

After 20 weeks seedlings were harvested and morphological data recorded. Seedling colours were noted and chlorotic seedlings identified. Tissue and bark (initial and final) samples were analysed for nutrient content (Section 2.3).

Table 3.2 Actual concentrations of the nitrogen forms, pH and EC of the nutrient solutions in the N trial determined by analysis.

N CONC. ($mg.\ell^{-1}$)	pH	EC ($\mu S.cm^{-1}$)	CONCENTRATION ($mg.\ell^{-1}$)	
			NO_3-N	NH_4-N
L.B.Urea 40	7.05	83	-	5.17
80	7.17	86	-	10.22
120	7.38	89	-	10.38
160	7.65	92	-	13.50
Calmag® 40	7.47	363	47.19	3.13
80	7.84	644	86.41	4.73
120	7.89	883	144.69	7.18
160	7.96	1263	205.47	9.87
NH_4NO_3 40	7.63	272	29.00	19.01
80	7.60	441	54.06	38.37
120	7.57	629	73.13	51.04
160	7.52	847	100.63	73.03

3.3 RESULTS

3.3.1 Plant Growth

The growth of *P. patula* seedlings was significantly increased ($P \leq 0.01$) by increasing the solution N concentration. This was representative for all forms of N applied and for all plant parameters (Fig. 3.1 a,b,c,d and Plates 3.1, 3.3 & 3.4).

Height (HT)

Control treatments, that received no N, had an average net HT of

62.4 ± 8.02mm, compared to the optimal mean HT of 208.6 ± 8.02mm for 80 mg.ℓ^{-1} NH_4-N, applied in the form of urea (Plate 3.1). This is in accordance with the 205mm which Donald (1992) recommended for maximum out-planting survival. The form of N applied revealed significant differences ($P \leq 0.01$), with urea producing taller seedlings, followed by NH_4NO_3 and lastly Calmag® (NO_3-N fertilizer). Differences in N concentration applied revealed no significant response in increasing the NO_3-N and NH_4-N above 80 mg.ℓ^{-1} N (Plates 3.3 & 3.4). Thus, a significant interaction occurred between the rate and form of N. NH_4NO_3 applied at 160 mg.ℓ^{-1} N resulted in greater HT of *P. patula* seedlings (205 ± 8.02mm) than when applied at low concentrations, i.e., seedlings responded linearly to NH_4NO_3 fertilizer rates (Fig. 3.1a and Plate 3.3). Seedlings, however, responded quadratically to increasing rates of urea and Calmag® (Fig. 3.1a).

Plate 3.1 Growth reduction caused by absence of N fertilizer, compared to optimal N application.

Collar diameter (CD)

The response of CD was the same as that for HT. The CD of seedlings grown without N was 1.66 ± 0.05mm (Fig. 3.1b). This increased to a maximum of 2.79 ± 0.03mm at 80 mg.ℓ^{-1} NH_4-N. Significant differences ($P \leq 0.01$) in CD were observed between the Calmag®, and the urea and NH_4NO_3 treatments. Calmag® produced seedlings of smaller mean CD (2.40 ± 0.03mm), compared to urea

(2.72 ± 0.03mm) and NH_4NO_3 (2.68 ± 0.03mm). The different rates applied revealed significant differences ($P \leq 0.01$) between 40 $mg.\ell^{-1}$ N (smallest CD) and the other rates. An increase in N rate resulted in seedlings responding linearly to the NH_4NO_3 treatments, and quadratically to urea and Calmag® (Fig. 3.1b).

Root and shoot dry mass (RDM and SDM)

Growth of seedlings at 0 $mg.\ell^{-1}$ N was poor with a mean SDM of 0.17 ± 0.05 $g.seedling^{-1}$, and RDM of 0.13 ± 0.02 $g.seedling^{-1}$ (Figs. 3.1c & d). Shoot dry mass and RDM were significantly different ($P \leq 0.01$) for the forms of N applied, with urea treatments producing greater mean SDM and RDM than NH_4NO_3. Calmag® treatments resulted in the smallest roots and shoots for all concentrations of N (Figs. 3.1c & d).

Highly significant differences existed between RDM and SDM for the various N concentrations. The higher the concentration above 40 $mg.\ell^{-1}$ N, the lower the RDM. Thus, increasing the N concentration in the nutrient solution led to a negative linear RDM response, for all three forms of N (Fig. 3.1d). NH_4NO_3 resulted in a linear SDM response to N concentration, with a 160 $mg.\ell^{-1}$ N producing a mean SDM of 1.01 ± 0.05 $g.seedling^{-1}$. Urea produced the second largest SDM (0.98 ± 0.05 $g.seedling^{-1}$) at 80 $mg.\ell^{-1}$ N, followed by a mean 0.04 $g.seedling^{-1}$ decrease at 120 $mg.\ell^{-1}$ N, and a 0.03 $g.seedling^{-1}$ increase at 160 $mg.\ell^{-1}$ N (Fig. 3.1c). Calmag® treatments responded quadratically to N concentrations (120 $mg.\ell^{-1}$ N produced highest SDM) (Fig..3.1c).

Root:shoot ratio

The larger the value of this ratio, the larger the root mass per unit mass of shoot. An increase in N concentration resulted in a significant decrease ($P \leq 0.01$) in the root:shoot ratio averaged for the three forms of N (Fig. 3.2a). Root:shoot ratio increased significantly ($P \leq 0.01$) with N deficiency, i.e., the control had a mean ratio of 0.78 ± 0.02 and the 160 $mg.\ell^{-1}$ N treatments had mean ratios of 0.30 ± 0.02, 0.27 ± 0.02 and 0.29 ± 0.02 for Calmag®, urea and NH_4NO_3, respectively. Root:shoot ratio was not significantly different for the forms of N applied.

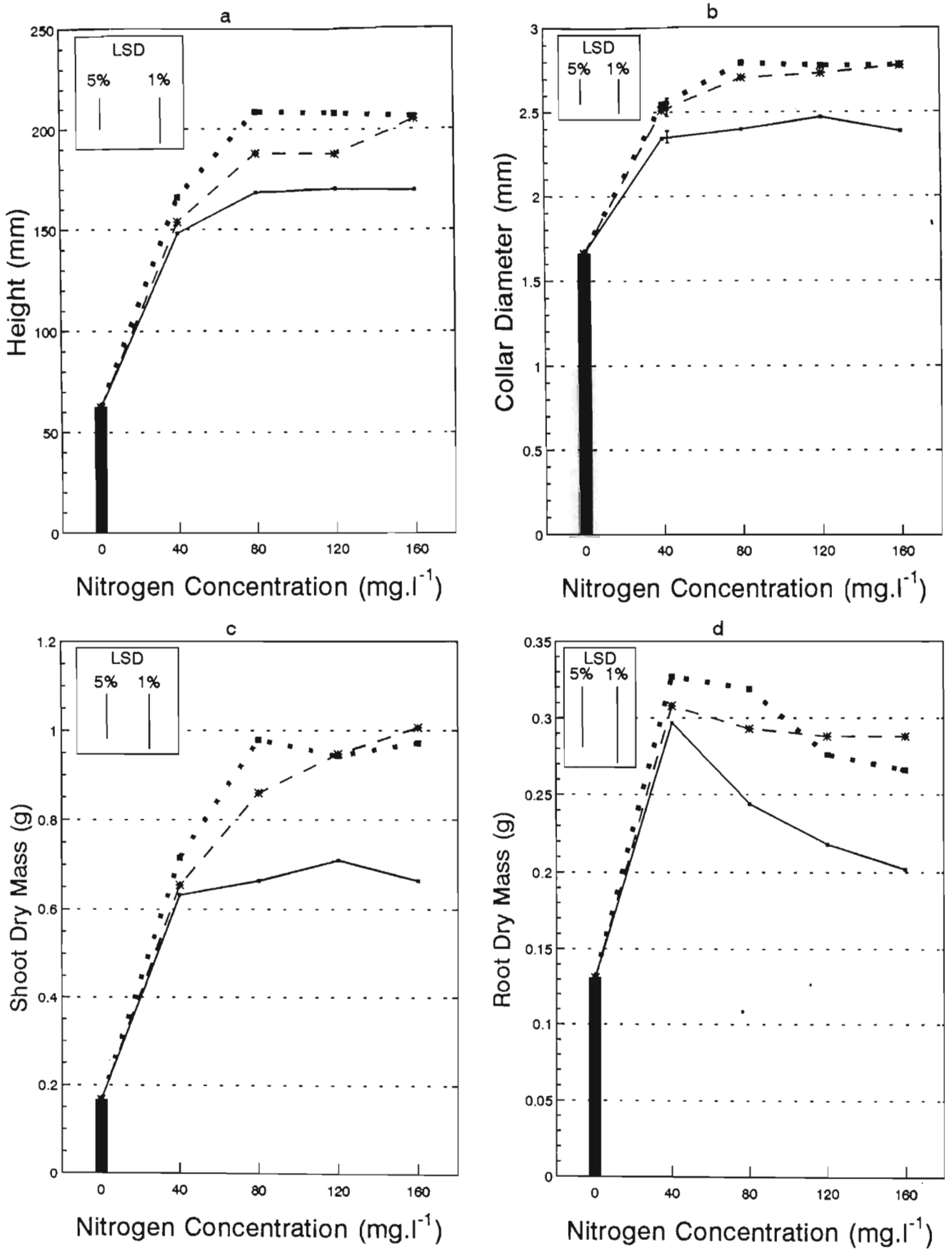


Fig. 3.1 Effect of N concentration and form on (a) height, (b) collar diameter, (c) shoot dry mass and (d) root dry mass of *P. patula* seedlings.

Seedling colour

At the end of the experiment seedlings varied in colour. Controls were bright yellow, and seedlings receiving high N were almost blue-green (Table 3.3). After five weeks of fertigation chlorosis was evident in plants that received greater than 40 mg.ℓ^{-1} NO_3-N, from the Calmag® treatments (Plates 3.2 & 3.4).

Table 3.3 Seedling colours at harvesting.
(1 = very yellow, 2 = chlorotic, 3 = greeny yellow, 4 = blue green)

TREATMENT	RATING
Calmag® - 40 mg.ℓ^{-1}	3
Calmag® - 80 mg.ℓ^{-1}	2
Calmag® - 120 mg.ℓ^{-1}	2
Calmag® - 160 mg.ℓ^{-1}	2
Urea - 40 mg.ℓ^{-1}	3
Urea - 80 mg.ℓ^{-1}	4
Urea - 120 mg.ℓ^{-1}	4
Urea - 160 mg.ℓ^{-1}	4
NH_4NO_3 - 40 mg.ℓ^{-1}	3
NH_4NO_3 - 80 mg.ℓ^{-1}	4
NH_4NO_3 - 120 mg.ℓ^{-1}	4
NH_4NO_3 - 160 mg.ℓ^{-1}	4
Control	1

Plate 3.2 *P. patula* seedlings showing evidence of chlorosis in the 120 mg.ℓ^{-1} N and 160 mg.ℓ^{-1} N Calmag® treatments.

Moisture content

As expected, plants grown under harsh conditions are hardier and more adaptable to varying moisture conditions. This was clearly evident in the experimental results. Control seedlings, subjected to no N fertilization, had the lowest mean moisture content of 64.91 ± 0.78% ($P \leq 0.01$)(Fig. 3.2c). Increased N concentrations significantly increased ($P \leq 0.01$) the overall moisture content, except for Calmag® treatments, which resulted in a quadratic response with 80 mg.ℓ^{-1} N having the greatest moisture content (74.55 ± 0.78%) (Fig. 3.2c). The Calmag® seedlings (73.63 ± 0.78%) had significantly ($P \leq 0.01$) lower moisture contents than the urea (74.99 ± 0.78%) and NH_4NO_3 (74.76 ± 0.78%) seedlings. Greatest moisture content was found in the 160 mg.ℓ^{-1} NH_4-N treatment (76.02 ± 0.78%), with 80 mg.ℓ^{-1} NH_4-N having a mean of 75.35 ± 0.78%. This was a 0.89% difference from the moisture contents of all treatments (74.46%), excluding the control.

Lateral shoot number (including buds)

The concentration of N applied had a large effect on total growth as well as influencing lateral bud development. Lateral shoot response to the different forms of N, was the same as that of the other plant parameters ($P \leq 0.01$), i.e., the control treatment had

an average of 1.2 ± 0.20 laterals per seedling, urea had 4.2 ± 0.20, NH_4NO_3 had 4.1 ± 0.20 and Calmag® had 3.4 ± 0.20 laterals per seedling (Fig. 3.2b). There was a non-significant response to the concentration of N, however, 120 > 160 > 80 > 40 mg.ℓ^{-1} N for all forms of N (Fig. 3.2b).

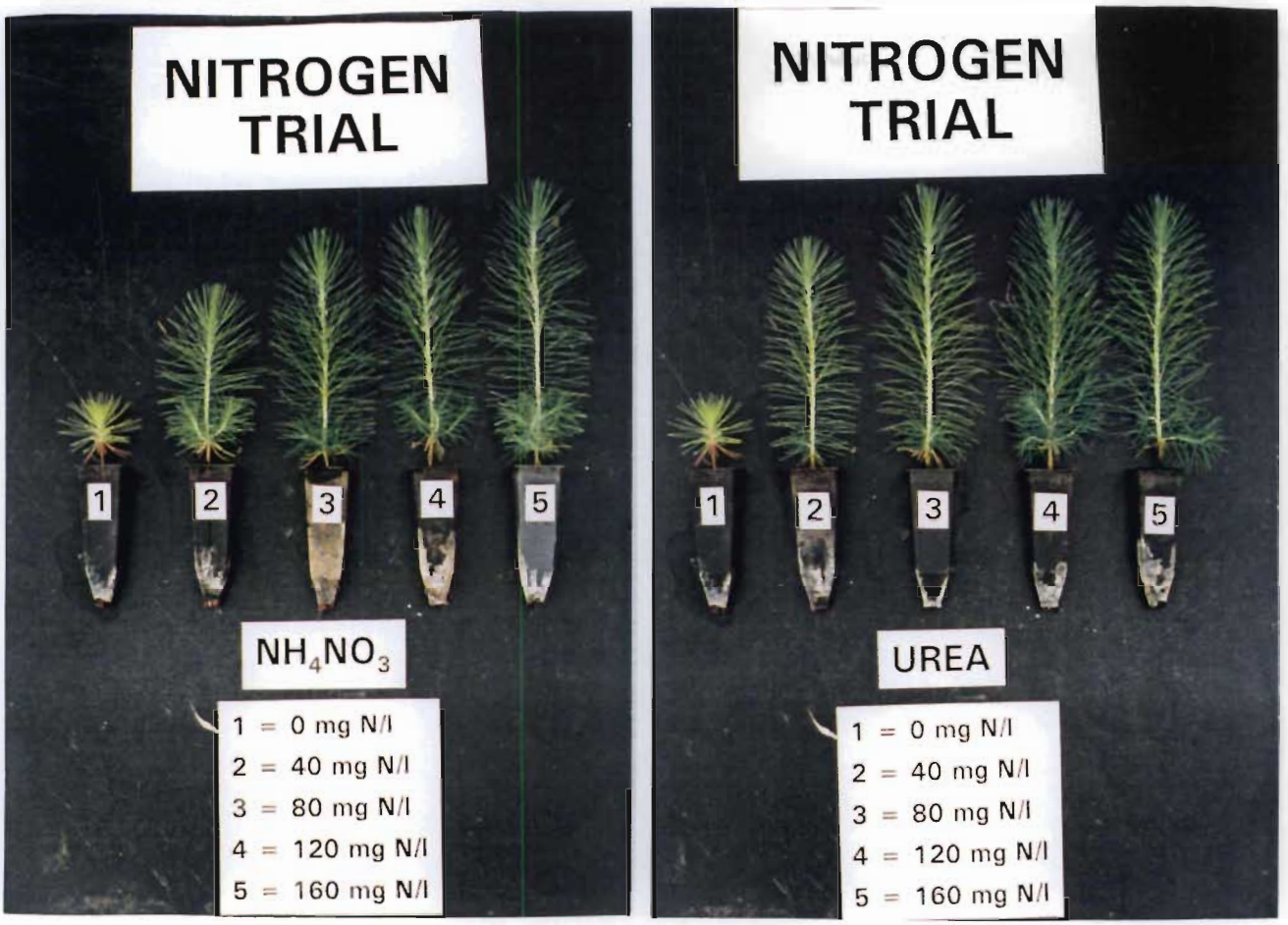


Plate 3.3 Response of increasing N concentration on the growth of *P. patula* seedlings (NH_4NO_3 resulting in maximal growth at 160 mg.ℓ^{-1} N and urea at 80 mg.ℓ^{-1} N).

Plate 3.4 Applications of high NO_3-N concentrations (Calmag®) showing marginal growth increases.

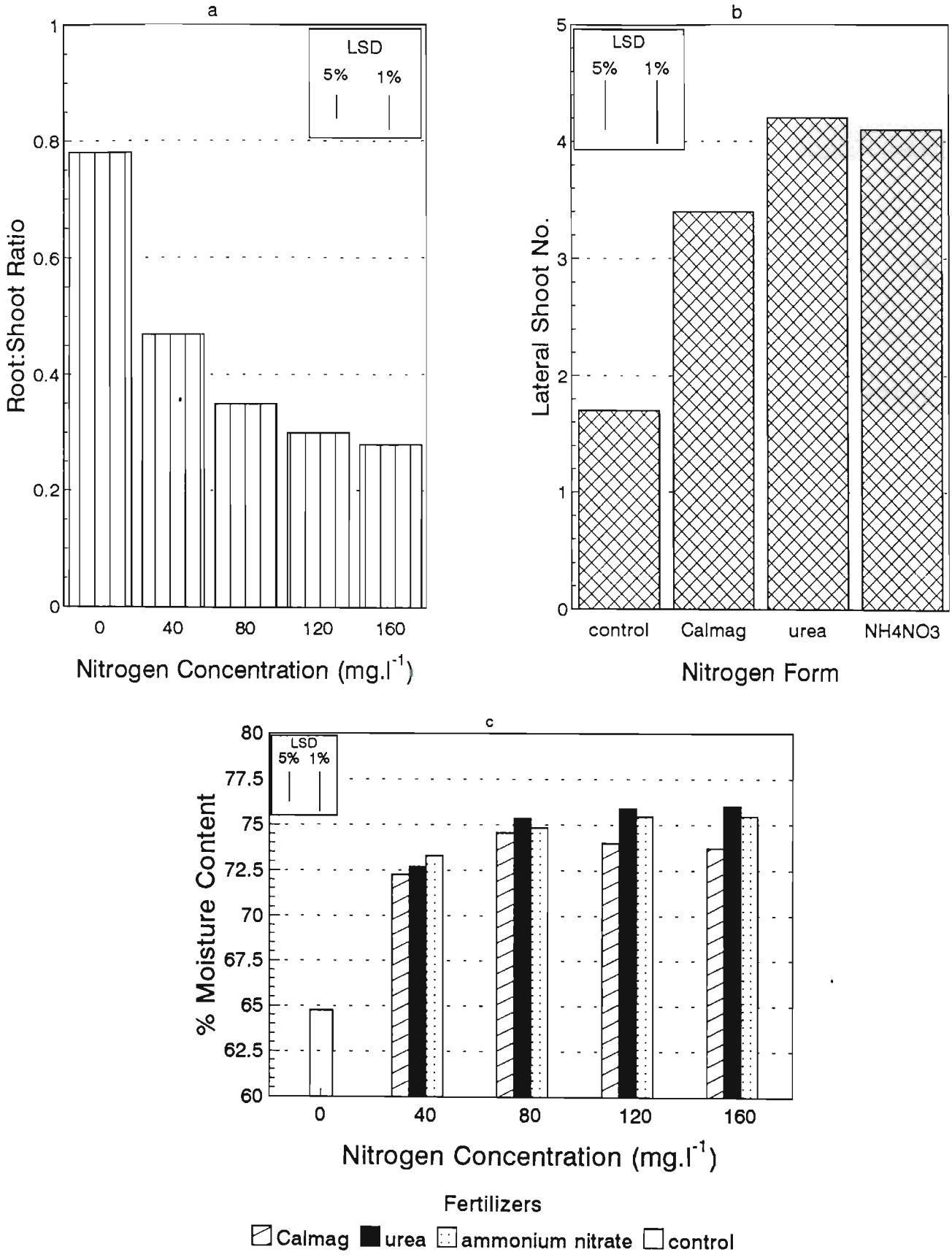


Fig. 3.2 Influence of N form and concentration on (a) root:shoot ratio (averaged over N forms), (b) lateral shoot number (averaged over N concentrations) and (c) % moisture content.

3.3.2 Leaf Analysis

Foliar leaf analysis was carried out to ascertain whether differences in growth response to N concentration and form was due to differential uptake of other nutrients, or entirely influenced by N nutrition.

Increasing concentrations of N in the liquid feed increased the foliar N concentration. This linear increase was constant for all the three fertilizers ($P \leq 0.01$). Highly significant differences in foliar N were found between the control and other treatments. The control had a foliar N concentration of 0.57 ± 0.19%; the maximum N concentration of 2.71 ± 0.22% occurred at 160 $mg.\ell^{-1}$ N with NH_4NO_3. Optimum seedling growth was associated with a foliar N level of 2.07 ± 0.22% at 80 $mg.\ell^{-1}$ NH_4-N. This is within the range of 1.4-2.2% recommended by Landis *et al.* (1992).

Highly significant differences in foliar P and K were observed between the controls and treatments that received N. Controls had far greater concentrations, most probably compensating for the lack of N and because K content in the control media was significantly higher than in the treated media. Differences were found for the macronutrients, Ca and Mg. These were significantly higher in the Calmag® treatments, 0.61 ± 0.04% Ca vs 0.46 ± 0.04% Ca and 0.45 ± 0.04% Ca, and 0.30 ± 0.03% Mg vs 0.23 ± 0.03% Mg and 0.22 ± 0.03% Mg, for urea and NH_4NO_3 respectively (Fig. 3.3). This can be explained by the fact that Calmag® is a Ca and Mg nitrate fertilizer, with 98 $g.kg^{-1}$ Ca and 48 $g.kg^{-1}$ Mg. Increasing amounts of N resulted in significant differences ($P \leq 0.01$) in foliar Ca levels. Foliar Ca levels were increased from 0.44-0.62 ± 0.05% with increase in N concentration from 40 to 160 $mg.\ell^{-1}$ N (Fig. 3.3). However, there was no significant interaction between the foliar Ca and Mg concentrations and concentration or form of N applied.

Tissue analysis revealed no significant differences in Fe and Zn status. Iron analysis revealed unusual results, e.g., chlorotic seedlings sometimes having higher Fe contents than non-chlorotic. However, due to the very large variability in the data, the

differences were statistically non-significant.

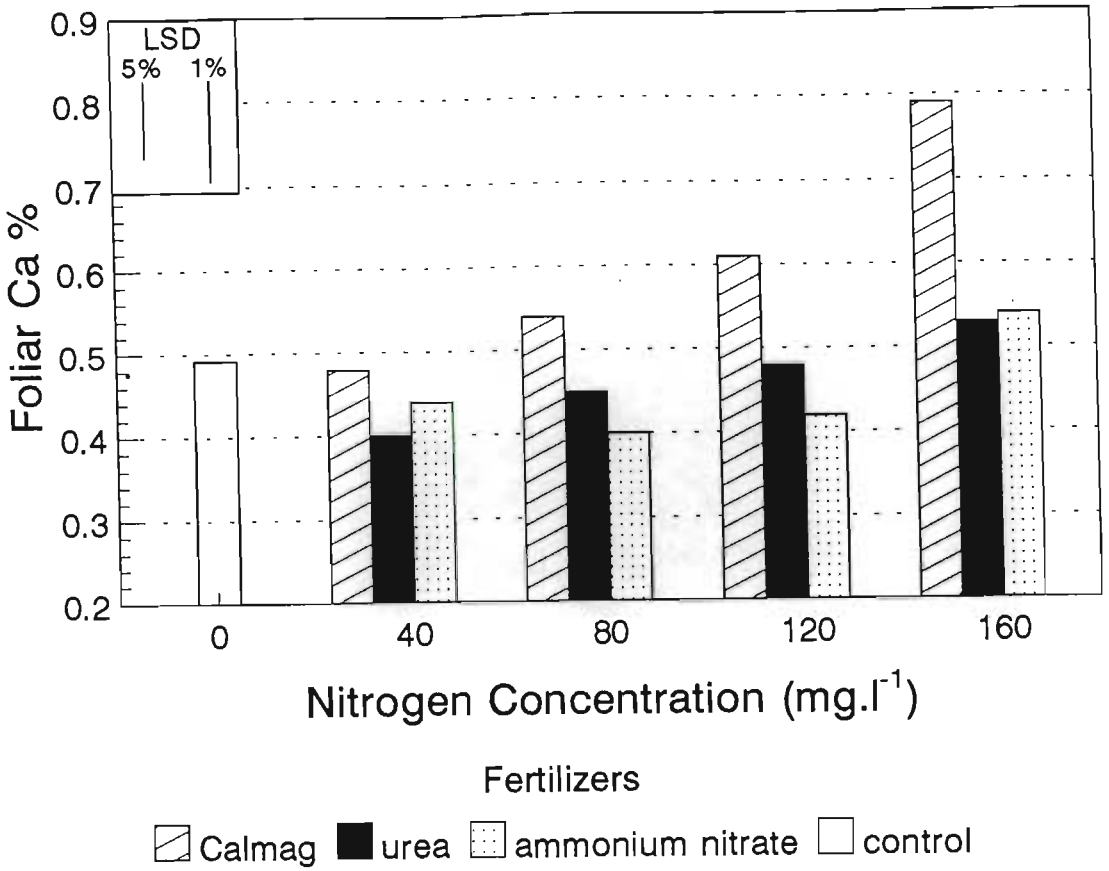


Fig. 3.3 Effect of N concentration on percentage foliar Ca content.

Plant Cu content revealed a significant difference ($P \leq 0.05$) between the forms of N, with NH_4NO_3 treatments containing 16.87 ± 1.24 $mg.kg^{-1}$ and urea and Calmag® each containing 5 ± 1.24 $mg.kg^{-1}$. Percent foliar Mn was higher ($P \leq 0.01$) in the controls than the other treatments, viz. 816 ± 86.25 $mg.kg^{-1}$ for the controls, and a mean Mn content of 535 ± 86.25 $mg.kg^{-1}$ for all the other treatments. Addition of N solutions to the media apparently resulted in Mn being easily displaced and leached from the bark particles, and therefore plant uptake of Mn was reduced.

It can therefore be concluded that the growth response to N concentration and form was mainly as a result of N, but also influenced by the differential uptake of Ca and Mg.

3.3.3 Medium Analysis

Analysis of the water extract of the initial pine bark was as follows:

EC = 2.94 $mS.cm^{-1}$	pH = 4.42	NO_3 = 2.96 $mg.\ell^{-1}$
NH_4 = 63.83 $mg.\ell^{-1}$	P = 89.04 $mg.\ell^{-1}$	K = 140 $mg.\ell^{-1}$
Ca = 460 $mg.\ell^{-1}$	Mg = 126 $mg.\ell^{-1}$	Fe = 0.09 $mg.\ell^{-1}$
Mn = 6.80 $mg.\ell^{-1}$	Zn = 0.42 $mg.\ell^{-1}$	Cu = 0.02 $mg.\ell^{-1}$.

The NH_4 concentration was higher than the NO_3, and Mn was higher than normal, both consequences of bark that is not fully composted.

Final bark analysis revealed that most elements were much lower than in the initial pine bark analysis, due to seedling uptake and leaching. However, on average, the pH of final bark was higher than the initial, due to the addition of NH_4NO_3 or Calmag® in most treatments. The urea treatments also increased the pH, no doubt due to leaching of certain ions responsible for low pH, such as Mn. Of interest is the fact that the Fe content increased in the final bark. The only explanation is that the water used to fertigate must have been contaminated with Fe from corroding pipes.

The pH of the final media was significantly reduced ($P \leq 0.01$) with increasing N in the nutrient solution. The control had the highest pH of 6.53 ± 0.51. In Calmag® and urea treatments, pH decreased linearly from 5.12 ± 0.51 to 4.80 ± 0.51 and from 6.17 ± 0.51 to 4.06 ± 0.51, respectively. Urea treatments, however, had a higher average pH, presumably due to the formation of NH_3 and NH_4^+ when urea hydrolysed. The reaction of urea in pine bark is the following (Lea-Cox, 1989):

$$CO(NH_2)_2 + 2H_2O + \text{urease enzyme} \rightarrow (NH_4)_2CO_3$$

$$(NH_4)_2CO_3 \rightarrow 2NH_3 + CO_2 + H_2O$$

$$NH_3 + H_2O \rightarrow NH_4OH$$

$$NH_4OH \rightarrow NH_4^+ + OH^-$$

NH_4NO_3 treatments showed a quadratic response to pH, with maximum medium pH (5.69 ± 0.51) at 80 $mg.\ell^{-1}$ N. Thus, the interaction between concentration and form of N was shown to be significant.

Analysis of final bark for NH_4 and NO_3 was as expected, showing that the control (0.51 ± 1.2 mg.ℓ^{-1} NH_4 & 0.35 ± 6.98 mg.ℓ^{-1} NO_3) had significantly less ($P \leq 0.01$) NH_4 and NO_3 than the other treatments, and that increasing N rates caused significant ($P \leq 0.01$) linear increases of NH_4 and NO_3 in the media for all treatments (Fig. 3.4). NH_4 availability in the media was also significantly ($P \leq 0.05$) greater in the NH_4NO_3 treatments (9.76 ± 1.2 mg.ℓ^{-1}), with less in the Calmag® (9.15 ± 1.2 mg.ℓ^{-1}) and urea (6.98 ± 1.2 mg.ℓ^{-1}) treatments (Fig. 3.4). Conversely, NO_3 in the media was significantly ($P \leq 0.05$) greater in Calmag® (28.56 ± 6.98 mg.ℓ^{-1}) and urea (27.47 ± 6.98 mg.ℓ^{-1}) treatments, than in NH_4NO_3 (17.89 ± 6.98 mg.ℓ^{-1}) treatments(Fig. 3.4). The linear interaction between concentration and form of N was significant ($P \leq 0.05$) for NH_4 and highly significant for NO_3.

Mean P availability was significantly different ($P \leq 0.05$) due to the amount of N applied. A quadratic and linear P response to N application was produced in all fertilizers, with 160 mg.ℓ^{-1} N the highest and 80 mg.ℓ^{-1} N the lowest. This can be explained by the fact that foliar P showed the opposite effect, i.e., the P content of the medium was lower due to increased P uptake by the plant. The potassium content of the media was significantly ($P \leq 0.05$) higher in the control treatments (2.47 ± 0.52 mg.ℓ^{-1}). Within Calmag® treatments, response to increasing N resulted in a significant ($P \leq 0.01$) increase in K availability in the media. This may have been due to increased competition for exchange sites by the NH_4^+ ions, which resulted in more K^+ being available in the medium solution.

Final bark Ca and Mg content were significantly less ($P \leq 0.01$) in the controls, and their availability increased significantly ($P \leq 0.01$) as N concentration increased. There was a linear response to all forms of applied N. The form of N applied also resulted in significant differences of Ca and Mg in the media. Calmag® treatments resulted in greater Mg in the media, followed by the urea and NH_4NO_3 treatments (Fig 3.5). Greatest Ca content was extracted from urea (37.90 ± 4.18 mg.ℓ^{-1}), then Calmag® (28.44 ± 4.18 mg.ℓ^{-1}) and NH_4NO_3 (27.13 ± 4.18 mg.ℓ^{-1}) treated media.

Zinc and Cu availability in the medium was not significantly affected by N nutrition. However, the availability of Fe and Mn was affected. The concentration of Mn in the controls was significantly lower than the urea and NH_4NO_3 treated media, with the opposite occuring for Fe. The Fe content in the bark was significantly affected ($P \leq 0.01$) by the amount of applied N. A negative linear response to Fe availability was observed for Calmag® and urea, and NH_4NO_3 caused a quadratic response. The reaction of Fe in the bark was therefore in accordance with foliar Fe uptake (Section 3.3.2). The concentration and form of N also significantly ($P \leq 0.01$) affected the availability of Mn in the bark. Manganese increased with increasing N application, and the greatest availability of Mn was found in the urea (0.35 ± 0.13 mg.ℓ^{-1}) treatments, followed by NH_4NO_3 (0.29 ± 0.13 mg.ℓ^{-1}) and Calmag® (0.07 ± 0.13 mg.ℓ^{-1}) (Fig. 3.5).

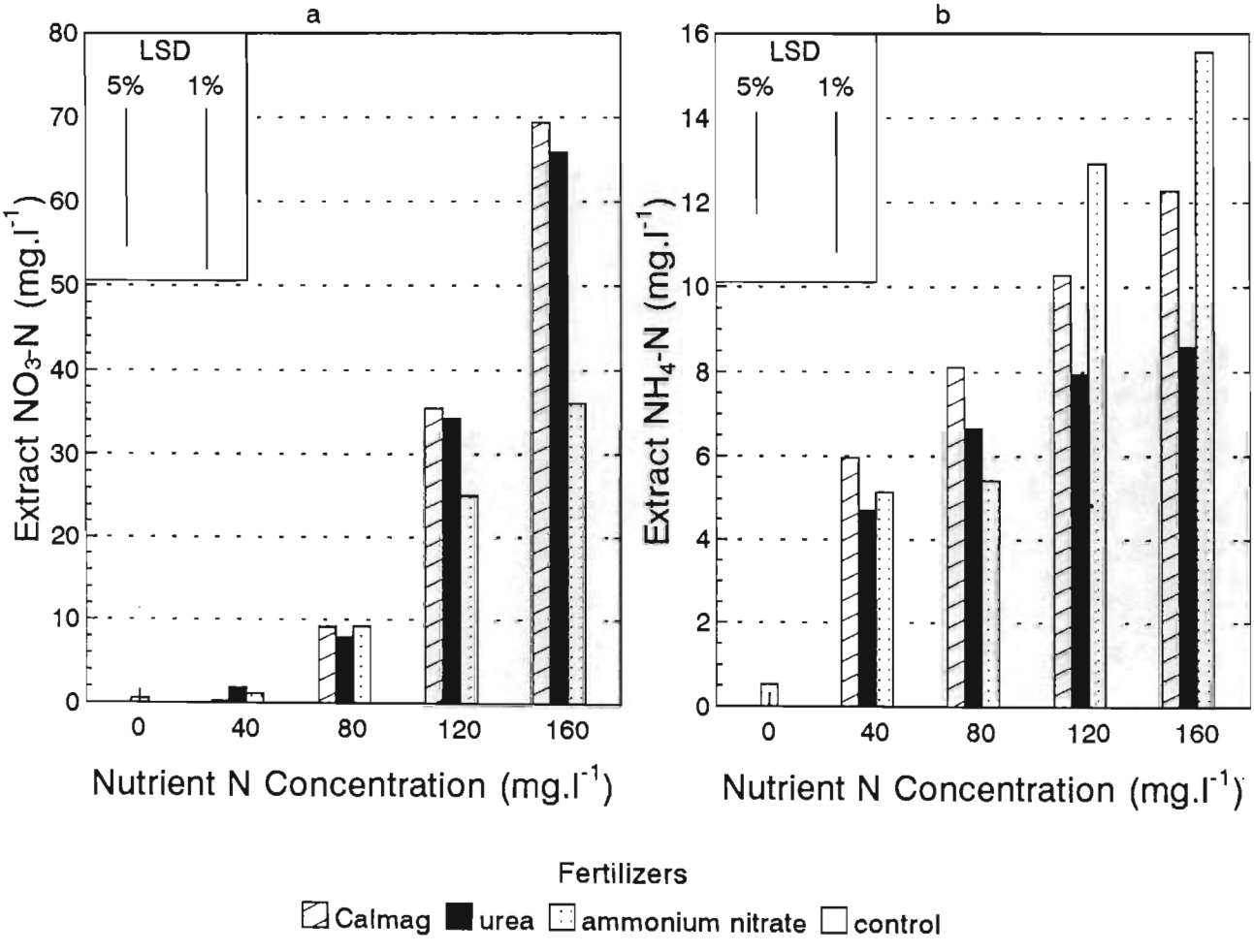


Fig. 3.4 Effect of increasing N in the nutrient solution on the media (a) NO_3-N and (b) NH_4-N concentrations.

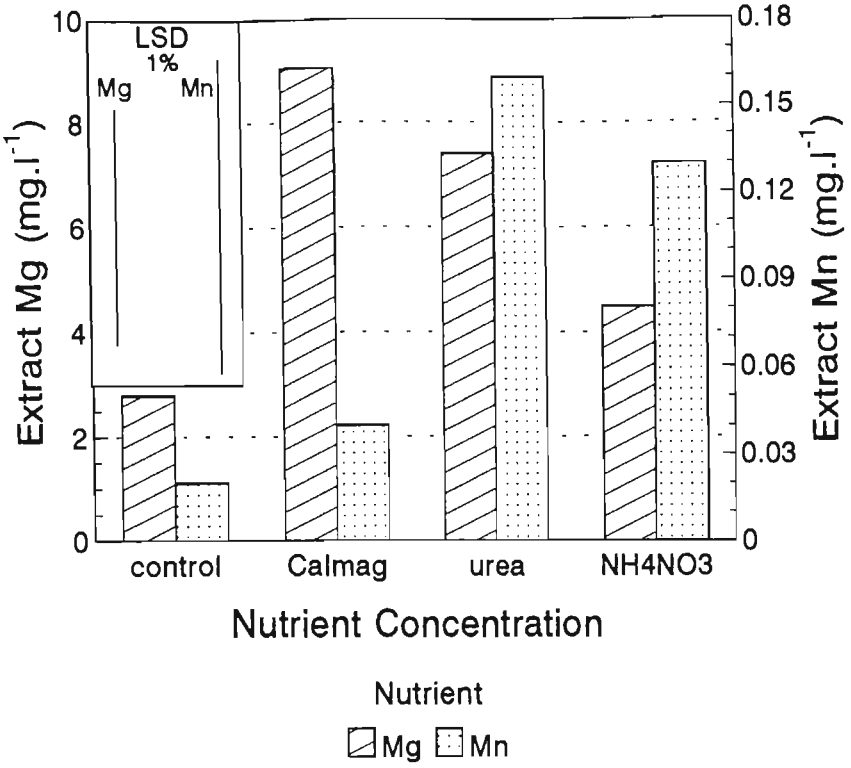


Fig. 3.5 Effect of 80 mg.ℓ^{-1} N on Mg and Mn concentrations in the media extracts, for all forms of N.

3.4 DISCUSSION AND CONCLUSIONS

The reduced growth rate and chlorotic symptoms observed when the NO_3^- form of N was applied at concentrations above 80 mg.ℓ^{-1} N confirm reports that NO_3-N alone is an inferior source of N for pines. Plant growth parameters measured were greater if NH_4-N was present, either alone or with NO_3. Optimal growth in HT and CD, and second best in SDM and RDM was obtained when 80 mg.ℓ^{-1} NH_4-N was applied. 160 mg.ℓ^{-1} N of NH_4NO_3 resulted in marginally greater SDM and thus maximum moisture content of 76.02%, but a lower root:shoot ratio than 80 mg.ℓ^{-1} NH_4-N. A very high moisture content of seedlings is undesirable as they tend to be softer, and their survival rate after transplanting is lower.

Lateral shoot number has been proposed as a measure of seedling quality; the greater the number, the better the seedling. Donald (1992) proposed that five laterals was a standard morphological parameter for seedlings produced in Unigro® trays, and Will

(1978) found that a range of 3-7 was normal when *P. radiata* seedlings received 100 mg.ℓ^{-1} N. In this trial, 80 mg.ℓ^{-1} NH_4-N produced an average of 4.3 laterals as did 160 mg.ℓ^{-1} of NH_4NO_3, both in the recommended range. However, too much N can cause excessive branching and a condition known as "retarded leader" affects tree form. This is an undesirable side-effect of too high an N concentration and results in the reduction of apical dominance in the main stem, which is not desired in forestry. Therefore, it can be concluded that 80 mg.ℓ^{-1} NH_4-N yielded the most suitable seedling for outplanting, i.e., a nutrient feed with only NH_4-N did not have a toxic effect on *P. patula* growth in pine bark, contrary to general belief.

Increasing N concentration in the solution increased foliar N and optimum growth occurred when foliar levels were above 2%. There was also a simultaneous increase in foliar Ca. NO_3-N is known to stimulate Ca uptake and this no doubt increased the Ca uptake in the treatments, which all had NO_3 in the final bark, due to NO_3 addition or nitrification. However, the different forms of N did not affect N uptake by *P. patula* seedlings, but did influence Ca, Mg and Cu uptake. The Calmag® treatments were responsible for greater Ca and Mg uptake than the other two treatments, since Calmag® is a Ca and Mg nitrate. Foliar Cu content was three times greater in the NH_4NO_3 treatments, than in the urea and Calmag® treatments. Although levels were very low, deficiency symptoms were not evident. Brafield-Rolfe (1992) working with *E. grandis* seedlings, also found that Cu content was influenced by N form, but no explanation was given.

It was interesting to note that foliar P and K contents of all treatments were below recommended percentages of 0.2-0.4% P and 0.7-1.5% K (half of the minimum recommendation) (Landis *et al.*, 1992). The final P and K contents of the bark were also very low, since the initial assumptions that bark had sufficient inherent K, and that 5 kg.m^{-3} of single superphosphate pre-enriched into the media would be enough for the duration of the experiment, were incorrect. The blue-green foliage observed with high N application could therefore have been slight symptoms of P and K deficiency. The mean foliar nutrient concentration of

seedlings from the 80 mg.ℓ^{-1} NH_4-N treatment are given in Table 3.4.

Table 3.4 Mean foliar nutrient concentration of *P. patula* seedlings grown in pine bark with a nutrient solution containing 80 mg.ℓ^{-1} NH_4-N.

CONCENTRATION	ELEMENT	80 mg.ℓ-1 NH4
%	N	2.07
	P	0.06*
	K	0.40*
	Ca	0.50
	Mg	0.20
mg.kg^{-1}	Fe	126.1
	Zn	330.0
	Mn	565.0
	Cu	4.8

* these nutrient concentrations are lower than recommended

Foliar Fe content was very variable and so any differences were non-significant. This was because leaf Fe analyses often yield inconsistent concentrations. However, from the data it can be seen that the Calmag® treatments generally resulted in much lower Fe concentrations than the other treatments. Coupled with the fact that chlorotic seedlings in the Calmag® treatments also contained greater Ca and Mg at maximum recommendations, chlorosis can be attributed to Ca and Mg interference with Fe uptake. Plant NH_4 nutrition results in the production of excess H^+ ions which are excreted, whereas NO_3 results in the production of excess OH^- ions which may be excreted or neutralized by organic acids produced within the plant (Van den Driessche, 1978). These form ion pairs with the cations, which enter the plant with NO_3^- (Van den Driessche, 1978). Thus, chlorosis in plants receiving NO_3 has been associated with higher organic acid concentration

and this has resulted in inactivation of Fe (Van den Driessche, 1978). Nelson & Selby (1974) also proposed that inactivation of Fe could be due to competitive chelation. This assumes that other metals (Ca, Mg), ligands such as OH^-, and chelating agents can compete with Fe for Fe-binding sites and thereby reduce the activity of Fe. This is probably the reason for the chlorosis. However, the high Ca and Mg concentrations do confound the effect of NO_3 nutrition. It is recommended that an experiment, using perhaps KNO_3, be performed to test the full consequences of NO_3 nutrition on pine seedlings.

The N content of pine bark media is inherently low, and therefore N must be supplied continuously to seedlings to ensure optimum growth rates. As most commercial forms of N are very soluble, application through the nutrient solution is considered the optimal method. Ammoniacal fertilizers are considerably cheaper than nitrate forms, and therefore provide an attractive alternative to high cost nitrate-based fertlizers.

Nitrate, ammonium and urea reactions in pine bark are very different. Nitrate is readily leached and NH_4^+ is adsorbed by pine bark. Ogden (1982) postulated that urea reacts directly with the bark particles (perhaps by covalent bonding), or is bound by microbial activity without prior conversion to NH_4^+. Chin & Kroontje (1962) surmised that adsorption of urea was by a different mechanism to cation adsorption. Since urea, when acted upon by urease, results in NH_4 hydrolysis within 24-40 hr, it would be expected to find high concentrations of this ion in the extract. Instead there was less recorded in the final pine bark. These data substantiate earlier thinking that the NH_4^+ is complexed on the bark and is not available for plant growth, except via the slow release mechanism of nitrification. Plants growing in pine bark media may, thus, be able to tolerate far higher concentrations of NH_4^+ in solution. Also, the slower rate at which ammonium is leached from acid media, compared with nitrate, may explain why 80 $mg.\ell^{-1}$ NH_4-N gave the best results. In addition, the greatest Mn content was found in the urea treatments, probably due to acidification of the medium by nitrification. This lowering of pH results in a greater

solubility of Mn (Mengel & Kirkby, 1982). The lower Mn content of the NO_3 seedlings may have been one of the factors contributing marginally to their lower growth compared to the NH_4 seedlings.

However, the possibility that the urea treatments could actually have been a mixed supply of N sources has also to be considered. In the final media, nitrate was present in the urea treatment, presumably due to nitrification. However, the greater dry matter production in the 80 $mg.\ell^{-1}$ NH_4-N treatment was due to the presence of ammonium-N, since larger quantities of nitrate-N were available in Calmag® treatments, in which growth was less.

It can therefore be concluded that *P. patula* seedling growth was enhanced more by ammonium-N application than by nitrate-N or NH_4NO_3. 80 $mg.\ell^{-1}$ N, supplied as urea, is recommended for the fertigation of *P. patula* seedlings.

CHAPTER FOUR

LIMING AMENDMENTS

4.1 INTRODUCTION

Even though tree seedlings are able to tolerate a relatively wide range of growing medium pH values, it has been well documented that conifers grow best at ± pH 5.5 (Landis *et al.*, 1992). Maintenance of the medium solution within one-half pH unit on either side of this target is therefore recommended. This generally involves increasing the pH. Any element that will bind with H^+ (compounds containing Ca, Mg and K supply useful cations to the plant and simultaneously bind H^+) will raise the pH of the medium. Liming is, therefore, a routine practice in the nursery industry. However, in South Africa lime amendment rates vary from 0-8 $kg.m^{-3}$, depending on the species grown and grower preference. Liming is most often performed as a "safety" precaution or because liming has traditionally always been done.

As pH determination is a measure of active acidity, it is often possible for two growing media to have the same pH, or active acidity, but different amounts of reserve or exchange acidity (Bunt, 1988). This is because of different buffering capacities which result in media having varying lime requirements. This is why the practice of liming composted bark media for pH control is questioned by several investigators, since fully composted pine bark has been shown to have a large buffering capacity due to a high CEC. With a large buffering capacity, small quantities of lime will be ineffective in raising the pH (Churstic & Wright, 1983; Wright, 1983; Whitcomb, 1984). Churstic & Wright (1983) concluded that there was no advantage in liming bark for the growth of holly and azalea, provided all the nutrients were supplied in sufficient quantities and that no element was present in toxic quantities. Several researchers found no growth improvement in certain types of plants with pre-plant lime additions, while others found positive growth responses (Ogden *et al.*, 1987). Brafield-Rolfe (1992) found that the pre-enrichment of composted pine bark with lime and micronutrients was

unnecessary for the growth of *E. grandis* seedlings. However, Wright & Hinesley (1991), using aged pine bark, found that it was beneficial to add 3 $kg.m^{-3}$ of dolomitic lime for eastern redcedar. It is not known if it is necessary to incorporate lime into pine bark media (which is often very acidic) for optimal growth of *P. patula* seedlings.

Liming is accomplished by adding dolomitic lime (Ca and Mg), calcitic lime (Ca only), calcium hydroxide or a combination of these and others to the media. Recommended incorporation rates range from 0-5 $kg.m^{-3}$ (Landis *et al.*, 1992). However, the addition of dolomitic lime has resulted in growth problems for some conifer seedlings. Dangerfield (1978) found that the addition of dolomite induced lime chlorosis in Douglas fir seedlings. Hathaway & Whitcomb (1984) reported that dolomitic lime significantly reduced shoot height and weight of Japanese black pine. Carter (1987) found that as the pH increased from 5.5-6.2, seedling weight and height of *Pinus sylvestris* decreased 15% and 37% respectively. Dolomitic lime has, therefore, been shown to reduce the growth of pines, but does it decrease *P. patula* growth? Do other liming products have the same effect or are they perhaps beneficial to the growth of *P. patula* seedlings?

In the field, liming is important on acid soils because it reduces the exchangeable Al and Mn to non-toxic levels. However, the pH of bark media is sometimes very low, just above 4, and does contain Al and Mn levels that are above the recommended values. This is because some bark producers compost with, and others without, lime, and some pre-enrich the composted media with lime and others do not. The bark producing industry is very secretive about their additions and so when a grower uses pine bark media it is not known what quantity of lime may have been added, and if the bark is well buffered with lime. Does this medium need to be limed or has it been limed, but still has a low pH due to reserve acidity?

The main objective of this experiment was to evaluate the recommendations for lime additions to pine bark, and to see whether the increase in pH due to these additions had significant

effects on improving the growth of pine seedlings grown in composted pine bark. It was also to test which of the pre-enriched lime products yielded the best quality seedling, and at what level of pre-enrichment. Another objective was to compare SAPPI's and Mondi's fertilization programmes, to see if timing and scheduling of fertilizers had an obvious effect on seedling quality.

4.2 MATERIALS AND METHODS

4.2.1 SAPPI - Experiment 2

Composted pine bark (fine seedling mix without pre-enrichment) was pre-enriched with five different lime types (dolomitic lime, calcitic lime, Langfos®, Calmafos® & Calmag®) at six rates (0, 2, 4, 6, 8 & 10 $kg.m^{-3}$). The percentage Ca and Mg present in the fertilizers is presented in Table 4.1. The percentage of the other nutrients in the fertilizers used in the experiment is given in Appendices 1 & 2.

Langfos® is a slow release rock phosphate (15.6% P), Calmafos® a basic phosphate (18.55% P) and Calmag® a Ca and Mg nitrate. All can be used as liming fertilizers, however Calmag® is far less effective as a liming source. Calcitic lime and Langfos® have the same liming effect, i.e., calcium carbonate equivalent, and Calmafos® and dolomitic lime are equivalent in their liming effect (Table 4.1).

No attempt was made to balance elemental contents of the various treatments, as the experiment aimed to compare the different fertilizers at the particular rates of incorporation. All treatments received a constant level of FRIT 504® at 250 $g.m^{-3}$ (Section 2.1.3). This was to ensure that there was adequate micronutrients in the media so that no deficiencies would occur when lime was added at high rates.

Table 4.1 Percentage Ca and Mg, and liming effects of the various fertilizers used in the experiment.

FERTILIZER	Ca	Mg	CALCIUM CARBONATE EQUIVALENT
	%	%	%
Dolomitic lime (Buhrmansdrif)	21.20	7.70	80.25
Calcitic lime (Lovedale)	28.00	0.28	83.80
Langfos®	29.00	0.32	83.80
Calmafos®	21.00	9.00	80.25
Calmag®	9.80	4.80	43.26

On 09.07.92 (Day 0) *P. patula* pre-germinated seeds (M4562) were sown into SAPPI trays (Section 2.1.2) and stacked in the shed at SAPPI, Richmond, until germination (Section 2.1.4). On 05.08.92 (Day 27) trays were laid out in the SAPPI nursery (Section 2.1.1). The experimental design was a 5 x 5 (+1 control) factorial random block design with two replications.

Seedlings received water until 24.09.92 (Day 77) and then fertigation was applied according to SAPPI's fertilization programme for pine seedlings. Table 4.2 gives the total fertilization that the trial received while in the nursery. Hortichem Blue® 3:1:2(43) was applied. This has an ammonium nitrate base and contains 21.2% N, 7.1% P and 14.2% K. Hortichem Orange® 1:2:1(43) contains 13.5% N, 18.0% P and 11.4% K and was also applied. Both are products sold by Ocean Agriculture (Pty) Ltd, Muldersdrift.

Table 4.2 Fertilizer applied in the nursery to Exp.2 - SAPPI seedlings.

DATE (DAY)	HORTICHEM BLUE®	HORTICHEM ORANGE®
	g.tray^{-1}	g.tray^{-1}
24.09(77)	0.50	
01.10(84)	0.86	
08.10(91)	0.75	
15.10(98)	0.89	
22.10(105)		0.81
29.10(112)	0.90	
05.11(119)		1.98
12.11(126)	0.81	
19.11(133)		0.86
26.11(140)		0.73
03.12(147)	0.83	
14.01(189)	0.90	
21.01(196)		1.45
TOTAL	6.44	5.83

Seedlings were hardened off with water, from 21.01.93 (Day 195) until 09.02.93 (Day 241) when they were harvested, data collected and samples stored according to Section 2.2. Bark samples (from initial and final stages of the experiment), foliar, and water samples were analysed for nutrient content (Section 2.3).

4.2.2 Mondi - Experiment 2

This experiment was prepared in exactly the same way as the experiment at SAPPI, described above. Experiment 2 was duplicated so that the trial could be placed at SAPPI and Mondi (Mountain Home, Hilton).

The Mondi experiment was sown on 09.07.92 (Day 0) and on 20.07.92 (Day 11) the 52 trays were moved to Hilton, having been stacked in the SAPPI shed (Section 2.1.4). Trays were laid out as at SAPPI, under a Uvidek® plastic tunnel. Temperatures experienced were very similar to that at Richmond (Section 2.1.1).

Seedlings received water until 09.09.92 (Day 62), and fertigation was then applied according to Mondi's fertilization programme. Mondi Orange® 1:2:1(44) made by Agrofert, Harvest Chemicals was applied at 0.5 $mS.cm^{-1}$. This contained 12% N, 16% P, 10.1% K and the N comprised of 7.7% NH_4 and 4.3% NO_3. It also contained trace elements: 1000 $mg.kg^{-1}$ Fe, 450 $mg.kg^{-1}$ B, 17 $mg.kg^{-1}$ Cu, 66 $mg.kg^{-1}$ Zn, 257 $mg.kg^{-1}$ Mn and 31 $mg.kg^{-1}$ Mo. Mondi Blue® 3:1:2(42) was unfortunately not applied to the seedlings, for reasons not known.

From 14.12.92 (Day 158) seedlings were hardened off, by alternating fertilizer applications with water, and on 03.02.93 (Day 210) they were harvested. Plant parameters were recorded and samples stored according to Section 2.2. Samples of water used for the irrigation were analysed for nutrients (Section 2.3). However, pine bark and foliar analysis were not done owing to the cost consideration, and it was considered sufficient to have just one trial analysed. Since the SAPPI trial was managed under a more representative fertilization programme, it was analysed.

4.3 RESULTS

4.3.1 Water Analysis

The irrigation water used at SAPPI and Mondi was analysed for nutrient content (Table 4.3) and were found to be very similar. The only differences were that the SAPPI water had a higher EC due to greater Ca and Na ion content, and a lower Zn content. However, these differences were marginal and it can be assumed that the irrigation waters were not significantly different, and therefore would not have influenced the results of the trials to any extent.

Table 4.3 Analysis of SAPPI and Mondi irrigation water.

DETERMINATION	MONDI	SAPPI
pH	6.64	6.62
EC ($\mu S.cm^{-1}$)	83	194
P ($mg.\ell^{-1}$)	1.2	1.3
NH_4 ($mg.\ell^{-1}$)	0	3.8
NO_3 ($mg.\ell^{-1}$)	0	0
K ($mg.\ell^{-1}$)	0.2	0.2
Ca ($mg.\ell^{-1}$)	5.0	13.1
Mg ($mg.\ell^{-1}$)	3.9	3.7
Na ($mg.\ell^{-1}$)	7.0	26.5
Fe ($mg.\ell^{-1}$)	<0.01	<0.01
Mn ($mg.\ell^{-1}$)	<0.01	<0.01
Cu ($mg.\ell^{-1}$)	<0.01	<0.01
Al ($mg.\ell^{-1}$)	<0.01	<0.01
Zn ($mg.\ell^{-1}$)	2.24	0.09

4.3.2 Plant Growth

Observations, at both SAPPI and Mondi, revealed that the Calmag® treatments (especially 6, 8 & 10 $kg.m^{-3}$) had very poor germination (Plate 4.1). Percentage germination for these treatments ranged from 20-56%. Thus, Calmag® at high rates was extremely toxic, i.e. the high EC (ranging from 1.09-7.98 $mS.cm^{-1}$) due to the nitrate appeared to inhibit seedling germination. It was not known that Calmag® would result in such toxic nitrate concentrations (269-1345 $mg.\ell^{-1}$) in the initial pine bark. It is, therefore, recommended that Calmag® not be pre-enriched into a pine bark medium, but rather used in the nutrient feed if needed. Thus, statistical analysis of the plant growth parameters was

performed with and without the Calmag® treatments. This was done because the Calmag® treatments were found to mask the influence of other treatment effects and interactions. All results are therefore presented without the Calmag® treatments being included.

Plate 4.1 The effect of Calmag® treatments at Mondi on the germination of *P. patula* seedlings.

4.3.2.1 SAPPI

Height (HT)

There was no significant difference in HT between the controls (mean of 218.3 ± 8.9mm) and the seedlings that had lime pre-enriched into the media, i.e., liming did not improve the HT of *P. patula* seedlings (Fig. 4.1a & Plates 4.2 & 4.3). In some cases liming actually reduced HT, e.g., all the dolomitic and calcitic treatments, and all the Calmafos® treatments, except at 6 & 8 $kg.m^{-3}$, had HT of less than 218.3 ± 8.9mm. The treatment with the greatest mean HT was 8 $kg.m^{-3}$ of Calmafos® at 244.1 ± 8.9mm, a 25.8mm increase over the control (Fig 4.1a & Plate 4.4). Calmafos® and Langfos® treated seedlings had mean HT significantly greater ($P \leq 0.01$) than those receiving calcitic and dolomitic lime.

Plate 4.2 Comparison of no lime versus 2 kg.m^{-3} of various lime products pre-enriched into pine bark media (SAPPI trial).

Plate 4.3 Effect of no lime versus 10 kg.m^{-3} of various liming products pre-enriched into pine bark media (SAPPI trial).

Collar diameter (CD)

Once again the controls (mean CD of 2.52 ± 0.08mm) were not significantly different to the other treatments (Fig. 4.1b). The control treatment ranked fourth. The greatest CD (2.59 ± 0.04mm) was produced with 4 kg.m^{-3} of Calmafos® and 6 kg.m^{-3} of Langfos®, followed by 8 kg.m^{-3} of Calmafos®, 0.01mm less (Fig. 4.1b).

Seedlings pre-enriched with Calmafos® and Langfos® therefore had significantly ($P \leq 0.01$) greater CD than the calcitic and dolomitic lime treatments, and the calcitic lime seedlings were also markedly ($P \leq 0.01$) larger than the dolomitic treatments. The liming rate of 10 $kg.m^{-3}$ for all treatments yielded significantly smaller ($P \leq 0.01$) CD (Fig. 4.1b).

Root and shoot dry mass (RDM and SDM)

There was no significant difference in total SDM and RDM between the unlimed and limed treatments (Fig.4.1c & d). However, all the dolomitic and calcitic treatments resulted in lower SDM and RDM than the control with 0.94 ± 0.04 $g.seedling^{-1}$ and 0.38 ± 0.01 $g.seedling^{-1}$, respectively. It was noticed at harvesting that all the dolomite treatments had poor root development, i.e., plugs were not solid and tended to collapse when removed from the trays. The control treatments were observed to have the most solid root plugs, i.e., removal from the trays was easy. Highly significant differences in dry mass between the various lime types were noted with Calmafos® = Langfos® > calcitic lime > dolomitic lime. All rates of lime yielded markedly greater ($P \leq 0.05$) RDM than when 10 $kg.m^{-3}$ was applied (Fig. 4.1d). A rate of 8 $kg.m^{-3}$ of Calmafos® resulted in seedlings with the greatest SDM and RDM of 1.10 ± 0.04 $g.seedling^{-1}$ and 0.48 ± 0.01 $g.seedling^{-1}$, respectively (Fig. 4.1c & d).

Plate 4.4 Effect of Calmafos® pre-enrichment on the growth of *P. patula* seedling (SAPPI trial).

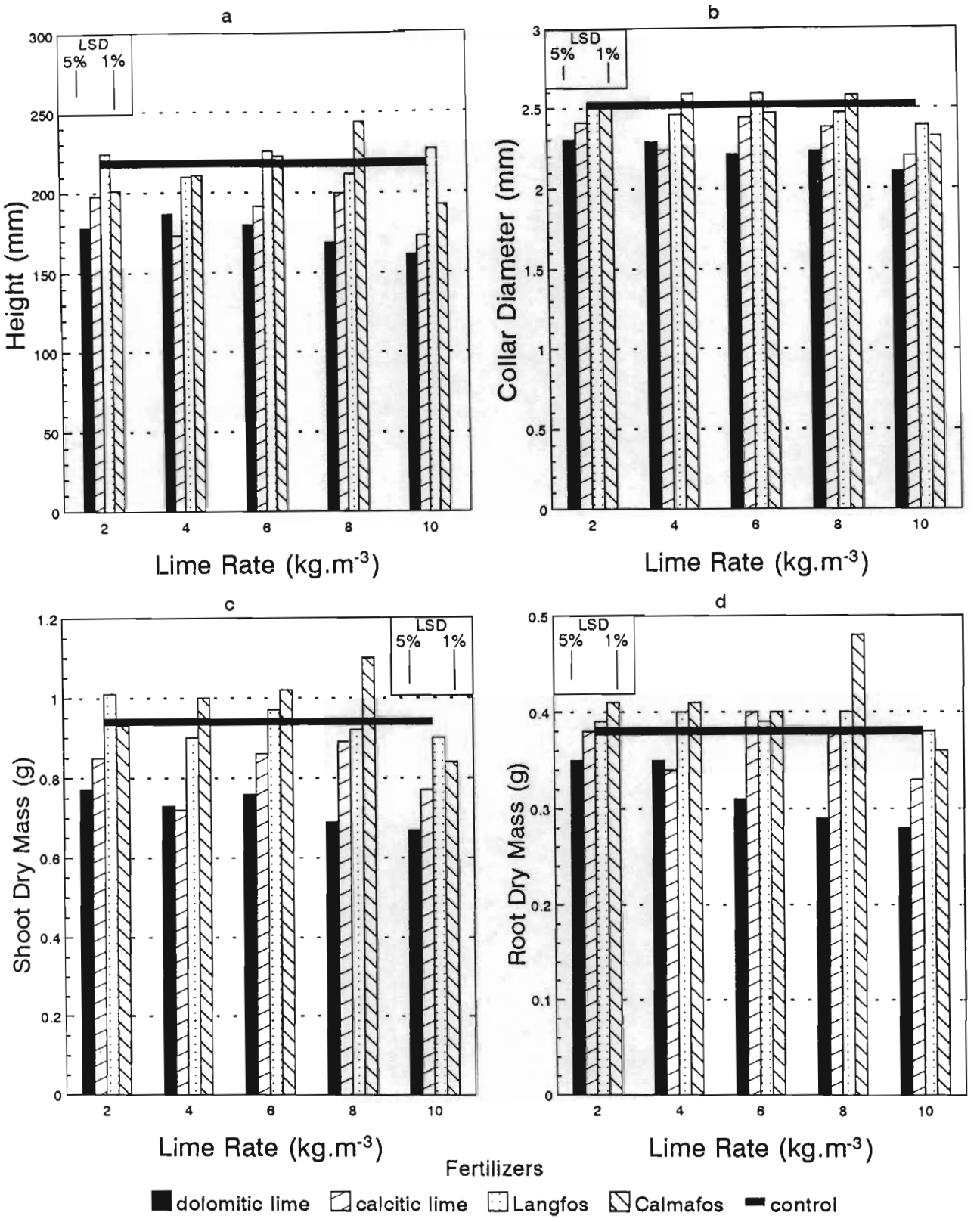


Fig. 4.1 Effect of liming with various products at different rates on the (a) height, (b) collar diameter, (c) shoot dry mass and (d) root dry mass of pine seedlings grown at SAPPI.

Root:shoot ratio

There was no significant treatment difference in root:shoot ratio. However, there was a significant block (replication) effect, indicating that an environmental gradient (temperature effect) existed in the tunnel, which was accounted for in the statistical model.

4.3.2.2 Mondi

Height (HT)

As at SAPPI, there was no significant difference between limed and unlimed treatments (Plate 4.5). The control seedlings had a mean HT of 166.2 ± 5.2mm which always exceeded the dolomitic treatments (Fig. 4.2a). Maximum HT was at 2 $kg.m^{-3}$ Calmafos® (182.5 ± 5.2mm mean), closely followed by 8 $kg.m^{-3}$ Calmafos® with a difference of 0.6mm (Fig. 4.2a). All Calmafos® treatments (171.7 ± 5.2mm mean) gave rise to significantly greater HT ($P \leq 0.05$) than dolomitic treatments (156.6 ± 5.2mm mean).

Collar diameter (CD)

Again, statistics proved that the control was not significantly different to the other treatments (Fig. 4.2a). There was however, a significant difference ($P \leq 0.01$) between the various liming fertilizers. Calmafos® (2.23 ± 0.04mm mean) and calcitic lime (2.25 ± 0.04mm mean) treatments resulted in larger CD than dolomitic lime (2.11 ± 0.04mm mean) (Fig. 4.2b). Calcitic lime at 8 $kg.m^{-3}$ produced the largest CD (2.32 ± 0.04mm) and 8 $kg.m^{-3}$ Calmafos® the next largest (Fig. 4.2b). For all treatments, the control had a greater CD than the dolomitic lime, however this was not statistically significant (Fig. 4.2b).

Root and shoot dry mass (RDM and SDM)

There was no significant RDM difference between any of the treatments (Fig. 4.2d). The control yielded a root system that was either better or similar to the other treatments, barring 2 $kg.m^{-3}$ calcitic lime which was greater (Fig. 4.2d). As at SAPPI, it was noticed that dolomitic lime treatments produced inferior roots.

The SDM was only significantly different ($P \leq 0.01$) between the lime products. Calcitic lime (0.80 ± 0.03 g.seedling^{-1}) and Calmafos® (0.78 ± 0.03 g.seedling^{-1}) were greater than dolomitic lime treatments with a mean of 0.69 ± 0.03 g.seedling^{-1} (Fig. 4.2c). The control SDM of 0.78 ± 0.03 g.seedling^{-1} was not markedly different to the other treatments, including the treatment producing the greatest mass, i.e, 6 kg.m^{-3} calcitic lime (0.85 ± 0.03 g.seedling^{-1}) (Fig. 4.2c).

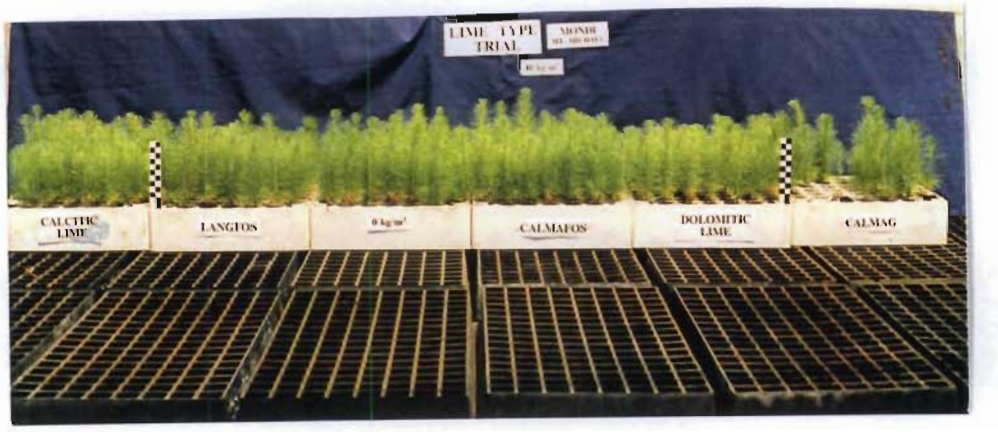


Plate 4.5 The effect of liming at 10 kg.m^{-3} versus no lime for the various lime products (Mondi trial).

Root:shoot ratio

There were no significant differences within the experiment, i.e., liming had no significant effect on the partitioning of dry mass between root and shoot.

Seedling colour

It was observed at Mondi that seedlings showed chlorotic symptoms for a period from October to December. These symptoms were most obvious in the 6, 8 & 10 kg.m^{-3} dolomitic lime treatments and in the 8 & 10 kg.m^{-3} calcitic lime treatments. However, approximately 1½ months after first appearing, the chlorosis disappeared. This phenomenon did not occur at SAPPI.

It was noticed at harvesting that Mondi seedlings were all a light green (yellowish) colour, and the SAPPI seedlings of the same age were much greener and of larger size. This can be attributed to the different fertilization regimes. SAPPI seedlings received more fertilizer in the nursery in the final growth stages than Mondi seedlings (Section 4.2).

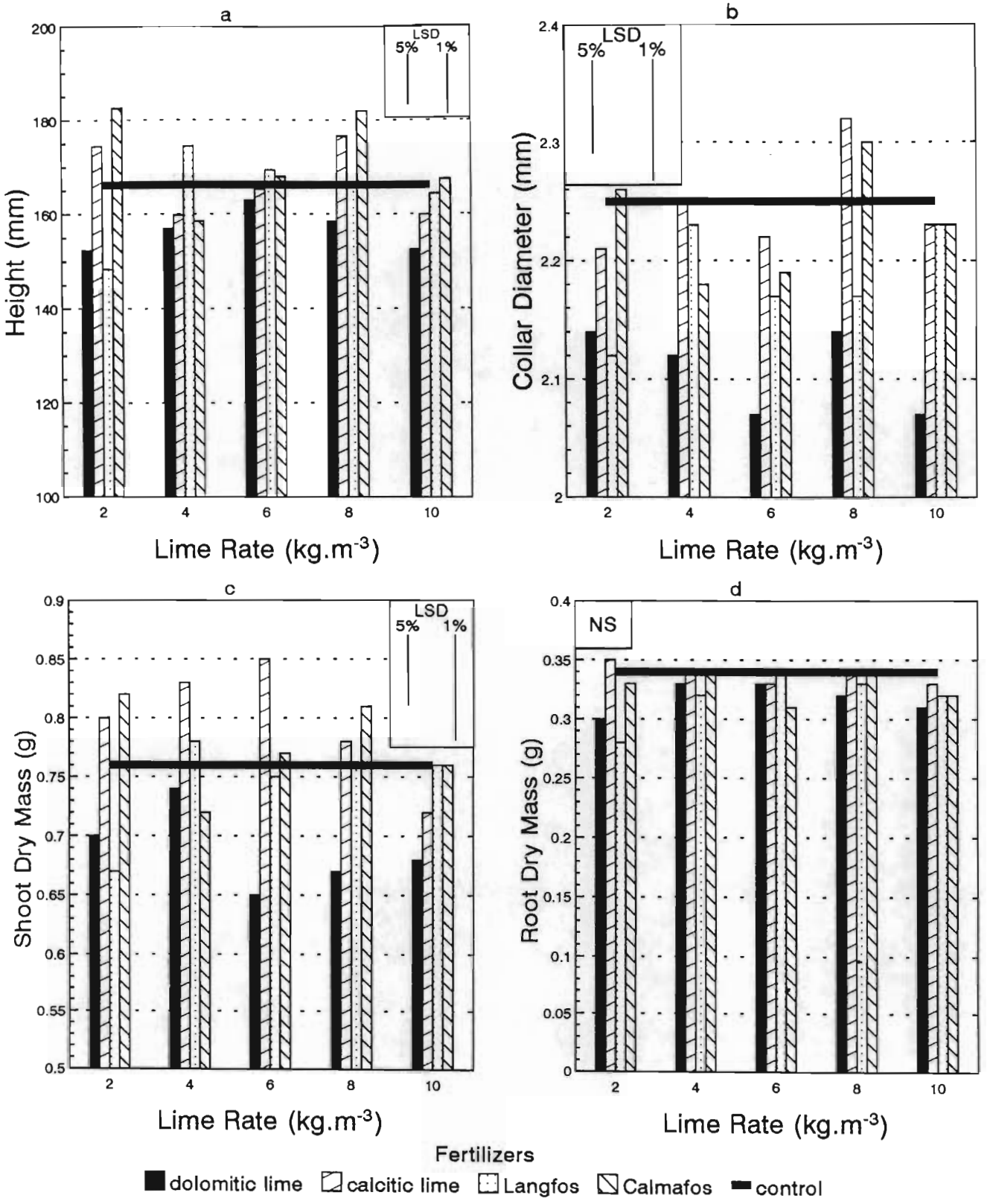


Fig. 4.2 Effect of liming with different fertilizers and rates on (a) height, (b) collar diameter, (c) shoot dry mass and (d) root dry mass of *P. patula* seedlings grown at Mondi.

4.3.3 Leaf Analysis

Foliar nutrient contents of the SAPPI seedlings were not statistically analysed using Genstat, since only one replication was chemically analysed. All comments relating to these data are therefore only made from observations.

Percentage foliar N was markedly greater in the control treatment (1.46% N) than in all the other treatments, which had a mean of 1.09% N. The Calmag® treatments did not vary much from the mean foliar N%. Foliar P revealed no great differences, ranging from 0.09-0.12% P. It was expected that the Calmafos® and Langfos® treatments would have a greater foliar P, both being phosphate fertilizers. However, this was not the case, as the control treatments (0.11% P) had as much P. Foliar K was 0.72% in the control, and increased with increasing lime rates, but decreased to 0.75% K when 10 $kg.m^{-3}$ of lime was pre-enriched. This trend was generally followed by all lime products and can be explained by the fact that K uptake mirrored the concentration of K in the pine bark. There was no increase in K% in the Langfos® and Calmafos® treatments which contained 0.25% and 0.04% K, respectively. Foliar P and K were not greater in the Langfos® and Calmafos® treatments because these are slow release fertilizers.

Foliar Ca and Mg followed the same trend, both increasing with increasing lime rate, and then reducing to a mean of 0.28% Ca and 0.20% Mg when 10 $kg.m^{-3}$ of lime was pre-enriched (Fig. 4.3). This was less than the control of 0.29% Ca and greater than the control of 0.18% Mg. This foliar accumulation can also be attributed to the influence of Ca and Mg in the pine bark on seedling uptake. However, Calmafos® treatments resulted in decreased foliar Ca as the liming rate increased, with the lowest Ca concentration of 0.16% at 10 $kg.m^{-3}$ (Fig. 4.3a). Thus, the Calmafos® seedlings did not take up all the Ca that was available, i.e., uptake seemed to be hindered, perhaps by another nutrient or perhaps the Ca was not in an available form.

No strong micronutrient trend was apparent, except for foliar Zn

which increased with increasing lime rate and then decreased at rates above 6 $kg.m^{-3}$ for the different lime products. This trend in seedling uptake equates to available Zn content in the pine bark. Foliar concentrations of these nutrients were very variable, with ranges from 2.8-7.0 $mg.kg^{-1}$ for Cu, 53-103 $mg.kg^{-1}$ for Zn, 70-650 $mg.kg^{-1}$ for Mn and 140-463 $mg.kg^{-1}$ for Fe. It was interesting to note that the Calmafos® treatments resulted in foliar Fe and Mn decreasing with increasing lime rates (Fig. 4.4). Calmafos® contains 7% Fe and 0.15% Mn, so increased availability could result in higher concentrations in the plant. This did not occur, since Fe and Mn must have been in an unavailable form, as it is known that increased pH decreases their availability and results in less plant uptake. This trend also occured with foliar Mn for the dolomitic and calcitic lime treatments (Fig. 4.4b). Therefore, foliar Mn was lower in all dolomitic and calcitic lime treatments than in the control.

Thus, treatment differences in foliar concentrations following tissue analysis were not apparent, except for N.

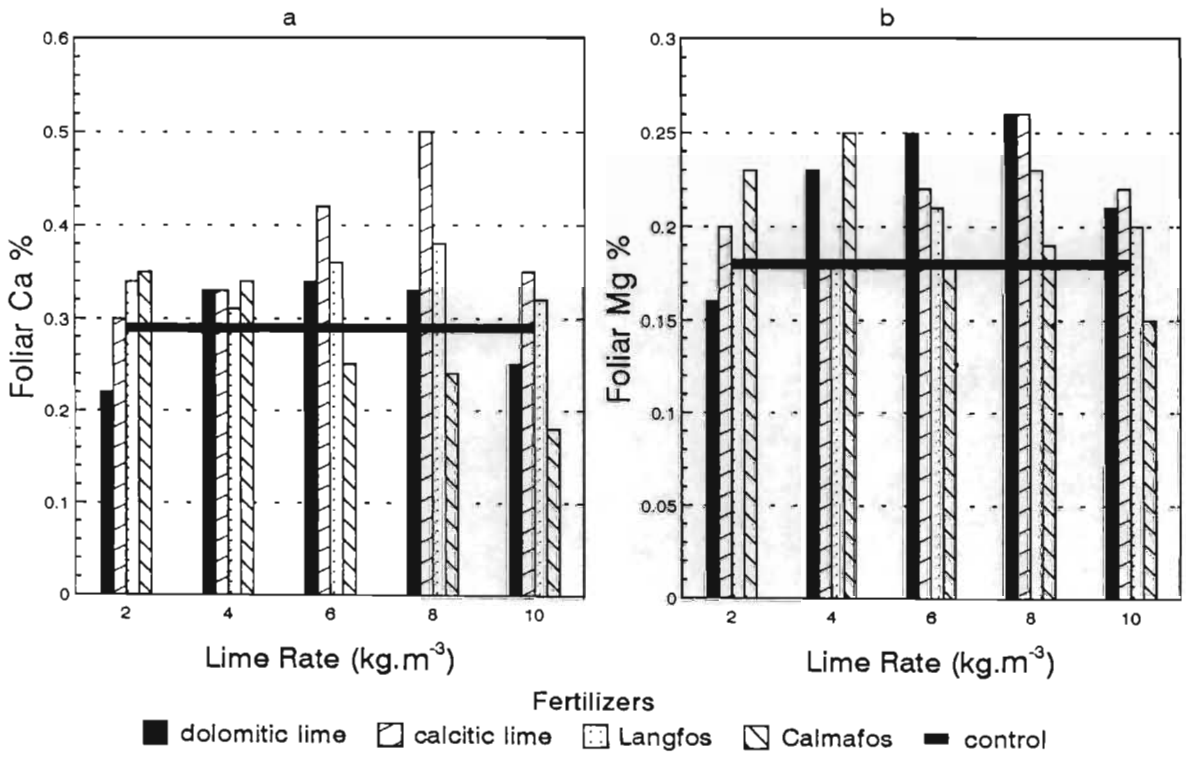


Fig. 4.3 Effect of increasing liming rates of the various fertilizers on (a) foliar Ca % and (b) foliar Mg % in *P. patula* seedlings.

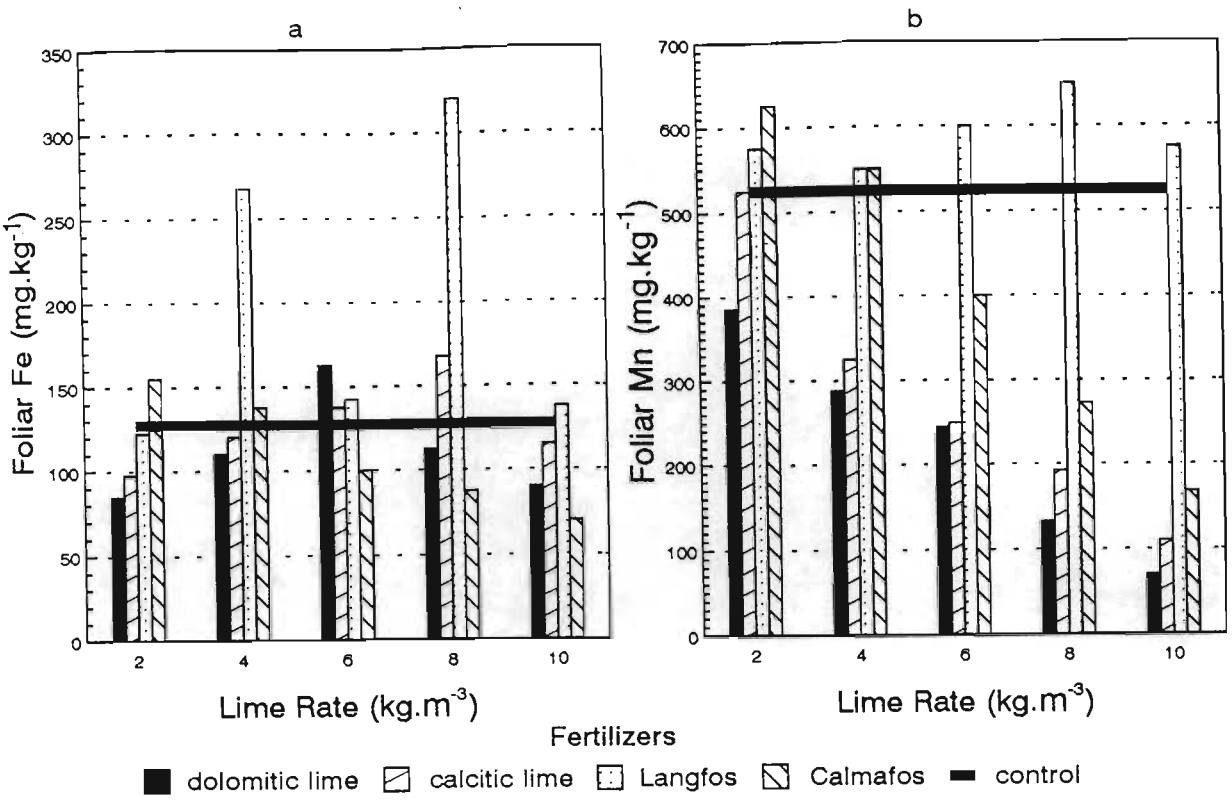


Fig. 4.4 Effect of increasing liming rates of the various fertilizers on (a) foliar Fe and (b) foliar Mn in *P. patula* seedlings.

4.3.4 Medium Analysis

As with the leaf analysis, only one replication of the experiment was chemically analysed, and so analysis of the pine bark can only reveal trends, since it was not analysed by Genstat.

Initial Pine Bark

As expected, the pH and EC of the treatments increased with increasing lime rates. However, not all showed a linear response. Calmafos® and calcitic lime had quadratic responses, probably due to sampling error. The highest pH of 5.26 was with 10 $kg.m^{-3}$ of calcitic lime compared to the lowest in the control at 4.38. However, this difference of 0.88 was much less than expected. This pH effect can be attributed to bark having a high buffering capacity so that the pH determination does not measure the reserve or actual acidity of the bark.

The quantity of NH_4-N in the bark was reasonably similar, ranging from 7.59-16.22 mg.ℓ^{-1}, with the control having marginally the highest NH_4-N and the calcitic and dolomitic lime treatments slightly lower NH_4 contents than the rest. The NO_3-N concentrations were also similar, with the control having 25.19 mg.ℓ^{-1} NO_3. However, the calcitic and dolomitic lime treatments had means of 44.42 and 41.69 mg.ℓ^{-1} NO_3-N respectively. This was due to NO_3 concentrations increasing with increasing rates of lime in these two treatments. This may be attributed to the higher pH in these treatments stimulating nitrification and therefore resulting in greater NO_3 availability in the medium. This, however, should not have occurred as samples were placed in a cold room to inhibit such microbiological reactions.

The availability of P and K did not differ between the various lime products and liming rates. However, P availability in the dolomitic lime treatments was the lowest for all products, with the lowest being 1.43 mg.ℓ^{-1} in the 8 kg.m^{-3} dolomitic lime treatment. Since Langfos® and Calmafos® are phosphate fertilizers, it was expected that an increasing lime rate would result in an increase in P content. This was not evident, indicating that the P was largely unavailable, i.e., both are slow release fertilizers.

Liming increased the availability of Ca and Mg in the initial bark for all lime products, but increases were negligible. This indicated that Ca and Mg were not readily available due to SRF such as Calmafos® and Langfos®, or because the bark had a high CEC which resulted in the nutrients being adsorbed and unavailable.

No marked differences in the availability of the micronutrients were identified. Copper and Zn showed no trends, ranging from 0.01-0.04 mg.ℓ^{-1} and 0.04-0.13 mg.ℓ^{-1}, respectively. Iron and Mn had ranges of 0.14-1.82 mg.ℓ^{-1} and 0.09-0.18 mg.ℓ^{-1}, respectively. However, the lowest Mn content was that of the control, but only marginally less. Thus, the incorporation of Langfos® and Calmafos®, which contain micronutrients, did not significantly increase the initial pine bark micronutrient contents for these

treatments.

Final Pine Bark

Analysis of the bark at the end of the experiment confirmed the perception that the nutrient status of bark does decrease with time due to leaching and seedling uptake of nutrients. Nitrate, NH_4, K, Ca, Mg, Mn, and therefore the EC were lower than in the initial bark. However, P and Cu were the same. The final N-P-K nutrient solution applied to the experiment contained a large amount of P (Table 4.2) and would have resulted in the P content being "topped up" to the initial amount. The unchanged Cu content could possibly have been due to the application of a fungicide, as did occur. The pH of the final bark was higher than that of the initial bark, due to the addition of lime and therefore the activation of nitrification in the medium during the duration of the experiment, resulting in NO_3 production. However, media Fe and Zn concentrations generally increased. The latter may have increased because the water supply had a high Zn content. Iron increased at all lime rates, except the 10 $kg.m^{-3}$ where Fe content was less than the initial bark samples. As mentioned in Exp.1, the increase in Fe content could be due to contamination of the irrigation water from corroding pipes. The fact that less Fe was available at the highest lime rates can be ascribed to Fe leaching, due to all cation exchange sites being dominated by Ca, or due to pH resulting in Fe being unavailable.

The pH and EC of the final media generally increased with liming. This trend was not very consistent for the different lime products. The highest pH and EC was 7.5 and 0.22 $mS.cm^{-1}$ when 8 $kg.m^{-3}$ calcitic lime was pre-enriched, and the lowest was 5.86 (4 $kg.m^{-3}$ Langfos®) and 0.11 $mS.cm^{-1}$ (2 $kg.m^{-3}$ calcitic lime). The control's pH was 6.17 and EC 0.13 $mS.cm^{-1}$.

The content of P in the final media was slightly lower in the dolomitic lime treatments and slightly higher in the Calmafos® and Langfos® treatments. However, these differences would probably not have been statistically different. Phosphorus, surprisingly, showed no decrease in availability with increased lime, as would happen with the formation of insoluble calcium

phosphates. Nitrate availability did not increase with liming, as expected with the stimulation of nitrification. Potassium increased in availability and then slightly decreased at rates above 6 $kg.m^{-3}$ for all treatments. The reduction in available K at the highest lime rates can be ascribed to K leaching, due to cation exchange sites being dominated by Ca. The opposite trend for N, P and K with liming, may be due to the nutrient solution suppling a constant amount of N, P and K to all treatments.

Calcium and Mg availability increased with increasing lime additions, but for Ca this dropped when 10 $kg.m^{-3}$ of lime was applied. This trend occurred across all lime products. The controls had the lowest Ca (2.2 $mg.\ell^{-1}$) and Mg (0.2 $mg.\ell^{-1}$) contents, since the bark was not pre-enriched with lime. The difference between the control and the treatments with the greatest mean availability of Ca and Mg, was 3.02 $mg.\ell^{-1}$ (8 $kg.m^{-3}$) and 1.00 $mg.\ell^{-1}$ (10 $kg.m^{-3}$), respectively. This was negligible when compared to the amounts of Ca and Mg that were added to the media.

The availability of Mn and Cu was not influenced by liming. All treatments had a Mn content of <0.01 $mg.\ell^{-1}$ and Cu ranged from 0.02-0.06 $mg.\ell^{-1}$, with the control having the lowest Cu content. However, Zn content in the media increased with liming and then generally decreased when lime rates increased above 8 $kg.m^{-3}$. Iron availability in the control was not markedly different to the limed treatments, but was greater in the 2, 4 and 10 $kg.m^{-3}$ treatments for all products. Therefore, it seems that only Zn was affected by liming the pine bark medium.

4.4 DISCUSSION AND CONCLUSIONS

The reduced growth rate or equal growth that resulted when the pine bark media were limed, confirms reports that there is no advantage to liming composted pine bark media. There was no significant improvement in the growth and quality of *P. patula* seedlings grown in media ammended with lime.

However, of the limed seedlings at SAPPI the treatment of 8 $kg.m^{-3}$

of Calmafos® resulted in the largest HT, RDM and SDM. The CD of this treatment ranked third. Plant parameters at Mondi also showed that 8 $kg.m^{-3}$ of Calmafos® was probably the best lime treatment for pre-enrichment, if liming was applied. For the plant parameters at Mondi this treatment always came out second best, with various treatments being the best for the different parameters measured. The fact that Calmafos® was the better of the limed treatments, can be attributed to it being a slow release rock phosphate that is high in macronutrients such as Ca, Mg and P and micronutrients such as Fe, Mn, Zn, Cu and Mo. However, in this experiment the effects of Calmafos® as a P and micronutrient source were negligible, i.e., foliar percentage and medium analysis were only marginally greater in the Calmafos® treatments. Thus, it can be concluded that even though Calmafos® was the best of the liming products, its use is not warranted in seedling production, where 8 $kg.m^{-3}$ has to be applied for only marginally better growth.

In some cases liming actually decreased growth on average, but this could not be proved statistically. This occurred with the dolomitic lime treatments, which had the smallest growth and produced roots that were not as developed as in all the other treatments. Finely ground dolomitic lime has been reported to produce poor plant growth, due to differences in the solubility of Ca and Mg carbonate of which it is comprised (Ogden *et al.*, 1987). Whitcomb (1984) found that this resulted in different release rates, and therefore in availabilities of Ca and Mg. He showed that soon after planting the Ca:Mg ratio, instead of being 2:1, approaches 1:50, and then slowly changes to 2:0.05 or less, i.e., initially plants are subjected to a Mg excess followed by a deficiency. Results obtained from the final bark analysis, showed that the dolomitic treatments did have a slight deficiency of Mg, i.e. the Ca:Mg ratio ranged from 5.5-3:1. This could have explained the poor root growth as Mg deficiency affects the roots more than the shoots (Lyle, 1969). Dolomitic lime treatments had a lower P content in the final bark that could also have resulted in decreased root growth.

The fact that the seedlings at Mondi, receiving 6, 8 and 10 $kg.m^{-3}$

of dolomitic and calcitic lime, showed chlorosis can perhaps be attributed to a Mg excess in the dolomitic treatments. The chlorosis disappeared after 1½ months, no doubt as a result of excessive leaching and fertigation by the nursery to neutralize the chlorotic problem. Therefore, the excess Mg was leached, as pine bark has a low attraction for Mg in the presence of large quantities of N and Ca, because of the competition for exchange sites. Chlorosis did not occur at SAPPI as excess Mg was leached before symptoms appeared. This experiment was planted in SAPPI trays, which have a larger volume than Mondi trays, but were fertigated under a Mondi fertilization programme. This could explain why chlorosis only occurred at Mondi. Plugs were probably not being thoroughly leached and a salt build-up resulted in chlorotic leaf symptoms. Chlorosis in the calcitic and dolomitic lime treatments could also be explained as an Fe deficiency, as seedlings were a pale greenish yellow colour in the tuft of the terminal needles. This temporary immobilization of iron in the media usually corrects itself within a few weeks and seedlings regain normal colour (Will, 1978). Chlorosis of this nature is induced by high concentrations of Ca, with an associated excess of Mg. Therefore, to compensate for the high solubility of Mg carbonate in finely ground dolomitic lime, a coarser dolomite is recommended in which the Mg release rate is slower (Whitcomb, 1984). Alternatively, Ca and Mg salts of similar solubility can be pre-enriched. Thus, dolomitic lime, which is frequently used for pre-enrichment in the nursery industry, should be applied with caution and not pre-enriched in media used to grow *P. patula* seedlings.

Comparison of Mondi and SAPPI seedlings revealed that Mondi seedlings were smaller in HT, CD, SDM and RDM, and did not look as healthy, i.e., they were not as green. SAPPI seedlings did not receive micronutrients, whereas Mondi seedlings were fertigated with micronutrients. However, this difference was not noticeable in the seedling growth responses. Mondi applied fertilizer 15 days earlier than SAPPI, but the results show that this did not benefit the seedlings. Fertilizer timing may, therefore, not be crucial in a long term crop. The reason for the size and quality factor can only be explained by the fact

that SAPPI applied a periodic fertigation schedule and Mondi supplied constant fertigation, with every sixth and seventh day leaching with water (Section 1.3.3.2). Thus, SAPPI's fertilization programme applied nutrients in relation to seedling development and size, whereas Mondi's programme was fixed, without taking into account seedling size or stage of development. This type of programme can result in young seedlings having excess nutrients and older seedlings insufficient nutrients. Thus, Mondi seedlings were not as green, an indication that the constant fertilization was not sufficient for the older seedlings with greater growth rates. This supports the theory that scheduling of nutrients is important if quality seedlings are required (Ingestad, 1979).

Pre-enrichment of Calmag® in the pine bark caused mortalities, i.e. the high ion concentration inhibited seedling germination. It is therefore recommended that Calmag® is not used for pre-enrichment in pine bark media. It can, however, be applied in a nutrient solution.

Dolomitic lime is often incorporated into pine bark for "safety" reasons at low rates of 2 $kg.m^{-3}$. Table 4.4 compares the resultant foliar concentrations of this treatment with a treatment receiving no lime and the best limed treatment (8 $kg.m^{-3}$ of Calmafos®).

All foliar percentages in Table 4.4 are similar, except for N% and Fe% which are much higher in the control, and Mn% which is lower. A lower Mn content in the control can be explained by the fact that the initial bark of the control inherently contained the least Mn. Higher Fe in the control is due to increasing pH resulting in Fe becoming less available. The fact that the control had the most N is attributed to nitrification. Liming stimulates nitrification, the conversion of NH_4^+ to NO_3^-. Thus, the rate of nitrification in the control would have been less than in any other treatment, and so leaching of nitrate would therefore have been the least. The seedlings grown without lime would not have had such a N depleted medium. This would explain why control seedlings had the highest foliar N. Any additional

N, i.e., in the limed treatments, added in the nutrient feed would have been rapidly converted to nitrate and leached with the following fertigation. This lack of foliar N may not have occurred if N was constantly applied in the nutrient solution. Thus, liming actually results in a lower foliar N concentration.

Table 4.4 Mean foliar nutrient concentrations of *P. patula* seedlings grown in pine bark pre-enriched with no lime, 2 $kg.m^{-3}$ dolomitic lime and 8 $kg.m^{-3}$ of Calmafos®.

CONCENTRATION	ELEMENT	NO LIME	2 $kg.m^{-3}$ DOLOMITIC LIME	8 $kg.m^{-3}$ CALMAFOS®
%	N	1.46	0.96	1.16
	P	0.11	0.10	0.09
	K	0.72	0.59	0.65
	Ca	0.29	0.22	0.24
	Mg	0.18	0.16	0.19
$mg.kg^{-1}$	Fe	127.5	85	87.5
	Zn	60	52.5	52.5
	Mn	105	385	272.5
	Cu	5.25	5.25	4.5

These comparisons show that a small and large amount of lime have a similar effect on the foliar nutrient concentrations of a pine seedling. Nutrient concentrations of the non-limed pine seedling are within the adequate nutrient range, however the limed treatments fall mostly in the non-adequate foliar nutrient range. Thus, from foliar concentrations it can be seen that liming has no beneficial effect on seedling tissue concentration and that foliar differences were not marked.

Incorporation of lime products did not significantly increase the pH or the Ca and Mg contents of the pine bark media. This is

because pine bark inherently has a high Ca and Mg content, and a high buffering capacity, which results in nutrients being adsorbed. Micronutrient availability was not markedly affected by liming, except for Zn which as expected decreased when the pH was too high. These data show how ineffective and wasteful it is to add large quantities of lime to alter the pH, in a medium that has a large buffering capacity.

Considering the small differences in nutrient availability in the media and in the leaf analysis, it is not surprising that there was a lack of significant differences in seedling growth due to liming and between the liming treatments. There may be a number of reasons for this. Firstly, it appears that pine bark media have a relatively high inherent base status with regard to Ca and Mg (the control treatments did not show any reduction in growth due to low Ca or Mg availability). Secondly, as all treatments were supplied with an N-P-K nutrient solution throughout the experiment, plants were not subjected to any other nutritional stress that may have been induced by high Ca concentrations in the media, e.g., P.

It can, therefore, be concluded that the liming of composted pine bark to improve growth of *P. patula* seedlings is an erroneous recommendation, and that lower pH of ±4.5 are acceptable in pine bark media for the growing of *P. patula* seedlings.

CHAPTER FIVE

LIME AND MICRONUTRIENT PRE-ENRICHMENT

5.1 INTRODUCTION

Composted pine bark is highly acidic, ranging in pH from 4.5-5.5 (Van Schoor *et al.*, 1990). It has been shown to contain most, if not all, of the micronutrients considered essential for plant growth, but their availability to plants is uncertain (Lunt & Clarke, 1959). Recently, Niemiera (1992) stated that the average water-extractable micronutrient content of pine bark, with the exception of Fe, is similar to Hoagland's micronutrient solution. He extracted bark with DTPA (an extractant that is a reliable indicator of growing media micronutrient status (Handreck, 1989)), and found that bark supplies sufficient Fe, Zn, Cu and Mn to satisfy a plant's requirements. Nevertheless, amending bark with micronutrients is a common nursery practice. Nurseries seem to apply micronutrients as insurance, rather than with assurance that they are actually helping (Wright & Niemiera, 1987). Both positive plant growth responses and no growth responses have resulted with the addition of micronutrients to container media (Niemiera, 1992). It is not known whether South African pine bark has sufficient inherent micronutrients for the growth of pine seedlings.

Studies have shown that maximum nutrient availability oçcurs at ± pH 6.5 in mineral soils, but is 1.0-1.5 units lower (pH 5.0-5.5) in organic soils and soilless media (Lucas & Davis, 1961). They pointed out that many organic soils and soilless media do not require liming to near-neutral pH to grow normally acid-sensitive plants, as at these pH ample Ca and Mg is available to plants because of the high exchange capacity of these soils and media. They stated that liming organic soils and soilless media to pH levels used for mineral soils may be a serious mistake due to reduced availability of essential elements. Lucas & Davis (1961) showed that raising the pH above 5.8 reduces P, Mn, B, Fe and Zn availability and that a very acidic pH causes K, Ca, N, B, Cu and Mo deficiency. The factors that account for reduced

growth at higher pH are many and interrelated to the extent that single cause-and-effect relationships cannot be established (Wright & Niemiera, 1987). An objective of this experiment was to test the effect of addition of lime on the availability of nutrients in pine bark medium.

In most forest nurseries in South Africa the addition of lime to bark has been common practice and is generally regarded as beneficial to seedlings. However, for many nurseries the amount of lime added has been a case of trial and error, as most recommendations are based on foreign results and norms for vegetable seedlings. The arguments for and against liming are very controversial. Thus, during the course of this experiment it was determined whether the addition of lime to pine bark is necessary for the growth of *P. patula* seedlings, and whether normal plant growth can be achieved by providing all the essential elements irrespective of pH.

It is generally accepted that the micronutrients Fe, Mn, Zn, Cu, B, Mo and more recently Ni, must be present to support normal plant growth. Researchers in America developed fritted trace elements (FTE®) and Micromax®, both micronutrient fertilizers, that can be pre-enriched into a medium to correct micronutrient deficiencies. FTE® consist of glass shards that contain the full range of micronutrients (Appendix 2) which are slowly released into the growing medium solution, providing a constant balanced nutrient supply (Bunt, 1988). The release period is the entire crop growing season, but is controlled by the pH of the medium (Bunt, 1988). Thus, there are many formulations of FTE®, to suit different crop growth periods. Micromax® is a soluble sulphate form of micronutrients (Appendix 2), that is slowly released (up to 18 months) when it is mixed at a rate of 0.5-0.8 $kg.m^{-3}$ for seedlings (Whitcomb, 1984). It is a specially patented formula of micronutrients in a well balanced ratio that maximises plant growth. The product Micromax® took 12 years to develop out of 81 formulations tested. Both these products have become important in the fertilizer market and are incorporated into many nutritional programmes. Therefore, both were applied in this experiment to see which of the two yielded a better quality pine

seedling with optimum growth.

Thus, this experiment had several objectives, but most importantly it aimed to find out which micronutrient-lime combination produced the best seedling.

5.2 MATERIALS AND METHODS

This experiment, Exp.3, compared two different micronutrient fertilizers at six levels of application with five rates of dolomitic lime (0, 2, 4, 8, 16 $kg.m^{-3}$). (Dolomitic lime was used not knowing that Exp.2 would show it to be inferior). FRIT 504® was applied at 0, 50, 100, 200, 400, 800 $g.m^{-3}$ and Micromax® at 0, 200, 400, 600, 800, 1000 $g.m^{-3}$ (Section 2.1.3). The exact quantities of elements applied are shown in Tables 5.1 and 5.2.

Table 5.1 Amount of micronutrients applied to the medium at the different levels of FRIT 504® and Micromax®.

FERTILIZER ($g.m^{-3}$)	**($g.m^{-3}$)**					
	Fe	**B**	**Mn**	**Zn**	**Cu**	**Mo**
FRIT 504® 50	7.2	1.9	3.8	3.5	3.5	0.04
100	14.3	3.8	7.5	7.0	7.0	0.08
200	28.6	7.6	15	14.0	14.0	0.16
400	57.2	15.2	30	28.0	28.0	0.32
800	114.4	30.4	60	56.0	56.0	0.64
Micromax® 200	24.0	0.2	5.0	2.0	1.0	0.1
400	48.0	0.4	10.0	4.0	2.0	0.2
600	72.0	0.6	15.0	6.0	3.0	0.3
800	96.0	0.8	20.1	8.0	4.0	0.4
1000	120.0	1.0	25.0	10.0	5.0	0.5

Table 5.2 Amount of Ca and Mg applied to the medium at the different levels of dolomitic lime and Micromax®.

FERTILIZER	($kg.m^{-3}$)	($g.m^{-3}$)	
		Ca	Mg
Dolomitic lime	2	424	154
	4	848	309
	8	1696	618
	16	3392	1235
Micromax®	0.2	12	6
	0.4	22	12
	0.6	32	18
	0.8	43	24
	1.0	54	30

P. patula seeds (M4562) were sown by hand (Section 2.1.4) on 21.08.92 (Day 0) into SAPPI trays described in Section 2.1.2. The trays were laid out in a plastic tunnel (Section 2.1.1) as a 5 x 11 factorial random block design with two replications. A standard nutrient solution of 3:1:3(38) at 1.0 $mS.cm^{-1}$ was applied to the seedlings through a boom system. Seedlings were harvested on 23-24.02.93 (Day 186 & 187) and once growth measurements had been taken they were dried and stored (Section 2.2). Foliar and pine bark samples were analysed for nutrient contents (Section 3.3), but not statistically as only one replication was chemically analysed.

5.3 RESULTS

5.3.1 Plant Growth

Height (HT)

Control treatments receiving no lime or micronutrients had a mean HT of 183.9 ± 14.3mm versus a mean of 223.0 ± 14.3mm for all the

other treatments, i.e., control seedling HT was significantly less ($P \leq 0.01$) than the other treatments. There were also significant HT differences ($P \leq 0.01$) between the lime rates, with no micronutrients, 4 $kg.m^{-3}$ > 0 $kg.m^{-3}$ = 2 $kg.m^{-3}$ > 8 $kg.m^{-3}$ > 16 $kg.m^{-3}$, with the HT ranging from 91.7 ± 9.7mm to 206.8 ± 9.7mm.

The HT parameter also showed that there was a significant interaction ($P \leq 0.05$) between the two micronutrient fertilizers and their respective levels. Micromax® gave a significantly larger mean HT (237.9 ± 20.3mm) than FRIT 504®, with a mean HT of 217.4 ± 20.3mm over all lime treatments (Fig. 5.1a). The treatment with the greatest HT was 1000 $g.m^{-3}$ Micromax® (258.3 ± 20.3mm) compared with the smallest HT of 202.1 ± 20.3mm when 50 $g.m^{-3}$ FRIT® was pre-enriched. This was over all lime treatments (Fig. 5.1a). Therefore, 1000 $g.m^{-3}$ Micromax® was significantly greater than 100 FRIT®, 600 Micromax®, 800 FRIT®, 200 Micromax® and 50 FRIT® (Fig. 5.1a). Seedlings receiving 50 $g.m^{-3}$ FRIT® were significantly shorter than all the other treatments, except 200 Micromax® and 800 FRIT® (Fig. 5.1a). However, individually, the best HT was at 8 $kg.m^{-3}$ lime + 200 $g.m^{-3}$ FRIT® and the worst at 16 $kg.m^{-3}$ lime + 0 $g.m^{-3}$ micronutrients.

Collar Diameter (CD)

The plant growth parameter CD revealed that there was a significant interaction ($P \leq 0.01$) between the various lime rates (Fig. 5.2) and the control and micronutrient treatments (Fig. 5.1b). Again the treatments with no micronutrients gave significantly smaller CD than the treated seedlings (Fig. 5.1b). In both the micronutrient treated and untreatment, 16 $kg.m^{-3}$ of dolomitic lime resulted in significantly smaller CD of 2.07 ± 0.19mm and 1.17 ± 0.19mm, respectively (Fig. 5.2). There was also a significant ($P \leq 0.01$) interaction between the micronutrient fertilizers and their respective levels of pre-enrichment (Fig. 5.1b). 1000 $g.m^{-3}$ Micromax® gave significantly greater CD than all the other pre-enriched micronutrient levels, except for the 400 and 800 $g.m^{-3}$ Micromax® treatments (Fig. 5.1b). 800 $g.m^{-3}$ FRIT® was significantly less than all other treatments, but similar to 200 $g.m^{-3}$ Micromax® and 50 $g.m^{-3}$ FRIT® (Fig. 5.1b). The following treatments were not significantly different: 400, 600,

800 g.m^{-3} Micromax® and 100, 200 and 400 g.m^{-3} FRIT® (Fig. 5.1b).

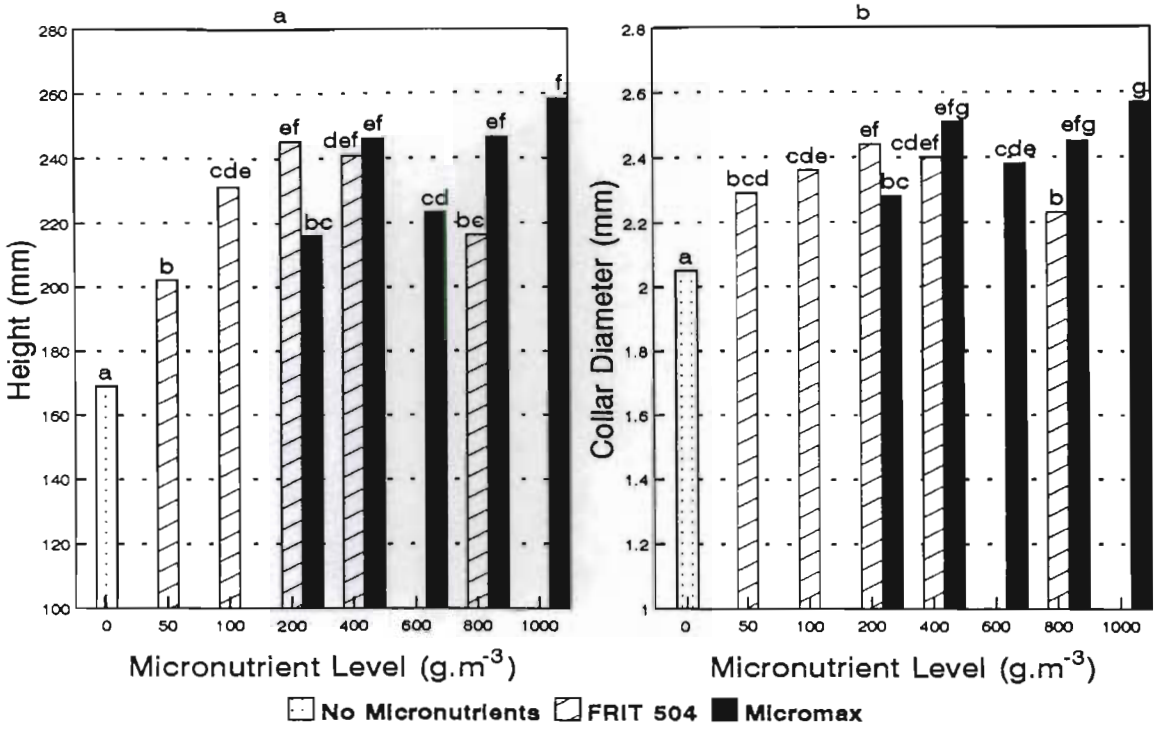


Fig. 5.1 Effect of Micromax® and FRIT 504®, averaged over all lime rates, at different levels of pre-enrichment in pine bark, on (a) height and (b) collar diameter of *P. patula* seedlings. Treatments with letters in common are not significantly different.

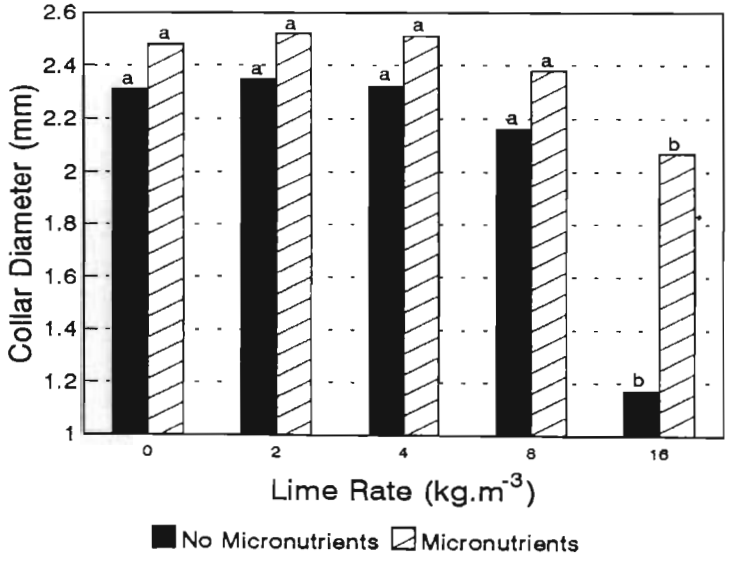


Fig. 5.2 Effect of dolomitic lime rates on the collar diameter of seedlings receiving micronutrients and those not treated with a micronutrient fertilizer. Treatments with letters in common are not significantly different.

Root and shoot dry mass (RDM and SDM)

The treatments with no micronutrients pre-enriched had significantly ($P \leq 0.01$) smaller RDM and SDM than the treatments that were pre-enriched with various micronutrient levels. The average RDM and SDM were 0.30 ± 0.02 $g.seedling^{-1}$ and 1.06 ± 0.17 $g.seedling^{-1}$, respectively, for the micronutrient treated seedlings and 0.20 ± 0.02 $g.seedling^{-1}$ and 0.73 ± 0.17 $g.seedling^{-1}$ for the untreated seedlings, respectively. The largest RDM and SDM were found in the treatment with 1000 $g.m^{-3}$ Micromax®; the smallest in the treatments with no micronutrient pre-enrichment (Fig. 5.3).

The RDM and SDM were also significantly different between the two pre-enriched micronutrient fertilizer. The RDM for the FRIT 504® treatments was significantly greater ($P \leq 0.01$) at 200 and 400 $g.m^{-3}$, than at 800 and 50 $g.m^{-3}$. The 50 $g.m^{-3}$ treatment resulted in markedly lower RDM than all other levels of FRIT 504® (Fig. 5.3a). The following significant responses ($P \leq 0.01$) were found for RDM in Micromax® treatments: 1000 $g.m^{-3}$ > 800 $g.m^{-3}$ = 400 $g.m^{-3}$ > 600 $g.m^{-3}$ > 200 $g.m^{-3}$ for all rates of lime (Fig. 5.3b). The SDM for the FRIT 504® treatments gave the following significant differences ($P \leq 0.05$): 200 $g.m^{-3}$ = 400 $g.m^{-3}$ = 100 $g.m^{-3}$ > 800 $g.m^{-3}$ = 50 $g.m^{-3}$ for all lime treatments (Fig. 5.3b). The Micromax® treatments resulted in significantly greater ($P \leq 0.05$) SDM in the 1000 and 400 $g.m^{-3}$ treatments, with significantly lower SDM in the 600 $g.m^{-3}$ and the lowest SDM in the 200 $g.m^{-3}$ treatments (Fig. 5.3b). The 800 $g.m^{-3}$ treatment was not significantly different to the 400 and 600 $g.m^{-3}$ Micromax® treatments (Fig. 5.3b).

There was a significant interaction ($P \leq 0.05$), for the plant parameter SDM, between the two micronutrient fertilizers and the various lime rates. 16 $kg.m^{-3}$ of dolomitic lime resulted in SDM that were significantly less than all other lime rates for the micronutrient FRIT 504® (Fig. 5.4). For Micromax®, 2 $kg.m^{-3}$ of lime resulted in greater SDM than all other lime rates, except when lime was not pre-enriched (Fig. 5.4). FRIT® + 16 $kg.m^{-3}$ of lime gave the lowest SDM, and Micromax® + 2 and 0 $kg.m^{-3}$ lime pre-enrichment the greatest SDM (Fig. 5.4).

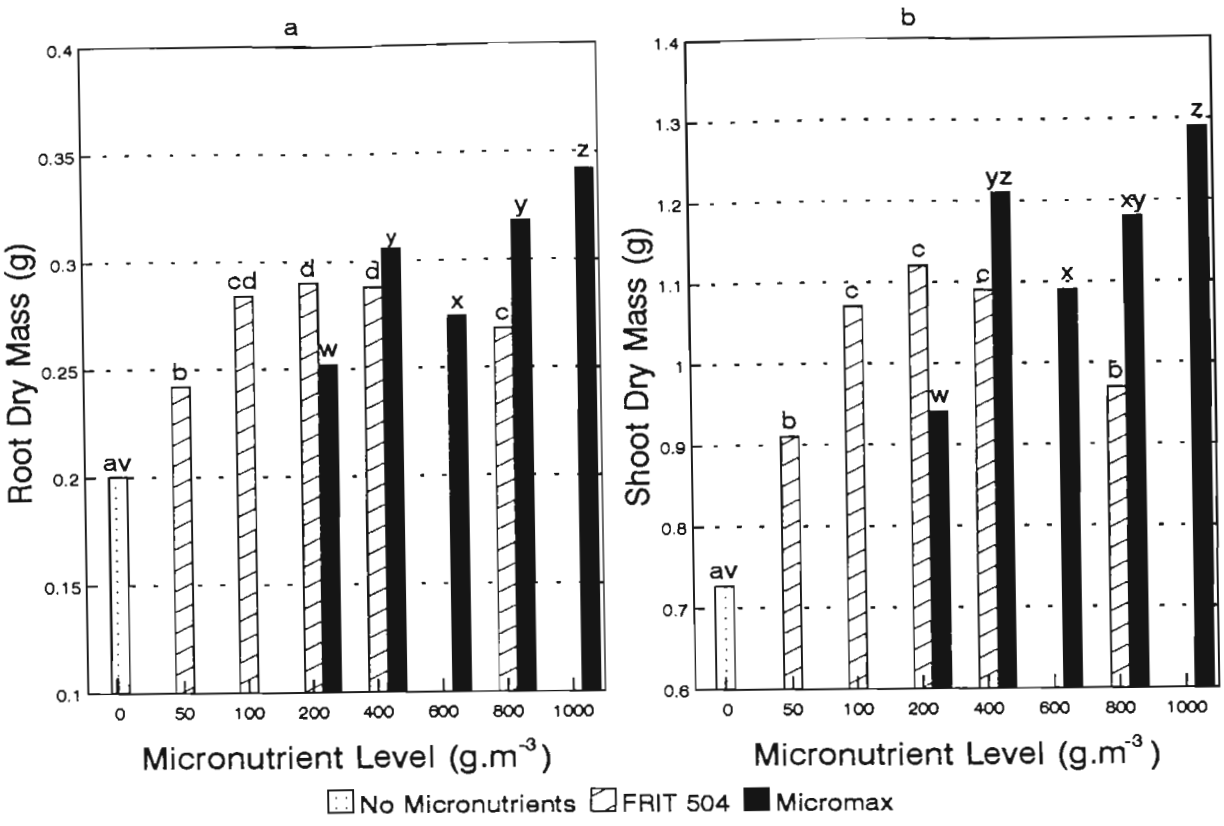


Fig. 5.3 Effect of FRIT 504® and Micromax® at different levels of pre-enrichment on (a) root dry mass and (b) shoot dry mass of pine seedlings grown in pine bark, averaged over the lime rates. (Treatments with letters in common are not significantly different).

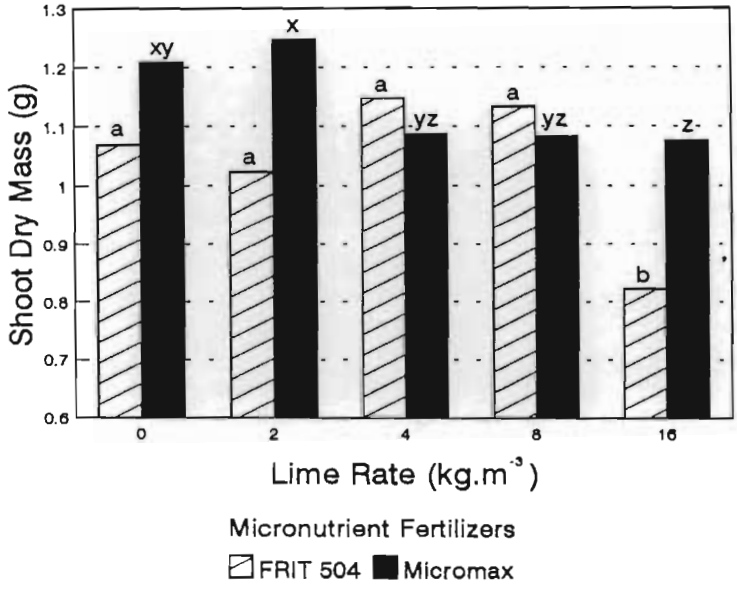


Fig. 5.4 Effect of different dolomitic lime rates and micronutrient fertilizers, FRIT 504® and Micromax®, on shoot dry mass of *P. patula* seedlings. (Treatments with letters in common are not significantly different).

The RDM was significantly smaller ($P \leq 0.05$) when FRIT 504® was pre-enriched (0.27 ± 0.01 g.seedling^{-1}), than when Micromax® was added (0.30 ± 0.01 g.seedling^{-1}). Significant RDM differences ($P \leq 0.01$) were also evident between the lime rates. Increased liming resulted in a linear decrease in RDM (Fig. 5.5).

Root:Shoot Ratio

Statistical analysis showed that only lime rates significantly ($P \leq 0.01$) affected seedling root:shoot ratios. Like RDM, increased addition of dolomitic lime resulted in decreasing root: shoot ratios (Fig. 5.5), with 0 kg.m^{-3} > 2 kg.m^{-3} > 4 kg.m^{-3} > 16 kg.m^{-3} > 8 kg.m^{-3} (Fig. 5.5). Root:shoot ratios ranged from 0.30-0.21 ± 0.01.

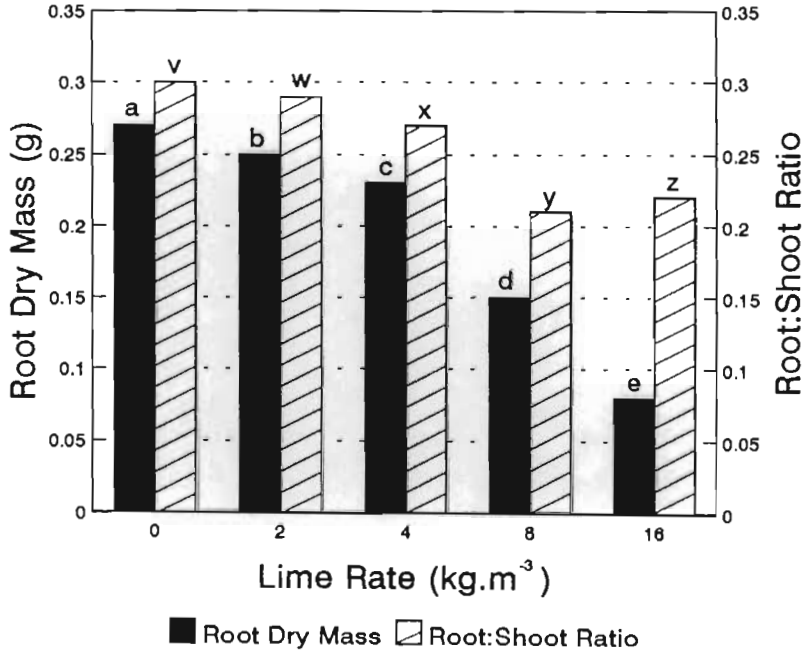


Fig. 5.5 Effect of increasing lime rate on root dry mass and root:shoot ratio of *P. patula* seedlings. (Treatments with letters in common are not significantly different).

Mortality

High rates of dolomitic lime incorporated into the media resulted in seedling mortality (Table 5.3). 16 kg.m^{-3} of dolomitic lime with no micronutrients resulted in the largest mortality of 28 seedlings - 57.14% (Plate 5.1) and FRIT 504® caused more deaths than Micromax® (Table 5.3).

Table 5.3 Mortality results of Exp.3 seedlings at harvest. (Treatments not shown had no mortalities).

TREATMENT		% DEAD
LIME ($kg.m^{-3}$)	MICRONUTRIENT ($g.m^{-3}$)	
8	0	6.12
8	50 FRIT®	10.20
8	400 FRIT®	2.04
8	800 FRIT®	12.24
8	800 Micromax®	2.04
16	0	57.14
16	50 FRIT®	33.20
16	100 FRIT®	12.24
16	200 FRIT®	14.29
16	400 FRIT®	30.61
16	800 FRIT®	32.65
16	200 Micromax®	14.29
16	400 Micromax®	6.12
16	600 Micromax®	14.29
16	800 Micromax®	8.16
16	1000 Micromax®	6.12

Thus, it can be seen that 16 $kg.m^{-3}$ of lime without micronutrients pre-enriched, resulted in the worst growth of *P. patula* seedlings in pine bark, followed by 16 $kg.m^{-3}$ lime + 50 $g.m^{-3}$ FRIT 504® (Plate 5.1 & Table 5.3). The best quality seedling was obtained when no lime was pre-enriched, as found in Exp.2 (Chapter 4) and when 1000 $g.m^{-3}$ of Micromax® was pre-enriched. 200 $g.m^{-3}$ of FRIT 504® with no lime produced the best growth when FRIT® was used (Plate 5.2). In some cases 200 $g.m^{-3}$ FRIT® and 1000 $g.m^{-3}$

Micromax® resulted in no significant effect, but 200 $g.m^{-3}$ FRIT 504® sometimes produced inferior seedlings. This could be attributed to Micromax® having the superior micronutrient ratio. Thus, this experiment showed the necessity for pre-enrichment with micronutrients if maximum growth is desired.

Plate 5.1 Growth reduction caused by high lime treatments, even with FRIT 504® pre-enrichment.

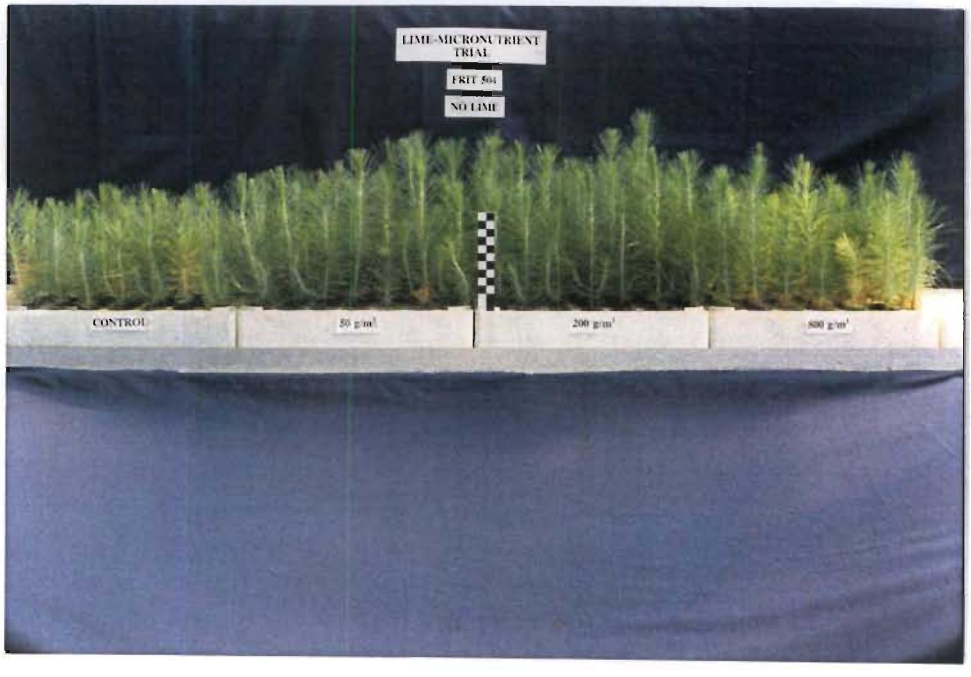


Plate 5.2 Effect of increasing levels of FRIT 504® on *P. patula* seedlings with 0 $kg.m^{-3}$ lime.

5.3.2 Leaf Analysis

Seedling observations at harvest indicated deficiency and/or toxicity symptoms (Plates 5.1 & 5.2). This was evident in the control, in which some needles were an orange, red and yellow colour (Plate 5.2). The treatments with high rates of lime and low micronutrient levels gave rise to nutrient imbalances. Needle tips showed necrosis and seedlings were often chlorotic due to micronutrient deficiencies at high pH. Seedlings which received high micronutrient levels and no lime (Plate 5.2) also showed chlorosis, presumably due to toxicity symptoms. The treatments which produced the best growth did not have these colourful symptoms.

Dolomitic Lime

Liming did not influence N, P and K foliar concentrations, but did affect the other nutrient concentrations. The leaf Mg levels increased with the addition of dolomitic lime, since there was more Mg available for plant uptake. The foliar Ca levels were mostly constant at 0.21% Ca and only increased to 0.29% when 16 $kg.m^{-3}$ was applied. This indicated that Ca concentrations in the pine bark were sufficient for plant growth. The concentration of leaf micronutrients was markedly affected by liming. Iron, Mn, Zn and Cu all decreased as the lime pre-enrichment rate increased (Fig. 5.6). These differences remained constant over the different micronutrient levels.

FRIT 504®

Increasing the amount of FRIT® resulted in an increase in leaf Cu and Zn (Fig. 5.7a), but caused no marked response in leaf N, P, Ca and Mg. Leaf Cu increased from 3.9-6.1 $mg.kg^{-1}$ and Zn from 48.3-87.0 $mg.kg^{-1}$ (Fig. 5.7a). This was the result of applying 0-56 $g.m^{-3}$ for both Cu and Zn to the pine bark. Leaf K decreased with increasing FRIT® from 1.16% to 0.87%, possibly due to the increased uptake of micronutrients in place of K^{+}. Foliar Mn and Fe initially increased and then decreased at levels greater than 100 $g.m^{-3}$ (Fig. 5.7b). Thus, the application of FRIT® only resulted in an increase of foliar Cu and Zn. The lack of increase in foliar Fe, at high levels of pre-enrichment, was

surprising as this is the single largest constituent of FRIT® (14.3%). This suggests that perhaps the Fe and Mn were either in unavailable forms, leached before being taken up by the plant, or the uptake of other more available nutrients was favoured over that of Fe and Mn.

Micromax®

Increasing amounts of Micromax® resulted in an increase in leaf micronutrient status. Zinc and Cu responded in a positive linear trend increasing from 48.3-70.5 $mg.kg^{-1}$ Zn and 3.9-5.8 $mg.kg^{-1}$ Cu (Fig. 5.8a). Foliar Fe increased from 34 $mg.kg^{-1}$ to 55 $mg.kg^{-1}$, but then decreased when more than 600 $g.m^{-3}$ of Micromax® was added (Fig. 5.8b). Manganese followed a similar trend, increasing from 234.5-356 $mg.kg^{-1}$ Mn, but decreased when more than 800 $g.m^{-3}$ Micromax® was added (Fig. 5.8b). This trend also occurred with FRIT® pre-enrichment and can be explained in the same way. No obvious change in leaf N, P and K was noticeable. However, foliar Ca and Mg followed a similar trend, i.e., concentrations increased when 200 $g.m^{-3}$ Micromax® was added, but then decreased and only increased with 600 $g.m^{-3}$ and greater pre-enrichment. The 200 $g.m^{-3}$ Micromax® treatment gave rise to the highest foliar Ca and Mg. Micromax® contains 5.4% Ca and 3.0% Mg, which explains the increase in foliar Ca and Mg, but the decrease at 600 $g.m^{-3}$ can be explained by a nutrient interaction, i.e., Ca and Mg were available for uptake, but not taken up due to competition.

Leaf analysis revealed that seedlings in the control treatment, with slight deficiency symptoms, had lower Cu, Zn, Mn and Fe contents than the seedlings with better growth and no obvious deficiencies. This suggests that these four micronutrients were at sub-optimal levels in the pine bark, which would affect the growth of *P. patula* seedlings.

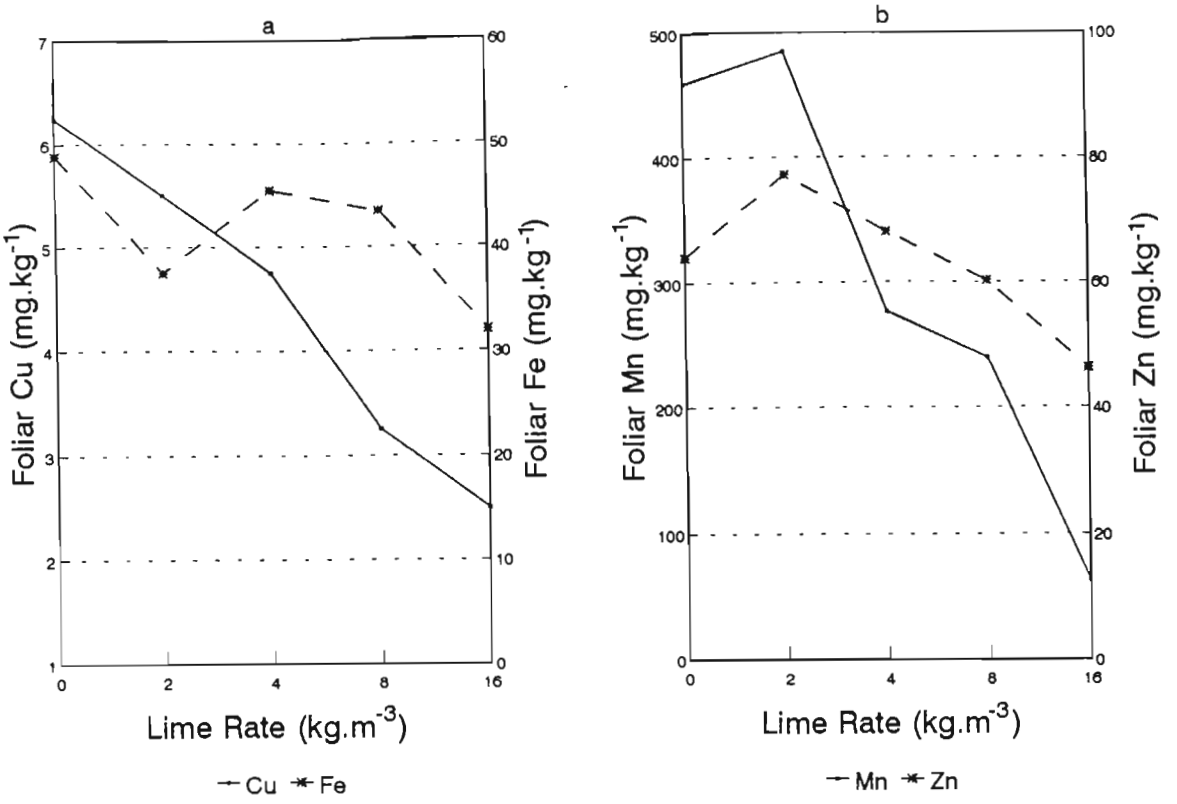


Fig. 5.6 Effect of increasing lime application to a pine bark medium on foliar (a) Cu and Fe (b) Mn and Zn concentrations in *P. patula* seedlings.

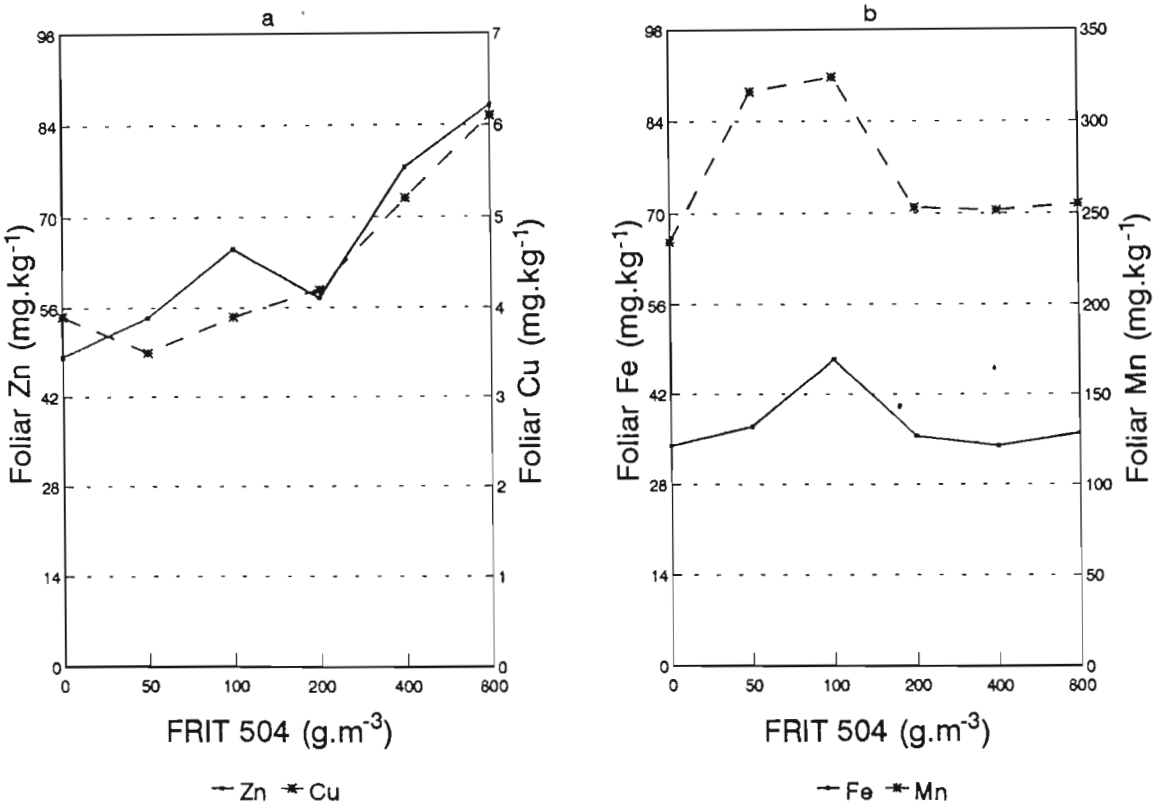


Fig. 5.7 Effect of increasing FRIT 504® applications to a pine bark medium on foliar (a) Zn and Cu (b) Fe and Mn concentrations in *P. patula* seedlings.

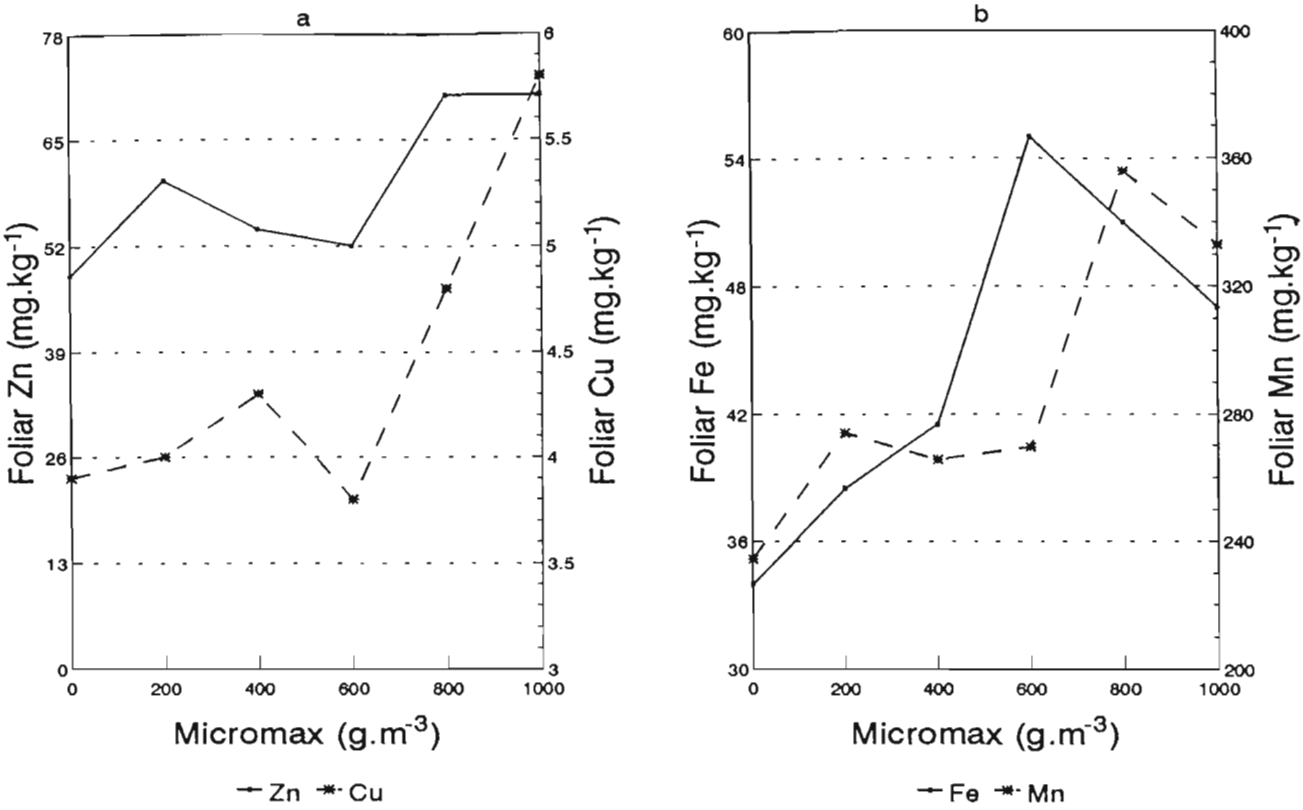


Fig. 5.8 Effect of increasing Micromax® applications to a pine bark medium on foliar (a) Zn and Cu (b) Fe and Mn concentrations in *P. patula* seedlings.

5.3.3 Medium Analysis

Initial Pine Bark

The pH increased with increasing amounts of dolomitic lime, from 4.42 (no lime) to 6.55 (16 kg.m^{-3} lime) over all micronutrient levels. No pH differences were observed between pre-enrichment levels of FRIT® and Micromax®, even though Micromax® contains Ca and Mg. The EC of the initial pine bark was higher in the Micromax® treatments, since Micromax® was added in higher amounts than FRIT®. Increasing additions of Micromax® resulted in increasing EC, as Micromax® is a more soluble fertilizer. FRIT® did not show any trend, probably as it is a SRF and not as soluble as Micromax®. With increasing lime additions the EC decreased (from 0.83 to 0.69 mS.cm^{-1}) and only increased again (from 0.71-0.74 mS.cm^{-1}) when 8 kg.m^{-3} and greater was applied. The EC was lower in the limed treatments, probably due to

available ions being bound by the excess Ca.

Availability of N and K in the pine bark was not affected by the addition of lime and micronutrients, having mean values of 62.01 and 76.40 mg.ℓ^{-1}, respectively. However, P availability decreased with increasing lime additions, presumably due to the formation of insoluble Ca-phosphates. Calcium and Mg increased with liming, although the differences were negligible. Availability of Ca and Mg ranged from 24.27-19.31 mg.ℓ^{-1} and from 19.81-16.04 mg.ℓ^{-1}, respectively. Availability of Ca and Mg was much greater in the Micromax® treatments than with FRIT®, and generally increased with increasing Micromax® additions, due to the Ca and Mg content of Micromax®.

Differences in micronutrient availability were identified in the pine bark. Manganese and Zn followed the same trends, both decreasing in availability with increased liming (Fig. 5.9). The former decreased from 1.14 mg.ℓ^{-1} to 0.31 mg.ℓ^{-1} and Zn from 0.10 mg.ℓ^{-1} to 0.03 mg.ℓ^{-1}. The Mn and Zn were more available in the Micromax® than FRIT® treatments for the same additions, even though FRIT® contained 3 times more Mn and 7 times more Zn. Thus, the Mn and Zn were in a more readily available form in the Micromax®, which is a more soluble slow release micronutrient fertilizer, than the FRIT® which is also a SRF, but is strongly affected by medium pH. The Mn and Zn content in the pine bark also increased with increasing additions of FRIT® and Micromax®. Copper availability decreased with liming, increased with increasing micronutrient additions and was more available in the Micromax® treatments, but differences were negligible. Iron availability did decrease with liming, but increased when pH rose above 6 with the addition of lime (Fig. 5.9). This follows the availability trend of Lucas and Davis (1961), who showed how pH greatly affected micronutrient availability. There were no marked differences and trends in Fe availability in the two micronutrient fertilizers nor in the levels of pre-enriched micronutrients. Contamination of Fe easily occurs and Fe concentrations can therefore often be inaccurate and very variable, resulting in non-significant trends.

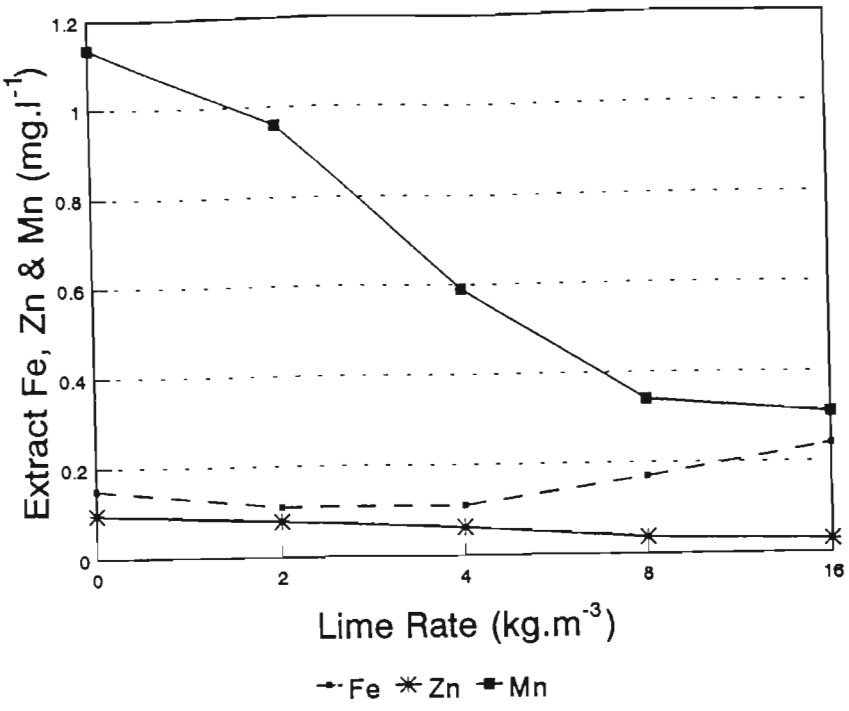


Fig. 5.9 Effect of liming on the Fe, Zn and Mn nutrient concentrations in initial pine bark.

Final Pine Bark

Analysis of the pine bark at the end of the experiment confirmed that bark nutrient status decreases due to leaching and seedling uptake of nutrients, i.e., N, Mn, Zn and pH were lower than in the initial bark. However, P, K, Fe, Ca, Mg and the EC were higher than in the initial bark. The increase in the P, K and EC was due to the fertigation of the seedlings with 3:1:3(38), which would have added to the inherent amounts in the bark and constantly "topped up" the concentrations. The fact that Fe increased by an average of 0.03 $mg.\ell^{-1}$ could be attributed to FRIT® being a SRF with a high Fe content that was made slowly available and not fully extracted at the start of the experiment. It is difficult to explain the greater Ca and Mg concentrations at the end of the experiment. The fact that the pH did not increase, suggests that the increase in Ca and Mg was not significant and that these readings might have been a laboratory error, i.e., the standards used for calibration were possibly incorrect. Thus, it can be assumed that normally the Ca and Mg contents would decrease, as occurred in Exp.2 (Chapter 4). The Cu content in the final bark was unchanged, perhaps from Cu contamination in the nutrient solution used for fertigation.

As expected the pH, EC, Ca and Mg all increased in the final medium with increasing rates of dolomitic lime. pH and Mg increased from 4.73-5.98 and 8.92-38.76 $mg.\ell^{-1}$, respectively. They were, however, not influenced by the level or type of micronutrient pre-enrichment. Calcium and EC increased from 12.7-57.82 $mg.\ell^{-1}$ and 0.68-1.09 $mS.cm^{-1}$, respectively. In both cases Micromax® treatments contained more Ca (44.32 $mg.\ell^{-1}$ vs 37.43 $mg.\ell^{-1}$) and therefore had larger EC (0.96 $mS.cm^{-1}$ vs 0.85 $mS.cm^{-1}$) than FRIT® treatments and for the treatments with the same level of pre-enrichment. This shows that the soluble Micromax® was not completely leached out of the pine bark after six months. No trends were observed in micronutrient pre-enrichment rates.

The availability of N, P and K in the final bark was not influenced by liming or micronutrient content. This was probably because the 3:1:3(38) nutrient solution supplied a constant amount of N, P and K to all treatments, and so masked any affects of liming, as occurred in Exp.2 (Chapter 4).

The availability of the micronutrients in the pine bark was influenced by liming, as also occurred in the foliage. Iron, Mn and Zn all decreased from 0.45 $mg.\ell^{-1}$, 0.35 $mg.\ell^{-1}$ and 0.16 $mg.\ell^{-1}$ to 0.16 $mg.\ell^{-1}$, 0.01 $mg.\ell^{-1}$ and 0.05 $mg.\ell^{-1}$, respectively. Copper was constant (0.01 $mg.\ell^{-1}$) and did not seem affected by liming or micronutrient additions. This was also found in Ogden's (1982) extensive research on pine bark. There were no obvious trends in Mn and Zn for the different levels of micronutrients added. However, the availability of Zn was greater in the FRIT® treatments, and that of Fe greater in the Micromax® treatments, but these difference were probably not statistically significant. Amounts of Mn were not significantly different between FRIT® and Micromax® and between the different pre-enrichment levels.

5.4 DISCUSSION AND CONCLUSIONS

The insignificant growth increases or reduced growth that occurred when the pine bark medium was limed, verify the findings

of Exp.2 (Chapter 4), viz. that liming pine bark is unnecessary, and that liming at rates greater than 2 $kg.m^{-3}$ is detrimental to pine seedling growth. However, addition of micronutrients is essential for optimum pine seedling growth in composted pine bark. This was clearly observed in the control treatments (no micronutrients), where seedlings were significantly smaller and displayed deficiency symptoms. The contention that pine bark contains sufficient micronutrients might apply to a short term crop, but does not apply to pine seedlings which are grown for 4 or more months in pine bark media.

Plant growth parameters clearly revealed that liming did not improve growth of *P. patula* seedlings, and that an addition of FRIT 504® or Micromax® was necessary for optimum growth. However, the availability of nutrients from FRIT® is pH dependent and liming is therefore an important factor in determining the micronutrient availability. Plants grown with FRIT® at low pH and at rates greater than 200 $g.m^{-3}$ showed symptoms of B and Mn toxicity, and Mo deficiency. Adams, Graves & Winsor (1986) obtained the same result in their experiment using aged pine bark. Optimal growth when FRIT® was pre-enriched occurred at 200 $g.m^{-3}$, but 100 $g.m^{-3}$ FRIT® was not significantly different to 200 $g.m^{-3}$. Thus, if FRIT 504® is used as a micronutrient fertilizer 100 $g.m^{-3}$ could be incorporated if the cost of the fertilizer is of prime concern. However, maximum growth of *P. patula* seedlings occurred when 1000 $g.m^{-3}$ Micromax® was applied to the pine bark. Thus, Micromax® was the better of the two micronutrient fertilizers, releasing micronutrients for the full duration of the seedling's nursery life.

It is therefore recommended that the lime-micronutrient combination to be pre-enriched into pine bark media for optimum growth of *P. patula* seedling is: no lime + 1000 $g.m^{-3}$ Micromax®. The initial pine bark and foliar nutrient content of this treatment is presented in Table 5.5.

Table 5.5 Medium and foliar nutrient content of *P. patula* seedlings from the treatment which resulted in the best growth.

ELEMENT	NO LIME + 1000 g.m^{-3} Micromax®	
	MEDIUM CONC.	FOLIAR CONC.
N	61.6 mg.ℓ^{-1}	1.70 %
P	0.4 mg.ℓ^{-1}	0.20 %
K	90.0 mg.ℓ^{-1}	1.10 %
Ca	40.0 mg.ℓ^{-1}	0.23 %
Mg	25.0 mg.ℓ^{-1}	0.20 %
Fe	0.39 mg.ℓ^{-1}	52.5 mg.kg^{-1}
Zn	0.23 mg.ℓ^{-1}	550.0 mg.kg^{-1}
Mn	2.74 mg.ℓ^{-1}	72.5 mg.kg^{-1}
Cu	0.01 mg.ℓ^{-1}	9.0 mg.kg^{-1}

Lucas & Davis (1961) stated that maximum nutrient availablity in soilless media, such as pine bark, occurs at pH 5-5.5. This experiment proved that this is mostly correct, i.e., pine bark and foliar analysis showed that the availability of nutrients decreased with liming and that maximum availability was from pH 4.7-5.4. Thus, liming beyond this limit will decrease micronutrient availability.

It must be noted, however, that seedlings grown in aged bark that is not composted would most probably require lime and micronutrient additions, as the medium is not as composted and broken down. Available Ca, Mg and micronutrients would be less and the medium would not be buffered against large additions of micronutrients, i.e., high rates of micronutrient addition would be toxic, unless high amounts of lime were added.

This experiment has shown that the micronutrient content of

composted pine bark is sometimes not sufficient for plant growth. Comparison of pine bark micronutrient content from this experiment with other pine bark analysed from the same producer, revealed variability in the micronutrient content of pine bark. Is micronutrient content fairly constant throughout the country, or is it largely affected by bark species composition, and/or is it greatly influenced by the land type and environment from which the bark is obtained? Can guidelines be set for the amount of micronutrients that should be available in a pine bark medium?

These questions illustrate the controversy surrounding micronutrient content in pine bark. The only obvious solution at present is to obtain a micronutrient analysis of the pine bark to be used, and adjust according to the recommendations for that particular plant or species.

CHAPTER SIX

SLOW RELEASE PRE-ENRICHMENT

6.1 INTRODUCTION

The most recent ideas, regarding the best method of nutrient supply to a bark medium, involve the pre-enriching of bark prior to planting with as many of the required nutrients as possible. The balance is then made up with a dilute nutrient feed. However, pre-enrichment with conventional fertilizers can result in problems, such as inefficiency due to rapid dissolution of the nutrients from the fertilizers. For optimum plant growth and development, the nutrient supply to the plant ideally should provide an initial concentration of nutrients for early development, followed by a constant nutrient release, which varies according to the plant's requirements throughout its growth cycle (Hawgood,1988). Thus, there has been ongoing research for improved fertilizer materials which have higher concentrations of nutrients, and which give a more effective and efficient availability of nutrients to plants. The result of this research has been the concept of controlled release or slow release fertilizers (SRF).

Slow release fertilizers are convenient and simple to use, in that they supply a continuous nutrient supply for a long time period, so that only water need be supplied after planting, thus saving on time and labour. They produce lower salinity than conventional fertilizers, because they gradually release nutrient ions into the growing medium solution, and therefore nutrients are used more efficiently and leaching is reduced. However, coated SRF, such as Osmocote® and Nutricote®, should not be mixed into the medium in advance of sowing seed, because leakage from the granules can raise the salt content of the medium to toxic levels. The disadvantages of SRF are that they are generally more expensive than conventional fertilizers, the time of depletion of nutrients is not reliably known, and uneven distribution of SRF can be a real concern, particularly with small containers. It is also impossible to control the

concentration and balance of nutrients in the growing medium solution. Research however, has shown that a SRF period of nutrient release conducive to optimum growth may be less than that claimed by the manufacturer (Wright & Niemiera, 1987). Thus, supplementary liquid feeds are often applied in combination with SRF. Results of this combined approach have shown that seedlings can be of better quality. Therefore, one of the aims of this experiment was to compare the combined and separate approaches, testing which yields the better seedling.

There are numerous types of SRF. Resin-coated products have been the most popularly used worldwide. The most commonly used coating is Osmocote®, an American product. However, use of SRF in South Africa has been limited to crops of high value, due to high importation costs. This is hopefully changing with South Africa looking to produce its own SRF. Thus, information on SRF used in the forestry industry is notably lacking.

Although some 14 elements are considered essential for healthy growth of conifers, the application of inorganic fertilizer in conifer nurseries is confined, with few exceptions, to three macronutrients: N, P and K (Landis *et al.*, 1992). Thus, Osmocote Mini® (an N-P-K SRF) was developed for the fertilization of plug and cell systems, as used for forestry seedlings, having ± 220 granules in 1g; 6 times as many as in normal Osmocote® (Anon., 1992). Osmocote Mini® is a unique organic resin-coated product, with a longevity of 2-3 months or 5-6 months. The coating controls the daily release during the entire built-in release period, which is determined by soil temperature and is not influenced by other factors. Other SRF are influenced by factors such as soil moisture, total volume of water applied, soil pH, microbial population and external salt concentration. Recommendations for forestry seedlings are 1.2-2.25 $kg.m^{-3}$ of 18-3-10 Osmocote Mini® and 2-3 $kg.m^{-3}$ of 18-3-9 Osmocote Mini®. Crowley *et al.* (1986) found that the best pine seedling growth and mycorrhizal formation was obtained with the (21-7-14) 8 to 9 month release Osmocote® fertilizer, at an application rate of 4.5 $kg.m^{-3}$. However, these recommendations are for American pine species. It is not known if these formulations will have

sufficient longevity in South African climatic conditions, or which rate of pre-enrichment will result in optimum *Pinus patula* growth.

The objectives of this experiment were to compare the efficiency of a slow release fertilizer (Osmocote Mini®) to fertigation; to determine the optimum rate of Osmocote Mini® pre-enrichment; and to test whether Osmocote Mini® needs to be supplemented with a nutrient solution. Thus, the main objective of this experiment was to test whether it is more economical to use only an SRF.

6.2 MATERIALS AND METHODS

Pre-germinated *Pinus patula* seeds were sown on 06.11.92 (Day 0) into Unigro® trays for Experiment 4 (Sections 2.1.2 & 2.1.4). The medium used was fine seedling mix, which was pre-enriched with different rates of Osmocote Mini® (Section 2.1.3). The seven pre-enrichment rates were 0, 0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 $kg.m^{-3}$. Micromax®, a micronutrient fertilizer, was also pre-enriched at a constant rate of 0.25 $kg.m^{-3}$, to ensure that micronutrient deficiencies would not mask the experimental results.

The Osmocote Mini® applied was the 18-6-12 formulation containing nitrate and ammoniacal N with a longevity of 2-3 months. (It was not initially intended to experiment with the 2-3 month formulation. Only near the end of the experiment when seedlings were not showing growth differences, was it discovered that the 2-3 month had been supplied, instead of the 5-6 month Osmocote Mini®). The release period or longevity of Osmocote Mini®, indicated in Table 6.1, applies to an average soil temperature of 21°C (Anon., 1992). Higher temperatures give a faster release and consequently decrease the longevity period, while lower temperatures have the opposite effect.

Table 6.1 Longevities of Osmocote Mini® at different soil temperatures (Anon., 1992).

Average soil temperature	18-3-10
	2 -3 months
16°C	3 - 4.5 months
21°C	2 - 3 months
32°C	1 - 1.5 months

Two levels of fertigation, water (EC of ± 0.1 $mS.cm^{-1}$) and 3:1:3(38) at a standard level (EC of ± 0.8 $mS.cm^{-1}$) were applied once or twice a day to the pre-enriched trays. An N-P-K feed treatment (only 3:1:3(38)) and a water treatment were included as controls. The experimental design used was 2 x 7 split-plot design with three replications.

Fertigation with 3:1:3(38) commenced on 11.12.92 (Day 35) and was applied by microjets to the respective treatments. The trial was leached with water approximately every two weeks to prevent salt build-up and, thus, toxic effects. 3:1:3(38) was used because it was found to be the most economical for the production of forestry seedlings (Brafield-Rolfe, 1992). 3:1:3(38) contains 48.1% NO_3-N and 51.2% NH_4-N. The complete composition of the fertilizers used in Exp.4 is given in Appendices 1 & 2.

The seedlings were harvested after 4 months on 11.03.93 (Day 125) and plant measurements taken (Section 2.2.2). Pine bark samples from the initial and final stages of the experiment were analysed for nutrients (Section 2.3). However, foliar analysis was not undertaken, since no obvious treatment effects were observed.

6.3 RESULTS

6.3.1 Plant Growth

Statistical analysis revealed that generally there were no significant treatment differences for all plant parameters measured, due to the use of the 2-3 month Osmocote Mini®. Thus, there were no obvious differences between the 7 different pre-enrichment rates of Osmocote Mini® (Plate 6.1), except for the control, which had no pre-enrichment or fertigation applied. The control seedlings were significantly ($P \leq 0.01$) smaller than all other treatments. However, differences were noted between the pre-enriched treatments that received fertigation and those that only received water, for the parameters SDM and lateral shoot number.

Shoot dry mass was significantly greater ($P \leq 0.05$) in the treatments that received fertigation (Plate 6.2). Fertigated treatments had a mean mass of 0.92 ± 0.03 g.seedling^{-1} and watered treatments 0.63 ± 0.03 g.seedling^{-1} (Fig. 6.1 & Plate 6.2). The number of lateral shoots was also markedly greater in the fertigation treatments, with a mean of 5 ± 0.12, compared to the watered treatments with a mean of 3.4 ± 0.12 (Fig 6.2).

The plant parameter, CD, was just not statistically significant ($P=0.051$), however, there was a difference between the treatments receiving fertigation and water - water treatments giving smaller CD.

The only other visible differences, although not significant, were in the roots and colour of the seedlings. Fertigation treatments were greener than water treatments, which were quite yellow (Plate 6.2). Also, plants receiving low rates of Osmocote Mini® were pale green but had larger root systems than those pre-enriched at higher rates, which were greener but had smaller root systems.

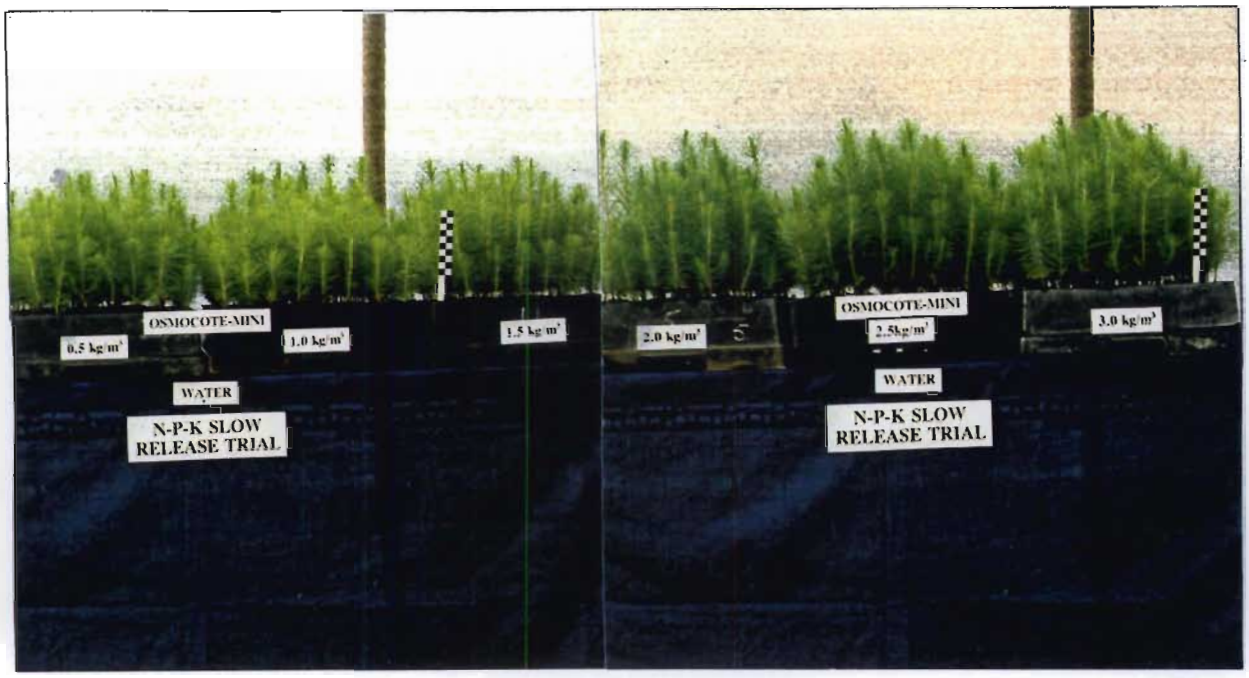


Plate 6.1 *P. patula* seedlings grown in pine bark pre-enriched with different rates of Osmocote Mini® showing no treatment effect.

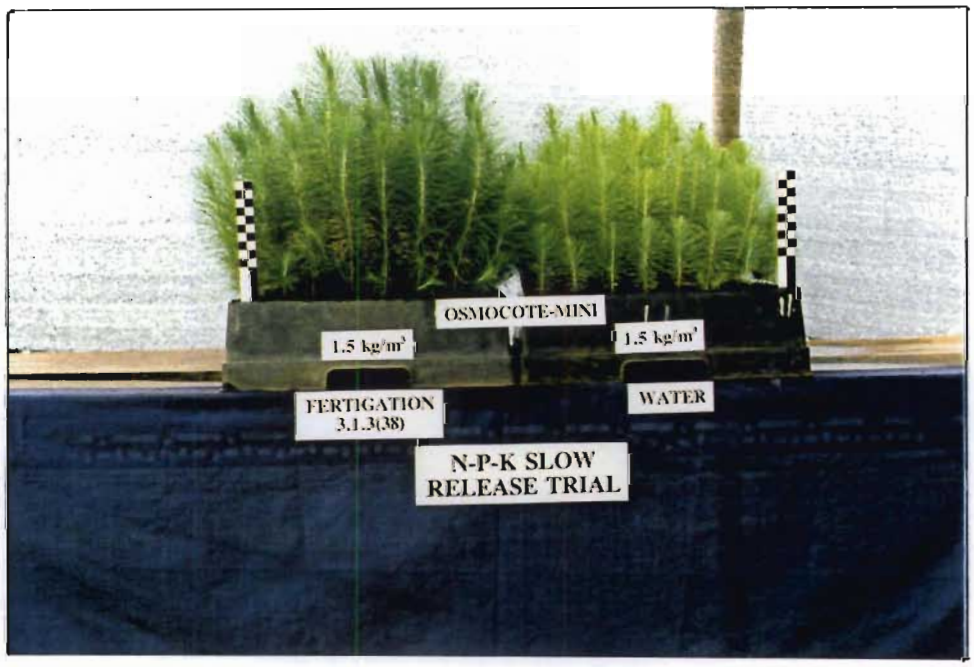


Plate 6.2 Effect of fertigation versus water on *P. patula* seedling growth.

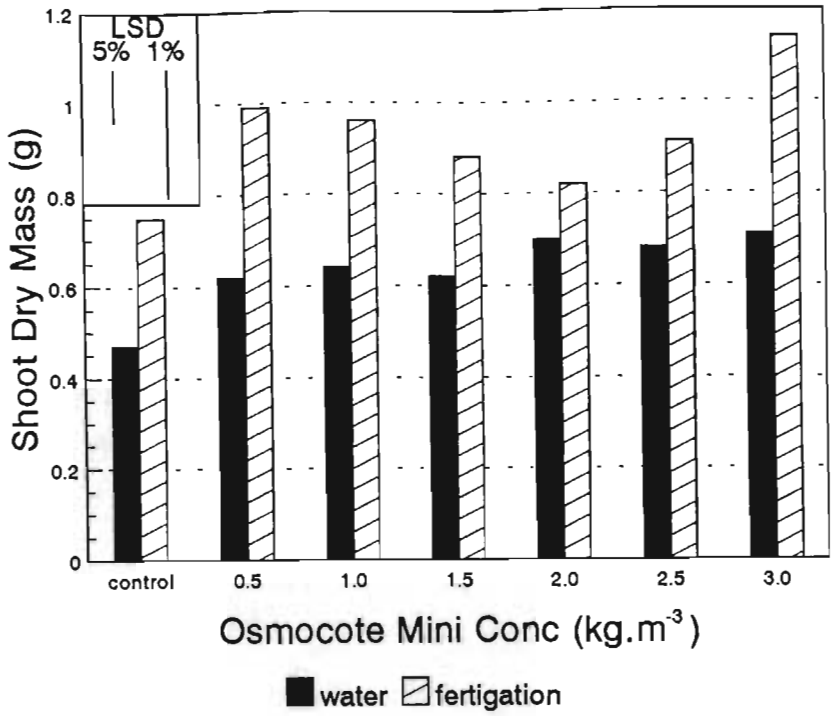


Fig. 6.1 Effect of fertigation and watering on the shoot dry mass (SDM) of seedlings grown in pine bark pre-enriched with different rates of Osmocote Mini®.

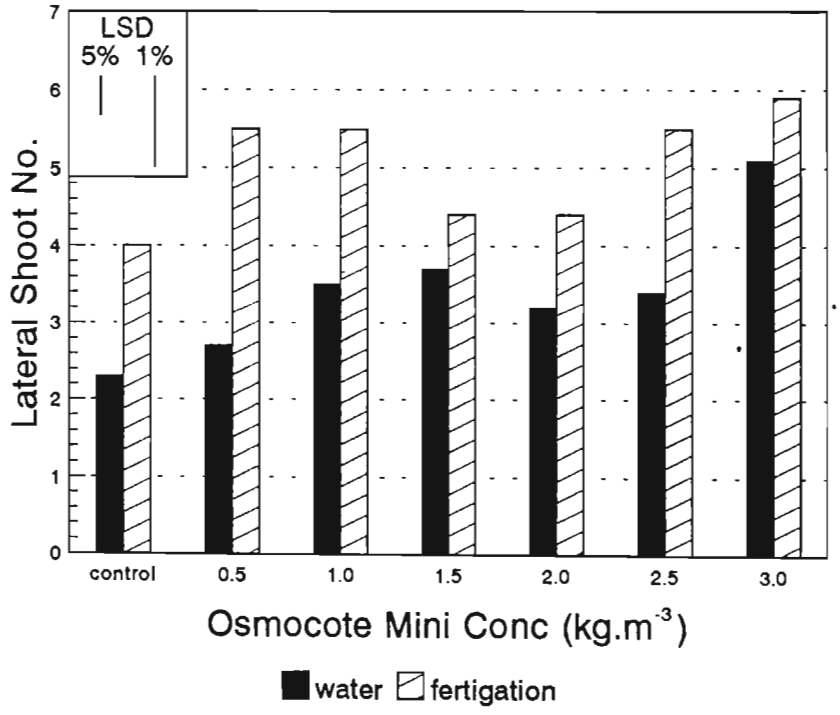


Fig. 6.2 Effect of fertigation and watering on the lateral shoot number of *P. patula* seedlings, grown in pine bark amended with Osmocote Mini®.

6.3.2 Medium Analysis

This experiment revealed that there were no marked fertilizer effects or unusual plant symptoms that could not be explained at the end of the experiment. In order to verify the inefficiency of the N-P-K SRF, only pH, EC, N, P and K were analysed in the medium.

Table 6.2 gives the initial and final nutrient concentrations of the pine bark medium that was pre-enriched with different rates of Osmocote Mini®. Results are as expected, with final bark having less nutrients due to leaching and plant uptake. Most of this effect must have been due to leaching, since seedlings were all of similar size.

The initial medium analysis was as expected, with N, P, K and EC all increasing with increasing pre-enrichment rates of Osmocote Mini® (Table 6.2). There was no correlation between treatment and resultant pH. The pH of 3.34 for the 1 $kg.m^{-3}$ of Osmocote Mini® was no doubt an experimental error (Table 6.2), as the mean pH was 5.26.

Final medium analysis revealed that nearly all the measurements had decreased since the start of the experiment, most of them to very low levels (Table 6.2). Phosphorus was the only nutrient which increased in the treatments that were fertigated with 3:1:3(38). However, the differences were marginal (Table 6.2), and were either due to experimental error (P contamination of glassware) or due to the fact that pine bark retains P. Prasad (1980) suggested that P retention in pine bark does occur and this may be evidence of it. The fertigation supplied a source of P and some of it was retained by the bark, whereas the water treatments would not have received this extra P and therefore had decreased P concentration.

Table 6.2 Initial and final concentrations of nutrients in pine bark pre-enriched with 7 rates of Osmocote Mini® and irrigated with water (W) or fertilizer (F).

TREATMENT	INITIAL CONCENTRATION ($mg.\ell^{-1}$)						FINAL CONCENTRATION ($mg.\ell^{-1}$)					
Osmocote M®	PH	EC- $mS.cm^{-1}$	NO_3	NH_4	P	K	PH	EC- $mS.cm^{-1}$	NO_3	NH_4	P	K
0 $kg.m^{-3}$ - W	5.47	0.78	35.2	28.8	2.3	70	4.95	0.13	0	0.63	1.67	12
0.5 $kg.m^{-3}$ - W	5.12	0.82	46.0	33.4	2.9	74	5.06	0.14	0	0.57	1.98	14
1.0 $kg.m^{-3}$ - W	3.34	0.99	57.5	34.9	3.0	78	4.68	0.17	0	2.53	2.18	21
1.5 $kg.m^{-3}$ - W	4.93	1.01	71.0	36.8	3.1	86	4.74	0.18	0	1.14	2.47	17
2.0 $kg.m^{-3}$ - W	5.56	1.03	76.6	39.5	3.1	90	4.88	0.18	0	0.65	2.44	14
2.5 $kg.m^{-3}$ - W	5.11	1.23	114.0	55.4	4.3	101	4.79	0.17	0	1.72	2.06	18
3.0 $kg.m^{-3}$ - W	5.37	1.34	124.3	59.3	4.9	114	4.23	0.22	0	1.29	2.93	24
0 $kg.m^{-3}$ - F	5.47	0.78	35.2	28.8	2.3	70	4.47	0.38	1.21	16.67	6.41	43
0.5 $kg.m^{-3}$ - F	5.12	0.82	46.0	33.4	2.9	74	4.27	0.33	1.21	16.71	6.65	38
1.0 $kg.m^{-3}$ - F	3.34	0.99	57.5	34.9	3.0	78	4.34	0.36	2.47	17.21	7.19	42
1.5 $kg.m^{-3}$ - F	4.93	1.01	71.0	36.8	3.1	86	4.38	0.36	3.83	15.96	6.80	38
2.0 $kg.m^{-3}$ - F	5.56	1.03	76.6	39.5	3.1	90	4.25	0.36	6.50	13.82	6.65	39
2.5 $kg.m^{-3}$ - F	5.11	1.23	114.0	55.4	4.3	104	4.20	0.36	9.48	14.93	7.17	41
3.0 $kg.m^{-3}$ - F	5.37	1.34	124.3	59.3	4.9	114	4.32	0.38	4.89	18.20	7.59	43

Thus, the final medium nutrient status showed no trend for pre-enrichment rate. The pH of the fertigated treatments were slightly lower than the watered treatments, since the addition of fertilizer would ultimately result in a more acidic medium (Table 6.2). The EC were higher in the fertigated than watered treatments and followed no trends, ranging from 0.33-0.38 $mS.cm^{-1}$, indicating that the pre-enriched fertilizer was not having an effect on EC, and only the fertigation was affecting the EC (Table 6.2). The water treatments for all rates had an extremely low nutrient content, which was lower than in the fertigation treatments, i.e., there was no NO_3 and very low NH_4 and P in the water treatments (Table 6.2). However, the K content in the water treatments was higher than would be expected (more than double), indicating that possibly there was still some K being made available from the SRF. Another explanation is that the pine bark contained relatively large quantities of K (Pokorny, 1979), and with time this could have become available as a slow release source.

These results were, therefore, an obvious indication that almost all the nutrients from the Osmocote Mini® had been leached or taken up by the plant, as only the treatments receiving additional fertigation had available nutrients in the medium.

6.4 DISCUSSION AND CONCLUSIONS

The insignificant differences in plant growth between the treatments can be explained by several hypotheses:

1. The SRF Osmocote Mini® had no effect on growth,
2. Osmocote Mini® did have an effect that was, however, equalised with time when most of the fertilizer had been leached and taken up by the seedlings,
3. Large variation in seedling growth parameters resulted in the differences being non-significant.

The second hypothesis seems most likely. The third was proven with statistical analyses showing very high coefficients of variability (CV).

Osmocote Mini® had an effect, i.e., initial pine bark analysis

showed an increase in nutrient content with increasing pre-enrichment rates, and if Osmocote Mini® had had no effect on growth, then the treatments receiving additional fertigation would have been significantly larger. The fact that the harvested seedlings after 4 months did not show any differences can be mainly attributed to the 2-3 month Osmocote Mini® applied, in the mistaken belief that it was the 5-6 month release formulation. This certainly proved that the longevity of the SRF was as predicted by the manufacturers. To make matters worse, this experiment was conducted over the hottest months of the year when soil temperatures were at their highest. Osmocote Mini®'s release was therefore maximized and the longevity of the SRF was probably approximately 1-1.5 months. This is the most obvious reason for non-significant growth effects. The plants took up what they could in the first month and then the effects were equalised when the nutrient source was depleted.

Very large variation in seedling sizes and uneven growth were noted from within single treatments. Short seedlings sometimes had taller seedlings in the plugs next to them, i.e., trays did not have uniform growth as is desired in seedling production. It was thought that a thorough mixing of Osmocote Mini® would ensure even distribution within the plugs, as advertised. This was apparently not the case. Thus, if SRF such as Osmocote Mini® are used, one must ensure that an equal number of granules are deposited in each plug. This is possible by the "dibble method", whereby, during sowing, the correct amount of SRF can be placed in the indentation made by the dibbler, after which the seed can be sown on top. This method should have been used in this experiment, but it was perceived that thorough mixing should be sufficient.

Another problem in this trial could have been the analysis of the pine bark medium. Pine bark was extracted with water at room temperature (not at a constant temperature) and Osmocote Mini®'s release of nutrients is only subject to temperature, i.e., the greater the temperature the more nutrients extracted. Thus, the analysis obtained might not have been a true reflection of the actual quantities available within the media. However, since

there were no significant differences in growth, this was probably not the case, but future analysis of SRF should take this factor into account.

Crowley *et al.* (1986) proved that pre-enrichment was not detrimental to mycorrhizal colonization of pine seedling roots. However, Hunt (1991) showed that 4.7 $kg.m^{-3}$ Osmocote® was detrimental to mycorrhizal intensity and diversity in *Picea engelmannii*. He suggested that rates of incorporation should be modest. Thus, it would be important to investigate the SRF effect on mycorrhizae in the nursery and the consequences once planted in the field.

It is therefore recommended that further trials be conducted on SRF, taking cognisance of the shortcomings of this trial. It is still not known if Osmocote Mini® is a viable alternative to fertilizer practices for forestry seedlings, or if it can be applied alone or should be supplemented with additional fertigation.

However, SRF such as Osmocote Mini® do have many potential advantages that were unfortunately not highlighted in this trial. Slow release fertilizers are an option that should be considered if a grower is looking to simplify management practices. The continual supply of nutrients provided by SRF may prove to be valuable when seedlings are planted in the field. This could have a carry-over effect and aid seedling establishment if planted in a poor soil. Also, if seedlings are held for a long time by the forester, SRF could tide the seedling over, until planted in the field. The main advantage with SRF is that they are environmentally friendly. The increasing environmental awareness through reduction in pollution will no doubt boost the use of these fertilizers.

CHAPTER SEVEN

SILICON NUTRITION

7.1 INTRODUCTION

Experiments have shown that silicon (Si) is necessary for the development of some plants, and may be considered an essential element for plants with a high Si content, such as the Graminaceae (Cheng, 1982), as well as having a beneficial effect on many others, including pine species. However, the majority of Si research has been done on the Graminaceae, so there is little information on conifer seedling optimum tissue Si concentration, conifer Si uptake or the effects of Si on conifer seedling growth.

It is interesting to note that the most successful plants in the ecology of land and sea, the grasses and the Pinaceae, are all distinguished by an obvious silica accumulation in cells (Werner & Roth, 1983). Possible functions of these silica structures are:

1. Protection against attacks by pathogens such as powdery mildew, blast disease and *Pythium*, which lack, perhaps, a silica metabolism (Cheng, 1982; Adatia & Besford, 1986; Menzies, Ehret & Bowen, 1992; Chérif, Menzies, Ehret, Bogdanoff & Bélanger, 1994).
2. Contribution to the mechanical stability and rigidity of cells, i.e., decreased lodging, better light interception and erectness of leaves (Yoshida, Navasero & Ramirez, 1969; Cheng, 1982).
3. Lowering of cuticular water loss due to epidermal accumulation of silica, i.e., plant-water relations (Lewin & Reimann, 1969; Emadian & Newton, 1989).
4. Increasing plant tolerance to high levels of Mn, Zn, Fe and Cu (Jones & Handreck, 1967; Vlamis & Williams, 1967; Kluthcouski & Nelson, 1980; Cheng, 1982; Mengel & Kirkby, 1982).

Members of the Pinaceae accumulate large quantities of Si in the leaves and stems (10-15% SiO_2 in the dry matter)(Werner & Roth,

1983), and are termed "silica accumulators" (Takahashi & Miyake, 1982), but it is not known which of these Si functions are influential in promoting pine seedling growth.

The effect of Si on the growth and water status of *Pinus taeda* seedlings was studied by Emadian & Newton (1989). They found that fresh and dry weights, and the water status of seedlings were enhanced by Si addition. The percentage increases in growth compared with the controls were greater under water stress. Also, Si-treated seedlings accumulated 3 times more Si than did the controls, substantiating the findings of Werner & Roth (1983) that pines absorb large quantities of Si. The enhanced growth was associated with higher water and osmotic potentials, a greater symplastic water volume, increased tissue elasticity and less turgor. They suggested that Si enhances pine seedling growth by increasing cell expansion and that Si-treated tissues become more elastic. Although Si was beneficial to plant growth in this case, other research has shown Si to be of no benefit (Van den Driessche, 1992).

It has been known for years that the application of silica-containing fertilizers, such as slags, can increase the availability of P. The silicates displace the P which is normally adsorbed by Al and Fe oxides, and promote plant growth (Fisher, 1929; Okuda & Takahashi, 1964), i.e., this is an indirect effect of Si on plant growth. However, will Si have the same effect in pine bark, where it has been suggested that P is bound by the bark or will it have no effect because P may be rapidly leached?

Because of the controversial evidence as to the importance of Si in pine nutrition, an attempt was made to study the effect of Si on the growth of *P. patula* seedlings.

7.2 MATERIALS AND METHODS

7.2.1 Silicon Pre-enrichment

In this experiment (Exp.5), composted pine bark, "wattle medium",

was pre-enriched with 7 levels of silicate in the form of two slags (Section 2.1.3). The levels of pre-enrichment were 0, 0.25, 0.50, 1.0, 2.0, 4.0 & 8.0 $kg.m^{-3}$. The slags used were EAF slag, a by-product from Columbus Stainless Steel; and Plaaslike Boeredienste - low carbon ferrochrome slag, a by-product of Middelburg Ferrochrome which is sold as a soil conditioner. The composition of the slags is given in Appendices 1 & 2.

P. patula seeds (M4609) were sown by machine (Section 2.1.4) on 12.03.93 (Day 0) into Unigro® trays described in Section 2.1.2. The trays were laid out in a plastic tunnel (Section 2.1.1) as a 6 x 2 (+1 control) factorial random block design with three replications. They received a nutrient solution feed containing 3:1:3(38) at 1 $mS.cm^{-1}$ through the boom system once or twice a day. The plug surfaces were very prone to algal growth, which frequently had to be scraped off. The seedlings were harvested on 27.08.93 (Day 168) and once the growth measurements were taken they were stored (Section 2.2). Seedlings were observed for needle rigidity and their moisture contents determined. Foliar and medium analyses were not undertaken because of the costs involved and because experimental results were generally non-significant.

Seedlings were subjected to a stress test at the end of the experiment, to see if Si content had an effect on drought tolerance. Seedlings were monitored for wilting (bent heads) and the number of wilted seedlings counted for a week. The seedlings did not receive water during this period.

7.2.2 Silicon Nutrient Feed

On 08.03.93 (Day 0) *P. patula* seeds (M4609) were sown into Unigro® trays (Section 2.1.2) for the start of Exp.6. The medium used for Exp.6 was "wattle medium" from Gromed, and it was pre-enriched with 0.5 $kg.m^{-3}$ of Micromax® (Section 2.1.3). The micronutrient fertilizer, Micromax®, was incorporated as it resulted in better growth than FRIT 504® in Exp.3. Single superphosphate at 5 $kg.m^{-3}$ was also pre-enriched, as a source of P.

Experiment 6 compared two silicate fertilizers, Silchem-K® and Fertigro-sil®, at four levels (0, 25, 50 & 100 mg.ℓ^{-1}) which were hand-applied daily (Tables 7.1 & 7.2).

Table 7.1 Quantities of fertilizers used to make up the nutrient solutions of the Si trial.

CONC. (mg.ℓ^{-1})	VOLUME (mℓ)	
	SILCHEM-K®	FERTIGRO-SIL®
25	8.368	10.204
50	16.736	20.408
100	33.480	40.816

Table 7.2 Actual concentrations of the important elements in the nutrient solutions of the Si trial determined by analysis.

CONC. (mg.ℓ^{-1} Si)	PH	EC (mS.cm^{-1})	CONCENTRATION (mg.ℓ^{-1})		
			K *	Na	Al
Fertigro-sil® 25	7.51	0.22	43.0	6.0	0.03
50	8.93	0.35	86.0	7.0	0.07
100	9.02	0.61	198.0	8.2	0.07
Silchem-K® 25	7.83	0.13	12.5	6.0	0.09
50	7.95	0.16	25.3	6.2	0.10
100	7.97	0.23	54.0	6.6	0.13
Control	7.39	0.10	0.7	5.5	<0.01

K * Fertigro-sil® and Silchem-K®, as can be seen from Appendices 1 & 2, do contain K. Thus, these actual values of K in Table 7.2 are representative of the K contents in the solution.

Fertigro-sil® is a potassium silicate made by Kemira in Sweden and is applied in the horticultural industry as a fertilizer. Silchem-K® (grade 2166) is also a potassium silicate, and is produced by Silicate and Chemical Industries in South Africa. It is used industrially and has not been applied in agriculture. The composition of these two fertilizers is given in Appendices 1 & 2. No attempt was made to balance the elemental K content in the two fertilizers, as the experiment aimed to compare the two products at various levels. 80 mg NH_4-N ℓ^{-1} was applied as the N source in the nutrient solutions, having been found in Exp.1 to be the optimum rate of N to apply to *P. patula* seedlings.

The 3 x 2 (+1 control) complete block design experiment with five replications was conducted in a tunnel at the University of Natal (Section 2.1.1). On 25.08.93 (Day 170) seedlings were harvested and observed for rigidity, and growth measurements taken (Section 2.2). Nutrient solutions applied to the seedlings were analysed for nutrient content (Table 7.2), as was the pine bark medium (Section 2.3), but not the seedling foliage.

It was advised by the CSIR and the Departments of Agronomy and Chemistry at the University of Natal, to determine the Si content of bark by the blue silicomolybdous acid procedure, a colorimetric determination of Si. Unfortunately all pine bark analyses resulted in similar Si contents, no matter what amount of Si was added. The control treatments had similar Si contents to the treatments that received Si. Thus, after 3 attempts the determination of Si was abandoned, as it became obvious that bark contains large quantities of Si and the addition of small amounts of Si were impossible to detect. No other literature could be found on Si determination in pine bark. Thus, the Si content of the pine bark is not presented.

As in Exp.5, seedlings were subjected to exactly the same stress test, and moisture contents of the seedlings were noted.

7.3 RESULTS

7.3.1 Silicon Pre-enrichment

Statistical analysis showed that the control (no slag) was not significantly different to any other treatments, for all plant measurements taken (Fig. 7.1). This, therefore, proved that the silicate slags, and therefore Si, had no beneficial effect on pine seedling growth, i.e., their use is not warranted in seedling nutrition.

Analysis did show that there were significant differences ($P \leq 0.05$) between the slag treatments, but only for the CD and SDM parameters. The EAF slag resulted in markedly greater CD and SDM than the Boeredienste slag, i.e., 2.52 ± 0.08mm vs 2.45 ± 0.08mm CD and 1.13 ± 0.08 $g.seedling^{-1}$ vs 1.06 ± 0.08 $g.seedling^{-1}$ SDM (Fig. 7.1a).

The various levels of slag pre-enrichment were also significantly different for CD and SDM. The SDM revealed at $P \leq 0.05$ that 1 $kg.m^{-3}$ of slag resulted in significantly greater mass, with a SDM of 1.30 ± 0.08 $g.seedling^{-1}$, than the treatments of 0.5, 2, 4 & 8 $kg.m^{-3}$ (Fig. 7.1b). Also, 8 $kg.m^{-3}$ of pre-enriched slag produced the lowest SDM (EAF having a mass of 0.97 ± 0.08 $g.seedling^{-1}$ and Boeredienste 0.96 ± 0.08 $g.seedling^{-1}$)(Fig. 7.1b). The CD showed the same trends as SDM, but the differences were more significant ($P \leq 0.01$). The CD of the worst treatment was 2.35 ± 0.08mm and the best treatment was 2.54 ± 0.08mm (Fig. 7.1b). There were no differences between the other levels of 0.25, 0.5, 2 & 4 $kg.m^{-3}$ of slag pre-enrichment.

The percentage shoot moisture and number of wilted seedlings (stress test) did not show any significant treatment effects. It was hoped that the treatments receiving Si would have been more tolerant to drought stress, indicative of a lower moisture content in the seedlings and a lower number of wilted seedlings. Thus, Exp.5 did not prove that Si reduces cuticular water loss.

At harvest, the seedling needles were compared for rigidity, but

no visible differences could be noted.

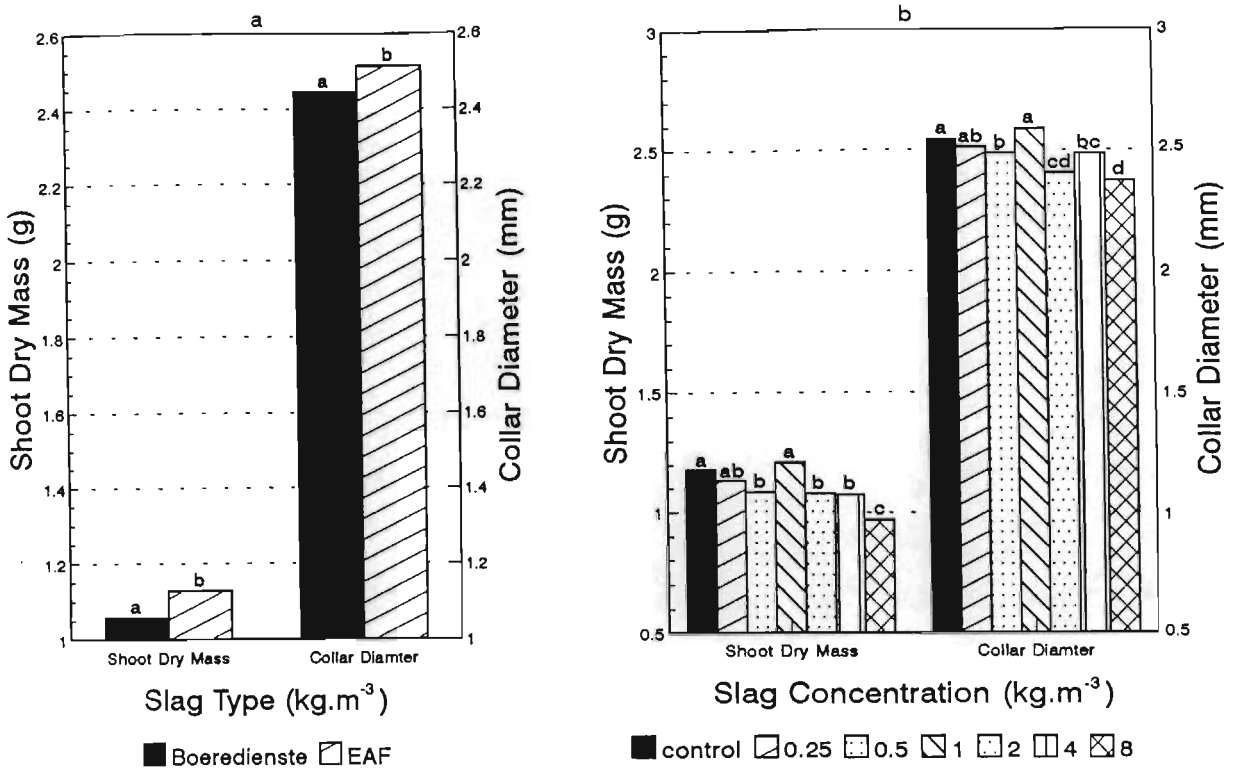


Fig. 7.1 The shoot dry mass and collar diameter of *P. patula* seedlings grown in pine bark amended with (a) EAF and Boeredienste slags and (b) at different slag concentrations. (Treatments with letters in common are not significantly different).

7.3.2 Silicon Nutrient Feed

Three months after planting it was noticed that certain treatments had foliar symptoms, where the ends of the needles were yellow, and in some cases orange and purple. However, the symptoms were scattered over the trial and not all seedlings in a tray were affected, i.e., it was not a treatment effect. The overall appearance was a "blotchiness". This was resolved when it was found that seedlings Mondi had planted at the same time, using the same medium, were also "blotchy". The symptoms were analysed as being characteristic of a medium that is not properly composted, as Maggs (1985) found for bark composting. Medium analysis verified this, i.e. the NH_4-N content was extremely high (282 mg.ℓ^{-1}) with a very low NO_3-N content (Table 7.3).

Table 7.3 Initial pine bark media analysis - Exp.6.

MEASUREMENT	CONCENTRATION
EC	4.62 $mS.cm^{-1}$
pH	5.33
NO_3	2.04 $mg.\ell^{-1}$
NH_4	281.98 $mg.\ell^{-1}$
P	26.96 $mg.\ell^{-1}$
K	70.0 $mg.\ell^{-1}$
Ca	241.0 $mg.\ell^{-1}$
Mg	50.9 $mg.\ell^{-1}$
Fe	0.08 $mg.\ell^{-1}$
Mn	1.44 $mg.\ell^{-1}$
Zn	0.17 $mg.\ell^{-1}$
Cu	0.07 $mg.\ell^{-1}$

Two months later when the trial was harvested there was no sign of this "blotchiness", indicating that most of the NH_4 had been converted to NO_3 (as was seen in the final pine bark analysis) which was subsequently leached.

Statistical analysis of the plant growth parameters at harvest showed that there was no significant difference between Fertigro-sil® and Silchem-K®, and between the three rates of 25, 50 & 100 $mg.\ell^{-1}$ (Plates 7.1 & 7.2; Fig. 7.2). Thus, the different rates of Si and K did not affect the growth or moisture content of *P. patula* seedlings. However, the growth parameters did show that there was significantly less growth in the control treatments, which had not received Si or K (Plates 7.1 & 7.2). However, the root dry mass did not reflect this difference (Fig. 7.2). It was concluded that this difference was not due to the effect of Si or K, but because the control treatments, in error, had not been given N in the solution from the beginning of the experiment, but

only from a month after the experiment had started. This does show, however, that N has an important effect on seedling growth and if timing of N application is delayed, the seedling never actually "catches up" in the nursery. Thus, Exp.6 showed, like Exp.5, that Si does not improve the growth of *P. patula* seedlings, and that it is not detrimental to their growth.

Results from the stress test and the observations of seedling needle rigidity were non-significant, as for Exp.5. Thus, Si addition did not have a marked effect on seedling growth, erectness or seedling drought tolerance.

Final media analysis revealed no marked differences between the nutrients, except for bark K content. This increased with an increase in Fertigro-sil® and Silchem-K® additions. The Fertigro-sil® treatments had greater K contents than the Silchem-K® treatments. This was expected as although both contained K, the Fertigro-sil® contained twice that of Silchem-K®. The control contained the least K, but this was only marginally less than the Silchem-K® at 25 mg.ℓ^{-1}. If K had been responsible for the increased growth in the treatments that received fertilizer, then the treatment of Silchem-K® at 25 mg.ℓ^{-1} would have been of similar growth to the control.

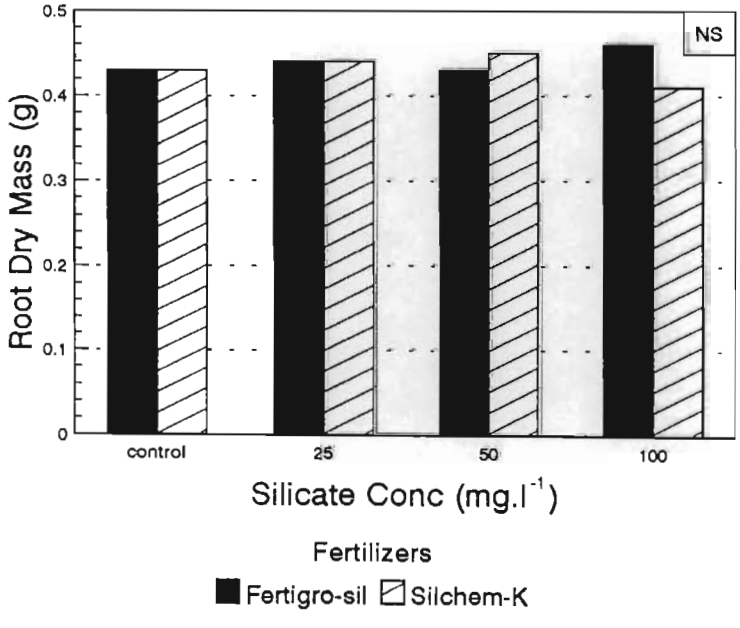


Fig. 7.2 Effect of Si concentration on root dry mass.

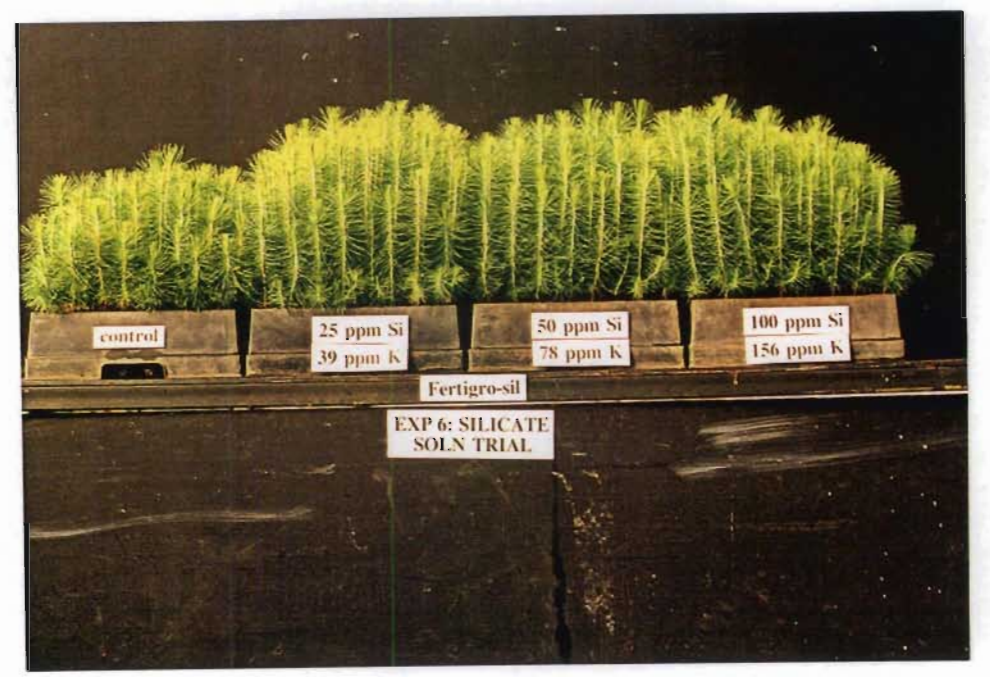


Plate 7.1 Effect of Fertigro-sil® at three rates on the growth of *P. patula* seedlings (the control not being representative of the experiment).

Plate 7.2 Effect of Silchem-K® at three rates on the growth of pine seedlings (the control not being representative of the experiment).

7.4 DISCUSSION AND CONCLUSIONS

The similar growth that resulted between treatments not receiving Si and treatments receiving Si, either as a pre-enrichment source in the pine bark medium or as a nutrient solution, adds further to the controversy surrounding Si as an essential element for conifer species. The two experiments conducted showed no advantage in supplying Si to pine seedlings to improve their growth by decreasing cuticular water loss and increasing plant rigidity. This could have been due to the fact that the levels of Si should have been higher or that other factors played a role in masking the Si effect.

However, further experimentation on the benefits of Si to pine seedling growth could be conducted. Emadian & Newton (1989) found that greater growth of *P. taeda* was promoted by Si when the seedlings were under water stress. Experiments 5 & 6 were not conducted under water stress and if repeated under such conditions might reveal significant growth differences. Silicon has also been shown to increase plant tolerance to high concentrations of Mn, Zn, Cu & Fe (Jones & Handreck, 1967; Cheng, 1982) and this fact, if proven correct, could be beneficial to the seedling industry which makes use of composted pine bark as a medium. Bark can often contain toxic concentrations of Fe, Cu, Zn and especially Mn, due to incomplete composting or because certain species such as *P. patula* contain greater amounts of these micronutrients. Thus, addition of Si could guarantee non-toxic concentrations of these micronutrients.

It has been shown that Si can increase the pathogen resistance of some plants (Cheng, 1982; Menzies *et al.*, 1992). Fertigro-sil® is applied overseas to horticultural crops for exactly this reason. However, disease resistance was not looked at in these trials because seedlings were not prone to disease. Thus, this factor should also be investigated for pine seedlings. If Si can increase pathogen resistance this will limit the dependency on fungicides, which are frowned upon by many environmentalists. However, the evidence that Si is the main factor in decreasing disease, is controversial. This effect may also be due to K, as

it has been proven that forest trees and other crops adequately supplied with K are also more resistant to fungal diseases (Mengel & Kirkby, 1982). Thus, the combination of Si and K should certainly benefit diseased crops. This is no doubt why fertilizers such as Fertigro-sil®, which contain both Si and K, are manufactured.

The classical approach to prove the essentiality of an element to higher plants, by growing them in a medium without that element, is very hard to achieve in the case of Si. No culture system could be claimed to be Si-free (Werner & Roth, 1983). Silica is present in significant quantities in water and air and was found to be abundant in pine bark. This would make the increased growth of pine seedlings in pine bark supplied with Si, difficult to determine. The non-significant results from Exp.5 & 6 verify this fact. It would, therefore, appear perhaps that pine bark has sufficient available Si, not to warrant the addition of Si in the form of fertilizers and slags.

OVERALL DISCUSSION AND CONCLUSIONS

The main concern of the forestry industry is the survival rate of seedlings in the field. Nurseries supplying these seedlings therefore have to ensure that they are hardy and able to survive transplanting stress. Several factors contribute to transplanting success and these can be controlled in the nursery by manipulating nutritional programmes. Thus, the focal point of interest in a nutrition programme is the availability of nutrients in the medium solution and the plant's ability to utilize these nutrients to produce optimum quality growth. This is influenced by medium properties, plant mineral nutrient requirements and type and method of fertilizer applied.

Properly composted pine bark has been shown to possess most of the necessary chemical properties to produce a containerised pine seedling of desired quality. Nevertheless, amending pine bark with lime and micronutrients is a common nursery practice. In this study pine bark was found to contain sufficient Ca and Mg, i.e., no liming of pine bark was required. However, pine bark was shown to contain insufficient micronutrients and K to satisfy the growth of *P. patula* seedlings grown for four months or more, as was previously believed. Total micronutrient requirement of *P. patula* seedlings can be satisfied by pre-enriching with 1000 $g.m^{-3}$ of Micromax®. However, bark that is not properly composted, with high NH_4 and Mn, can cause toxicity problems. Even though the South African bark composting industry is 15 years old, growers frequently experience problems. Further research is definitely needed on the issue of bark quality standards. Unlike South Africa, countries such as Australia have already set standards (Handreck & Black, 1984). The only obvious solution at present is to obtain a nutrient analysis of the pine bark to be used, and adjust according to the recommendations for that particular plant or species. However, *in lieu* of the wide range of extraction methods currently being used for the analysis of pine bark, this may be difficult to interpret and adopt. Thus, the development and adoption of standardized extraction procedures in South Africa is of utmost importance, before nurseries can compare and develop nutrient sufficiency guidelines

from pine bark extracts.

For decades fertilizers have been applied in the nursery and the results merely noted as "increased growth" or "reduced, distorted growth". Although these results have been useful in defining, in general terms, the best fertilizer regimes to suit individual nurseries and species, further advances can only be expected once the precise roles that nutrient elements play in physiological processes are known and understood.

The identification and correction of nutrient deficiencies is important in the production of healthy seedlings. Seedling nutrient analyses should be performed regularly to monitor mineral nutrient utilization. Conventional diagnoses are based on evaluating plant tissue concentrations and comparing these with predetermined critical nutrient concentrations. Interpretations may be confounded by variation in age, season and environment, concentrations of other nutrients, dilution effects and nutrient interactions. There is also some controversy as to which plant part to use when testing for deficiencies, as some parts, like shoots, may be chlorotic and yet foliar nutrient analyses can reveal normality. This is often the case with foliar Fe. Very little information exists for critical element concentrations for pines grown in pine bark. Literature available is American or European, with vastly different growing conditions. Thus, in this study foliar concentration standards were established and found to be similar to American standards. However, the development of ratios, indices and vector analyses should be the future method of comparing foliar nutrient concentrations. Most importantly each nursery should begin to develop its own seedling nutrient analysis standards.

The theory that tree seedlings are best adapted to nutrient poor sites if grown with a low supply of nutrients is rapidly becoming replaced by an increasing awareness that vigorous seedlings, resulting from an ample and well balanced nutrient supply, are likely to out-perform those raised with limited nutrition. Experimental results have shown that nursery fertilization greatly increases final volume in the field. In the past, most

forestry seedling nutrition research was carried out overseas and so results did not apply to the conditions and growing media used in South Africa.

Post-sowing nursery N-P-K fertilization is essential for maximum pine seedling production. Slow release forms of N-P-K fertilizers, such as Osmocote Mini®, did not, however, prove beneficial, but their use may be warranted when the cost of SRF is similar to that of conventional fertilizers, and when the "teething" problems are overcome. Problems of incorporation into the media, analysis of pre-enriched SRF media and effect on mycorrhizae are still topics that need to be researched. Although incorporation of conventional and slow release fertilizers into the medium can be justified in some instances, direct application of liquid fertilizers is recommended whenever possible. The benefits of this technique include precise control of both the concentration and balance of all the essential nutrients, the ability to completely change the nutrient solution at any time, and a very low chance of over-fertilization and resultant salt injury. However, growers should regularly monitor mineral nutrient levels at various phases during fertilization operations. This should start with nutrient salts in the irrigation water and end with leachate solutions that drain from the bottom of the container, i.e., the pour-through (PT) method.

It is apparent that N is the key element in controlling the growth of containerised pine seedlings in pine bark. The concentration and form of N in the nutrient solution dramatically influenced growth and hardiness. Optimum growth, moisture content and lateral bud development occurred at 80 $mg.\ell^{-1}$ NH_4-N, which yielded the most suitable seedling for out-planting performance. Thus, the dramatic effect of N on plant growth has made it an important tool in the manipulation of growth cycles as well as seedling hardiness.

The controversial topic of Si nutrition was addressed, but both experiments concluded that Si was not beneficial in improving growth, reputedly by decreasing cuticular water loss and increasing needle rigidity of *P. patula* seedlings. It appeared

that the pine bark had sufficient available Si, to not warrant the addition of Si in the form of nutrient solutions and slags. However, it has been shown that Si can increase pathogen resistance (Cheng, 1982), and if this could be proven for pine seedlings, this would limit dependency on fungicide. If not proven, this would highlight the advantages of pine bark as a growing medium.

Not only fertilizer amount, but correct fertilizer scheduling is important to achieve better results. Growers often fertilize with a constant nutrient solution during the seedling's entire nursery life, not scheduling fertilizer ratios in relation to plant growth. This is wasteful and can hinder plant growth in its later stages, as was seen with the Mondi seedlings in Exp.2. Much research is needed on scheduling under South African conditions, as nurseries in this country need positive evidence that this is the better fertilizer regime.

In conclusion, the ability to design and apply a well balanced nutrition programme can result in better quality seedlings, and ultimately result in improved planting stock and therefore greater yields. Growers must therefore consider their overall situation before formulating a nutritional programme based on these guidelines. The combination of cultural practices that produce the best quality seedling in the shortest period of time, and at an acceptable cost, will be the most economical in the final evaluation. Although there has been much research on conifer nursery fertilization in the past few years, there are still many unanswered questions and much research is still needed on the subsequent field performance of nursery trials. Nursery trials which test out-planting survival, by means of RGP and/or stress tests, are not adequate testing methods and need to be supplemented by improved versions of these methods and by field trials.

SUMMARY

Pine trees are an integral part of reforestation and afforestation. The expansion of the forestry industry has resulted in a greater demand for seedlings. Thus, the chemical factors affecting mineral nutrition of pine seedlings are of utmost importance, since they determine seedling quality and therefore field performance. The nutrition can be influenced by the growing medium used (the chemical properties), the mineral nutrient requirements of the seedling, and the method and type of nutrient addition.

The use of composted pine bark as a growing medium for containerised pine seedlings has created some difficulties for growers, mainly due to a lack of understanding of chemical properties and reactions occurring in pine bark. Bark contains reasonable quantities of the elements required by plants; however, N and P contents are low and need to be supplemented with fertilizers. The CEC of bark is initially low but increases with composting. The pH of bark is an important factor requiring continual monitoring. Variations in the pH have marked effects on nutrient availability and can often be the cause of nutrient deficiencies. Composting of pine bark is essential, in order to raise the pH to ± 5 (the optimal range for seedling growth), and to reduce the C:N ratio from an initial value of ± 100 to ± 30, and therefore stabilise the media. Composting also reduces toxic components such as high concentrations of Mn and phenols, which if present in the bark can be detrimental to pine seedling growth. Composted bark is very different to soil and other soilless media and so extrapolation must be viewed with caution.

The mineral nutrient requirements of pine seedlings are difficult to determine, since the required concentrations depend on many factors. However, there are 14 essential elements required for normal plant functioning. They comprise the macronutrients (N, P, K, Ca, Mg and S) and the micronutrients (Fe, Cu, Mn, Zn, B, Mo, Cl and Ni). Nitrogen is the element, when applied to pines, that elicits the most growth, and therefore fertilizer programmes

are designed according to the N concentration and form of N. Other elements, such as Si, Na and Co, are considered to be important for certain plants, but current scientific evidence prohibits the addition of these to the list of essential elements.

Nutrient deficiencies have become increasingly noticeable in nurseries and correct identification is an important aspect of nursery management. Nitrogen and P deficiencies are the easiest of the macronutrients to identify. The other macronutrients, K, Ca, Mg and S, produce characteristic symptoms, displaying some form of chlorosis. Micronutrient deficiency symptoms are, however, extremely variable between nutrients and species. They are difficult to diagnose because symptoms are often a result of an imbalance between micronutrients.

Fertilization, the addition of nutrients, controls the rate and type of growth in container tree seedling nurseries. Thus, a fertilization programme for a seedling nursery should be designed to maintain specific concentrations of nutrients in the media, keep them in balance, and it should allow the necessary scheduling of fertilizers during the growth of a seedling. Nutrient application can be applied in the form of a liquid feed, or pre-enriched into the growing media, either by the incorporation of SRF or conventional fertilizers. The correct timing and scheduling of fertilizer applications to seedlings conserves fertilizers, minimizes the imposition of environmental stresses and more importantly, results in nutrient additions paralleling plant growth. Different nurseries use and recommend various fertilizer formulations and regimes, all based on overseas or vegetable seedling recommendations, due to the lack of information on pine seedling nutrition. There being almost as many methods of fertilizer addition as nurseries.

Nutrient N levels were tested to determine what form and concentration resulted in optimum growth of *P. patula* seedlings. Optimum concentration for the production of good quality seedlings was with the application of 80 $mg.\ell^{-1}$ N in solution. The best form of N to be applied was NH_4^+ as *P. patula* growth was

enhanced more by ammonium-N application than by nitrate-N or NH_4NO_3. This experiment also showed that pine bark does not contain sufficient P and K to meet the requirements of pine seedlings grown in pine bark for a duration of four months. Thus, P and K, like N must be applied to containerised pine seedlings grown in pine bark.

Pre-enrichment of pine bark is commonly practised to provide many of the essential elements. In two trials using various lime products, and dolomitic lime and micronutrients at different concentrations, it was found that there is no advantage to liming pine bark media. This was due to the fact that pine bark contains sufficient Ca and Mg to satisfy the seedling's requirements and because it has a high CEC which buffers lime addition. In some cases liming was detrimental to pine seedling growth and it is recommended that dolomitic lime, which is often used for pre-enrichment in nurseries, be avoided when growing *P. patula* seedlings. Of the lime products tested, 8 $kg.m^{-3}$ Calmafos® was the best lime pre-enrichment treatment, if liming was required. However, pre-enrichment of micronutrients, preferably 1000 $g.m^{-3}$ Micromax® was necessary for optimal pine seedling growth, i.e., pine bark does not contain sufficient micronutrients for the duration of a pine seedlings' growth in the nursery. A lower pH of ± 4.5 is acceptable in pine bark media for the growth of *P. patula* seedlings, as maximum availability of nutrients was shown to occur from pH 4.7-5.4.

The addition of an N-P-K fertilizer and scheduling of nutrients, to correlate with the seedling's growth stage, are essential for maximum pine seedling production. The application of a periodic fertilization schedule resulted in seedlings of better quality than when constant fertilization was applied. Slow release forms of N-P-K, such as Osmocote Mini®, did not prove beneficial, although their use may be warranted when the cost of SRF equals that of conventional fertilizers.

The addition of Si, in the form of pre-enriched silicate slags and Si nutrient solutions, to improve growth, reputedly by decreasing cuticular water loss and increasing plant rigidity and

growth, provided no observed advantage to pine seedling growth. It was suggested that sufficient Si was available from the pine bark growing medium.

Thus, from results of this thesis, foliar norms recommended are: 2% N, 0.2% P, 1% K, 0.3% Ca, 0.2% Mg, 100 $mg.kg^{-1}$ Fe, 550 $mg.kg^{-1}$ Zn, 100 $mg.kg^{-1}$ Mn and 9 $mg.kg^{-1}$ Cu.

Further research is definitely required on SRF, nutrient scheduling and timing, and on the benefits of Si to disease resistance. There is also a need to extend nursery experiments into the field to determine survival rate and performance, as RGP and stress tests often show inconclusive results and are thus inadequate testing methods.

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APPENDICES

Appendix 1. Percentage concentrations of macronutrients in fertilizers used in the experiments.

FERTILIZER	%					
	N	P	K	Ca	Mg	S
Low biuret urea	46.0	-	-	-	-	-
Calmag®	13.2	-	-	9.8	4.80	-
NH_4NO_3	35.0	-	-	-	-	-
Dolomitic lime	-	-	-	21.2	7.72	-
Calcitic lime	-	-	-	28.0	0.28	-
Langfos®	-	12.6	0.25	29.0	0.32	0.08
Calmafos®	-	9.5	0.04	21.0	9.00	-
Single supers	-	10.5	-	20.3	-	10.20
Agrofert Orange®	12.0	16.0	10.10	-	-	-
Hortichem Orange®	13.5	18.0	11.40	-	-	-
Hortichem Blue®	21.2	7.1	14.20	-	-	-
3:1:3(38)	16.2	5.5	16.20	-	0.60	5.00
Osmocote-M®	18.0	3.0	10.00	-	-	-
Micromax®	-	-	-	5.4	3.00	-
EAF Slag	-	-	-	31.3	11.70	-
Boeredienste	-	-	-	37.4	13.20	-
Fertigro-sil®	-	-	25.70	-	-	-
Silchem-K®	-	-	11.11	-	-	-

Appendix 2. Percentage concentrations of micronutrients in fertilizers used in the experiments.

FERTILIZER	%						
	Fe	**Zn**	**B**	**Mn**	**Cu**	**Mo**	**Si**
FRIT 504®	14.30	7.00	3.80	7.50	7.00	0.08	-
Micromax®	12.00	1.00	0.10	2.50	0.50	0.05	-
Langfos®	1.26	0.005	0.035	0.03	0.005	0.001	5.2
Calmafos®	7.00	0.016	-	0.15	0.005	0.005	12.0
Agrofert Orange®	0.10	0.007	0.045	0.026	0.002	0.003	-
EAF Slag	1.30	-	-	5.80	-	-	29.4
Boeredienste Slag	0.10	-	-	0.50	-	-	33.0
Fertigro-sil®	0.002	-	-	-	-	-	19.6
Silchem-K®	-	-	-	-	-	-	23.9

Appendix 3. Quick 1:1.5 Volume Method (Sonneveld & van Elderen, 1994).

Extraction method:

1. Remove any roots and large pieces that are not representative.
2. Take a subsample of 100g and dry it at 150°C to constant mass.
3. Re-weigh subsample to obtain percentage moisture.
4. Weigh out a 50g sample of pine bark at a representative moisture content for planting, i.e., ± 35%.
5. Place into a 250ml conical flask and add 150ml of distilled water.
6. Shake for 1 hr at 180 revs.min^{-1}.
7. Filter through tea strainers to remove bark pieces.

8. Centrifuge ± 100ml of the suspension at 5000rpm for 5 min or until clear.
9. Pour solution through a Whatman No. 1 filter paper.
10. Measure pH, conductivity and elemental content of the extract (Section 2.3.3.).

Appendix 4. Plant Ashing Method.

1. Weigh out ± 1g (not > 1.002g) of dried, milled plant material into a porcelain crucible.
2. Put in furnace and switch on to 500°C.
3. Leave in furnace for 16-24 hr.
4. Remove and cool.
5. Add 6-8 drops of distilled water.
6. Add 2ml of concentrated (conc.) HCl and evaporate on a hot plate, stirring occasionally.
7. Cool and then add 15ml of 10% HCl.
8. Filter through filter paper into 30ml plastic vials and then add a further 10ml of 10% HCl to the crucible to rinse.
9. Pour through filter paper to obtain a concentrated sample.
10. Make up diluted samples for analysis.
11. Analyse for all elements, except N, using the methods described in Section 2.3.3.

Correction factor (CF) (leaf sample)

$$= (a \times 25/\text{mass of sample})/10000 = \%$$

or

$$CF \text{ (leaf sample)} = a \times 25/\text{mass of sample} = mg.kg^{-1}$$

Appendix 5. Digestion Method (to determine total-N in the plant).

1. Switch Labcon D60 digestion block on 1 hr before use at 360°C.
2. Weigh out 0.1g of milled plant material and brush down

funnel into digestion tube.

3. Add a 1g scoop of Kelpak, the catalyst (include a tube with just Kelpak as a blank).
4. Add 4ml conc. H_2SO_4 to each tube and swirl to mix.
5. Place tubes in preheated digestion block.
6. After 15 min rinse down the sides of the tubes with 1ml H_2O_2.
7. After 30 min rinse again with H_2O_2.
8. Leave for 1 hr until digests are clear.
9. Remove tubes from block and cool for 30 min.
10. Make up to 50ml with distilled water and mix.
11. Analyse for total-N, using the Kjeldahl method (Appendix 6).

CF (leaf sample) = ([concentration of reading - blank reading] x 500)/10000
= (concentration x 500)/10000
= concentration/20 = % N

or

CF (leaf sample) = concentration x 500 = $mg.kg^{-1}$

Appendix 6. Ammonium (Kjeldahl) Determination (Bremner & Mulvaney, 1982).

Reagents:

Sodium hyperchlorite (NaOCl) - 2.5ml 15% solution in 100ml H_2O. Made up fresh each time.

20% Sodium hydroxide (5M NaOH) - 200g NaOH dissolved in 600ml H_2O. Cooled and made up to 1ℓ in volumetric flask.

Sodium salicylate/sodium nitroprusside - 150g Na salicylate and 0.3g Na nitroprusside dissolved in 600ml H_2O. Made up to 1ℓ in volumetric flask.

20% Potassium sodium tartrate - 200g KNa tartrate dissolved to make up 1ℓ.

Stock buffer solution - 134g disodium hydrogen phosphate added to

66ml 20% NaOH and made up to 1ℓ with H_2O.

Working buffer solution - Make up 200ml stock buffer, 250ml 20% potassium sodium tartrate and 120ml 20% NaOH to 1ℓ.

Colour solution - 200ml working buffer, 200ml distilled water and 80ml sodium salicylate/sodium nitroprusside (or any other volume with the same ratio) in a dispenser bottle and set at 12ml.

3N H_2SO_4 - 83.3ml conc. H_2SO_4 added to water slowly and made up to 1ℓ.

Standards - 1.908g ammonium chloride dissolved in 3N H_2SO_4 and made to volume (500ml) with 3N H_2SO_4. Standard stock solution diluted for given concentrations (Table 2.1).

Table 2.1 Amount of stock solution to be diluted to 100ml for a given concentration.

$mg.\ell^{-1}$ N	ml of stock standard soln.
25	2.5
50	5.0
75	7.5
100	10.0

Method:

1. Take 0.1ml of solution to be analysed, and 0.1ml of each standard and 0.1ml water for a blank.
2. Add 12ml of colour solution.
3. Add 1ml of sodium hypochlorite and mix.
4. Allow solutions to stand for 30 min and then read on spectrophotometer at 660nm.