# The effect of Phosphorus on the growth, plant mineral content and essential oil composition of Buchu (*Agathosma betulina*)

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**Declaration**

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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## ABSTRACT

An increase in the demand of buchu (*Agathosma betulina*) oil has lead to an increase in the commercial cultivation of buchu in fields and also in hydroponic systems. A nutrient solution for hydroponically grown buchu is still required to ensure optimal growth and yield. ASNAPP (Agribusiness in Sustainable Natural African Plant Products) South Africa has done some trials to achieve optimal EC and pH in the nutrient solution. Phosphate concentrations in the nutrient solution might play a significant role due to reports by a variety of researchers on the sensitivity of Protea plants to phosphate. Buchu and Proteas are both part of the Fynbos biome and are found in regions with similar soil (sandy soils with a low pH and mineral contents) and climatic conditions.

Two separate experiments were conducted to determine the effect of increasing phosphate concentrations (ranging from 0.00 to 1.40 me $L^{-1}$) in the nutrient solution on buchu growth. The first experiment was done in a plastic covered structure with a pad and fan and the objective of this trial was to determine the effect of increasing phosphate concentrations in the nutrient solution on the general growth, biomass production, oil composition, mortality rate and chemical composition of the buchu plants. The second experiment was done in a glasshouse with mechanical temperature control and the aim of this trial was to determine the response of buchu to increasing concentrations of P at two different root temperatures. A chemical analysis of the plants was done and the general growth, yield and root mass were recorded to determine the response of buchu plants to the phosphate and temperature treatments.

In the greenhouse experiment an optimum growth and yield response of buchu plants was found at a phosphate concentration of 0.7 me $L^{-1}$ in the nutrient solution. Phosphate concentrations lower or higher than 0.7 me $L^{-1}$ lead to a decrease in growth and yield. An increase in the phosphate concentration in the nutrient solution lead to a general increase in N, P, K, Ca, Mg and B content in the buchu plants and a decrease in Fe content. The mortality rate of the buchu plants increased with an increase in the phosphate concentration from 0.0 to 1.4 me $L^{-1}$ in the nutrient solution. The phosphate concentration in the nutrient solution only made a significant difference on one major

component of the buchu oil which was Ψ-Diosphenol, but no general trend with Ψ-Diosphenol content and P concentration could be found and the significant difference in Ψ-Diosphenol observed in this trial may only have been due to genetic variation between the plants.

The effect of the different root temperatures in the glasshouse experiment was very clear. The buchu plants grown at the high root temperature (20°C) produced a higher yield and better overall growth than the plants grown at lower (10°C) temperatures. The buchu plants grown at 20°C had a significantly higher N, K, Na and B content than plants grown at 10°C. Buchu plants grown at 10°C showed no significant response in terms of growth and yield to the phosphate concentration in the nutrient solution, but plants grown at 20°C exhibited growth and yield peaks at phosphate concentrations of 0.35 and 1.4 me $L^{-1}$. The peak observed in the plants growth at high phosphate concentrations is unexplainable and can possibly be ascribed to the limitation of the plants per experimental unit and/or amount of replications. The increase in P concentration in the nutrient solution caused a general increase in N, P and K content in the buchu plants. A significant interaction between the phosphate concentration and root temperature was observed for the P, Mn en Zn contents of the plants which meant that the buchu plants respond differently towards phosphate concentrations at different root temperatures.

# UITTREKSEL

‘n Toename in die aanvraag na boegoe (*Agathosma betulina*) olie het gelei tot `n toename in boegoe veldaanplantings sowel as boegoe wat hidroponies verbou word. Die voedingsoplossings se samestelling wat gebruik word in die hidroponiese stelsels moet nog getoets word om optimale groei en opbrengs van boegoe plante te verseker. Proewe onder die leiding van ASNAPP Suid Afrika is al gedoen om optimale EC en pH waardes te verkry. Fosfaat konsentrasie in die voedingsoplossing kan `n belangrike rol speel omdat baie navorsers al die nadelige effekte van hoë fosfaat konsentrasies op Proteas gemeld het. Proteas en boegoe is albei deel van die Fynbos bioom en kom natuurlik in dieselfde habitat voor met soortgelyke grondtipes (sanderige gronde met ‘n lae pH en minerale inhoud).

Twee afsonderlike eksperimente is gedoen om die effek van toenemende fosfaat konsentrasies wat wissel van 0.00 tot 1.40 me $L^{-1}$ op boegoe te toets. Die eerste eksperiment is gedoen in ‘n plastiek tonnel, wat verkoel is m.b.v ‘n natmuur en waaiers, om die effek van toenemende fosfaat konsentrasies op die groei, opbrengs, chemiese samestelling, mortaliteit en olie samestelling van boegoe te toets. Die tweede proef is gedoen in ‘n meganies verkoelde glashuis om die effek van toenemende fosfaat konsentrasies by twee verskillende worteltemperature te toets op die loof en wortel groei, opbrengs en chemiese samestelling van boegoe plante.

In die tonnel proef is gevind dat ‘n fosfaat konsentrasie van 0.7 me $L^{-1}$ die beste groei en hoogste opbrengs tot gevolg gehad het en dat fosfaat konsentrasies hoër of laer as 0.7 me $L^{-1}$ tot ‘n afname in groei en opbrengs lei. Daar is ‘n positiewe korrelasie gevind tussen die fosfaat inhoud in die voedingsoplossing en die N, P, K, Ca, Mg en B inhoud van die boegoe plante en ‘n negatiewe korrelasie met Fe inhoud. ‘n Toename in fosfaaat konsentrasie vanaf 0.0 tot 1.4 me $L^{-1}$ in die voedingsoplossing het gelei tot ‘n toename in die mortaliteit van die boegoe plante, wat moontlik kan dui op ‘n toksiese effek by hoë fosfaat konsentrasies. Die fosfaat inhoud van die voedingsoplossing het slegs een hoof komponent (Ψ-Diosfenol) van die olie betekenisvol beinvloed, maar geen korrelasie tussen fosfaat konsentrasie en Ψ-Diosfenol kon gevind word nie.

Die effek van die verskillende worteltemperature in die glashuis proef was baie duidelik. Beter groei, hoër wortelmassas en hoër opbrengs per plant is waargeneem by boegoe plante wat by ‘n gemiddelde worteltemperatuur van 20°C verbou is in vergelyking met plante wat by ‘n gemiddelde worteltemperatuur van 10°C verbou is. Hoër N, K, Na en B inhoude is ook gevind in boegoe plante wat by 20°C verbou is. By die lae temperatuur behandeling is daar geen verskil in groei en opbrengs waargeneem vir die verskillende fosfaat behandelings nie terwyl die beste groei en hoogste opbrengs verkry is by fosfaat konsentrasies van 0.35 en 1.40 me $L^{-1}$ by die hoë temperatuur behandeling. Die groeipiek wat waargeneem is by 1.40 me P $L^{-1}$ is egter onverklaarbaar en kan moontlik toegeskryf word aan die beperking in die aantal herhalings en plante per perseel. ‘n Positiewe korrelasie is waargeneem tussen fosfaat konsentrasie in die voedingsoplossing en die N, P en K inhoud van die plante. ‘n Betekenisvolle interaksie is waargeneem tussen die worteltemperatuur en fosfaat konsentrasie op die P, Mn en Zn inhoud van plante wat beteken dat die boegoe plante verskillend reageer teenoor die fosfaat konsentrasie by verskillende worteltemperature.

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# CONTENTS

# CHAPTER 1
# INTRODUCTION

Buchu (*Agathosma betulina*) is one of the most important export products developed from the indigenous flora of South Africa. The primary use of buchu is as a flavoring agent due to its black currant flavour and smell (Posthumus *et al*., 1996; Roberts, 1997). Its cosmetic and pharmaceutical applications (shampoo, mouthwash, natural medicines, skin care products etc) are next in importance. Fragrances came third and alternative health products like aromatic oils, for instance, are at the bottom of the list (McVeigh, 2001).

When Jan van Riebeeck landed in the Cape in 1652 the native people already used buchu as an ointment and for various medicinal purposes. In the past buchu has mainly been harvested from its natural environment, which limited the production and ensured high prices. In recent years buchu oil became very popular and the high demand for consistent amounts of good quality oil has put pressure on buchu production from its natural habitat. This has lead to commercial buchu plantations that have proven to be extremely profitable. In 2001, 40% of buchu on the markets was already from plantations (Coetzee, 2001) and some growers have already started to produce buchu hydroponically in an effort to increase production. The problem however is that not much research has till now been done on the production of buchu and this can be a serious limitation for the sustainable production of buchu in South Africa.

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# CHAPTER 2
# LITERATURE REVIEW

## 2.1 General information on Buchu

### 2.1.1 Taxonomy and geographical distribution

Buchu belongs to the Rutaceae family, and the general name, buchu, includes different genera such as *Diosma, Barosma* and *Agathosma* (Spreeth, 1976; Blomerus, 2003). Before the two genera *Barosma* and *Agathosma* were united in 1950 by Pillans, commercial important buchu species were classified under the genus *Barosma*. Three types were recognized, *B. betulina, B. crenulata* and *B. serratifolia.* Two types of buchu species are of commercial importance namely *A. betulina* and *A. crenulata*. *Agathosma betulina* is generally known as Roundleaf buchu or "Bergboegoe", whereas *A. crenulata* is known as Oval leaf Buchu (Spreeth, 1976).

The following diagnostic traits distinguish between *A. betulina* and *A. crenulata. Agathosma betulina* is a small, multi stemmed shrub which contains the greatest number of oil glands in its smaller, light green tinted leaves. They grow about one meter high (von Wielligh, 1913). The leaves are more round and the ratio of the length to width of the lamina is 1.95 ± 0.06 (Spreeth, 1976). The flowers of *A. betulina* are white or purplish pink (Goldblatt & Manning, 2000). The malic acid content of the leaves is 7.6 mg $g^{-1}$ wet material, with diosphenol strongly represented in the oil (Spreeth, 1976).

*Agathosma crenulata* is a shrub with a height of up to 2.5 meters and can grow as high as 5 meters. They have single stems and the leaves have little or no hair and are oval shaped. The average length/width ratio of the lamina is 2.34±0.07 (Spreeth, 1976). *Agathosma crenulata* has white or mauve flowers (Goldblatt & Manning, 2000). The malic acid content of the leaves is 1.2 mg $g^{-1}$ wet material, with diosphenol at very low concentrations (Spreeth, 1976).

According to Collins *et al.* (1996) different end users require varying qualities of oil, thus the identification, propagation and selection of desirable chemotypes appears to

have considerable merit. The chemical composition of the oils of *A. betulina* and *A. crenulata* varies and it is thus important to distinguish the two species from each other. Bean (1993) reported that the classification can lead to confusion due to the hybridization of the two species.

Posthumus *et al.* (1996) analyzed the essential oils of *A.betulina* and *A. crenulata* and their hybrids to determine if their taxa could be distinguished by their monoterpene chemistry. Pulegone was found to be the key component for the identification of the oils. *Agathosma betulina* is identified by a pulegone content of 2.4% to 4.5%, the hybrids have 7.6% to 27.8% and *A. crenulata* has 31.6% to 73.2% pulegone. Pulegone is a hepatotoxin and can be over stimulating to the kidneys, which make oil with high pulegone levels less desirable (Pers. Communication Allen Harris, 2006. Buchu Farming Consultant. Palmiet Valley, Wellington. Tel/Fax 021 864 3317). *A. betulina* and the hybrids have a higher content of 8-mecapto-p-menthan-3one while the reverse is true for *A. crenulata* (Collins *et al.,* 1996; Posthumus *et al.*, 1996).

Spreeth (1976) described the geological distribution as follows:
*Agathosma betulina* grows naturally in mountain areas, usually in nutrient poor, acidic, sandy red soils. It can be found from Ceres Northwards to the Pakhuisberg near Clanwilliam and have been found in the following districts, Ceres, Tulbagh, Piketberg, Citrusdal, Clanwilliam and Calvinia. It is also commonly found in the Cederberg Mountains.

*Agathosma crenulata* is found from Tulbagh southwards to Bettysbay. It is found in the Stellenbosch, Paarl and Wellington areas in the West and the eastern border of its distribution is Ceres, Wolseley, Worcester and Caledon. It can also be found in Jonkershoek and Bainskloof.

### 2.1.2 Oil composition and quality

The oil produced by buchu plants consist of volatile oils (Endenburg, 1972). The six major components of buchu oil are d-limonene, d-menthone, l-isomenthone, l-pulegone, Ψ-diosphenol and diosphenol (Endenburg, 1972; Kaiser, Lamparsky &

Schudel, 1975; Posthumus *et al.*, 1996). These form part of a group of compounds known as terpenes, which include primary and secondary metabolites. The primary metabolites include caretenoids, growth regulators, dolichols, quinines and proteins, which is essential for normal growth and development of the plant. Secondary metabolites include monoterpenes, sesquiterpenes, and diterpenes which play a role in the interaction of the plant and its environment (Jones, Somerville & Walbot, 1995 in Karsen, 2003).

According to Kaiser *et al.* (1975) the compounds cis- and trans-8mercapto-p-menthan-3-one is responsible for the characteristic odour of the buchu. This compound is low in concentration (<3%) in buchu, with *A. betulina* having the highest concentration (Posthumus *et al*, 1996). The oil content depends on the species, environmental conditions, seasonal variation, physical factors, cultivation practices, extraction method and the method used in oil analysis (Karsen, 2003).

The main essential oil components of *A. betulina* are diosphenol (22.3%), isomenthone (19.91%), Ψ-diosphenol [Diosphenol] (18.58%), limonene (11.64%), menthone (9.82%) and low levels of pulegone (Posthumus *et al.*, 1996). Two chemotypes of *A. betulina* were identified by Collins *et al.* (1996), a diosphenol and an isomenthone chemotype. The Diosphenol chemotype is characterized by high Ψ-diosphenol (>10%) and diosphenol (>12%), and low isomenthone (<29%) concentrations. The Isomenthone chemotype is characterized by high isomenthone (>31%) and low Ψ-diosphenol (<0.16%) and diosphenol (0.14%) concentrations. The isomenthone chemotypes are restricted to the Piketberg mountain range. The oil of the isomenthone chemotype is less desirable than the diosphenol chemotype due to the low diosphenol content (Pers. Communication Allen Harris, 2006. Buchu Farming Consultant. Palmiet Valley, Wellington. Tel/Fax 021 864 3317). *Agathosma crenulata* contains pulegone (53.75%), limonene (11.7%) and trans-8-acelylthio-p-menthan3-one (6.83%), 8-hydroxymenthone (4.67%) isomenthone (3.58%) and menthone (2.91%). The main components of the hybrid are isomenthone (33.42%), limonene (21.96%) and pulegone (15.75%) (Posthumus *et al.*, 1996).

### 2.1.3 Cultivation

The high demand for buchu has lead to a strong tendency towards commercial buchu plantations, which requires large quantities of suitable material. There is an increasing interest in more intensive plant production of buchu, especially higher production on smaller areas. At present buchu is harvested in its natural habitat or produced in open field plantations. The best time to transplant the seedlings is between March and June (von Wielligh, 1913; Blomerus, 2003) after the first winter rain. During the winter the plants establish their root system before the dry summer.

### 2.1.4 Nutritional requirements

Not much is known about the fertilization needs of Buchu plants. To better understand the nutritional needs of Buchu it is important to look at the environment and soil conditions where it naturally grows. Buchu grows naturally in environmental conditions similar to those where the Proteaceae family grows. It grows in sandy soils which are well drained, nutrient deficient and acidic with a phosphorus concentration less than 16 mg $kg^{-1}$ (Bray1l) (Blomerus, 2003; Unpublished data, ASNAPP, 2007). This gives an indication that buchu prefers soils with low phosphorus content and that high phosphate treatments may have a negative effect on the growth of buchu plants. Introducing buchu to hydroponics requires a proper nutrient solution with an optimum macro nutrient composition, EC and pH.

Nichols & Beardsell (1981) proved that high calcium content in Proteas can enhance leaf necroses due to high phosphorus content, while increased nitrogen and potassium contents can reduce it. They also mentioned that normal levels of phosphorus which are generally in the range of 0.95 and 1.91 me $L^{-1}$ for the most plants are toxic for many members of the Proteaceae family and that 0.48 me $L^{-1}$ is the maximum level that they can tolerate.

Handreck (1991) grew *Banksia ericifolia* (Proteaceae) in a soil-less medium having eight levels of iron and a range of phosphorus added to the medium. Symptoms of Fe deficiency and chlorosis of shoot apices appeared in combination with high phosphorus and low iron treatments. Proteaceae seedlings treated with high

phosphorus applications also developed symptoms like necrosis and premature shedding of oldest leaves about 2.5 months after pricking out. After a couple of months total defoliation occurred on the lower half of the stems. He also found that there was no indication of a phosphorus deficiency when no phosphorus was added to the medium in combination with high iron. These results contrast sharply with very poor growth and death through phosphorus deficiency when many other species are grown in the same medium without added phosphorus.

Montarone & Allemand (1995) suggest that a P concentration of 0.12 me $L^{-1}$ in a nutrient solution will be optimal for growing various Protea species in a soil-less medium. Heinsohn & Pammenter (1986) also found that the relative growth rate of *Leucadendron salignum* (Proteaceae) is the highest at phosphorus concentrations of 0.12 me $L^{-1}$. They also found that the specific utilization rate of phosphorus is the highest at 0.12 me $L^{-1}$. The plant phosphorus concentration of *L. salignum* was 1.5 mmol $g^{-1}$ when supplied with a phosphorus concentration of 0.12 me $L^{-1}$. This literature indicates that phosphorus may be a very important nutritional factor when buchu is grown hydroponically.



## 2.2 Role of Phosphorus in Plants

### 2.2.1 General

Phosphate in the plant occurs in inorganic form as orthophosphate and pyrophosphate. When the orthophosphate is esterfied with hydroxyl groups of sugars and alcohols or bound by a pyrophosphate, organic phosphate compounds are formed. These organic phosphates are intermediary compounds of the metabolism (Mengel & Kirkby, 1987; Marschner, 1995).

### 2.2.2 Phosphorus as a structural element

The function of Phosphorus function in macromolecular structures can best be observed in nucleic acids like DNA, which are carriers of genetic information and RNA, which are the structures responsible for the translation of genetic material. Phosphate forms a bridge between ribonucleoside units to form macromolecules. The

proportion of phosphorus in nucleic acids to total organically bound phosphorus differs among tissues and cells, it is high in meristems and low in storage tissues (Marschner, 1995).

The bridging form of phosphorus diester is also abundant in the phospholipids of biomembranes. It forms a bridge between a diglyceride and another molecule (amino acid, amine, or alcohol) (Marschner, 1995).

### 2.2.3 Phosphorus in Plant Physiology

***Role in energy transfer***

Although phosphorus is present in low concentrations in plant cells, the phosphate esters and energy-rich phosphates represent the metabolic machinery of the cells (Epstein, 1972). Most phosphate esters are intermediates in metabolic pathways of biosynthesis and degradation. The esters function and formation are directly related to the energy metabolism of the cells and to energy rich phosphates (Marschner, 1995).

ATP is the most important compound in which phosphate groups are linked by pyrophosphate bonds. ATP can be used for various endergonic processes as active ion uptake and the synthesis of various organic compounds. In these processes there is an initial phosphorylation reaction which involves the transfer of the phosphoryl group from ATP to another compound. This phosphorylated compound is loaded with energy which enables it to participate in further metabolic processes (Mengel & Kirkby, 1976). ATP is the principal energy rich phosphate required for starch synthesis. The energy rich pyrophosphate bonds of ATP can also be transmitted to other coenzymes like uridine triphosphate (UTP) and guanosine triphosphate (GTP), which are required for synthesis of sucrose and cellulose respectively (Marschner, 1995).

***Regulatory role of Inorganic Phosphate***

Vacuolar phosphate serves as a reserve supplying the cytoplasm with phosphate when necessary. This maintains the level in the cytoplasm. Low concentrations of cytoplasmic inorganic phosphate (Pi) depress growth at a concentration of 0.3 mM inorganic phosphate or below, growth is completely inhibited (Rebeille, Blgny & Douce, 1984).

Inorganic phosphate in the cytoplasm has a regulatory function by influencing the activity of various enzymes like phosphofructokinase which is the key enzyme in the regulation of substrate flux into the glycolytic pathway (Mengel & Kirkby, 1976). Thus an increase in the release of Pi from vacuoles can initiate the respiratory burst correlated with fruit ripening (Woodrow & Rowan, 1979). Delayed fruit ripening in phosphorus deficient tomato plants may be related to this function of Pi (Pandita & Andrew, 1967).

***Phosphorus fractions and the role of phytate in seeds***

The amount of phosphorus being supplied to a plant affects the various phosphorus fractions in a typical manner. The phosphorus fraction in the leaves increases with an increase in the supply from a suboptimal to an optimal level. Above this level only the Pi increases, reflecting the storage function of Pi in highly vacuolated tissues (Marschner, 1995).

In grains and seeds the level of Pi at maturity is very low and the phosphate is present as an organic compound called phytate. Phytin in plant seeds occurs as the Ca and Mg salts of phytic acid is formed during seed formation. After pollination there is an increase in Pi transport towards the young developing seeds and the Pi levels during this stage is low. Phosphorus in phytin of the seed is a P reserve and during seed germination phytin is mobilized and converted into other phosphate forms (phytate) needed in the metabolism of young plants (Marschner, 1995).

Some phosphorus is associated with the starch fraction and is incorporated into starch grains. In cereals only a small proportion is involved but in potato tubers up to 40% of the total phosphorus may be incorporated in starch. Starch bound phosphorus may be a part of the compartementation of Pi and control of its concentration (Marschner, 1995).

### 2.2.4 Phosphorus deficiencies and toxicities

Phosphorus requirements for optimal growth are in the range of 0.3 to 0.5% of the plants dry weight during the vegetative stages of growth (Marschner, 1995). A lack of phosphorus in the plant causes cells to become smaller, with thicker walls and greater lignification of the walls and these factors may lead to the plant being darker green (Ellis & Swaney, 1947). Retarded growth and reddish coloration also occurs due to anthocyanin formation. Under conditions of phosphorus deficiency, cell and leaf expansion are more retarded than chlorophyll formation and therefore the chlorophyll content per unit leaf area being higher (Hecht-Buchholz, 1967 in Marschner, 1995). Although the chlorophyll content is higher the photosynthetic efficiency is much lower. The inhibition of leaf expansion is expressed during the daytime and caused by decreased root hydraulic conductance in the phosphorus-deficient plants (Radin & Eidenbock, 1984). Other symptoms include slow and stunted growth, dark green coloration with tips of leaves dying, delayed maturity and poor grain, fruit or seed development and quality. Symptoms occur in older leaves first (Barry, 1996).

Phosphorus function in the growth and metabolism of plants and therefore a deficiency leads to a general reduction of most metabolic processes such as cell division and expansion, respiration, and photosynthesis. It is also difficult to diagnose a phosphorus deficiency effectively due to the many processes it influences (Epstein, 1972).

Chemical changes due to phosphorus deficiencies are an increase of nitrate-nitrogen and lowering of phosphorus content in the plant cells. In the early stages carbohydrate accumulation occurs, with a resulting increase of anthocyanin pigment. During later stages the carbohydrate content decreases (Ellis & Swaney, 1947). The regulatory

function of Pi in photosynthesis is one of the mayor factors limiting growth, particularly during the reproductive stage.

An excess of phosphorus causes iron and/or zinc deficiencies which lead to yellowing, interveinal chlorosis of younger leaves, stunted plant growth and leaves that dies and fall off (Barry, 1996). Loneragan & Asher (1967) found that very high uptake rates of phosphate were associated with reduced growth rates in some plant species like *Erodium spp.*, clover and silver grass. They concluded that such effects may be dependant on phosphate retarding the uptake and translocation of some of the micronutrients such as Zn, Fe and Cu.

### 2.2.5 Phosphorus uptake and translocation in plants

Plant roots are able to absorb phosphate from solutions of very low phosphate concentrations and the phosphate content of root cells and xylem sap can be up to 100 to 1000 fold higher than that of the soil solution. Phosphate is thus taken up by plants against a very steep concentration gradient and this uptake is active. Phosphate uptake is mediated by $H^+$ cotransport. A plasmalemma-located ATPase pumps $H^+$ into the apoplast to protonate the phosphate carrier. ATPase activity is expected to have an impact on phosphate uptake because of the close relationship between root respiration and phosphate uptake which relates to the provision of respiratory ATP to the ATPase (Ullrich-Eberius *et al*., 1981).

The capability for active uptake of phosphate differs between plant species and may even differ between cultivars of the same species. Barber & Thomas (1972) found considerable differences in the rate of uptake of phosphate by various maize cultivars and concluded that this uptake is fixed genetically. This phosphate uptake efficiency can be an asset when phosphate is limited but can also be a liability when the plants are subjected to Fe and Cu stresses since the more efficiently the plant utilizes phosphate the more susceptible it is to Fe and Cu deficiency (Brown, Clark & Jones, 1977).

When phosphate is taken up by the plants there is an effective and rapid incorporation of the inorganic radical into organic compounds. Within 10 seconds of uptake 30% of

the phosphorus has been incorporated into nucleotides and within 50 seconds 80% has been so converted. The phosphate content of the vacuole is normally very low and in most cells the phosphate in its organic form remains in the cytoplasm but when excess of phosphates is available the inorganic phosphate content in the vacuole rises (Hall, 1976). Phosphate is quite mobile in plants and can be translocated in an upward or downward direction (Mengel & Kirkby, 1987). Young leaves are supplied by phosphates taken up from the soil and with phosphate originating from the older leaves (Bouma, 1967).

The rate of transport of phosphate from root to shoot is not related to the phosphate content of the root but it is the level of carbohydrate nutrition of the plant that appears to control the rate at which the plant can translocate its phosphate supplies upwards. The link between phosphate transport and photosynthetic activity is probably a result of the fact that phosphate moves through the plant in nucleotide form. Phosphate is usually accumulated in the younger leaves and as the plant grows the element continuously moves from older to younger tissues (Hall, 1976).

### 2.2.6 Phosphorus availability and crop requirements

Soil phosphate can easily be rendered unavailable for plant roots and phosphorus is the most immobile of the major plant nutrients. Available phosphate is often used as an indicator for a soil phosphorus fraction that can be utilized by the plants. This availability can be measured by measuring the phosphate concentration in the soil solution and the ability of the soil to maintain the soil solution concentration (phosphate buffer capacity). Concentrations of about $10^{-4}$ M phosphate in the soil solution are considered as high while phosphate concentration of $10^{-6}$ M is generally too low to supply crops with adequate phosphorus. Soil phosphate buffer capacity also plays a role in the supply of phosphorus to crops. Optimum soil solution phosphate concentrations differ for individual crops, cropping systems, and particular sites (Mengel & Kirkby, 1987).

With plants that absorb high quantities of phosphate it is important that the phosphates in the soil solution must be replenished several times per day by mobilization of phosphate from the labile pool. The rate of desorption is higher in soils with a higher phosphate buffer capacity and soils are better able to buffer the phosphate concentration of the soil solution during the growing season (Mengel & Kirkby, 1976). According to Olsen & Watanabe (1970) the phosphate concentration of the soil solution and the phosphate buffer capacity are the most important parameters controlling the phosphate supply to plant roots.

Soils with strong phosphate fixation usually require higher phosphate fertilizer applications to alleviate the effects of fixation. If the quantity of available soil phosphate is in the normal range, the rate of phosphate application required should correspond to the amount of phosphorus removed by the crop. If labile phosphorus is rendered immobile the phosphorus applications should be about 10 to 50% higher than the quantity being taken up by the plant. For arable crops the rates range from 20 to 80 kg P $ha^{-1}$ (Mengel & Kirkby, 1987). In a common nutrient solution the phosphorus concentration should be between 0.48 me $L^{-1}$ and 2.55 me $L^{-1}$ (Barry, 1996).

De Kreij *et al.* (1999) (in Combrink, 2005) proposed different nutrient solution recipes to optimally meet the nutritional needs of a variety of plants. Table 2.1 shows the phosphate concentrations needed by these plants and at which EC it should be applied. Levels of phosphate that is currently being used in the nutrient solution of buchu are 0.7 $mmol_c$ $L^{-1}$ at an EC of 0.85 (Unpublished data, ASNAPP, 2007).

**Table 2.1** Phosphate concentrations and EC levels of a nutrient solution expected to suite South African conditions

| Plant | $H_2PO_4$ ($me.L^{-1}$) | EC ($mS.cm^{-1}$) |
|---|---|---|
| Amaranthus/Imbuya | 1.5 | 2.3 |
| Amaryllis | 1.0 | 1.55 |
| Alstromeria/Inca lily | 1.0 | 1.52 |
| Anthurium | 0.8 | 0.85 |
| Beans | 1.0 | 1.6 |
| Carnation & Chrysanths | 1.2 | 1.6 |
| Cherry tomato | 1.5 | 2.3 |
| Courgette | 1.0 | 1.65 |
| Cucumber | 1.0 | 1.65 |
| Cymbidium/Orchid | 0.8 | 0.73 |
| Disa | 0.7 | 0.6 |
| Gerbera | 1.2 | 1.5 |
| Gypsophila | 1.0 | 1.5 |
| Lettuce | 1.0 | 1.3 |
| Melon & water melon | 1.5 | 2.2 |
| Pepper | 1.2 | 1.8 |
| Rose | 1.1 | 1.4 |
| Spinach | 1.5 | 2.3 |
| Strawberry | 1.0 | 1.5 |
| Tomato | 1.5 | 2.0 |

Modified from Combrink (2005)

## 2.3 Soil Phosphorus

### 2.3.1 Phosphorus fractions and phosphate minerals

Phosphorus in soils is mostly found as orthophosphate and the total content range from 0.02 to 0.15%. A substantial amount of this P is associated with the soil organic matter (Williams, 1959 in Mengel & Kirkby, 1987) and in mineral soils the proportion of organic P lies between 20 to 80% of the total P (Mengel & Kirkby, 1987). There are three main soil phosphate fractions of importance in the soil solution, the labile pool and the non-labile fraction. The phosphorus in the soil solution is accessible to the plant. In the labile pool solid phosphate is held on surfaces and is in rapid equilibrium with soil solution phosphate. The phosphate in the non-labile pool is insoluble and can only be released very slowly into the labile pool. Important soil phosphate minerals include hydroxyapatite [$Ca_5(PO_4)_3OH$], fluorapatite [$Ca_5(PO_4)_3F$], dicalciumphosphate [$CaHPO_4$], tricalciumphosphate [$Ca_3(PO_4)_2$], variscite [$AlH_2PO_4(OH)_2$], strengite [$FeH_2PO_4(OH)_2$ (Mengel & Kirkby, 1987)

### 2.3.2 Phosphate adsorption, desorption and mineralization

The labile phosphate fraction consists mainly of soluble Ca phosphates and adsorbed phosphate. The labile fraction is in equilibrium with the soil solution. Soil pH influences the rate at which ions are adsorbed, at low pH anions are adsorbed more strongly. As the pH is raised $OH^-(HCO_3)$ ions are able to exchange with adsorbed phosphate and release it into the soil solution, this is called desorption (Mengel & Kirkby, 1987).

The phosphate availability is increased by raising the soil pH, as can be seen in Figure 2.1. Iron oxides adsorb phosphate more strongly than layer silicate minerals and the non specific adsorption of nitrate stabilizes phosphate adsorption on the surface of iron oxides (Mengel & Kirkby, 1987).

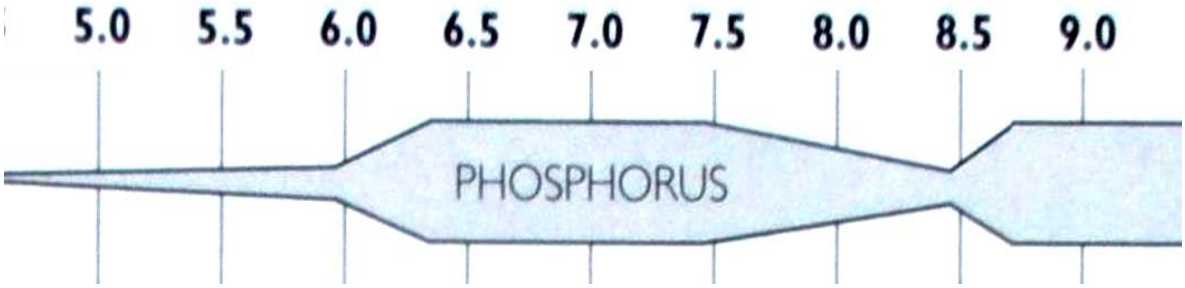



**Figure 2.1** Phosphate availability to plants at different pH levels [Modified from Barry (1996)].

Adsorption of phosphate to soil particles is frequently not an ideal adsorption process but rather a combination of adsorption and precipitation (Larsen, 1967). Calcium carbonates can adsorb phosphate which is then slowly converted into apatites (Parfitt, 1979). This causes phosphates of the labile pool being rendered immobile and so transferred to the non labile phosphate fraction. This process is rapid in acid soils with a high adsorption capacity. Adsorption depends not only on the type of adsorbing minerals but also on their specific surfaces (Mengel & Kirkby, 1987).

Phosphate adsorption is not the only factor depressing phosphate availability but also the formation of low solubility precipitates (Ca-Fe- and Al phosphates). High pH and $Ca^{2+}$ concentrations in the soil solutions may lead to the precipitation of Ca phosphates. A high soil pH can thus also lead to the opposite effect of phosphate availability and phosphate desorption. To determine the phosphate availability in soils it is important to know which process, Ca phosphate precipitation or phosphate desorption is of greater importance. In Al- and Fe oxide rich soils and in clay minerals phosphate desorption is usually dominant and in poor sandy soils, in calcareous soils and in organic soils, phosphate precipitation plays the mayor role (Mengel & Kirkby, 1987).

Organic matter decomposition also influences phosphate adsorption. Soil organic matter contains phosphorus and the mineralization of organic matter releases phosphate into the soil solution. These phosphates are involved in the equilibrium between free and adsorbed phosphate ions. The microbial breakdown of organic soil

matter also leads to an increase in $CO_2$ production which could increase the solubility of soil phosphates (Mengel & Kirkby, 1987).

### 2.3.3 Phosphorus in solution and plant root interaction

Available soil phosphorus may only be 1% or less of the total amount present. The most important phosphate ions in soil solution are $H_2PO_4^-$ and $HPO_4^{-2}$ and the ratio of these two ions in the soil solution is pH dependant. High $H^+$ concentrations shift the equilibrium to a more protonated form as can be seen in the equation: $HPO_4^{-2}+H^+ \leftrightarrow H_2PO_4^-$ (Mengel & Kirkby, 1987). At a pH of 5, $HPO_4^{-2}$ is almost absent and at pH 8 both phosphate species are present in almost equal proportions, as can be seen in Table 2.2. Maximum availability of soil phosphorus occurs between pH of 6.5-7 (Ellis & Swaney, 1947).

**Table 2.2** Ratio of $H_2PO_4^-$ and $HPO_4^{-2}$ as affected by pH

| | $H_2PO_4^-$ | $HPO_4^{2-}$ |
|---|---|---|
| pH=5.0 | 100 % | 0 % |
| pH=6.0 | 90 % | 10 % |
| pH=7.0 | 78 % | 22 % |
| pH=8.0 | 50 % | 50 % |
| pH=9.0 | 15 % | 85 % |

Source: Steiner, 1984.

As plant roots grow through the soil it comes into contact with the phosphate of the soil solution and absorb these phosphates, which lead to a depletion of phosphates in the soil solution. The depletion of phosphates leads to a gradient between the phosphate concentration near the root surface and the phosphate concentration in the bulk soil and this concentration gradient regulates the rate of phosphate diffusion towards the plant root (Olsen & Watanabe, 1970). During rapid growth the phosphorus in the soil solution may be replaced 10 times a day or more from the solid phase phosphorus (Ellis & Swaney, 1947).

Plant roots also have an effect on phosphorus availability in the soil by inducing pH changes in the rhizosphere. The changes in pH are brought about by differential rates of uptake of cations and anions and $OH^-$ or $H^+$ effluxes. In plants supplied with mostly $NO_3^-$ the anion uptake will exceed cation uptake and $OH^-$ or $HCO_3^-$ is released from the roots and the rhizosphere pH becomes more alkaline than the bulk soil. Plants supplied with $NH_4^+$-N or molecular $N_2$ take up more cations than anions and releases $H^+$ into the soil, which leads to the rhizosphere being more acid and a lower phosphate availability (Mengel & Kirkby, 1987).

According to Cooper (1973) the root temperature of various plants has an influence on the phosphorus content of the plant and this indicates that the availability, absorption and use of phosphorus may be affected by environmental conditions such as plant root temperature.

## 2.4 Root temperature and plant growth

### 2.4.1. The influence of root temperature on plant growth

#### Dry mass production

Optimum root temperatures for plant growth differ between genera, species and cultivars. In general there is an increase in dry weight of the whole plant with rising root temperatures, until it reaches an optimum above which the dry weight declines (Cooper, 1973). He also made a generalization about the influence of temperature on shoot dry weight, based on a couple of studies involving different plants. He found that the response curve below and above the optimal temperature for most rapid growth may be sigmoid as can be seen in Figure 2.2. The change of shoot dry weight with unit change in root temperature above the optimum is steeper than below it, this may be due to different mechanisms limiting dry weight gain above and below the optimal root temperature. He also proposed that there is a band of optimal root temperatures over which temperature change has a relatively small effect on shoot dry weight.

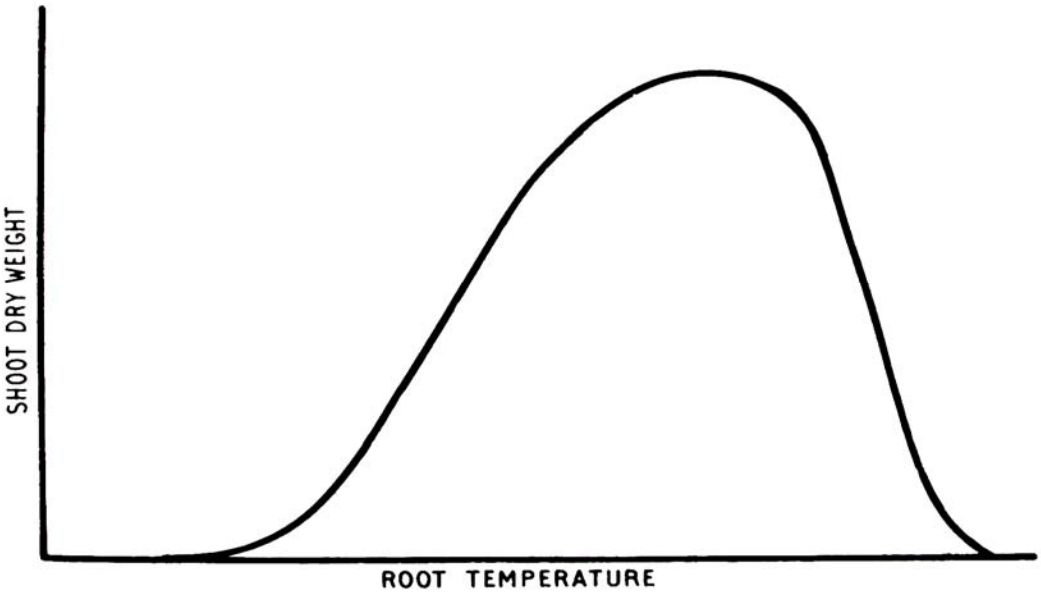



**Figure 2.2** Generalized influence of root temperature on shoot dry weight (Cooper, 1973).

Plants in different genera respond differently to the influence of root temperature on root dry weight. According to Brouwer (1962) (in Cooper, 1973) the response of root dry weight to root temperature is similar in form to the generalized response curve for shoot growth. Root extension and root branching also follows the generalized response shown in Figure 2.2.



**Plant height**

Plant height also increases with an increase in temperature until it reaches an optimum. The response curve for plant height is in many species similar to that of Figure 2.2. The optimum root temperature varies between 18°C and 30°C (Cooper, 1973).

### 2.4.2. Influence of root temperature on plant processes

**Photosynthesis and respiration**

At a root temperature of 12°C the net photosynthesis and respiration by maize plants is less at 25°C. The concentration of chlorophyll per unit dry weight of leaves is much

less at low temperatures, thus influencing the intensity of photosynthesis (Andreenko & Kerechki, 1966 in Cooper, 1973).

According to Brouwer, 1963 (in Cooper, 1973) the net assimilation rate to root temperature differed between species. There is a broad optimal temperature band suggesting that assimilation rate may be independent of root temperature except at extremes of root temperatures.

**Water absorption**

Root temperature history of a plant affects its response to a subsequent root temperature and reducing root temperature reduces water absorption. This reduction of water uptake can be due to the influence of temperature on the viscosity of the water as well as the effect on the water permeability of the cytoplasmic membranes (Kuiper, 1964 in Cooper, 1973).

**Water movement and transpiration**

The root temperature at which transpiration and water movement in the plant is the greatest differs between species, as well as the optimal temperature band. This optimum temperature band seems to be between 20 and 30°C for the most plants (O`Leary, 1966 in Cooper, 1973).

### 2.4.3 Influence of root temperature on mineral content of plant tissue

When roots are cooled, their nutrient uptake capacity is initially reduced compared to plants maintained at higher temperatures. After long periods of cooling the nutrient uptake capacity is restored to the levels of warm grown controls (White, Clarkson & Earnshaw, 1987). Low temperatures cause a physical change in the membrane lipids which results in a change in properties of the cell membranes and associated proteins. These changes may adversely affect growth of the shoots at low or high temperatures by secondary effects on nutrient deficiency or toxicity (Lyons, 1973).

According to Cooper (1973) data suggests that there is a general relationship between root temperature and the percentage mineral content of plant tissue as can be seen in Figure 2.3. Curves A, B, C and D indicate four representative species. In A the percentage mineral content decreases with increasing root temperature between 0 to 40°C. In D there is an optimal root temperature at which mineral concentration is the greatest. The response curve moves between these two extremes. A high temperature peak develops along the line YZ. High concentration at low temperature decreases along the line VW and a low concentration of minerals are shown by line WX. The majority of species relate to D as far as nitrogen, phosphorus and potassium are concerned.

According to Chaudhary & Ghildyal (1970) the uptake of various nutrients was optimum between 20 and 32°C and decreased progressively as the soil temperatures deviated from 20 and 32°C to either side. The deviation from these temperatures leads to a greater reduction in the uptake of nitrogen and phosphorus than potassium.

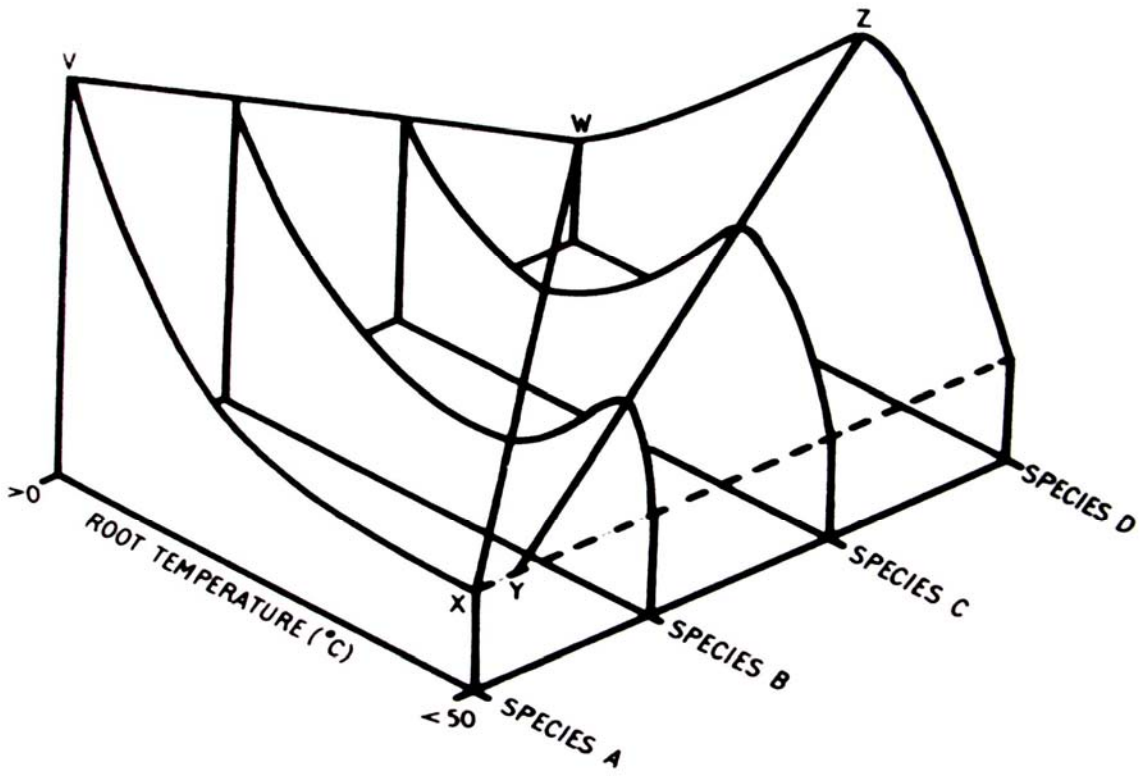



**Figure 2.3** General relation for all species between root temperature and percentage mineral content of the plant tissue (Cooper, 1973).

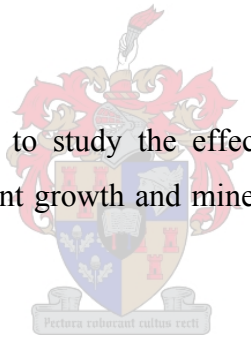
## 2.5 Objectives of the study

It is clear that phosphorus plays an important role in the nutrition of buchu. The objective of this study is to determine the effect of different concentrations of phosphorus in a nutrient solution and root zone temperature on the growth, mineral content and essential oil quality of buchu. This objectives was accomplished as follow:

2.5.1 A thorough literature review on general information regarding buchu, the role of phosphorus in plants, the influence of soil phosphorus and root temperature on plant growth as presented in Chapter 2;

2.5.2 A experiment conducted to study the effect of different P concentrations on plant growth, mineral content and essential oil quality of buchu as presented in Chapter 3;

2.5.3 An experiment conducted to study the effect of root zone temperature and different P concentrations on plant growth and mineral uptake of buchu as presented in Chapter 4.



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# CHAPTER 3
# PLASTIC COVERED GREENHOUSE TRIAL
## Reaction of hydroponically grown buchu (*Agathosma betulina*) to increasing phosphorus concentrations

### Introduction

Arnon & Hoagland (1940) developed some of the first well balanced nutrient solutions required for plant growth and modern nutrient solution compositions do not differ much from these older ones. Various plants have different nutrient requirements (mineral concentrations and ratios) and it is important for producers to use a nutrient solution that is optimal for the specific plant specie that is grown to enable them to achieve optimal crop yield and quality.

In their natural habitat, buchu and Proteas grow in nutrient poor, sandy soils which indicate that it doesn't need a high concentration of nutrients to survive (Heinsohn & Pammenter, 1986). To be able to survive in these conditions Proteas possess dense clusters of hairy rootlets called proteoid roots which enhance nutrient and water uptake. The formation of these rootlets is suppressed by high nutrient availability, clayey soils, water logging and drought. Nichols, Jones & Beardsell (1979) found that high concentrations of P suppress proteoid root production and thus causes poor growth in various Protea species.

Research done by several researchers provides a good indication of required P concentrations. Claassens (1986) found that a P concentration of 0.5 me $L^{-1}$ could be harmful to some Protea species. Nichols & Beardsell (1981) found that Proteas respond the best to P concentrations of below 0.47 me $L^{-1}$ when grown in solution and sand culture. Prassad & Dennis (1985) reported that P concentrations above 0.8 me $L^{-1}$ of available P can result in satisfactory growth for *Leucadendron* grown in containers, but most researchers found best results with low P concentrations in the nutrient solutions (Montarone & Allemand, 1995; Montarone & Ziegler, 1997).

Although optimal P concentrations for the growth of a variety of Protea species have been established, no information with regard to the growth of buchu is available. Because buchu became increasingly popular as a commercial crop, requirements for maximum growth are needed. The aim of this experiment is to determine the effect of increasing P concentrations in a nutrient solution on the growth and oil composition of buchu plants in a hydroponic system.

## Material and Methods

### Locality

The experiment was conducted in a greenhouse at the Department of Agronomy of Stellenbosch University, Republic of South Africa during the period of August 2006 to April 2007. A pad and fan system was used to cool down the plastic covered greenhouse when the temperature inside exceeded 25°C.

### Cultivation Practices

Seedlings of *A. betulina* were used for this trial. Before transplanting, the seedlings were grown in a mixture of vermiculite, peat moss and polystyrene balls as a medium and watered with a nutrient solution with an EC of 0.8 to 1.1 $mS.cm^{-1}$. Seedlings (one per pot) were transplanted on the 16$^{th}$ of August 2006 in 9.5 liter white pots filled with a growth medium that consisted of 7 parts acid washed nr.1 sand and 3 parts coco-peat treated with calcitic lime ($CaCO_3$) at a rate of 5 kg $m^{-3}$. The $CaCO_3$ was used to increase the buffering capacity of the medium which helps to slow down the acidification from ammonium fertilizers in the root zone. The sand was acquired from Console Minerals and can be classified as coarse sand (Table 3.1), with very few nutrients (Table 3.2). After one month the growth tips of all the plants was removed.

The nutrient solutions were stored in 1500 liter plastic tanks. Netafim drippers (pressure compensated, non-leakage), with a capacity of 2.0 L $hr^{-1}$ were used to irrigate each pot individually. Each treatment in the experiment had its own separate irrigation system to prevent the mixing of nutrient solutions. The total volume of

nutrient solution supplied per day was the same for all the treatments and increased gradually over the growing season from 120 ml to 600 ml per day per plant.

**Table 3.1** Particle size analyses of Nr. 1 sand from Console Minerals used as growth medium

| Aperture (mm) | % Retained |
|---|---|
| 2.000 | 0.4 |
| 1.700 | 0.2 |
| 1.400 | 0.4 |
| 1.180 | 2.2 |
| 1.000 | 8.3 |
| 0.850 | 18.3 |
| 0.710 | 21.4 |
| 0.600 | 21.1 |
| 0.500 | 19.9 |
| 0.425 | 5.6 |
| 0.355 | 1.9 |
| 0.300 | 0.2 |
| 0.250 | 0.1 |

**Table 3.2** Chemical analysis for Nr. 1 sand from Console Minerals used as growth medium

| Compound | %/mg $kg^{-1}$ |
|---|---|
| $SiO_2$ | 99.75/997500 |
| $Al_2O_3$ | 0.07/700 |
| $Fe_2O_3$ | 0.023/230 |
| $TiO_2$ | 0.024/240 |
| $ZrO_2$ | 0.005/50 |
| CaO | 0.003/30 |

**Treatments and Experimental design**

Five nutrient solutions with different phosphorus concentrations were evaluated (Table 3.3). The different P concentrations in the nutrient solution were tested with treatments at the same EC levels and by neutralizing the effects of associated ions ($NO_3$ and $SO_4$) (Steiner, 1984). To keep the EC of all the treatments and the nitrate/sulphate ratio the same, the nitrate and sulphate concentration were decreased as the phosphate concentration increased. Analyses of the nutrient solution were done each month to ensure that the P concentration and the $NO_3/SO_4$ ratio in the nutrient solution were correct (Addendum, Table 6.1-6.5). The pH and EC of the nutrient solution were also recorded every time a new nutrient solution was prepared. All the treatments received the same micro nutrients (Table 3.4). The pH of the treatments varied between 5.8 and 6.5. After 3 months the pH of all the treatments were adjusted with hydrochloric acid (HCl) to levels between 4.7 and 5.4. This was done due to the high pH of the drainage water (Addendum, Table 6.6).

**Table 3.3** Macro nutrient composition of the nutrient solutions containing different P concentrations used to irrigate the buchu plants in the greenhouse

| $K^+$ | $Ca^{++}$ | $Mg^{++}$ | $NH_4^+$ | $NO_3^-$ | $H_2PO_4^-$ | $SO_4^=$ |
|---|---|---|---|---|---|---|
| | | | me.$L^{-1}$ | | | |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.72 | 0 | 1.98 |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.46 | 0.35 | 1.89 |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.2 | 0.7 | 1.8 |
| 3.3 | 2.73 | 1.4 | 0.27 | 4.94 | 1.05 | 1.71 |
| 3.3 | 2.73 | 1.4 | 0.27 | 4.68 | 1.4 | 1.62 |

**Table 3.4** Micro nutrient composition of the different micro nutrients of the nutrient solution used to irrigate the buchu plants in the greenhouse

| Micro Nutrient Source | ppm | Amount added (g $1000L^{-1}$) |
|---|---|---|
| Fe:Libfer (Fe EDTA) | 0.65 | 5.03 |
| Mn: Manganese Sulphate | 0.42 | 1.71 |
| Zn: Zink Sulphate | 0.25 | 1.13 |
| B: Solubor | 0.25 | 1.15 |
| Cu: Cupper Sulphate | 0.04 | 0.11 |
| Mo: Ammonium-Mo | 0.04 | 0.07 |

**Data collected**

Plant height was measured every month (every 4 weeks) starting at the end of the first month after transplanting by recording the difference between the soil surface and to the highest point on the plant. Plants (one per treatment per replication) were harvested every 3 months (12 weeks) from all the treatments to determine the fresh and dry weight as well as the chemical composition of the plants. After the first 3 months three plants per treatment were harvested randomly in each replication. After six months, two plants of each treatment per replication were harvested. These plants were selected to represent the average growth per treatment of the specific replication. The plants were cut at the soil surface and both the stems and leaves were weighed after harvesting to determine the fresh weight. The plants were then oven dried at 80°C for 48 hours and the dry weight was also recorded. Fresh and dry weights were used to calculate the dry matter index (DMI) as follows:

$$DMI\ (\%) = (Dry\ weight \div Fresh\ weight)*100$$

The dried plant material was taken to Bemlab laboratory for mineral analyses. During the last harvest nine months after transplanting the fresh plant material was frozen in order to extract the oil from the plants over time. Approximately 100g of each treatment per replication was oven dried to calculate the DMI and to determine the mineral composition of the plants. The oils were extracted from the buchu plants using steam distillation. The plant material sample was too small to determine the essential oil yield accurately. The oil samples were taken to Puris Natural Aroma

Chemicals (Pty) Ltd for analyses. Oil analyses were carried out by means of gas chromatographic methods and the results are reported in terms of relative percentages of the integral peak areas relative to the total integrated peak areas on the chromatogram.

**Statistical analyses**

Five treatments were arranged in a randomized block design, using eight replicates (Addendum, Plate 6.1). Ten plants represented an experimental unit. Data was analyzed using SAS statistical software version 9.1 (SAS 2001). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means and to determine significant differences.

**Results and Discussion**

**Plant height**

On average plant height increased from 80 mm after 1 month to about 330 mm during the nine month growth period of the experiment (Figure 3.1), which indicated that growth conditions generally favoured the growth of the buchu plants. The P concentration of the nutrient solution did not affect the plant height significantly (P0.05), but differences in plant height seemed to develop and increased with time especially after six months of growth (Figure 3.1). From this, it is clear that plants receiving P at 0.7 me $L^{-1}$ tended to be the tallest and plants that received either no P or 1.4 me $L^{-1}$, the smallest. Should the trial be continued for a longer period these differences might have been significant.

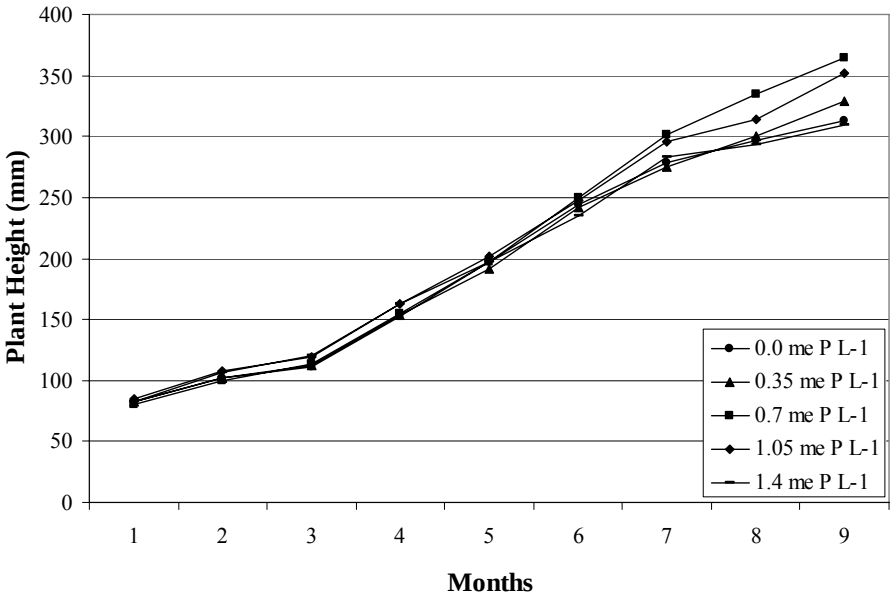


**Figure 3.1** The effect of the P concentration in the nutrient solution on the buchu plant height over the nine month period of the trial.

**Biomass Yield**

The different P concentrations in the nutrient solution affected the DMI (dry matter index) of the buchu plants only at the early growth stages (three months of growth), while the fresh weight was only significantly affected by the P concentration after six and nine months of growth and dry weight after nine months (Table 3.5).

**Table 3.5** Analyses of variance (ANOVA) of the effect of P concentration in the nutrient solution on the DMI, fresh and dry weight of buchu plants three, six and nine months after transplanting

| Months after transplanting | Fresh Weight Pr>F | | Dry Weight Pr>F | | DMI Pr>F | |
|---|---|---|---|---|---|---|
| | Block | P | Block | P | Block | P |
| 3 | 0.0025 | 0.4146 | 0.0014 | 0.1386 | 0.5802 | 0.0037 |
| 6 | 0.0937 | 0.0413 | 0.0489 | 0.0783 | 0.1549 | 0.3435 |
| 9 | 0.0065 | 0.0004 | 0.0019 | 0.0001 | 0.3665 | 0.0754 |

*Fresh Weight*

Fresh weights of between 34.76 and 52.65 g plant$^{-1}$ were on average produced after six months of growth (Table 3.6). At this stage, buchu plants receiving 0.7 me P L$^{-1}$ produced the highest fresh weight (52.65 g plant$^{-1}$) followed by plants receiving a P concentration of 0.35 me L$^{-1}$ (48.82 g plant$^{-1}$) or no P at all (46.34 g plant$^{-1}$) (Table 3.6). No significant differences were found between these three P treatments, but plants receiving 1.4 (36.78 g plant$^{-1}$) and 1.05 me.P.L$^{-1}$ (34.76 g plant$^{-1}$) produced significantly less (P0.05) fresh weight than plants receiving 0.7 me P L$^{-1}$ (Table 3.6, Figure 3.2).

After nine months of growth the buchu plants receiving 0.7 me P L$^{-1}$ produced significantly more fresh weight (192.56 g plant$^{-1}$) than all the other P treatments, followed by the 0.35 me P L$^{-1}$ (145.98 g plant$^{-1}$), 1.05 me P L$^{-1}$ (145.70 g plant$^{-1}$), 1.40 me P L$^{-1}$ (124.40 g plant$^{-1}$) and 0.0 me P L$^{-1}$ (94.08 g plant$^{-1}$) treatments (Table 3.6, Figure 3.2). This data suggested an optimum P concentration of about 0.70 me L$^{-1}$, while high concentrations can have a negative effect on buchu yield as was also shown for a variety of Protea species (Nichols & Beardsell, 1981; Montarone & Allemand, 1995; Montarone & Ziegler, 1997).

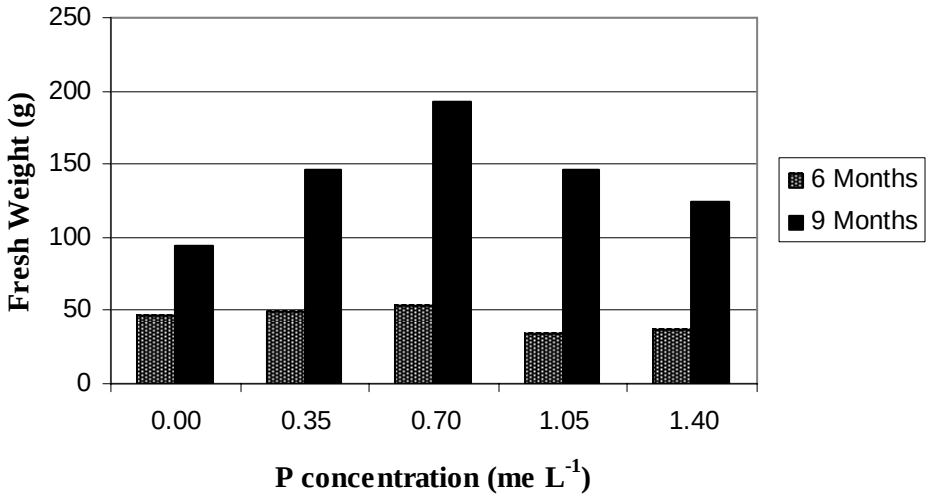


**Figure 3.2** The effect of different P concentrations in the nutrient solution on the fresh weight (g plant$^{-1}$) produced by buchu plants 6 and 9 months after transplanting (LSD: Month 6 = 13.89; Month 9 = 38.84).

*Dry weight*

After nine months of growth plants that received a concentration of 0.7 me P $L^{-1}$ in the nutrient solution yielded a dry weight of 64.14 g $plant^{-1}$, which was significantly higher than that produced by the other P concentrations (Table 3.6). Plants receiving 0.35 me P $L^{-1}$ produced the second highest dry weight (48.29 g $plant^{-1}$) followed by 1.05 me.P.$L^{-1}$ (47.06 g $plant^{-1}$), 1.4 me $L^{-1}$ (40.73 g $plant^{-1}$) and 0.0 me $L^{-1}$ (33.78 g $plant^{-1}$). Maximum dry weight production was therefore clearly obtained with a concentration of 0.7 me.P.$L^{-1}$ in the nutrient solution (Figure 3.3).

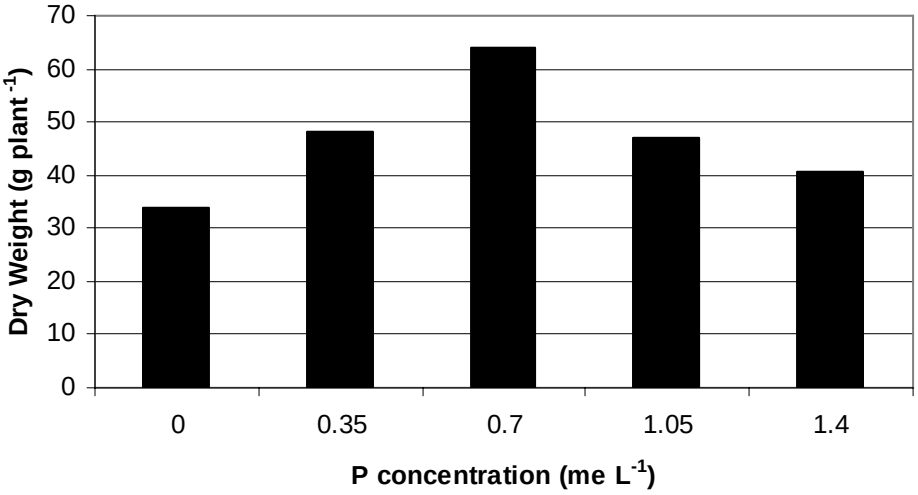


**Figure 3.3** The effect of different P concentrations in the nutrient solution on the dry weight (g $plant^{-1}$) produced by buchu plants 9 months after transplanting (LSD = 11.23)

*Dry matter index (DMI)*

Dry matter index (DMI) indicated a significant (P0.05) response to P concentration in the nutrient solution only after three months of growth (Table 3.5). At this stage DMI decreased with an increase in P concentration (Figure 3.4). P concentrations of 0.7, l.05 and 1.4 me $L^{-1}$ in the nutrient solution resulted in significant lower DMI values compared to the control (0 me P $L^{-1}$ in the nutrient solution) with a value of 0.28 (Table 3.6). This decrease in DMI with increasing P concentrations at early growth stages may indicate a need for higher P concentration at this stage, because high DMI values are in general an indication of less vigorous plant growth.

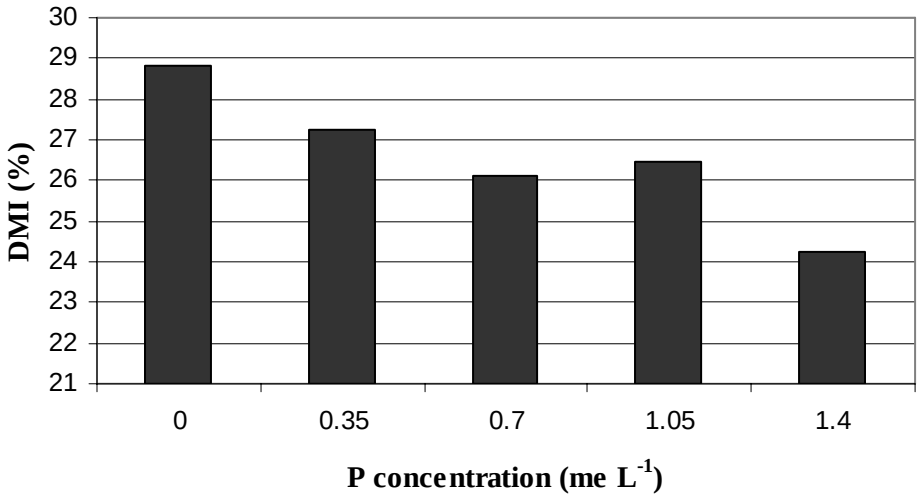


**Figure 3.4** The effect of different P concentrations in the nutrient solution on the Dry Matter Index (DMI) of buchu plants 3 months after transplanting (LSD = 2.17).

**Table 3.6** The significant effects of different P concentrations in the nutrient solution on the Dry Matter Index (DMI), fresh and dry weight produced by buchu plants

| P concentration me $L^{-1}$ | Fresh Weight (g $plant^{-1}$) 6 Months | 9 Months | Dry Weight (g $plant^{-1}$) 9 Months | DMI (%) 3 Months |
|---|---|---|---|---|
| 0.0 | 46.34abc | 94.08c | 33.78c | 28.00a |
| 0.35 | 48.82ab | 145.98b | 48.29b | 27.00ab |
| 0.7 | 52.65a | 192.56a | 64.14a | 26.10bc |
| 1.05 | 34.76c | 145.70b | 47.06b | 26.40b |
| 1.4 | 36.78bc | 124.40bc | 40.73bc | 24.20c |

Means followed by the same letter in a column do not differ significantly at P = 0.05 (LSD)

**Mineral analyses**

The P concentration in the nutrient solution had a significant (P0.05) effect on the N, P, K and Zn content in the plants analyzed after three, six and nine months of growth in this experiment (Table 3.7). The Mg, Na, Ca and B content of the plants were significantly influenced (P0.05) by P concentration in the nutrient solution when harvested after three and six months of growth, while the Mn and Fe content were

only significantly affected when harvested after three and nine months. The Cu content indicated a significant response to the P concentration in the nutrient solution only when harvested after nine months of growth (Table 3.7). Discussion of the results will focus on the significant effects only and a complete mineral analysis of the buchu plants are given in the Addendum (Table 6.7-6.8).

**Table 3.7** Analyses of variance (ANOVA) of the mineral composition of buchu plants in response to P concentration in the nutrient solution

| | Block (Pr>F) | | | P concentration (Pr>F) | | |
|---|---|---|---|---|---|---|
| Mineral | 3 Months | 6 Months | 9 Months | 3 Months | 6 Months | 9 Months |
| N | 0.3795 | 0.2643 | 0.4318 | 0.0289 | 0.0089 | <0.0001 |
| P | 0.2202 | 0.0582 | 0.4076 | <0.0001 | <0.0001 | <0.0001 |
| K | 0.3280 | 0.3494 | 0.4429 | <0.0001 | <0.0001 | 0.0001 |
| Ca | 0.5768 | 0.0108 | 0.6767 | <0.0001 | <0.0001 | 0.0580 |
| Mg | 0.2951 | 0.0764 | 0.087 | <0.0001 | <0.0001 | 0.0775 |
| Na | 0.0947 | 0.3231 | 0.5188 | 0.0412 | 0.0165 | 0.1166 |
| Mn | 0.0316 | 0.3044 | 0.4739 | 0.0280 | 0.1712 | 0.0131 |
| Fe | 0.1120 | 0.9381 | 0.4548 | <0.0001 | 0.0744 | 0.0008 |
| Cu | 0.0031 | 0.0786 | 0.6010 | 0.6432 | 0.2404 | 0.0500 |
| Zn | 0.0870 | 0.0756 | 0.0053 | <0.0001 | 0.0117 | <0.0001 |
| B | 0.066 | 0.7499 | 0.0261 | <0.0001 | 0.0018 | 0.0777 |

*Nitrogen*

Generally the N content in the plants decreased with an increase in growth period (Figure 3.4). At all sampling times (three, six and nine months), N content in the plants tended to increase with an increase in the P concentration in the nutrient solution (Figure 3.5) with the lowest N contents where no P was applied. After a growth period of three and six months, N content obtained with a concentration of 1.4 me P $L^{-1}$ did not differ significantly from N contents obtained with concentrations of 0.35 and 0.7 me P $L^{-1}$ in the nutrient solution (Table 3.8), but after nine months the N content obtained with a concentration of 1.4 me P $L^{-1}$ were significantly higher than all other P treatments. This trend indicated that the effect of P concentration in the

nutrient solution on the N content of the buchu plants may increase with time. Cecil *et al.* (1995) also found a positive correlation between N and P in *Leucadendron* during seasonal trends in mineral concentrations.

In its natural habitat in the Cederberg Mountains, the N content of buchu plants one year after a "veldfire" was found to be between 0.60% and 0.68% (Unpublished data, ASNAPP, 2007). The N content of the leaves in this trial was found to be substantially higher after 3 months (1.6-1.8%) compared to values ranging from 0.99% to 1.37% after nine months. The N content found in the buchu plants were lower than that reported for *Leucadendron* "Safari Sunset", which consisted of 1.5 to 2.1% N (Ran *et al.*, 2001). The sufficient range for citrus crops, which is also part of the Rutaceae family ranges from between 2.3 to 2.9% and the range of 2.0 to 2.2% is considered to be low while levels above 3.3% are considered high (Barker & Bryson, 2007). The N content of nine month old buchu plants in this experiment were therefore higher than that of buchu plants of similar age (12 months) in its natural environment, but lower than some Protea species. Because values for a sufficient and/or toxic N content in the buchu leaves are yet to be established, it is difficult to say whether the increase in N due to an increase in P was beneficial for growth or not.

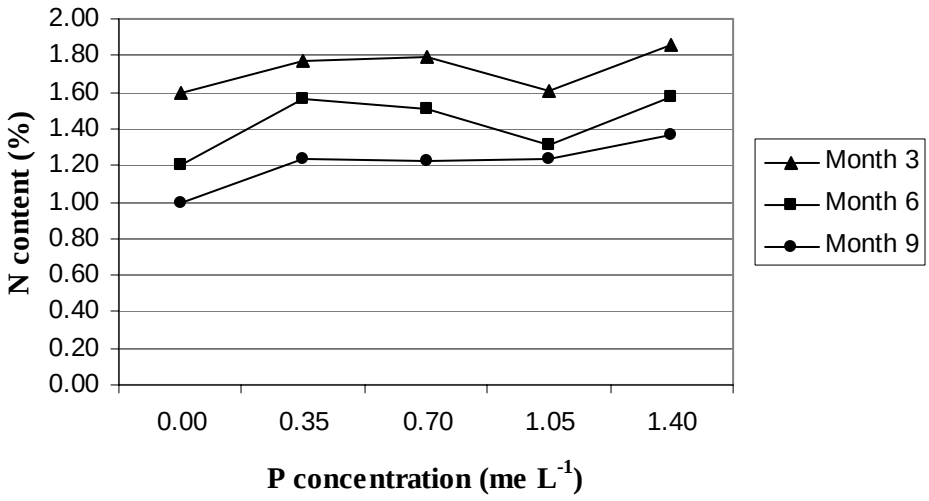


**Figure 3.5** The effect of different P concentrations in the nutrient solution on the N content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 0.20; Month 6 = 0.23; Month 9 = 0.12).

*Phosphorus*

Although the response was somewhat smaller and the P contents in the buchu plants lower after nine months compared to three and six months of growth, P content in the plants increased with an increase in P concentration in the nutrient solution at all sampling times (Figure 3.6). Highest P contents of 1.48% after three and six months and 0.83% after nine months of growth were therefore recorded in plants irrigated with a nutrient solution that contains 1.4 me P $L^{-1}$, while P contents of 0.11%, 0.08% and 0.04% were respectively recorded where no P was applied (Table 3.8).

In its natural habitat a P content of 0.02% to 0.08% was found in buchu plants (Unpublished data, ASNAPP, 2007). Buchu irrigated with nutrient solution containing no P had similar low values (Table 3.8). According to Cresswell (1991), the P content in recently matured leaves of *Leucadendron* cultivars could be used to describe the P status of the plants. He classified plants with less than 0.15% P as low or deficient, 0.15 – 0.42% P as desirable, 0.42 – 0.45% as high and plants with a P content higher than 0.57% as supra optimal. According to this classification plants receiving no P were deficient and plants receiving 0.35 me P $L^{-1}$ had a desirable P content while plants receiving higher P concentrations had high or supra optimal P contents, which could lead to a negative influence on growth. It should however be taken into consideration that in this study all the leaves (young and old) were sampled.

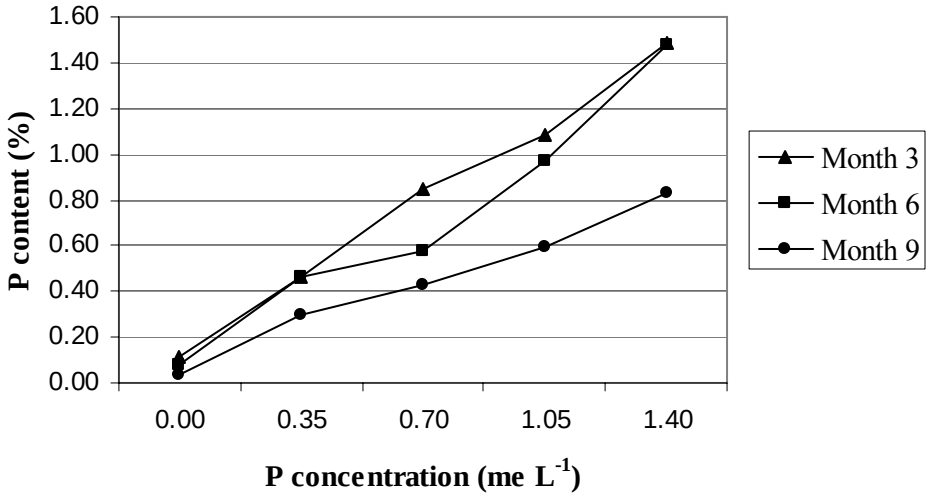


**Figure 3.6** The effect of different P concentrations in the nutrient solution on the P content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 0.16; Month 6 = 0.26; Month 9 = 0.13).

*Potassium*

The K content of the plants generally increased with time with an increase in P concentration of the nutrient solution (Figure 3.7; Table 3.8). Cecil *et al.* (1995) also found a positive correlation between P and K in *Leucadendron* during seasonal trends in mineral concentrations. Plants receiving the highest P concentration (1.4 me $L^{-1}$) indicated a significantly higher K content after three, six and nine months of growth compared to plants receiving 0, 0.35 and 0.7 me P $L^{-1}$ (Table 3.8). Values after six and nine months of growth were not significantly higher compared to plants receiving 1.05 me P $L^{-1}$. The K content in the plants receiving 0.35 me.P.$L^{-1}$ did not differ significantly after three and nine months from those receiving no P (Figure 3.7).

The K content of one year old buchu leaves growing in its natural habitat varied between 0.55% and 0.62% (Unpublished data, ASNAPP, 2007), while Madakadze, Nyamangara & Mahenga (2006) recorded a K content of 0.30% in *Leucadendron* "Coniferum". The K content of buchu plants in this experiment was found to be considerably higher. As the P concentration increased in the nutrient solution, the K content increased as well. Increasing K contents coupled with the already high K contents could have a negative effect on the growth of the buchu plants.

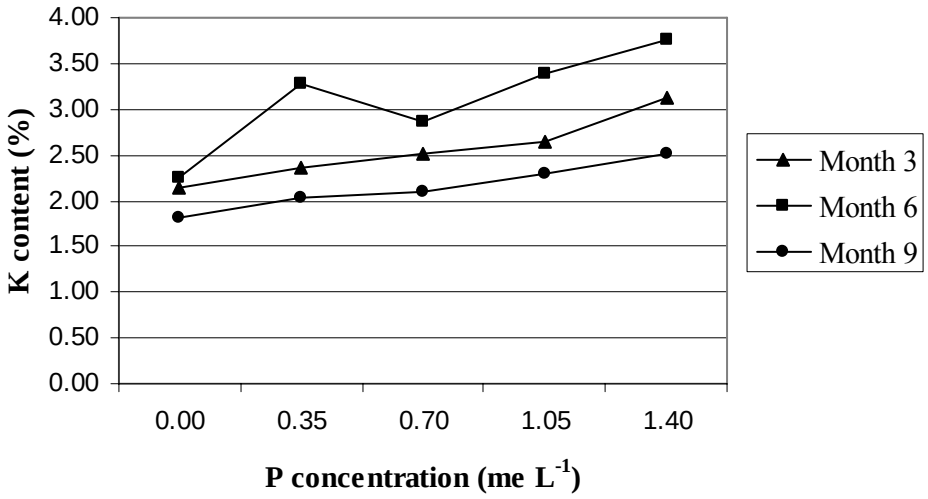


**Figure 3.7** The effect of different P concentrations in the nutrient solution on the K content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 0.31; Month 6 = 0.48; Month 9 = 0.26)

*Calcium*

After three and six months of growth the Ca content increased significantly with an increase in P in the nutrient solution causing plants that received the highest P concentration (1.4 me $L^{-1}$) to have a significantly higher (P0.05) Ca content than plants receiving low P concentrations in the nutrient solution (Figure 3.8; Table 3.8). Although the Anova analyses (Table 3.7) indicated no significant difference (Pr>0.058) in the Ca content between plants receiving different P concentrations were observed after nine months, the trend were similar to that after three and six months of growth. Nichols & Beardsell (1981) also found that P stimulates Ca uptake in *Grevillea* spp., but that the increase in Ca content observed with an increase in P concentration did not cause toxicity symptoms, but rather the increase in P.

In its natural habitat (Unpublished data, ASNAPP, 2007), values for Ca in buchu leaves one year after a "veldfire" varied between 0.38% and 0.42%. When the plants were young, the Ca contents were higher (Figure 3.8) than these values mentioned above, but after nine months of growth the Ca content of the buchu plants started to decrease and this indicates that the Ca content of older plants, at all P concentrations, was normal compared to buchu growing in its natural environment.

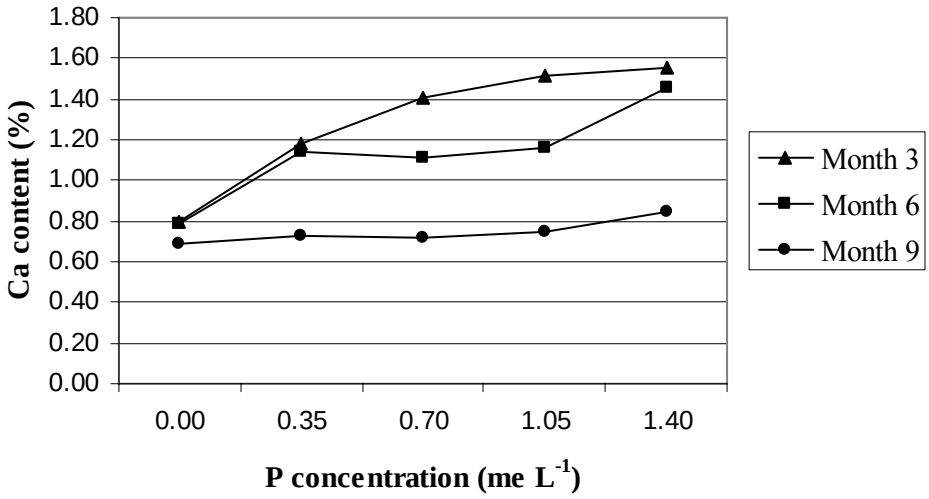


**Figure 3.8** The effect of different P concentrations in the nutrient solution on the Ca content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 0.19; Month 6 = 0.17; Month 9 = 0.11).

*Magnesium*

In general an increase in Mg content was observed with an increase in P concentration in the nutrient solution after three and six months of growth (Figure 3.9). When harvested after three months of growth, buchu plants receiving 0.7, 1.05 and 1.4 me P $L^{-1}$ in the nutrient solution, had a significantly (P0.05) higher Mg content than plants receiving 0.35 and 0 me P $L^{-1}$ (Table 3.8). After six months of growth, it was found that plants receiving 1.4 me P $L^{-1}$ had a significantly (P0.05) higher Mg content than all the other P-treatments. After nine months no significant response due to P concentration in the nutrient solution was found.

The Mg values found for buchu in this experiment (Table 3.8) corresponded with values found for buchu in its natural habitat (Unpublished data, ASNAPP, 2007) and for Proteas (Madakadze *et al.,* 2006). No extremely high or low Mg contents were observed.

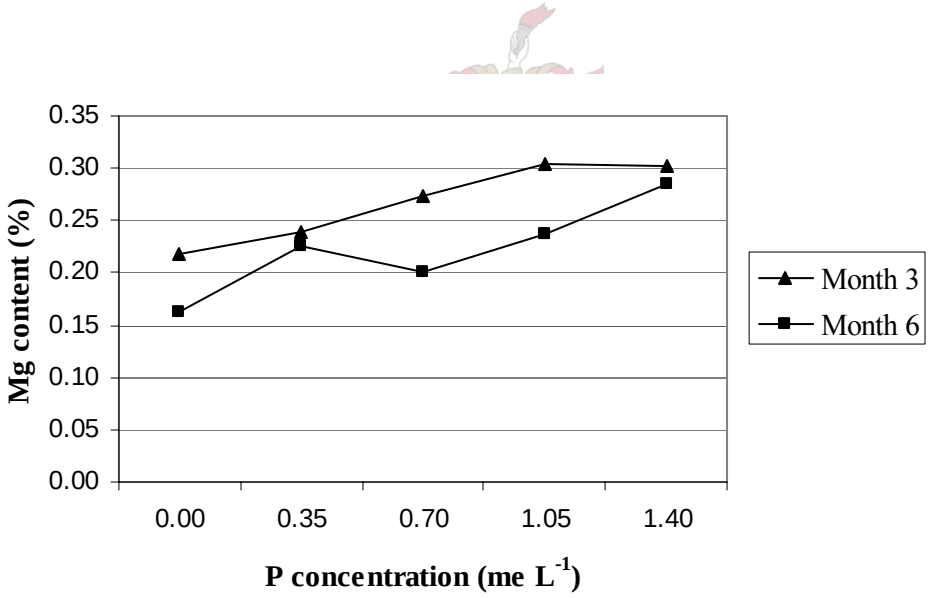


**Figure 3.9** The effect of different P concentrations in the nutrient solution on the Mg content of buchu plants three and six months after transplanting (LSD: Month 3 = 0.03; Month 6 = 0.04).

*Sodium*

Although significant differences in Na content of the buchu plants were found after three and six months due to the different P treatments, results were not convincing (Figure 3.10).

After three months the lowest Na content was found in the plants that were receiving P concentrations of 1.05 and 1.4 me $L^{-1}$, while the same P concentrations, together with the 0.35 me $L^{-1}$ treatment resulted in the highest Na contents after six months of growth (Table 3.8).

The values obtained from this trial (Table 3.8) is lower than values recorded for buchu in its natural habitat (Unpublished data, ASNAPP, 2007), which was found to vary between 920 $mg.kg^{-1}$ and 3931 $mg.kg^{-1}$.

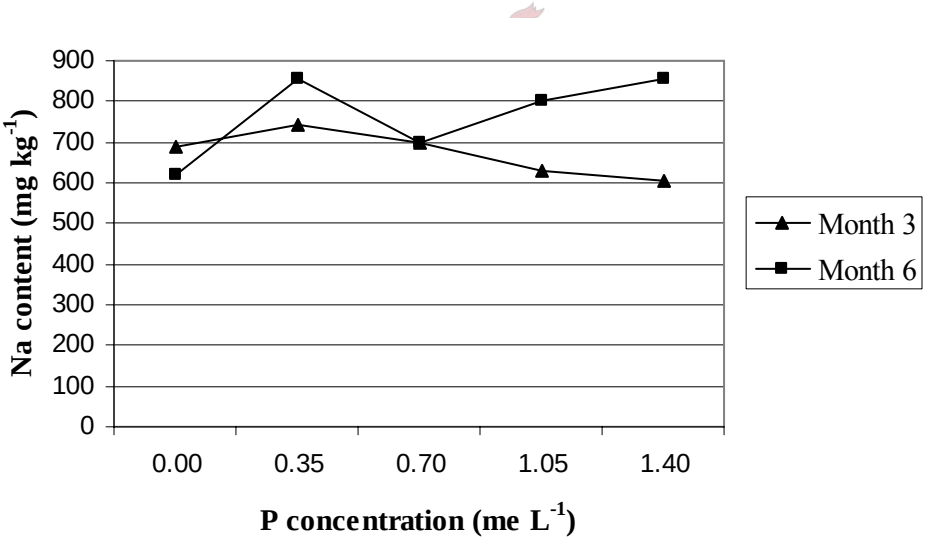


**Figure 3.10** The effect of different P concentrations in the nutrient solution on the Na content of buchu plants three and six months after transplanting (LSD: Month 3 = 96.84; Month 6 = 157.48)

*Manganese*

After 3 months of growth a general increase in Mn was observed with an increase in P concentration in the nutrient solution to reach a maximum value at a P concentration of 1.05 me $L^{-1}$ in the nutrient solution (Figure 3.11). Plants receiving no P in the nutrient solution had a significantly lower Mn content than plants receiving higher

concentrations of P (0.7, 1.05 and 1.4 me P $L^{-1}$) (Table 3.8) after three months. In contrast to this, plants receiving no P in the nutrient solution had a significantly higher Mn content than all the other P treatments after nine months of growth. For this reason, no general trend in Mn content in response to P concentration in the nutrient solution could be found.

The sufficiency range of Mn for most plant species ranges between 20 and 500 mg $kg^{-1}$ (Jones, 1991). The Mn content of buchu plants growing in its natural habitat one year after a "veldfire" was found to be between 179 mg $kg^{-1}$ and 183 mg $kg^{-1}$ (Unpublished data, ASNAPP, 2007). After nine months, similar values were found for buchu plants receiving 0.35 and 1.05 me P $L^{-1}$ in this experiment, but at P concentrations of 0, 0.7 and 1.4 me P $L^{-1}$ much higher values were obtained (Table 3.8).

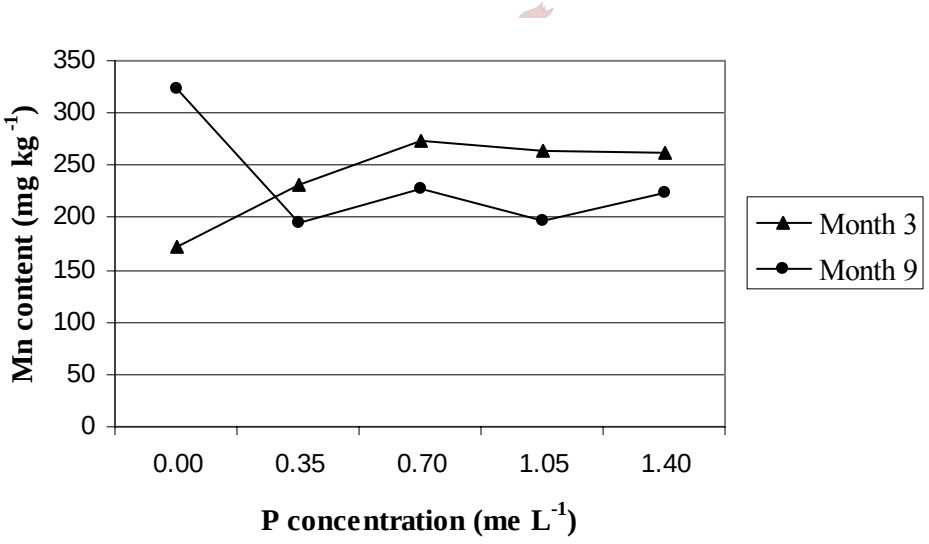


**Figure 3.11** The effect of different P concentrations in the nutrient solution on the Mn content of buchu plants three and nine months after transplanting (LSD: Month 3 = 66.20, Month 9 = 77.97).

*Iron*

The Fe content in the buchu plants indicated a significant response to the P concentration in the nutrient solution only after three and nine months of growth. After three months plants receiving 0 and 0.35 me P $L^{-1}$ had a significantly higher Fe content than plants receiving 0.7, 1.05 and 1.4 me P $L^{-1}$ (Table 3.8), while all

treatments that received P in the nutrient solution indicated a significantly lower Fe content compared to the control (no P) after nine months of growth. In general it can therefore be concluded that the iron content of the buchu plants indicated a negative correlation with an increasing P concentration in the nutrient solution (Figure 3.12).

In this experiment, some of the buchu plants receiving high P concentrations in the nutrient solution (1.05 and 1.4 me $L^{-1}$) displayed signs of chlorosis and necrosis, especially on the younger leaves after three months of growth. This is typical Fe deficiency symptoms (Marschner, 1995). Barry (1996) also indicated that an excess of phosphorus can cause iron deficiencies which leads to yellowing, chlorosis of younger leaves, stunted plant growth and leaves that die and abscise.

In its natural habitat, the Fe content of buchu plants one year after a "veldfire" was found to be between 77 mg $kg^{-1}$ and 92 mg $kg^{-1}$ (Unpublished data, ASNAPP, 2007). Cecil *et al*. (1995) found Fe contents of five and six year old *Leucadendron* "Safari sunset" and "Silvan Red" to be between 42 mg $kg^{-1}$ and 92 mg $kg^{-1}$. Buchu plants receiving a P concentration higher than 0.35 me $L^{-1}$ generally had Fe contents lower than 40 mg $kg^{-1}$ (Table 3.8), that could have been deficient if compared to the values mentioned above.

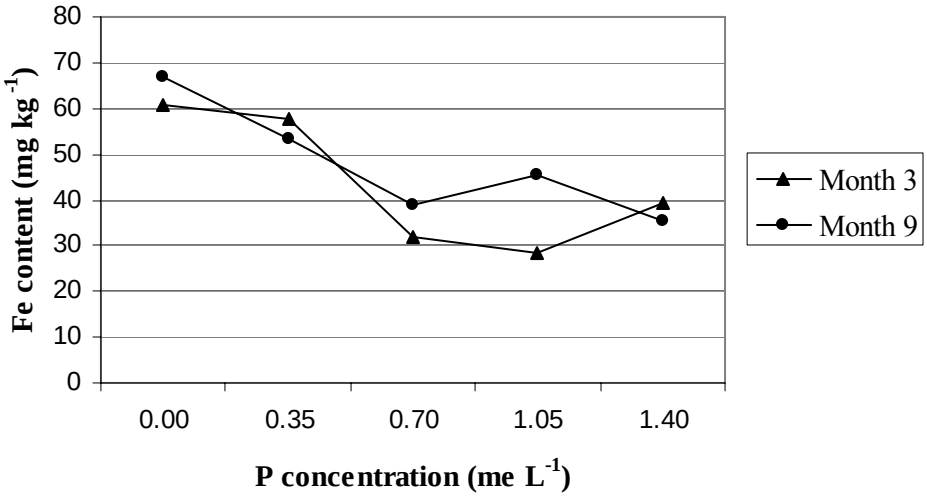


**Figure 3.12** The effect of different P concentrations in the nutrient solution on the Fe content of buchu plants three and nine months after transplanting (LSD: Month 3 = 9.40; Month 9 = 14.38).

*Copper*

The only significant difference (P0.05) in plant Cu content due to P concentration in the nutrient solution was observed after nine months with a significantly higher Cu content in plants which did not receive any P compared to plants receiving 0.35 and 0.7 me P $L^{-1}$ (Table 3.8). Although the Cu content seemed to increase again at P concentrations of 1.05 and 1.4 me $L^{-1}$, the general trend indicated a reduction in Cu content where P was applied (Figure 3.13).

Cu contents of 2 to 3 mg $kg^{-1}$ were recorded for buchu in its natural habitat (Unpublished data, ASNAPP, 2007) and similar values were recorded for Proteas (Cecil *et al*., 1995). According to Jones (1991) the Cu concentration in plants generally ranges from 5 to 20 mg $kg^{-1}$, but deficiency symptoms may appear at different concentrations. From Figure 3.13 it appears that there might be a decline in Cu content as the P concentration increases, although the lowest Cu content was found at 0.7 me P $L^{-1}$. The Cu content observed in this experiment (Table 3.8) therefore did not differ much from the values found in its natural habitat (Unpublished data, ASNAPP, 2007), except at P concentrations of 0.7 me $L^{-1}$, where it was less and in the control (0.00 me P $L^{-1}$) where the Cu content was considerably higher.

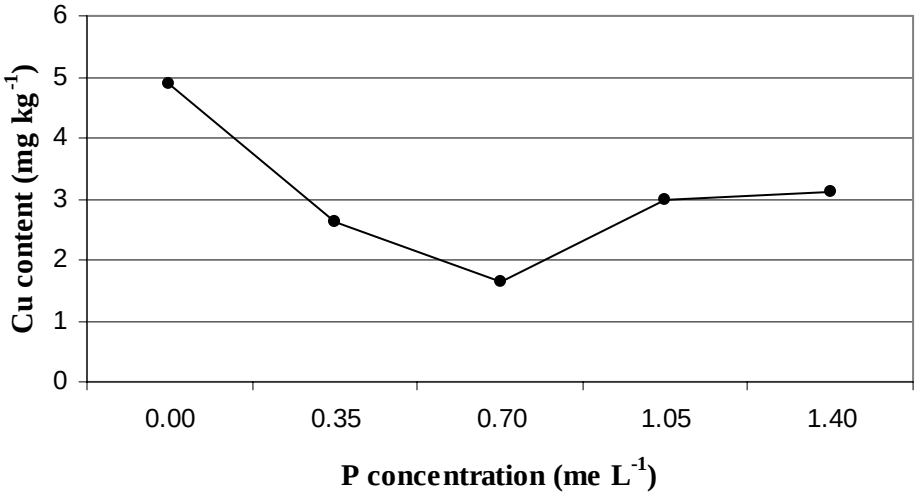


**Figure 3.13** The effect of different P concentrations in the nutrient solution on the Cu content of buchu plants nine months after transplanting (LSD = 2.07).

*Zinc*

Responses in Zn content of buchu plants to P concentration in the nutrient solution varied with time (Figure 3.14). After three months of growth Zn content tended to increase with an increase in P concentration with significantly (P0.05) higher Zn contents where 1.05 and 1.4 me P $L^{-1}$ were applied compared to 0, 0.35 and 0.70 me P $L^{-1}$ (Table 3.8). After six months no significant difference was found between P concentrations of 0, 0.35, 0.70 and 1.05 me $L^{-1}$, but significantly higher Zn contents was found with 1.4 me P $L^{-1}$. In contrast to this the highest Zn content after nine months was found where no P was applied.

After three months the Zn concentration of the buchu plants varied between 30.13 and 48.48 mg $kg^{-1}$ (Table 3.8), which were substantially higher than values of 5 to 20 mg $kg^{-1}$ found for buchu plants in its natural habitat (Unpublished data, ASNAPP, 2007) , and for Proteas (Cecil *et al.,* 1995). After nine months, values varied between 21.75 and 33.25 mg $kg^{-1}$ which indicated that Zn concentrations decreased with time. For most plants, Zn deficiencies are however likely to occur at Zn concentrations lower than 20 mg $kg^{-1}$ (Jones, 1991).

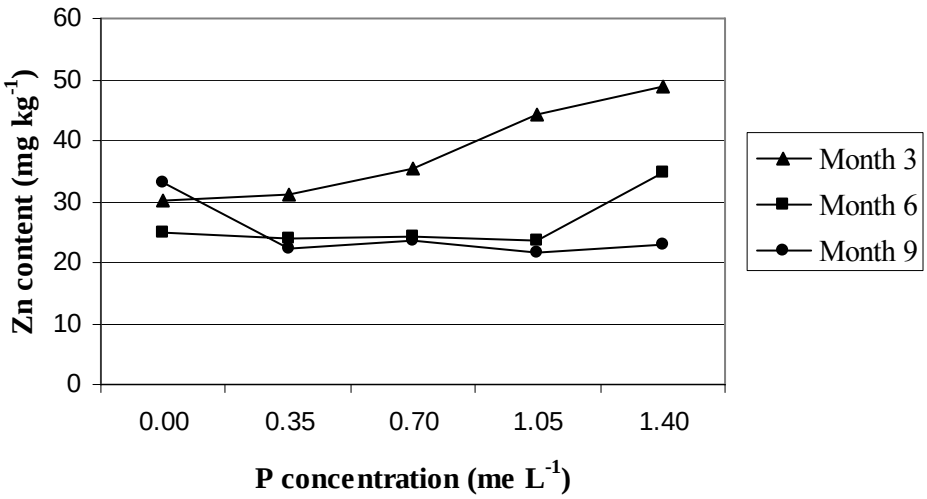


**Figure 3.14** The effect of different P concentrations in the nutrient solution on the Zn content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 6.15; Month 6 = 6.81; Month 9 = 4.16).

*Boron*

The B content in buchu plants was significantly affected due to the P concentration in the nutrient solution after three, six and nine months of growth (Table 3.8). Although trends were somewhat distorted, the highest B content during sampling times after three and six months were found where 1.4 me P $L^{-1}$ was applied (Figure 3.15). After three months a significant increase (P0.05) in B content in the buchu plants were observed with an increase in P concentration in the nutrient solution (Table 3.8). During the six month harvest it was found that B content in the plants increased significantly (P0.05) from a P concentration of 0 me $L^{-1}$ to 0.35 me $L^{-1}$, where-after it decreases significantly (P0.05) again when the P concentration was increased to 0.7 me $L^{-1}$. From a P concentration of 0.7 me $L^{-1}$ and higher the B content increased. The response seemed to decrease with time and no increasing trend with an increase in P concentration was found after nine months (Figure 3.15).

In this experiment B contents varied between 20.50 and 41.75 mg $kg^{-1}$ (Figure 3.15), almost similar to values of 20 to 50 mg $kg^{-1}$ found for buchu in it's natural habitat (Unpublished data, ASNAPP, 2007), but considerably higher than B concentrations found for Proteas, ranging from 11 to 14 mg $kg^{-1}$ (Cecil *et al.,* 1995). According to Gupta (1979) the B sufficiency range in many plants lies between 20 and 100 mg $kg^{-1}$.

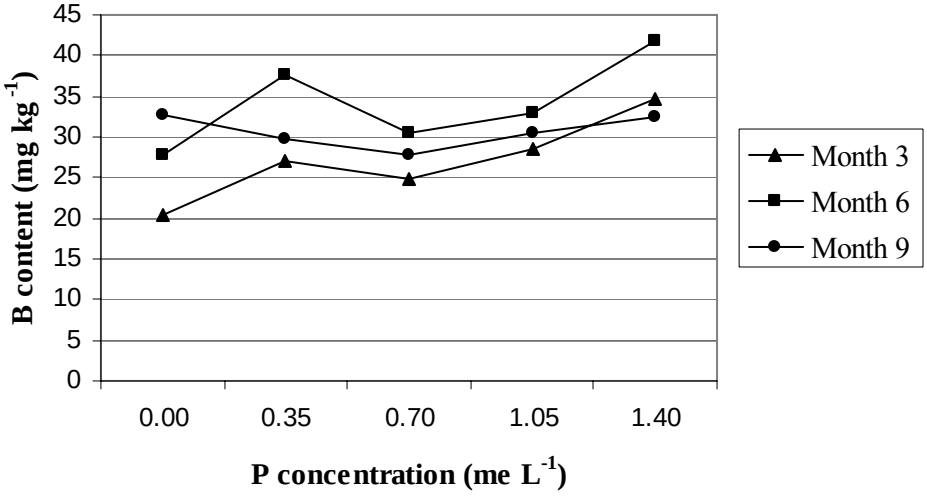


**Figure 3.15** The effect of different P concentrations in the nutrient solution on the B content of buchu plants three, six and nine months after transplanting (LSD: Month 3 = 3.81; Month 6 = 6.77; Month 9 = 3.79).

**Table 3.8** Effects of different P concentrations in nutrient solutions on the mineral content of buchu plants

| Mineral | Month | P concentration (me $L^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 0.00 | 0.35 | 0.70 | 1.05 | 1.4 |
| N (%) | 3 | 1.59c | 1.77abc | 1.79ab | 1.60bc | 1.86a |
| | 6 | 1.20c | 1.56a | 1.51ab | 1.32bc | 1.57a |
| | 9 | 0.99c | 1.23b | 1.23b | 1.24b | 1.37a |
| P (%) | 3 | 0.11e | 0.47d | 0.85c | 1.08b | 1.48a |
| | 6 | 0.08d | 0.47c | 0.58c | 0.97b | 1.48a |
| | 9 | 0.04e | 0.30d | 0.43c | 0.50b | 0.83a |
| K (%) | 3 | 2.13c | 2.35bc | 2.51b | 2.65b | 3.13a |
| | 6 | 2.25d | 3.27bc | 2.86c | 3.40ab | 3.75a |
| | 9 | 1.82c | 2.03bc | 2.11b | 2.29ab | 2.51a |
| Ca (%) | 3 | 0.80c | 1.18b | 1.41a | 1.51a | 1.56a |
| | 6 | 0.79c | 1.11b | 1.14b | 1.16b | 1.46a |
| | 9 | 0.69b | 0.72b | 0.72b | 0.75ab | 0.85a |
| Mg (%) | 3 | 0.22b | 0.24b | 0.27a | 0.30a | 0.30a |
| | 6 | 0.16c | 0.23b | 0.20bc | 0.24b | 0.29a |
| Na | 3 | 687.50ab | 744.75a | 697.63ab | 629.00b | 603.63b |
| (mg $kg^{-1}$) | 6 | 620.75c | 856.50a | 697.75bc | 802.63ab | 854.0ab |
| Mn | 3 | 172.88b | 232.25ab | 272.88a | 363.00a | 261.38a |
| (mg $kg^{-1}$) | 9 | 323.63a | 195.00b | 228.00b | 196.50b | 222.88b |
| Fe | 3 | 60.23a | 57.63a | 33.00bc | 28.25c | 39.38b |
| (mg $kg^{-1}$) | 9 | 67.00a | 53.88bc | 38.88bc | 45.38bc | 35.50c |
| Cu (mg $kg^{-1}$) | 9 | 4.88a | 2.63b | 1.63b | 3.00ab | 3.13ab |
| Zn | 3 | 30.13b | 31.25b | 35.38b | 44.13a | 48.88a |
| (mg $kg^{-1}$) | 6 | 25.00b | 23.88b | 24.38b | 23.75b | 34.63a |
| | 9 | 33.25a | 22.38b | 23.50b | 21.75b | 23.00b |
| B | 3 | 20.50d | 27.13bc | 24.75c | 28.63b | 34.75a |
| (mg $kg^{-1}$) | 6 | 27.88c | 37.63ab | 30.50c | 33.00bc | 41.75a |
| | 9 | 32.75a | 29.88ab | 27.88b | 30.50ab | 32.50a |

Means in a row followed by the same letter do not differ significantly at P = 0.05 (LSD)

**Mortality**

The mortality rate is the percentage of the original ten plants in a block that died throughout the trial. The mortality of the buchu plants were significantly affected ($P<0.001$) by the P concentration in the nutrient solution. The mortality rate increased with an increase in P concentration in the nutrient solution. *Grevillea* as well as other species of the Proteaceae family (Nichols & Beardsell, 1981) is affected by P toxicity, and it appears that high P concentrations in the nutrient solution of buchu plants grown hydroponically are also toxic, probably due to an induced Fe deficiency.

**Table 3.9** The effect of P concentration in the nutrient solution on the mortality of the buchu plants

| P concentration (me $L^{-1}$) | Mortality (%) |
|---|---|
| 0.00 | 1.25d |
| 0.35 | 3.75dc |
| 0.70 | 11.25bc |
| 1.05 | 17.50ab |
| 1.40 | 23.75a |

Means followed by the same letter do not differ significantly at P = 0.05 (LSD)

**Oil composition**

The oil composition of buchu plants receiving different P concentrations revealed that only two components were significantly affected by the P concentration in the nutrient solution. These two components were the Ψ-diosphenol and the (E)-b-Ocimene content of the oil (Table 3.10). Discussion of the results will focus on the significant effects only and an analysis of the buchu oil is given in the Addendum (Table 6.9).

**Table 3.10** Analysis of variance (ANOVA) of buchu essential oil components extracted from nine month old plants that were significantly influenced by P concentration in the nutrient solution

| | Block | P concentration |
|---|---|---|
| | Pr>F | |
| Ψ-diosphenol | 0.0360 | 0.0388 |
| (E)-b-Ocimene | 0.0700 | 0.0209 |

No general trend could be found for the Ψ-diosphenol content (Figure 3.16; Table 3.11) of the buchu oil, but at P concentrations of 0.35 and 1.4 me $L^{-1}$ the Ψ-diosphenol content was significantly lower than plants receiving no P in the nutrient solution (Table 3.11). From these results it will not be possible to determine the P concentration which will result in the highest Ψ-diosphenol content, because no significant differences between the Ψ-diosphenol content at low (0 me $L^{-1}$), medium (0.7 me $L^{-1}$) and high (1.05 me $L^{-1}$) P concentrations were found. Differences observed in Ψ-diosphenol content must for this reason be attributed to variation between the buchu plants.

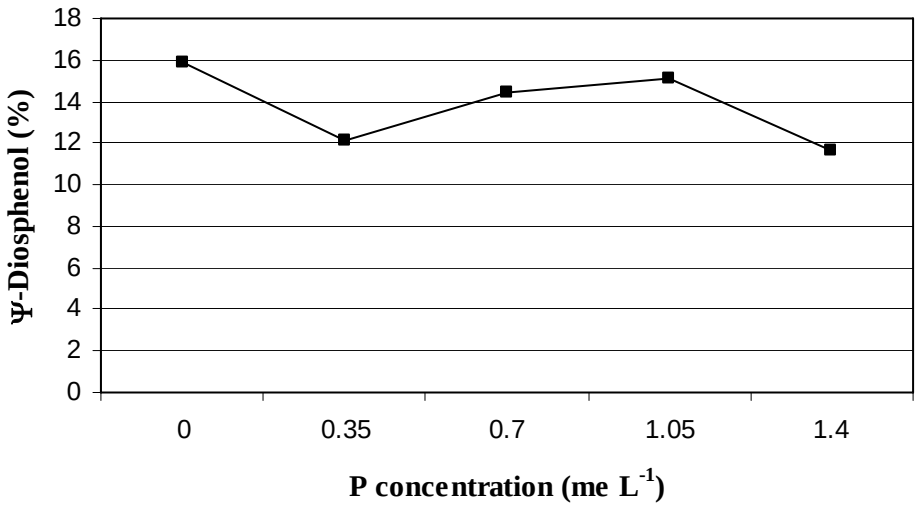


**Figure 3.16** The effect of P concentration in the nutrient solution on the Ψ-Diosphenol content of the buchu oil (LSD = 3.18).

(E)-b-Ocimene is not a mayor constituent of buchu oil (Endenburg, 1972; Kaiser, Lamparsky & Schudel, 1975; Posthumus *et al*., 1996) and is not essential in determining the oil quality. Plants receiving no P in the nutrient solution had a significantly (P0.05) lower (E)-b-Ocimene content than plants receiving 0.35, 0.7 and 1.4 me P $L^{-1}$ (Table 3.11, Figure 3.17), but no general trend for (E)-b-Ocimene content as a result of P concentration in the nutrient solution could be found.

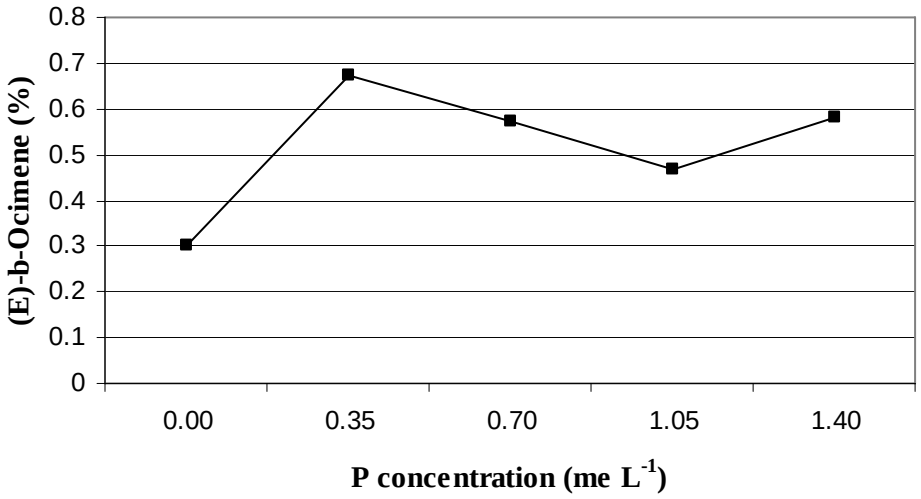


**Figure 3.17** The effect of P concentration in the nutrient solution on the (E)-b-Ocimene content of the buchu oil (LSD = 0.24).

**Table 3.11** The effect of P concentration in the nutrient solution on the Ψ-diosphenol and (E)-b-Ocimene components of the buchu oil

| | P concentration (me.$L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0.00 | 0.35 | 0.70 | 1.05 | 1.4 |
| Ψ-diosphenol (%) | 15.863a | 12.138bc | 14.471abc | 15.086ab | 11.600c |
| (E)-b-Ocimene (%) | 0.300b | 0.675a | 0.571a | 0.467ab | 0.583a |

Means in a row followed by the same letter do not differ significantly at P = 0.05 (LSD)

These results suggest that the different P concentrations had a very small and almost insignificant effect on the composition of the Buchu oil. According to the Agricultural Research Council (ARC) (Unpublished data, 2004) different fertilizers can have a great influence on buchu growth, but it doesn't affect the oil composition. This data suggests that the buchu oil is not influenced by fertilization but possibly by other factors such as the environment, growth stage and genetic composition of the plants.

**Conclusion**

In this experiment the P concentration did not have a significant effect on the plant height after nine months, although differences in plant height were starting to emerge after six months. The variation between plants in a block was found to be very high due to the lack of genetic uniformity. If this variation between plants would have been smaller, or the trial went on for longer, the differences in plant height between the different P concentrations might have been significant. One month after transplanting the plants growth tips were removed to induce lateral growth for a more "bushy" plant, as done in practice. This restricted plant height to the detriment of shoot growth as parameter. Despite all these circumstances mentioned above, it would appear that the buchu plants showed the best response at a P concentration of 0.7 me $L^{-1}$.



The fresh weight produced by the plants is very important, since producers are paid according to the fresh weight delivered. In this experiment it is evident that plants receiving 0.7 me P $L^{-1}$ produced significantly (P0.05) more fresh weight after nine months than all the other P concentrations. An increase in fresh weight was observed with an increase in P concentration until the optimum value was reached at 0.7 me P $L^{-1}$, where after the fresh weight declined with further increases in the P concentration. Nichols & Beardsell (1981) found that Proteas perform the best at low P concentrations in nutrient solutions.

An increase in P concentration in the nutrient solution led to a higher mortality rate of buchu plants. Nichols, Jones & Beardsell (1979) suggested that high P in the root zone of Proteas suppresses proteoid root production that can cause poor growth, but buchu does not posses proteoid roots (Goldblatt & Manning, 2000) which exclude this explanation for the high mortality rate. A possible explanation for the increasing

mortality at high P concentrations may be the induction of mineral imbalances in the plants. The N, P and K concentrations in buchu plants recorded in this experiment was considerably higher than concentrations measured in buchu growing in its natural environment. The already high N, P and K concentrations also increased as the P concentration in the nutrient solution increased, causing higher concentrations of these minerals in the buchu plants. No toxic level of minerals in buchu has been described, which makes it difficult to identify certain minerals that might be toxic. It is however possible that a deficiency of Fe was induced at the high P concentrations. The Fe concentration in the buchu plants ranged from 35.5 to 67.0 mg $kg^{-1}$ after nine months. These values were lower than the values of 77 to 92 mg $kg^{-1}$ found in plants growing in its natural environment (Unpublished data, ASNAPP, 2007). The lowest Fe concentration was found in plants receiving 1.4 me P $L^{-1}$ and in general the Fe concentration in the plants decreased with an increase in P in the nutrient solution. According to Barry (1996), an excess of P causes Fe and/or Zn deficiencies which leads to yellowing, interveinal chlorosis of younger leaves, stunted plant growth and leaves that die and abscise. Loneragan & Asher (1967) found that high uptake rates of phosphate were associated with reduced growth rates in some plants. They concluded that the uptake and translocation of some of the micronutrients such as Zn, Fe and Cu were retarded. Symptoms of dying plants in this study were yellowing and loss of leaves, where after the branches became naked and the plants died back (Addendum, Plate 6.2-6.3). These symptoms were mostly displayed by buchu plants receiving 1.05 and 1.4 me P $L^{-1}$, and could be a possible explanation for the high mortality rates found at these high P concentrations.

The quality of the buchu oil was poor due to the high pulegone content (Addendum, Table 6.9) and this might be due to the fact that the plants were still young when harvested since the pulegone content of young plants tend to be high and that the pulegone content ideally needs to be below 5% (Pers. Communication Allen Harris, 2006. Buchu Farming Consultant. Palmiet Valley, Wellington. Tel/Fax 021 864 3317). Increasing concentrations of P in the nutrient solution had no effect on the mayor constituents of the buchu oil except for Ψ-Diosphenol. No general trend between increasing P concentration and Ψ-Diosphenol concentration could be found. The P concentration in the nutrient solution also had a significant effect on the (E)-b-

Ocimene content of the buchu oil. (E)-b-Ocimene is however not a major constituent of the buchu oil and not important in determining oil quality. The P level in nutrient solutions had little, or no effect on the buchu oil composition, although it had a significant effect on the mineral composition of the plants. This raises the question of which factors do play a role in determining oil composition? According to Karsen (2003), a variety of factors influence the oil content including the species, environmental conditions, seasonal variation, physical factors, cultivation practices, extraction method and the method used in oil analysis. It is well known that the pulegone concentration is high during the active growing period of buchu plants and decreases as the buchu plants reach maturity. This indicates that the plant's age and growth stage are factors influencing the oil composition. More studies are however necessary to determine the effects of factors such as harvesting time (season of the year) on the essential oil quality.

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# CHAPTER 4
# GLASSHOUSE TRIAL
## Reaction of hydroponically grown buchu (*Agathosma betulina*) to increasing phosphorus concentrations at different root temperatures

### Introduction

Phosphorus is one of the essential macro nutrients required for plant growth. Optimum P concentrations for many crops have been identified, but research on the P requirements of fynbos species, especially buchu has been limited. Nichols & Beardsell (1981) reported that high P levels can have a negative effect on Fynbos species such as Proteas. They also found that Proteas respond the best to P concentrations below 0.47 me $L^{-1}$ when grown in solution and sand culture, while a P concentration of 0.7 me $L^{-1}$ is currently being used by growers producing buchu hydroponically. Achiecing an optimum P concentration for buchu is but one problem, there are various other factors influencing the uptake and utilization of P by plants such as pH (Mengel & Kirkby, 1987), temperature (Zsoldos & Karvaly, 1979) as well as ammonium and nitrate concentrations (Arnon, 1939). This particular experiment was planned to investigate the effect that temperature has on buchu growth, yield and mineral uptake, as well as the role temperature plays in the utilization of P by buchu.

Cooper (1973) reviewed some research done on the effect of temperature on plant growth and concluded that low temperatures can have a negative effect on a variety of plant species and may interact with the absorption of nutrients. These effects include lower dry weight gain by the whole plant, less leaf expansion, less root branching and smaller plants. Sensitive plants are generally injured at temperatures below 10 to 12°C (Olien & Smith, 1981). Because little is known about the growth and nutrient requirements of buchu, an experiment was conducted under controlled conditions to study the effect of temperature on the growth and yield of hydroponically grown buchu, but more importantly, the role it plays in P-uptake and utilization. The aim of this experiment was to determine the growth response of buchu grown at different root temperatures to different phosphate concentrations.

## Material and Methods

### Locality

The experiment was conducted in a mechanically temperature controlled glasshouse at the Department of Agronomy, Stellenbosch University, Republic of South Africa, during the period of October 2006 to March 2007.

### Cultivation Practices

Buchu (*A. betulina*) seedlings were transplanted in 2 litre pots with drainage holes at the bottom in a temperature controlled (20/15°C day/night) glasshouse on the 5$^{th}$ October 2006. Nr. 1 acid washed sand from Consol Minerals was used as growth medium and can be classified as coarse sand (Table 4.1) that contains almost no nutrients (Table 4.2). No $CaCO_3$ was added to the root medium in this trial. One seedling was transplanted per pot and pots were arranged in one double row in the length of the glasshouse. The spacing was 50 cm between the rows and 40 cm between the pots in each row.

The nutrient solutions with different P concentrations were stored in 500 litre asbestos tanks painted on the inside. Netafim drippers (pressure compensated, non-leakage), with a capacity of 2L $hr^{-1}$ were used to irrigate each pot individually. Each treatment in the experiment had its own separate irrigation system to prevent the mixing of nutrient solutions. The total volume of irrigated water per day was the same for all the treatments and increased gradually over the growing season from 60 ml to 160 ml per day per plant. The growth tips of the buchu plants were removed one month after transplanting.

**Table 4.1** Particle size analyses of Nr. 1 sand from Console Minerals used as growth medium

| **Aperture (mm)** | **% Retained** |
|---|---|
| 2.000 | 0.4 |
| 1.700 | 0.2 |
| 1.400 | 0.4 |
| 1.180 | 2.2 |
| 1.000 | 8.3 |
| 0.850 | 18.3 |
| 0.710 | 21.4 |
| 0.600 | 21.1 |
| 0.500 | 19.9 |
| 0.425 | 5.6 |
| 0.355 | 1.9 |
| 0.300 | 0.2 |
| 0.250 | 0.1 |

**Table 4.2** Chemical analyses of Nr. 1 sand from Console Minerals used as growth medium

| Compound | %/mg $kg^{-1}$ |
|---|---|
| $SiO_2$ | 99.75/997500 |
| $Al_2O_3$ | 0.07/700 |
| $Fe_2O_3$ | 0.023/230 |
| $TiO_2$ | 0.024/240 |
| $ZrO_2$ | 0.005/50 |
| CaO | 0.003/30 |

**Treatments**

The same five complete nutrient solutions with different P concentrations, as used in Chapter 3, were used in this experiment (Table 4.3-4.4), but in this experiment, the plants were subjected to two different root temperatures. Analyses of the nutrient solutions were done each month to ensure that the P concentrations and the $NO_3/SO_4$ ratios in the nutrient solutions were correct (Addendum, Table 6.10-6.14) and the pH and EC of the nutrient solution were also recorded every time a new nutrient solution was prepared. For the cold root treatment a water bath was used to cool the water to temperatures between 6°C and 10°C (Addendum, Plate 6.4). This cold water was then pumped into glass spirals running through the pots (Addendum, Plate 6.5), cooling down the rooting medium in the pots to temperatures between 6°C and 14°C, the average temperature being 10°C. For the control temperature treatment the containers were kept at room temperature. The root temperature inside the pots varied between 14°C (night) and 26°C (day), the average being 20°C.

**Table 4.3** Macro nutrient composition of the nutrient solutions containing different P concentrations used to irrigate the buchu plants in the glasshouse

| $K^+$ | $Ca^{++}$ | $Mg^{++}$ | $NH_4^+$ | $NO_3^-$ | $H_2PO_4^-$ | $SO_4^=$ |
|---|---|---|---|---|---|---|
| | | | me $L^{-1}$ | | | |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.72 | 0 | 1.98 |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.46 | 0.35 | 1.89 |
| 3.3 | 2.73 | 1.4 | 0.27 | 5.2 | 0.7 | 1.8 |
| 3.3 | 2.73 | 1.4 | 0.27 | 4.94 | 1.05 | 1.71 |
| 3.3 | 2.73 | 1.4 | 0.27 | 4.68 | 1.4 | 1.62 |

**Table 4.4** Micro nutrient composition of the different micro nutrients in the nutrient solution used to irrigate the buchu plants in the glasshouse

| Micro Nutrient Source | ppm | Amount added (g $1000L^{-1}$) |
|---|---|---|
| Fe:Libfer (Fe EDTA) | 0.65 | 5.03 |
| Mn: Manganese Sulphate | 0.42 | 1.71 |
| Zn: Zink Sulphate | 0.25 | 1.13 |
| B: Solubor | 0.25 | 1.15 |
| Cu: Cupper Sulphate | 0.04 | 0.11 |
| Mo: Ammonium-Mo | 0.04 | 0.07 |

**Data collected**

Plant height measurements were recorded monthly, by measuring the distance from ground level to the highest point on the plant. The number of branches on each plant as well as the total length of the branches was also recorded monthly. After 6 months the plants (leaves and stems) were cut at the soil surface level and the fresh weight was recorded. Dry mass was measured after the plants were oven dried for 48 hours at 80°C, where-after the dry plant material was sent to Bemlab laboratory to do mineral analyses. Fresh and dry weights were used to calculate the dry matter index (DMI) as follows: DMI (%) = (Dry weight ÷ Fresh weight)*100.

To determine the root dry mass, the sand was washed from the roots before drying for 48 hours at 80°C.

**Experimental design and statistical analyses**

Split plot analyses were done for each month individually with temperature as the main block factor and P concentration as the sub block factor. Shortage of space and effective cold water circulation lead to a restriction in the total amount of plants that could be used in this experiment and therefore a single plant was considered as an experimental unit. Three blocks were used in total with ten plants in a block (five different P concentrations at two root temperatures). Data was analyzed using SAS statistical software version 9.1 (SAS 2001). Student's t-Least Significant Differences

(LSD) was calculated at a 5 % level to compare the treatment means. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965).

## Results and Discussion

### Branching and plant height after different periods of growth

The temperature (T) of the root medium affected (P0.05) the number of branches (month 2-6), total branch length (month 2-6) and plant height (month 3-6) (Table 4.5). The phosphate content (P) of the nutrient solution did not affect the plant height, but had a significant (P0.05) effect on the number of branches and the total branch length as measured 5 and 6 months after transplanting. Significant (P0.05) TxP interactions were only found with regard to number of branches after 6 months of growth and the total branch length as measured after 5 and 6 months of growth. Discussion of the results will focus on these significant effects only.

*Number of branches*

The number of branches of buchu plants grown hydroponically in pots were significantly (P0.05) increased during months 2-6 due to an increase in the root temperature from 10 to 20˚C (Table 4.5). On average 70.80 branches were produced per plant after six months of growth at 20 ˚C, compared to 16.53 branches per plant at 10˚C (Figure 4.1, Table 4.6).

The P concentration in the nutrient solution affected the number of branches per plant only after 5 and 6 months of growth (Table 4.5). Six months after transplanting the number of branches increased with an increase in P concentration in the nutrient solution to reach a peak at P concentrations of 0.35 me $L^{-1}$, where-after it declined with a further increase in P concentration to 1.05 me $L^{-1}$. An unexpected and difficult to explain high value was however also found with a phosphate concentration of 1.4 me $L^{-1}$ in the nutrient solution. There was no significant difference between the number of branches of buchu plants receiving 0.35 and 1.4 me $L^{-1}$ six months after transplanting (Figure 4.2). Results obtained indicated a significant interaction between root temperature and P concentration with regard to the number of branches after 6

months of growth (Table 4.5). When grown at a root temperature of 10˚C, the number of branches of buchu plants was not affected by P concentration in the nutrient solution in this experiment (Table 4.8). Buchu plants grown at 20°C, however, showed a significant increase in the production of branches per plant as the P concentration was raised from 0 me $L^{-1}$ to 0.35 me $L^{-1}$. With further increases in P concentration to 0.70 and 1.05 me $L^{-1}$ the number of branches decreased significantly, but at concentrations of 1.4 me P $L^{-1}$ an increase was again observed.

**Table 4.5** Analyses of variance (ANOVA) of the number of branches, total branch length and plant height as affected by the temperature (T) in the root zone, P concentration (P) in the nutrient solution and the interaction between T and P

| | Factor | | | |
|---|---|---|---|---|
| | Block | Temperature (T) | P concentration (P) | T*P |
| | Pr>F | Pr>F | Pr>F | Pr>F |
| Number of branches | | | | |
| Month 1 | 0.0636 | 0.1994 | 0.5611 | 0.8646 |
| 2 | 0.1512 | 0.0328 | 0.7519 | 0.3900 |
| 3 | 0.2321 | 0.0016 | 0.6998 | 0.7528 |
| 4 | 0.9373 | 0.0186 | 0.1866 | 0.4942 |
| 5 | 0.4222 | 0.0248 | 0.0233 | 0.1839 |
| 6 | 0.3915 | 0.0065 | 0.0015 | 0.0010 |
| Total branch length | | | | |
| Month 1 | 0.0924 | 0.0515 | 0.5758 | 0.9772 |
| 2 | 0.3606 | 0.0431 | 0.9519 | 0.7127 |
| 3 | 0.4198 | 0.0091 | 0.8298 | 0.7367 |
| 4 | 0.7252 | 0.0118 | 0.3142 | 0.3352 |
| 5 | 0.5189 | 0.0332 | 0.0016 | 0.0096 |
| 6 | 0.5569 | 0.0185 | 0.0037 | 0.0056 |
| Height | | | | |
| Month 1 | 0.1314 | 0.1401 | 0.1473 | 0.8791 |
| 2 | 0.3447 | 0.1291 | 0.0799 | 0.6510 |
| 3 | 0.0026 | 0.0003 | 0.3576 | 0.7958 |
| 4 | 0.5926 | 0.0138 | 0.1752 | 0.9478 |
| 5 | 0.8258 | 0.0180 | 0.1297 | 0.8291 |
| 6 | 0.5773 | 0.0333 | 0.0836 | 0.7836 |

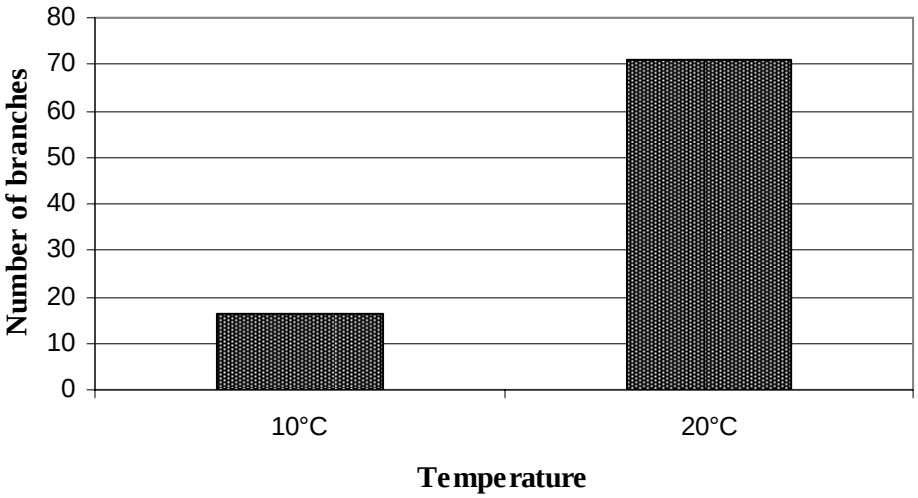


**Figure 4.1** The effect of root temperature on the number of branches produced by buchu plants (average per plant) six months after transplanting (LSD = 18.97).

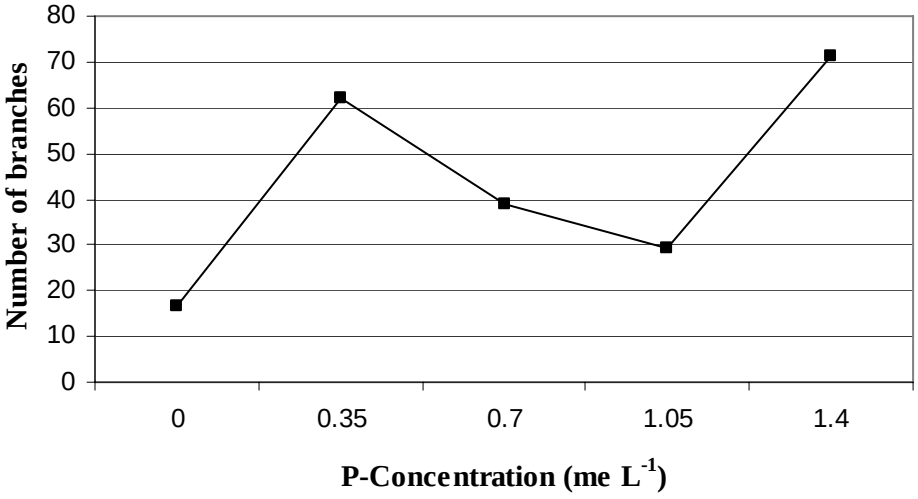


**Figure 4.2** The effect of phosphate concentration in the nutrient solution on the number of branches produced by buchu plants (average per plant) six months after transplanting (LSD = 25.08).

*Total length of branches*

The total length of branches of buchu plants grown in pots showed similar responses to the temperature of the root medium and P concentration of the nutrient solution than that recorded for the number of branches. The total length of branches from plants grown at a temperature of 20˚C was therefore significantly (P0.05) more than that of plants grown at 10˚C for all months 2-6 (Table 4.6). After a growth period of 6 months at 20˚C, a total length of 5151.67 mm was produced per plant, compared to 710.33 mm at 10˚C (Figure 4.3).

In response to increasing P concentrations in the nutrient solution, total branch length increased significantly from 0 to 0.35 me $L^{-1}$ but showed a significant decline when P concentration was increased to 1.05 me $L^{-1}$ (Table 4.7). An unexpected high value was again obtained with a P concentration of 1.4 me $L^{-1}$ (Figure 4.4). As was also shown with regard to the number of branches, total length of branches per plant did not show any significant response to P concentration when grown at 10˚C (Table 4.8). The high values found at 1.4 me P $L^{-1}$ are in direct contrast with other research that has been done on the P requirements of different *Protea spp.* Nichols (1981) found that P concentrations that are considered normal for most plants (between 1 and 2 me $L^{-1}$) can be harmful to many members of the *Proteaceae* family. He stated that 0.47 me P $L^{-1}$ is the maximum level the Protea plant can tolerate. Prassad & Dennis (1985), however, reported that levels above 0.8 me $L^{-1}$ of available P can result in satisfactory growth for *Leucadendron* grown in containers, but most researchers found best results with P concentrations between 0.12 (Montarone & Allemand, 1995) and 0.3 me $L^{-1}$ (Montarone & Ziegler, 1997). An optimum P concentration for buchu growth cannot be derived from the data from this trial due to the high values found at both P concentrations of 0.35 and 1.4 me $L^{-1}$. Further research is necessary to achieve an optimum P concentration for buchu, although the literature cited suggests that low P concentrations might be more favorable for buchu growth.

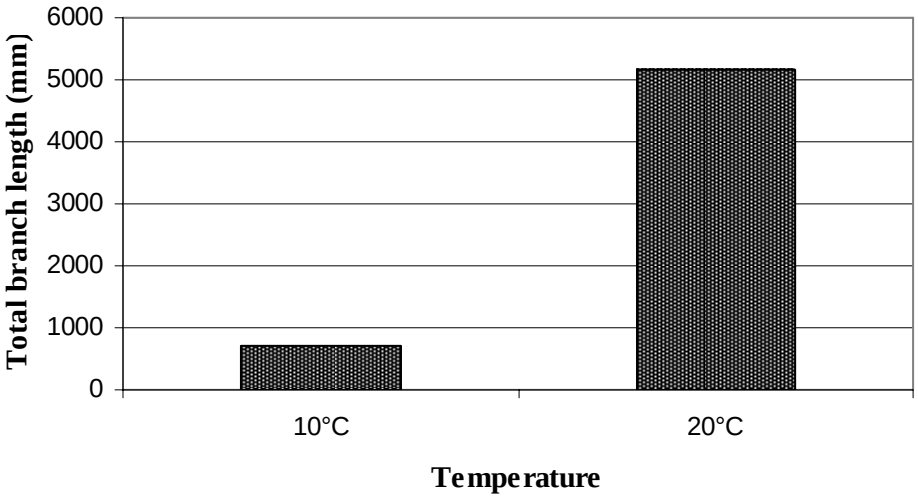


**Figure 4.3** The effect of temperature on the total branch length produced by buchu plants (average per plant) six months after transplanting (LSD = 2632.3).

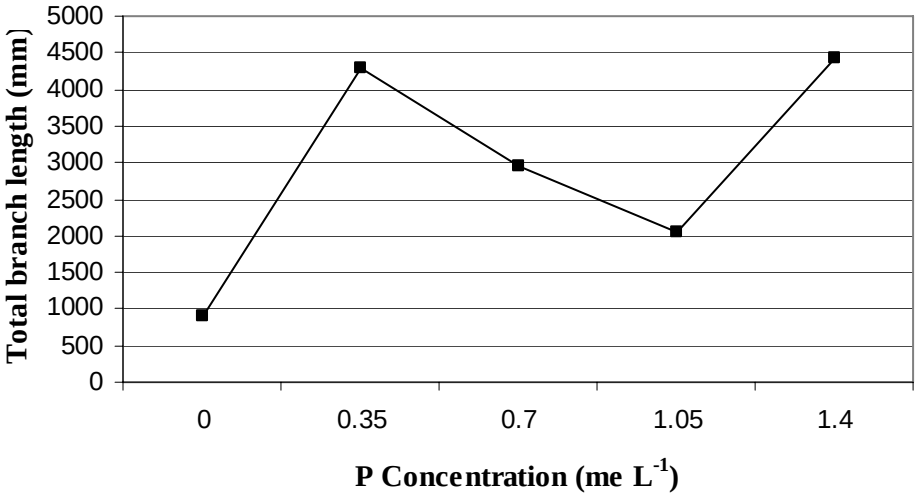


**Figure 4.4** The effect of phosphate concentration in the nutrient solution on the total branch length produced by buchu plants (average per plant) six months after transplanting (LSD = 1824).

*Plant height*

Plant height was only significantly affected as a result of temperature of the root medium after 3 to 6 months of growth (Table 4.6). High root temperatures of 20˚C resulted in increased plant heights compare to that at 10˚C, with the result that an average plant height of 261.94 mm after 6 months at 20˚C was obtained in comparison to an average plant height of 160.67 mm at 10˚C (Figure 4.5).

According to Mckersie & Leshem (1994), plants that experience chilling may exhibit a loss of vigour and reduced growth rates. This statement is supported by the poor branching and plant height of the buchu plants grown at a low root temperature.

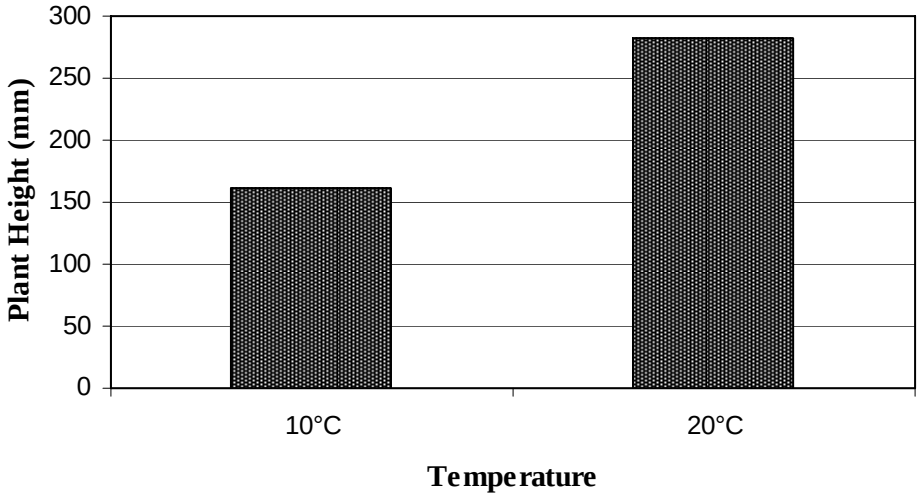


**Figure 4.5** The effect of temperature on the average plant height of buchu plants six months after transplanting (LSD = 97.86).

**Table 4.6** The effect of temperature on the average number of branches, total branch length and height of buchu plants measured during the growth period

| Month | Temperature | Number of branches | Total branch length | Height |
|---|---|---|---|---|
| | °C | | mm | |
| 1 | 10 | 1.27 a | 28.80 a | 92.93 a |
| | 20 | 1.60 a | 53.00 a | 98.87 a |
| 2 | 10 | 1.80 a | 45.00 a | 100.07 a |
| | 20 | 6.87 b | 183.67 b | 111.33 a |
| 3 | 10 | 2.87 a | 67.00 a | 106.33 a |
| | 20 | 19.00 b | 647.67 b | 139.33 b |
| 4 | 10 | 6.40 a | 166.00 a | 118.13 a |
| | 20 | 29.07 b | 1573.53 b | 170.69 b |
| 5 | 10 | 12.07 a | 443.67 a | 139.00 a |
| | 20 | 45.30 b | 3172.10 b | 221.36 b |
| 6 | 10 | 16.53 a | 710.33 a | 160.67 a |
| | 20 | 70.80 b | 5151.67 b | 261.94 b |

Means followed by the same letter do not differ significantly at P = 0.05 (LSD)



**Table 4.7** The effect of phosphate concentration on the average number of branches and total branch length produced by buchu plants after five and six months of growth

| P concentration | Number of branches | | Total branch length | |
|---|---|---|---|---|
| me $L^{-1}$ | Month 5 | Month 6 | Month 5 | Month 6 |
| 0.00 | 14.83 c | 16.50 c | 755.83 c | 913.30 c |
| 0.35 | 29.33 abc | 62.17 ab | 2248.33 a | 4301.70 a |
| 0.70 | 31.33 ab | 39.00 bc | 1915.83 ab | 2945.00 ab |
| 1.05 | 24.17 bc | 29.50 c | 1303.17 bc | 2061.70 bc |
| 1.40 | 40.00 a | 71.17 a | 2456.67 a | 4433.30 a |

Means followed by the same letter in a column do not differ significantly at P = 0.05 (LSD)

**Table 4.8** The effect of P concentration in the nutrient solution on the number of branches and total branch length produced by buchu plants at different temperatures in the root medium after six months of growth

| Temperature °C | P concentration (me $L^{-1}$) | Number of branches | Total branch length (mm) |
|---|---|---|---|
| 10 | 0.00 | 8.67 c | 298 d |
| | 0.35 | 16.33 bc | 888 d |
| | 0.70 | 35.67 bc | 1680 bcd |
| | 1.05 | 8.00 c | 223 d |
| | 1.40 | 14.00 c | 462 d |
| 20 | 0.00 | 24.33 bc | 1528 cd |
| | 0.35 | 108.00 a | 7715 a |
| | 0.70 | 42.33 bc | 4210 b |
| | 1.05 | 51.00 b | 3900 bc |
| | 1.40 | 128.33 a | 8405 a |

Means followed by the same letter in a column do not differ significantly at P = 0.05 (LSD)



**Biomass Yield**

The temperature of the root medium affected (P0.05) the fresh and dry weight, DMI and the root mass of the buchu plants, but not the root/shoot mass ratio (Table 4.9). The P concentration of the nutrient solution had a significant (P0.05) effect on the fresh and dry weight and the DMI but not on the root mass and the root/shoot mass ratio (Table 4.9). T*P interaction was only significant with regard to the fresh and dry weight produced.

**Table 4.9** Analyses of variance (ANOVA) of the wet and dry weight, dry mass index (DMI), root mass and the root to shoot mass ratio as affected by the temperature in the root zone, P concentration in the nutrient solution and the interaction between temperature and P concentration

| | Factor | | | |
|---|---|---|---|---|
| | Block | Temperature (T) | P Concentration (P) | T*P |
| | Pr>F | Pr>F | Pr>F | Pr>F |
| Fresh weight | 0.6592 | 0.0247 | 0.0014 | 0.0041 |
| Dry weight | 0.7032 | 0.0262 | 0.0340 | 0.0121 |
| DMI | 0.2437 | 0.0338 | 0.0096 | 0.4772 |
| Root mass | 0.5915 | 0.0308 | 0.2578 | 0.2386 |
| Root mass/ Shoot mass | 0.3102 | 0.3025 | 0.4248 | 0.6214 |

*Fresh and dry weight*

The fresh and dry weight of the buchu plants were significantly (P0.05) increased after six months of growth due to an increase in the root temperature from 10 to 20˚C (Table 4.10). Buchu plants grown at 20˚C produced an average fresh weight of 56.73g and dry weight of 3.26g per plant after six months, while buchu plants grown at 10˚C produced 9.69g of fresh and 0.7g of dry weight per plant (Figure 4.6).

In general, the fresh and dry weight increased with an increase in P concentration from 0.00 to 0.35 me $L^{-1}$ (Figure 4.7), where after it declined with a further increase in P concentration of 1.05 me $L^{-1}$. Similar unexpected high values as for branching were observed with a P concentration of 1.4 me $L^{-1}$ in the nutrient solution.

A significant interaction between root temperature and P concentration were observed with regard to the fresh and dry weight. When grown at a root temperature of 10˚C, the fresh and dry weight of buchu plants was not affected by P concentration in the nutrient solution in this experiment (Table 4.10). Buchu plants grown at 20°C,

however, showed a significant increase in the production of fresh and dry weight when the P concentration was raised from 0 me $L^{-1}$ to 0.35 me $L^{-1}$. With further increases in P concentration to 0.70 and 1.05 me $L^{-1}$ the fresh and dry weight decreased significantly, but at P concentrations of 1.4 me $L^{-1}$ an increase was again observed.

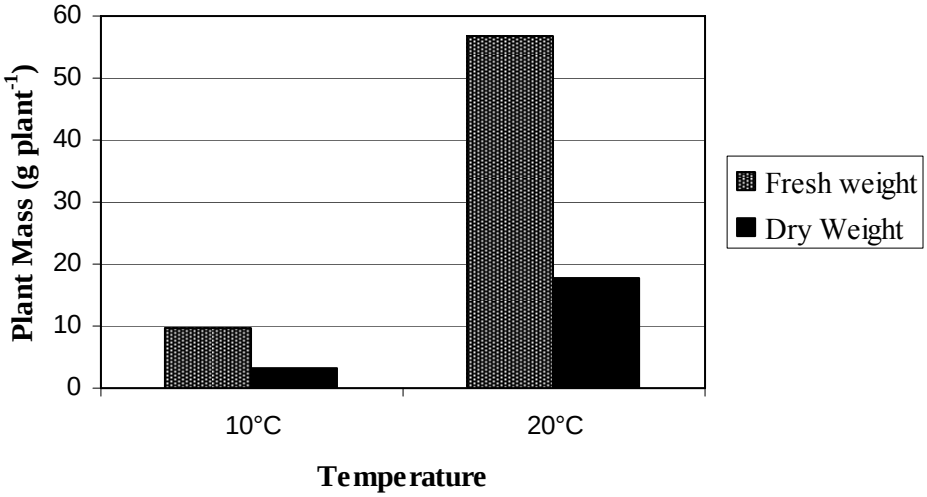


**Figure 4.6** The effect of the root medium temperature on the wet and dry weight produced by buchu plants six months after transplanting (LSD: Fresh weight = 32.39; Dry weight = 10.28).

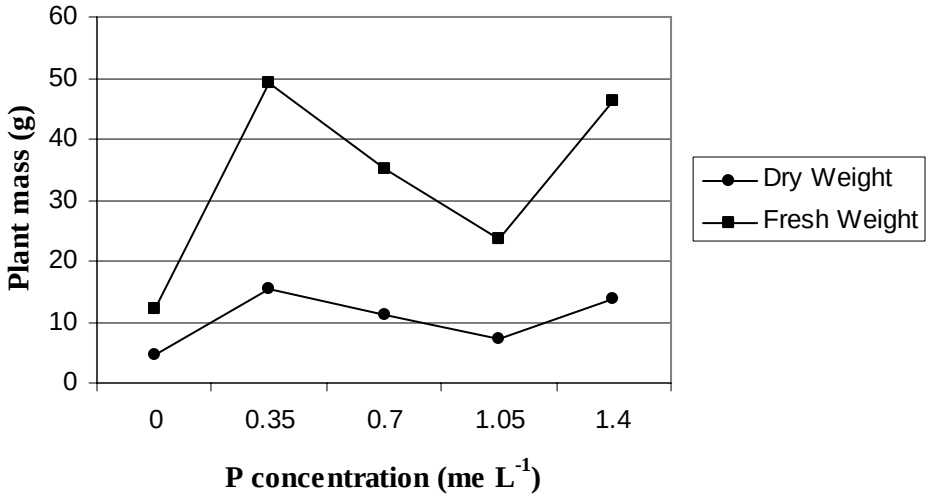


**Figure 4.7** The effect of P concentration in the nutrient solution on the fresh and dry weight produced by buchu plants 6 months after transplanting (LSD: Fresh weight = 17.12; Dry weight = 5.44).

**Table 4.10** The effect of P concentration in the nutrient solution on the fresh and dry weight produced by buchu plants at different root temperatures

| Temperature °C | P-Treatment (me $L^{-1}$) | Fresh Weight (g) | Dry Weight (g) |
|---|---|---|---|
| 10 | 0 | 5.93 c | 2.25 d |
| | 0.35 | 13.63 c | 4.87 d |
| | 0.7 | 17.94 c | 5.48 dc |
| | 1.05 | 4.38 c | 1.62 d |
| | 1.4 | 6.57 c | 2.08 d |
| 20 | 0 | 18.03 c | 7.04 dc |
| | 0.35 | 84.70 a | 26.24 a |
| | 0.7 | 52.53 b | 16.84 b |
| | 1.05 | 42.51 b | 13.06 bc |
| | 1.4 | 85.88 a | 25.45 a |

Means followed by the same letter in a column do not differ significantly at P = 0.05 (LSD)

*DMI*

Buchu plants grown at 10°C produced an average DMI of 36.6% while plants grown at 20°C produced an average DMI of 32.7% which is significantly (P0.05) lower (Figure 4.8). The DMI significantly (P0.05) decreases with an increase in P concentration in the nutrient solution from 0 me $L^{-1}$ to 0.7 me $L^{-1}$ (Figure 4.9). At 1.05 me P $L^{-1}$ the DMI increases again to a level not significantly different (P0.05) from that of 0 me P $L^{-1}$, but decreases again at a P concentration of 1.4 me $L^{-1}$.

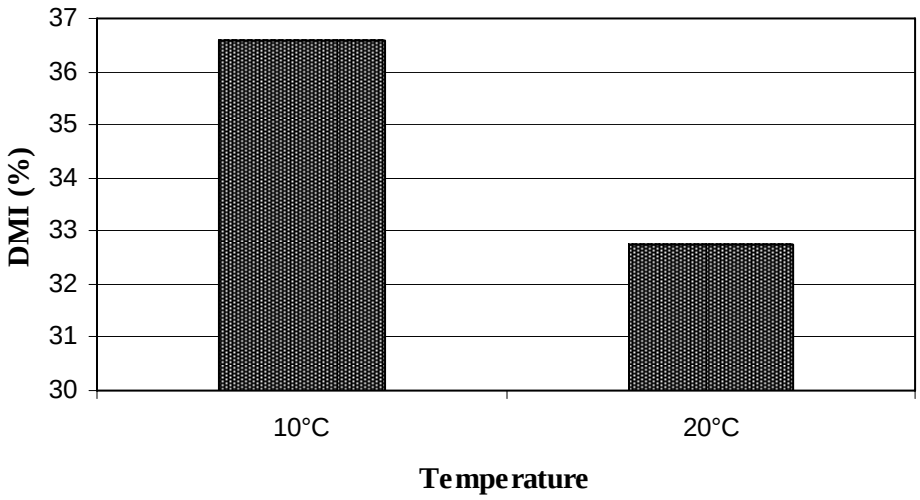


**Figure 4.8** The effect of temperature in the root medium on the dry mass index (DMI) of buchu plants six months after transplanting (LSD = 3.12).

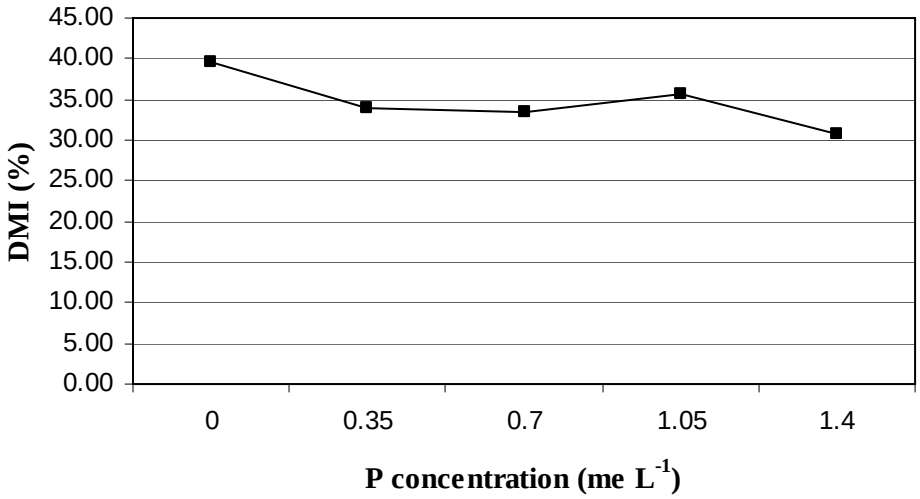


**Figure 4.9** The effect of P concentration in the nutrient solution on the dry mass index (DMI) of buchu plants 6 months after transplanting (LSD = 4.4).

*Root dry mass*

The root mass produced by the buchu plants grown at 20°C was significantly (P0.05) higher than the root mass of plants grown at 10°C. On average 7.12g of dry root mass were produced per plant grown at 20°C compared to1.36g per plant at 10°C (Figure 4.10).

Cooper (1973), who reviewed research that has been done on the effects of temperature on plants, concluded that a general decrease in root and shoot dry weight of plants was observed at root temperatures below 25°C. This tendency was also shown in this experiment.

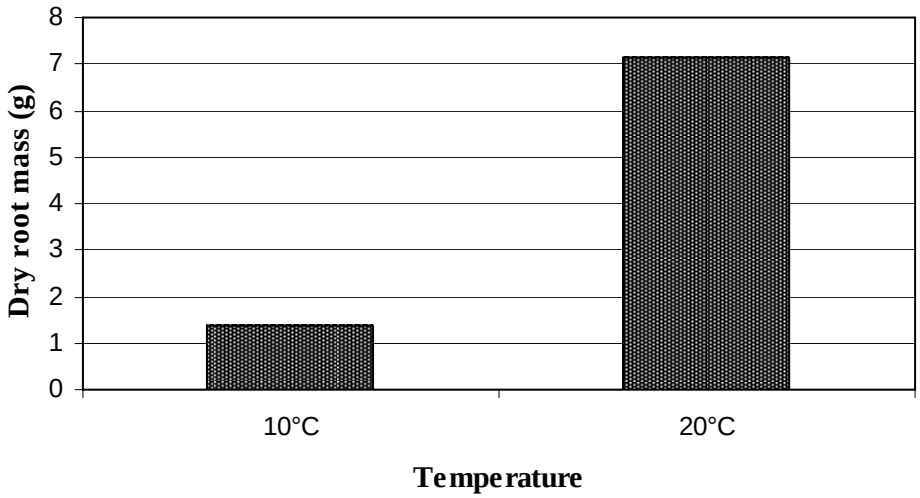


**Figure 4.10** The effect of root temperature on the root dry mass produced by buchu plants 6 months after transplanting (LSD = 4.46).

**Mineral analyses**

Temperature and P concentration had no significant effect on the Calcium (Ca), Magnesium (Mg), Copper (Cu) and Iron (Fe) content of the plants, but both temperature and P concentration affected the Nitrogen (N) and Potassium (K) content of the plant while the T*P interaction had a significant effect on the Phosphorus (P), Calcium (Ca), Manganese (Mn) and Zink (Zn) content of the plants. Temperature had a significant effect on Sodium (Na) and Boron (B) and P concentration affected the P, Mn and Zn content of the plants (Table 4.11).

A positive correlation between elements with high mobility (N, P and K) was found as the P concentration in the nutrient solution was increased from 0 me $L^{-1}$ to 1.4 me $L^{-1}$ (Figures 4.13-14, Figure 4.16). Cecil *et al.* (1995) found a positive correlation between N, P and K in *Leucadendron* during seasonal trends in mineral concentrations. He also found that N, P and K were negatively correlated with elements of low mobility e.g. Ca, Mg, Fe and Mn. This negative correlation was however not found in this experiment. Discussion of the results will focus on the significant effects only and a complete mineral analysis is given in the Addendum (6.15-6.16).

**Table 4.11** Analyses of variance (ANOVA) of the chemical composition of the buchu plants as affected by the temperature (T) in the root zone, P concentration (P) in the nutrient solution and the interaction between T and P

| | | Factor | | |
|---|---|---|---|---|
| | Block | Temperature (T) | P Concentration (P) | T*P |
| Mineral | Pr>F | Pr>F | Pr>F | Pr>F |
| N | 0.186 | 0.0132 | 0.0218 | 0.818 |
| P | 0.4125 | 0.1049 | <0.0001 | 0.0115 |
| K | 0.4066 | 0.0312 | 0.0097 | 0.0667 |
| Ca | 0.1114 | 0.0718 | 0.0725 | 0.0040 |
| Mg | 0.6490 | 0.2058 | 0.4462 | 0.6534 |
| Na | 0.0748 | 0.0034 | 0.6077 | 0.5619 |
| Mn | 0.8529 | 0.7695 | 0.0003 | 0.0271 |
| Fe | 0.3496 | 0.1311 | 0.0763 | 0.3301 |
| Cu | 0.9946 | 0.7007 | 0.6203 | 0.6663 |
| Zn | 0.6784 | 0.8174 | <0.0001 | 0.0006 |
| B | 0.0465 | 0.0021 | 0.0607 | 0.0956 |

*Nitrogen*

Plants grown at root temperatures of 20°C had a significantly higher (P0.05) N content (1.42%) than plants grown at 10°C (1.06%) as can be seen in Figure 4.12. A general increase in the N content of the plants was found with an increase in P concentration in the nutrient solution (Figure 4.13). Between concentrations of 0 me P $L^{-1}$ and 1.05 me P $L^{-1}$ the increase in N content was not significant (P0.05), but at a P concentration of 1.4 me $L^{-1}$ the N-content of the plants was significantly higher (P0.05) than at 0 me $L^{-1}$.

The N content of one year old buchu plants growing in its natural habitat was found to be between 0.60% and 0.68% (Unpublished data, ASNAPP, 2007). The N content of the leaves in this experiment was found to be higher, even at low temperatures. The reason for the high N content of the buchu plants might be due to the fact that the plants were still actively growing during the harvest.

Marschner (1995) reported that N deficiencies in plants will lead to stunted growth. Plants grown at 10°C in this study, clearly displayed stunted growth which could among others, be the result of inhibited N uptake by the plants due to the cold root temperature.

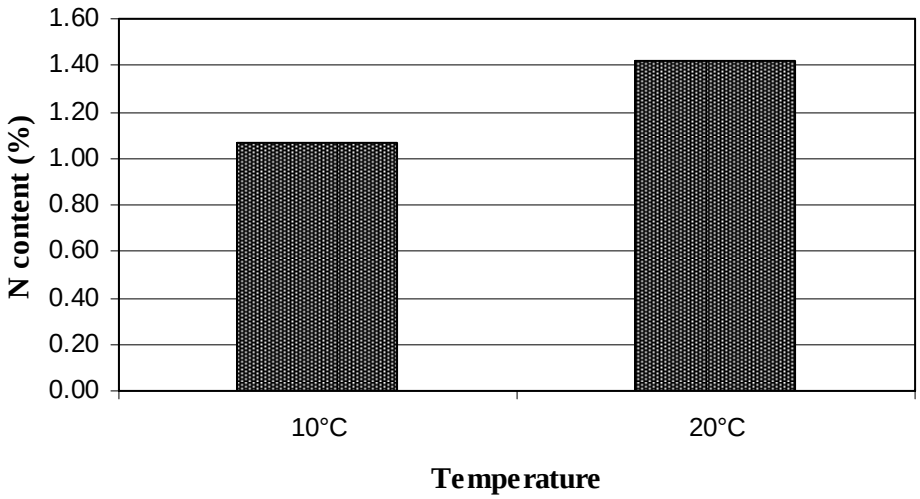


**Figure 4.12** The effect of root medium temperature on the N content of the buchu plants (LSD = 0.18).

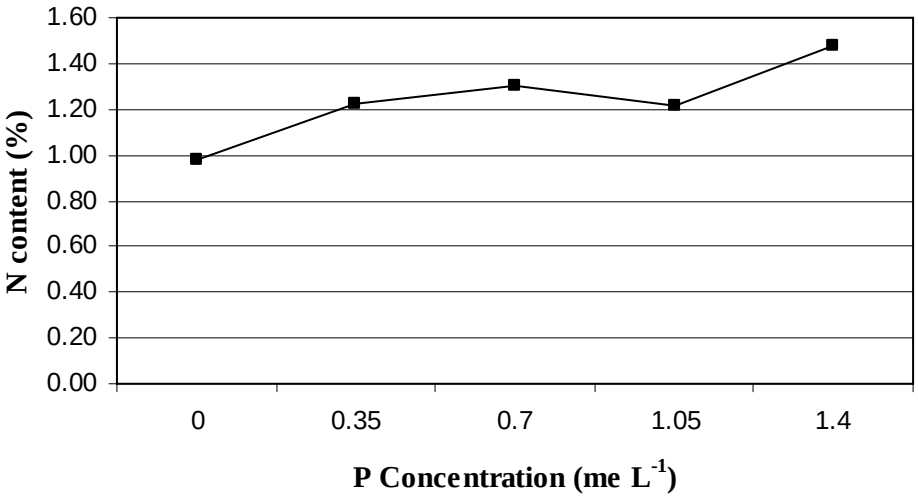


**Figure 4.13** The effect of P concentration in the nutrient solution on the N content of the buchu plants (LSD = 0.27).

*Phosphorus*

The significant interaction between root temperature and P indicated that response to different P concentrations in the nutrient solution was not the same when buchu was grown at a root temperature of 10°C compared to 20°C (Figure 4.14). Buchu plants grown at 20°C showed a significant (P0.05) increase in P content of the plants as the P concentration increased from 0 me $L^{-1}$ to 1.4 me $L^{-1}$ in the nutrient solution (Figure 4.14). Buchu plants grown at 10°C also indicated a significant increase in plant P content when the P concentration was increased from 0 me $L^{-1}$ to 0.7 me $L^{-1}$, but with further increases in P concentrations to 1.05 me $L^{-1}$, plant P content tend to decrease (Figure 4.14). Between concentrations of 1.05 and 1.4 me P $L^{-1}$ the P content of the plants, however increased again.

A P content of 0.04% was found in one year old buchu plants growing in its natural habitat (Unpublished data, ASNAPP, 2007). Buchu plants grown at 20°C and irrigated with nutrient solution containing no P had similar low values, but all the other P treatments had higher P contents. According to Cresswell (1991) a P content of 0.15 to 0.42% in *Leucadendron* cultivars can be described as desirable. The P contents of all the buchu plants fall in this category except for the plants grown at 20°C and receiving no P in the nutrient solution.

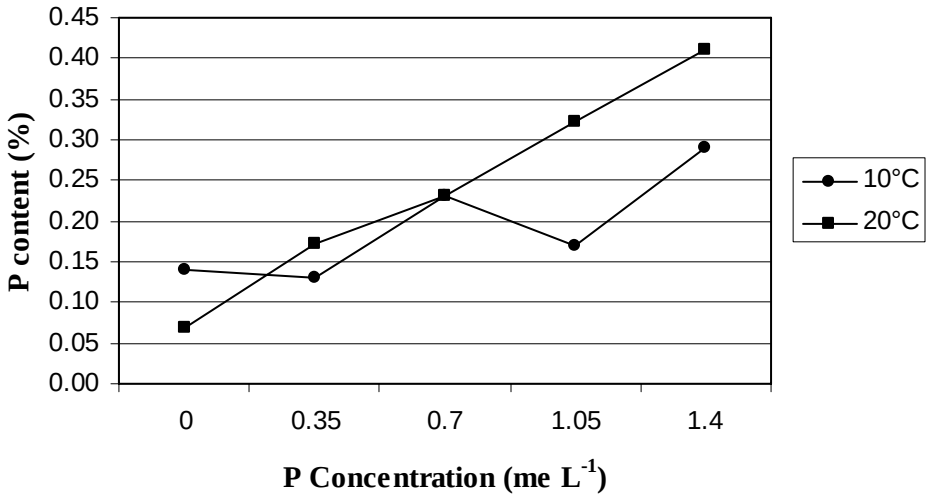


**Figure 4.14** The effect of P concentration in the nutrient solution on the P content of buchu plants at different temperatures (LSD = 0.08).

*Potassium*

Plants grown at 20°C had on average a K content of 2.13%, that was significantly higher (P0.05) compared to the 1.32% of plants grown at 10°C (Figure 4.15). According to Bennet (1993) K is very important in the water relations of the plant and K deficiency will lead to retarded growth. This might also be a reason for buchu plants to grow less vigorously when the root medium is cooled down. A significant increase (P0.05) in plant K content was observed with an increasing P concentration in the nutrient solution from 0 me $L^{-1}$ to 1.4 me $L^{-1}$ (Figure 4.16).

The K content of buchu plants growing in its natural habitat varied between 0.23 and 0.63% (Unpublished data, ASNAPP, 2007). The K content of Buchu plants in this experiment was found to be much higher than these values (Figure 4.15-4.16).

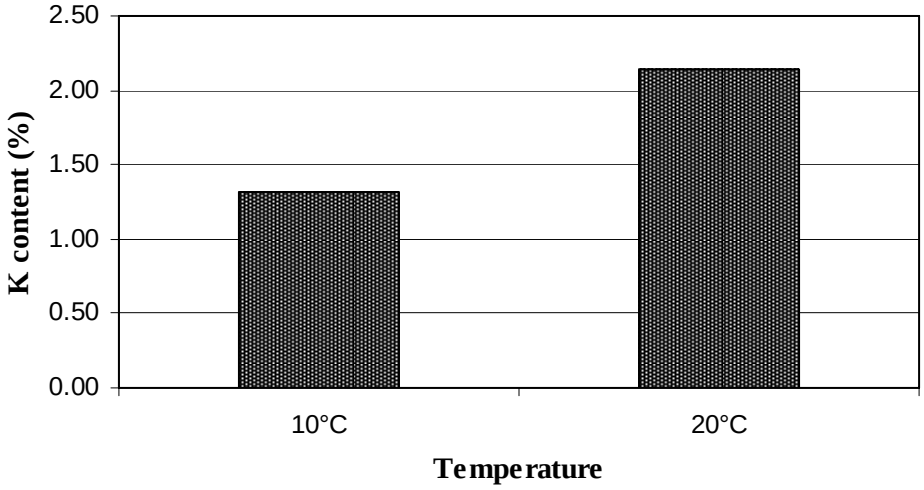


**Figure 4.15** The effect of root medium temperature on the K content of buchu plants (LSD = 0.63).

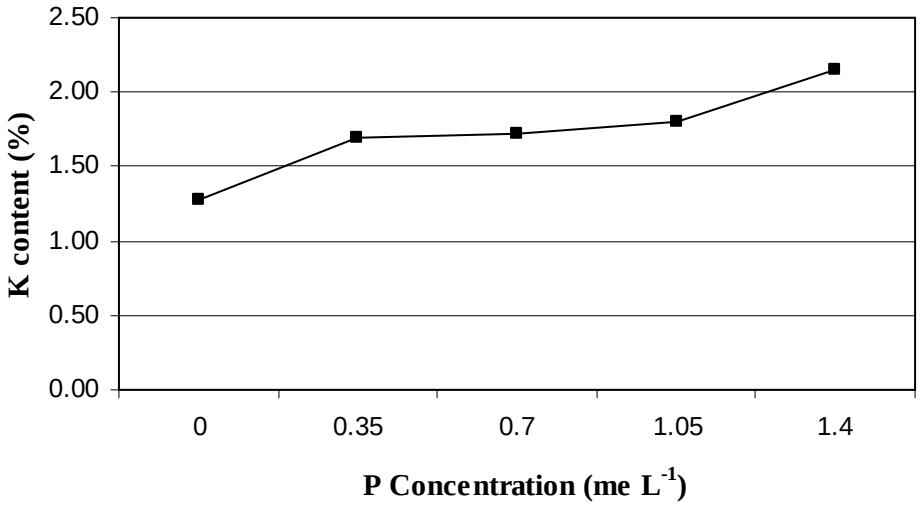


**Figure 4.16** The effect of P concentration in the nutrient solution on the K content of buchu plants (LSD = 0.42).

*Calcium*

Although plants receiving 0 me P $L^{-1}$ had a significantly lower (P0.05) Ca content than plants receiving 0.35, 1.05 and 1.4 me P $L^{-1}$, no clear trend with regard to Ca content of buchu plants was found at 20°C. No significant differences in Ca content were induced by P treatments in buchu plants grown at 10°C (Figure 4.17).

Values of Ca content found in this experiment are similar to values of Ca content in buchu plants growing in its natural habitat, which was found to be between 0.25 and 1.0% (Unpublished data, ASNAPP, 2007).

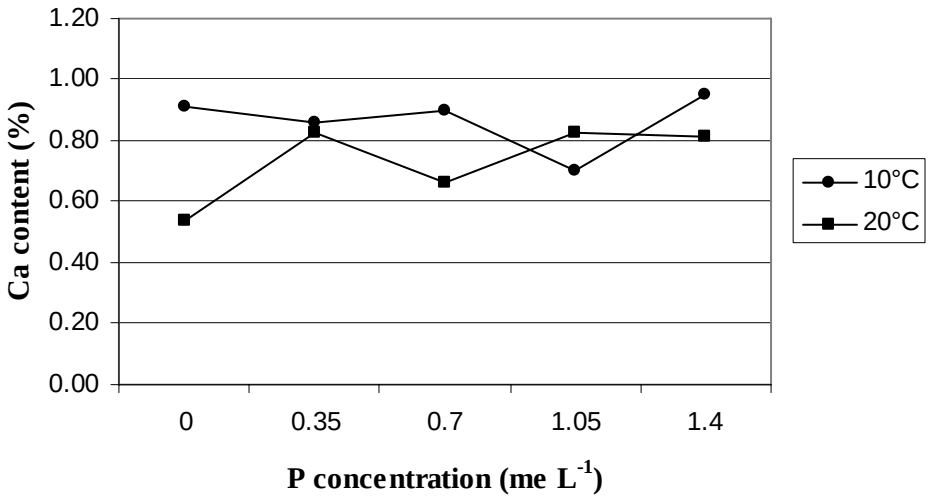


**Figure 4.17** The effect of P concentration in the nutrient solution on the Ca content of buchu plants at different root temperatures (LSD = 0.17).

*Sodium*

Phosphorus concentration of the nutrient solution did not affect (P0.05) the Na content of the buchu plants, but plants grown at 10°C contained only 584.47 mg Na $kg^{-1}$ dry plant material compared to 879.80 mg Na $kg^{-1}$ of plants grown at 20°C. Na contents varying between 920 mg $kg^{-1}$ and 3931 mg $kg^{-1}$ were obtained from buchu growing in its natural habitat and the Na contents observed in this trial at low temperatures are much lower than these values.

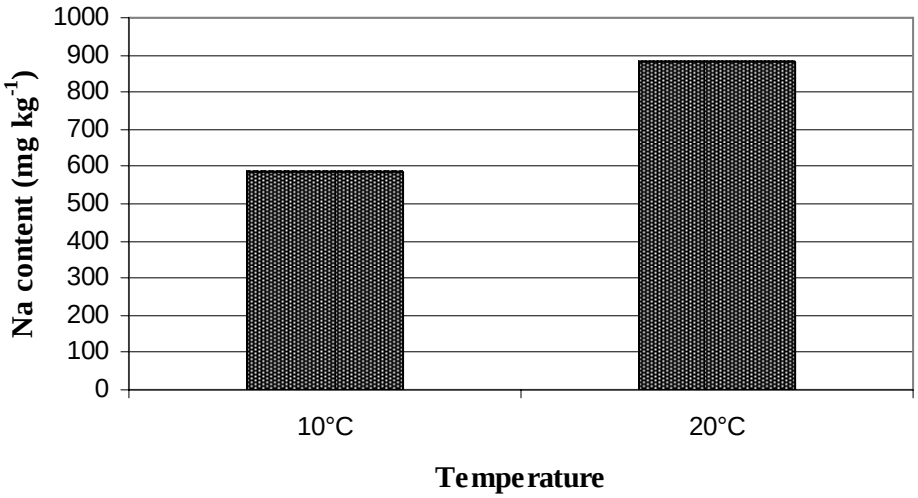


**Figure 4.18** The effect of root medium temperature on the Na content of buchu plants (LSD = 73.57)

*Manganese*

Buchu plants grown at different temperatures responded differently to P concentrations in the nutrient solution in terms of Mn content of the plant tissue. Plants grown at 10°C were not significantly affected by an increase in P concentration from 0 me $L^{-1}$ to 0.7 me $L^{-1}$, but showed a significant reduction in Mn content, followed by an increase when P concentration was increased from 0.7 to 1.05 and to 1.4 me P $L^{-1}$ (Figure 4.19). This trend is difficult to explain as plants grown at 20°C showed no significant difference in Mn content as the P concentration increased.

According to Jones (1991) the sufficiency range of Mn for most plant species ranges between 20 and 500 mg $kg^{-1}$. The Mn content of one year old buchu leaves growing in its natural habitat was found to be between 179 mg $kg^{-1}$ and 183 mg $kg^{-1}$ (Unpublished data, ASNAPP, 2007).

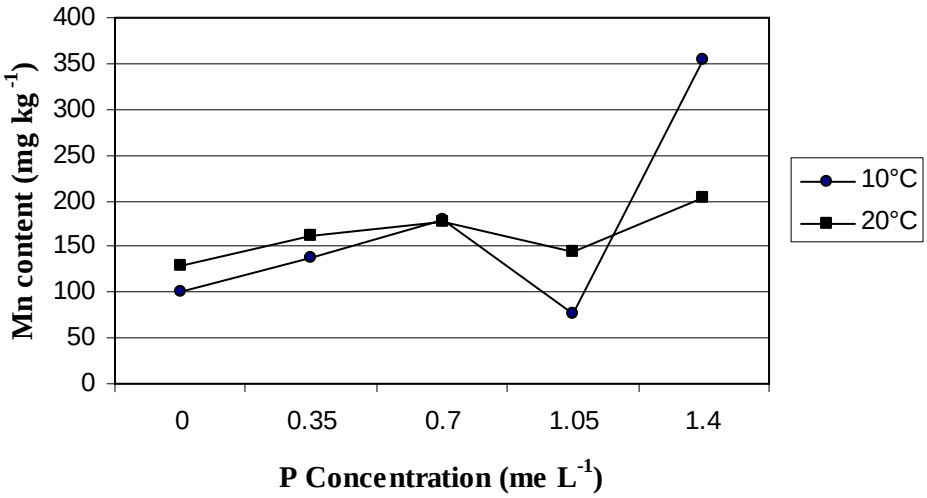


**Figure 4.19** The effect of P concentration in the nutrient solution on the Mn content of buchu plants at different root temperatures (LSD = 93.29).

*Zinc*

Although P concentration in the nutrient solution had a significant effect on the Zn content of the buchu plants (P0.0001), this response was affected by root temperature as well (Figure 4.20). Plants grown at 10°C showed a significant increase in Zn content as the P concentration increased from 0 me $L^{-1}$ to 0.35 me $L^{-1}$ to reach a maximum Zn content of 37.67 mg $kg^{-1}$ at 0.35 me P $L^{-1}$. The Zn content decreased significantly again between P concentrations of 0.35 and 1.05 me $L^{-1}$, but between 1.05 and 1.4 me P $L^{-1}$ the Zn content increased significantly again to reach a Zn content of 33.67 mg $kg^{-1}$.

Plants grown at 20°C showed higher Zn contents at P concentrations of 0 and 0.35 me $L^{-1}$ than at 0.7, 1.05 and 1.4 me $L^{-1}$ (Figure 4.20). For most plants Zn deficiencies are likely to occur at Zn concentrations lower than 20 mg $kg^{-1}$.

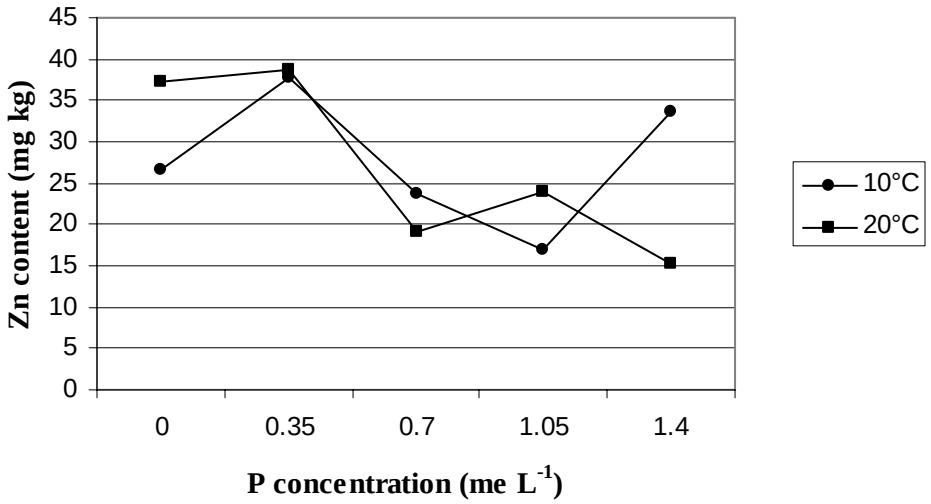


**Figure 4.20** The effect of P concentration in the nutrient solution on the Zn content of buchu plants at different root temperatures (LSD = 8.10).

*Boron*

Boron content of buchu plants were significantly (P0.05) affected by root temperature. At 20°C the B content was 42.62 mg $kg^{-1}$ on average, compared to a B content of 27.07 mg $kg^{-1}$ at 10˚C.

B contents varying between 20 and 50 mg $kg^{-1}$ were found for buchu growing in its natural habitat (Unpublished data, ASNAPP, 2007) and similar values were found in this trial. The plants in this experiment were still actively growing when it was harvested and the contents of some minerals (N, P, K and Zn) of the buchu plants were found to be higher in this trial than in its natural habitat. Although the B contents recorded in this experiment are similar to B contents of buchu growing in its natural habitat the B contents of buchu plants in this experiment might still be too low, especially at low temperatures.

According to Marshner (1995) B plays a role in cell development as well as in sugar and starch formation and translocation. A deficiency will for this reason retard new growth and development and the lower B contents found at low temperatures in this study might have contributed to the significantly (P0.05) reduced number of branches and branch length obtained with plants grown at root temperatures of 10˚C compared to 20°C.

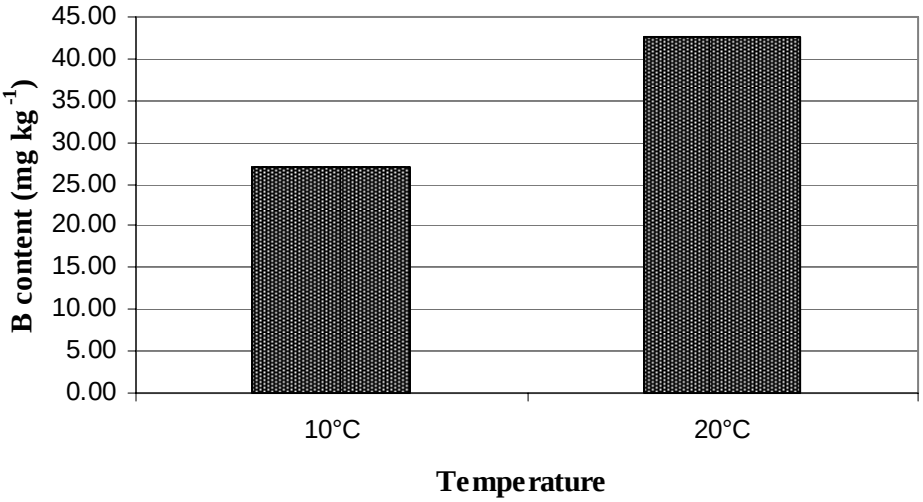


**Figure 4.21** The effect of root medium temperature on the B content of buchu plants (LSD = 3.10).

**Conclusion**

It is clear that temperature plays a significant role in the growth and development of buchu plants. Plants grown at root temperatures of 10°C in this study, indicated less vigorous growth and produced less fresh and dry weight than plants grown at 20°C. Low temperatures have a negative effect on various plant processes including photosynthesis and respiration (Andreenko & Kerechki, 1966 in Cooper, 1973), water absorption (Kuiper, 1964 in Cooper 1973) and water movement in plants (O`Leary, 1966 in Cooper 1973). To add to these straining effects on plant growth, low root temperature may also reduce the absorption of many important minerals including N, K, Na and B (Marschner, 1995). All these factors may have contributed to the observed reduced growth at 10˚C.

A phosphorus concentration of 0.35 me $L^{-1}$ in the nutrient solution resulted in more vigorous branching and dry matter production of buchu when compared to P concentrations of 0 me $L^{-1}$. Montarone & Ziegler (1997) and Nichols (1981) recommended low P-levels of respectively 0.47 and 0.3 me $L^{-1}$ in the nutrient solution for certain Protea *spp.* From this experiment it is difficult to suggest optimal P concentrations, because of the unanticipated and difficult to explain increase in branch

number, total branch length and dry matter production at P concentrations of 1.4 me $L^{-1}$. This vigorous growth at high P concentrations is in contrast to the results of Nichols (1981), Montarone & Allemand (1995) and Montarone & Ziegler (1997). The most probable explanation for this contradiction in results may be the limited number of plants (one), used as an experimental unit in this experiment. Plants propagated from seeds harvested from buchu plants growing wild in nature, as was done in this experiment, showed large variation between plants due the lack of genetic uniformity. More plants per experimental unit or more replications should have been used to reduce the effect of variation between plants. This was however not possible due to space limitations in the glasshouse.

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# CHAPTER 5
# GENERAL CONCLUSIONS

In the past buchu has mainly been harvested in its natural habitat, but the high demand for buchu oil has lead to many commercial buchu plantations as well as producing buchu hydroponically. No research has yet been done on the production of buchu in intensive production systems and the lack of knowledge can be a serious limitation for producers. ASNAPP (Agribusiness in Sustainable Natural African Plant Products) has started a program in order to formulate a nutrient solution optimal for buchu growth, starting with finding an optimum pH and EC.

High P concentrations in the root medium of Proteas have a negative and toxic effect on its growth (Nichols, 1981) and since buchu and Proteas are found in regions of South Africa with similar soil (nutrient poor, acidic) and climatic conditions (Heinsohn & Pammenter, 1986), it is possible that the same can be true for buchu plants. It is important to determine optimal concentrations and ratios of all the nutrients in the nutrient solution especially the P concentration since research proved the negative effect of high P on Proteas. Two separate experiments were done to determine the effect of P levels in the nutrient solution on buchu growth.



The first experiment was done in a climatically controlled greenhouse and the objective of this experiment was to determine the effect of increasing P concentrations in the nutrient solution on the general growth, biomass production, oil composition, mortality rate and chemical composition of the buchu plants. Five nutrient solutions with different P concentrations varying from 0 me $L^{-1}$ to 1.4 me $L^{-1}$ were tested. The pH and EC of the different nutrient solutions were the same as well as the N: S ratio in order to accommodate the increasing P concentrations in the nutrient solution. Height measurements were taken monthly and plants were harvested on a three monthly basis to determine the fresh and dry weight, as well as the chemical composition. The final harvest took place after nine months where all the plants were harvested and the essential oil were extracted and analyzed.

It was found that the P concentration in the nutrient solution had no significant effect on plant height during the experiment although differences in plant height started to emerge after six months. It appears as if buchu plants receiving 0.7 me P $L^{-1}$ in the nutrient solution were taller than plants receiving higher and lower P concentrations. Plants receiving 0.7 me P $L^{-1}$ also produced a significantly higher fresh weight than all the other P treatments after nine months. The increase in P concentration in the nutrient solution has lead to a general increase in N, P, K, Ca, Mg and B content in the buchu plants and a decrease in Fe content. Most of the mineral concentrations found in the buchu plants in this experiment were higher than mineral concentrations of buchu plants growing in its natural habitat.

The mortality rate of the buchu plants also increased with an increase in P concentration in the nutrient solution. The cause of this observation is not clear and it can be speculated that the high P concentration in the nutrient solution caused an imbalance and/or toxically high mineral content in the buchu plants. The decrease in Fe content of the buchu plants observed with an increase in P concentration in the nutrient solution may also have played a role in the high mortality rates found at high P concentrations and typical Fe deficiency symptoms like stunted growth, yellowing and loss of leaves (Barry, 1996) were observed during this trial (Addendum, Plate 6.2-6.3).



The different P concentrations in the nutrient solution only made a significant difference on one major component of the buchu oil which was Ψ-Diosphenol. No general trend with Ψ-Diosphenol content and P concentration could be found and the significant difference in Ψ-Diosphenol may only have been due to variation between the plants. The overall essential oil quality was poor due to the high pulegone (13.37% to 16.31% on average) content (Addendum, Table 6.9).

The second experiment was done in a mechanical temperature controlled glasshouse and the aim of this experiment was to determine the response of buchu to increasing concentrations of P at different temperatures. The temperature of the glasshouse was kept at a day/night temperature of 20/15°C. The plants in this trial were subjected to two different root temperatures. For the cold root treatment a water bath was used to

cool the water to temperatures between 6°C and 10°C. This cold water was then pumped into glass spirals running through the pots, cooling down the rooting medium in the pots to temperatures between 6°C (night) and 14°C (day), the average temperature being 10°C. For the control temperature treatment the containers were kept at room temperature and the root temperature inside the pots varied between 14°C (night) and 26°C (day), the average being 20°C. The same nutrient solutions were used as in the greenhouse experiment to determine the effect of increasing P concentrations in the nutrient solution. Each month the plant height, number of branches and total branch length was taken to compare the effect of the different P treatments. After six months the plants were harvested and the fresh-, dry- and root weight were determined as well as the chemical composition of the plants.

It was found that the temperature had a significant effect on the branching, plant height, yield, root mass and chemical composition of the plants. The buchu plants grown at the higher temperature (20°C) produced more branches, taller plants and a higher total branch length than the plants grown at lower (10°C) temperatures. A significant increase in fresh, dry and root weight was observed at 20°C compared to plants grown at 10°C. The buchu plants grown at 20°C had significantly higher N, K, Na and B contents than plants grown at 10°C and significant interactions between temperature and P concentration were found for the P, Ca, Mn and Zn contents of the plants.

A peak in the production of branches and the total branch length was found at P concentrations of 0.35 me $L^{-1}$ and 1.4 me $L^{-1}$ in the nutrient solution of plants grown at 20°C. The yield (fresh and dry weight) of the buchu plants grown at 20°C also displayed peaks at 0.35 me P $L^{-1}$ and 1.4 me P $L^{-1}$. Buchu plants grown at 10°C showed no significant response in terms of growth and yield to the P concentration in the nutrient solution. The peak observed in the plants growth at high P concentrations is unexplainable and in direct contrast with research done by Nichols (1981), Montarone & Allemand (1995) and Montarone & Ziegler (1997), who found that Proteas respond best to low P concentrations. The unexpected high values found at high P concentrations might be due to the lack of genetic uniformity together with the limitation of the plants per experimental unit and/or amount of replications. The

increase in P concentration in the nutrient solution caused a general increase in N, P and K content in the plants and also affected the Mn and Zn content, but no general trend could be found between the P concentration in the nutrient solution and the Mn and Zn content in the plants.

Several interactions between P and temperature were observed in this trial and it can be concluded that the temperature in the root zone affected the response of buchu plants to different P concentrations in the nutrient solution. The P concentration in the nutrient solution had for example no significant effect on plants grown at a root temperature of 10°C in terms of growth and yield. Plants grown at a root temperature of 20°C in the glasshouse exhibited best results at 0.35 me P $L^{-1}$ and 1.4 me P $L^{-1}$ in the nutrient solution, while buchu plants in the greenhouse displayed optimum growth at a P concentration of 0.7 me P $L^{-1}$ in the nutrient solution. The results of this experiment point out that buchu plants may react differently towards P at different temperatures.

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# CHAPTER 6
# ADDENDUM

**Plate 6.1** The layout of the water cooled, plastic cladded greenhouse experiment. Each row running down the length of the greenhouse represented a replication.

**Plate 6.2** Visible yellowing and loss of leaves of buchu plants in the plastic cladded greenhouse experiment receiving high P concentrations (1.4 me $L^{-1}$) in the nutrient solution after 3 months.

**Plate 6.3** Visible yellowing and loss of leaves of buchu plants after 7 months in the plastic cladded greenhouse experiment receiving high P concentrations (1.4 me $L^{-1}$) in the nutrient solution.

**Table 6.1** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the plastic cladded greenhouse experiment for the treatment where no P was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.2 | 1.02 | 0.00 | 2.95 |
| 2 | 6.2 | 1.03 | 0.00 | 2.56 |
| 3 | 6.4 | 1.07 | 0.00 | 2.84 |
| 4 | 5.4 | 1.03 | 0.16 | 2.68 |
| 5 | 5.4 | 1.05 | 0.16 | 3.17 |
| 6 | 5.0 | 1.05 | 0.00 | 3.09 |
| 7 | 5.3 | 1.09 | 0.00 | 2.68 |
| 8 | 5.4 | 1.11 | 0.00 | 2.60 |
| 9 | 5.5 | 1.10 | 0.00 | 2.52 |

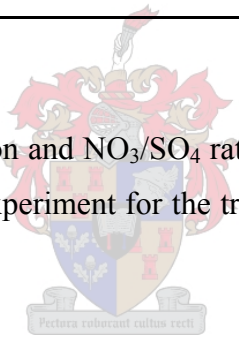

**Table 6.2** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the plastic cladded greenhouse experiment for the treatment where 0.35 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.2 | 1.01 | 0.29 | 2.98 |
| 2 | 6.2 | 1.01 | 0.31 | 2.64 |
| 3 | 6.1 | 1.02 | 0.31 | 2.93 |
| 4 | 5.3 | 1.10 | 0.40 | 2.87 |
| 5 | 4.8 | 1.09 | 0.41 | 3.15 |
| 6 | 4.7 | 1.03 | 0.32 | 3.20 |
| 7 | 5.3 | 1.07 | 0.32 | 2.60 |
| 8 | 5.3 | 1.11 | 0.33 | 2.58 |
| 9 | 5.5 | 1.13 | 0.33 | 2.56 |

**Table 6.3** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the plastic cladded greenhouse experiment for the treatment where 0.7 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.0 | 0.99 | 0.65 | 2.89 |
| 2 | 5.8 | 1.01 | 0.65 | 2.55 |
| 3 | 6.1 | 1.01 | 0.63 | 2.85 |
| 4 | 5.2 | 1.12 | 0.74 | 2.88 |
| 5 | 5.1 | 1.11 | 0.75 | 3.15 |
| 6 | 4.7 | 1.01 | 0.64 | 3.09 |
| 7 | 5.2 | 1.07 | 0.66 | 2.64 |
| 8 | 5.2 | 1.09 | 0.66 | 2.56 |
| 9 | 5.4 | 1.11 | 0.68 | 2.54 |

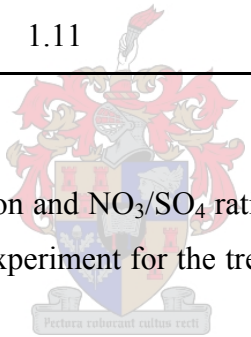

**Table 6.4** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the plastic cladded greenhouse experiment for the treatment where 1.05 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 5.8 | 0.98 | 0.96 | 2.87 |
| 2 | 5.8 | 1.01 | 1.04 | 2.72 |
| 3 | 6.1 | 1.00 | 0.95 | 2.95 |
| 4 | 5.4 | 1.10 | 1.18 | 3.00 |
| 5 | 5.2 | 1.11 | 1.17 | 3.18 |
| 6 | 4.9 | 1.00 | 0.98 | 3.15 |
| 7 | 4.8 | 1.06 | 0.99 | 2.78 |
| 8 | 4.9 | 1.10 | 1.04 | 2.58 |
| 9 | 5.4 | 1.09 | 1.01 | 2.69 |

**Table 6.5** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the plastic cladded greenhouse experiment for the treatment where 1.40 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 5.7 | 0.96 | 1.25 | 2.78 |
| 2 | 6.0 | 1.03 | 1.40 | 2.83 |
| 3 | 6.0 | 0.97 | 1.27 | 3.00 |
| 4 | 5.3 | 0.99 | 1.43 | 2.45 |
| 5 | 5.2 | 1.03 | 1.44 | 3.00 |
| 6 | 4.9 | 0.97 | 1.31 | 3.19 |
| 7 | 4.9 | 1.02 | 1.32 | 2.85 |
| 8 | 5.0 | 1.08 | 1.36 | 2.53 |
| 9 | 5.3 | 1.08 | 1.35 | 2.56 |

**Table 6.6** Monthly pH and EC (mS $cm^{-1}$) measurements of the different P treatments recorded in the drainage water of the plastic cladded greenhouseexperiment

| | P-concentration (me $L^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.00 | | 0.35 | | 0.70 | | 1.05 | | 1.40 | |
| Month | pH | EC | pH | EC | pH | EC | pH | EC | pH | EC |
| 1 | 7.8 | 1.20 | 7.7 | 1.15 | 7.7 | 1.27 | 7.7 | 1.21 | 7.6 | 1.05 |
| 2 | 7.8 | 1.18 | 7.7 | 0.94 | 7.7 | 1.05 | 7.6 | 0.95 | 7.6 | 0.98 |
| 3 | 7.5 | 1.52 | 7.7 | 1.47 | 7.6 | 1.34 | 7.4 | 1.50 | 7.1 | 1.26 |
| 4 | 7.2 | 1.42 | 7.3 | 1.37 | 7.2 | 1.24 | 7.3 | 1.31 | 7.1 | 1.28 |
| 5 | 7.2 | 1.47 | 7.3 | 1.51 | 7.2 | 1.38 | 7.3 | 1.41 | 7.0 | 1.44 |
| 6 | 7.0 | 1.39 | 7.0 | 1.34 | 6.9 | 1.37 | 7.0 | 1.32 | 6.9 | 1.38 |
| 7 | 6.9 | 1.52 | 6.9 | 1.49 | 7.0 | 1.50 | 6.9 | 1.56 | 6.8 | 1.45 |
| 8 | 6.8 | 1.54 | 6.6 | 1.44 | 6.8 | 1.54 | 6.7 | 1.43 | 6.5 | 1.50 |
| 9 | 6.7 | 1.44 | 6.7 | 1.36 | 6.8 | 1.40 | 6.8 | 1.45 | 6.7 | 1.37 |

**Table 6.7** Mineral analyses (macro nutrients) of buchu plants in the plastic cladded greenhouse experiment after 3, 6 and 9 months as affected by the P concentration in the nutrient solution

| | | P concentration (me $L^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Mineral | Months | 0.00 | 0.35 | 0.70 | 1.05 | 1.40 |
| N (%) | 3 | 1.59 | 1.77 | 1.79 | 1.60 | 1.86 |
| | 6 | 1.20 | 1.56 | 1.51 | 1.32 | 1.57 |
| | 9 | 0.99 | 1.23 | 1.23 | 1.24 | 1.37 |
| P (%) | 3 | 0.11 | 0.47 | 0.85 | 1.08 | 1.48 |
| | 6 | 0.08 | 0.47 | 0.58 | 0.97 | 1.48 |
| | 9 | 0.04 | 0.30 | 0.43 | 0.50 | 0.83 |
| K (%) | 3 | 2.13 | 2.35 | 2.51 | 2.65 | 3.13 |
| | 6 | 2.25 | 3.27 | 2.86 | 3.40 | 3.75 |
| | 9 | 1.82 | 2.03 | 2.11 | 2.29 | 2.51 |
| Ca (%) | 3 | 0.80 | 1.18 | 1.41 | 1.51 | 1.56 |
| | 6 | 0.79 | 1.11 | 1.14 | 1.16 | 1.46 |
| | 9 | 0.69 | 0.72 | 0.72 | 0.75 | 0.85 |
| Mg (%) | 3 | 0.22 | 0.24 | 0.27 | 0.30 | 0.30 |
| | 6 | 0.16 | 0.23 | 0.20 | 0.24 | 0.29 |
| | 9 | 0.13 | 0.15 | 0.14 | 0.15 | 0.17 |

**Table 6.8** Mineral analyses (micro nutrients) of buchu plants in the plastic cladded greenhouse experiment after 3, 6 and 9 months as affected by the P concentration in the nutrient solution

| | | P concentration (me $L^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Mineral | Month | 0.00 | 0.35 | 0.70 | 1.05 | 1.4 |
| Na | 3 | 687.50 | 744.75 | 697.63 | 629.00 | 603.63 |
| (mg $kg^{-1}$) | 6 | 620.75 | 856.50 | 697.75 | 802.63 | 854.00 |
| | 9 | 586.86 | 665.00 | 609.00 | 699.75 | 710.00 |
| Mn | 3 | 172.88 | 232.25 | 272.88 | 363.00 | 261.38 |
| (mg $kg^{-1}$) | 6 | 249.13 | 227.88 | 249.25 | 216.13 | 343.50 |
| | 9 | 323.63 | 195.00 | 228.00 | 196.50 | 222.88 |
| Fe | 3 | 60.23 | 57.63 | 33.00 | 28.25 | 39.38 |
| (mg $kg^{-1}$) | 6 | 36.38 | 34.50 | 29.38 | 26.88 | 39.88 |
| | 9 | 67.00 | 53.88 | 38.88 | 45.38 | 35.50 |
| Cu | 9 | 4.88 | 2.63 | 1.63 | 3.00 | 3.13 |
| (mg $kg^{-1}$) | 6 | 7.50 | 11.75 | 7.63 | 13.78 | 8.50 |
| | 9 | 4.88 | 2.63 | 1.63 | 3.00 | 3.13 |
| Zn | 3 | 30.13 | 31.25 | 35.38 | 44.13 | 48.88 |
| (mg $kg^{-1}$) | 6 | 25.00 | 23.88 | 24.38 | 23.75 | 34.63 |
| | 9 | 33.25 | 22.38 | 23.50 | 21.75 | 23.00 |
| B | 3 | 20.50 | 27.13 | 24.75 | 28.63 | 34.75 |
| (mg $kg^{-1}$) | 6 | 27.88 | 37.63 | 30.50 | 33.00 | 41.75 |
| | 9 | 32.75 | 29.88 | 27.88 | 30.50 | 32.50 |

**Table 6.9** Analysis of the buchu oil composition receiving different P concentrations in the nutrient solution

| | P concentration (me $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| Oil component (%) | 0.00 | 0.35 | 0.70 | 1.05 | 1.40 |
| α-pinene | 0.425 | 0.725 | 0.543 | 0.3667 | 0.538 |
| Sabinene | 0.625 | 0.950 | 0.840 | 0.580 | 0.880 |
| β-pinene | 0.163 | 0.250 | 0.200 | 0.200 | 0.217 |
| Myrcene | 0.838 | 1.700 | 1.386 | 0.929 | 1.450 |
| Limonene | 13.563 | 18.625 | 16.314 | 13.171 | 14.600 |
| (E)-b-Ocimene | 0.300 | 0.675 | 0.571 | 0.467 | 0.583 |
| γ-Terpenine | 0.167 | 0.133 | 0.217 | 0.125 | 0.100 |
| Linalool | 0.775 | 0.700 | 0.614 | 0.571 | 0.588 |
| Menthone | 6.075 | 6.313 | 6.571 | 7.514 | 6.475 |
| Isomenthone | 18.275 | 18.100 | 16.571 | 16.085 | 20.025 |
| cis-/trans-Isopulegone | 3.663 | 4.000 | 3.900 | 4.071 | 3.786 |
| Pulegone | 14.325 | 15.563 | 13.371 | 14.914 | 16.313 |
| Ψ-Diosphenol | 15.863 | 12.138 | 14.471 | 15.086 | 11.600 |
| LΨ-Diosphenol | 2.736 | 2.465 | 2.628 | 2.695 | 2.427 |
| 2-Hydroxyisomenthone | 0.100 | 0.100 | 0.114 | 0.100 | 0.143 |
| Diosphenol | 17.038 | 12.688 | 15.957 | 15.586 | 11.812 |
| 1-Hydroxydiosphenol | 0.338 | 0.275 | 0.150 | 0.233 | 0.250 |
| t-8-Mercapto-p-menthan-3-one | 0.613 | 0.738 | 0.814 | 0.850 | 0.743 |
| c-8-Mercapto-p-menthan-3-one | 4.150 | 4.650 | 5.300 | 6.429 | 5.428 |

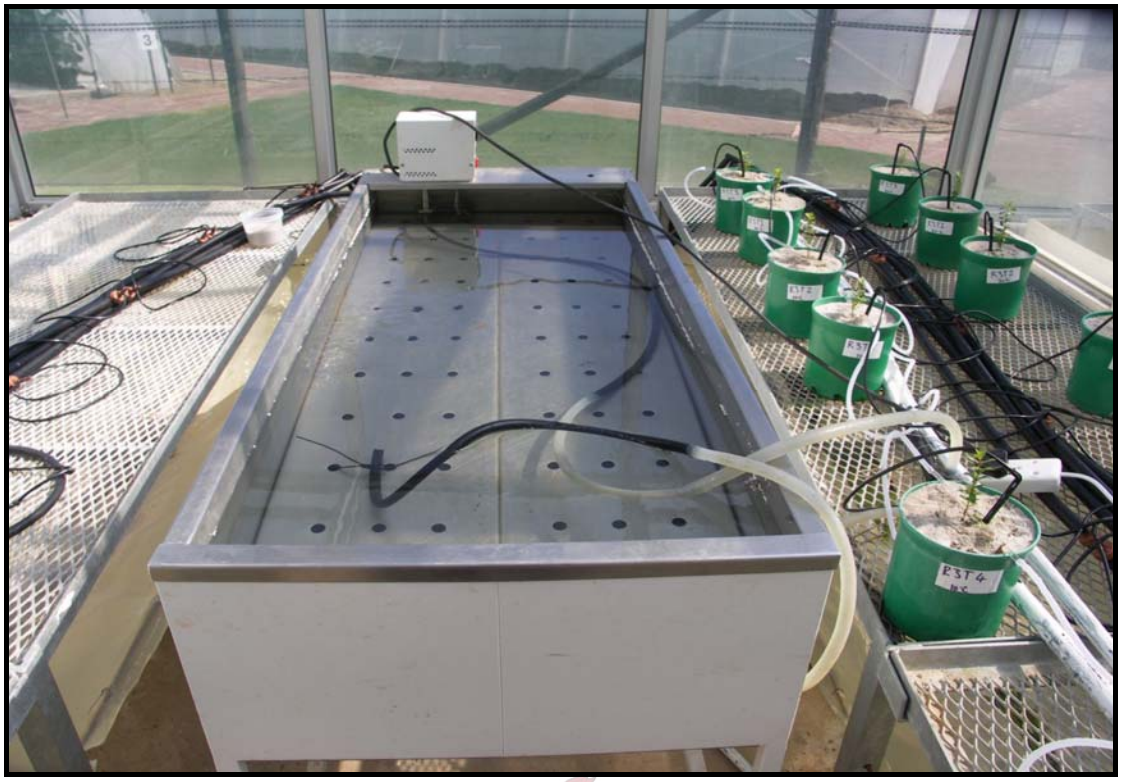

**Plate 6.4** The cold bath that was used in the mechanically cooled glasshouse experiment to cool down water.


**Plate 6.5** Cold water was pumped through glass spirals running through the pots to cool down the root zone.

**Table 6.10** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the glasshouse experiment for the treatment where no P were applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.4 | 1.04 | 0.00 | 3.05 |
| 2 | 6.3 | 1.07 | 0.01 | 2.59 |
| 3 | 6.2 | 1.11 | 0.00 | 2.74 |
| 4 | 5.5 | 1.08 | 0.02 | 2.55 |
| 5 | 6.2 | 1.07 | 0.00 | 3.01 |
| 6 | 6.3 | 1.04 | 0.01 | 3.12 |

**Table 6.11** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the glasshouse experiment for the treatment where 0.35 me P $L^{-1}$ was applied



| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.2 | 1.02 | 0.30 | 3.10 |
| 2 | 6.3 | 1.08 | 0.33 | 2.62 |
| 3 | 6.2 | 1.04 | 0.32 | 2.90 |
| 4 | 5.9 | 1.12 | 0.34 | 2.56 |
| 5 | 6.1 | 1.04 | 0.32 | 3.03 |
| 6 | 6.3 | 1.02 | 0.31 | 3.15 |

**Table 6.12** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the glasshouse experiment for the treatment where 0.7 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.3 | 1.01 | 0.63 | 3.07 |
| 2 | 6.0 | 0.98 | 0.70 | 2.73 |
| 3 | 6.0 | 1.01 | 0.63 | 2.92 |
| 4 | 5.8 | 1.08 | 0.69 | 2.50 |
| 5 | 6.1 | 1.04 | 0.67 | 2.86 |
| 6 | 6.2 | 1.01 | 0.63 | 3.08 |

**Table 6.13** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the glasshouse experiment for the treatment where 1.05 me P $L^{-1}$ was applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.1 | 1.00 | 0.95 | 3.11 |
| 2 | 6.0 | 1.01 | 0.96 | 2.58 |
| 3 | 5.9 | 0.98 | 0.97 | 3.02 |
| 4 | 5.6 | 1.10 | 1.07 | 2.81 |
| 5 | 6.0 | 1.00 | 0.99 | 2.99 |
| 6 | 6.1 | 1.00 | 1.02 | 3.09 |

**Table 6.14** pH, EC, P concentration and $NO_3/SO_4$ ratio of the nutrient solution used in the glasshouse experiment for the treatment where 1.40 me P $L^{-1}$ were applied

| | Factor | | | |
|---|---|---|---|---|
| Month | pH | EC (mS $cm^{-1}$) | $PO_4$ (me $L^{-1}$) | $NO_3/SO_4$ |
| 1 | 6.2 | 0.98 | 1.31 | 3.13 |
| 2 | 5.9 | 0.94 | 1.35 | 2.67 |
| 3 | 5.7 | 0.99 | 1.25 | 3.02 |
| 4 | 5.5 | 1.05 | 1.33 | 2.63 |
| 5 | 6 | 1.00 | 1.35 | 2.99 |
| 6 | 6.1 | 0.98 | 1.32 | 3.12 |

**Table 6.15** Mineral analyses (macro nutrients) of buchu plants in the glasshouse experiment after 6 months as affected by the P concentration in the nutrient solution at different root temperatures

| | P concentration | Mineral concentrations (%) | | | | |
|---|---|---|---|---|---|---|
| Temperature | (me $L^{-1}$) | N | P | K | Ca | Mg |
| 10°C | 0.00 | 0.84 | 0.14 | 1.16 | 0.91 | 0.26 |
| | 0.35 | 1.05 | 0.13 | 1.24 | 0.86 | 0.18 |
| | 0.70 | 1.16 | 0.23 | 1.47 | 0.90 | 0.19 |
| | 1.05 | 0.94 | 0.17 | 1.08 | 0.70 | 0.19 |
| | 1.40 | 1.32 | 0.29 | 1.65 | 0.95 | 0.20 |
| 20°C | 0.00 | 1.11 | 0.07 | 1.40 | 0.54 | 0.17 |
| | 0.35 | 1.40 | 0.17 | 2.14 | 0.82 | 0.17 |
| | 0.70 | 1.44 | 0.23 | 1.97 | 0.66 | 0.13 |
| | 1.05 | 1.49 | 0.32 | 2.52 | 0.82 | 0.17 |
| | 1.40 [1] | 1.63 | 0.41 | 2.64 | 0.81 | 0.17 |

**Table 6.16** Mineral analyses (micro nutrients) of buchu plants in the glasshouse experiment after 6 months as affected by the P concentration in the nutrient solution at different root temperatures

| Temperature | P concentration (me $L^{-1}$) | Mineral concentrations (mg $kg^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Na | Mn | Fe | Cu | Zn | B |
| 10°C | 0.00 | 697.67 | 100.00 | 54.67 | 4.33 | 26.67 | 24.33 |
| | 0.35 | 499.00 | 136.67 | 41.67 | 3.00 | 37.67 | 26.00 |
| | 0.70 | 620.67 | 178.67 | 43.33 | 3.67 | 23.67 | 31.00 |
| | 1.05 | 504.67 | 77.33 | 43.67 | 3.33 | 17.00 | 23.33 |
| | 1.40 | 610.33 | 354.33 | 47.00 | 2.00 | 33.67 | 30.67 |
| 20°C | 0.00 | 829.67 | 128.00 | 113.33 | 4.00 | 37.33 | 25.00 |
| | 0.35 | 805.00 | 161.33 | 48.00 | 2.33 | 38.67 | 46.00 |
| | 0.70 | 729.00 | 176.67 | 51.67 | 2.33 | 19.00 | 38.33 |
| | 1.05 | 929.67 | 143.33 | 53.00 | 6.00 | 24.00 | 52.33 |
| | 1.40 | 1105.67 | 203.67 | 56.00 | 3.67 | 15.33 | 51.67 |