

**MINERAL NUTRITION OF CULTIVATED SOUTH AFRICAN
PROTEACEACE**

By

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Declaration

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

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SUMMARY OF THESIS

Protea, *Leucadendron* and *Leucospermum* belong to the Proteaceae family. These three genera form an important part of the floriculture industry in South Africa and are commonly used as fresh cut flowers or dried flowers for both the local and international market. The distribution of macro and micro - nutrients was investigated in *Protea* 'Cardinal' using rooted cuttings grown from October 2001 to March 2002. The plants were divided into 1st flush leaf and stem, 2nd flush leaf and stem, basal leaf and basal stem, roots and the flower bud. These tissues were analysed to determine N, P, K, Ca, Mg, Fe, S, Na, B and Mn concentration.

Results indicated that N decreased over time in all tissues and accumulated more in leaves than in stems. P in leaves and stems increased with time, while K increased in leaf tissues and remained stable in stem tissue. Ca did not change in young leaves but was high in old leaves. Mg in leaves increased but in basal stem, 1st flush stem and roots Mg decreased over time. Fe in leaves and roots increased with time and not a less significant change occurred in stems. In most tissues, B and Na decreased with time. S increased in leaves and decreased in stems with time. Mn was relatively stable in most tissues except in basal leaves where it increased. Nutrient concentration in tissues, especially in leaves, showed no distinct pattern in the distribution of nutrients.

Eleven cultivars from the three genera, namely *Protea*, *Leucadendron* and *Leucospermum*, were used to develop deficiency symptoms of macro - nutrients by using different nutrient compositions. The plants were grown in 20cm pots from

December 2002 to September 2003. The eleven cultivars were chosen because of their high market value. Visual symptoms were recorded in two stages with a five - month interval for each stage. The first stage was recorded from December 2002 to April 2003 and the second stage was recorded from May 2003 to September 2003.

Observed symptoms indicated significant differences between the control and treatments in which specific nutrients were withheld. Some cultivars exhibited some symptoms that are commonly noticed under field conditions, e.g. in the N deficiency treatment the upper leaves of *Protea* 'Sylvia' were reddish and the lower leaves were chlorotic especially at the later stage. *Protea* 'Red Rex' in the P deficiency treatment had random red tints around the leaf including the petiole, these symptoms are also commonly noticed in *Protea* 'Red 'Rex' in field conditions. *Leucadendron* 'Rosette' also showed some uncommon symptoms of accumulating "sugar" on leaf tips in Ca deficiency treatment that was not observed in any other cultivar. In some cultivars the symptoms were systematic e.g. *Leucadendron* 'Chameleon' while in other cultivars the deficiency affected a certain leaf age e.g. *Leucospermum* 'High Gold' in the Ca deficiency treatment. The buds in 'High Gold' died prematurely in the Ca deficiency treatment.

Protea 'Cardinal' was used to determine the optimal N and P source and concentrations for optimal growth. *Protea* 'Cardinal' was grown in a temperature-controlled glasshouse for seven months using silica sand as growth medium. Different levels and sources of N and P were applied. The N was applied in a complete nutrient solution as NH_4^+ , NO_3^- , $\text{NH}_4^+ : \text{NO}_3^-$ (1: 1, 1: 4 and 4: 1 ratios) and Urea, at different concentration levels: 5 mM, 1 mM and 0.1 mM. P was applied at 1

mM, 0.1 mM and 0.01 mM. The plants were fertigated manually with 1L nutrient solution on every second day of the week.

P at 0.01 mM and 0.1 mM resulted in optimal plant growth. The 1 mM P resulted in marginal leaf scorching or dryness. When N was applied at 5 mM plant growth was more optimal than when N was applied at 1 mM and 0.1 mM. More dry weight was accumulated at 5 mM than at 1 mM or 0.1 mM N. The dry weight of leaves, stem and roots tissues at 5 mM were higher in the NO_3^- treatment.

OPSOMMING VAN TESIS

Protea, *Leucadendron* en *Leucospermum* behoort aan die Proteaceae familie.

Hierdie drie genera vorm 'n belangrike deel van die blomme industrie in Suid-Afrika en word algemeen gebruik as vars sny blomme of gedroogte blomme vir die plaaslike, sowel as internasionale markte.

Gewortelde steggies van *Protea* 'Cardinal' is vanaf Oktober 2001 tot Maart 2002 gebruik om die verspreiding van makro-en mikro voedingselemente te bepaal. Die plantmateriaal vir ontleding is verdeel in blare en stam van eerste die groeifase, blare en stam van die tweede groeifase, basale blare en basale stam, wortels en blomknoppe. Hierdie weefsels is ontleed om N, P, K, Ca, Mg, Fe, S, Na, B en Mn konsentrasie te bepaal.

Resultate het getoon dat N oor tyd in al die weefsel afgeneem het, maar het eerder in die blare as in die stam akkumuleer. P het toegeneem in die blare en stam oor tyd, terwyl K stabiel gebly het in die stamweefsel, maar toegeneem het in die blaarweefsel. Ca het nie in die jong blare verander nie, maar was hoog in die ou blare. Alhoewel Mg in die blare toegeneem het, het dit in die basale stam, die eerste groeifase van die stam en in die wortels oor tyd verminder. Fe het oor tyd in die blare verhoog, maar min betekenisvolle verandering in die stamme en wortels het voorgekom nie. In meeste van die weefsel het B en Na oor tyd verminder. S het in die blare verhoog, maar in stamme oor tyd verminder. Mn was relatief stabiel in meeste van die weefsel, behalwe in die basale blare het dit verhoog. Die konsentrasies van die voedingselement het geen duidelike verspreidingspatroon in die weefsel, veral in blare, getoon nie.

Elf kultivars van drie Proteaceae genera, nl. *Protea*, *Leucadendron* en *Leucospermum*, is gebruik om tekort simptome te induseer, wat veroorsaak word deur die weglating van makro-voedingselemente uit in andersins volledige voedingsmengsels. Die plante is gekweek in 20cm potte vanaf Desember 2002 tot September 2003. Die elf geselekteerde kultivars het hoë bemarkings waarde.

Visuele simptome is in twee fases van 5 maande intervalle, vir elke fase, aangeteken. Die eerste fase was aangeteken vanaf Desember 2002 tot April 2003 en die tweede fase was aangeteken vanaf Mei 2003 tot September 2003.

Betekenisvolle verskille is waargeneem tussen die kontrole en die behandelings waarin voedingselemente weerhou is. Meeste kultivars het simptome getoon wat dikwels onder veldtoestande waargeneem word, bv. die boonste blare van *Protea* 'Sylvia' het in die gekontroleerde N tekort behandeling baie rooi vertoon en die laer blare was bleek van kleur in die latere fase. *Protea* 'Red Rex' in die P tekort behandeling het 'n rooi tint ontwikkel op die blaarstele en tussen die blaarnerwe. Hierdie simptome is soortgelyk aan dié wat dikwels op *Protea* 'Red Rex' plante onder veldtoestande waargeneem word. *Leucadendron* 'Rosette' het ook ongewone simptome getoon: nektaar het op die punte van die blare gevorm indien Ca weerhou is uit voedingsmengsel. Die simptome was sistemies in sommige van die kultivars (bv. *Leucadendron* 'Chameleon'), terwyl in ander kultivars het behandelings net blare van bepaalde ouderdomme beïnvloed, bv. *Leucospermum* 'High Gold' in Ca tekort behandeling. Die onvolwasse blomknoppe van *Leucospermum* 'High Gold' het ook in die Ca tekort behandeling geaborteer.

Protea 'Cardinal' is gebruik om die optimale N en P bron en konsentrasie vir optimale plantgroei te bepaal. *Protea* 'Cardinal' is vir sewe maande gekweek in 'n

temperatuur gekontroleerde glashuis in silika sand as groeimedium. Verskillende konsentrasies en bronne van stikstof en fosfaat is toegedien. Die stikstof was toegedien in 'n volledige voedingsoplossing as NH_4^+ , NO_3^- , $\text{NH}_4^+ : \text{NO}_3^-$ (verhoudings van 1:1, 1:4 en 4:1) of Ureum teen verskillende konsentrasies: 5mM, 1mM en 0.1mM. Fosfaat was toegedien teen konsentrasie van 0.01 mM, 0.1 mM en 1 mM. Die plante het elke tweede dag 1L voedingsoplossing met die hand ontvang.

Die laagste P konsentrasies (0.01 mM en 0.1 mM) het tot optimale plantgroei gelei, terwyl die hoogste P konsentrasie (5 mM) gelei het tot uitdroging of brand van die blaarrande. Stikstof toediening teen die hoogste konsentrasie (5 mM) het gelei tot optimale plantgroei, as teen 1 mM en 0.1 mM. Die droë massa van die blare, stamme en wortels was hoër in die 5 mM NO_3^- behandeling as in enige ander behandeling.

CONTENTS

	Page
CHAPTER ONE	
OPTIMIZATION OF MINERAL NUTRITION IN POTS ON PROTEA PLANTS	1
1. INTRODUCTION	1
1. 1. Proteaceae	1
1. 2. Economic Importance	3
1. 3. AIMS AND OBJECTIVES	4
CHAPTER TWO	
2. LITERATURE REVIEW	5
2. 1. General adaptation to growing conditions of Proteaceae.	5
2. 2. Root system of Proteaceae	7
2. 2. 1. The characteristics and functions of cluster roots	7
2. 3. pH INFLUENCE ON PLANT MINERAL NUTRION	10
2. 4. SOIL AND PLANT ANALYSIS TO EVALUATE PLANT MINERAL STATUS	11
2. 4. 1 The leaf analysis as a mineral nutrient indicator in Proteaceae plants	14
2. 4. 2. Growth as a measure of nutrient availability in plants	15
2. 5. PLANTS SENSITIVITY TO N	16
2. 6. PLANT SENSITIVITY TO P	17
2. 7. PLANT NUTRIENT DIFICIENCIES AND TOXICITIES	19
2. 7. 1 Nitrogen (N)	21
2. 7. 2 Phosphorus (P)	22
2. 7. 3 Potassium (K)	22

2. 7. 4 Calcium (Ca)	22
2. 7. 5 Magnesium (Mg)	22
2. 8. CURRENT FERTILIZATION MOTIVATIONS	24
2. 8. 1. Claassens' s motivation (1986)	25
2. 8. 2. Harré' s motivation (1995)	25
2. 9. Leake' s motivation (1996)	25
2. 10. REFERENCES	26

CHAPTER THREE

TO INVESTIGATE THE DISTRIBUTION OF MACRO AND MICRO NUTRIENTS WITHIN PROTEA CULTIVAR 'CARDINAL' PLANTS GROWN IN SAND CULTURE	32
ABSTRACT	32
3. 1. INTRODUCTION	33
3. 2. MATERIALS AND METHODS	34
3. 2.1. Plant material	34
3. 2. 2. Harvest	35
3. 2. 3. The sampling plan	36
3. 2. 4. Chemical Analysis	36
3. 2 5. Statistical Analysis	36
3. 3. RESULTS	37
3. 3 1. N concentration in plant tissue	37
3. 3. 2 P concentration in plant tissue	37
3. 3. 3. K concentration in plant tissue	37
3. 3. 4. Ca concentration in plant tissue	38
3. 3. 5. Mg concentration in plant tissue	38

3. 3. 6. Fe concentration in plant tissue	38
3. 3. 7. Na concentration in plant tissue	38
3. 3. 8. B concentration in plant tissue	38
3. 3. 9. S concentration in plant tissue	39
3. 3. 10. Mn concentration in plant tissue	39
3. 4. DISCUSSION	39
3. 5. CONCLUSION	43
3. 6. REFERENCES	44
3. 7. GRAPHS/FIGURES	46

CHAPTER FOUR

THE SYMPTOMS CAUSED BY DEFICIENCY OF MACRO NUTRIENTS IN PROTEA, LEUCADENCROON AND LEUCOSPERMUM	51
ABSTRACT	51
4. 1. INTRODUCTION	52
Table 1. Summary of general deficiency symptoms in plants	55
4. 2. MATERIALS AND METHODS	58
4. 2. 1 plant material	58
4. 2. 2. Visual symptoms sampling plan	60
4. 3. RESULTS AND DISCUSSION	60
4. 4. CONCLUSION	64
4. 5. REFERENCES	65
Table 2a. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Control treatment	66

Table 2b. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Nitrogen treatment	67
Table 2c. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Phosphorus treatment	69
Table 2d. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Potassium treatment	71
Table 2e. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Calcium treatment	72
Table. 2f. Description of deficiency symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) in Magnesium treatment	74
4. 6. FIGURES	75
 CHAPTER FIVE	
OPTIMISATION OF PHOSPHORUS AND NITROGEN NUTRITION ON PROTEA 'CARDINAL': - CONCENTRATIONS AND FORMS OF APPLICATIONS	87
ABSTRACT	87
5. 1. INTRODUCTION	88
5. 2. MATERIALS AND METHODS	89
5. 2. 1 Experimental site and plant material	89
5. 2. 2 Harvest	90

5. 2. 3 Sample analysis	90
5. 2. 4 Statistical Analysis	91
5. 3. RESULTS	91
5. 3. 1 Visual observations on 'Cardinal'	91
5. 3. 2 Plant dry weight accumulation	92
5. 3. 3 Nutrients accumulation	93
5. 4. DISCUSSION	93
5. 5. CONCLUSION	97
5. 6. REFERENCES	98
5. 7. PICTURES/PHOTOGRAPHS	100
5. 8. GRAPHS	104
CHAPTER SIX	
GENERAL DISCUSSION OF THESIS	110

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CHAPTER ONE

MINERAL NUTRITION OF CULTIVATED SOUTH AFRICAN

PROTEACEACE

1 INTRODUCTION

1.1 Proteaceae

Members of the Proteaceae are one of the main components of the vegetation found in the Cape floral Kingdom (CFK). The CFK occupies 0.04% of the earth surface, yet it enjoys equal status with kingdoms such as the Boreal Forest Kingdom (Bond and Goldblatt, 1984) due to the great species diversity found in the CFK. Proteaceae occur in Africa, Australia, Asia, and in South America. The most important genera used internationally, as cut flowers are *Leucospermum*, *Leucadendron*, *Protea*, *Banksia*, and *Grevillea* and these plants are also used as landscape materials (Criley, 2000). In local markets in floriculture industry the genera that are commonly used are *Leucospermum*, *Leucadendron*, *Protea*.

Proteaceae are woody evergreen perennial shrubs with sclerophyllous leaves that withstand dry and hot weather conditions. The growing seasons for *Protea* are winter (March to August), spring (September to November), and autumn (February to March) (Malan, 1993), although this is dependent on several environmental factors. Some species in Proteaceae family such as *Leucospermum cuneiformei*, *Leucadendron salignum* and *Protea cynaroides* can regenerate after disturbance (e.g. fire) through sprouting from the lignotuber. Members of the genus *Protea* grow in spurts, called flushes, which are produced seasonally, from spring to autumn. The environmental conditions and species characteristics influence growth flushes. Plants from the Proteaceae may differ widely in morphological features. The genus *Protea* is distinguished from other genera of the Proteaceae, by its large inflorescence, enclosed by involucre bracts and long period over which it flowers. The involucre bracts of *Protea* vary in colour and contribute aesthetic appeal and broader leaves. Long woody styles and the

absence of the woody bracts characterize *Leucospermum*. *Leucadendron* is a dioecious species with female woody cones, while male cones are devoid of woody bracts with thin leaves (Rebello, 1995).

Intense wild flower picking and frequent fires deplete nutrient reserves. Nutrients removed from cultivated fynbos stands give annual losses of nutrient pool of between 1. 6% (w/w) P and 2. 3% (w/w) K (Low and Lamont, 1986). As a consequence there is a need to replace what the plant has removed from the soil. Flower quality is at least in part determined by the nutritional status of the plant. Good control of plant nutritional status is necessary to deliver quality fresh cut flowers to the market.

Little is known about the nutrition and nutritional requirements of the South African Proteas. Proteaceae are regarded as difficult crops to manage with regard to the rates of application and types of fertilizers applied. Without proper guidelines, growers have difficulties with plant performance and also with determining and maintaining fertilizer management programs (Barth *et al.*, 1996). A major limitation in cultivated production at present revolves around the difficulties of replacing nutrients lost when harvesting cut flower stems.

Plant mineral nutrition refers to the mineral elements a plant is able to take up, utilize and that are required for plant survival (Marschner, 1997). Mineral nutrition is also influenced by factors such as translocation (movement of solutes in a plant) and allocation of solutes to different tissues and organs of the plant. Greulach (1973) described mobilization and translocation as the movement of solutes from a region where they are absorbed or synthesized to regions where they are utilized through diffusion processes in plants.

A mineral nutrient is regarded as essential when three criteria are met within a plant's life: 1) These plants will not be able to complete its life cycle without it. 2) The element must not be replaceable by another element. 3) It must be directly involved in plant metabolism in that it must be required for a specific physiological function (Salisbury and Ross, 1992; Taiz and Zeiger, 1991). Thus, when the essential element is omitted it should cause some abnormal growth in the plant, or premature senescence or death (Hewitt and Smith, 1975).

Husbandry of plants requires meeting the requirement of nutrients by plants when nutrient imbalances and salinity exist. This challenge will differ with plant age and the environmental conditions such as drought and temperatures. Production optimisation in general is based on plant characteristics, such as a good rooting percentage to form strong roots without antagonistic effects on plant growth.

In plant nutrition, N, P and K are the common elements that receive most attention in most crops. This does not mean that other elements are not of importance to a plants nutritional need. This trio (N, P and K) differs in mobility within plants and the fact that they are often limiting in most plants when they are short supplied. N plays an important role in photosynthesis because N is part of the chlorophyll molecule, without which the plant will not be able to functions normally.

1 2 Economic importance

F.C. Batchelor set a trend in commercially cultivating *Protea* plants in Western Cape, Stellenbosch. He identified research needs and inspired people in other countries to investigate Proteaceae cultivation for fresh cut flowers. Batchelor founded the forerunner of the association known today as South African Protea Producer and Exporters Association (SAPPEX), which currently have 300 producers as members. Australia has over 150 members

of the Australian Flora and Protea Growers Association. South African Proteaceae are cultivated in many countries around the world such as Australia, Zimbabwe, USA, Portugal, Israel Chile, and New Zealand. The South African wild flower industry originated with street hawkers selling flowers harvested from the wild, a practice that still continues (Coetzee and Littlejohn, 2001; Criley, 2001). Of the economically important genera, *Protea* and *Leucospermum* are extensively cultivated, but about 50% of all *Leucadendron* sold still derive from wild plants and from broadcast sown fields.

The cultivation of indigenous Proteaceae has great economic potential since the flowers are exportable commodity. The industry can create job opportunities especially in coastal areas where these plants are mostly grown. Secondly can help to alleviate unemployment, poverty, and uplift communities. The industry supports over 20 000 (estimation) people, this estimation is based on production chains that involves packaging, airfreight services, researchers as well as farmers and export agents. The export market value for the industry in 2002 was estimated at R94.91 million, 56% of which was derived from fresh cut flowers and 44% was from sale of dried products (SAPPEX 2002). These show a great potential contribution by the indigenous products industry to the economic upgrading of the country.

1.3. Aims and objectives:

The main objectives of this study were to:

- (a) Determine the nutrient requirements for *Protea* plant growth.
- (b) To determine the distribution of micro- and macro- nutrients within the plant.
- (c) To observe the effects of nutrient deficiency on the plants.
- (d) To determine plant sensitivity to different N forms and concentrations and also to different P concentrations.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 General adaptations to growing conditions of Proteaceae

Some members of the Proteaceae can tolerate temperatures of between -5°C to a maximum of 45°C , but they are generally sensitive to frost, especially when they are young (Rebelo, 1995). These plants when grown outside their natural habitat ranges, can be sensitive to new environmental conditions including temperature, moisture levels and the soil texture quality. Under cultivation a row spacing of between 3.5 m to 4.5 m and within row spacing of 8.0 m to 1.0 m is currently commonly used (Coetzee and Littlejohn, 2001) giving a plant density of 2 500 to 3 560 plants/ha.

The soils of South African Fynbos Biome in which the South African Proteaceae grow are generally low in mineral nutrients. Most of these plants grow at soil pH of between 4 and 6, but there are species that can grow on soils with up to pH of 8, e.g. *Protea obtusifolia*, *Protea susannae*, and *Protea laurifolia* (Criley, 2000). Matthews and Carter (1983) suggested that the ideal pH for the soils used for *Leucospermum spp*, was 5.5. Clay content of less than 20% is acceptable for most species, but up to 50% can be tolerated by some species as long as the soil drainage system is good (Coetzee and Littlejohn, 2001).

Although the plants can survive long hot dry summers, a water supply is necessary for good optimum shoot growth and for high biomass production (Coetzee and Littlejohn, 2001).

Water requirements among the three cultivars differed significantly with regard to the quantity of water required for shoot growth development. Both *Protea* 'Cardinal' and *Leucospermum* 'Succession 11' grown under 20% (w/w) water depletion had longer shoot growth than *Leucadendron* 'Inca Gold' (Van Zyl and Myburgh, 2000). This indicates that

Protea and *Leucospermum* need more water than *Leucadendron* 'Inca Gold'. These authors suggested that irrigation should be implemented, especially for *Protea* and *Leucospermum*, since these plants at 40% (w/w) level water depletion they became sensitive to water stress. This will also ensure sufficient soil water levels for nutrient uptake and consequently optimal growth.

Proteaceae species differ in their water requirements; e.g. *P. cynaroides* required twice the quantity of water of *P. eximia* for optimal growth (Manders and Smith, 1992). Mortimer *et al.* (2003) found that irrigation did not influence vegetative growth and flush length on five-year-old *Protea* 'Sylvia'. However, in this study the deep root system had access to water lower in the profile. Plant water requirements also depend on plant age. Seedlings and young plants need more water than matured plants. The common requirement of the Proteaceae is high light interception, good air circulation and frost-free conditions (Criley, 2000). These environmental conditions interact with water availability. For instance, when humidity is low, high air temperatures may result in leaf scorch, especially during summer drought periods.

The South African Proteaceae grows in a wide range of temperature conditions. For example, *Protea magnifica* grows well within the snowline of the intermittent winter snowfall of the CFK. *Leucospermum cordifolium* in comparison grows in lower lying hilly areas near the coast where snow conditions never occur. *Leucadendron salignum* occurs from the west of the CFK to the east, growing mainly on the slopes of the mountains, as well as lowlands (Rebelo, 1995). Thus it is to be expected that the various species of Proteaceae will have vastly different environmental requirements. This is of pertinence not only to the water requirements of the plants, but also to the nutrient requirements.

2.2 ROOTS SYSTEM OF THE PROTEACEAE

2.2.1 The characteristics and functions of cluster roots

The root system of the Proteaceae is dimorphic, consisting of lateral roots and sinker roots. Sinker roots are strongly attenuate; they extend vertically downward to absorb water even as far as the water table. During the early summer period and late spring season, the sinker roots can transport minerals to the lateral roots (Jescheke and Pate, 1995). The lateral roots radiate outwardly into the upper soil layer and abstract nutrients through the help of the "cluster roots". These lateral roots also serve for the acquisition of water.

Proteaceae differ from most other plants, because they have a root specialization called "cluster" or "proteoid" roots. The term "proteoid" root was initially used to describe the dense cluster of fine roots that occur in longitudinal rows along the ordinary roots of the Proteaceae family (Lamont, 1986). Most plants do not have cluster roots, but do have lateral roots to transport solutes and the taproot for water transport. The "cluster roots" are crowded together along the axis of the lateral roots, they are longitudinally diarch in shape, and are found at the upper level of five to ten centimeters of the soil surface (Criley, 2000; and Coetzee and Littlejohn, 2001). The "cluster roots" are most prominent when P supply is restricted and decline in number and activity when P is made increasingly available to the roots (Watt and Evans, 1999).

The cluster roots extract nutrients by being in close contact with the soil surface and by being able to exude organic acids for mobilization of sparingly soluble nutrients (especially P) from complexes in the soil. The large surface area of these roots enables both the exudation and absorption of these nutrients. With a sufficient supply of nutrients in the *Leucadendron*-hybrid 'Safari Sunset', Silber *et al*, (1997) found fewer cluster roots. In a low nutrient environment these authors found extensive cluster root development. This indicates that

cluster roots might develop at low solute concentration thus explaining why Proteaceae survive and flourish in poor substrates (Vorster and Jooste, 1986b).

Members of other plant families also form cluster roots: *Casuarina cunninghamiana* (Casuarianaceae) is a woody plant known to form cluster roots and also has the ability to fix N₂ through nodules. The *Casuarina cunninghamiana* roots are also capable of forming symbiotic and mutualistic relation with arbuscular mycorrhizae and ectomycorrhizae fungi (Reddell *et al.*, 1997). Like infection with mycorrhizal fungi, cluster roots enable more efficient use of soil P mobilisation in these plants (Lambers *et al.*, 2003). The Proteaceae and *C. cunninghamiana* share the characteristic of forming cluster roots in response to low P accumulation (Reddell *et al.*, 1997). In Australian heathland regions, many species accumulate P from low concentrations and use P efficiently for normal plant growth (Reddell *et al.*, 1997). Members of the South African Proteaceae exhibit a similar pattern to those of the Australian heathland plants. Proteoid roots do not only enhance P acquisition in plants, but are also able to reduce Fe³⁺ (Marschner, 1997).

In *Lupinus albus*, P and to a lesser extent Fe deficiencies enhance and promote formation of cluster root development (Lambers *et al.*, 2003). However, for *Protea*, *Leucadendron* and *Leucospermum*, the Fe uptake enhancement by cluster root growth development is not yet well understood. Furthermore, Watt and Evans (1999) reported that other cluster root forming species do not produce cluster roots under Fe stress. Some cluster roots may also be able to access complex forms of organic N (Lambers *et al.*, 2003).

The ability to mobilise sparingly soluble nutrient such as P and Fe, and possibly to access organic forms of N are all highly desirable traits. Jeschke and Pate (1995) found that *Banksia prionotes* formed a dense mat of cluster roots in the presence of organic matter and can take

up large amounts of N, P and micronutrients. Vorster and Jooste (1986a) reported that cluster roots could absorb N, P, and K in greater amounts than ordinary roots. Watt and Evans (1999), however, reported that cluster roots do not take up cations such as K^+ extensively.

In crop production system, plants that exhibit cluster roots can be used with crops that are non-cluster root formers to enhance availability of poorly soluble nutrients such as P. Thus cluster root producing plants can be intercropped with non-cluster root producing plants. Watt and Evans (1999) found that when wheat was intercropped with *Lupinus albus* the content of P, N and Mn in intercropped wheat was higher than in monoculture wheat planting. As Lambers *et al.* (2003) further anticipated, with increased P fertilizer prices and the likely future scarcity of P fertilizer, intercropping with P sensitive crops will assist in mobilising and accessing P to crops that most need P, such as wheat.

Cluster roots synthesize larger amounts of citrate and malate for exudation than most other root types (Johnson *et al.*, 1996; Shane *et al.*, 2004). The concentrations of carboxylate increase with cluster root age (Shane *et al.*, 2004). After a prolonged period of accumulation these carboxylates are released from the cluster roots in what has been termed an “exudative burst”. The organic acids exuded by roots under P deficiency solubilise various P complexes such as Al, Fe and Ca (Engels, 1999).

Temperature and pH play an important role in nutrient absorption by the cluster roots. Cluster roots showed greater metabolic activity in P and K absorption than ordinary roots at 35 °C compared to most plant’s roots which show active P absorption between 15 °C to 35 °C temperatures (Smith and Jooste, 1986). Since Proteaceae prefer soils with acidic pH, alkaline pH can negatively influence plant growth (Silber *et al.*, 2000). The low pH and warmer temperatures of soils to which the Proteaceae are native enhance nutrient uptake.

Hanekom *et al.* (1973) found with *P. cynaroides* that active the growth period and active release of organic chelating compounds from the cluster roots immediately preceded vegetative bud differentiation and floral bud development, thus correlating with possible periods of nutrient uptake in plants. Thus the plant has to combine the provision of resources for cluster root development and functioning with the provision of resources for growth and flowering. This must impose considerable demands on the available photosynthate.

2. 3 pH INFLUENCE ON PLANT MINERAL NUTRITION

The concentration of hydrogen ions in a solution determines the pH in the soil or in solution. The pH influences the availability and solubility of nutrients in a specific way. Both macro-elements and micro - elements interact with soil pH. Elements such as Ca and Mg are commonly absorbed in large amounts at pH's 6.5 to 8; while at pH of 3 to 6 the uptake of N and P may be favored.

Application of NH_4^+ at higher concentration rates to cultivar 'Safari Sunset' reduced soil pH below 5 while application of NH_4^+ at low concentrations resulted in pH being increased to above 7 (Silber *et al.*, 2001). Thus there is a strong interaction between the form of N supplied and the soil pH. In the soils with low pH, N, K^+ , Ca^{2+} , Mg^{2+} , P, and S are less available amounts (Epstein 1972). At low pH, roots poorly absorb elements such as Ca; Mg, K, and P. Furthermore, P availability might be reduced when elements such as Al, Fe, or Mn are available in large amounts (Epstein 1972) since insoluble complexes form between P and di- or tri-valent cations. In soils with higher pH (7 and 7.5) elements such as Fe^{2+} , Mn^{2+} , Cu^{2+} , and Zn^{2+} become less available and others such as B become extremely unavailable.

Accumulation of Mn in *Leucadendron* 'Safari Sunset' was high at low pH and was reduced at higher pH (Ran *et al.*, 2001). Thus Mn accumulation is pH dependent, and may be associated with the activity of cluster roots, which liberate cations from insoluble complexes.

Some crops of other family members such as of the Brassicaceae are sensitive to excessive concentrations of Mn when the soil pH is low, but sugar beet could tolerate high concentrations of Mn (Hewitt and Smith, 1975). The Mn concentration in *Protea* 'Pink Ice' and *Leucadendron* 'Safari Sunset' (Maier, 1995 and Ran *et al.*, 2001) was considerable, but no toxicity effects on plants were reported.

Root growth in *Leucadendron* 'Safari Sunset' at pH of 7.5 was restricted and resulted in inhibition of root hairs and poor branching, but at lower pH root growth was normal (Silber *et al.*, 2001). These authors concluded that pH might be an important factor in the rhizosphere. This showed that high pH was detrimental for *Leucadendron* 'Safari Sunset'. Some other species such as of the Solanaceae family which prefer acidic soils and do well in acidic soils, may have poor growth provided that Ca content is not a limiting factor, while other crops like some members of the *Protea* are limited to grow in soils with acidic pH and with low nutrient status. In acid soils nutrient availability may be influenced by the impaired absorption of elements such as Ca, Mg, K, P and Mo which might lead that these nutrients becoming deficient in the plant tissue (Hewitt and Smith, 1975).

2. 4 SOIL AND PLANT ANALYSIS TO EVALUATE PLANT MINERAL STATUS

Soil maintains and governs plant growth since nutrients and water is mostly applied to the soil. The plant accesses these nutrients and water through the roots systems. Soil and plant analysis therefore gives the indications of nutrient status of both the soil and the amount the plant has removed after harvesting and how much can be added in the next growing season. Engels (1998) defined soil and plant analysis as the chemical/physical treatment of the soil or plant samples and subsequent determination of the nutrient concentration. Soil and plant analysis, as mineral nutrient indicators are important techniques to evaluate soil and plant nutrient status.

These techniques can also be used to determine specific nutrient requirements of crops in terms of nutrient deficiencies. Plant analysis is based on sampling of plant tissue organs such as leaves, stem, roots, sometimes including seed, fruit and grain. Soil and plant analysis data provide the basis for the fertilizer recommendations, and thus form an essential part of soil and plant management programmes. Soil and plant analysis should be used to assess the correlation between the amount of nutrient extracted and crop yield. In most cases the results obtained represent classes or categories such as stages of deficiency, luxurious critical level and toxicity of nutrient.

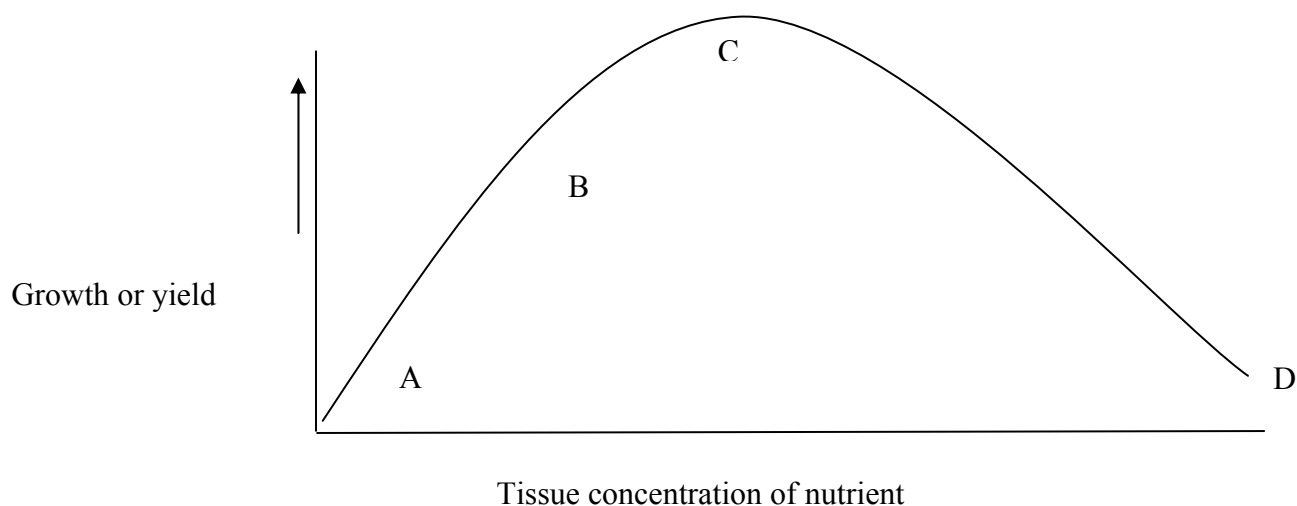


Figure 1. The correlation of tissue nutrient concentrations with yield. A = severe deficiency B = mild deficiency C =Luxury range D = toxic range. (Modified from Engels, 1998).

In plant nutrition, the Law of diminishing returns states, “when equal increment of a nutrients are applied to a crop, the yield response becomes smaller for each increment" (Fig 1). This means that application of nutrient will reach the stage where further application will no longer benefit the plant and may in fact limit yield.

Since leaves are the first to show nutrient deficiency in plants, Bierman and Rosen (2005) adopted the ‘Key’ technique chart. The ‘Key’ technique is used to identify visual symptoms

and to compare nutrient deficiencies associated with a specific symptom shown on chart. It is a useful tool that can help to diagnose a specific nutritional problem in crops (Fig. 2) (Bierman and Rosen, 2005).

The 'Key' consists of different alternative statements about the plant structures and their appearance and if possible it will be helpful to have a healthy plant for comparison (McCauley *et al.*, 2003). The 'Key' gives choice to choose the visual symptom observed whether was on 'Upper leaves' or 'Lower' leaves. The visual box below explains the description of the symptoms that are likely to be associated with nutrients on leaves.

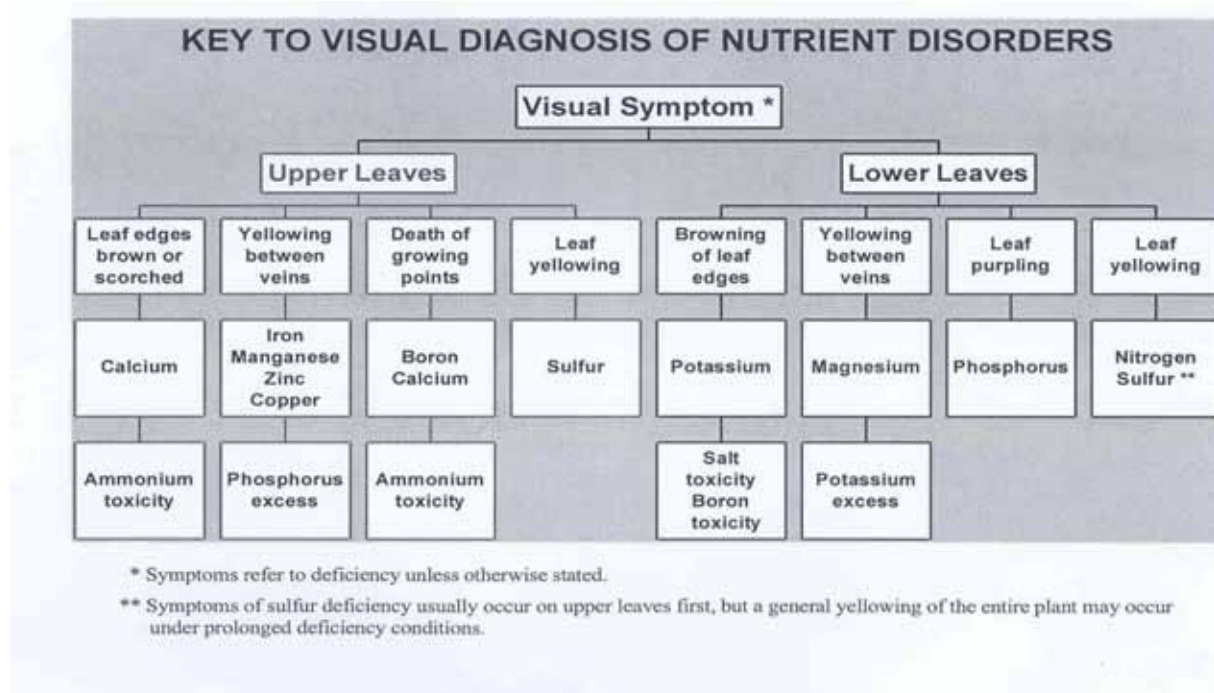


Fig. 2. Key technique chart to diagnose visual diagnosis of nutrient disorders (from Bierman and Rosen, 2005).

2. 4. 1. Leaf analysis as a mineral nutrient indicator in Proteaceae plants

Plant leaf analysis is nutrient management tool effectively used to determine the nutrient sufficiency levels and is useful for diagnosing many of the suspected nutrient disorder such as deficiencies (Bierman and Rosen, 2005). Plant analysis is used in a wide range of annual and perennial agricultural and horticultural crops (Maier *et al.*, 1995).

In most cases matured leaves that have fully expanded or stems are usually used.

Interpretation of leaf analysis assists in designing fertilizer programmes and in determining the deficiencies when noticed in plants. It is of importance that when leaves are used to determine nutrients, leaf age and time of sampling be taken into consideration. These two factors can have a negative effect on interpretation of results. For instance, when older leaves are used, it is possible that mobile nutrients have been mobilized out of the tissue prior to harvest thus false interpretation may result. Plant growth stages, like vegetative growth tissue development like leaf maturation and nutrient mobility are important for proper tissue analysis.

Leaf chemical analysis in *Leucadendron* 'Safari Sunset' and *Leucadendron* 'Silvan Red' was used to define seasonal nutrient trends so as to identify a suitable time for leaf analysis and nutrient removed by stems. The main nutrients removed by harvest of marketable stems were found to be Na, N, Ca, K and Mg (Cecil *et al.*, 1995). The concentration of N, P, K, and Na increased through the growing season, corresponding to the flush of spring growth, after which nutrient concentration decreased, especially in summer and autumn season (Cecil *et al.*, 1995). Thus nutrient reserves may be stored and then re-mobilized during the growing seasons.

Seasons influence nutrient element uptake in plants. For example, in *Protea* 'Pink Ice' the P concentration was higher during spring and summer, while in winter, P and K concentration was low in young fully expanded leaves (Barth *et al.*, 1996). Distinct seasonal changes in leaf analysis values highlight the importance of coordinating the growth status of the plants with leaf sampling times (Barth *et al.*, 1996).

Furthermore the tissue sampled is of importance in interpreting the nutritional status of the plants. For example young fully developed leaves of *Protea* 'Pink Ice' showed strong seasonal changes, reflecting seasonal and also flowering patterns (Barth *et al.*, 1996). In contrast, concentrations of N, P, K, Ca, Na, S, Cu, Zn, Mn and Fe were relatively stable during May to August and from December to February period in fully developed leaves of *Protea* 'Pink Ice'.

2. 4. 2 Growth as a measure of nutrient availability in plants

Protea plants grow in spurts called flushes, which are determined seasonally as either spring or autumn flush. The environmental conditions and species characteristics influence growth flush. Growth flushing can be used to determine nutrients accumulation according to the flush growth stages (Malan, 1993). Growth flushing has been studied in *Protea* species principally in relation to time of flowering and to understand site-dependant seasonal growth patterns in stems. Growth flushing patterns can be used to determine optimal periods for fertilizer application and leaf nutrient monitoring (Barth *et al.*, 1996). Monitoring growth flushing can assist to assess stem length requirements in terms of marketable stem and flower quality.

Protea neriifolia 'Kouga' and *Protea* 'Pink Ice' both have different peaks in their growth seasons. *Protea neriifolia* 'Kouga' showed peak growth in August and September (early spring) and *Protea* 'Pink Ice' showed peak growth in October (Barth *et al.*, 1996). Different peaks in growth activity may be strongly related to the availability of certain nutrients. For

instance, Harré (1988) found that N available to plants in late winter or spring promoted strong terminal vegetative growth during the early part of the summer.

However, when N was available in late spring it only promoted formation of a soft pendulous type of the terminal growth, especially for *Leucospermum* and *Proteas*. If N was available in late summer multiple lateral secondary shoots (bypass) growth are formed. Thus the season significantly modified the influence of nutrition on the type of growth that was produced. The seasonality of growth and nutrient uptake also influences the degree to which nutrients are removed from plants by harvesting of stems.

Nutrients removed by harvesting flowering stems were less than those removed by young fully developed leaves (Maier *et al.*, 1995). The following nutrients Ca, N, K, Na, S, Mg, P, Fe, Mn, Zn, B, and Cu were all lost to the plant through the harvesting of stems. However, Maier *et al.*, (1995) and Cecil *et al.*, (1995) found that N, Ca and K in stems were the main nutrients that were lost. Significant quantities of Na were also removed by harvesting.

Nutrient concentrations in leaves were influenced by the photosynthetic activity-taking place in the leaves and thus young active foliage is associated with greater concentrations of nutrients.

2. 5 SENSITIVITY TO NITROGEN

Nitrogen is absorbed as both NO_3^- and NH_4^+ forms in plants. Plant preference depends on species tolerance to the nutrient uptake. For *Protea* plants it seemed that the preferred N source is NH_4^+ , rather than NO_3^- . This is supported by the work of Heinesohn and Pammenter (1986) and Harré (1988). Heinesohn and Pammenter (1986) reported that growth was promoted on *Leucadendron salignum* when NH_4^+ was a source, but when N was supplied as NO_3^- , toxic effects resulted. Harré (1988) indicated that N should not be given to *Proteas* in

the form of NO_3^- because a 70 mg kg^{-1} , level is toxic and 150 mg kg^{-1} resulted in plant death. *Protea* 'Ivy' and *Leucospermum cordifolium* also tolerated NH_4^+ and *Protea* 'Ivy' failed to grow with NO_3^- only as an N source (Claassens, 1986). However, *Leucospermum patersonni* and *Protea repens* tolerated NO_3^- more than NH_4^+ as N source; the plants were chlorotic with few flower buds than when grown with NH_4^+ (Claassens, 1986).

Correct timing of fertiliser application is important for shoot growth. Poor timing of fertilizer application, especially when shoot growth has already commenced or had fully developed could have negative influenced on plant growth. *Protea repens* was found not to respond well to the application of nutrients in August and October since vegetative growth of these species had commenced in September (Lamb and Klausner, 1988). Should the application have been done before commencement of vegetative growth, nutrient application could have had a positive effect on growth of *Protea repens*. This means that nutrient applied after vegetative growth had no significant importance for plant growth. This late application might, however, benefit the subsequent vegetative growth of the following season.

2. 6 SENSITIVITY TO PHOSPHORUS

Plants absorb P either as $(\text{H}_2\text{PO}_4^{2-})$ or (HPO_4^{2-}) . P fertilization, considered normal for other woody plants, often causes phyto-toxicity in Proteaceae. Therefore it is generally recommended that P fertilizer should not be included in fertilizer programs (Criley, 2000). Silber *et al.*, (1997), in their experiment to determine optimal fertilization for *Leucadendron* 'Safari Sunset', found that the addition or increasing P improved both plant and root growth, and yield was also increased. Silber *et al.*, (1997) suggested that as long as the micronutrients are provided in irrigation with up to 20 mg l^{-1} P, growth would be improved without toxicity symptoms developing in *Leucadendron* 'Safari Sunset' since it is a vigorously growing plant. *Leucadendron* 'Safari Sunset' is the mostly widely grown crop of all Proteaceae, primarily

because it is highly adaptable. No significant reasons for this adaptability have ever been identified, but it is not representative of the majority of Proteaceae. It has been extensively researched in Israel because it survives well on the high pH Israeli soils.

Other researchers, (Claassens, 1986; Nichols, 1988; Hendreck, 1991a and 1991b), observed that high P in a solution tends to affect root growth, especially cluster root formation.

Claassens (1986) suggested that since plants are sensitive to high P, P application should be applied at very low rates. The chemical reaction that takes place between N and P in the soil seems to promote P uptake in the plant especially in mycorrhizae kind of plants. P seems to influence the availability of microelements such as Zn and Fe, but when P is available in high concentrations in a solution, P may mask Fe chlorosis in plants (Mortvedt *et al.*, 1972).

The boundary between toxicity and deficiency is remarkably narrow in Proteaceae (Montarone and Allemand, 1993). The reasons for the sensitivity to P are not really well known. However, P inactivates Fe in that it complexes with it within the cell. This may have profound effects, on metabolism for instance on photosynthesis. P might also compete with other chelating agents for Fe. A high phosphorus level generally thus causes chlorotic symptoms. Leake (1996) observed that some *Protea* plants, when supplied with high P, developed an apparent iron deficiency, mostly associated with interveinal chlorosis developing in young leaves and red colour in older leaves, but extending later to the whole plant. Toxicity symptoms, such as leaf necrosis in the presence of high P concentration, have been reported on numerous Proteaceae species (Claassens, 1986; Lamont, 1986; Goodwin, 1983; Silber *et al.*, 1998).

Al toxicity is one of the major problems in acidic soils because it inhibits root growth elongation and it also has inhibition blockage effects on Ca^+ , K^+ and P uptake (Buchanan *et*

al., 2000). There is no evidence of Al toxicity in *Protea* that has been reported or published. Montarone (2001) reported that the *Protea* plants remained healthy, although Al was available in large quantities (the large quantities were not explained - how large were the quantities) in Proteaceae leaves.

Although these plants can grow in acidic soils where Al commonly affects P availability, it seems that Al has no detrimental effects in *Protea* plants. In white lupin (*Lupinus albus*), Al toxicity has been reported to reduce plant growth with older leaves becoming chlorotic with necrotic spots developing in midrib and margins (Snowball and Robson, 1986). Thus, although Al is not harmful to *Protea*, it is harmful to *Lupinus*, which also grow in nutrient poor soils and also form "cluster roots".

2. 7 PLANT NUTRIENT DEFICIENCIES AND TOXICITIES

Salisbury and Ross (1992) classified N, P, K, Mg and Cl as mobile elements, which readily move through phloem from old to young leaves. Boron, Fe, and Ca were classified as immobile; lastly the intermediate elements were identified as Zn, and Mn. In general, leaves are sensitive indicators of nutrient deficiency. Tiaz and Zeiger (1991) define nutrient deficiency symptoms as the expression of a metabolic disorder, resulting from insufficient supply of essential nutrient element.

An essential element should be of central importance with specific functions in plant growth and shortage thereof will limit growth. The nutrients regarded as essential element to plants maybe due to the demand on that element and its shortage causing an abnormal development in the plant. Deficiencies of mobile elements are commonly visible in older leaves, whereas with the deficiency of immobile elements, symptoms are more visible in younger leaves (Marschner, 1997).

With nutritional deficiency leaves tend to be reduced in size, pale in colour, develop dead areas on tips and margins, or between veins with some abnormal shapes or structures on leaves (Kramer and Kozlowski, 1979). Other common nutrient deficiencies are stunted growth of plants, lodging, browning and bronzing of leaves, dryness, dying of internodes and terminal buds. Other examples include small roots (e.g. deficiency of P) or large root mass (e.g. deficiency of N), thin stems, flower formation failure, necrotic spots or death plant tissue, leaf lesions occurrence and irregularities in leaf shape (Salisbury and Ross, 1992; Epstein, 1972).

In deficient plants terms such as chlorosis, interveinal chlorosis, necrosis, stunting and abnormal coloration are used to describe or to define the extension of deficiency caused by a particular nutrient or element. Chlorosis in leaves is caused by interference with chlorophyll synthesis thus resulting in chlorophyll being reduced and therefore causing yellowing in leaves Kramer and Kozlowski (1979). Chlorosis varies with leaf age, which is mostly noticed in older than younger leaves involving the degree of deficiency severity (which can be at an early or late stage), and species.

Interveinal chlorosis is commonly termed “striping” because of the stripes like appearances in the internal parts of the leaves. Interveinal chlorosis is when leaf tissue between the veins turns yellow while veins remained green. Necrosis is death of plant tissue caused by complete dryness. It commonly starts from the tip and edges of older leaves. Stunting is when growth rate is reduced that usually results with plant having weak thin short stems.

Abnormal coloration on leaves of the plant reflects uncommon colour than the normal colours the plant is known with. Red, dark green, purple brown are common in deficient

plants (Bennett, 1993). In *Protea*, *Leucadendron* and *Leucospermum* there has been no definitive work on the visual deficiencies symptoms.

The information on the effects of macro elements deficiencies presented below is based on general or common indicative symptoms of deficiencies observed in other plants. Macro - nutrient element essentiality is determined by a requirement for vitality and the large quantities that are needed by the plants. The following nutrient elements are regarded as macro - elements: N, P, K, Ca, Mg and their functions and deficiency symptoms are described below.

2. 7. 1. Nitrogen. Nitrogen is part of the chlorophyll molecule and its deficiency causes leaves to become chlorotic. When N is a limiting factor to plant, limited chlorophyll availability restricts photosynthesis. As a mobile nutrient N deficiency is initially restricted to older leaves, but when the deficiency is severe the whole plant will also be affected. In most cases growth is restricted and stunted and the stem becomes woody when the deficiency is prolonged.

2. 7. 2 Phosphorus. Phosphorus is a constituent of plant compounds such as enzymes and proteins. It is an integral part of chlorophyll synthetic process and is involved in energy transfer and genetic information (Bennett, 1993). Triose phosphates are both substrate and activator of starch synthesis in stroma (Engels, 1999).

When P is deficient a smaller amount of triose phosphate is transferred from chloroplast with little starch being synthesised. Therefore P deficient leaves results in high starch accumulation (Engels, 1999). Since P is centrally involved in respiration especially in formation of sugar-phosphates in plants (Bennett, 1993) growth will be affected by P

deficiency. Common symptoms associated with P deficiency include dark green colour in leaves with large root mass but poor plant growth. When the deficiency is severe necrotic spots are also common with leaves having some malformation shapes.

2. 7. 3 Potassium. K maintains osmotic potential in cells thus making K an important element for water uptake, water retention in plant tissue and water transporter in cells. K stabilises pH in the guard cells, and is also required for maintenance of turgor and the opening and closing of the stomata.

Plants that have sufficient K have thicker cell walls and have more tissue stability because K is involved in cell growth through its role in turgor and cell expansion (Bennett, 1993, Salisbury and Ross, 1992).

Leaves that are K deficient have high susceptibility to light, which causes chlorotic and necrotic spots (Engels, 1999), and may later cause the leaf to develop leaf tip burn caused sensitivity to light. Leaf scorching especially on margins and on leaf tips of older leaves, is a very common symptom of K deficiency (Hewitt and Smith, 1975). Other common symptoms of K deficiency include lodging (easy bending of the stem) because of weak stems.

2. 7. 4 Calcium. Calcium is involved in cell elongation and cell division and is also a structural component of cell membranes. Ca lacks mobility; hence deficiency is often first observed in younger tissue. Failure of buds and internodes to develop in plants is a sign of Ca shortage (Bennett, 1993). Young leaves can be severely distorted with hooked tips and margins curling backward or forward. When the deficiency is severe the leaf margins start browning and are scorched.

2. 7. 5 Magnesium. When Mg is deficient, the plants usually exhibit similar symptoms to those of N deficiency, since Mg is central element of chlorophyll constituent, and Mg is thus important in light absorption. During the electron flow between photosystems II and I, the light driven pumping of protons into thylakoid lumens from stroma is counter balanced by transportation of Mg element (Engels, 1999). This might explain how the leaves become chlorotic when Mg is in shortage in leaves. The difference between the symptoms of N and Mg deficiency is that in Mg there is interveinal chlorosis and with N the whole leaf becomes chlorotic.

Both the essential and non-essential elements can produce toxic effects when in excess. According to Wallace (1961), an excess of one element may lead to a deficiency of another, which ultimately results in abnormal metabolic function. Excess availability of N or P may for instance; lead to insufficient availability of K, while excess K may lead to a deficiency of Ca and Mg (Wallace, 1961). Excess Cu, Zn and Mn may likewise induce Fe deficiency (Wallace, 1961). Excess Cl causes necrosis of leaf tip and outer edges that quite similar to K deficiency (Bennett, 1993). When Mn is excessive it inhibits the uptake of K and competes with Fe, Ca, and Mg, and excess Cu also competes with Fe and also inhibits root elongation and damages root cells (Bennett, 1993).

Excess Mo forms molybdocatechol complexes in vacuole and this complex compete with essential elements that are similar in valency and reaction. In this regard this competition of complexes and essential elements disrupts essential metabolic processes in plants. As a result of these interactions and often-similar visual symptoms, diagnosing toxicities is as complex as diagnosing deficiencies. Mineral element deficiencies and toxicities are both problematic and relatively unknown in the Proteaceae, although recent work by Shane *et al.*, (2004) has documented the influences of P toxicity.

Deficiency and toxicity of an essential element will generally first limit plant growth and then produce a specific symptom associated with the deficient element. In many cases the specific symptoms are not clearly diagnostic. For instance N and S deficiencies both cause leaf chlorosis. Excess and deficiency of Mn produced similar symptoms in barley with leaves showing chlorosis on young leaves. Nitrogen deficiency in legumes can also easily be associated with Mo or S deficiencies since N fixation in legumes prevented when Mo and S are deficient (Hewitt and Smith, 1975). Calcium deficiency in apples causes bitter pit or corky pit, which can easily be confused with drought spot and corky core in apples when B is deficient. Thus visual symptoms, although useful, are hard to use as a diagnostic tool.

Plant deficiency and plant disease also have the same or similar symptoms. The diseases such as like *Phyllochora* (leaf spots), *Coniothyrium* (leaf tip disease), and *Botrytis cinerea* in *Protea* plants (Coetzee and Littlejohn, 2001), can be easily confused with plant deficiency disorder such as necrosis and chlorosis in *Protea* plants. In other crops similar problems exist. For instance, alfalfa wilt disease may induce similar symptoms to K deficiency (Sprague, 1951). Furthermore nutritional deficiencies may interact with disease. For instance leaf spot disease was increased and was most severe when K and Ca levels were deficient, but not always when N was deficient (Marschner, 1997). Leaf spot and soft rot disease are common when B is deficient. Thus a disease and a deficiency can be seen as the cause of one another.

2. 8 CURRENT FERTILIZATION RECOMMENDATIONS.

Optimal plant growth in terms of nutrient application can be obtained when actual or recommended applications are known. Fertiliser programmes serve as information guide in order to understand the rates needed by the plant. The following recommendations give the basic knowledge of how much fertiliser application can be used on *Protea* plants.

2. 8. 1 Claassens motivation (1986):

Claassens (1986) had the following suggestions on application of N, P, and K fertilizers.

N: N is variable in all soil types and from one locality to the other, however, ammonium nitrogen and ammonium sulfate fertilizers were currently recommended.

P: P should not be given to plants because of the sensitivity to P nutrients and avoid damage to the rhizosphere.

K: Little is required because most of South African soils have sufficient K in them.

Proteaceae plants can tolerate a high K content provided NO_3^- and P are kept at low concentrations. Claassens (1986) recommendations on N, P and K were based on sand cultured *Protea* plants using modified half strength Hoagland No. 2 nutrient solution.

2. 8. 2 Harré's motivation on N fertilizer application (1995)

Harré (1995) recommended that N, P and K should be applied in the following doses: total N should be in the range of 40 mg kg^{-1} , but NO_3^- should not exceed 30 mg kg^{-1} . Phosphorus should be applied at 25 mg kg^{-1} level and lastly K at a level of 300 mg kg^{-1} . However, some cultivars can use double the amount of the nutrients; e.g. *Leucadendron* 'Safari Sunset'. Some cultivars, such as *P. cynaroides*, are very sensitive and 25% (w/w) extra of these elements can be toxic or cause problems such as leaf scorching to the plant.

2. 8. 3 Leake's P fertilizer recommendation (1996)

Leake (1996) suggested that where P is at 15 mg kg^{-1} in the soil, P should not be applied to plants. Lower than 5 mg kg^{-1} a moderate to low addition can be applied. Where P is equal or less than 1 mg kg^{-1} even the most sensitive plants can benefit from a P fertilizer program in this range.

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CHAPTER THREE

THE DISTRIBUTION OF MACRO- AND MICRO - NUTRIENTS WITHIN *PROTEA* (CULTIVAR) 'CARDINAL' PLANTS GROWN IN SAND CULTURE.

Abstract

The concentration of macro-elements (N, P, K, Ca and Mg) and micro - elements (Mn, Fe, B, S, Na) were monitored in roots, leaves, stems and flower buds. Rooted cuttings of *Protea* 'Cardinal' (*P. eximia* x *P. susara*) were grown from July 2001 to March 2002 in 10 L pots filled with an inert silica sand (4-8mm Ø) in a temperature controlled glasshouse in a randomised block design. The plants were harvested monthly from November 2001 to March 2002 and mineral contents of the different plant parts analysed.

The concentration of N decreased while P increased over time within tissues. K was more stable in stem than in leaf tissues. Ca was higher in basal leaves than in any other tissues. Mg increased over time in leaves and stems. Fe in leaf tissue increased over time and in stem tissue decreased with time. S increased over time in leaves and roots and decreased with time in stem tissues. Mn increased with growth stages in leaves but not in stems. B and Na decreased in leaves, stem and root tissues. Thus the mineral content changed with growth stages in roots, stems, leaves and flower bud tissues. Nutrient accumulation in *Protea P.* 'Cardinal' showed that leaves are not the best part of the plant for sampling because of nutrient variability in distribution pattern. The stem of *Protea* 'Cardinal' showed that nutrient sampling could be possible since most nutrients were relatively stable during growth period.

3. 1 INTRODUCTION

Cut flower production has increased because of an increase in demand for flowers with improved quality (Heinesohn and Pammenter, 1986). This demand is influenced by the changes in market expectations driven by the quality of flowers and the consistent supply of flowers to the market. In order to cultivate *Protea* plants for successful marketing the following serve as desirable traits: long stems of marketable quality, high yields, disease resistance, long vase life and suitable flowering time for the export market.

The genera of South African Proteaceae commonly used as cut flowers in the flower industry are: *Leucospermum*, *Leucadendron* and *Protea* (Criley, 2001). Proteaceae are perennials, usually sclerophyllous plants and are found on nutrient poor soils, which are oligotrophic as a result of being highly leached (Lamb and Klaussener, 1988, Coetzee and Littlejohn, 2001).

The Proteaceae originate from Australia and South Africa (Silber *et al.*, 1997). Most cultivated Fynbos plants prefer cool soil temperature with some mulching and soils with good water holding capacity and good aeration (Coetzee and Littlejohn, 2001). Proteaceae are woody evergreen perennial shrubs with sclerophyllous leaves that withstand dry and hot weather conditions. The growing season in *Protea* plants are winter (March to August), spring (September to November), and autumn (February to March). Fynbos under general translation terms it means “finebush”. These plants are indigenous plants to South Africa. Some genera in Proteaceae family such as *Leucospermum*, *Leucadendron salignum* and *Protea cynaroides* can generate vegetative growth through sprouting known as lignotuber.

Leaves from the youngest flushes, but that are fully expanded, and also basal stem leaves have been used for nutritional analyses in Proteaceae (Cecil *et al.*, 1995, Barth *et al.*, 1996). The use of nutrient analyses of leaves to indicate when *Protea* plants require nutrient application has not been particularly useful (Barth *et al.*, 1996). This is partially because

nutrient concentration in tissue, especially in leaves, fluctuates seasonally. However, analysis of other plant parts may be good indicators of when plants require nutrient application and this could serve as a guide for nutritional programs.

In this study nutrient contents of the plant parts were monitored over a growing season. The aims of this study were to determine the distribution of macro- and micro - nutrients within different plant parts and whether the nutrient content of different plant tissues varied over time in association with a specific growth stage.

3. 2 MATERIALS AND METHODS

3. 2. 1 *Plant material*

The experiment was conducted at Agricultural Research Council (ARC) at Elsenburg, Stellenbosch, Western Cape, (35° 50' 80" S, 18° 50' 10" E, 200 m above sea level) in a polycarbonate covered glass house equipped with a wet wall temperature control system. Rooted cuttings of *Protea* 'Cardinal', a cross between *Protea eximia* and *Protea susannae* were planted in 10 L containers in July 2001 in inert silica sand (4-8mm Ø) in a block design with six blocks and five replicates per block in three lines adjacent to each other. The plants were fertigated with nutrient solution made from Kingpin and Supercal (Agrofert, South Africa) using an automatic irrigation system. Kingpin consisted of (g/kg) NH₄⁺ 83, P 37, K 189, Mg 81, S 192, B 0.17, Fe 0.265, Mn 0.115, Zn 0.105, Cu 0.055, Mo 0.035. Supercal contained (g/kg) NO₃⁻ 136, Ca 220, Cl 80. A 0.375 mS cm⁻¹ EC solution made up from 274 g Kingpin with 138 g Supercal in 2250 L water was used for the first month and then a 0.75 mS cm⁻¹ EC solution containing a 549 g of Kingpin and 276 g of Supercal in 2250 L were used thereafter. This resulted in the final nutrient solution of (µM) NH₄⁺ 148.21, P 29.87, K 120.84, Mg 83.30, S 149.53, B 0.39, Fe 0.12, Mn 0.05, Zn 0.04, Cu 0.02, Mo 0.01, NO₃⁻ 388.57, Ca 219.57 and Cl 90.27. The pH was adjusted to 5.5 using 0.1 M sulphuric acid

(H₂SO₄). The pH was checked after making up solutions in the tanks and also at the outlets to the pots in the greenhouse and corrected in the tank when necessary. A dripper-cleaning agent (Drip-a-tron) was included (1.2 L per 2250 L) and a sterilant (Sporekill) was also included (40 ml per 2250 L) to control fungal growth in nutrient tanks.

3. 2. 2. *Harvest*

Three replicates of two plants of each were harvested at monthly intervals from November 2001 to March 2002. This time frame coincided with the production of two growth flushes and a flower head. Plants were divided into 1st flush leaves, 2nd flush stems, basal leaves, basal stems and roots. The roots were washed in distilled water to remove growth medium and thereafter all the plant materials were oven dried for 48 h at 75^oC.

3. 3. 3 *The sampling plan*

For nutrient analyses, plants were sampled for first flush leaves, second flush leaves, first flush stems, second flush stems, basal stem, basal leaves, immature and mature flower buds and also roots. The various stages sampled are shown in Table 3. 1.

Table 3. 1. The sampling dates, growth stages and number of plants sampled at each stage. Note that each repetition was comprised of 2 plants combined to comprise a “bulked” sample.

Date	Growth stage sampled	No replicate plants
July 2000	Planted trial	
April 2001	No flush developed	3
May 2001	No flush developed	3
June 2001	1 flush	3
July 2001	1 flush	3(2 plants/replicate)
August 2001	1 flush	3(2 plants/replicate)
September 2001	1 flush	3(2 plants/replicate)
September 2001	2 flushes	3(2 plants/replicate)
October 2001	2 flushes	3(2 plants/replicate)
November 2001	3 flushes	3(2 plants/replicate)
December 2001	3 flushes + flower bud	3 (2 plants/replicate)
January 2002	3 flushes +flower bud	3 (2 plants/replicate)
February 2002	3 flushes +flower bud	3 (2 plants/replicate)
March 2002	3 flushes +flower bud	3 (2 plants/replicate)

3. 2. 4 *Chemical Analyses*

The dried and milled samples were analysed for N, P, K, Ca, Mg, Mn, Na, S, B, Fe using a dry ash method and the inductive coupled plasma (ICP) for detection purposes methods. N was analysed using Kjeildahl digestion. ICP was used to analyse P, K, Ca, Mg, Mn, Na, and Fe (Giron, 1973). Total sulphur present as sulphate was dissolved in HCL and the concentration was determined using turbidimeter with spectrophotometer (Wimberley, 1968). All analysis was performed by the Analytical Laboratory of the Provincial Department of Agriculture, Western Cape, Muldersvlei Road, Elsenburg, South Africa.

3. 2. 5. *Statistical analysis*

Analysis of variance was performed on data from chemical analysis for each nutrient element using the General Linear Model (GLM) procedure of SAS (SAS 2000). Student's t Least Significant Difference was calculated at 5% level to compare interaction means. The Shapiro-

Wilk test (1965) was performed to test for normality, and after transformation into normality all data was normally distributed.

3. 3 RESULTS

3. 3. 1. *N concentration in plant tissue*

Generally N concentration seemed similar in all tissues and decreased over time in leaves, stems, roots and flower buds, especially towards the end of the growth period (Fig.1). Despite the overall trend in N concentration, there was a short-lived increase in roots, basal leaves and basal stems in February. Overall, though, N was decreasing with time, the concentration of N in the stems was lower than that in leaves.

3. 3. 2. *P concentration in plant tissue.*

The P concentration in 1st and 2nd flush leaves, basal leaves, 1st and 2nd flush stems increased over time while that in the root tissue was relatively unchanged (Fig. 2). Although P was highest at the end of the growth period in both stems and leaves, P was relatively low in absolute terms in both stems and leaves ranging between 125 and 25 $\mu\text{mol g}^{-1}$ DW in leaves and between 125 and 50 $\mu\text{mol g}^{-1}$ DW in stems.

3. 3. 3. *K concentration in plant tissues*

The K in leaf tissues increased towards the period of flower bud development, but this was not true for stem tissue. The concentration of K was relatively stable in stems. The roots had the lowest *ca.* 81 $\mu\text{mol g}^{-1}$ DW in January (Fig.3) although; there was a peak in February (*ca.* 150 $\mu\text{mol g}^{-1}$ DW). Potassium was generally more stable and higher in stems than in leaves and was lowest in roots.

3. 3. 4. *Ca concentration in plant tissues*

There was no strong pattern of changes in Ca concentration in the 1st flushes and 2nd flushes leaves, although it did seem to slightly increase over time (Fig. 4). Stem and root tissue Ca concentrations were highest at the start of the growth period. Ca was lower in root tissues than in other tissues *Protea* cv. 'Cardinal' (ca. 40 $\mu\text{mol g}^{-1}$ DW).

3. 3. 5. *Mg concentration in plant tissue*

Magnesium increased over time in leaves, particularly at the stage when the flower head had fully developed in March (Fig.5). In stems and roots Mg decreased over time, although, as in leaves, there was a trend of increase towards the end of the growth period. The Mg concentrations of the 2nd flush stems were anomalously high.

3. 3. 6. *Fe concentration in plant tissue*

Iron concentrations in leaf and stem tissues were significantly lower than those in roots (Fig 6). The Fe in the leaves increased strongly over time. The increase of Fe in flower buds over time was not significant. The Fe concentrations in the basal stems also increased over time. Fe concentration in roots was not significantly different between sample times.

3. 3. 7. *Na concentration in plant tissue*

Na concentration was not significantly different between the different leaf classes and did not vary significantly over time (Fig. 7). Na in roots and stems had a pattern of being low in January and high in February and decreasing in March. The overall Na concentration decreased over time in roots and stem tissues.

3. 3. 8. *B concentration in plant tissue*

In leaves, stems and roots B decreased over time and was lowest at the end of the growth period (Fig. 8). Basal leaves had the highest B concentrations throughout the growing period. There were no strong differences between any of the other tissues measured.

3. 3. 9. *S concentration in plant tissue*

Apart from an anomalous low in S concentration for basal leaves in February, the S concentrations of the leaves seemed to decrease slightly during the initial growth period and then increase towards the end of the growing period (Fig. 9). The S concentration in stems and roots was lower than that in leaves, although roots had consistently higher S concentrations than stems. The S concentrations in roots and stems did not change strongly over growing time.

3. 3. 10. *Mn concentration in plant tissue*

The Mn concentrations of the basal leaves were consistently higher than those of the other tissues, with an anomalous increase at the end of the growing period (Fig. 10). Apart from this, there were relatively small changes in concentration of Mn in leaves, stems and roots, over time.

3. 4. DISCUSSION

The results showed that N concentration was higher in leaves than in stems and generally decreased over the growing period. Ran *et al.* (2001) found that N decreased over time from 2.1 to 1.5% (w/w) (1500 to 1071 $\mu\text{mol g}^{-1}$ DW) during the summer period in the leaves of *Leucadendron* 'Safari Sunset'. In the basal leaves of *Protea* 'Cardinal', N decreased from ca. 600 - to 325 - $\mu\text{mol g}^{-1}$ DW over the growth period. These values are considerably lower than those of Ran *et al.* (2001) whose values are at the top end of the scale for conventional crop

plants. The decrease over the season reported here differs from the findings of Maier *et al.* (1995) who found that N in stems of *Protea* 'Pink Ice' was higher than that in leaves. N is a mobile element and can easily move from older tissues to young tissue; this may be the reason for N being higher in the younger leaf and stem tissue than in basal leaves and basal stem and roots.

Cecil *et al.* (1995) found that mobile elements (N, P, K and Mg) usually decline during the season, thus allowing concentrations to be retranslocated from older tissues to nutrient sinks such as fruits, seed or storage organ. In woody plants and deciduous trees the same trend of N declining from older tissues was noticed during growth season (Kramer and Kozlowski, 1979). The N decrease was in accordance with the notion that mobile nutrients decrease from older tissues to young tissues through the season. Therefore N decline or decrease, especially at the end of the growing season does not imply that N is deficient or N is in short supply to the plant. In general terms, N should be applied at the beginning of the growing period. This is to ensure that N is being utilised optimally during plant growth since N is a leachable element.

Phosphorus concentration in *Protea* 'Cardinal' increased over time in stems and in leaves. Maier *et al.* (1995) reported that a P value of $>0.57\%$ (w/w) ($184 \mu\text{mol g}^{-1}$ DW) was a toxic concentration in *Protea* 'Pink Ice' and a P value of $0.52\text{-}0.13\%$ (w/w) ($168 - 42 \mu\text{mol g}^{-1}$ DW) was considered tolerable for most *Protea* plants. In this study no toxicity or any form of deficiency was observed in *Protea* 'Cardinal', though, leaves and stems accumulated high P concentrations. In the leaves of *Protea* 'Cardinal', P increased in the range of $25 - \mu\text{mol g}^{-1}$ DW to $150 - \mu\text{mol g}^{-1}$ DW. Ran *et al.*, (2001) found that P in *Leucadendron* 'Safari Sunset' increased over a range of $0.05 - 0.16\%$ (w/w) (16 to $52 \mu\text{mol g}^{-1}$ DW) over time. From the present study it seemed that the leaves and stems accumulated more P as growth continued.

The high P in stems and leaves might be an indication that *Protea* plants tend to accumulate P in large quantities when present in a solution; this might be the cause of the sensitivity of Proteaceae to toxicity and their reputation as P sensitive plants.

K in leaves increased from 0.32% to 0.47% (w/w) (82 to 121 $\mu\text{mol g}^{-1}$ DW) over time in leaves of *Leucadendron* 'Safari Sunset' (Ran *et al.*, 2001). No strong increase was observed in the present investigation, although K did accumulate in leaves towards the end of the growing period. In contrast, the level of K in the roots was higher initially (November 178 $\mu\text{mol g}^{-1}$ DW) and lowest finally (March 92 $\mu\text{mol g}^{-1}$ DW). K is a highly mobile element within the xylem and phloem, thus making K a transient element in stems. K is important in buffering anions and stabilizing the internal pH and functions in transport of metabolites (Bennett, 1993) thus having a direct related function in regulating osmotic pressure of the guard cell (Läuchli and Bielecki, 1983) in plants, which makes K easily translocated in tissues.

Ca is an immobile nutrient that seems to be "trapped" within older tissues, thus becoming less available to young tissues. Mengel and Kirby (1982) reported that Ca couldn't remobilize to growing tips or young leaves. The high Ca in basal leaves can be encouraged by suberisation, thus restricting Ca from moving upward through the xylem and resulting in high Ca pools in basal leaves. The accumulation of N and Ca in plant tissues seemed to behave differentially in the reverse order, in that N tended to decline and Ca tended to increase in older parts. Since these cuttings were rooted from field material, high accumulation of Ca in basal leaves in the beginning of growth period could be from stored Ca in cuttings. Hewitt and Smith (1975) found that Ca tends to accumulate in basal leaves rather than in apical leaves. These findings correspond with the results from *Protea* 'Cardinal' in that Ca was more highly accumulated in basal leaves than in 1st and 2nd flush leaves.

The Mg, Mn, S and Na concentrations in leaves, stems and roots did not vary strongly over time. However, S and Mn tended to accumulate in older leaves. Kramer and Kozlowski (1979) reported that Fe and Zn are sometimes precipitated in xylem and thus may fail to reach the young shoot growth. Higher Fe concentration was accumulated both in the older leaves and roots than in young tissues in *Protea* 'Cardinal'. In young leaves (1st flush leaves) and young stems (1st flush stem) Fe was lower in concentration than in basal leaves and stems.

Boron is a relatively immobile element, but B concentration is likely to be higher in older leaves compared to young leaves (Mengel and Kirby, 1982), as it was in the case of basal leaves of *Protea* 'Cardinal' where B was higher than in other tissues. B in roots and stems decreased over time and was rather stable in 1st flush leaves.

In general, N decreased towards the end of growth period while P increased in leaves, stems and root tissue. K was higher in leaves and stable in stems and variable in roots. Ca was higher in basal leaves than in 1st and 2nd flush leaves. Fe increased with time in leaves, but in stem tissue decreased with time. B decreased with time in tissues and Mn was stable in 1st and 2nd flush leaves and increased with time in basal leaves. In stem and root tissue Mn was low and decreased with time. S in basal leaves increased with the same pattern as the Mn, high towards the end of growth period and was lower in stems than leaves.

Thus the patterns of nutrient distribution over time were complex and it is hardly possible to proclaim which would be the best time or tissue to sample. In many cases the leaves are appropriate for sampling. Although the concentrations of some of the nutritional elements in the leaves may be more variable over time than in the stems, it is the leaves that are the centers of metabolic activity and the site at which nutritional disorders are most likely to be manifested.

3. 5. CONCLUSION

Does tissue sampling as a tool for nutrient determination provide a good indication for *Protea* 'Cardinal'? The data indicates that sampling for nutritional determination is possible but fraught with complexities of interpretation due to variability between tissues and in mobility and immobility of the various elements. Therefore it is not possible to identify one particular strategy that is appropriate for all circumstances. Preliminary visual diagnosis (see following chapter) might aid in identification of potential problems and then strategies of sampling should be applied according to suspected problems.

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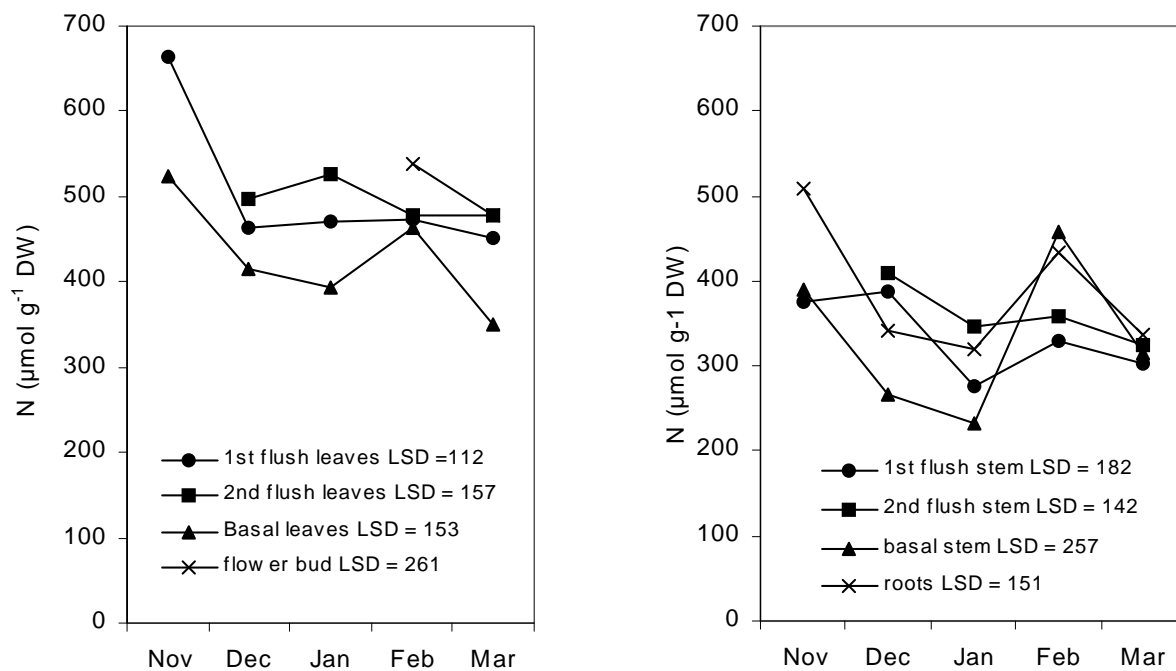


Figure 1. The nitrogen (N) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

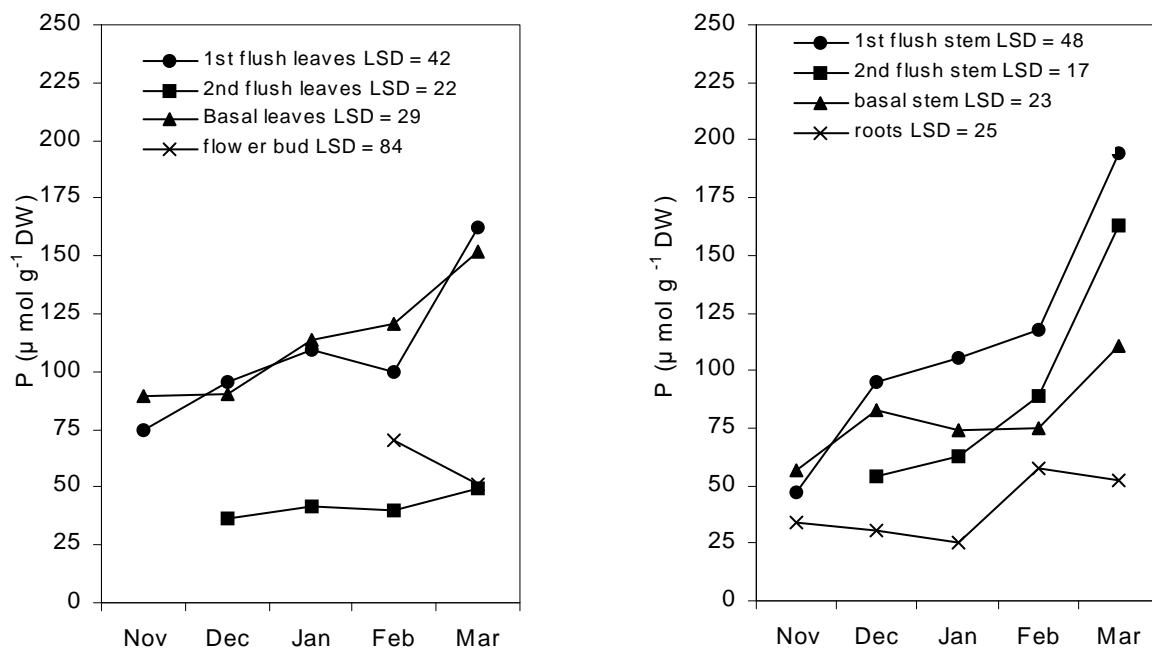


Figure 2. The phosphorus (P) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

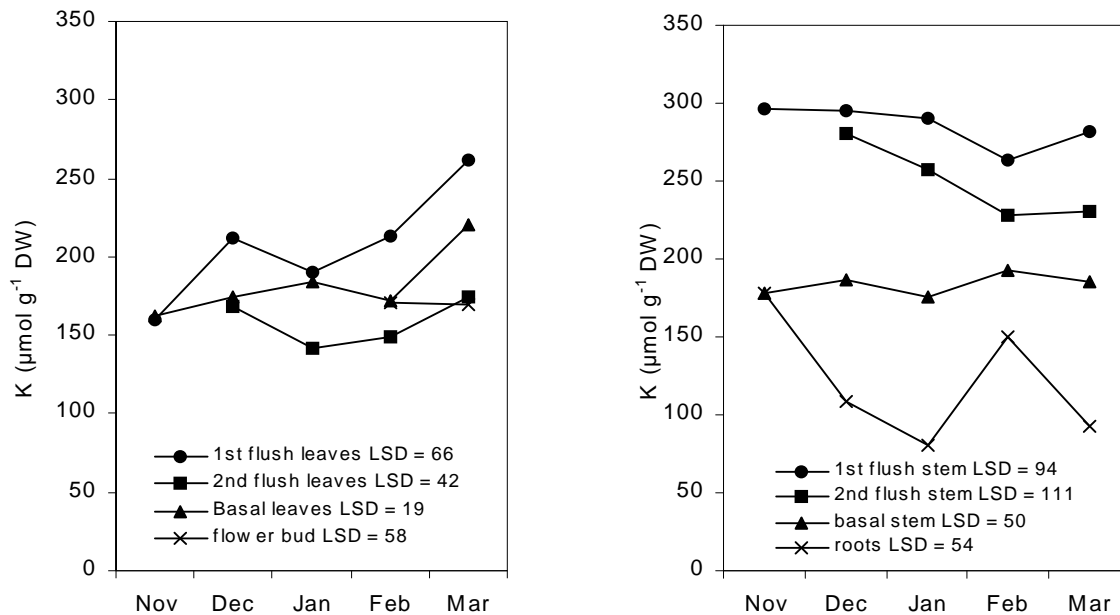


Figure 3. The potassium (K) concentration in the tissue of *Protea* 'Cardinal' grown in sand culture with fertigation. The plants were grown for nine months and individuals (n=3) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the LSD_{0.05} are shown on the graphs.

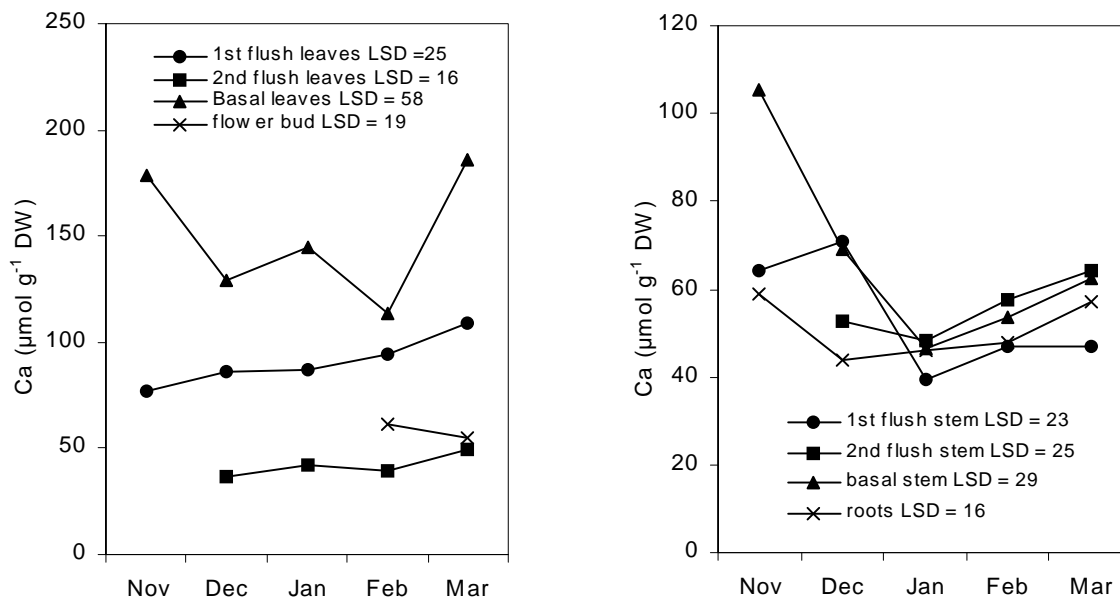


Figure 4. The calcium (Ca) concentration in the tissue of *Protea* 'Cardinal' grown in sand culture with fertigation. The plants were grown for nine months and individuals (n=3) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the LSD_{0.05} are shown on the graphs.

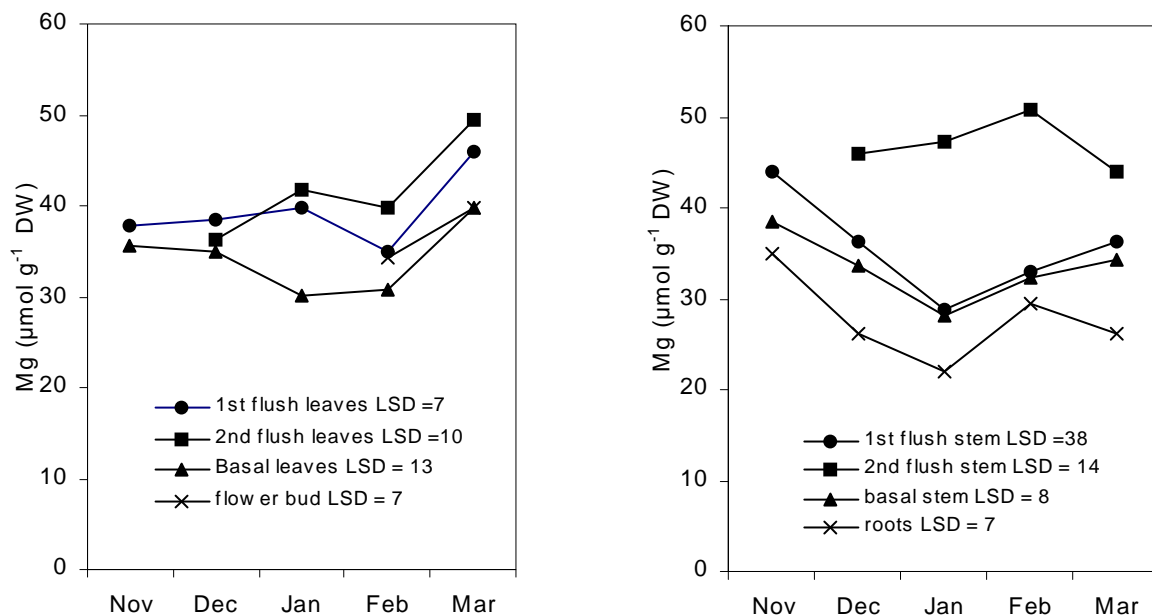


Figure 5. The magnesium (Mg) concentration in the tissue of *Protea* 'Cardinal' grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five period (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

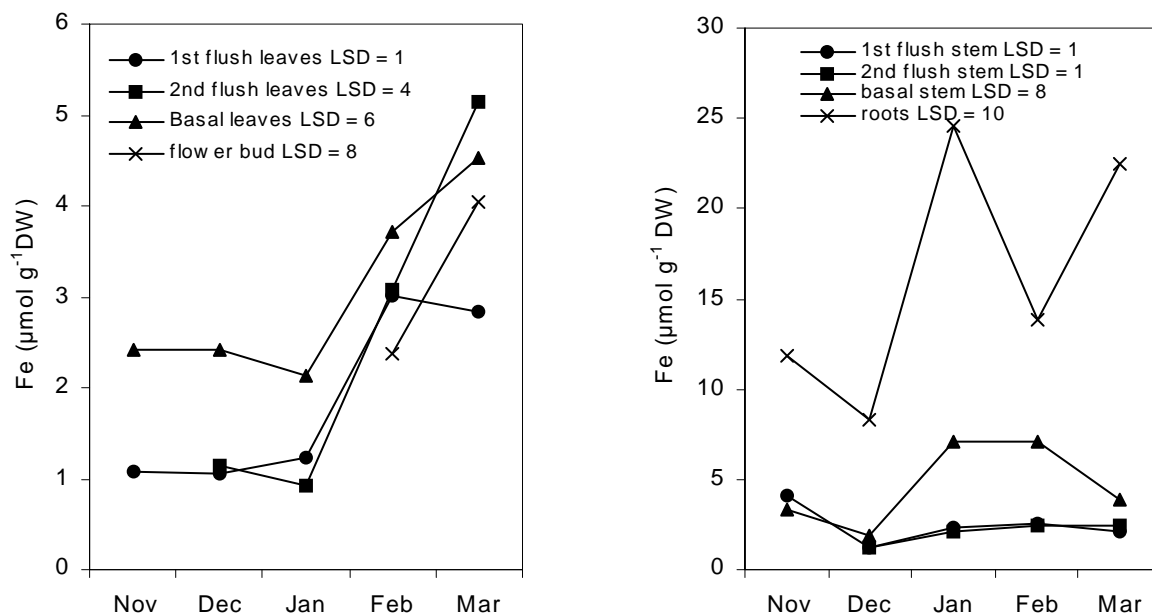


Figure 6. Iron (Fe) concentrations in the tissue of *Protea* 'Cardinal' grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five period (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

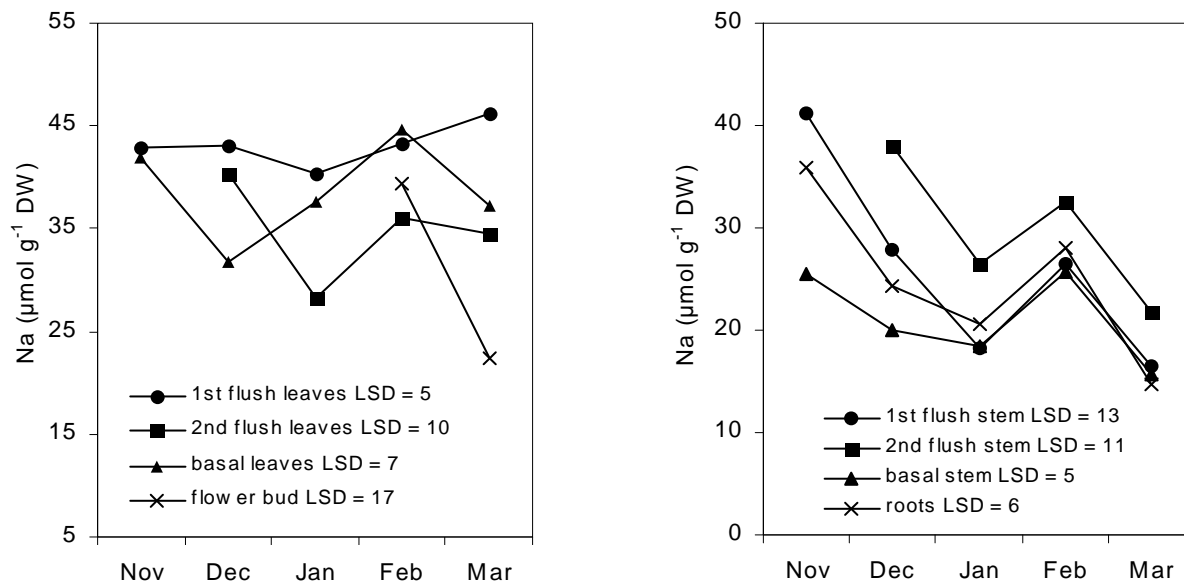


Figure 7 The sodium (Na) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

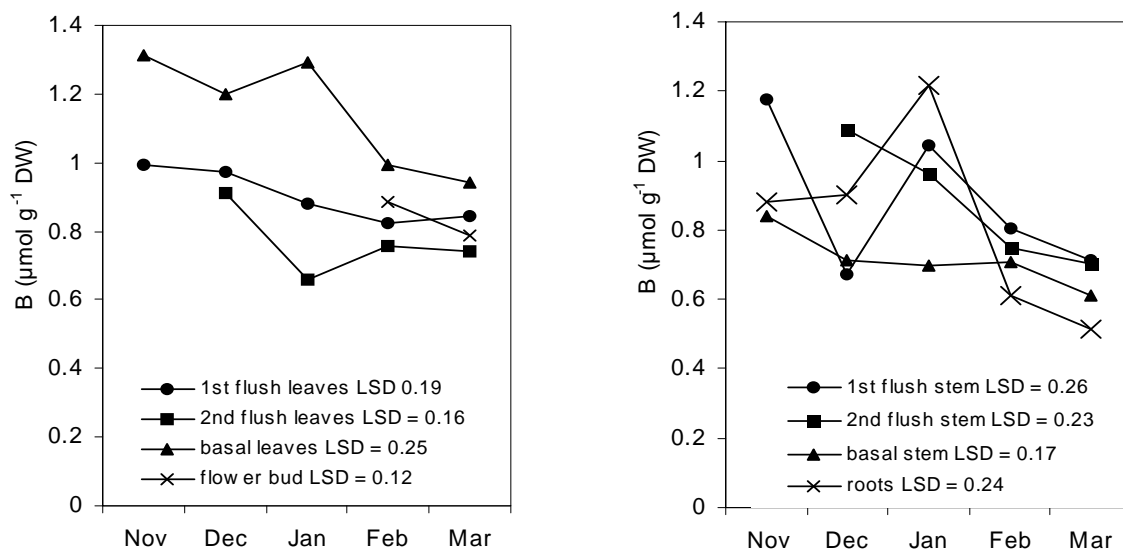


Figure 8. The Boron (B) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

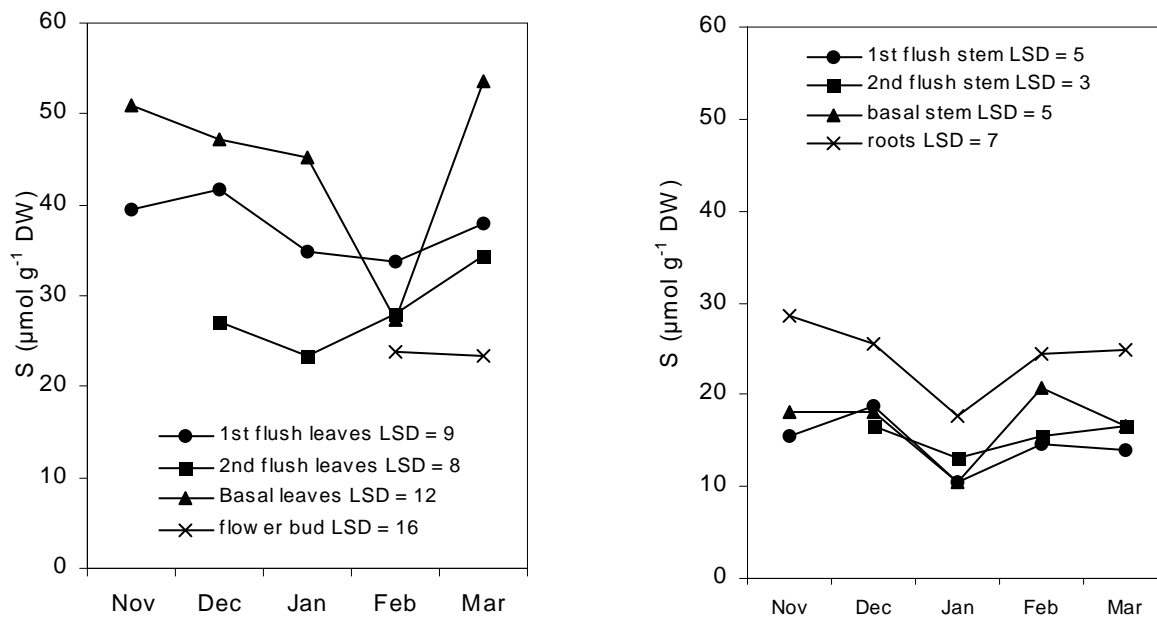


Figure 9. The sulphur (S) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five period (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

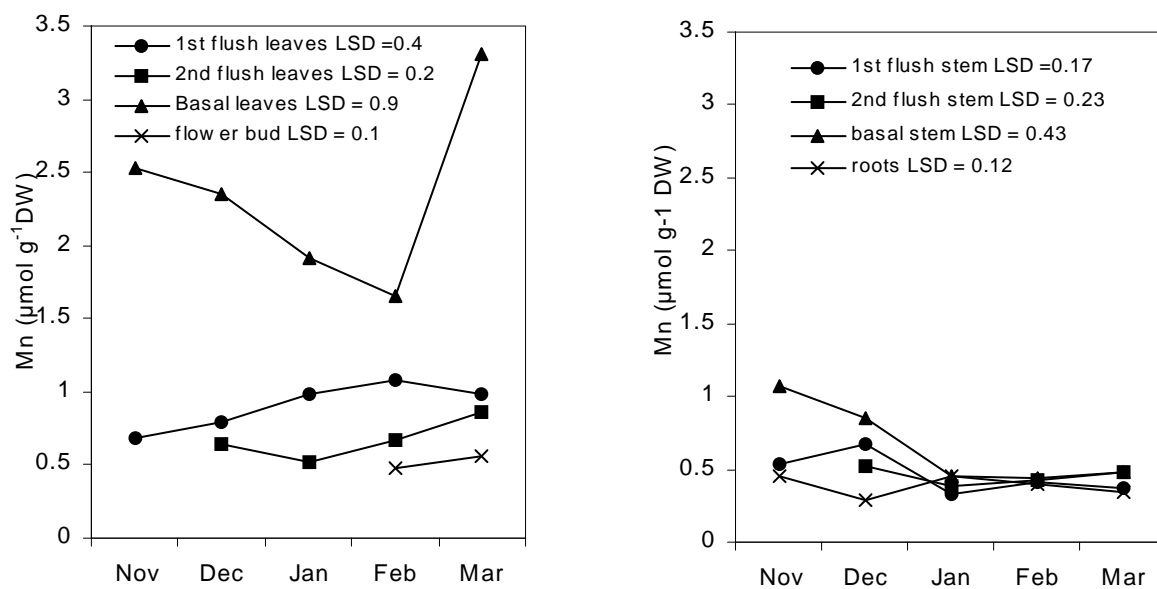


Figure 10. The manganese (Mn) concentration in the tissue of *Protea* "Cardinal" grown in sand culture with fertigation. The plants were grown for nine months and individuals ($n=3$) harvested over five periods (Nov-March). The plants were sub-divided into 1st and 2nd flush leaves and stems basal stems and basal leaves, roots and flower buds. The values for the $\text{LSD}_{0.05}$ are shown on the graphs.

CHAPTER FOUR

THE SYMPTOMS CAUSED BY DEFICIENCY OF MACRO- NUTRIENTS IN *PROTEA, LEUCADENDRON AND LEUCOSPERMUM.*

Abstract

The aim of the study was to investigate the visual nutrient deficiency symptoms on three genera of Proteaceae *Protea*, *Leucospermum* and *Leucadendron*, on eleven different cultivars grown in siliceous inert sand under controlled temperature conditions in a tunnel. The cultivars were chosen because of their high market value.

The treatments consisted of a complete nutrient solution "Control" and five withheld macro - nutrients (N, P, K, Ca or Mg). Visual symptoms were recorded on two different growth stages. The early stage was recorded from December 2002 to April 2003 and the late stage was recorded from May 2003 to September 2003.

Withholding nutrients of N, P, K, Ca and Mg on eleven cultivars showed the classical deficiency symptoms that are common in most crops. In N deficiency, the lower leaves were chlorotic and the upper leaves were still green at the early stages of deficiency. At the late stage in N deficiency the lower leaves' veins formed a chlorotic "V" shape and the whole plant was chlorotic. The cultivars were dark green in P deficiency treatments and later leaf scorch developed in some of the cultivars. Leaf burn was noticed in K deficient plants.

Leucospermum 'High Gold' showed typical Ca deficiency symptoms such as terminal buds dying off. The Mg deficient plants had interveinal chlorosis, especially *Protea* cultivars. The cultivars *Leucospermum* 'Tango', *Leucadendron* 'Chameleon' and *Leucospermum* 'Succession 11' did not grow well in complete nutrient solution; and the leaves had leaf tip burn.

4. 1. INTRODUCTION

A nutrient deficiency occurs when an essential nutrient is not available in sufficient quantity to meet the needs of the growing plant (McCauley *et al.*, 2003). A plant's sufficiency range is defined as the range of nutrient necessary to meet the plants nutritional needs and to maximise growth (McCauley *et al.*, 2003). The nutrient level not within the plant sufficiency range will cause growth reduction either due to deficiency or toxicity. Toxicity in plants occurs when the nutrient element is in excess of what the plant needs. Imbalanced supply of nutrients in a solution can cause growth reduction and visual symptoms may be expressed (Beardsell, 1995). Therefore, plants need a correct combination of nutrients to live and to grow (Hosier and Bradley, 1999).

Nutrients in nutrient solution should be available in balanced ratios in such a manner that plants will not be injured or killed by a nutrient in solution. Certain elements should not be available in higher quantities as this may cause antagonistic to other elements thus giving a false interpretation. Wallace (1961) reported that an excess of one element might lead to a deficiency of another and this can cause confusion when diagnosing these elements. For example, excess N or P may lead to lower availability of K or excess K may lead to a deficiency of Ca and Mg. Excess of Cu, Zn and Mn may induce Fe deficiency.

Translocation (movement of solutes in a plant) and mobilization (transportation) of solutes to different tissues and organs influence nutrition in plants. Greulach (1973) describes mobilization and translocation as movement of solutes from a region where they are absorbed or synthesized to a region where they are utilized. These two processes govern the mobility and immobility of solutes in a plant. A “mobile” nutrient is a nutrient that can translocate easily from old tissue to young tissues or to aerial parts of the plant. An “immobile” element

cannot easily be translocated to other parts of the plant once absorbed within the root zone (Bierman and Rosen, 2005; McCauley *et al.*, 2003).

When an element is mobile its deficiency is predominant or more visible in older leaves, whereas with immobile elements, the deficiency will be more visible in younger leaves and in terminal buds (Beardsell; 1995; Hosier and Bradley, 1999; and Wong, 2005). Elements such as N, P K, Mg, Mo and Cl are regarded, as mobile elements because they can readily move through phloem and from older to young leaves, and their symptoms are more visible in older leaves than in younger leaves. For those that are less mobile or immobile, for example B, Fe, Ca and S the symptoms are more visible in younger leaves than in older leaves. Deficiency of intermediate mobile elements such as Zn results in growth reduction of young leaves and stem internodes. And the intermediate elements are likely to cause interveinal chlorosis on young and old leaves (McCauley *et al.*, 2003).

Leaves are usually sensitive indicators of nutrient deficiency in plants. The "Key" method is the descriptive technique used to identify and to describe the visual deficiency symptoms in plants. The "Key" consists of different alternative statements about the plant structures and their appearance and it is advisable, if possible, to have a healthy plant for comparison (McCauley *et al.*, 2003). In the "Key" technique the focus is based mostly on leaves, either "Upper" or "Lower" leaves, to identify the deficiency, and nutrients are classified as either mobile elements or immobile elements.

The common characteristics of nutrient deficiencies are stunted growth of plants, lodging, browning, and dryness, dying of internodes and terminal buds. Other symptoms include thin stems, failure of flower formation, necrotic spots, and irregularities in leaf shape (McCauley *et al.* (2003); Hosier and Bradley, 1999; Wong, 2005). McCauley, *et al.*, 2003 defined the

deficiency terminology: 1) necrosis is death of a plant tissue eventually resulting in plant death. 2) Interveinal chlorosis is yellowing between leaf veins, yet veins remained green. 3) Generalized symptoms are symptoms not limited to one area of a plant, but rather spread over the entire plant. 4) Localized symptoms are symptoms limited to one area or section of the plant or leaf. 5) Burning is explained as severe localized yellowing; scorch appearance mostly is noticed on the leaf tips or on leaf margins. 6) Chlorosis is defined as general yellowing of the plant tissue due to lack of chlorophyll. Kramer and Kozlowski (1979) reported that this chlorosis in leaves is caused by interference with chlorophyll synthesis thus resulting in chlorophyll concentration being reduced, and yellowing of leaves.

Use of visual symptoms as a diagnostic tool to identify nutrient deficiencies in plants has the disadvantages that deficiency might not be easy to address or to correct on time. Crop quality and yield can be affected, especially when deficiency is noticed at the late stage. The corrective measures might not be useful when deficiency is noticed at the late stage and might also be difficult to correct. Furthermore, some visual symptoms look similar; for example the deficiency of Mg and Fe, which both cause interveinal chlorosis (Wallace, 1961). Crop species, and even some cultivars of the same species, can differ in their ability to adapt to a nutrient (McCauley *et al.*, 2003), and false interpretations of deficiencies based on species knowledge might disable diagnosis.

Plant nutrition deficiency problems should also be considered with other environmental conditions in which the plant lives. Apart from nutrient elements, factors such as environmental conditions, water, farm activities, pests and bird injuries can cause disorders that might appear to be nutrient deficiency problem in plants. Shortage of water can cause the leaves to be small and curly. Leaf burn or scorching due to high light intensity and high salinity in water can easily be confused and associated with K or S deficiency (Wallace, 1961;

Cramer *et al*, 2004). Visual diagnosis alone, therefore, might not be enough to conclude that the plant is deficient. Better results in diagnosing plant nutrient deficiency symptoms could be obtained by combining the visual observations with tissue and soil analysis.

Nevertheless, visual diagnosis is effective for rapid indication of potential problems and guidelines as to potential deficiencies are important tools for crop management. These do not currently exist for the Proteaceae. Table 1. Summary of general deficiency symptoms observed in plants.

Deficiency	General Symptoms
Nitrogen	<p>N plays an important role in many functions of the plant. It is needed for formation of DNA and RNA and for chlorophyll.</p> <p>The general symptoms of nitrogen deficiency are restricted or stunted growth with woody stem. Growth is slow. Shoots and leaves are short, thin and small. When deficiency is starting lower leaves are chlorotic while the upper leaves remain green and when the symptoms becomes severe the veins become chlorotic in a “V” shape form. The "V" shape veins might be caused by the leaf trying to draw N from the veins thus causing dryness in veins. Defoliation becomes premature in older leaves (Beardsell 1995; Epstein, 1972; McCauley <i>et al.</i>, 2003; Wallace, 1961, and Wong, 2005). Greulach (1973) reported that when N is deficient abnormal production of anthocyanin (red tints) do develop, which is caused by sugar accumulated in the plant that cannot be used in synthesizing metabolic product activities of the plant.</p>

Phosphorus P plays an important role in plant metabolism, 6-sugar-phosphate in respiration processes, photosynthesis and in DNA and RNA formation. P is necessary for seed germination; flower and fruit formation; therefore poor fruit size and quality in flowers may result. The dark green, purple tints or dull bronzing with browning spots are common in P deficient plants. In maize as symptoms start to develop, the plant loses its chlorophyll allowing the purple colour to dominate. The purple colour starts to accumulate because of the sugar content dropping off (Sprague, 1951). Growth may not be restricted but leaf scorching at a later stage is possible. Leaf margins might also have some scorching effects. Leaves may curl forward. The root mass becomes small unlike in P deficiency and in N deficiency the root mass is large. (Bennett, 1993, Wallace, 1961).

Potassium K plays an important role in regulation of plant turgor and osmotic potential. It is important for opening and closing of stomates and is abundantly found in the guard cell. Plants that are K deficient are likely to be susceptible to disease and fungus like blossom end rot in tomatoes, and lodging is common in K deficient plants. Leaf burning, marginal leaf scorching, and leaf curling and leaf browning are common, thus affecting the luster and brightness of leaf resulting in dull leaves. Growth can possibly be restricted. K symptoms are mostly exhibited in older leaves of the plant (Beardsell, 1995; Bennett, 1993 and McCauley *et al.*, 2003).

Calcium Calcium is an immobile element that is not easily redistributed from old tissue once stored in the plant tissue. The plant uses Ca for the formation of new cells particularly middle lamella, which is rich with pectin for separating the newly divided daughter cells from the mother cells. It also acts as a second messenger to many plant membranes. It can bind with Ca-calmodulin, a protein in plant cytosol. Ca influences water movement in cells. Young leaves are affected mostly by calcium deficiency than matured leaves. Terminal bud may fail to grow, or to open and when they do grow it is possible that they become out small. Marginal scorching with interveinal chlorotic spots as well as necrosis is common. Root systems are poorly developed and lack fiber. The root system symptom is not an easy diagnosis, unless the diagnosis is done on plants grown in hydroponic systems. Appearance of foliage near the shoot tips is striking green colour (Beardsell, 1995, McCauley *et al.*, 2003, Wallace, 1961).

Magnesium Mg is the central molecule in chlorophyll and is an important co - factor for the production of ATP. Mg is necessary for the functioning of plant enzymes to produce carbohydrates, sugars and fats. It is a very critical nutrient in fruit and nuts formation and is also essential in seed germination. Common symptoms in Mg deficient plants are that the leaves become randomly chlorotic and necrotic spots or lesions do occur but not affecting the veins and the midrib. Marginal leaf scorching and defoliation is common on fully matured leaves. Magnesium symptoms in most cases are similar to nitrogen deficiency symptoms, since both elements deficiencies are noticed first in older leaves than in young leaves (Beardsell, 1995, McCauley *et al.*, 2003, Wallace, 1961).

4. 2. MATERIALS AND METHODS

4. 2.1. Plant material

The cultivars were chosen for their high market value. The following *Protea* cultivars were used: 'Sylvia' (*P. eximia* x *P. susannae*), 'Cardinal' (*P. eximia* x *P. susannae*), 'Red Rex' (*P. cynaroides*), 'Susara' (*P. magnifica* x *P. susannae*) and 'Pink Ice' (*P. compacta* x *P. susannae*). The following *Leucadendron* cultivars were used: 'Chameleon' (*L. salignum* x *L. eucalyptifolium*), 'Safari Sunset' (*L. salignum* x *L. laureolum*) and 'Rosette' (*L. laureolum* x *L. elimense*). The following *Leucospermum* cultivars were used: 'Tango' (*L. lineare* x *L. glabrum*), 'Succession II' (*L. lineare* x *L. cordifolium*) and 'High Gold' (*L. cordifolium* x *L. patersonii*).

The plants were cultivated from September 2002 until September 2003 and were grown in 20cm pots filled with sterilized fine river sand (grade one sand, 2 to 4mm Ø) in a temperature controlled polycarbonate tunnel. Each treatment consisted of five plants and plants were fertigated daily with either a complete nutrient solution or a solution in which N, P, K, Ca or Mg were withheld from the nutrient solution. The automatic irrigation system used prevented randomisation of treatments. The following nutrient composition was used to make up stock solution: 0.375 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mM K_2SO_4 , 1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$. Iron source: 0.0776 mM FeEDTA. Nitrogen source: 1 mM $\text{NH}_4^+\text{NO}_3^-$, 1 mM NaNO_3 , 0.5 mM $(\text{NH}_4)_2\text{SO}_4$, Micro - nutrients: 0.01345 mM H_3BO_3 , 0.0026 mM, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.00066 mM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0009 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 0.000165 mM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

The final solution per treatment was prepared using the stock solutions. The final micro - nutrients solution applied was the combination of all micro - nutrients to make one solution. For the Control treatment the final solution used was 2.5 ml/L MgSO_4 , 2.5ml/L K_2SO_4 , 2.5 ml/L CaCl_2 , 0.17 ml/L NaH_2PO_4 , 0.83 ml/L $\text{NH}_4^+\text{NO}_3^-$, 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrient solution from the stock solution. For the N deficiency treatment the following stock solutions were used: 2.5 ml/L MgSO_4 , 2.5 ml/L K_2SO_4 , 2.5 ml/L CaCl_2 , 0.17 ml/L NaH_2PO_4 , 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrient solution from the stock solution. N source nutrients were withheld from the N deficiency treatment. For the P deficiency treatment the following stock solution were used: 2.5 ml/L MgSO_4 , 2.5 ml/L K_2SO_4 , 2.5 ml/L, CaCl_2 , 0.83 ml/L $\text{NH}_4^+\text{NO}_3^-$, 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrient solution from the stock solution. For the K deficiency treatment the following stock solutions were used: 2.5 ml/L MgSO_4 , 2.5 ml/L, CaCl_2 , 0.83 ml/L $\text{NH}_4^+\text{NO}_3^-$, 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrient solution from the stock solution. For the Ca deficiency treatment the following stock

solution were used: 2.5 ml/L MgSO₄, 2.5 ml/L K₂SO₄, 0.83 ml/L NH₄⁺NO₃⁻, 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrient, solution from the stock solution. For the Mg deficiency treatment the following stock solutions were used: 2.5ml/L K₂SO₄, 2.5 ml/L CaCl₂, 0.83 ml/L NH₄⁺NO₃⁻, 12.51 ml/L FeEDTA as iron source, and 0.17 ml/L micro - nutrients solution from the stock solution.

4. 2.2. *Visual symptoms*

The visual symptoms were recorded on monthly bases during two stages. The first stage was five months from December 2003 to April 2003 (three months after plant establishment) and was regarded as an early stage for description of deficiency. The second stage was the five months following the first stage, May 2003 to September 2003 and was regarded as the late stage for description of deficiency. A Pentax camera was used to take photographs. A light box was used to photograph since this magnifies the appearance of the visual symptoms.

The "Key" technique was used to describe the deficiency in *Protea*, *Leucospermum* and *Leucadendron*. In the "Key" technique the deficiency is described according to the appearance of deficiency on "Upper Leaves" or in "Lower Leaves". Authors such as Beardsell, 1999; Bierman, 2005 and McCauley, 2003) used the "Key" method to describe the appearance of deficiency on plant. The following terms were used 1) chlorosis, 2) necrotic, 3) interveinal chlorosis, 4) leaf tip burn and 5) leaf scorching. These terminologies are explained on (page 54).

4. 3. RESULTS AND DISCUSSION

At the early stage plants did not show vivid or clear deficiency symptoms. At the later stage most plants started to show some differences when compared to control treatments. Cultivars such as *Leucadendron* 'Rosette', *Leucadendron* 'Chameleon', *Leucospermum* 'Tango' and

Leucospermum 'Succession II' did not perform well in the “control” treatment. These cultivars had either chlorotic or necrotic spots on leaves. Necrosis and chlorosis are common signs in plants that have nutrient deficiencies. Therefore it was difficult to conclude whether the cultivars were sensitive to nutrients withheld (i. e. N, P, K, Ca and Mg deficiency treatment).

In the N deficiency treatment all cultivars showed classical symptoms that are common in both agronomic and horticultural crops. Symptoms such as older leaves becoming chlorotic and stunted growth were noticed in *Protea*, *Leucospermum* and *Leucadendron* cultivars. At the early stage in these three genera, the older leaves were chlorotic, while the upper leaves were still green. Only at the later growth stage, did the older leaves veins bulge forming a chlorotic “V” shape especially in the *Protea* cultivars. The "V" shape in veins is explained as stress exerted by a plant trying to draw nutrients from the leaves (Bennett, 1993). This might be due to the fact that the vascular bundles are transporters of nutrients and water. Growth stopped, stems were woody and the leaves were brittle and hard. Brittle and hard leaves lack a waxy coat and break easily.

At the later stage of deficiency the upper leaves in *Protea* 'Pink Ice', *Protea* 'Cardinal' and *Protea* 'Susara' (Fig. 1a, b and c) showed some classic symptoms that are common in either potted or field plants. This leaf-reddening colour is common in young plants. This classic symptom from these cultivars was reddening of the upper leaves with very chlorotic lower leaves. During winter period, *Protea* cultivars do have some leaf reddening which can be due to physiological changes such as leaf hardening. These plants are able to recover from this redness in springtime when vegetative spring flush commences. This leaf reddening under these conditions might thus be the result of plants not being supplied with optimal N concentrations or forms. This leaf reddening in winter might be confused with leaf reddening noticed in N deficiency.

Cultivar such as *Leucadendron* 'Safari Sunset', which is known to grow optimally in most conditions, was also affected by nitrogen deficiency especially at the late stage (Fig.1c).

Leucadendron 'Chameleon' showed N deficiency at a later stage, the leaves were chlorotic with stunted growth (Fig.1e). *Leucadendron* 'Rosette' showed the symptoms at the early stage, the leaves were slightly chlorotic. *Leucospermum* 'High Gold' (Fig.1f) started to show chlorotic signs at the later stage of deficiency, and at the early stage the plants had no chlorotic signs.

The dark green colour is mostly associated with P deficiency symptoms. This was noticed in cultivars such as *Protea* 'Cardinal' (Fig.1a), and *Protea* 'Sylvia', (Fig.1b). Leaves at the early stage were dark green and later the leaves were either necrotic or chlorotic. *Leucospermum* 'Succession II' (Fig1d) *Leucadendron* 'Chameleon' (Fig 1e), and *Leucospermum* 'High Gold' (Fig.1f), had leaf tip burns in P deficiency treatments. The thinness and sharp pointed shape of leaves of these cultivars might influence the effect of leaf tip burn. *Protea* 'Susara' (Fig. 1b) showed no P deficiency at the early stage but at the late stage the leaves were irregular in shape.

Cultivar *Protea* 'Red Rex' under P deficiency (Fig. 1a) showed distinct red to dark purple tints in fully expanded leaves (FEL), which also affected the petiole. When the deficiency began it first affected the leaf margin that became reddish, thereafter the red coloration intensified interveinally when deficiency became severe.

The response to K deficiency was slow and the symptoms only became visible at the later stage. In K deficiency treatments *Protea* 'Red Rex' (Fig.1a), *Protea* 'Susara' (Fig.1b) and *Protea* 'Pink Ice' (Fig.1c) did not show K deficiency symptoms especially at the early stage. *Leucospermum* 'Succession II' (Fig. 1d) *Leucadendron* 'Rosette' (Fig.1e), *Leucadendron*

'Chameleon' (Fig. 1.e), and *Leucospermum* 'High Gold' (Fig.1f) were either chlorotic or had interveinal necrosis and leaf tip burn and the symptoms were apparent at the early stage.

Leucospermum 'Tango' (Fig.1d) also showed leaf tip burn in K deficiency treatment in the early stage of deficiency. *Leucadendron* 'Safari Sunset' did not had leaf tip burn but the leaves were chlorotic (Fig.1c).

K is usually abundant around stomatal guard cells, which makes it an important element in stomatal regulation. Therefore K deficiency might influence plant-water relations. The commonly observed leaf tip burn might thus be caused by the stomata not being able to control transpiration.

Calcium is a structural element and important in membranes and also activates many enzymes. Ca is important in formation of new cells. In most cases when Ca is deficient, the young growth is more affected than the older growth because of the immobility of Ca in the plant. When the plants are grown without Ca, the cell wall appears to become 'leaky' and loses the effectiveness as a barrier to the diffused ions (Bennett, 1993). *Leucadendron* 'Rosette' (Fig.1e) under Ca deficiency showed symptoms that were not noticed in other cultivars, namely sugar accumulation on upper leaves. The sugar caused problems to plants because insects became common. At a later stage when the leaves had matured, the leaves showed either marginal or interveinal necrotic black spots that were difficult to associate with deficiency and might have been of fungal origin. The 'leaky' concept of Bennett (1993) might be the causes of this cultivar to have the sugar.

Protea 'Red 'Rex' and *Protea* 'Pink Ice' did not have any signs of Ca deficiency during the growing period. Most cultivars such as *Protea* 'Cardinal' (Fig.1a), *Protea* 'Susara' (Fig.1b) *Protea* 'Sylvia' (Fig.1b), *Leucospermum* 'Tango' (Fig.1d) and, *Leucadendron* 'Chameleon' (Fig.1e) started to show the symptoms only at the later stage in lower leaves, rather than in

upper leaves. Other cultivars such as *Leucadendron* 'Safari Sunset' (Fig.1c), *Leucadendron* 'Chameleon' (Fig.1e), and *Leucospermum* 'High Gold' (Fig1.f) showed Ca deficiency both at the early and the late stage of deficiency, the leaves were either chlorotic, or necrotic with leaf tip burn. In most cases, it is stated that the deficiency of Ca is more visible in young tissue than in older tissues (Table 1). However cultivars of *Protea*, *Leucadendron* and *Leucospermum* started showing Ca deficiency symptoms more in older leaves than in young leaves.

With Mg deficiency *Leucadendron*, *Leucospermum* and *Protea* cultivars showed common symptoms similar to other horticultural crops. The older leaves had interveinal chlorosis, without affecting the veins. In *Leucadendron*, *Leucospermum* and *Protea* cultivars symptoms were more noticeable on the older leaves than in upper leaves (Fig. 1a-f). The Mg deficiency symptoms share some commonality with N deficiency symptom, such as the older leaves being chlorotic. This is because both elements are part of the chlorophyll molecule and therefore leaf yellowing is common with both these elements.

4. 4. CONCLUSION

In conclusion the visual deficiency symptoms could assist in the detection of nutritional problems under field conditions. This collection of notes regarding deficiency development and the collection of photographs should provide a valuable resource for growers and those interested in nutrition of native plants growing in the wild.

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Table 2a-f describes the deficiency symptoms at an early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003). The descriptive deficiency symptoms below are of eleven cultivars of three genera: *Protea*, *Leucospermum* and *Leucadendron*

Table 2a. Description of control during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) of

Cultivar	Symptoms Upper leaves	Symptoms lower leaves	Growth
'Cardinal'	The leaves had no chlorotic or necrotic spots at the early stage but at the later stage the leaves became chlorotic. (Fig. 2a)	Leaves were chlorotic both at the early and the late stage of deficiency (Fig. 2a)	Growth continued but was stunted with small and thin stems
'Red Rex'	No chlorotic or necrotic spots on leaves both at the early and the late stage of deficiency (Fig. 2b)	No chlorotic or necrotic spots on leaves both at the early and the late stage of deficiency (Fig. 2b)	No new shoots formed, growth was stunted.
'Sylvia'	Both at the early and the late stage of deficiency the leaves had interveinal necrotic spot, along the leaf margins. (Fig. 2c)	Localised interveinal and marginal necrotic spots and leaf tip burn were present (Fig. 2c)	Growth was stunted
'Pink Ice'	There were no deficiency symptoms noticed both at the early and the late stage of deficiency (Fig. 2d)	No deficiency symptoms noticed both early and the late stage of deficiency. (Fig. 2d)	Growth was stunted
'Susara'	Not chlorotic or necrotic. There were no symptoms noticed both at the early and late stage of deficiency (Fig.2e)	No chlorotic or necrotic spots noticed both at the early and the late stage of deficiency (Fig.2e)	Growth was stunted.
'Chameleon'	Only at the later stage leaves were chlorotic with necrotic spots present (Fig. 2f)	Leaf tip burn, necrotic with chlorotic spots were centered mainly at the bottom of the midrib mainly at the later stage of deficiency. (Fig. 2f)	Growth was stunted
'Rosette'	Leaves had marginal necrotic spots with red margins and interveinal chlorotic spots. These symptoms were noticed both at the early and the late stage of deficiency (Fig. 2g)	Interveinal and marginal chlorotic and necrotic spots. Leaf tip burn was present. Both at the early and the late stage of deficiency (Fig. 2g)	Growth was stunted
'Tango'	Chlorotic margins progressing from the top downward with red spots was present both at early and late stage of deficiency (Fig.2 h).	Interveinal necrotic spots at the early and late stage were present (Fig.2 h)	Growth was stunted
'Safari Sunset'	No chlorotic or necrotic spot that was present. Leaves showed no deficiency symptoms both at the early and the late stage of deficiency (Fig. 2i)	The leaves were chlorotic with leaf tip burn present only at the later stage. (Fig. 2i)	Growth was slow but later many new shoots were formed
'High Gold'	Not chlorotic or necrotic spots or leaf tip burn on leaves both at early and late stage of deficiency (Fig. 2j)	No chlorotic or necrotic spots or leaf tip burn on leaves noticed both at the early and the late stage of deficiency. (Fig. 2j)	Growth continued, with the plants forming many shoots at the later stage of deficiency
'Succession II'	Necrotic spots were centered along the midrib with chlorotic leaves. This was noticed in the early stage and the late stage of deficiency (Fig. 2k)	Leaves first became chlorotic and later leaf tip burn developed (Fig. 2k)	Growth was stunted

Table 2b. Description of symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) of nitrogen deficiency.

Cultivar	Symptoms Upper leaves	Symptoms middle & lower leaves	Growth
'Cardinal'	The upper leaves were not affected by deficiency at an early stage. Later the leaves were thin, hard and very red (Fig. 2a)	Chlorotic spots were present interveinally on middle and lower leaves. At the later stage the leaves were very chlorotic (Fig. 2a).	No new growth was formed both at an early and late stages.
'Red Rex'	Not affected by deficiency at an early stage, leaves were still green. Later the red tints developed marginally on leaves (Fig. 2b).	Both middle and lower leaves the veins were slightly chlorotic and were hard. When the deficiency was severe the leaves were very chlorotic and were hard (Fig. 2b).	The stems were thin and hard, the petiole of older leaves were red tinted but not of upper leaves. There were no new shoots formed at later stage.
'Sylvia'	At early stage there were no signs of deficiency. When the deficiency was severe at the later stage leaves were hard and very red (Fig. 2c).	Middle and lower leaves had -N deficiency earlier, later the leaves were chlorotic hard and red as upper leaves (Fig. 2c).	No new growth was formed even at later stages. Growth stopped.
'Pink Ice'	The leaves were still green at the early stage. At the later stage were very red and chlorotic (Fig. 2d).	The interveinal chlorotic spots were present at an early stage. At the later stage the leaves developed a "V" shape on veins and the leaves were very chlorotic (Fig. 2d)	The growth had stopped.
'Susara'	Not affected by deficiency stress at the early stage. Leaves were later chlorotic with some red tints on leaves when deficiency advanced (Fig. 2e)	Deficiency affected the plants at the later stage but not in early stages of deficiency. At the later stage leaves had interveinal necrotic spots and marginal leaf blotching (Fig. 2 e).	No new shoots were formed on plant since terminal buds were dead.
'Chameleon'	Not affected by deficiency stress at an early stage. At the later stage the leaves were chlorotic with interveinal red tints (Fig. 2f)	Not affected by deficiency stress at the early stage but later the leaves were chlorotic with interveinal red tints (Fig. 2f)	No new growth was formed on plants
'Rosette'	Slight interveinal chlorosis at an early stage. At the later stage necrotic spots were randomly centered on leaves. Marginal leaf blotching that started from the top to the bottom was present. Leaf tip burn was also noticed. At the later stage the leaves were chlorotic (Fig. 2g))	Interveinal chlorotic spots were present both at early and late stage. At later stage necrotic spots along margins were also noticed and the leaves were chlorotic (Fig. 2g).	At the later stage new growth was formed but the leaves were thin and curled inwards.
'Tango'	Deficiency affected the leaves at the later stage. The leaves were chlorotic with reddish tints at margins and at tips (Fig. 2h)	Leaves were chlorotic especially the lower leaves. Later the leaves had red or brown necrotic spots centered marginally or interveinally on leaves (Fig. 2h).	Growth had stopped and was stunted.
'Safari Sunset'	Chlorotic spots were present at early stage but at the later stage leaves were hard and red (Fig. 2i)	Lower leaves were interveinally chlorotic (Fig. 2j).	No new buds were formed on plants.
'High Gold'	Plants showed symptoms at the later stage. Leaves were chlorotic with interveinal necrotic spots (Fig. 2j).	Lower leaves were chlorotic. At the later stage interveinal necrotic spots were mainly centered at midrib (Fig. 2j)	Growth stopped, no new shoots were formed.

'Succession II'	Leaves were still green, at the early stage. At the later stage, leaf veins were chlorotic and the midrib had interveinal necrotic spots. Leaf blotching was also present (Fig.2k).	The lower leaves were chlorotic but the veins were not chlorotic. At the later stage necrotic spot (Fig. 2k)	There were no new shoots formed
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Table 2c Description of symptoms during the early stage (Dec. 2002 to April 2003) and the late stage (May 2003 to September 2003) of phosphorus deficiency

Cultivar	Symptoms Upper leaves	Symptoms Middle & lower leaves	Growth
'Cardinal'	There were no chlorotic or necrotic spots but leaves were dark green in colour both at early and late stage of the deficiency (Fig. 2a).	Only chlorotic spots were present interveinally along midrib with thin and small leaves (fig. 2a).	New shoots were formed at the later stage, but the stems were thin.
'Red Rex'	Purple or red tints at leaf margins were present both at early and late stage of deficiency (Fig. 2b).	Leaf size was normal but older leaves had purple or reddish spot both interveinally and at the margins. The symptoms start affecting leaf margin before purple spots are visible in older leaves. Red tints also affect the leaf petiole (Fig. 2b)	Growth continued as new stems were formed.
'Sylvia'	No necrotic or chlorotic spots present. The leaves were twisted and bend outwardly with leaf tip burn present and very dark green (Fig. 2c)	When deficiency was severe necrotic spots developed marginally with interveinal chlorotic spots (Fig. 2c)	New leaves were thin, small and greener.
'Pink Ice'	The leaves had interveinal red spots towards the margin. Leaves were dark green (Fig. 2d)	Twisted with red or purple tints along midrib and leaf margin, leaves not chlorotic but were dark green (Fig. 2d).	Growth was not affected, as new shoots were formed
'Susara'	Leaves were chlorotic were curling outwards (Fig. 2e)	At the early stage the leaves were dark green and the later stage leaves were chlorotic (Fig. 2e).	The leaves were small and thin and stems were not straight, the plants were lodging.
'Chameleon'	They were small, bend outwardly with red tints at the tips and margin at the early stage. Chlorotic or necrotic spots were only present at the later stage starting from the top (Fig. 2f).	There were no chlorotic or necrotic spot present or any curling or twisted leaves. Leaf tip burns developed at later stage and the leaves were dark green (Fig. 2f).	No new shoots were formed.
'Rosette'	Leaf size was normal but the leaves were dark green with red tints along the leaf margin. (Fig. 2g)	Lower leaves were darker green than the upper leaves (Fig. 2g).	New shoots were formed.
'Tango'	The leaves had malformed shapes but no chlorotic or necrotic spots were present on leaves even at late stage of deficiency leaves were dark green (Fig. 2h)	Both at early and late stage necrotic spot along the leaf margin with leaf blotching at leaf tip and margins were present. Interveinal chlorotic spots on leaves were noticed and the leaves bent outwards. Middle leaves had red tints scattered around the leaf. Lower leaves were more dark green than the middle leaves (Fig. 2h).	No new shoots were formed.
'Safari Sunset'	No necrotic or chlorotic spots were present even at later stage of deficiency but the leaves were dark red in colour (Fig. 2i).	Interveinal chlorotic spots along midrib were noticed at the early and late stage especially in lower leaves. Leaf tip burn was present mostly in middle leaves (Fig. 2i).	New growth developed but the leaves and stem were thin
'High Gold'	Leaf tip burn and necrotic spots were present. Leaves were twisted and bending outwardly. This was noticed both at early and late stage (Fig. 2j)	Leaf tip and marginal necrotic spots were present both at early and late stage. The leaves bent outwardly (Fig. 2j)	Growth continued but the stems were thin and small in size.

'Succession II'	Chlorotic and necrotic spots were present at the early stage, the leaves curled inwards and were thin, at the later stage the leaves developed leaf tip burn (Fig. 2k)	Necrotic spots were present interveinally mostly at the late stage of deficiency (Fig. 2k).	Growth continued as new shoots were formed
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Table 2d Description of symptoms during the early stage (Dec. 2002 to April 2003) and the late stage (May 2003 to September 2003) of potassium deficiency

Cultivar	Symptoms Upper leaves	Symptoms Middle & lower leaves	Growth
'Cardinal'	Upper leaves were thin and small with no chlorotic spots at early stage but at the later stage chlorotic spots along the midrib were noticed (Fig.2a)	Interveinal chlorotic and necrotic spots as well as leaf tip burn on leaves was noticed (Fig.2a).	No new shoots were formed.
'Red Rex'	No chlorotic or necrotic spots noticed both early and the late stages of deficiency (fig.2b).	No chlorotic or necrotic spots noticed. Plants looked growing well (Fig. 2b).	No new shoots were formed
'Sylvia'	Interveinal necrotic and chlorotic spots were present (Fig.2c)	Leaf tip burn along the margin was noticed (Fig.2c).	Terminal buds were present.
'Pink Ice'	Leaves had reddish tints at the tip and along the leaf margin and were twisted and curled outwardly but no chlorotic or necrotic spots were present both at early and late stage (Fig. 2d)	No chlorotic or necrotic spot on leaves (Fig. 2d).	Growth had stopped and leaves and stem were thin.
'Susara'	Interveinal chlorotic and necrotic spots were noticed mostly at the late stage of deficiency (Fig 2e)	Leaves had black spots on leaf tips with interveinal chlorotic spots. Later the leaves developed localised necrotic spots at midrib and leaf tip burn was also present (Fig 2e)	No new shoots were formed. Growth was stunted
'Chameleon'	No chlorotic or necrotic spots were present at early stage. At the later stage leaves developed interveinal chlorosis (Fig. 2f)	There were no chlorotic or necrotic spots present on leaves at early stage. At the late stage leaf tip burn and interveinal chlorosis were present (Fig. 2f)	New terminal buds were formed that died later and growth had stopped
'Rosette'	No leaf tip burn or blotching was noticed at the early stage. At the later stage localised necrotic spots were present (Fig.2g)	Leaf blotching and leaf tip burn were present (Fig.2g).	Terminal buds were formed and stems were thin
'Tango'	No necrotic or chlorotic present earlier stages. Localised necrotic spots along leaf margin, midrib and interveinally with leaf blotching and leaf tip burn were noticed. (Fig. 2h).	Interveinal necrosis along the leaf margin and was present. This was noticed both at early and the late stage of deficiency (Fig. 2h).	Growth had stopped.
'Safari Sunset'	Leaves were only chlorotic without necrotic spots at the early stage but lately interveinal chlorotic spots with marginal necrotic spots and leaf tip burn were present (Fig. 2i).	Interveinal chlorotic and necrotic spots, and leaf tip burn were noticed both at early and late stage. (Fig. 2i)	New terminal buds were formed at early stage that died at later stage and growth stopped.
'High Gold'	Necrotic, interveinal chlorotic spots were present and leaf blotching along the margin was seen both at the early and the late deficiency stages both early and late stage of deficiency (Fig.2j).	Necrotic, interveinal chlorotic spots, leaf blotching along the margin were present and noticed both at the early and late deficiency stage (Fig.2j).	No new shoots were formed.
'Succession II'	Leaf tip burn was noticed with no chlorotic or necrotic spots noticed both at the early and the late stage of deficiency. (Fig. 2k)	Localised chlorotic spots and marginal necrotic was present with but no leaf tip burn at the later stage was present. The leaves were curling inwardly. (Fig. 2k)	No new shoots were formed

Table 2e Description of symptoms during the early stage (December, 2002 to April 2003) and the late stage (May 2003 to September 2003) of calcium deficiency

Cultivar	Symptoms Upper leaves	Symptoms Middle & lower leaves	Growth
'Cardinal'	Not chlorotic or necrotic spots were present at the early stage but at the later stage interveinal chlorotic spots and necrotic spots were present (Fig. 2a).	Leaves were thin and small but the leaves were chlorotic from the top along the midrib in the early stage of deficiency and later leaves developed marginal leaf blotching on them (Fig. 2a).	No new shoots were formed at the early stage of deficiency but later new shoots were formed.
'Red Rex'	Leaves were small but without chlorotic or necrotic spots on leave both at early and late stage (Fig. 2b).	Leaves looked normal with no chlorotic or necrotic spots on them both at early and late stage of deficiency (Fig. 2b).	Growth was slow No new shoots were formed at the beginning but new shoots were formed lately during growth.
'Sylvia'	Symptoms were visible at later stage than at early stage Leaves were twisted, with random chlorotic spot and very red leaves (Fig. 2c).	Visibility of symptoms was more noticed at late than at early stage of deficiency. Margins were chlorotic with centered interveinal necrotic spots at the later stage (Fig. 2c).	No new growth was formed on plants
'Pink Ice'	No chlorotic or necrotic spots were noticed both at early and late stage of deficiency (Fig. 2d)	Only interveinal chlorotic spot was present at the later stage of deficiency (Fig. 2d)	Stems of new growth was small and thin.
'Susara'	Leaf chlorosis was present, at the later stage of deficiency (Fig. 2.e)	Marginal interveinal chlorotic spots was present at the later stage of deficiency (Fig. 2e)	No new shoots were formed growth was slow.
'Chameleon'	The leaves were small but not chlorotic or necrotic at early stage but at the later stage the leaves were chlorotic with leaf tip burn (Fig. 2f).	Necrotic lesion and leaf tip burn was present with no leaf chlorosis. Symptoms were more noticeable at the later stage (Fig. 2f)	New shoots were formed.
'Rosette'	No necrotic or chlorotic spots were present when deficiency was at early stage but at the later stage brown random necrotic spots and leaf tip burn were present (Fig. 2g).	Interveinal and marginal chlorotic and brown necrotic spots were present (Fig. 2g)	No new shoots were formed at the late stage but growth of new stems formed was weak, thin and small.
'Tango'	Deficiency was more visible at the later stage. Necrotic and marginal leaf burning was notice. Leaf margins were red (Fig. 2h).	Leaf blotching, interveinal chlorotic and necrotic spots starting from the margin progressing downwardly were present at the later stage of deficiency (Fig. 2h).	No new shoots were formed yet. Growth was stunt
'Safari Sunset'	Marginal and interveinal necrotic and chlorotic spots were present both at early and late stage of deficiency (Fig. 2i).	Leaves were small with interveinal, marginal chlorotic and necrotic spots, along midrib especially at the later stage of deficiency (Fig. 2i).	No new shoots were formed at early stage but growth of new shoots was thin and small.
'High Gold'	Leaf tip burn, and centered interveinal necrotic spots were present both at early and late stage of deficiency (Fig. 2j).	Leaf tip burn, interveinal and marginal necrotic spots were present both at early and late stage of deficiency (Fig. 2j)	Growth was slow No new shoots were formed at early stage but late stage the stems formed were thin and small

'Succession II'	Leaves were marginally chlorotic at early stage but at later stage necrotic lesion developed (Fig. 2k).	Interveinal chlorotic and necrotic spots along mid rib was present and the leaves were small both at early and young stage of deficiency. Leaf tip burn was noticed. (Fig. 2k)	No new shoots were formed.
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Table 2f. Description of symptoms during the early stage (December 2002 to April 2003) and the late stage (May 2003 to September 2003) of magnesium deficiency.

Cultivar	Symptoms Upper leaves	Symptoms middle & lower leaves	Growth
'Cardinal'	Marginal leaf-tip burns and leaf blotching were present Chlorosis and necrosis on leaves were noticed both at early and late stage of deficiency. Symptoms were more visible at the later stage than at the early stage (Fig. 2a)	Interveinal chlorosis, necrosis, and leaf tip burn were progressing along the leaf margin. Symptoms were more common at the later stage (Fig. 2a)	Growth New growth was stunted with thin leaves and stem
'Red Rex'	Symptoms were more visible at the late stage than at the early stage of deficiency. The leaves were interveinally and marginally chlorotic at late stage (Fig. 2b)	Interveinal chlorosis and necrosis spots with red tints at the tip of the leaf were present both at early and late stage of deficiency (Fig. 2b).	Stunted growth, no new shoots formed.
'Sylvia'	Interveinal chlorosis and necrosis spots both at the early and late stage of deficiency (Fig. 2c).	Marginal and interveinal chlorosis and necrosis starting from the top to bottom both at early and late stage of deficiency (Fig. 2c).	Stems and leaves of new growth were small and thin
'Pink Ice'	No deficiency noticed both at the early and the late stages (Fig. 2d)	Interveinal chlorotic and necrotic spots with marginal necrotic lesion and leaf tip burn both at the early and the late stage of deficiency (Fig. 2d).	Growth was stunted.
'Susara'	Interveinal chlorosis was noticed both at the early and the late stage of deficiency (Fig.2e)	Marginal chlorosis was noticed both at the early and late stage (Fig.2e).	Stunted growth
'Chameleon'	At the early stage the leaves were chlorotic but at the later stage centered and marginal chlorotic, necrotic spots and leaf tip burn were present (Fig. 2f).	Both at the early and late deficiency stage leaf tip burn were present. Brown necrotic spots were centered at the bottom of the leaf at the late stage (Fig. 2f)	Growth was stunted
'Rosette'	Marginal interveinal necrotic spots starting from the top of the leaf were noticed both at the early and the late stage of deficiency (Fig. 2g).	Interveinal chlorosis and necrosis with leaf tip both at early and late stages of deficiency were noticed (Fig. 2g).	Growth was stunted.
'Tango'	Marginal and interveinal chlorosis was noticed both at the early and the late stage of deficiency (Fig. 2h).	Interveinal chlorotic and necrotic spots and leaf tip burn was present both at the early and the late stage of deficiency (Fig. 2h).	Growth was stunted
'Safari Sunset'	Interveinal chlorosis was noticed both at the early and late stage of deficiency. Leaves were reddish especially the tips (Fig. 2i).	Interveinal and marginal chlorosis both at beginning and at the late stage of deficiency were noticed (Fig. 2i).	Stunted growth with small and thin stems
'High Gold'	Interveinal chlorosis and necrosis with no leaf tip burn were noticed at the early stage. At the later stage marginal necrosis tips with leaf blotching were noticed (Fig. 2j)	Chlorosis and centered interveinal necrosis were noticed especially on mid rib starting from the top progressing downwardly. Leaf tip burn was present. Deficiency symptoms were similar both at early and at the late stage (Fig. 2j)	New shoots formed were small and thin.
'Succession II'	The leaves had no chlorotic or necrotic spots at the early stage of deficiency. At the late stage localised interveinal and necrotic spots were present (Fig.2k)	Interveinal chlorosis was present both at early and the late stage of deficiency (Fig. 2k).	Growth was stunted with no new shoots formed.

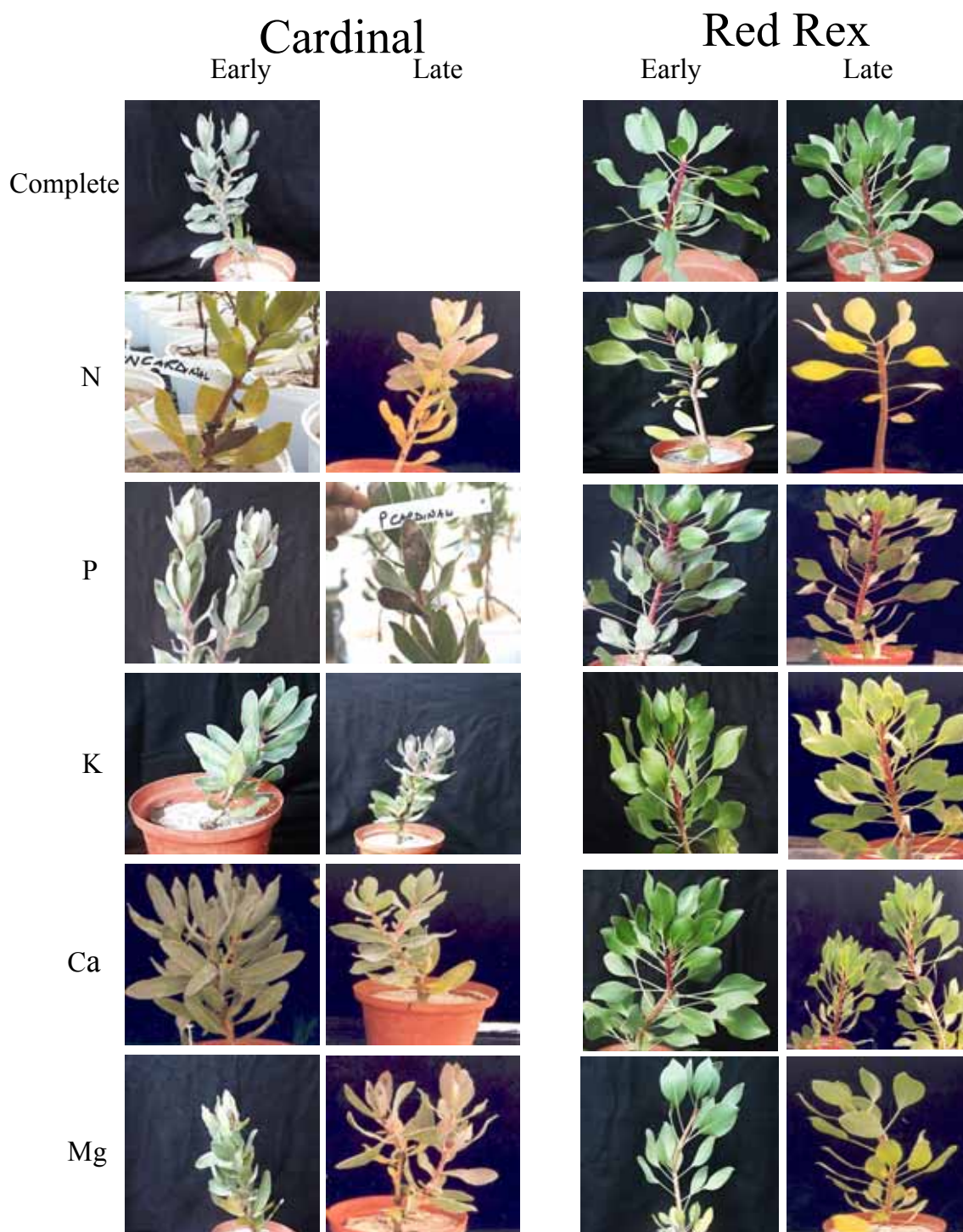


Figure 1a. Photographs of 'Cardinal' and 'Red Rex' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

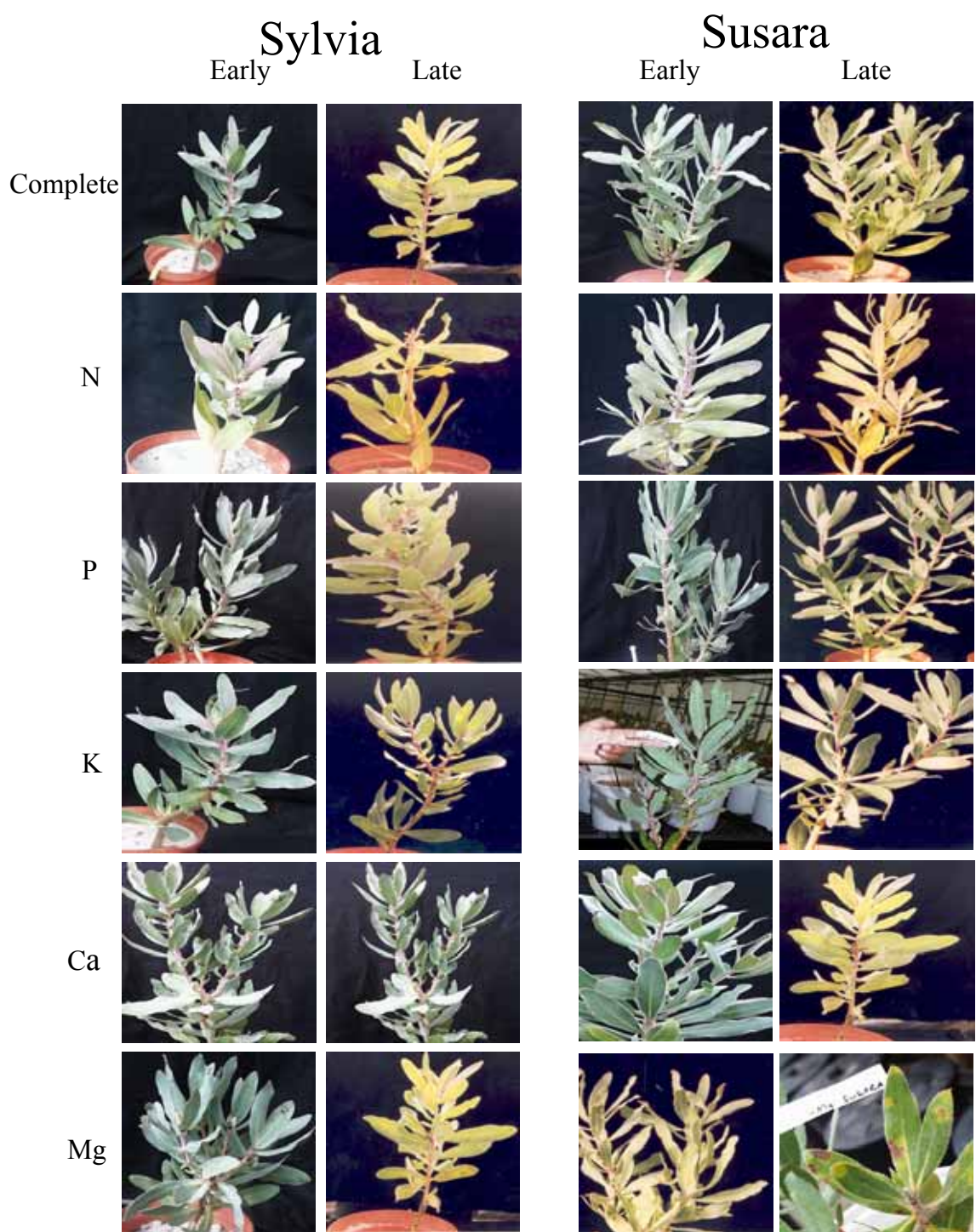


Figure 1b. Photographs of 'Sylvia' and 'Susara' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

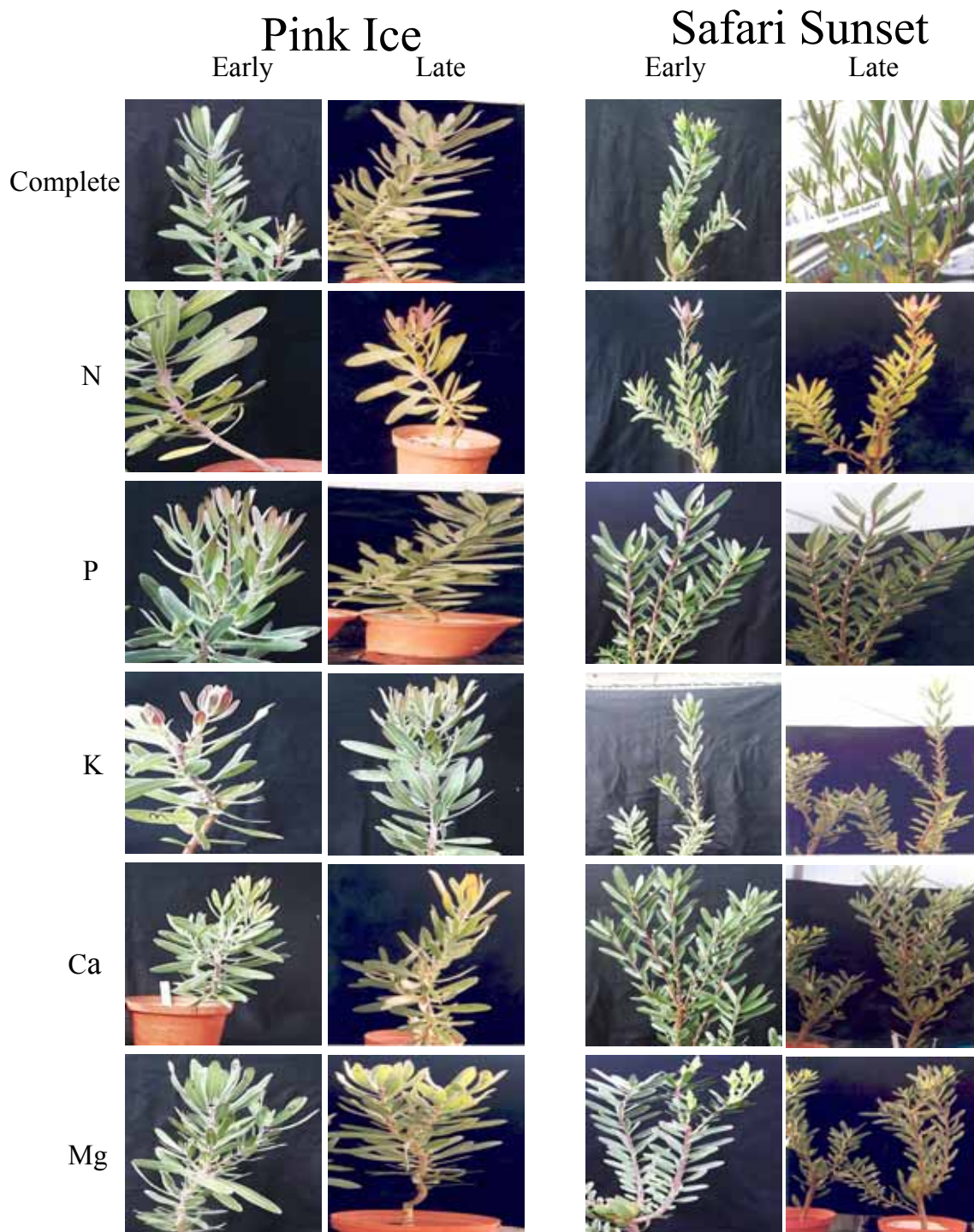


Figure 1c. Photographs of 'Pink Ice' and 'Safari Sunset' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

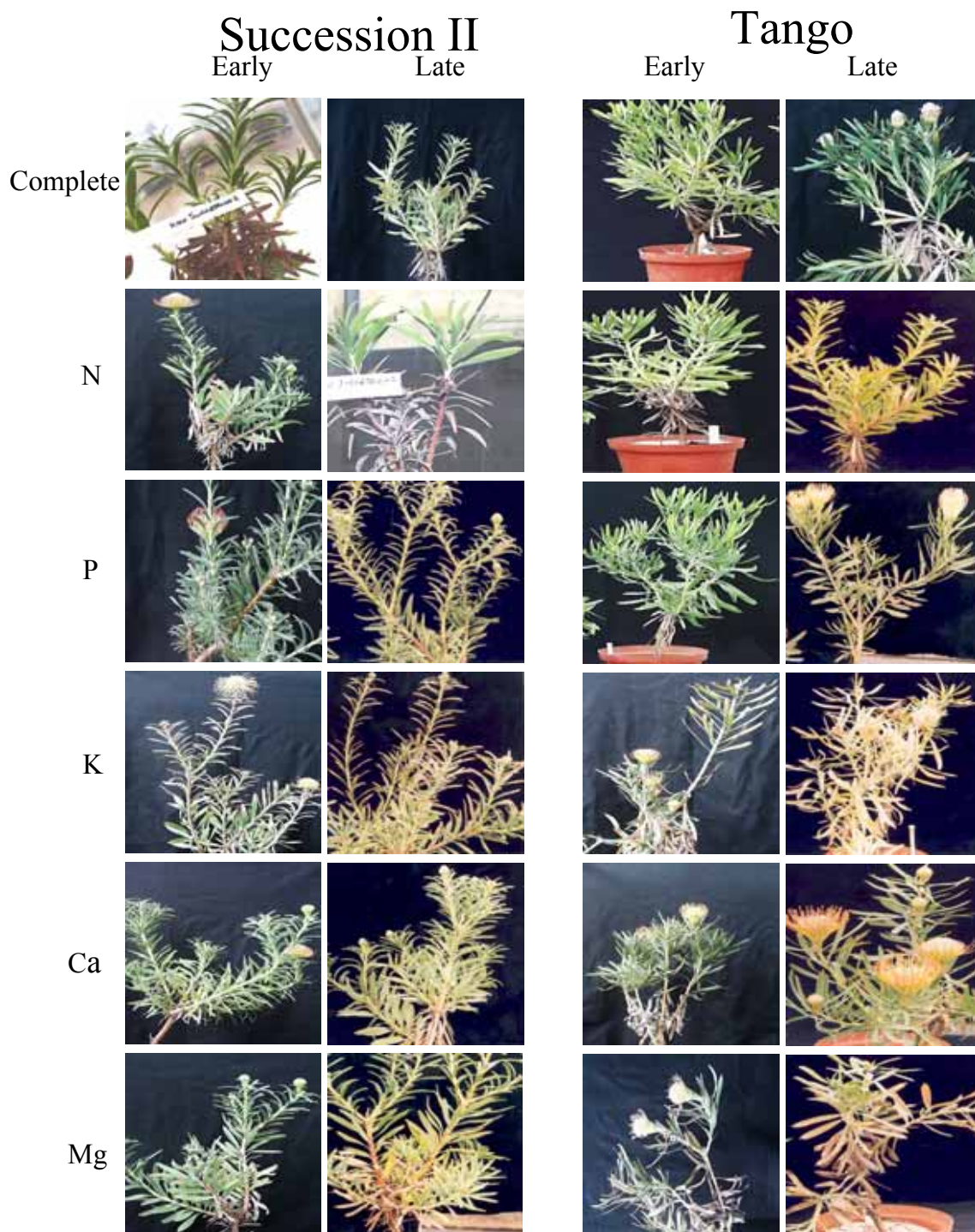


Figure 1d. Photographs of 'Succession II' and 'Tango' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

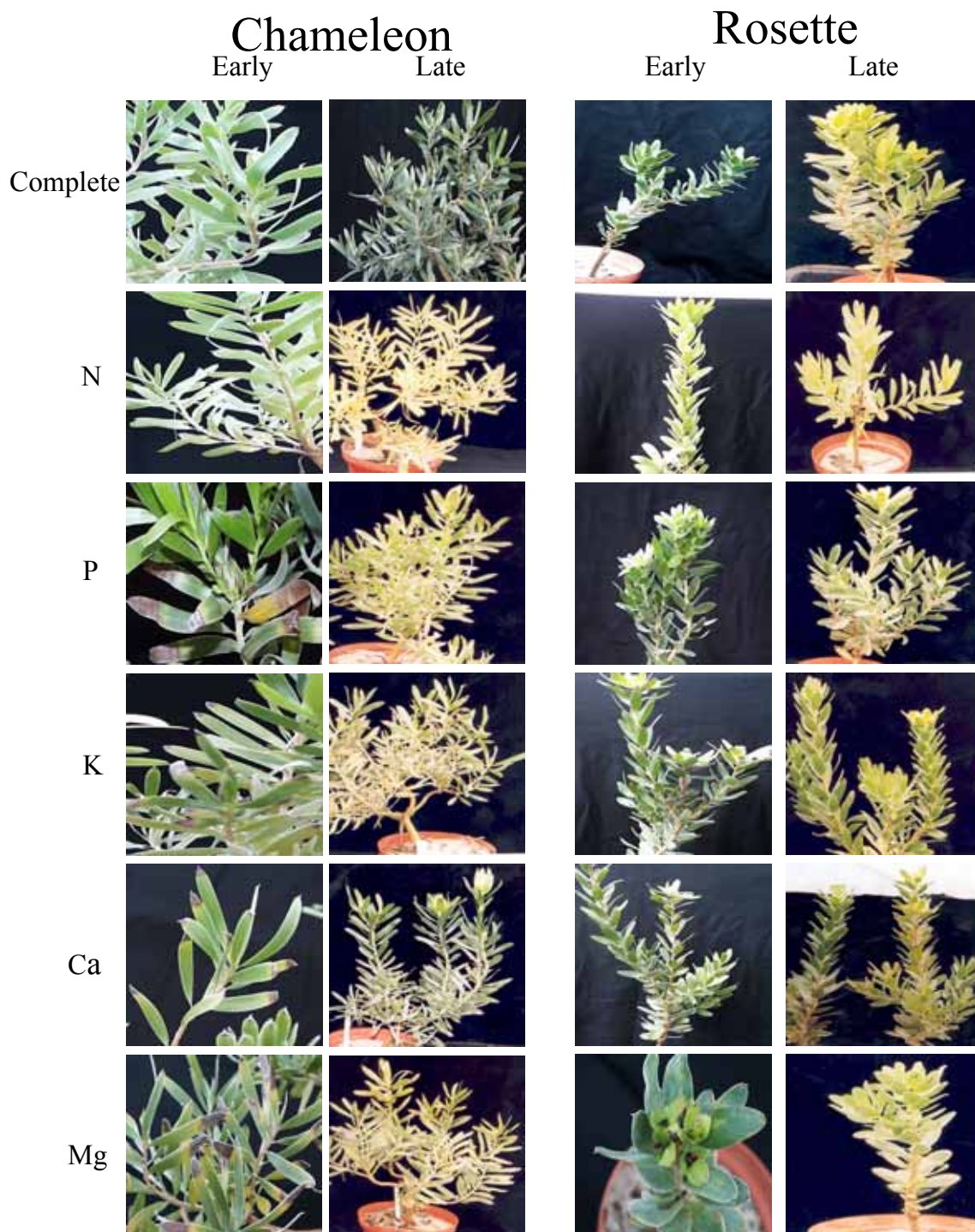


Figure 1e. Photographs of 'Chameleon' and 'Rosette' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

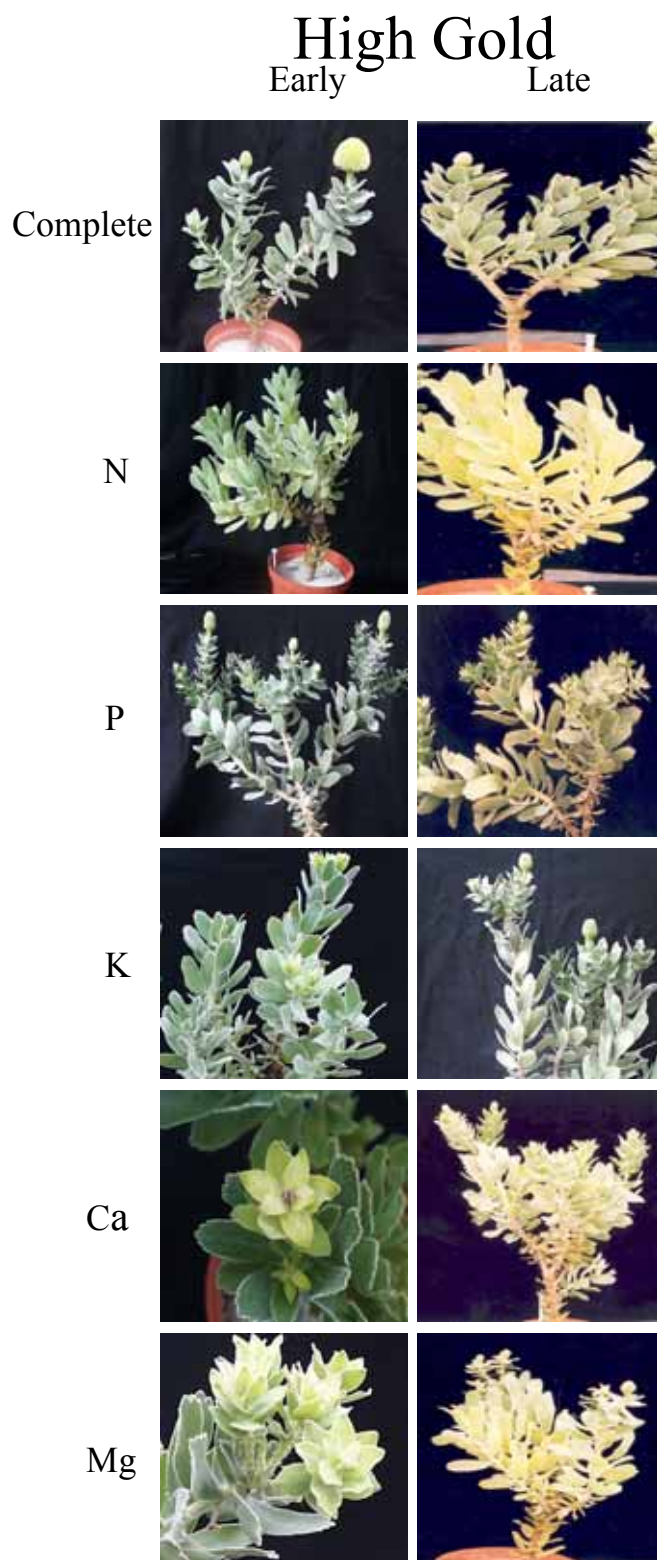


Figure 1f. Photographs of 'High Gold' plants showing deficiency symptoms due to lack of N, P, K, Ca or Mg in the nutrient solution in comparison to plants supplied with a complete nutrient solution. Photographs show both an early stage (December 2002 to April 2003) and a late (May 2003 to September 2003) stage of deficiency symptoms.

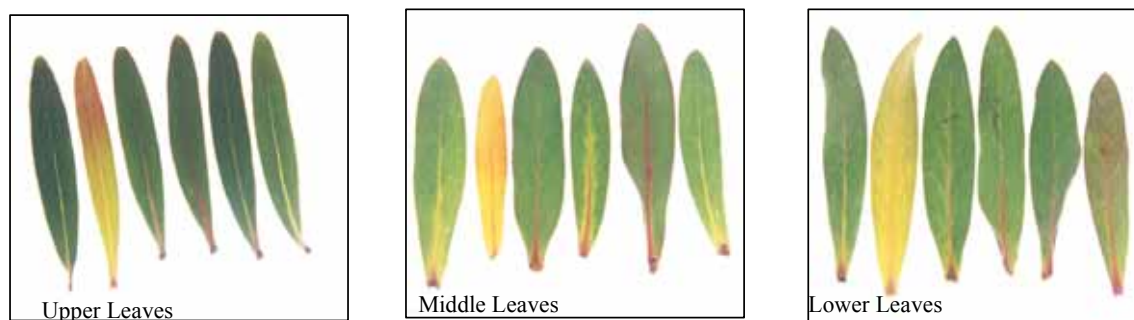


Figure 2a. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of cultivar 'Cardinal'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

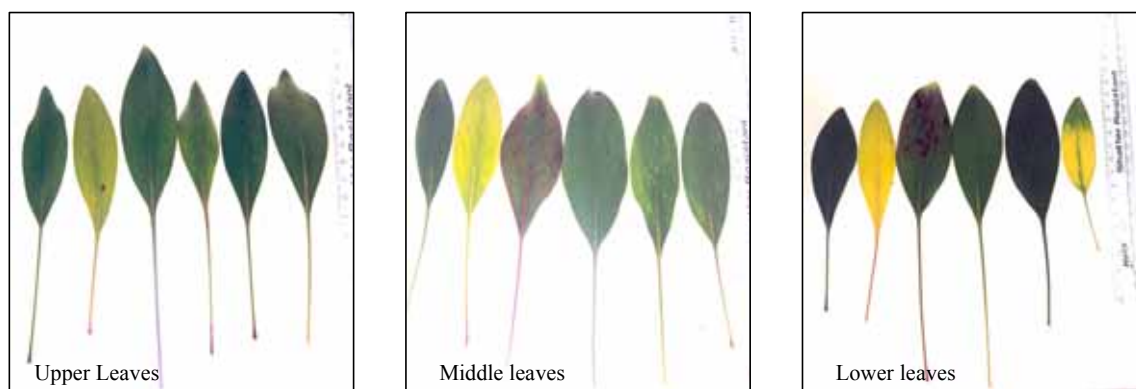


Figure 2b. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Red Rex'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.



Red spots on middle leaves in -P treatment in 'Red Rex'



Figure 2c. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Sylvia'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

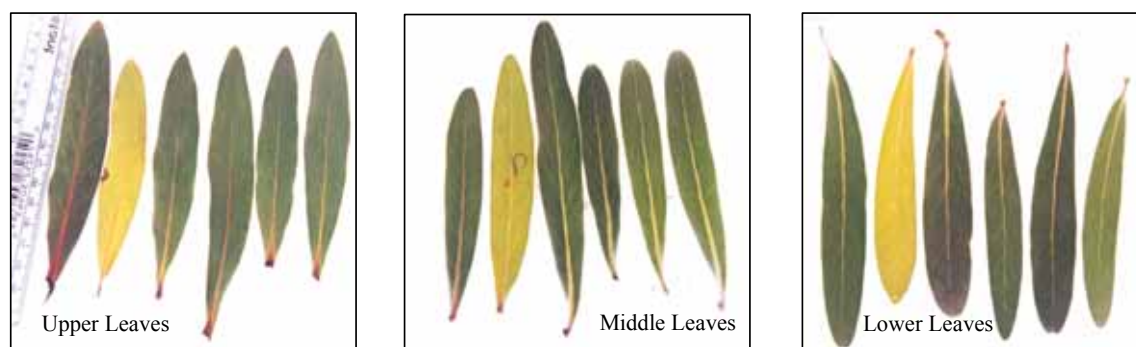


Figure 2d. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Pink Ice'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.



Figure 2e. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Susara'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

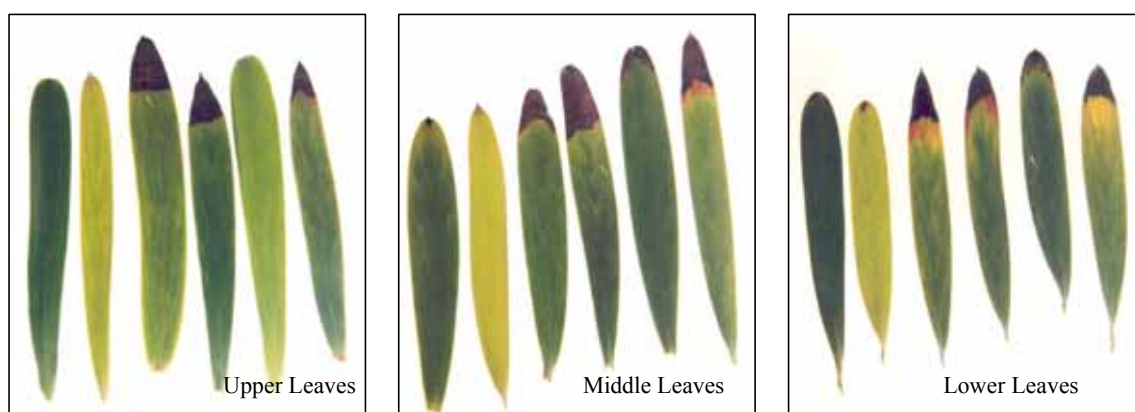


Figure 2f. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Chameleon'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

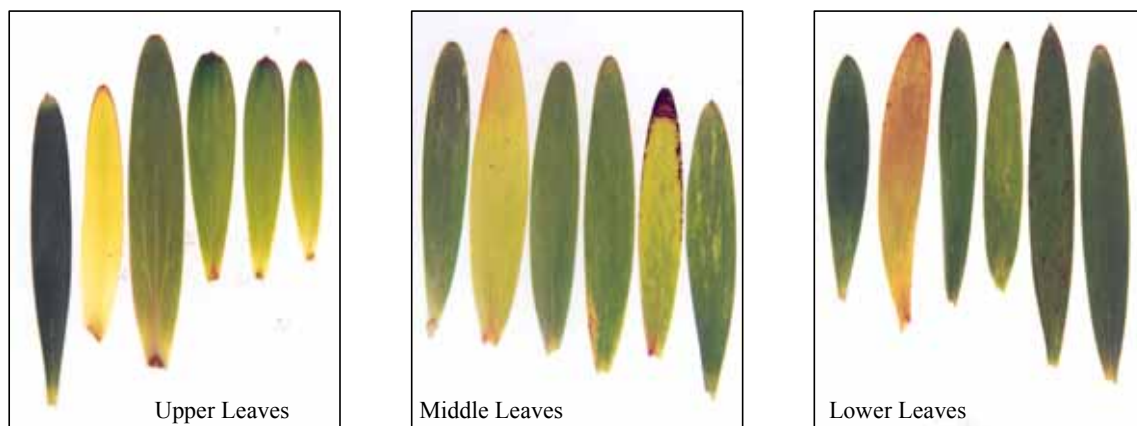


Figure 2g. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Rosette'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.



"Sugar" -
build up on
upper leaves
of 'Rosette'



Figure 2h. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Tango'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

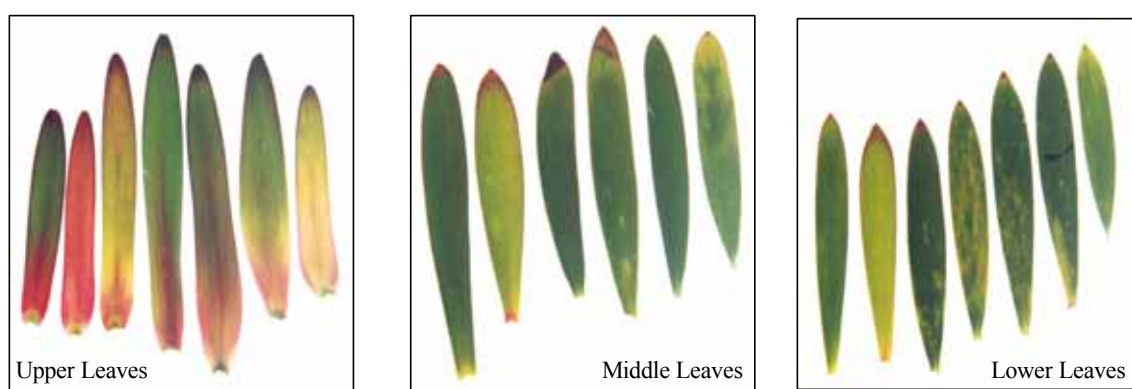


Figure 2i. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'Safari Sunset'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.



Figure 2j. Symptoms of mineral nutrient deficiencies in the upper, middle and lower leaves of the cultivar 'High Gold'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.



Figure 2k. Symptoms of mineral nutrient deficiencies in the upper, and lower leaves of the cultivar 'Succession 11'. Treatments were (from left to right) Control, -N, -P, -K, -Ca, -Mg.

CHAPTER FIVE

OPTIMIZATION OF P AND N NUTRITION FOR *PROTEA* 'CARDINAL': CONCENTRATIONS AND FORMS OF APPLICATION.

ABSTRACT

The objective of this study was to determine the sensitivity to different N and P concentrations and forms for *Protea* 'Cardinal'. A complete nutrient solution with different levels and sources of N and P were applied on rooted cuttings of *Protea* 'Cardinal'. N concentrations used were 5 mM, 1 mM, and 0.1 mM and P concentrations of 1 mM, 0.1 mM and 0.01 mM. N was also applied in different forms NO_3^- , NH_4^+ , $\text{NH}_4^+:\text{NO}_3^-$ (1:4, 4:1 and 1:1 ratios) and Urea. The dry matter of leaves, stem and roots was higher in the NO_3^- treatment at 5 mM than at 1 mM and 0.1 mM N. Total N concentration was higher in $\text{NH}_4^+:\text{NO}_3^-$ (1:1), NH_4^+ and Urea treatments at 5 mM than at other concentrations. In P treatments, dry of matter leaves, stem and roots was higher at 0.1 mM P, and not significantly different at 0.01 mM and 1 mM P. The tissue P concentration was significantly higher at 0.01 mM than at 0.1 mM P. This strange result may be due to differences in cluster root development between the various P concentrations. The K concentration was significantly higher in $\text{NH}_4^+:\text{NO}_3^-$ (4:1) treatment at 0.1 mM N and at higher P concentrations. Application at 5 mM N and P at 0.01 mM yielded better growth for *Protea* 'Cardinal'. The form of N applied was important in determining the response to N concentrations.

5. 1 INTRODUCTION

Plant mineral nutrient optimization refers to the supply of certain nutrient elements to the plant in order to determine the correct quantity required by the plants without being over or under-supplied. Correct nutrient supply can promote rapid growth and improves the quality of the plants. N and P are the most growth limiting nutrients in most crop production. P readily binds to cations and particles in the soil forming sparingly soluble precipitates, thus making P the least accessible macronutrient to plants (Lambers *et al*, 2003).

Quantitatively, N is the most important nutrient for plants due to its important function in chlorophyll, amino acids, DNA and many other constituents of plants. NO_3^- and NH_4^+ are the major sources of inorganic N for plants (Marschner, 1995). NO_3^- is believed to be a preferred source of N for many plants since NH_4^+ toxicity rapidly develops in some situations (Mengel and Kirby, 1979). When both forms of N are supplied, it is easier for the plant to regulate intracellular pH (Marschner, 1995).

Claassens (1986) reported that *Protea* 'Ivy' responded better to NH_4^+ than to NO_3^- nutrition. Claassens (1986) reported that most *Protea* species did not grow well when NO_3^- was a sole nutrient supplied. However, good yield was obtained for *Protea* 'Ivy' and *Leucadendron salignum* (Berg) with a combination of NH_4^+ and NO_3^- at higher levels (Heinsohn and Pammenter, 1986). Silber *et al.* (2000) found that altering $\text{NH}_4^+:\text{NO}_3^-$ ratios did not affect development of 'Safari Sunset' root growth, regardless of pH.

Protea plants are generally fertilized with very low P since P is often toxic at high concentrations to the Proteaceae. *Protea* plants generally grow on nutrient deficient soils with a pH of 5 to 6 (Heinsohn and Pammenter, 1986; Silber *et al*, 2000; Criley 2000). Application of P at higher application rates restricts cluster root formation (Watt and Evans, 1999). Silber

et al. (1998) suggested that a soil P concentration of 15 mg P kg⁻¹ was optimal for *Protea* growth.

These plants have special temporary root systems called proteoid roots or cluster roots, which are likely to appear during the rainy season or winter period (Lamont, 1986). These roots act to mobilize and take up P (Jescheke and Pate, 1995). Although these plants are able to utilize limited available soil P very effectively, continual removal of marketable stems under cultivation will deplete available P. Therefore there is a need to replace what the plant has removed from the soil.

5. 2 MATERIALS AND METHODS

5. 2.1 Experimental site and plant material.

The experiment was conducted at Agricultural Research Council at Elsenburg, Stellenbosch, Western Cape, (35° 50' 80" S, 18° 50' 10" E, 200m above sea level) in polycarbonate-covered greenhouse, equipped with awet wall temperature control system. Rooted cuttings of *Protea* 'Cardinal' were grown for seven months (May to October 2002) in 10 L containers in inert sand as a growth medium. The plants were fertigated manually with 1 L nutrient solution every second day. Complete nutrient solutions with different levels and sources of N and P were applied to plants. The following nutrient composition was used: 0.375 mM Mg SO₄.7H₂O, 0.5 1 mM K₂ SO₄, 1 mM CaCl₂.2H₂O and 1 mM NaH₂PO₄.2H₂O. The iron source: 0.0776 mM FeEDTA. The nitrogen source: 1 mM NH₄NO₃, 1 mM NaNO₃, 0.5 mM (NH₄)₂SO₄, 1 mM Urea. The micro - nutrients were supplied as 13.45 μM H₃BO₃, 2.6 μM MnSO₄ 4H₂O, 0.66 μM ZnSO₄.7H₂O, 0.9 μM CuSO₄.5H₂O, 1.65 μM Na₂Mo₄.2H₂O. The N concentration levels used were 5 mM, 1 mM, and 0.1 mM and phosphorus concentration levels of 1 mM, 0.1 mM and 0.01 mM. N was applied in different forms: NO₃⁻, NH₄⁺, Urea and NH₄⁺: NO₃⁻ (1:4, 4:1 and 1:1 ratios). The stock solutions were made and kept in a cool

room at 6°C. Solutions were kept in 60 L drums and maintained at a constant pH 5.5. There were twenty-one treatments with five replicates in a randomised block design layout.

5. 2. 2 Harvest

The visual symptoms on leaves and roots of plants were recorded using a digital camera. The plants were harvested after the completion of the trial in October 2002. The plants were divided into leaves, stems and roots and were washed in distilled water to remove growth medium before they were oven dried at 75°C for 72 h.

5. 2. 3 Sample analysis

The Kjeldahl method was used to analyse N in which samples were dried for 5 h at 90°C and left for 1 h to cool. Dried samples of 1.4 g were placed in Kjeldahl flask with 5 g of Na₂SO₄ and 5 glass beads and the content were thoroughly mixed. Then 25 ml of H₂SO₄ was added to the flask. Once the mixture cleared, it was boiled for further 50 min and 450 ml of H₂O was added after cooling. The distillate from the Kjeldahl distillation apparatus was collected in boric acid with a colour indicator. The distillate was then titrated until the distillate turned from grey to light yellow. The Analytical Laboratory of the Department of Agriculture Western Cape, Muldersvlei Road, Elsenburg, South Africa, performed these analyses.

P, K, Ca and Mg were analysed using a dry ash method and the inductive coupled plasma (ICP) method (Analytical Laboratory of Department of Agriculture Western Cape, Muldersvlei Road, Elsenburg, South Africa). Dry ashing was done using air-dried sample of 3 g that was ground to pass through a 0.5 to 1 mm sieve and was placed in a porcelain crucible. The crucible was placed in a cooled muffled furnace and ashed at 550°C overnight. 5 ml 6 M HCl was added to dissolve the sample and the sample was evaporated to dryness on a water bath. After cooling the sample, 5 ml HNO₃ was added and the crucible was heated again on a

water bath and removed as soon as the sample started to boil. The solution was filtered through a Whatman number 40 filter paper into 100 ml volumetric flasks. The filter paper was washed with warm water and the sample was diluted to volume with deionized H₂O. The Analytical Laboratory of the Department of Agriculture Western Cape, Muldersvlei Road, Elsenburg, South Africa, performed these analyses.

5. 2 4 *Statistical analysis*

Multivariate analysis of variance (ANOVA) was performed on the data for each nutrient element per tissue type, per treatment; the General Linear Model (GLM) procedure of SAS was used and LSD was calculated at a 5% significance level to compare treatment means.

5. 3 RESULTS

5. 3. 1 *Visual observation on 'Cardinal'.*

The leaves of *Protea* 'Cardinal' were much greener and plant height was greater at 5 mM than at 1 mM and 0.1 mM N concentrations, irrespective of the N form (Fig. 1). The plants at 5 mM differed among treatments; they were either dark green as in the Urea treatment, or lighter green as in the case of NO₃⁻ treatment. Most plants at 1 mM and 0.1 mM did not form new buds shoots and growth had stopped. The colour of the leaves and stems also were affected at 1 mM and 0.1 mM N concentration with the leaves and stems being reddish (Fig. 1). This leaf reddening was also noticed in N deficiency treatment (Chapter 2) for many of the Proteaceae cultivars.

The plants at 1 mM P formed new shoots and second flush with bright green leaves, but at the later stage the plants developed necrosis and the leaves were scorched, especially the older leaves (Fig. 2). This could indicate that P at higher levels is detrimental to growth and could

have been the result of incipient toxicity. Best plant shoots and leaf growth and plant height was attained at intermediate (0.1 mM) and at low (0.01 mM) levels of P.

It is of interest to note that in these results at high N levels and at high P levels, cluster roots were noticed (Fig. 3 & 4). Cluster roots were present at 5 mM $\text{NH}_4^+ : \text{NO}_3^-$ (4:1), $\text{NH}_4^+ : \text{NO}_3^-$ (1:4), NO_3^- , NH_4^+ , and Urea (Fig. 3) and also at a P concentration of 1 mM (Fig. 4). Cluster roots were also present at 0.01 mM and at 0.1 mM P concentration (Fig. 4). High concentrations of nutrients are normally regarded as detrimental for normal proteoid roots growth and can cause roots formation to fail. In 5 mM 1:1 $\text{NH}_4^+ : \text{NO}_3^-$ the plants formed a thick mat of red lateral roots with some cluster root (Fig. 3). Nutrient availability might have influenced the colour change in these roots. Application of N at higher concentration might have enhanced the metabolic activities around the rhizosphere and further increased active uptake of N nutrient by these roots.

5. 3. 2 Plant dry weight accumulation

The total dry weight of leaves, stems and roots was significantly higher at 5 mM than at 1 mM or 0.1 mM N, especially in the NO_3^- and there was no statistical difference among these treatments and $\text{NH}_4^+ : \text{NO}_3^-$ treatment (Fig. 5). Urea resulted in higher biomass accumulation than NO_3^- at 1 mM. There was no significant difference in the dry mass accumulation among N forms at 0.1 mM N (Fig. 5). The leaves and stems dry weight was significantly higher in the NO_3^- and $\text{NH}_4^+ : \text{NO}_3^-$ (1:4) treatments at 5 mM level and significantly lower in NH_4^+ treatment at 0.1 mM and 1 mM (Fig. 7a & b). The dry weight of the roots was significantly higher in NO_3^- and significantly lower in NH_4^+ treatments at 5 mM (Fig. 7c).

The P treatment resulted in highest (39 g) dry weight at 0.1 mM and lowest dry weight (27 g) at 0.01 mM (Fig. 6). Total plant dry mass was not significantly different between 0.01 mM and 1 mM P indicating an optimum P concentration of 0.1 mM in this investigation. The leaf,

stem and root tissue biomass accumulation was also greater in the 0.1mM P treatment, than 0.01 mM and 1 mM levels (Fig. 8).

5. 3. 3 Nutrient accumulation

The total NH_4^+ concentration in plant tissue (Fig. 9a) was significantly higher with 1:1 NH_4^+ : NO_3^- , NH_4^+ and Urea than with NO_3^- and 1(: 4) NH_4^+ : NO_3^- treatments at 5 mM. At 1 mM N the total N concentration was significantly lower in the NO_3^- treatments and significantly higher in the 1:1 NH_4^+ : NO_3^- treatment than other treatments (Fig. 9a). Differences in tissue N accumulation at 0.1 mM of supplied N were small.

The K concentration at 5 mM N did not differ significantly between treatments and was lower at 5 mM than at 0.1 mM and 1 mM N (Fig. 9b). The K concentration at 1 mM NH_4^+ was significantly lower than any other treatments (Fig. 9b). Overall, the K concentration at 0.1 mM of supplied N was significantly higher than that at 5 mM N. The K concentration in P treatments was significantly higher at 1 mM than at 0.1 and 0.01 mM P (Fig. 10).

There were few significant differences in tissue P concentration between supplied N forms except at 5 mM N (Fig. 11a). The tissue P concentration was significantly higher with treatments rich in NH_4^+ (NH_4^+ and 4:1 NH_4^+ : NO_3^-) at 5 mM N. Tissue P concentration in P treatments was significantly higher at 0.01 mM supplied P than at 0.1 mM and 1mM P levels (Fig. 11b).

5. 4. DISCUSSION

Application of N at 5 mM levels resulted in leaves that were much greener than N application at either 0.1 mM or 1 mM concentrations. At 0.1 mM and 0.1 mM, N, the leaves and stems were red. This red leaf colour was also noticed in N deficiency treatment, (Chapter four). The

plants formed many shoots and best plant height was attained at 0.01 mM P. At 1 mM P plants formed new shoot, but at the later stage necrosis spots developed on the leaves. Cluster roots are known to be sensitive to high rates of P application (Claassens, 1981 and 1986; Goodwin, 1983; Nichols *et al.*, 1988; Silber *et al.*, 2000). These roots are also known to grow in nutrient poor soils (Montarone and Allemand, 1995) and are mostly present in wet winter months and senesce after the winter period (Jescheke and Pate 1995).

Some nutrients elements such as P seem to inhibit cluster roots development (Cramer *et al.*, 2004) when available in nutrient solution in higher quantities. High P concentration does not only restrict growth, it also decreases the number of these roots present (Watt and Evans, 1999). Lamont (1986) reported that cluster roots developed only when N and P were omitted from nutrient solution. However, at 1 mM P concentration, cluster roots were present in *Protea cv. 'Cardinal'*. Cluster roots development in the presence of high N (5 mM) and high P (1 mM) concentration, might indicate that these plants were able to distinctively access the nutrients. Therefore, this may indicate that the levels of N and P used were acceptable for the development of cluster roots.

The dry weight of leaves, stems and roots increased significantly when the plants were treated with 5 mM N (Fig. 5), especially in the form of NO_3^- (Fig. 7a, b and c), when compared to plants treated with 0.1 or 1 mM N. The form of N supplied had a large influence on dry weight accumulation. Proteaceae species are thought to utilize NH_4^+ as the predominant N source (Stock and Lewis, 1984). The plants in these experiment fared well with NO_3^- nutrition showing that in pots, at least, NO_3^- can be an effective N source. In fact NH_4^+ was the N source to which the plants responded the least across all concentrations. This might indicate that the ineffective response by these plants to NH_4^+ is possibly due to pH problems associated with use of NH_4^+ as a sole N source. It is well known that NH_4^+ nutrition elicits

acidification of the rhizosphere. Thus the relative lack of response of the plants to NH_4^+ nutrition may be attributable to associated pH problems.

The tissue N concentration was high at 5 mM supplied N in $\text{NH}_4^+:\text{NO}_3^-$ (1:1), NH_4^+ and Urea treatments (Fig. 9a) and also high with 0.01 mM P (Fig. 11a). These results are in accordance with Claassens (1986) that *Protea* plants can tolerate high NH_4^+ concentrations. Heinesohn and Pammenter (1986) also found that when NH_4^+ was the N source at high concentrations growth of *Leucadendron salignum* was promoted. NH_4^+ was more preferred when NO_3^- was included as a source of N, especially at higher concentration (5mM). The concentration of tissue N in the NO_3^- treatment was very low (Fig. 9a).

In general it seems that cluster root producing plants grow well with NH_4^+ as an N source and this might be related to the acidic pH of the soils to which they are native and the relatively cool Mediterranean climate under which that they grow. However, in this study *Protea* 'Cardinal' did well with NO_3^- as N source. Mengel and Kirby (1979) reported that when NH_4^+ was N source, the uptake of NO_3^- was reduced in young wheat. Even though wheat is not cluster root bearing crop, but both cluster root bearing plants and wheat fared well in soils with low pH or acidic soils. Wheat crop also do well with NH_4^+ as N source.

When the pH was in the range of 4 to 6, NH_4^+ source was preferred over NO_3^- by rice, sugar beet and wheat crops (Hewitt and Smith, 1975). The low NO_3^- accumulated in the *Protea* 'Cardinal' may due insufficient light, since NO_3^- uptake, reduction and assimilation is more dependent on light energy. The low NO_3^- could also be attributed to cation-anion competition with NH_4^+ , since NO_3^- accelerates the uptake of cations (Mengel and Kirby, 1979). When NH_4^+ is present at higher concentration in the solution it usually inhibits NO_3^- uptake (Abrol, 1990).

In P treated plants, greater accumulation of dry weight was obtained at 0.1 mM P than at 0.01 or 1 mM P (Fig. 6). This indicates that the lowest concentration was not sufficient for growth while the highest concentration was toxic.

The K concentration in cv. 'Cardinal' was negatively influenced by increased N concentration, probably because high N concentrations stimulated growth and result in K dilution accumulation in the tissue. Inhibition of K accumulation in NH_4^+ treatment at 1mM N was particularly strong and probably indicates that cation-cation competition was involved in excluding K. K accumulation with 1 mM P concentration may reflect the inhibition of plant growth by this concentration of P. It is possible that the release of organic anions from cluster roots is accompanied by K^+ efflux from white *Lupin* (Sas *et al.*, 2001). There is no clarity on the role of K in relation to cluster roots and N and P sources in *Protea* plants. This deserves further investigation.

The P concentrations were significantly higher in NH_4^+ treatments and significantly lower in NO_3^- treatments at 5 mM N concentration (Fig. 11a). This might indicate cation-anion interactions with P uptake and NH_4^+ uptake. This is born out by the fact that higher concentrations of NO_3^- limited P accumulation.

From these results it is clear that Proteaceae are able to utilize P at low concentrations (0.01 mM and 0.1mM). This concentration is a fraction of that normally supplied to crop plants. However, in these experiments 0.01 mM P was apparently sufficient for growth. It seems that tissue P is a good indicator of the P-status of these plants. This was not in agreement with the results of Silber *et al* (1997) who found that low P inhibited growth of 'Safari Sunset' and was in accordance with Claassens (1981, 1986); Goodwin (1983); Nichols *et al.* (1988), and Silber *et al.* (2000) and also who showed that low P could be tolerated by *Protea*.

5. 5. CONCLUSION

In these results the form of N supplied was found to be of critical importance, although plants fared surprisingly well with NO_3^- as N source. Plants responded well to relatively high concentrations of N (5 mM). *Protea* 'Cardinal' plants tolerated very low levels (0.01mM and 0.1mM) of P, and growth was inhibited under high levels (1 mM).

5. 6. REFERENCE

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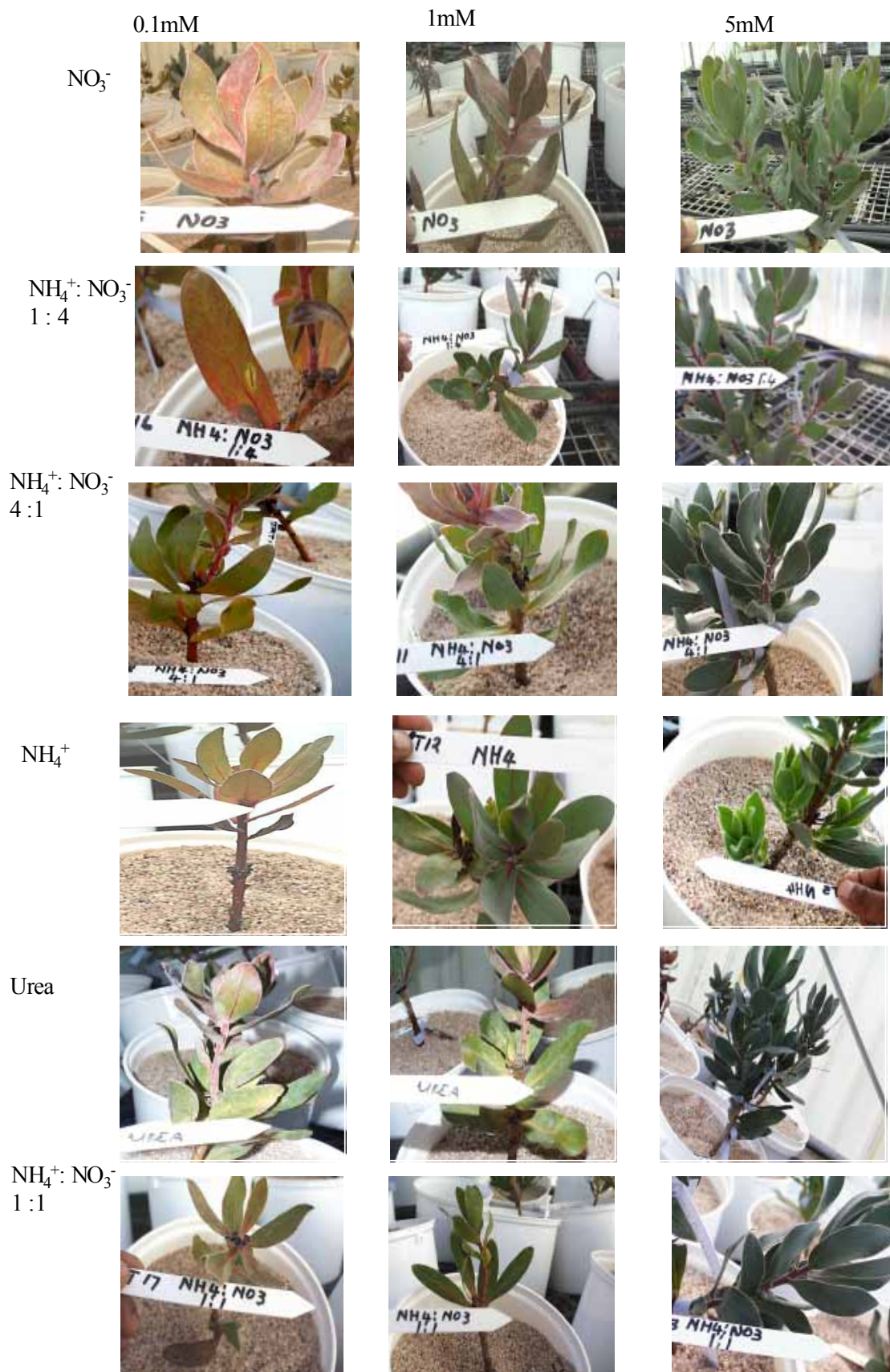


Fig. 1. Visual observations on *Protea* 'Cardinal' treated with different nutrient combinations of $\text{NH}_4^+ : \text{NO}_3^-$ (1:4) $\text{NH}_4^+ : \text{NO}_3^-$ (4:1) $\text{NH}_4^+ : \text{NO}_3^-$ (1:1) $\text{NH}_4^+ : \text{NO}_3^-$ and Urea (different forms of nitrogen) at 5, 1 and 0.1 mM.

0.01 mM



0.1 mM



1 mM



Fig. 2 Visual observations on *Protea* 'Cardinal' treated with P at 0.01 mM, 0.1 mM and 1 mM.

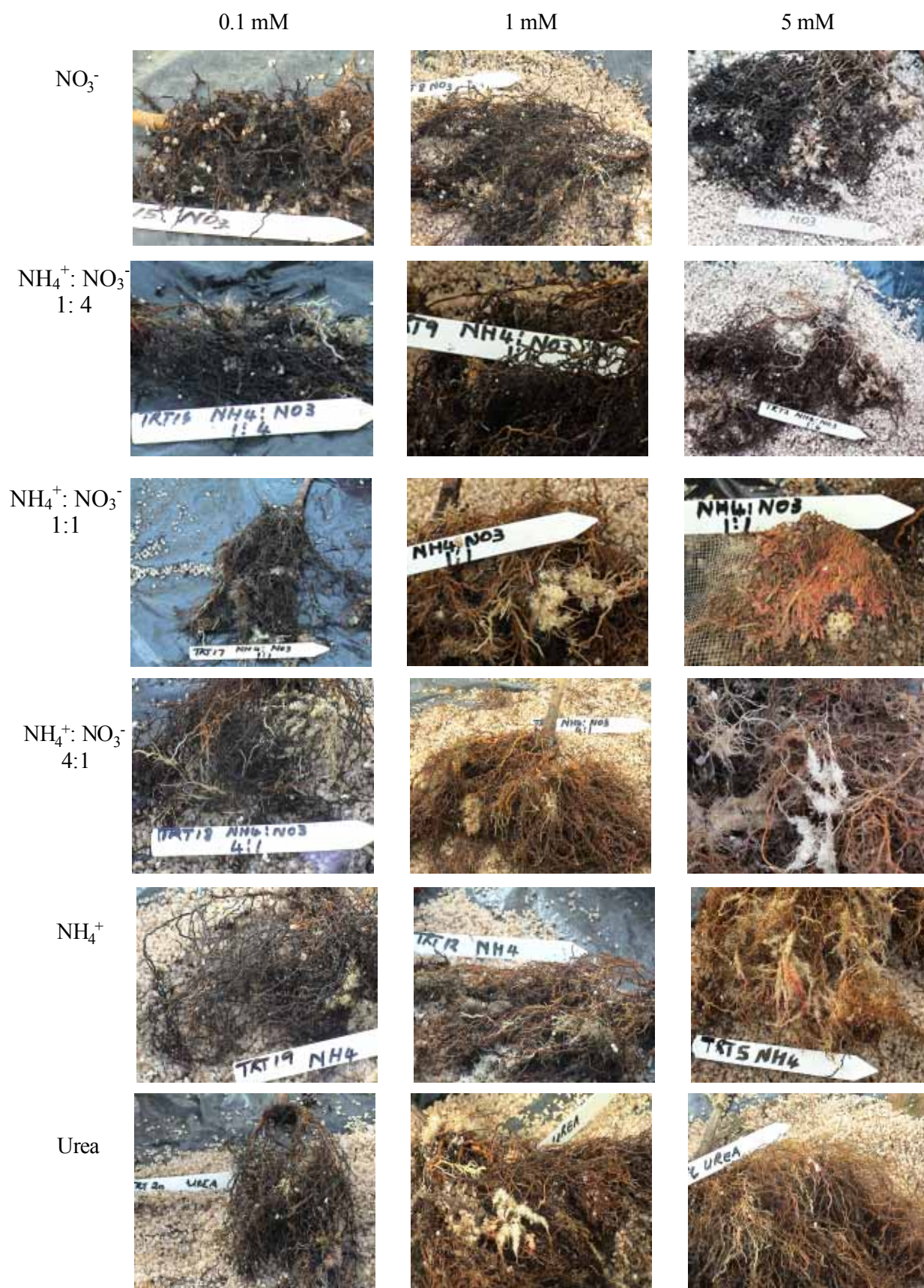


Fig. 3 Observations on roots of cultivar 'Cardinal' grown in sand for seven months in temperature controlled and treated with seven nutrient combination of NO_3^- , $\text{NH}_4^+ : \text{NO}_3^-$ (1:4), $\text{NH}_4^+ : \text{NO}_3^-$ (4:1), $\text{NH}_4^+ : \text{NO}_3^-$ (1:1), urea at 0.1 mM, 1 mM, and 5 mM

0.01mM



0.1mM



1mM



Fig. 4 Observations on roots of cultivar 'Cardinal' grown in sand for seven months in temperature controlled and treated with phosphorus at 0.01 mM, 0.1 mM and 1 mM.

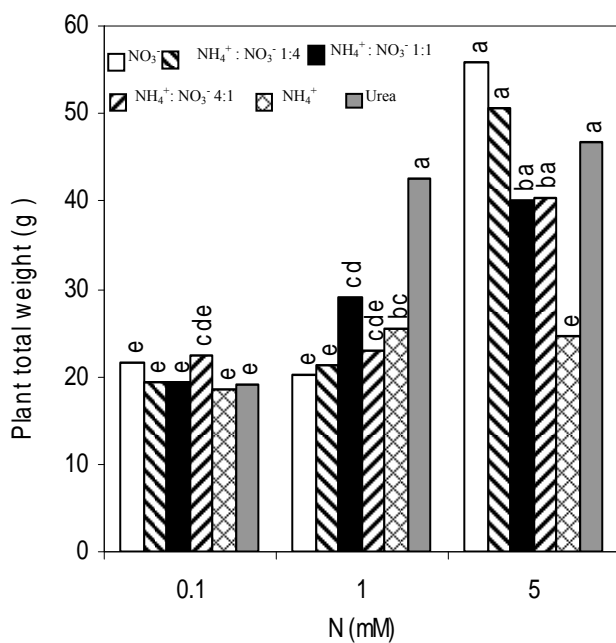


Fig. 5 Total plant dry weight of *Protea* 'Cardinal'. *Protea* 'Cardinal' was grown in sand for seven months in temperature controlled glasshouse and treated with seven nutrients combination of NO_3^- , NH_4^+ , $\text{NH}_4^+:\text{NO}_3^-$ (1:1), $\text{NH}_4^+:\text{NO}_3^-$ (1:4), $\text{NH}_4^+:\text{NO}_3^-$ (4:1) and Urea at 0.1 mM, 1 mM or 5 mM.

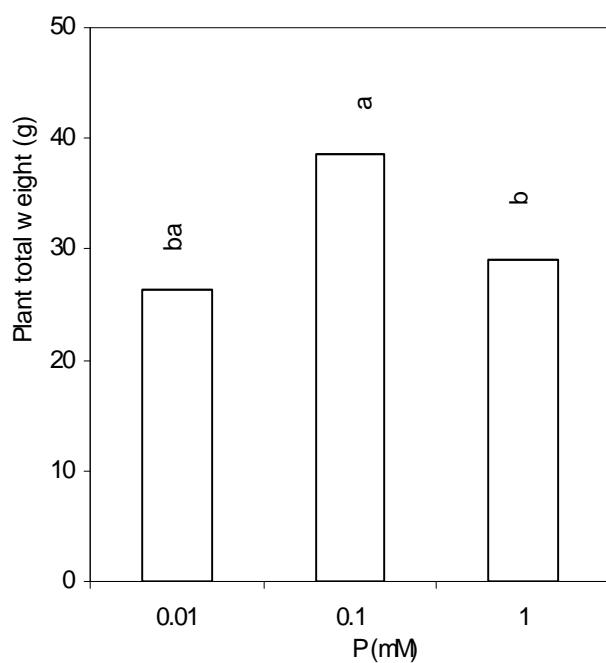


Fig. 6 Total plant dry weight in *Protea* 'Cardinal' plant tissue. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with P at 0.01 mM, 0.1 mM, or 1 mM.

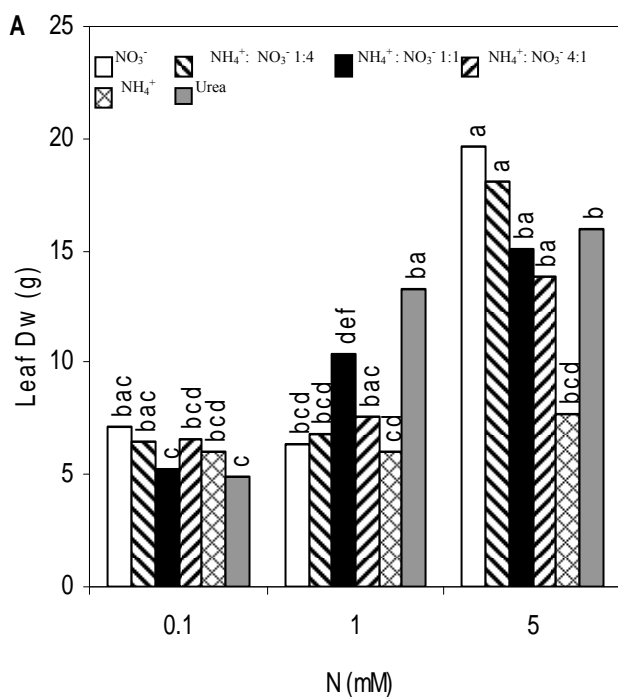


Fig.7a Dry weight of leaves in *Protea* 'Cardinal'. *Protea* 'Cardinal' was grown in sand for seven months in temperature controlled glasshouse and treated with seven nutrients combination of NO₃⁻, NH₄⁺, NH₄⁺:NO₃⁻ (1:1), NH₄⁺:NO₃⁻ (1:4), NH₄⁺:NO₃⁻ (4:1) and Urea at 0.1 mM, 1 mM or 5 mM.

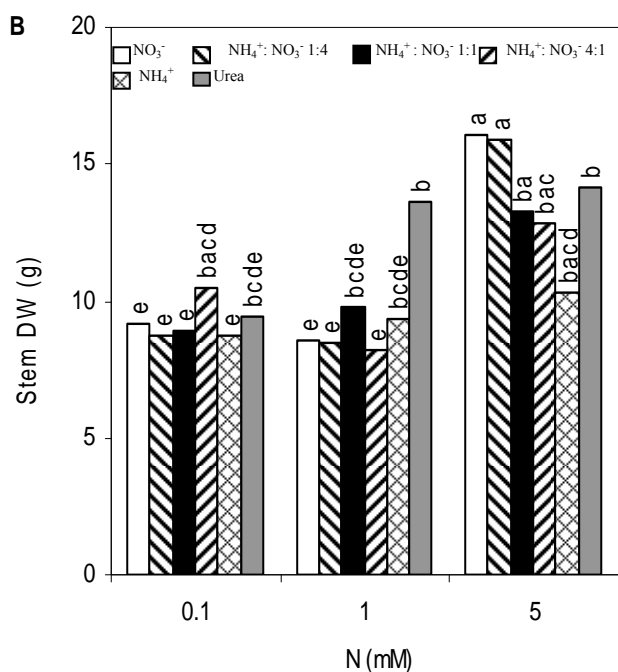


Fig. 7b Dry weight of stems in *Protea* 'Cardinal'. *Protea* 'Cardinal' was grown in sand for seven months in temperature controlled glasshouse and treated with seven nutrients combination of NO₃⁻, NH₄⁺, NH₄⁺:NO₃⁻ (1:1), NH₄⁺:NO₃⁻ (1:4), NH₄⁺:NO₃⁻ (4:1) and Urea at 0.1 mM, 1 mM or 5 mM.

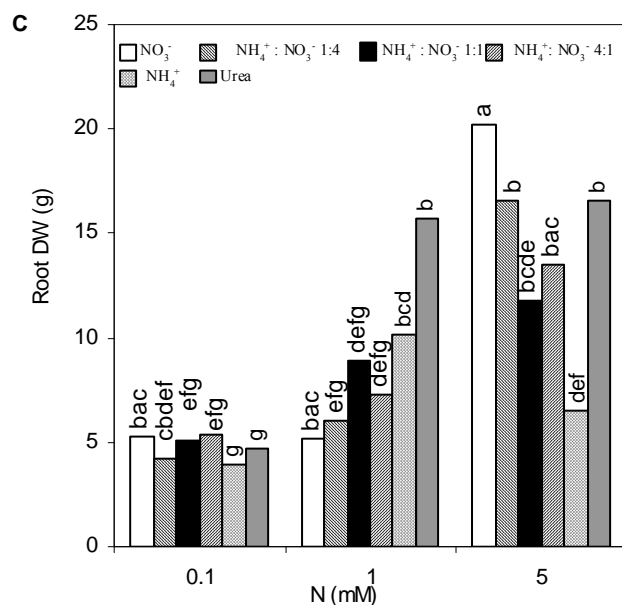


Fig. 7c Dry weight of roots in *Protea* 'Cardinal'. *Protea* 'Cardinal' was grown in sand for seven months in temperature controlled glasshouse and treated with seven nutrients combination of NO₃⁻, NH₄⁺, NH₄⁺:NO₃⁻ (1:1), NH₄⁺:NO₃⁻ (1:4), NH₄⁺:NO₃⁻ (4:1) and Urea at 0.1 mM, 1 mM or 5 mM.

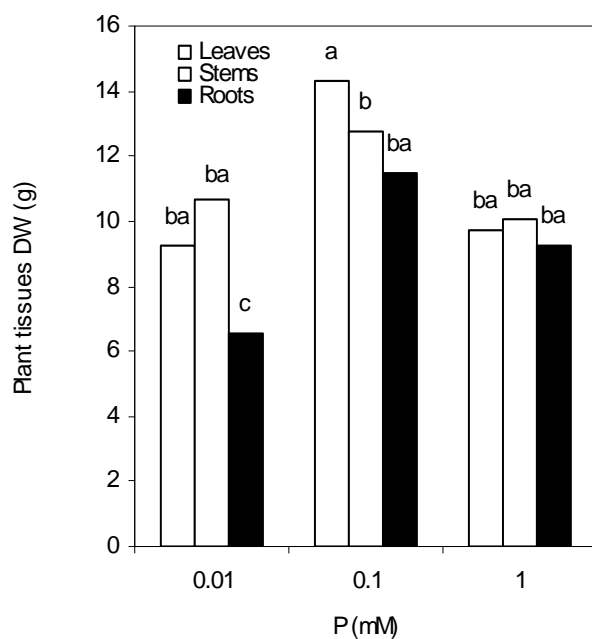


Fig. 8 Dry weight of leaves, stem and roots of *Protea* 'Cardinal'. *Protea* 'Cardinal' was grown in sand for seven months in glasshouse environment and treated with P at 0.01 mM, 0.1 mM or 1 mM.

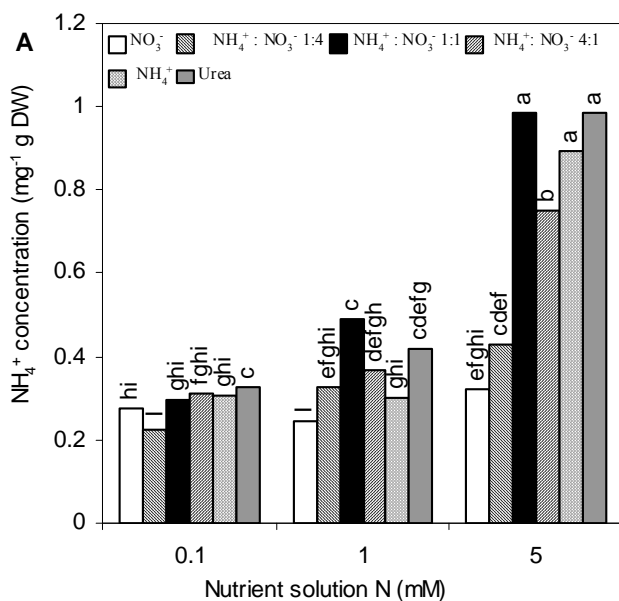


Fig. 9a The accumulation of NH_4^+ concentration in *Protea* 'Cardinal' plants tissue. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with seven nutrients combinations of NO_3^- , NH_4^+ , $\text{NH}_4^+:\text{NO}_3^-$ (1:1), $\text{NH}_4^+:\text{NO}_3^-$ (1:4), $\text{NH}_4^+:\text{NO}_3^-$ (4:1) and Urea (different forms of nitrogen) at different concentration. N at 0.1 mM, 1 mM or 5 mM.

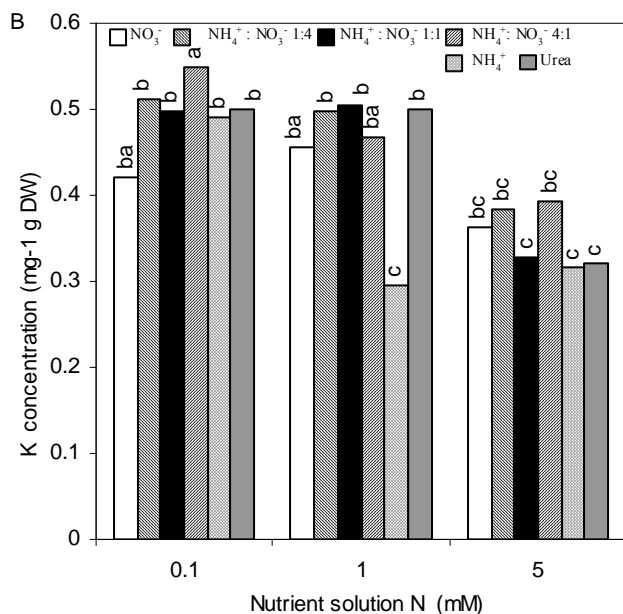


Fig. 9b The accumulation of K^+ concentration in *Protea* 'Cardinal' plants tissue. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with seven nutrients combinations of NO_3^- , NH_4^+ , $\text{NH}_4^+:\text{NO}_3^-$ (1:1), $\text{NH}_4^+:\text{NO}_3^-$ (1:4), $\text{NH}_4^+:\text{NO}_3^-$ (4:1) and Urea (different forms of nitrogen) at different concentration. N at 0.1 mM, 1 mM or 5 mM.

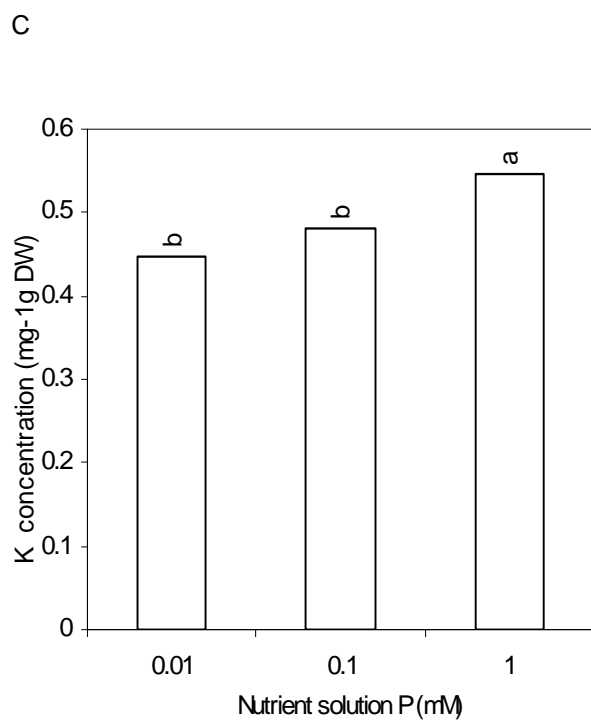


Fig. 10 The K^+ concentration in *Protea* 'Cardinal' plants tissues. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with P at 0.01 mM, 0.1 mM, or 1 mM.

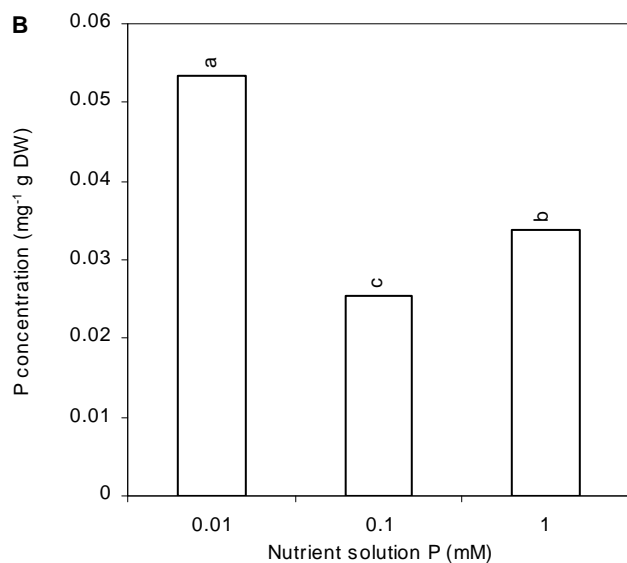


Fig. 11b The accumulation of P concentration in *Protea* 'Cardinal' plants tissue. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with P at 0.01 mM, 0.1 mM, or 1 mM.

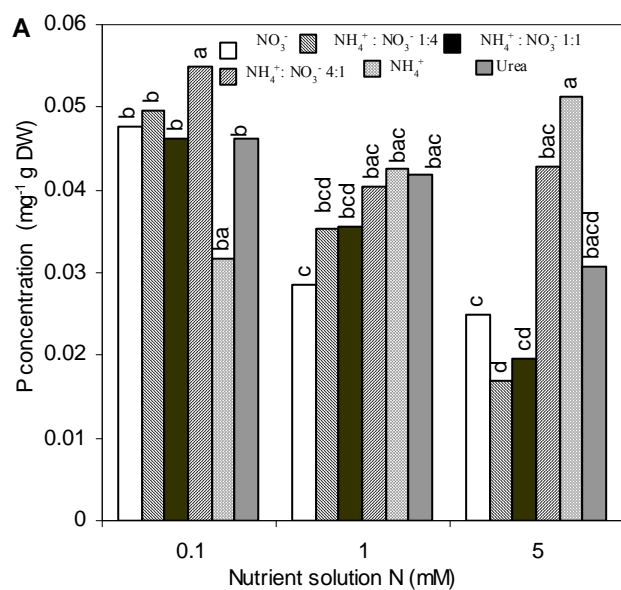


Fig. 11a The accumulation of P concentration in *Protea* 'Cardinal' plants tissue. *Protea* 'Cardinal' was grown in sand for seven months in a temperature controlled glasshouse and treated with seven nutrients combinations of NO₃⁻, NH₄⁺, NH₄⁺:NO₃⁻ (1:1), NH₄⁺:NO₃⁻ (1:4), NH₄⁺:NO₃⁻ (4:1) and Urea (different forms of nitrogen) at different concentration. N at 0.1 mM, 1 mM or 5 mM.

CHAPTER SIX

1. GENERAL DISCUSSION

Growth flushing in *Protea* species has been studied principally in relation to time of flowering and to understand the environmental dependence of seasonal growth. Leaf sampling has been used to investigate seasonal uptake of nutrients in order to coincide with fertilizer management programs. Cecil *et al.* (1995) used the youngest fully expanded leaves of *Leucadendron* 'Silvan Red' to determine the most suitable time for leaf analysis and the magnitude of differences in leaf nutrient composition between *Leucadendron* and *Protea* hybrids.

In this study (Chapter 2) *Protea* 'Cardinal' was used to determine nutrient distribution on first flush leaves and stem, second flush leaves and stems, basal leaves and stems, roots and flower buds. The results showed that N decreased with time, while P, K, and Ca were either stable or increased with time in plant tissue towards the end of growth. There was a distinct pattern on nutrient distribution on leaves but stems proved to have a more stable nutrient composition than leaves. In most cases stems are not regarded as a nutrient indicator to provide necessary information for nutrient analysis. From the results of this work, the nutritional information available from the various tissues was dependent on the nutrient uptake and the distribution thereof in various tissues with relation to growth stage and time.

The results in Chapter 3 are based on observations of visual symptoms of eleven cultivars of the three genera *Protea*, *Leucadendron* and *Leucospermum*. In these results most cultivars did not show signs of deficiency at an early stage especially in Ca deficiency treatment. At the later stage most cultivars started to show symptoms that were either classical (common) or unique. Most cultivars including *Protea* 'Red Rex', *Protea* 'Pink Ice', *Protea* 'Sylvia', *Protea*

'Cardinal', *Protea* 'Susara', *Leucadendron* 'Safari Sunset', *Leucospermum* 'Tango', and *Leucospermum* 'Succession 11' in the N deficiency treatment responded accordingly to classic N deficiency symptoms at the early stage of deficiency. The older leaves in the N deficiency treatment showed common symptoms with other agronomic plants in that the older leaves were chlorotic while the upper leaves were still green at the early stage of deficiency. At the later stage the lower leaves became very chlorotic especially the *Protea* cultivars.

Cultivar *Protea* 'Red Rex' in the early and the late stage of P deficiency, showed symptoms that were not similar to other cultivars under P deficiency treatment. Most cultivars became dark green, but in *Protea* 'Red Rex' the older leaves had red to purple spots interveinally and also on the leaf petiole. The young or upper leaves were marginally red, which later developed into red spots as the leaf matured. This P deficiency observed in *Protea* 'Red Rex' is also common in this cultivar under field conditions. Growth was also not affected as new shoots were formed. *Protea* 'Cardinal' at the later stage developed leaf dryness, especially on the upper leaves.

The common symptom associated with K deficiency is leaf tip burn, especially of older leaves (Chapter 3). The *Leucadendron* and *Leucospermum* cultivars seemed to be more affected by K deficiency than the *Protea* cultivars. Most cultivars at the early stage did not show Ca deficiency symptoms. Only at the later stage were leaves chlorotic, especially the older leaves. However, cultivars *Protea* 'Cardinal', *Leucospermum* 'Tango', and *Leucospermum* 'High Gold' showed the Ca deficiency symptoms at an early stage.

In cultivar *Protea* 'Cardinal' the lower leaves and mid-rib were chlorotic, but this did not affect the interveinal area of the leaf. In *Leucospermum* 'Tango' the upper leaves had leaf tip burn and cultivar *Leucospermum* 'High Gold' had dead buds and the leaves surrounding the

bud were chlorotic at the early stage of deficiency. Cultivar *Leucadendron* 'Safari Sunset' did not show the signs of Ca deficiency; the plant growth was good with leaves showing no chlorosis or necrosis spots on them.

For cultivar *Leucadendron* 'Safari Sunset' it was difficult to conclude that Ca nutrient is not important or Ca is not needed since the plant had no Ca deficiency symptoms. *Leucadendron* 'Safari Sunset' seem to adapt to variable conditions either be of nutritional or environmental stress because (Silber *et al.*, 1997) reported that high P, improved growth of *Leucadendron* 'Safari Sunset'.

In conclusion, the observed visual deficiency symptoms documented here can be used to explain some unknown phenomena noticed under field conditions, such as the upper leaves becoming reddish in N deficiency treatments on *Protea* 'Sylvia', *Protea* 'Pink Ice' and *Protea* 'Cardinal' plants. It is intended that these photographs and notes will be made available to growers to serve as a means of identifying nutritional disorders in the field.

Observations (Chapter 4) of visual symptoms on N optimisation treatments on leaves, showed that at 5 mM level *Protea* 'Cardinal' leaves were greener than at 1 mM and 0.1 mM levels of N. At 0.1 mM leaves were reddish for example in NO_3^- treatment. In P treatment at 1 mM though, the leaves were greener and the plant formed new shoots, but at the later stage leaves developed necrotic spots. The 0.01 mM and 0.1 mM gave better growth for *Protea* 'Cardinal', and the leaves had no necrotic spots.

Cluster roots were present in $\text{NH}_4^+ : \text{NO}_3^-$ (1:4), $\text{NH}_4^+ : \text{NO}_3^-$ (4:1), NO_3^- and NH_4^+ at 5 mM in N and P source treatments. The $\text{NH}_4^+ : \text{NO}_3^-$ (1:1) at 5 mM had a thick mat of lateral roots that had unusual red colour with no presence of cluster roots on them. Watt and Evans (1999) reported that the morphology of proteoid roots evolves independently and different

physiological thresholds, including nutrients, might trigger production of these roots. This red colouration was only noticed in this treatment. The cause of these at this stage is unknown.

There was no significant difference in Ca and Mg accumulations in both the N and P treatments; hence this data was not included with the results. Overall, the results indicate that the NO_3^- treatment resulted in higher dry weight of leaves, stem and roots at the 5 mM level. The form of N applied had a greater effect on plant growth and plant nutrient concentration availability than the N source.

Greater dry weight was accumulated in the P treatment at the 0.1 mM level. The root mass was lower at both the 0.01 mM and 1 mM levels. These results will be used to inform nutrition trials in the field. It is unusual for NO_3^- to be identified as an appropriate N source for Proteaceae. This might be an artifact of the highly controlled addition of nutrients at a particular pH as conducted in these experiments.

It will be of interest to discover whether NO_3^- can be confirmed as a beneficial source of N in the field. However, the results of this work do encourage the view that fertigation of Proteacea is possible and desirable for increased vegetative growth, at least. It will be interesting to discover how the fertigation practices interact with other environmental constraints such as temperature and water availability in field conditions.

Furthermore, the role of different soils in the supply and retention of nutrients still needs to be examined. Although the soils to which Proteacea are native are highly leached, strong variability exists between different species in their ability to withstand differences in nutrition. For instance, some Proteaceae are known to occur exclusively on calcareous soils and not on non-calcareous soils.

In conclusion, this study has taken the understanding of Protea nutrition forward by establishing the distribution of nutrients within plants, the symptoms of nutritional deficiencies and a first step towards optimizing nutritional supplementation. There is still much work required to bring nutritional understanding of the Proteaceae used in floriculture to the level of that of other crops. This is likely to be a complex endeavour considering the huge variety of cultivars for diverse parentage being used within the industry.**6.**

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