Soil Acidity and Eucalypt Growth: Aluminium Toxicity and Its Interactions with Calcium, Phosphorus and Moisture Levels

by

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Declaration

Except where otherwise noted, the work contained in this thesis is my own work.

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Abstract

Aluminium toxicity is today considered to be the most critical factor limiting plant growth in vast areas of acid soils of the world. But very little research has been conducted on the impact of Al toxicity on tree species in general, and eucalypts in particular.

In this study, a series of experiments was conducted aimed at investigating the impact of Al toxicity on the growth of *Eucalyptus camaldulensis*. The experiments included interrelationships of Al with Ca, P and moisture stress.

Initially, using glasshouse pot trials and 3 acid soils, the growth of 22 eucalypt species from various provenances was found to vary in shoot height and shoot weight in three acid soils. Two better performing tropical species (*E. camaldulensis* and *E. citriodora*) and two poor performing species (*E. gummifera* and *E. saligna*) were selected from the 22 species for experiments at the next phase.

These four species differed in their response to conventional liming and to soil moisture stress in a different selected acid soil high in exchangeable Al and low in exchangeable Ca and available P. Although the growth of *E. camaldulensis* was poor, in terms of percentage increase in dry matter produced, this species demonstrated a maximal response to liming and was selected for the remaining experiments. In the same acid soil, *E. camaldulensis* also responded to Ca and P application (and also to their interaction) and not at all to K, S or Mo. The application of Ca alone did not increase the growth of *E. camaldulensis* but the higher levels of Ca resulted in an increase in soil pH and a decrease in exchangeable Al. Phosphorus increased growth earlier in treatments receiving both Ca and P.

Considering the importance of Ca supply to plants in an acid soil, an attempt was made to raise soil Ca levels while having a minimum impact on other acidity related factors. It was found that the addition of Ca from $CaCO_3$ and $CaSO_4$ at a ratio of 2 : 1 raised soil Ca to the same level as does an addition of equal amounts of Ca from a single source of $CaCO_3$ or $CaSO_4$ but affects the soil pH and exchangeable Al and Mn much less than does the single source.

When liquid media was used high Al in general had a negative effect while Ca had a positive effect on root and shoot growth of *E. camaldulensis*. Adverse effects of Al toxicity were most severe for fine root growth. The root peripheries of seedlings grown in a high Al nutrient solution were heavily thickened. These adverse effects on root and shoot growth and the thickening of the root periphery were partly ameliorated by the application of higher amounts of Ca. Low levels of Al accompanied by high Ca improved shoot height, shoot weight and total biomass to a greater extent than treatments with nil Al. Fineness of fine root [defined as (fine root length)/(fine root weight)], and Al concentration in mature leaves of seedlings, appeared to be two good phytoindicators of Al toxicity in *E. camaldulensis* seedlings.

When P was included along with Ca and Al, both Ca and P ameliorated Al toxicity affecting seedling growth and improved the nutrient absorption rate. The beneficial effects of Ca and P treatments are related to increased concentrations of Ca and P in the roots rather than a decrease in Al concentrations in the seedlings.

In pots using soil as a lower layer under sand, high Al levels and moisture stress both independently and interactively, affected root growth and the development of *E. camaldulensis* seedlings. Mineral

concentrations of Ca, Mg, Al and P differed between shoot, top root and bottom roots and also for different treatment combinations. The effect of moisture stress on Ca, Mg and Al concentrations in the seedling shoots was statistically significant. In terms of mineral concentrations in the seedlings grown in sand pots, Al had a lesser effect in sand than it had on seedlings grown in nutrient solution under the same or comparable treatment combinations.

Eucalyptus camaldulensis displays an intermediate level of tolerance to Al when compared to other tree species. There are many tree species both more tolerant and more sensitive to Al toxicity than this species.

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

The problem of soil acidity and its harmful effects on agricultural production has been known since early times but research in this area did not begin until the 19th century. To date many aspects of soil acidity have not been researched adequately, when compared to the pervasiveness of the problem. The problem of soil acidity can be fully analysed only in relation to its impact on plant growth and in such research many interrelated factors affecting plant growth need to be taken into consideration.

Vast areas of the world are covered by acid soils. They are more common in the tropical regions (FAO, 1975); occupying about 81% of humid tropical America, about 56% of humid tropical Africa and about 38% of humid tropical Asia and the Pacific land masses. The importance of the problem for Australia may be realised from the fact that in humid tropical Queensland, almost all soils have a pH of less than 5.5.

On strongly acidic soils most plants produce less than their full potential due to one or more of the following factors: low pH *per se*, toxicities of Al and Mn; deficiency or low availability of Ca, Mg, P, Mo and/or restricted microbiological activity. Under natural conditions, acid soil toxicity is not a single factor, but rather a complex of these factors in combination, affecting the growth of plants. Though acid soil is defined in terms of low pH, one of the major growth limiting factors for plants is Al toxicity in the acid soil. However, Al solubility is highly pH dependent. In general, in a simple system such as aluminium hydroxide with water, Al exists as Al^{3+} at a pH < 4.0 and above that as hydroxy aluminium complexes. With a decrease in pH, Al solubility increases and

basic cations are released from the exchange complex and become vulnerable to leaching loss.

Because of the high economic return per hectare from agricultural crops, the influence of these factors on arable soils has been more widely studied during the past two decades. In agricultural systems, Al toxicity may be alleviated by liming, or avoided by growing Al tolerant plant varieties (Pratt, 1966; Adams and Moore, 1983). Studies have also concentrated on the influence of Ca and other ions in alleviating Al toxicity of soils (Hoyt et al, 1974; Alva et al, 1987). The interaction of some of these soil acidity related factors with moisture conditions has also received some attention for agricultural soils (Horsnell, 1984).

However forest soils, which usually have management problems which are at least as serious as agricultural soils and are less suitable for agricultural purposes, have not been studied. For example most Australian forest soils are acid to highly acid. Although Australia has not suffered from artificial soil acidification as countries in the northern hemisphere (Blackburn and McLeod, 1983), many acid forest soils in Australia are high in Al and low in Ca with the potential for Al toxicity and Ca deficiency (Humphreys and Truman, 1964; Khanna et al, 1986). Similarly in Bangladesh, where although very few forest soils have been surveyed, a major part of those mapped have a pH less than 5.0 (Hossain et al, 1979). Studies on the influence of acid soil factors on the growth of tree crops have been few and there has been even less monitoring of eucalypt species on these soils.

In order to examine these issues a number of hypotheses have been postulated; these are based on an extensive literature review (Chapter 2).

1) Despite the fact that eucalypts appear to be adapted to Australian conditions, their growth is adversely affected by soil acidity and different species differ in their responses to soil acidity.

2) Aluminium toxicity is the most crucial aspect of the soil acidity problem. It is possible to demonstrate the adverse effects of Al toxicity on eucalypt growth.

3) The mechanism of depressed growth in high Al systems is through the effects of Al on root growth and especially on fine root growth. The growth of fine roots by eucalypts requires an optimum ratio of Ca and Al in the growth medium and high Al concentrations depress fine root growth.

4) Other nutrients are also affected by acidity and in addition to Ca, the levels of P in the growth medium will interact with Al and will change the toxic effect of the Al.

5) The nutrient absorption rate will vary due to Al, Ca and P concentrations.

6) Soil moisture levels will influence the effects of Al on growth and mineral uptake by *Eucalyptus camaldulensis*.

The objective of this research is to test these hypotheses and by so doing to further analyse the ways in which Al toxicity affects the growth of eucalypts. Therefore, a number of experiments were conducted. From amongst nearly 600 endemic species one, *E. camaldulensis*, which is widely present in Australia and is grown commercially in other tropical and subtropical countries, was selected. The subsequent experiments were used to make a detailed study of the performance of this species, as affected by the soil acidity factors. The performance of *E. camaldulensis* was studied by focussing on the following aspects of the growth of seedlings: (a) seedling growth parameters, (b) mineral concentration in

different parts of the seedlings, (c) nutrient uptake rate by the seedlings, (d) growth and development of roots in the subsoil and (e) accumulation of Al and other cations in the root periphery of Al stressed seedlings.

An outline of the sequence of experiments and the objective of each experiment:

1) **The modification of Ca and Al levels in a soil by amendments.**

In order to examine just how Al or Ca affects growth, these characteristics must be varied whilst holding as many other soil variables as constant as possible. Soils with differing Ca and Al levels under natural conditions are likely to differ in other characteristics and the usual liming treatment changes several more characteristics simultaneously. Therefore, attempts were made in this study to amend soil Ca and Al levels whilst having a minimum impact on the other soil characteristics (Chapter 3).

2) **Selection of an eucalypt species.**

In order to select a tree species which would adequately demonstrate the effects of acidity a number were tested. The performance of 22 eucalypt species in three natural acid soils and the responses of 4 selected species to liming and moisture levels were monitored. They were all grown in an acid soil. Insufficient information on the growth of eucalypts in acid soils necessiated this screening process (Chapter 4).

3) **The growth responses of *E. camaldulensis* in Al rich acid soil.**

The growth responses of *E. camaldulensis* to additions of Ca, P, K, S and Mo were evaluated when the seedlings were grown in a fourth

selected acid soil – high Al, low pH, low Ca and low available P. This experiment was conducted to examine which nutrients limit the growth of *E. camaldulensis* in an acid soil so that the growth of the seedlings may be boosted by fertilization (Chapter 5).

4) **The response of *E. camaldulensis* to varying Al and Ca levels.**

The response of this species to varying Al and Ca levels in water culture was examined. Various growth parameters were monitored. The mineral concentration in young leaves, mature leaves and fine roots of *E. camaldulensis* were examined to establish a suitable phytoindicator for Al (Chapter 6).

5) **How do Al stressed *E. camaldulensis* seedlings respond in terms of root efficiency ?**

Since Al in the plant tissues appears to inhibit root growth, a study on the efficiency of Al stressed *E. camaldulensis* roots was undertaken in nutrient culture solution. Adverse effects of Al on the efficiency of roots of *E. camaldulensis* was examined with respect to nutrient absorption. Nutrient absorption rates by seedlings were measured at different times and under different treatment combinations. The ameliorating role of Ca and P against Al toxicity was also examined. Successive harvests of seedlings from different treatment combinations allowed an examination of how the treatment effects changed as the seedlings grew older (Chapter 7).

6) **The accumulation of Al in the root periphery.**

If the adverse effects of Al on root morphology and total seedling growth is ameliorated by high Ca and high P, it is important to examine whether the thickening of roots by Al stressed seedlings of *E.*

camaldulensis was caused by the accumulation of high amounts of Al in the root periphery and further, if high Ca and P reduced these Al levels. Therefore, the influence of high Ca and P on the concentration of Al and other cations in the root periphery of Al stressed *E. camaldulensis* was examined (Chapter 8) using nutrient solution.

7) **The effects of Al in a lower layer on growth and mineral concentration.**

Once the effects on roots in nutrient solution were demonstrated the next stage was to examine the changes as roots came in contact with higher Al concentrations in the soil. Therefore this experiment was designed to demonstrate the effects of Ca, Al and P on the development and growth of *E. camaldulensis* roots in a soil high in Al. Since different measures of root growth were found to vary significantly due to Al toxicity (Chapter 6), this experiment was planned to examine the effects on root growth and development of *E. camaldulensis* as its roots enter lower layers with high Al toxicity in the soil growth medium. The effects were examined in both sand and soil as growth mediums (Chapter 9).

8) **The effects of Al and moisture stress in a lower layer on growth and mineral concentration.**

The severity of Al toxicity is affected by moisture stress. Therefore, the effects of Ca, Al, P and moisture stress on growth and mineral uptake by *E. camaldulensis* were examined in a two layer sand medium. The earlier experiments were conducted in nutrient solution and did not include the effect of moisture levels. This experiment was conducted using sand as the growth medium and the interaction of moisture stress along with Al, Ca and P on growth parameters and nutrient uptake in *E. camaldulensis* were demonstrated (Chapter 9).

In conclusion, from these experiments it was hoped to show the adverse effects of Al toxicity on different aspects of *E. camaldulensis* growth. It was also expected to reveal interactions among Al, Ca, P and moisture stress affecting *E. camaldulensis* growth. Thus, it was expected to highlight the mechanism by which Al toxicity was ameliorated by high Ca and P levels through interpretation of mineral concentration, nutrient uptake rate, accumulation of cations in the root periphery, and root growth and development.

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Introduction

Acid soils are widespread throughout the world. They develop from silicic parent material, from intense weathering or from human activities through inappropriate management. Acid soils occupy about 81% of humid tropical America, about 56% of humid tropical Africa and about 38% of humid tropical Asia and the Pacific. Problems associated with acid soil infertility have engaged the attention of agriculturists since early historical times.

The objective of the present chapter is to review previous research on soil acidity relating to Al toxicity, alleviation of Al toxicity by Ca and P and the impact of soil moisture status on these variables as revealed by plant growth. This review highlights different aspects of Al toxicity and related problems to identify the issues which have not been adequately studied. Sections 2.2 to 2.6 relate to aspects of soil acidity and Al toxicity and form the general background to this study. Sections 2.7 to 2.9 are more directly related to the present research and include detailed discussion to highlight the contributions made by previous research so that gaps can be identified to better design the present research.

2.2. Sources of acidity in soil.

Sources of acidity in soil are many and they may be discussed under two broad categories: natural acidification and man made acidification.

2.2.1. Natural acidification

Acid soils may develop from parent material poor in bases and/or from intense weathering (Adams, 1981 b). Generally the older and more

weathered soils are more acidic than young soils. Most soil and plant processes involve the production and consumption of protons and acidification of a soil is the consequence of a net production of protons (Khanna and Ulrich, 1985). Some of the processes such as uptake of nutrients, mineralization of organic substances, weathering of minerals, leaching of ions etc. were termed "internal" processes. Supplementary sources of protons to the forest ecosystem are from sources such as atmospheric inputs and these were termed "external" to the system (Khanna and Ulrich, 1985).

When rainfall exceeds evapotranspiration for a larger part of the year, soil leaching takes place. Such leaching gradually removes soluble salts, more readily soluble minerals and bases and consequently the soil becomes progressively more acidic (Conyers, 1986). For a range of soil types from different rainfall zones of south-eastern New South Wales, Conyers established that at a given pH, the amount of exchangeable Al in surface soil tends to increase with increasing average annual rainfall.

2.2.2. Man-made acidification

Compared to natural processes, man-made acidification is much more extensive and generally results from the mismanagement of soil. There are a variety of ways that a soil can be rendered acid by human activity. Local and extremely acidic conditions can also arise from mine spoils containing iron pyrites (FeS_2) or other sulfides being exposed to the air. The pyrite oxidizes to H_2SO_4 and $Fe(OH)_3$ (Bohn et al, 1979). Use of acid forming nitrogenous fertilizers may cause soil acidification (Pierre et al, 1971). In rank order the acidifying effect of different nitrogenous fertilizers was reported to be ammonium sulphate > ammonium nitrate > urea > calcium nitrate (Helyar and Porter, 1989). In coming to such a

conclusion they assumed that nitrification was complete in the soil and losses of N were similar for each fertilizer. Also heavy application of liquid N P K fertilizers to a slightly acidic forest soil has recently been reported to lower the pH below the critical value 4.2 at which Al begins to be released onto exchange sites (Khanna et al, 1991). Acidity produced by agricultural practices is much more extensive geographically.

In more general terms, removal of basic cations in harvested crops may develop soil acidity (Kamprath and Foy, 1971). This occurs if the cations removed are not replaced by adequate fertilization. In that case acidic cations (H and Al) are left to dominate the available sites on soil particles. Removal of greater amounts of inorganic cations than anions in plant products (Riley and Barber, 1969) has also been identified as potentially important factor in the development of acid soils. This is more important in the case of nitrogen fixing *rhizobium* since legumes absorb more cations than anions from the soil when nitrogen is obtained almost entirely from the atmosphere (Nyatsanga and Pierre, 1973; Haynes, 1983). Growth of subterranean clover pasture over a long period in southern Australia has accumulated organic matter in soil and decreased the soil pH (Kohn et al, 1977; Lee, 1980; Williams, 1980; Bromfield et al, 1983). More recently Ritchie and Dolling (1985) reported that the initial soil pH and organic anion concentration (i.e., the percent dissociation of soluble organic acids when released into the soil) determine the acidifying effect of organic matter. Introduction of certain tree species can result in increased soil acidity. In Australia, Feller (1978) reported that a greater quantity of acid was found in the soil under *Pinus radiata* compared to *Eucalyptus obliqua*. In the former case, soil also contained lower quantities of exchangeable cations.

In recent years increasing concern about the effect of acid rain on soils has developed. It is more critical for heavily industrialized regions or areas under prevailing weather from such industrialized regions. The combustion of large amounts of fuel result in the discharge of nitrous oxide and sulphur dioxide gases which in contact with rainfall are converted to acid and return to earth. Ulrich et al (1980) and Fowler et al (1985) reported acid inputs from the atmosphere. The extent to which acid rain will induce decrease in pH or increase in Al saturation will depend on soil conditions. In Australian agricultural ecosystems acid inputs other than carbonic acid in rainfall are minor (Blackburn and McLeod, 1983).

In agricultural ecosystems acidification results from agriculturally induced increases in losses of the products of acid reactions in the C and N cycles. The important losses are of nitrate (e.g., leaching, runoff) and of organic anions (e.g., product and waste product removal). In agricultural ecosystems the rate of soil acidification can be more rapid than that in natural ecosystems (Siman et al, 1974; Helyar, 1976; Haynes, 1981). Cregan and Helyar (1986) estimated acid additions of 3 - 5 kmoles H^+/ha/year for fertilized annual legume based pasture or pasture crop rotation in the 700 - 900 mm rainfall zone.

2.3. Distribution of Aluminium in soils

Aluminium is ubiquitous in the earth's crust, being the third most abundant element, second most plentiful oxide and the most plentiful metallic element. It occurs as primary minerals (micas, feldspars), secondary minerals (clay minerals) and ores (bauxite). The total Al concentration in surface soils is generally of the same magnitude as that of the earth's crust. Exceptions occur where the soils have a predominance of silica sands due to the influence of sandy parent

material or where the soil has lost much of its Al by intensive weathering. Ritchie (1989) described the distribution of Al in soil as the net result of two sets of reactions. The first of these is the competition between ligands for Al where the ligands may be soluble species, or ligands on particles that can react with cations (i.e., adsorption) or species that can precipitate Al ions. The second is the competition between Al and other cations for ligands.

The forms of Al in soils may be summarized as follows:

A. Solid phase Al

i. That which is freely exchangeable with other cations.

ii. Organic complexes.

iii. Non crystalline coatings on soil particles.

iv. Discrete amorphous phases.

v. Structural Al in primary and secondary minerals.

vi. Oxides and hydroxides of Al.

B. Soil solution Al.

i. The monomeric and polymeric hydroxy species of Al.

ii. Ion associations of Al with sulphate [$AlSO_4^{2+}$, $Al(SO_4)_2^{+}$] and Al with fluoride (AlF^{2+}, AlF_2^{+}, AlF_3^{o}, AlF_4^{-}) and

iii. Soluble Al-P, Al-P-Si, Al-OH-Si and Al-organic matter complexes.

In aqueous solution Al^{3+} does not remain as a free ion, but it is surrounded by six molecules of water forming $Al(H_2O)_6^{3+}$. As pH increases, protons are removed from the coordinated waters giving a series of hydrolysis products:

pK values

$$Al(H_2O)_6^{3+} + H_2O \rightleftharpoons Al(H_2O)_5(OH)^{2+} + H_3O^+ \quad - 5.02$$

$$Al(H_2O)_5(OH)^{2+} + H_2O \rightleftharpoons Al(H_2O)_4(OH)_2^{+} + H_3O^+ \quad - 9.30$$

$$Al(H_2O)_4(OH)_2^{+} + H_2O \rightleftharpoons Al(H_2O)_3(OH)_3^{0} + H_3O^+ \quad - 14.99$$

$$Al(H_2O)_3(OH)_3^{0} + H_2O \rightleftharpoons Al(H_2O)_2(OH)_4^{-} + H_3O^+ \quad - 23.33$$

$$Al(H_2O)_2(OH)_4^{-} + H_2O \rightleftharpoons Al(H_2O)(OH)_5^{2-} + H_3O^+ \quad - 34.24$$

Each hydrolysis reaction liberates hydrogen ions and lowers the solution pH unless a source of hydroxyl is present with which the hydrogen can react. This stepwise production of hydrogen ions is similar to that which occurs in the dissociation of polyprotonated acids. In addition to hydrolysis, Al may also precipitate as solid phase gibbsite, $Al(OH)_3$ whenever the solubility product of this mineral is exceeded.

In addition to the above mentioned monomeric Al species, Al may also form soluble polymers with hydroxyl ions alone or in conjunction with phosphate or silicate ions (Bache, 1963; Hsu, 1968; Blamey et al, 1983). When the solution OH : Al is low, most of the Al remains as the monomeric form (Wada and Wada, 1980) although the equilibrium between monomeric and polymeric forms is slowly established (Hsu, 1968). Some of the polymeric forms of Al identified by different authors are summarized below (Nair, 1978; Huang, 1988).

Author(s) and year	Polymeric Al form
Bottero et al (1980)	$Al_2(OH)_2(H_2O)_8^{4+}$
Matijevic et al (1961)	$Al_8(OH)_{20}^{4+}$ $Al_8(OH)_{22}^{2+}$ $Al_8(OH)_{24}$
Brosset et al (1954)	$Al_6(OH)_{15}^{3+}$
Raupach (1957)	$Al_3(OH)_8^{+}$
Fripiat et al (1965)	$Al_4(OH)_8^{4+}$ $Al_7(OH)_{16}^{5+}$
Aveston (1965)	$Al_2(OH)_2^{4+}$ $Al_{13}(OH)_{32}^{7+}$

2.4. Phytotoxicity of Aluminium

During the early days of soil acidity research, criteria for acidity removal were focussed on pH control (Truog, 1946). In this long held view, lime recommendations were based on the amount of lime required to bring a soil to a given pH (Brady, 1984; Thompson and Troeh, 1978) and a number of methods have been developed to determine the lime requirement of an acid soil (Coleman and Thomas, 1967). Reeve and Sumner (1970) reported exchangeable Al to be a suitable criterion for the measurement of lime requirement and found much less lime was required for Al control (for maximum crop production) than was needed to raise soil pH to 6.5. The inadequacy of soil pH as a single predictor of lime requirements became evident with progressive research on soil acidity and liming. Once the position of Al in soil acidity had been established, liming of acid soil began to be aimed at neutralizing Al (and Mn) rather than to raising the soil pH.

Traditionally, Al toxicity to plants has been evaluated by measuring total concentration of Al in soil or by applying known

amounts of Al to the plant growth medium (Munns, 1965; Andrew et al, 1973; Carvalho et al, 1980; Bouma et al, 1981). This total soil Al may comprise both monomeric and polymeric Al and the fate of Al added to the growing medium is decided by factors such as pH, ionic strength, and P and Ca concentration of the growing medium. Total quantity of either of these forms of Al may be misleading as a measure with respect to toxicity. The question obviously arises which form of Al is more toxic. This debate persisted for rather a long time and innumerable studies were carried out using different forms of Al as a criterion for measuring Al toxicity and/or lime requirements of acid soils (Table 2.1).

Exchangeable Al has been used by many researchers as a measure of phytotoxic Al in soil (For example, Reeve and Sumner, 1970; Anandan et al, 1985; Flores et al, 1988). Pearson (1975) advocated using the soil solution rather than exchangeable cations on the grounds that the soil solution more directly reflects the soil chemical environment to which the plant root system is exposed. Alva et al (1986 b) compared:

(i) the concentration of total Al;

(ii) the concentration of monomeric Al; and

(iii) the sum of activities of monomeric Al species ($\Sigma a_{Al\ mono}$)

in solution as an index of Al toxicity to 5 different crops and concluded that $\Sigma a_{Al\ mono}$ was the best index. Alva et al (1986 d) reported that among the individual Al monomers relative root length of soybean was correlated to (in order): $Al(OH)^{2+} > AlSO_4^+ > Al(OH)_2^+ > Al^{3+}$. Cameron et al (1986) concluded from the measurement of barley roots in nutrient solution that root elongation correlated better with Al^{3+} when compared with Al complexed with SO_4^{2-} and F. Tanaka et al (1987) confirmed these results with barley roots while adding that the degree of retardation in root length elongation was controlled by the activity of Al^{3+} rather

than its concentration. Very recently Conyers et al (1991) reported that in pot trials with barley, exchangeable Al expressed as: Al/effective CEC, 0.01M $CaCl_2$ extractable total and monomeric Al, Al^{3+} activity in 0.01M $CaCl_2$ were all better indicators of the infertility of acid soils than soil pH.

The toxic levels of metal cations in the soil solution were reported to depend primarily on their ratio to base cations, especially of Ca (Meiwes et al, 1986). Rost - Siebert (1985) also reported that the molar ratio of Ca/Al in the nutrient solution determined the growth and activity of roots of spruce and beech. The ratio Ca/Al in fine (less than 2 mm diameter) and medium (2 - 5 mm diameter) roots of spruce was also used to characterize soil acidity (Murach, 1984; Ulrich et al, 1984). Wright and Wright (1987) suggested that the ratio of $a_{Ca^{2+}}/\Sigma a_{Al\ mono}$ was a better predictor of Al toxicity. In contrast to monomeric Al, however Wagatsuma and Kaneko (1987) reported that polymeric Al ions are preferentially absorbed by roots and finally considerably inhibit root elongation. Foy (1987) summarized the best measure of potential Al toxicity to a given plant as the molar activity of Al (monomeric) in the soil solution. However, this determination is too complicated for routine use and Foy (1987) concluded that from a practical point of view percentage Al saturation is the most useful predictor of Al toxicity. Meiwes et al (1986) indicated that when the equivalent fraction of Ca on the exchange sites falls to values lower than 0.15, the soil solution ceases to be dominated by Ca. A decrease in the equivalent fraction of Ca is accompanied by a corresponding increase in the equivalent fraction of Al. A list of the different indices of Al toxicity used and the authors using them on different plants is shown in Table 2.1. From the table it may be seen that more recent studies use different Al species or their combination while earlier studies relied on exchangeable Al, Al saturation, or Al added to the growing medium as indices of Al toxicity.

Table 2.1. Some examples of indices of Al toxicity used by different authors.

Index	Author and year
pH	Magistad (1925)
Exchangeable Al	Evans and Kamprath (1970), Reeve and Sumner (1970), Farina et al, (1980), Anandan et al (1985), Foy (1987), Foy et al (1987 b), Hoyt and Nyborg (1987), Miranda and Rowell (1987), Flores et al (1988), Foy et al (1989).
Total added Al	Cate and Sukhai (1964), Foy and Brown (1964), Munns (1965), Foy et al (1969), Andrew et al (1973), Mullette (1975), McCormick and Steiner (1978), Huett and Menary (1980), Carvalho et al (1981), Duncan et al (1983), Arp and Ouimet (1986), Ryan et al (1986), Truman et al (1986), Bilski and Foy (1987), Hecht-Buchholz et al (1987), Paganelli et al (1987), Wagatsuma et al (1987b), Fageria et al, (1989 a), Hai et al (1989), McLaughlin and James (1989), Keltjen (1990).
Total soil solution Al	Gonzalez-Erico et al (1979), Carvalho et al (1980), Adams and Moore (1983), Adams and Hathcock (1984), Foy (1987), Joslin and Wolfe (1988).
Al^{3+}	Cameron et al (1986), Jarvis and Hatch (1986), Tanaka et al (1987), Conyers et al (1991), Edmeades et al (1991).
$a_{Al^{3+}}$	Adams and Lund (1966), Brenes and Pearson (1973), Pavan et al (1982), Lee and Pritchard (1984), Bruce (1986), Shuman et al (1990), Dahlgren et al, 1991.
$\Sigma a_{Al\ mono}$	Blamey et al (1983), Alva et al (1986 b), Alva et al (1986 d), Hetherington et al (1986), Alva et al (1987); Wright and Wright (1987).
Solution Ca/Solution Al	Rost-Siebert (1983), Meiwes et al (1986), Truman et al (1986).
Exch. Ca/Exch.Al	Wright and Wright (1987)
Exch. Ca/ (Exch. Ca + Exch. Al)	Smit et al (1987 a).

2.5. Factors affecting aluminium toxicity

2.5.1. Mineralogy and weathering

By far the largest portion of Al in most soils and sediments is in Al bearing clay mineral crystals in octahedral and to a lesser extent in tetrahedral coordination with oxygen (McLean, 1976). During weathering of primary minerals Al is released and then crystalised as secondary minerals, largely aluminosilicates. Even a relatively highly weathered soil usually has the bulk of its Al remaining as part of the aluminosilicate minerals (McLean, 1976). Clay type and proportion in soil, and the structural and surface properties of clay minerals have enormous influences on Al transformation. The concentration of Al in the soil solution is determined by the solubility of Al containing minerals (Lindsay, 1979). Gibbsite is important in controlling Al solubilities in highly weathered soils, whereas other aluminosilicates are more likely to control Al solubilities in more moderately weathered soils (Marion et al, 1976). Some of the Al containing minerals may be amorphous or crystalline (e.g., Al oxide, kaolinite) and the solubility of the amorphous form of these minerals is about 100 times that of the crystalline form (Adams, 1981 a). Adams (1981 a) ranked Al containing clay minerals according to the concentration of Al that each would maintain in soil solution at equilibrium and at the same pH as follows: $Al(OH)_3$ (amorph.) > $Al_2Si_2O_5$ $(OH)_4$ (amorph.) > halloysite > gibbsite > kaolinite > montmorillonite. The mineralogy of a soil also influences the supply of Al to the soil solution through its effect on cation exchange capacity (CEC) and thus on the potential level of exchangeable Al (Bell and Edwards, 1987). As weathering continues, the CEC of soils decreases as higher capacity minerals such as montmorillonite, vermiculite and illite are replaced by kaolinite and then by iron and aluminium oxides.

Therefore, the amount of Al has been found to decrease with intensity of weathering (Tessens and Shamsuddin, 1983) but Al saturation is often high in weathered soils such as Oxisols and Ultisols (Sanchez, 1976).

2.5.2. pH

Aluminium concentration is highly affected by the pH of the environment. This has been clearly demonstrated by Magistad (1925) who dissolved a defined quantity of $Al_2(SO_4)_3$ in a series of flasks, added different amounts of NaOH to bring the solution to desired pH levels and then measured the concentration of Al remaining in solution. He found that in the pH range of 4.7 - 7.5 the solubility of Al was quite low. This is the pH range where Al is precipitated and remains so as the relatively insoluble $Al(OH)_3$. Tanaka et al (1987) also reported that total Al concentration and the ratios of Al containing ions to the total Al in a solution vary, depending upon the pH. They reported that total Al concentration starts to decrease when the pH increases above about 4.0 and reaches below 1 ppm at about pH 4.8. A slight change in the pH of the environment changes the relative proportion of different Al species which in turn affects Al toxicity to plants. Figure 2.1 shows the relative distribution of different soluble Al species as a function of pH at an ionic strength of 0.1 M (after Marion et al, 1976). For any given exchange capacity, the actual amount of exchangeable Al is strongly influenced by pH and decreases rapidly to very low values at about pH 5.5 (Juo, 1977). In conventional liming practice aimed at raising soil pH, the beneficial effects of lime are largely related to the precipitation of phytotoxic levels of soluble and exchangeable Al (Haynes, 1982).

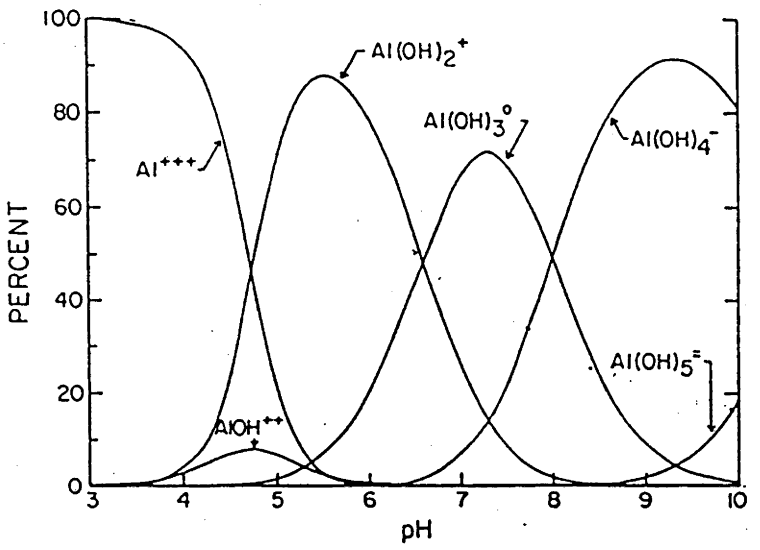

Figure 2.1. The relative distribution of the soluble Al species as a function of pH (Marion et al, 1976).

Although Al solubility is highly pH dependent, soil pH is a poor measure of Al toxicity and acid soil infertility (Adams and Lund, 1966; Richburg and Adams, 1970; Conyers et al, 1991). This is because increasing soil pH by liming may simultaneously ameliorate several other acid soil infertility factors. Therefore, there is no 'critical' pH value with respect to Al toxicity. However, McCormick and Amendola (1983) reported that for soils having similar parent materials and clay minerals, pH alone may be useful in predicting Al toxicity to a given plant.

In solutions with low OH : Al ratios, most of the Al occurs as monomeric Al species. Aluminium polymers are formed under certain conditions, particularly on addition of OH^- ions although a rapid equilibrium does not exist between monomeric and polymeric Al ions (Hsu, 1968). Wada and Wada (1980) found that the proportion of polymeric Al species in solution increased linearly from ~ 2% with an OH : Al ratio of 0, to ~ 95% when the OH : Al ratio was 2.7. Blamey et al (1983) suggested that the ameliorating effect of OH^- resulted from the

reduction in the concentration of monomeric Al in solution, either through polymerization or precipitation of Al. They further reported that Al polymers were formed with the additions of OH^- only when Al concentrations originally were less than 50 μM in solution but not at concentrations greater than 100 μM.

2.5.3. Presence of other ions

Toxicity of Al is affected by the presence of other ions in soil solution. There are many reports of alleviation of Al toxicity by the presence of P and Ca (P and Ca will be discussed in section 2.8). Recently Alva et al (1986 a) suggested that K, Mg or NH_4 also directly alleviated Al toxicity in soybean and subterranean clover.

Ions already present in the root medium, or changes in their concentration, may affect Al toxicity through effects on the ionic strength of the medium. When a salt or a fertilizer is added to soil, it increases ionic strength of the soil solution and thus decreases the activity coefficients of the ions already present in soil solution due to an increase in the ionic strength. Therefore, addition of salt and/or fertilizer to high Al soils will result in a decrease in the activity of Al. However, for soils having solution Al in equilibrium with exchangeable, structural or organic combination Al, a decrease in the ionic strength will affect the solubility of these forms of Al. For example, polymeric forms of Al may be transformed into monomeric forms at high ionic strength and may outweigh the decreasing effect in the activity of Al (Bell and Edwards, 1987). Blamey et al (1983) illustrated the importance of ionic strength as a factor affecting plant response to Al concentration. They reported that in a nutrient solution with ionic strengths less than 900 μM, the Al polymers formed, remained in solution, but the polymers were not detected in solutions of higher ionic strengths. Alva et al, (1986 a)

reported that the effect of Al toxicity on the root elongation of subterranean clover was better alleviated by Ca when ionic strength of the solution was raised. They added the required amounts of ionic strength adjusting' solution containing 1.25 M KNO_3, 0.5 M Mg $(NO_3)_2$. 6 H_2O and 0.25 M. NH_4NO_3 to raise the ionic strength of the nutrient solution to the same level as one of the high Al treatment levels.

2.5.4. Organic matter

The role of organic matter in ameliorating the toxic effect of soil acidity was recognised in very early research (Mattson and Hester, 1933) and similar results have been published during the last two decades (Evans and Kamprath, 1970; Reeve and Sumner, 1970; Hoyt, 1977; Bloom et al, 1979; Hargrove and Thomas, 1981).

Organic matter reduces the availability of Al in soil solution by forming Al organic matter complexes (Thomas, 1975) and this is the reason why plants grow more satisfactorily in organic soils at a considerably lower pH than on mineral soils. This is true in organic soils even when the total Al is quite high (Farina et al, 1982). Therefore, attempts had been made to correlate soil organic matter with lime requirements for acid soils. Keeney and Corey (1963) found organic matter to be significantly related to lime requirements (r = 0.62) while a function of a pH organic matter interaction defined as (pH 6.5 - soil pH) x (% organic matter) correlated even better with the lime requirement (r = 0.884).

The mechanism for the interaction of organic matter and Al toxicity was reported as the carboxyl groups. These include the main functional groups involved in cation exchange and in acidity in organic matter (Broadbent and Bradford, 1952). This was later confirmed by

Schnitzer and Skinner (1963) who found that Al would react with organic matter up to a 6 : 1 molar ratio indicating six carboxyl groups per organic matter molecule. Hue et al (1986) grouped carboxylic acids on the basis of their effectiveness as Al detoxifiers: (i) strong (citric, oxalic, tartaric); (ii) moderate (malic, malonic, salicylic); and (iii) weak (succinic, lactic, formic, acetic, phthalic). The Al detoxifying capacities of these acids were positively correlated with the relative position of OH/COOH groups on their main C chain; positions that favoured the formation of stable 5 or 6 bond ring structures with Al were least toxic.

There are reports of depressions in plant growth when organic rich acid soils were limed (Friesen et al, 1980; Hargrove and Thomas, 1981; Anandan et al, 1985). Such depressions were generally considered as a pH induced micronutrient deficiency. However, Farina et al (1982) found that the yield depression correlated with a simultaneous increase in Al uptake by maize. A positive relationship existed between organic matter content and growth response pattern: marked depression only occurred on highly organic soils. Hargrove (1986) has shown that the Al organic matter complex may be solubilized in the pH range of 5 to 7 and thereby become available to plant roots and subject to plant uptake. This may be considered as a likely mechanism for explaining Al uptake and plant growth depression at around neutral pH values.

2.6. Effects of soil acidity

2.6.1. Effects on nutrient uptake by plants

The most important infertility factor in acid soil is Al toxicity and its effects will be reviewed in section 2.7. The second most important growth limiting factor is Mn toxicity and it can occur at a slightly higher critical pH limits than does Al toxicity and in some cases it may be the dominant problem (Bromfield et al, 1983). In addition to soil pH, Mn toxicity in soil is determined by: the amount of easily reducible Mn present in soil, microbial activity and soil aeration. Therefore, it can occur even at a pH of 6.0 or above in poorly drained or compacted soils where reducing conditions bring divalent Mn into solution (Foy, 1983). Symptoms of Mn toxicity are confined to the plant tops whereas roots are more affected by Al toxicity (Foy, 1974). Plant species may vary markedly in their susceptibility to Mn (Robson and Loneragan, 1970; Culvenor, 1985). Soil acidity *per se* has marked effects on nutrient uptake. Excess H^+ ions affect root membrane permeability, compete with other cations for absorption sites and interfere with their uptake (Foy, 1984). Acid soils are usually deficient in Mo too. Molybdenum is strongly adsorbed on hydrous Fe oxides and with decreasing pH, adsorption increases reaching a maximum at pH 4.0 (Barrow, 1978).

2.6.2. Effects on microbial activity and nitrogen fixation

The activity of microorganisms is responsible for the decomposition of organic matter and this is one of the important mechanisms by which inorganic nitrogen is made available for plant growth. Microorganisms are sensitive to their environment and their activity can be severely curtailed at low pH or increased by liming (Alexander, 1977; Edmeades et al, 1981; Hojito et al, 1987). However, the magnitude of this lime

response depends on the initial pH, rate of liming and soil organic matter level (Singh and Beuchamp, 1986). Several researchers have suggested that lime controls the mineralization of organic matter and this is the mechanism for lime response in the field (Cullen and Grigg, 1971; Awad and Edwards, 1977; Sarathchandra and Edmeades, 1985). Soil acidity can limit biological fixation of atmospheric N. A wide range of temperate and tropical pasture legumes have been shown to grow well in acid conditions provided adequate N and Ca are supplied (Loneragan and Dowling, 1958; Munns, 1965; Andrew, 1976).

Edmeades et al (1981) attributed the increase in microbial activity to a change in pH whereas Nyborg and Hoyt (1978) concluded from their incubation and field studies that the mineralization of nitrogen is generally a temperature effect and nitrification was not statistically related to base saturation, soluble Fe or Al or Mn. Cook et al (1985) suggested that an increase in microbial activity after liming may result from an increase in the amount of water in the soil due to liming, since microbial activity is related to the moisture status of soil (Wilson and Griffin, 1975; Orchard and Cook, 1983). Sarathchandra and Upsdell (1981) also reported that liming with $Ca(OH)_2$ increased biomass C level and CO_2 evolution reflecting increases in biological activity in the soil.

2.6.3. Effects on plant growth

Whenever there is a change in pH of the plant root zone, many factors are changed simultaneously. Some of the changes may have no significant effect on plants, but many may be crucial depending on circumstances. The direct effects of the H^+ ion on plant growth are difficult to determine because of the other confounding factors in soil acidity. Howard and Adams (1965) found a drastic reduction in the growth rate of cotton taproots below pH 4.2. Lund (1970) found that H^+

was toxic to soybean taproots at pH 4.75 and below, when solution Ca was very low. With increasing Ca levels, H^+ toxicity was increasingly ameliorated. Foy (1984) considered that in most acid soils (pH < 4.0) , Al^{3+} and Mn^{2+} toxicities were more important than H^+ toxicity, particularly for non legumes. However, H^+ ion toxicity may restrict the survival and activity of *rhizobium* or of other soil microorganisms (Moore, 1974). Excess H^+ ions can affect root membrane permeability and thus interfere with nutrient uptake and transport. Foy (1984) reported a reduction in uptake of Ca, Mg, Mn, Zn, P and Cu due to excess H^+ ions.

2.7. Effects of aluminium

2.7.1. Effects on plant nutrients

Polymer Al species exert important influences on soil physical properties, particularly in maintaining the stability of soil aggregates. Hydroxy Al and Fe interlayers restrict the swelling of Na montmorillonite (El Rayah and Rowell, 1973) and in this respect, Al species are more effective than Fe. The tensile strength, liquid limit and shear stress are also significantly affected by hydroxy Al interlayers (Davey and Low, 1971). With an increase in H^+ levels in soil solution there is an increase in the solubility of Al which may occupy cation exchange sites. As the process continues, Al dominates the exchange positions replacing Ca and Mg and ultimately the soil becomes very low in base cations necessary to support plant growth (Kamprath and Foy, 1971). This process is crucial for the humid tropics where weathering processes have already resulted in a low CEC (Adams, 1981 b).

The chemistry of soil Al can play a dominant role in controlling both P solubility and its uptake by plants. At low pH, soil P availability is decreased through precipitation of Al, Fe and Mn

phosphates and through P adsorption onto the surfaces of hydrated Al and Fe oxides (Juo and Fox, 1977) and on the weathered edges of clay particles (Barrow, 1978). Liming acid soil often increases P uptake by plants by decreasing Al toxicity rather than increasing phosphate availability *per se* (Haynes and Ludecke, 1981). On the otherhand precipitation of exchangeable Al^{3+} as polymeric hydroxy Al cations, following liming, creates new highly active phosphate adsorbing surfaces (Haynes, 1982). Therefore, liming acid soil may even reduce P uptake by plants. To get rid of P adsorption by polymeric hydroxy Al cations, Haynes (1982) suggested drying of limed acid soil before P application so that crystallization of the amorphous hydroxy Al polymers may occur.

2.7.2. Effects on microbial activity and nitrogen fixation

Aluminium toxicity can restrict biological fixation of atmospheric nitrogen by limiting the growth of host plants (Jarvis and Hatch, 1985), the growth of *rhizobium* (Thornton and Davey, 1983; Wood et al, 1984; Wood and Cooper, 1988) and/or its nodulation (Carvalho et al, 1981, 1982 b; Murphy et al, 1984; Coventry et al, 1985; Jarvis and Hatch, 1986; Alva et al, 1987; Simpson et al, 1987). Nodule numbers may be reduced and/or nodulation may be delayed (Hartel and Alexander, 1983; Jarvis and Hatch, 1985; Kim et al, 1985 b; Alva et al, 1987). However, Al concentrations which have strong inhibitory effects on nodulation, were found to have no effect on the functioning of nodules in N fixation (Carvalho et al, 1982 a). Carvalho et al showed that N fixation by well nodulated plants of three *Stylosanthes* species was independent of solution Al concentration as high as 100 µM. Growth of *rhizobium* was markedly restricted by Al concentrations to as low as 10 µM (Thornton and Davey, 1983; Wood et al, 1984) or even lower (6.4 µM, Kim et al, 1985 a). It is now well documented that *rhizobium* is more sensitive to Al

toxicity than is the growth of host plants (Robson and Loneragan, 1970; Carvalho et al, 1982 b; Kim et al, 1985 a; Alva et al, 1987) although Munns et al (1981) reported that the susceptibility of the host plant to Al toxicity was the effective limitation to growth and nitrogen fixation. Carvalho et al (1980) reported that Al toxicity depressed growth more severely in six *Stylosanthes* which depended on N fixation for N supply than in those which received fertilizer N. In this case Al delayed nodulation in 5 out of 6 species, while it reduced the number and the dry weight of nodules in all species.

2.7.3. Effects on plant growth

There are a number of studies summarizing the effects of Al on plant growth (Table 2.2) where indicator crops were related to maximum Al level used and growth media. In most of these studies, the indicator plants were agricultural crops, but in a few, tree species were included. Most research has been carried out using plants grown in nutrient solution, in pots in glasshouse (using soil or sand), or field experiments. Some researchers used a combination of growing media to confirm findings between projects. Experiments vary widely in terms of the length of growth period for the plants. The period ranges from less than a week in some experiments (Kinraide and Parker 1987 - 2 days; Alva et al 1987 - 4 days; Lee and Foy 1986 - 3-15 days) to using existing trees in a forest in some studies (Joslin et al, 1988).

Table 2.2. Findings from previous studies related to Al toxicity and plant growth.

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Adams and Hathcock	1984	Cotton	2	0.24 cmol/ kg	Soil classification may not be helpful in predicting Al toxicity and Ca deficiency.
Adams and Lund	1966	Cotton	1, 2	7.3 me/ 100 gm	Subsurface Al in soil or nutrient solution significantly reduced root penetration.
Adams and Moore	1983	Cotton	2	2.26 me/ 100 gm	In an illuviated horizon soil solution Al of < 0.4 µM was toxic to cotton roots, but 9 - 134 µM Al was not toxic in an eluviated horizon.
Alva et al	1986(a)	Soybean & subclover	1	40 µM	Existence of a protective action of Ca against Al toxicity in case of soybean and subterranean clover.
Alva et al	1986(b)	Soybean, alfalfa, subclover, sunflower	1	16 µM	Evaluated different Al indices in terms of root length in nutrient solution. They concluded that $\Sigma a_{Al\ mono}$ was the best index of Al toxicity.
Alva et al	1986(d)	Soybean	1	40 µM	Relative root length of soybean in nutrient solution highly correlated with $\Sigma a_{Al\ mono}$ ($R^2 = 0.91$). Among the individual monomers relative root length highly correlated with calculated activity of $Al(OH)_2^+$ ($R^2 = 0.87$).
Alva et al	1987	Soybean	1	200 µM	Nodule formation in soybean and dry weight of nodule were much more sensitive to Al stress than the host plant.
Andrew et al	1973	Legumes (11) *	1	2 ppm	Eleven species of legume were studied for Al tolerance and chemical composition. Al tolerant species grew better with 0.5 ppm Al than control. Al reduced top/root weight ratio of sensitive species and Ca concentration in tops of all species.

Continued on next page

Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Baligar et al	1987	Red clover (23)	1	100 µM/l	The cultivars differed with respect to growth, uptake and mineral nutrient efficiency. Overall shoot nutrient contents showed an inverse relation with treatment Al level and shoot Al concentration.
Bilski and Foy	1987	Oat (11)	1,2,4	15 mg/l	Based on relative root yield, 11 oat cultivars significantly differed in Al tolerance. The order of tolerance was similar under field conditions and in nutrient solution.
Blamey et al	1983	Soybean	1	200 µM	Effects of Al concentration, OH:Al, P:Al ratios and ionic strength on soybean root length was studied. Soybean root elongation was reduced severely with Al concentration > 10 µM. The toxic effect was ameliorated by the addition of OH or P.
Bouma et al	1981	Lucerne, subclover	1,2	200 µM	In pot experiments, inverse relations were found between relative yield and leaf Al concentration.
Cambraia et al	1989	Sorghum (2)	1	5 ppm	Al reduced the nitrate uptake and had a direct effect on the nitrate reductase and consequently on nitrate reduction.
Cameron et al	1986	Barley	1	100 µM	Root elongation was correlated with Al^{3+} concentration but not with total soluble Al or Al complexes with F^- and SO_4^{2-}.
Carvalho et al	1980	Stylosanthes (6)	2	55 µM	Al toxicity restricted growth in 3 acid soils. Maximum yield was associated with reduction in Al to < 5% of the effective CEC.
Carvalho et al	1981	Stylosanthes (6)	1	125 µM	Al toxicity depressed growth more severely in plants dependent on N fixation than in those supplied with N.
Cate and Sukhai	1964	Rice	1,2	1000 ppm	In the absence of nutrient cations, water soluble Al (1-2 ppm) markedly inhibited growth of rice. Higher Al prevented growth altogether.

Continued on next page

Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Duncan et al	1983	Sorghum (8)	1,4	93 µM	The genotype most tolerant to acid soil field conditions was also the least affected by Al toxicity in nutrient solution.
Fageria et al	1989(a)	Rice (2)	1	2226 µM	Al reduced root and shoot growth. The effect was greater in roots. The uptake and use efficiency of P was highly correlated with the growth of rice plants.
Fageria et al	1989(b)	Alfalfa and bean	2	4.62 cmol/ kg	Root and shoot growth of both species was positively correlated with soil pH, exchangeable Ca and Mg and negatively correlated with exchangeable Al.
Foy and Brown	1964	Variety of plants (4)	1,2	6 ppm	Al tolerance was closely associated with the ability of plants to absorb and utilize P in the presence of excess Al.
Foy and Campbell	1984	Amaranthus (9)	2	80% Al saturation	In general, strains that were most tolerant to Al toxic soil were also among the most tolerant to the Mn toxic soil.
Foy et al	1987(a)	Barley (2)	1	110 µM	Al stress significantly reduced concentrations of organic acids in the roots of Al sensitive species but not in Al tolerant species.
Foy et al	1969	Soybean (2)	1	12 ppm	Al toxicity was associated with decrease in concentration of Ca in the tops and roots of both varieties, but this effect was much more pronounced in the Al sensitive variety.
Foy et al	1987 (b)	Oat (48)	2	4.95 cmol/ kg	Relative top yields (pH 4.3/pH 5.7 %) ranged from 34.2 to 13.1% and relative root yields from 50 to 16.9%.
Hai et al	1989	Rice	1	40 mg/l	Al stimulated dry matter production and nutrient uptake until a concentration threshold was reached. The sensitive cultivars tended to accumulate more Al than resistant ones. Effect of P on the alleviation of Al toxicity was slight.

Continued on next page

Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Haynes and Ludecke	1981	Legumes (2)	2	12.8 me/ 100 gm	Effect on secondary plus lateral roots was more than on the tap and primary lateral roots. Relative yield correlated with Al content of shoots. Nodulation increased on liming.
Hecht-Buchholz et al	1987	Spruce	1	1700 µM	With low nutrient supply, the plants were affected even at low Al concentration. Excess Al and Mn decreased Ca and Mg concentration in needles and roots.
Hetherington et al	1986	Sugarcane (34)	1,2	371 µM	In general sugarcane has a high tolerance to Al. A 50% reduction in root length of the varieties occurred with 10 to 160 µM Al in solution.
Horst	1987	Cowpea (55)	1	185 µM	Increasing Ca supply completely eliminated inhibition of root elongation by Al and increasing Al supply depressed Ca concentration in all root tips. In general there was a positive relationship between Al tolerance and Ca efficiency.
Huett and Menary	1980	Cabbage, lettuce and kikuyu grass	1	0.11 mM	Al treatment decreased uptake of cations by tops and roots of all species. Higher Ca treatments reduced Al concentrations in tops for all species.
Humphreys and Truman	1964	Pine	1	20 ppm	High Al^{3+} in the growing medium also required higher P to attain good growth of primary roots in nutrient solution.
Jarvis and Hatch	1986	White clover	1	100 µM	Al in nutrient solution resulted in a reduction in root extension. Uptake and transport of P and NO_3 was also affected. Presence of Al in nutrient solution prevented nodulation.
Joslin and Wolfe	1988	Spruce	2	1650 µM	Soil Al was the major cause of biomass reduction. Soil solution inorganic monomeric Al and total Al were superior predictors of root biomass.

Continued on next page

Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Joslin et al	1988	Spruce (2)	4	372 µM	Root Al was much higher than foliage, fine roots from B horizons had the highest Al and were a better indicator of toxic Al. High Al in soil resulted in low Ca and Mg uptake.
Keltjens and van Loenen	1989	Tree species (5)	1	30 mg/l	The species were highly tolerant to Al up to the highest Al level (30 mg/l). Al did not reduce nutrient concentration or the Ca/Al ratio below the critical level.
Kim et al	1985(a)	Subclover	1	49.5 µM	*Rhizobium* survival and nodulation was more sensitive to Al than the host plant.
Kinraide and Parker	1987	Wheat	1	35 µM	Competition between the cations and Al for external binding sites may account for most of the amelioration.
Konishi et al	1985	Tea	1	6.4 mM	Maximum growth of roots and shoots occurred with 0.4 mM Al in the presence of 0.1 mM P and with 1.6 mM Al in the presence of 0.8 mM P.
Krizek and Foy	1988	Barley (2)	2	High Al	Drought exacerbated the stress effects of Al in plants. Increases in soil moisture level slightly reduced stress effects of Al.
Lance and Pearson	1969	Cotton	2	0.30 ppm	Uptake of nutrients was reduced in seedlings by Al. Inhibition of Ca uptake was reduced by increasing Ca in the nutrient solution.
Lee and Foy	1986	Snapbean (2)	1	8 mg/l	The Al tolerant cultivar has a higher potential for Al chelation and detoxification than does the Al sensitive cultivar.
Lee and Pritchard	1984	White clover	1	1180 µM	Uptake of divalent cations was inhibited but Al enhanced the concentration of K and N in both leaves and roots.
Manrique	1987	Cassava	4	6.5 cmol/kg	Early growth stages were more sensitive to Al.
McCormick and Steiner	1978	Tree species (11)	1	280 ppm	Some tree species were tolerant to as high as 80 - 160 ppm Al.

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Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
McLaughlin and James	1989	Wheat (4)	1,2	200 µM	Increased supply of P at surface enhanced subsurface root growth, only in the absence of Al.
Miranda and Rowell	1987	Wheat (2)	2	49% saturation	Addition of P to the topsoil caused good growth regardless of subsoil acidity. Root growth increased in both layers and P^{32} taken up from the topsoil was translocated to roots in the subsoil.
Mullette	1975	Eucalypt	3	10 ppm	Low Al stimulated eucalypt growth independent of P concentration.
Munns	1965	Lucerne and alfalfa	1	200 ppm	Al toxicity depressed yields, root elongation and Ca and P concentration in shoots and roots. P alleviated Al toxicity only when added P caused Al to precipitate.
Murphy et al.	1984	Tropical legumes (4)	1	125 µM	Solutions containing > 25 µM Al delayed nodule appearance and reduced the percentage of plants nodulated and dry weights of nodules produced by all four legumes.
Paganelli et al	1987	Pine	3	80 mg/l	Root regeneration potential was found to be very sensitive to Al.
Rhue and Grogan	1977	Corn	1	0.25 mM	Calcium and Mg equally protected seedlings from Al toxicity.
Ryan et al.	1986	Conifers (3)	1	175 ppm	All conifers were tolerant of the acid solutions and high levels of Al.
Sangalang and Bouwkamp	1988	Sweet potato (379)	3,4	2000 µM	The clones which were tolerant to Al toxicity had greater relative root length.
Schier	1985	Red spruce and balsam fir	1	200 mg/l	Small reductions in shoot growth were observed and symptoms in root growth were well developed.
Shuman et al	1990	Sorghum	2	1.45 cmol/ kg	Correlation between plant growth and soil Al levels was significant for top soils but nonsignificant for subsoils. Soil solution Al^{3+} activity was the best predictor of Al toxicity.

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Table 2.2. Continued

Author	Year	Indicator crop	Growing medium **	Max. Al level	Main finding/conclusion
Smit et al	1987 a	Douglas fir	4	Not mentioned	At very low pH, root length was correlated with Ca/(Ca + Al). At higher soil pH, parameters other than exchangeable Al and Ca were more important in determining total root length.
Thornton et al	1986	Honeylocust	1	1500 $mmolm^{-3}$	Root Al was 50 - 100 times that of shoots. Low Al increased Ca, Mg and P concentration in shoots but higher Al levels reduced plant nutrient contents.
Truman et al.	1986	Pine	1	0.35 me/l	Al treatments increased uptake of P and K and decreased uptake of Ca and Mg. Higher P increased root Al but decreased shoot Al.
Webber et al.	1982	Barley	2	15.3 me/ 100 gm	Percent base saturation correlated better with yield than solution Al, exchangeable Al and pH.
White	1976	Lucerne	1	50 µM	Uptake of Al was higher at pH 5 than at 4.5 although Al was less toxic at pH 5. Also, P/Al was critical.
Wright and Wright	1987	Subclover	2	6.86 cmol/ kg	Exchangeable Ca, Ca saturation and solution Ca correlated positively and solution Al correlated negatively with the growth of snapbean. Interaction between Ca and Al affected both growth and uptake of Ca. Authors suggest consideration of both Ca and Al in studying Al toxicity.
Wright et al	1987	Snapbean (2)	2	7.5 cmol/kg	Root and shoot growth was negatively correlated with soil and soil solution Al and positively correlated with soil pH and measures of soil and soil solution Ca. They found $\Sigma a\ Al_{mono}$ to be a better predictor of root growth than any individual Al species.

* Number in parentheses indicate total number of species/cultivars used in the study if more than one.

** 1, 2, 3 and 4 in this column indicate different growth medium: 1 = Nutrient solution, 2 = Pots using soil, 3 = Pots using sand, and 4 = Field experiments.

The fact that Al toxicity affects plant growth adversely is well documented (Table 2.2) although there are a few reports of beneficial effects of Al on plant growth and these will be discussed later in this Section. According to the existing literature, the net effect of Al on plant growth can be discussed in terms of:

(a) effects on roots,

(b) effects on nutrient uptake and plant growth, and

(c) beneficial effects of Al.

These will be discussed separately despite the fact that it may be argued that effects on nutrient uptake and plant growth may be manifestations of effects on roots.

Effects on roots

Symptoms of Al toxicity are first and most acutely observed in roots. Many plant growth studies related to soil acidity/Al toxicity have dealt with the study of roots (e.g.; Simpson et al, 1977; Pinkerton and Simpson, 1981; Blamey et al, 1983; Lee and Pritchard 1984; Cameron et al, 1986; Hetherington et al, 1986; Smit et al 1987 a; Wagatsuma et al 1987; Joslin and Wolfe, 1988; Aitken et al, 1990). The roots of Al affected plants are characteristically stubby and thick in appearance. The root tips and lateral roots may become thickened and turn brown. In addition, the whole root system may develop a coralloid appearance with many thickened lateral roots and lacking in fine branching (Bell and Edwards, 1987). Various characteristics of roots such as total root length, root elongation, root branching, fine root length, root regeneration potential etc have been taken into consideration. Whatever the measure of root growth used, less roots are produced as a result of Al toxicity and less

volume of soil will be exploited by the plants under Al toxic conditions thereby resulting in a decrease in the uptake of water and nutrients.

Aluminium toxicity curtails root elongation (Adams and Lund, 1966; Blamey et al, 1983; Alva et al, 1986 d; Cameron et al, 1986), and the tip of the roots where cell division and elongation growth are localized, is the region most affected. Cotton seedling root penetration was significantly reduced by subsurface Al in strongly acid soil or in nutrient solution (Adams and Lund, 1966). Jarvis and Hatch (1986) reported that high Al levels in the nutrient solution resulted in a reduction in root extension of white clover. Hetherington et al (1986) exposed 9 cultivars of sugarcane which differed in Al tolerance, to a range of Al concentrations and found that a 50% reduction in root length occurred with 10 to 160 μM Al in solution. Adams and Moore (1983) reported that Al in the illuviated horizon was more toxic to cotton root penetration than were higher amounts of Al in eluviated horizons. They assumed that this contradiction was due to Al chelation in eluviated horizons with organic matter rendering them less harmful to plants. Adams and Pearson (1970) compared the effects of subsoil acidity on root penetration in cotton and peanut. Cotton root growth was almost completely inhibited by subsoil acidity but no apparent detrimental effect on peanut roots were observed. To explain these results, they noted that cotton roots created a more acid root environment in the nutrient solution and that the peanut had a greater propensity for preferential absorption of lower valency ions to the exclusion of higher valency ions.

Alva et al (1986 d) studied the relationships between root length of soybean and the calculated activities of Al monomers in nutrient solution. By varying the amounts of Al at different pH's, they

established a range of different Al monomers. Relative root length of soybean was highly correlated with $\Sigma a_{Al\ mono}$ ($R^2 = 0.91$). Also, among the individual monomers, relative root length was highly correlated with the calculated activity of $Al(OH)_2^+$ ($R^2 = 0.87$). Alva et al (1986 b) proposed that $\Sigma a_{Al\ mono}$ was the best index of Al toxicity in terms of root length of soybean, alfalfa, subterranean clover and sunflower, all grown in nutrient solution and under a wide range of other characteristics. Wright and Wright (1987) assessed soil acidity related limitations to growth in limed and unlimed acid soil. They found that $\Sigma a_{Al\ mono}$ was a better predictor of root growth than any individual Al species. Cameron et al (1986) reported that root elongation of barley correlated (negatively) with Al^{3+} concentration but not with total soluble Al or Al complexes with F^- and SO_4^{2-}.

Cate and Sukhai (1964) reported that in the absence of nutrient solution, water soluble Al as low as 1 - 2 ppm markedly inhibited the growth of rice roots. Higher concentrations prevented root growth altogether. Similar effects of Al on growth of Norway spruce seedlings under low nutrient supply was reported by Hecht-Buchholz et al (1987). Joslin and Wolfe (1989) reported that fine root branching in red oak seedlings in a glasshouse study was more sensitive than either root biomass production or root elongation to levels of soil Al. Paganelli et al (1987) in their experiment with sensitivity of loblolly pine to Al used another measure of root growth: 'root regeneration potential' (RRP), which is the total length of new roots greater than 2 mm in length produced over a specific period of time. They found that both total number and total length of new white roots produced declined with as little as 5 mg Al per litre of solution.

Pinkerton and Simpson (1981) assessed the root and shoot growth of four tropical and two temperate summer legumes in deep acid soil profiles. They found that liming resulted in a larger and more immediate effect on root growth, particularly on fine root length, as compared to the effect on shoot growth. They suggested that the ratio of fine root length to shoot weight is a better indicator of tolerance to subsoil acidity. The effects of differing levels of soil acidity and P deficiency on root growth and P absorption by Townsville Stylo and Desmodium Greenleaf were studied in acutely P deficient soil in columns by Pinkerton and Simpson (1983). The species differed in their response to lime and P and there was little response to lime by either species at low P rates. At the highest P rate, there was a large interaction between lime and P placement for Desmodium Greenleaf. McLaughlin and James (1989) reported that subsurface Al affected the growth and uptake of surface applied P by wheat seedlings in a split root sand/solution culture experiment. They found that increased supply of P on the surface enhanced subsurface root growth, only in the absence of Al. In contrast to this study, Miranda and Rowell (1987) reported that the addition of P to topsoil caused good growth of wheat regardless of subsoil acidity: root growth increased in both layers and P (labelled with ^{32}P) taken up from the topsoil was translocated to roots in the subsoil. They explained that the translocated P inactivated the root Al and allowed the roots to grow and take up more P from the acid subsoil despite a reduction in inflow. Shuman et al (1990) also reported a negative correlation between sorghum root weight and soil Al levels which was significant for topsoils but nonsignificant for subsoils.

Attempts were also made to find relationship between root growth of Al stressed seedlings and effects on root morphology. Fleming and Foy (1968) reported that differential Al tolerance of two wheat

varieties was associated with morphological damage to root tips and lateral roots. Morphological abnormalities in onion root systems (measured over a period of 24 hours) treated with Al were explained by an inhibitory effect of Al on either cell division or cell extension (Clarkson, 1965). He concluded that cell division was highly sensitive to Al. Similar results were also reported for snapbean and cotton roots (Naidoo et al, 1978). They further reported that Al and P coprecipitated on or in the outer cells of the root cap and major elements detected in spot analysis of nuclei, cytoplasm and cell walls were Al, P, S and Ca. Matsumoto et al (1976) reported that pea root elongation was considerably inhibited by $Al^{3+} > 10^{-4}M$ and absorbed Al^{3+} in roots was localized in the epidermis and region where cell division is active (e.g., root tips) as revealed under microscope and electromicroprobe X-ray analysis (EMX). Wagatsuma et al (1987 a) reported that cell damage occurred only in the epidermis in the Al tolerant oat plants, epidermis and outer cortex in the Al sensitive maize plants, and epidermis and almost all of the cortex in barley plants highly sensitive to Al. In very Al sensitive species, cells at the root tip develop necrotic patches and in young roots the root tip may die back completely as reported by Edwards et al (1976) for peach seedlings and Thornton et al (1986) for loblolly pine seedlings.

Effects on nutrient uptake and plant growth

Presence of Al in the root environment and its uptake by roots tends to reduce the concentration of other mineral nutrients especially Ca, Mg and P in the plant (Foy et al, 1978). Andrew et al (1973) studied the growth and chemical composition of legumes in nutrient solution with various levels of Al. Aluminium treatments reduced the Ca concentration in the tops of all species. The effects of Al on growth,

uptake and mineral nutrient efficiency ratios (defined as dry shoot weight/element in shoot) were investigated by Baligar et al (1987) on 23 barley cultivars. The cultivars differed with respect to shoot and root weights. Overall shoot contents of Ca, Mg, Mn, Fe, P, S, K and Na showed significant positive correlations with shoot and root dry weight and an inverse relation with treatment Al levels and shoot Al concentration. Except for Ca, Mg and Mn, overall inverse relationships were observed between levels of Al and efficiency ratios.

Fageria et al (1989 a) demonstrated reduced root and shoot growth in rice due to high Al levels. The effect was greater in roots while the uptake and P use efficiency was highly correlated with the growth of the rice plant. Fageria et al (1989 b) studied growth and nutrient utilization of alfalfa and bean. Bean plants were far less sensitive to acidity than alfalfa. Root and shoot growth of both species were negatively correlated with exchangeable Al. Bean was more efficient than alfalfa in taking up Ca and Mg. Foy and Brown (1964) studied Al tolerance in 4 different plant species grown in nutrient solution or soil. The activity of Al was controlled by adding a chelating agent to the nutrient solution and lime to the soil. Al tolerance was closely associated with the ability of plants to absorb and utilize P in the presence of excess Al.

Oat cultivars differed significantly in tolerance of acid soil (Foy et al, 1987 b). The sensitive cultivars tended to accumulate higher concentrations of P, Al and Fe and lower concentration of K and Mn. Hai et al (1989) also reported that an Al tolerant rice cultivar accumulated more Al than the resistant cultivar. Al tolerance of two soybean varieties were studied by Foy et al (1969). Al toxicity was associated with a decrease in concentrations of Ca in the tops and roots of both varieties, but this

effect was much more pronounced in the Al sensitive variety. Al interfered in the uptake and use of Ca to different degrees.

On the basis of the Al level in soil solution, toxicity effects of Al restricted growth in six *Stylosanthes* species (Carvalho et al, 1980). Maximum yield was associated with a reduction in Al saturation to less than 5% of the effective CEC. Joslin and Wolfe (1988) suspected Al was the major cause of biomass reduction in red spruce and also found that soil parameters of Al and biomass response had a good correlation. Haynes and Ludecke (1981) reported that decreasing the Al toxicity by liming resulted in substantial increases in shoot yield of two legumes but the increase in root yield was small. Relative yield of both species significantly correlated with the Al content of shoots. Compared to limed soil, plants grown in unlimed soil had a greater percentage of P, Al, Mn and N accumulated in roots of both species.

Huett and Menary (1980) reported the uptake of less cations in tops and roots of cabbage, lettuce and kikuyu grass from nutrient solution with high Al treatments. Hecht-Buchholz et al (1987) reported that excess Al and Mn decreased Ca and Mg concentration in spruce needles and roots. Joslin et al (1988) also reported that high Al in soil resulted in low Ca and Mg uptake in spruce. Root Al was much higher than in foliage, while the roots from the B horizon had the highest Al and such measurement was a better indicator of Al. Thornton et al (1986) reported that concentrations of Al in the roots were 50 to 100 times higher than that in shoots of honeylocust. Low levels of Al increased Ca, Mg and P concentrations in shoots while higher Al levels reduced plant nutrient contents.

Duncan et al (1983) reported that in sorghum the most tolerant plants to acid soil field stress were also the least affected by Al toxicity in

nutrient solutions. Keltjens and Loenen (1989) reported that 5 tree species grown in nutrient solution were highly tolerant to Al up to the highest Al levels (30 mg/l). The species differed in their mineral uptake but in none did Al reduce other nutrient concentrations or the Ca/Al ratio to values below a critical level.

Cambraia et al (1989) reported that Al reduced the nitrate uptake and had a direct effect on nitrate reductase and consequently on nitrate reduction. A correlation between nitrate reductase tolerance and plant tolerance to Al was observed. However, the soil solution concentration of Ca in many acid soils is sufficient for plant growth under Al free conditions (Kamprath, 1978).

Beneficial effects of Al

Aluminium is generally considered to be phytotoxic to plants. But various claims have been made for its beneficial effects when it is present or applied at low levels. Macleod and Jackson (1965) reported that Al concentrations of 0.1 to 0.2 mg/l in nutrient solution increased the growth of alfalfa and red clover seedlings. Ryan et al (1986) reported that Douglas fir and western red cedar displayed similar or better growth in nutrient solutions containing 175 mg/l Al than in solutions without Al at pH 3.0. Mclean and Gilbert (1927) reported that 3 to 13 mg/l Al stimulated plant growth but higher concentrations were toxic. Truman et al (1986) observed an increase in root and shoot P in *Pinus radiata* with an increase in solution Al level. The beneficial effects of low levels of Al (usually < 20 mg/l) have also been attributed to facilitated uptake of Al-P complexes (Humphrey and Truman, 1964; Bartlett and Riego, 1972; Mullette, 1975). Stimulatory effects of Al (1.6 mM) on the growth of tea plants in the presence of 0.8 mM of P were reported by Konishi et al

(1985). They concluded that Al plays a regulatory role in the effective absorption and utilization of P.

The effect of Al concentration on the uptake of mineral nutrients by rice plants from dilute nutrient solution was studied by Hai et al (1989). Aluminium stimulated dry matter production and nutrient uptake below a threshold. The value of this threshold was influenced by the nutrient solution composition and the cultivar studied. Foy (1974) suggested increased Fe solubility and availability in the growth medium resulted from Al hydrolysis and suggested therefore, that a lower pH benefits plant growth.

2.8. Role of Calcium and Phosphorus in alleviating Aluminium toxicity

There have been many reports describing a decrease in Al toxicity induced by high levels of other elements. Calcium and P are the most important elements with a strong capacity for alleviating Al toxicity. Calcium performs an essential role in maintaining selective ion absorption by roots (Epstein, 1961). Aluminium uptake by roots is passive and the initial process involves exchanging Ca from free space (Huett and Menary, 1979). Therefore, a high Ca treatment is likely to reduce Al transport to the stele of plant roots.

Lance and Pearson (1969) reported an Al induced inhibition of Ca uptake from nutrient solution was avoided by increasing Ca concentrations in the nutrient solution. Huett and Menary (1980) reported that a higher Ca treatment reduced Al concentrations in tops of cabbage, lettuce and kikuyu grass. Horst (1987) reported that increasing the Al supply depressed the Ca concentration in all root tips in cowpea seedlings and an increasing Ca supply completely eliminated inhibition of root elongation by Al. In general, there is a positive relationship

between Al tolerance and Ca efficiency. Rhue and Grogan (1977) reported that Ca and Mg equally protected seedlings from Al toxicity and they used this criteria to screen corn for Al toxicity. An increase in Ca concentration in acid soil was reported to decrease Al toxicity (Munns, 1965; Rhue and Grogan, 1977). Alva et al (1986 c) reported that increasing the Ca concentration over a range of 500 - 15,000 μM substantially decreased Al toxicity in different plants. They viewed the protection of roots by Ca against Al toxicity to be a 'partial exclusion of Al by Ca'.

The amelioration of Al toxicity by Ca application has also been analysed as the relationship Ca/Al (or other forms of a ratio between the two) and plant growth. From their limited data Wright and Wright (1987) suggested that $a_{Ca^{2+}}/\Sigma a_{Al\ mono}$ could be a promising predictor of Al toxicity and suggested additional measurements of root growth covering more values of Ca/Al. Smit et al (1987 a) reported that at very low pH, root length was correlated with Ca/(Ca + Al), but at a higher soil pH, exchangeable Al and exchangeable Ca were not good predictors in determining total root length. Recently the counteracting effect of Al and Ca has been analysed in terms of a Ca - Al balance (CAB) (Noble et al, 1988). There have been various criticisms of the theoretical validity of the CAB (Kinraide and Parker, 1989; Grauer and Horst, 1991) and very recently Noble and coworkers (Fey et al, 1991) accepted some criticism and produced a new formulation which indicates that this concept is not yet widely accepted.

There are also reports where Ca did not alleviate Al toxicity. Horsnell (1985) reported that the addition of $CaSO_4$ increased the Al concentration in soil solution and reduced the plant growth. However, when $CaSO_4$ salt was used to increase the Ca status of soil it also lowered the soil pH, increased the Mn concentration, and EC and Al levels did

not fall as much as they did with the usual lime treatments. Due to an increase in ionic strength and the lowering of pH, monomeric Al concentration may even be increased in the system (Marion et al, 1976). Rios and Pearson (1964) reported that increasing the Ca concentration to 5000 μM did not overcome the toxic effects of 19 μM Al on cotton root weights. Clarkson and Sanderson (1971) found that 15000 μM Ca did not alleviate the toxic effects of 50 and 150 μM Al on barley root growth. There are probably other reasons for the conflicting effects of Ca on Al toxicity such as: use of applied nominal Al as a measure of Al concentration, the variety of plants grown under different conditions and locations, the differing nutrient solution composition, the different growth parameters measured, and the adjustment of nutrient solution pH and thereby the concentration of monomeric Al (Marion et al, 1976).

Humphreys and Truman (1964) reported that high Al in the nutrient solution also required higher P to attain good growth of *Pinus radiata* seedlings which indirectly means P has an ameliorating effect against Al at low concentration. Bartlett and Riego (1972) considered P to serve a double function in acid soils as:

(i) an essential nutrient and

(ii) a precipitator of Al.

Hsu (1968) found that the addition of phosphate to an Al solution formed soluble complexes with monomeric Al species and precipitated polymeric Al species. Blamey et al (1983) reported that the ameliorating effect of P resulted from the reduction in the concentration of monomeric Al in solution, either through polymerization or precipitation. Alva et al (1986 c) observed that with an increasing P/Al molar ratio, concentrations of total and monomeric Al decreased and root elongation of different crops increased.

Research on the effect of P in alleviating Al toxicity has given divergent results. Hai et al (1989) reported that the alleviating effect of P on Al toxicity was slight and Munns (1965) reported that depressing the effect of Al toxicity in alfalfa and lucerne could not be remedied by increasing the P supply, until the added P actually caused Al to precipitate. Other studies on the interaction between Al and P in influencing plant growth have been mentioned (Section 2.7.3) and they also support the different interactions between Al and P for different crops.

With regard to cation amelioration of Al toxicity in wheat, Kinraide and Parker (1987) speculated that competition between the cation and Al for external binding sites on the roots may account for most of the amelioration. Lee and Foy (1986) determined changes in organic acid concentration of Al tolerant and Al sensitive cultivars in nutrient solution. Results indicated that the Al tolerant cultivar has a higher potential for Al chelation and detoxification than does the Al sensitive cultivar. Therefore, they suggested that an Al chelation mechanism may be involved in differential Al tolerance within the species.

2.9. Soil water as a factor in the response to lime

Liming is the usual practice prescribed for the amelioration of soil acidity and like all other experiments related to plant growth, sufficient water is usually made available for liming experiments. Water is seldom used as a variable. In fact, the dissolution of lime and the movement of Ca^{2+} (and Mg^{2+}) down into the subsoil is dependent on the presence of water. Neutralization of acid subsoils and the rate of neutralization is strongly dependent on the rate of dissolution and hydrolysis of added lime to enable the formation of OH^- ions (Adams, 1981 b). This is further

governed by various reactions such as acid-base equilibria, complexation with organic and inorganic ligands, oxidation-reduction and ion exchange adsorption (Mattigod et al, 1981). At any instant, Al concentration will, therefore, depend on the rate at which these reactions occur and the rate of biological uptake. Although it is quite obvious that the presence of water is essential for the action of lime on soil and plant growth there is no definitive data on the specific amount and distribution of water required for such action. Simpson et al (1979) observed root and shoot response in lucerne to liming in acid soil at a soil moisture level of pF 2 to 2.5. In a pot experiment carried out with high Al soil, Horsnell (1984) found an increase of 50% in the dry weight of clover tops when lime was added to soil at a low moisture (70% field capacity) level but very little response was observed when lime was applied at 100% field capacity. Growth of roots was not considered in this experiment. Similarly a pasture response to lime was reported in New Zealand by Shannon et al (1984) who found the largest relative responses to liming in summer and/or autumn and the smallest responses or even a depression in spring. Rowe (1982) reported similar results from Tasmania. Further, Simpson et al (1987) reported a decline in numbers of *Rhizobium trifoli* in limed acid soil during the dry summer period. This phenomena was termed 'the Seasonal Pattern' to lime response. Very few researchers have investigated this aspect of soil acidity and liming in detail although a few explanations have been put forward.

(i) An increase in soil moisture content due to liming was suggested to be a possible cause of this seasonal pattern (Thomson, 1982; During et al, 1984).

(ii) Available phosphate accumulated in the soil during the winter period of slow growth and this is then better able to support the burst of spring growth (Scott and Cullen, 1965).

(iii) The high phosphorus status of soil in spring is due to release of phosphate from organic residues and soil organic matter (Saunders and Metson, 1971).

(iv) And by alleviating Al toxicity, liming increases rooting volume which results in better plant growth during times of moisture stress (Shannon et al, 1984).

2.10. Conclusions

Complex acid soil factors affect the growth of higher plants either independently or often together. They may also promote or inhibit the survival and functions of microorganisms in soil. It is now well known that Al toxicity severely affects root growth while total plant growth is also reduced. The latter is assumed to be due to less roots and thereby the exploitation of less soil for nutrients and water. However, there is no report yet available as to whether morphological abnormalities of Al effected root systems cause a loss in root efficiency in the absorption of plant nutrients or simply whether the lower volume of root is the main cause of poor plant growth. As a result of Al toxicity, there is a reduction in the amount of mineral nutrients absorbed and this is well established. It is difficult to separate the effects of Al toxicity and Ca deficiency because high levels of Al are usually associated with low levels of Ca in acid soils, and reliable diagnostic techniques particularly for assessing phytotoxicities of Al, which apply across a range of soils, are not available (Adams and Lund, 1966; Carvalho et al, 1980).

Studies on the effect of Al toxicity on plants have individually covered different aspects of root and shoot growth and mineral nutrient absorption. Aluminium toxicity has also been examined in relation to Al quantity in the growing medium relative to other elements such as Ca and P. Concentrations of Al relative to Ca in plants have also been

considered. However, it is difficult to come to a conclusion regarding the whole process of Al toxicity for a particular species from the same study. A comprehensive study of more aspects of the effects of Al on the same species may reveal more insights into Al toxicity effects.

The interrelationship between Al and Ca has been widely studied. The problem related to low Ca is undoubtedly a major problem associated with Al toxicity, but for a comprehensive treatment of the problem, other important factors which usually pose infertility problems in Al toxic soils need to be identified and studied. The other important factor in relation to Al toxicity which has received inadequate attention in past research is the influence of P. Some studies looked at the ameliorating effect of P on the adverse effects of Al toxicity on plant growth. But the role of P needs to be analysed in association with Ca, both of which are common deficiency problems in many acid soils such as the soil used for this study.

Indirect evidence (e.g., effects of season of application) suggests that important interactions occurred between soil moisture levels and acidity problems. Therefore, direct and detailed study of this interaction and its effect on different aspects of plant growth is important.

Most research related to soil acidity and Al toxicity has been carried out with agricultural crops. Very little information is available on the effect of soil acidity and Al toxicity on tree species. There are some limited reports on the growth of native eucalypts under acid conditions and changes consequent upon liming. But no such specific report is available on Al tolerance/sensitivity nor its alleviation by Ca or P addition. This oversight may be due to the fact that tree species are considered to be more tolerant to high levels of Al when compared to agricultural crops. This thesis, therefore analyses the acidity problem and

specifically the Al toxicity problem taking into account interrelationships with Ca, P and moisture levels. These analyses are based on a tree species widely grown in Australia and other tropical countries —— *Eucalyptus camaldulensis*.

CHAPTER 3

GENERAL METHODS

3. GENERAL METHODS

In this Chapter only general aspects of materials and methods will be described. Materials and methods specific to particular Chapters will be described therein.

3.1. Soil selection

The soil selection for the preliminary experiments is described in Section 4.2; acid soils were collected from sites 1, 2, 3, 4, 5, 6, 7 and 8 of the Cotter catchment area (Figure 4.1 presented on P 78) as identified by Talsma (1983). From these, three sites were selected which will be described in detail in Section 4.2. Later an acid soil even higher in exchangeable Al and lower in exchangeable Ca than the previous soils was selected (Section 4.3). For this purpose, 14 soil samples from 6 new sites in the Cotter catchment area and 7 samples from three sites from compartment 152 of the Uriarra forest were previewed. One site from compartment 152 of the Uriarra forest was finally selected (Section 4.3).

3.2. Soil collection and processing

Soils were collected from carefully examined sites and depths in polythene bags. If the field moisture content was high (much more than about 20%) then the soil was air dried in the shade to about 20% moisture content before being sieved. Soil samples used for site selection were passed through a 2 mm sieve, while soil collected for plant growth experiments was sieved through 5 mm sieves. Following sieving, soils were mixed thoroughly either in a small cement mixer when bulk collection was made or by hand for smaller quantities. After sieving and mixing, soils for chemical analysis were stored in a cold room until the completion of analyses. All analyses on soil were carried out at about

20% moisture conditions and then results expressed on an oven dried basis after oven drying a sub sample taken at the time of analysis. Soils used in plant growth experiments were stored in open heavy duty polythene bags in a cool, dry place.

3.3. Soil analysis

3.3.1. General

pH

Soil pH was measured both in water (pH_w) and in 0.01 M $CaCl_2$ solution (pH_s) with a glass electrode pH meter (Anax) after 30 minutes of shaking. The sample was made up at a soil : water or salt solution ratio of 1 : 5.

Electrical conductivity

Electrical conductivity (EC) (mS/cm) was measured using a conductivity meter (Radiometer, CDM 3). For soils, the measurement was made on the same extract as for pH_w (i.e., at a soil : water ratio of 1 : 5). For soil solutions, EC was measured on a 1 : 1 extract.

Particle size analysis

Particle size distribution was determined by the hydrometer method (Bouyoucos, 1928).

Organic matter

Soil organic matter was determined by a chromic acid digestion method which is based on spontaneous heating by dilution of H_2SO_4 (Walkley and Black, 1934).

Field capacity

Field capacity was determined by packing soil above gravels in a large cylinder. The soil surface was flooded by adding water and then allowed to drain freely for 48 hours while its surface was kept covered to check evaporation. The moisture content was determined as the field capacity (Wilde et al 1979). Moisture content at 0.1 bar was also determined, but only for compartment 152 soil using a pressure plate. The moisture content thus obtained was lower (27.7%) when compared to the field capacity determined (46.6%) for the same soil.

3.3.2. Extraction methods

Extractable cations

For the initial soil selection, exchangeable cations were extracted by shaking the soil with 1M KCl. Three extractants were compared for determining exchangeable Ca, Al, Mn, Mg, K and Na for compartment 152 soils. These were: 1M KCl, 1M NH_4Cl and 0.1M $BaCl_2$. Values for Ca, Al, Mn, Mg and K were not significantly different between those obtained with 1M KCl and 1M NH_4Cl although 0.1M $BaCl_2$ extracted significantly less Al and Mn than the other two. The amount of Na extracted was significantly different for each extractant (Figure 3.1, for display values of cations were multiplied by factors). Since 1M NH_4Cl extract has the advantage of allowing K measurement from the same extract it was decided to use it for soil analysis for the remaining study. Recently Shuman and Duncan (1990) also reported that 1M KCl and 1M NH_4Cl compared favourably in extracting Al from 248 soil samples. Slightly more Al was extracted by 1M NH_4Cl which is also true in the present case. Finally soil samples were shaken for 1 hour with 1M NH_4Cl at a soil to solution ratio of 1 to 20 and then the suspension was filtered

through Whatman No 42 filter paper. Different cations were estimated from the filtrate by methods described in the Section 3.3.3.

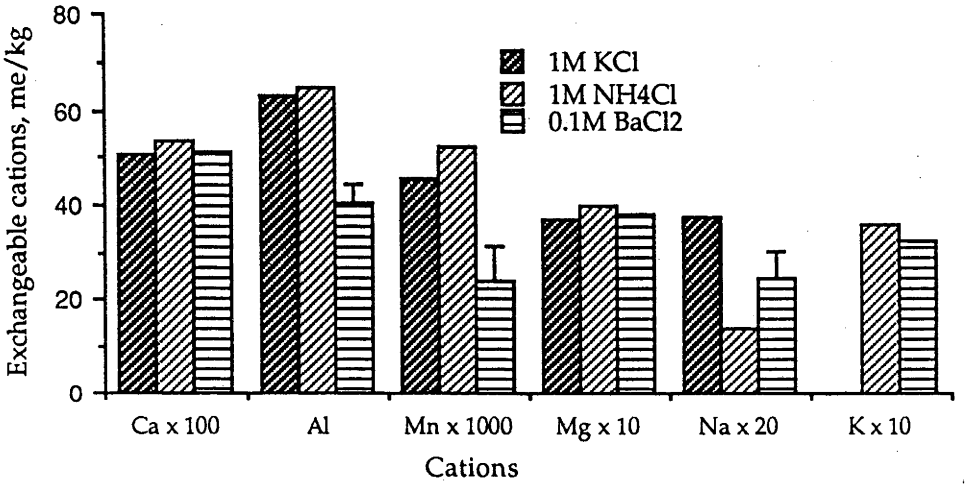


Figure 3.1. Exchangeable cations extracted by different extractants. For significant differences, vertical bars represent l.s.d. ($P < 0.01$).

Extraction of soil solution

Soil solutions were extracted at a soil : water ratio of 1 : 1 (w/v). Since the proportion of water in soil will alter the actual soil : water ratio for such a small amount of water, separate aliquots were oven dried at 105°C beforehand to estimate the amount of water already present in soil. This figure for water content was accounted for when calculating the amount of water required to make a 1 : 1 soil : water ratio. The suspension was shaken for 1 hour, centrifuged and then filtered through Whatman No 42 filter paper. The soil solution was stored in a cold room until completion of all analyses. Cations were analysed by the same methods used for extractable cations.

Available phosphorus

Available phosphorus was extracted by shaking the soil for 30 minutes with 0.5 M $NaHCO_3$ at pH 8.5 at a soil : solution ratio of 1 : 20 (Olsen et al, 1954). The concentration of P in the extract was then determined by the methods below (Section 3.3.3). (Determination of P by the ammonium lactate method also gave similar results).

Extractable mineral nitrogen

Ammonium and nitrate ions were extracted by shaking soil for 1 hour with 2M KCl at a soil : solution ratio of 1 : 10 (Bremner, 1965). The extracts were analysed for ammonium and ammonium plus nitrate and then nitrate was calculated by the difference.

3.3.3. Estimation methods

Aluminium

Aluminium was determined by several methods. During the earlier soil selection, the ferron method (Belyaeva, 1966), which was being followed in the Forestry Department, Australian National University, was used. This method is based on the principle that ferron forms a colourless complex with Al and a green complex with Fe. These complexes are stable. The Al ferron complex has an absorption maximum at 370 mμ while the Fe ferron complex has maxima at 370 mμ and 600 mμ. Therefore, spectrophotometrically Fe is measured at 600 mμ and the total Fe plus Al at 370 mμ. The amount of Al is then calculated by difference. Based on this principle, Al was measured using an UV spectrophotometer (Pye Unicam SP 1800). However, considering the large number of samples to be analysed in the remaining experiments, another method, the aluminon method (Hsu, 1963) on an autoanalyzer, was tested. To eliminate the interference from iron in the aluminon

method, ascorbic and thioglycollic acids were used (Cabrera et al, 1981). Finally an atomic absorption method or an ICP method was followed. Monomeric Al was determined by ion chromatography (Willett, 1989).

Other cations

Calcium, Mg, Na, K and Mn now as extract concentraton, were determined by atomic absorption spectrophotometer (Varian SpectrAA-20).

Inductively coupled plasma (ICP) emission spectroscopy was used for estimating mineral concentration in plant samples described in Chapter 7. Ashing and H_2O_2-H_2SO_4 digestion were compared beforehand using Division of Forestry and Forest Products standard samples and some shoot and root samples from the current study which had a wide variation in mineral concentration were included. Both extracts were analyzed for Ca, Mg, K, Al and P on the ICP and both digests gave comparable results (Figure 3.2).

Extractable mineral nitrogen

Total mineral N (ammonium + nitrate) was measured from extracts (Section 3.3.2) by an automated colorimetric procedure (Heffernan, 1985). For ammonium analysis, the extract was made alkaline with sodium hydroxide and ammonia, continuously distilled and absorbed in hydrochloric acid. The ammonium chloride was continuously sub sampled and then reacted with hypochlorite and sodium phenate (containing acetone as a catalyst) to form an indophenol blue complex. Nitrate plus ammonium were measured from extracts which were made alkaline with sodium hydroxide and then titanous sulphate was immediately added prior to continuous distillation. Nitrate and

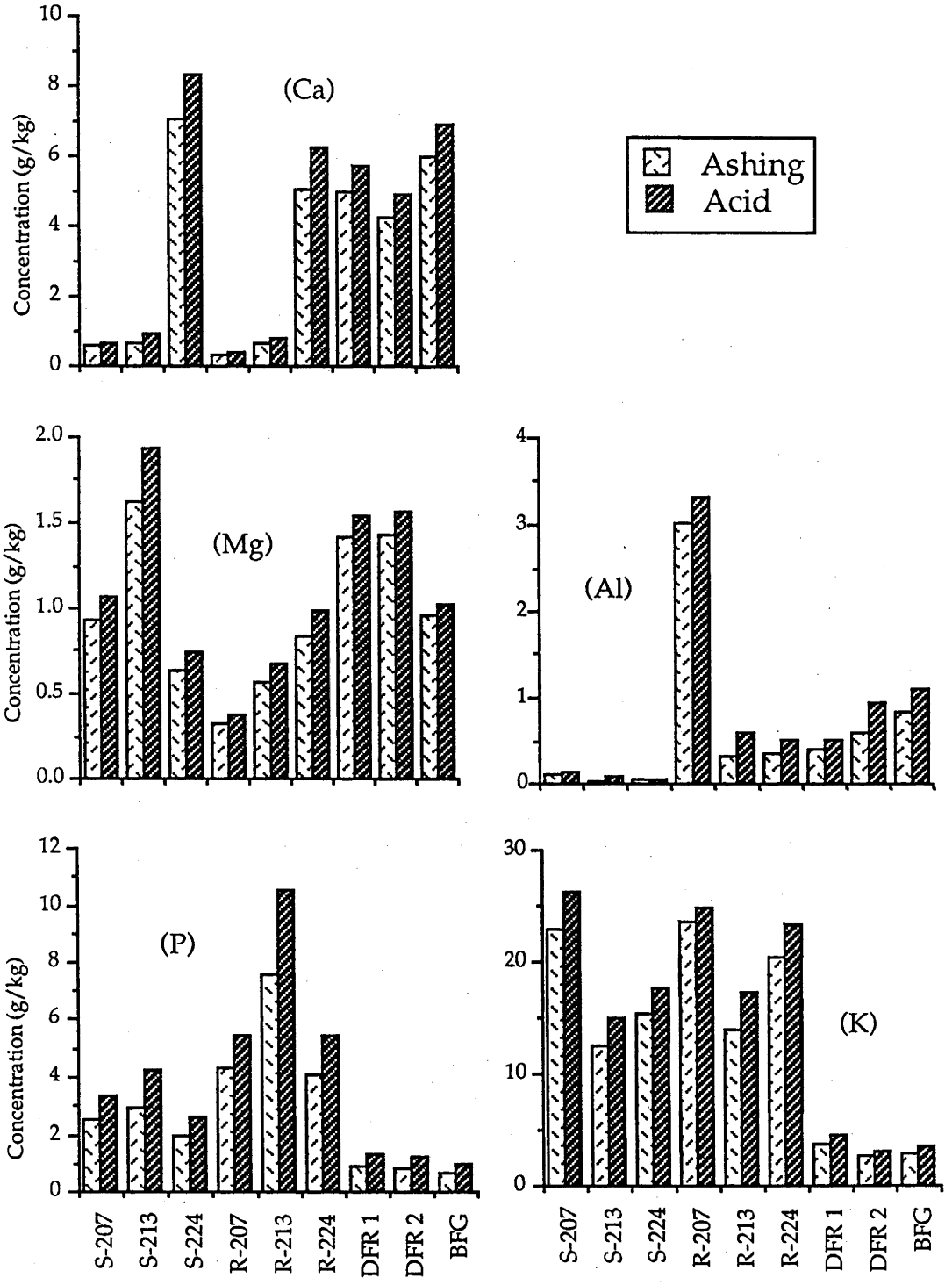


Figure 3.2. Comparative values of mineral elements determined with ICP after digestion with $H_2O_2 - H_2SO_4$ or ashing.

ammonium ions were reduced to ammonia so that the sub sample contained ammonium plus nitrate as the ammonium ion.

Available Phosphorus

Phosphorus was extracted (Section 3.3.2) and then estimated by an automated procedure which develops colour at 95°C with ammonium molybdate in H_2SO_4 and ascorbic acid (Colwell, 1965).

3.4. Plant growth

Seed source

All seeds used in this series of experiments were provided by the Australian Tree Seed Centre, Division of Forestry and Forest Products, CSIRO, Canberra, A C T. Towards the end of the study there were no more seeds of *E. Camaldulensis* from the seedlot 10886 (used in initial experiments) and therefore its closest substitute (seedlot 10885) was used for further studies. These seeds were collected from approximately the same place as 10886.

Raising of seedlings

Initially seeds were germinated in a 5 cm deep germination tray containing a vermiculite perlite mixture (1 : 1). Due to a high pH in some later vermiculite, germination was then carried out in a mixture of perlite, sand and peat moss at a ratio of 2 : 1 : 1. From two weeks after germination the seedlings were watered with dilute, modified Hoagland's solution (Thomson, 1988).

Transplantation of seedlings

At the time of transplantation (usually between 3 and 6 weeks old) healthy and uniform sized seedlings were uprooted with as little

distrubance to the roots as possible, transplanted immediately and watered. Tranplanting usually took place during the late afternoon. During hot sunny periods freshly transplanted pots were placed under shade for a few days before transferring them to the glasshouse.

Plant growth environment

All plant growth experiments were conducted either in a controlled environment glasshouse or in phytotrons and the pots were arranged in a completely randomized design.

3.5. The nutrient solution

The composition of nutrient solution used throughout the tenure of the study is shown in Table 3.1. This is a modified Hoaglands' solution which was successfully used by Thomson (1988) for *E. camaldulensis* and related species. Wherever necessary, modifications were made to this composition to include nutrient treatments and these are shown in the respective Chapters.

3.6. Chemical analysis of seedlings

Cations

Initially plant samples were wet digested in an HNO_3 - HCl mixture (3 : 1) (Heffernan, 1985) using 0.25 gm samples. In some cases (Chapter 7, 8 and 9) an H_2O_2-H_2SO_4 digest was made (Heffernan, 1985) using a 0.1 g sample for estimating the cations with ICP. When sufficient samples were available, they were ground to pass through a 20-mesh screen on a Wiley mill. Otherwise the whole sample (leaf/root/shoot/or the whole seedling) was digested. Cations were estimated by methods described earlier in this Chapter.

Phosphorus was estimated from the same extract by an automated procedure using ammonium vanadate (Jackson, 1958).

Nitrogen

Plant samples were digested with H_2SO_4 using K_2SO_4 as a catalyst to raise the boiling point of the H_2SO_4 (Jackson 1958). The digest was diluted and an aliquot analyzed for N using an autoanalyzer. The principle of this N determination is based on a colorimetric method in which an emerald green colour is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at a pH of 12.8 to 13.0. The ammonia salicylate complex is read at 660 nm.

3.7. Calculation of Aluminium species

In an aqueous medium Al exists in different forms depending on the solution chemistry, mainly solution pH. The quantities of the different important forms of Al for the different treatments (Al levels in the nutrient solution experiments) were calculated using the computer program 'Titrator' version 2.2 (Cabaniss, 1987) on an IBM PC.

After preparing nutrient solutions pH and electrical conductivity (EC) were measured. Where necessary pH was adjusted to a predetermined value by adding dilute acid or alkali, EC was measured after adjusting the pH. Ionic strength was calculated by using the relationship $\mu = 0.013$ EC where μ is the ionic strength based on concentrations expressed in mole litre^{-1} and EC was expressed in mS (Griffin and Jurinak, 1973).

Table 3.1. Composition of the nutrient solution used.

Chemical	Requirements	ml stock per 10 L nutrient solution
KNO_3	75.83 g/L	20
$Ca(NO_3)_2.4H_2O$	118.08 g/L	40
$NH_4H_2PO_4$	57.52 g/L	10
$MgSO_4.7H_2O$	61.62 g/L	20
$MnCl_2.4H_2O$	0.197 g/L	10
$ZnSO_4.7H_2O$	1.15 g/L	10
$CuSO_4.5H_2O$	0.626 g/L	10
$Na_2MoO_4.2H_2O$	0.242 g/L	10
H_3BO_3	0.744 g/L	10
Fe EDTA*		4

* EDTA: 5.0 gm NaOH was dissolved in 800 ml distilled water. Then 33.2 gm EDTA (disodium salt) and 24.9 gm $FeSO_4.7H_2O$ were added, made up to 1L volume and aerated overnight.

Nominal levels of Ca, Al, P, S, Mg and P were used as their total concentrations in the system. The following are the equilibrium reactions (Lindsay, 1979) for the important forms of Al which had substantial proportions of the element in the system:

Equilibrium reaction			log K
$Al^{3+} + H_2O$	$\rightleftharpoons$	$AlOH^{2+}$	-5.02
$Al^{3+} + 2H_2O$	$\rightleftharpoons$	$Al(OH)_2^+$	-9.30
$Al^{3+} + 3H_2O$	$\rightleftharpoons$	$Al(OH)_3$	-14.99
$Al^{3+} + SO_4^{2-}$	$\rightleftharpoons$	$AlSO_4^+$	3.20

3.8. Calculation of activities of Calcium and Aluminium.

To calculate the activities of Ca, the relationship used was:

$a_i = c_i \gamma_i$. (Equation 3.1)

Where: a_i = activity of Ca;

c_i = concentration of Ca;

γ_i = activity coefficient.

Ionic strengths of the nutrient solutions were calculated as:

Ionic strength = 0.013 EC (Griffin and Jurinak, 1973).

Values of the activity coefficient (γ_i) were obtained from the Debye-Huckel equation (as mentioned by Lindsay, 1979) for different ionic strengths. A graph was then plotted (shown in Appendix 3.1) for activity coefficients against ionic strengths and then particular ionic strengths of different nutrient solutions were extrapolated to obtain the corresponding activity coefficient values. Then the activity of Ca was calculated using those values of activity coefficients in equation 3.1.

For calculating the activity of the sum of the monomeric Al species ($\Sigma a_{Al\ mono}$), concentrations of individual Al species were calculated using the computer programme 'Titrator' (Section 3.7), then activities of the species were calculated using Debye-Huckel equation (Lindsay, 1979). Individual activities were added to obtain $\Sigma a_{Al\ mono}$. The symbol a_{Al} in this thesis refer to $\Sigma a_{Al\ mono}$.

3.9. Calculations, graphics and statistical analysis

Most calculations were carried out using the program 'Excel'; graphs were plotted using the software 'Cricket graph', sometimes in

combination with 'MacDraw'. All programs were mounted on an Apple Macintosh microcomputer. Statistical analyses were carried out either using the program 'Statworks' on an Apple Macintosh microcomputer or by using the program GENSTAT on an Australian National University VAX main frame computer.

3.10. Amendments in soil

3.10.1. Introduction

Calcium and Al levels in soil form an essential component of this project. It was therefore, necessary to either find soils in nature with varying Ca and Al levels or alter soil Ca and Al levels under laboratory conditions. Soils with differing Ca and Al levels under natural conditions are likely to differ in other characteristics as well, making it difficult to isolate differences in plant responses which could be solely assigned to levels of Ca and Al in the soils. Soils rich in Ca are generally high in pH and *vice versa*. The pH level reflects the solubility of mineral elements, mineralization of soil organic matter and element uptake by plants. Further, the levels of soluble Al in soils are determined by various factors such as soil mineralogy, pH, solution ionic strength, organic matter, and the presence of other elements such as P, Ca, and/or Mg. Furthermore, there may be variations in soil physical and biological conditions even at sites close to one another. Therefore, in experiments which involve the variation of Al and Ca in soils, it is more convenient to alter the levels of these elements in the same soil under laboratory conditions.

The use of soil amendments to alter soil acidity by liming is an ancient and common practice. Recently many experiments have included soils where Ca and Al levels were altered. For example, Adams

and Moore (1983) treated a soil: with $CaSO_4$ to correct a Ca deficiency; with $Ca(OH)_2$ to correct a Ca deficiency and Al toxicity; and with MgO to correct an Al toxicity and aggravate a Ca deficiency.

The objective of the present experiment was to devise appropriate treatments which would vary the levels of Al and Ca in a single original soil without affecting other chemical characteristics in any significant way. Initially the experiments were conducted to produce soils of varying Al and Ca levels to grow *E. camaldulensis*. To change soil Ca a combination of lime and $CaS0_4$ (in addition to their separate treatments) was tested and the accompanied changes in soil pH, exchangeable Ca, Al, Mn, K, Mg and available P were compared to single separate additions of $CaSO_4$ and $CaCO_3$.

The soil used for experiments described in Chapters 4.3, 4.4 and 5 was high in exchangeable Al, but was found to have no monomeric Al in soil solution (as revealed by ion chromatography, Willett, 1979). Therefore, exchangeable and solution Al were increased in the soil to levels where some monomeric Al appeared in solution.

3.10.2. Materials and methods for amending soil Calcium levels

The same soil used for the experiments described in Section 4.3 was used for this experiment. Soil was collected in bulk and four hundred and eighty three gms of field moist soil (equivalent to 400 gm of oven dry soil) was transferred to each preweighed polythelene bag inside a plastic pot. Soil was treated with pure $CaCO_3$ at the rate of 4940, 9880 and 14820 kg/ha. This covers a range from little to excess lime as revealed by the Shoemaker et al (1961) method. This method showed that 8650 kg/ha and 10380 kg/ha of lime were required for this soil to reach a pH of 6.0 and 6.4 respectively. Identical treatments were also made with $CaSO_4$

which contained the same amounts of Ca as was added by $CaCO_3$ treatments of the soil. Three additional treatments were included with equal amounts of Ca to 9880 kg $CaCO_3$/ha from a combination of $CaCO_3$ and $CaSO_4$ at a proportion of:

(a) 1 : 2,

(b) 1 : 1, and

(c) 2 : 1.

All treatments were replicated 4 times. The chemicals were mixed thoroughly with the soil. The soil was wetted to 70% field capacity and the pots were placed in a glasshouse with controlled temperature facilities (day/night = 25/15^{o}C).

Pots were sampled at the end of 1, 2, 4, 8, 12 and 16 weeks for chemical analysis. Before sampling, soil in each pot was mixed thoroughly. At the end of each week (whether sampling was made or not), soil in each pot was mixed thoroughly and wetted back to 70% field capacity. Soils were analysed for pH_w, pH_s, EC, exchangeable Ca, Mg, K, Mn and Al at field moisture conditions (Section 3.3) and the results were converted to an oven dry basis. At the end of 16 weeks the soil solution (1 : 1) was extracted (Section 3.3) and pH, EC and total cations were analyzed in addition to exchangeable data. Values for 0.5M $NaHCO_3$ extractable P were extremely low in all treatments and were not continued after the second week.

3.10.3. Materials and methods for amending soil Aluminium levels

After a preliminary trial to select the treatment range, field moist soils were treated with 0, 10, 25, 50 and 75 mg Al/kg soil in the form of $AlCl_3.6H_2O$ in solution. Treated soils were wetted to field capacity and kept on a laboratory bench. An electric fan was used to expedite drying of the soil. Soil samples were mixed individually and wetted again to field

capacity when they dried to ~ 40% field capacity. After four such cycles of drying and wetting over about three weeks, they were analysed in a moist condition for soil solution (1 : 1) pH, EC, cations and monomeric Al (Section 3.3.2).

3.10.4. Results

Figures 3.3, 3.4 and 3.5 are presented to show the changes in some chemical characteristics of soil with different Ca sources over a period of 16 weeks. The treatments selected for the figures were: control, intermediate levels of $CaSO_4$ and $CaCO_3$ sources, and the combined treatments [(a), (b) and (c) from Section 3.10.2]. It may be seen from Figure 3.3a that pH_s increased from week 1 to week 2 and then decreased in all treatments until the 4th week; in some treatments pH_s decreased further up to the 6th week and after that were almost stable for all treatments. The maximum decrease in pH_s was in the case of the $CaCO_3$ treatment of soil where pH_s was the highest.

There was not much variation in the EC of the 1 : 5 soil slurry over the 16 week period (Figure 3.3. b). All treatments raised EC; the rise being very little in the case of the $CaCO_3$ treatment and a maximum rise occurred in the case of the $CaSO_4$ treatment. In combined treatments, the proportion of $CaSO_4$ determined the rise in EC.

Concentrations of exchangeable Ca were also fairly steady up to 12 weeks and there was a little rise in Ca concentration between 12 and 16 weeks (Figure 3.4 a). All treatments raised the Ca levels to almost the same values.

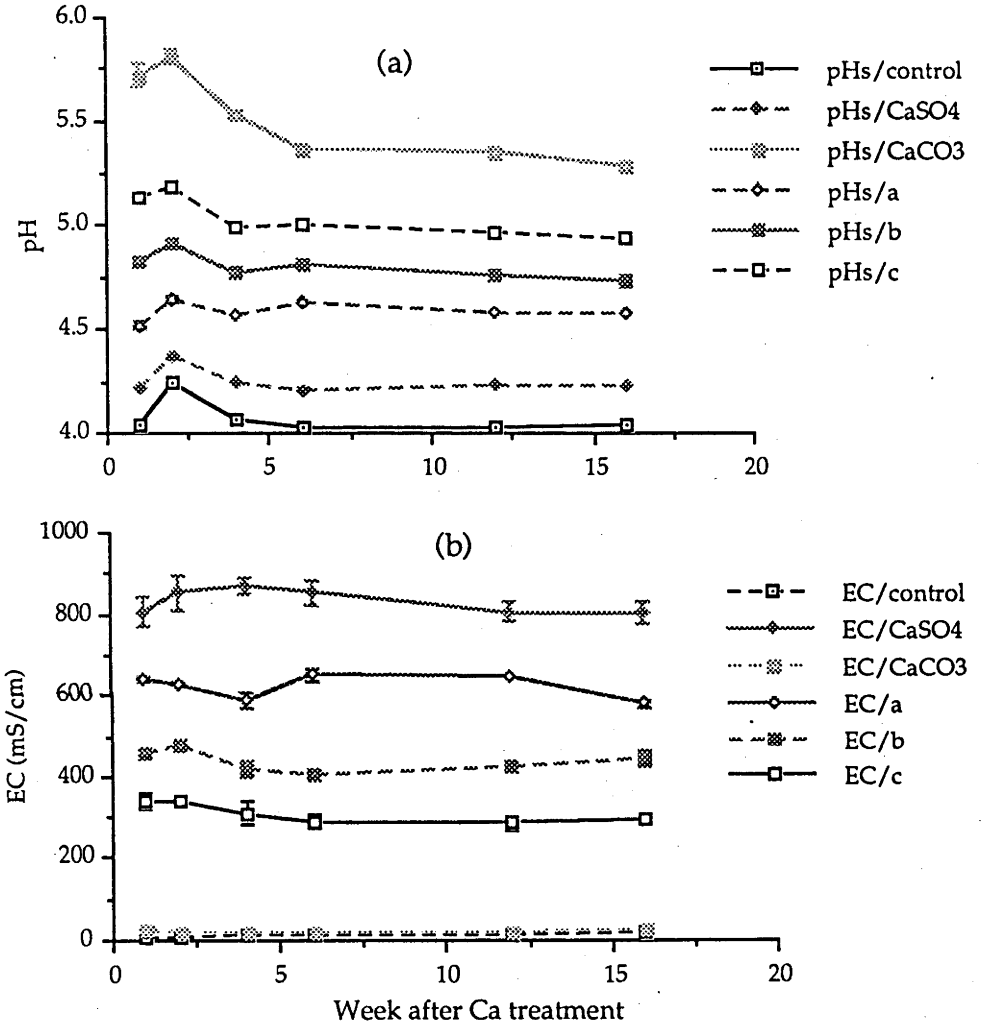


Figure 3.3. Changes in (a) pHs and (b) EC of soils of selected treatments over the incubation period. Bars represent SE of the mean.
a, b and c indicate treatment combinations of $CaCO_3$ and $CaSO_4$ at a ratio of 1 : 2, 1 : 1 and 2 : 1 respectively.

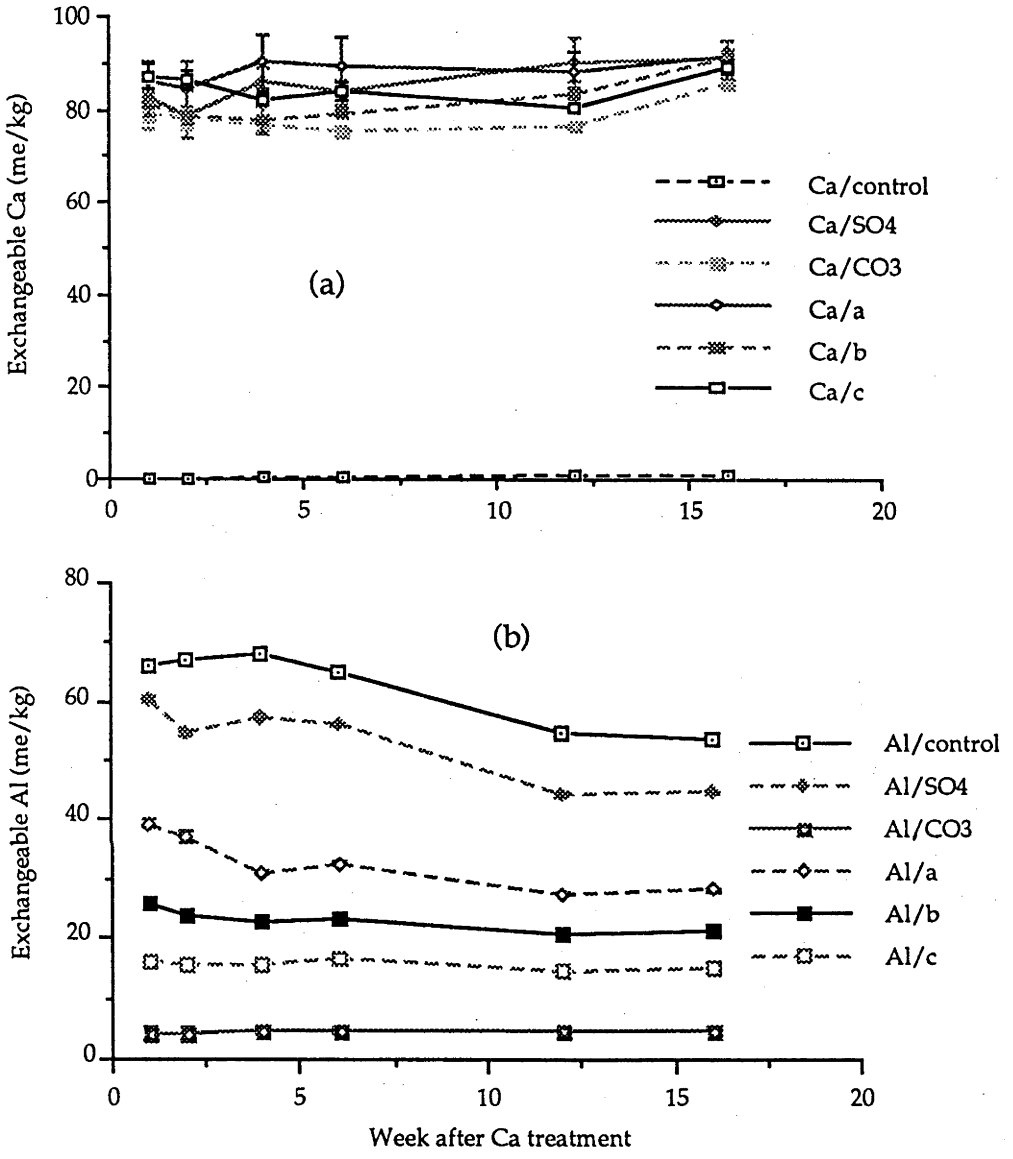


Figure 3.4. Changes in (a) Exchangeable Ca and (b) Exchangeable Al of soils of selected treatments over the incubation period. Bars represent SE of the mean. a, b and c indicate treatment combinations of $CaCO_3$ and $CaSO_4$ at a ratio of 1 : 2, 1 : 1 and 2 : 1 respectively.

Calcium treatments which maintained high exchangeable Al levels have shown a slight decrease in Al levels up to the 12th week (e.g.; In control, Al declined from 66.0 me/kg after one week to 54.9 me/kg after 12 weeks and in $CaSO_4$ treated soils Al declined from 60.2 me/kg after one week to 44.4 me/kg after 12 weeks) (Figure 3.4 b). There was very little decline after that. Treatments which resulted in low Al levels have shown little or no decline. Since all the treatments under comparison had equal amounts of Ca, it is the source of Ca which affected Al levels.

Figure 3.5 shows total changes in exchangeable Ca, Al, Mg, K and Mn over the incubation period, compared to their respective initial (1 week) control values. Soil solution values for each cation after 16 weeks was subtracted from the corresponding exchangeable values to calculate the actual amount of each cation in exchangeable positions. To calculate initial exchangeable values, soil solution values were taken to be zero. There was an increase in the exchangeable Ca for all the Ca treatments; the increase was more with $CaCO_3$ treatments and for combined treatments where the proportion of $CaCO_3$ was higher (Figure 3.5). There was a decrease in the exchangeable Al for all Ca treatments; the decrease was smaller with the $CaSO_4$ treatments. Since there was a slight decrease in the soil pH over this period, it is not possible to explain the behaviour of Al with the available data. There was not much variation in the exchangeable Mg, K and Mn.

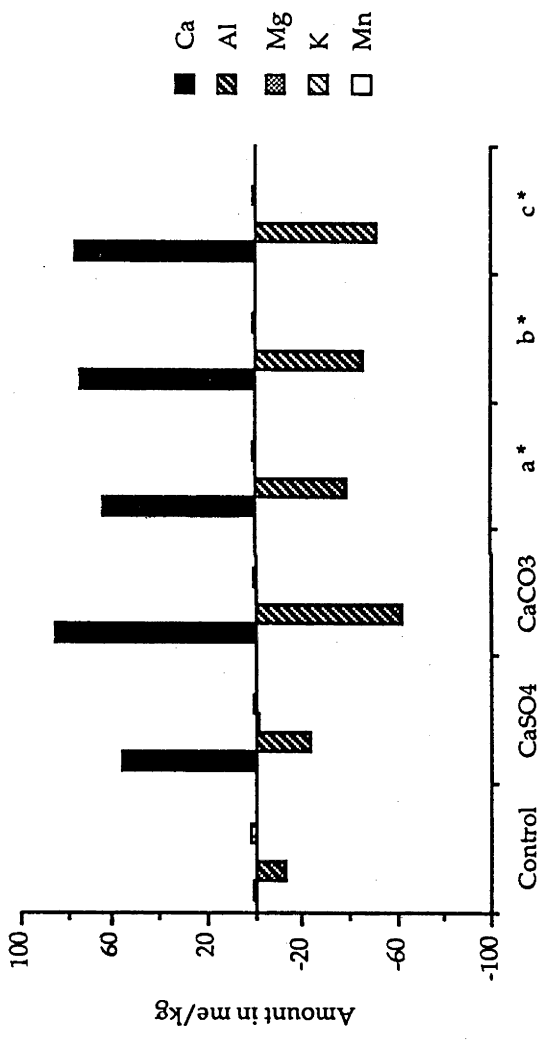


Figure 3.5. Increase (+) or decrease (-) in the exchangeable cations after 16 weeks compared to initial control.
* a, b and c indicate treatment combinations of $CaCO_3$ and $CaSO_4$ at a ratio of 1 : 2, 1 : 1 and 2 : 1 respectively.

The characteristics of the soil solution were considered in the case of Al treated soil since the main aim was to examine the presence and quantity of monomeric Al in soil solution as a result of Al treatment. The effects of Al treatments on soil solution characteristics are shown in Table 3.2. An increase in Al levels caused a consistent decrease in pH from 5.64 (control) to 3.72 (highest Al). Also there was an increase in EC of the soil solution from 62 mS/cm to 503 mS/cm. Likewise there was an increase in the concentration of the cations Ca, Mg, K, Mn and Al. The increase was small for Ca and Mn but Mg, K and Al concentrations increased substantially.

Table 3.2. Effects of Al treatment on chemical characteristics of soil solution.

	Al treatment level (mg/kg)				
Characteristics	0	10	25	50	75
Soil solution pH	5.64	4.28	3.97	3.77	3.72
Soil solution EC, mS/cm	62	105	210	365	503
Cations in soil solution (mg/kg):					
Ca	0.5	0.7	1.4	1.4	2.6
Mg	0.1	2.7	8.3	14.8	22.5
Na	3.5	9.5	14.0	12.0	13.5
K	2.0	7.2	12.1	16.2	22.6
Mn	0.0	0.1	0.4	0.6	1.3
Al	0.8	0.3	0.8	3.2	10.1
Al (monomeric)	-	-	0.7	2.8	8.1

Most importantly, monomeric Al began to appear in soil solution beginning with a treatment level of 25 mg Al/kg soil. The quantities of monomeric Al varied significantly. They were 8.1 mg/kg when Al was added at 75 mg Al/kg soil which is about 10% of the added amount whereas the amount of monomeric Al in soil solution only reached 5% when Al was added at 50 mg Al/kg soil.

3.10.5. Discussion and conclusion

The main aim of the experiments described in this Section was to select a Ca source which would raise Ca levels without affecting other parameters significantly. A second aim was to select an Al treatment which would raise soil Al status to the desired levels of monomeric Al in soil solution. Since it is obvious that addition of more Ca salts will raise soil Ca status to higher values, emphasis was given to which Ca source would have the minimum effect on other characteristics (rather than which treatment raised Ca, and by how much). Therefore, treatments containing the same amount of Ca were compared (Figures 3.3, 3.4 and 3.5).

It is difficult to compare the results of the present experiment with those in the literature. Attempts such as this to raise soil Ca levels and subsequently examine the impact on other chemical characteristics are not very common. Generally lime is added to an acid soil by a level which is predetermined by a 'lime requirement' assessment (e.g., Shoemeker et al, 1961). In screening studies for acid or Al tolerance, usually acid soil or Al toxic soil is limed at different rates to obtain a range of acidity (Carvalho et al, 1980; Smit et al, 1987 b) or Al toxicity (Krizek and Foy, 1988; Joslin and Wolfe, 1988) and the plants are grown on for tolerance tests. This entanglement of factors leads to a great deal of confusion. Further there are also reports of the effects of liming on

plant growth which include simultaneous nutrient availability evaluation during (Martini and Mutters, 1985 a; b) or after plant growth (Anandan et al, 1985). Reports are also available describing specifically the raising of soil Ca to predetermined levels by adding $CaCO_3$, $Ca(OH)_2$ etc (Simpson et al, 1977; Howard and Adams, 1965), but again the side effects of such treatments are not considered.

Anandan et al (1985) analyzed the liming of an acid sandy loam which then had peanuts grown for 105 days. They reported a rise in pH from 4.3 to 6.9 and a rise in exchangeable Ca from 4.3 to 24.3 me/kg. Although these changes were reported under field conditions as well as in pot cultures where plants were grown and irrigated, these results may be compared to the present data in terms of variation in pH, Ca and Al. Their effects (pH, exchangeable Ca and Al) are all smaller compared to the present experiment. For example, in the present experiment, pH_w and exchangeable Ca increased by 0.52 units and 47.57 me/kg respectively under a lime treatment of 4990 kg/ha compared to 2.6 units and 20 me/kg respectively under a lime treatment of 2250 kg/ha (Anandan et al, 1985). The differences in post treatment Ca levels were quite high. However, their soil was initially more acid (by about one unit of pH) and the soils were subjected to plant growth, irrigation and possible leaching. A substantial part of the Ca (added as lime) may have been absorbed by the growing plants or may have been leached out [(Jarvis (1987) reported loss of Ca from soil columns after irrigating limed acid soil)]. A treatment of 1723 kg $CaCO_3$/ha reduced the exchangeable Al to a trace (Ananadan et al, 1985) whereas in the present experiment exchangeable Al was present even at 9880 kg $CaCO_3$/ha treatment. However, the effect of a Ca addition would have been different had this study been conducted under field conditions where downward movement of Ca would have occurred (Pearson et al, 1961; Juo and Ballaux, 1977).

The stability of pH, exchangeable Ca and Al during the present experiment may be compared with that of Martini and Mutters (1985 a; b). In their experiment both pH and exchangeable Al became stable earlier than in the present experiment. In their experiment Ca levels increased up to 16 weeks for the high lime treatment soils whereas lime rates were less in the present experiment and Ca here remained fairly steady up to 12 weeks and then increased slightly until 16 weeks.

When all the analyses for Ca treated soils in the present experiment are reviewed together, the combined 3 treatment, (c) did not alter the Mn level at all. The pH_s under the $CaSO_4$ treatment was closer to the control but this treatment increased the Mn status in the soil which is an important acid soil factor. The $CaCO_3$ treatment raised the pH_s and lowered Mn and Al levels more than treatment (c). This treatment, although it lowered the exchangeable Al level, had Al which was still higher than the $CaCO_3$ treatment. In conclusion, soil Ca levels in low Ca acid soils such as the present one can be raised by adding Ca from a combined $CaCO_3$ and $CaSO_4$ source at a ratio of 2 : 1. This will raise Ca levels but will not alter other characteristics to a significant extent.

In order to bring small amounts of monomeric Al into the soil solution $AlCl_3.6H_2O$ was added to soil. Higher Al treatments resulted in greater quantities of other cations (including Al) in soil solution. This effect may be due to the displacement of other cations by Al in the exchange complex. Since the addition of Al causes a change in soil chemical characteristics, including the pH and concentrations of Mg, K and Na in soil solution, emphasis must be given to the selection of an Al treatment which brings monomeric Al into soil solution while other chemical characteristics are not significantly affected.

CHAPTER 4

THE PERFORMANCE OF EUCALYPT SPECIES IN ACID SOILS

4. THE PERFORMANCE OF EUCALYPT SPECIES IN ACID SOILS

4.1. Introduction

Plant species, and cultivars within species, vary widely in their sensitivity and tolerance to acid soil infertility factors. Australian native eucalypts grow right across a vast range of edaphic factors. Very subtle changes in species distribution mirror a changing mosaic of land with very gradual changes of soil nutrients and soil moisture regimes (Florence, 1963). The distribution of each species or association of species reflects a difference in preference for environmental conditions. However, reports on the performance of different eucalypt species in natural acid soils are rare.

One of the objectives of the research described in this Chapter was to select species for future experiments on the effects of Al toxicity and related factors on the growth of eucalypts. For this purpose two experiments were carried out. In the first (Section 4.2) 22 eucalypt species were grown in three soils of differing acidity. On the basis of their performance in these soils a preliminary selection of four species was made. In the second experiment (section 4.3) the response of these four selected species to lime amendments was investigated in another soil which was higher in exchangeable Al and lower in exchangeable Ca and available P than the soils used in the first experiment. Thus, on the results of these experiments, one species was finally selected for future experiments.

Though the objective of this research was the selection of particular species, a subsidiary aim was to begin to fill the gap in basic information on the growth of eucalypts in Australian acid soils. The first experiment showed the comparative pattern of growth of a variety of

eucalypt species in acid soils, meaning that the influences of a variety of acidity factors could begin to be observed. The second group of experiments (Sections 4.3 and 4.4) showed how the performance of four selected species varied with lime or moisture levels respectively.

4.2. Growth of 22 eucalypt species in three different acid soils

4.2.1. Selection of soils and eucalypt species

A soil survey by Talsma (1983) on the Cotter catchment area, A. C. T provided the data for a preliminary selection of soils. Soil samples were collected from sites 1, 2, 3, 4, 5, 6, 7 and 8, (Figure 4.1) all of which were identified as acid soil sites by Talsma (1983). Because of the well known interaction of Al with soil organic matter, this complication was excluded by removing organic matter rich surface soils and these horizons were excluded from the collection. Soils were collected from a variety of depths below the A_h horizon. After collection, soil material was sieved (2 mm sieve) and stored in a cold room until the completion of analyses.

Soil description including pH in water (pH_w), pH in 0.01M $CaCl_2$ solution (pH_s), particle size distribution, organic matter content, and exchangeable Ca, Mn and Al, were made by the methods described in Chapter 3. The characteristics which were used for selection of the three soils for this experiment are presented in Table 4.1. The following further criteria (once acidity was established) were considered in the selection of soils: (a) similar parent materials, (b) low in organic matter content, (c) low in clay content, (d) low in exchangeable Mn, (e) low in gravel and/or stone content and (g) a wide range in exchangeable Ca and Al. Vehicular access to the site for bulk soil collection was also taken into consideration in the final selection.

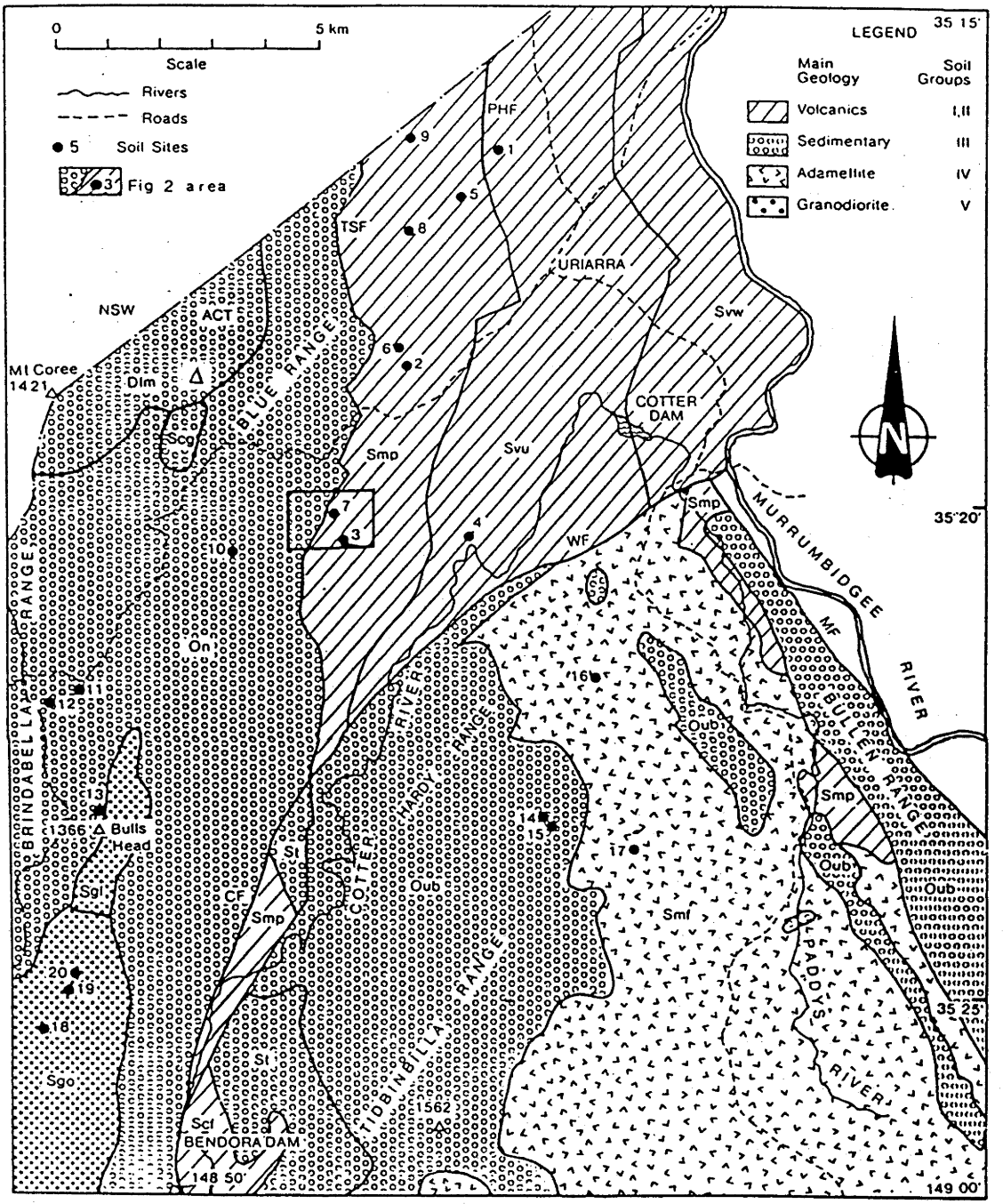


Figure 4.1. Location map showing the selected sites from the Cotter area, A. C. T. (Talsma, 1983). Compartment 152 of the Uriarra forest is also shown as Δ on the map.

Table 4.1. Characteristics of the soils collected. These were used for selection of the soil for experiments described in Section 4.2 *.

Site No.	Depth (cm)	pH_w	pH_s	Ca me/kg	Al me/kg	Ca/Al	Mn me/kg	Org. matter (g/100g)	Clay (g/100g)	gravel/stone (g/100g)
1	10 - 20	5.42	4.70	62.23	0.60	103.72	0.69	3.64	10.00	0.00
	20 - 30	**5.94**	**4.73**	**40.97**	**0.84**	**48.77**	**0.06**	**1.40**	**13.75**	**0.00**
2	10 - 20	5.27	3.91	6.35	23.20	0.27	1.21	2.22	13.75	41.90
	20 - 30	5.15	3.92	5.50	27.04	0.20	1.00	2.90	15.00	30.80
3	10 - 20	5.00	4.05	15.59	10.04	1.55	0.52	1.69	11.25	8.70
	20 - 30	**5.34**	**4.28**	**14.86**	**6.81**	**2.18**	**0.08**	**0.86**	**13.75**	**1.80**
4	10-20	5.43	4.40	15.59	5.81	2.68	3.09	1.41	15.00	0.00
	20-30	5.35	4.39	11.83	5.63	2.10	1.67	1.41	16.25	0.00
5	10 - 20	5.55	4.48	78.25	1.09	71.79	0.75	4.23	7.50	31.80
6	**10 - 20**	**5.53**	**4.43**	**15.42**	**4.71**	**3.27**	**0.12**	**2.20**	**15.00**	**3.80**
	20 - 30	5.36	3.97	2.26	18.60	0.12	0.18	1.21	20.00	6.80
7	10 - 20	5.51	4.05	17.27	16.64	1.04	1.05	2.61	13.75	3.40
	20 - 30	5.17	4.03	12.67	16.04	0.79	0.33	2.36	16.25	7.80
8	10 - 20	5.34	4.55	39.52	0.87	45.69	0.63	2.24	18.75	0.00
	20 - 30	5.51	4.52	19.42	1.11	17.56	0.47	2.14	18.75	1.50

* Soils selected for the experiment are shown in bold.

The final selection of sites and then soils ensured that all belonged to the same volcanic rocks parent material (Talsma, 1983) . Clay content of these soils varied from 7.5 g/100 g to 20.0 g/100 g. Soils from sites 8 (both depths) and 6 (20 - 30 cm) had high clay contents and were excluded from selection. Soils from sites 2 (both depths), 3 (10 - 20 cm) and 7 (20 - 30 cm) were high in gravel and/or stone and were excluded from selection. Organic matter content of the soils also varied widely; from 0.86 to 4.23 g/100 g. Soils from sites 1 (10 - 20 cm), 2 (20 - 30 cm) and 5 were excluded because of their high organic matter content. Exchangeable Mn content of the soils ranged from 0.06 to 3.09 me/kg. Soil from site 4 (10 - 20 cm) had the highest Mn content followed by those from site 4 (20 - 30 cm), site 2 (both depths) and 7 (10 - 20 cm). Therefore, these soils were also excluded from selection. After elimination of soils as described so far, soils from sites 3 (20 - 30 cm), 6 (10 - 20 cm) and 1 (20 - 30 cm) remained. These soils varied widely in exchangeable Ca (14.86 me/kg to 40.97 me/kg), exchangeable Al (0.84 me/kg to 6.81 me/kg) and the ratio of exchangeable Ca : exchangeable Al (2.18 to 48.77) which fulfilled the selection criteria. The sites 3, 6 and 1 were located at an elevation of 710, 750 and 710 m respectively. Talsma (1983) classified the soil from site 6 as a red podzolic and from sites 1 and 3 as yellow earths. Soils from sites 3, 6 and 1 were termed soil 1, 2 and 3 respectively and this nomenclature is used throughout this and later Chapters.

Bulk quantities (approximately 150 kg) of each soil were collected for the pot experiment, air dried and sieved through a garden sieve (approximately 5 mm mesh size). Soils were analyzed for pH, organic matter, exchangeable Ca, Al, K and Mg, available P, extractable mineral N and field capacity.

The eucalypt species used:

The eucalypt species selected for this experiment included those growing naturally under conditions of varying soil pH. Some species (*E. camaldulensis, E. citriodora* and *E. tereticornis*) which are being grown in Bangladesh (the authors home country) were also included. Table 4.2 provides a list of the eucalypt species (arranged alphabetically) and a description of the area from which seeds were collected. These species represent a good geographical coverage (Table 4.2) and six Australian states were covered. The species selected cover a range of altitudes from 0 to 1225 m, latitudes from 17°38' to 38°4'S and longitudes from 115°45' to 153°12'E.

4.2.2. The pot experiment

Air dry soil, in amounts equivalent to 1940 gm oven dry weight, was placed in each pre weighed polythene bag inside a plastic pot. Seven week old seedlings were transplanted at a rate of three seedlings per pot and the pots were placed in the glasshouse. The glasshouse environment was controlled to give day and night temperatures of 25 and 16°C respectively. There were five replicates. When the seedlings were established (after two weeks), the pots were thinned to two seedlings per pot and the soil in each pot was covered with 40 g of plastic beads (as mulch). Since the soil was very poor a basal dose of urea dissolved in water was applied at a rate of 30 mg N per kg of soil. Otherwise very poor growth would have made it difficult to observe any treatment effect. Shoot heights were recorded initially and then weekly, beginning with the 8th week after transplantation. Seedlings were harvested at 20 weeks after transplantation and shoot weight was recorded after oven drying the seedlings at 70°C for 48 hours.

Table 4.2. List of eucalypt species used and a description of the source of seeds.

Serial No.	Seedlot number	Species	Locality	Altitude Metre	Latitude Deg.	Latitude Min.	Longitude Deg.	Longitude Min.
1	S 17348	*Eucalyptus albens*	Temora Cootamundra, NSW	0	34	25	147	53
2	S 11835	*E. Blakelyi*	South of Mendooran, NSW	450	31	50	149	6
3	S 13852	*E. calcicola*	Boranup Block, WA	50	34	9	115	2
4	S 15021	*E. camaldulensis* (a)	Wimmera R Elmhurst, Vic.	325	37	13	143	16
5	S 10886	*E. camaldulensis* (b)	Mt. Wedge, SA	25	33	28	135	5
6	S 14712	*E. citriodora*	North of Mt. Garnet, Qld	710	17	38	145	8
7	S 12264	*E. decipiens*	Near Kwinana, WA	0	32	16	115	45
8	S 12915	*E. dives*	South of Jerangle, NSW	1225	35	53	149	22
9	S 12285	*E. gummifera*	Termeil, Nowra, NSW	30	35	28	150	23
10	S 14853	*E. macrorhyncha*	Cotter/Uriarra, A.C.T.	650	35	15	149	0
11	S 12159	*E. mannifera*	Yass Dalton, NSW	0	34	51	148	54
12	S 15290	*E. melliodora*	SSE Goulburn, NSW	610	34	58	149	52
13	S 15084	*E. microtheca*	Walget/Mungindi, NSW	155	29	20	148	35
14	S 15416	*E. occidentalis*	N Esperance, WA	200	33	20	121	43
15	S 13903	*E. pilularis*	Newfoundland SF	60	29	55	153	12
16	S 15340	*E. polyanthemas*	South of Wagga, NSW	400	35	24	147	22
17	S 10418	*E. populnea*	Warren area, NSW	180	31	42	147	49
18	S 12464	*E. rossii*	Mt. Ainslie, A.C.T.	800	35	17	149	13
19	S 11676	*E. saligna*	N Batesman Bay, NSW	30	35	36	150	10
20	S 17430	*E. tereticornis*	Loch Sport, Vic	0	38	4	147	35
21	S 11978	*E. viminalis*	Blundells Flat, A.C.T.	685	35	19	148	50
22	S 12240	*E. yalatensis*	N of Cooktown, Qld.	30	31	27	130	30

Source: Tree Seed Centre, Division of Forestry and Forest Products, C.S.I.R.O., Canberra, A.C.T.

Chemical analysis of harvested seedlings

Since the total sample size was too large for complete analysis, chemical analyses were carried out on only six selected species. These six species were the best, intermediate and the worst performing species in these soils in terms of shoot weight (Table 4.5). The species chosen were, best: *E. camaldulensis, E. pilularis*; intermediate: *E. dives, E. decipiens*; worst: *E. gummifera* and *E. yalatensis*. However, the performance of each species varied in each of the three soils and the same species was not ranked identically in all three soils.

In some cases the seedlings were very small and there was insufficient biomass available for analysis from the two seedlings grown in a single pot. Therefore, seedlings from more than one replicate pot were combined to make one sample. Where the shoot weighed in excess of the required amount, it was ground and sub sampled for digestion. Otherwise the whole shoot was digested for analysis. For N, plant samples were digested with H_2SO_4 (Jackson, 1958). For cations and P, samples were digested with HNO_3-HCl (Heffernan, 1985). From the digest, N was estimated colorimetrically using an autoanalyzer (Heffernan, 1985). Phosphorus was also estimated by an automated procedure using ammonium vanadate (Jackson, 1958). Potassium, Ca, Mg, Mn and Na were estimated using an atomic absorption spectrophotometer.

4.2.3. Results and discussion

Table 4.3 compares the characteristics of the three soils selected for the experiment. Soil 1 was the most acid and soil 2 intermediate and soil 3 least acid. Soil 1 had pH_w and pH_s of 5.34 and 4.28 respectively, its exchangeable Al content was 6.81 me/kg and the highest of all these soils

while its exchangeable Ca level of 14.86 me/kg was the lowest. The percentage of Al saturation was also the highest in soil 1 (22.94). Exchangeable Mg in soil 1 and 2 were similar (6.78 and 7.66 me/kg respectively) but was much higher in soil 3 (21.28 me/kg). The organic matter content was low in all three soils, and lowest in soil 1 (0.86 g/100 g). Extractable mineral N followed the same trend as organic matter.

Table 4.3. Important characteristics of the three soils used.

Characteristics	Soil 1	Soil 2	Soil 3
Clay (g/100 g soil)	13.75	15.00	13.75
Field capacity (g /100 g)	22.50	20.90	27.80
pH_w	5.34	5.53	5.94
pH_s	4.28	4.43	4.73
Organic matter (g/100 g)	0.86	2.20	1.40
NH_4-N (mg/kg)	1.50	4.20	2.12
NO_3-N (mg/kg)	0.43	1.48	0.52
Available P (mg/kg)	0.40	14.43	0.19
Exch. K (me/kg)	1.16	2.15	0.91
Exch. Ca (me/kg)	14.86	15.42	40.97
Exch. Mg (me/kg)	6.78	7.66	21.28
Exch. Al (me/kg)	6.81	4.71	0.84
Exch. Mn (me/kg)	0.08	0.12	0.06
Ca (me/kg)/Al (me/kg)	2.18	3.27	48.77

Soils varied in available P (0.19 to 14.43 mg/kg) and soil 2 had the highest available P (14.43 mg/kg). Thus soil 2 had the highest organic matter, mineral N and available P for the three soils studied. These

variations in the acidity related properties of soils were useful in the comparison of the performance of the eucalypt species.

Performance of the tree species:

Performance is discussed in terms of two parameters: shoot height (cm) and dry shoot weight (mg/seedling). Table 4.4 shows data on shoot height at harvest from the three soils. Data are arranged in descending order of performance in soil 1 which is the most acidic. The least significant difference (LSD) in shoot height among the soils is also shown in the table. Shoot heights ranged from 3.89 cm for *E. melliodora* in soil 3 to 44.08 cm for *E. camaldulensis* (a) in soil 2. Taking the average for all soil types, shoot height ranged between 11.19 and 21.36 cm. On the basis of average shoot height, *E. occidentalis*, *E. camaldulensis* (a) and *E. viminalis* were the best performing species and *E. saligna*, *E. yalatensis* and *E. blakelyi* were the poorest ones. Appendix 4.1 shows fortnightly shoot heights beginning at the 8th week after transplantation. This table shows that the species which grew better had, in general, consistently higher shoot heights during the entire period although they were very similar at the 8th week after transplantation. The opposite was true for species growing slowly. The variation in shoot height among the three soils was significant ($P = 0.05$) for all the species indicating that species varied not only in their growth rate but also in their erectness.

Table 4.4. Shoot heights (cm) at harvest of different species in the three soils (arranged in order as shoot height in soil 1).

Species	Soil 1	Soil 2	Soil 3	LSD (0.05)	Average
E. viminalis	14.57	32.78	8.43	4.83	18.59
E. citriodora	13.40	31.29	10.20	4.68	18.30
E. occidentalis	13.09	38.98	12.02	4.04	21.36
E. pilularis	11.39	24.93	8.98	2.23	15.10
E. mannifera	11.35	36.26	6.69	3.53	18.10
E. albens	10.84	21.60	7.32	2.24	13.25
E. camaldulensis (a)	10.82	44.08	6.31	4.29	20.40
E. camaldulensis (b)	10.77	29.35	5.57	5.24	14.34
E. decipiens	10.47	28.90	7.27	3.99	15.55
E. gummifera	9.61	17.38	8.64	3.53	11.88
E. dives	9.55	24.88	5.41	3.28	13.28
E. rossii	8.82	28.55	5.75	3.59	14.37
E. macrorhyncha	8.81	26.54	5.40	4.02	13.58
E. blakelyi	8.80	21.74	4.56	4.51	11.70
E. calcicola	8.08	24.48	4.19	2.69	12.25
E. populnea	8.04	27.29	4.48	2.36	13.27
E. saligna	7.67	20.31	5.60	4.32	11.19
E. tereticornis	7.33	29.10	4.90	2.38	13.78
E. polyanthemas	7.27	25.08	4.67	1.81	12.34
E. yalatensis	6.60	21.49	5.68	4.43	11.26
E. melliodora	5.74	26.79	3.89	2.67	12.14
E. microtheca	5.72	31.77	5.09	3.19	14.19
Average	**9.49**	**27.77**	**6.41**		**14.56**

Table 4.5 shows shoot weights (mg/seedling) of the species in the three soils. The species are again arranged in descending order of

shoot weights observed in soil 1 and values of LSD for shoot weight are also shown. The shoot weight varied from 2344 mg/seedling for *E. camaldulensis* (b) in soil 2 to only 21 mg/seedling for *E. melliodora* in soil 3. The last column in the table shows the average shoot weight of the species in three soils and this ranged from 909 to 211 mg/seedling. Based on Table 4.5 it is possible to group the species on their performance in soil 1 into good, medium and poor. Some of the species did not perform equally well in all the soils. Rejecting those that have major inconsistencies in their performance the species may be grouped as:

Good:	*E. camaldulensis* (b), *E. pilularis*, *E. macrorhyncha*, *E. citriodora*, and *E. occidentalis*.
Intermediate:	*E. calcicola*, *E. rossii*, *E. dives*, and *E. saligna*.
Poor:	*E. blakelyi*, *E. melliodora*, *E. polyanthemas*, and *E. yalatensis*.

In all soils the species *E. gummifera* and *E. saligna* showed physiological disorders in their leaves from about 12 weeks after transplanting. After about 16 weeks, *E. rossii*, *E. occidentalis* and *E. calcicola* also developed similar symptoms in some soils (necrosis and purple blotches on older leaves). The symptoms were similar to those reported for phosphorus deficiency in *E. pilularis* (Truman and Turner, 1972). Before harvest, leaves of *E. saligna* were shed in soil 1 and 3 and leaves of *E. gummifera* were shed from seedlings in soil 3.

From Table 4.5 the variation in performance of the species across the three soils can be seen. The variation is significant. This inter soil difference in growth indicates that the factors that accompany acid soil characteristics are important in determining seedling growth. This is a fundamentally important conclusion.

Table 4.5 Shoot weights (mg/seedling) of different species in the three soils (arranged in order of shoot weight in soil 1).

Species	Soil 1	Soil 2	Soil 3	LSD (0.05)	Average
E. camaldulensis (b)	305	2344	78	452	909
E. pilularis	291	2093	140	349	841
E. dives	206	1410	73	158	563
E. macrorhyncha	189	1948	80	536	739
E. decipiens	183	1400	79	342	554
E. albens	178	974	85	146	412
E. rossii	169	1414	73	324	552
E. calcicola	159	1481	46	193	562
E. viminalis	154	1090	47	5	430
E. gummifera	146	596	137	237	293
E. occidentalis	132	1572	116	251	607
E. camaldulensis (a)	129	1915	47	412	697
E. citriodora	126	1577	96	510	600
E. blakelyi	121	1084	42	374	416
E. populnea	101	1233	31	188	455
E. tereticornis	100	1563	59	141	574
E. mannifera	97	1159	22	257	426
E. saligna	80	1224	42	305	449
E. polyanthemas	60	782	26	62	289
E. yalatensis	46	557	31	189	211
E. microtheca	41	1468	32	227	514
E. melliodora	37	1019	21	183	359
Average	**139**	**1359**	**64**		**521**

The soils varied in a number of characteristics. Soil 2 was severalfold higher in available P than soil 1 and soil 3. Also soil organic

matter and extractable mineral N were much higher in soil 2. Soil 3 was higher than soil 1 with respect to organic matter, extractable mineral N, pH, exchangeable Ca and Mg, but lower in available P. Growth of all the species was best in soil 2 and was poorest in soil 3. There is thus reason to hypothesize that low P is the reason for such poor growth in soils 1 and 3. Ashton (1976) found that the levels of P determined by both total digestion and $NaHCO_3$ extraction correlated strongly with the growth of *E. regnans* and *E. sieberi* (shoot height and shoot dry weight) in pot experiments. McColl (1969) reported changes in the eucalypt species growing on the south coast of New South Wales depending on a gradient of moisture and nutrient status. For instance these characteristics varied from ridge to gully sites and parallelled changes in eucalypt association. He found that concentrations of leaf P and bark Ca of dominant trees on various sites correlated strongly with the corresponding soil nutrients. In pot experiments with soil from these sites he found that 6 eucalypt species had better growth in the soil with high P levels. These findings are similar to the present study: the species differed widely in their performance in the three acid soils which varied in other properties, and especially their P levels.

Mineral concentrations in selected eucalypt species are shown in Table 4.6. The table also shows the ANOVA for individual elements with respect to the three soils and 6 species. Aluminium concentrations were very low (below the detection limit of atomic absorption spectrophotometer). It can be seen that the mineral composition for all elements significantly differed between species. Except for N, seedling mineral composition for all other elements differed significantly across the soils. It should be mentioned that equal amounts of N were added to all soils during the growth of seedlings and therefore it is likely that seedling N results do not reflect the soils natural levels.

Except for Mg and Na, there were significant soil by species interactions in mineral composition. However, the difference in P concentrations in seedlings grown on different soils is quite high. Seedlings grown in soil 2 were high in P compared to seedlings in soils 1 and 3; probably due to higher P and organic matter in that soil. Available P levels of the soils were highly correlated ($r^2 = 0.81$) with seedling P concentration. In general, the species *E. dives, E. gummifera* and *E. yalatensis* showed higher mineral accumulation. Since in *E. gummifera* and *E. yalatensis* shoot yield was very low and the material consisted of succulent leaves and stems, their mineral concentration was likely to be high. For *E. dives*, it may have been the characteristics of the species.

Concentrations of Ca, Mg and P were much lower in *E. camaldulensis* than in the other species particularly in soil 2 where overall growth was better. This is probably due to the large size of the seedlings. On the other hand *E. pilularis* was of about the same size in soil 2 but the mineral concentrations were quite different. A striking difference in mineral accumulation is the very low Na content in all the soils by *E. decipiens* which may also be a characteristic of the species.

Table 4.6. Mineral concentrations of selected eucalypt species grown in the three acid soils.

Soil and species	N	P	K	Ca	Mg	Mn	Na
	Percent						mg/kg
Soil 1							
E. camaldulensis	1.50	0.07	1.22	0.35	0.16	453	577
E. pilularis	1.31	0.05	0.75	0.40	0.25	309	1429
E. dives	2.56	0.09	1.98	0.44	0.20	590	2285
E. decipiens	1.68	0.07	0.92	0.54	0.26	572	68
E. gummifera	1.84	0.10	1.08	0.65	0.26	480	1529
E. yalatensis	2.55	0.09	1.29	0.58	0.37	759	1146
Soil 2							
E. camaldulensis	1.19	0.13	1.22	0.33	0.13	391	405
E. pilularis	1.13	0.18	0.95	0.46	0.30	359	992
E. dives	1.66	0.28	1.89	0.45	0.21	442	1048
E. decipiens	1.89	0.19	1.11	0.54	0.30	755	157
E. gummifera	2.53	0.24	1.67	1.09	0.40	719	1229
E. yalatensis	2.92	0.23	2.04	1.11	0.44	1364	910
Soil 3							
E. camaldulensis	1.59	0.05	1.01	0.40	0.19	571	876
E. pilularis	1.24	0.03	0.71	0.37	0.28	332	1909
E. dives	1.83	0.04	1.28	0.43	0.21	517	1432
E. decipiens	1.55	0.04	0.69	0.42	0.28	461	97
E. gummifera	1.90	0.08	0.89	0.48	0.22	374	1804
E. yalatensis	1.69	0.05	0.71	0.37	0.29	362	1697
ANOVA:							
Between soil	2.94	252.97**	37.67**	7.87**	4.25*	4.48*	6.06**
Between species	12.00**	11.05**	22.28**	5.67**	12.58**	4.27*	15.13**
Between soil x species	3.00*	4.46**	4.06**	2.42*	1.74	2.50*	1.40

** and * indicate the F-ratio is significant at <1 % and < 5% level respectively.

4.2.4. Conclusions for the selection of species for the liming experiment

The lime response experiment which follows was conducted to judge whether eucalypt species improve their performance when soils are conventionally limed. For this purpose *E. camaldulensis* (b) and *E. citriodora* were chosen because they are useful for Bangladesh and showed a reasonable high level of growth in all soils. *Eucalyptus gummifera* and *E. saligna* were selected to represent the poorer performing species. In addition to poor growth, these two species also showed symptoms of physiological disorders. If only acidity related problems were responsible for poor growth and physiological disorders, it could be expected that they would respond to liming more than the species which grew better in the acid soils. From this point in the thesis *E. camaldulensis* will mean *E. camaldulensis* (b) [the parentheses (a) or (b) after *E. camaldulensis* will be dropped].

4.3. Responses of four eucalypt species to lime

4.3.1. Selection of soil

For this experiment it was planned to use a highly acid soil (and high in exchangeable Al) which was specially selected. In order to illustrate fully the response to liming of different eucalypt species, the soil was chosen so that it was low in exchangeable Ca. Other characteristics such as Mn and soil organic matter levels could complicate and confuse the effects. Therefore, variations in other acid soil factors were minimized to better define the effects of the chosen (important) variables.

Twenty one soil samples (from 9 locations) from the Cotter catchment area (Talsma, 1983) and compartment 152 of Uriarra forest were previewed. Soils from three profiles from compartment 152 of

Uriarra forest and six profiles from Cotter catchment area were collected. Soils were sampled at various depths, sieved (2 mm sieve) and stored in the cold room until the completion of analyses for pH_w, pH_s, organic matter, exchangeable Ca, Mg, Mn and Al (methods described in Chapter 3). All the soils are high in exchangeable Al and low in exchangeable Ca contents (Table 4.7). Exchangeable Al varied from 31.44 me/kg to 72.74 me/kg and exchangeable Ca varied from 0 me/kg to 0.26 me/kg (Table 4.7). The pH_w varied from 4.95 to 5.67 and pH_s from 3.74 to 4.20. All the soils from Uriarra and a few from Compartment 152 were high in organic matter (varied from 0.40 to 8.34 g/100 g). Exchangeable Mn for all the soils were below 0.06 me/kg and exchangeable Mg ranged from 0.17 me/kg to 11.37 me/kg (Table 4.7).

All the soils from Uriarra and samples 4, 5 and 6 from Cotter (Table 4.7) were excluded from selection due to their high organic matter content. From the remaining 4 soils (No. 1, 2, 3 and 7), organic matter was lowest (0.40 g/100 g) in soil 3, but its Al content was much lower than the others and therefore this soil was also excluded; emphasis in this experiment was on high Al content. Samples 1 and 2 had lower Al contents. Sample 7 had the highest level of Al (66.00 me/kg) and lowest pH_w (5.35). Therefore sample 7 (site 3, depth 20 - 30 cm, compartment 152) was selected for use in this experiment.

This soil is a yellow earth developed on Paddys River volcanics which include dacite and mudstone. The site is located at the Blue Range, Brindabella, A C T about 25 km west of Canberra (35°17"S and 148°52"E). The site is at an altitude of 850 m, and has a slope of approximately 3° to 5° to the south-west (location shown on Figure 4.1).

Table 4.7. Charactersitics of the soils from which the soil for the liming experiment was chosen (Section 4.3).

Location and depth (cm)	Sample No	pH_w	pH_s	Organic matter (g/100 g)	Ca (me/kg)	Al (me/kg)	Ca/Al ratio	Mn (me/kg)	Mg (me/kg)
Compartment 152									
1(10-20)	1	5.43	3.74	1.95	0.17	49.85	0.00	0.04	4.20
1(20-30)	2	5.63	4.01	1.09	0.02	42.48	0.00	0.02	7.08
1(30-40)	3	5.67	4.07	0.40	0.01	31.44	0.00	0.00	11.37
2(10-20)	4	5.58	4.10	3.17	0.15	53.23	0.01	0.16	1.45
2(20-30)	5	5.62	4.18	2.17	0.08	43.48	0.00	0.10	1.98
3(10-20)	6	5.51	4.12	2.71	0.06	47.23	0.00	0.02	4.51
3(20-30) *	**7**	**5.35**	**4.04**	**1.69**	**0.18**	**66.00**	**0.002**	**0.04**	**4.02**
Uriarra									
1(10-20)	8	5.28	3.88	5.34	0.26	48.83	0.01	0.06	1.16
1(20-30)	9	5.13	3.97	4.69	0.17	48.90	0.00	0.04	1.11
2(10-20)	10	5.06	3.96	6.15	0.31	59.82	0.01	0.07	2.42
2(20-30)	11	5.09	4.03	5.21	0.07	57.10	0.00	0.05	1.80
3(10-20)	12	5.09	3.92	7.33	0.13	71.86	0.00	0.03	1.12
3(20-30)	13	5.24	4.04	5.64	0.02	57.57	0.00	0.01	0.78
3(30-40)	14	5.22	4.09	3.72	0.01	46.13	0.00	0.01	0.41
4(10-20)	15	5.02	3.97	7.88	0.09	65.42	0.00	0.03	1.56
4(20-30)	16	5.04	4.02	6.88	0.04	58.42	0.00	0.02	1.18
4(30-40)	17	5.06	4.08	5.78	0.01	44.41	0.00	0.01	0.93
5(10-20)	18	4.95	3.99	9.86	0.06	72.74	0.00	0.06	0.73
5(20-30)	19	5.02	4.09	8.14	0.19	59.78	0.00	0.06	1.01
6(10-20)	20	5.00	4.10	8.34	0.10	55.18	0.00	0.04	0.61
6(20-30)	21	5.09	4.20	3.95	0.01	35.48	0.00	0.02	0.17

* Soil selected for the experiment.

The soil collected was further analysed for exchangeable K and Na, available P, extractable mineral N, and the lime requirement and field capacity determined (Table 4.8). Available P in this soil was extremely low, extractable mineral N was 16.10 mg/kg and the lime requirement to achieve a pH of 6.0 was 8650 kg/ha. The percentage of Al saturation was also very high in this soil (91.24).

Table 4.8. Characteristics of the soil used for the liming experiment.

Properties	Values
Clay (g/100 g)	28.60
Organic matter (g/100 g)	1.69
pH_w	5.35
pH_s	4.04
Exchangeable Ca (me/kg)	0.18
Exchangeable Mg (me/kg)	4.02
Exchangeable Na (me/kg)	Tr.
Exchangeable K (me/kg)	2.08
Exchangeable Mn (me/kg)	0.06
Exchangeable Al (me/kg)	66.00
Extractable mineral N (mg/kg)	16.10
Available P (mg/kg)	Tr.
Lime requirement to attain pH 6.0	8650 kg/ha
Field capacity (g/100 g)	46.60
Ca (me/kg)/Al (me/kg)	0.006

4.3.2. The pot experiment

Soil was collected in bulk and transferred at one kg/pot (equivalent to 812 gm oven dry soil) to pre weighed polythelene bag lined plastic pots. The soil was treated with reagent grade $CaCO_3$ at 0, 4940 and 9880 kg/ha. This covered a range from deficient to excess lime requirement for this soil to reach a pH of 6.0. Thirty five day old seedlings of *E. camaldulensis*, *E. citriodora*, *E. gummifera* and *E. saligna* were transplanted at three seedlings/pot on 28/10/88. After one week in the shade, the pots were thinned to two seedlings/pot and were transferred to a glasshouse. During the growth period 60 mg N/kg soil was applied as urea in solution in two split doses of 30 mg N/kg soil each time.

Shoot heights were recorded during growth (Appendix 4.2) and at harvest. The seedlings were harvested after 8 months; shoots and roots were collected, washed in distilled water, dried in an oven at 70°C for 48 hours and weighed.

4.3.3. Results, discussion and conclusion

Table 4.9 includes the effects of liming on some growth parameters of the seedlings. Percentage increases in the growth parameters on the highest lime level are shown in the last column. Shoot height, shoot dry weight and the ratio (root dry weight)/(shoot dry weight) of *E. camaldulensis* was significantly affected ($p < 0.05$) by liming. Shoot height and shoot weight responded positively and increased from 3 to 5.3 cm and from 0.12 g/seedling to 0.2 g/seedling respectively. Although the absolute increases were small, percentage increases over control were quite high. The effect on root weight was very small and as shoot weight increased on liming, the root/shoot ratio decreased progressively from 1 to 0.7.

Table 4.9. The response of selected eucalypt species to liming.

Species	Growth parameter	Treatment			% rise in Lime 2 over control
		Control	Lime 1	Lime 2	
E. camaldulensis	Shoot height (cm)*	3.00	3.00	5.30	77
	Shoot weight (gm/seedling)*	0.12	0.13	0.20	67
	Root weight (gm/seedling)	0.12	0.11	0.11	(-) 8
	Total biomass (gm/seedling)	0.24	0.24	0.31	29
	Root/shoot*	1.00	0.85	0.55	(-) 45
E. citriodora					
	Shoot height (cm)**	10.50	9.90	14.50	38
	Shoot weight (gm/seedling)	0.32	0.22	0.33	3
	Root weight (gm/seedling)	0.18	0.15	0.23	28
	Total biomass (gm/seedling)	0.50	0.37	0.56	12
	Root/shoot	0.56	0.68	0.70	25
E. gummifera					
	Shoot height (cm)**	9.20	6.60	8.10	(-) 12
	Shoot weight (gm/seedling)	1.08	0.87	1.06	(-) 2
	Root weight (gm/seedling)	0.52	0.41	0.40	(-) 23
	Total biomass (gm/seedling)	1.61	1.28	1.46	(-) 9
	Root/shoot	0.48	0.47	0.38	(-) 21
E. saligna					
	Shoot height (cm)	4.00	4.50	5.80	45
	Shoot weight (gm/seedling)	0.06	0.06	0.10	67
	Root weight (gm/seedling)	0.07	0.04	0.05	(-) 29
	Total biomass (gm/seedling)	0.13	0.10	0.15	15
	Root/shoot	1.17	0.67	2.00	71

** and * indicate significant differences among treatments at < 1% and < 5% levels respectively.

In the case of *E. citriodora* shoot height was affected significantly ($p < 0.01$) by liming and it increased from 10.5 cm in the control to 14.5 cm under lime at level 2. Other growth parameters also increased at the highest lime level, but the differences were not statistically significant. The lower level of increased lime resulted in a decrease in all growth parameters.

In the case of *E. gummifera*, all growth parameters responded negatively to liming. However, only the effect on shoot height was significant ($p < 0.01$) decreasing from 9.2 cm in the control to 8.1 cm at the highest lime level. Thus this species responds negatively to high lime contents in soils.

The growth of *E. saligna* was similar to that of *E. camaldulensis*, responding positively to liming. Most growth parameters of *E. saligna* showed an increase with increasing lime. The increases were however, smaller than those of *E. camaldulensis* in both absolute values and percentage terms and were not statistically significant for any of the parameters.

In the present experiment, some growth parameters of *E. camaldulensis* responded both positively and significantly. Since the percentage increase in these growth parameters were high, this species was considered to be more sensitive to acid soil factors than the other three species. Further, it is an important species grown extensively in overseas tropical areas. Therefore, *E. camaldulensis* was selected for future experiments.

The shoot weights of *E. camaldulensis* in this soil were much less when compared to the acid soils described in section 4.2. The reason for this difference probably lies in the soil properties. The present soil is much more acidic, its exchangeable Al and % Al saturation are much higher and available P and exchangeable Ca much lower. The shoot dry weight of *E. camaldulensis* was much less than that of *E. gummifera* and *E. citriodora* in this soil whereas its performance in the two most acidic soils of the previous experiment (section 4.2) was substantially better compared to *E. gummifera* and *E. citriodora*. Therefore, *E. camaldulensis* is more sensitive (than *E. gummifera* and *E. citriodora*) to soil conditions

in this soil compared to the conditions in the previous experiment (i.e., high Al, low P and Ca).

The response to lime varies widely from species to species but generally demonstrates positive effects on the growth of plants in acid soil. For example, Carvalho et al (1980) reported a positive lime response in six *Stylosanthes* species; Hoyt and Nyborg (1987) reported a positive lime response in barley, rape, red clover and alfalfa and Sing et al (1986) reported the same in maize. These lime responses are usually considered to be the result of a decrease in Al concentration in soil. Reports on the effect of liming as such are not available for tree species although a population of *E. obliqua* from an acidic soil provenance showed severe chlorosis, slow growth and low survival rates when grown in more alkaline calcareous soil, but grew very well on fertile acid soil (Anderson and Ladiges, 1978).

On the otherhand there are also reports of lime induced yield depressions in maize (Farina et al, 1982; Friesen et al, 1980). In some cases yield depressions have been attributed to lime induced P deficiency (Sumner, 1979) although deficiencies of Mg, K and some trace elements have also been reported (Sumner et al, 1978).

Organic matter acts as an important Al source in highly weathered soils (Juo and Kamprath, 1979), therefore an increase in the decomposition of Al rich organic matter may release Al to bind soil P. This may cause an imbalance in P nutrition. Also Hargrove (1986) has shown that the Al organic matter complex may be solubilized in the pH range 5 to 7 making released Al available to plant roots and thus cause toxicity.

These studies have shown contrasting performances of different species to liming. However they were based on widely different experiments. The present research shows that the response to liming is quite different for various eucalypt species on different soils. This confirms the fact that contrasting results observed in other studies were due to both differences in the soil and experimental conditions and to the ways in which individual species respond to otherwise similar conditions.

4.4. The response of eucalypt species to soil moisture stress

The analysis of the response of eucalypt species to soil moisture stress consists of two experiments. First, the four eucalypt species for which the lime response was examined (section 4.3) were tested for their response to soil moisture stress in the same acid soil without any amendments. In the second experiment the response of the selected species, *E. camaldulensis*, to soil moisture stress was examined in the same soil after the soil was amended with lime and the addition of P.

4.4.1. The response of four eucalypt species to soil moisture stress

4.4.1.1. Material and methods

One kilogram of air dry (812 g oven dry weight), sieved (5 mm mesh size) soil was transferred to each pre weighed bag inside a plastic pot. A plastic access tube (16 mm internal diameter) was inserted vertically in the centre of each pot before packing the soil. The lower ends of the tubes were filled with cotton wool to prevent soil entry and the upper ends were covered with a cap to prevent evaporation via the tube. As it is difficult to water soils in pots to a uniform moisture regime which is below field capacity, the plastic access tube was used to overcome problems (Bachelard, 1986) when moisture stress was implemented. Five

week old seedlings of *E. camaldulensis, E. citriodora, E. gummifera* and *E. saligna* were transplanted at three seedlings to a pot. After establishment, the seedlings were thinned to two per pot. After 12 weeks, 30 mg N/kg soil was applied in solution as urea. After another 15 weeks, a further 30 mg N/kg soil was applied in the same way. The soil surface of the pots were covered with about 5 mm of plastic pellets as a mulch.

Three pots were allocated to each of the four moisture stress levels 23 weeks after transplantation. At this stage shoot heights were measured and the pots were selected to provide seedlings of identical heights and vigour for each moisture stress level. All the pots were watered to field capacity. The estimated pot weights required for specified moisture levels of 40, 53, 67 and 80% of field capacity were calculated. Thereafter the pots were watered daily to moisture stress weights. About half the water was added through the tube and the remaining half was added on the soil surface. Daily losses of water were recorded for the levels and an average minima of 35.5, 46.5, 58.4, 70.1% (of field capacity) respectively were reached before watering. Shoot heights were recorded before the moisture stress was imposed and then every two weeks until harvest at 18 weeks after the moisture stress began (Appendix 4.3). The seedlings were cut at the soil surface, the shoots were washed in distilled water and dried in an oven for 48 hours at 70^{o}C. The roots were washed out of the soil and then dried in the same manner.

4.4.1.2. Results and discussion

The parameters used to evaluate the effects of soil moisture stress on the eucalypt species were shoot height at harvest, shoot dry weight, total seedling biomass and the ratio root/shoot (Figure 4.2). The species differed in their response to moisture stress. At the lowest moisture level, average shoot height ranged from 2.9 cm to 3.7 cm for *E.*

camaldulensis, 11.7 cm to 14.6 cm for *E. citriodora*, 6.8 cm to 7.8 cm for *E. gummifera* and from 4.8 cm to 5.2 cm for *E. saligna* (values of shoot height before the beginning of moisture stress are not shown).

Shoot height of all the species increased slightly with an increase in soil moisture level except for *E. citriodora*, in which shoot height declined beyond a field capacity of 67% (i.e., at 80%). Shoot dry weight and total seedling biomass increased in *E. camaldulensis* and *E. saligna* with an increase in the soil moisture level. Shoot weight and total biomass increased first, and then declined beyond 67% and 53% of field capacity for *E. citriodora* and *E. gummifera* respectively (i.e., 80% and 67% respectively). In the case of *E. gummifera* both shoot weight and total biomass declined with moisture levels at 67% or wetter and in the case of *E. citriodora* these parameters declined beyond a moisture level of 53% of field capacity. In *E. citriodora* and *E. saligna* total root weight decreased at higher moisture levels as did the root/shoot ratio which declined for all species. A decrease in root/shoot ratio resulted from an increase in moisture level; it was greater in absolute terms in *E. camaldulensis* and *E. saligna* than for the other two species.

In general, growth of all the species was very poor and they produced a low biomass even over the long growing period. Some of the *E. camaldulensis*, *E. citriodora* and *E. gummifera* and many *E. saligna* seedlings showed some physiological disorders. The disorders occurred in older leaves and after about 12 weeks from transplantation. The symptoms were brown to dark brown spots in older leaves; in a few cases edges of leaves became purple. In *E. gummifera* some leaves became yellowish and in *E. saligna* some developed signs of necrosis and purple edges.

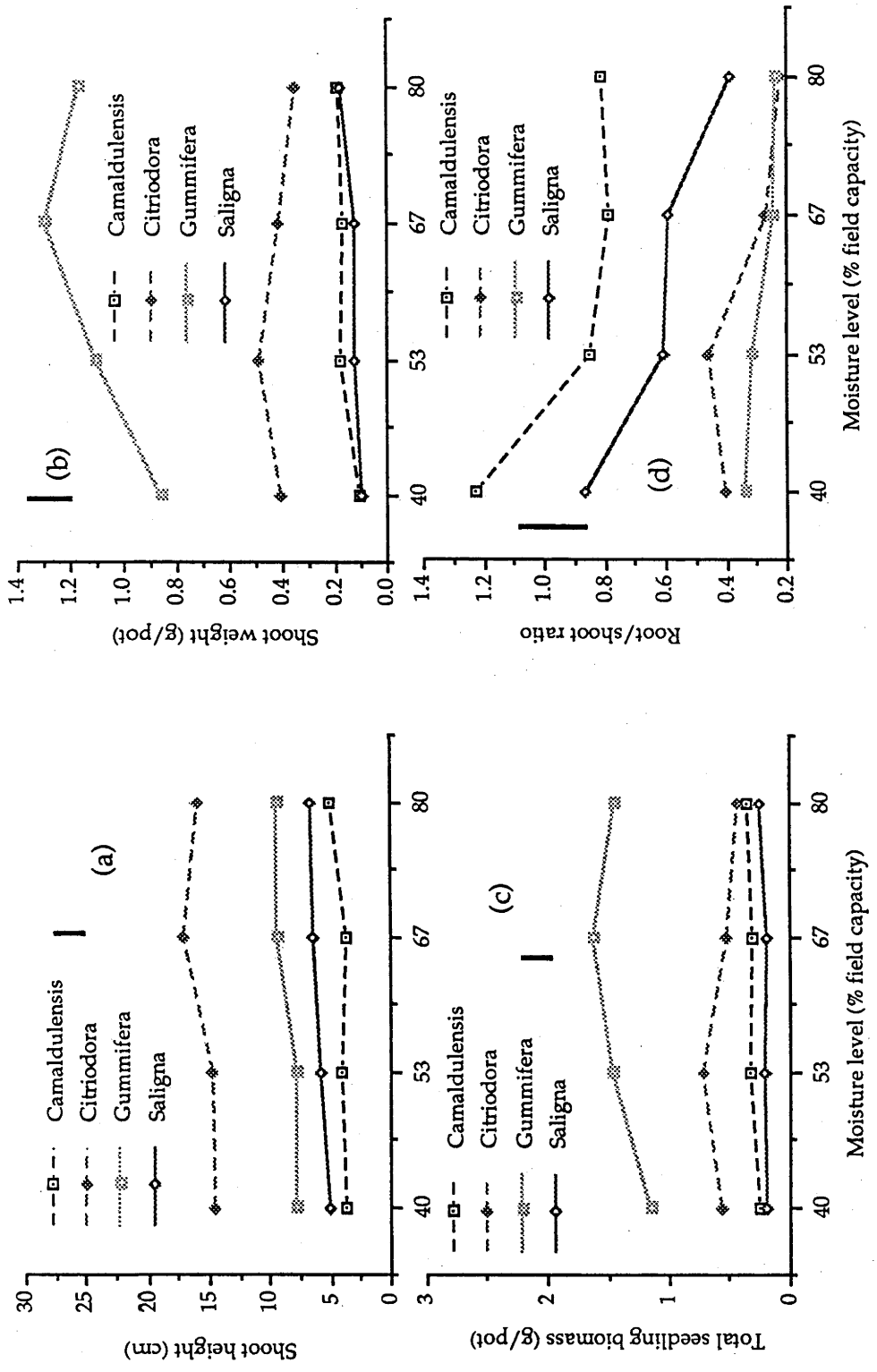


Figure 4.2. Effect of soil moisture stress on the growth parameters of *E. camaldulensis, E. citriodora, E. gummifera* and *E. saligna;* (a) shoot height, (b) shoot weight, (c) total biomass and (d) root/shoot ratio. Vertical bars represent l.s.d. ($P < 0.05$).

The generally very poor growth of seedlings, may have concealed the small response of the species to soil moisture stress. Therefore, to further study the influence of soil moisture stress, only *E. camaldulensis* was selected for later experiments. This time the moisture stress was imposed after the growth was boosted by adding lime, P and N to the soil.

4.4.2. The response of *E. camaldulensis* to soil moisture stress

4.4.2.1. Materials and methods

This experiment was conducted along similar lines to the previous moisture stress experiment. The differences were that there was only one species but at five moisture levels and seedling growth was boosted by adding lime and P to the soil. Lime was added to the soil at a rate of 9880 kg pure $CaCO_3$/ha and thoroughly mixed. After three wetting and drying cycles which took about two weeks and then thorough mixing, one kilogram of air dry soil (equivalent to 852 gm oven dry weight) was transferred to each pre weighed bag inside a plastic pot. A plastic access tube was inserted vertically. Three week old *E. camaldulensis* seedlings were transplanted, three seedlings per pot. After establishment, the seedlings were thinned to two per pot and 100 mg P and 45 mg N/kg soil were added in the form of $NH_4H_2PO_4$ dissolved in water. After 5 weeks, a second dose of N at 35 mg N/kg soil was applied in the form of NH_4NO_3. The soil surface of the pots were covered with approximately 5 mm of plastic pellets as mulch.

Nine weeks after transplantation 15 pots with healthy and uniformly sized seedlings were selected. Three pots were allocated to each of the 5 moisture stresses so that identical heights and vigour were

represented in the different moisture stress levels. Watering of pots proceeded in the same way as earlier experiments except that the specified moisture levels were now 40, 50, 60, 70 and 80% of field capacity. Daily losses of water were recorded and for these same levels an average minimum of 35, 43, 48, 56 and 63% of field capacity respectively was reached just before watering. Shoot heights were recorded before the stress was imposed and then every two weeks until harvest (Appendix 4.4).

Seedlings were cut off at the soil surface after 6 weeks of stress, washed in distilled water and dried in oven for 48 hours at 70°C. Roots were washed free of soil, cleaned and fine root lengths were measured using the 'Comair root length scanner' before drying in an oven.

4.4.2.2. Results and discussion

Soils were analyzed after seedlings were harvested and found to have a pH_s of 4.99, exchangeable Ca of 66.9 me/kg and exchangeable Al of 8.9 me/kg. To evaluate the effects of soil moisture stress on *E. camaldulensis* growth seedling parameters examined included shoot height at harvest, shoot weight, root weight, fine root length, total biomass and root/shoot ratio (Figure 4.3). Shoot height, shoot weight and root weight all increased as moisture levels increased. At a moisture level of 40% of field capacity the seedlings did not die, but the most recently initiated leaf pairs in those pots died and shoot height increments almost ceased. Overall growth of the seedlings in this treatment was almost completely inhibited after the moisture stress was applied. All moisture levels over 40% and up to 60% of field capacity resulted in a steep rise in shoot height, shoot weight, total biomass and fine root length. Increases in seedling growth parameters between 60 and 80% field capacity were less steep. Between 70 and 80% all parameters

except shoot height almost levelled out. Fine root length showed a decline beyond the 60% field capacity level.

In the previous experiment (Section 4.4.1), the response of growth parameters to soil moisture stress were found to be much lower in *E. camaldulensis*. One important reason for this is that the overall growth of seedlings in the unamended soil was very poor. *Eucalyptus camaldulensis* responded much more strongly to soil moisture stress in this experiment, due to boosting growth by applying P and lime.

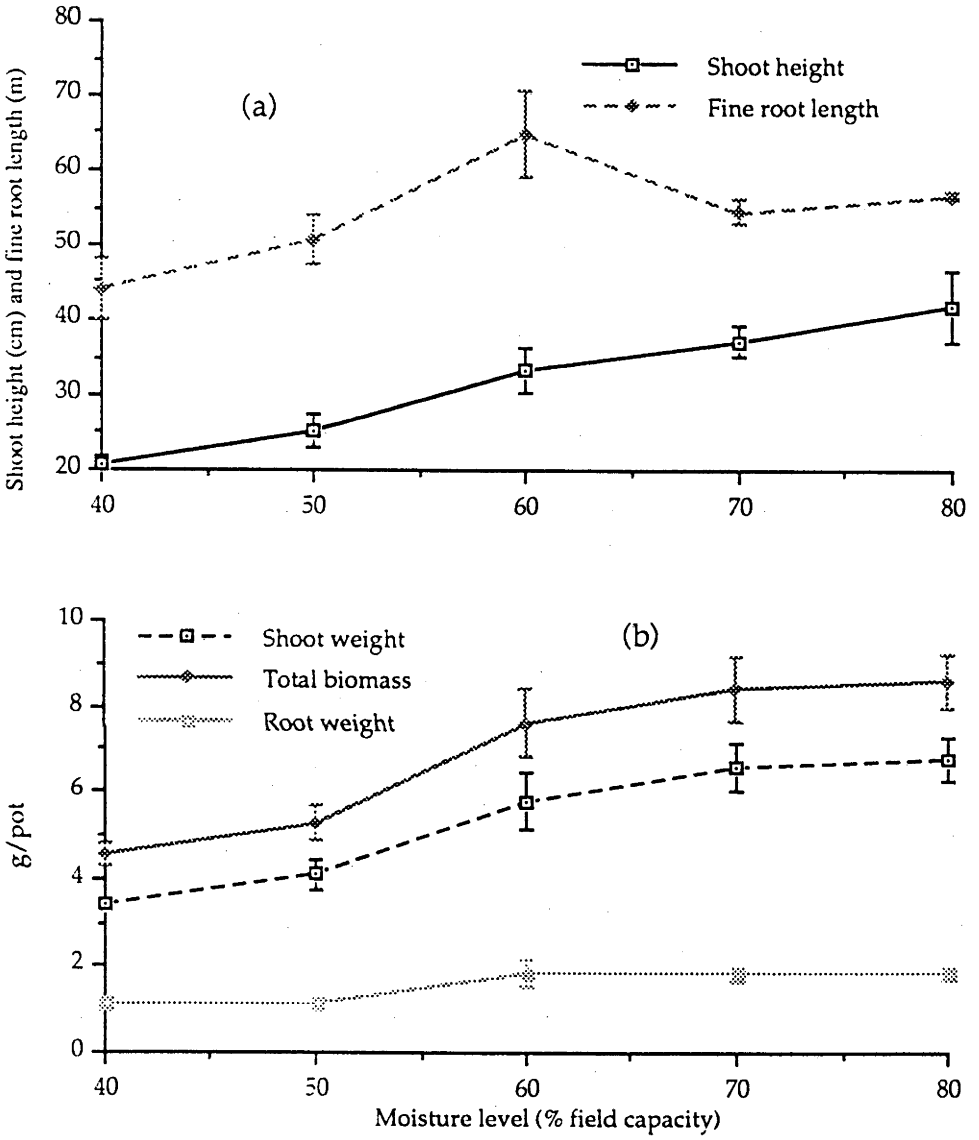


Figure 4.3. Effect of soil moisture stress on the growth paramaters of *E. camaldulensis* (a) Shoot height and fine root length and (b) shoot weight, root weight and total biomass. Bars represent SE of the mean; in some cases they are small and lie within the symbol.

CHAPTER 5

IDENTIFICATION OF NUTRIENT DEFICIENCY FOR *E. CAMALDULENSIS* IN THE SELECTED SOIL

5. IDENTIFICATION OF NUTRIENT DEFICIENCY FOR *E CAMALDULENSIS* IN THE SELECTED SOIL

5.1. Introduction

The growth of the four selected eucalypts (*E. camaldulensis, E. citriodora, E. gummifera and E. saligna*) was extremely poor in the acid soil (Section 4.3) despite amendment with N and different rates of lime. Therefore, it became important to examine what the nutrient limiting factors for the growth of *E. camaldulensis* were in this soil. This information is useful for boosting the growth of the seedlings for other experiments. The nutrients which may improve the growth of *E. camaldulensis* will probably show an additional interaction with Ca and thereby stimulate the growth.

Therefore there were two experimental phases: In the first the response of *E. camaldulensis* to different nutrients which are usually deficient in acid forest soils (P, K, S and Mo) was examined. In the second, nutrients found to stimulate *E. camaldulensis* growth in the first phase, were applied in combination with Ca.

5.2. The response of *E. camaldulensis* to phosphorus, potassium, sulphur and molybdenum

5.2.1. Materials and methods

Characteristics of the soil used were reported previously (Table 4.8). Soil was transferred to plastic pots at equivalent amounts to 315 gm oven dry soil per pot and six week old *E. camaldulensis* seedlings were transplanted. Four seedlings were planted per pot and pots replicated three times. Ten days after transplantation, an initial fertilization using solutions containing nutrients were added (Table 5.1). The chemical salts used for P,

K and S also contained N and this was included in the calculations for the amount of NH_4NO_3 required. The pots were transferred to a phytotron. Conditions for growth included 16 hour day length and day and night temperatures of 25 and 15°C respectively. After 7 weeks a second fertilization, this time containing only N was applied in solution at 40 mg N/kg soil. Twelve weeks after transplantation the seedlings were harvested at ground level, washed and dried in the oven at 70°C for 48 hours and oven dry weight recorded.

Table 5.1. The treatment levels applied and chemicals used.

Nutrient	Treatment levels (mg/kg soil)			Chemical used (A.R.)
	Control	T 1	T 2	
P	0	10	50	$NH_4H_2PO_4$
K	0	40	80	KNO_3
S	0	10	40	$(NH_4)_2SO_4$
Mo	0	2	10	$Na_2MoO_4.2H_2O$
N	100	100	100	NH_4NO_3

5.2.2. Results

Average weights of shoot produced for different treatments during the 12 weeks are given in Table 5.2. None of the K, S and Mo treatments showed significantly different values from the control. Application of 10 and 50 mg P/kg soil increased shoot weight production to 0.156 and 0.325 g/seedling respectively (from 0.041 g/seedling for the control). Application of 50 mg P/kg soil resulted in an increase of about eight fold in shoot weight production. No visual deficiency symptoms were observed for any seedling during this short period of growth. On the basis of these results, the response of *E. camaldulensis* to P applications was tested in factorial combinations with Ca in the second phase of this experiment.

Table 5.2. Effect of P, K, S and Mo treatments on the shoot weights (g/seedling) of the *E. camaldulensis* seedlings.

Nutrient	Treatment			F-ratio
	Control	T1	T2	
P	0.041	0.156	0.325	29.41**
K	0.041	0.045	0.043	0.26
S	0.041	0.036	0.039	1.82
Mo	0.041	0.039	0.038	0.20

** indicates that the F ratio is significant at < 1% level.

5.3. The response of *E. camaldulensis* to calcium and phosphorus

5.3.1. Materials and methods

5.3.1.1. Soil calcium treatment

Calcium was added in this experiment from a combination of $CaCO_3$ and $CaSO_4$ at a ratio of 2 : 1 for all the Ca treatments (on the basis of findings presented in Chapter 3). Levels of Ca used were equivalent amounts of Ca to 0, 5,000, 10,000 and 15,000 kg pure $CaCO_3$/ha. These treatment levels will be referred to as Ca_0, Ca_5, Ca_{10} and Ca_{15} respectively in subsequent discussion.

The required amounts of Ca were added to air dry soil and thoroughly hand mixed. Soils were wetted to field capacity inside plastic pots (bottom sealed) in the glasshouse and allowed to dry and mixed again. This wetting and drying was repeated three times over a period of 11 weeks. Soil samples from each Ca treatment level were collected and stored in the cold room until the completion of analyses.

5.3.1.2. The pot experiment

At the end of 11 weeks, soils from each of different bulk treatments were mixed (separately) and transferred to plastic pots at a rate equivalent to 315 gm oven dry soil/pot. Four week old *E. camaldulensis* seedlings from same seedlot (Chapter 4) were transplanted, three seedlings per pot on 23/7/89.

5.3.1.3. Treatment with phosphorus

Twelve days after transplanting, P was applied to each pot at rates of 0, 10, 25, 50 and 100 mg P/kg soil (oven dry basis) in the form of $NH_4H_2PO_4$ dissolved in water. At this stage seedlings were of a similar size and vigour in all P treatments. Because all pots received different amounts of N (45% of P) corresponding to the different P levels from $NH_4H_2PO_4$; supplementary N was applied using NH_4NO_3 to make total application of N up to 45 mg N/kg soil. After four days of P treatment the pots were transferred to the phytotron under conditions of 16 hours day length and day and night temperatures of 25°C and 15°C respectively. A second dose of N (45 mg N/kg soil) was added 6 weeks after transplanting (NH_4NO_3). Seedlings were harvested 10 weeks and 4 days after transplantation.

5.3.2. Results

The effects on the shoot height and shoot dry weight of Ca and P application are presented in Figure 5.1 while the effects on root weight and total seedling biomass are presented in Figure 5.2. There is a similarity between responses to Ca and P treatments for all the growth parameters. Responses to the P treatments were different in the presence or the absence of Ca. In the presence of Ca (all levels) all the growth parameters increased sharply up to 25 mg P/kg soil treatment. Beyond

that, the increase in growth parameters was small up to 50 mg P/kg soil and after that increases were negligible. There were even small decreases in the shoot weight, root weight and total biomass beyond 50 mg P/kg soil when the P treatment was accompanied by Ca. In contrast to these, when P was applied alone, growth parameters increased consistently even up to the highest P level, producing almost straightline relationships (Figures 5.1 and 5.2).

In general, a large increase in all the growth parameters were recorded for the $Ca_{10}P_{50}$ treatment. This combination resulted in a maximum for both shoot yield and total seedling biomass production. When accompanied by Ca, the application of P beyond 50 mg P/kg soil resulted in a decline of total biomass yield. In some cases other parameters slightly increased . All the growth parameters were significantly different (at varying levels) for different Ca and P treatments (Table 5.3).

Table 5.3. F ratios for the ANOVA on the effects of Ca and P treatments on growth parameters of *E. camaldulensis* seedlings.

Parameters	Between Ca	Between P	Between Ca x P
Shoot height	16.94**	112.26**	2.36*
Shoot weight	27.13**	125.20**	3.44**
Root weight	14.77**	111.18**	2.49*
Total biomass	27.38**	136.50**	3.37**
Root/shoot	6.14**	10.20**	1.86

** and * indicate significance levels of < 1.0% and < 5.0% respectively.

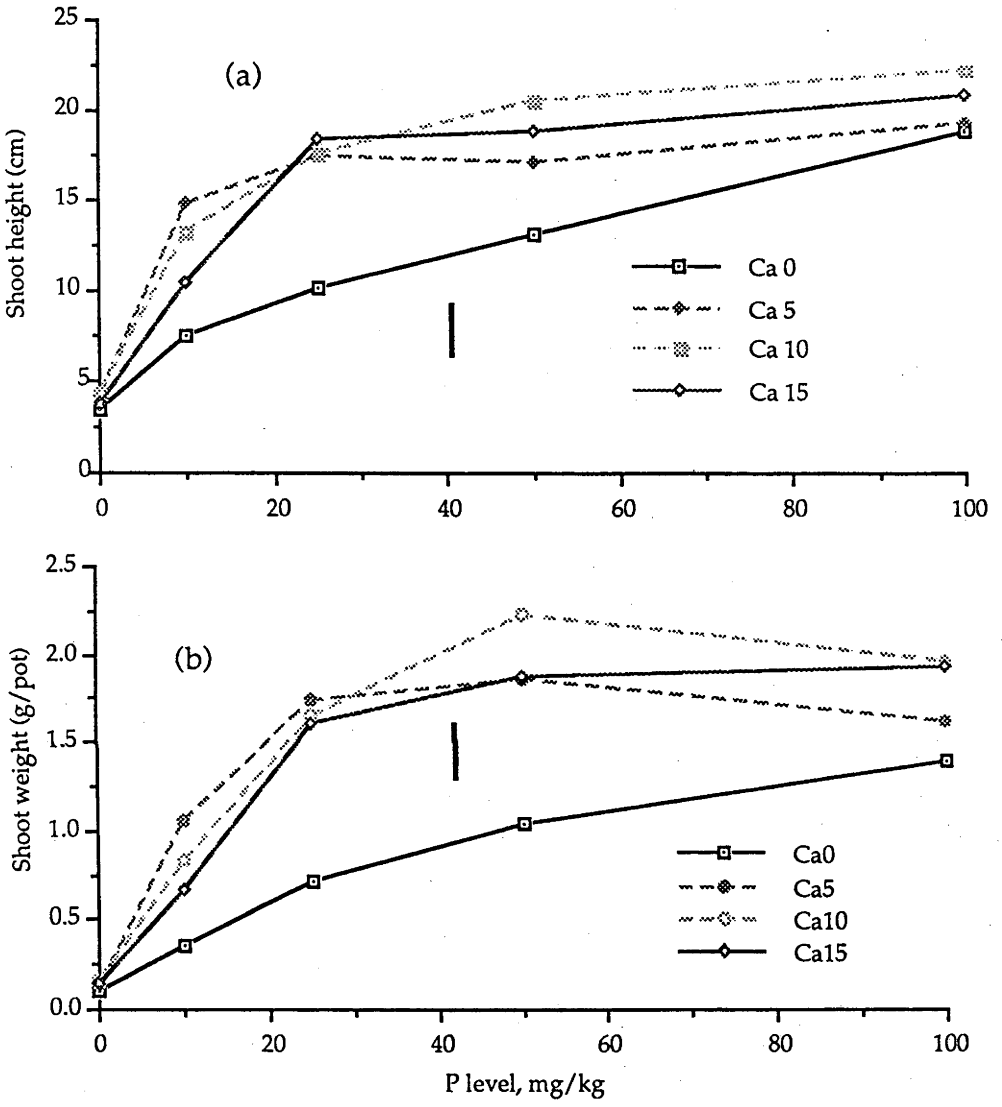


Figure 5.1. Effect of P at different Ca levels on the (a) shoot height and (b) shoot weight of *E. camaldulensis*. Vertical bars represent l.s.d. ($P < 0.05$).

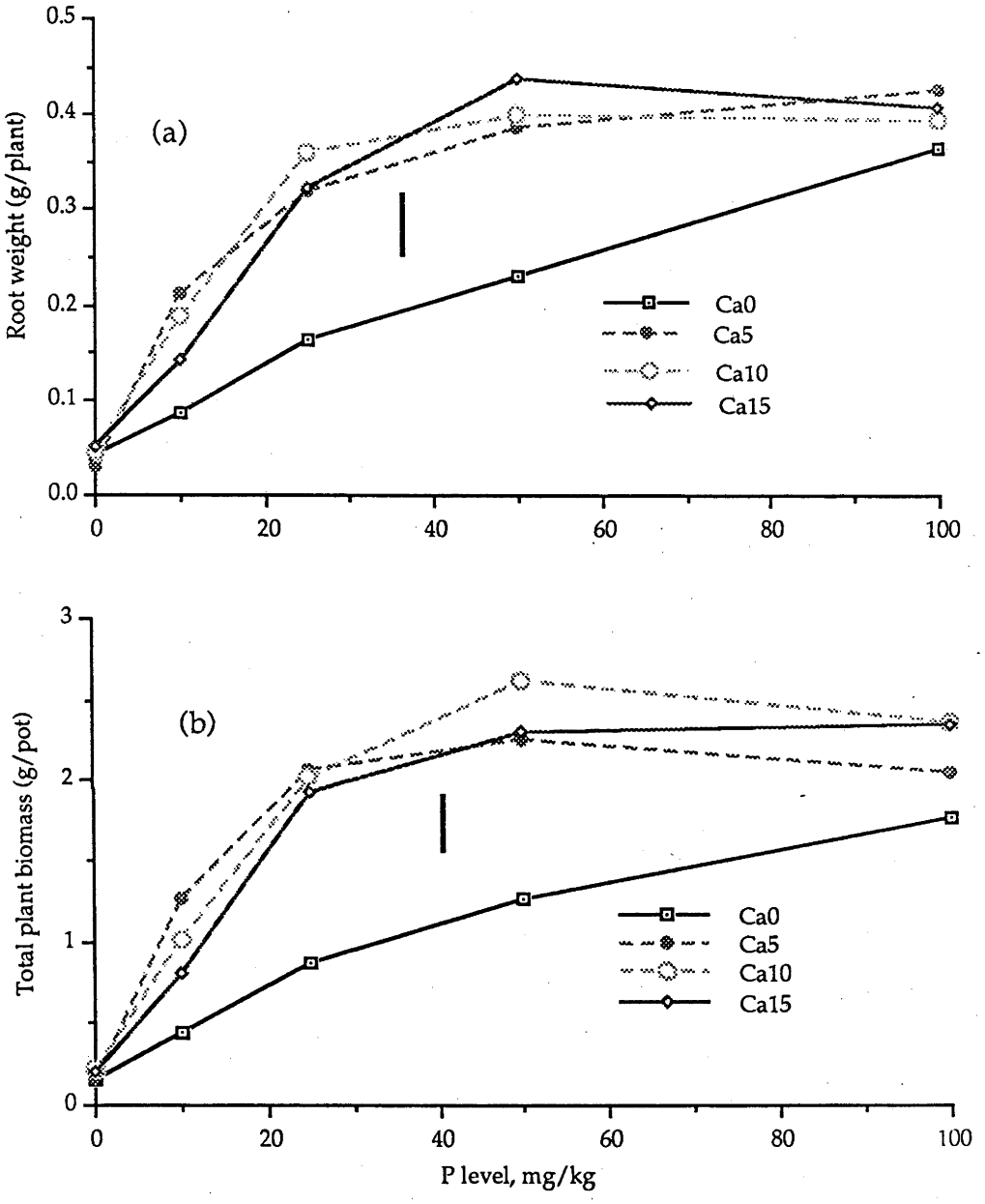


Figure 5.2. Effect of P at different Ca levels on the (a) root weight and (b) total biomass of *E. camaldulensis*. Vertical bars represent l.s.d. ($P < 0.05$).

Results of the chemical analyses of soil 11 weeks after the Ca treatment are presented in Table 5.4. They are quite similar to those presented in Chapter 3. With an increase in the level of Ca, pH_s, EC and exchangeable Ca increased consistently and exchangeable Al decreased consistently. There was very little effect on exchangeable Mg, K and Mn. It may be mentioned that Ca treatment is not expected to have any effect on soil physical conditions within such a short time.

Table 5.4. Effect of Ca treatments on soil chemical characteristics.

Treatments	pH_s	EC mS/cm	Exchangeable cations (me/kg)				
			Ca	Al	Mn	Mg	K
Ca_0	4.01	10	0.5	45.8	0.08	3.8	3.0
Ca_5	4.44	97	32.9	27.2	0.09	4.5	3.0
Ca_{10}	4.81	240	70.9	13.8	0.05	4.6	2.9
Ca_{15}	5.22	585	110.2	1.2	0.03	3.9	2.9

5.3.3. Discussion and conclusion

Plant growth responses to Ca treatments in a low Ca acid soil may vary (Chapter 2). In the present experiment, the soil Ca level was very low (exchangeable Ca in control was 0.5 me/kg soil). But the response of *E. camaldulensis* seedlings to an addition of Ca alone was also small. Available soil P was also extremely low (below detection limits) in the soil and the addition of P alone gave a marked response.

When Ca treatments were accompanied by additional P, even greater increases in growth were recorded. Therefore, many low Ca, acid soils may appear to have adequate Ca (Kamprath, 1978) but when overall plant growth is considered, an application of other nutrients, may make

the Ca contents inadequate. In the present experiment the seedlings did not seem to suffer from Ca deficiency as such, since Ca alone did not increase *E. camaldulensis* growth to any extent, but increased addition of Ca to soil resulted in a decrease in exchangeable Al and an increase in soil pH (Table 5.4). These changes may have enhanced growth because P became effective in growth earlier in treatments receiving both Ca and P. But when P was applied alone, it had to counter the toxic effect of Al (Blamey et al, 1983; Alva, 1986) and then improve growth. Therefore, the effect of P was slow when applied in isolation. Dell et al (1983) examined the response of 5 glasshouse grown eucalypt species to P in clay (pH 4.0) treated with or without lime. They reported that in the presence of a complete fertilizer, except for lime and P, seedlings made poor growth. Application of P (as calcium phosphate) promoted root and shoot growth in all species. Lime alone had no positive effect. Their $CaPO_4$ treatment may be compared to the combined Ca and P treatment in the present experiment and the responses were also both very high.

It was concluded from the two experiments described in this chapter that in addition to N, application of Ca equivalent to 10,000 kg $CaCO_3$/ha accompanied by 50 mg P/kg soil will boost the growth of *E. camaldulensis* in this acid soil.

CHAPTER 6

THE RESPONSE OF *E. CAMALDULENSIS* TO DIFFERENT CALCIUM AND ALUMINIUM LEVELS IN NUTRIENT SOLUTION

6. THE RESPONSE OF *E. CAMALDULENSIS* TO CALCIUM AND ALUMINIUM LEVELS IN NUTRIENT SOLUTION

6.1. Introduction

In acid soils Al toxicity and Ca deficiency are the two main factors limiting the growth of many plants (Chapter 2). Experiments described in Chapter 5 showed that the growth of *E. camaldulensis* in an Al rich acid soil improved significantly with the addition of Ca. Detailed analyses are therefore required to examine the effects of Al and Ca on the growth of *E. camaldulensis;* one of the objectives of the present study. It is also necessary to establish suitable indicator(s) describing the phytotoxicity of Al in *E. camaldulensis,* since these are not well defined. Seedling growth in nutrient solution media was chosen for this study since it made manipulattion of treatment levels more efficient without affecting other elements. More importantly it made monitoring of roots during the growth of the seedlings more practical.

6.2. Materials and methods

6.2.1. Treatments

This experiment had four Ca (5, 10, 50 and 100 mg/l, equivalent to 0.125, 0.25, 1.25 and 2.5 mmol) and five Al (0, 0.25, 2.5, 20 and 50 mg/l, equivalent to 0, 0.009, 0.09, 0.74 and 1.85 mmol) treatments in combination. There were four replicates per treatment in each of 20 treatment combinations. In addition four bottles were kept as blanks to determine water loss through evaporation. The nutrient solution used for this experiment was a slight modification of Hoaglands' solution (Chapter 3, Table 3.1) which was successfully used by Thomson (1988) for *E. camaldulensis* and related species. Final nutrient solutions were

made up in 10 litre buckets from concentrated solutions (Table 6.1) using deionized water (EC = ~ 2 μS/cm). Aluminium in the solution came from reagent grade $AlCl_3.6H_2O$ and Ca from $Ca(NO_3)_2.4H_2O$. The normal Ca concentration of the nutrient solution was 80 mg/l. Therefore, when the Ca level was lower in the treatment, consequently N was also lowered. The difference in the amount of N was compensated for by adding N in the form of NH_4NO_3 and to get higher Ca levels extra Ca was added in the form of $CaCl_2.2H_2O$ so that the total concentration of N remained the same throughout the treatments while varying the Ca concentrations. The pH of the nutrient solutions varied from 5.39 to 3.06. Nutrient solutions were changed initially after 11 days and then the period between changes was reduced to 7 days as the seedlings grew in size.

6.2.2. Glasshouse conditions and experimental set up

On 26/6/89 two six week old seedlings were transferred to each 2.5 litre plastic bottle containing nutrient solution (half normal strength). Round holes were cut into the screw cap lid of the plastic bottle to accommodate corks which provided mechanical support for the seedlings. Another small hole was made in the lid to allow a polythene tube for aeration. The seedlings were supported by wrapping a short section of the stem with cotton wool and then wedging the stem through one side of the cork which was pre cut for the purpose. After one week the nutrient solution was changed to full strength and the seedlings were thinned to one seedling per pot. After one more week of full strength nutrient solution, treatments were imposed. At this time seedling heights were measured and the mean height was made as uniform as possible by selecting pots across the various treatment combinations.

Table 6.1. The composition of nutrient solution for different treatment combinations.

Stock solutions	Requirements	ml stock per 10 L nutrient solution
Al stock for 0.25 mg/l Al:	1.12 gm $AlCl_3.6H_2O$/500 ml.	10
Al stock for 2.5 mg/l Al:	11.18 gm $AlCl_3.6H_2O$/500 ml.	10
Al stock for 20 mg/l Al:	89.42 gm $AlCl_3.6H_2O$/L.	20
Al stock for 50 mg/l Al:	89.42 gm $AlCl_3.6H_2O$/L.	50
Ca stock for 5 mg/l Ca:	3.69 gm $Ca(NO_3)_2.4H_2O$/500 ml 18.75 gm NH_4NO_3/500 ml	40
Ca stock for 10 mg/l Ca:	7.38 gm $Ca(NO_3)_2.4H_2O$/500 ml 17.50 gm NH_4NO_3/500 ml	40
Ca stock for 50 mg/l Ca:	36.90 gm $Ca(NO_3)_2.4H_2O$/500 ml 7.50 gm NH_4NO_3/500 ml	40
Ca stock for 100 mg/l Ca:	59.04 gm $Ca(NO_3)_2.4H_2O$/500 ml 9.19 gm $CaCl_2.2H_2O$/500 ml.	40
Composition of nutrients common to all treatments:		
Chemical	**Requirements**	**ml stock per 10 L nutrient solution**
KNO_3	75.83 g/L	20
$NH_4H_2PO_4$	28.76 g/500 ml	10
$MgSO_4.7H_2O$	30.81 g/500 ml	20
$MnCl_2.4H_2O$	0.197 g/L	10
$ZnSO_4.7H_2O$	1.15 g/L	10
$CuSO_4.5H_2O$	0.626 g/L	10
$Na_2MoO_4.2H_2O$	0.242 g/L	10
H_3BO_3	0.744 g/L	10
Fe EDTA*		4

* EDTA: 5.0 gm NaOH was dissolved in 800 ml distilled water. Then 33.2 gm EDTA (disodium salt) and 24.9 gm $FeSO_4.7H_2O$ were added, made up to 1L volume and aerated overnight.

The experiment was conducted during the winter months (July-August) in a glasshouse. The glasshouse had an average maximum day temperature of 26^0C and an average minimum night temperature of 18^0C during the tenure of the experiment. Extra light was provided in the morning and in the evening by fluorescent tubes to extend the day length to 12 hours. The nutrient solutions were aerated for 15 minutes every hour using an air pump.

When nutrient solutions were replaced, the lid of the bottle with the seedling in it was put aside and the weight of the bottle including solution was recorded. Each time, before the nutrient solution was changed, the weight of the bottle was recorded. Water loss was calculated as the difference between these two weights and then the loss of water from bottles which contained no seedling were subtracted from this difference to get the transpiration loss.

Shoot heights and root lengths were recorded for each seedling at the beginning of the treatment. Thereafter, shoot heights and root lengths were measured for about each two week interval on a date which coincided with the date for change of nutrient solution.

Seedlings were photographed twice: 25 days after the beginning of treatments and at harvest. Two seedlings from each treatment combination were photographed together. Some close up photographs of roots and shoots severely affected by Al treatment were also taken. Photographs taken at harvest were unfortunately damaged beyond use during development so those taken after 25 days are presented here. However, the same trends in treatment effects were followed.

The outline of the topmost expanded leaf of three seedlings of each treatment was traced at harvest. Leaf area and length were

measured from the tracing by an Image Analyser system using Sigma scan version 3.10 software on an IBM PC AT. The mean width of each leaf was calculated as leaf area/length.

6.2.3. Harvest

After 47 days of imposing treatments the seedlings were harvested. The shoots were partitioned into: lower leaves (3rd and 4th leaf pairs from the base), top two immature leaf pairs and the rest of the leaves and stems. Roots were harvested in two fractions: coarse (> 1 mm diameter) and fine (< 1 mm diameter). The number of root branches (> 1 cm in length) in the middle 5 cm of the primary roots were counted. Fine roots were cut into small pieces and total lengths were measured using the 'Comair Root Length Scanner'. Samples were dried at 70°C for two days before recording their weights.

6.2.4. Thin sections of roots

Root tips (3 - 4 cm long) were cut and preserved in formalin acetic acid (FAA). They were fixed in 2.5% glutaraldehyde, dehydrated and embedded in spruce resin. One micron transverse thin sections of the embedded roots were cut (at about 1 cm distance from the tip) with an ultramicrotome and stained with toluodine blue in Na phosphate buffer. The thin sections were examined under a light microscope and photographs were taken to show the effects of treatments on root anatomy.

6.2.5. Chemical analyses of harvested seedlings

Young leaves, mature leaves and fine roots from harvested seedlings were digested with HNO_3 and HCl. From the digest, P was determined by an automated procedure using ammonium vanadate. Calcium, Mg, K,

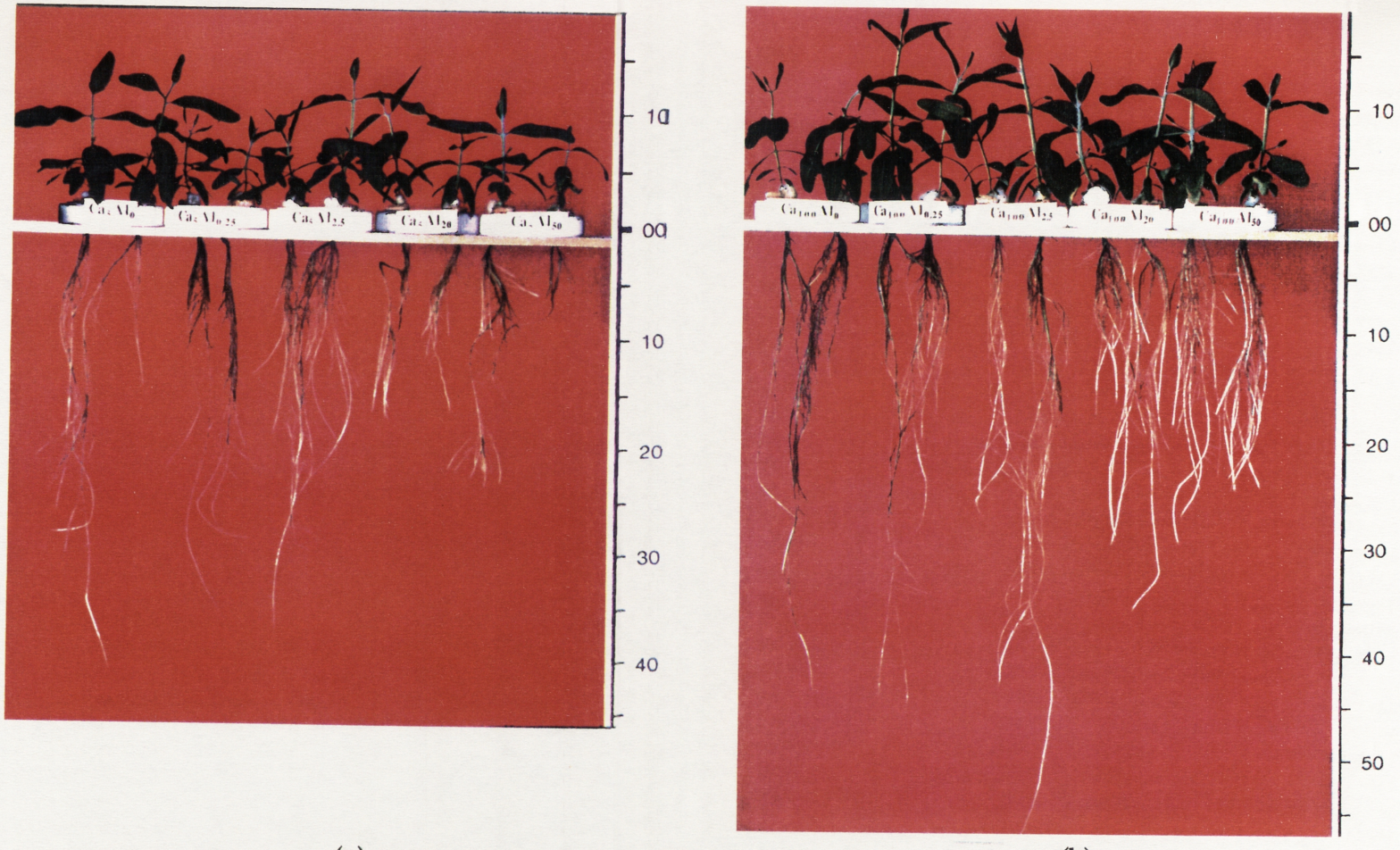
Mn, and Al were measured using an atomic absorption spectrophotometer (details of analytical methods are presented in Chapter 3).

6.3. Results

6.3.1. Effects of treatments on seedling growth

Plate 6.1. shows the effects of Al treatments on the growth of seedlings 25 days after treatment at two levels of Ca while varying Al levels: (a) 5 mg Ca /l and (b) 100 mg Ca/l. It can be seen from the plates that when the Ca level was low (Plate 6.1.a), growth of both shoot and root was severely affected with an increase in Al level, particularly at the two highest Al levels. At high Ca levels (Plate 6.1.b), the growth of shoot and root was restricted by the high Al level, but not as severely as in case of low Ca. Overall, shoot and root growth was better in the case of high Ca.

Plate 6.2 shows a close up of the adverse effects of Al on the seedlings at an Al concentration of 50 mg/l accompanied by (a) lowest (5 mg/l) and (b) highest (100 mg/l) Ca treatments. At low Ca levels (Plate 6.1 a) the roots of the seedlings were thickened and fine roots were lacking. At high Ca levels (Plate 6.1 b) seedlings had some fine roots and they also showed root branching. In both cases [(a) and (b)], mature leaves showed toxicity symptoms although the seedlings in (b) grew bigger in terms of both shoot and root size. Within about two weeks of imposing the treatments, symptoms of Al toxicity appeared in older leaves (3rd and the 4th leaf pairs from the base) in seedlings of the high Al treatment started to show loss of turgor at leaf tips and margins. Gradually this symptom progressed to the midrib after which the affected parts became necrotic. No apparent toxicity symptoms were observed in young leaves. This symptom may be assumed to be due to Al toxicity.



(a) (b)

Plate 6.1. *E. camaldulensis* seedlings after 25 days of treatment at varying Al levels (0, 0.25, 2.5, 20 and 50 mg/l) and constant Ca level, (a) 5 mg Ca /l and (b) 100 mg Ca/l.

Plate 6.2. *E. camaldulensis* seedlings at (a) Ca $_5$ Al $_{50}$ and (b) Ca $_{100}$ Al $_{50}$ treatments after 25 days of treatment.

Shoot growth was measured in terms of shoot height and shoot weight. Root related parameters were: primary root length, fine root length, fine root weight, ratio of (fine root length)/(fine root weight), and root branching. In addition, total biomass, ratio of shoot weight/root weight, transpiration, transpiration/total biomass ratio and leaf width were also examined. Shoot height and root length were measured 5 times during the growth period and these two parameters will be presented on a time scale. Other growth parameters were measured at harvest. Table 6.3 includes the F ratios of the ANOVA to examine the effects of Al and Ca treatments on various growth parameters. Since it was observed that the effects of Al and Ca were not proportional to their treatment levels, the effects of both linear and quadratic forms of Al and Ca were included in the ANOVA.

Shoot height and weight

Figure 6.1 is a graphical display of the shoot height of the seedlings under different Ca and Al treatments over the growing period. In figures treatment Al levels were used and ranges of corresponding $\Sigma a_{Al\ mono}$ values are are shown in Table 6.2. For convenience, one graph was plotted for each of the four Ca levels but they have the same l.s.d. bars. From the figure it can be seen that shoot height increased consistently over the growing period. Initially the shoot height increment was slow and for the period up to 14 days after the treatments began, there was little difference between shoot heights for various treatments. After that seedlings with low Ca and high Al lagged behind the other treatment combinations with respect to shoot height. At each Ca level, lower shoot heights were observed for increasing Al levels. The adverse effect of Al gradually declined as higher levels of Ca were applied. For example at a

Ca level of 5 mg /l, shoot height decreased from 39.6 cm to 20.6 cm when the Al level was raised from 0 to 50 mg/l. But at a Ca level of 100 mg/l the shoot height was 50.5 cm at 0.25 mg Al/l and 44.7 cm at 50 mg Al/l. With higher Ca, the shoot height of high Al seedlings became almost equal to the shoot height of seedlings in the nil Al treatment. Shoot heights were higher at 0.25 mg Al/l treatment than under the nil Al treatment. The ANOVA table (Table 6.3) includes data which show that shoot heights were significantly different for Al and Ca treatments and their interaction (Al x Ca and Al quadratic x Ca).

Table 6.2. Correspondence of treatment Al levels with calculated $\Sigma a_{\text{Al mono}}$.

Treatment Al level (mg/l)	$\Sigma a_{\text{Al mono}}$ (μM)
0.25	6.58 - 6.71
2.5	61.13 - 62.07
20.0	369.30 - 390.30
50.0	708.60 - 759.40

The response of the seedlings to Ca and Al treatments in terms of shoot weight (Figure 6.2 a) showed results similar to those reported for shoot heights. Under high Al conditions lower shoots were produced. When the Ca level was raised this effect was less prominent. For example, at a Ca concentration of 5 mg/l, shoot weight decreased from 2.67 g/seedling at low Al to 1.11 g/seedling at 50 mg Al/l. At high Ca concentrations (100 mg/l) shoot weight decreased from 3.45 g/seedling at 0.25 mg Al/l to 2.77 g/seedling at 50 mg Al/l. The differences in shoot weights were statistically significant for both Al and Ca treatment levels but not for their interaction.

Total biomass

The effect of Al and Ca treatments on total biomass of the seedlings is presented in Figure 6.2 c. The effect on total biomass is similar to that for shoot weight which is the major component of biomass. Biomass was also significantly different for Al and Ca treatments but not for their interaction.

Table 6.3. F ratios for the ANOVA on the effects of Al and Ca treatments on different growth parameters of *E. camaldulensis* seedlings.

	Factors							
Parameter	Al	Al^2	Ca	Ca^2	Al x Ca	Al^2 x Ca	Al x Ca^2	Al^2 x Ca^2
Shoot height	39.90**	0.37	34.49**	3.84	9.55**	4.42*	0.46	0.45
Root length	63.43**	1.49	18.52**	4.11*	11.00**	0.19	0.41	1.53
Shoot weight	9.60**	0.01	10.59**	0.36	0.87	2.02	0.15	0.92
Root weight	1.09	0.59	4.42*	0.87	1.26	1.02	0.26	1.47
Total biomass	7.80**	0.06	9.67**	0.47	1.00	1.91	0.17	1.06
Fine root length	24.15**	1.88	86.23**	0.01	1.97	5.82*	0.00	2.30
Fine root length/ fine root weight	184.79**	7.38**	197.49**	6.87*	0.17	0.47	2.83	2.39
Root branching	8.07**	0.76	0.34	0.43	0.12	0.33	0.01	0.01
Leaf width	13.09**	1.45	6.35*	1.22	1.29	0.00	1.18	5.19**
Transpiration	25.84**	1.23	12.13**	2.43	12.08**	1.10	1.61	1.02
Transpiration/ total biomass	5.66*	4.17*	0.87	1.52	2.40	0.52	1.47	0.14

** and * indicate level of significance at < 1 % and < 5% level respectively.

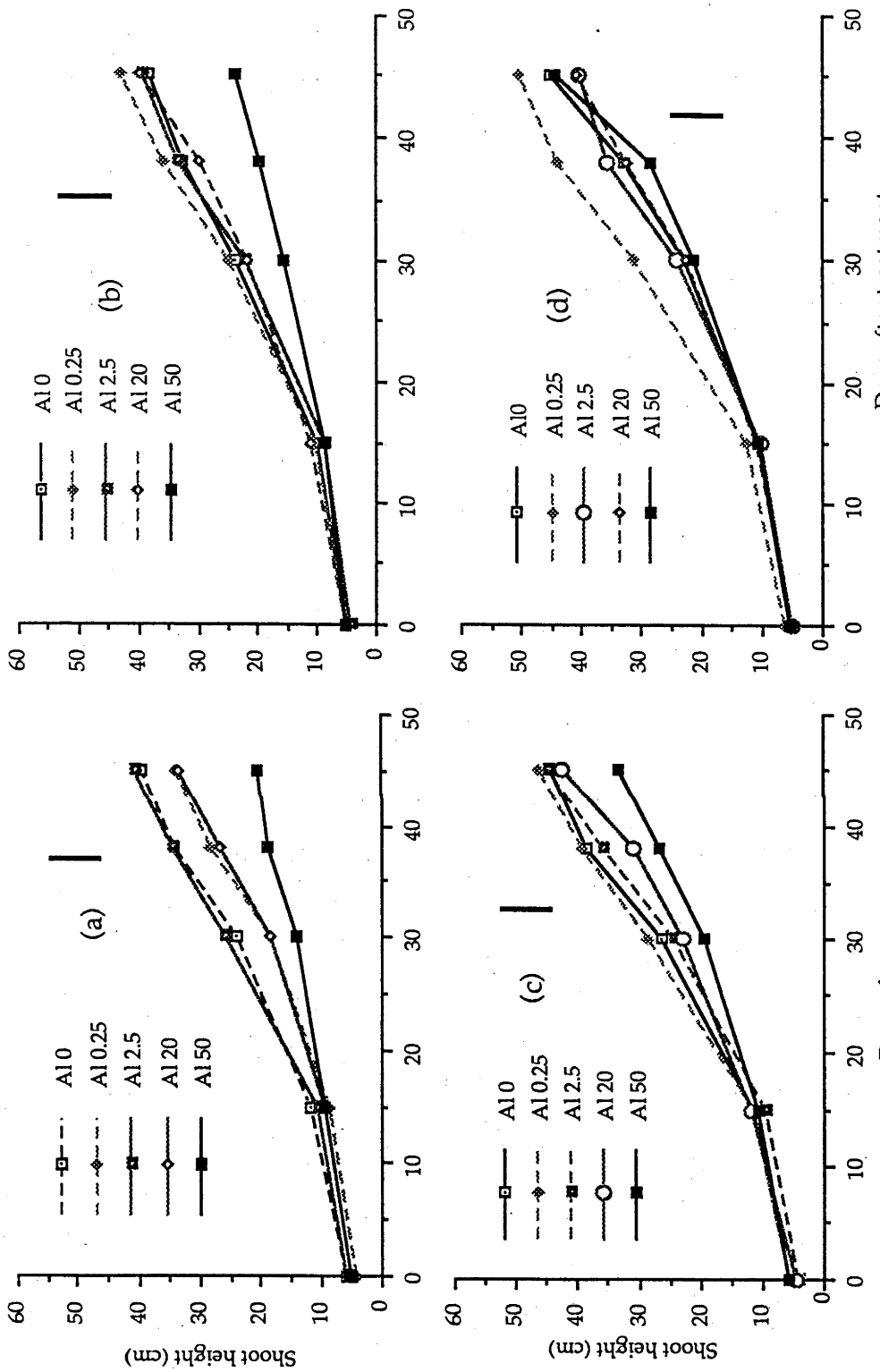


Figure 6.1. Shoot height of *E. camaldulensis* on different dates as affected by Al levels: (a) at 5 mg Ca/l, (b) at 10 mg Ca/l, (c) at 50 mg Ca/l and (d) at 100 mg Ca/l. Vertical bars represent l.s.d. for the last harvest ($P < 0.05$).

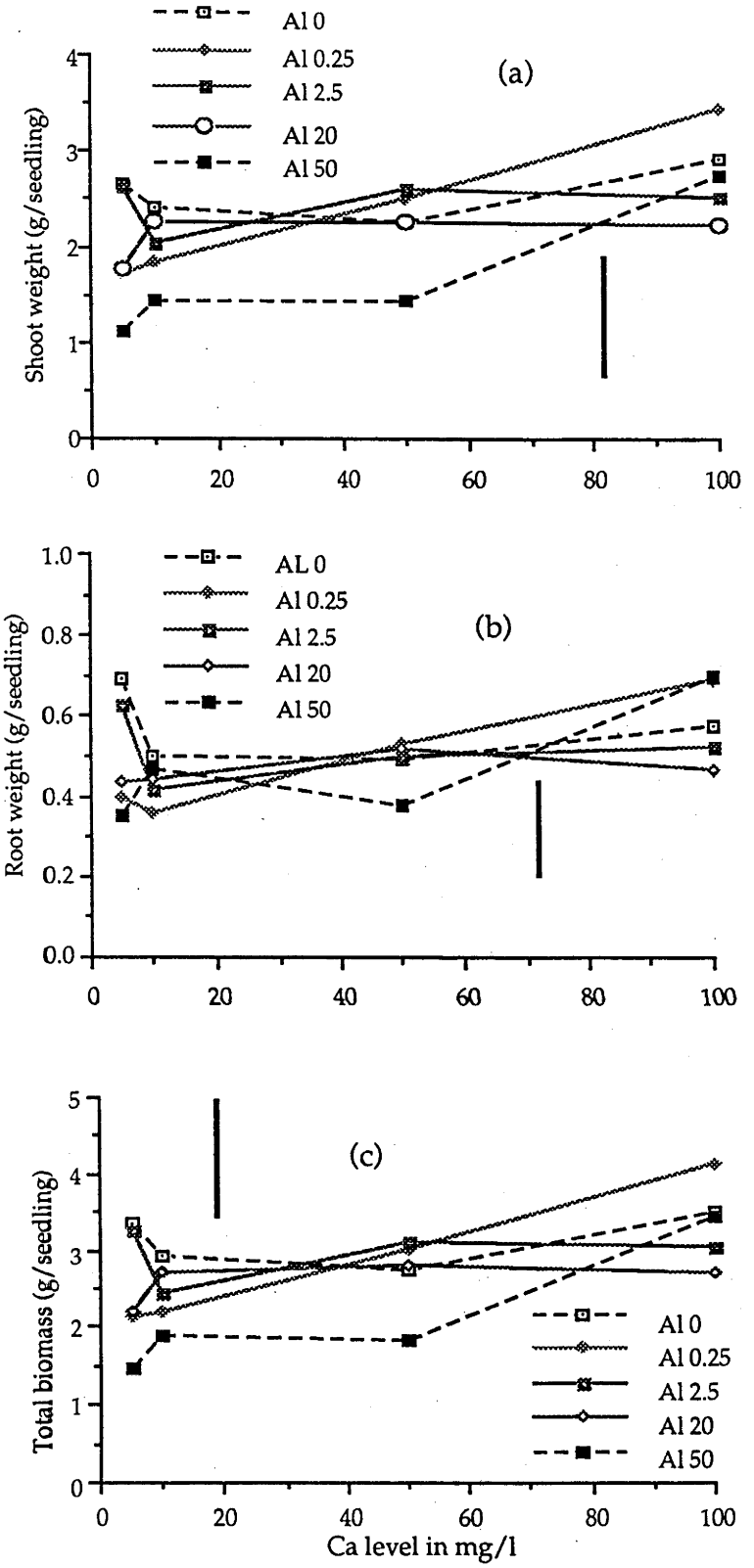


Figure 6.2. Effects of Ca and Al levels on (a) shoot weight, (b) root weight and (c) total biomass of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

Root length and root branching

Primary root lengths measured during the growth period are shown in Figure 6.3. As in the case of shoot height, four graphs were plotted, one for each Ca level. It can be seen from the graphs that the primary roots were shorter for high Al treatments. This effect was more prominent at lower Ca levels. At Ca concentrations of 5, 10, 50 and 100 mg/l, the difference in primary root lengths between 0.25 mg Al/l and 50 mg Al/l were 33, 38, 26 and 9 cm. However, an Al treatment of up to 2.5 mg/l did not have any adverse effect on primary root length even at the lowest Ca level (Figure 6.3 a). Root lengths at 20 and 50 mg Al/l progressively became closer to the situation at lower Al levels as Ca concentration increased; at the highest Ca level root lengths of different levels of Al treatment were only slightly different from each other. Differences in primary root length due to Al and Ca treatments (both linear and quadratic) were significant but only their linear interaction was statistically significant (Table 6.3).

The number of root branches in the middle 5 cm of the primary root are presented in Figure 6.4 a. It may be seen from the figure that the number of root branches decreased from 22 at nil Al, to 13 at the highest Al level. The number of root branches was significantly different only for Al treatments (Table 6.3). Even the lowest Al level caused a large reduction in the number of root branches as compared to the nil Al treatment. Unlike other shoot and root growth parameters, a decrease in the root branching (due to high Al) was not ameliorated by an increase in the Ca level.

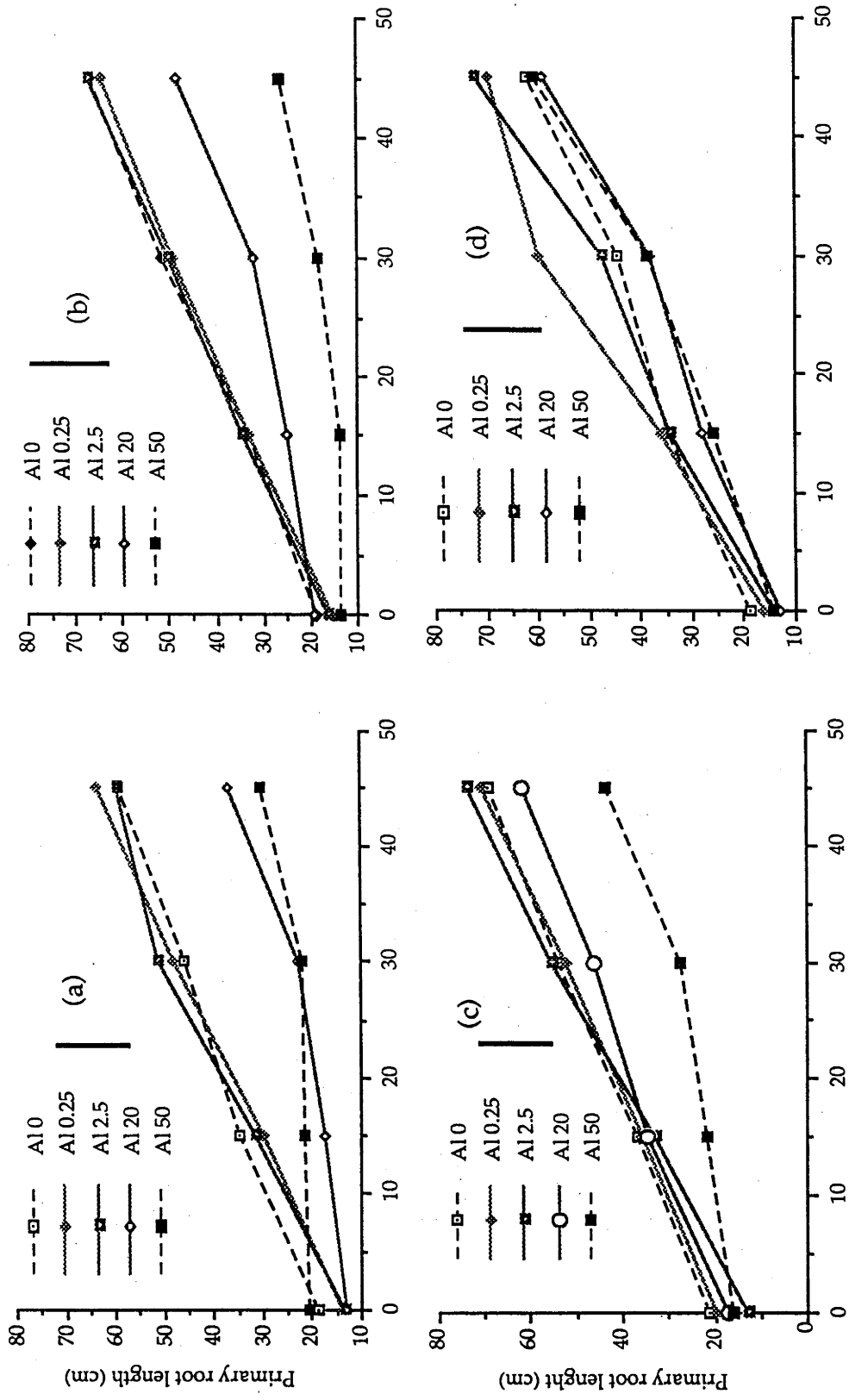


Figure 6.3. Primary root length of *E. camaldulensis* on different dates as affected by Al levels: (a) at 5 mg Ca/l, (b) at 10 mg Ca/l, (c) at 50 mg Ca/l and (d) at 100 mg Ca/l. Vertical bars represent l.s.d. for the last harvest ($P < 0.05$).

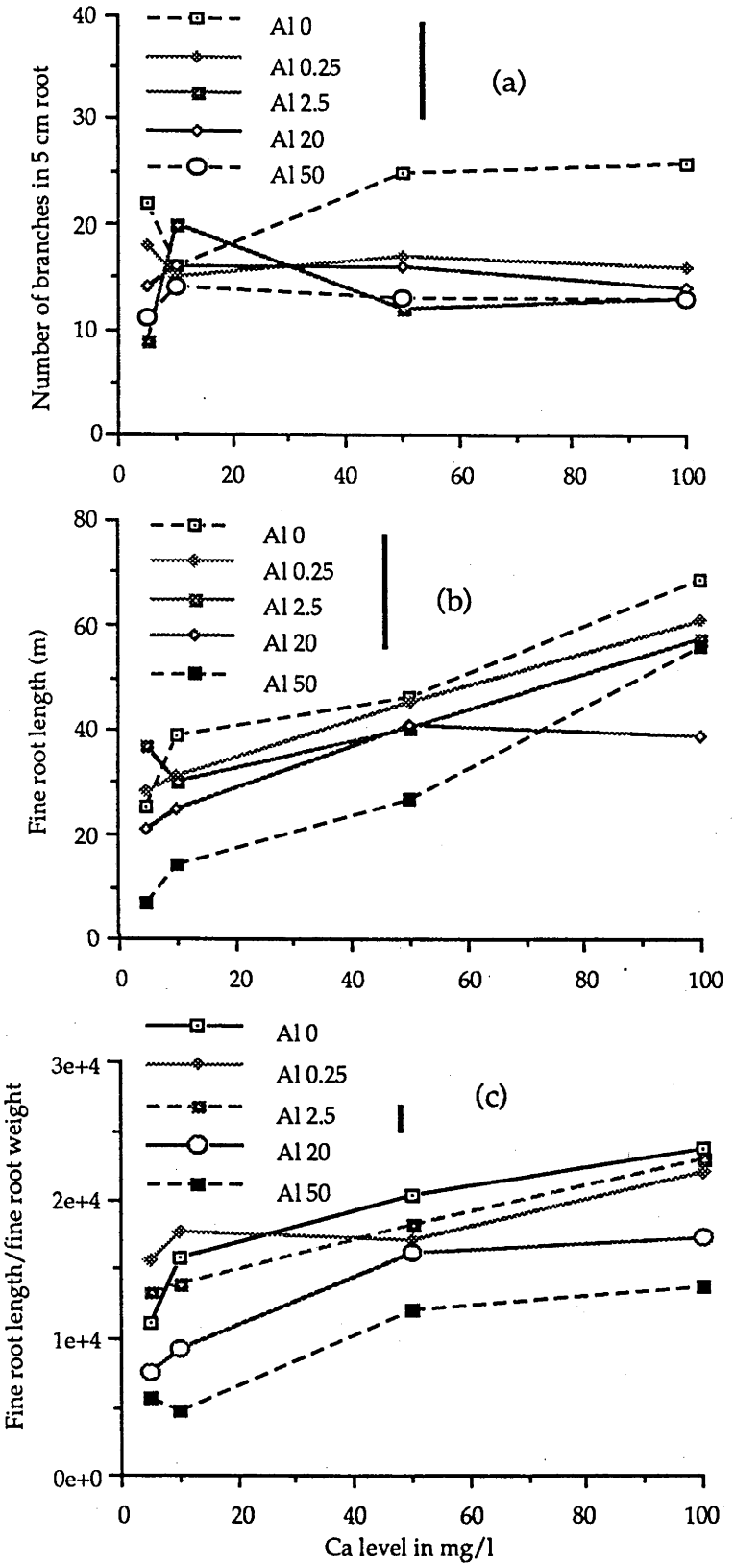


Figure 6.4. Effects of Ca and Al levels on (a) number of branches in 5 cm root, (b) fine root length and (c) (fine root length)/(fine root weight) of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

Fine root length

The effect of Al and Ca treatments on the total length of fine roots is presented in Figure 6.4 a. Fine root length was found to be highly sensitive to both Al and Ca levels. The fine root length decreased with an increase in the Al level and increased with an increase in the Ca level. An increase in the Ca level however, did not bring the primary root length to the same levels as for a low or nil Al treatment. Fine root length was significantly different for Al and Ca levels and also for their interaction (Al quadratic x Ca) (Table 6.3).

Fine root length/fine root weight (FRL/FRW)

This ratio defines a measure of the fineness of fine roots. It was highly sensitive to Al and Ca treatment levels (Figure 6.4 c). It may be seen from the figure that as Al treatment levels rise, the lines representing FRL/FRW at various Ca levels shift downwards. The downward shift was consistent and pronounced at all levels of Al above 0.25 mg/l. At each Al level, as the Ca level was increased, the index showed a rise. The ameliorating effect of Ca was however, lower for this parameter when compared to the other parameters discussed so far. Values for FRL/FRW were significantly different for both Al (linear and quadratic) and Ca (linear and quadratic) but not for their interaction (Table 6.3).

Total root weight

The effect of Al and Ca levels on root weight were less systematic than on other root parameters. In most cases, a higher Al was associated with lower root weight and higher Ca level significantly increased it and counteracted the effect of high Al. This parameter is not considered as a good indicator of the effects of Al and Ca treatments (Table 6.5). The

reason is that an increase in Al level makes the root thicker and this causes root weight to increase. This counteracts the negative effect of Al on root weight which is caused by the growth of fewer roots. Similar reasons hold for the effect of Ca which ameliorates the effect of Al and enhances root growth but makes them finer.

Relative growth reduction due to Al

In general, an increase in the Al level reduced the growth of the seedlings as measured by different growth parameters. This adverse effect was not the same for all parameters. Those which were reduced more due to high Al may be considered more sensitive to Al. To compare this reduction among the growth parameters, Table 6.4 presents 'relative growth reduction due to Al' (RGR) for the parameters which were found to differ significantly for Al and Ca treatments. The RGR was calculated by the technique of Baligar et al (1987).

$$RGR = [1 - (\text{growth with Al})/(\text{growth without Al})] \times 100.$$

Table 6.4. Relative growth reduction of *E. camaldulensis* seedlings due to Al.

Treatment Al level (mg/l)	Relative growth reduction in					
	Shoot height	Shoot weight	Primary root length	Fine root length	Fine root length/ fine root weight	Root branching
0.25	- 3.82	7.39	- 4.19	7.32	- 2.20	22.73
2.5	1.20	4.67	- 6.06	7.77	3.65	36.36
20	6.22	16.73	19.88	29.50	29.05	31.82
50	26.79	34.24	37.27	40.60	49.02	40.91

The maximum decrease in RGR occurred in fine root length and (fine root length)/(fine root weight) and these parameters of *E. camaldulensis* may be considered most sensitive to Al toxicity (Table 6.4).

Relationship of growth parameters with a_{Al}/a_{Ca}

In the above analysis, the effects of Al and Ca levels on the seedling growth parameters have been shown as individual effects. In general, all the growth parameters responded negatively to Al levels and positively to Ca levels. That is, they affected seedling growth in opposite directions. Further, the effects of each of these were also influenced by the levels of the other, that is, by their interaction.

Since the effects of Al and Ca were opposed, an analysis of whether a_{Al}/a_{Ca} acts as a single factor and if it can explain the variance in growth parameters satisfactorily, is presented. Some recent studies used different forms of relationship between Ca and Al for this purpose (e.g., Ca - Al balance, Noble et al, 1988), but these are not yet widely accepted and were criticised on theoretical validity (Kinraide and Parker, 1989; Grauer and Horst, 1991). Therefore in this analysis, the simple relationship between a_{Ca} and a_{Al} were used. However, in many recent studies the ratio a_{Ca}/a_{Al} was used whereas in the present study a_{Al}/a_{Ca} was used. It was chosen since otherwise the values of the ratio in some cases would have been indeterminate (because of nil Al treatment level). For this purpose correlation coefficients were calculated for the growth parameters against a_{Al}/a_{Ca} and the values of R^2 are presented in Table 6.5. For all the growth parameters, polynomial regression (2nd order) explained the variance better than a simple regression. Therefore, the values of R^2 in Table 6.5 are from respective polynomial regression equations.

The relationships between the growth parameters and a_{Al}/a_{Ca} are presented in Figure 6.5 along with the equations best fitted to the relationships and associated R^2.

Table 6.5 Values of R^2 for the variation due to a_{Al}/a_{Ca} for different growth parameters.

Growth parameter	R^2
Fine root length/ fine root weight	0.61
Root length	0.56
Shoot height	0.42
Fine root length	0.33
Shoot weight	0.15
Total biomass	0.11
Root branching	0.04
Root weight	0.02

6.3.2. Effects of treatments on water transpiration

Transpiration loss was measured during the treatment period to examine whether Ca and Al treatments had any effect on the amount of water transpired by the growing seedlings. The effect of Al and Ca concentrations on transpiration was highly significant but their interaction was not. The amount of water transpired by seedlings (ml/seedling/day) in each treatment is presented in Figure 6.6 b. The amount of water transpired was highest (59 ml/seedling/day) in the low Al plus high Ca treatment and lowest (18 ml/seedling/day) in the high

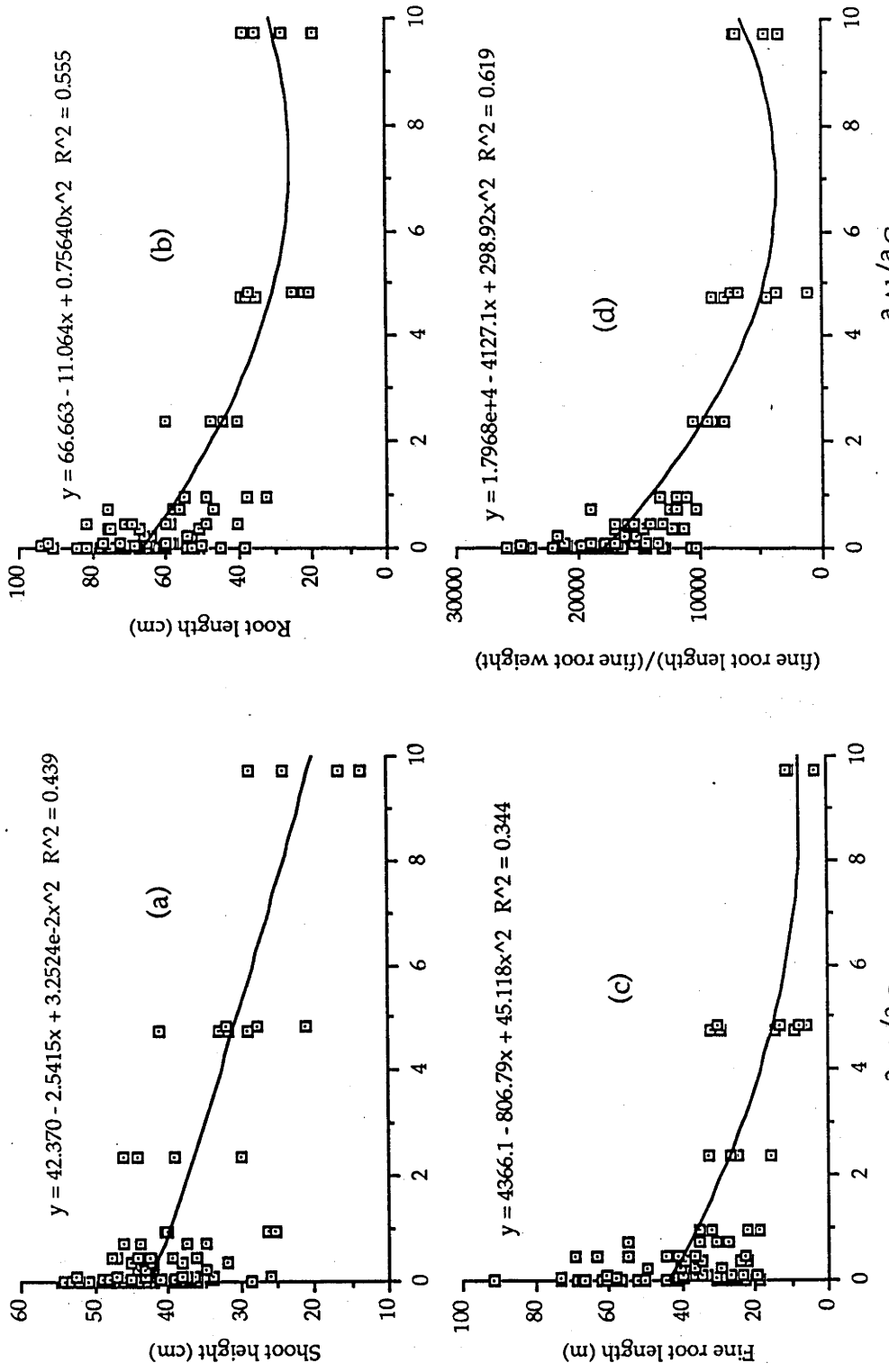


Figure 6.5. Relationship between (a) shoot height, (b) primary root length, (c) fine root length and (d) (fine root length)/(fine root weight) with a_{Al}/a_{Ca} .

Al plus low Ca. In general an increase in Al levels decreased transpiration and an increase in Ca level increased transpiration. When the size of the seedlings (dry matter produced) was considered as a covariant the amount of water transpired varied only among Al treatments (at 5% level) which indicate that the effects of Ca and interaction between Ca and Al on transpiration were primarily related to the size of the seedlings in each treatment.

6.3.3. Effects of treatments on leaf width

From the leaves traced at harvest, toutlines from nil Al and maximum Al are shown in Plate 6.3. With an increase in Al levels the leaves became more linear as opposed to a lanceolate shape. Leaves were wider when the Ca concentration increased at a given Al level. As an index to quantify leaf width, the ratio between mean leaf width and maximum leaf width is used. A high value indicates that the leaves are narrower. The values of this index are plotted against Al and Ca treatments in Figure 6.6 a. The change of leaves to a linear form as Al levels increased, indicates a stress effect on the seedlings; this effect is similar in nature to that which occurs with leaves under moisture stress (Andrew, 1973) and moisture and nutrient stresses (Gibson and Bachelard, 1989). Leaf width differed significantly for Al treatments but not for the Ca or Ca by Al interaction.

6.3.4. Effects of treatments on the anatomy of roots

The primary roots of *E. camaldulensis* stopped growing when high Al concentrations were accompanied by low Ca, (i.e, in the $Al_{50}Ca_5$ treatment) (Plate 6.2). This resulted in slow secondary root formation. All the roots became thicker, particularly at the tips which appeared

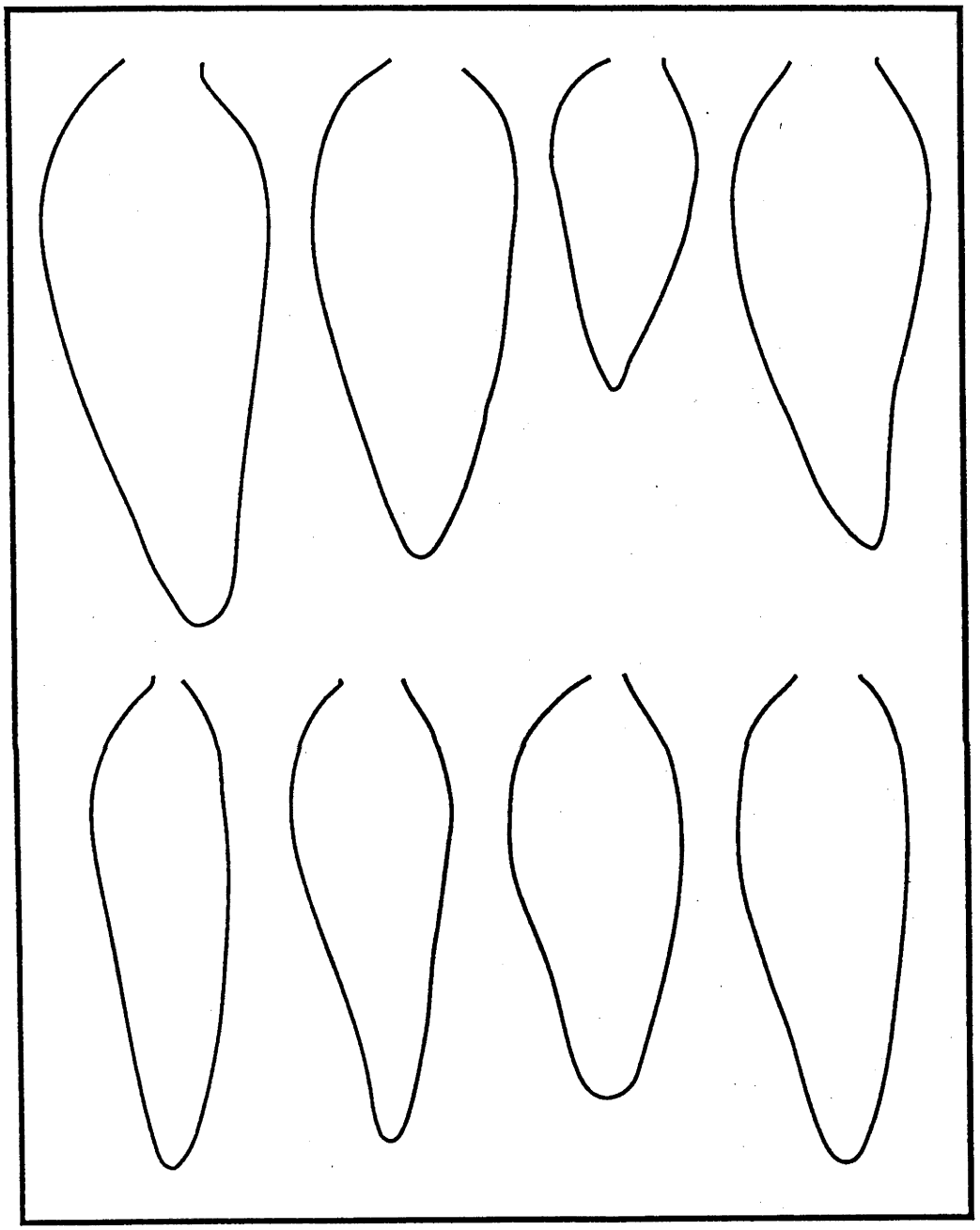


Plate 6.3. Tracings of young leaves (actual size) of seedlings from no Al (top row) and maximum Al (bottom row) at different Ca levels (mg/l). From left to right: Ca_5, Ca_{10}, Ca_{50} and Ca_{100}.

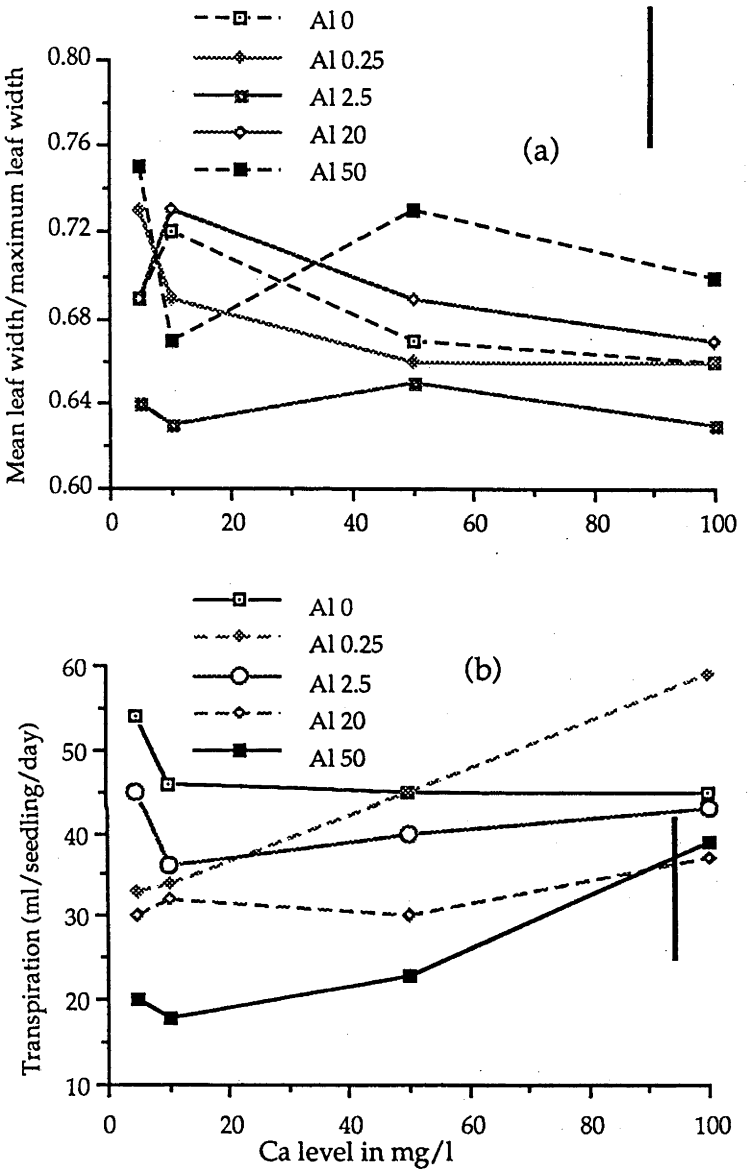


Figure 6.6. Effects of Ca and Al levels on (a) leaf width and (b) transpiration of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

brownish in colour. To examine whether these effects on roots were accompanied by changes in the root periphery, transverse thin sections of the roots of seedlings from treatments with a contrasting Ca and Al level were prepared. Light microscope photographs of the root thin sections are presented in Plate 6.4. On the basis of these photographs the following observations may be made about root peripheries.

i. A treatment constituting high Al in the presence of low Ca (Ca_5Al_{50}) results in a thickening of the root periphery, damage to the epidermis and development of the hypodermal layer.

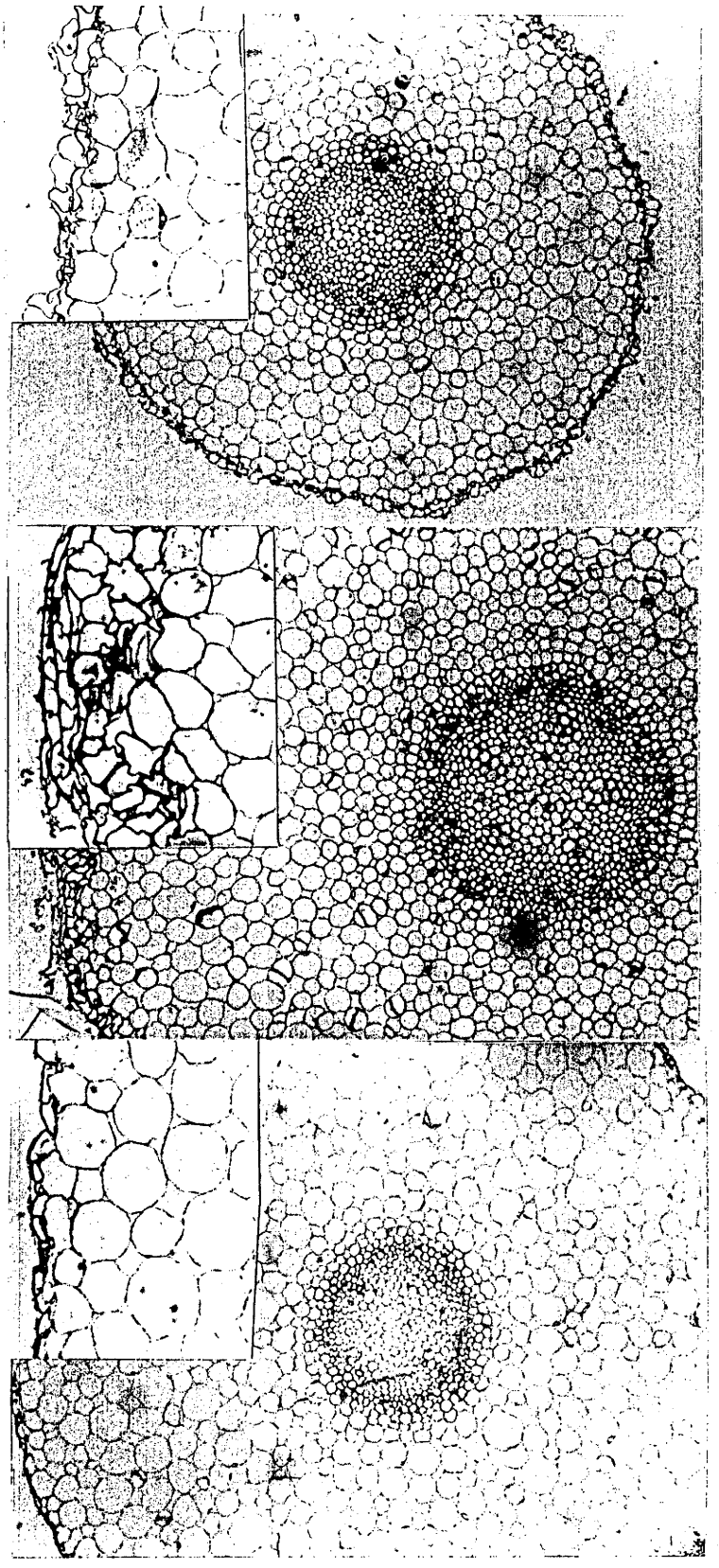
ii. At the same distance from the tips, roots of the $Ca_{100}Al_{0.25}$ treatment were not thickened and the epidermis was not damaged.

iii. Increasing the Ca level in the presence of high Al ($Ca_{100}Al_{50}$) improves the situation although there remains some thickening of the root periphery.

6.3.5. Effects of treatments on mineral concentrations in seedlings

Aluminium

Aluminium concentrations in mature leaves and fine roots at different Al and Ca treatments are presented in Figure 6.7. Analysis showed that there was no Al in young leaves. In mature leaves (Figure 6.7 b), the Al concentration was very low at the shown Al levels of 0.25 and 2.5 mg/l. There was not much difference in the tissue Al concentration between these two treatments. At a treatment Al level of 20 mg/l, the Al concentration in mature leaves increased several fold and at 50 mg/l, the tissue Al concentration increased even more than proportionately. These increases were statistically significant for treatment Al levels and for the Al by Ca interaction, but not for the Ca treatment level.



(a) (b) (c)

Plate 6.4. Effects of Al and Ca treatments on root anatomy of *E. camaldulensis* seedlings at harvest. (a) $Al_{0.25}$ Ca_{100}, (b) Al_{50} Ca_{5} and (c) Al_{50} Ca_{100}. Inset shows an enlarged portion of root periphery in each plate.

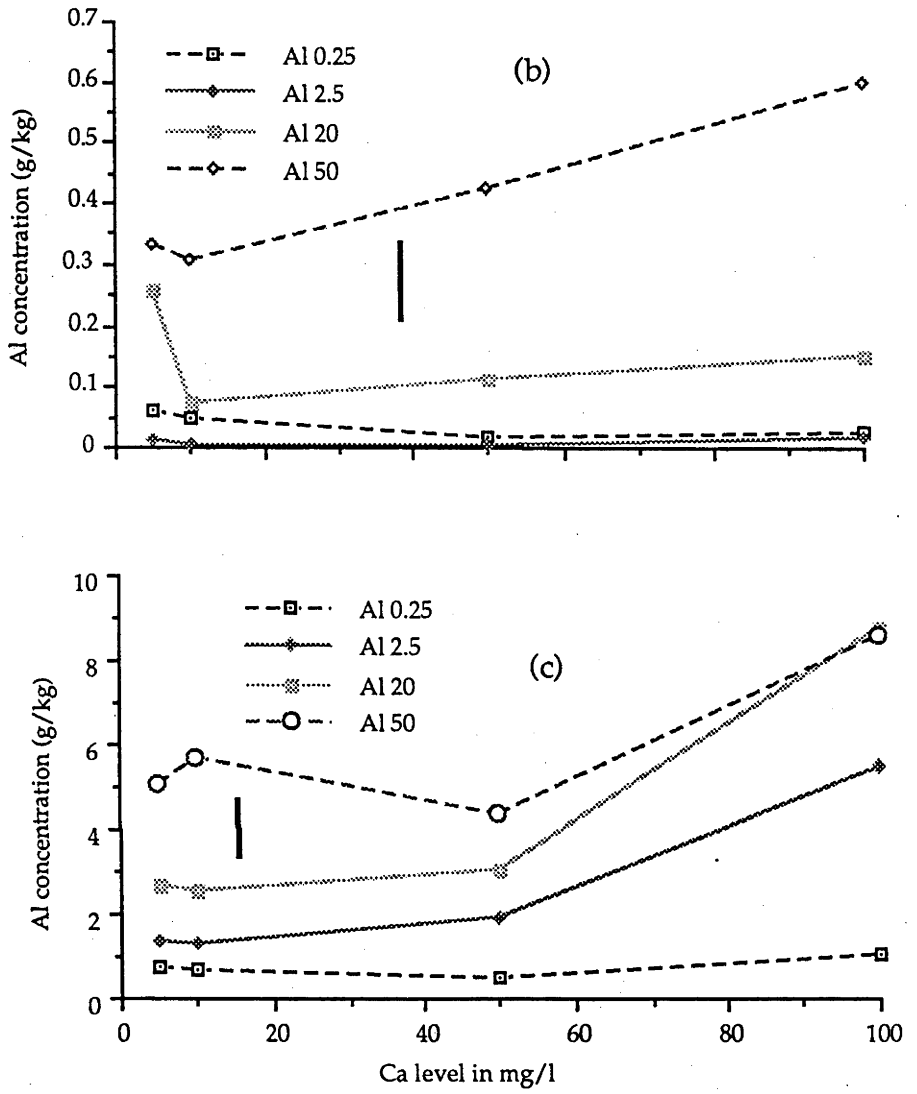


Figure 6.7. Effects of Ca and Al levels on Al concentrations in *E. camaldulensis* seedlings, (a) Al concentration in young leaves were zero and are not plotted, (b) in mature leaves and (c) in fine roots. Vertical bars represent l.s.d. ($P < 0.05$).

The Al concentrations in fine roots (Figure 6.7 c) were more sensitive to treatment levels of both Al and Ca than Al concentration in mature leaves. The Al concentration in fine roots clearly increased for all Al levels. At all Al levels, the Ca treatment increased Al concentration in fine roots. The only exception was the lowest Al level (0.25 mg/l). These increases in Al concentration were significant for both Al and Ca treatments and also for their interaction. Correlation coefficients (R^2) for Al concentration in mature leaves were 0.66 and 0.70 when compared with $\Sigma a_{Al\ mono}$ and total added Al respectively, whereas the R^2 for the Al concentration in fine roots were 0.41 and 0.40 respectively. The concentration of Al in fine roots was much higher (maximum of 9 mg Al/kg root tissue compared to a maximum of 0.6 mg Al/kg mature leaf) than the Al concentration in mature leaves (note the difference in the scale of the y axis in the two graphs).

Calcium

Calcium concentrations in young leaves, mature leaves and fine roots are presented in Figure 6.8. Aluminium treatment levels of 0.25 and 2.5 mg/l significantly increased the Ca concentration in young leaves (Figure 6.8 a); the increase was larger in the case of 0.25 mg/l than for 2.5 mg/l of Al. This increase occurred at all levels of Ca. Further increases in the Al level (20 and 50 mg/l) decreased the Ca concentration, but the decrease was not significant. At all Al levels, the Ca levels significantly increased Ca concentration in young leaves. The absolute increase was however, larger in the presence of lower Al treatments (0.25 and 2.5 mg Al/l).

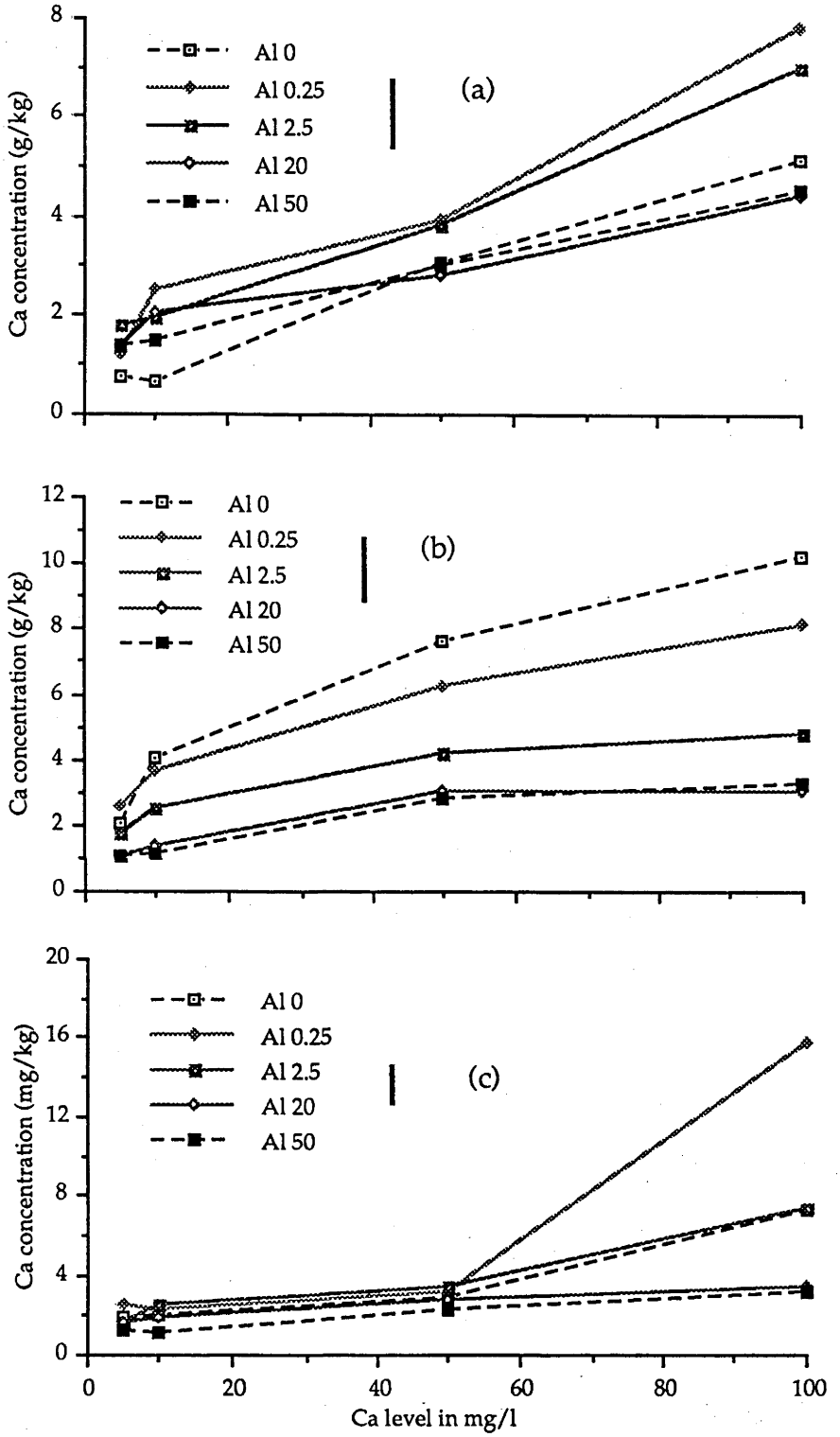


Figure 6.8. Effects of Ca and Al levels on Ca concentrations in *E. camaldulensis* seedlings, (a) in young leaves, (b) in mature leaves and (c) in fine roots. Vertical bars represent l.s.d. ($P < 0.05$).

In mature leaves (Figure 6.8 b) all Al levels significantly decreased the Ca concentration except in the case of 0.25 mg Al/l. Further, Ca concentrations at the two highest Al levels were almost the same as at all Ca levels. An increase in the treatment Ca level significantly increased the Ca concentration at all Al levels although in absolute terms, the increases were smaller in the presence of high Al compared to nil or low Al.

Calcium concentrations in fine roots were significantly higher in the lowest (0.25 mg/) Al treatment. The next higher Al level (2.5 mg/l) did not have any effect but the two highest (20 and 50 mg/l) Al levels significantly reduced the Ca concentration in fine roots. The Ca concentrations at these two Al levels were almost the same as at all Ca levels. Calcium treatment levels significantly increased the Ca concentration in fine roots although the increase was small at higher Al levels.

In general, Ca concentrations were highest in mature leaves followed by young leaves and then fine roots (except for the Ca concentration in fine roots at the $Ca_{100}Al_{0.25}$ treatment).

Magnesium

Magnesium concentrations in the seedlings are shown in Figure 6.9. In young leaves the effect of Al was different for low and high Ca levels. At Ca levels of up to 50 mg/l, low Al levels (0.25 and 2.5 mg/l) increased the Mg concentration while higher Al levels decreased the Mg concentration. The decrease in Mg concentration at 20 mg Al/l however, was not significant. At a Ca concentration of 100 mg/l, Mg concentrations were lowered when accompanied by 0, 0.25 and 2.5 mg Al/l, but when

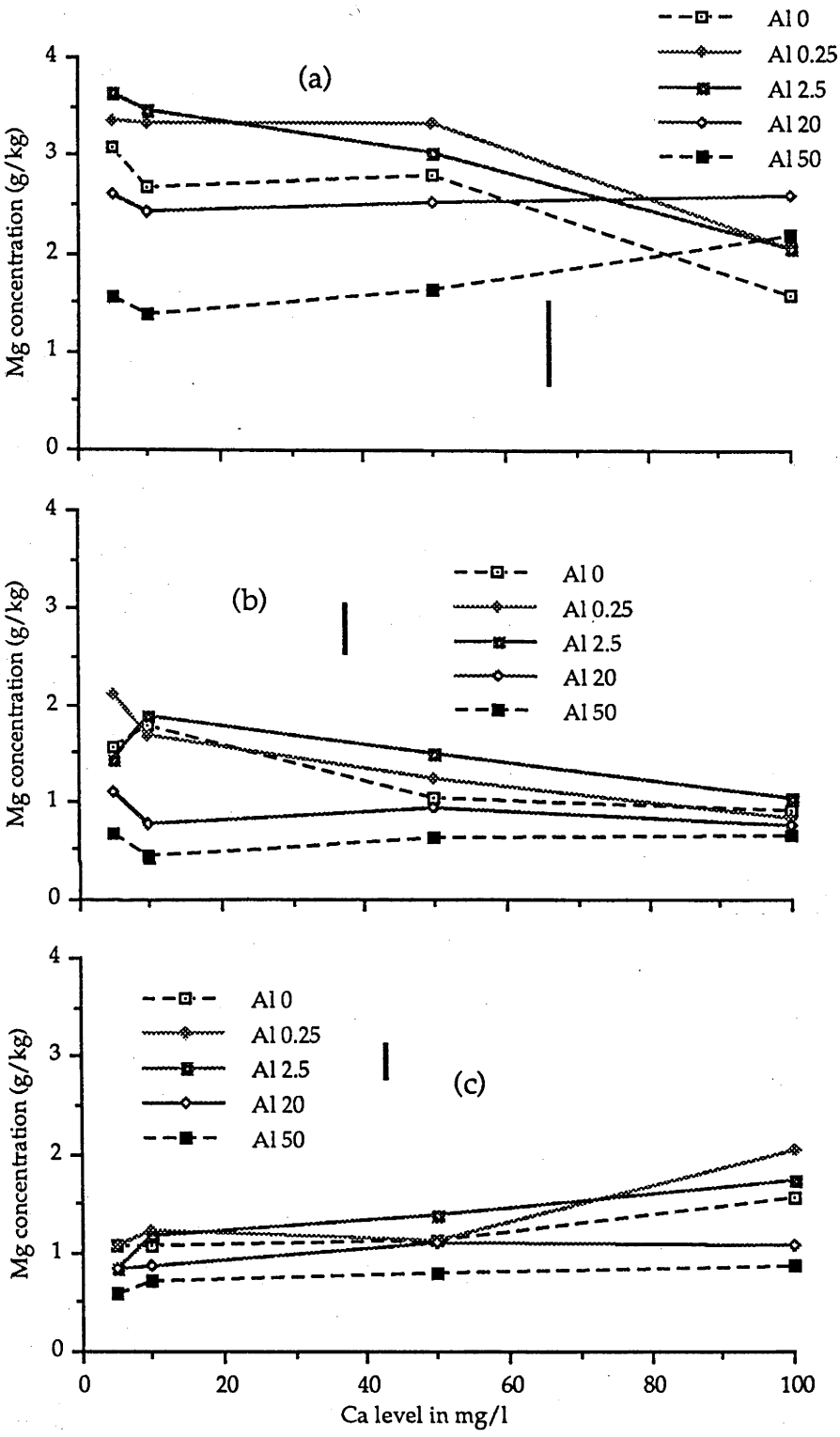


Figure 6.9. Effects of Ca and Al levels on Mg concentrations in *E. camaldulensis* seedlings, (a) in young leaves, (b) in mature leaves and (c) in fine roots. Vertical bars represent l.s.d. ($P < 0.05$).

accompanying Al levels were 20 and 50 mg/l, Mg concentrations increased further but not significantly.

In mature leaves (Figure 6.9 b) in the presence of low Ca (5 and 10 mg/l), Mg concentrations at the two highest Al levels (20 and 50 mg/l) decreased significantly. When Ca treatment levels were also raised, the Mg concentration declined with low Al levels but at 20 and 50 mg Al/l, the Mg concentration increased further. At the highest Ca treatment level, the Mg concentration at different Al treatments was not significantly different.

In fine roots, lower Al treatment levels (0.25 and 2.5 mg/l) increased the Mg concentration (Figure 6.9 c). Higher Al levels (20 and 50 mg/l) significantly decreased Mg concentrations. The effects of Al treatments on Mg concentrations were more prominent at higher than at lower Ca levels. The increase in the treatment Ca level increased the Mg concentration at all Al levels, but at the two highest Al levels the increases were not significant. In general, Mg concentrations were highest in young leaves followed by mature leaves and then fine roots.

Phosphorus

Phosphorus concentrations in the seedlings are presented in Figure 6.10. In general, intermediate levels of Al (0.25 to 20 mg/l) resulted in maximum P concentrations in young leaves at different Ca levels. The differences in P due to Al were significant although in absolute terms the differences were small. Phosphorus concentrations were not significantly different for Ca treatment levels or for the Al by Ca interaction.

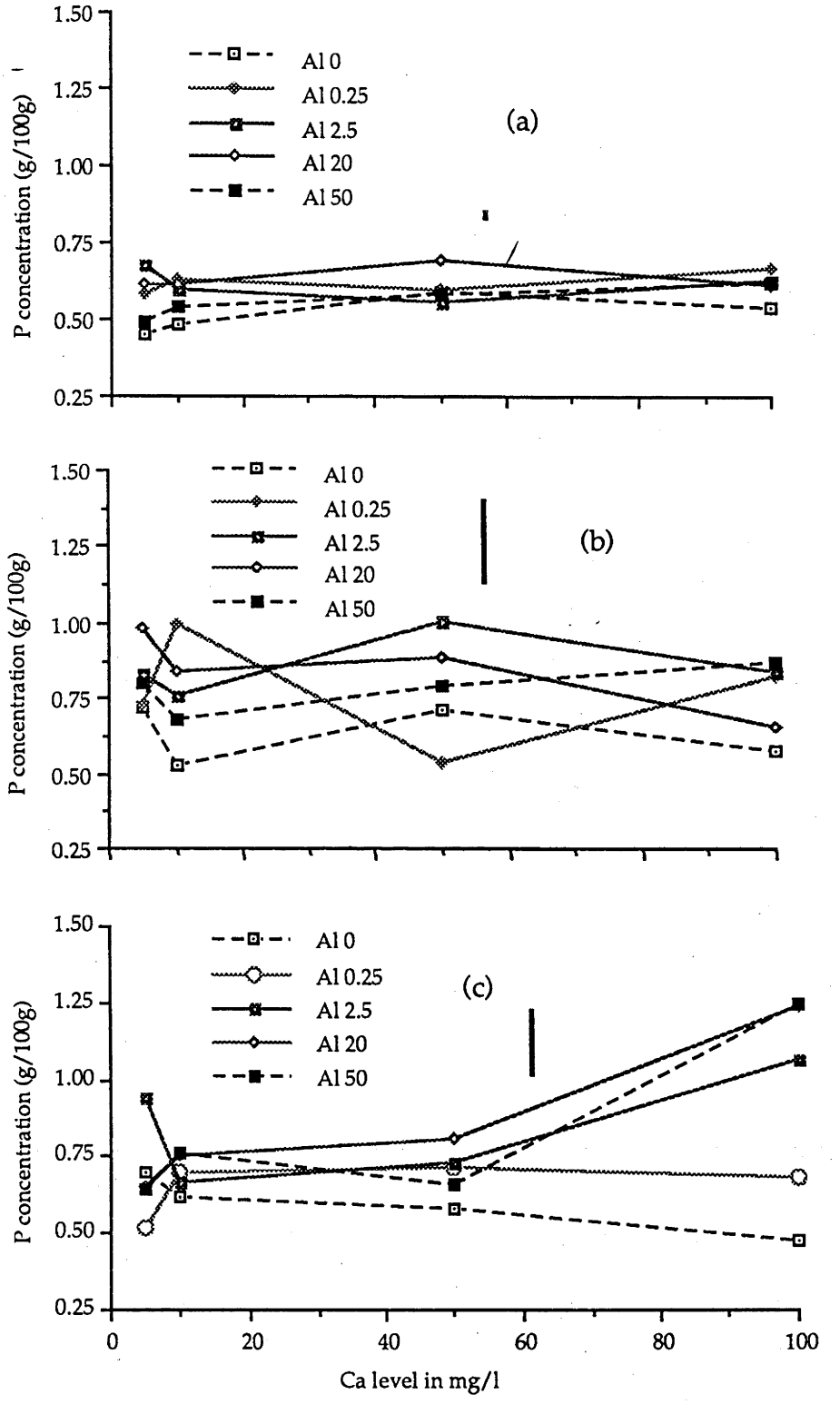


Figure 6.10. Effects of Ca and Al levels on P concentrations in *E. camaldulensis* seedlings, (a) in young leaves, (b) in mature leaves and (c) in fine roots. Vertical bars represent l.s.d. ($P < 0.05$).

Table 6.6. F ratios for the ANOVA on the effects of Al and Ca treatments on concentrations of Al, Ca, Mg and P in different parts of *E. camaldulensis* seedlings.

Parameters	Factors							
	Al	Al^2	Ca	Ca^2	Al x Ca	Al^2 x Ca	Al x Ca^2	Al^2 x Ca^2
Al (mature leaves)	326.92**	1.37	4.54*	1.76	24.64**	5.67*	0.26	1.08
Al (fine roots)	476.68**	47.59**	136.04**	37.65**	15.09**	60.79**	14.13**	5.99*
Ca (young leaves)	10.23**	1.66	280.02**	0.43	10.66**	5.47*	0.43	0.00
Ca (mature leaves)	64.24**	26.66**	116.09**	7.87**	12.55**	8.06**	0.23	0.00
Ca (fine roots)	48.61**	8.79**	218.48**	18.62**	46.06**	21.20**	11.93**	6.12*
Mg (young leaves)	50.83**	1.18	16.78**	1.65	28.03**	1.99	2.95	0.49
Mg (mature leaves)	78.59**	3.90	29.55**	0.25	17.17**	2.26	0.27	0.51
Mg (fine roots)	75.20**	2.31	68.07**	1.05	9.62**	2.59	3.23	3.50
P (young leaves)	0.36	8.60**	4.81*	0.55	0.74	1.58	0.40	0.88
P (mature leaves)	0.54	2.51	0.26	0.03	0.40	2.94	0.00	0.74
P (fine roots)	9.65**	9.53**	25.20**	5.91*	19.46**	8.08**	4.37*	0.07

** and * indicate level of significance at < 1 % and < 5% level respectively.

In mature leaves P concentration showed an increasing trend due to higher Al treatment levels. Except in one case, P concentrations were always higher at higher Al levels compared to the nil Al treatment. However, these differences in P concentration were not significant, probably due to greater variation between replicates. The differences in P concentration were also not significant for Ca or for the Al by Ca interaction.

Phosphorus concentrations in fine roots (Figure 6.10 c) were significantly increased by higher Al levels. At higher Ca concentrations, the increases were larger than at low Ca. When Ca treatment levels were increased beyond 50 mg/l, P concentration slightly decreased at 0 and 0.25 mg Al/l. The differences in P concentration in fine roots were highly

significant for Al and Ca treatments and also for their interaction. The P concentration was lower in young leaves and more or less similar in mature leaves and fine roots.

6.4. Discussion

6.4.1. Growth

The present experiment examined the effects of Al and Ca on growth parameters of *E. camaldulensis*. Different parameters did not respond to Al and Ca levels in the same way and some growth parameters were more sensitive than others. Shoot height, shoot weight and total biomass are the growth parameters most commonly used to evaluate seedling performance under any treatment. Existing studies on Al toxicity have focussed mainly on roots: root elongation, root length, root branching etc.

In the present study the growth of *E. camaldulensis* seedlings was significantly influenced by Ca and Al treatments as measured by shoot height, shoot weight and total biomass. There are very few studies which discuss the effects of Al and Ca levels on shoot growth of tree species and could provide a basis of comparison for eucalypt growth with the present study. Keltjen and Loenen (1989) studied the effects of Al on 5 tree species (Douglas fir, Scots pine, larch, oak and birch). In the case of Douglas fir a significant increase in shoot and root growth was observed at 5 mg Al/l, while beyond that shoot dry matter was unaffected and root growth was significantly reduced. Larch and oak showed a positive shoot and root dry matter response to Al at high levels (Al up to 30 mg/l). Birch and Scots pine were unaffected by Al treatments. Compared to all these species, *E. camaldulensis* may be considered more sensitive to Al

because its total biomass declined when Al reached more than 2.5 mg/l unless the Ca was raised to a very high level. However, compared to *Populus* (Steiner et al, 1984), black spruce (Hutchinson et al, 1986) and honeylocust (Thornton et al, 1986), *E. camaldulensis* may be considered more tolerant to Al as these species were adversely affected at a lower Al concentration. Joslin and Wolfe (1988) also found a reduction in shoot weight in red spruce when soil solution Al levels were increased to 1.65 mM in forest soil.

In the present study, measures of root growth were also examined. Among these, total root weight was less affected by Al or Ca although other measures such as primary root length, fine root length, the ratio (fine root length)/(fine root weight) and root branching were significantly different for various Al and Ca treatments. The very low fine root length under the high Al treatment and its significant increase with increasing Ca level indicates that in the presence of high Ca, Al is not so toxic. Calcium is required in the meristematic regions (root and shoot) of young seedlings for growth and development. Roots are the organ in contact with the growth medium and fine roots have a much large number of exchange sites for Al than do tap roots or stems in young seedlings (Thornton et al, 1986). Therefore, the presence of high Al in the root environment limits fine root growth by reducing the Ca intake. In the present experiment Ca concentrations in fine roots were much lower at low Ca when the Al treatment was high. With increasing Ca, both fine root length and the Ca concentration in fine roots increased and this supports the above explanation. Kinraide and Parker (1987) also reported competition between Al and other cations for binding sites in wheat roots.

Blamey et al (1983) found that in 4 days relative root length of soybeans sharply declined up to the $\Sigma a_{Al\ mono}$ of 40 μM, then remained fairly steady up to the highest level of 160 μM. In the present experiment, root length remained unaffected at a $\Sigma a_{Al\ mono}$ of 62 μM even when the Ca level was low. Root length decreased at and above a $\Sigma a_{Al\ mono}$ of 380 μM when Ca levels were low, indicating that *E. camaldulensis* is more tolerant to Al levels than are soybeans. This experiment shows that root length increased consistently with Ca levels even when accompanied by high Al levels and this result agrees with those of Alva et al (1986 a) and Rhue and Grogan (1977).

The interaction effect of Al and Ca was examined as the ratio between the two. The value of R^2 was significant for all the equations but the variance in different growth parameters explained by the equations varied widely. Thus the ratio a_{Al}/a_{Ca} is a significant factor in explaining the performance of the growth parameters. However, the values of R^2 were sometimes low indicating that a_{Al}/a_{Ca} is not the only explanator of variation for the growth parameters. Wright and Wright (1987) reported that $a_{Ca^{2+}}$ in soil solution did not successfully explain root and shoot growth of subterranean clover but $a_{Ca^{2+}}/\Sigma a_{Al\ mono}$ in soil solution correlated better with the relative growth index. Rost-Siebert (1983) reported that roots of Norway spruce seedlings grown in nutrient solution were adversely affected only when the Ca/Al was low (< 1). Some studies used the Ca/Al ratio separately for each Ca level (Alva and Edwards, 1990) while others used a single Ca/Al for the whole range (Wright and Wright, 1987). Although the former approach may obtain a better relationship, the latter approach was used in this study because it is then possible to obtain a generalised effect over a wide range.

An examination of root thin sections indicated the presence of an epidermis in the $Ca_{100}Al_{0.25}$ treatment which implies that the roots were fast growing. The damaged epidermis in the high Al treatments with low Ca implies that root elongation was adversely affected. The effect of high Al on root morphology is in conformity with the existing literature. Wagatsuma et al (1987 a) reported that cell damage occurred in the epidermis in Al tolerant oat plants, while the adverse effect in sensitive plants (e.g. barley) was found throughout the whole cortex. Due to a lack of similar studies, it is not possible to compare the effects of increased Ca in alleviating the effect of Al on root anatomy. A thickened root periphery in low Ca plus high Al treated seedlings is likely to restrict the movement of water (including nutrients) into the cortex.

6.4.2. Mineral concentrations

The effect of treatments on the mineral concentration of some elements known to be affected by Al was examined. Rather than analyzing the whole seedling or shoot and root as is usually done, in this study young leaves, mature leaves and fine roots were analyzed separately to examine which part of the seedling was more sensitive to Al toxicity and to find a suitable phytoindicator of Al toxicity for *E. camaldulensis*.

In general, higher Al levels increased Al and P concentrations and decreased Ca concentration. Magnesium concentration was increased at low Al levels and decreased at high Al. Higher Ca levels increased Ca and Al concentration and decreased Mg concentration. The P concentration in leaves was not affected by Ca, while root P was increased by Ca at high Al levels only.

A decrease in Ca and Mg concentration in leaves was a result of the high Al in the root environment. Alternatively Al already absorbed

may be due to excess Al reducing Ca and Mg transport to the tops (Foy et al 1978). The latter effect was not completely overcome by high Ca levels. Under similar conditions with wheat, Johnson and Jackson (1964) suggested that high Al levels completely inactivated a portion of the Ca accumulating mechanism. In mature leaves (a major component of the total shoot), higher Al treatments reduced the Ca concentration, but Al beyond 20 mg/l did not reduce the Ca concentration any further indicating a limitation to this effect. Competition between Al and Ca or Mg for binding sites in the roots is considered a possible mechanism for reduced Ca uptake in the presence of high Al (Kinraide and Parker, 1987). Similar reductions in Ca and Mg concentrations by higher Al treatments were reported for other plants. For example, cabbage, lettuce and kikuyu grass (Huett and Menary, 1980), pine (Truman et al, 1986), and spruce (Joslin et al, 1988) showed this effect. Magnesium concentration was also used as a criteria for screening Al tolerance in corn (Rhue and Grogan, 1977).

Phosphorus concentrations were significantly increased by higher Al levels, although in many cases the absolute increase was small. In existing studies, the effect of Al on P concentrations is contrary to this finding. There are many reports of adverse effects of Al on P concentration in plants (McCormick and Borden, 1974; Naidoo et al, 1978; Foy et al, 1978; Fageria et al, 1989 a; Alva and Edwards, 1990). Foy et al (1978) noted that Al tolerance may be closely related to the efficient use of P. Fageria et al (1989 a) also expressed similar views. However, White (1976) reported a stimulatory effect of Al on P uptake. Using ^{32}P to study the transport of P in lucerne, he found that the stimulus to uptake of P by Al was confined to the acid extractable pool which comprised mainly free space P.

Among the elements, Al concentrations in fine roots were much higher than in leaves (more particularly in the mature leaves). Root Al concentration was about 15 times higher than Al in leaves indicating that only a small portion of the absorbed Al was translocated to the mature leaves. This also suggests that once taken up, Al becomes immobilized in the tissues and is not translocated to the more actively growing parts (Attiwill, 1981). This ratio would have been even wider if the whole shoot was analyzed as a composite sample, since young leaves were free from any Al. The higher Al in roots when compared to shoots, is in agreement with many agricultural crops (Andrew et al, 1973; Huett and Menary, 1980; Fageria et al, 1989 a) and tree species (Arp and Ouimlet, 1986; Thornton et al, 1986; Joslin et al, 1988). Thornton et al (1986) reported 50 to 100 times higher Al in roots compared to shoots in honeylocust. Keltjen (1990) reported 5 to 10 times higher Al in nutrient solution grown roots of Douglas fir than in shoots. Joslin et al (1988) also reported much higher Al in roots from the B horizon than in shoots in the case of spruce.

In the present experiment increases in Ca treatment level increased both Ca and Al concentration in the leaves and at the same time increased total seedling growth which implies that when Ca concentrations in seedlings are high, higher Al concentrations are not so toxic. This also implies that lower Ca concentrations are the main problem with Al toxicity in *E. camaldulensis* seedlings, a conclusion which is similar for kikuyu grass (Awad et al, 1976). There are many reports of decreasing Al toxicity when applying large amounts of Ca (e.g., Rhue and Grogan, 1977; Alva et al 1986 a; Alva et al, 1986 c). All these authors report shoot and root growth (different measures) and the uptake of nutrient elements such as Ca, Mg, N, P, K etc at higher Ca.

However, reports on whether higher Ca actually reduced Al concentration in plants are rare. Huett and Menary (1980) studied the growth and nutrient uptake of cabbage, lettuce and kikuyu grass as affected by pH, Al and Ca. They found that high Ca treatments increased root Al concentrations in kikuyu grass and reduced shoot Al in all three. In the present study root Al increased at higher Ca levels but shoot Al significantly increased only at the highest Al treatment level.

6.4.3. Phytoindicators of Aluminium for *E. camaldulensis* seedlings.

The conclusions above help in the search for a suitable phytoindicator of Al toxicity in *E. camaldulensis*. Some measures of root growth (fine root length, fineness of fine root, and root branching) were significantly reduced by increasing Al levels. Among them, fineness of the fine roots was found to be an important growth parameter most sensitive to Al and Ca. Very few earlier studies focussed on this parameter. Pinkerton and Simpson (1981, 1983) considered fine root length to be one of the most important parameters affected by high levels of Al. However the assessment of fineness can be much more sensitive than attempting actually to measure diameters. For instance the measure of fineness used in this experiment was defined as the root length per unit weight, i.e, (fine root length)/(fine root weight). Thus the less the root weight per unit of root length, the finer the root and hence this measure provided a means to overcome any fineness measurement problem.

Aluminium concentrations in fine roots correlated well with $\Sigma a_{Al\ mono}$ or nominal Al (R^2 of 0.45 and 0.40 respectively). And Al concentrations in mature leaves correlated more strongly with both $\Sigma a_{Al\ mono}$ and nominal Al (R^2 of 0.65 and 0.69 respectively). Joslin et al (1988) reported that Al concentrations in fine roots from the B horizon

strongly correlated with different measures of soil Al but shoot Al was a weak phytoindicator of soil Al. Hutchinson et al (1986) also reported a weak correlation of shoot Al with Al in the growth medium. However, in these reports on shoot Al, a composite shoot sample was analyzed in contrast to the present study where mature and young leaves were analyzed separately. Therefore, under similar conditions mature leaf Al rather than shoot Al may be considered to be a suitable phytoindicator of Al toxicity in studies with small seedlings of *E. camaldulensis*. The use of mature leaf Al as a phytoindicator of Al toxicity may be even more useful when the collection of fine roots is difficult. This conclusion is also supported by Al toxicity symptoms which appeared in mature leaves after about two weeks of Al treatment.

CHAPTER 7

EFFECTS OF ALUMINIUM, CALCIUM AND PHOSPHORUS ON THE GROWTH OF, AND NUTRIENT ABSORPTION RATE BY *E. CAMALDULENSIS* SEEDLINGS

7. EFFECTS OF ALUMINIUM, CALCIUM AND PHOSPHORUS ON THE GROWTH OF, AND NUTRIENT ABSORPTION RATE BY *E. CAMALDULENSIS* SEEDLINGS.

7.1. Introduction

In the previous experiment (Chapter 6) high Al adversely affected the growth of *E. camaldulensis* seedlings, especially their roots. These effects were less severe when Ca levels were high. Also high P levels significantly increased the growth of *E. camaldulensis* seedlings in an acid soil, low in P and exchangeable Ca and high in exchangeable Al (Chapter 5). Therefore, it was necessary to include P along with various levels of Al and Ca when examining the interaction of P, Al and Ca on the growth of *E. Camaldulensis:* the objective of this experiment. Further, the performance of seedlings needs to be evaluated sequentially over a period of time through successive harvests (whereas in the previous experiments only one final harvest was made).

A reduction in seedling growth was associated with stunted fine root growth, so the question was raised whether the stunted roots are less efficient in their functioning. One important measure of root efficiency is the nutrient absorption rate (Brewster and Tinker 1972). Therefore, a secondary objective of these experiments was to examine the effects of Al, Ca and P on root efficiency of the seedlings as measured by nutrient absorption rate at different points in time.

Eucalyptus camaldulensis seedlings were grown in nutrient solution using treatment combinations including two levels of Al, two levels of Ca and two levels of P. Successive harvests of seedlings from each treatment combination were made and growth parameters were recorded and mineral nutrient absorption rates determined.

7.2. Materials and methods

The general set up of the experiment and environmental conditions in the glasshouse were identical to those described in Chapter 6.

7.2.1. Treatments

As the aim of the experiment was to investigate the effect of high Al on the root efficiency of *E. camaldulensis* and the role of high levels of Ca and P in alleviating effects of Al, only one high and one low level each of Al, Ca and P were included in the experiment. These were 0.25 and 50 mg Al/l; 5 and 100 mg Ca/l and 7.5 and 30 mg P/l. For Ca and Al, these levels correspond to the lowest and highest levels used in the previous experiment (Chapter 6). For P, the two levels were half and double the normal P level in nutrient solution so that the two P levels varied widely. In all there were (2 X 2 X 2 =) 8 treatment combinations and five replicates. To allow for successive harvests, initially each replicate had two bottles of four seedlings. Therefore, after harvesting T_0 samples (before the beginning of treatments), there were 80 bottles to begin treatments.

Deionized water (EC approximately 2 μS) was used to prepare nutrient solutions. Chemicals used to obtain the desired levels of treatments are shown in Table 7.1.

Table 7.1. The composition of nutrient solutions used for different treatment combinations.

Stock solutions	Requirements	ml stock per 50 L nutrient solution
Al stock for 0.25 mg Al/l:	1.12 gm $AlCl_3.6H_2O$/500 ml.	50
Al stock for 50 mg Al/l:	89.42 gm $AlCl_3.6H_2O$/L.	200
Ca stock for 5 mg Ca/l:	3.69 gm $Ca(NO_3)_2.4H_2O$/500 ml 18.75 gm NH_4NO_3/500 ml	200
Ca stock for 100 mg Ca/l:	59.04 gm $Ca(NO_3)_2.4H_2O$/500 ml 9.19 gm $CaCl_2.2H_2O$/500 ml.	200
P stock for 7.5 mg P/l:	13.92 gm $NH_4H_2PO_4$ /L. 5.16 gm NH_4NO_3 75.75 gm KNO_3	100
P stock for 30 mg P/l:	55.68 gm $NH_4H_2PO_4$ /2L 50.56 gm KNO_3 18.63 gm KCl 80.64 gm NH_4NO_3	200

Composition of nutrients common to all treatments:

Chemical	Requirements	ml stock per 50 L nutrient solution
$MgSO_4.7H_2O$	30.81 g/500 ml	100
$MnCl_2.4H_2O$	0.197 g/L	50
$ZnSO_4.7H_2O$	1.15 g/L	50
$CuSO_4.5H_2O$	0.626 g/L	50
$Na_2MoO_4.2H_2O$	0.242 g/L	50
H_3BO_3	0.744 g/L	50
Fe EDTA*		20

* EDTA: 5.0 gm NaOH was dissolved in 800 ml distilled water. Then 33.2 gm EDTA (disodium salt) and 24.9 gm $FeSO_4.7H_2O$ were added, made up to 1L volume and aerated overnight.

7.2.2. Nutrient solution pH adjustment

The two levels of Al used in this experiment differed greatly in nutrient solution pH which is another acid soil factor by itself. It was therefore decided to maintain the same pH for all the treatments. Chosing a pH level is complicated because a lower pH affects seedling growth itself and increasing the pH reduces the proportion of monomeric Al in the system. Therefore, a compromise between these two had to be made. The computer program 'Titrator', version 2.2 (Cabaniss, 1987) was used to detail the effect pH would have on Al species as it changed from 3 to 6. The treatment combination used was the one with the highest levels of of Ca, Al and P (i.e. $Ca_{100}P_{30}Al_{50}$) in nutrient solution (Figure 7.1). A pH of more than 3.7 decreases the amount of monomeric Al sharply. It may be seen from the figure that at a pH of 3.7 most of the Al is present in the monomeric form, thus implying the absence of any precipitation of Al. Therefore, it was decided to maintain the nutrient solution at a pH of 3.7 by adding either dilute HCl or dilute NaOH. After adding the required amounts of all chemicals including acid or alkali, the solution pH was finally checked and adjusted on the following day.

7.2.3. Seedling growth

Five week old seedlings of *E. camaldulensis* were transferred to 2.5 litre size plastic bottles at four seedlings per bottle and bottles filled with half strength nutrient solution. After one week, these dilute nutrient solutions were replaced by normal strength solutions; the actual treatment started two weeks after that. At the start of the treatment, seedling heights were recorded and by swapping seedlings from one bottle to another before treatments began it was possible to ensure that bottles for each treatment had seedlings of a similar size and vigour. From the time treatments began seedlings were grown for a maximum period of six weeks.

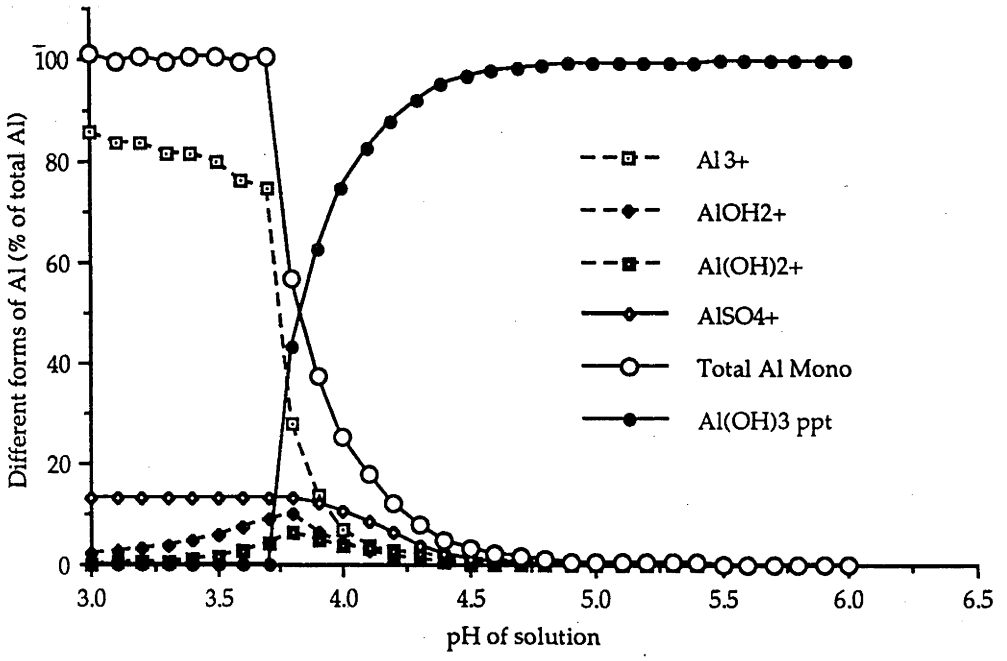


Figure 7.1. Change in the concentration of different forms of Al with a change in solution pH.

7.2.4. Harvest and agronomic data

Shoot heights were recorded weekly. Seedlings were harvested at the start of treatments (T_0), after two days and then at the end of one, two, four and six weeks. At T_0, three seedlings were harvested to make up one sample to ensure enough material for chemical analyses. After two days and one and two weeks two seedlings were harvested to make up one sample. After 4 and 6 weeks each seedling included sufficient material for a sample.

7.2.5. Chemical analysis of seedlings

Two digestion methods were compared: ashing or an H_2SO_4 and H_2O_2 digestion method (Chapter 3). The results were comparable (Figure 3.2) and the H_2SO_4 and H_2O_2 digestion method was chosen for its simplicity.

Shoot and root samples were ground to pass through a 20 mesh screen on a Wiley mill and then digested. Phosphorus, Ca, Mg, and Al were analysed by Inductively Coupled Plasma (ICP) spectroscopy as described in Chapter 3.

7.3. Results

7.3.1. Seedling growth

Figures 7.2 to 7.6 show the change in seedling growth parameters during the 6 week period after treatments began. The Al levels of 0.25 mg/l and 50 mg/l shown in the figures correspond to ranges of 6.58 to 6.71 and 733 to 827 μM in terms of $\Sigma a_{Al\ mono}$. In each figure, the upper portion shows the effect when P was at the lower level (7.5 mg/l) and the lower portion represents the higher P level (30 mg/l) respectively for various Ca and Al combinations. Values of l.s.d. (< 5% level) are also shown in each figure. Since both the figures were drawn from the same sample, they have the same l.s.d. bars.

Shoot weight, root weight and total biomass have a common pattern of change with the course of time. During the first week, the rate of growth was very slow. During the second to fourth weeks seedlings started to grow better; during the 5th and 6th weeks the rate of growth was steeper.

Figure 7.2a shows that in the presence of 7.5 mg P/l, shoot weight growth was fastest for high Ca plus low Al seedlings and at the end of the 6 week period it was significantly higher than in others. Among the other 3 treatment combinations shoot weight was minimum in the low Ca plus high Al treatment though they did not differ significantly. In the presence of 30 mg P/l (Figure 7.2 b) the curves for high Ca plus high Al and the curves for high Ca plus low Al almost

overlapped, they were significantly higher than the other two treatment combinations. At the last harvest, shoot weight in the Ca_5Al_{50} treatment was significantly higher than in the $Ca_5Al_{0.25}$ treatment.

The patterns for the response of root weight (Figure 7.3) were similar to shoot weight, except that in the presence of 30 mg P/l, the root weights after 28 days were higher for high Ca plus high Al than for high Ca plus low Al; for shoot weights these two overlapped. Initially the root weight increment was slower than in case of shoot weight and treatment effects on root weights were visible only after the 4th harvest. But the relative position of different treatment combinations in terms of shoot weight and root weight were similar. The difference in root weight between the treatments Ca_5Al_{50} and $Ca_{100}Al_{50}$ was significant which was not the case in shoot weight.

The pattern for total biomass (Figure 7.4) was similarly influenced by Ca, Al and P as for the case of shoot weight. However, biomass was influenced more by shoot weight than by root weight.

The pattern of change of shoot height (Figure 7.5) and root length (Figure 7.6) have some similarities with shoot weight and root weight, but sometimes the differences due to treatment combinations did not show any clear trend. Shoot height demonstrated a change even during the first 7 days. The steepness of increase during the 5th and 6th weeks was less for shoot height than for shoot weight. The relative position of the curves for shoot height during the period 2 to 6 weeks was similar to the curves for shoot weight. In the initial two weeks, there was little difference in shoot height increments among the

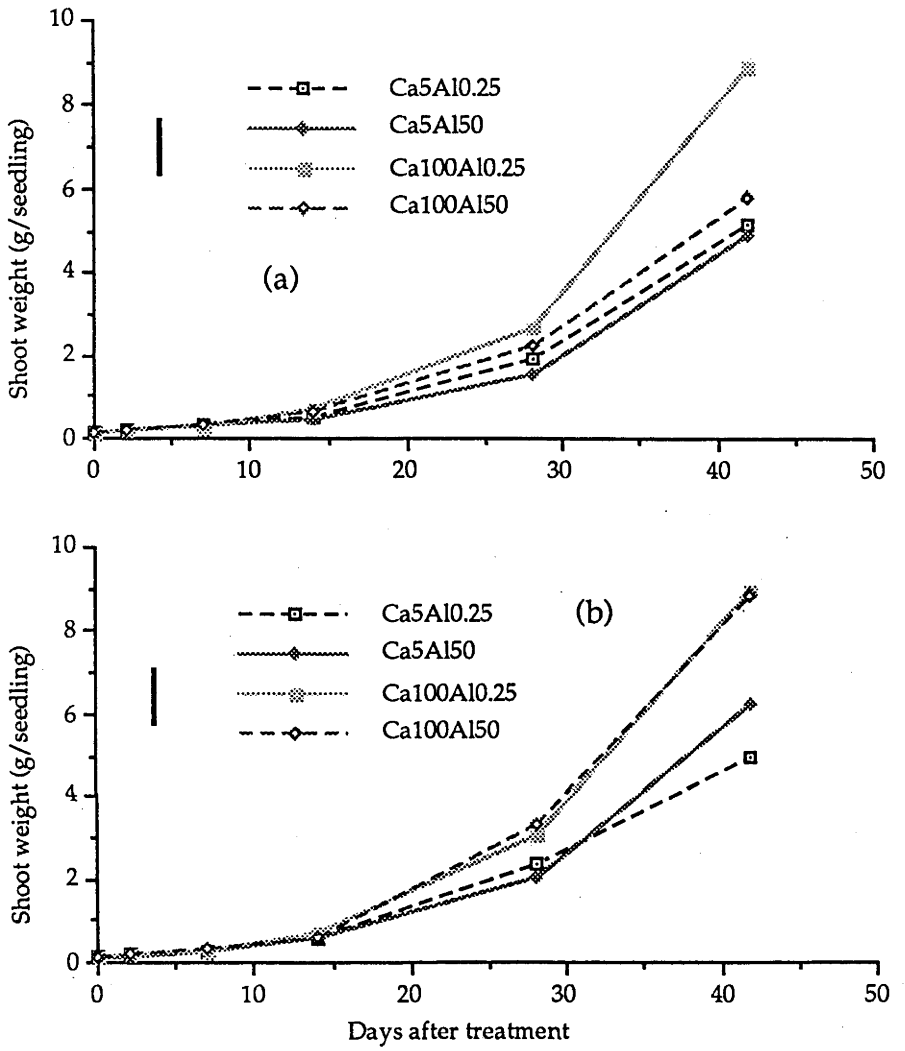


Figure 7.2. Shoot weight increment of *E. camaldulensis* seedlings at different Ca and Al treatments when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

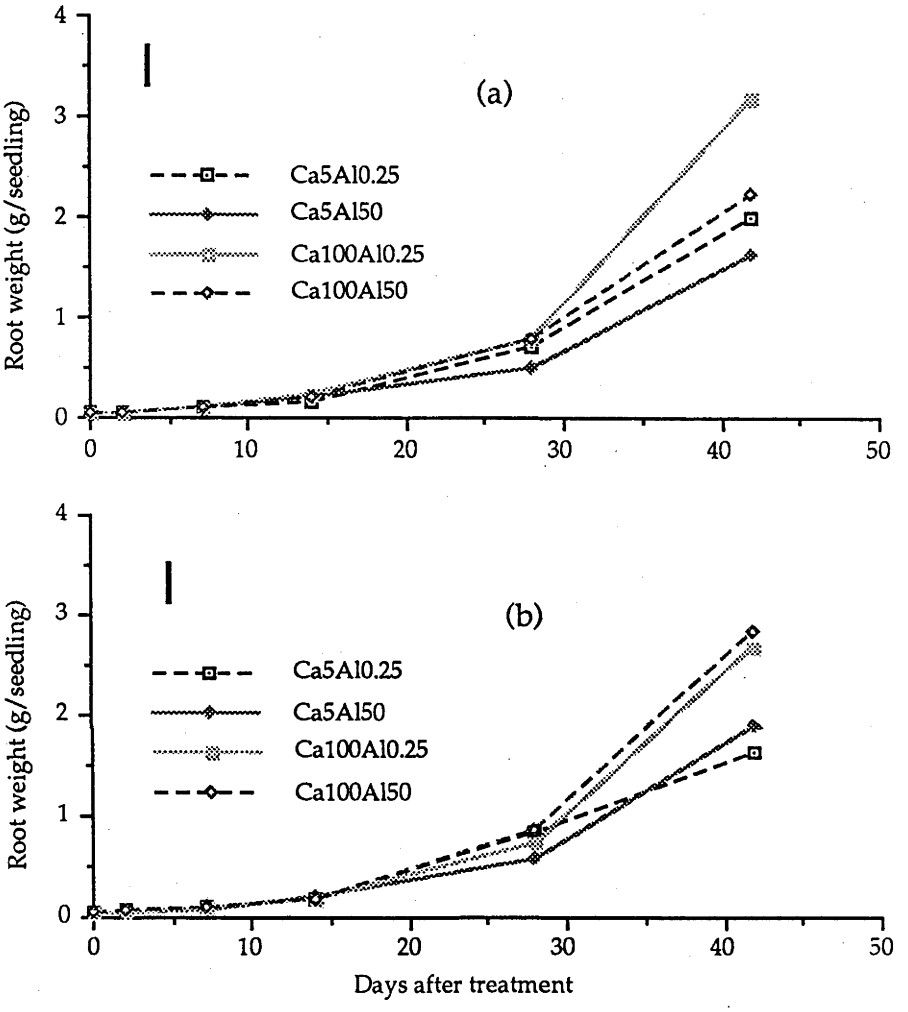


Figure 7.3. Root weight increment of *E. camaldulensis* seedlings at different Ca and Al treatments when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

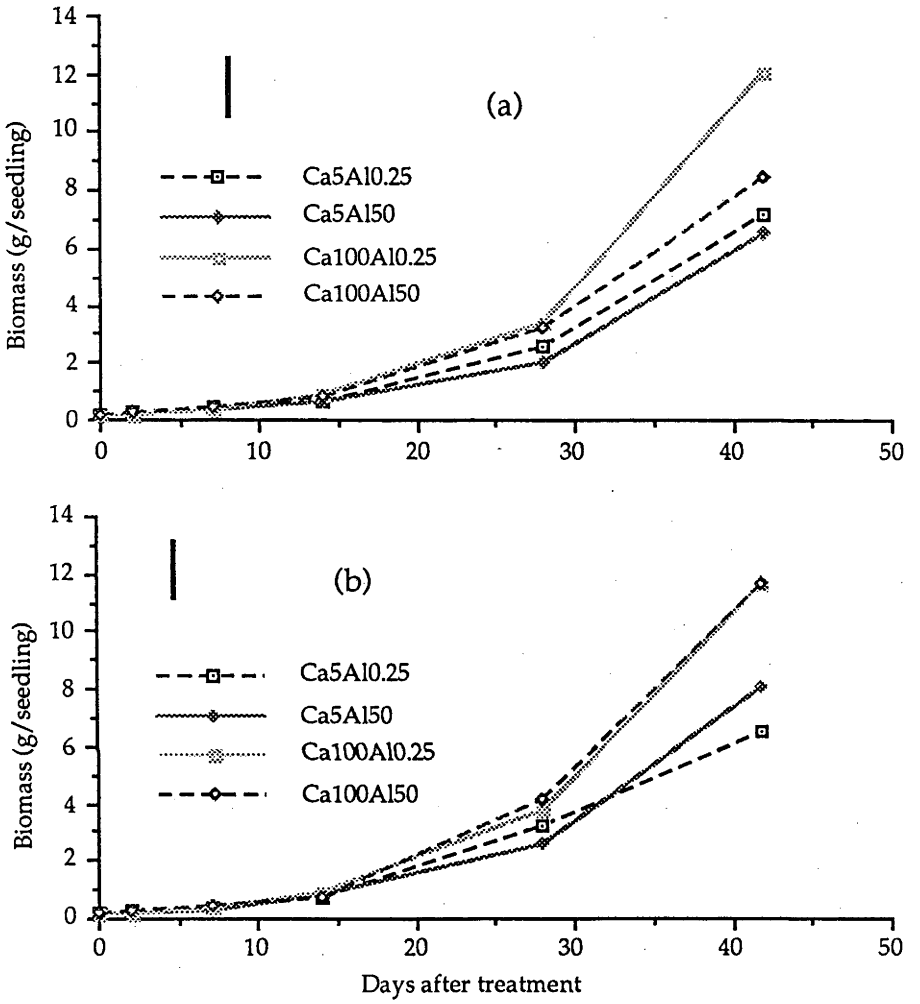


Figure 7.4. Total biomass increment of *E. camaldulensis* seedlings at different Ca and Al treatments when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

treatments. Shoot heights were significantly higher in high Ca treatments irrespective of Al and P levels.

The pattern of effects on primary root lengths was not as systematic as for the other parameters, though the general pattern was a rise with time and the low Al plus high Ca curve was the highest. At 6 weeks, the case of 7.5 mg P/l, high Ca plus low Al produced the longest root while the roots growing in low Ca plus low Al were the shortest. This pattern was similar for root weight. One point needs to be clarified about the pattern of change of root length over time. That is, for some treatment combinations, the root length showed an absolute decline over time. This happened because root length in any treatment combination at a particular instant was the mean root length of seedlings harvested at that time and therefore the root length of the same group of seedlings were not compared.

These differences in the responses of different growth parameters of *E. camaldulensis* to Al at different Ca and P levels indicate that Al interacts with Ca and P to influence seedling growth parameters.

The statistical significance of the influence of treatment levels and their interactions on the seedling growth parameters are presented in Table 7.2. The data show that with the exception of shoot height, Ca had a significant F value for all the growth parameters. The F values of the interaction of Al and P were significant for most of the parameters.

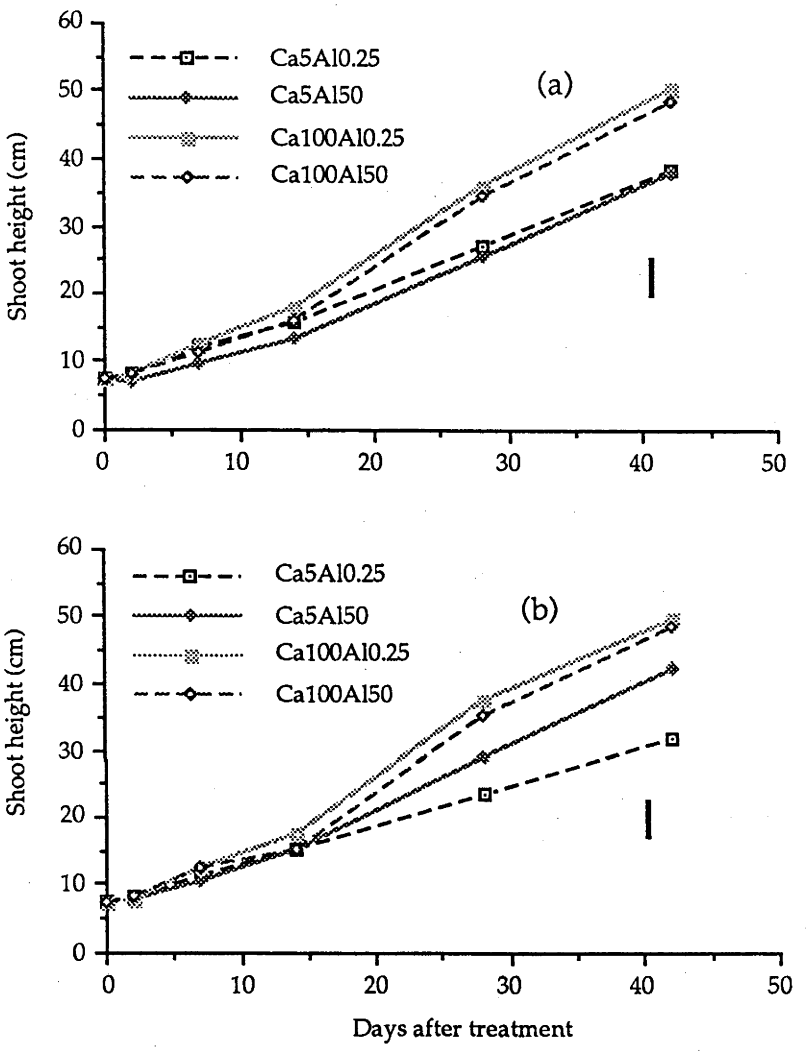


Figure 7.5. Shoot height increment of *E. camaldulensis* seedlings at different Ca and Al treatments when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

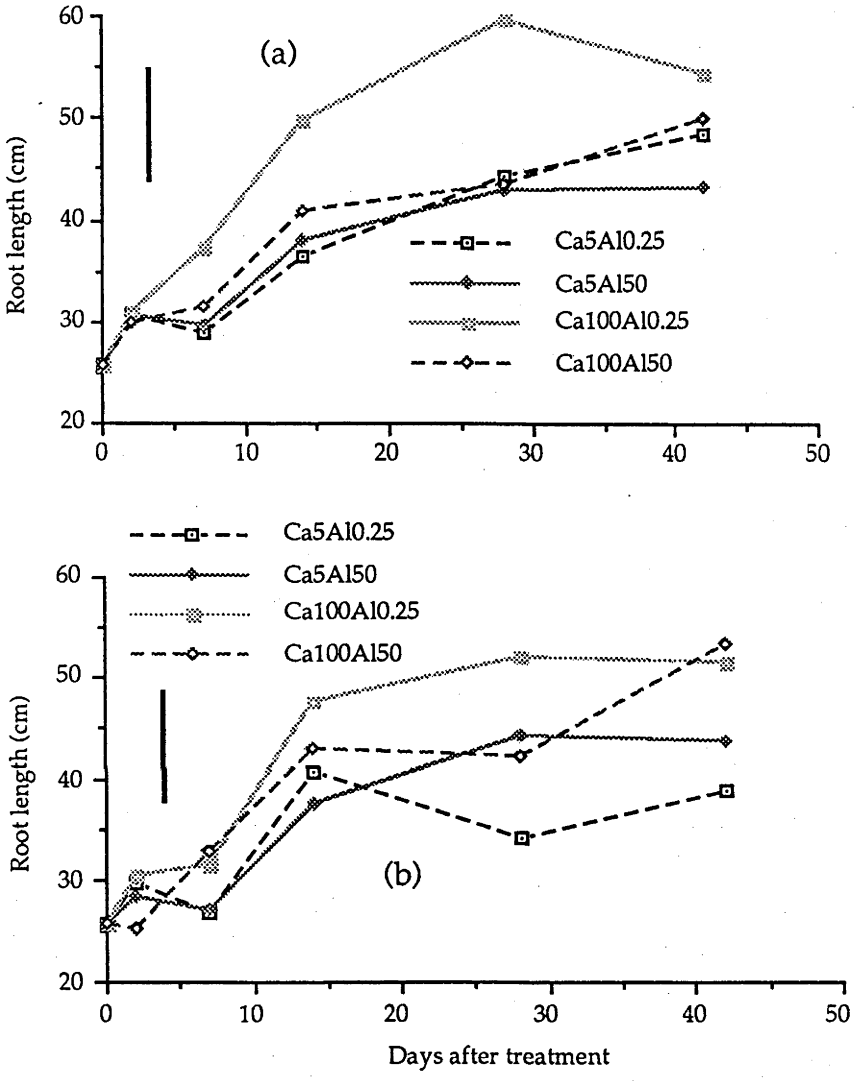


Figure 7.6. Primary root length increment of *E. camaldulensis* seedlings at different Ca and Al treatments when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

Table 7.2. F ratios for the ANOVA on different growth parameters with respect to treatments.

Source of variation	Shoot weight	Root weight	Total biomass	Shoot height	Root length
Al	1.30	2.47	1.67	0.39	3.56
Ca	29.32**	22.20**	29.76**	0.25	19.56**
P	5.71*	0.19	4.01*	0.07	2.71
Time: Lin.	1076.51**	1218.27**	1198.27**	2075.90**	122.05**
Quad.	130.80**	172.90**	151.49**	4.11*	13.52**
Al x Ca	1.59	0.14	1.20	3.21	5.48*
Al x P	3.70	5.16*	4.34*	5.32*	2.02
Ca x P	0.83	0.04	0.59	0.04	0.07
Al x Time (lin.)	1.99	4.69*	2.73	1.97	0.00
Al x Time (quad.)	0.16	0.64	0.26	1.22	1.11
Ca x Time (lin.)	49.54**	47.21**	52.91**	59.71**	4.76*
Ca x Time (quad.)	7.29**	14.36**	9.45**	0.04	1.35
P x Time (lin.)	8.76**	0.30	6.16*	0.06	0.12
P x Time (quad.)	0.32	0.05	0.15	0.26	0.00
Al x Ca x P	0.56	1.01	0.71	2.97	0.01
Al x Ca x Time (lin.)	5.54*	1.47	4.69*	8.01**	0.23
Al x Ca x Time (quad).	3.08	2.60	3.20	0.01	3.39
Al x P x Time (lin.)	7.53**	12.91**	9.37**	3.38	2.37
Al x P x Time (quad).	1.99	4.63*	2.72	0.22	0.22
Ca x P x Time (lin.)	1.60	0.28	1.28	0.40	0.52
Ca x P x Time (quad)	0.42	0.50	0.48)	0.01	0.13
Al x Ca x P x Time (lin)	0.97	1.64	1.20	1.93	0.35

** and * indicate level of significance at < 1% and < 5% respectively.

The difference in growth parameters over time was always significant; the seedlings are obviously expected to grow over time. Both linear and quadratic effects of time were significant for all the growth parameters. The significant quadratic effect of time implies that the rate of growth of the parameters, change significantly as they grew older.

7.3.2. Nutrient absorption rate

The absorption rates of Al, Ca, Mg and P by the seedlings during the 6 week period of growth were examined. The absorption rates were calculated as total uptake (shoot + root) of an element (mg) per unit of fresh root weight (g) at any time. Separate figures are presented for each element at the two levels of P. Table 7.3 shows the F ratios of the multivariate ANOVA for different growth parameters with respect to treatments.

The absorption rate for Al increased with an increase in the Al level (Figure 7.7). At low Al treatment level, an increase in the Ca or P level did not affect the already low Al absorption rate. But at high Al level, an increase in the Ca level raised the Al absorption rate when the P level was low. When the P level was higher, this effect was less prominent. In most cases, the absorption rate of Al was lower when the P level was high. As the seedlings grew bigger, the absorption rate of Al remained fairly constant when treatment Al level was low.

Table 7.3. F ratios for the ANOVA on absorption rate of different nutrient elements with respect to treatments.

Source of variation	Al	Ca	Mg	P
Al	710.52**	59.10**	225.62**	25.48**
Ca	1.36	418.70**	49.52**	7.55**
P	11.01**	11.94**	13.06**	96.56**
Time: lin.	12.72**	344.98**	57.53**	27.14**
quad	16.95**	143.61**	13.12**	2.50
Al x Ca	1.34	48.17**	37.02**	0.00
Al x P	6.98**	4.80*	0.00	0.33
Ca x P	3.58	17.02**	4.65*	5.40*
Al x Time (lin.)	19.26**	13.47**	26.65**	7.04**
Al x Time (quad.)	11.96**	2.32	23.62**	4.43*
Ca x Time (lin.)	6.02*	70.87**	9.83**	4.24*
Ca x Time (quad.)	0.25	13.06**	3.76	1.99
P x Time (lin.)	6.65*	15.32**	1.82	3.63
P x Time (quad.)	2.53	0.64	19.57**	10.95**
Al x Ca x P	2.98	1.83	0.01	3.79
Al x Ca x Time (lin.)	4.72*	9.69**	1.16	3.71
Al x Ca x Time (quad).	0.03	0.01	4.32*	0.66
Al x P x Time (lin.)	7.55**	2.75	3.61	2.98
Al x P x Time (quad).	4.87*	0.73	0.40	1.28
Ca x P x Time (lin.)	0.56	21.61**	3.16	1.17
Ca x P x Time (quad)	0.36	0.08	3.05	3.19
Al x Ca x P x Time (lin)	1.08	2.96	0.14	0.37

** and * indicate level of significance at < 1% and < 5% respectively.

When the Ca level was high the absorption rate for Ca was significantly reduced by high Al levels (Figure 7.8). At a low Ca level, high Al did not influence the Ca absorption rate throughout the treatment period. High P also reduced the Ca absorption rate and when both Al and P were higher, their total effect was less than their additive reduction. For the first two weeks, the absorption rate of Ca declined sharply with time and thereafter declined very slowly. The adverse effect of Al on the Ca absorption rate remained more or less stable after two weeks.

The Mg absorption rate was significantly reduced by high Al levels (Figure 7.9) at any level of Ca or P. The absorption rate of Mg was reduced in most cases by the increase of Ca and P treatments. When Ca, Al and P levels were low, the absorption rate of Mg was significantly higher than other treatment combinations and remained fairly constant at 0.78 mg Mg/g of fresh root. The Mg absorption rate for the remaining treatment combinations in Figure 7.9a declined sharply during the first two weeks and did not change much after two weeks. When the P level was high (Figure 7.9 b) there was a slow declining trend in the absorption rate except for the $Ca_5Al_{0.25}$ treatment combination in which case the absorption rate increased from the 4th week. The absorption rate was significantly lower when the Ca level was high.

The P absorption rate was significantly reduced by high Al levels (Figure 7.10) and this effect was more prominent at low, than at high P levels. At identical Ca and Al levels, the P absorption rate was slightly higher at the high P treatment level. With time, the P absorption rate generally declined, but in many cases the decrease was small and in a few even a contrary pattern was observed.

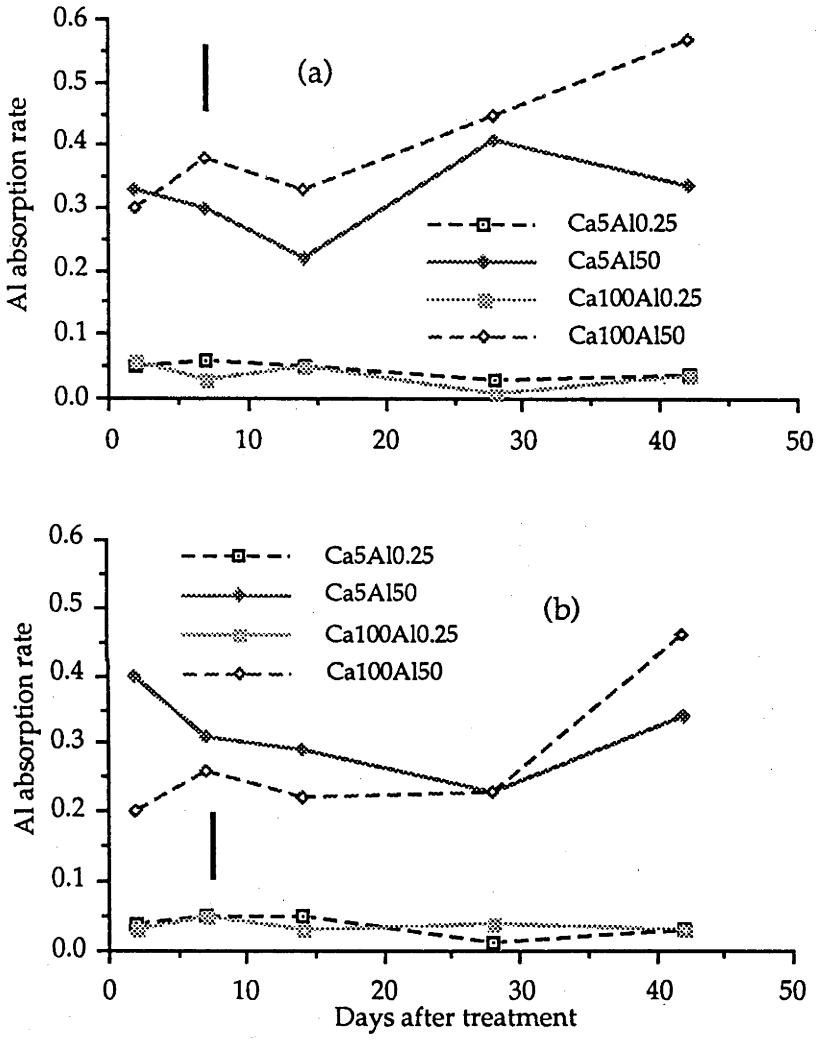


Figure 7.7. Absorption rate of Al (mg Al/g fresh root) at different dates after treatment, at different Ca and Al levels when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

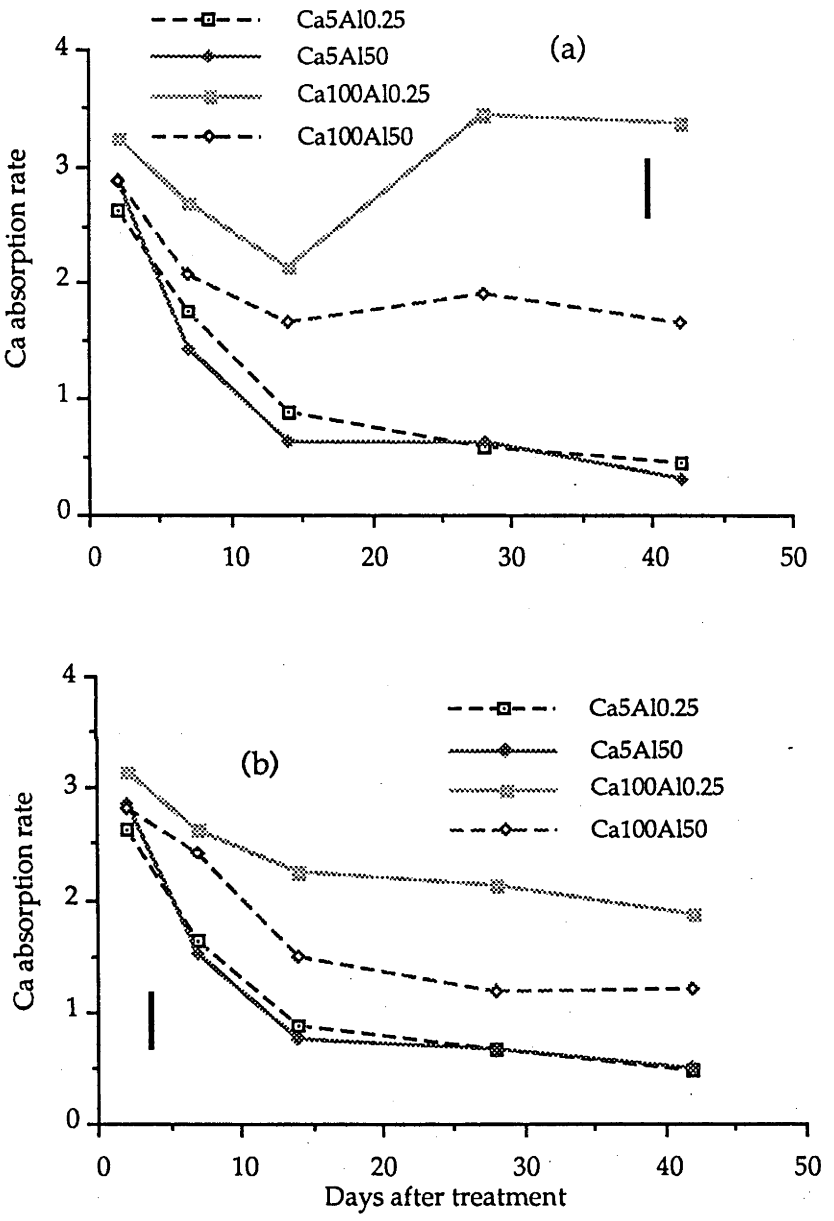


Figure 7.8. Absorption rate of Ca (mg Ca/g fresh root) at different dates after treatment, at different Ca and Al levels when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

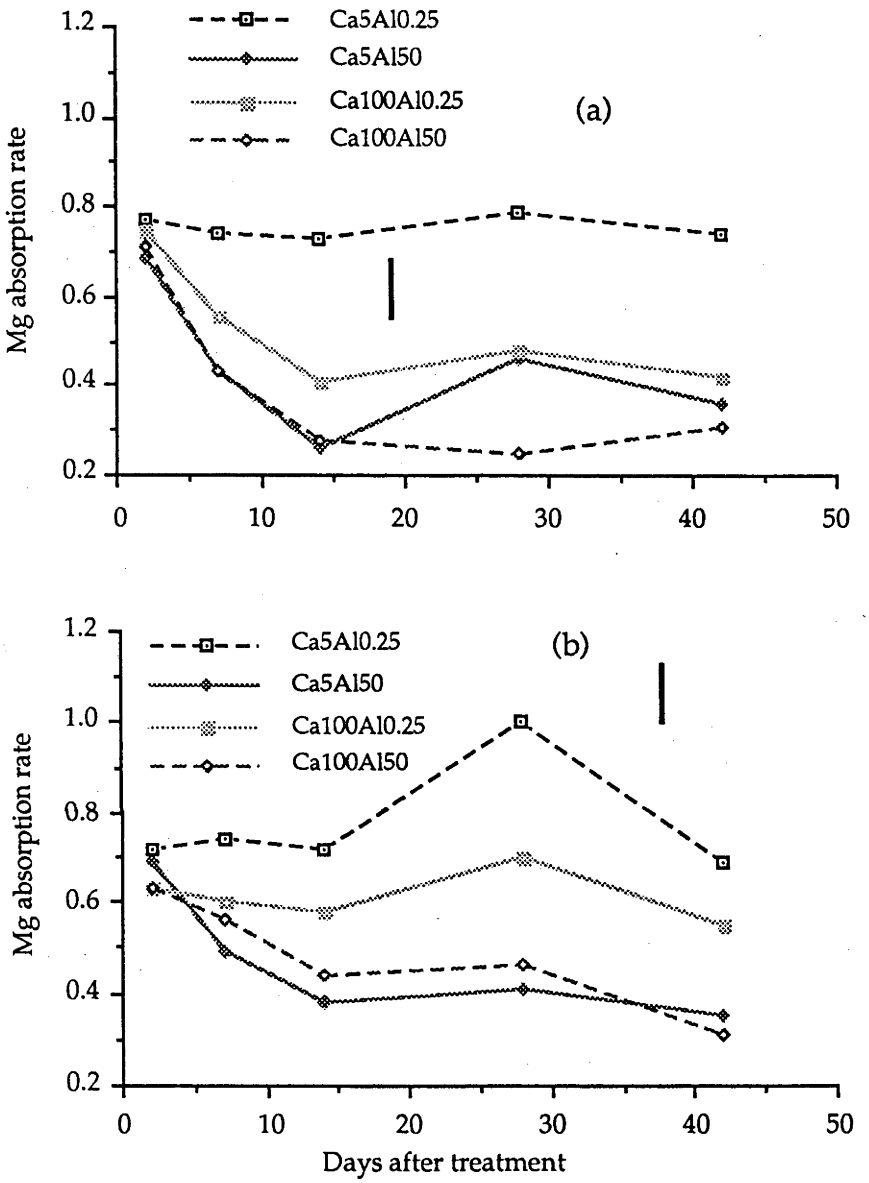


Figure 7.9. Absorption rate of Mg (mg Mg/g fresh root) at different dates after treatment, at different Ca and Al levels when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$)

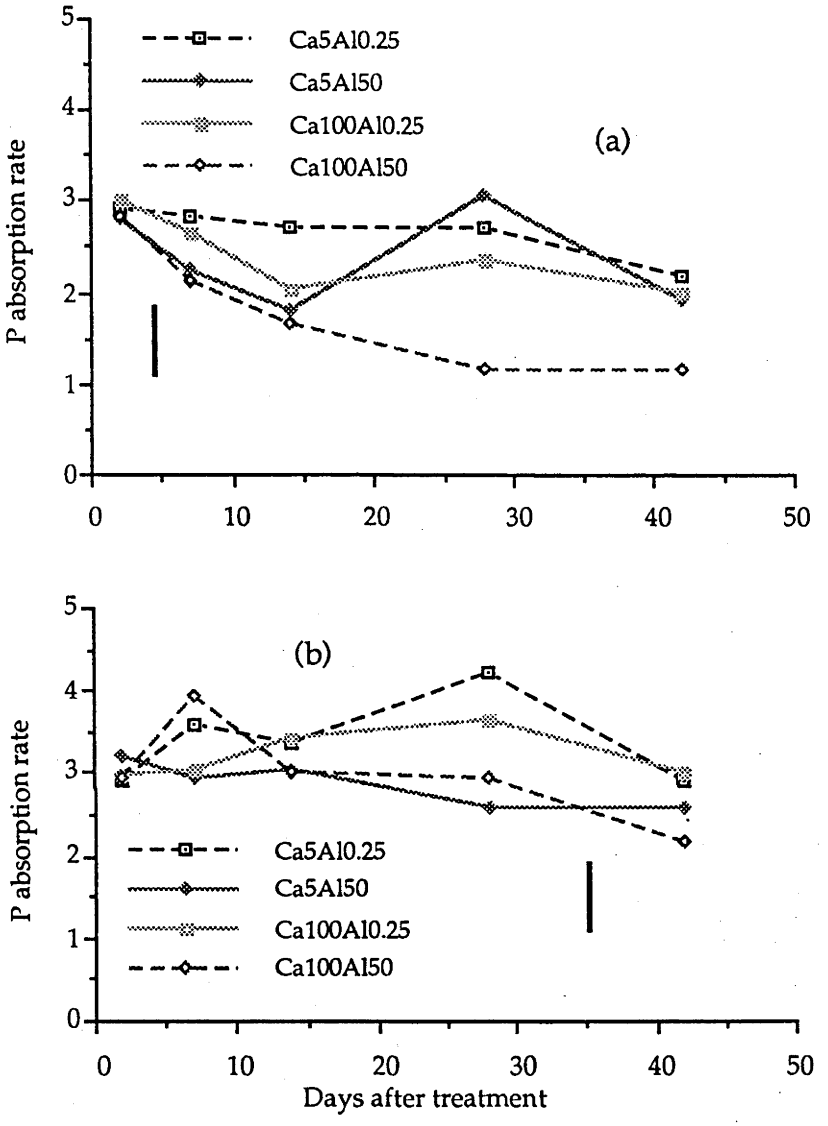


Figure 7.10. Absorption rate of P (mg P/g fresh root) at different dates after treatment, at different Ca and Al levels when (a) P level was fixed at 7.5 mg/l and (b) P level was fixed at 30 mg/l. Vertical bars represent l.s.d. ($P < 0.05$).

The relationship of nutrient absorption rate with a_{Al}/a_{Ca}

In Chapter 6 it was found that the ratio a_{Al}/a_{Ca} explained the variation of some of the growth parameters quite well. Here a further attempt is made to analyze whether the a_{Al}/a_{Ca} ratio can explain the variation in the absorption rate for Al, Ca, Mg and P. Figure 7.11 shows the scatter diagram and best fit equations for these analyses. However, the R^2 was in general, small though it was significant for Ca and Mg. Therefore, in terms of their effect on nutrient absorption rate, Al and Ca levels may be more relevant as independent factors than as a ratio.

7.4. Discussion

7.4.1. Seedling growth

The effects of Ca, Al and P treatments on growth parameters of *E. camaldulensis* seedlings for 6 weeks period revealed that in general, high Al significantly reduced the growth of *E. camaldulensis* seedlings when Ca and P levels were low. An increase in Ca and/or P levels ameliorated the deleterious effects of Al. An increase in the Ca level increased the growth of *E. camaldulensis*, but an increase in the P level decreased the growth when Ca and Al were low and improved growth when Ca and/or Al were high.

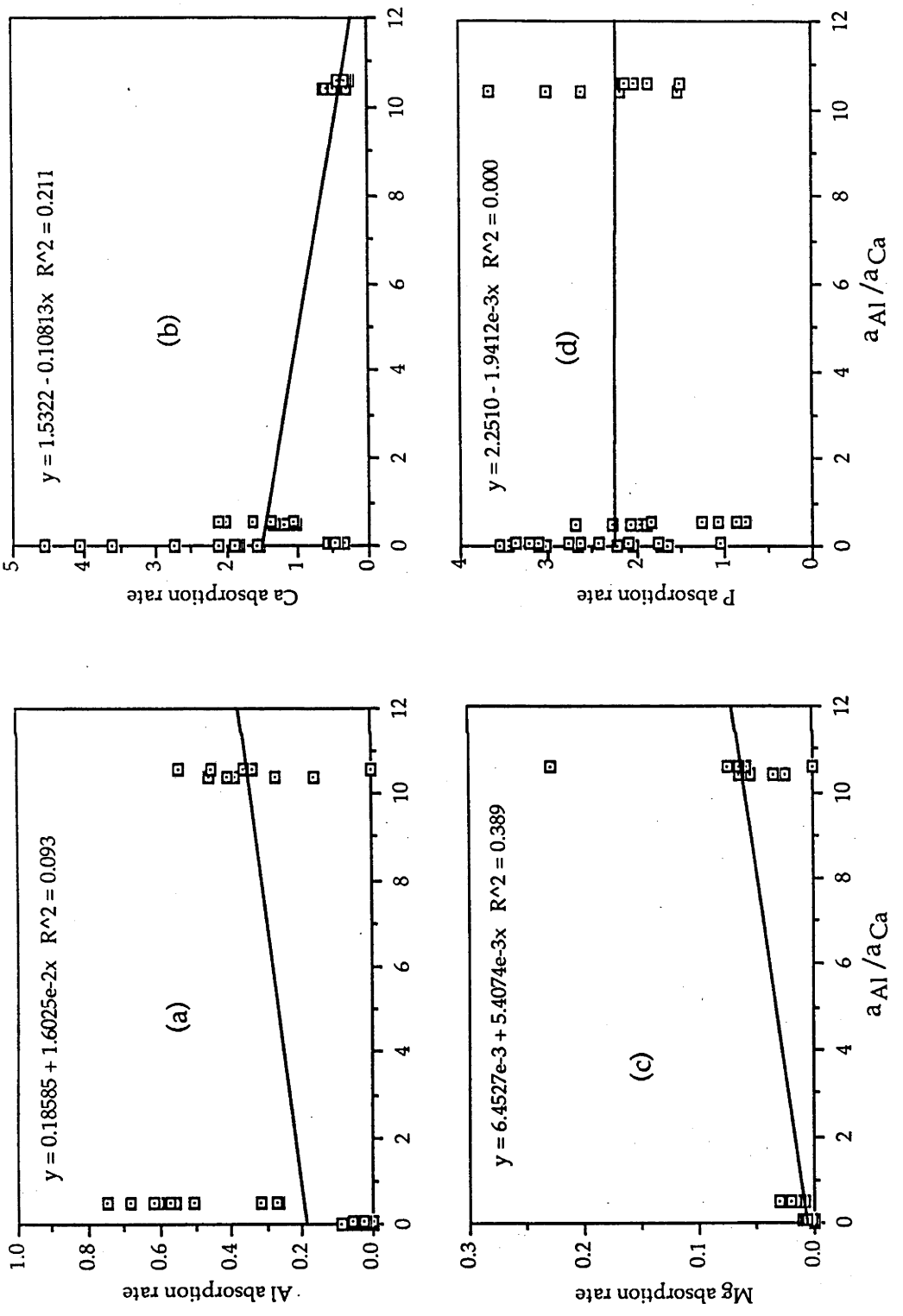


Figure 7.11. Relationship between a_{Al}/a_{Ca} and absorption rates (mg/g fresh root) of (a) Al, (b) Ca, (c) Mg and (d) P.

Table 7.4. Increase in growth parameters with an increase in the P level at different Al and Ca levels.

Growth parameter	Increase from $Ca_5Al_{0.25}P_{7.5}$ to $Ca_5Al_{0.25}P_{30}$	Increase from $Ca_5Al_{50}P_{7.5}$ to $Ca_5Al_{50}P_{30}$	Increase from $Ca_{100}Al_{50}P_{7.5}$ to $Ca_{100}Al_{50}P_{30}$
Shoot height (cm)	* - 6.7 (21.1)**	4.30 (11.3)	0.20 (0.4)
Root length (cm)	- 9.7 (25.0)	0.50 (1.2)	3.30 (6.6)
Shoot dry wt. (g/seedling)	- 0.22 (4.4)	1.33 (27.1)	2.62 (41.9)
Root dry wt. (g/seedling)	- 0.37 (22.7)	0.27 (16.6)	0.60 (26.8)
Total biomass (g/seedling)	- 0.59 (9.0)	1.60 (24.5)	3.22 (37.9)

* Negative sign preceding a number means that growth actually declined.

** Figures in the parenthesis indicate percent increase or decrease over lower P level.

The effect of P on seedling growth parameters at different Ca and Al levels is compared (for the last harvest) in Table 7.4. It can be seen from the table that high P alone is in fact harmful in terms of the growth of *E. camaldulensis* seedlings. In fact the increased level of P is about double the optimum level of P (15.5 mg/l) for *E. camaldulensis* grown in nutrient solution (Thomson, 1988) and was included in this study to assess the ameliorative role of high P on Al toxicity.

These results imply that high P did not affect *E. camaldulensis* growth in nutrient solution by removing a P deficiency because increased P did not improve growth at low Ca or low Ca plus low Al treatments. Since P improved growth only at high Al or high Al in combination with high Ca, it may be assumed that the beneficial effects of P on growth parameters is via the amelioration of Al. Furthermore the role of P was more effective in the case of high Al plus high Ca than in case of high Al alone indicating an interdependence between the three elements.

Where P ameliorates the toxicity of Al and improves growth this research is in conformity with other findings on maize (Bartlett and Reigo, 1972) and soybean, sunflower, subterranean clover and alfalfa (Alva et al, 1986 c). Positive growth responses in the presence of high Al and high P were reported for other plants. Konishi et al (1985) reported that the toxic effect of high P (0.8 mM when 0.1 mM was optimum) was ameliorated and the growth and P uptake of tea plants grown in a nutrient solution maintained at a pH of 4.5 were enhanced by 1.6 mM Al. Bartlett and Riego (1972) reported that P prevented Al toxicity in maize only when P in the solution precipitated Al.

Another possible explanation for the positive growth response of P only when Al and/or Ca were high, is that when both P and Al were high, some P was used to counteract the toxic effects of Al and therefore toxicity of both Al and P were reduced. However, this argument is difficult to conclude because the equilibrium species calculated do not suggest any precipitation of aluminium phosphate under the set conditions. An alternative explanation in support of the present findings may be drawn from the study by Blamey et al (1983) who reported that even in solutions which were undersaturated with respect to aluminium phosphate, progressive losses of monomeric Al have occurred. They also reported that losses due to the formation of soluble polymeric complexes of Al and P, are essentially non phytotoxic. Alva (1986) studied the effect of aging on a solution containing different amounts of P, Al and Ca for a period of 21 days. He found that at high P levels, total and monomeric Al decreased markedly after 3 days. After 21 days of aging, losses of up to 34 and 60% respectively of total and monomeric Al had occurred. At high Ca and Al levels the P concentration decreased by up to 50 percent.

Unlike P, Ca may have improved growth both by removing a Ca deficiency and by ameliorating an Al toxicity. In fact the lower Ca level in this experiment (5 mg/l) was much lower than the optimum Ca level for *E. camaldulensis* in nutrient solution (80 mg Ca/l, Thomson, 1988). In Chapter 6 also, a Ca level of 5 mg/l was found to be insufficient for *E. camaldulensis* growth. The ameliorating role of Ca on *E. camaldulensis* seedlings was discussed earlier (Chapter 6).

The interactions over time of some of the treatments were also significant. The significant increase in seedling growth parameters with time is obvious, but more importantly there are differences due to an interaction between time and the other treatments. An interaction between Al, Ca and time and between Al, P and time were significant for most parameters (Table 7.3). These imply that as the seedlings grow older, the influence of Ca, Al and P and their interactions change. For example, it was seen from Figure 7.4b that at the end of 6 weeks when both Ca and P were higher, seedlings treated with high Al produced the same biomass as those under low Al. Low Ca plus high Al seedlings even superseded the low Ca plus low Al seedlings after 4 weeks in terms of biomass production. Therefore, it may be hypothesized that as the seedlings grow, they become more tolerant of high Al.

The change in the impact of Al on plants with longer growth periods has also been reported in other studies though the interaction among Al, time and other factors ameliorating Al (Ca and P especially) were not included. To explain higher Al sensitivity in cassava at early growth stages, Manrique (1987) suggested that the sensitivity of Al shifts among plant components as plants develop, with leaves and storage components (stems and roots) as the most sensitive tissues to Al at early plant growth and root enlargement stages respectively.

However, it should be mentioned that in the present study, there were only two treatment levels of each of Ca, Al and P which were widely spaced and the effect of any intermediate level(s) could have been different. The experiments at only two levels were useful to fulfil the aim of this section which was to examine if and how, high Ca and P levels protect the seedlings from Al toxicity as measured by seedling growth.

7.4.2. Nutrient absorption rate

The growth of a plant is a rate process and the absorption of nutrients is an essential part of that growth. If the absorption rate does not keep pace with the growth rate, the plant becomes deficient (Brewster and Tinker, 1972). However, the proportion of root to whole plant, the distribution of nutrients in the plant and the utilization of nutrients may also modify the relationship between absorption rates and plant growth. Therefore, absorption rates at any time may not have a strong relationship with growth performance. The crucial role of the absorption rate lies in the ability of a plant to utilize absorbed nutrients.

Williams (1948) first suggested that root activity should be expressed as uptake rate per unit dry weight, a measure also used by Welbank (1962), who called it the 'specific absorption rate'. Scott Russell and Saunderson (1967) found that an uptake rate in solution culture was related more closely to root volume than to surface area, which suggests that the parameter based on fresh weight may be preferable and some other studies also (Brewster and Tinker, 1972) used fresh root weight for calculating absorption rates. In this study absorption rates were calculated on the basis of fresh root weight.

The effect of Al on the absorption rate of Al was different at low levels of Ca and P than at high levels of Ca and/or P. High P decreased the Al absorption rate of seedlings and checked the decrease in the Mg absorption rate resulting from high Al. The effect of Ca on the absorption rate of Al was in general positive in the presence of high Al. It appeared that P had a greater effect when compared to that of Ca in influencing the absorption rate of the seedlings.

Although these effects on the absorption rates of Ca, Mg and P are related to Al levels, there are reports that an increase in the uptake of some cations may not be a direct treatment effect but brought about by the capacity of the plants to preserve a cationic balance (van Itallie, 1948). There may also be an interaction effect between some of the elements or an effect over time which influences the absorption rates.

Another major interest of this analysis was to examine how the effect of Al on nutrient absorption rate was influenced by time. The effect of Al on absorption rates of Ca, Mg and P was found to decline over time which is quite to be expected as the seedlings grow older. The interaction between time and Al treatment levels was found to be significant when considering the absorption rates of Ca, Mg and P (Table 7.3).

This Chapter tested one of the hypotheses that followed from Chapter 6 relating to Al toxicity effects on root efficiency. In the following Chapter another hypothesis relating to accumulation of cations in Al stressed *E. camaldulensis* seedlings will be tested.

CHAPTER 8

EFFECTS OF CALCIUM AND PHOSPHORUS ON ALUMINIUM AND OTHER CATIONS IN THE ROOTS OF *E. CAMALDULENSIS* GROWN IN HIGH ALUMINIUM MEDIA

8. EFFECTS OF CALCIUM AND PHOSPHORUS ON ALUMINIUM AND OTHER CATIONS IN THE ROOTS OF *E. CAMALDULENSIS* GROWN IN HIGH ALUMINIUM MEDIA

8.1. Introduction

High levels of Al in the growth medium adversely affect root growth of *E. camaldulensis* causing a reduction in fine root length and the fineness of fine roots (Chapter 6). Roots grown in a high Al medium were found to be thickened with layers of small cells in the root periphery. When high Al was accompanied by high Ca these malfunctions were less adverse. An increase in the treatment Ca level increased Ca concentration in fine roots by up to about 8 times and increased Al concentration by up to about three times thus changing the ratio of (concentration of Al)/(concentration of Ca) in the root (Chapter 6). The ratio of a_{Al}/a_{Ca} in treatments explained a large percentage of variation in root related growth parameters of *E. camaldulensis* seedlings. Therefore Al had a negative effect and Ca had a positive effect on growth parameters.

The objective of the experiment described in this Chapter is to understand the physiological role of various elements in terms of their accumulation in the root periphery and their implications for the thickening of that periphery. Therefore, the experiment was designed to find how the total and desorbable forms of Ca, Al and Mg in the root periphery are changed with varying Ca levels. These Ca levels when applied with a high Al level resulted in a different a_{Al}/a_{Ca} ratio in the growth medium. Phosphorus significantly improves *E. camaldulensis* growth and also interacts with Ca in high Al acid soil (Chapter 5). For *E. camaldulensis* grown in nutrient solution, various Ca, Al and P levels

interacted to influence the growth of the shoot and root (Chapter 7). Therefore, P was also included as a treatment in the present experiment.

8.2. Materials and methods

Seedlings were propagated by cutting from a single seedling of *E. camaldulensis* which was in turn grown from the seedlot 10886 (Table 4.2). The aim was to eliminate any genetic variability between seedlings. When the cuttings developed roots, they were transplanted into potting mix in small pots and when these cuttings reached a size big enough for fresh cuttings, the process was repeated until sufficient seedlings were available for the experiment[1]. In the last lot, when the cuttings developed roots, they were transplanted and placed in half strength nutrient solution (Table 3.1) instead of potting mix. After one week the half strength nutrient solution was changed to full strength.

After two weeks in full strength nutrient solution, uniform and healthy seedlings were selected for experimentation. Since high Al was found to be responsible for an adverse effect on root growth, the high Al level in this experiment was chosen to match the highest Al level (50 mg/l) used in Chapter 6. Two levels of Ca treatments (5 and 100 mg/l) were applied, so that the effect of the ratio a_{Al}/a_{Ca} could be examined. Phosphorus levels were 7.5 and 30 mg P/l. Each treatment had four replications and the same nutreint solutions as in Chapter 7 were used (Table 7.1).

After 30 days under the treatments, the seedlings were harvested. The fine roots were separated and washed twice quickly in distilled water to remove nutrient solution. To desorb cations from the

[1] This process took about 9 months to produce sufficient cuttings for this experiment. For this reason, it could not be used for earlier experiments.

root periphery the roots were then soaked in 3 mM citric acid (Ownby and Popham, 1989), first for 5 minutes and then for another 25 minutes (30 minute) in fresh solution. These two desorption times were assumed to represent extracellular (5 minutes) and cellular (next 25 minutes) desorption of cations. The roots were washed with distilled water and dried in the oven at 70° C for two days and then analysed for total cations with ICP (Section 3.6.) after digesting the root tissues with H_2SO_4 - H_2O_2 (Heffernan, 1985).

8.3. Results

Cations desorbed in the first 5 minutes and in the additional 25 minutes were calculated separately. These amounts when added to the cation concentrations in the H_2SO_4 - H_2O_2 digest represent the total concentration of each cation in the root tissue.

The association between treatment a_{Al}/a_{Ca} ratio and the amount of cations desorbed in the 5 minutes and in the additional 25 minutes are shown in Figure 8.1. The amount of Al desorbed was not affected by the treatment a_{Al}/a_{Ca} while the amount of Ca desorbed showed a negative association. Magnesium showed a positive correlation although the R^2 was small.

Figure 8.2 shows the effects of P and Ca treatments on the amounts of cations desorbed from the root periphery. Calcium and P significantly enhanced the desorption of Ca at 5 and additional 25 minutes. When both Ca and P treatment levels were raised, Ca desorption increased several fold (both at 5 minutes and 25 minutes, although the Ca desorbed in 5 minutes was much higher).

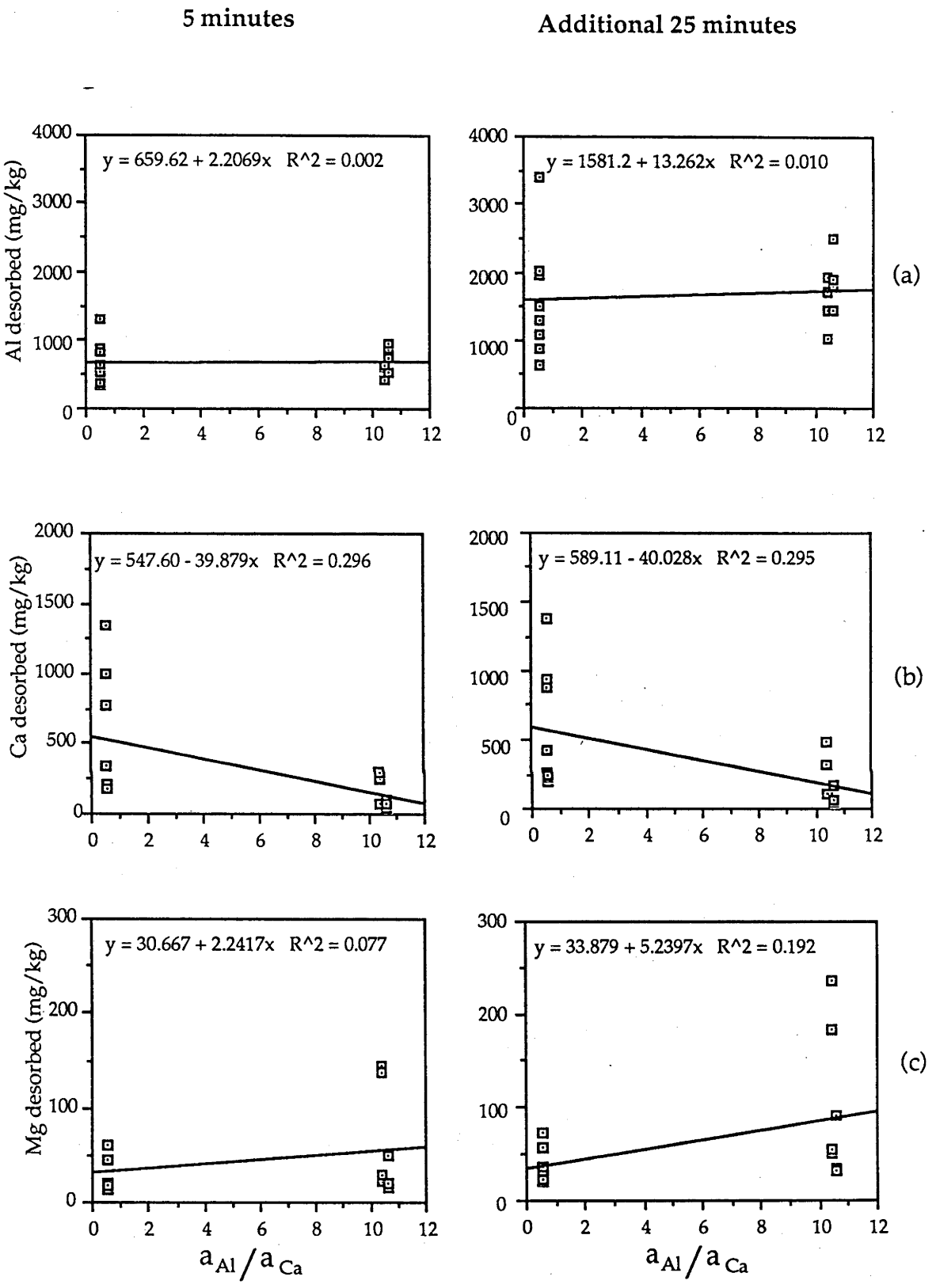


Figure 8.1. Relationship between a_{Al}/a_{Ca} in the treatment with root tissue concentrations of (a) Al, (b) Ca and (c) Mg desorbed in 5 minutes (left hand figures) and an additional 25 minutes (right hand figures).

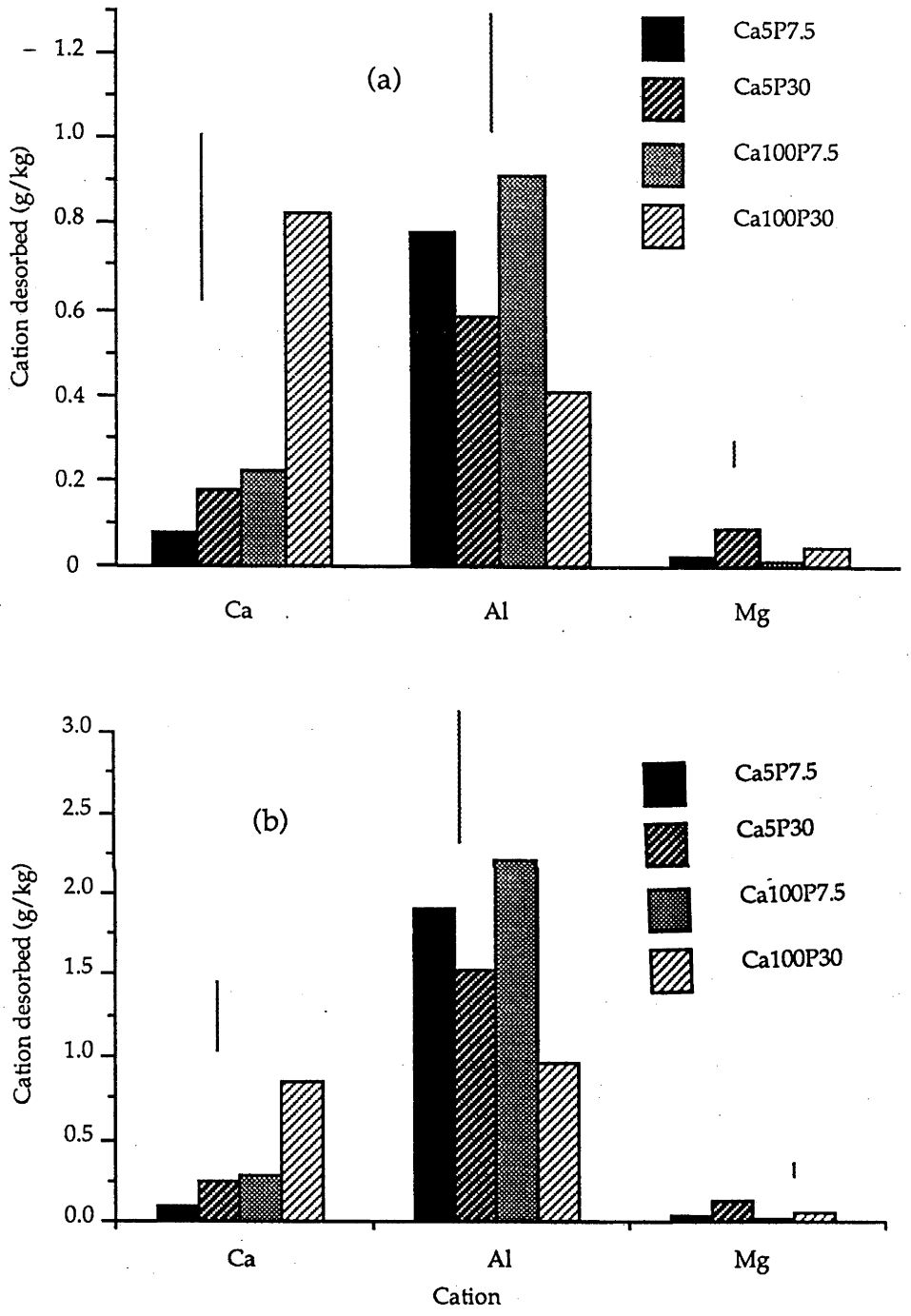


Figure 8.2. Cations desorbed in (a) 5 minutes and (b) an additional 25 minutes. Vertical bars represent l.s.d. ($P < 0.05$).

The amount of desorbed Al was not reduced by higher Ca levels, when P was at 7.5 mg/l; it in fact slightly increased, at both 5 minutes and the additional 25 minutes desorption (Figure 8.2). Aluminium desorbed at both 5 and 25 minutes was significantly lower for high P treatments when high P was accompanied by high Ca.

Magnesium concentrations were much lower compared to other cations at both desorption times. It was reduced by an increase in Ca level and was increased by a higher P level when the higher P level was accompanied by higher Ca.

Table 8.1 gives the results of an ANOVA on the effects of Ca and P treatments on 5 and the additional 25 minute desorption of the cations and the total concentration of elements in the root. Calcium desorption was significant for both Ca and P treatments; Al and Mg (5 minutes only) desorptions were significantly different for P treatments only.

To examine the relative amounts of each of the three cations in the extracellular spaces and cellular materials, the desorbed amount of each cation is expressed as a percentage of the total of the three cations (Al, Ca and Mg); both for 5 minutes and for the additional 25 minutes (Figure 8.3). This figure therefore shows directly the effects of the different treatments on the extracellular and cellular cations. At any treatment combination a large proportion of the total cations both in the extracellular and cellular positions is occupied by Al. At higher Ca and P, Ca occupies the large proportion mostly at the cost of Al. Therefore at higher Ca plus P treatments, Al was much reduced in the root periphery; although total Al concentration in the root did not differ significantly.

Table 8.1. F ratios for the ANOVA on variation due to Ca and P treatments in Ca, Al and Mg desorbed and total Ca, Al, Mg and P in Al injured roots of *E. camaldulensis*.

Element	Desorption time (min.)	Form of expression	Variation due to		
			Ca	P	Ca x P
Ca	5	Conc. (mg/kg)	10.10**	8.08*	4.24
		% total in root	0.32	7.40*	5.18*
	Additional 25	Conc (mg/kg)	8.93**	7.47*	2.21
		% total in root	0.03	4.48*	0.05
		Total (mg/kg)	13.42**	5.56*	2.01
Al	5	Conc (mg/kg)	0.05	15.24**	2.8
		% total in root	0.13	7.38*	0.08
	Additional 25	Conc (mg/kg)	0.24	9.44**	2.72
		% total in root	0.43	8.94**	0.25
		Total (mg/kg)	0.04	0.23	1.00
Mg	5	Conc (mg/kg)	1.66	6.15*	0.62
		% total in root	0.92	4.00	0.13
	Additional 25	Conc (mg/kg)	4.49	4.69	1.46
		% total in root	5.87*	1.83	0.87
		Total (mg/kg)	1.57	14.42**	0.10
P		Total (mg/kg)	0.00	18.52**	0.82

** and * indicate level of significance at < 1% and < 5% respectively.

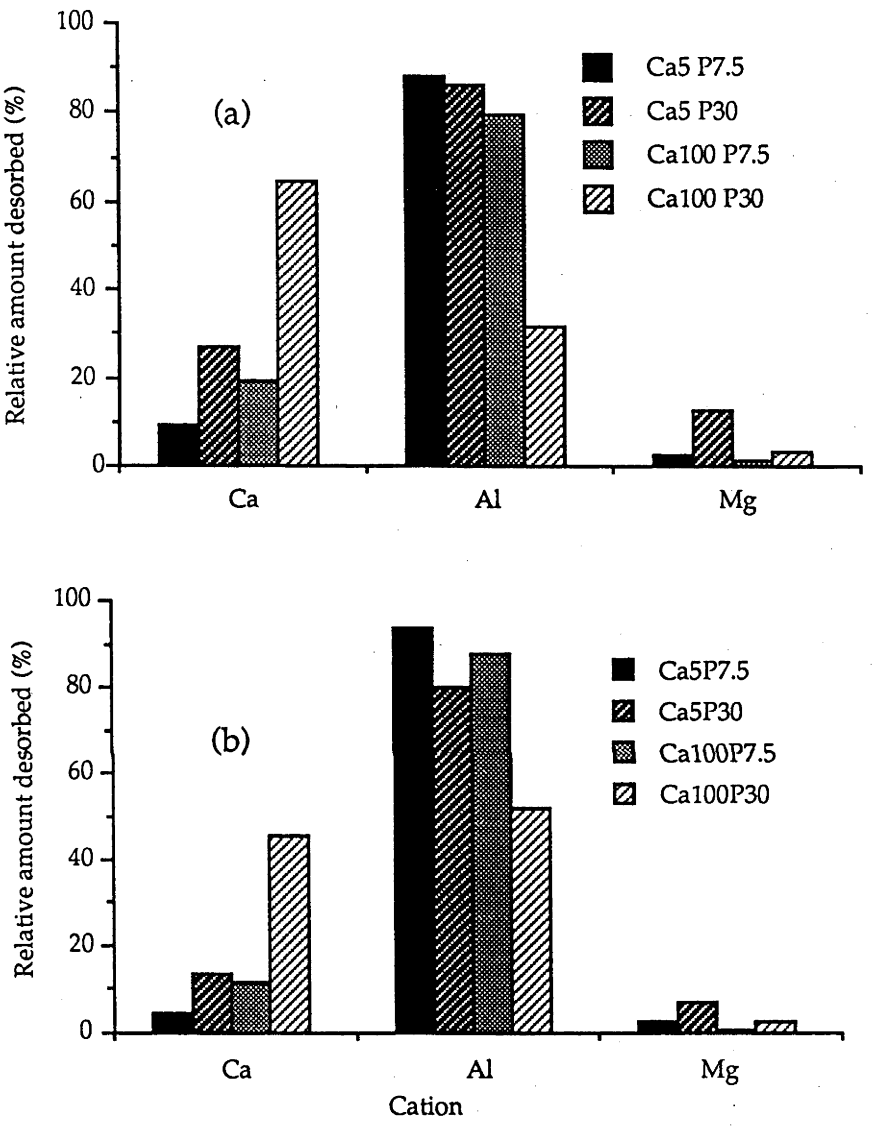


Figure 8.3. Cations (Ca, Al and Mg) desorbed in (a) 5 minutes and (b) an additional 25 minutes expressed as percentage of total of these cations.

To examine the influence of treatments on the cations desorbed from the root periphery, it is also useful to look at the relationship between the extent of cations desorbed and the total concentration of Ca, Al, Mg and P in the root. The influence of elements in the root that are not part of the treatments in the present experiment may also be examined through this procedure. Moreover the influence of elements deliberately changed by the treatments can be shown directly rather than as associated with the treatment levels. To examine these relationships, Table 8.2 presents a summary of the values of R^2 between total concentration of each element in the root and the quantity of desorbed cations at 5 minute and at the additional 25 minute reading (actual scatter diagrams are presented in Appendices 8.1 to 8.5). The relationship of desorbed cations with total P was included since the quantity of cations desorbed was significantly different for the P treatments alone as well as in combination with Ca (Table 8.1).

Table 8.2. Correlation coefficients (R^2) between desorbed cations and total concentrations of different elements in the roots.

Desorption time and element	Total Ca	Total Al	Total P	Total Mg	Total Al/Ca
5 min. Al	0.16	0.27*	0.30*	0.34*	0.13
Additional 25 min. Al	0.13	0.38*	0.28*	0.21*	0.16
5 min. Ca	0.97**	0.00	0.12	0.07	0.42*
Additional 25 min. Ca	0.96**	0.01	0.09	0.10	0.45*
5 min. Mg	0.04	0.12	0.14	0.82**	0.03
Additional 25 min Mg	0.00	0.14	0.10	0.69**	0.00

** and * indicate level of significance at < 1% and < 5% respectively.

All the cations desorbed, correlated strongly with their respective total concentrations in the roots (Table 8.2). For Ca, the correlation coefficient was close to one, whereas for Al, they were 0.27 and 0.38 for 5 and additional 25 minute desorptions respectively, indicating that Al in the root periphery is only moderately related to its total concentration in the root. The correlation between Mg desorbed and total Mg was quite high (0.82 and 0.69 for 5 and 25 minute desorptions respectively). Desorbed Al correlated positively with total Al but negatively with total P and total Mg levels in the root. Aluminium desorption was negatively influenced by total Ca concentrations in the roots, and this effect was similar to the effect of Ca levels. The effect of total Al concentrations on Ca desorption was not significant. In contrast, the values of R^2 for the ratio (total Al concentration)/(total Ca concentration) against Ca desorption were significant.

8.4. Discussion

These results provide an explanation of the differences in the thicknesses of root peripheries in terms of the differences in concentrations of elements in those peripheries. The hypothesis, that a high Ca level in situations of high Al protects the root periphery by reducing the Al concentration thereby reducing thickening, is only weakly established. Such a reduction in Al in the root periphery occurred only when P was high. The results from Chapter 6, where Ca helped to protect the root periphery from thickening, does not contradict the present conclusions because the P level in that experiment was 15.5 mg/l, which was double the lesser treatment in this experiment. However, the presence of a higher P level in that nutrient solution helped the roots to discriminate between Ca and Al in the root periphery (Figure 8.2). When comparing the percentage of Al desorbed in 5 minutes and at the additional 25 minutes reading with the desorption of the other cations, more Al was desorbed both from intercellular spaces (5 minutes desorption) and cellular material (additional 25 minutes desorption). In regards to the absorption of Al into the roots, it is possible that the damage to the cell membrane reduces its barrier function and Al passively permeates into protoplasts (Wagatsuma 1984).

The protection of the root periphery due to higher Ca levels and thus a lower a_{Al}/a_{Ca} ratio, may also be due to the larger concentration of Ca in the root periphery and in the total root; these reflect the lower a_{Al}/a_{Ca} ratios (Chapter 6 and also in the present experiment). However, to some extent the protective effect of Ca occurred even at lower P levels though it was reinforced by a high P treatment. The effects of P may also be similar in nature. In addition P

may play a role by significantly increasing the Ca and Mg concentrations in the roots.

Though it has not been tested directly, the root may be protected from developing a thick periphery by the presence of higher concentrations of Ca, P, and/or Mg in the root (not only in the periphery) since total concentrations of these elements in the roots reduced the concentrations of Al in the root periphery.

CHAPTER 9

GROWTH OF *E. CAMALDULENSIS* AS AFFECTED BY ALUMINIUM APPLIED IN A LOWER LAYER OF THE GROWTH MEDIUM

9. GROWTH OF *E. CAMALDULENSIS* AS AFFECTED BY ALUMINIUM APPLIED IN A LOWER LAYER OF THE GROWTH MEDIUM

9.1. Introduction

High Al affects root growth of *E. camaldulensis* in nutrient solution and the adverse effects of a high Al and high a_{Al}/a_{Ca} ratio are larger for roots than other growth parameters (Chapter 6). Also Al, Ca and P interact to influence root efficiency (as measured by nutrient absorption rate) of *E. camaldulensis* seedlings in nutrient solution (Chapter 7). In addition a high percentage of Al accumulates in the root periphery of Al injured *E. camaldulensis* roots and this effect was significantly reduced when high Al was accompanied by high Ca and P (Chapter 8). Further, the growth of *E. camaldulensis* responded negatively to soil moisture stress when grown on a high Al soil and especially when P and Ca were applied on that soil (Chapter 4). Variation in moisture levels could not be included in experiments conducted in nutrient solution (Chapters 6 and 7). Therefore, the present experiments were designed to examine the role of moisture stress, Ca and P in modifying the effect of Al toxicity on root growth and development, total seedling growth and nutrient uptake by *E. camaldulensis*. The established seedlings were confronted with the Al and other treatments in the lower layer of a two layer growth medium to demonstrate the changes.

One experiment was conducted using soil as the treatment medium (lower layer) after establishing the seedlings in sand (used as top layer). The soil was the same as that used in other experiments in the initial phase (Chapters 3, 4.3 and 5) and was modified with Ca, Al and P. This modified soil was used to demonstrate the effect of treatments on seedling growth parameters and specifically on the roots in the soil.

In the second experiment in this series, the effect of Al and other factors were tested by using sand as the growth medium in both layers. Sand has the advantage of being a solid medium of growth where other factors including moisture stress can be easily controlled and known. This is true particularly for monomeric Al. In soil, monomeric Al is affected by organic matter, pH and other factors which are not complications in the sand. The experiment with sand had two parts: the first part consisted of Ca, Al and P treatments and the seedlings were harvested early (after four weeks). The second part included moisture stress in addition to the treatments mentioned above and these were harvested three weeks later (i.e., after 7 weeks).

9.2. Materials and methods for soil pots

Pots used for this experiment were made out of PVC pipe (85 mm internal diameter). Each pot was made by combining two separate pieces of pipe and these "pots" were paired vertically. The top pot was 11.7 cm long and held one kg of the river sand used. The bottom half was 15.1 cm long and held 0.76 kg of soil (oven dry weight basis). Both top and bottom pots were split vertically into two halves and joined again with tape before filling. This was done to make root separation from the growing medium easy at harvest. The bottom of the lower pots was covered with fine shade cloth to allow drainage while holding in the growth medium. River sand was used for the initial transplantation and establishment of the seedlings. The sand was sieved with a garden sieve (about 5 mm mesh) and soaked in 3% HCl for 3 days, washed until Cl^- free (tested with dilute $AgNO_3$ solution) and dried. The particle size distribution of sand used was determined by dry sieving and is shown in Table 9.1. The pots were filled with sand and five week old seedlings were transplanted at a rate of three seedlings per pot. After establishment

(in about a weeks time), the seedlings were thinned to two per pot and were given dilute nutrient solution twice a week until the beginning of treatments. To avoid the complication of a transplantation shock to roots being confused with treatments, the seedlings were transplanted into sand in the top pot and grown there for 5 weeks when the roots had started to appear at the bottom. Then the actual treatment began by joining a treated soil pot underneath. Prior to this joining, the top pots were flushed several times with water so that nutrient solutions in the upper pots were washed out. Seedling heights were measured and pots were distributed throughout the treatments so that at the beginning total seedling heights were almost the same in all the treatments. Both halves of the pots were then combined and the joint sealed by packaging tape making the two pots into a single long pot (26.8 cm). Before combining top and bottom pots the perforated polythene sheet from the bottom of the upper pot was carefully removed.

Table 9.1. Particle size distribution of the river sand used.

Size group (mm)	% sand by weight
5.00 - 2.00	10.37
2.00 - 1.00	54.40
1.00 - 0.50	26.18
0.50 - 0.25	7.96
< 0.25	1.09

There were two Ca treatments, 0 and an equivalent amount of Ca to 9880 kg pure $CaCO_3$/ha. Soils requiring a Ca treatment were treated with $CaCO_3$ and $CaSO_4$ at a ratio of 2 : 1. After three wetting and drying cycles over two weeks, soils were ready for potting. Aluminium and P treatments were applied after potting. Levels of Al (from $AlCl_3$. 6 H_2O)

were 0, 25 and 75 mg Al/kg soil and levels of P (from $NH_4H_2PO_4$) were 0 and 50 mg P/kg soil. Calcium and P levels selected for this experiment were based on the results in Chapter 5. The Al levels were selected so that some monomeric Al remained in soil solution (Section 3.10). The soil solution in this natural soil was free from monomeric Al although exchangeable Al was quite high. In all there were (2 Ca x 3 Al x 2 P =) 12 treatment combinations each with 5 replicates.

Initially N was added at 40 mg N/kg soil including the amount which came from the $NH_4H_2PO_4$. Later N was added in a solution made up from NH_4NO_3 at 40 mg/kg soil at both 3 weeks and 6 weeks after the treatments started. Watering was done with tap water and no other nutrient was added. Seedlings were harvested 7 weeks after imposing treatments; the shoots, the top and bottom pots were separated and the top pot roots and bottom pot roots separated, washed and dried.

9.3. Materials and methods for sand pots

Except for the use of sand in the lower pot this experiment parallels that for soil pots (Section 9.2). Nutrient solution was used for watering once treatment began. The effect of soil moisture stress on the seedlings was included in this experiment.

The levels of treatment were the same as for experiments described in Chapter 7 (i.e., 0.25 and 50 mg Al/l, 5 and 100 mg Ca/l and 7.5 and 30 mg P/l) and the nutrient solutions were made exactly the same way (Table 7.1). An adjusted nutrient solution pH of 3.7 ensured that Al was in monomeric form and P in solution. There were (2 Ca x 2 Al x 2 P =) 8 treatment combinations and 5 replicates of each. To begin with treatments were the same as for soil pots except that the lower pots also contained sand. These lower pots were watered with the different

treatment nutrient solutions. The pots were placed on saucers and thereafter nutrient solutions were added to these saucers. The treatment was started with 15 pots for each treatment combination. Seedlings in one third of the pots were harvested after 4 weeks. At this stage moisture stress was imposed on half of the remaining pots in combination with other treatments. At the end of another three weeks all the remaining seedlings were harvested. After harvesting, the shoots, the top and bottom pots were separated and the top pot roots and bottom pot roots separated, washed and dried.

9.3.1. Moisture stress

With sand as a growing medium, it is difficult to specify a moisture stress situation in terms of (percentage of) field capacity. Further, since two pots were combined to make one it was not possible to put in an access tube to partly water the pots (Chapter 4). Since the imposition of moisture stress was the main objective rather than a quantification of the stress, an arbitrary technique was used to impose moisture stress.

When first applying the moisture stress the saucers were watered with excess nutrient solution. Next morning excess solution was removed from the saucer and the pots including saucer were weighed (W_1). Then no nutrient solution was added until the seedlings showed clear signs of wilting and the pots were weighed again (W_2). From that point the pots were watered to a level well below field capacity $[W_2 + (W_1 - W_2)/2]$ whenever the pot weights approached the weight at wilting point (W_2).

9.3.2. Chemical analysis of harvested seedlings

Shoots and roots (top and bottom) of seedlings from sand pots (both harvests) were separately analyzed for mineral concentration. Samples

were ground in a Wiley mill and then digested with H_2O_2 and H_2SO_4. Aluminium was analyzed by ICP, Ca and Mg by atomic absorption spectrophotometer and P was determined colorimetrically using an autoanalyzer (Chapter 3).

9.4. Results and discussion

9.4.1. Treatment effects in soil pots

Aluminium, Ca and P treatments affected growth parameters of *E. camaldulensis* (Figure 9.1). Both shoot and root parameters varied in a similar fashion. A comparison of growth parameters between the zero and highest Al levels show that in general, nearly all the measured growth parameters were negatively affected by higher Al. The exceptions are the bottom roots of the Ca_1P_1 treatment and the shoot heights of the Ca_0P_0 treatment.

Higher Ca levels raised the values of each of the growth parameters, especially when Al was at the highest level. Similarly, higher P levels also improved seedling growth and caused an upward shift in growth parameters. These shifts occurred for top root, bottom root, shoot weight and biomass. The F ratios from the ANOVA for different growth parameters with respect to treatments are presented in Table 9.2.

Though all the growth parameters showed a downward trend with an increase in Al levels, the trends were in general steeper for bottom root and top root as compared to shoot weight and biomass. A comparison of the effects of Al levels on bottom root and top root may be made from the relative reduction in these parameters due to an increase in Al from zero to the highest level. The relative reduction in bottom root (RRBR) being defined as:

$$RRBR = \frac{\text{(bottom root at zero Al level) - (bottom root at the highest Al level)}}{\text{(bottom root at zero Al level)}} \times 100.$$

Similarly, the relative reduction for top root growth was also calculated and Table 9.3 includes these values. The relative reduction due to an increase in Al was found to be greater for bottom root growth for each combination of Ca and P treatments, as well as when all Ca and P treatments were combined. For the whole sample, the relative reduction due to increase in Al was 26% for bottom root growth and 21% for top roots. Since the bottom roots received the treatments directly, the adverse effects of Al were more prominent here compared to top root where the treatment effects were transmitted.

Table 9.2. F ratios for the ANOVA on different growth parameters in soil pots with respect to treatments.

Source of variation	Shoot height	Shoot weight	Top root	Bottom root	Total root	Total biomass
Ca	53.40**	86.63**	19.02**	3.64	11.25**	58.09**
P	9.93**	10.09**	4.98*	5.27*	5.47*	9.03**
Al	1.19	4.60*	2.28	2.90	2.68	4.19*
Ca x P	0.73	6.20*	0.59	0.14	0.07	3.33
Ca x Al	0.08	0.29	0.10	0.33	0.15	0.06
P x Al	0.87	0.25	0.12	0.14	0.03	0.14
Ca x P x Al	0.41	0.26	0.42	0.74	0.56	0.36

** and * indicate level of significance at < 1% and < 5% respectively.

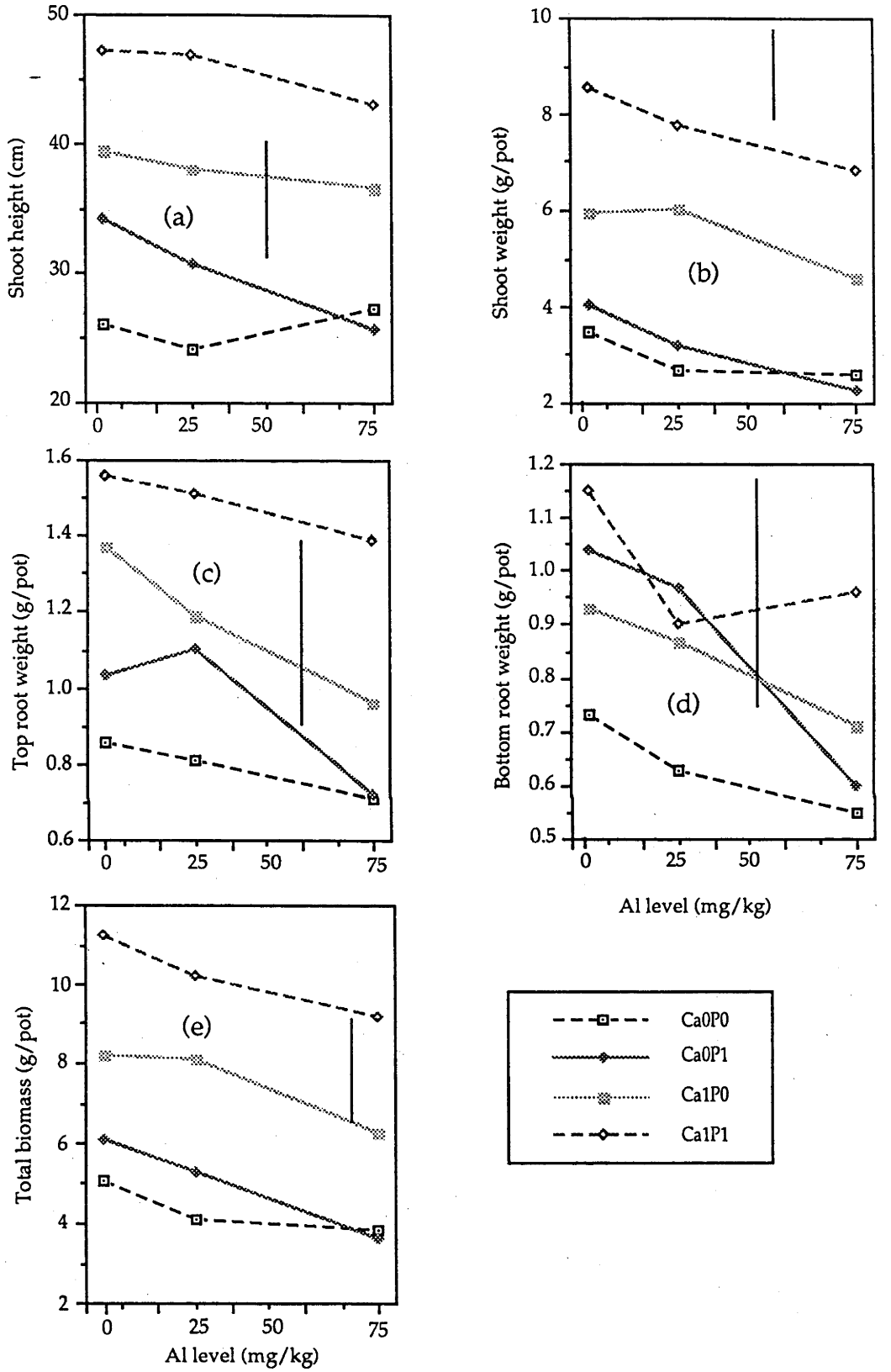


Figure 9.1. Effects of Al treatment levels under different Ca and P treatment combinations in soil pots on the (a) shoot height, (b) shoot weight, (c) top root weight, (d) bottom root weight and (e) total biomass of *E.camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

Table 9.3. The relative reduction in bottom and top roots of *E. camaldulensis* due to an increase in Al from nil to the highest level under each Ca and P treatment combination in the soil pots.

Ca and P level	Relative reduction (%) in	
	Bottom root	Top root
Ca_0P_0	25	17
Ca_0P_1	42	30
Ca_1P_0	24	30
Ca_1P_1	17	11
All Ca & P treatment combinations	26	21

An increase in Ca or Ca and P levels reduced the relative reduction in root growth. Phosphorus alone increased the relative reduction. However, P alone or in combination with Ca increased root growth, but when P treatments were accompanied by Al treatments, the decrease in root growth was more than in case of treatments without P (Figure 9.1). Although the relative reduction in bottom root due to high Al was higher at Ca_0P_1 treatment, it was influenced by higher root growth when Ca_0P_1 treatment was not accompanied by Al (Table 9.3). In fact the effect of P on all the growth parameters was significant (Table 9.2).

Some studies which have dealt with agricultural crops and subsoil acidity, reported an improvement in root growth in the subsoil after liming (Pinkerton and Simpson, 1981, 1983; Simpson et al, 1979). However, liming simultaneously improves soil Ca and eliminates other acidity factors (pH, Mn etc) in their research. In the present study an

improvement of soil Ca alone increased root growth of *E. camaldulensis* in the subsoil. Under similar conditions of high Al and low Ca in the subsoil, root elongation of lucerne was reported to be adversely affected (Simpson et al, 1979) and to improve on liming. The results of the present experiment show that the adverse effects of Al toxicity on root growth of *E. camaldulensis* is similar to that for agricultural crops. The findings of the present study are also more conclusive in outlining the importance of subsoil Ca for root growth and development.

9.4.2. Treatment effects in sand pots

9.4.2.1. Growth parameters

The results for the seedlings harvested after four weeks (Figure 9.2) demonstrate that the effect of Al on bottom and top roots was negative at treatment levels of Ca_5P_{30} and $Ca_{100}P_{7.5}$. When the treatment level was $Ca_5P_{7.5}$ and $Ca_{100}P_{30}$, there was no clear pattern. In some cases, top root weight showed a slight increase at higher Al levels. The negative effect of Al was observed to be systematic for shoot height, shoot weight and biomass; the effect on biomass was mainly due to the contribution of shoot weight. Table 9.4 gives ANOVA data for the difference in growth parameters due to differences in treatment levels.

These data imply that the effect of treatments were immediately transmitted to the shoot even in cases where the effect was not felt on root weight itself. The relative reduction due to the high Al treatment was greater for bottom roots (Table 9.5). Thus even within this short period, development of bottom roots was more adversely affected (as compared to top roots) by high Al.

Table 9.4. F ratios for the ANOVA on different growth parameters of seedlings in sand pots (harvested after 4 weeks) with respect to treatments.

Source of variation	Shoot height	Shoot weight	Top root	Bottom root	Total root	Total biomass
Ca	0.33	0.00	0.08	0.02	0.01	0.00
P	0.00	1.72	0.00	0.23	0.07	1.13
Al	7.59**	5.21*	0.01	2.09	0.58	3.72
Ca x P	5.26*	0.02	0.22	0.00	0.09	0.00
Ca x Al	1.48	0.04	0.01	0.00	0.01	0.02
P x Al	0.01	0.13	0.03	0.29	0.02	0.05
Ca x P x Al	0.45	0.42	1.01	1.28	1.29	0.64

** and * indicate level of significance at < 1% and < 5% respectively.

Table 9.5. The relative reduction in bottom and top roots of *E. camaldulensis* due to an increase in Al treatments when seedlings were grown in sand and harvested after 4 weeks.

Level of Ca and P Treatment	Relative reduction (%) in	
	Bottom root	Top root
$Ca_5P_{7.5}$	10	- 11
Ca_5P_{30}	21	11
$Ca_{100}P_{7.5}$	36	9
$Ca_{100}P_{30}$	- 2	- 9
All Ca and P treatment combinations	17	1

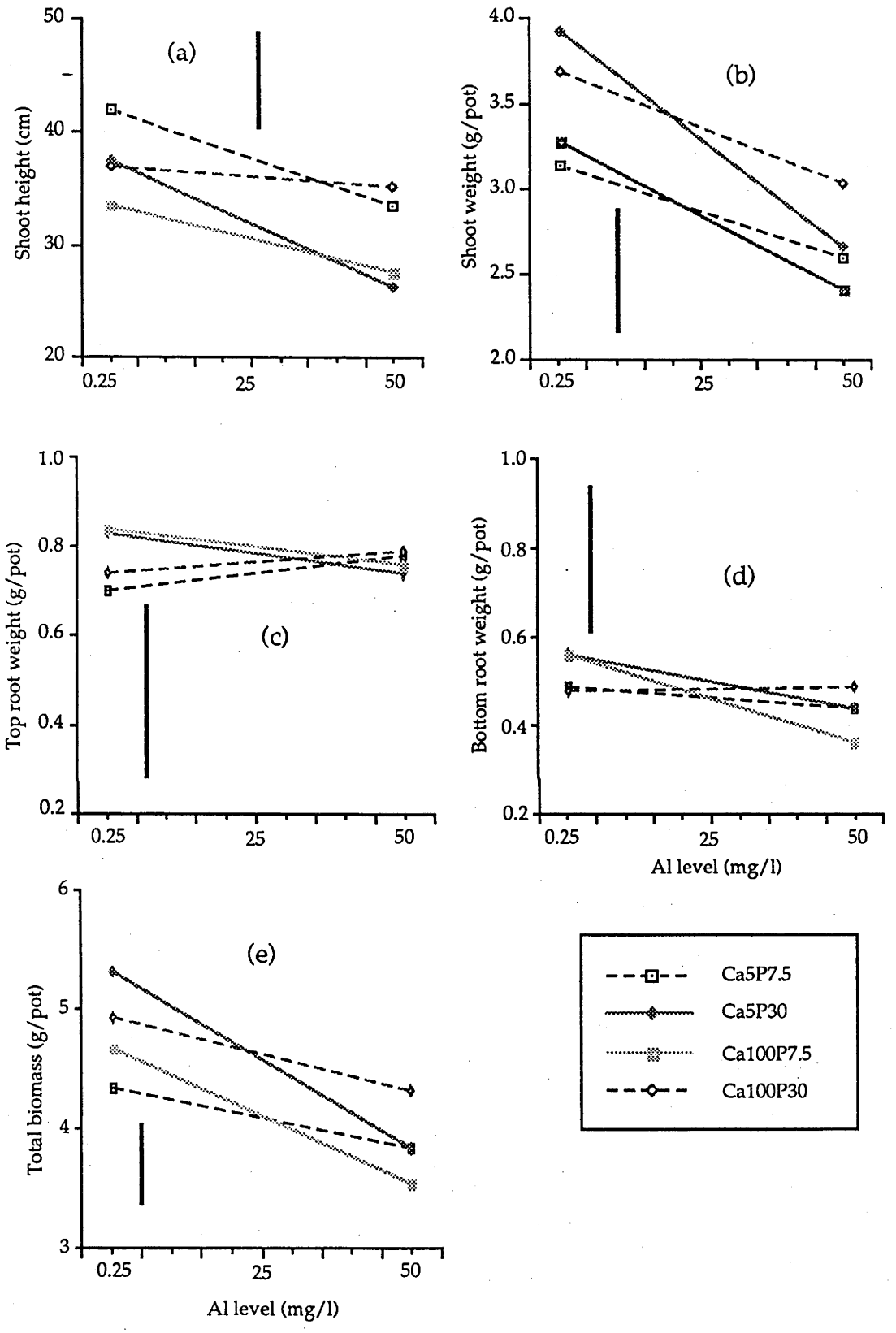


Figure 9.2. Effects of Al treatment levels under different Ca and P combinations in sand pots after 4 weeks on (a) shoot height, (b) shoot weight, (c) top root weight, (d) bottom root weight and (e) total biomass of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

The effect of Al levels on the growth parameters of sand pot seedlings were measured on material harvested 7 weeks after treatment began (Ca and P levels are shown in Figures 9.3 and 9.4). Treatment effects on any growth parameter with or without moisture stress are shown side by side in the figures. The results of ANOVA for different growth parameters with respect to treatments are presented in Table 9.6.

All growth parameters showed a decline with Al (except for the top roots in only one treatment combination) (Figures 9.3 and 9.4). With an increase in either P or Ca, the value of growth parameters increased and the increase was greater when the Al level was higher. The adverse effects of Al on the growth parameters were less severe under moisture stress conditions.

The effect of treatments on root growth in each layer, the relative reduction in top and bottom root weights due to increase in Al, for two groups of seedlings, with and without moisture stress are presented in Table 9.7.

It can be seen that the relative reduction in bottom root weight is much larger than in top root weight for both moisture stress and nil moisture stress situations (with the exception of the $Ca_{100}P_{30}$ treatment).

The nil moisture stress picture from this harvest can be compared to Table 9.5 which shows the situation from the earlier harvest which did not include moisture stress. The relative reduction in both top and bottom roots is much larger in the second harvest. This implies that when Al toxicity occurs at a lower layer its adverse effects in relation to the initial situation is larger after a longer growth period.

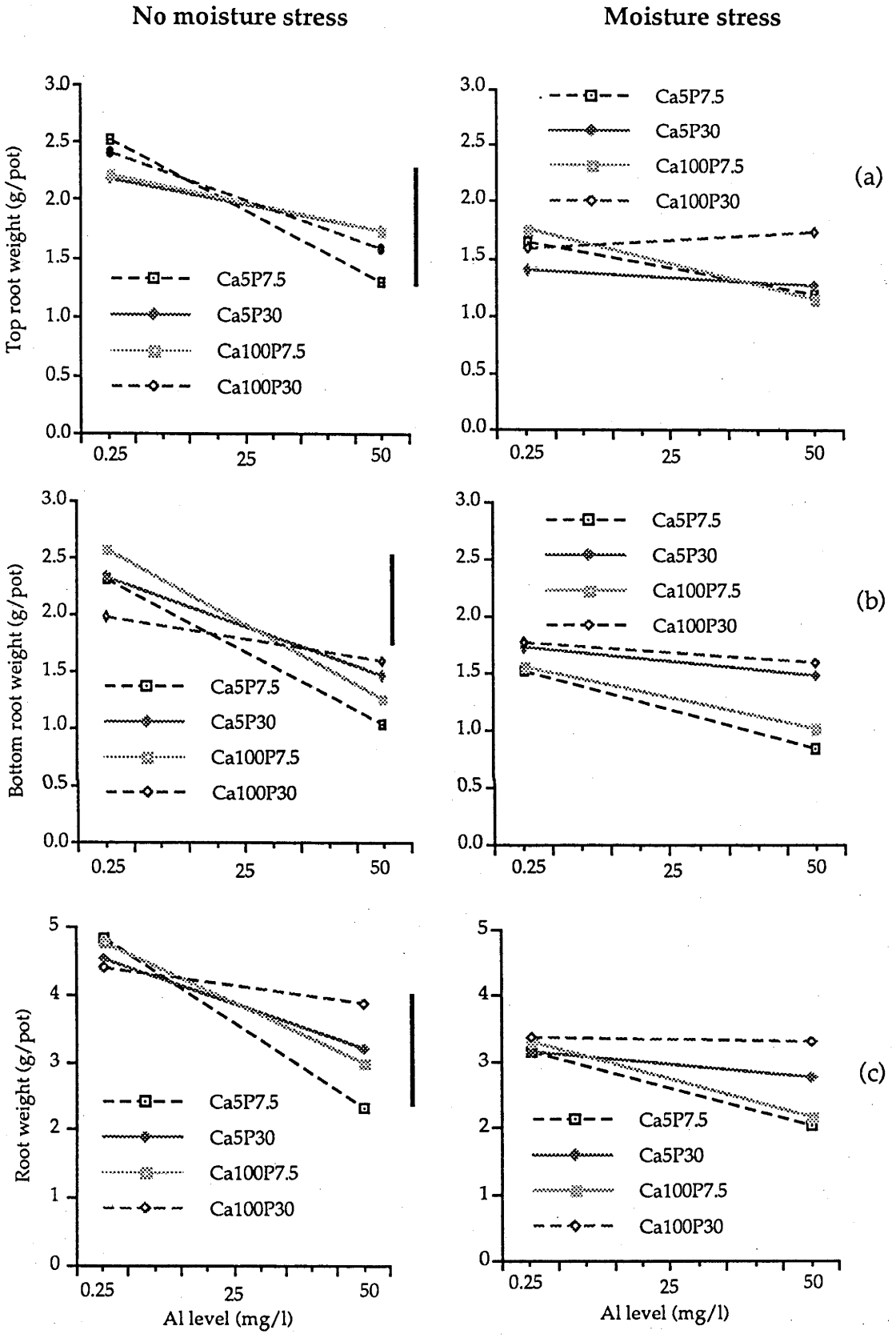


Figure 9.3. Effects of Al treatment levels under different Ca and P combinations in sand pots after 7 weeks on (a) top root weight, (b) bottom root weight and (c) total root weight of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

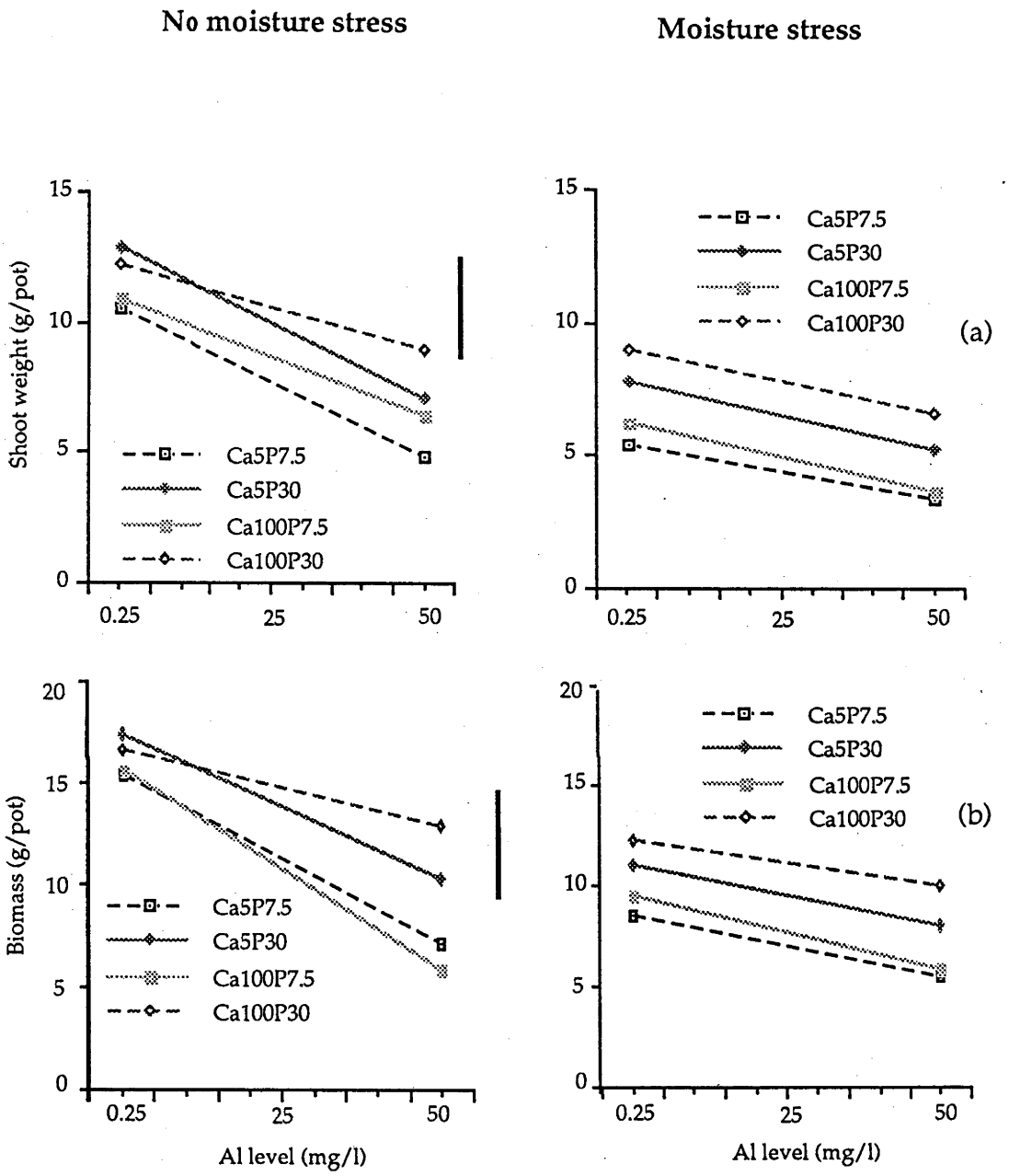


Figure 9.4. Effects of Al treatment levels under different Ca and P combinations in sand pots after 7 weeks on (a) shoot weight and (b) biomass of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

Table 9.6. F ratios for the ANOVA on different growth parameters of seedlings in sand pots with respect to treatments (harvested 7 weeks after treatment began).

Source of variation	Shoot height	Shoot weight	Top root	Bottom root	Total root	Total biomass
Ca	0.70	3.20	2.96	6.72*	5.40*	4.05*
P	7.79**	23.34**	1.21	7.79**	4.10*	17.23**
Al	58.73**	58.09**	11.61**	55.10**	32.43**	53.36**
WS	30.02**	49.71**	20.54**	35.00**	32.28**	47.37**
Ca x P	0.18	0.04	1.52	0.82	0.12	0.06
Ca x Al	0.56	0.88	1.46	1.04	1.56	1.13
P x Al	2.35	0.04	5.08*	11.03**	9.04**	1.11
Ca x WS	0.15	0.02	0.00	2.20	0.49	0.01
P x WS	3.24	0.14	0.14	3.45	0.43	0.22
Al x WS	5.69*	0.65	2.10	8.12**	5.17*	6.64*
Ca x P x Al	0.16	0.33	0.00	0.86	0.18	0.30
Ca x P x WS	0.41	0.33	0.02	0.31	0.12	0.28
Ca x Al x WS	0.00	1.21	0.61	0.08	0.39	0.99
P x Al x WS	1.46	0.17	0.02	1.20	0.18	0.19
Ca x P x Al x WS	1.60	0.01	1.16	1.68	0.00	0.00

** and * indicate level of significance at < 1% and < 5% respectively.

Table 9.7. Relative reductions in bottom root and top root as Al increased, for seedlings in sand pots harvested 7 weeks after treatments began.

Level of Ca and P treatment	Relative reduction (%) in			
	No moisture stress seedlings		Moisture stress seedlings	
	Bottom root	Top root	Bottom root	Top root
$Ca_5P_{7.5}$	55	9	44	28
Ca_5P_{30}	37	21	14	9
$Ca_{100}P_{7.5}$	51	22	34	35
$Ca_{100}P_{30}$	16	9	10	-9
All Ca and P treatment combinations	41	26	25	17

Another point of interest from this part of the experiment was an analysis of the effects of moisture stress on seedling growth. The effects of moisture stress include top, bottom and total root weights (Figure 9.3) and shoot weight and total biomass (Figure 9.4). Seedlings under stress show lower growth in terms of each parameter for any treatment combination; the position of the lines representing stressed seedlings are in general lower. It can also be seen from Table 9.6 that the variation in all the growth parameters were significantly different for the moisture stress treatment. But of more interest and the reason for including moisture stress in this experiment was the chance to examine the interaction of moisture stress with other treatments, particularly Al. The data in Table 9.6 also show that the variations in shoot height, bottom root, total root and total biomass were significantly different for the Al and moisture stress interaction.

Shoot heights were recorded each week and these are shown for various treatment combinations and moisture stress (Figure 9.5). After the stress was imposed (from week 4), the shoot height increment lines are much less steep for all treatment combinations, reflecting a lower growth rate.

The relative reduction in root weight and shoot weight due to an increase in Al in situations with or without moisture stress are shown in Table 9.8. It can be seen that the relative reduction due to Al is less for total root and shoot weight under moisture stress. There is no obvious explanation for the lessened effect of high Al under moisture stress than under an adequate moisture supply. Krizek and Foy (1988) and Krizek et al (1988) reported that moisture stress exacerbated the stress effects of Al (in high Al acid soil) in the case of sunflower and barley respectively which is in contrast with the findings of the present study. A decrease in accessibility to water of Al injured roots and/or a decrease in the availability of essential nutrient elements were suggested to be the possible reasons behind this. But these factors are not applicable to the present situation since watering was made with nutrient solution. This may partly explain the difference in the results obtained. Chemical analyses of these seedlings were carried out (Section 9.4). The results of the chemical analyses may suggest reasons for the lessened effect of Al toxicity under a moisture stress situation.

Table 9.8. The relative reduction in shoot weight and total root weight as Al increased, for seedlings in sand pots harvested 7 weeks after treatment.

Level of Ca and P treatment	Relative reduction (%) in			
	No moisture stress		Moisture stress	
	Shoot weight	Root weight	Shoot weight	Root weight
$Ca_5P_{7.5}$	54	52	37	35
Ca_5P_{30}	45	60	34	12
$Ca_{100}P_{7.5}$	41	37	41	34
$Ca_{100}P_{30}$	27	12	26	- 41
All treatments	41	33	34	21

9.4.2.2. Mineral nutrient concentration

Shoot, top roots and bottom roots of seedlings harvested after 4 and 7 weeks of treatment were chemically analyzed for their Ca, Mg, Al and P concentrations. Figures 9.6 and 9.7 include the results of chemical analysis of seedlings harvested after 4 weeks and Figures 9.8 to 9.11 represent seedlings harvested after 7 weeks of treatment. Table 9.9 and 9.10 present the F ratios from the multivariate ANOVA for treatment effects on the mineral concentration of seedlings harvested after 4 weeks and 7 weeks of treatment respectively.

The Ca concentration in the bottom roots is not affected by higher levels of Al (Figure 9.6). In the top roots and shoots, Ca concentrations slightly decreased due to higher Al in all treatment combinations. The only exception was the $Ca_{100}P_{7.5}$ treatment in the case of top roots. The Mg concentration in shoot and bottom roots decreased in all treatment combinations due to higher Al. The only exceptions

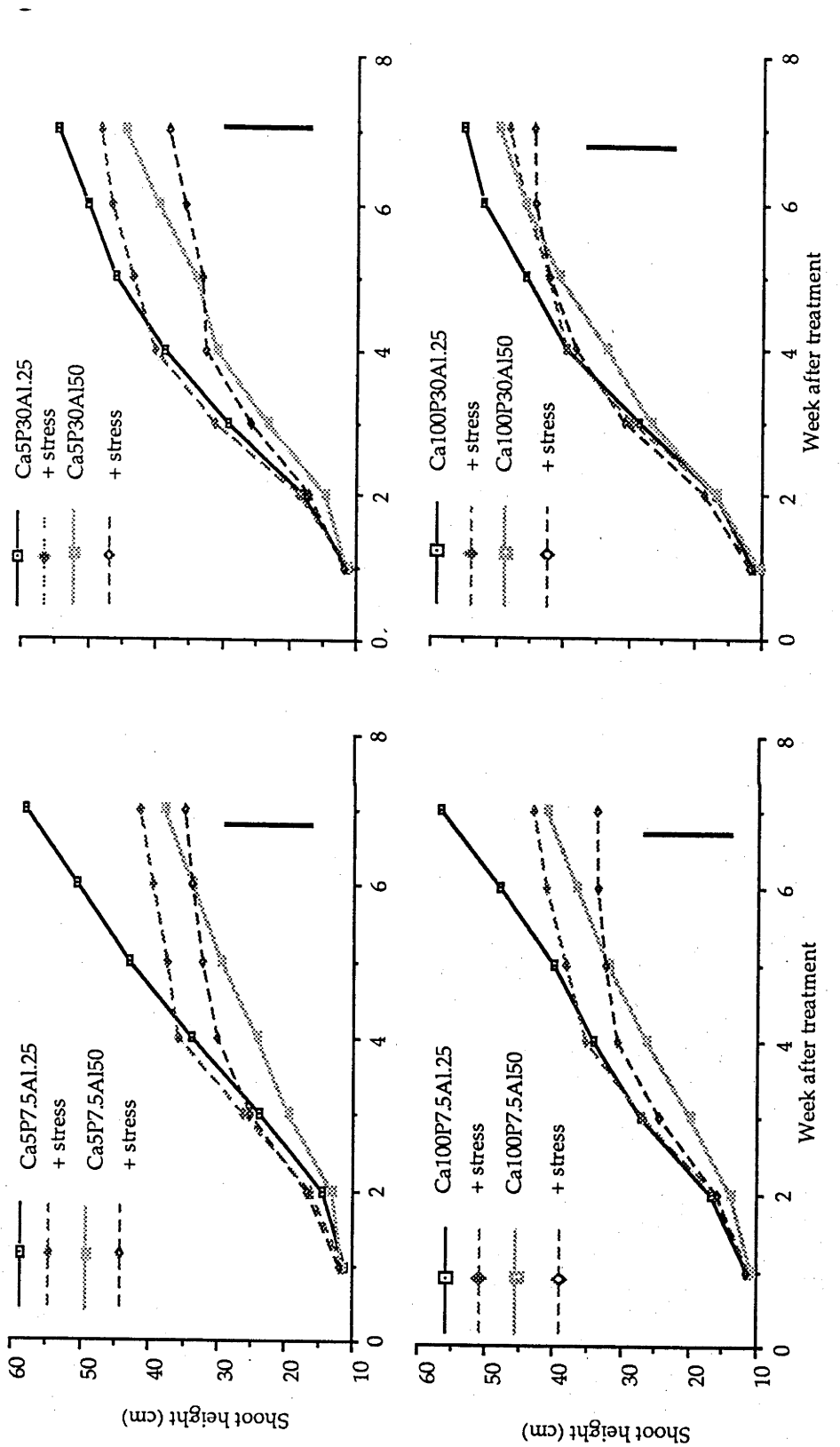


Figure 9.5. Shoot height increments under different treatment combinations including moisture stress. Vertical bars represent l.s.d. ($P < 0.05$) for the last week.

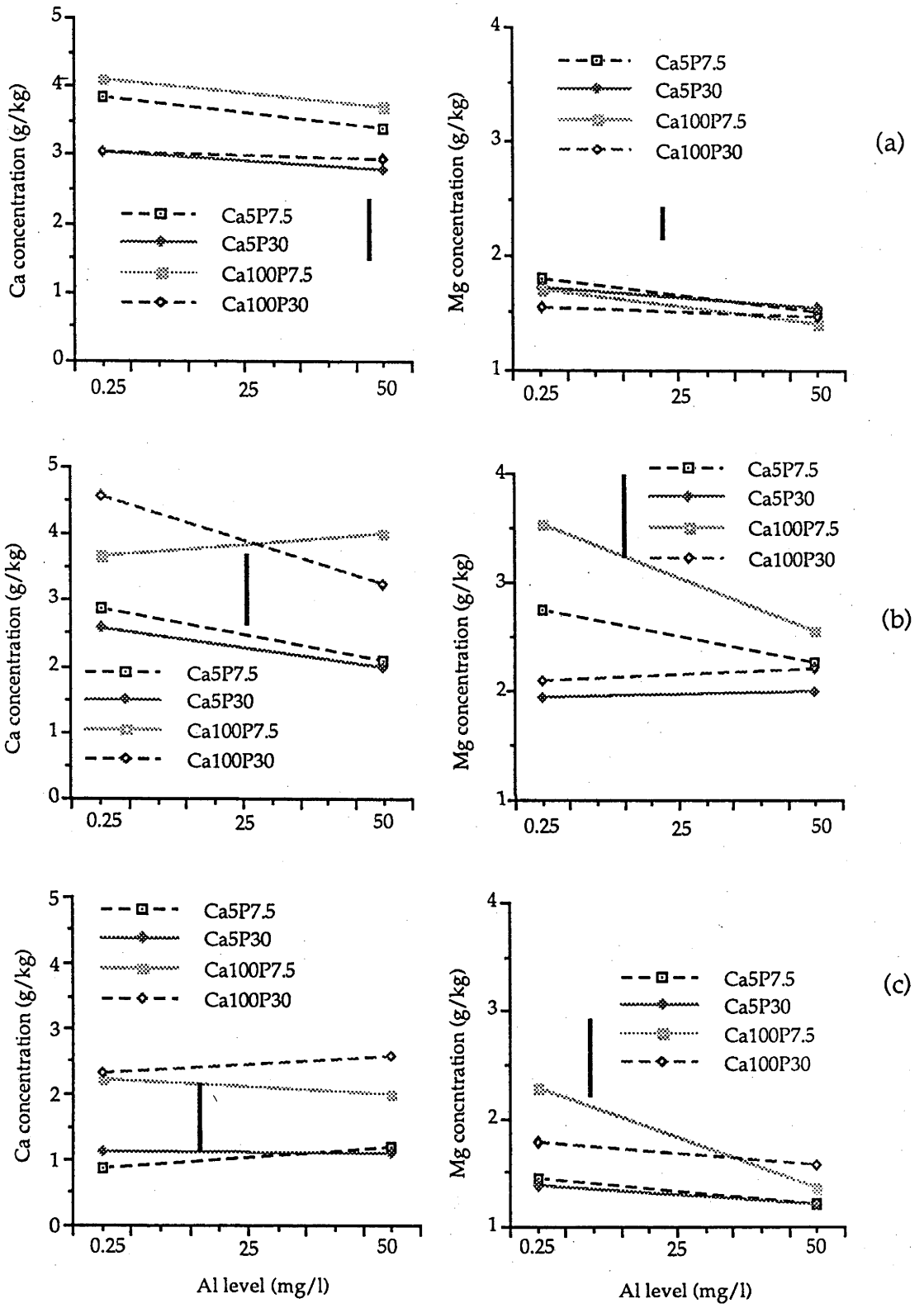


Figure 9.6. Effects of Al treatment levels under different Ca and P combinations in sand pots after 4 weeks. Calcium and Mg concentration in (a) shoot, (b) top root and (c) bottom root of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

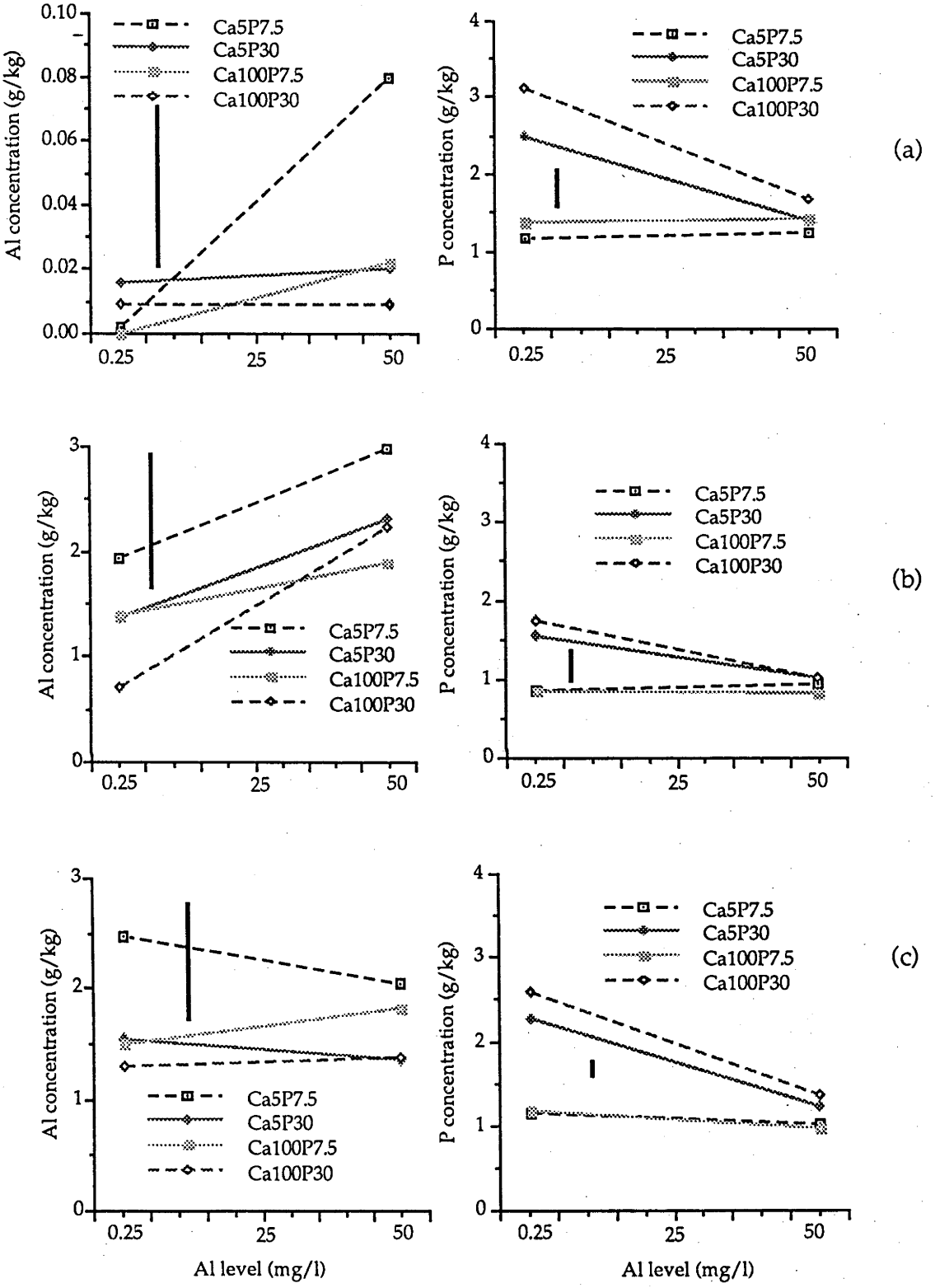


Figure 9.7. Effects of Al treatment levels under different Ca and P combinations in sand pots after 4 weeks. Aluminium and P concentration in (a) shoot, (b) top root and (c) bottom root of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

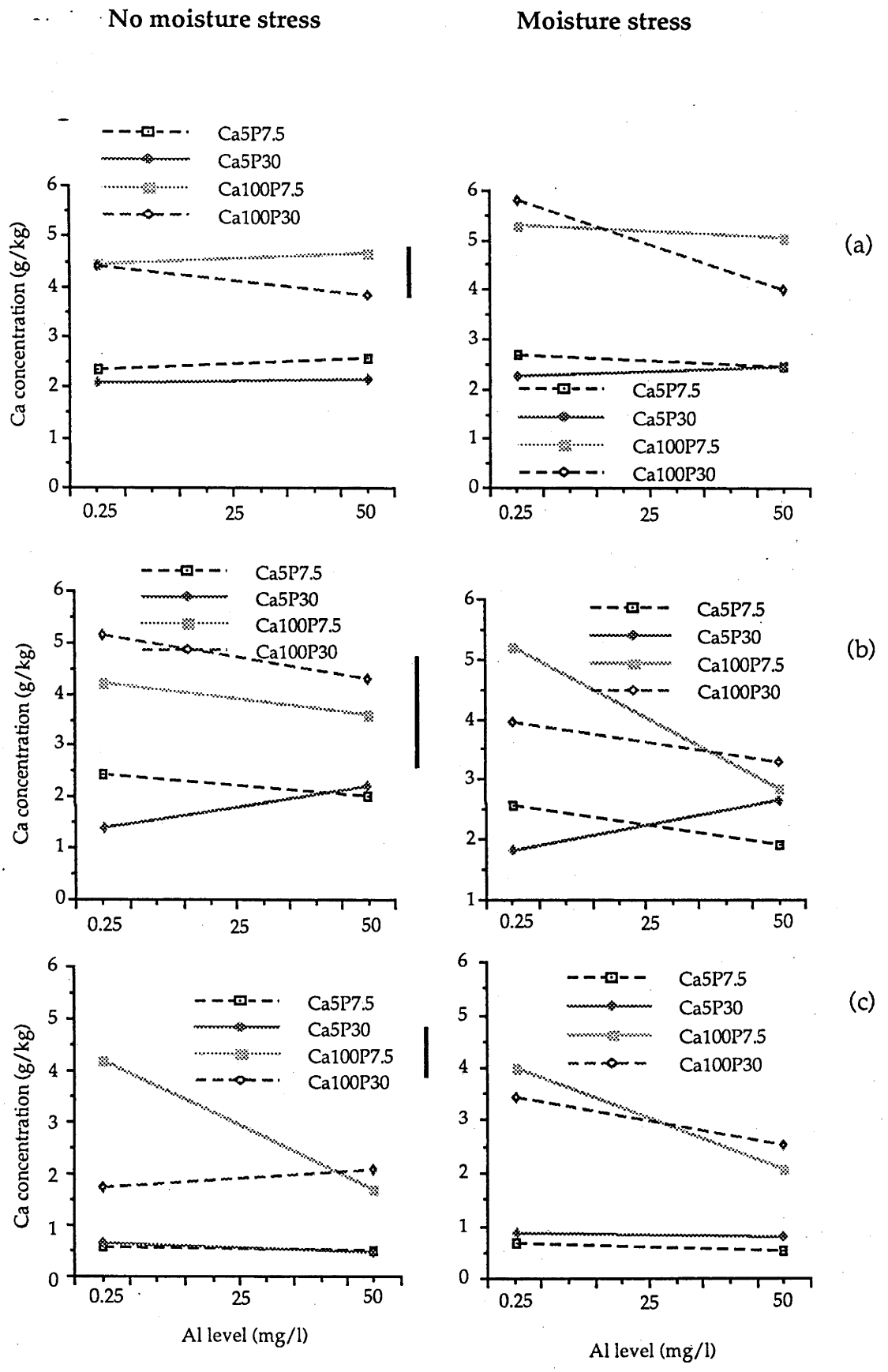


Figure 9.8 Effects of Al treatment levels under different Ca and P combinations in sand potsafter 7 weeks. Calcium concentration in (a) shoot, (b) top root and (c) bottomroot of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

were the top roots when P was high. These results indicate that, although lesser in amount, higher Al levels limit the Ca and Mg translocation to shoots.

The Al and P concentrations in the seedlings harvested after 4 weeks of treatment show that Al concentrations in the bottom and top roots were higher when both Ca and P were low (Figure 9.7). Higher levels of Al increased the concentration of Al in top roots in all treatment combinations, but the concentration of Al in bottom roots remained more or less the same. Shoot Al concentrations were much lower when compared to top and bottom roots. This is in agreement with the findings presented in Chapters 6 and 7 and also of others (Arp and Ouimlet, 1986; Thornton et al, 1986; Joslin et al, 1988). Shoot Al concentrations increased severalfold at higher levels of Al but only when both Ca and P treatment levels were low. There was little increase in Al concentrations when only Ca was high.

The results on Al concentrations in shoot and roots imply that the movement of Al from the bottom root (comparatively recently developed) to the top root was not limited by higher Ca and P levels, but movement of Al to the seedling top was restricted by Ca and P levels, both individually and collectively.

Phosphorus concentrations in shoot, top and bottom roots were similarly affected by different treatments. At low Al levels, the P concentration was higher at higher P levels. But at the high Al level, higher P treatment did not result in an increase in P concentrations in any of the shoots and roots.

Figure 9.8 shows the Ca concentration in seedlings harvested 7 weeks after treatment. Concentrations of Ca were consistently greater

under high Ca treatments in both shoots and roots. In general, root Ca concentrations were reduced more than shoot Ca by higher Al levels. The shoot Ca concentration was higher in moisture stressed pots compared to pots without, particularly when treatment Ca levels were high.

The Mg concentration of the seedlings is in most cases lower in higher Al treatments (Figure 9.9). The shoot Mg concentration was slightly increased in pots both with and without moisture stress, at higher Al levels when Ca levels were high.

The Al concentrations in shoots, top and bottom roots are presented in Figure 9.10. Aluminium concentration was lowest in the shoots and highest in bottom roots while the Al concentration in the top roots is at an intermediate level (note the difference in the y axis scale of the figures). The increase in Al concentrations due to higher Al treatment levels was less affected by Ca and P levels in bottom roots than in top roots and shoots. This difference in root and shoot Al concentrations indicates that high Ca and P levels more greatly restrict Al translocation to the shoot than the absorption of Al by the roots.

Aluminium concentrations in bottom roots in the moisture stressed pots were higher compared to those pots without moisture stress under otherwise identical treatments. Shoots in moisture stressed pots had higher Al concentrations as compared to the no stress situation.

Phosphorus concentrations in the seedlings (Figure 9.11) show that in all cases higher P resulted in higher P concentrations in the seedlings. Higher Al treatments resulted in a decrease in P concentration

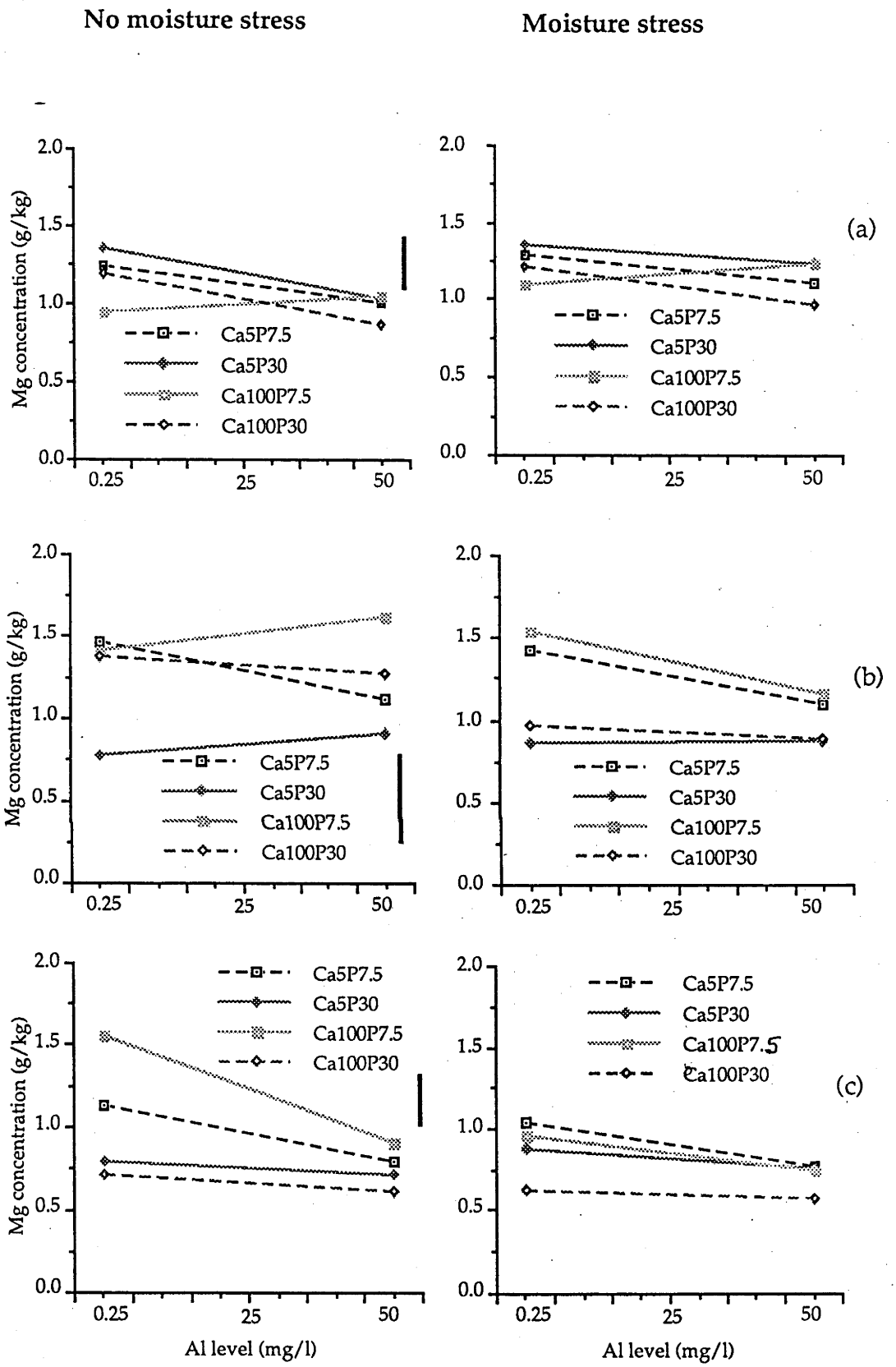


Figure 9.9. Effects of Al treatment levels under different Ca and P combinations in sand pots after 7 weeks. Magnesium concentration in (a) shoot, (b) top root and (c) bottom root of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

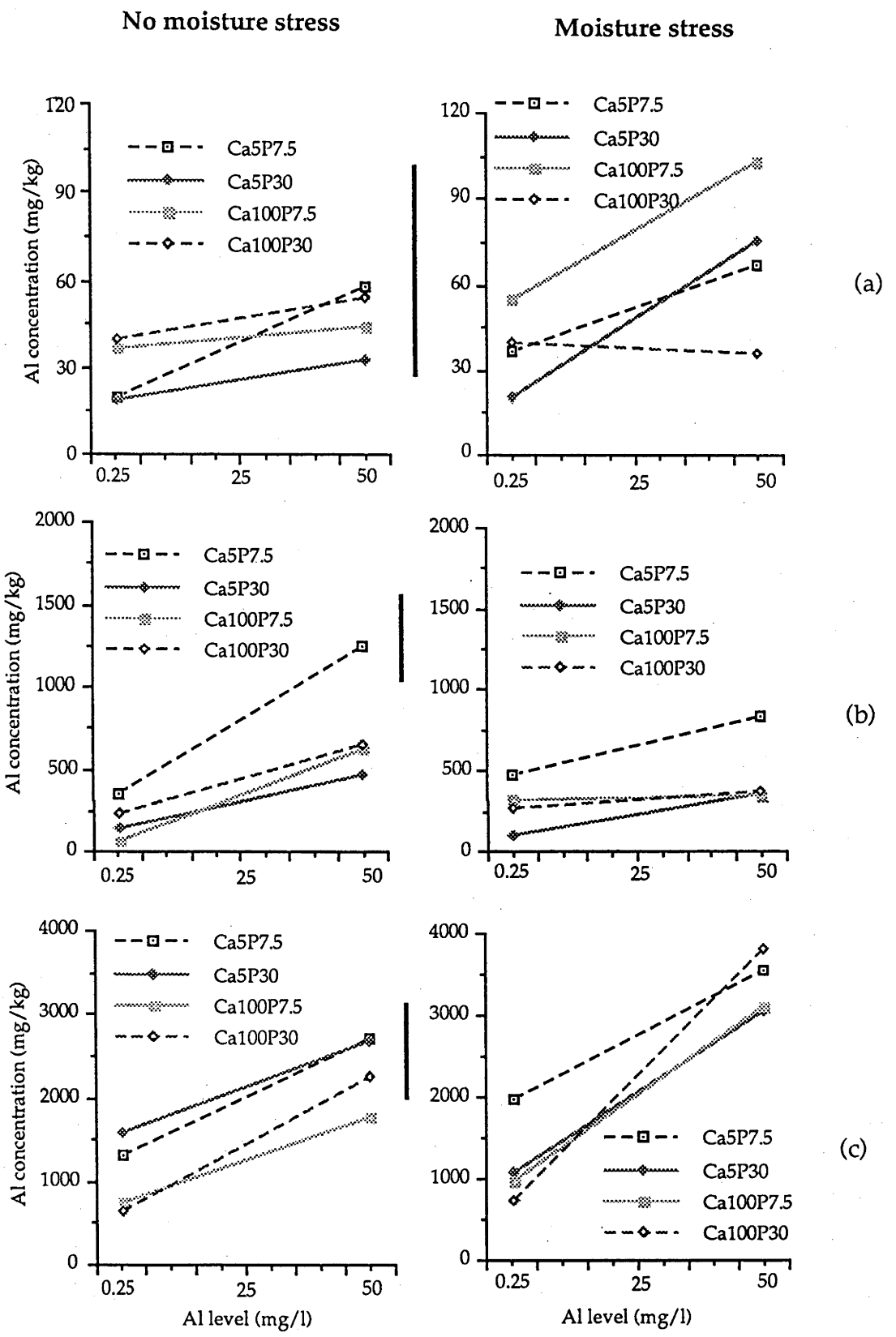


Figure 9.10. Effects of Al treatment levels under different Ca and P combinations in sand pots after 7 weeks. Aluminium concentration in (a) shoot, (b) top root and (c) bottom root of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

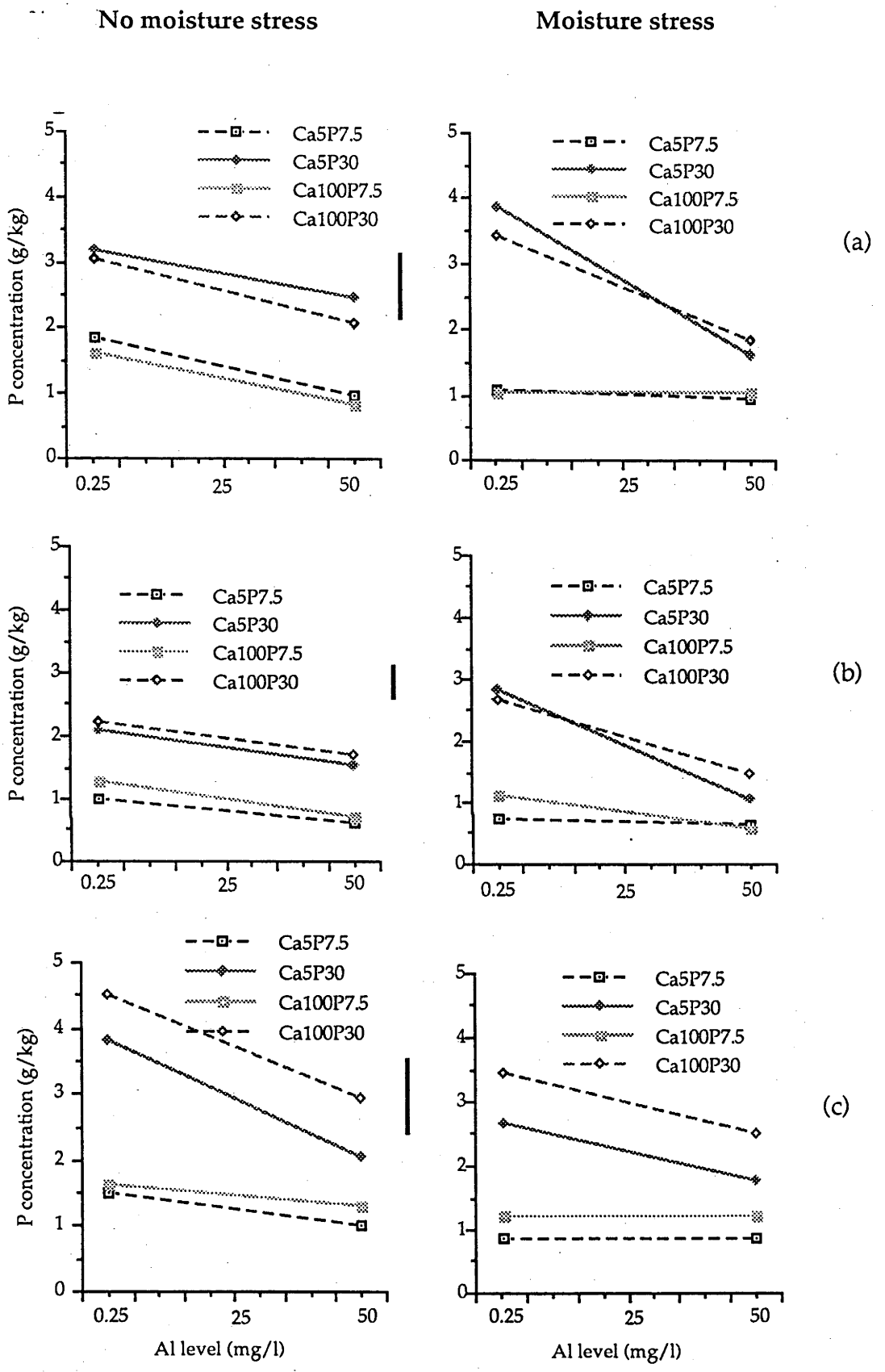


Figure 9.11. Effects of Al treatment levels under different Ca and P combinations in sand pots after 7 weeks. Phosphorus concentration in (a) shoot, (b) top root and (c) bottom root of *E. camaldulensis* seedlings. Vertical bars represent l.s.d. ($P < 0.05$).

Table 9.9. F ratios for the ANOVA on mineral concentration of seedlings in sand pots (harvested after 4 weeks) with respect to treatments.

Sample	Source of variation	Mineral concentration			
		Ca	Mg	Al	P
Shoot	Ca	1.27	2.54	3.45	14.80**
	P	24.24**	0.26	1.45	105.88**
	Al	3.40	9.56**	5.16*	50.07**
	Ca x P	0.46	0.05	1.23	2.34
	Ca x Al	0.11	0.15	2.67	1.21
	P x Al	0.46	1.59	6.13*	59.32**
	Ca x P x Al	0.02	0.09	1.61	0.79
Top root	Ca	56.03**	6.24*	5.64*	0.04
	P	0.13	25.11**	2.56	35.42**
	Al	8.81**	5.02*	16.55**	15.37**
	Ca x P	0.44	1.55	0.83	0.98
	Ca x Al	0.27	0.55	0.00	1.08
	P x Al	3.43	8.13**	0.83	18.74**
	Ca x P x Al	5.50*	0.89	1.23	0.13
Bottom root	Ca	39.61**	9.84**	2.78	1.09
	P	1.32	0.45	7.18*	54.94**
	Al	0.16	7.58**	0.07	39.20**
	Ca x P	0.44	0.16	1.27	0.39
	Ca x Al	0.13	1.84	1.47	0.39
	P x Al	0.06	1.92	0.00	21.55**
	Ca x P x Al	1.14	1.27	0.31	0.06

** and * indicate level of significance at < 1% and < 5% respectively.

Table 9.10. F ratios for the ANOVA on mineral concentrations in seedlings (harvested after 7 weeks of treatments) with respect to treatments.

Source of	Mineral concentration in shoot				Mineral concentration in top root				Mineral concentration in bottom root			
variation	Ca	Mg	Al	P	Ca	Mg	Al	P	Ca	Mg	Al	P
Ca	413.18**	12.32**	2.68	1.30	50.38**	9.93**	4.79*	6.40*	338.06**	0.34	7.10**	14.69**
P	7.58**	0.74	0.91	165.08**	0.00	28.50**	11.38**	336.40**	3.59	50.57**	1.32	179.44**
Al	5.17*	14.00**	11.61**	61.19**	3.15	2.74	34.80**	135.42**	36.81**	33.48**	183.78**	32.48**
W S	14.92**	6.57*	4.80*	1.71	0.23	4.30*	2.01	0.03	10.82**	7.71**	10.63**	14.96**
Ca x P	0.17	1.60	0.01	0.18	0.59	0.64	16.36**	0.29	8.17**	11.45**	0.82	4.33*
Ca x Al	8.38**	2.32	0.02	0.52	5.20*	0.10	2.18	0.06	24.40**	0.62	6.47*	0.00
P x Al	5.01*	7.10**	0.02	15.19**	3.61	2.15	2.40	24.76**	18.06**	12.47**	0.35	12.49**
Ca x WS	5.50*	0.08	0.18	0.61	1.70	4.29*	0.15	0.01	3.40	8.23**	0.67	0.00
P x WS	0.44	0.25	0.06	1.74	0.56	0.34	0.04	4.33*	6.59*	8.02**	0.26	1.47
Al x WS	5.07*	1.18	2.43	0.36	0.63	1.35	8.52**	10.42**	0.53	2.74	6.00*	3.94
Ca x P x Al	8.25**	5.22*	0.00	0.03	0.33	2.45	1.42	6.41*	18.04	0.68	0.12	0.34
Ca x P x WS	0.01	0.60	0.87	0.09	2.15	1.09	0.60	0.01	2.77	1.48	6.32*	0.01
Ca x Al x WS	1.84	0.20	0.22	0.83	0.38	0.75	0.25	0.47	0.67	1.99	0.20	0.20
P x Al x WS	0.03	0.46	0.26	15.25**	1.05	0.66	2.02	20.84**	3.14	2.57	3.39	1.15
Ca x P x Al x WS	2.46	0.08	0.44	1.07	0.57	1.84	0.27	2.94	4.74*	0.75	0.36	0.09

** and ** indicate level of significance at < 1% and < 5% respectively.

in all cases in pots without any moisture stress. On the other hand, in moisture stressed pots, P concentrations in seedlings were lower at low P levels compared to those without moisture stress. In these latter cases, higher Al treatments did not affect P concentrations.

A consideration of the concentration of Ca, Mg, Al and P in seedlings of different treatment combinations for pots with and without moisture stress did not yield a plausible explanation as to why moisture stressed pots suffered less adverse effects. There were no obvious abrupt differences in the concentration of these elements between seedlings from moisture stress and unstressed treatments.

A comparison of seedling shoot Ca concentrations in sand pots here and those described in Chapter 7 at the same treatment levels, shows that the effect of Al was less pronounced in the present case. Also Mg concentrations (at low Ca and P levels when Mg concentration was high) were less affected by Al. In Chapter 7 an increase in the P at high Al levels increased shoot P concentrations which did not occur here. These differences in the concentration of Ca, Mg and P between nutrient solution (Chapter 7) and the present experiment (where the same nutrient solutions were used) was probably due to the difference in growth media. Recently Horst et al (1990) reported that in comparison with solution culture, in sand culture a ten times higher Al supply was necessary to inhibit root elongation of soybean to a comparable degree. These authors suggested that enhanced exudation of organic complexors in the sand culture, reduced activities of monomeric Al species and therefore the seedlings can withstand higher Al level. These explanations are in conformity with the data in the present study.

CHAPTER 10

GENERAL DISCUSSION AND SUMMARY

10. GENERAL DISCUSSION AND SUMMARY

Vast areas of the world are covered by acid soils and soil acidity is a major growth limiting factor for both agricultural crops and forest trees. Aluminium toxicity is the most critical aspect of soil acidity. In recent years extensive research has been directed towards the problem posed by Al toxicity in relation to agricultural crops. However, despite the fact that forest soils are often highly acid, the problem of Al toxicity has received little attention in relation to trees.

The present research examined the major aspects of Al toxicity on the growth and nutrient absorption of tree seedlings by using experiments on an important eucalypt species namely, *E. camaldulensis*. It focussed on the adverse effects of Al toxicity and its interrelationships with Ca, P and moisture stress. The experiments were undertaken in the glasshouse and took into account various combinations of the above factors. This chapter provides a general discussion of the findings and on this basis, some suggestions are also made for further research in this area.

To begin with, preliminary experiments were conducted with 22 eucalypt species using glasshouse pot trials to select a species whose performance in acid forest soils would demonstrate the important interrelationships of soil acidity factors (Chapter 4). The species showed a wide variation in their performance in acid soils. Some selected species also differed significantly in their ability to absorb mineral elements. Four eucalypt species (*E. camaldulensis, E. citriodora, E. gummifera* and *E. saligna*) selected from the 22, were tested for their response to conventional liming in a soil high in exchangeable Al whilst low in exchangeable Ca and available P (Section 4.3). From amongst these four,

E. camaldulensis showed a maximum response to liming by increasing growth despite the fact that total growth remained very poor in the acid forest soil under study. The poor performance of *E. camaldulensis* in Al rich acid soil and the large percentage response to liming demonstrated the adverse effects of Al on growth, as suggested in the hypothesis in Chapter 1. This adverse effect was further confirmed in the second phase experiments where it was dealt with in greater detail. It appears from these findings that *E. gummifera* is more tolerant to acid conditions including high Al, low P and Ca which usually accompanies soil acidity. Such a conclusion confirms the findings of McColl (1969) who reported that *E. gummifera* is well adapted to harsh conditions like high Al and low P and Ca levels. Therefore, this important species may be considered suitable for growing in similar acid soils.

The growth of *E. camaldulensis* responded significantly to added Ca and P when grown in a pot trial using the same acid soil in which the liming experiment was conducted (Chapter 5). This finding was utilized in a later experiment for boosting the growth of *E. camaldulensis* for examining root growth and development as affected by Ca, P and Al treatments applied in a lower layer of a soil (Section 9.2).

A conventional remedy for both soil acidity and Al toxicity is to lime (for example, Coleman et al, 1958; Anandan et al, 1985; Bromfield et al, 1987). For agricultural production and land management, this convention is widely accepted. In the usual liming procedure there is a simultaneous addition of Ca and reduction in the toxicities of Al and Mn, improved availability of Mg, P and Mo and improved microbial activity. Thus for research purposes, the specific mechanism by which liming improves growth remains unresolved; due to the difficulties in isolating the effect of each of these individual factors

from the liming. Therefore, in Chapter 3 of this thesis (Section 3.10) a treatment combination was established so that the soil Ca level could be raised while causing a minimal impact on the other characteristics, particularly pH and exchangeable Al and Mn. The addition of Ca from $CaCO_3$ and $CaSO_4$ at a ratio of 2 : 1 raised the soil Ca to an identical level as that of a single Ca source such as $CaCO_3$, but there was a minimal change in pH, exchangeable Al or Mn. This technique could then be used for soil modification in the remaining experiments such as those described in Chapter 9. This methodological finding will be very useful for research in soil acidity dealing with Ca. However, the quantity and proportion of $CaCO_3$ and $CaSO_4$ required under any specific set of soil conditions may be different and will need to be determined.

In the next phase, an experiment was conducted using water culture to intensively investigate the effects of Al toxicity and the ameliorative role of Ca on the growth of *E. camaldulensis*. In general, Al has a negative effect and Ca a positive effect on the root and shoot growth of *E. camaldulensis*; these changes were evaluated by different measures of root and shoot growth. Low levels of Al accompanied by high Ca levels improved some growth parameters to an even greater extent than treatments involving no Al. The adverse effects of high Al were partly ameliorated by applying high levels of Ca. Primary root length, fine root length and fineness of the fine root [as defined by (fine root length)/(fine root weight)] were among the parameters most sensitive to high levels of Al. The root periphery of seedlings in high Al treatments were heavily thickened. The thick periphery of roots grown in this high Al medium were able to recover when the Ca levels were increased. Although the high Ca level protected (at least partly) the seedlings from Al toxicity, in terms of growth and thickened root periphery, the high Ca level did not reduce Al concentrations in fine roots or in mature leaves. The

reduction due to Al reported in other studies (e.g., Huett and Menary, 1980). Therefore, in the present research the protection against Al toxicity given by high Ca did not operate by reducing the Al uptake, but by increasing the Ca concentration in the seedlings. Therefore, the main problem with Al toxicity is the low Ca content in the seedlings which has in turn resulted from low Ca contents or higher Al contents, in the growth medium. The ratio of a_{Al}/a_{Ca} in the growth medium was significant in explaining the performance of growth parameters of *E. camaldulensis*, but its explanatory power varied between the parameters. The order in which they correlated with a_{Al}/a_{Ca} were: (fine root length)/(fine root weight) > root length > shoot height > fine root length > shoot weight > total biomass > root branching > root weight. The parameters where the explanatory power was small demonstrate that other factors are also responsible for toxicity.

In earlier studies, fine root length (Pinkerton and Simpson, 1981) and Al concentration in fine roots (Joslin et al, 1988) were considered to be more sensitive to Al levels while shoot Al (whole shoot) concentration was reported to be unsuitable as a predictor of Al toxicity (Hutchinson et al, 1986; Joslin et al, 1988). However, in the present study young and mature leaves were analyzed separately and the Al concentration in mature leaves appeared to be a suitable phytoindicator for Al toxicity in *E. camaldulensis* seedlings. Fine root length responded significantly to Al level but fineness of fine roots responded even better to Al treatment levels. Between these two parameters (fineness of fine root and Al concentration in mature leaves), the Al concentration in mature leaves would be a useful phytoindicator of Al toxicity in *E. camaldulensis* seedlings, particularly when separation of fine roots or measurement of roots is difficult (e.g., from a solid growth medium). Thus this experiment established the hypothesis that

Al toxicity adversely affects *E. camaldulensis* growth and Ca ameliorates it. This experiment further shows the responsiveness of various growth parameters and mineral concentration in young leaves, mature leaves and fine roots. Not only are previous studies on these aspects of *E. camaldulensis* lacking, but a comprehensive study dealing with several interrelated aspects of the effect of Al toxicity is not available for any tree species. However, these findings, particularly on the phytoindicator for *E. camaldulensis* needs confirmation in varying growth conditions, across seedlings of varying ages and Al tolerance levels and also for trees in a forest stand.

Based on the findings described so far, it became necessary to conduct additional experiments to examine specific aspects of the impact of Al toxicity on the growth of *E. camaldulensis.*

i. Whether P and Ca interact with each other and with Al levels to ameliorate Al toxicity and what is the nature of such an interaction over a growth period ?

ii. Do Al affected roots become less efficient in terms of nutrient absorption rates ?

iii. How do Ca and P ameliorate the problem of the thickening of root peripheries of seedlings grown in a high Al medium ?

iv. How do subsoil Al, Ca and P levels affect the growth and the mineral concentrations of *E. camaldulensis* and how does moisture stress interact with these factors ?

Three further experiments were conducted where both Ca and P levels were varied to examine their effects and interactions in ameliorating Al toxicity. The first of these three experiments saw seedlings grown in nutrient solution and harvested successively. This meant that the effect of growth period may be examined with other

treatments while monitoring seedling growth and nutrient absorption rate (the first two questions above). The adverse effects of high Al was ameliorated by either or both P and Ca and further the adverse effects decreased over time as the seedlings grew older. Although liming is not prescribed for forest soils (Schaedle et al, 1989) on economic grounds, for initial establishment of *E. camaldulensis* seedlings, at least localized liming may be useful, since the seedlings were found to increase Al resistance as they grew. The ameliorating effect of P and Ca were interrelated and reinforced each other. These interrelated effects occurred for the seedling growth parameters as well as for absorption rates of Al, Ca, Mg and P. The absorption rate is a measure of root efficiency and this has important implications for the sustained growth of a plant. In this experiment the absorption rate of Al by the roots increased with an increase in the Ca treatment level. Yet, an increase in Ca led to a boost in the growth of both roots and shoots. These findings provide a test for the hypothesis that the effect of Al was dependent on the growth period and that such an effect is influenced by the interaction of Ca and P treatment levels (hypothesis 4 and 6 in Chapter 1).

To answer the third question above (the role of the high Ca and P levels in ameliorating the thickening of the root periphery), seedlings were grown in an high Al media. It was found that the higher Ca levels alone did not reduce Al in the root periphery or in the total root to any large extent but they increased the concentration of Ca in the periphery and in the total root. When both Ca and P were higher, the Ca concentration in the root periphery increased at the cost of Al. From these results it may be suggested that in a high Al growth medium, seedlings benefit from increased Ca and P levels through increased Ca and P concentration in the whole root as well as in the periphery. This is

another reflection that higher Ca and P levels interact to accentuate their ameliorating influence against Al toxicity.

To answer the fourth question (the effect of Al, Ca and P and moisture stress in the lower layer on root growth and mineral concentration), another experiment examined the effects of Al and other treatments (Ca and P and moisture stress) imposed in the bottom layer of a two layer growth medium. In one phase, soil was used in the bottom layer and in another sand was used. Aluminium toxicity reduced root development and thereby reduced the root weight in the subsoil by a greater extent than the reduction caused to roots in the upper layer. However, adverse effects of high Al in the bottom layer did spread to the shoot height and weight. In addition, moisture stress adversely affected the seedlings growth in both high and low Al treatments and the extent of the adverse effects were higher in the low Al situation. Mineral concentrations of Ca, Mg, Al and P in the shoot, top root and bottom root which were analyzed separately, did not indicate any possible explanation for the lessened effect of moisture stress on high Al seedlings. However, it may be noted that the growth of seedlings under moisture stress was already very poor and therefore, the stress effect of Al was likely to be smaller in magnitude. Further, since the seedlings were provided with nutrient solution, the question of nutrient availability and movement as in soil (Krizek and Foy, 1988; Krizek et al, 1988) does not arise. These experiments thus established the hypothesis that soil moisture levels influence the effect of Al on growth of *E. camaldulensis* (hypothesis 7 in Chapter 1) and also demonstrated how the adverse effects of subsoil Al are transmitted to both root and shoot growth. Since the less severe effect of Al toxicity in a stressed situation was due to the fact that moisture stress did not affect nutrient availability, this aspect needs further study where moisture stress may have such an effect. Therefore,

the response to Al toxicity in interaction with moisture stress may be extended to field conditions to include more levels of both Al and moisture stress.

The harmful effects of Al as reflected in decreased seedling growth was associated with a simultaneous decrease in the Ca concentration in the seedlings (Chapter 6) and Ca absorption rate (Chapter 7). The decrease in the Ca concentration in seedlings resulting from higher Al may therefore, be viewed as a major cause of growth reduction (Keltjen, 1990) and this is more acute when the Ca level in the growth medium is low. These conclusions are also supported by the results (Section 9.4.1) where Al treated soils were used as a subsoil and a comparatively lower Al level (compared to experiments with nutrient solution and with sand as subsoil) resulted in a significant reduction in seedling growth (since soil Ca level was very low).

When all the results on the amelioration of Al toxicity by Ca are reviewed together it is evident that higher levels of Ca do not significantly reduce Al uptake from the growth medium but they do significantly improve seedling growth. Higher levels of Ca increased Ca concentration in all parts of the seedlings (Chapter 6), improved the absorption rate of Ca (Chapter 7), increased the Ca concentration and relative amounts of Ca in the root periphery (Chapter 8) and in addition led to growth improvements in all experiments.

Presence of a high level of P in Al toxic situations helped to reduce the adverse effect of Al on the growth of eucalypt species. This was indicated in the first instance by better growth of most species in one of the acid soils which had a higher available P in addition to high Al (Chapter 4). This finding was supported by another experiment on one acid soil with low P where raising the P level improved seedling growth

(Chapter 5). The final experiments further confirmed this by varying P, Al and Ca in nutrient solution and in sand. When consideration is given to the ameliorating role of P, its high level actually reduce Al concentration in the growth medium by forming soluble polymeric Al complexes on aging (Alva, 1986). These are non phytotoxic (Blamey et al, 1983) and are associated with the better growth performance of *E. camaldulensis*. In addition, P may play an indirect role by significantly increasing Ca concentration in roots which then protects seedlings from Al toxicity. Thus even though both Ca and P help to ameliorate Al toxicity, their modes of action are different.

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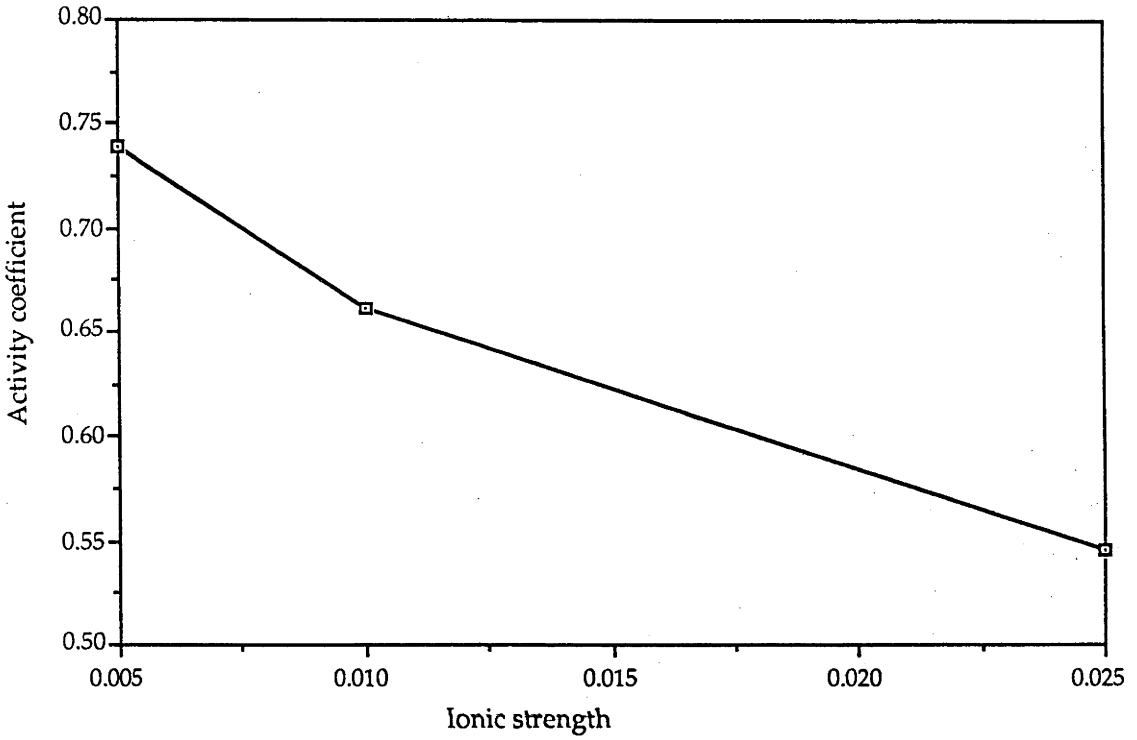
APPENDICES



Appendix 3.1. Relationship between ionic strength (μ) and activity coefficient (γ_i) for Al (Values for ionic strength and activity coefficient were obtained from Lindsay, 1979).

Appendix 3.2. Change in soil pH_w, pH_s and EC with time after Ca treatment.

Treatment	Wk 1	Wk 2	Wk 4	Wk 6	Wk 12	Wk 16	Wk 16 (soil solution)
	pH_w						
Control	5.45	5.57	5.43	5.55	5.34	5.26	5.90
$CaSO_4$ - 1	4.48	4.62	4.51	4.53	4.49	4.48	4.22
$CaSO_4$ - 2	4.39	4.53	4.41	4.42	4.39	4.37	4.13
$CaSO_4$ - 3	4.36	4.49	4.37	4.38	4.37	4.36	4.12
$CaCO_3$ - 1	6.07	6.20	5.94	5.94	5.82	5.78	5.43
$CaCO_3$ - 2	6.69	6.75	6.47	6.40	6.34	6.14	5.45
$CaCO_3$ - 3	7.22	7.40	7.07	6.90	6.85	6.69	5.27
Combined - 1	4.76	4.86	4.87	4.77	4.79	4.83	4.47
Combined - 2	5.09	5.19	5.08	5.05	5.05	5.00	4.46
Combined - 3	5.42	5.49	5.37	5.30	5.25	5.23	5.25
	pH_s						
Control	4.04	4.25	4.07	4.03	4.02	4.04	
$CaSO_4$ - 1	4.20	4.34	4.21	4.18	4.20	4.19	
$CaSO_4$ - 2	4.22	4.37	4.25	4.21	4.23	4.23	
$CaSO_4$ - 3	4.24	4.39	4.25	4.24	4.24	4.26	
$CaCO_3$ - 1	4.82	4.91	4.77	4.71	4.68	4.67	
$CaCO_3$ - 2	5.72	5.81	5.53	5.37	5.35	5.29	
$CaCO_3$ - 3	6.41	6.65	6.27	6.08	6.04	5.95	
Combined - 1	4.52	4.64	4.57	4.63	4.58	4.57	
Combined - 2	4.83	4.92	4.77	4.81	4.76	4.73	
Combined - 3	5.14	5.19	4.99	5.01	4.97	4.94	
	EC (mS/cm)						
Control	9	9	11	12	15	19	56
$CaSO_4$ - 1	413	349	376	338	307	309	1031
$CaSO_4$ - 2	805	853	868	854	807	803	2393
$CaSO_4$ - 3	1273	1248	1220	1315	1200	1298	2478
$CaCO_3$ - 1	17	31	15	15	19	19	58
$CaCO_3$ - 2	24	21	21	18	20	24	77
$CaCO_3$ - 3	53	36	35	28	29	36	73
Combined - 1	640	630	590	653	645	581	1850
Combined - 2	460	478	418	405	425	444	1240
Combined - 3	340	338	310	290	285	297	960

Appendix 3.3. Change in exchangeable Ca, Al and Ca/Al with time after Ca treatment.

Treatment	Wk 1	Wk 2	Wk 4	Wk 6	Wk 12	Wk 16	Wk 16 (soil solution)
Ca (me/kg)							
Control	0.17	0.19	0.38	0.47	0.73	1.10	0.00
$CaSO_4$ - 1	41.46	35.33	42.22	40.52	45.78	52.82	9.91
$CaSO_4$ - 2	82.88	79.07	85.92	83.95	90.30	91.17	34.48
$CaSO_4$ - 3	122.05	125.12	132.43	120.67	133.64	140.93	37.94
$CaCO_3$ - 1	40.89	40.44	40.22	40.33	41.35	48.67	0.09
$CaCO_3$ - 2	79.00	78.22	76.75	75.40	76.78	86.05	0.31
$CaCO_3$ - 3	108.08	114.84	110.86	108.23	109.16	120.43	0.67
Combined - 1	86.19	84.40	90.42	89.44	88.69	91.75	26.89
Combined - 2	81.09	78.80	77.92	79.44	83.70	91.20	16.04
Combined - 3	87.16	86.70	82.35	84.14	80.73	89.41	12.17
Al (me/kg)							
Control	66.00	66.90	68.00	64.80	54.90	53.90	0.03
$CaSO_4$ - 1	63.90	61.80	60.40	56.60	44.80	45.30	0.28
$CaSO_4$ - 2	60.20	54.80	57.50	56.10	44.40	44.80	1.13
$CaSO_4$ - 3	60.70	58.00	61.40	54.80	42.30	44.40	1.19
$CaCO_3$ - 1	29.50	28.10	26.30	25.90	24.00	24.30	0.04
$CaCO_3$ - 2	4.00	4.10	4.60	4.70	4.60	4.90	0.09
$CaCO_3$ - 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Combined - 1	39.40	37.20	31.10	32.70	27.60	28.20	0.13
Combined - 2	25.90	24.00	22.60	23.20	20.50	21.20	0.04
Combined - 3	16.10	15.50	15.50	16.70	14.20	15.10	0.01
(Ca/Al)							
Control	0.00	0.00	0.01	0.01	0.01	0.02	0.02
$CaSO_4$ - 1	0.65	0.57	0.70	0.72	1.02	1.17	35.39
$CaSO_4$ - 2	1.38	1.44	1.49	1.50	2.03	2.04	30.51
$CaSO_4$ - 3	2.01	2.16	2.16	2.20	3.16	3.17	31.88
$CaCO_3$ - 1	1.39	1.44	1.53	1.56	1.72	2.00	2.25
$CaCO_3$ - 2	19.75	19.08	16.68	16.04	16.69	17.56	3.44
$CaCO_3$ - 3	*	*	*	*	*	*	*
Combined - 1	2.19	2.27	2.91	2.74	3.21	3.25	206.85
Combined - 2	3.13	3.28	3.45	3.42	4.08	4.30	401.00
Combined - 3	5.41	5.59	5.31	5.04	5.69	5.92	1217.00

* Al levels in these cases were zero, respective Ca values may be seen in paragraph above.

Appendix 3.4. Change in exchangeable Mg, Mn and K with time after Ca treatment.

Treatment	Wk 1	Wk 2	Wk 4	Wk 6	Wk 12	Wk 16	Wk 16 (soil solution)
Mg (me/kg)							
Control	4.02	3.68	3.96	4.02	4.38	4.35	0.02
$CaSO_4$ - 1	4.27	3.76	3.86	4.55	5.15	5.28	1.43
$CaSO_4$ - 2	4.19	3.52	3.94	4.55	5.17	5.29	2.44
$CaSO_4$ - 3	4.17	3.54	3.94	4.72	5.09	5.34	2.54
$CaCO_3$ - 1	3.81	3.19	3.35	4.08	4.74	4.93	0.04
$CaCO_3$ - 2	2.68	2.09	2.09	2.92	3.56	3.74	0.05
$CaCO_3$ - 3	1.92	0.99	0.83	1.59	2.02	2.33	0.04
Combined - 1	3.98	3.48	4.41	4.62	4.97	5.65	1.77
Combined - 2	3.72	3.12	4.06	4.38	4.84	5.17	1.17
Combined - 3	3.62	2.97	3.73	4.14	4.45	4.92	0.87
Mn (me/kg)							
Control	0.06	0.07	0.03	0.03	0.04	0.04	0.00
$CaSO_4$ - 1	0.10	0.10	0.04	0.05	0.07	0.07	0.09
$CaSO_4$ - 2	0.09	0.14	0.05	0.06	0.07	0.07	0.17
$CaSO_4$ - 3	0.10	0.17	0.05	0.06	0.07	0.07	0.17
$CaCO_3$ - 1	0.05	0.05	0.02	0.02	0.03	0.03	0.00
$CaCO_3$ - 2	0.04	0.05	0.02	0.02	0.02	0.02	0.00
$CaCO_3$ - 3	0.03	0.02	0.01	0.01	0.01	0.01	0.00
Combined - 1	0.06	0.02	0.04	0.04	0.05	0.05	0.08
Combined - 2	0.05	0.02	0.03	0.03	0.04	0.04	0.04
Combined - 3	0.05	0.02	0.02	0.02	0.03	0.04	0.02
K (me/kg)							
Control	2.08	3.51	3.05	3.72	3.81	3.88	0.05
$CaSO_4$ - 1	2.33	3.21	3.01	3.71	3.78	3.90	0.38
$CaSO_4$ - 2	2.14	2.15	2.95	3.71	3.75	3.87	0.47
$CaSO_4$ - 3	2.16	1.94	3.06	3.67	3.67	3.94	0.49
$CaCO_3$ - 1	2.11	1.97	3.09	3.68	3.66	3.87	0.05
$CaCO_3$ - 2	2.10	2.06	3.03	3.60	3.49	3.82	0.05
$CaCO_3$ - 3	2.02	2.08	2.79	3.51	3.39	3.61	0.05
Combined - 1	3.14	3.12	3.75	3.82	3.64	3.83	0.32
Combined - 2	3.10	3.07	3.64	3.81	3.67	3.78	0.24
Combined - 3	3.18	3.20	3.51	3.88	3.59	3.72	0.20

Appendix 4.1. Shoot height (in cm) of 22 eucalypt species at two week intervals beginning at week 8.

Soil 1	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20
Speies							
E. albens	5.08	5.88	6.46	7.81	8.77	9.74	10.84
E. blakelyi	3.41	4.16	4.64	5.65	6.82	7.97	8.80
E. calcicola	3.08	4.12	5.23	6.21	7.09	7.81	8.08
E. camaldulensis	5.35	6.14	6.96	7.96	9.04	9.80	10.82
E. camaldulensis	4.11	4.87	6.45	8.51	9.86	10.49	10.77
E. citriodora	7.79	9.12	9.82	11.16	11.60	12.80	13.40
E. decipiens	3.48	4.65	5.92	7.48	8.78	9.38	10.47
E. dives	4.75	5.73	6.63	7.42	8.47	9.25	9.55
E. gummifera	5.79	6.69	7.14	8.21	8.68	9.32	9.61
E. macrorhyncha	3.11	4.32	5.45	6.52	7.82	8.35	8.81
E. mannifera	4.66	5.73	6.85	8.10	8.97	10.42	11.35
E. melliodora	2.88	3.39	3.72	4.19	4.77	5.09	5.74
E. microtheca	3.37	3.89	4.15	4.42	5.04	5.44	5.72
E. occidentalis	4.86	6.05	7.30	9.20	11.00	12.43	13.09
E. pilularis	5.36	7.29	8.09	9.57	10.35	10.82	11.39
E. polyanthemas	3.26	4.08	4.61	5.35	6.16	6.73	7.27
E. populnea	3.21	4.00	4.53	5.43	6.39	7.33	8.04
E. rossii	4.28	5.70	6.19	7.01	7.45	8.11	8.82
E. saligna	3.56	4.44	4.89	5.51	6.17	6.73	7.67
E. tereticornis	3.89	4.55	4.82	5.24	5.89	6.63	7.33
E. viminalis	6.22	6.99	8.43	9.94	11.57	13.15	14.57
E. yalatensis	2.83	3.40	3.97	4.43	5.25	5.95	6.60

Appendix 4.1. contd.

Soil 2	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20
Species							
E. albens	5.14	6.61	9.10	12.14	15.51	18.07	21.60
E. blakelyi	5.92	8.48	11.77	16.11	18.83	20.08	21.74
E. calcicola	5.58	10.04	14.89	19.13	21.60	22.80	24.48
E. camaldulensis	10.46	16.38	22.00	31.78	37.22	40.34	44.08
E. camaldulensis	8.89	12.48	16.47	21.79	24.17	25.64	26.67
E. citriodora	9.89	13.10	17.56	21.97	25.76	27.89	31.29
E. decipiens	7.64	12.08	17.53	22.48	25.28	26.85	28.90
E. dives	7.61	11.81	16.00	19.87	22.17	23.40	24.88
E. gummifera	6.38	7.95	9.99	13.13	14.01	15.22	17.38
E. macrorhyncha	6.65	9.92	13.01	17.13	20.63	22.55	26.54
E. mannifera	8.20	11.74	17.04	23.70	29.08	32.50	36.26
E. melliodora	5.59	8.00	11.57	15.68	20.91	23.88	26.79
E. microtheca	6.81	10.17	14.93	21.08	25.92	28.31	31.77
E. occidentalis	11.96	18.71	24.50	30.74	34.45	36.65	38.98
E. pilularis	12.51	16.56	19.22	22.37	23.69	24.53	24.93
E. polyanthemas	5.37	8.06	12.03	16.81	20.55	22.40	25.08
E. populnea	7.53	10.70	15.05	19.90	23.25	25.37	27.29
E. rossii	8.58	13.61	19.34	24.01	26.82	27.97	28.55
E. saligna	5.43	8.17	11.53	16.38	18.25	18.85	20.31
E. tereticornis	6.23	12.28	16.90	21.98	24.87	26.30	29.10
E. viminalis	12.94	18.71	22.68	26.41	29.24	30.81	32.78
E. yalatensis	4.82	6.93	9.88	13.29	15.90	18.23	21.49

Appendix 4.1. contd.

Appendix 4.1 contd.

Soil 3	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20
Species							
E. albens	5.25	5.77	6.27	6.43	6.65	7.24	7.32
E. blakelyi	3.43	3.83	4.03	4.13	4.28	4.56	4.56
E. calcicola	2.63	3.26	3.63	3.75	3.97	4.07	4.19
E. camaldulensis	4.64	5.16	5.68	5.77	5.97	6.13	6.31
E. camaldulensis	4.07	4.34	4.76	4.89	5.05	5.30	5.57
E. citriodora	7.37	8.23	8.67	9.07	9.57	9.71	10.20
E. decipiens	4.42	5.20	5.75	6.12	6.61	6.99	7.27
E. dives	4.07	4.93	5.28	5.28	5.35	5.35	5.41
E. gummifera	6.06	6.67	7.31	7.79	8.00	8.30	8.64
E. macrorhyncha	3.73	4.50	4.75	4.90	5.03	5.33	5.40
E. mannifera	5.08	5.89	6.12	6.13	6.25	6.33	6.69
E. melliodora	2.83	3.01	3.39	3.39	3.52	3.72	3.89
E. microtheca	4.04	4.25	4.50	4.50	4.71	4.94	5.09
E. occidentalis	6.91	8.14	9.39	10.68	11.39	11.87	12.02
E. pilularis	5.54	6.54	7.13	7.38	8.05	8.52	8.90
E. polyanthemas	3.32	3.70	4.00	4.13	4.33	4.59	4.67
E. populnea	3.19	3.71	3.89	4.04	4.27	4.44	4.48
E. rossii	4.51	4.93	5.28	5.36	5.49	5.54	5.75
E. saligna	4.47	4.58	4.95	5.01	5.30	5.53	5.60
E. tereticornis	3.85	4.23	4.49	4.67	4.81	4.85	4.90
E. viminalis	6.20	6.57	7.10	7.25	7.41	8.23	8.43
E. yalatensis	3.35	3.99	4.65	4.90	5.23	5.43	5.68

Appendix 4.2. Shoot height increments (cm) of lime treated eucalypt seedlings beginning at week 12.

Species	Lime level	Week 12	Week 14	Week 16	Week 18	Week 20	Week 22	Week 24	Week 26	Week 28
E. camaldulensis	0	2.0	2.3	2.5	2.6	2.9	2.8	2.9	2.9	3.0
	1	2.5	3.0	3.2	3.4	3.3	3.3	3.4	3.4	3.6
	2	3.3	4.0	4.4	4.6	4.9	4.7	4.9	5.0	5.3
E. citriodora	0	8.4	9.2	9.5	9.6	9.9	9.8	10.2	10.2	10.5
	1	9.0	9.2	9.4	9.5	9.6	9.5	9.7	9.8	9.9
	2	10.8	11.7	12.4	12.8	13.2	13.3	13.9	14.0	14.5
E. gummifera	0	7.6	8.2	8.3	8.3	8.8	8.9	9.0	9.1	9.2
	1	5.5	5.8	6.2	6.2	6.3	6.3	6.4	6.4	6.6
	2	6.7	7.2	7.4	7.4	7.5	7.5	7.8	7.9	8.1
E. saligna	0	2.8	3.3	3.4	3.5	3.7	3.7	3.9	3.9	4.0
	1	3.4	3.7	4.0	4.0	4.2	4.2	4.3	4.3	4.5
	2	4.0	4.4	4.8	4.9	5.0	5.1	5.3	5.4	5.8

Appendix 4.3. Shoot height increments (cm) of eucalypt seedlings after beginning of soil moisture stress.

Species	Moisture level (% field capacity)	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18
E. camaldulensis	40	2.8	2.9	3.1	3.1	3.4	3.5	3.6	3.6	3.7	3.7
	53	2.7	2.7	2.9	3.1	3.3	3.5	3.7	3.8	4.1	4.2
	67	2.8	2.8	2.8	2.9	3.0	3.1	3.3	3.3	3.8	3.8
	80	2.8	3.3	3.3	3.6	3.9	4.0	4.3	4.5	4.8	5.1
E. citriodora	40	11.9	12.2	12.4	12.7	13.1	13.3	13.6	13.8	14.3	14.6
	53	11.8	12.3	12.5	12.9	13.1	13.4	13.6	13.9	14.5	14.9
	67	11.8	12.8	13.3	13.7	14.1	14.8	15.6	16.2	16.9	17.2
	80	12.0	12.6	13.1	13.7	14.2	14.6	14.6	15.0	15.7	15.9
E. gummifera	40	6.8	7.0	7.1	7.2	7.6	7.6	7.5	7.7	7.8	7.8
	53	6.2	6.8	6.9	6.9	7.2	7.2	7.4	7.5	7.6	7.9
	67	6.7	7.4	7.5	7.8	7.9	8.2	8.4	8.7	9.1	9.4
	80	6.8	7.3	7.6	7.9	8.1	8.4	8.6	8.9	9.1	9.4
E. saligna	40	4.7	4.8	4.7	4.7	4.8	4.9	5.0	5.1	5.1	5.2
	53	4.7	5.1	5.0	5.1	5.6	5.6	5.8	5.8	6.1	6.0
	67	4.7	4.8	5.0	5.4	5.7	5.9	6.4	6.3	6.6	6.6
	80	4.7	4.9	5.0	5.4	5.7	5.9	6.2	6.3	6.7	6.8

Appendix 4.4. Shoot height increments (cm) of *E. camaldulensis* after beginning of soil moisture stress.

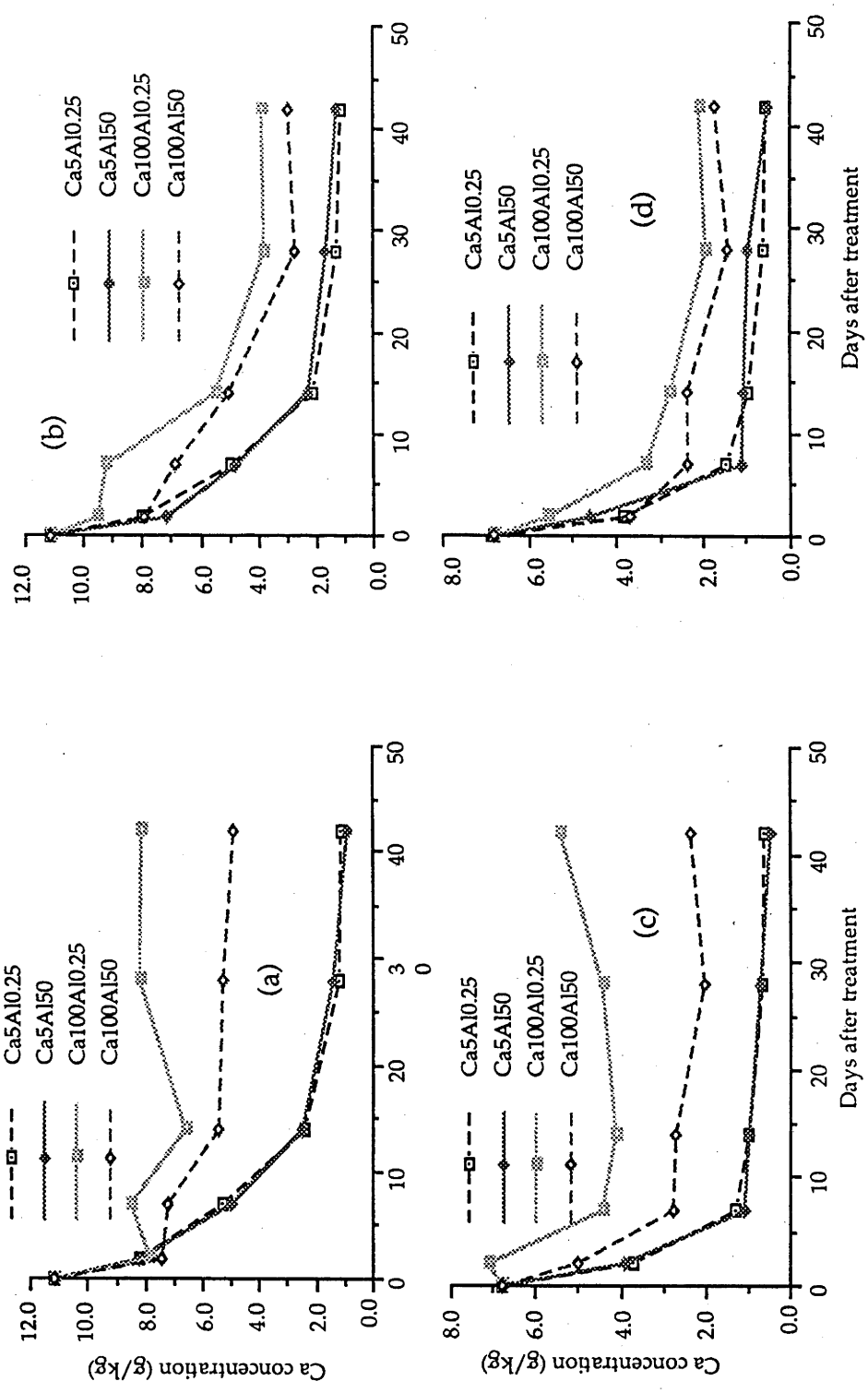
Moisture level (% field capacity)	Week 2	Week 4	Week 6	Week 12
40	19.6	20.4	20.5	20.6
50	19.5	22.4	23.4	25.3
60	19.7	24.3	28.5	33.3
70	19.7	25.8	32.1	37.1
80	19.6	27.3	35.3	41.9

Appendix 6.1. Calculated concentrations and activities of main forms of Al at different Ca and Al treatment combinations.

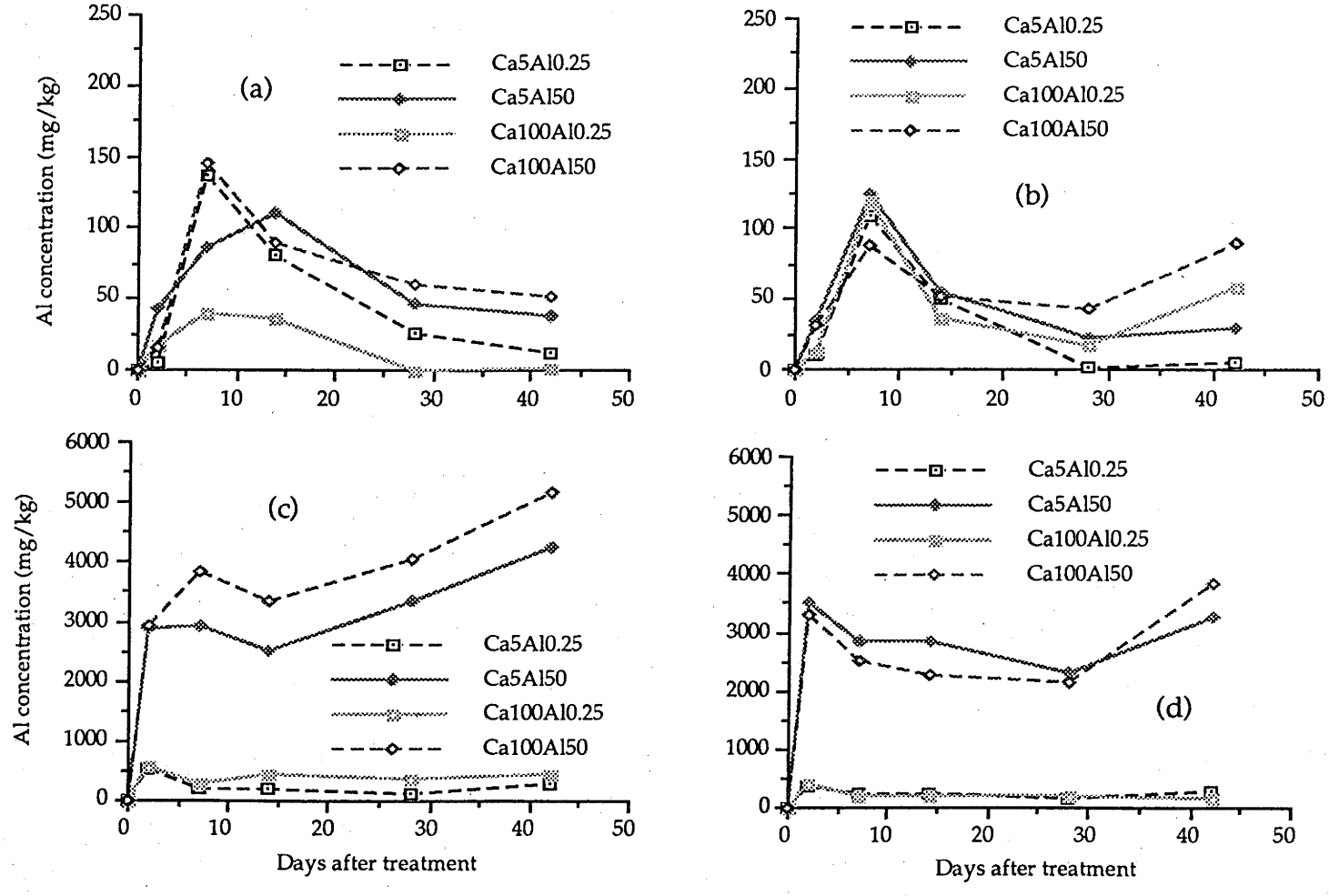
Treatment combination	Concentration (μM)					Activity (μM)				
	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$AlSO_4$	Total monomeric	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$AlSO_4$	Total monomeric
$Al_{0.25}Ca_5$	3.42	0.83	0.93	4.08	9.27	1.55	0.59	0.85	3.74	6.71
$Al_{0.25}Ca_{10}$	3.43	0.82	0.90	4.13	9.27	1.54	0.57	0.82	3.78	6.71
$Al_{0.25}Ca_{50}$	3.36	0.78	0.81	4.33	9.28	1.44	0.53	0.74	3.94	6.65
$Al_{0.25}Ca_{100}$	3.15	0.76	0.80	4.58	9.27	1.24	0.50	0.72	4.12	6.58
$Al_{2.5}Ca_5$	43.55	1.65	0.28	47.54	93.03	17.69	1.11	0.25	43.01	62.07
$Al_{2.5}Ca_{10}$	43.45	1.62	0.27	47.69	93.03	17.55	1.08	0.24	43.12	62.00
$Al_{2.5}Ca_{50}$	42.27	1.49	0.23	49.00	92.98	16.29	0.97	0.21	44.07	61.55
$Al_{2.5}Ca_{100}$	40.27	1.46	0.23	51.05	93.01	14.45	0.93	0.20	45.55	61.13
$Al_{20}Ca_5$	529.70	16.22	2.19	193.00	741.10	204.10	10.62	1.97	173.60	390.30
$Al_{20}Ca_{10}$	529.70	16.27	2.20	193.30	741.40	203.10	10.63	1.98	173.80	389.50
$Al_{20}Ca_{50}$	527.20	15.97	2.11	195.90	741.20	193.60	10.23	1.89	175.30	380.90
$Al_{20}Ca_{100}$	522.40	16.61	2.27	199.90	741.20	179.40	10.33	2.01	177.50	369.30
$Al_{50}Ca_5$	1570.00	39.75	4.28	236.80	1851.00	521.70	24.36	3.79	209.50	759.40
$Al_{50}Ca_{10}$	1570.00	39.20	4.15	237.10	1851.00	515.50	23.89	3.67	209.50	752.60
$Al_{50}Ca_{50}$	1570.00	38.32	3.93	237.90	1851.00	499.70	23.04	3.46	209.50	735.70
$Al_{50}Ca_{100}$	1567.00	39.83	4.20	239.40	1850.00	472.00	23.37	3.68	209.50	708.60

Appendix 7.1. Calculated concentrations and activities of main forms of Al at different Ca, Al and P treatment combinations.

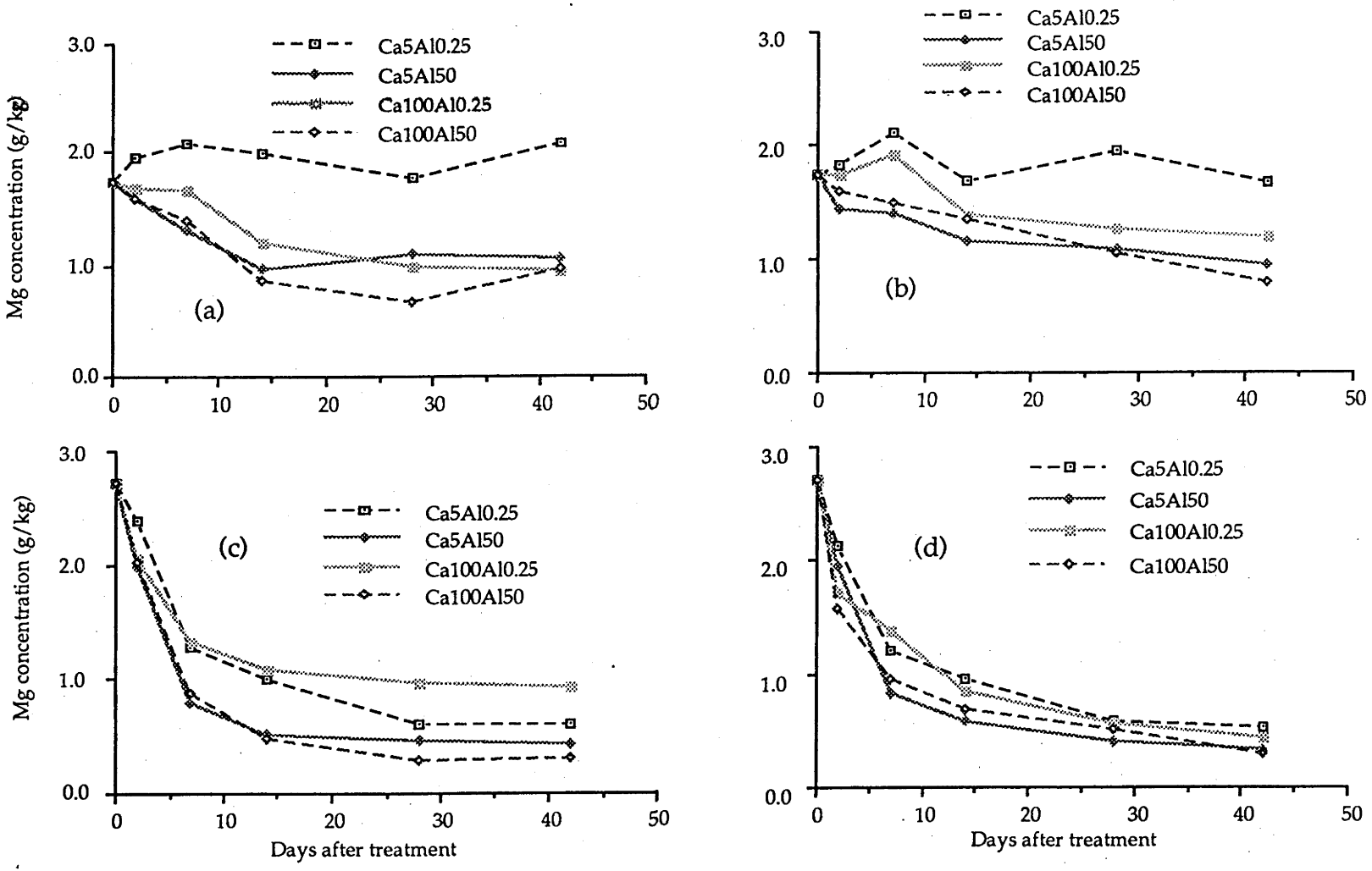
Treatment combination	Concentration (μM)					Activity (μM)				
	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$AlSO_4$	Total monomeric	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$AlSO_4$	Total monomeric
$Ca_5 P_{7.5} Al_{0.25}$	3.96	0.34	0.13	4.87	9.30	1.73	0.23	0.12	4.44	6.52
$Ca_5 P_{7.5} Al_{50}$	1409.00	143.90	62.74	236.00	1852.00	473.40	88.61	55.58	209.10	826.60
$Ca_5 P_{30} Al_{0.25}$	3.47	0.33	0.14	5.37	9.30	1.29	0.21	0.12	4.81	6.43
$Ca_5 P_{30} Al_{50}$	1387.00	153.80	70.79	239.00	1850.00	418.10	90.27	61.96	209.20	779.50
$Ca_{100} P_{7.5} Al_{0.25}$	3.53	0.33	0.14	5.30	9.30	1.33	0.22	0.12	4.76	6.43
$Ca_{100} P_{7.5} Al_{50}$	1390.00	152.80	69.97	238.70	1851.00	423.70	90.12	61.32	209.20	784.30
$Ca_{100} P_{30} Al_{0.25}$	3.16	0.32	0.14	5.68	9.30	1.05	0.20	0.13	5.03	6.41
$Ca_{100} P_{30} Al_{50}$	1371.00	152.80	69.97	238.70	1832.00	379.00	86.29	60.66	206.90	732.90



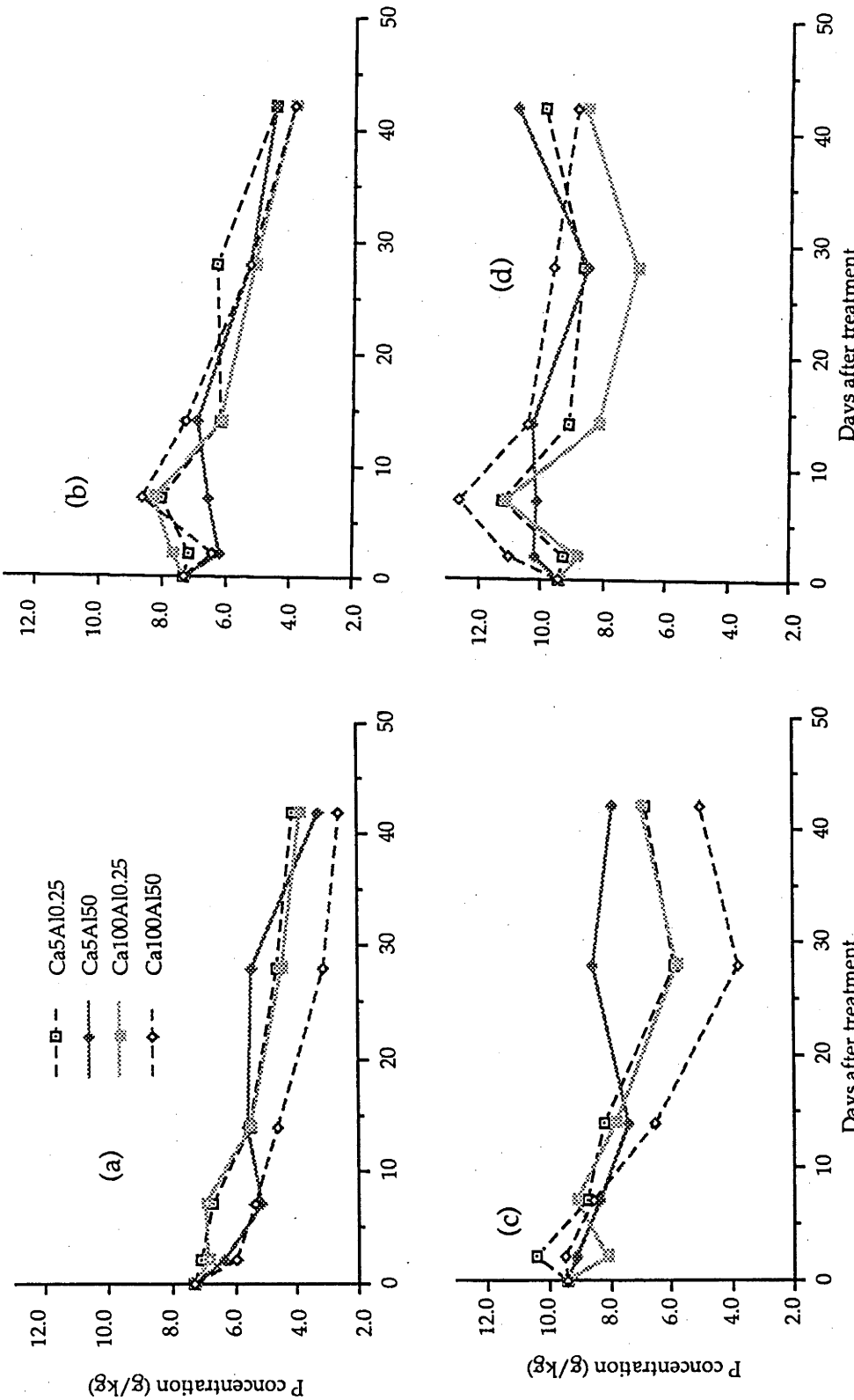
Appendix 7.2. Treatment effects on the change in Ca concentration with time. (a) Shoot Ca at 7.5 mg P/l, (b) shoot Ca at 30 mg P/l, (c) Root Ca at 7.5 mg P/l and (d) Root Ca at 30 mg P/l.



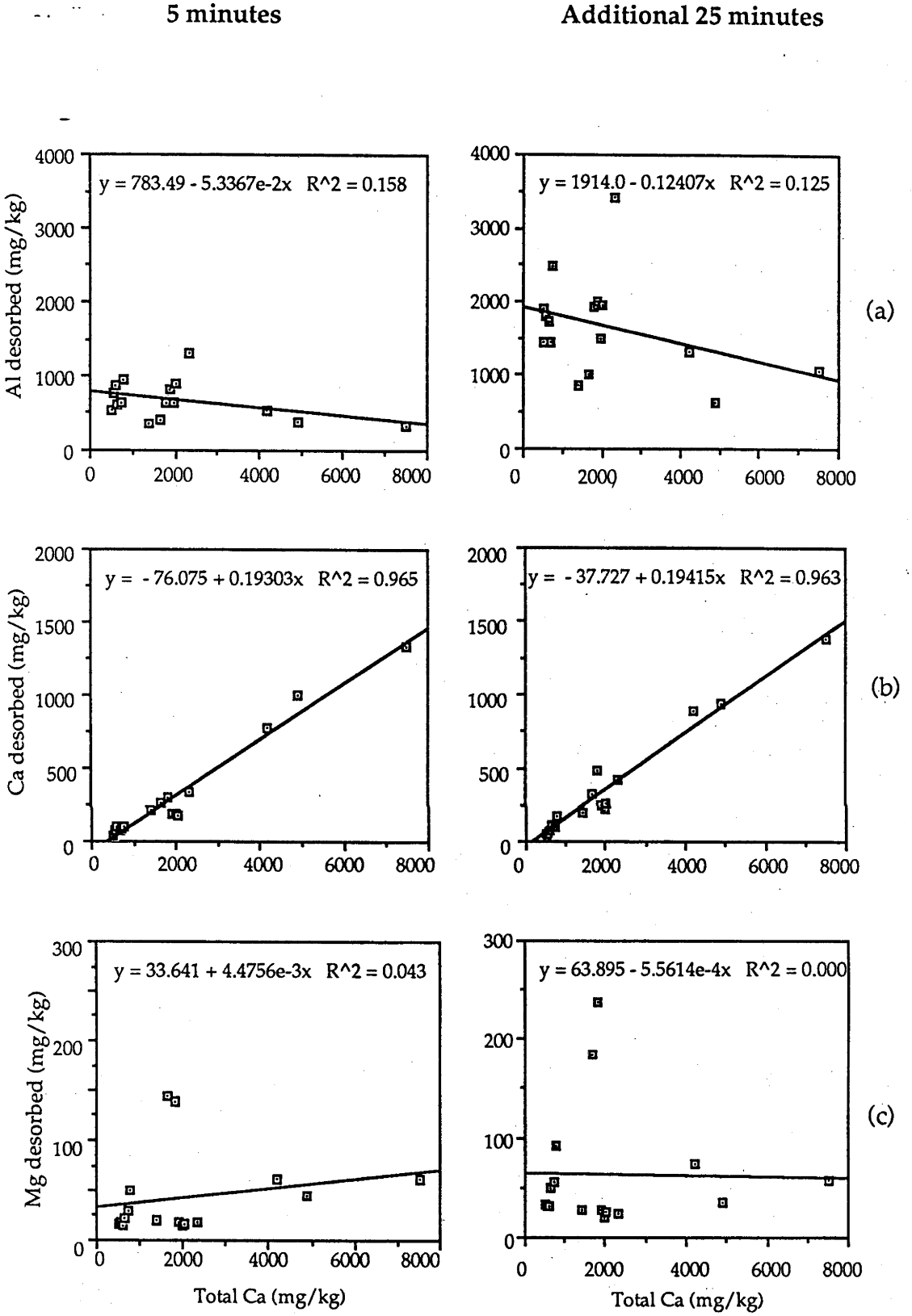
Appendix 7.3. Treatment effects on the change in Al concentration with time. (a) Shoot Al at 7.5 mg P/l, (b) shoot Al at 30 mg P/l, (c) Root Al at 7.5 mg P/l and (d) Root Al at 30 mg P/l.



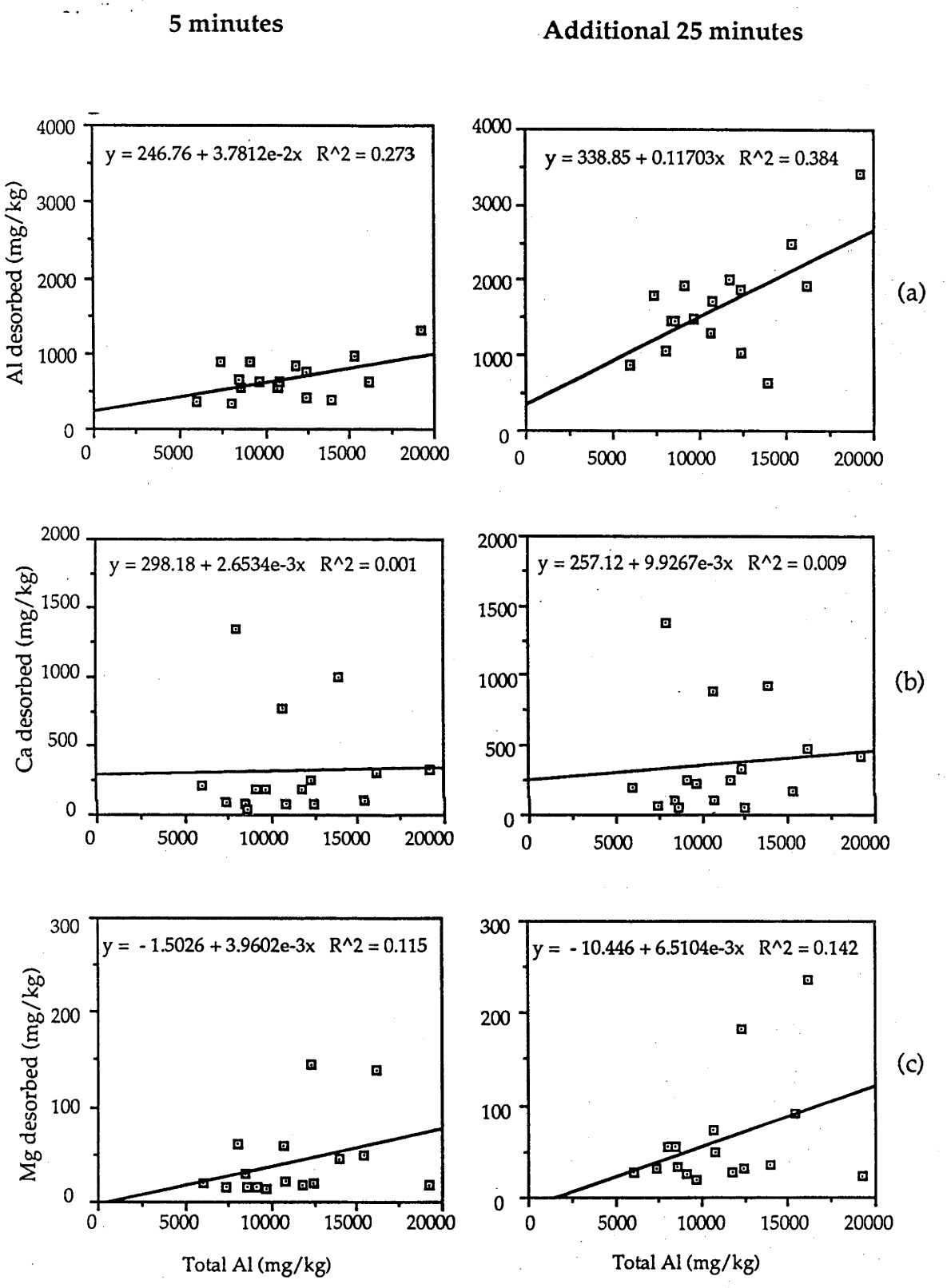
Appendix 7.4. Treatment effects on the change in Mg concentration with time. (a) Shoot Mg at 7.5 mg P/l, (b) shoot Mg at 30 mg P/l, (c) Root Mg at 7.5 mg P/l and (d) Root Mg at 30 mg P/l.



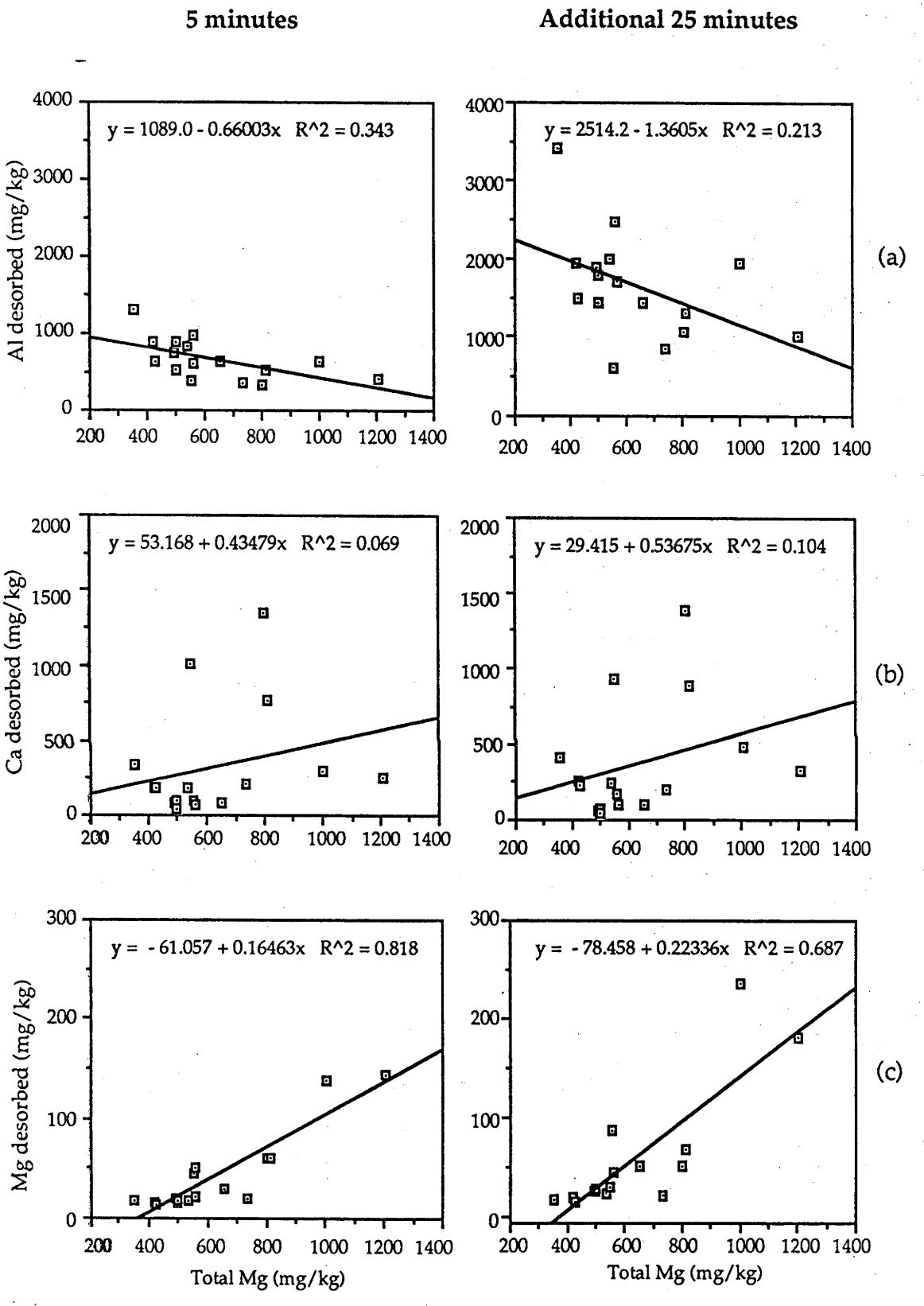
Appendix 7.5. Treatment effects on the change in P concentration with time. (a) Shoot P at 7.5 mg P/l, (b) shoot P at 30 mg P/l, (c) Root P at 7.5 mg P/l and (d) Root P at 30 mg P/l.



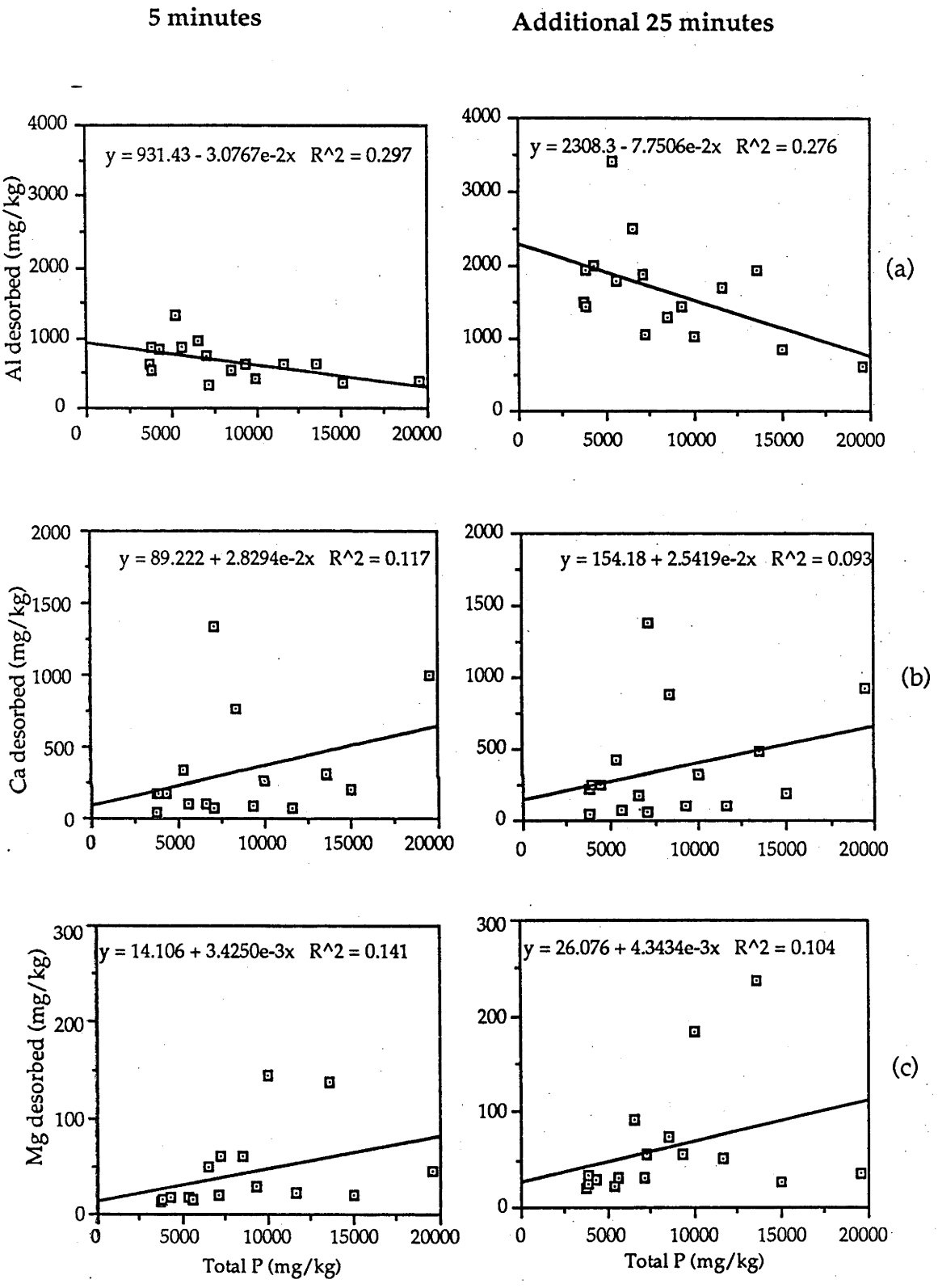
Appendix 8.1. Relationship between total Ca concentration in the root and (a) Aluminium (b) Calcium and (c) Magnesium desorbed in 5 minutes (left hand scatters) and an additional 25 minutes (right hand scatters).



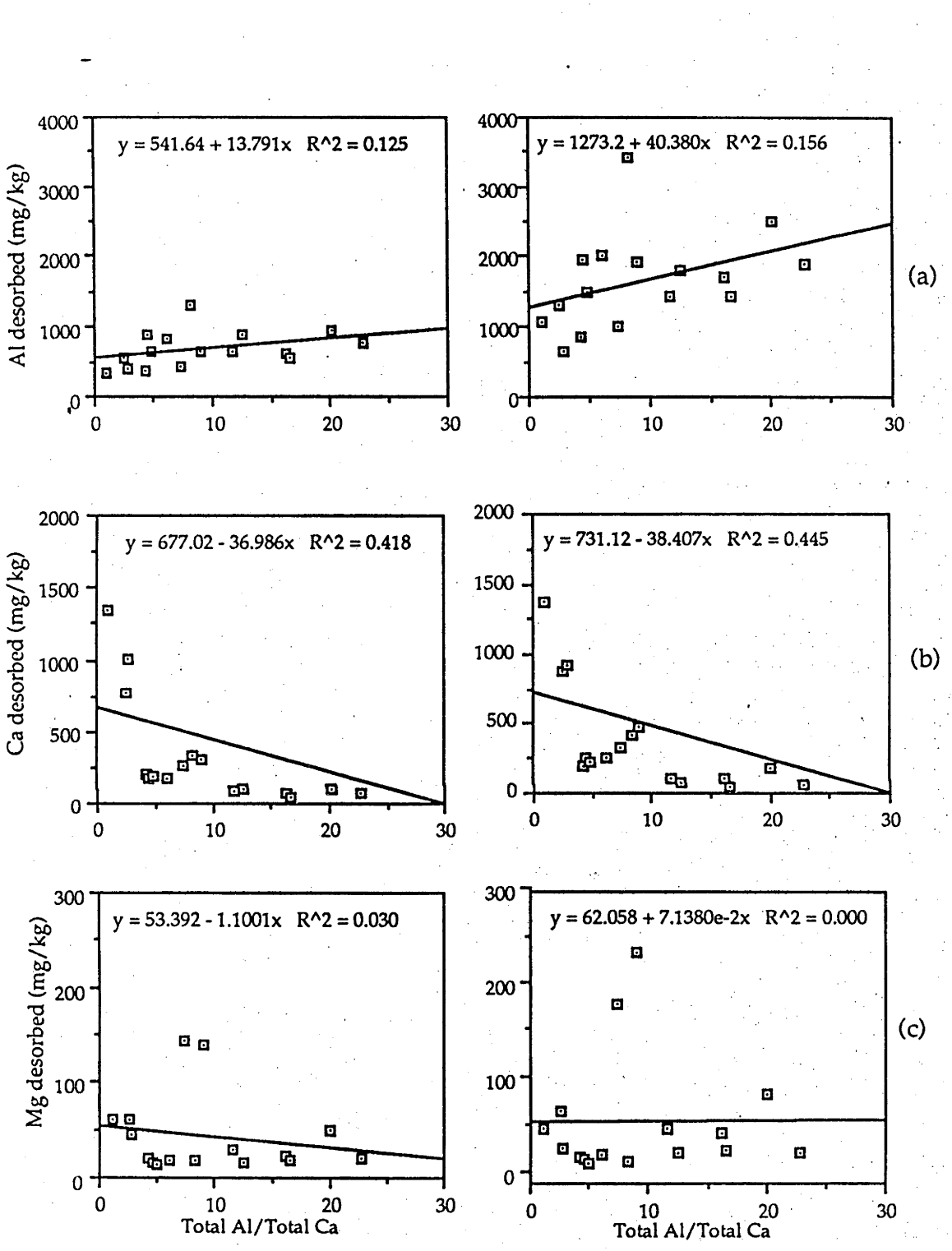
Appendix 8.2. Relationship between total Al concentration in the root and (a) Aluminium (b) Calcium and (c) Magnesium desorbed in 5 minutes (left hand scatters) and an additional 25 minutes (right hand scatters).



Appendix 8.3. Relationship between total Mg concentration in the root and (a) Aluminium (b) Calcium and (c) Magnesium desorbed in 5 minutes (left hand scatters) and an additional 25 minutes (right hand scatters).



Appendix 8.4. Relationship between total P concentration in the root and (a) Aluminium (b) Calcium and (c) Magnesium desorbed in 5 minutes (left hand scatters) and an additional 25 minutes (right hand scatters).



Appendix 8.5. Relationship between total Al/total Ca concentration in the root and (a) Aluminium (b) Calcium and (c) Magnesium desorbed in 5 minutes (left hand scatters) and an additional 25 minutes (right hand scatters).