A STUDY OF SOME FACTORS ASSOCIATED WITH ALUMINIUM UPTAKE BY THREE PLANT SPECIES

by

D.O. HUETT, M.Sc.Agr., SYD.

Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

UNIVERSITY OF TASMANIA

HOBART

AUGUST 1979

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

D.O. HUETT

University of Tasmania,
Hobart.

August, 1979.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

ACKNOWLEDGEMENTS

I wish to sincerely thank my supervisor, Dr. R.C. Menary, Senior Lecturer in Horticultural Science of the Faculty of Agricultural Science, for his advice, constructive criticism and encouragement throughout this project.

I express my thanks to Mr. G. McPherson, Department of Mathematics for his considerable assistance with statistical analyses and to Dr. A.N. McKee and Mr. A.R. Eastgate for assistance with Scanning Electron Microscopy and Energy Dispersive X-ray Microanalyses.

I wish to thank Mr. R.H. Cruickshank for helpful discussion and advice on various aspects of this project, Mr. G. Richardson and Mr. W. Peterson for their technical advice and assistance and Mrs. S. Jones who typed the manuscript.

The backing of the New South Wales Department of Agriculture who provided part salary and study leave is gratefully acknowledged.

Finally, I extend appreciation to my wife, Kay, for her encouragement and assistance throughout the project.

SUMMARY

Growth of many plant species may be limited in acid soils by aluminium excess which may be alleviated by applications of lime (calcitic or dolomitic) and phosphate fertilizers. The nature of the aluminium response is not fully understood because the factors associated with low pH-aluminium excess on plant growth and the processes involved in aluminium uptake are not completely documented. The aim of this project was to examine these factors and provide evidence which would account for aluminium uptake and translocation using three plant species, cabbage (*Brassica oleracea* var. capitata (L.) Alef. cv. Ballhead hybrid), lettuce (*Lactuca sativa* L., cv. Pennlake) and kikuyu (*Pennisetum clandestinum* Chiov. cv. Whittet).

Aluminium uptake by excised roots consisted of two phases, rapid adsorption where most of the calcium was exchanged, followed by a slow accumulation phase that was pronounced for cabbage and lettuce and almost absent for kikuyu. Aluminium uptake in Phase I was considerably higher at pH 4.2 than at 4.0; this could have resulted from a decrease in net charge per aluminium atom, which could be expected at the higher pH. Greater dissociation of carboxyl groups at the higher pH may have also contributed to higher aluminium uptake.

The effect of temperature and a metabolic inhibitor indicated that the entire uptake process was non-metabolic.

Succinic-tartaric acid buffer desorbed most of the aluminium from roots. The small amount remaining was either associated with the cytoplasm and/or irreversibly bound to exchange sites.

EDX-analyses (cell wall region) of freeze-fractured, dried roots from all species demonstrated that aluminium was present in all tissues throughout the epidermis, cortex and stele and along the

entire length of roots. The highest concentrations were recorded in the epidermis followed by the cortex. Aluminium was also recorded in the stele and in the protoplasm of cortical cells for all species. The distribution was consistent with transport in the symplasm where aluminium was present in the radial wall(cytoplasm) of the endodermis and also with passive movement through meristematic cells hence bypassing the barrier at the endodermis. High calcium application reduced aluminium levels in the protoplasm of some xylem parenchyma and cortical cells. There was a poor correlation between aluminium and phosphorus levels in the cell walls of all tissues.

The yield of roots and tops of kikuyu, in contrast to cabbage and lettuce, was relatively unaffected by low pH (4.0 vs. 4.6) and aluminium compared with the yield of control plants. The control treatment level of calcium was markedly lower and the magnesium level markedly higher for kikuyu compared with cabbage and lettuce.

The tolerance of kikuyu to aluminium was not associated with lower aluminium levels of roots than cabbage and lettuce but was associated with significantly lower levels of tops. Aluminium levels of roots were higher at pH 4.6 than 4.0 which was consistent with the excised root results. Results for tops were also consistent for all species where levels were lower at the higher pH. High calcium application had no effect on aluminium levels of roots but reduced levels of tops. This supports the previous results where calcium had little effect on aluminium adsorption during Phase I, but

reduced accumulation during Phase II where passive movement into the cytoplasm and transport to the stele occurs.

High calcium increased the root yield of cabbage and lettuce and reduced top yield of kikuyu. This treatment overcame the inhibitory effect of aluminium on the root and top yield of cabbage and the root yield of lettuce. The magnesium levels of roots and tops were reduced by high calcium for all species.

Aluminium increased phosphate levels of roots for cabbage and kikuyu, but had no consistent effect on levels of tops.

I. INTRODUCTION

I. INTRODUCTION

Plant growth may be limited by excess levels of available aluminium in acid soils such as krasnozems. Tolerance appears to be associated with ecological adaptation where plants derived from acid soils tolerate much higher levels of aluminium than those derived from neutral or alkaline soils. Despite wide differences in aluminium tolerance, all plants absorb and translocate aluminium to tops.

Only limited information is available on the nature of the processes involved in aluminium uptake. Some documentation is available on aluminium adsorption by roots, reaction with phosphate and interaction with cation uptake. The interpretation of results of many of these studies has been confounded by the failure to control pH and nutrient concentration as this often leads to precipitation of aluminium and phosphate in the nutrient solution. The pH of a solution not only controls the solubility of aluminium, but it also controls the ionic species of aluminium and valence of aluminium ions in acid aqueous media. These latter properties have usually been ignored in aluminium studies with plants. The interpretation of the results of aluminium soil studies is far more difficult than that of solution culture studies because where pH adjustments are made to soil, lime is generally used. Hence, in addition to raising soil pH, additional calcium is supplied, thus confounding the interpretation of the pH effect. The solution culture technique has been used exclusively in the present study to effect a control of variables and improve interpretation of results.

Despite the presence of aluminium in plant tops, even when exposed to moderate levels of aluminium where minimal inhibition of plant growth occurs, little attempt has been made to account for the movement of aluminium into the stele of plant roots beyond the adsorption process in free space. A classical method of studying ion uptake utilizes excised roots and this technique was adopted in the present study to elucidate the nature of the aluminium absorption processes. The effect of pH on aluminium uptake was also studied.

The excised root study was complemented by short term whole plant solution culture experiments where pH and nutrient concentration were frequently adjusted to minimise salt precipitation. The aim of these experiments was to extend the interpretation of the aluminium uptake processes by excised roots to whole plants where not only aluminium translocation to plant tops could be measured,but the effects of aluminium on plant growth and interactions with nutrient uptake could be determined.

Cations, particularly calcium, have been shown to play an important role in maintaining selective cell membrane function and there is some evidence that aluminium significantly inhibits calcium uptake. The extrapolation of these effects to account for possible processes by which aluminium moves into the cytoplasm of root cells and subsequent movement into the stele has been ignored in most studies on aluminium uptake. The interaction between aluminium and calcium uptake has been considered in both the present excised root and whole plant studies in the light of this information. The effect of pH on aluminium absorption and translocation was recorded in the whole plant study.

Energy dispersive X-ray (EDX) analyses were used to investigate the distribution of major elements, particularly aluminium and phosphorus in transverse sections of roots. The histology and ultrastructure of tissues affects the radial transport of some ions to the stele, particularly those absorbed non-metabolically. The major barrier to mass flow of ions in roots lies at the extremity of the stele, the endodermis, where secondary and tertiary thickening has been shown to affect this process. While the present study was not concerned with cytology, EDX-analyses allowed inferences to be drawn on the nature of the aluminium uptake processes. Root material for these analyses was obtained at harvest of the whole plant study where a simple rapid method of tissue preparation was required which avoided redistribution of elements during the preparation process.

For all experiments, three species were used: a sub-tropical grass, kikuyu (*Pennisetum clandestinum* Chiov. cv. Whittet), which is well adapted to acid krasnozem soils, and two vegetable crop species, cabbage (*Brassica oleracea* var. capitata (L.) Alef. cv. Ballhead hybrid) and lettuce, (*Lactuca sativa* L. Pennlake), which are susceptible to aluminium and prefer neutral soils. All species are vegetative producers and hence over the short duration of experiments reported in this study, top growth consisted entirely of stem, leaf and petiole.

II. LITERATURE REVIEW

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

II.A. INTRODUCTION

The manifestation of aluminium excess in plants depends on the tolerance of their physiological and biochemical processes. A pre-requisite involves the absorption of aluminium by roots and this can lead to translocation to tops.

Some aspects of the initial aluminium uptake processes by roots have been studied in reasonable depth. However, this work does not adequately explain the uptake processes leading to translocation to plant tops. The behaviour of aluminium in solution is complex due to the effects of pH on solubility, ionic species and reaction with other nutrients, particularly phosphate. The importance of this behaviour has not been fully appreciated in many studies involving aluminium uptake and has confounded the interpretation of plant response to aluminium.

In this review, emphasis will be placed on the interpretation of data which could account for aluminium uptake processes by plant roots. Misinterpretation of data due to the complexity of nutrient interactions in solution, particularly in relation to aluminium uptake, has also been emphasised.

II.B. ALUMINIUM EXCESS IN SOILS

II.B.1. Aluminium Excess

Poor growth of plants on many acid soils has been attributed to aluminium excess (Ahmed 1960; Foy and Brown 1963; Munns 1965a; Foy *et al.* 1967a;Armiger *et al.* 1968; Hutchinson and Hunter 1970; Helyar and Anderson 1971). On other soils, excess levels of plant available aluminium only occur when the soil pH has been reduced below pH 5.0. Awad *et al.* (1976) reported that aluminium excess inhibited the growth of kikuyu grass on a krasnozem where the soil pH had been reduced from 5.0 to 4.4 following four years of continuous heavy nitrogen fertilizer application. The solubility of aluminium increases sharply below pH 5.0 accompanied by an increase in the valence of monomeric aluminium species (McLean 1976).

The displacement of exchangeable aluminium into the soil solution by non-nitrogenous fertilizers can also aggravate the problem (Ragland and Coleman 1962). Aluminium excess is particularly serious in strongly acid subsoils that are difficult to lime resulting in a restricted root system (Adams and Lund 1966) where the only feasible solution is frequent irrigation (Doss and Lund 1975). While aluminium causes injury as a cation in soils, an anionic form causing similar injury has been reported in alkaline fly ash deposits (Jones 1961).

II.B.2. pH and Nutrient Availability

Soil acidity or low pH is the underlying basis of aluminium excess. In some plants, the foliar symptoms of aluminium excess resemble those of phosphorus deficiency and in others, aluminium excess appears as an induced calcium deficiency as a result of reduced calcium transport from roots to shoots (Foy *et al.* 1978).

Stunted and thickened roots have been reported for wheat (Fleming and Foy 1968; Foy *et al.* 1969). In general, young seedlings are more susceptible to aluminium than older plants(Thawornwong and Van Diest 1974).

The ability of lime to alleviate the inhibitory effects of low pH in soils high in aluminium is well-documented (Munns 1965a, c; Helyar and Anderson 1971; Awad *et al.* 1976; Howeler and Cadavid 1976; Awad and Edwards 1977). Awad *et al.* (1976) reported that aluminium concentrations causing severe yield reductions of kikuyu grass were associated with reduced calcium concentrations in tops approaching deficiency levels. Liming reduced the soluble soil and plant aluminium levels (Awad *et al.* 1976; Awad and Edwards 1977). Similarly, Hutchinson and Hunter (1970) and Vickers and Zak (1978) overcame the inhibitory effects of aluminium on plant growth by raising soil pH by lime application .

II.B.2.1. Phosphate application

The precipitation of aluminium with phosphate is a principle used in reducing wastewater phosphate concentrations (Ferguson and King 1977). The same principle applies in soils where low pH - aluminium excess is often associated with phosphorus deficiency in plants (Foy and Brown 1963, 1964; Chiasson 1964). In most acid soils the amount of exchangeable and water scluble aluminium rather than high H^+ concentration and low calcium is the primary problem (Blue and Dantzman 1977). In highly weathered acid soils, phosphate is often extremely deficient and marked improvements in root development result from the application of phosphate. Aluminium is neutralized when soil pH is adjusted to 5.5.

On an acid sandy soil, aluminium excess and phosphorus deficiency in lucerne were overcome by the addition of large

quantities of phosphate (Munns 1965c). Both lime and phosphate lowered the concentrations of aluminium in the soil solution and in plants. Aluminium effects on kikuyu grass growth on a krasnozem soil at pH 4.4 were alleviated by raising soil pH or by application of high rates of phosphate (Awad *et al.* 1976; Awad and Edwards 1977). Both treatments decreased the concentration of soluble soil aluminium on which the concentration of aluminium in plant tops was linearly dependent.

II.B.2.2. Confounding of effects

Several factors are confounded when studying the inhibitory effects of low pH - aluminium excess on plant growth and nutrient uptake in soils. Low pH itself due to the inhibitory effect of high H^+ concentration on plant growth; the increased supply of calcium resulting from lime application; the increase in available soil aluminium resulting from a pH decrease and the reduced levels of available soil phosphate due to aluminium phosphate precipitation resulting from a pH decrease.

There are other nutritional effects that are confounded in the low pH - aluminium excess soil situation. Siman *et al.* (1971) attributed the stunting of French beans on a kraznozem soil below pH 4.8 to manganese excess. Linear relationships were found between plant manganese and both water soluble and exchangeable soil manganese and were reduced by raising pH through lime application. The authors did not examine the possibility of aluminium contributing to the winter stunting problem in beans. The levels of available soil aluminium at similar pH values on a krasnozem soil recorded by Awad *et al.* (1976) and Awad and Edwards (1977) would indicate that aluminium was present in sufficient amounts to inhibit bean growth.

Similarly, Jones and Fox (1978) presented evidence that manganese and aluminium occur concurrently at low pH. These effects were alleviated by high phosphate application. Neenan (1960) also reported manganese and aluminium injury to wheat and barley cultivars on an acid brown loam which could be alleviated by liming. In sand culture, barley was more susceptible to injury from manganese than aluminium and wheat was more susceptible to aluminium than manganese.

II.B.2.3. Phosphatase

The ability of plants to utilize soil phosphate often depends on the activity of acid phosphatases in roots. Bieleski (1971) suggested that low phosphate levels in root zones induced root phosphatase activity and enabled plants to extract phosphate from organic sources in soils. Woolhouse (1969) reported that the phosphatase activity of an acid soil ecotype of *Agrostis tenuis* was inhibited less by aluminium than that of a calcareous soil ecotype. Hence differential phosphatase activity would further confound the interpretation of effects associated with plant response to low pH - aluminium excess, particularly the phosphate effect.

Certain aluminium tolerant wheat cultivars (Fleming 1975) and maize inbreds (Clark 1975) had higher activity of root phosphatases than aluminium sensitive genotypes. Similarly, Bilde (1977) found that root surface acid phosphatase activity of calcifuge ecotypes of *Silene nutans* was higher than that of the calcicolous ecotype. Plants adapted to acid soils where phosphate availability is reduced by reaction with aluminium have therefore overcome this problem by a well-developed root phosphatase system. A significant proportion of insoluble phosphates, including salts of aluminium, occur as organic compounds which can be hydrolysed by phosphatase to produce orthophosphate (Woolhouse 1969, Bilde 1977). Aluminium stimulates root acid phosphatase activity in some aluminium genotypes (Bilde 1977).

II.C. ALUMINIUM EXCESS IN SOLUTION CULTURE

II.C.1. Control of Nutrient Concentration

In solution culture, aluminium phosphate precipitation can be avoided by precise control over nutrient concentrations and pH so that aluminium and pH effects on plant growth and nutrient uptake can be studied without confounding these effects. Munns (1965b) demonstrated that phosphate concentrations above 1μM were adequate for lucerne growth, and if phosphate was kept below 50μM at pH 4.0 or below 10μM at pH 4.5, then aluminium concentrations in the order of 100μM could be obtained without evident reaction between aluminium and phosphate in solution. White (1976) presented solubility product data to indicate that precipitation had been avoided in studies on the interaction between aluminium, phosphate and pH on lucerne growth. Despite clear warnings in the literature on the need for precise control of pH, phosphate and aluminium concentrations, many papers have been published where results have been confounded as a result of aluminium phosphate precipitation. Examples will be presented in the relevant sections of the review.

A major problem associated with nutrient solution experiments is the maintenance of nutrient concentrations and pH at predefined levels. This is particularly critical where very low concentrations are used, hence low intensity and high capacity conditions exist which can be maintained using a high volume of nutrient solution per plant. Munns (1965b) used 20ℓ nutrient solution per 20-24 plants and regularly adjusted phosphate, aluminium and pH to keep them close to nominal values. An improved method for controlling the ionic environment of plant roots was presented by Asher *et al.* (1965). This was achieved by having a high volume of nutrient solution per plant (275ℓ per 256 plants), continuously recirculating

the nutrient solution and continually readjusting nutrient concentration and pH to nominal values. For experiments on phosphate uptake at very low concentrations, the volume of nutrient solution per plant was increased (2800 ℓ per 256 plants) and all species tested made appreciable growth at 0.2μM phosphate (Asher and Loneragan 1967). Because of the size of a continuous flow through system, it is restricted to a glasshouse where the degree of environmental control depends on the sophistication of the equipment available. Where growth chambers are available and hence precise environmental control can be achieved, limited space results in a need to use relatively smaller nutrient solution volumes. This would be suitable for short term experiments using young seedlings where frequent adjustments to nutrient concentration and pH can be made.

Modifications of this technique were used by Kerridge *et al.* (1971), Howeler and Cadavid (1976), Mugwira *et al.* (1976) and Rhue and Grogan (1977) where the response of young seedlings to aluminium in a complete nutrient solution was measured after exposures ranging from 12 to 24 days. Small numbers of seedlings were used in each experiment and hence insufficient plant material was available for the determination of nutrient concentrations on plant material. Kerridge *et al.* (1971) were the only authors to maintain pH, aluminium and phosphate concentrations within the range suggested by Munns (1965b) to avoid aluminium phosphate precipitation. Mugwira *et al.* (1976) grew plants in 10μM phosphate and 220μM aluminium at pH 4.8, exceeding the solubility product. Rhue and Grogan (1977) grew plants in 100μM phosphate and 125μM aluminium at pH 4.6 which also exceeded the solubility product. In both studies, no adjustment to pH or nutrient concentration was

made and this would have enhanced aluminium phosphate precipitation. Howeler and Cadavid (1976) used 130μM phosphate and two aluminium treatments of 110 and 1100μM at pH 4.0, the latter aluminium concentration greatly exceeding the solubility product.

Root growth appears to be the most sensitive indicator of aluminium excess (Kerridge *et al.* 1971). Moore (1974) modified the experimental procedure of these authors to evaluate the tolerance of wheat cultivars to aluminium by measuring root elongation. Wheat plants were started in an aluminium-free nutrient solution until the root length was 3-5cm. The plants were then transferred to identical nutrient solutions containing aluminium but free of phosphate for 48 hours. The length of the primary root was recorded and plants returned to their original aluminium-free solutions where the length of the primary root during the recovery period was used as an indicator of the tolerance of species to aluminium. Moore (1974) found this technique to be very sensitive since irreversible aluminium damage could be readily evaluated. Clarkson (1965) and Fleming and Foy (1968) had shown that primary roots did not recover when exposed to excess levels of aluminium. This technique has recently been used by Henning (1975) and Rhue (1976) to examine the tolerance of wheat cultivars to aluminium.

II.C.2. Low pH

Arnon and Johnson (1942) reported that roots of bermuda grass, tomato and lettuce failed to grow in a nutrient solution at pH 3 and soon became necrotic. Maximum root growth of bermuda grass occurred at pH 4 whereas tomato and lettuce root growth was about half that at pH 6. Calcium additions resulted in a substantial improvement in growth which was not evident at pH 6 suggesting that calcium may offset the deleterious effects of H^+ excess. In contrast, Kerridge

(1969) found negligible differences in root weight between wheat cultivars when nutrient solution pH was reduced from 5.0 to 4.0.

In solution culture where nutrients are readily available, pH over the range of 4-8 had little effect on calcium, magnesium, potassium, phosphate and nitrogen uptake by tomato, lettuce and bermuda grass (Arnon *et al.* 1942). In short-term uptake studies with excised roots, cation uptake is sharply reduced below pH 5. This effect has been recorded for potassium (Fawzy *et al.* 1954; Nielsen and Overstreet 1955; Jacobson *et al.* 1957; Murphy 1959) for lithium, sodium, rudibium and calcium (Jacobson *et al.* 1960), for manganese (Maas *et al.* 1968) and for calcium (Maas 1969).

The inhibitory effect of low pH on cation absorption is mainly associated with H^+ antagonism. Anion absorption is relatively less affected by H^+ but more strongly affected by OH^- where Jacobson *et al.* (1957) reported that bromide uptake by barley roots was maximal at pH 5 and declined steadily as the pH was increased to 10.5. Bromide uptake decreased below pH 5, but not to the same extent as potassium uptake. Maas (1969) reported similar results for chloride uptake by maize roots in comparison with calcium uptake.

Calcium and other polyvalent cations apparently maintain the integrity of ion absorption, especially in the acid pH range. These cations strongly stimulated potassium absorption by excised barley roots below pH 6 (Viets 1944; Fawzy *et al.* 1954; Jacobson *et al.* 1960). Hence, calcium appeared to decrease the competitive effects of H^+ on absorption. In addition to this effect, calcium is probably the most important polyvalent cation in maintaining the integrity of the absorption mechanism (Epstein 1961; Jacobson *et al.* 1961; Rains *et al.* 1964).

In addition to its competitive effects on ion absorption, damage to roots caused by H^+ excess is generally manifested by a loss of nutrients which suggests an increase in cell membrane permeability. Significant losses of potassium from roots exposed to low pH in short-term experiments have been reported (Fawzy *et al.* 1954; Nielson and Overstreet 1955; Jacobson *et al.* 1957, 1960). Similar results were reported for magnesium (Moore *et al.* 1961a) and calcium (Jacobson *et al.* 1950; Moore *et al.* 1961b). Hence independent treatments examining both the pH effect and aluminium effect are required in solution culture experiments.

II.C.3. Aluminium Species in Acid Aqueous Media

The full significance of the effect of pH on aluminium reaction at low pH and its subsequent effect on aluminium uptake have been ignored in most studies. Moore (1974) reported that the inhibition of root elongation caused by a particular aluminium concentration to a wheat cultivar sensitive to aluminium and to those of a moderately tolerant cultivar increased as the pH of the solution increased from 4.0 up to the pH at which aluminium was no longer soluble. He suggested that aluminium injury was probably caused by a hydrolysed form of aluminium rather than Al^{3+}. Moore's paper omitted to recognise the behaviour of aluminium in solution as detailed by Hem (1968) who showed that over the pH range 4.5 to 6.5, hydrated aluminium monomers exist which polymerize, particularly at higher pH, forming gibbsite crystals. The subject was more thoroughly investigated by Smith (1971) who confirmed and extended Hem's results by showing that in solution, aluminium hydroxy complexes occur, composed of monomeric species of valence 1-3, as well as polynuclear species and solid particles of gibbsite.

The monomeric species can be simply represented by Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$ and $Al(OH)_4^-$ although it is likely that they become more complex as the solution ages. Polynuclear aluminium hydroxide probably consists of a six-membered ring structure in which each aluminium is bonded to its neighbour through shared pair of OH^-. The individual rings tend to coalesce into larger structures with time until they ultimately become large enough to be filtered out and identified by electron microscopy and X-ray diffraction as gibbsite crystals. The manner in which the rings coalesce appears to be governed by a first order rate law relative to polynuclear aluminium material. Polynuclear aluminium particles appear to range in size from around $Al_{24}(OH)_{60}^{12+}$ to $Al_{96}(OH)_{264}^{24+}$ and perhaps larger. The mean net charge density per aluminium atom decreases as the pH increases (Hsu and Bates 1964; Smith 1971).

Nair and Prenzel (1978) calculated that the relative amounts of aluminium species existing at a given pH was dependent on total aluminium concentration with the polynuclear ions, $Al_7(OH)_{17}^{4+}$ and $Al_{13}(OH)_{34}^{5+}$ predominating at aluminium concentrations as low as $10^{-4.5}$M. At an aluminium concentration of 10^{-6}M, Al^{3+} is predominant up to pH 4 while its predominance is only up to pH 3 at 10^{-3}M. The 'neutral species', $Al(OH)_3$ readily forms above pH 4 at a total aluminium concentration of 10^{-6}M whereas at higher concentrations, higher pH's are required for its formation.

II.C.4. Effect of Aluminium on Phosphate Uptake

II.C.4.1. Inhibition

Under the conditions described by Munns (1965b), uncomplicated by precipitation or phosphate deficiency in the nutrient solution, aluminium excess depressed yields, root elongation and calcium and

phosphorus concentrations in shoots and roots, and it made the shoots look phosphorus deficient, but it could not be remedied by increasing phosphate supply even when this restored plant phosphorus to high levels. Andrew *et al.* (1973) found that aluminium reduced the phosphorus levels in roots and tops of sensitive species; in some tolerant species the intermediate aluminium treatment increased the phosphorus concentration in plant tops; however, the high aluminium treatment reduced the phosphorus concentration. Similarly, Clarkson (1966a) recorded phosphorus deficiency symptoms in shoots of three *Agrostis* species moderately and highly susceptible to aluminium excess.

The precise nature of the aluminium induced phosphorus deficiency has been extensively studied. Wright (1943) proposed that aluminium caused internal precipitation of phosphate in roots as it could not be removed by a dilute sulphuric acid rinse. Wright and Donahue (1953) showed that aluminium reduced ^{32}P translocation to barley tops and caused accumulation in roots. The latter could only be desorbed with 0.05M sulphuric acid and the authors concluded that much of the phosphate was internal to the root. In a similar study conducted by Wallihan (1948) using ladino clover, aluminium and phosphate accumulated in roots but the concentration in tops was not reduced and he concluded from desorption studies that aluminium and perhaps phosphate were held to root surfaces by a mechanism such as ionic exchange. Macleod and Jackson (1965) grew several plant species in a nutrient solution containing aluminium and phosphate at a pH exceeding the solubility product of Munns (1965b) and found that both aluminium and phosphate accumulated in roots. The accumulation process would have been enhanced by a precipitation reaction in nutrient solution. However, where aluminium and phosphate concentrations and pH were strictly controlled, Andrew and Vandenberg

(1973) found that aluminium increased phosphate sorption by a range of tropical legume species. Many studies avoid precipitation of aluminium and phosphate in the nutrient or absorption solution by exposing roots separately to each of the nutrients, both at higher concentrations than could be used in a combined nutrient solution within the physiological range, and hence have questionable value. Under these conditions high concentrations of aluminium and phosphate accumulate in roots (Wright 1943; Wright and Donahue 1953). Ragland and Coleman (1962) reported that aluminium stimulated phosphate uptake by excised bean roots with both an aluminium pretreatment and aluminium in the presence of phosphate. Uptake was linear for short periods (5 min) only, hence they concluded that phosphate accumulated in free space. Andrew and Vandenberg (1973) found that an aluminium pretreatment significantly enhanced phosphate sorption by a wide range of tropical species.

The site of the aluminium enhanced phosphate uptake was demonstrated by Clarkson (1967) who reported that aluminium pretreated isolated cell wall material of barley roots adsorbed appreciable quantities of phosphate which was completely exchangeable. Clarkson (1966b) similarly found that aluminium pretreatment increased the rate of phosphate accumulation by barley roots as inorganic phosphate which was completely exchangeable. White (1976) also found that aluminium substantially increased phosphate uptake by lucerne roots; 70% of which could be extracted with 0.1M $HClO_4$ after a 15 min wash. The phosphate remaining in the root was taken to represent metabolically-accumulated phosphate. The aluminium treatments reduced this fraction as well as inhibiting phosphate translocation to tops. As discussed previously, H^+ excess leads to plasmalemma damage and hence a severe treatment such as 0.1M $HClO_4$ would lead to

leakage of metabolically accumulated phosphate out of roots. The commonly reported effect of stimulated phosphate uptake in the presence of aluminium is misleading, as shown by White (1976) where aluminium,which enhanced total phosphate uptake by roots, inhibited phosphate absorption across the plasmalemma of root cells and subsequent translocation to tops. The formation of alumino-phosphate complexes was maximal at around pH 5 (White *et al.* 1976) and the low net charge density led to higher aluminium absorption by roots and greater amounts translocated to tops than at pH 4.5 (White 1976). The inhibitory effect on plant growth was greater at the lower pH in contrast to the results of Moore (1974). Irrespective of the effects of aluminium on phosphate uptake,inhibition of root growth by aluminium (Morimura and Matsumoto 1978) was due to the inhibition of cell division and not phosphorus deficiency (Matsumoto and Hirasawa 1979).

II.C.4.2. Stimulation

There is evidence that for some species adapted to acid soils, aluminium stimulates growth and phosphate translocation. Mullette *et al.* (1974) reported that *Eucalyptus gummifera*, which grows on highly weathered, low phosphate acid sandstone soils, showed a marked growth response to aluminium and iron phosphates. They proposed a model which involves Fe^{3+} and Al^{3+} blocking the negative sites on the cell wall, thus enhancing phosphate absorption across the plasmalemma. A second study by Mullette (1975) showed that *Eucalyptus gummifera* responded to increasing levels of aluminium up to 1.0µg ml^{-1} in the presence of varying phosphate concentrations. Enhanced growth in the presence of aluminium has also been reported for sweet potato, taro, ginger and soybean (Guratilaka *et al.* 1977).

Totev (1977) found that unlike lucerne and clover, growth of timothy was stimulated by additions of aluminium and manganese. Andrew *et al.* (1973) similarly found that an intermediate aluminium treatment increased phosphorus concentrations in the tops of aluminium-tolerant tropical legume species.

Kumar (1979) reported that aluminium concentrations of 8-16μg ml^{-1} significantly increased shoot phosphorus concentrations. He concluded that aluminium had mobilised phosphate from root to shoot as corresponding root phosphorus concentrations were significantly reduced by aluminium. This interpretation cannot be fully accepted as the corresponding shoot dry weights decreased in the presence of aluminium so that the total amount of phosphate translocated to tops remained relatively constant for all treatments except at 4μg ml^{-1} aluminium where total phosphate translocated to tops increased. Both root and shoot dry weight increased with this treatment which represents a similar situation to that reported by Mullette (1975) where optimum yield and phosphate uptake were recorded at a specific level of aluminium. Kumar (1979) omitted to compare his data to that of Mullette *et al.* (1974) and Mullette (1975) and hence failed to fully appreciate the nature of the aluminium stimulation of phosphate uptake.

A mechanism by which aluminium stimulates phosphate incorporation into roots would have to be specific to species such as *Eucalyptus gummifera*. The model proposed by Mullette *et al.* (1974) does not seem plausible as the work of Rorison (1965), Clarkson (1966b, 1967) and Guerrier (1978) indicates that the screening of negative sites in the free space of roots by aluminium is universal to all species. In addition, the model does not take into account the ability of plants such as lucerne, which is aluminium-sensitive, to absorb aluminium phosphate as a complex polymer (White 1976; White *et al.* 1976).

Nissen (1977) reviewed the models presented in the literature to account for the complex kinetics of ion uptake by higher plants and presented substantial evidence that the concept was consistent with multiphasic uptake mechanisms. KCl stimulation of plasmalemma-bound ATPases was shown to obey multiphasic kinetics, thus strengthening the correlation between ion uptake and membrane bound ATPases. Klimashevskii and Bernatskaya (1973) reported a greater increase in ATPase activity of aluminium-tolerant than aluminium-sensitive pea cultivars and this may account for stimulated phosphate absorption and subsequent translocation recorded for *Eucalyptus gummifera* by Mullette *et al.* (1974) and Mullette (1975).

II.C.5. Effect of Aluminium on Calcium Uptake

Adjustments to the pH of nutrient solution cultures are made with either dilute acid or alkali, hence the pH-calcium confounding that occurs in soil experiments following lime application is avoided. Chamura (1967) was able to demonstrate that the growth of Italian ryegrass and vetch was depressed by low pH, low calcium and added aluminium to the nutrient solution. The inhibitory effect of aluminium on calcium uptake and translocation is well-documented. Andrew *et al.* (1973) reported that aluminium reduced the calcium levels in tops of a range of tropical and temperate legumes with differential tolerance to aluminium. Kotze *et al.* (1977) found that the efficiency of calcium uptake by roots of apple and translocation to tops was decreased by the presence of aluminium whereas Edwards and Horton (1977) concluded that aluminium toxicity in peach may have been related to a reduction in the calcium uptake rate and not the rate of translocation.

Kotze (1979) confirmed the results of Kotze *et al.* (1977) that aluminium depressed the yield of apple plants with various

combinations of NO_3^- and NH_4^+. The greatest reduction in total yield and calcium uptake by roots occurred when 100% of the nitrogen was supplied as NO_3^-. The fraction of calcium translocated to tops was substantially reduced by this treatment only. A suitable explanation for this response was not available and the literature indicates that this may be an isolated example.

Species tolerance to aluminium is closely related to calcium nutrition. Foy *et al.* (1969) reported that the effects of aluminium excess in soybean was associated with a decrease in the calcium concentrations in roots and tops of both tolerant and susceptible cultivars, but the effect was much more pronounced in susceptible cultivars. Similarly, in bean cultivars, the ability to resist aluminium induced calcium deficiency resulting from reduced calcium uptake by roots was associated with aluminium tolerance (Foy *et al.* 1972).

There is good evidence that the normal calcium levels in plants reflect their ability to tolerate aluminium. *Chlorella pyrenoidosa*, a green alga which has no measurable calcium requirement, tolerated much higher aluminium concentrations in solution than higher plants that require considerable calcium (Foy and Gerloff 1972). Tomato cultivars showing the greatest tolerance to aluminium excess tended to contain lower concentrations of aluminium, calcium and phosphorus in tops than did sensitive cultivars (Foy *et al.* 1973). In contrast, Oullette and Dessureaux (1958) reported that lucerne cultivars tolerant to aluminium contained more calcium than non-tolerant cultivars.

The nature of the aluminium-calcium antagonism was demonstrated by Johnson and Jackson (1964) who studied the time-course of calcium uptake by attached and excised wheat roots. Uptake consisted of an initial rapid adsorption phase followed by a linear rate of accumulation,

both phases being reduced by an aluminium treatment. The reduction in the accumulation phase could not be overcome by supplying additional calcium and transport to the shoots of intact seedlings was also restricted by aluminium although appreciable transport still occurred when root uptake was inhibited completely. Similar results were obtained by Clarkson and Sanderson (1971) who found that the aluminium reduced levels of exchangeable calcium in roots and amounts of calcium transported to the shoots of barley. The authors proposed that the effect of aluminium in restricting calcium entry to the cortex also reduced the amount of calcium available for transport to the stele. The inhibition of calcium uptake caused by 1.4 and 2.8μg ml^{-1} aluminium sulphate could be overcome if the calcium chloride concentration in the absorption solution was raised to 15mM although growth was still inhibited by 50%. Similarly, the inhibitory effect of 0.3μg ml^{-1} aluminium on calcium uptake by cotton was overcome by increasing the calcium concentration of the nutrient solution to 15mM (Lance and Pearson 1969).

Rhue and Grogan (1977) also reported that high calcium concentrations in the nutrient solution reduced the inhibitory effects of aluminium excess on maize inbreds. The ability of calcium to ameliorate these effects varied markedly with the inbred lines. At equal concentrations magnesium was as effective as calcium in protecting maize roots from aluminium excess. Ali (1973) obtained similar results for wheat cultivars and found that potassium and sodium were also effective in overcoming the effects of aluminium excess. The non-specific effect of high cation concentration alleviating heavy metal excess in plants is not restricted to aluminium. Osawa and Ikeda (1979) found that both potassium and calcium overcame the inhibitory effects of zinc on the growth of eight species of vegetable crops.

Clarkson and Sanderson (1971) required a minimum calcium/aluminium ratio of 215/1 to restore calcium concentrations in barley roots when growth was still inhibited by aluminium whereas Khue and Grogan (1977) overcame the inhibitory effects of aluminium on the root growth of most wheat cultivars by a calcium/aluminium ratio of 12/1. A calcium/aluminium ratio of 20/1 had no effect in ameliorating the inhibitory effects of aluminium on yields and phosphate uptake by lucerne (White 1976). The large differences reported in the literature in the calcium/aluminium ratio required to overcome the inhibitory effects of aluminium on calcium uptake and plant growth require further investigation, particularly with a range of species with differential aluminium tolerance.

Wallace *et al.* (1966) investigated aspects of the role of calcium in higher plants. They showed that plants accumulated considerably higher levels than needed to maintain normal metabolic function. The residual calcium buffered plants against heavy metal excess. The fact that the initial reaction between calcium and aluminium in roots involves ionic exchange, confirms the buffering effect of calcium in ameliorating the effects of aluminium excess. However, this does not explain the aluminium tolerance of plants having a low calcium requirement. Ultimate control of aluminium injury and absorption by root cells could lie with the plasmalemma.

II.C.6. Differential Ion Uptake and pH Change

Plant tissues are required to maintain electrical neutrality and cation-anion balance for normal metabolic function (Moore 1974). The net result of excess cation absorption is the net release of H^+ from the root, while the result of a net excess of anion absorption is the release of OH^- or HCO_3^-. On the basis of measurements of H^+ fluxes and cation/anion balance during salt accumulation Jackson

and Adams (1963) suggested that H^+ efflux and OH^- efflux could be driving forces for cation and anion uptake respectively. The pH changes recorded by Hoagland and Broyer (1940) and Dodge and Hiatt (1972) when plants were grown in a complete nutrient solution were attributed to differential cation and anion uptake.

The effect of nutrient absorption on the resultant pH of a salt solution depends on the differential rate of cation and anion absorption. Monovalent cations, generally are absorbed rapidly (Jacobson *et al.* 1960), whereas divalent cations, particularly calcium, are more slowly absorbed (Maas 1969; Moore *et al.* 1961a, b). The moncvalent anions, generally are absorbed more rapidly than polyvalent anions (Hagen and Hopkins 1955; Leggett and Epstein 1956; Jacobson *et al.* 1957). Pitman (1970) reported that H^+ efflux from barley roots in K_2SO_4 solutions was about twice as rapid as from roots in KCl solutions. This indicates that K^+ is absorbed more rapidly than Cl^-. The pH of a $CaCl_2$ solution increased during nutrient absorption (Hiatt 1967) while little change in the pH of $CaSO_4$ was recorded (Pitman 1970).

The problem of pH drift in nutrient solution culture experiments is accentuated when NO_3^- is the sole source of nitrogen. Dodge and Hiatt (1972) found that under these conditions, the pH of the nutrient solution consistently increased from the initial level. However, solution pH decreased when NH_4^+ was present in concentrations as low as 0.5% of the total nitrogen. The pH of the system controls the distribution of ammoniacal nitrogen between the NH_4^+ form and the NH_3 form. The latter is quite toxic to roots (Warren 1962; Colliver and Welch 1970), apparently because it is a neutral molecule and can readily penetrate cell membranes.

Many plants that are adapted to acid soils and hence tolerate aluminium, also tolerate NH_4^+ in concentrations that inhibit growth of other plants. Greidanus *et al.* (1972) found that aluminium-tolerant cranberry plants absorbed NH_4^+ preferentially to NO_3^- and when grown with the latter as the sole source of nitrogen, were nitrogen deficient. Nitrate reductase activity was absent from the shoots. Other species that prefer NH_4^+ to NO_3^- are sugar cane, blueberry and certain grasses such as *Paspalum notatum* and *Lolium rigidum* (Townsend and Blatt 1966; Wiltshire 1973; Presad 1976). Species that do not tolerate acid soils such as lima bean, consistently produced higher dry weights when NO_3^- was 75% of the total nitrogen supplied (McElhannon and Mills 1978).

The form of nitrogen preferred by plant species is not always associated with acid tolerance and this complicates the design of nutrient solution experiments, particularly in relation to pH control. Havill *et al.* (1974) reported that certain calcifuge species, notably members of the Ericaceae, had low nitrate reductase activity and limited ability to utilize nitrate. Other calcifuge species and all species from calcareous soils had detectable nitrate reductase activity and responded to nitrate addition by large increases in enzyme activities. Gigon and Rorison (1972) noted that among a wide ecological range of herbaceous species, some calcifuge species grew better when nitrogen was available as NH_4^+, some calcicoles grew better when it was available as NO_3^- and the growth of widely-distributed species showed tolerance to either form. There was no indication that calcifuge species lacked a nitrate reductase system. The apparent disagreement between Havill *et al.* (1974) and Gigon and Rorison (1972) suggests that over the whole ecological range of plant species; one extreme can be represented by plants that tolerate low pH have an ineffective

nitrate reductase system and require NH_4^+ as the major nitrogen source; at the other extreme, plants have the opposite requirements and in between these extremes plants have a range of requirements.

The interaction between plant species, ion uptake and H^+-OH^-(HCO_3^-) extrusion emphasises the need for frequent adjustments to the pH of the nutrient solution for whole plant studies. This is particularly important where aluminium is present in the nutrient solution as large upward changes in pH will lead to either the precipitation of aluminium as gibbsite (Smith 1971) where phosphate is not present in the nutrient solution, or precipitation of aluminium with phosphate where it is present (Munns 1965b).

II.D. ALUMINIUM UPTAKE

II.D.1. Uptake Processes

The nature of aluminium uptake by excised barley roots was studied by Clarkson (1967) who showed an initial rapid absorption phase after which little additional uptake occurred. Kinetics were similar at either 23°C or 3°C and at either temperature most of the aluminium was recovered in the cell wall fraction indicating non-metabolic uptake. Confirmation was provided by the similarity in aluminium uptake between excised roots and cell wall material and once bound it was not readily exchanged by calcium or sodium. Cell wall material pretreated with aluminium was able to absorb appreciable amounts of phosphate, almost all of which was completely exchangeable. Clarkson (1967) proposed that free carboxyl groups of polygalacturonic acid chains in the middle lamella were the most likely sites of aluminium adsorption. Matsumoto *et al.* (1977) investigated the possibility of adsorbed aluminium being associated with pectin in pea roots and observed no distinct association after gel filtration of the pectinase-digested cell wall material.

Clarkson and Sanderson (1969),using ^{46}Sc as a tracer for aluminium, described uptake by attached barley roots as consisting of two phases; superficial adsorption that was characterised by rapid initial uptake and was unaffected by low temperature; the second phase was slower but remained constant for 24 hours and was highly dependent on temperature. The amount of isotope associated with dividing cells increased steadily over a six hour period and possibly represented Phase II uptake. The primary endodermis restricted the entry of scandium into the stele at a very early stage in its development. Clarkson and Sanderson (1969) concluded

that migration of the ion across the root was primarily in free space. The exchange of calcium in free space by aluminium and scandium (Clarkson and Sanderson 1971) confirmed that Phase I aluminium (scandium) uptake involved exchange-adsorption. Rorison (1965) also reported that aluminium uptake by excised sanfoin roots was into free space and was almost completely exchangeable with a dilute organic acid buffer.

More recently, Guerrier (1978) studied aluminium uptake by attached roots of broad bean (aluminium-susceptible) and yellow lupin (aluminium-tolerant) and described the time-course of aluminium uptake as consisting of an initial rapid passive phase during which the former species absorbed four times as much aluminium as the latter species. Broad bean continued to accumulate aluminium beyond this phase at a much faster rate than lupin. The amount of aluminium accumulated during the latter phase was proportional to the external concentration. Aluminium exchanged divalent cations (calcium, magnesium) and monovalent cations (potassium) during both uptake phases. Guerrier (1978) made no attempt to interpret the processes involved in aluminium uptake beyond that already stated and made no reference to the work of Clarkson (1967) and Clarkson and Sanderson (1969, 1971) who had shown that Phase I uptake consisted of exchange-adsorption in free space and Phase II uptake represented transport through free space and into the meristematic zone of roots. There was universal agreement on the effect of aluminium in exchanging calcium from roots but this wasn't discussed in the light of the importance of calcium in maintaining normal cell membrane function (Viets 1944; Epstein 1961).

Guerrier (1978) demonstrated that the second phase of aluminium uptake was linear with time for both lupin and broad bean and this suggests a possible active component. Clarkson and Sanderson (1969) demonstrated that this phase for barley was dependent on temperature but involved passive movement in free space. Further work is required to separate the aluminium uptake processes and to determine whether there is any dependence on metabolism. The use of a synthetic cation-exchange resin would characterise the exchange-adsorption process and assist in the interpretation of these results.

Henning (1975) elucidated aluminium uptake during Phase II by sequentially treating roots with dyes and showed that aluminium absorbed by wheat roots penetrated the boundary between the root apex and root cap and accumulated in meristematic cells and adjacent cells of the central cylinder. Hence, the barrier at the endodermis, which prevented radial aluminium movement from the cortex to the stele, was bypassed by transport into the central cylinder from the root apex. Henning also found that aluminium penetrated the plasmalemma of both sensitive and tolerant wheat cultivars, provided the concentration used for the latter was 100-200 times that used for the former. From this evidence he concluded that aluminium tolerance in wheat was due to aluminium exclusion at the root plasmalemma and that cultivar differences in aluminium tolerance were due to differences in the molecular structure of this membrane. Rhue (1976) also showed that aluminium uptake involved passive movement across the plasmalemma and was supported by Klimashevskii *et al.* (1976) who reported that disrupted membrane permeability caused greater aluminium accumulation in sensitive than tolerant pea cultivars.

There is a weight of evidence to support passive movement of aluminium across the plasmalemma, coinciding with absorption during Phase II. The few studies examining the nature of the aluminium uptake processes have made little attempt to identify all the steps involved and this is particularly evident in the work of Guerrier (1978). Additional data are required to elucidate these processes, preferably with a range of species with differential aluminium tolerance.

II.D.2. Interaction of Aluminium and Calcium on Membrane Function

Simon (1978) reviewed the symptoms of calcium deficiency where tissues become water-soaked as a result of cell breakdown and loss of turgor. This apparently involves increased membrane permeability which would account for a loss of turgor and permit cell fluids to invade intercellular spaces. Van Steveninck (1965) reported that beetroot storage tissue became leaky when EDTA (ethylenediaminetetraacetic acid) removed 69-76% of the calcium present in tissue.

Calcium performs an essential role in maintaining selective ion absorption by roots (Viets 1944; Epstein 1961). This role is non-specific as other divalent and polyvalent cations can replace calcium, but generally less efficiently. Aluminium reduces the adsorption phase of calcium uptake and transport to shoots (Johnson and Jackson 1964; Clarkson and Sanderson 1971). Hence it follows that an aluminium treatment would eventually lead to a disruption of normal membrane function and allow passive movement of aluminium into the protoplasm as proposed by Henning (1975), Klimashevskii *et al.* (1976) and Rhue (1976).

II.D.3. Aluminium Effects on Phosphate Uptake and Metabolism

Clarkson (1967) reported that cell wall material and roots of barley pretreated with aluminium absorbed appreciable quantities of phosphate which was completely exchangeable. Rorison (1965) also reported aluminium uptake into root free space of sanfoin which was almost completely exchangeable with dilute buffer. Subsequent treatment of roots with ^{32}P indicated that aluminium inhibits phosphorylation, either by binding phosphate in Donnan Free Space, hence reducing the amount able to enter the protoplasm, or by interfering with sites of esterification. Clarkson (1966b) similarly found that an aluminium pretreatment increased the rate of phosphate accumulation by barley roots as inorganic phosphate that was completely exchangeable. The aluminium treatment markedly decreased the incorporation of ^{32}P into sugar phosphates but increased the pool size of ATP and other nucleotide triphosphates present in roots. Preliminary results indicated that aluminium inhibits hexokinase, thus blocking sugar phosphorylation. Matsumoto and Hirasawa (1979) using pea, found no evidence to support the results of Rorison (1965) and Clarkson (1966b)which indicated that aluminium effects on phosphate esterification vary with species.

Subsequent transport of phosphate to shoots appears to depend on prior incorporation into organic forms through esterification followed by hydrolysis and translocation of inorganic phosphate in the xylem (Loughman 1966; White 1973). This would account for the reduction in phosphorus levels in shoots following aluminium treatment. Clarkson (1966b) concluded that there are two reactions between aluminium and phosphate: at; the cell surface or free space of roots, which results in the fixation of phosphate by an

adsorption-precipitation reaction and; within the cell possibly within the mitochondria which results in a marked decrease in the rate of sugar phosphorylation, probably as a result in inhibition of hexokinase. An aluminium-sensitive barley cultivar was used in these experiments and the effects of aluminium on phosphorus metabolism with tolerant cultivars and species should be less pronounced, either through exclusion of aluminium at the plasmalemma of epidermal and cortical cells, or inactivation in the protoplasm.

Randall and Vose (1963) also reported stimulated phosphate uptake by perennial ryegrass with an aluminium pretreatment or with aluminium present with phosphate in the absorption solution. These results should be treated with some caution as anomalies can be found, particularly in the experimental procedure. For example, the concentrations of aluminium and phosphate used in the combined nutrient solution exceeded the solubility product and would have significantly contributed to the reduced total plant uptake of phosphate by eight week old plants in the presence of 500μg ml^{-1} aluminium. In a four hour uptake experiment the same aluminium and phosphate levels substantially increased phosphate uptake by plant tops. The authors concluded that the aluminium-induced increase in phosphate uptake was largely metabolic. Caution is required when considering this interpretation as KCN, one of the metabolic inhibitors used, forms a precipitate with aluminium and this would have inhibited phosphate uptake. Clarkson (1966b) reported that phosphate uptake by barley roots was as inorganic phosphate and almost completely exchangeable, and was not affected by DNP (2,4 dinitrophenol) or low temperature.

II.E. ALUMINIUM DISTRIBUTION IN ROOTS AND TRANSLOCATION

II.E.1. Aluminium and Phosphorus Distribution and Fixation

Plant roots accumulate large concentrations of aluminium when exposed to water soluble or exchangeable forms. In most species, only a small fraction of this aluminium is translocated to tops, irrespective of tolerance (Foy *et al.* 1967b;Medappa and Dana 1970; Kirkpatrick *et al.* 1975; Edwards *et al.* 1976; White 1976; Clark 1977; Kotze *et al.* 1977; Vickers and Zak 1978).

Wright and Donahue (1953) used hematoxylin stain to show that aluminium did not penetrate beyond the endodermis of barley roots. Keser *et al.* (1977) using susceptible sugar beet cultivars and a red staining precipitate showed that aluminium mainly occurred in the root cap, epidermis and cortex but some was detected in the stele. In maize, from Electron microprobe X-ray (EMX) analyses, Rasmussen (1968) found aluminium on the surface of epidermal cells and in the root tip with no penetration to the cortex and stele providing the root surface remained intact. The localization of phosphorus was the same as aluminium. The apparent disparity in aluminium distribution between plant species and cultivars could have been related to differential species and cultivar tolerance and experimental techniques, which included culture conditions for plants, methods of tissue preparation and aluminium detection. Despite these differences, Klimashevskii *et al.* (1972), Matsumoto *et al.* (1976a)and Naidoo *et al.* (1978) all reported that aluminium distribution within cells was mainly confined to the nucleus.

Evidence supporting the presence of aluminium and phosphate as aluminium phosphate in the free space of roots was presented by McCormick and Borden (1972, 1974) using a specific molybdenum staining technique. They showed that aluminium phosphate occurred

in the root cap, epidermal and cortical region extending from the tip to 105mm. The precipitate appeared to be associated with the cell wall and cytoplasmic membrane. The co-precipitation of aluminium and phosphate in free space, mainly in the root cap, has been supported by EMX-analyses (Rasmussen 1968; Naidoo *et al.* 1978) and by staining (Keser *et al.* 1977). In these studies the formation of an aluminium phosphate precipitate was enhanced by a high pretreatment concentration of aluminium (20µg ml^{-1}) followed by a high concentration of phosphate (30µg ml^{-1}) (McCormick and Borden 1972, 1974) or by growing plants in a complete nutrient solution where the concentrations of aluminium and phosphate were such that their solubility product, based on the data of Munns (1965b), was exceeded (Rasmussen 1968; Keser *et al.* 1977; Naidoo *et al.* 1978). Waisel *et al.* (1970) could find no correlation between aluminium and phosphate in cortical cells. However, they grew plants in a complete nutrient solution at pH 9.5, hence aluminium was present as an anion and this would have prevented aluminium phosphate precipitation in the nutrient solution and inhibited precipitation in the free space of roots.

Despite some anomalies in the literature, particularly where excessive levels of aluminium and phosphate have been used, there is general agreement on aluminium phosphate fixation in free space of roots of most species from both excised root and whole plant studies. Very few studies have examined the effect of pH on aluminium or phosphate uptake by either excised roots or whole plants. Soluble polymeric complexes of aluminium and phosphate have been shown to exist in dilute solutions, with maximal formation around pH 5 (White *et al.* 1976). White (1976) studied the effect of aluminium and phosphate on lucerne growth and recorded 3-4 times as much aluminium

in roots and shoots of plants grown at pH 5 with less inhibition of growth than at pH 4.5. This demonstrated the tolerance of plants to aluminophosphate complexes. As in other studies, aluminium enhanced phosphate uptake by roots, most of which could be removed by dilute acid, and reduced phosphate uptake by shoots.

II.E.2. Histology and Ultrastructure of Tissues

Passive movement of aluminium through meristematic cells to the stele (Henning 1975) would allow access to xylem vessels and translocation to shoots. This process coincided with irreversible damage to meristematic cells of wheat roots (lethal treatment) and root elongation ceased, but it may not account for translocation to shoots following a sub-lethal treatment in susceptible species or a non-lethal treatment in tolerant species.

Two other possibilities could account for lateral transport of aluminium to the stele. As already discussed, aluminium has been shown to move across the plasmalemma of root cells, hence it could enter the symplasm at the cortex and bypass the endodermis. Rasmussen (1968) proposed that the penetration of a lateral root through the endodermis, cortex and epidermis provided a channel of entry for aluminium into the cortex and conducting tissues of both the lateral and main root. Support for this proposal was presented by Dumbroff and Pierson (1971), who found that endodermal cells of the parent root of tomato, morning glory and oats maintained a continuous, unbroken, suberized layer over the surface of a very young lateral root, but with continued elongation, there was a period when formation of the Casparian strip lagged behind division of endodermal cells. The authors suggested that at this stage, water and other ions would enter the stele of the parent root by mass flow. If this hypothesis is correct, a peak of passive ion

uptake would occur at the zone of lateral root initiation.

Calcium uptake by barley is non-metabolic (Moore *et al.* 1961b) and has been shown to be related to root structure. Robards *et al.* (1973) identified three successive states of endodermal development in nodal axes and primary lateral roots of barley. Uptake of calcium was correlated with the primary state of endodermal development where no suberin lamellae were present. Similarly for *Cucurbita pepo*, calcium uptake was absent where secondary thickening of the endodermis occurred through suberization (Harrison-Murray and Clarkson 1973). This severely restricted direct access of the endodermal plasmalemma to the apoplast. Radial lead transport in the cortex of radish was also restricted to the apoplast where it accumulated at the endodermis, indicating that the Casparian strip provided a barrier to transport in the apoplast from cortex to stele (Lane and Martin 1977). However, the endodermis only acts as a partial barrier as some lead was detected in the vascular tissues. The pathway available for radial lead transport to the stele may be also available for aluminium due to its ability to exchange calcium and cause leakiness of membranes.

Maas (1969) reported that calcium uptake by maize was metabolically mediated. Uptake occurred over the entire root length except where a suberized hypodermis occurred at the base (Ferguson and Clarkson 1975, 1976). A maximum in calcium translocation occurred 12cm from the root tip coinciding with the region of lateral root initiation.

Apart from the EMX work of Rasmussen (1968) and the sequential staining work of Henning (1975), little attempt has been made to relate the histology and ultrastructure of tissues to aluminium absorption and translocation. Rasmussen's work has already been

criticised for growing plants in a nutrient solution where the solubility product of aluminium and phosphate was exceeded and can be further criticised for the method of tissue preparation used for EMX analyses. The standard technique of infiltrating and embedding tissue in paraffin was used which involves fixing in FAA (formalin, acetic acid, alcohol), and would have removed some aluminium and altered its distribution in tissues. Where root samples were frozen they were subsequently allowed to thaw and this would have led to both redistribution of nutrients and damage to tissues.

II.E.3. Effect of Aluminium on Cell Division

Complete and permanent inhibition of onion root elongation was achieved by exposure to 10^{-4}M aluminium sulphate for 6-8 hours (Clarkson 1965). Cessation of root elongation was closely correlated with the disappearance of mitotic figures, hence cell division was highly sensitive to short exposures to aluminium. DNA synthesis continued but the type of DNA had an unusual base composition and was metabolically labile (Sampson *et al.* 1965). Morimura and Matsumoto (1978) similarly showed that the template activity of DNA *in vitro* was altered by aluminium. Sampson and Davies (1966) reported that DNA from aluminium-treated barley roots consisted of two fractions; the usual 'genetic' DNA which is stable and has a high molecular weight; the second is a DNA of low molecular weight which is metabolically labile and is found characteristically in young actively growing tissue.

Henning (1975) could find no evidence for an alteration in DNA composition as changes in the genetic code would be expected to cause gross abnormalities in the morphology of regrowth root tips, but none was present in his study. The major effect of aluminium was degeneration of nuclei and cytoplasm (plasmolysis)

and hence cells were unable to carry out normal physiological functions such as cell division by meristematic cells. If the aluminium stress was removed before the onset of plasmolysis, the mitotic cycle would proceed again. These observations are based on paraffin infiltration of root tissue and as Cruickshank(*personal communication*) has frequently observed plasmolysis of plant tissue when prepared in this manner, the effects observed by Henning may be an artifact rather than an aluminium effect *per se*.

More recently, Morimura *et al*. (1978) found that aluminium inhibited cell division of onion roots and there was a distinct association between aluminium and nuclei after a one day treatment with 10^{-3}M $AlCl_3$. Examination of their photographs of aluminium-treated root tips revealed some evidence of plasmolysis which superficially supports Henning (1975), but may also be an artifact due to the method of tissue preparation used. However, Aimi and Murakami (1964) showed that the effects of aluminium excess start with dehydration of the protoplasm, hence the question as to whether the inhibition of DNA metabolism by aluminium is a primary or secondary effect requires elucidation.

The fact that aluminium does interfere with DNA replication supports the evidence previously discussed that aluminium can gain access to the symplasm and is therefore available for translocation to plant tops.

II.F. DIFFERENTIAL TOLERANCE TO ALUMINIUM

II.F.1. Plant Species and Cultivars

Tolerance of wheat to aluminium is controlled by one or more recessive genes (Lafever and Campbell 1978) whereas tolerance in maize is a dominant trait, controlled at a single locus by a multiple allelic series (Rhue *et al.* 1978) and in soybean by a single dominant gene (Kerridge and Kronstad 1968). Hence, differences in tolerance to aluminium among plant species would be expected simply because of natural selection. McLean and Gilbert (1927) reported large differences in aluminium tolerance among many crop species as a result of mutation and natural selection. Ramakrishnan(1968) concluded that the greater tolerance to aluminium and manganese excesses of an acidic population of *Melilotus alba* was partly responsible for its occurrence on acid soils and the absence of the calcareous population from acidic habitats. Among dicotyledons, the ability to accumulate large quantities of aluminium is statistically correlated with seven primitive characters (Chenery and Sporne 1976).

Cultivar differences in aluminium-tolerance have been reported in lucerne (Dessureaux 1969), cereals (Neenan 1960), barley (Maclean and Chiasson 1966; Macleod and Jackson 1967; Reid *et al.* 1969), wheat (Foy *et al.* 1965a; Kerridge and Kronstad 1968; Kerridge *et al.* 1971), *Agrostis* (Clarkson 1966a), soybean (Armiger *et al.* 1968), sunflower (Foy *et al.* 1974) and dry bean, French bean and lima bean (Foy *et al.* 1972). Hence, the importance of using a range of cultivars or species is emphasised when studying plant response to aluminium.

II.F.2. Characterization of Differential Response to Aluminium

II.F.2.1. Differential amounts of aluminium absorbed

Plants absorb aluminium to varying degrees and their tolerance can be related to this phenomenon. Tolerance can be defined as the ability of a plant to grow normally in the presence of a given aluminium concentration and is not simply related to differential aluminium uptake and distribution between roots and tops. Foy *et al.* (1978) divided aluminium-tolerant plants into three groups based on these criteria. In the first group, aluminium concentrations in tops are not consistently different from those in aluminium-sensitive plants, but the roots of tolerant plants often contain less aluminium than those of sensitive plants; in the second group, aluminium tolerance is associated with lower aluminium levels in tops and entrapment of excess aluminium in roots; in the third group, aluminium tolerance is directly associated with aluminium accumulation by tops. The first group includes several cultivars of wheat, barley, soybean and French bean (Foy *et al.* 1974). The second group also includes some French bean cultivars (Foy *et al.* 1972), and wheat and barley cultivars (Foy *et al.* 1967b). Tolerant cultivars of triticale, wheat and rye accumulate higher aluminium concentrations in roots than sensitive cultivars but there was little difference in the aluminium concentrations in tops (Mugwira *et al.* 1976). Chenery and Sporne (1976) regard aluminium accumulators, which represent the third group, as those that contain greater than 1000μg g^{-1} aluminium in leaves. Among the dicotyledons, 37 of 259 families contain aluminium-accumulating members, all of which have primitive traits. Tea is another example of an aluminium accumulator where Matsumoto *et al.* (1976b)recorded more than 30,000μg g^{-1} aluminium in old leaves.

II.F.2.2. Aluminium induced pH change in the root zone

The increase in pH of the nutrient solution by aluminium-tolerant cultivars of wheat, triticale, rye and barley has been demonstrated when they were grown in the presence of aluminium (Foy *et al.* 1965a; Foy *et al.* 1967b;Foy 1974; Mugwira *et al.* 1976; Mugwira and Patel 1977). In contrast, aluminium-sensitive cultivars of the same species decreased or had no effect on the pH of their nutrient solutions and thus were exposed to higher concentrations of aluminium for longer periods.

The question arises as to whether differential pH changes are a cause or effect of aluminium tolerance. The factors responsible for the pH change were discussed previously where an excess of anion over cation uptake leads to a pH increase in the nutrient solution. The source of nitrogen (NO_3^- *vs* NH_4^+) is the most important factor and this is further complicated by differential aluminium tolerance being related in some cases to the preferred form of nitrogen in the nutrient solution. The importance of pH control in nutrient solution experiments has also been discussed and has particular relevance to evaluation of aluminium tolerance. For example, Foy *et al.* (1967b)showed that aluminium-sensitive Kearney barley cultivar induced lower pH in the growth media than did aluminium-tolerant Dayton. Clarkson (1969) observed that when the nutrient solution pH was maintained at 4.2, Dayton and Kearney barley cultivars appeared equally sensitive to aluminium. In the experiment by Foy *et al.* (1967b),plants were grown in the aluminium treatment for 20 days with no change of nutrient solution or pH adjustment. The increase in pH by tolerant cultivars would have precipitated aluminium and hence overcome any inhibitory effects on growth. When sensitive and tolerant cultivars were grown separately in

control nutrient solutions, similar increases in pH were noted after 20 days. Mugwira *et al.* (1976) obtained similar results with differentially aluminium-tolerant cultivars of triticale, wheat, rye and barley. More recently, Mugwira *et al.* (1978) reported that differences in aluminium tolerance between cultivars of triticale, wheat and rye were greater when the pH of the nutrient solution was adjusted to 4.8 only on the first day compared with daily adjustments of pH. Accumulation of aluminium by roots was greater under the latter conditions confirming that upward drift in pH by tolerant cultivars precipitates aluminium and reduces its inhibitory effects. Henning (1975) proposed that the inability of sensitive plants to alter the pH of an aluminium-treated nutrient solution resulted from the inactivity of roots associated with death of tissues and cells.

Differential aluminium tolerance between Perry and Chief soybean cultivars (Foy *et al.* 1969) and Dade and Romano French bean cultivars (Foy *et al.* 1972) were not associated with differential pH changes in the nutrient solution. This indicates that differential pH changes are results, rather than causes, of differential aluminium tolerance and highlight the need to control pH and nutrient concentration in studies measuring aluminium tolerance of plants.

II.F.2.3. Aluminium - organic acid complexes

Organic acids form soluble complexes with aluminium and have been used by Rorison (1965) to remove exchangeable aluminium from the free space of roots. Jones (1961) also showed that aluminium was soluble in oxalic and citric acids and proposed that because the pH of xylem sap was within the range where aluminium was insoluble, it was likely that aluminium was translocated as an

organic acid complex, most likely in combination with phosphate.

Mathys (1977) analysed zinc-resistant and sensitive ecotypes of *Silene cucubalus*, *Rumex acetosa*, *Thlaspi alpestre* and *Agrostis tenuis* for malate, oxalate and mustard oil glucosides. He generally demonstrated higher concentrations in resistant ecotypes and postulated that malate acts as a complexing agent for zinc within the cytoplasm whereas malate and mustard oils may function as terminal receptors for very large amounts of zinc in the vacuole. Similarly, the tea plant, which accumulates high concentrations of aluminium in tops (Matsumoto *et al.* 1976b), contains appreciable amounts of organic acids and polyphenols which could render aluminium harmless by chelation and account for aluminium tolerance of the species (Sivasubramaniam and Talibudeen 1972). Similar mechanisms would be expected to operate in other aluminium accumulating plants to account for their tolerance.

III. MATERIALS AND METHODS

TABLE OF CONTENTS

III.A. PLANT SPECIES

The plant species used in all experiments were cabbage, *Brassica oleracea* var. *capitata* L., CV. Ballhead Hybrid, lettuce, *Lactuca sativa* L., CV. Pennlake, and kikuyu, *Pennisetum clandestinum* Hochst., CV. Whittet. All species are vegetative producers and can be compared over short growth periods. Lettuce in particular, and cabbage are susceptible to low pH and aluminium, whereas kikuyu appears to be tolerant to these conditions as it grows well on acid krasnozem soils in north-eastern New South Wales. Hence the three species represent a range of aluminium tolerance. Plants in all experiments were grown from one batch of seed/species.

III.B. NUTRIENT SOLUTION

A nutrient solution based on that described by Hoagland and Arnon (1950) was used at 1/10 strength for all solution culture experiments. The composition of the full strength solution is presented in Table III.B. The solution was modified to include various nutrient treatments in the whole plant study. With the exception of sequestrene NaFe, analytical grade chemicals were used throughout the course of this study. The nutrient solution will be referred to as Hoagland's solution.

Solutions were made up with deionized water produced by passing water through a sand bed, then twin bed cation and anion exchange resins and finally through a 5 μm cartridge filter. (Deionizer unit manufactured by Commando Products, Aust., St. Marys, South Australia.) The deionized water was stored in two 450 l light proof polythene reservoirs and the exchange beds were regenerated when conductance approached 5 μ mho m^{-1}.

Table III.B.

Composition of nutrient solution (Hoagland and Arnon 1950).

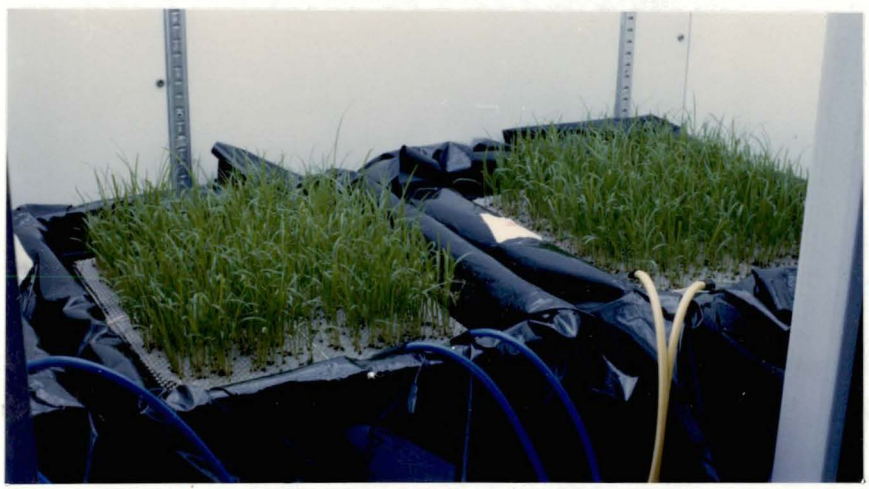
Salt	Concentration
KH_2PO_4	1 mM
$MgSO_4.7H_2O$	2 mM
KNO_3	5 mM
$Ca(NO_3)_2.4H_2O$	5 mM
H_3BO_3	46.2 µM
$MnSO_4.H_2O$	9.1 µM
NaFe Sequestrene	8.9 µM
$ZnSO_4.7H_2O$	0.76 µM
$CuSO_4.5H_2O$	0.32 µM
$Na_2MoO_4.2H_2O$	0.11 µM

III.C. PLANT GROWTH AND CABINET CONDITIONS

Plants for all experiments were grown in growth cabinets (Plates III.C.(i)-(ii)) ("Controlled Environments", Model No. EF7H - Winnipeg, Canada) at a quantum flux (400-700 nm) at plant height of approximately 165 $\mu E\ m^{-2}s^{-1}$ and a 12 h photoperiod. All species were grown at constant temperatures, kikuyu at 25°C and cabbage and lettuce at 20°C. The growth cabinets maintained precise control over temperature and the deviation was less than 0.5°C. Nutrient solutions in these cabinets were continuously aerated using small rubber diaphragm pumps (Kiho Special V-2, Japan).

A weighed quantity of seed of each species was surface sterilised in 7% $Ca(OCl)_2$ filtrate for 20 min then rinsed in five changes of deionized water. The seed was then placed in cheesecloth 'tea bags' and soaked in aerated 0.5 mM $CaSO_4$ solution for 6 h at 25°C for kikuyu and 20°C for cabbage and lettuce. The seed was then uniformly spread out over the cheesecloth on stainless steel screens (30 x 25 cm) supported over 10 ℓ of continuously aerated 0.5 mM $CaSO_4$ in growth cabinets at the pre-defined temperatures. The containers holding the $CaSO_4$ solution were lined with black polythene. The seed was covered with a piece of Sarlon mesh which itself was covered with cheesecloth, sufficiently large to dip into the solution and act as a wick to ensure that the seed remained moist during germination. The two layers of cloth were removed at germination when radicles were approximately 1 cm long, and this took three, four and five days respectively for cabbage, lettuce and kikuyu. The $CaSO_4$ solution was then replaced by Hoagland's solution, the details of

(i)



(ii)

Plate III.C.

Cabbage (i) and kikuyu (ii) plants growing in controlled environment growth cabinets.

which are given in Section III.F.1.1. for the excised root experiments and Section III.F.3.1. for the whole plant experiments.

III.D. PREPARATION OF TISSUE FOR CHEMICAL ANALYSES

II.D.1. Drying and Weighing of Tissue

Plant material from both excised root and whole plant experiments was dried at 65^{o}C for 48 h in a forced air oven (Qualtex, Watson Victor Ltd., Australia). A standard procedure was adopted for weighing all plant material where upon removal of each sample separately from the oven it was immediately transferred to the weighing room, placed on a tared holder and the weight recorded using a Mettler H10Tw balance (accuracy 0.1 mg).

II.D.2. Digestion of Tissue

It was not necessary to grind plant material to improve the rate of digestion as in all experiments it was that of 10 day old seedlings, which was relatively non-fibrous, and was easily and rapidly digested with perchloric and nitric acids. In addition, it was felt desirable to avoid grinding of plant material where whole samples were used in the digestion as it avoided an additional source of contamination, particularly where trace concentrations of elements were being determined.

For the excised root study, dry weight of root samples was generally about 0.05 g and these were weighed directly into test tubes for wet digestion. For the whole plant study, the dry weight of tops exceeded 0.1 g and they were ground using a glass mortar and pestle and a representative subsample of about 0.1 g was taken, weighed and transferred to a test tube for digestion.

Where the dry weight of roots exceeded 0.1 g, a subsample was also taken after grinding. The "Pyrex" test tubes (1.5 cm diameter, about 28ml volume) were precisely graduated at 5, 10 and 20ml.

Digestions commenced the same day to further minimise possibility of contamination by dispensing 5ml of a perchloric-nitric acid mixture (1 volume 70% perchloric acid - 5 volumes concentrated nitric acid) into the test tubes. The tubes were heated to 110^{o}C and left to digest overnight in a fume cupboard fitted with a large exhaust fan and a wash down water trap to dissolve fumes, which in this case were dense brown NO_2 fumes. Overnight digestion was found to be critical to efficiency as a too rapid an increase in temperature would lead to excessive frothing and boiling and loss of digestate. Considerable time was saved and safety achieved by a low temperature overnight digestion. The following morning digestion was almost complete and this was achieved by increasing the temperature to 180-200^{o}C whereby after about 3 h, the digestate became colourless, the volume reduced and dense white fumes of perchloric acid were emitted. The digestate was diluted with deionized water while still warm to avoid the formation of potassium perchlorate crystals.

After the perfection of this technique, a similar method was published by Zasoki and Burau (1977) where the acids were added to the plant material separately. This had the disadvantage that after the initial nitric acid digestion,samples must be cooled before perchloric acid can be added for the final digestion.

III.E. CHEMICAL ANALYSES

III.E.1. Atomic Absorption Spectroscopy

The aluminium, calcium and magnesium content of plant material was determined from an aliquot of diluted digestate using an Atomic Absorption Spectrophotometer (AAS - SP1800 Pye Unicam Ltd., Cambridge, England). Acetylene was the fuel used for each element, air was the oxidant used for calcium and magnesium and nitrous oxide was used for aluminium determinations. This required two different burners. Analyses were based on an integration time of 1 sec and the mean of 10 analyses recorded on a digital printout used for each sample. Operating conditions for each element are shown in Table III.E.1. Thorough mixing of diluted digestate was ensured using a vortex stirrer.

The AAS was run for about 20 min before the commencement of analyses to ensure stability of measurements. In addition, blanks (duplicate) and standards were analysed at the beginning and end of a run for unknown samples to minimise the error associated with drift. For the analyses of all elements, the drift from the start to the end of a run rarely exceeded 5%. Readings for standards were checked periodically during the course of a run as an additional check against malfunction. Deionized water was run through the atomizer between samples to eliminate contamination.

The extent of dilution used for the various elements depended on the nature of the experiment (treatment affected the final concentration of elements in plant tissue) and the dry weight of plant material.

Table III.E.1.

Operating conditions for Pye Unicam SP1800 Atomic Absorption Spectrophotometer.

Element	Wavelength (nm)	Slit width (mm)	Lamp Current (mA)	Burner height (cm)	Fuel flow rate (ℓ min^{-1}) acetylene	Oxidant flow rate (ℓ min^{-1}) Air	Nitrous Oxide
Aluminium	309.3	0.22	8	1.0	1.8	5	
					4.2		5
Calcium	422.7	0.20	8	0.8	1.4	5	
Magnesium	285.2	0.20	4	0.5	1.4	5	

III.E.1.1. Aluminium

A final dilution of from 1:60 to 1:250 in deionized water was used for aluminium determinations. Because of the relatively low concentrations of aluminium in the samples analysed, a scale expansion was used to increase the value of the readouts by a factor of 10. Aluminium content of the solution was calculated from a standard curve prepared in the concentration range 0-100 μg ml^{-1} aluminium.

III.E.1.2. Calcium

A final dilution of from 1:310 to 1:1250 in deionized water was used for calcium determinations. Calcium absorption is subject to interference from aluminium, phosphate and silicate; lanthanum was added to overcome or minimise this interference (Christian and Feldman 1970). The final conconcentration of lanthanum was 0.04% in both unknowns and standards which also contained 0.01% H_2SO_4. Calcium content of the solution was calculated from a standard curve prepared in the concentration range 0-20 μg ml^{-1} calcium.

III.E.1.3. Magnesium

A final dilution of from 1:1600 to 1:6250 in deionized water was used for magnesium determinations. Magnesium is also subject to interference from aluminium, phosphate and silicate (Christian and Feldman 1970). The solution used for magnesium determinations was obtained by dilution of that used for calcium, hence lanthanum had been added to suppress interference. Proportional amounts of lanthanum and H_2SO_4 were added to magnesium standards. Magnesium content of the solution was calculated from a standard curve prepared in the concentration range 0-5 μg ml^{-1} magnesium.

III.E.2. Flame Photometry

The potassium and sodium content of plant material was determined from an aliquot of diluted digestate using an EEL Flame Photometer (Evans Electroselenium Ltd., Hallstead, Essex, England). Optical filters isolated emitted light into the characteristic wavelength bands of the two elements. Propane was used as the fuel and air as the oxidant, the latter being pumped into the burner at a constant pressure of 0.69 bar. Deionized water was run through the atomizer between samples to eliminate contamination.

III.E.2.1. Potassium

A final dilution of from 1:7800 to 1:25000 in deionized water was used for potassium determinations. Potassium can be subject to interference from other ions but this is usually overcome by the optical filter. In the present study dilutions were made from the solution used for calcium determinations, hence possible interference from aluminium, phosphate and silicate was suppressed by lanthanum. Potassium content of the solution was calculated from a standard curve prepared in the concentration range 0-10 $\mu g\ ml^{-1}$ potassium.

III.E.2.2. Sodium

A final dilution of from 1:1600 to 1:6250 in deionized water was used for sodium determinations. Sodium can also be subject to interference from other ions but this is usually overcome by the optical filter. In the present study dilutions were made from the solution used for calcium determinations, hence possible interference from aluminium, phosphate and silicate was suppressed by lanthanum. Sodium content of the solution was

calculated from a standard curve prepared in the concentration range 0-2 μg ml^{-1} sodium.

III.E.3. Colorimetry

The phosphorus content of plant material was determined on an aliquot of diluted digestate using a Spectrophotometer (Hitachi 101 fitted with a flow through cell). Deionized water was used between samples to flush out the flow through cell and to check on the 0% absorbance setting.

III.E.3.1. Phosphorus

A final dilution of from 1:780 to 1:6250 in deionized water was used for phosphorus determinations using ammonium molybdate - ammonium metavanadate reagent as described by Chapman and Pratt (1961). Colour was allowed to develop for 30 min before the optical density was measured at 470 nm. Phosphorus content of the solution was calculated from a standard curve prepared in the concentration range 0-20 μg ml^{-1} phosphorus.

III.F. EXPERIMENTAL PROCEDURES

III.F.1. Aluminium Uptake by Excised Roots

III.F.1.1. Plant growth and root excision

A weighed quantity of seed (about 2000 seed weight) was prepared for germination as described in Section III.C. Plants were grown in standard 1/10 strength Hoagland's solution adjusted to pH 5.6 with 0.1 M H_2SO_4. The nutrient solution was changed every second day and this prevented algal contamination. Plants were harvested after 10 days' growth (Plate III.F.1.1.)

Plate III.F.1.1.

Stainless steel stand and screen with kikuyu seedlings for root excision.

and roots excised immediately below the stainless steel screen for experimentation. At this stage roots were 6-8 cm in length.

III.F.1.2. Short-term uptake technique

III.F.1.2.1. Excised roots

Excised roots were rinsed in deionized water and immersed in aerated 0.5 mM $CaSO_4$. Approximately 1 g samples (fresh weight) were removed and placed in a square (20 x 20cm) of nylon mesh (1 mm aperture) which was formed into a 'tea bag', tied and a label attached, similar to the method described by Epstein *et al.* (1963). The 'tea bags' with their root samples were transferred to aerated 0.5 mM $CaSO_4$ for 45 min for temperature equilibration. The temperature of this solution was identical to that of the absorption solution and maintained at a constant temperature by an immersion thermostat unit (Thermomix II - B. Braun, West Germany). When a temperature of 1^oC was required for the absorption solution, this was achieved by bathing the containers holding the solution in ice. A temperature of 1^oC for the desorption solution was similarly achieved.

The pH of the absorption solution was adjusted immediately prior to the commencement of an experiment with 0.1 M H_2SO_4 or 0.1 M NaOH where 2,4-dinitrophenol (DNP) was used. Deionized water was used in all experimental solutions.

After the temperature equilibration period root samples were removed, shaken to remove excess solution and immersed in the aerated absorption solution containing both aluminium and calcium (Plate III.F.1.2.1.). They were withdrawn after the treatment absorption periods, shaken to remove excess solution, rinsed for

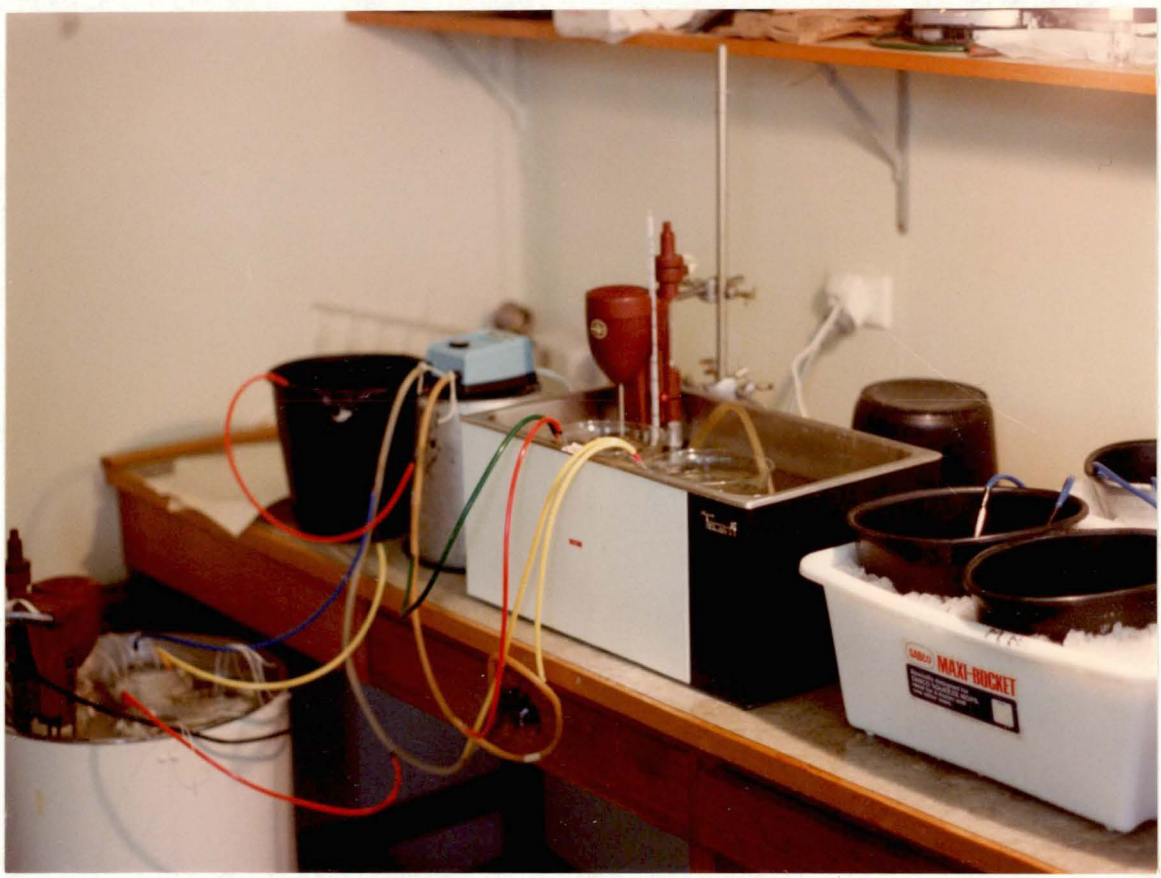


Plate III.F.1.2.1.

Apparatus used for conducting short-term uptake experiments with excised roots.

10 sec in cold deionized water then immersed in deionized water at $1^{o}C$ for 20 min for further desorption.

In specific desorption experiments, water was initially used followed by 22.5 mM succinic-tartaric acids plus triethylamine, pH 4.5 (Rorison 1965). After the treatment desorption periods, roots were removed, shaken, then placed in a forced air drying oven.

All experimental solutions were of sufficient volume that depletion was less than 10% of the initial aluminium level. The pH change at the end of an experiment was <0.05. Desorption solutions were maintained at a ratio of 12 g fresh weight of roots per 12 l or less.

Duplicate samples of root (and resin) were used in each experiment except where triplicate samples were used in the temperature response experiments and when measuring endogenous levels of aluminium and calcium.

III.F.1.2.2. Cation exchange resin

A cation exchange resin was used in absorption experiments as a comparison with excised roots. Amberlite IRC-50 (Rohm and Haas Co., U.S.A.), which is a weakly acidic (acrylic) carboxylic cation exchanger (cation exchange capacity (C.E.C.) of 10^{3} m. equiv. per 100 g dry weight) was prepared by rinsing in two bed volumes of deionized water (after an initial soaking until fully swollen) followed by two bed volumes 4% NaOH then two bed volumes deionized water, five bed volumes 10% HCl and a final rinse with 10 bed volumes deionized water to give a final pH of the effluent of about 4.2. Amberlite was used in uptake experiments similar to roots

where it was initially bathed in 0.5 mM $CaSO_4$ for 45 min for temperature equilibration and hence was in the calcium form prior to aluminium absorption. About 2 g samples of resin were used similarly to excised roots except that microfine nylon gauze was used for 'tea bags'.

III.F.1.3. Chemical analyses

To the dried Amberlite, 20 ml 20% HCl was added and allowed to stand for 2 h with intermittent stirring to exchange aluminium and calcium before chemical analyses were conducted. A final dilution of about 1:10 and 1:100 for aluminium and calcium determinations was used respectively. Details of chemical analyses used for plant tissue were described previously. C.E.C. of roots was measured by the method of Crooke (1964).

III.F.2. Aluminium Distribution in Roots by Energy Dispersive X-Ray Analysis

III.F.2.1. Root preparation and freeze-drying

Roots were obtained at harvest from the whole plant study, immersed in 0.5 mM $CaSO_4$ and prepared for freeze-drying. The six treatments are described in Section V.B.2.

Roots were removed from the $CaSO_4$ solution, the primary root sectioned into 1 cm segments from the apex (tip - proximal to the meristematic zone), the area of lateral root initiation (mid) and the base. The segments were inserted into brass holders that contained sufficient 0.5 mM $CaSO_4$ to bathe the roots. The brass holders containing the root segments were carefully immersed in liquid nitrogen together with a new, clean razor blade for about 10 s, removed and the razor blade run along the surface of the

block to fracture the roots transversely. The holders were returned to the liquid nitrogen within 5 sec to ensure that there was no thawing of roots. The glass beaker containing the brass holders covered with liquid nitrogen was placed in a freeze-drying flask and the fractured root segments were freeze-dried for 24 h (Plate III.F.2.1.).

III.F.2.2. EDX-analysis

The freeze-dried roots were cut 1 mm below the fractured surface and mounted on brass stubs with a colloidal graphite - epoxy resin mixture. The adhesive ensured electrical conductance between the specimen and the brass stub. Its main disadvantage was that it contained sulphur and when epidermal cells were being analysed, the sulphur peak of the Energy Dispersive X-Ray (EDX) spectrum was enhanced by the incident electron beam striking the epoxy resin. However, colloidal graphite - epoxy resin was found to be a more suitable adhesive for freeze-dried root segments than colloidal silver that also interfered with the EDX spectrum for epidermal cells where the silver peak overlapped the potassium peak. Specimens for EDX-analyses were vacuum coated with carbon and where micrographs from secondary electron images were required, gold coating was used.

The analyses (86 sec analysis time) were carried out at an accelerating voltage of 25 kV using a JEOL JXA-50A scanning electron microscope with an EDAX 707B multichannel analyser. The count rate was held at about 800 sec^{-1} by varying the beam current from 0.5 to 1.0^{-10} A.

The two pathways available for radial ion movement to the stele are the apoplast and the symplasm. This involves the

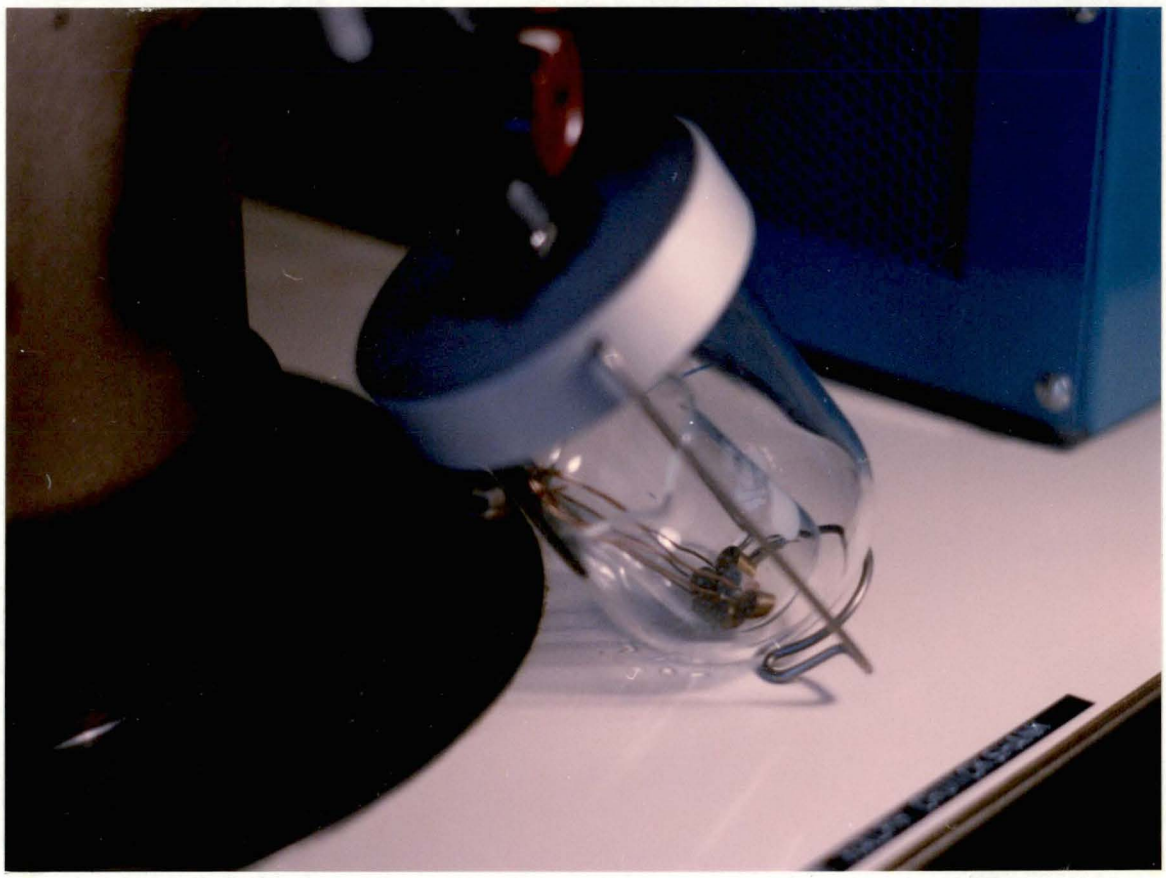


Plate III.F.2.1.

Vacuum flask on freeze-drier with brass holders containing root segments.

cell wall and the thin strip of cytoplasm closely associated with the wall. Point analyses were taken from the cell wall region (but will have included some cytoplasm as the two were indistinguishable and beam scattering is inevitable) of the epidermis, cortex, endodermis, xylem parenchyma, protoxylem, metaxylem and phloem. Limited data are presented for the protoplasm as dehydration of tissue left little intact.

Results are presented as X-ray spectra consisting of histograms where the number of X-ray quanta in each 20 eV band (channel) of a relevant part of the spectrum is shown. For the elements being analysed, aluminium, silicon and phosphorus, seven channels per window were used. Windows were chosen to include most of the counts in a peak, hence centroids were taken as the K_α levels rounded to the nearest 20 eV. Integrated counts under the silicon, aluminium and phosphorus peaks plus backgrounds were recorded so that peak to background ratios, as described in Section V.B.2., could be calculated.

III.F.3. Effect of Aluminium Excess on Growth and Nutrient Uptake of Plant Species in Nutrient Solution

III.F.3.1. Plant growth

A weighed quantity of seed (¼ x 2000 seed weight) was prepared for germination as described in Section III.C. Each stainless steel screen was divided into four equal parts onto which the unit quantity of seed was spread for germination.

Plants were grown in modified 1/10 strength Hoagland's solution representing various nutrient treatments (Section VI.B.). The phosphate concentration was reduced to 50 μM so that treatment

aluminium concentrations and pH corresponded to the guidelines of Munns (1965b) in an attempt to avoid aluminium phosphate precipitation in solution. Nutrient solutions were changed daily and pH adjusted with 0.1M H_2SO_4 to minimise changes in pH and nutrient concentration.

III.F.3.2. Harvesting and tissue analysis

Plants were harvested after 10 days' growth after rinsing in deionized water. Roots were excised immediately below and tops immediately above the stainless steel screen. Plant material was then dried, weighed and wet digested for chemical analyses as described in Section III.D.2.

IV. ALUMINIUM UPTAKE BY EXCISED ROOTS

TABLE OF CONTENTS

IV. ALUMINIUM UPTAKE BY EXCISED ROOTS

IV.A. Introduction

The nature of aluminium uptake by excised roots was studied by Rorison (1965) and Clarkson (1967) and they concluded that almost all the absorption was into free space associated with pectins of the cell wall. Clarkson and Sanderson (1971) showed that aluminium reduced the amount of calcium held in the free space of roots. This reduction was due to more than simple exchange-adsorption onto free carboxyl groups as high concentrations of calcium, sodium and disodium EDTA failed to desorb aluminium (Clarkson 1967). Matsumoto *et al.* (1977) investigated the possibility of adsorbed aluminium being associated with pectin in pea roots and observed no distinct association after gel filtration of the pectinase-digested cell wall material.

The importance of pH in studies on aluminium uptake has been largely ignored. Smith (1971) reported that three separate types of aluminium exist in solution, a monomeric species, polynuclear aluminium hydroxide species and small insoluble aluminium hydroxide particles. The monomeric species are hydrated with valences of 1-3. As pH increases, the mean valence per monomer decreases, polymerization occurs and the average charge per aluminium atom decreases (Hsu and Bates 1964; Smith 1971). White (1976) suggested that higher aluminium uptake by lucerne roots at pH 5 than 4.5 from a complete nutrient solution resulted from polymerization of alumino-phosphate at pH 5 with low net charge density. The existence of these polymers was confirmed by White *et al.* (1976) using paper electrophoresis.

There is indirect evidence for a second component for aluminium uptake which would account for its occurrence in protoplasts of susceptible species, generally in the root cap and meristematic zone and largely associated with the nucleus (Klimashevskii *et al.* 1972; Matsumoto *et al.* 1976b; Keser *et al.* 1977; Naidoo *et al.* 1978). Henning (1975) confirmed that the endodermis prevented aluminium entering the stele but with a lethal treatment this occurred by movement through meristematic cells of the root tip.

This study was undertaken to characterise aluminium uptake by plants using three species, cabbage, lettuce and kikuyu.

IV.B. Treatments

The time course of aluminium uptake was measured for cabbage, lettuce, kikuyu and Amberlite from 1.0 mM $Al_2(SO_4)_3$ (54 $\mu g\ g^{-1}$) in the presence of both normal (0.5 mM $CaSO_4$) and high (0.6737 M $CaCl_2$) calcium concentrations for intervals up to 180 min at 25°C and with the three plant species, normal calcium level, at 25°C with 0.2 mM DNP and at 1°C. Normal calcium levels were used in all other experiments.

Aluminium absorption-temperature response studies were undertaken using absorption times of 0-60 and 60-120 min and temperatures of 1, 10, 20, 30, 40 and 50°C for the three plant species.

Separate aluminium desorption experiments were also conducted on roots which had an absorption time of 120 min at 25°C. They were initially desorbed in deionized water at 1°C for 20 min followed by succinic-tartaric acid buffer at 1°C for intervals up to 240 min.

Aluminium absorption for all experiments was conducted at pH 4.2 and 4.0. The effect of aluminium absorption on calcium levels in roots (and resin) was also measured for each experiment. Preliminary experiments were conducted and confirmed the reproducibility of results. The experiments reported in the study involving pH comparisons were conducted concurrently.

IV.C. Results

IV.C.1. Time course of aluminium uptake

The time course of aluminium uptake (normal calcium) for cabbage, lettuce and kikuyu at 25^{o}C is given in Figs. IV.C.1. (i), (ii) and (iii) respectively. The rapid initial phase (Phase I) was more pronounced and more extensive for cabbage and lettuce than for kikuyu. The second phase (Phase II) was represented by linear (steady state) uptake for cabbage and slightly curvilinear uptake for lettuce. Phase I was complete after 60 min, Phase II represented 28% of the total uptake after 180 min for both cabbage and lettuce (mean pH 4.2 and 4.0). Phase II was almost completely absent for kikuyu indicating that after an initial rapid uptake very little additional aluminium was absorbed. Total uptake by kikuyu was about 21% of that by cabbage and 25% of that by lettuce which does not coincide with a comparison of the C.E.C. of roots which are 23.5, 49.0 and 59.5 m. equiv. per 100g dry weight respectively. Temperature had little effect on aluminium uptake by the three species in contrast to the effect of a metabolic inhibitor, DNP, which substantially enhanced uptake (Figs. IV.C.I. (i) - (iii)).

Figure IV.C.1. (i)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ by excised roots of cabbage at 25^oC ——— pH 4.2 (△) and pH 4.0 (▲), at 1^oC - - - - - pH 4.2 (□) and pH 4.0 (■), in the presence of 0.2 mM DNP —— —— pH 4.2 (○) and pH 4.0 (●).

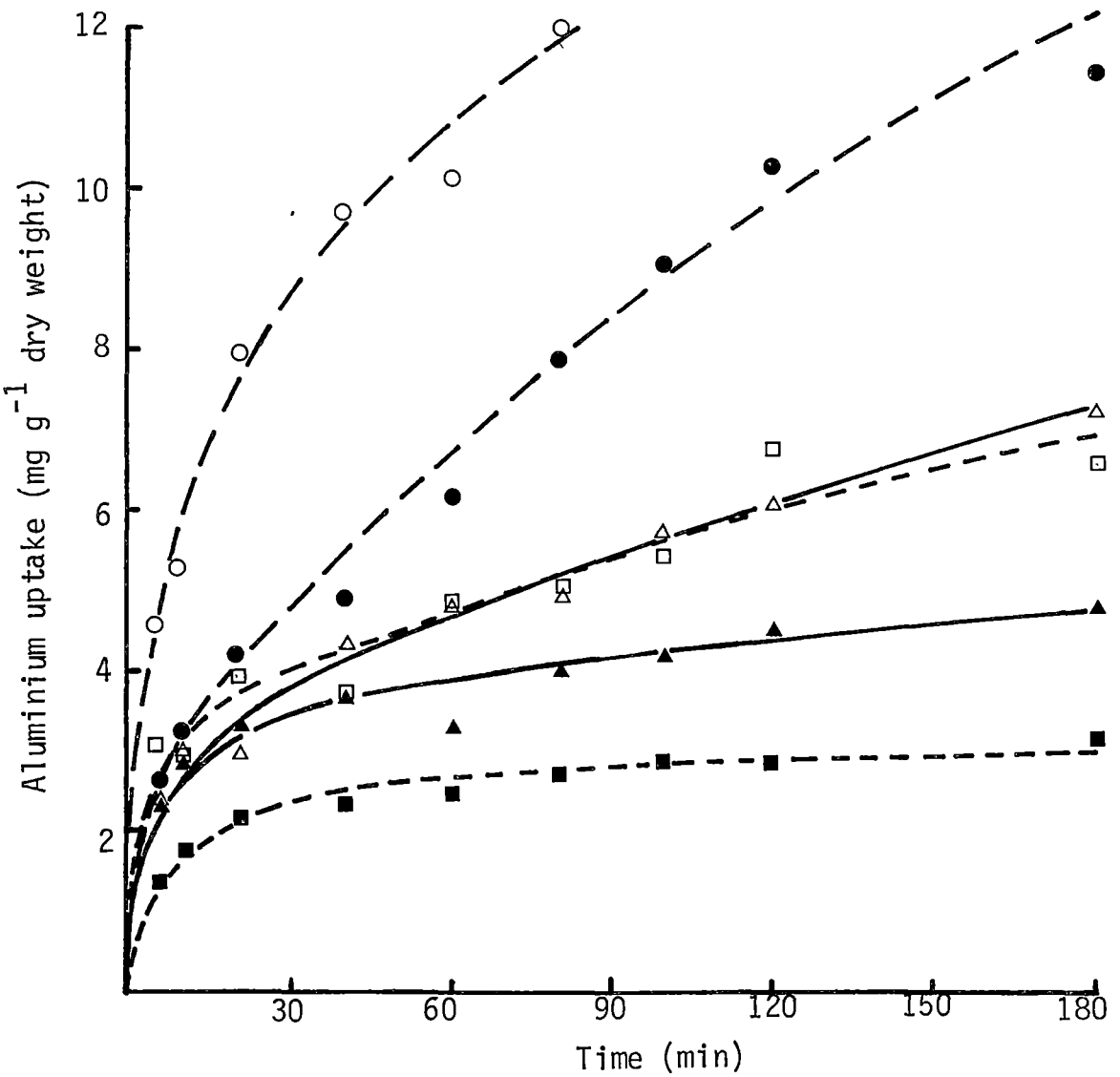

12
10
8
6
4
2
Aluminium uptake (mg g^{-1} dry weight)
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (ii)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ by excised roots of lettuce at 25°C ——— pH 4.2 (△) and pH 4.0 (▲), at 1°C – – – – pH 4.2 (□) and pH 4.0 (■), in the presence of 0.2 mM DNP —— —— pH 4.2 (○) and pH 4.0 (●).

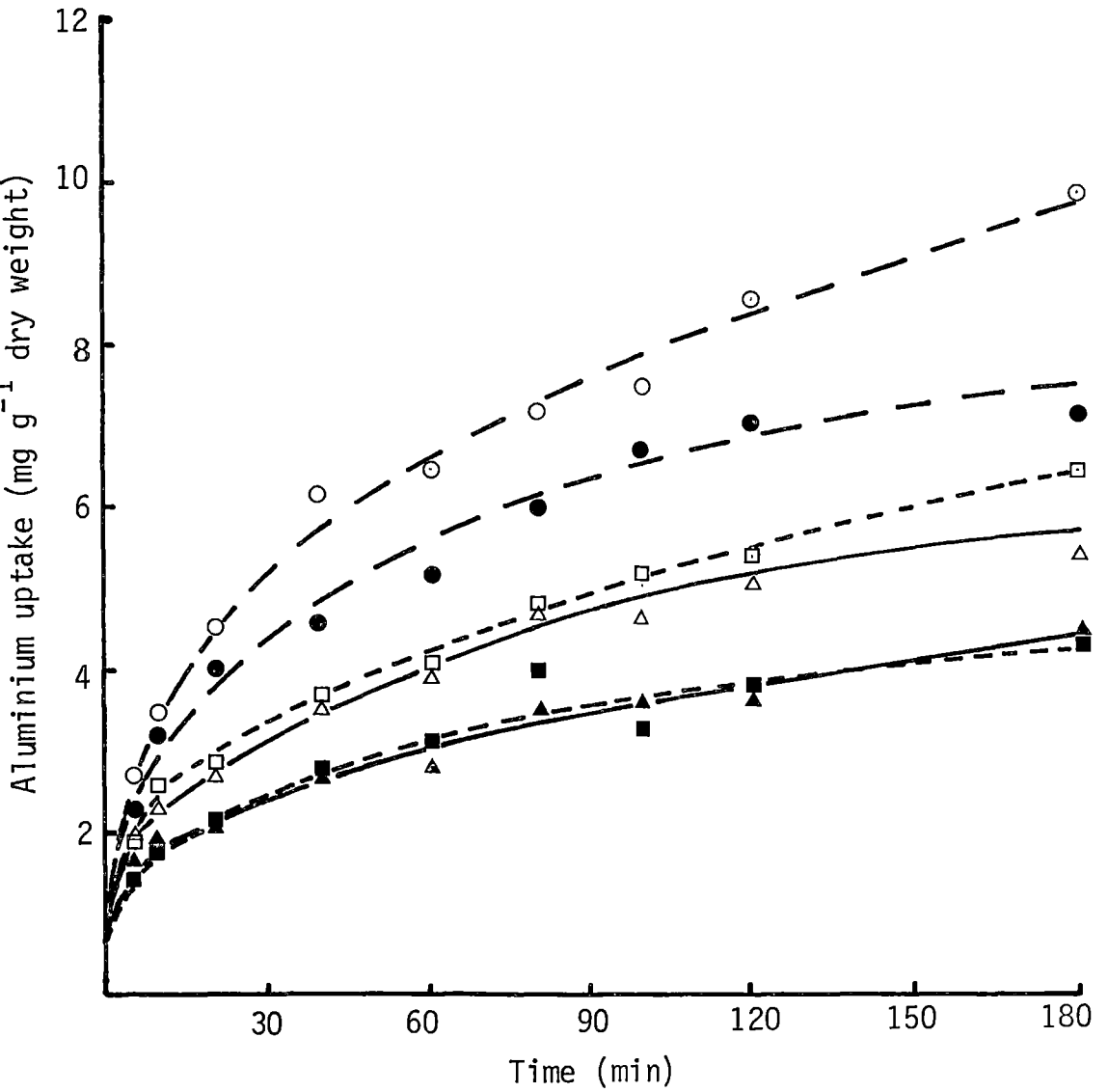
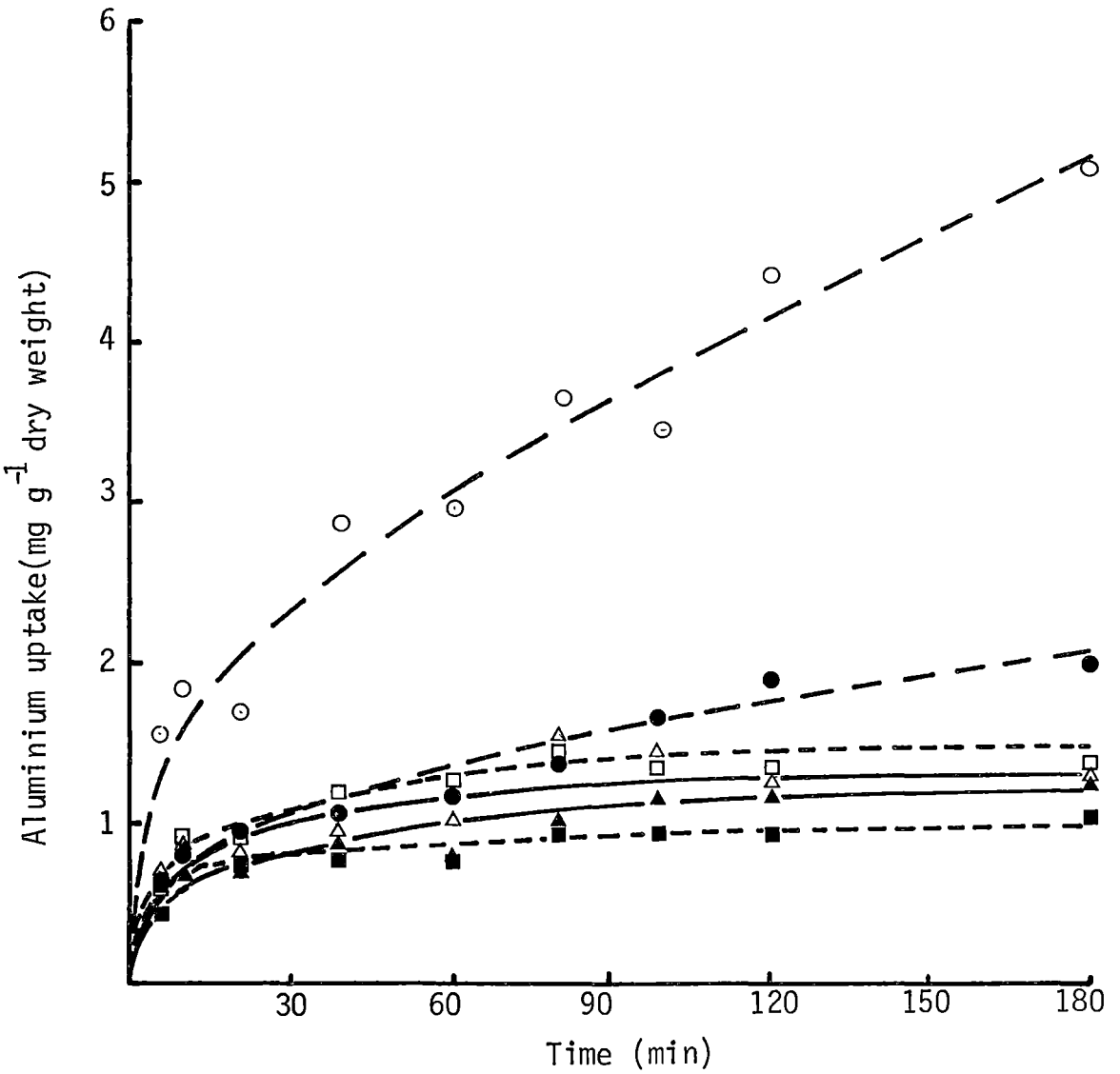

Aluminium uptake (mg g^{-1} dry weight)
12
10
8
6
4
2
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (iii)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ by excised roots of kikuyu at 25°C ——— pH 4.2 (△) and pH 4.0 (▲), at 1°C – – – – pH 4.2 (□) and pH 4.0 (■), in the presence of 0.2 mM DNP —— —— pH 4.2 (○) and pH 4.0 (●).


Aluminium uptake(mg g^{-1} dry weight)
6
5
4
3
2
1
30
60
90
120
150
180
Time (min)

The time course of aluminium uptake by Amberlite (Fig. IV.C.1. (iv)) followed a slightly different pattern to excised roots with the absence of the rapid uptake phase. The initial uptake phase was slow and took 120 min for equilibration to occur after which no further uptake occurred.

High calcium had little effect on aluminium uptake by cabbage and kikuyu, it substantially increased uptake by lettuce (Fig. IV.C.1. (v)), and substantially reduced uptake by Amberlite (Fig. IV.C.1. (vi)).

In experiments at normal calcium levels, aluminium uptake was directly associated with calcium desorption. Examples for roots (Fig. IV.C.1. (vii)) and Amberlite (Fig. IV.C.1. (viii)) at 25^{o}C show rapid calcium desorption during the initial 60 min uptake phase with little desorption thereafter. The endogenous calcium levels for cabbage, lettuce and kikuyu after a 10 sec rinse and 20 min desorption in deionized water corresponding to the previous examples were 6.85, 5.52 and 0.99 $\mu g\ g^{-1}$(dry weight) indicating that aluminium had exchanged most of the calcium from roots.

In experiments at high calcium levels, both aluminium and calcium uptake occurred concurrently. Examples for roots (Fig. IV.C.1. (ix)) and Amberlite (Fig. IV.C.1. (x)) at 25^{o}C show rapid calcium uptake for cabbage, kikuyu and Amberlite during the initial phase followed by a plateau,whereas there was some increase for lettuce during the second phase. The relative differences in calcium uptake in the presence of high calcium were similar to that for aluminium with normal calcium where lettuce had the highest uptake.

Figure IV.C.1. (iv)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ by Amberlite at 25°C, pH 4.2 (△) and pH 4.0 (▲).

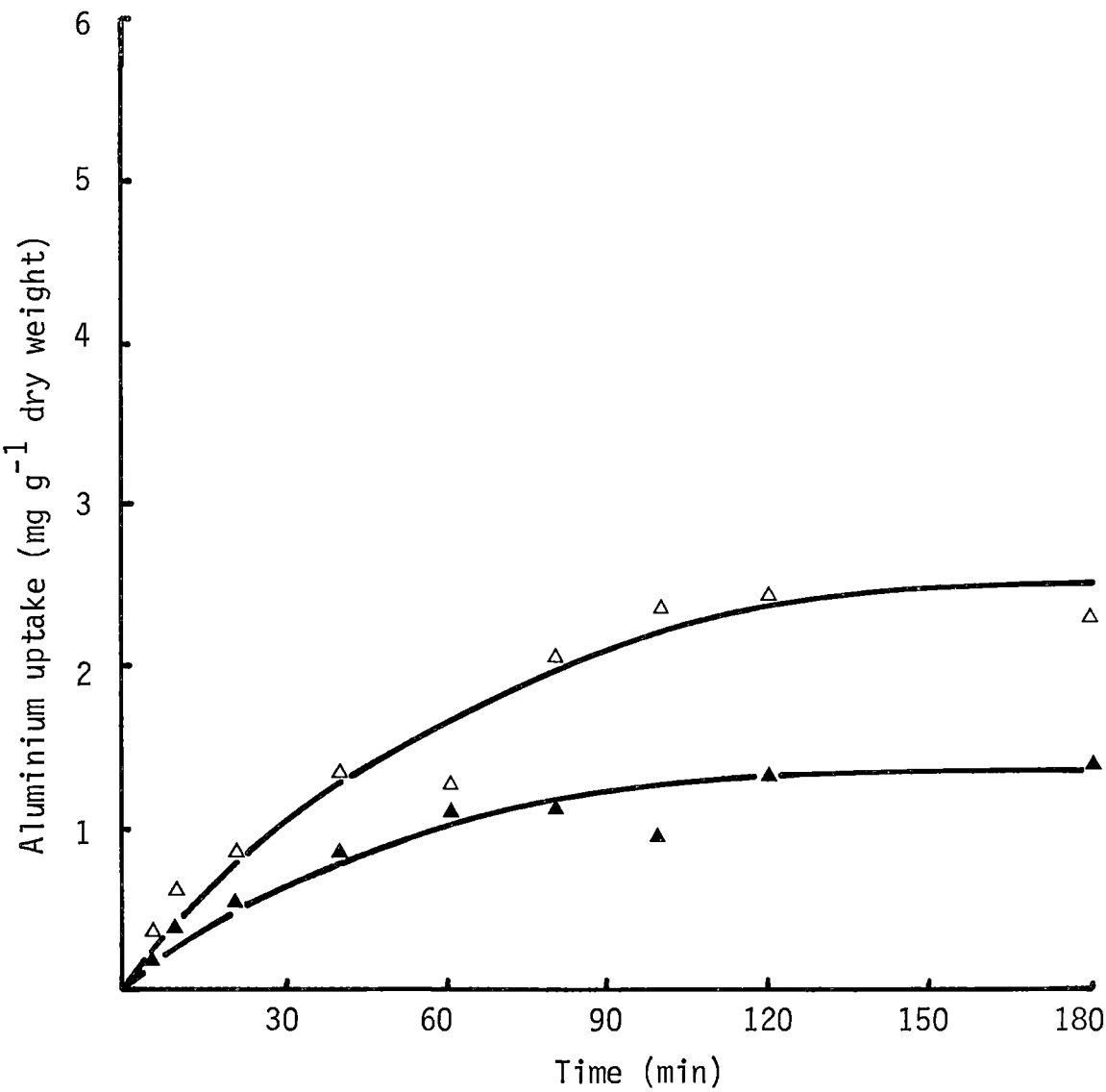

Aluminium uptake (mg g^{-1} dry weight)
6
5
4
3
2
1
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (v)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.6737 M $CaSO_4$ at 25°C by cabbage —— —— pH 4.2 (△) and pH 4.0 (▲), lettuce – – – – pH 4.2 (□) and pH 4.0 (■), kikuyu ———— pH 4.2 (○) and pH 4.0 (●).

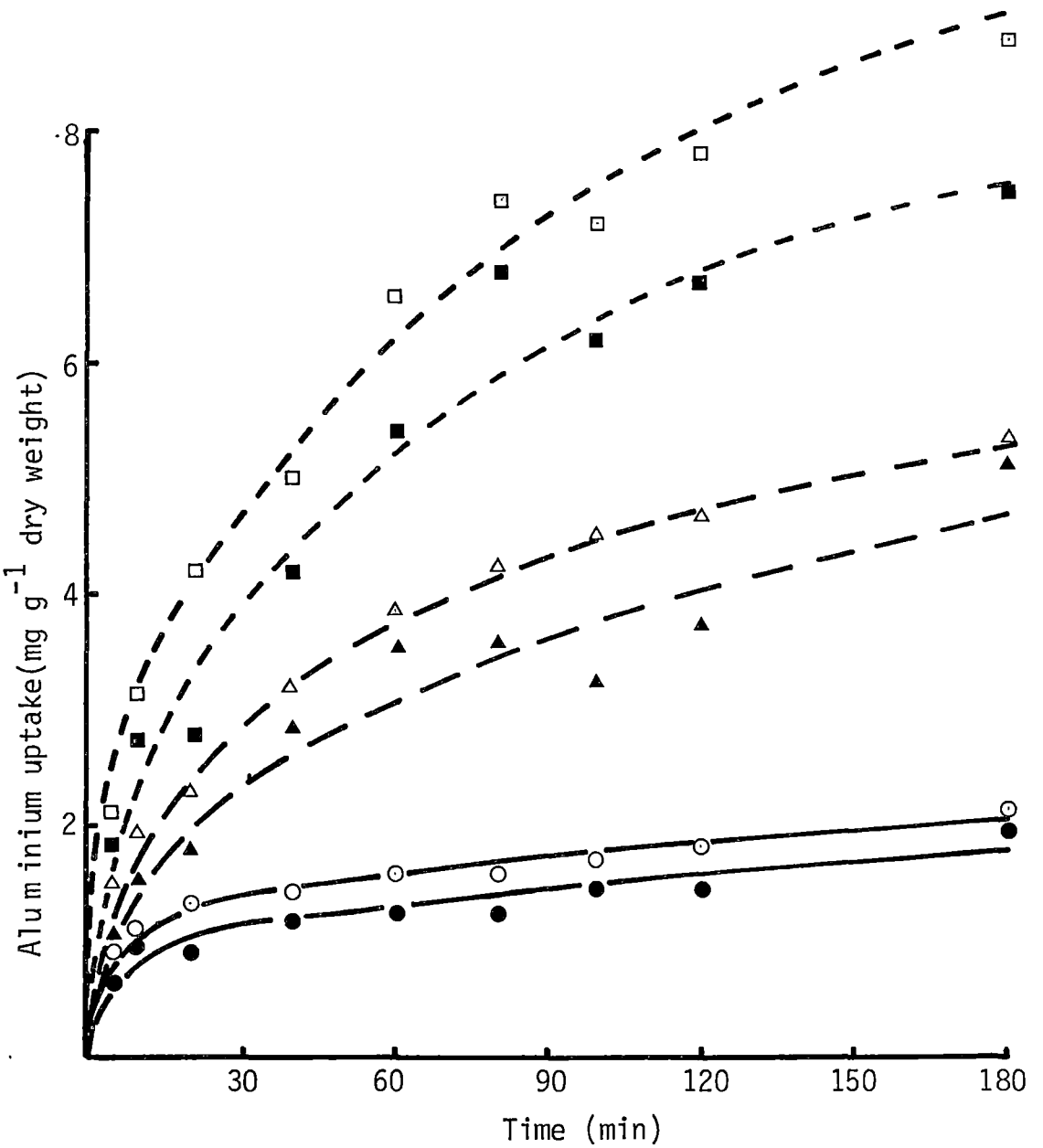

·8
6
4
2
Aluminium uptake(mg g^{-1} dry weight)
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (vi)

Time course of aluminium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.6737 M $CaCl_2$ by Amberlite at 25°C, pH 4.2 (△) and pH 4.0 (▲).

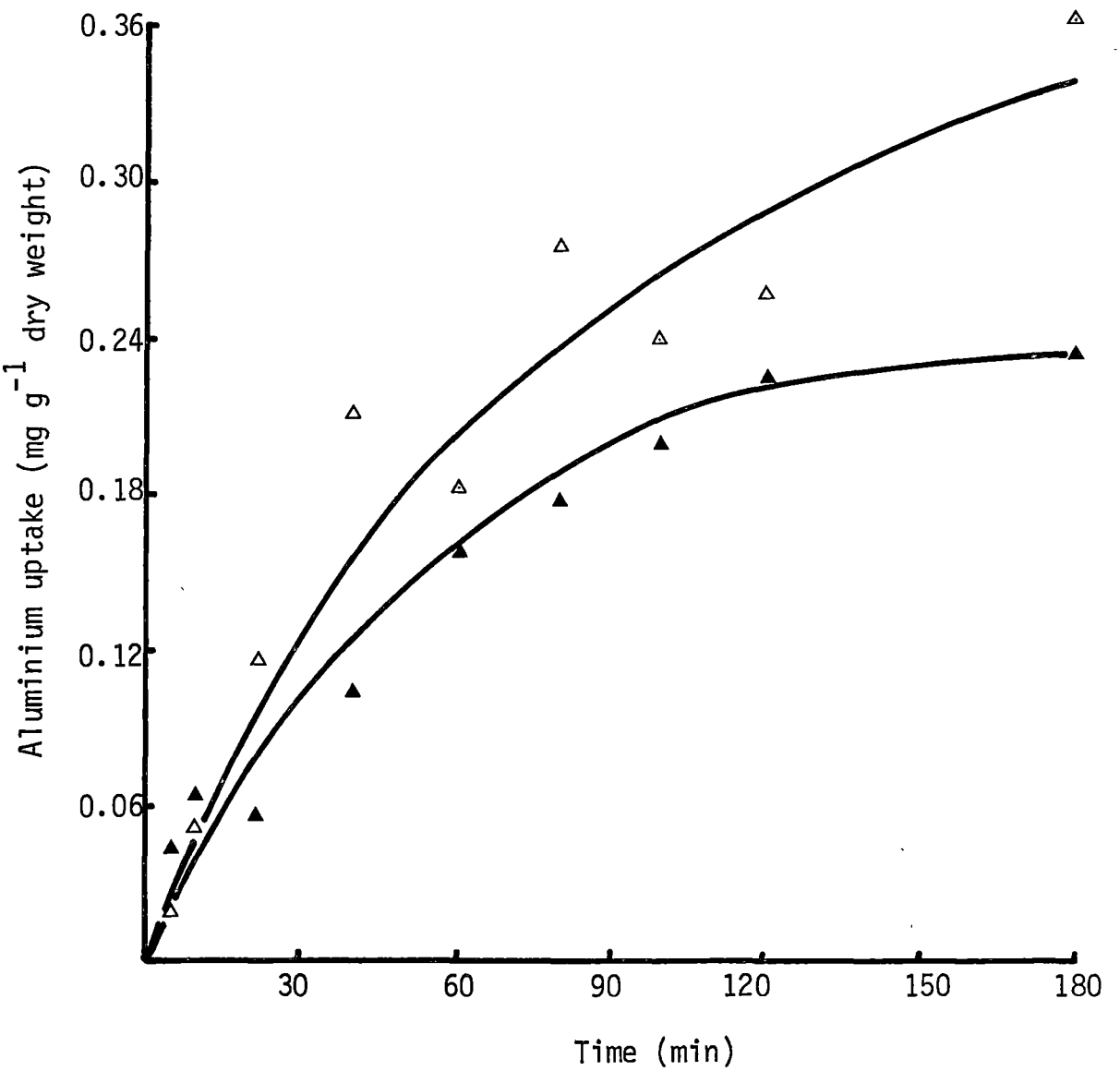

0.36
0.30
0.24
0.18
0.12
0.06
Aluminium uptake (mg g^{-1} dry weight)
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (vii)

Time course of calcium desorption by 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ at 25°C from excised roots of cabbage —— —— pH 4.2 (△) and pH 4.0 (▲), lettuce — — — — pH 4.2 (□) and pH 4.0 (■), kikuyu ———— pH 4.2 (○) and pH 4.0 (●).

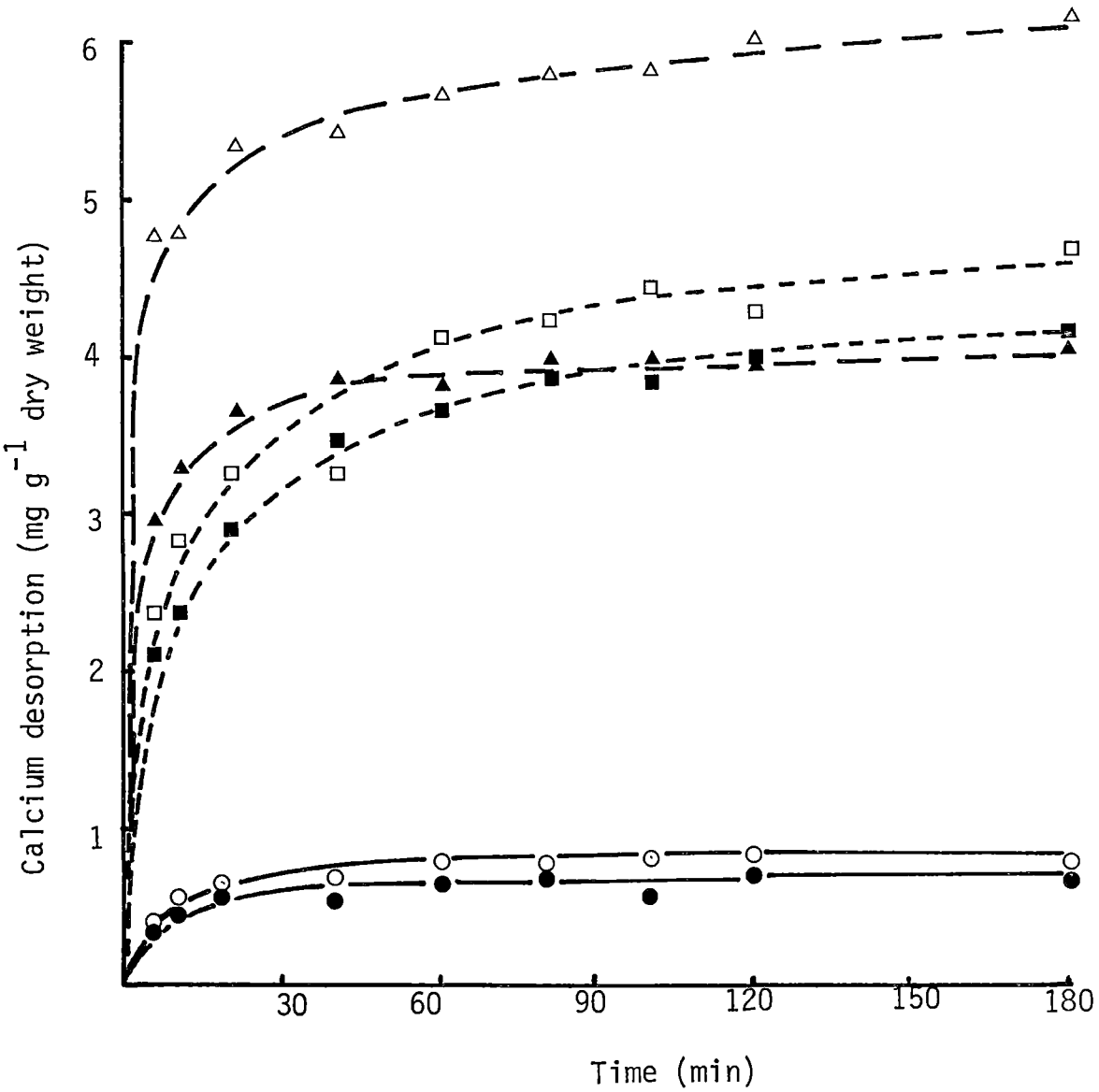

Calcium desorption (mg g^{-1} dry weight)
6
5
4
3
2
1
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (viii)

Time course of calcium desorption by 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ from Amberlite at 25°C, pH 4.2 (△) and pH 4.0 (▲).

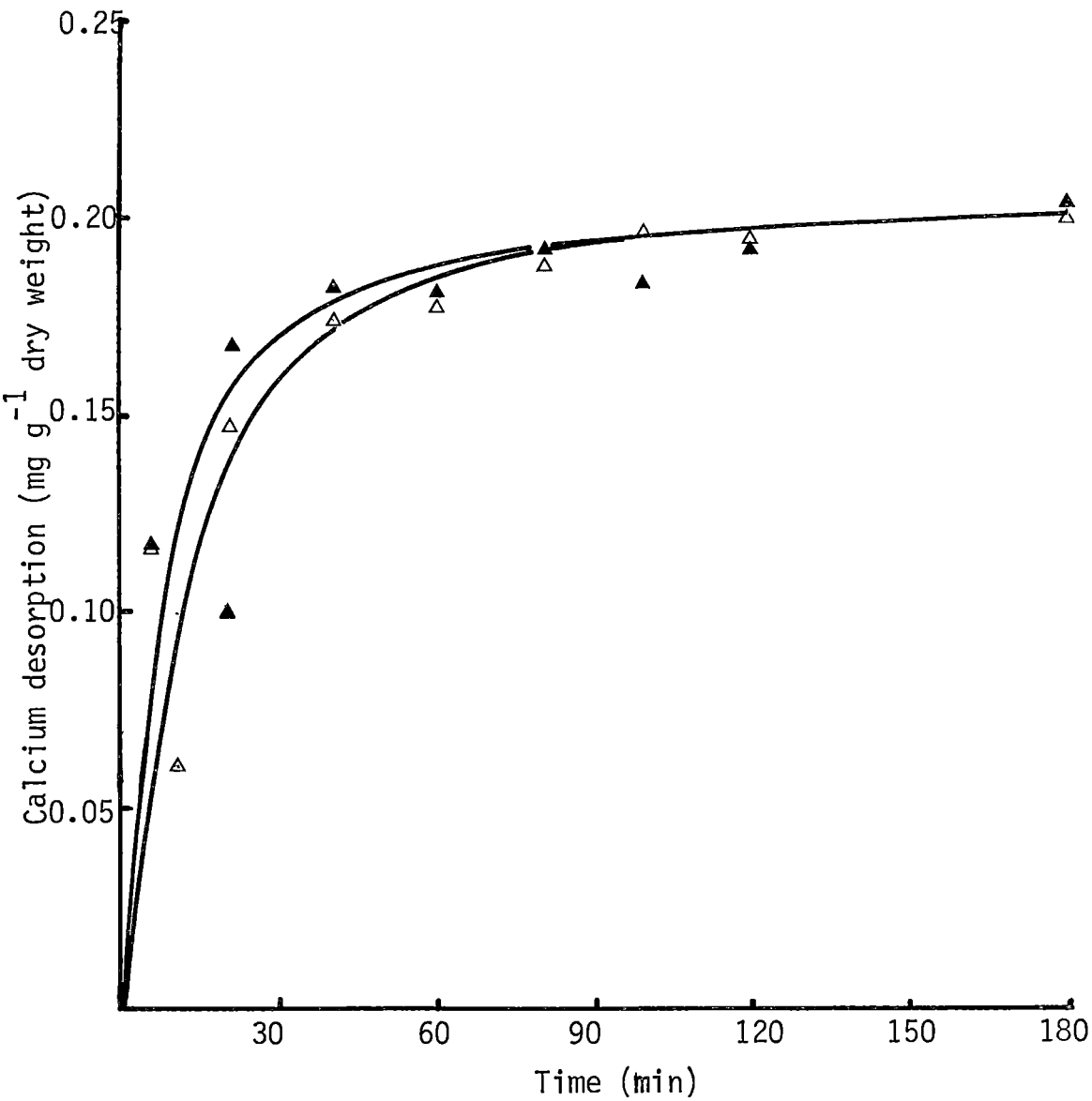

0.25
0.20
0.15
0.10
0.05
Calcium desorption (mg g^{-1} dry weight)
30
60
90
120
150
180
Time (min)

Figure IV.C.1. (ix)

Time course of calcium uptake from 1.0 mM $Al_2(SO_4)$, 0.6737 M $CaCl_2$ at 25°C by excised roots of cabbage —— ——pH 4.2 (△) and pH 4.0 (▲), lettuce – – – – –pH 4.2 (□) and pH 4.0 (■), kikuyu ——— pH 4.2 (○) and pH 4.0 (●).

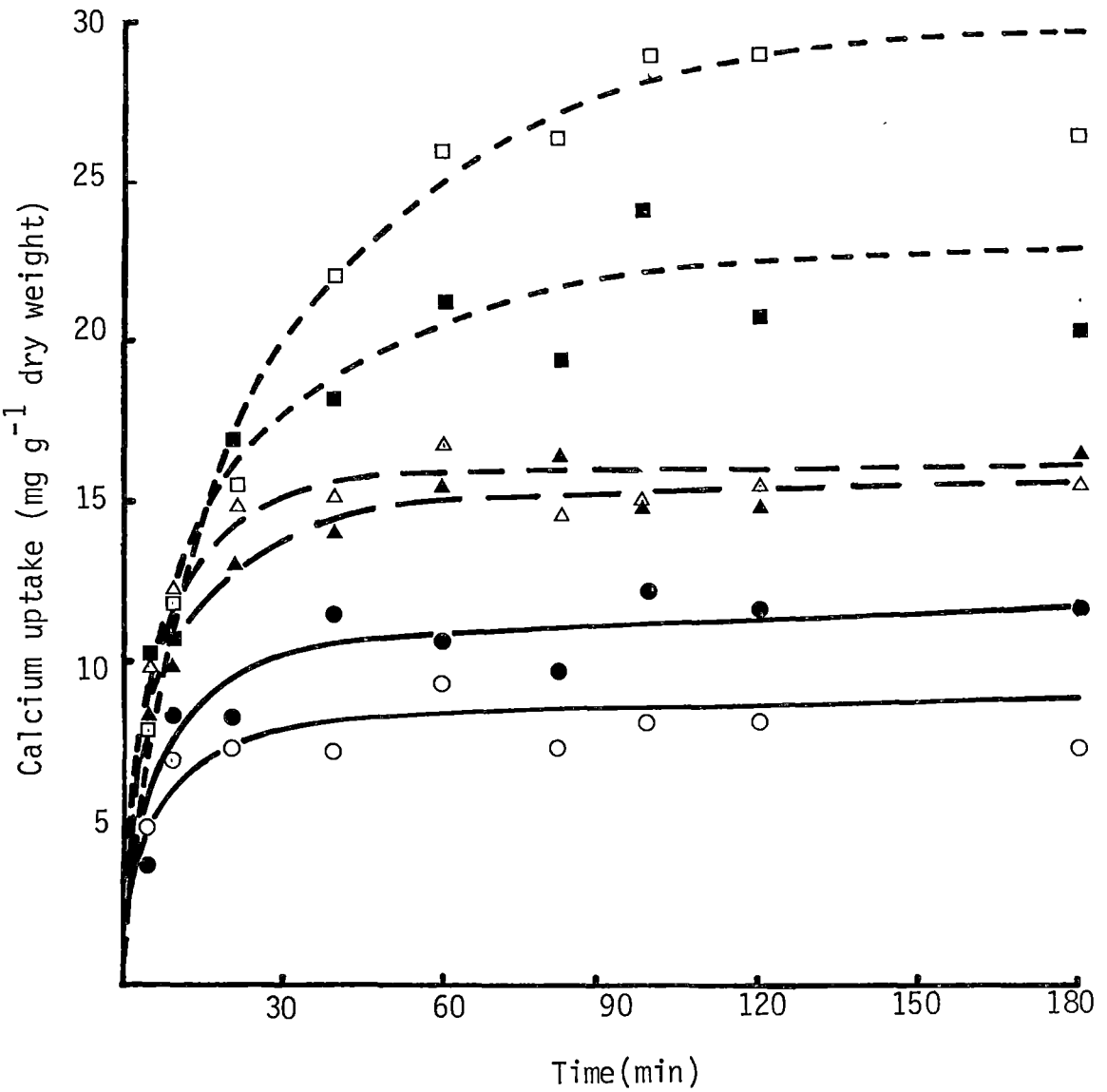
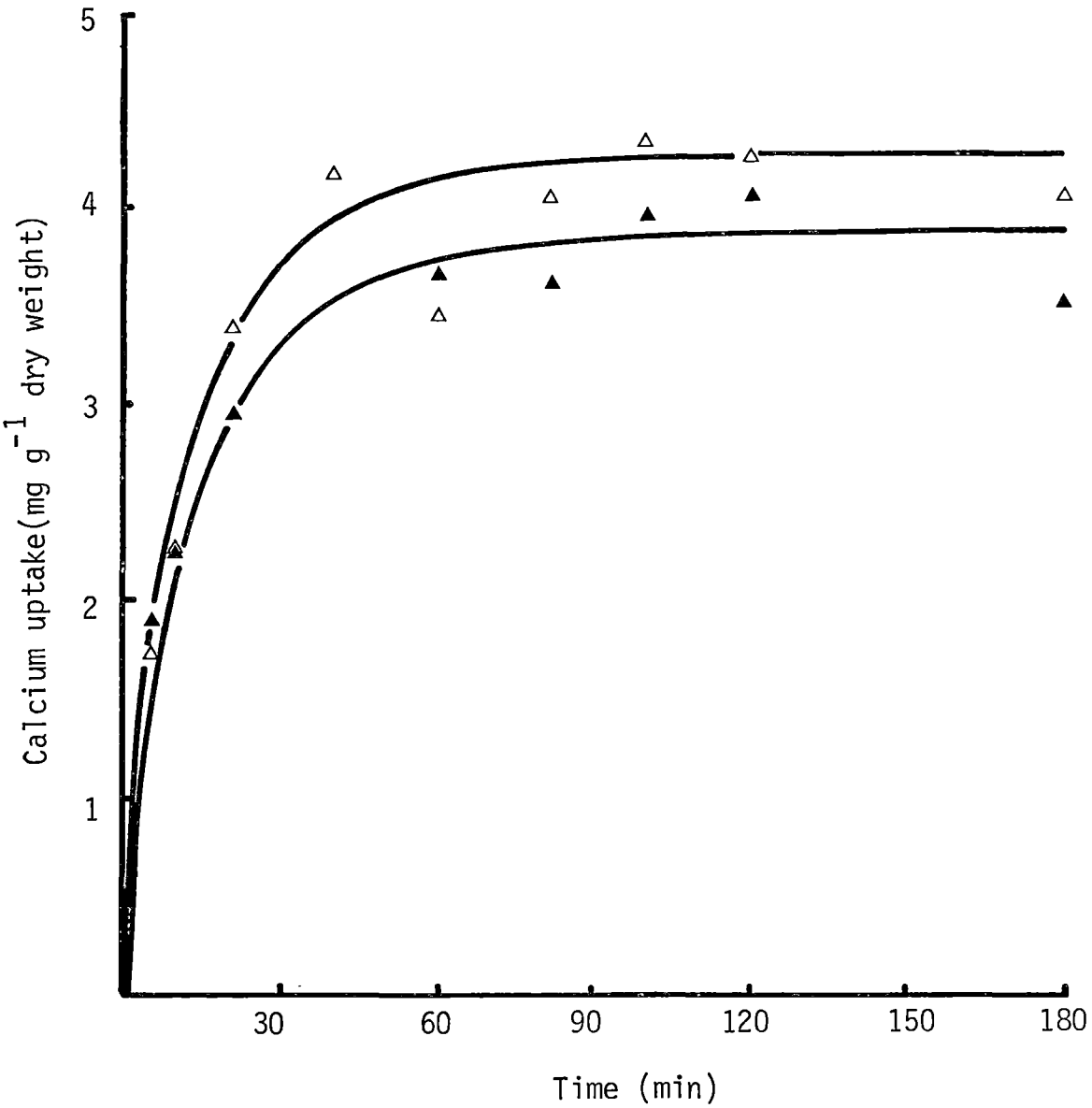

30
25
20
15
10
5
Calcium uptake (mg g^{-1} dry weight)
30
60
90
120
150
180
Time(min)

Figure IV.C.1. (x)

Time course of calcium uptake from 1.0 mM $Al_2(SO_4)_3$, 0.6737 M $CaCl_2$ by Amberlite at 25°C, pH 4.2 (▵) and pH 4.0 (▲).


5
4
3
2
1
Calcium uptake(mg g^{-1} dry weight)
30
60
90
120
150
180
Time (min)

Aluminium uptake was consistently higher at pH 4.2 than 4.0 in all time course experiments irrespective of the calcium concentration of the absorption solution. Where both aluminium and calcium uptake occurred concurrently with the high calcium treatment, pH had no consistent effect on uptake of the latter. Calcium uptake was higher at pH 4.0 than 4.2 for kikuyu, similar for cabbage and the reverse occurred for lettuce (Fig. IV.C.I. (ix)). There was little difference in calcium uptake between pH 4.2 and 4.0 for Amberlite (Fig. IV.C.1. (x)).

IV.C.2. Effect of temperature on aluminium uptake

The effect of a range of temperatures on aluminium uptake was examined from 0-60 min and 60-120 min. These time intervals were chosen to separate Phase I from Phase II absorption. Temperature had little effect on aluminium uptake in the physiological range (1-30^{o}C) during both phases (Figs. IV.C.2. (i) - (ii)). The significantly enhanced uptake at the high temperatures would have resulted from membrane damage. During the 60-120 min phase, aluminium uptake by kikuyu at 40^{o}C remained constant indicating its tolerance to higher temperatures than cabbage and lettuce which showed substantially enhanced uptake.

The ratio of Al absorbed/Ca desorbed reflected the nature of aluminium absorption (Table IV.C.2.). The ratio was higher at pH 4.2 than 4.0 for all species during both uptake phases due to the lower net charge density of aluminium at the higher pH. The ratio was also higher during the 60-120 min phase than the 0-60 min phase except for kikuyu at pH 4.0. As exchange was the dominant process during the first phase (Fig. IV.C.1. (vii)), either alternative or additional processes were operating during the second phase.

Figure IV.C.2. (i)

The effect of temperature on aluminium uptake for a 0-60 min uptake period from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ at 25^oC by excised roots of cabbage —— ——pH 4.2 (△) and pH 4.0 (▲), lettuce — — — — pH 4.2 (□) and pH 4.0 (■), kikuyu ———— pH 4.2 (○) and pH 4.0 (●).

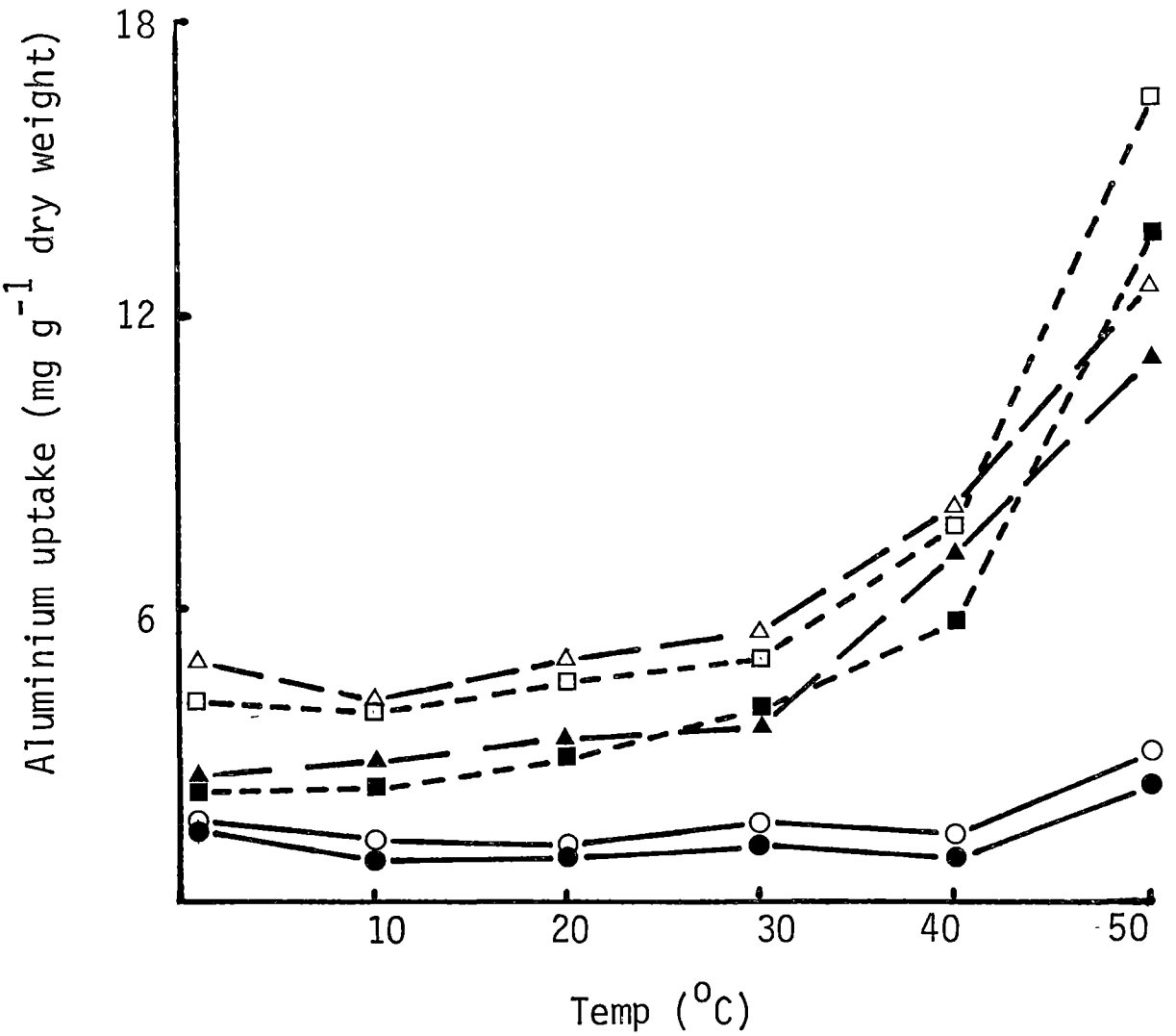

18
12
6
Aluminium uptake (mg g^{-1} dry weight)
10
20
30
40
50
Temp (^{o}C)

Figure IV.C.2. (ii)

The effect of temperature on aluminium uptake for a 60-120 min uptake period from 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ at 25°C by excised roots of cabbage —— —— pH 4.2 (△) and pH 4.0 (▲), lettuce – – – – pH 4.2 (□) and pH 4.0 (■), kikuyu ——— pH 4.2 (○) and pH 4.0 (●).

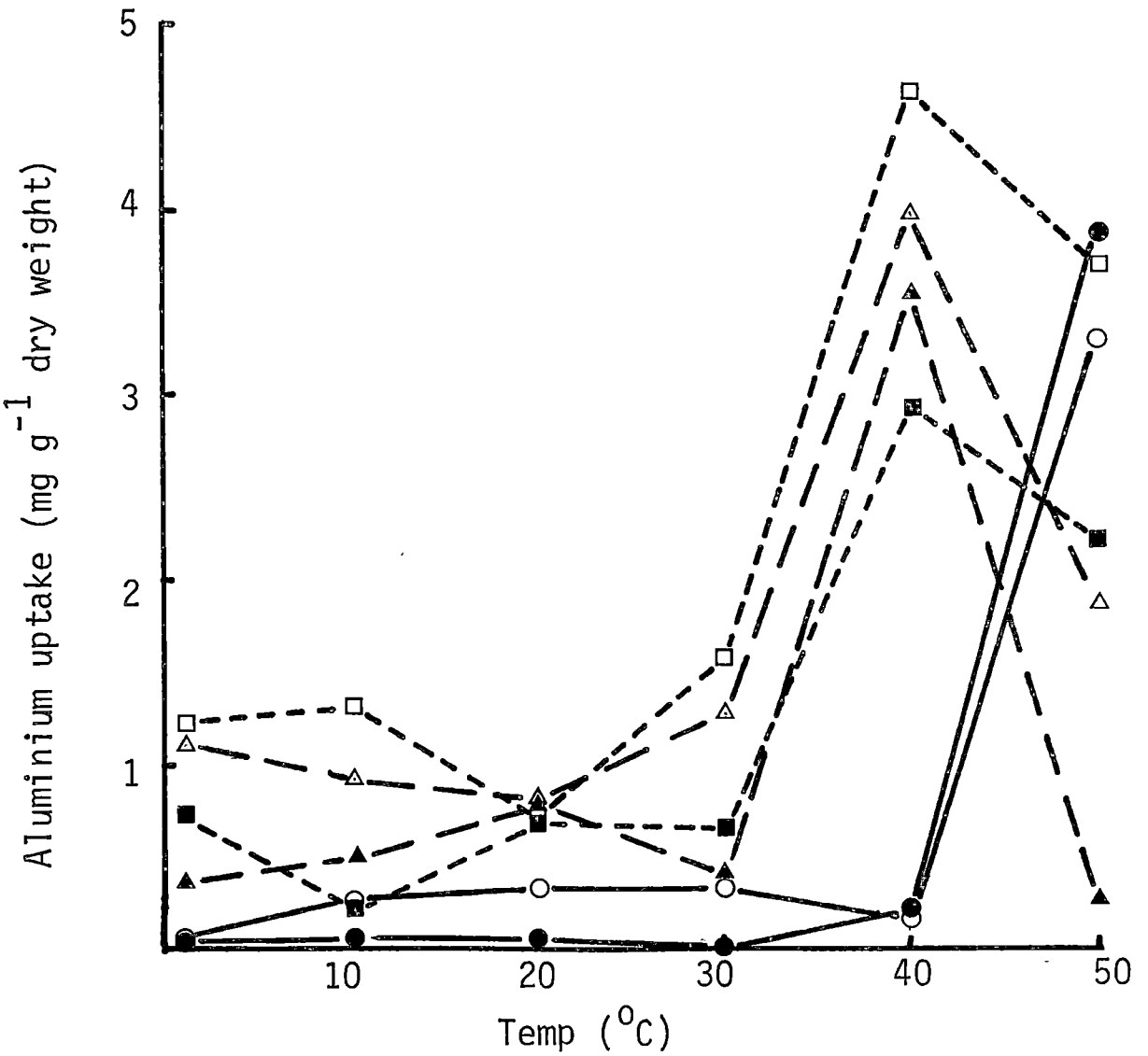

Aluminium uptake (mg g^{-1} dry weight)
5
4
3
2
1
10
20
30
40
50
Temp (^{o}C)

Table IV.C.2.

Mean ratios mg Al absorbed/mg Ca desorbed (1-30°C).

Species	Time (min)	pH 4.2	pH 4.0
Cabbage	0-60	1.35	0.82
	60-120	6.21	4.20
Lettuce	0-60	1.05	0.96
	60-120	3.13	1.22
Kikuyu	0-60	2.43	1.93
	60-120	4.73	1.37

IV.C.3. Desorption of aluminium by buffer

Water removed a small proportion of the aluminium absorbed by all species after a two hour uptake period (Fig. IV.C.3.). However, 22.5 mM succinic-tartaric acids plus triethylamine pH 4.5 which chelates aluminium (Rorison 1965) desorbed a large fraction of the remaining aluminium. There was no further desorption after 120 min suggesting that the small but significant fraction remaining was either irreversibly bound to exchange sites or it had diffused into the protoplasm. The amount desorbed exceeded 75% for all plant species.

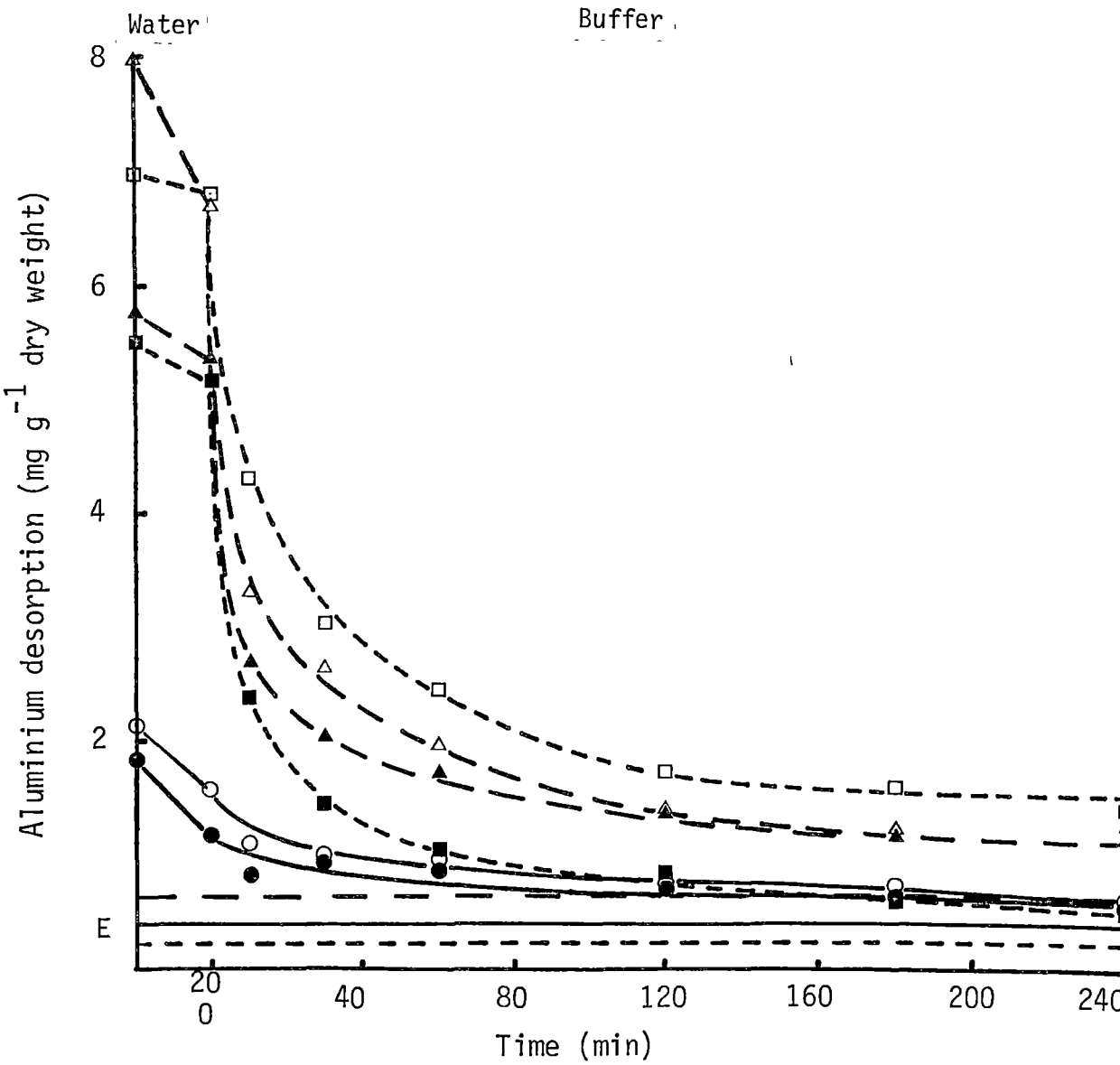
IV.D. Discussion

The time course of aluminium uptake by excised roots of cabbage (Fig. IV.C.1. (i)), lettuce (Fig. IV.C.1. (ii)) and kikuyu (Fig. IV.C.1. (iii)), particularly for the rapid uptake phase, was similar to that reported by Clarkson (1967) for excised barley roots. The similarity in the uptake patterns between excised barley roots and isolated cell wall material led Clarkson to support Rorison (1965) in suggesting that in the initial phase, most of the aluminium becomes bound to adsorption sites in the cell wall. This was supported by the fact that there was some similarity in the aluminium uptake pattern between excised roots and Amberlite and as carboxyl groups are the active exchange sites for the latter, this suggested that carboxyl groups of pectins are involved in cation adsorption by roots.

The difficulty in comparing ion uptake between Amberlite and excised roots is that the carboxyl groups are on acrylic acid for the former with a pKa of 4.25 (Weast 1973) compared with roots

Figure IV.C.3.

Time course of aluminium desorption from excised roots of cabbage —— ——pH 4.2 (△) and pH 4.0 (▲), lettuce – – – – pH 4.2 (□) and pH 4.0 (■), kikuyu ———— pH 4.2 (○) and pH 4.0 (●), and corresponding endogenous (E) aluminium levels. Roots were initially placed in 1.0 mM $Al_2(SO_4)_3$, 0.5 mM $CaSO_4$ at 25°C for 120 min, then desorbed in deionized water at 1°C for 20 min followed by desorption in 22.5 mM succinic-tartaric acids plus triethylamine pH 4.5 at 1°C for periods up to 240 min.


Water
Buffer
8
6
4
2
E
Aluminium desorption (mg g^{-1} dry weight)
0
20
40
80
120
160
200
240
Time (min)

where the active groups are on, for example, glucuronic acid and have a pKa of about 2.8 (Walker and Pitman 1976). The carboxyl groups of Amberlite are almost entirely in the hydrogen form below pH 2.5 (Vogel 1961) and despite its markedly higher C.E.C. than roots adsorbed no more aluminium, presumably because at a pH of 4.0 to 4.2, most of the active groups remained in the hydrogen form.

A pH increase from 4.0 to 4.2 may have resulted in increased dissociation of carboxyl groups and contributed to higher aluminium uptake at pH 4.2 than 4.0 by both Amberlite and roots. Their pKa values indicate that this effect would be far more pronounced for the resin. Moore *et al.* (1961b) showed a negligible effect of a pH increase from 4.0 to 4.2 on non-metabolic calcium uptake (adsorption) by excised barley roots. Most authors have shown a large decrease in charge with an increase in pH (Hsu and Bates 1964; Smith 1971) which would account for significantly higher aluminium adsorption at pH 4.2 than pH 4.0.

There is some evidence which negates the latter argument. Nair and Prenzel (1978) reported that at a pH and aluminium concentration similar to that used in the present study, all the aluminium was present as polynuclear species where net charge increased with increase in pH. However, Hsu and Bates (1964), Hem (1968) and Smith (1971) confirmed that monomeric, polynuclear and solid aluminium hydroxide particles occur between pH 4.0 and 5.0. The formation of the particles and the decrease in net charge is associated with an increase in pH.

If higher aluminium uptake at the higher pH can be attributed to lower net charge of the ions, the number of aluminium equivalents adsorbed would be similar at both pH 4.0 and 4.2. Hence the amount of calcium exchanged should be relatively constant. The higher calcium uptake at pH 4.2, particularly for cabbage, may reflect greater dissociation of carboxyl groups. The differential species response may also reflect different pKa values.

The inability of the high calcium treatments to reduce aluminium uptake by roots (Fig. IV.C1. (v)) was similar to the results of Guerrier (1978) who reported a small reduction, although the Ca/Al ratio of the absorption solution was considerably lower

than that of the present study. The high calcium concentration of 0.6737 M probably resulted in membrane damage to roots and this was reflected in higher aluminium uptake by lettuce than at normal calcium levels. The marked reduction in aluminium uptake by high calcium for Amberlite (Fig. IV.C.I. (vi)), despite differences in pKa between roots and resin, suggests that where membrane damage can be avoided,a high calcium treatment would reduce aluminium uptake by ion exchange. The ability of a high calcium treatment to overcome the inhibitory effect of aluminium on calcium uptake, particularly the absorption phase (Johnson and Jackson 1964; Clarkson and Sanderson 1971) was supported in the present study for both excised roots and Amberlite where the desorption process was reversed to an adsorption process. Calcium uptake was not consistently higher at pH 4.2 than 4.0 in contrast to aluminium, supporting a lower net charge for the latter at the higher pH.

The absorption of aluminium by excised roots apparently involved three components. The first and largest was characterised by exchange-adsorption where aluminium desorbed most of the calcium from roots of all species and Amberlite. C.E.C. did not account for the differences in the amount of aluminium adsorbed by excised roots, supporting Matsumoto *et al.* (1977) who reported that the chemical nature of exchange sites was obscure and C.E.C. did not reflect the extent of aluminium adsorption.

The reduction in calcium levels of roots and tops by aluminium has been widely reported (Munns 1965b; Foy *et al.* 1969; Clark 1977) and is most likely a consequence of the initial aluminium uptake process. Clarkson and Sanderson (1971) studied

the nature of this inhibition with barley and showed from elution experiments that polyvalent cations reduced the amount of calcium held in water free space and Donnan free space and suggested that the basis of the inhibition was exchange with calcium in free space and hence reduction in the amount of calcium available to enter the symplast.

Aluminium uptake does not simply involve adsorption onto exchange sites in the cell wall as suggested by Clarkson and Sanderson (1971) as a small but significant proportion adsorbed by roots could not be desorbed by the organic acid buffer at pH 4.5. This pH should favour dissociation of carboxyl groups and the amount remaining could have resulted from precipitation. Matsumoto *et al.* (1977) could show no distinct association between aluminium and pectins in cell walls and suggested that precipitation of aluminium may have resulted from polymerization of adsorbed hydroxy aluminium monomers due to a pH increase in the free space of the root. Evidence for polymerization of aluminium ions in solution was presented by Hem (1968) and Smith (1971) and supported in whole plant studies by White (1976) and White *et al.* (1976) where the formation of alumino-phosphate polymers of low net charge density accounted for higher aluminium uptake by lucerne roots at pH 5 than 4.5.

Two possible additional uptake components are represented by the small aluminium fraction remaining after desorption in buffer for all species. Aluminium could be irreversibly bound to exchange sites in the cell wall and the fact that Matsumoto *et al.* (1977) could show no distinct association between aluminium and pectins may be due to the small size of this fraction. Passive movement across the plasmalemma would also

account for the non-exchangeable nature of this fraction. The size of this fraction may have been reduced in the desorption study if the buffer had removed aluminium from the cytoplasm.

The steady or near steady state for the second phase of aluminium uptake for cabbage (Fig. IV.C.I. (i)) and lettuce (Fig. IV.C.1. (ii)), which is unlikely to represent exchange-adsorption as no further desorption of calcium occurred after Phase I (Fig. IV.C.1. (vii)), do not represent metabolic uptake because of insensitivity to temperature and a metabolic inhibitor. Cutler and Rains (1974) recorded near linear cadmium uptake with time for short periods and also concluded that uptake was non-metabolic based on the effects of temperature, metabolic inhibitors and oxygen levels on the rate of uptake.

The tolerance of kikuyu to higher temperatures ($40^{o}C$) than cabbage and lettuce reflects the subtropical origin of the former compared with the temperate origin of the latter. Carter and Lathwell (1967) demonstrated active uptake of orthophosphate by maize at $40^{o}C$. Membrane damage at high temperatures would allow passive uptake into the whole root and would account for the high rates of uptake recorded in the present study by cabbage and lettuce at 40 and $50^{o}C$ and kikuyu at $50^{o}C$ (Figs. IV.C.2. (i) - (ii)).

The small magnitude of the second phase for kikuyu (Fig. IV.C.I. (iii)) which is absent for Amberlite (Fig. IV.C.1. (iv)) suggests that little movement of aluminium across the plasmalemma occurred and this may represent a tolerance mechanism. The higher aluminium/calcium ratios for the second uptake phase (Table IV.C.2.) confirm that uptake processes other than exchange-adsorption are involved and

both precipitation through polymerization and passive uptake would account for these higher ratios.

Viets (1944), Epstein (1961) and Van Steveninck (1965) have shown the importance of calcium in maintaining selective ion absorption and cell membrane permeability. The exchange of most of the calcium from roots of cabbage, lettuce and kikuyu by aluminium via the initial uptake process may lead to a loss in plasmalemma permeability and movement of aluminium into the symplast. Support for this proposal comes from Wallace *et al.* (1966) who reported that plants can survive on much lower calcium levels than usually provided in nutrient solutions. The high levels normally found in plants reflect the ability of calcium to ameliorate toxicity of other ions. Henning (1975), working with several wheat cultivars, showed that aluminium entered the stele of roots by passing through meristematic cells, hence bypassing the endodermis. Tolerant cultivars required 100-200 times as much aluminium in the medium as did sensitive cultivars before it penetrated the plasmalemma of meristematic cells, and he concluded that cultivar tolerance was due to differences in molecular structure of the membrane. Klimashevskii *et al.* (1976) similarly concluded that disrupted membrane permeability caused a greater accumulation of aluminium in sensitive pea cultivars.

DNP can lead to an alteration in membrane permeability allowing leakage of inorganic ions (Johnson and Jackson 1964; Hiatt and Lowe 1967; Maas 1969) and metabolites (Maas 1969). Drew and Biddulph (1971) recorded a 30% increase in calcium uptake by bean roots in the presence of 1.0 mM DNP at pH 5.0.

Evidence has been presented in this study for a possible passive component of aluminium uptake into the symplasm as a result of a loss in membrane selectivity due to the exchange of calcium by aluminium. The extent of membrane damage by DNP is enhanced as the concentration is increased and pH reduced (Maas 1969), hence the greatly enhanced aluminium uptake in the presence of 0.2 mM DNP at pH 4.2 and 4.0 would have been due to increased membrane permeability. Ali (1973) reported enhanced aluminium inhibition of seedling root growth of wheat in the presence of DNP from which he concluded that aluminium uptake was non-metabolic. The evidence suggests that the enhanced inhibition would have been due to increased movement of aluminium into meristematic cells due to the effect of DNP on membrane permeability.

The importance of pH in studies involving aluminium uptake was shown where uptake at pH 4.2 was higher than at pH 4.0 due to the effect of increasing pH in reducing the net charge density of aluminium (Hsu and Bates 1964; Hem 1968; Smith 1971). Hence, both the exchange-adsorption and irreversible binding processes would be affected by a small shift in pH.

Cutler and Rains (1974) conducted a similar study to the present one to characterise cadmium uptake by barley roots. They concluded that uptake was characterised by three mechanisms, exchange-adsorption, irreversible sequestering to exchange sites, and diffusion. The observation that cadmium is transported to the shoots of intact plants indicated that it must at some point follow a symplasmic pathway.

V. ALUMINIUM DISTRIBUTION IN ROOTS BY ENERGY DISPERSIVE X-RAY ANALYSIS

TABLE OF CONTENTS

V. ALUMINIUM DISTRIBUTION IN ROOTS BY ENERGY DISPERSIVE X-RAY ANALYSIS

V.A. Introduction

Electron microprobe X-ray (EMX) analyses have frequently been used to determine the localization and distribution of elements in biological material where the energy dispersive system has usually been used in preference to the wavelength dispersive system. The fundamental physical aspects influencing these techniques have been discussed by Coleman (1978). EMX-analyses of the aluminium distribution in plant roots have not been consistent with that expected from the nature of the uptake processes proposed by Clarkson (1967), Clarkson and Sanderson (1969) and supported in the previous section where it accumulated in the free space of the epidermis and cortex with a small amount moving into the stele.

Rasmussen (1968) specifically examined the mode of aluminium entry and its distribution in roots of maize and found that it was precipitated on the surface of epidermal cells with penetration into the cortex and stele only where a lateral root provided a channel of entry. In another study aluminium was found in the cell lumen and not associated with the cell wall (Waisel *et al.* 1970) and in studies with the root tip, it has been located in meristematic cells, mainly associated with cell walls and nuclei (Matsumoto *et al.* 1976a; Keser *et al.* 1977). Evidence for the presence of aluminium in meristematic cells by specific staining has also been supported by Klimashevskii *et al.* (1972) and Keser *et al.* (1977) and is consistent with the results of Henning (1975) who reported that

the endodermis, which offered a partial barrier to lateral passive aluminium movement, was bypassed by entering meristematic cells and thence into the stele.

The co-distribution of aluminium and phosphorus from EMX-analyses, reported by Rasmussen (1968) and Naidoo *et al.* (1978), and interpreted as representing aluminium phosphate precipitation, was not supported by Waisel *et al.* (1970), but supported by McCormick and Borden (1972, 1974) using a specific staining technique.

The aim of the present experiments was to examine the distribution of aluminium in roots of cabbage, lettuce and kikuyu using EDX-analyses. Possible uptake processes to account for this distribution are discussed.

V.B. Methods of Data Presentation

V.B.1. Theory

If an element is present in a sufficiently high concentration in biological material, a peak will be present in the X-ray spectrum corresponding to its principal emission line. The presentation of X-ray spectra has been used to demonstrate the location of elements in a specimen (Chino and Hidaka 1977; Lott and Buttrose 1977; Yeo *et al.* 1977a, b). A visual estimate of peak height has been used to indicate the location and relative concentration of an element throughout a specimen (Chino and Hidaka 1977).

Sample geometry, which affects X-ray generation, is a problem with biological material, particularly where freeze fracturing has been used which invariably leaves an irregular

surface (Yeo *et al.* 1977a, b). However, in both papers the authors considered that comparisons between peak heights of different elements within a spectrum were justifiable, as were comparisons between spectra where large differences existed. This method of interpretation of EMX-data should be treated with caution because X-ray intensity is not only influenced by factors such as absorption and flourescence, but also by atomic number (Coleman 1978). The comparison of peak heights or integrated counts under a particular peak for elements with large differences in atomic number will be difficult without correction as outlined by Buttrose (1978). There may be some justification in comparing peaks for the same element providing a background correction has been made and even then a semi-quantitative interpretation only may be justified where large differences in peak heights or integrated counts exist.

In an attempt to improve the method of data presentation, background levels were estimated for a particular element and subtracted from the total integrated count under the peak and the results expressed as a total peak minus background to background ratio (P_T - B/B = P_A/B) (Sangster and Parry 1976; Van Steveninck *et al.* 1976; Buttrose 1978; Findlay and Pallaghy 1978; Lott *et al.* 1978). Lott *et al.* (1978) indicated that peak minus background to background compensated for variations in sample thickness and differences in sample density. An important additional advantage of this method over the presentation of X-ray spectra to indicate peak heights is that the data can be numerically presented, hence mean values and standard errors can also be presented.

Buttrose (1978) corrected peak (peak minus adjacent background) to background (continuum at K_α 4.94 keV) for P values that compensated for differences in peak heights and total counts between elements when present in equal concentrations (atomic number correction) and found close agreement between these values expressed as a percentage of the total group (of six elements) to the percentage based on chemical analyses. Lott and Buttrose (1978) used a similar method of data presentation except background levels were calculated under actual peaks from an EDIT window programme.

Line scans from EMX-analyses have been used to determine the localization and distribution of aluminium in roots where a peak confirmed its presence (Rasmussen 1968; Waisel *et al.* 1970; Matsumoto *et al.* 1976a, b; Naidoo *et al.* 1978). The variability associated with this method of data presentation placed doubt on some of the interpretations derived from these studies, particularly on the semi-quantitative analyses of Rasmussen (1968).

V.B.2. Methods used in present study

In the present study, the data have been mainly used for qualitative analyses where the distribution of aluminium in particular and phosphorus has been recorded. Peak to background (P_A/B) ratios were calculated for both aluminium and phosphorus largely to facilitate ease of data presentation and to allow means of several values (and treatments) and confidence limits to be presented. The peak to background ratio for a particular element gave an indication of concentration and where large differences in the value existed the interpretation was extended to a semi-quantitative analysis to indicate a possible concentration difference.

The two treatments for each of three experiments are summarised below:

1 ± Al (3 μg ml^{-1}) pH 4.0, Normal (N) Ca

2 ± Al (1 μg ml^{-1}) pH 4.6, Normal (N) Ca

3 ± Al (1 μg ml^{-1}) pH 4.6, High (H) Ca

As the two treatments were identical except for aluminium, the ratio of integrated counts for a 140 eV energy range with the K_α emission line as the centroid (to the nearest 20 eV), corresponding to an aluminium peak when present for the aluminium treatment (+Al) to that for the control treatment (-Al) was calculated for each tissue and each root segment (Section III.F.2.2.).

P_A = intensity of counts due to the element, B = background

$$\text{Ratio} = \frac{P_A + B}{P_A + B} \quad \begin{matrix} \text{- aluminium treatment} \\ \text{- control treatment} \end{matrix} \qquad \text{(1)}$$

For the control treatment $P_A = 0$

It is reasonable to assume that B will be nearly the same in both control and aluminium treatments.

$$\text{Ratio} = \frac{P_A}{B} + 1 \qquad \text{(2)}$$

$$\text{Ratio} - 1 = \frac{P_A}{B} \quad \text{that is peak to background ratio.}$$

Similarly, the ratio of the integrated counts under the silicon peak for the aluminium treatment to that of the control treatment was calculated as per equation (1). Silicon peaks were occasionally present in both aluminium and control treatments. This silicon evidently came from seeds, because no silicon was added in nutrient solutions, and none was detected as a contaminant in specimens prepared for EDX-analyses. The colloidal

graphite - epoxy resin used to mount sections of freeze-dried roots produced a single sulphur peak (Plate V.B.2.).

The mean silicon ratios (equation 1) for all species were close to 1.00 based on nine measurements, three treatments x three root segments (tip. mid, base). The silicon ratios and confidence intervals ($t_{0.05}\ S\bar{x}$) for cabbage, lettuce and kikuyu were respectively 0.97 ± 0.14, 1.10 ± 0.13 and 1.20 ± 0.29. Large deviations in the silicon ratio from 1.00 would be expected to lead to similar deviations in the aluminium ratio. Hence the aluminium ratio (equation 1) was corrected for a silicon ratio of 1.00 and this should lead to smaller errors associated with the aluminium peak to background ratio. The same assumptions apply, that is $P_A = 0$ for control, B can be assumed as being nearly the same for both control and aluminium treatments.

$$\text{Si corrected Ratio} = \frac{P_A}{B} + 1 \qquad \text{——————(3)}$$

$$\text{Si corrected Ratio} - 1 = \frac{P_A}{B}$$ that is, Si corrected peak to background ratio.

Buttrose (1978) estimated the phosphorus background from an adjacent non-peak portion of the spectrum. In the present study this was not possible and the background was estimated by measuring the X-ray counts mid-way between the phosphorus and sulphur peaks using three channels per window and adjusting this value by 7/3 (as phosphorus was measured using 7 channels per window). This would have overestimated the background due to the contribution from the phosphorus and sulphur peaks. Peak to background values were calculated ($P_T - B/B = P_A/B$).

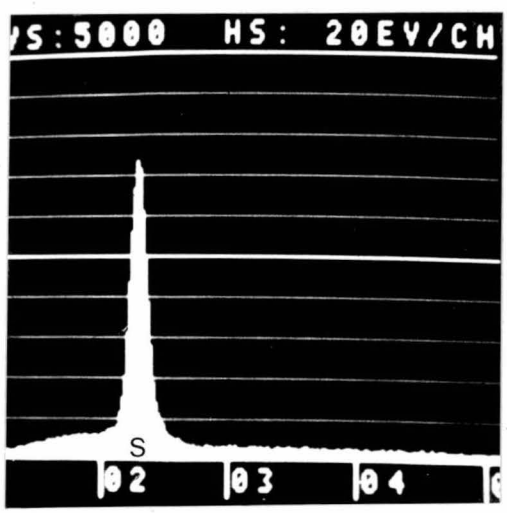


Plate V.B.2.

EDX-spectrum of colloidal graphite - epoxy resin used to mount segments of freeze-dried roots.

V.C. Results

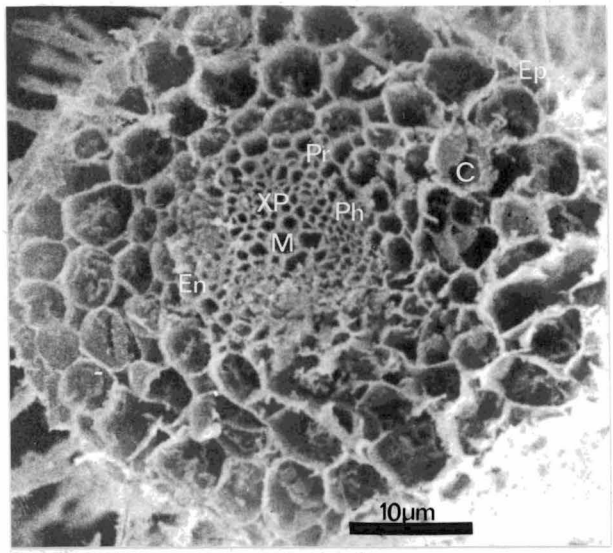
V.C.1. Micrographs of roots

Secondary electron images of the transversely fractured surface of typical freeze dried roots of cabbage, lettuce and kikuyu are presented in Plates V.C.1. (i) - (iii). Specimens were tilted so that the surface was reasonably perpendicular to the electron beam within the limitations imposed by the freeze fracturing technique that resulted in some irregularities in surface topography.

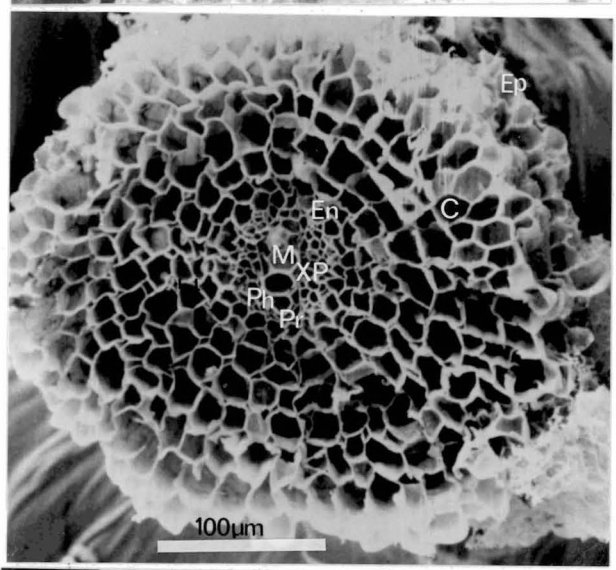
V.C.2. Aluminium distribution in roots

Most of the analyses conducted in the present study were for six treatments (three experiments x two (±Al)) for each of cabbage, lettuce and kikuyu. For each treatment, three root segments (tip, mid, basal) were analysed and for each segment, seven tissues (cell types) were analysed. The aluminium and silicon adjusted aluminium peak to background (aluminium (+)/ control (-) treatments) and phosphorus peak to background ratios (aluminium treatment) for each species and experiment are presented in Appendix II.1.-9. The use of silicon corrected aluminium peak to background ratios reduced the variation for means in most cases.

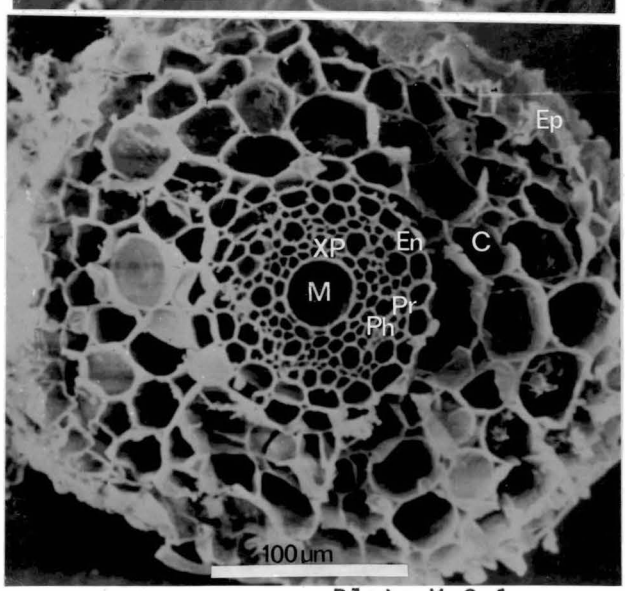
The preferred method of presenting results for aluminium (phosphorus) distribution in roots was to take several readings of the integrated counts for each element on adjacent cells for each tissue and present mean peak to background values. This reduced differences in geometry which can be large when comparing different specimens and improved the precision of the measurements.



(i) cabbage



(ii) lettuce



(iii) kikuyu

Plate V.C.1.

Scanning electron micrographs of transverse sections of freeze-dried roots.

Ep = Epidermis; C = Cortex; En = Endodermis; Pr = Protoxylem; M = Metaxylem; XP = Xylem Parenchyma; Ph = Phloem.

The use of silicon corrected aluminium peak to background ratios also reduced the variation for means in most cases (Appendix II.10.-11.).

In isolated cases the protoplasm remained intact following freeze fracturing and drying and a high degree of precision was achieved by taking several readings on the protoplasm of adjacent cells (Appendix II.12.-13.).

V.C.2.1. Mean aluminium distribution for each species

There was no consistent trend in silicon adjusted aluminium peak to background ratios between tip, mid and basal sections of roots, either within or between species or experiments (Appendix II.1.-9.), hence the mean values and confidence limits for each species have been presented in Table V.C.2.1. Aluminium was present in most tissues with the highest ratios recorded in the epidermis followed by the cortex. These values were markedly higher than that for tissues of the stele although the presence of aluminium in the stele was confirmed for all species.

Linear correlation analyses were performed between phosphorus and silicon corrected aluminium peak to background ratios on the data presented in Appendix II.1.-9. for each species. Correlation coefficients for cabbage, lettuce and kikuyu were -0.13, -0.26 and -0.05 respectively.

V.C.2.2. Specific examples of aluminium distribution

Small sampling errors were involved in the determination of silicon corrected aluminium peak to background ratios for tissues of cabbage (Table V.C.2.2.(i)) and lettuce (Table V.C.2.2.(ii)). Representative EDX-spectra from the aluminium (+) treatment for

Table V.C.2.1.

Silicon corrected aluminium peak to background ratios and confidence limits for tissues of cabbage, lettuce and kikuyu (mean 3 treatments x 3 segments).

Tissue	Cabbage		Lettuce		Kikuyu	
	Mean	$t_{0.05}\ S\bar{x}$	Mean	$t_{0.05}\ S\bar{x}$	Mean	$t_{0.05}\ S\bar{x}$
Epidermis	0.92	0.41	0.61	0.23	0.61	0.40
Cortex	0.46	0.21	0.50	0.30	0.51	0.24
Endodermis	0.28	0.17	0.67	0.23	0.37	0.15
Protoxylem	0.16	0.10	0.23	0.23	0.27	0.19
Metaxylem	0.04	0.07	-0.04	0.21	0.29	0.21
Xylem parenchyma	0.16	0.13	0.24	0.24	0.29	0.17
Phloem	0.15	0.07	0.15	0.21	0.33	0.26

Table V.C.2.2.(i)

Silicon corrected aluminium peak to background ratios and confidence limits for tissues of cabbage ± Al (1) pH 4.6 H Ca, mid root segment.

Tissue	Mean	$t_{0.05}\ S\bar{x}$
Epidermis	1.40	0.26
Cortex	1.05	0.11
Endodermis	0.47	0.09
Protoxylem	0.25	0.06
Metaxylem	0.29	0.08
Xylem parenchyma	0.29	0.05
Phloem	0.29	0.03

Table V.C.2.2.(ii).

Silicon corrected aluminium peak to background ratios and confidence limits for tissues of lettuce ± Al (1) pH 4.6 N Ca, mid root segment.

Tissue	Mean	$t_{0.05}\ S\bar{x}$
Epidermis	1.09	0.13
Cortex	1.49	0.24
Endodermis	0.48	0.08
Protoxylem	0.40	0.05
Metaxylem	0.30	0.05
Xylem parenchyma	0.23	0.10
Phloem	0.19	0.07

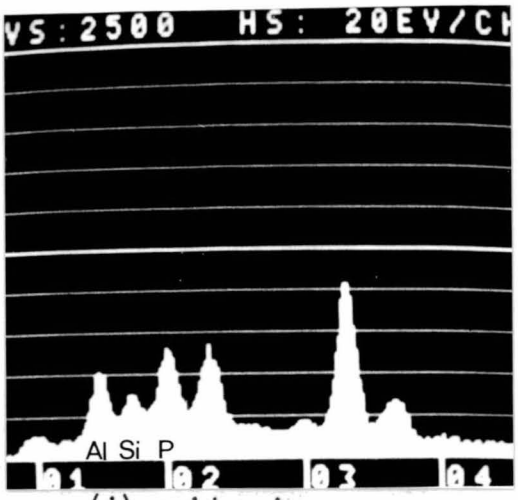
lettuce and from both aluminium (+) and control (-) treatments for cabbage are presented in Plates V.C.2.2.a.(i)-(vi) and Plates V.C.2.2.b.(i)-(vi) respectively. The silicon corrected aluminium peak to background ratios represent the means of 10 analyses (from about three cells) and correspond reasonably well with the height of the aluminium peaks (P_A). Aluminium peaks are absent in control (-) treatments (Plates V.C.2.2.b.(ii), (iv), (vi)). Both species were grown at pH 4.6; lettuce at the normal calcium level and cabbage at the high calcium level.

Aluminium was present in all tissues for both species (aluminium (+) treatments) with the highest ratios in the epidermis and cortex and the lowest ratios in the stele. The ratios for epidermis and cortex were 2-5 times higher than those for tissues of the stele. The presence of aluminium in the stele was confirmed for both species.

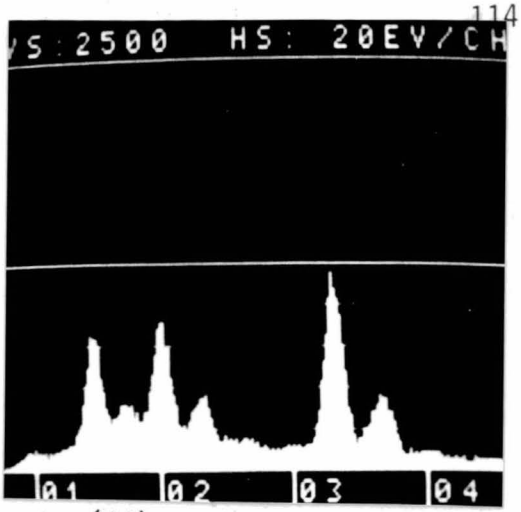
V.C.2.3. Aluminium distribution in protoplasm

Silicon corrected aluminium peak to background ratios for the protoplasm of the cortex and xylem parenchyma cells are presented in Table V.C.2.3.(i). Representative EDX-spectra for aluminium (+) treatments, on which these ratios are based, are presented in Plates V.C.2.3.(i)-(vi). The ratios represent the means of 10 analyses (from about three cells) and correspond reasonably well with the height of the aluminium peaks (P_A). The results were taken from the pH 4.6 ± Al (1) N Ca treatment, mid root segment.

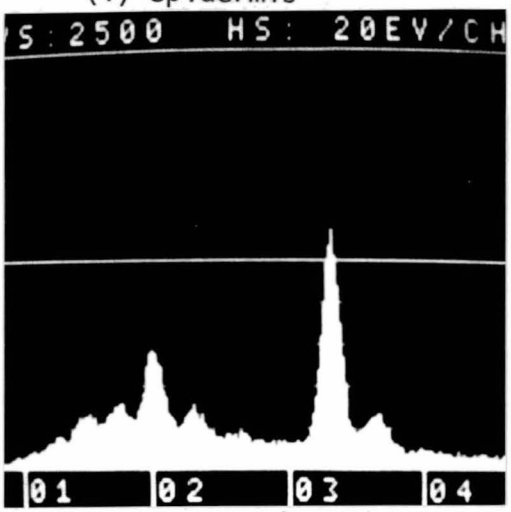
As indicated previously, no treatment effects were evident from the EDX-analyses (Appendix II.1.-9.) and hence mean values have been presented (Section V.C.2.1.). There was one exception



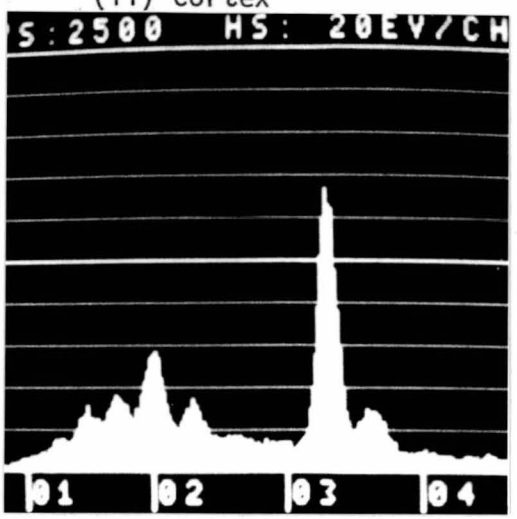
(i) epidermis



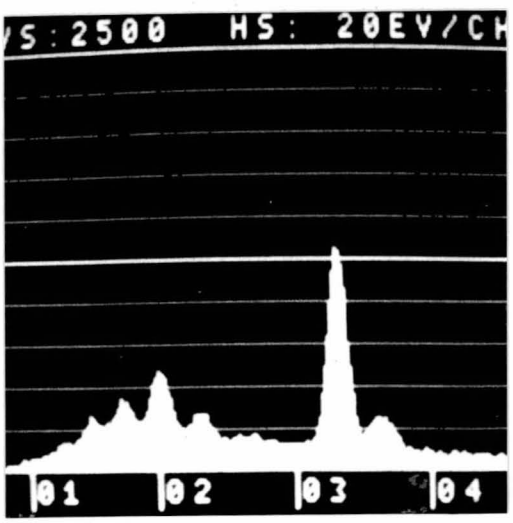
(ii) cortex



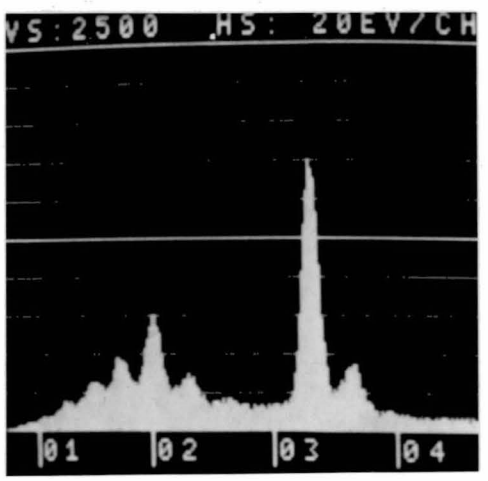
(iii) endodermis



(iv) protoxylem



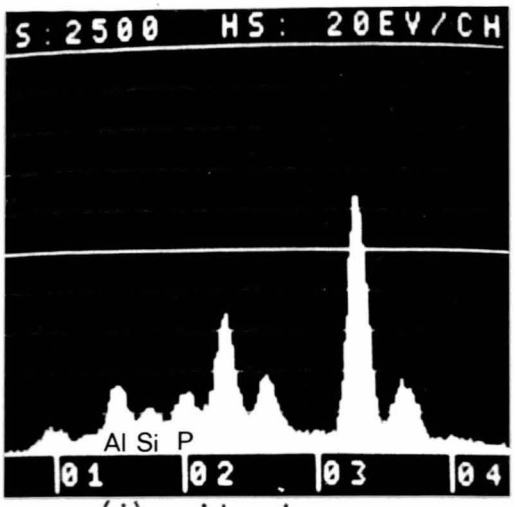
(v) metaxylem



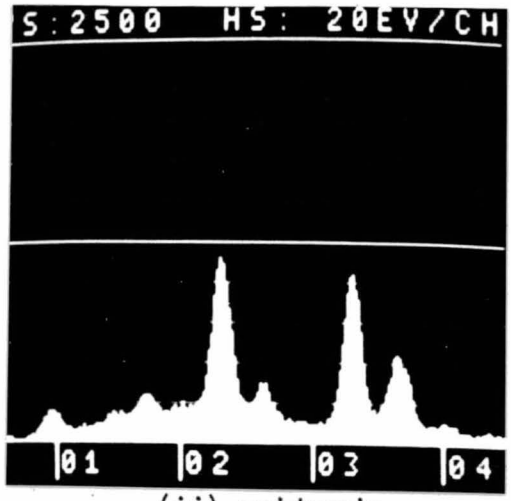
(vi) xylem parenchyma

Plate V.C.2.2.a.

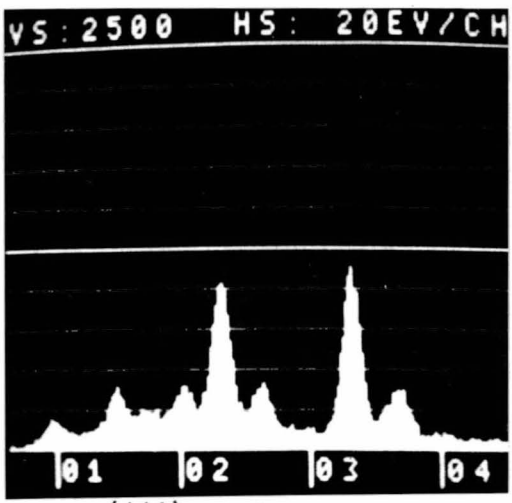
EDX-spectra of tissues of freeze-dried roots for lettuce, aluminium (+) treatment, pH 4.6 N Ca, mid root segment.



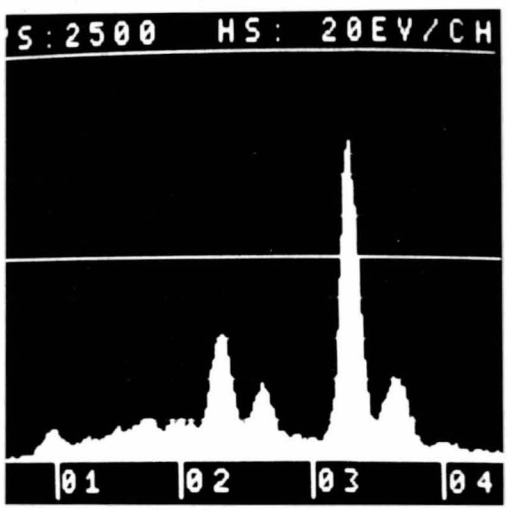
(i) epidermis



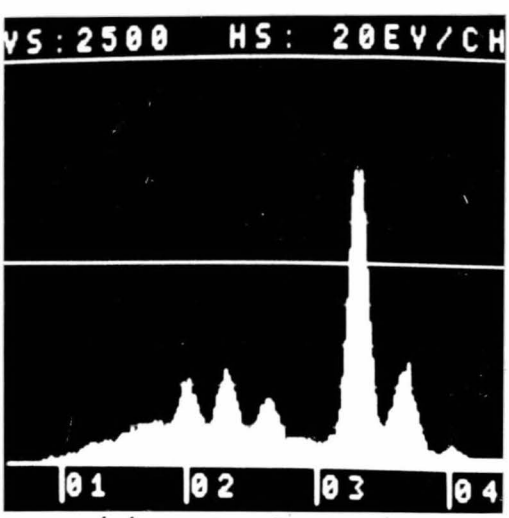
(ii) epidermis



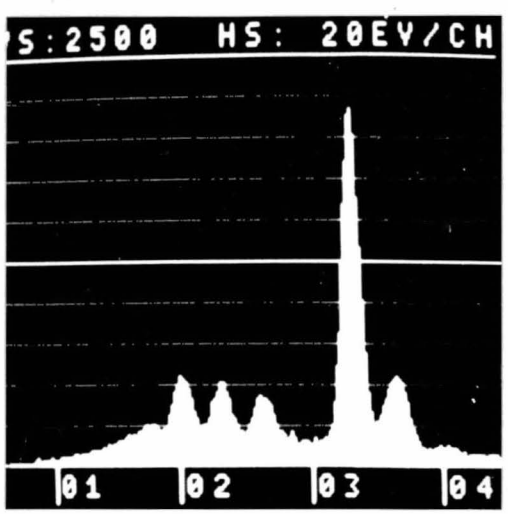
(iii) cortex



(iv) cortex



(v) protoxylem



(vi) protoxylem

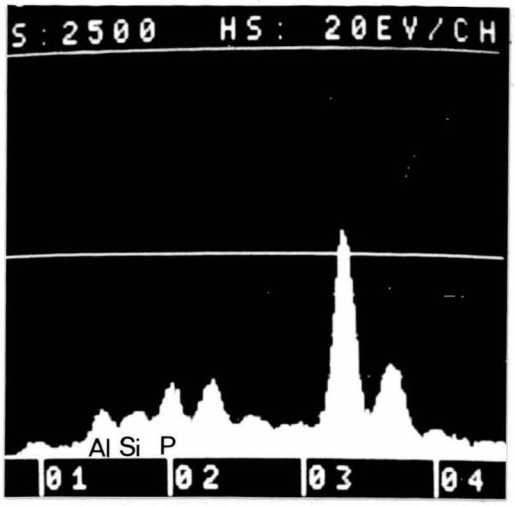
Plate V.C.2.2.b.

EDX-spectra of tissues of freeze-dried roots for cabbage, aluminium (+) (i), (iii), (v), and control (-) (ii), (iv), (vi) treatments, pH 4.6 high Ca, mid root segment.

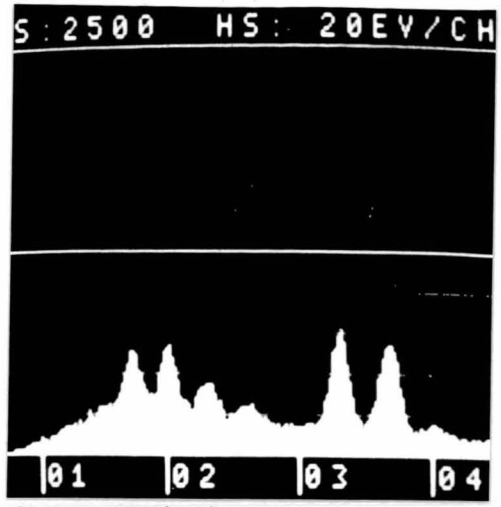
Table V.C.2.3.(i)

Silicon corrected aluminium peak to background ratios and confidence limits for the protoplasm of cortical and xylem parenchyma cells of cabbage, lettuce and kikuyu, ± Al (1) pH 4.6 N Ca, mid root segment.

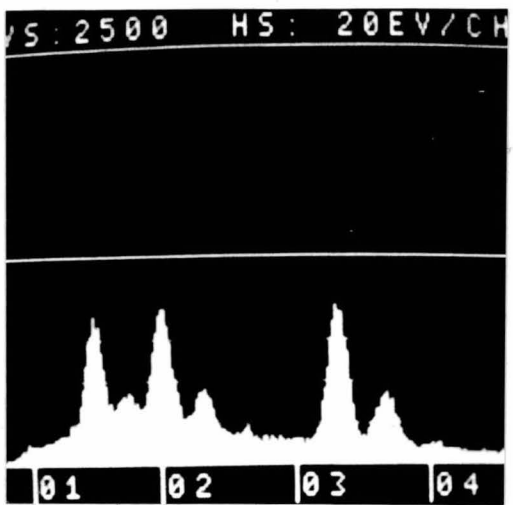
Protoplasm	Cabbage		Lettuce		Kikuyu	
	Mean	$t_{0.05}$ $S\bar{x}$	Mean	$t_{0.05}$ $S\bar{x}$	Mean	$t_{0.05}$ $S\bar{x}$
Cortex	0.34	0.07	0.47	0.16	0.76	0.17
Xylem parenchyma	-0.24	-0.08	0.15	0.09	0.39	0.10



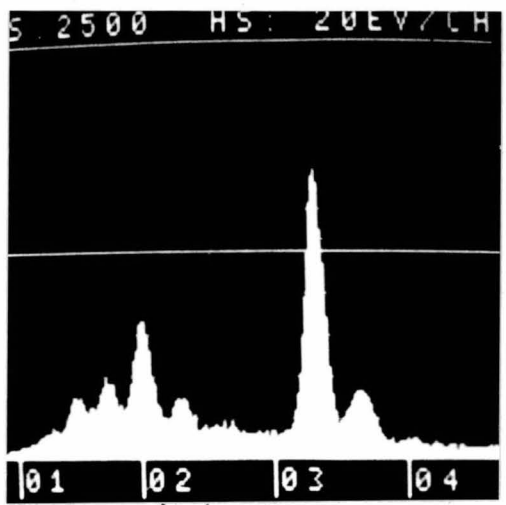
(i) cortex



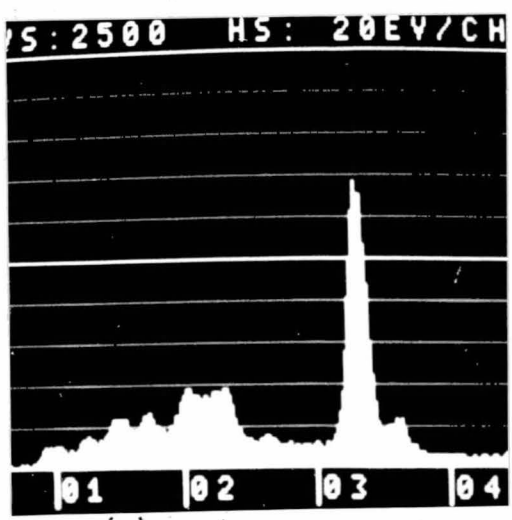
(ii) xylem parenchyma



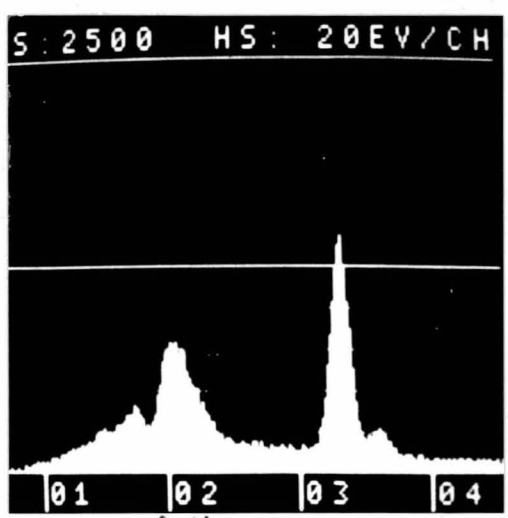
(iii) cortex



(iv) xylem parenchyma



(v) cortex



(vi) xylem parenchyma

Plate V.C.2.3.

EDX-spectra of the protoplasm of cortex and xylem parenchyma cells for cabbage (i), (ii), lettuce (iii), (iv) and kikuyu (v), (vi), aluminium (+) treatment, pH 4.6, N Ca, mid root segment.

where the silicon corrected aluminium peak to background ratios for the protoplasm of cortex and xylem parenchyma cells for each species, mid root segment were compared for the ± Al (1) pH 4.6 N Ca and ± Al (1) pH 4.6 H Ca experiments. High calcium application reduced ($p < 0.05$) the ratio for the protoplasm of the cortex and xylem parenchyma for lettuce and the protoplasm of xylem parenchyma for kikuyu (Table V.C.2.3.ii.).

V.D. Discussion

Rapid freezing of roots, transverse fracturing then freeze-drying the frozen segment produced specimens relatively free from structural distortion (Plates V.C.1. (i)-(iii)) which are comparable to that of a transverse fractured surface obtained for a maize root by Yeo *et al.* (1977b) using fully frozen specimens and a cryostage. Both methods avoided the use of chemical fixation and dehydration and hence retain the distribution and concentration of elements for X-ray microanalysis. Lott *et al.* (1978) demonstrated that glutaraldehyde fixation without subsequent washing or dehydration produced no significant changes in elemental composition of cotyledon globoid crystals and had the advantage over freeze dried tissue powders of a more uniform thickness and somewhat less variability in the EDX-analysis. Freeze fracturing and drying had the advantage of being a simple and very rapid technique and was well suited to the present study where a large number of specimens were prepared at the harvest of each experiment.

As discussed previously, the use of peak to background (P_A/B) ratios have been widely reported in the literature, particularly in recent publications, to indicate the localization

Table V.C.2.3.(ii).

Effect of high calcium on the silicon corrected aluminium peak to background ratios for the protoplasm of cortical and xylem parenchymacells, cabbage, lettuce and kikuyu, ± Al (1) pH 4.6.

Species	Tissue	Mean		p value from computed t value
		N Ca	H Ca	
Cabbage	Cortex	0.34	0.31	0.840
	Xylem parenchyma	-0.24	-0.25	0.320
Lettuce	Cortex	0.94	0.47	0.007
	Xylem parenchyma	0.34	0.15	0.013
Kikuyu	Cortex	0.76	0.78	0.890
	Xylem parenchyma	0.39	0.24	0.008

of elements in specimens and have been used in conjunction with chemical analyses for semi-quantitative analyses. Background estimations by measuring adjacent non-peak portions of the X-ray spectrum (Buttrose 1978) or using computer estimations of background (Lott *et al.* 1978) provided greater precision than the method used in the present study, but the latter was considered acceptable because of the largely qualitative nature of the work.

The use of a silicon correction for the aluminium peak to background ratios can be criticised because the integrated counts under the peak would contain some counts from the adjacent aluminium and phosphorus peaks. However, this is a problem in measuring any element and was not considered a major problem because windows were chosen to include most counts in a peak or non-peak (corresponding to the principal emission line as the centroid). Silicon was absent from the nutrient solution and absent as a contaminant and its distribution should have been relatively unaffected by aluminium as was confirmed by the mean ratios (aluminium (+)/control (-)) being close to 1.00 for all species. Both aluminium and silicon ratios were calculated on identical specimens and hence the many factors contributing to variability in X-ray emission (Coleman 1978) were cancelled out. Silicon corrections did not alter the interpretation of the data but reduced the variability of the aluminium ratios and thus increased their precision.

The present method of interpreting results was considerably better than that used in previous studies involving aluminium distribution in roots (Rasmussen 1968; Waisel *et al.* 1970; Matsumoto *et al.* 1976a; Naidoo *et al.* 1978) where the presence of a peak in the X-ray spectrum indicated the element's presence

and an estimate of peak height indicated relative differences in concentration. Without at least a background correction and preferably numerical presentation as peak to background ratios which corrects for variations in sample thickness and differences in sample density (Lott and Buttrose 1977) the interchange of peak height with concentration is not valid. A statistical comparison is also preferred because of inherent variability in X-ray microanalysis. The errors associated with the peak to background ratios in the present study were small when measurements were taken on adjacent areas of the same specimen and compared favourably with those of Lott and Buttrose (1977).

For all species, aluminium was recorded in the cell walls of the epidermis, cortex, endodermis and tissues of the stele and there was no consistent trend along the entire length of the root (tip, mid, base). These results contrasted with those of Rasmussen (1968) who found that no aluminium penetrated the cortex of maize roots when the epidermis remained intact. Where lateral roots emerged, aluminium was recorded in the cortex and stele. Dumbroff and Pierson(1971) suggested that penetration of the endodermis by a lateral root provided a transient site for mass flow of ions to the stele. This was supported by Ferguson and Clarkson (1975) who showed that the zone of maximum calcium uptake in maize coincided with the zone of lateral root initiation. The presence of aluminium in xylem vessels distal to the zone of lateral root initiation for all species in the present study was evidence that a transient break in the endodermis was not necessary for radial movement of aluminium to the stele.

The markedly higher aluminium peak to background ratios in the epidermis and cortex than the stele should reflect

differences in aluminium concentration. These results are consistent with the processes involved in aluminium uptake where exchange-adsorption in free space, most likely associated with the cell wall, is the dominant process, and a small amount is transported into the stele (Clarkson 1967; Clarkson and Sanderson 1969, 1971). Henning (1975) reported that aluminium was able to bypass the endodermis by penetrating the boundary between the root apex and root cap and accumulated in meristematic and adjacent cells. He concluded that the plasmalemma controlled movement into these cells as the effect could be repeated in both susceptible and tolerant cultivars by adjusting solution aluminium concentrations. Aluminium has also been shown to occur in the protoplasm of cortical cells (Waisel *et al.* 1970), mainly associated with the nucleus (Matsumoto *et al.* 1976a).

If the plasmalemma of meristematic cells became leaky and likewise cortical cells, aluminium could bypass the barrier at the endodermis via the symplasm. This was confirmed by the presence of aluminium in the radial wall of the endodermis and the protoplasm of cortical cells, and to a lesser extent, xylem parenchyma cells. Both passive movement into the symplasm via the cortex and meristematic cells would have accounted for the uniform distribution of aluminium in roots. The significantly lower aluminium ratios in the stele than both epidermis and cortex for all species indicated that the endodermis provided a partial barrier to lateral aluminium transport as proposed by Clarkson and Sanderson (1969).

A significant reduction in the aluminium peak to background ratios of protoplasm for cortical and xylem parenchyma cells, particularly of lettuce by high calcium application, suggested a possible

reduction in aluminium concentration. This implied that calcium reduced passive aluminium movement across the plasmalemma and was consistent with storage root tissue becoming leaky after removal of most of the calcium (Van Steveninck 1965).

The presence of an aluminium phosphate precipitate in roots, mainly in free space, has been reported by several authors (Rasmussen 1968; McCormick and Borden 1972, 1974; Keser *et al.* 1977; Naidoo *et al.* 1978). These authors either used an excessive level of aluminium to pretreat roots followed by a high concentration of phosphate or grew plants in a nutrient solution containing aluminium and phosphate at concentrations exceeding the solubility product data of Munns (1965b) and White (1976). In the present study, where aluminium and phosphate concentrations and pH were controlled to avoid precipitation in the nutrient solution, the correlation between the phosphorus peak to background ratio and the silicon corrected aluminium peak to background ratio for all species was very poor. This suggested that if an aluminium phosphate precipitate did occur in the free space of roots, it was not widespread and it was less likely that the precipitate occurred in the protoplasm. Similarly, Waisel *et al.* (1970), who avoided precipitation in the nutrient solution by using anionic aluminium, found it localized inside the cell-lumen with no correlation between aluminium and phosphorus.

Additional criticism can be levelled against the methods of interpreting results used by Rasmussen (1968) and Naidoo *et al.* (1978), the former using wavelength dispersive and the latter energy dispersive analyses. They concluded that aluminium and phosphorus occurred as a precipitate from the concurrence of

peaks for these elements in a line scan across roots. In the present study, phosphorus was detected in all root tissues and aluminium in most tissues from aluminium treated roots. If an aluminium phosphate precipitate occurred there should have been a reasonable correlation between respective peak to background ratios and this was not the case. Naidoo *et al.* (1978) calculated ratios between aluminium and phosphorus for total integrated counts under the peaks (P_T) and concluded that ratios indicated the relative concentrations of these elements in combination. They made no background and atomic number corrections; hence invalidating their interpretations.

VI. EFFECT OF ALUMINIUM EXCESS ON GROWTH AND NUTRIENT UPTAKE OF PLANT SPECIES IN NUTRIENT SOLUTION

TABLE OF CONTENTS

VI. EFFECT OF ALUMINIUM EXCESS ON GROWTH AND NUTRIENT UPTAKE OF PLANT SPECIES IN NUTRIENT SOLUTION

VI.A. Introduction

Results obtained in previous sections demonstrated that the processes involved in aluminium uptake by plant roots are non-metabolic and lead to its widespread distribution throughout the cortex and stele. The dominant uptake process involves exchange-adsorption which is not markedly affected by a high calcium treatment, supporting the results of Guerrier (1978). A small increase in pH led to an increase in the amount of aluminium adsorbed by roots which tends to confirm the effect of an increase in pH in decreasing the net charge density per aluminium atom (Hsu and Bates 1964; Smith 1971). An extension of the model proposed by Henning (1975) and supported by Klimashevskii *et al.* (1976) whereby the plasmalemma of root cells ultimately controls passive movement of aluminium into the cytoplasm has been presented.

The aim of the present experiments was to examine the effect of aluminium on growth and nutrient uptake of cabbage, lettuce and kikuyu and to extend the interpretation of the processes involved in aluminium uptake, particularly the effect of pH and calcium on the extent of uptake and differential species tolerance to aluminium. As EDX-analyses were conducted on roots obtained from these experiments, the presence of aluminium in the stele of all species confirmed the passive component of aluminium into the cytoplasm detected in the excised root study. Aluminium present in the stele should be available for translocation to tops and the amount reflects the relative aluminium tolerance of some plant species (Foy *et al.* 1967b;Foy *et al.* 1972). Despite the presence of aluminium in xylem vessels of cabbage, lettuce and kikuyu from EDX-analyses, the

inability to quantify these results prevented differentiation between species. The levels in plant tops should reflect the extent to which aluminium is transported into the stele.

Calcium performs an essential role in maintaining selective ion absorption by roots and membrane integrity (Viets 1944; Epstein 1961), hence a high calcium treatment should reduce aluminium transport into the stele of plant roots. Both the excised root and EDX-studies were restricted in their ability to demonstrate this effect. The short term nature and limited application to studying movement into the cytoplasm of the former and the relative imprecision and the inability to quantify data from the microprobe for the latter were the major shortcomings. The whole plant study should complement the interpretations provided by the two previous studies.

VI.B. Experimental Design and Treatments

Each of two treatments per experiment was replicated three times in a completely random design. Each replicate (tray) was divided into four sub-plots. The three experiments and six treatments are summarised below.

1 ± Al ($3\mu g\ ml^{-1}$) pH 4.0, Normal (N) Ca

2 ± Al ($1\mu g\ ml^{-1}$) pH 4.6, Normal (N) Ca

3 ± Al ($1\mu g\ ml^{-1}$) pH 4.6, High (H) Ca

Aluminium was added as $Al_2(SO_4)_3.16H_2O$ to give the appropriate final treatment concentrations. The normal calcium concentration in experiments 1 and 2 was that of 1/10 strength Hoagland's solution. The high calcium concentration in experiment 3 was achieved by adding $CaCl_2.2H_2O$ to give a 500/1 Ca/Al ratio, the same ratio as used in the excised root study.

VI.B.1. Statistical analyses

A large number of measurements was made on each plot in each experiment and a degree of correlation (covariance) can be expected between some of these. This study was mainly interested in independent treatment effects and the data have been analysed accordingly. Analyses of variance for the 14 variables for each of these experiments is presented in Appendix III. Because of the volume of data and the need to compare treatment effects between different experiments, the results have been summarised in Figs. VI.C. 1-14, where the means of each of five treatments have been separately compared with that of the sixth treatment, -Al pH 4.6 N Ca (which has been treated as a control), for each of the 14 variables.

The code used to denote each of the five treatments in each figure is presented below.

a -Al pH 4.0 N Ca

b -Al pH 4.6 H Ca

c +Al pH 4.6 N Ca

d +Al pH 4.0 N Ca

e +Al pH 4.6 H Ca

Treatment comparisons were made using a t test for means of unequal variance (Snedecor and Cochran 1967; pp. 114-5) where the probabilities corresponding to the computed t values have been presented. The 5% level of significance is indicated by horizontal lines on each figure and treatment differences, including a stated increase or decrease resulting from a particular treatment in the text refer to a significance level of $p \leq 0.05$. Additional treatment comparisons are presented in the tables using the same t test as described previously.

VI.C. Results

VI.C.1. Dry weight yield roots

Treatment comparisons of the dry weight yield of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.1. Kikuyu was more tolerant to low pH (4.0) and aluminium than cabbage and lettuce. The yields of cabbage and lettuce were reduced at low pH in both the presence and absence of aluminium. The roots of lettuce were necrotic and very stunted with these two treatments as they were with aluminium at pH 4.6 where yield was also reduced. The only treatment to reduce kikuyu yield, plus cabbage and lettuce, was aluminium at pH 4.0. The extent of reduction was cabbage 59%, lettuce 70% and kikuyu 20%. Plate VI.C.1.a. compares whole plant growth of cabbage and lettuce, ±Al (1) pH 4.6 N Ca.

High calcium application increased the yield for cabbage and lettuce in the presence of aluminium but had no effect for kikuyu (Table VI.C.1.). In the case of lettuce, high calcium overcame the inhibitory effect of aluminium on root yield. High calcium also increased the yield of cabbage and lettuce in the absence of aluminium (Fig. VI.C.1.). Plate VI.C.1.b. compares whole plant growth of cabbage, lettuce and kikuyu, ±Al (1) pH 4.6 H Ca.

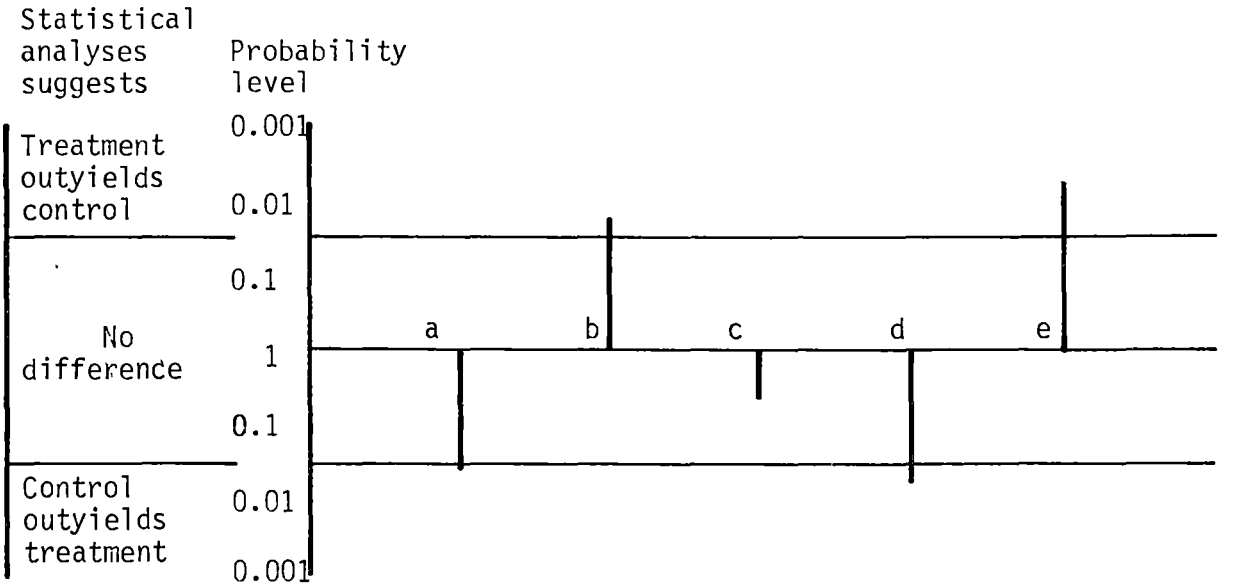
VI.C.2. Dry weight yield tops

Treatment comparisons of the dry weight yield of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.2. Kikuyu was more tolerant to low pH and aluminium application than cabbage and lettuce. The yields of cabbage and lettuce, in contrast to kikuyu, were reduced at low pH in both the presence and absence of aluminium. The yields of all species were reduced at pH 4.6 in the presence of aluminium at the normal calcium level. The extent of reduction was cabbage 27%, lettuce 99% and kikuyu 16%.

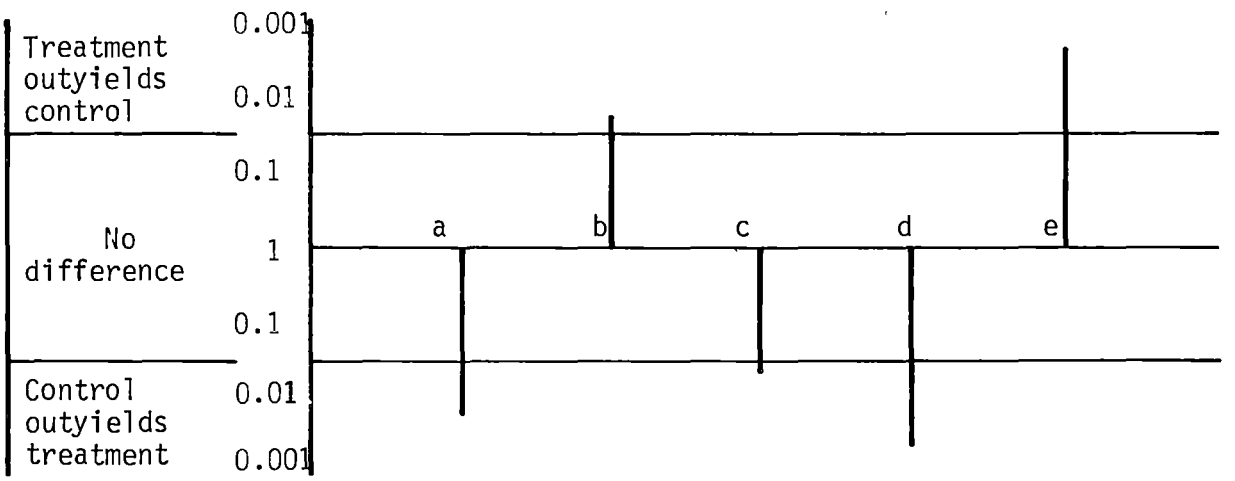
Fig. VI.C.1.

Dry weight yield roots

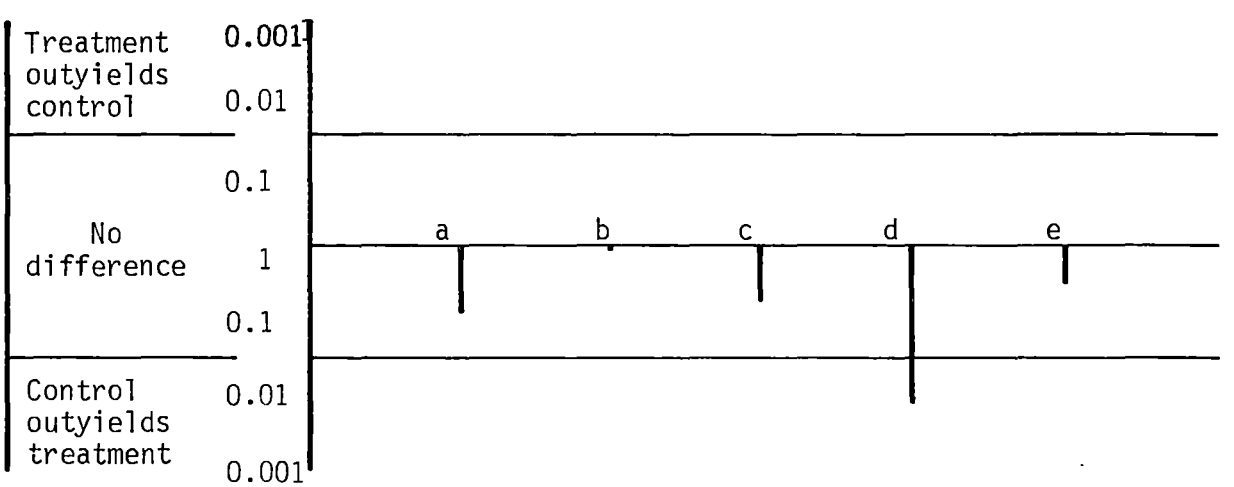
Cabbage



Lettuce



Kikuyu



(i)

(ii)

Plate VI.C.1.a.

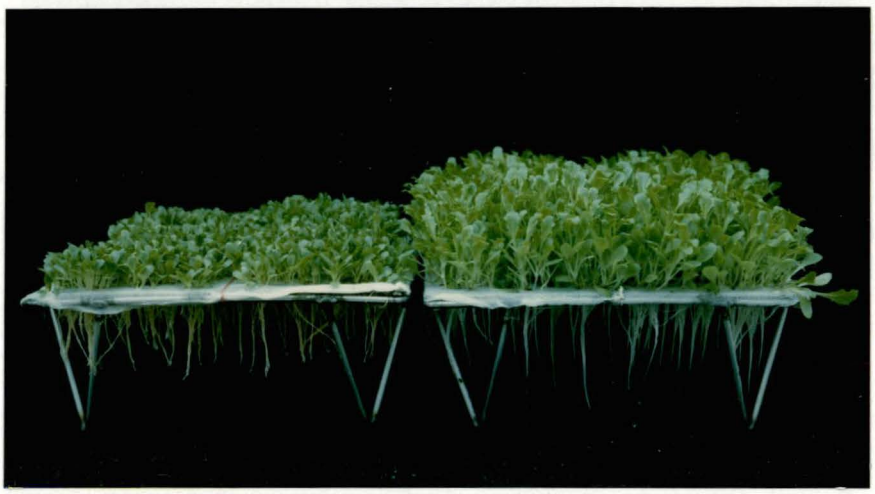
Cabbage (i) and lettuce (ii) grown at pH 4.6 normal calcium; + aluminium (left) and - aluminium (right).

Table VI.C.1.

Effect of high calcium on the dry weight yields of roots and tops (g sub plot^{-1}) for cabbage, lettuce and kikuyu.

Species	Plant part	Treatment (+Al pH 4.6)	
		N Ca	H Ca
Cabbage	Roots	0.0633	0.1509
p value from computed t value		0.001	
	Tops	1.6913	2.3816
p value from computed t value		0.025	
Lettuce	Roots	0.0391	0.1412
p value from computed t value		0.001	
	Tops	0.5379	0.5250
p value from computed t value		0.660	
kikuyu	Roots	0.1488	0.1540
p value from computed t value		1.800	
	Tops	0.7354	0.5911
p value from computed t value		0.004	

(i)



(ii)

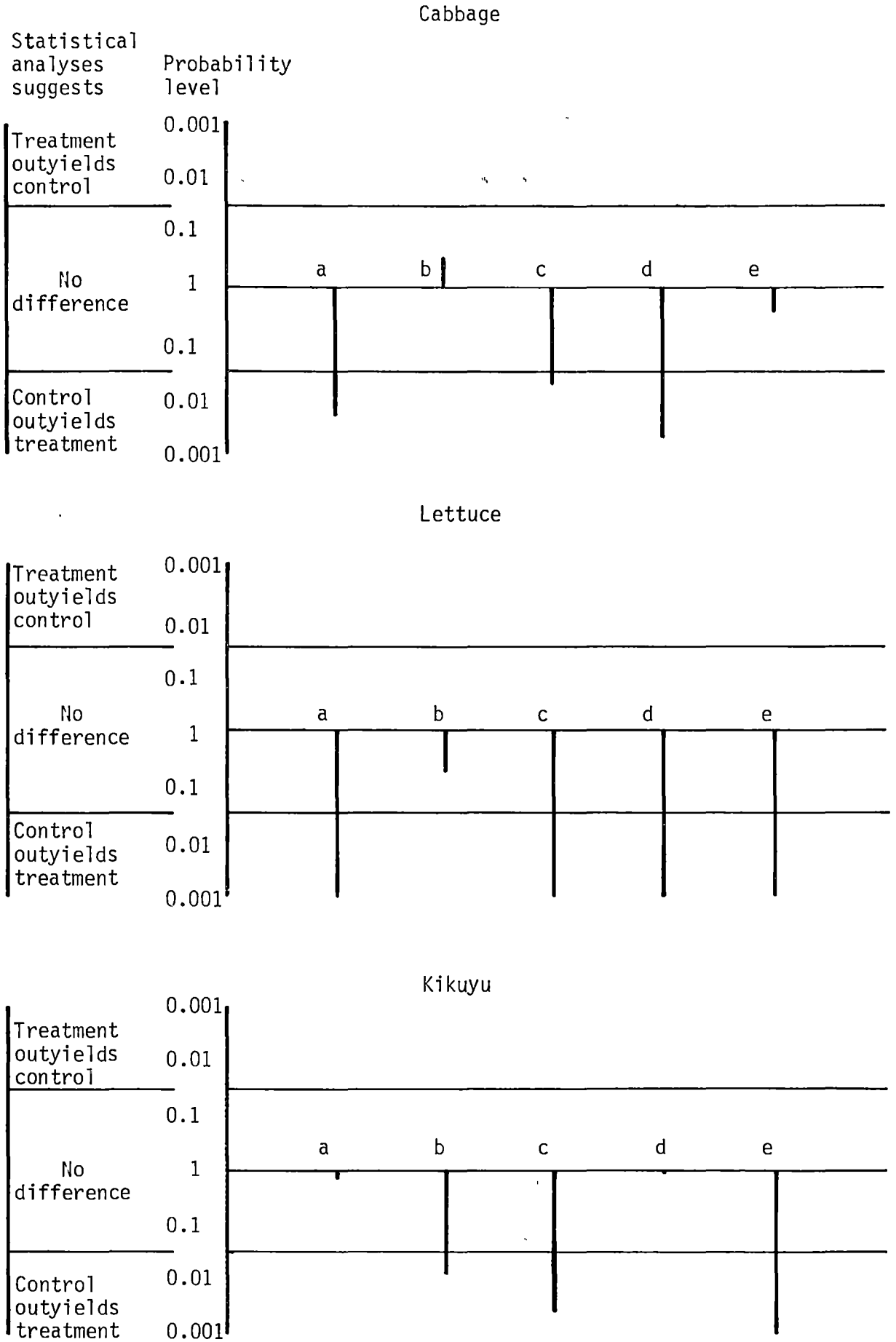
(iii)

Plate VI.C.1.b.

Cabbage (i), lettuce (ii) and kikuyu (iii) grown at pH 4.6 high calcium; + aluminium (left) and - aluminium (right).

Fig. VI.C.2.

Dry weight yield tops



The high calcium treatment overcame the inhibitory effect of aluminium on the yield of cabbage, had no effect on lettuce and further reduced the yield of kikuyu (Table VI.C.1.). The yield of lettuce was reduced by all three aluminium treatments, negating the high calcium effect in the absence of aluminium. High calcium application reduced the yield of kikuyu in the absence of aluminium (Fig. VI.C.2.) which was further reduced in the presence of aluminium (Appendix III 21).

VI.C.3. Aluminium concentration roots

Treatment comparisons of the aluminium concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.3. The aluminium levels of roots were higher at pH 4.6 than pH 4.0 for cabbage and kikuyu (Table VI.C.3.). High calcium application had no effect on these levels except for kikuyu where the aluminium levels were increased.

VI.C.4. Aluminium concentration tops

Treatment comparisons of the aluminium concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.4. The aluminium levels of tops were higher at pH 4.0 than pH 4.6 for cabbage and kikuyu (Table VI.C.3.). High calcium application reduced levels for all species.

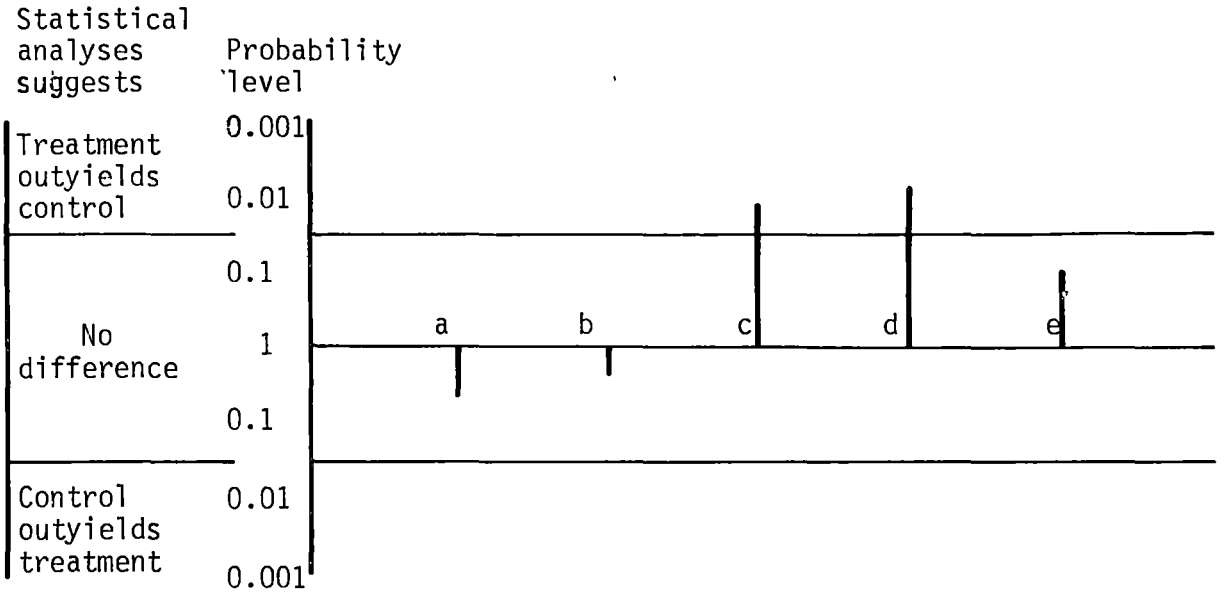
VI.C.5. Calcium concentration roots

Treatment comparisons of the calcium concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.5. Low pH and aluminium reduced the calcium levels for all species except where low pH had no effect on lettuce levels. High calcium application increased the levels for all species in the absence of aluminium and overcame the inhibitory effect for cabbage and lettuce in its presence.
A comparison of the calcium levels of roots and tops for cabbage, lettuce and kikuyu for the control treatment is presented in Table VI.C.5. The levels of roots for kikuyu were lower than those for cabbage and lettuce by 512% and 298% respectively.

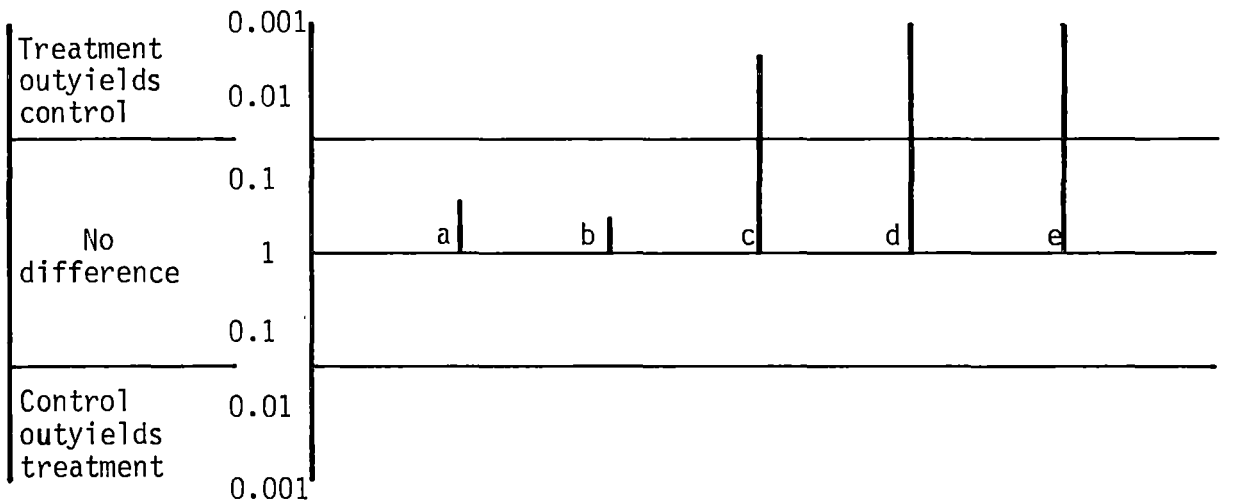
Fig. VI.C.3.

Aluminium concentration roots

Cabbage



Lettuce



Kikuyu

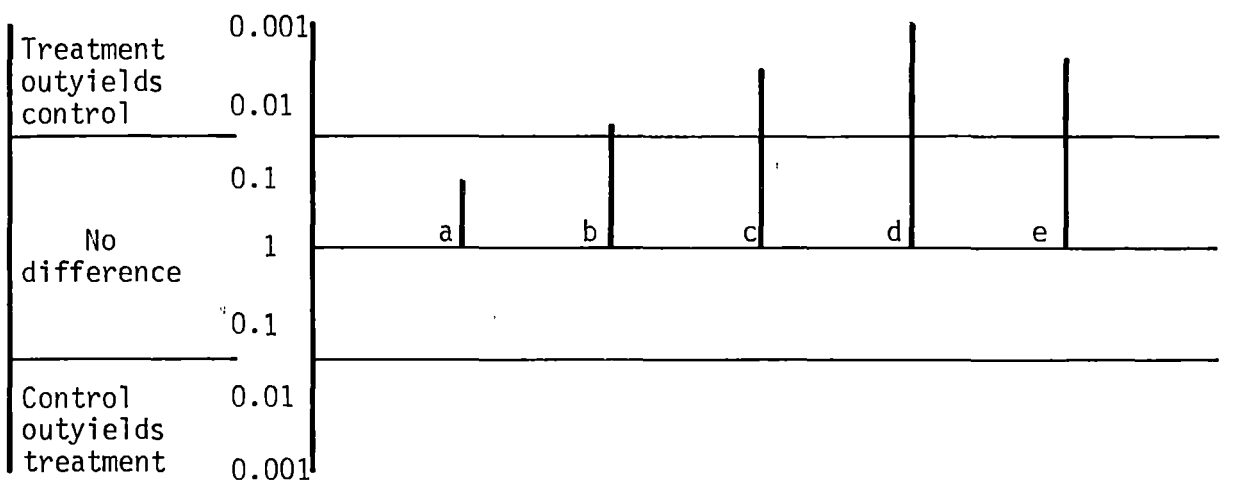


Table VI.C.3.

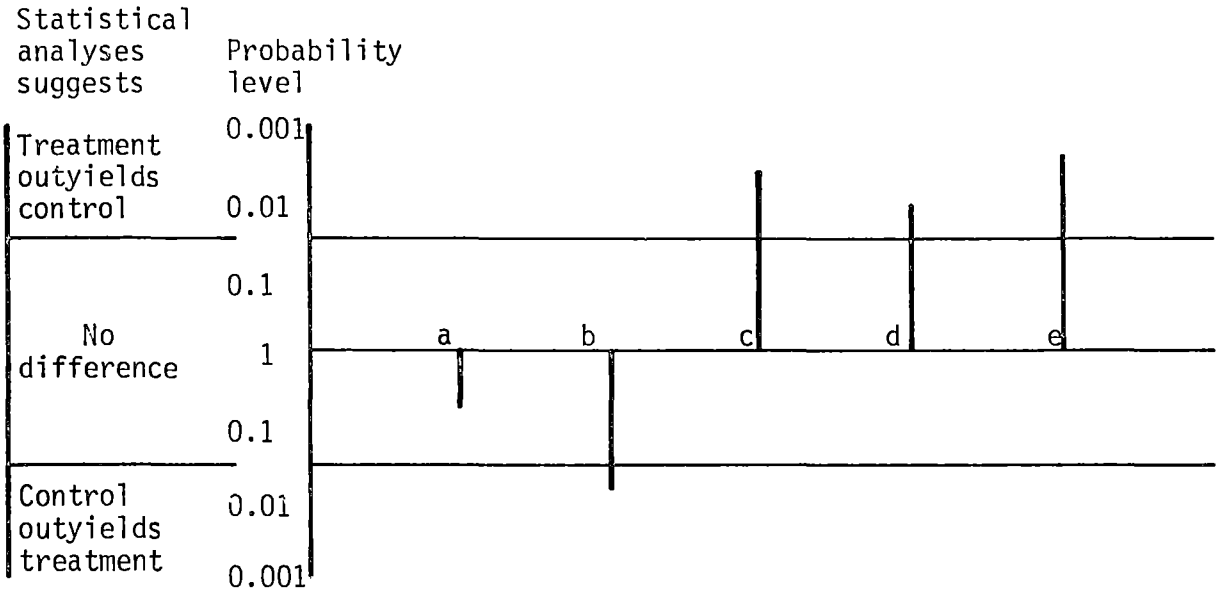
Effect of pH and high calcium on aluminium concentrations of roots and tops ($\mu g\ g^{-1}$ dry weight) of cabbage, lettuce and kikuyu.

Species	Plant part	Treatment (+ Al)		
		pH 4.0 N Ca	pH 4.6 N Ca	pH 4.6 H Ca
Cabbage	Roots	9439	18297	14132
p value from computed t value		0.008	0.130	
	Tops	572	288	93
p value from computed t value		0.014	0.013	
Lettuce	Roots	6410	8747	5530
p value from computed t value		0.150	0.072	
	Tops	644	449	241
p value from computed t value		0.150	0.020	
Kikuyu	Roots	5658	16401	20362
p value from computed t value		0.008	0.018	
	Tops	272	111	44
p value from computed t value		0.000	0.007	

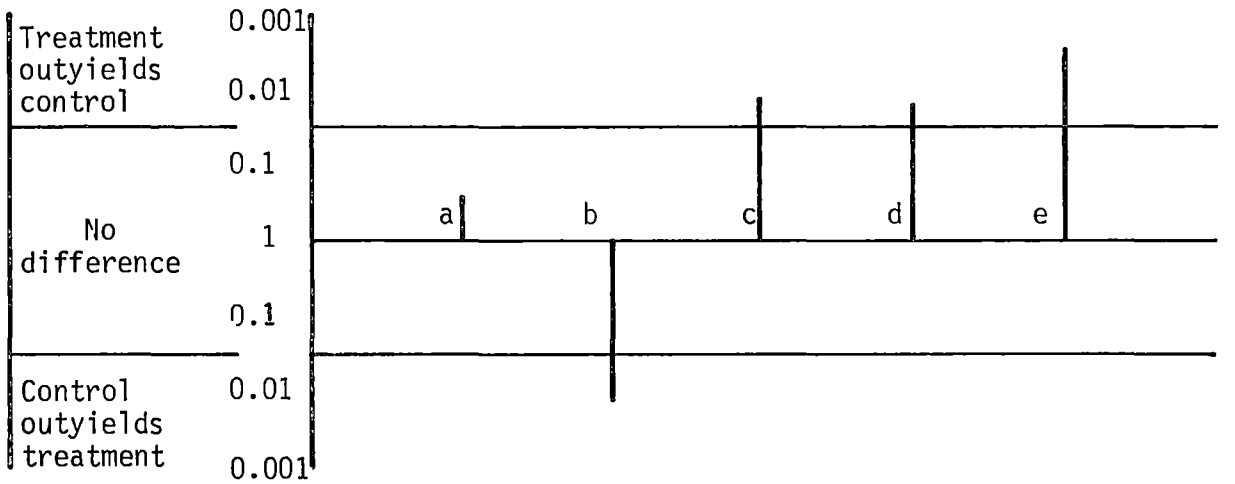
Fig. VI.C.4.

Aluminium concentration tops

Cabbage



Lettuce



Kikuyu

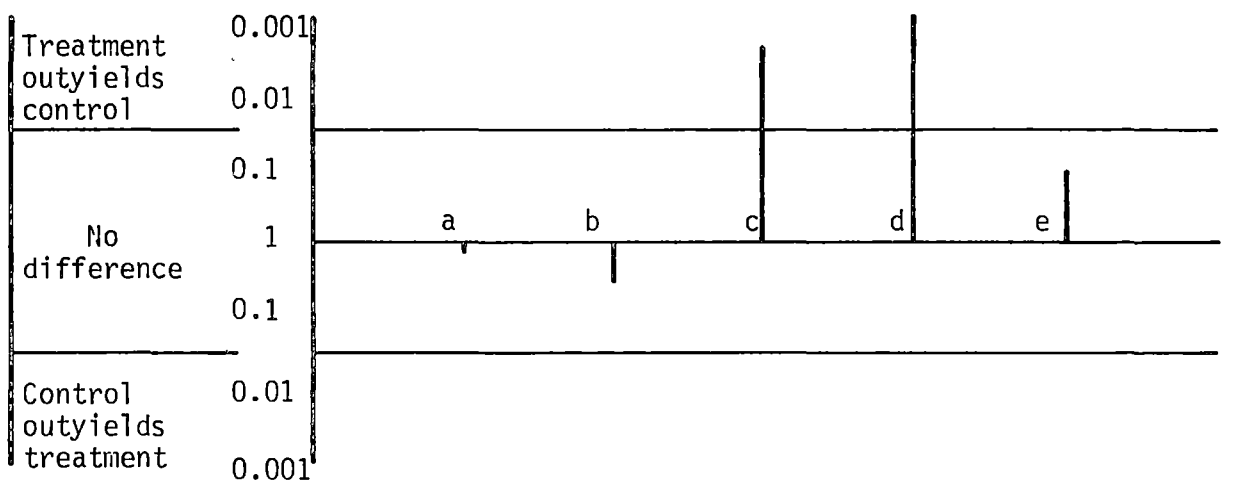
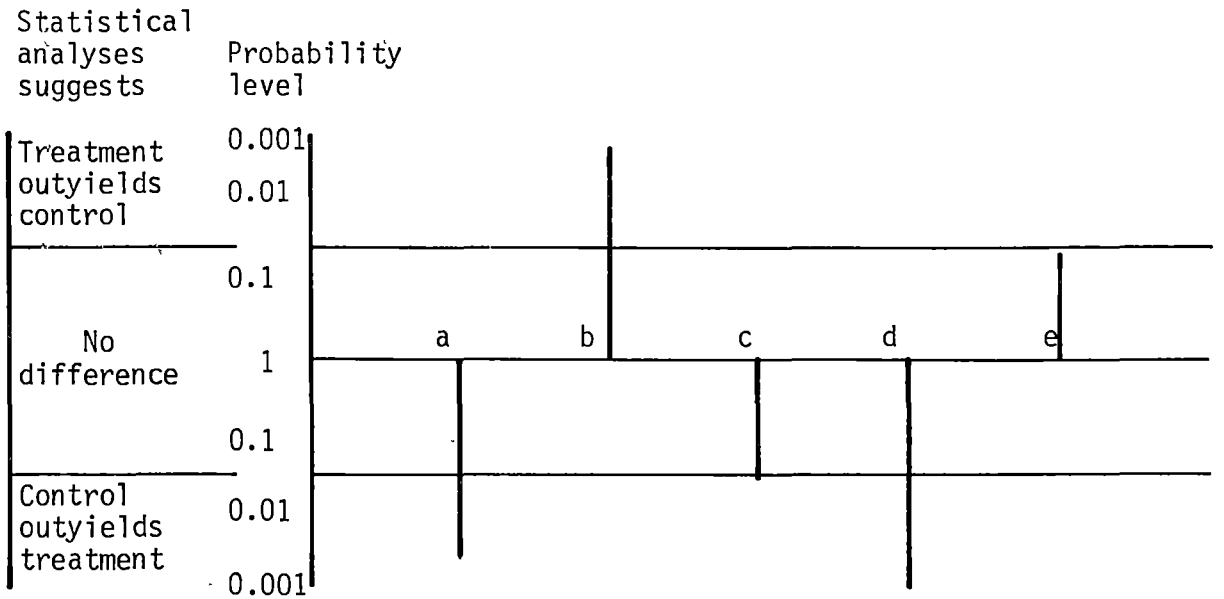


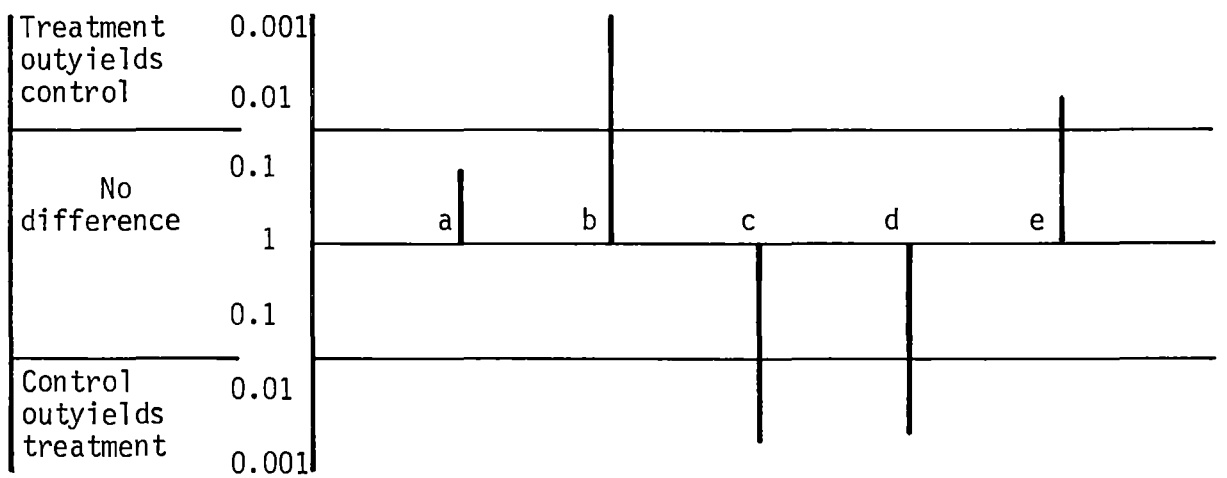
Fig. VI.C.5.

Calcium concentration roots

Cabbage



Lettuce



Kikuyu

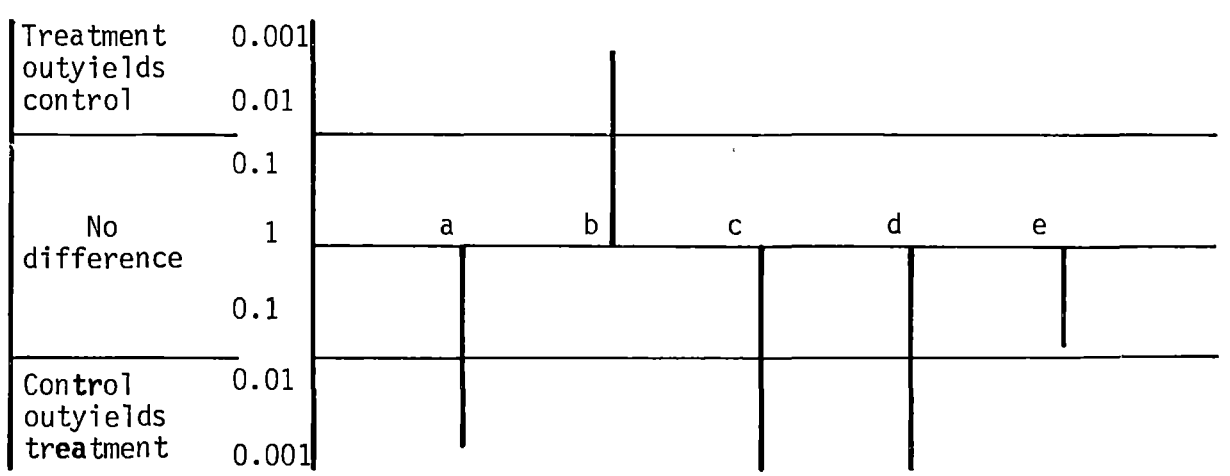


Table VI.C.5.

Comparison of calcium concentrations of roots and tops (% dry weight) for kikuyu with cabbage and lettuce, -Al pH 4.6 N Ca.

Plant part	Species		
	Cabbage	Kikuyu	Lettuce
Roots	0.300	0.049	0.195
p value from computed t value		0.000	0.000
Tops	2.110	0.348	0.651
p value from computed t value		0.000	0.000

VI.C.6. Calcium concentration tops

Treatment comparisons of calcium concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.6. Low pH and aluminium reduced the calcium levels of tops for all species except where aluminium at pH 4.6 had no effect on kikuyu levels. High calcium application increased the levels in both the presence and absence of aluminium and overcame the inhibitory effect of aluminium for cabbage and lettuce. As for roots, the calcium levels of tops for kikuyu were lower than that for cabbage and lettuce (Table VI.C.5.) by 507% and 87% respectively.

VI.C.7. Magnesium concentration roots

Treatment comparisons of magnesium concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.7. Low pH and aluminium reduced the magnesium levels of roots for cabbage and kikuyu except where aluminium at pH 4.0 had no effect on kikuyu levels. High calcium application reduced the levels for all species in both the presence and absence of aluminium.

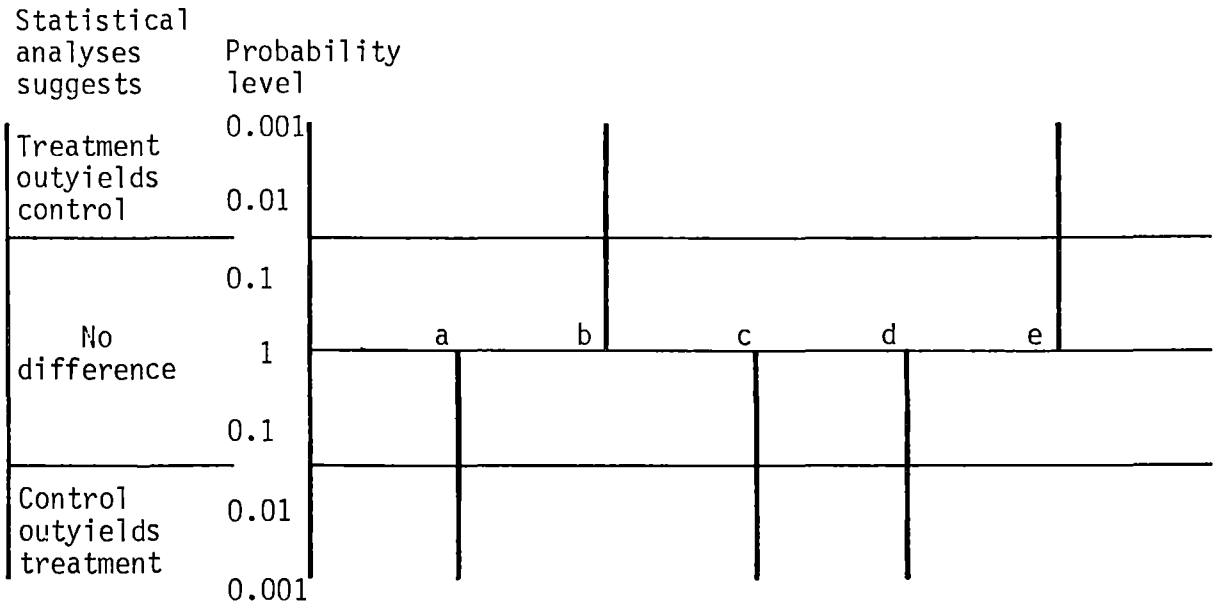
A comparison of the magnesium levels of roots and tops for cabbage, lettuce and kikuyu for the control treatment is presented in Table VI.C.7. The levels of roots for kikuyu were higher than that for cabbage and lettuce by 338% and 768% respectively.

VI.C.8. Magnesium concentration tops

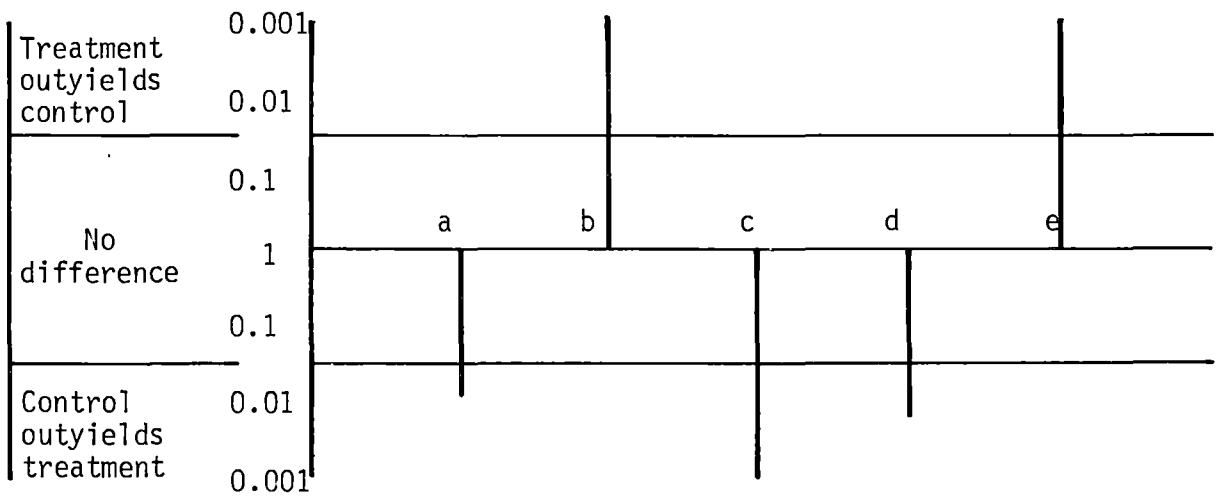
Treatment comparisons of magnesium concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.8. Low pH, aluminium and high calcium application reduced the magnesium levels for all species.

Fig. VI.C.6.
Calcium concentration tops

Cabbage



Lettuce



Kikuyu

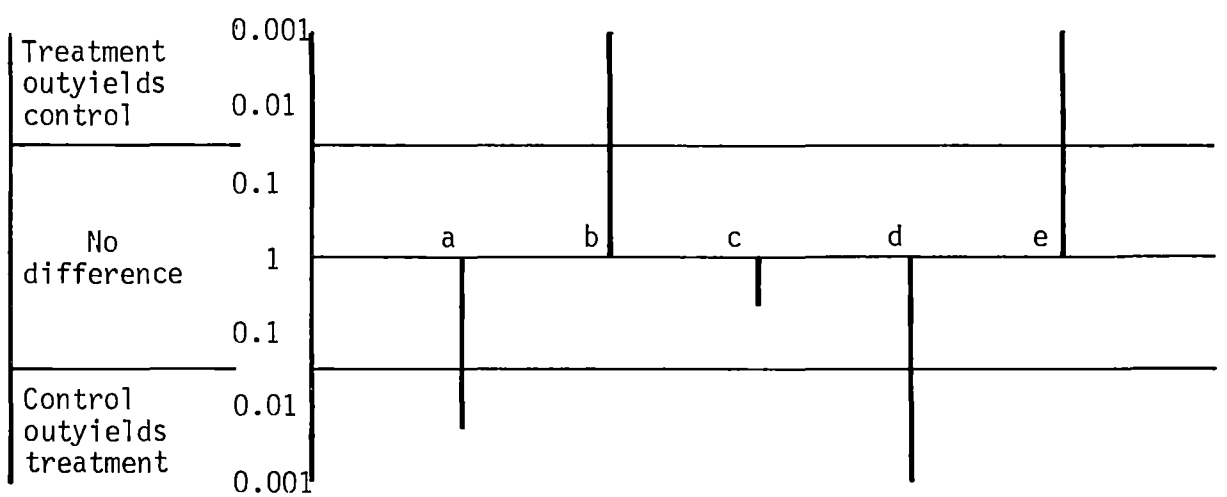
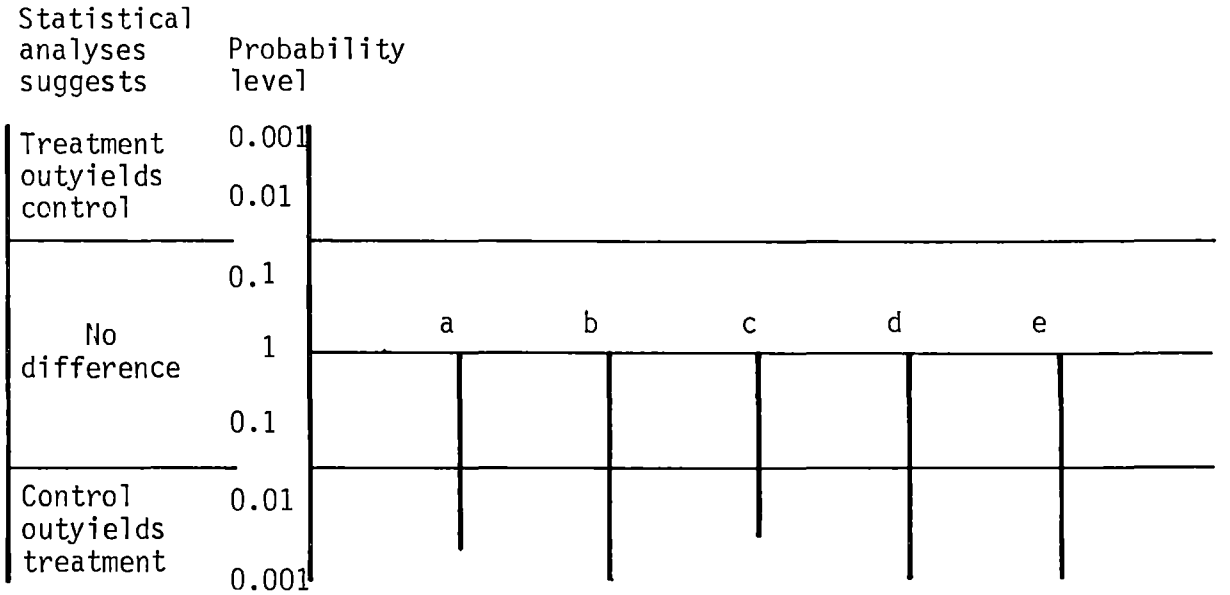
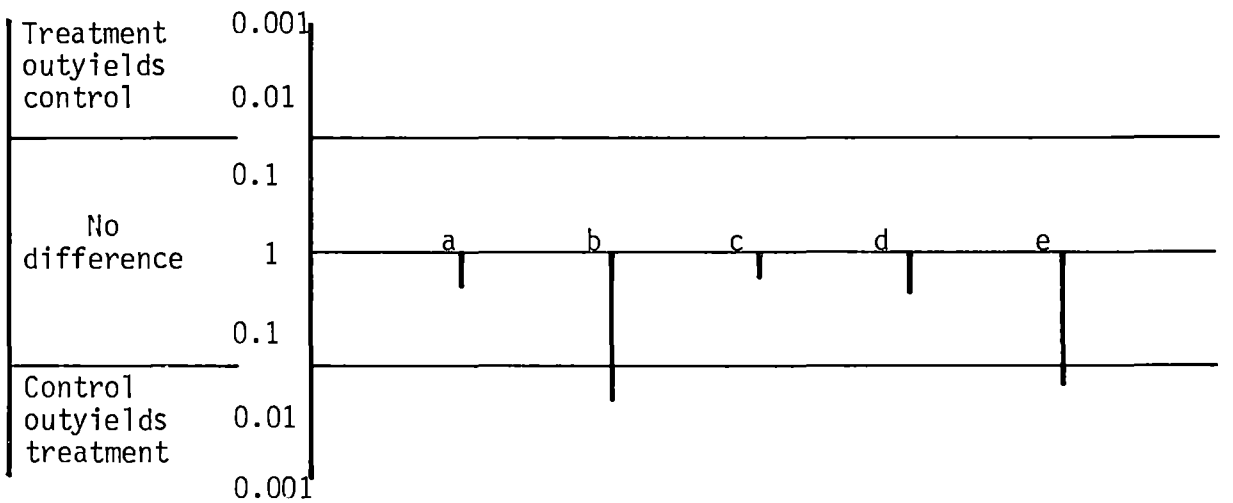


Fig. VI.C.7.
Magnesium concentration roots

Cabbage



Lettuce



Kikuyu

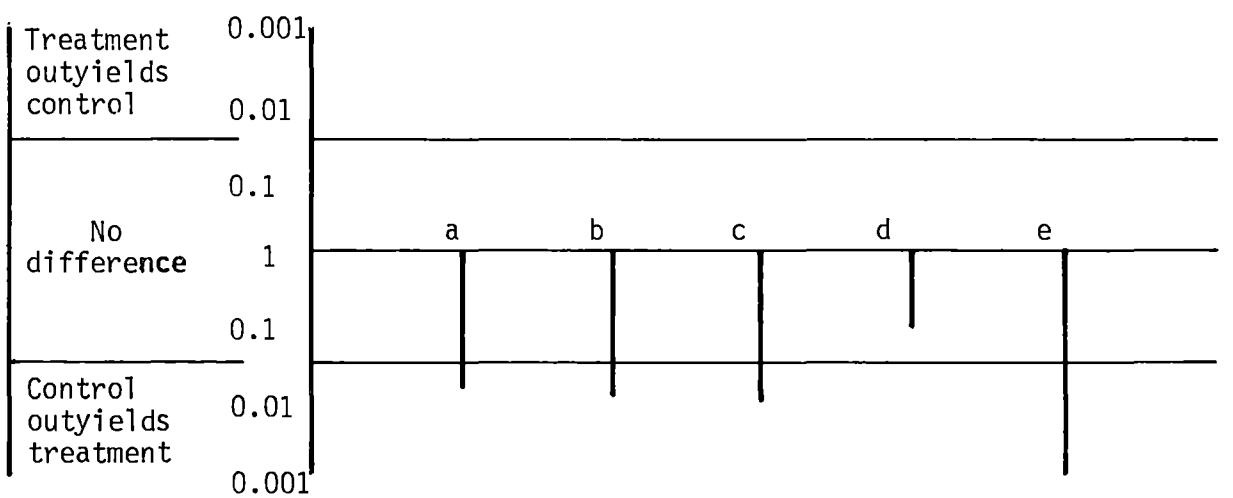


Table VI.C.7.

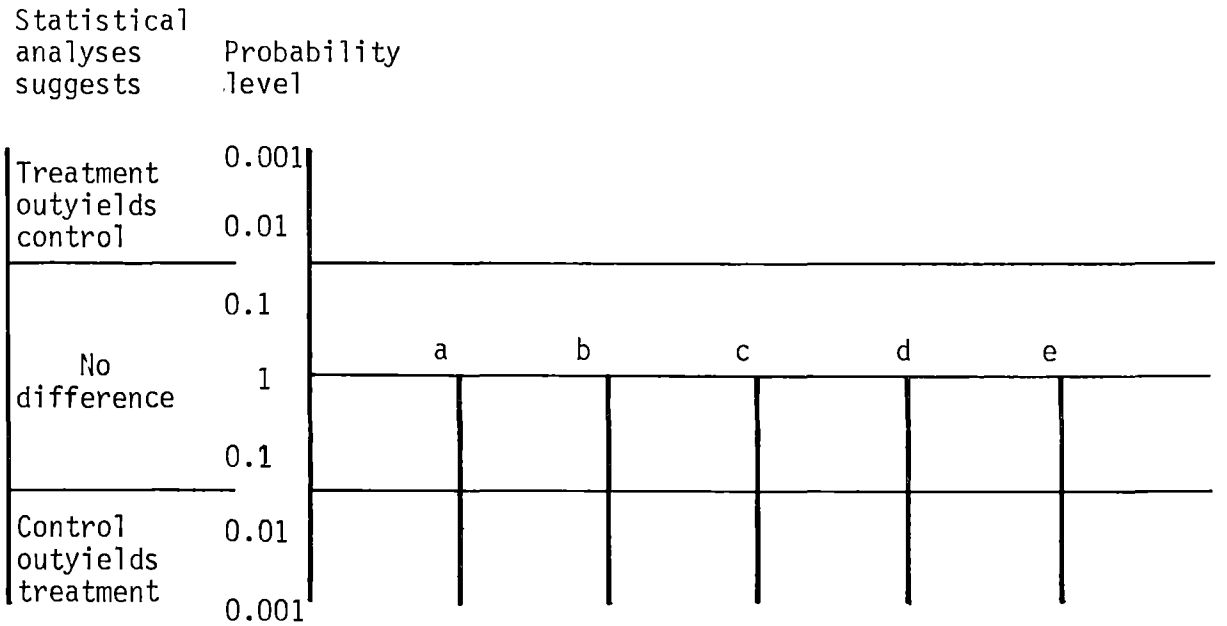
Comparison of magnesium concentrations of roots and tops (% dry weight) for kikuyu with cabbage and lettuce, -Al pH 4.6 N Ca.

Plant part	Species		
	Cabbage	Kikuyu	Lettuce
Roots	0.226	0.989	0.114
p value from computed t value		0.000	0.000
Tops	0.647	0.533	0.410
p value from computed t value		0.000	0.000

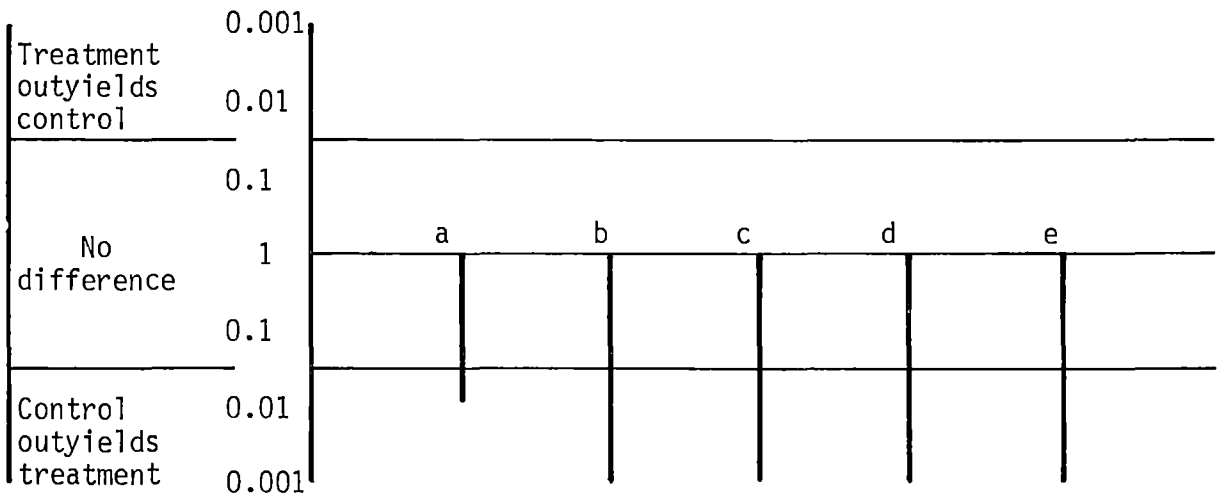
Fig. VI.C.8.

Magnesium concentration tops

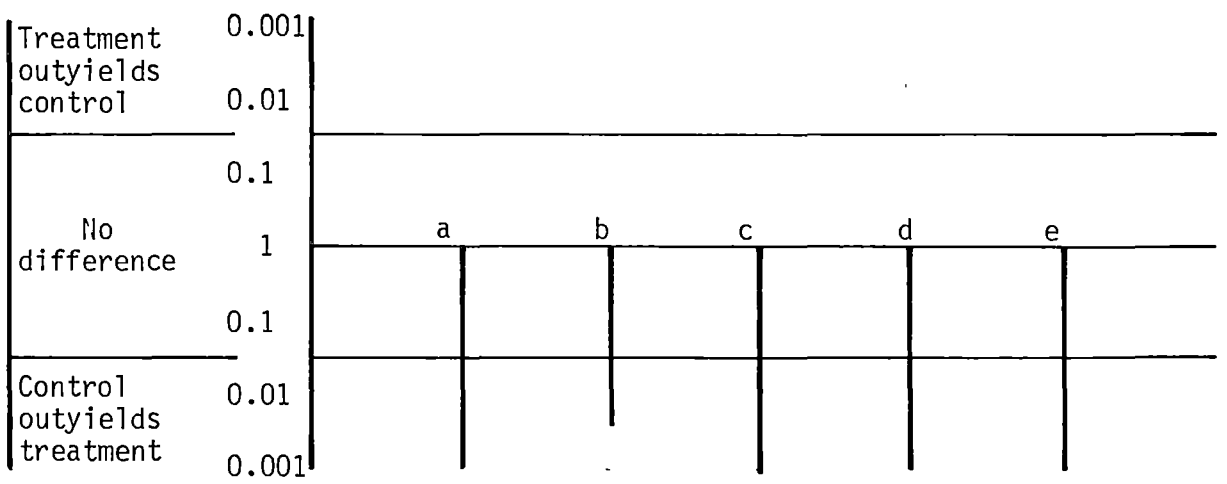
Cabbage



Lettuce



Kikuyu



The magnesium levels of tops for kikuyu were lower than that for cabbage and higher than that for lettuce (Table VI.C.7.) by 21% and 23% respectively.

VI.C.9. Potassium concentration roots

Treatment comparisons of potassium concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.9. Low pH and aluminium reduced the potassium levels for cabbage and lettuce. Aluminium at pH 4.0 increased the level for kikuyu.

High calcium application reduced the potassium level for lettuce in both the presence and absence of aluminium, had no effect for cabbage and increased the levels in the presence of aluminium for kikuyu.

VI.C.10. Potassium concentration tops

Treatment comparisons of potassium concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.10. Low pH and high calcium application reduced the potassium levels for lettuce and kikuyu and had no effect for cabbage. Aluminium at pH 4.0 reduced the levels for all species as well as at pH 4.6 for lettuce.

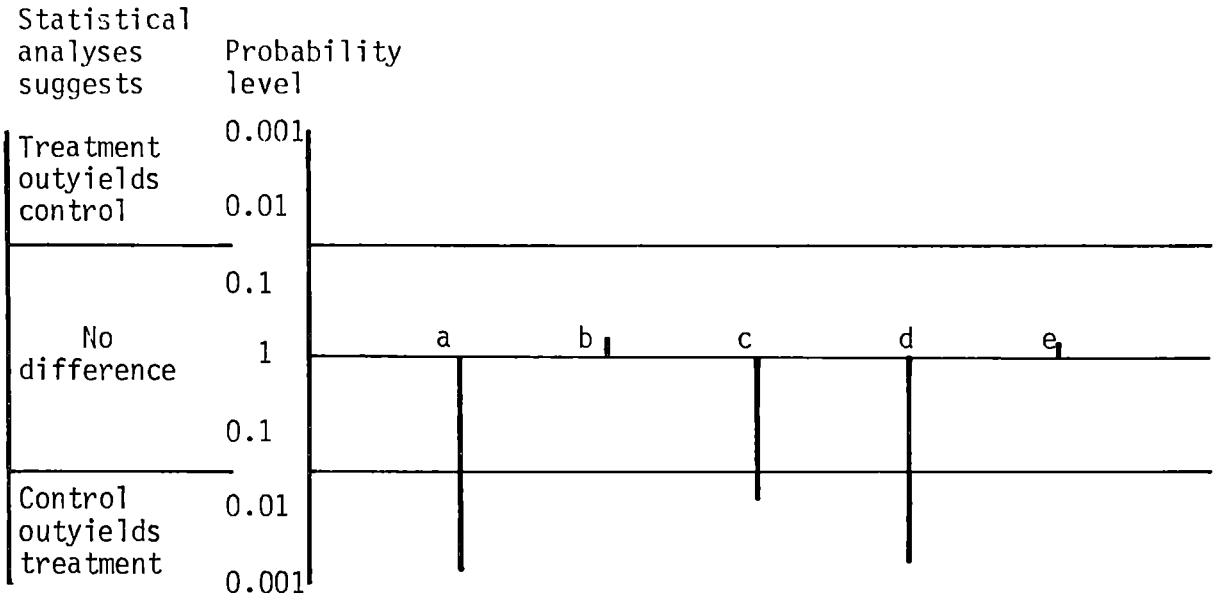
VI.C.11. Phosphorus concentration roots

Treatment comparisons of phosphorus concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.11. Low pH and high calcium application in the absence of aluminium had no effect on the phosphorus levels for cabbage and kikuyu but were decreased by these treatments for lettuce. Aluminium treatments consistently increased the levels for cabbage and kikuyu and reduced the levels for lettuce.

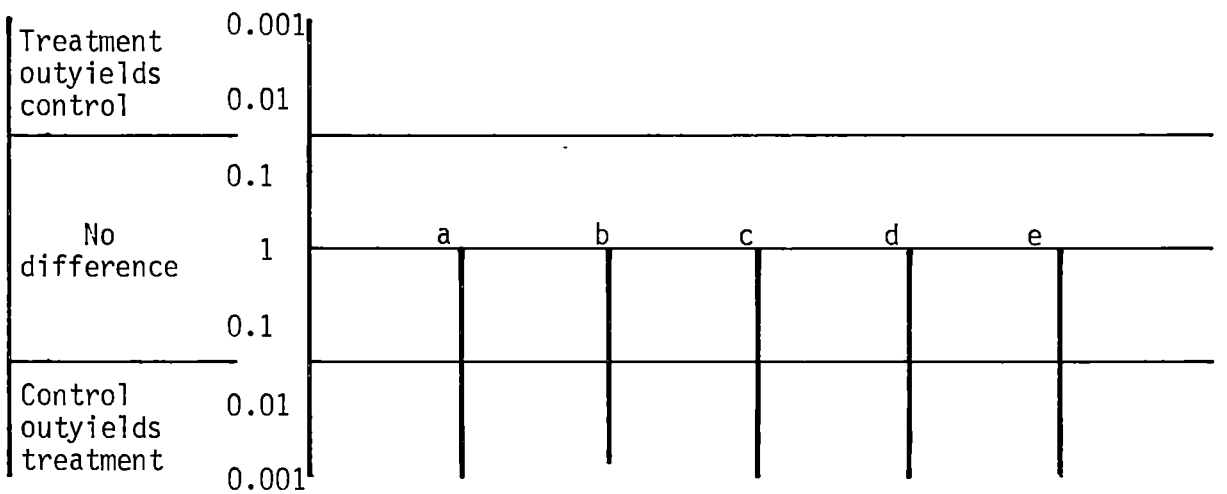
Fig. VI.C.9.

Potassium concentration roots

Cabbage



Lettuce



Kikuyu

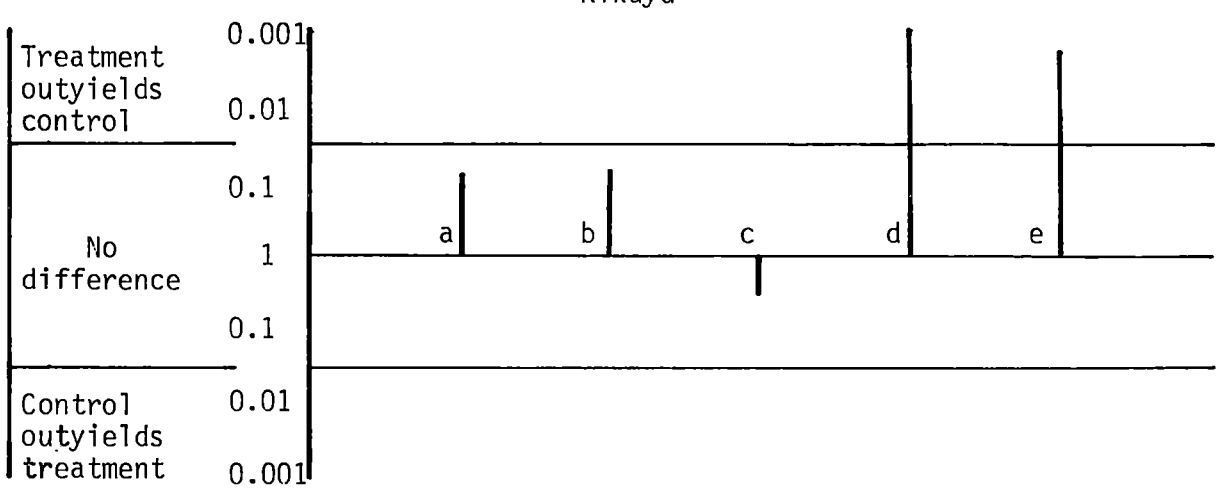
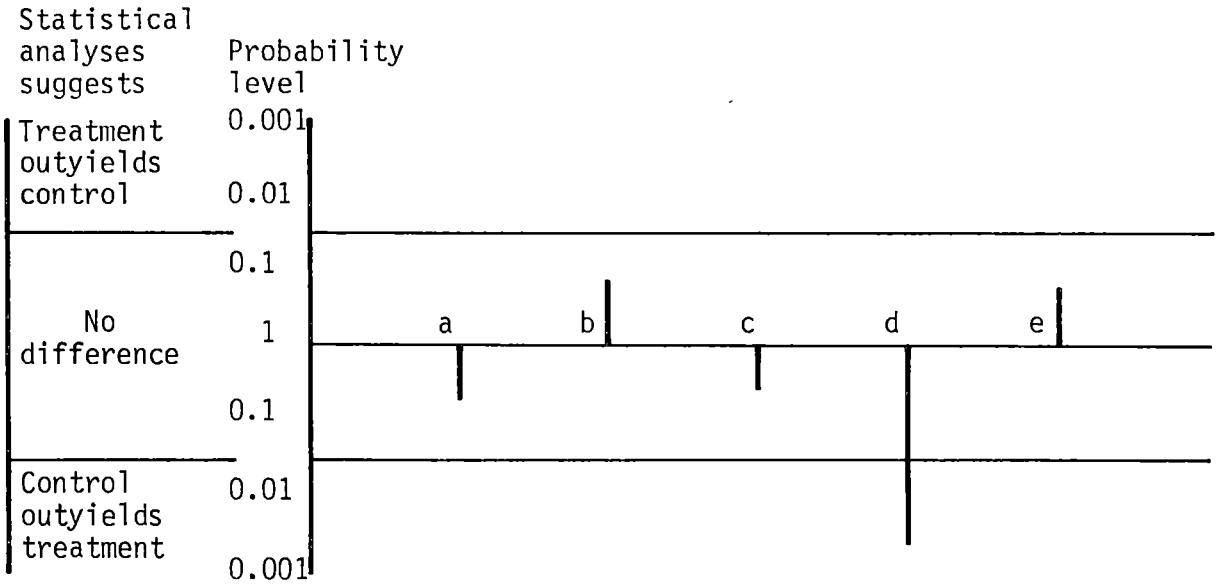


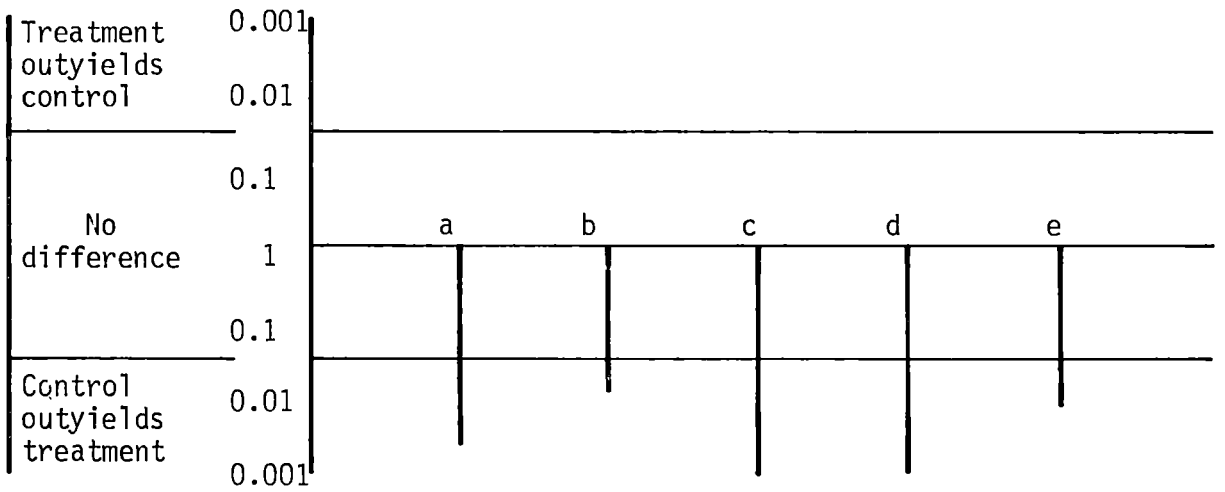
Fig. VI.C.10.

Potassium concentration tops

Cabbage



Lettuce



Kikuyu

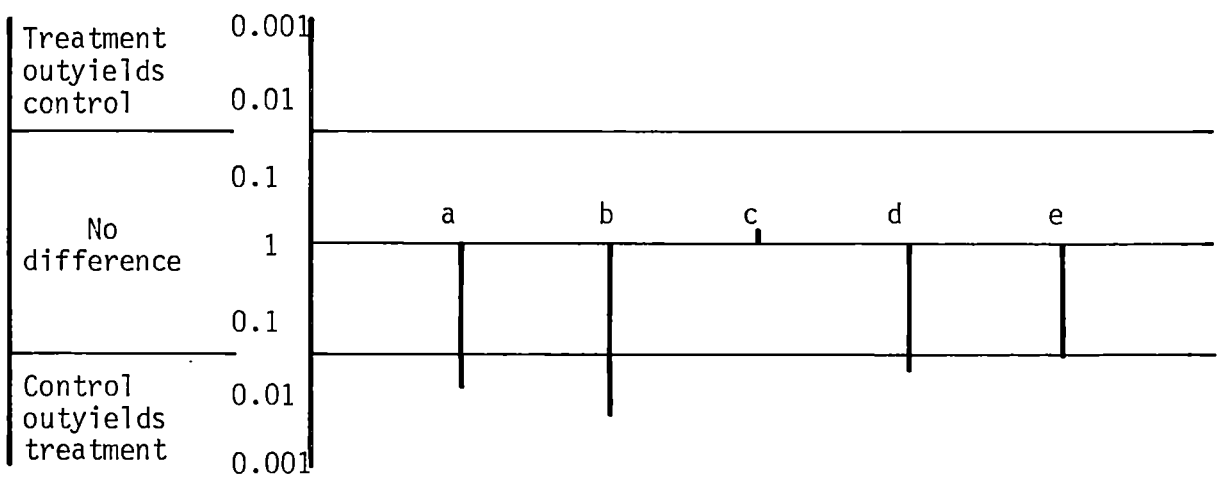
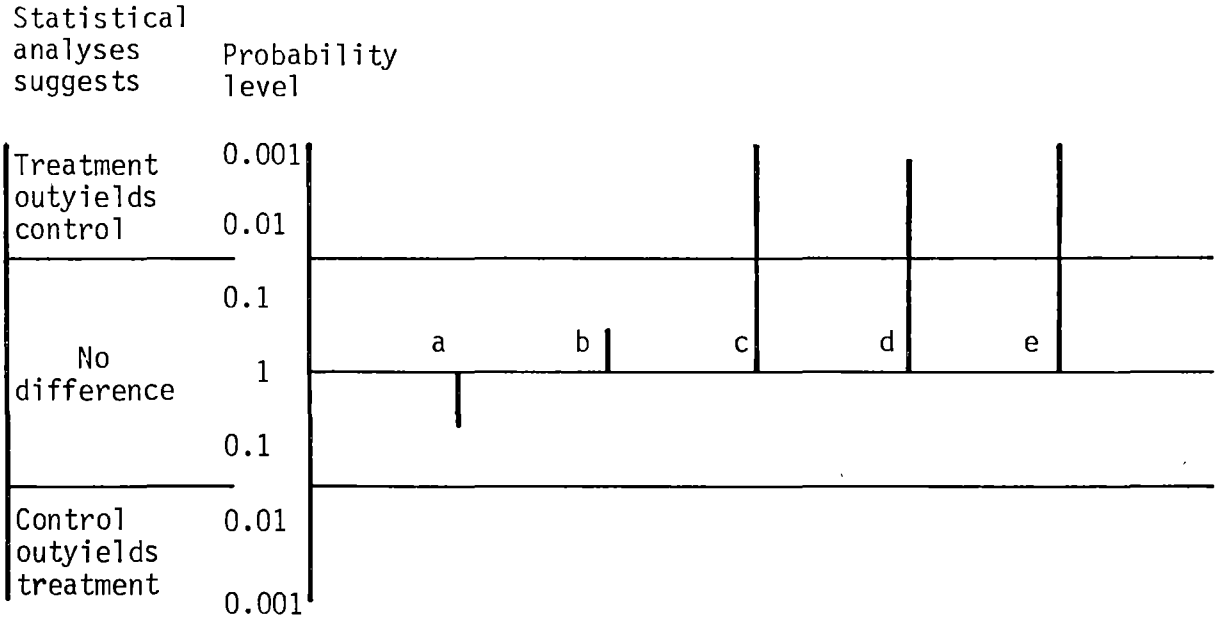
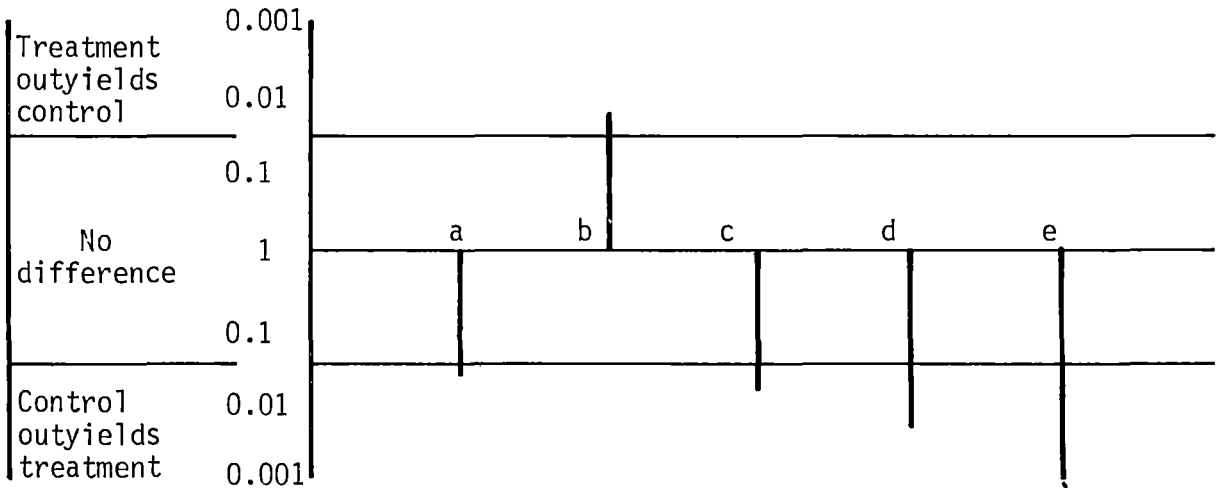


Fig. VI.C.11.
Phosphorus concentration roots

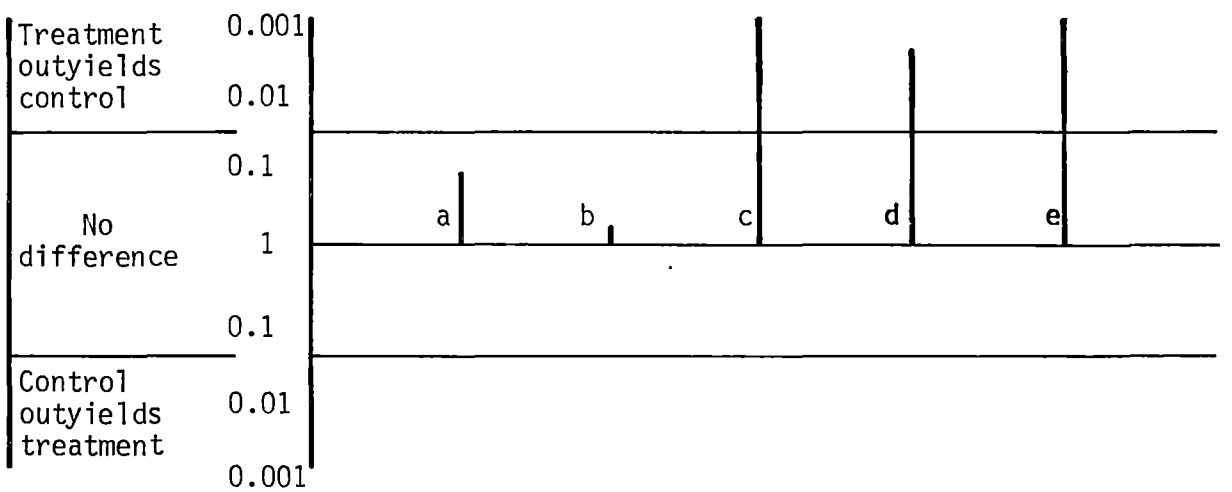
Cabbage



Lettuce



Kikuyu



VI.C.12. Phosphorus concentration tops

Treatment comparisons of phosphorus concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.12. Low pH and high calcium application in the absence of aluminium increased and decreased respectively the phosphorus levels for cabbage, but had no effect for lettuce and kikuyu. All aluminium treatments reduced the levels for lettuce, had no effect for kikuyu and increased the level for cabbage at pH 4.0.

VI.C.13. Sodium concentration roots

Treatment comparisons of sodium concentrations of roots for cabbage, lettuce and kikuyu are presented in Fig. VI.C.13. Low pH had no effect on sodium levels in both the presence and absence of aluminium for all species. Aluminium reduced the levels for cabbage and lettuce at pH 4.6 with and without high calcium applications, whereas these treatments had no effect on the levels for kikuyu. High calcium application in the absence of aluminium also reduced the levels of roots for lettuce.

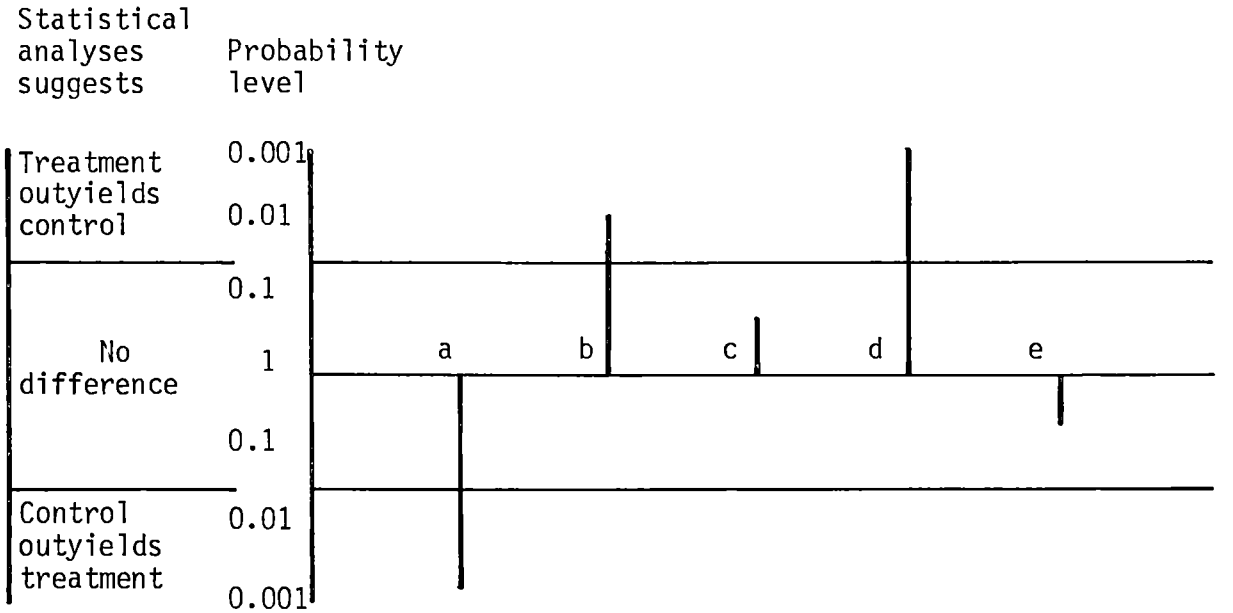
VI.C.14. Sodium concentrations tops

Treatment comparisons of sodium concentrations of tops for cabbage, lettuce and kikuyu are presented in Fig. VI.C.14. Low pH in both the presence and absence of aluminium reduced sodium levels for cabbage and kikuyu. The levels were increased at low pH in the absence of aluminium and reduced in the presence of aluminium for lettuce. High calcium application overcame the inhibitory effect of aluminium on sodium levels for cabbage and lettuce, but reduced the levels for kikuyu in both the presence and absence of aluminium.

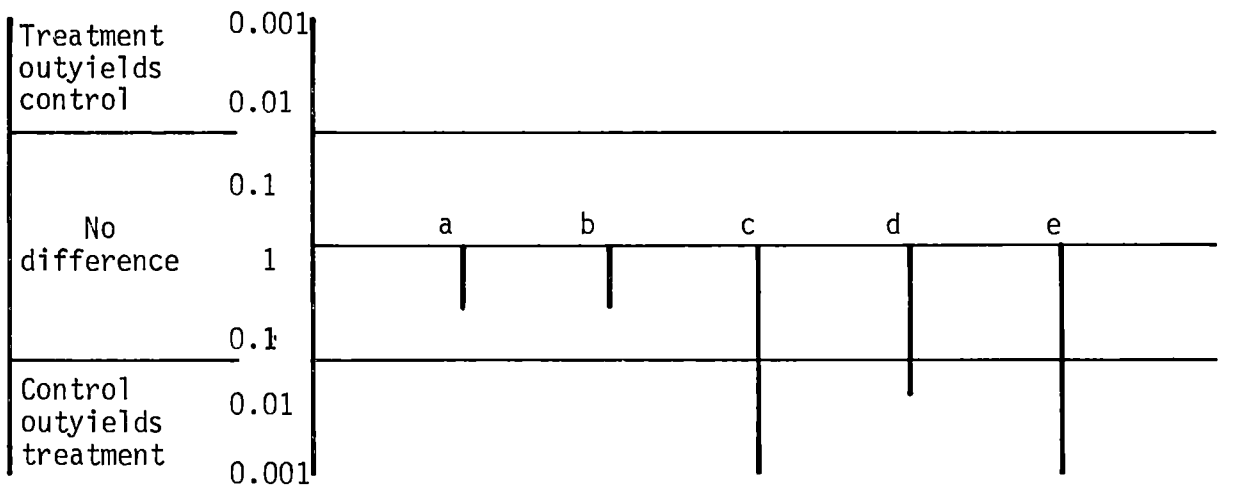
Fig. VI.C.12.

Phosphorus concentration tops

Cabbage



Lettuce



Kikuyu

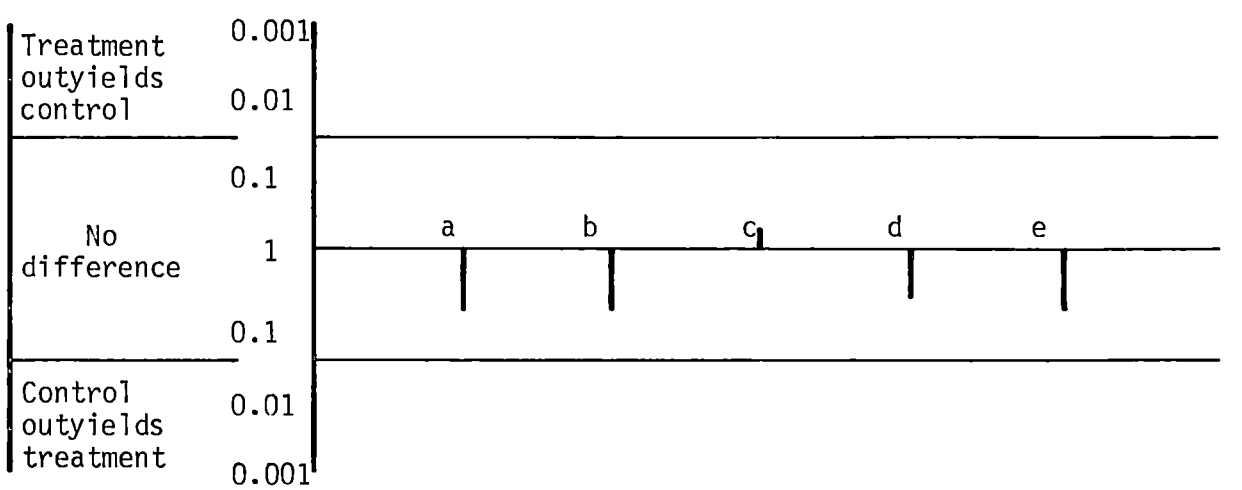
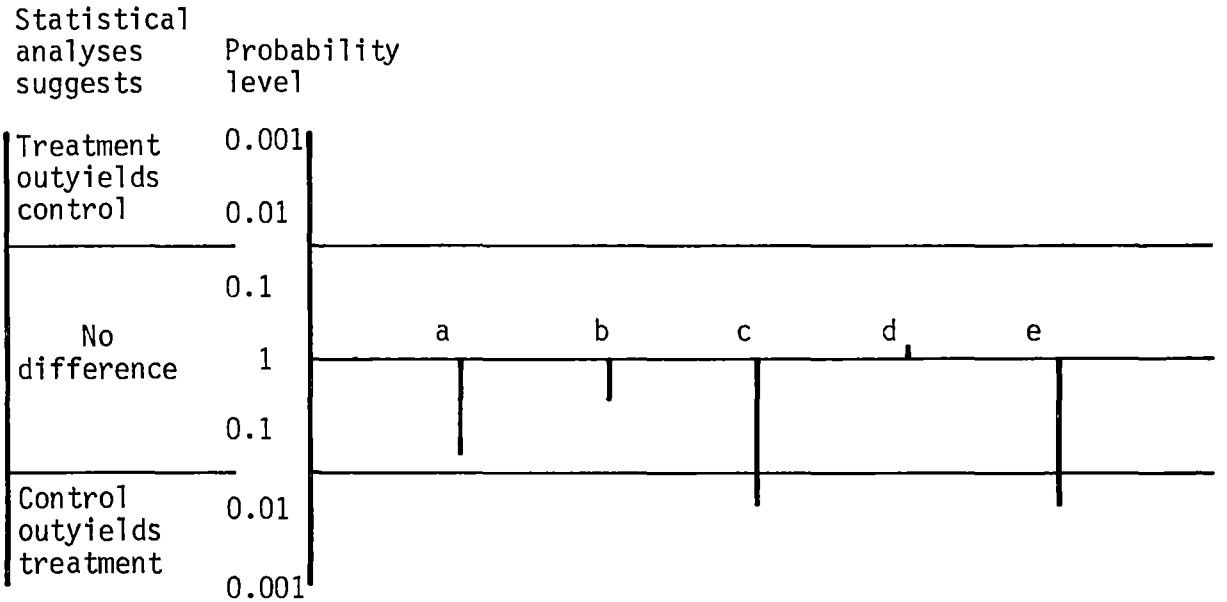


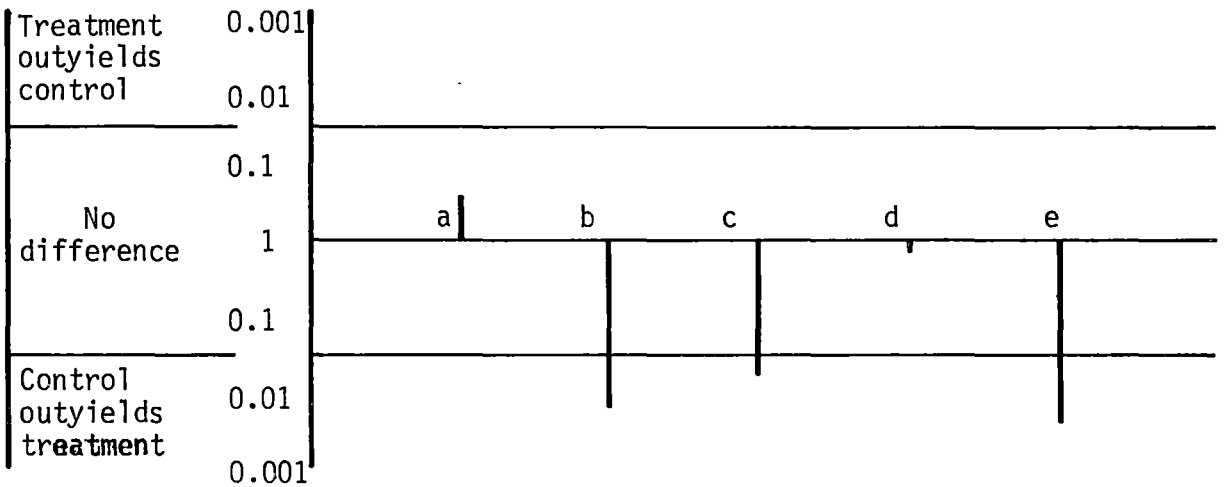
Fig. VI.C.13.

Sodium concentration roots

Cabbage



Lettuce



Kikuyu

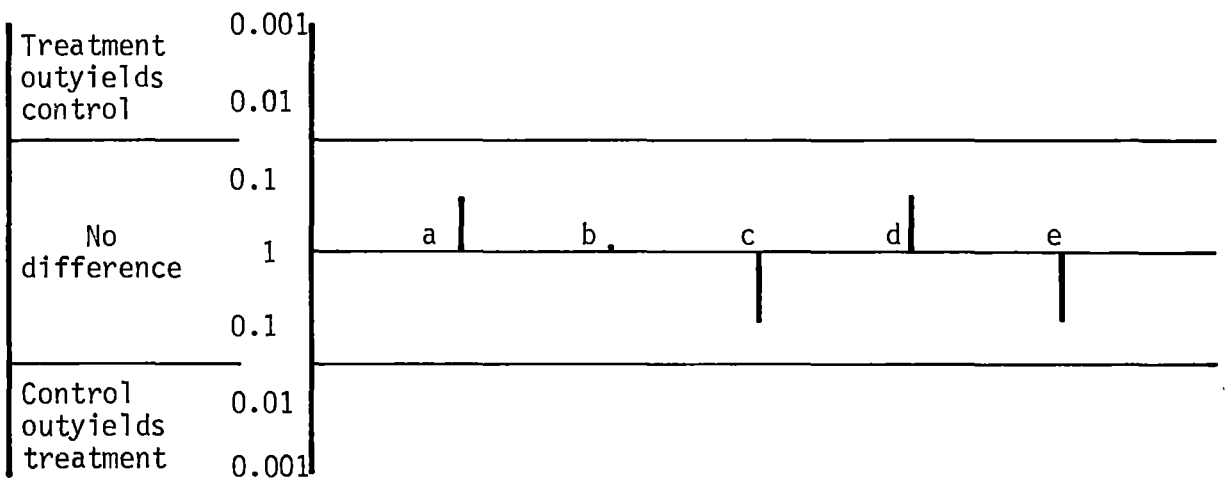
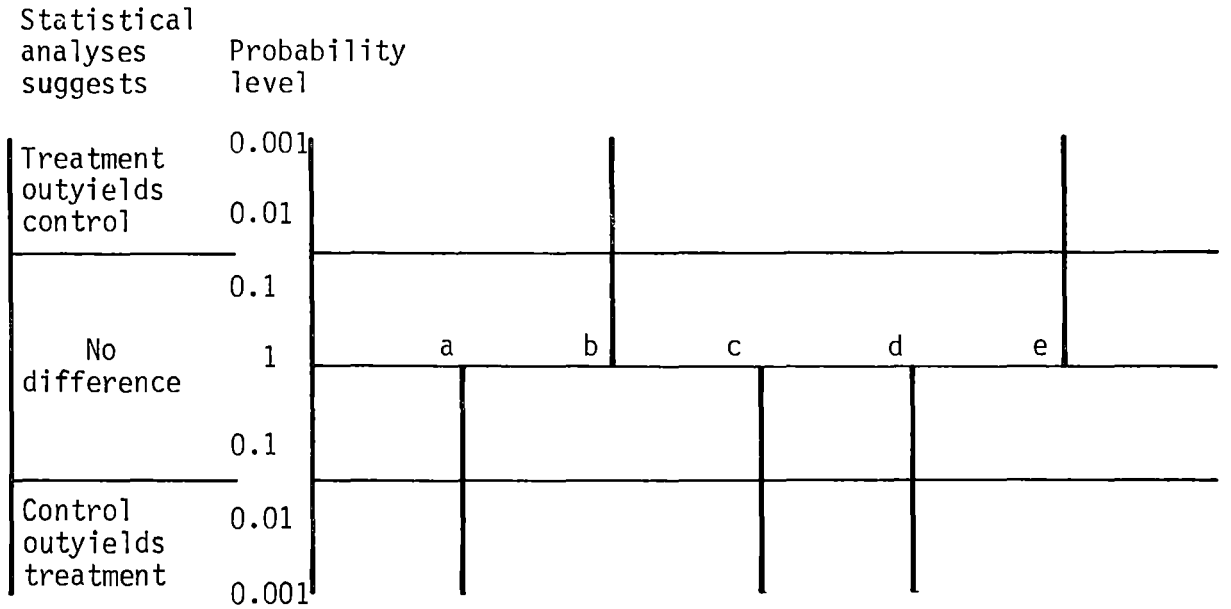
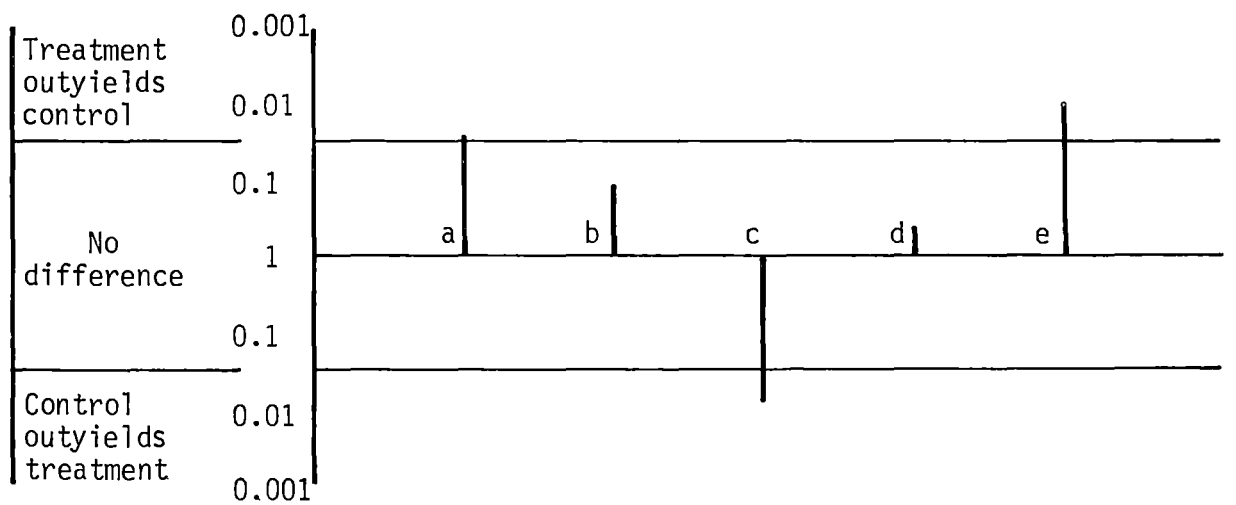


Fig. VI.C.14.
Sodium concentration tops

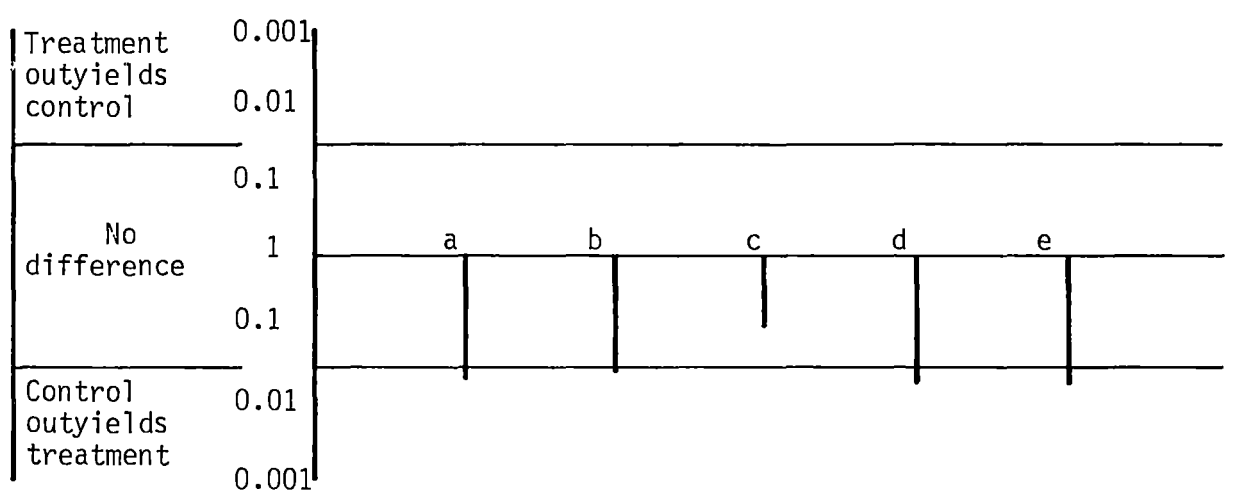
Cabbage



Lettuce



Kikuyu



VI.D. Discussion

Kikuyu, in contrast to cabbage and lettuce, was relatively tolerant to low pH and aluminium. Lettuce was the most susceptible species where roots were necrotic and severely stunted in the presence of these treatments at normal calcium levels. The extent of reduction of dry weight yield of tops exceeded that for cabbage which also displayed considerable susceptibility to low pH and aluminium. High calcium application generally overcame the inhibitory effect of aluminium on growth and nutrient uptake for these species except for the yield of lettuce tops which further emphasised its high susceptibility to aluminium. An additional exception was the calcium-magnesium antagonism and this was universal to all species.

Awad *et al.* (1976) reported significant yield reductions of kikuyu in a soil experiment where aluminium concentrations exceeded 1.5µg g^{-1} in soil and 90µg g^{-1} in plant tops. In the present study aluminium concentrations of 3µg ml^{-1} in nutrient solution and 272µg g^{-1} in tops had no effect on top growth, whereas 1µg ml^{-1} in solution and 111µg g^{-1} in tops reduced top growth (Fig. VI.C.1. and 2., Table VI.C.3.). These results suggested that either the critical aluminium levels for yield reduction provided by Awad *et al.* (1976) are of questionable significance or that they only apply to a soil situation. The higher aluminium uptake by plant tops in nutrient solution was recorded at the lower pH despite higher uptake by roots. In the soil experiment, a reduction in pH over the same range resulted in excess of a 100-fold increase in the soluble aluminium concentration. Awad and Edwards (1977) confirmed the dry weight yield reduction of kikuyu tops with increasing aluminium uptake. Despite the confounding of treatment

effects in soil studies with aluminium, the exponential increase in soluble soil aluminium with a pH decrease from 4.6 to 4.0 would negate the increased aluminium uptake by roots in nutrient solution at the higher pH. In nutrient solution the increased aluminium uptake negated any possible treatment concentration effect which was insignificant compared with the difference recorded in soil over the same pH range (Awad *et al.* 1976).

The inhibitory effect of both low pH and aluminium on cabbage and lettuce growth confirmed the difficulty in interpreting effects in soil studies involving aluminium excess. The ability of a high calcium application to ameliorate the inhibitory effects of aluminium on the growth of susceptible species in solution culture was interpreted as a calcium response *per se*, whereas in the soil situation a response to lime application (Munns 1965a, c; Helyar and Anderson 1971; Howeler and Cadavid 1976) was associated with a pH increase, an increase in available calcium and a reduction in available aluminium (Awad *et al.* 1976). In the present study, high calcium application reduced the dry weight yield of kikuyu tops whereas in soil, the yield response to lime application was attributed to increased exchangeable calcium and reduced soluble aluminium from the resultant pH increase which was reflected in similar changes in levels in plant tops (Awad *et al.* 1976).

The higher aluminium uptake by roots of cabbage and kikuyu at pH 4.6 and the relatively small amounts translocated to tops were consistent with adsorption being the dominant uptake process as proposed by Rorison (1965), Clarkson (1967) and Clarkson and Sanderson (1969). The lowering of net charge density per aluminium atom with increasing pH (Hsu and Bates 1964; Smith 1971) accounted

for greater adsorption at higher pH. The inability of calcium to exchange significant amounts of aluminium adsorbed by roots was consistent with the results of Clarkson (1967) and Guerrier (1978), where the former also used sodium salts with little effect.

The greater inhibitory effect of aluminium with increasing pH on root growth (Moore 1974) was not confirmed in the present study nor by White (1976). Aluminium reduced root yield of cabbage and lettuce at pH 4.0 but had no effect at pH 4.6. The higher treatment solution concentration at the lower pH was associated with considerably lower aluminium uptake by roots and hence the inhibition of root yield at the lower pH was unlikely to be due to the concentration effect. Low pH itself, which reduced yield, may have been the dominant effect. The lower aluminium uptake by tops at the higher pH for cabbage and kikuyu was the opposite response to that recorded for lucerne by White (1976). However, these experiments were conducted at a higher pH and the formation of polymeric aluminophosphate complexes was maximal at pH 5 (White 1976; White *et al.* 1976), which had low toxicity and moved more readily into roots, resulted in greater translocation of aluminium to tops than at pH 4.5.

The dominant effects of aluminium on cation uptake were to reduce both calcium and magnesium uptake. This effect on calcium uptake has been widely reported in soil studies (Foy and Brown 1964; Munns 1965a, c; Macleod and Jackson 1967; Foy *et al.* 1969; Awad *et al.* 1976; Awad and Edwards 1977; Foy *et al.* 1978). As indicated previously, a decrease in soil pH was associated with a decrease in available soil calcium or conversely lime application which raises soil pH and available calcium also reduces soluble aluminium. Hence, reduced calcium uptake in the presence of

aluminium in soil was accentuated by low pH and low calcium availability. In the present study low pH was as effective as aluminium in reducing both calcium and magnesium uptake by roots and tops, irrespective of effects on plant growth. Despite the difficulty in interpreting the aluminium-calcium antagonism in soil studies, there was widespread evidence in the literature supporting this antagonism in solution culture where confounding of treatments effects were avoided (Munns 1965; Andrew *et al.* 1973; Kotze *et al.* 1977; Mugwira *et al.* 1976; Clark 1977; Edwards and Horton 1977). The nature of the aluminium-calcium antagonism was demonstrated by Johnson and Jackson (1964) and Clarkson and Sanderson (1971) where aluminium reduced the amount of exchangeable calcium in roots and the amount transported to shoots.

Low pH was as effective as aluminium in reducing cation levels in roots and tops and this appeared to be due to non-specific cation competition. These treatments reduced calcium levels in both roots and tops of kikuyu and despite its very low requirement in comparison with cabbage and lettuce (Table VI.C.5.), it had little effect on yield. Awad *et al.* (1976) attributed one of the main inhibitory effects of low pH-aluminium excess to reduced calcium uptake and suggested that calcium was limiting to kikuyu growth when concentrations in tops were less than 0.11%. Despite the relative tolerance of kikuyu to aluminium in solution culture, it reduced the dry weight yield of tops at pH 4.6, corresponding to a calcium concentration in tops of 0.26%, well in excess of the critical level reported in the soil study. These results, together with the reduction in calcium levels of tops at low pH with no effect on yield and the reduction in yield following high calcium application in both the presence and

absence of aluminium, indicated that aluminium excess *per se*, rather than aluminium induced calcium deficiency, accounted for reduced kikuyu yield under conditions of low pH-aluminium excess in soil.

Aluminium tolerance was also associated with low calcium requirement where *Chlorella pyrenoidosa*, a green alga which grew well in a medium containing magnesium but no calcium (Gerloff and Fishbeck 1969), tolerated very high levels of aluminium (Foy and Gerloff 1972). The very low calcium levels of roots and tops of kikuyu were associated with high magnesium levels, particularly in roots (Table VI.C.7.), a situation parallel to that for *Chlorella*. The role of calcium in buffering against heavy metal toxicity in plants (Wallace *et al.* 1966) may have been fulfilled by magnesium for kikuyu.

The importance of adequate calcium nutrition of plant species susceptible to low pH-aluminium excess was highlighted by necrosis of lettuce roots in the presence of these treatments, a symptom associated with calcium deficiency (Loneragan *et al.* 1968; Simon 1978). Both cabbage and lettuce had a considerably higher calcium requirement than kikuyu and the increased root yield of the former two species in the presence of high calcium suggested that a pH of 4.6 may be sufficiently low to reduce calcium uptake beyond that required for normal growth.

The ability of high calcium to ameliorate the inhibition of root growth by aluminium for cabbage and lettuce was also reported for wheat (Ali 1973) and maize (Rhue and Grogan 1977) and extended to top growth in the present study. This response was associated with increased calcium uptake by roots and tops as reported for wheat (Lance and Pearson 1969) and barley (Clarkson and Sanderson 1971). This effect, together with the high calcium requirement of

cabbage and lettuce and the reduction in aluminium levels in tops by high calcium, probably accounted for the yield response.

The control of aluminium movement into root cells by the plasmalemma (Henning 1975; Klimashevskii *et al.* 1976), the reduction in calcium levels of roots by aluminium in the present study and the essential role of calcium in maintaining selective ion absorption and membrane integrity (Viets 1944; Epstein 1961), provided evidence that the reduction in aluminium levels of tops of cabbage, lettuce and kikuyu by high calcium application was due to reduced passive transport into the stele. Other cations, particularly magnesium, were effective in maintaining selective ion absorption (Viets 1944) and overcoming the inhibitory effect of aluminium on root growth (Ali 1973; Rhue and Grogan 1977). These effects suggested that for kikuyu, because of its low calcium and high magnesium requirement, magnesium may play a dominant role in controlling aluminium transport into the stele.

In addition to low pH and aluminium, high calcium application reduced cation levels in roots and tops of cabbage, lettuce and kikuyu as was reported for maize inbreds (Clark 1978). The most pronounced effect for the former species was the reduction in magnesium levels. Hara *et al.* (1977) found that high calcium levels in tops of cabbage following calcium application were liable to cause magnesium deficiency where a critical level of 0.1% was determined. The high calcium treatment used in the present study was identical to that used by Hara *et al.* (1977) and reduced magnesium levels in cabbage tops to 0.19%, suggesting that calcium induced magnesium deficiency was unlikely to be a problem, particularly as the yields of cabbage and lettuce were increased by this treatment. The reduction in yield of kikuyu

tops in the presence of high calcium may have been due to reduced magnesium levels.

Aluminium had a predominant effect on phosphate uptake by cabbage, lettuce and kikuyu. Response was related to species tolerance where aluminium increased phosphorus levels in roots of kikuyu and had no effect on tops in contrast to lettuce where the opposite occurred for roots and levels were reduced in tops. Cabbage followed a similar pattern to kikuyu except for an increase in tops at pH 4.0. Increased phosphate uptake by roots in the presence of aluminium was consistent with an adsorption-precipitation reaction in free space (Rorison 1965; Clarkson 1966b) and has been supported by histological studies using specific stains (McCormick and Borden 1972, 1974; Keser *et al.* 1977) and EMX-analyses (Rasmussen 1968; Naidoo *et al.* 1978). These studies used high pre-treatment and post-treatment concentrations of aluminium and phosphate respectively to demonstrate their co-precipitation. However, White (1976), who maintained aluminium and phosphate concentrations and pH within the range defined by Munns (1965b) to avoid aluminium phosphate precipitation in solution, also reported increased phosphate uptake by roots for aluminium-sensitive lucerne.

Andrew and Vandenberg (1973) grew plants under similar culture conditions to that of White (1976) and to that in the present study and also reported increased phosphate sorption in the presence of aluminium by a range of tropical legume species displaying varying degrees of aluminium tolerance. In contrast to the results of White(1976), aluminium had no effect on phosphate uptake by lucerne roots and whole plants (Munns 1965b; Andrew and Vandenberg 1973), whereas it consistently increased phosphate sorption by excised roots (Andrew

and Vandenberg 1973). Culture conditions and species appeared to play an important role in the aluminium-phosphate response by roots and may have accounted for some of the differences reported in the literature and in the present study.

Apart from the reaction between aluminium and phosphate in the cell wall, once inside the cell, aluminium has been shown to interfere with phosphate metabolism. A prerequisite for phosphate transport to tops was prior incorporation into organic forms (Loughman 1966; White 1973),and the inhibition of esterification by aluminium (Rorison 1965; Clarkson 1966b) may have accounted for reduced phosphate uptake by tops of sensitive species (Andrew *et al.* 1973) and lettuce in the present study. However, Matsumoto and Hirasawa (1979) found no effect of aluminium on phosphate esterification by an aluminium-sensitive pea cultivar and this may have accounted for the effect of aluminium on phosphorus levels in tops of cabbage which were unaffected except for an increase at pH 4.0 and for kikuyu with all aluminium treatments.

The fixation of phosphate in lucerne roots by aluminium (White 1976) was unlikely to account for reduced metabolic accumulation and transport to tops. This principle did not apply to lucerne in other studies where reduced transport to tops was associated with reduced uptake by roots (Munns 1965b; Andrew *et al.* 1973; Andrew and Vandenberg 1973) as was the case for lettuce in the present study. Aluminium may have interfered with active transport of orthophosphate into roots, the predominant form at low pH (Edwards 1970) and differential species response may have been associated with differences in the carrier system at the plasmalemma. Calcium has been shown to play an important role in maintaining selective ion absorption (Viets 1944; Epstein 1961) and the

reduction in calcium levels in roots by aluminium in species such as lettuce and cabbage that were shown to have a high calcium requirement, suggested a possible explanation for reduced phosphate uptake. However, this explanation did not hold for lettuce where phosphate uptake by both roots and tops was still reduced by aluminium in the presence of high calcium, where calcium levels were higher than those in the control treatment.

Edwards (1968) demonstrated that calcium exerted an important synergistic effect on phosphate absorption by *Trifolium subterranean* and was supported by Robson *et al.* (1970) for *Medicago* and *Trifolium* species. The latter indicated that the response resulted from calcium screening electronegative charges on roots. A similar response was recorded for lettuce roots and cabbage tops at pH 4.6 in the present study, however, as discussed previously, this pH may have been sufficiently low to reduce calcium to sub-optimal levels, hence an increased calcium supply may have stimulated metabolic accumulation of phosphate. Because the response was not recorded for lettuce tops and cabbage roots the explanation is undoubtedly more complex and some of the inconsistent interactions between aluminium and phosphate reported in the literature and in the present study would be related, at least in part, to the explanations provided. Further research is required before the nature of these responses can be fully understood.

VII. GENERAL DISCUSSION

VII. GENERAL DISCUSSION

Factors associated with aluminium uptake by cabbage, lettuce and kikuyu were studied by examining some of the processes involved in absorption and transport. An excised root study was complemented by whole plant studies and the extent to which they describe uptake and translocation is discussed in this section.

The time course of aluminium uptake by excised roots involved initial rapid uptake (Phase I) followed by a slower rate of accumulation (Phase II) which was pronounced for aluminium-sensitive cabbage and lettuce and was almost completely absent for aluminium-tolerant kikuyu. The response to temperature and a metabolic inhibitor indicated that the entire uptake process was non-metabolic. During Phase I aluminium exchanged most of the calcium from excised roots (Section IV.C.1.) and significantly reduced calcium and magnesium levels of whole roots (Section VI.C.). This process involved exchange-adsorption and was supported by the results of Clarkson and Sanderson (1971) and Guerrier (1978). The cation exchange behaviour of roots was proposed by Walker and Pitman (1976) and Wuytath and Gillett (1978) where negative sites are associated with carboxyl groups. Clarkson (1967) similarly reached this conclusion from excised root studies with barley.

Wuytath and Gillett (1978) examined the nature of exchange reactions in cell walls and found that normal kinetics of ion exchange apply where monovalent cations compete with each other so that at low pH, carboxyl groups tend to be in the hydrogen form. The reduction in calcium and magnesium levels of whole

roots at low pH would have involved exchange-adsorption as a result of hydrogen ion competition. Polyvalent cations readily compete with monovalent cations, where competition by the former is favoured by low concentration and competition by the latter is favoured by high concentration (Vogel 1961). Wuytath and Gillett (1978) found that calcium forms a stable complex with carboxyl groups and this factor, in addition to its higher valence, accounted for the ease in which it could exchange monovalent cations from cell walls (Gillett and Lefebvre 1978). A similar explanation would account for the ease in which aluminium exchanged calcium from both excised roots and whole roots in the present study. Clarkson and Sanderson (1971) used scandium as a tracer for aluminium where it inhibited calcium uptake when the ratio of scandium:aluminium was as low as 1:1000.

Aluminium uptake was consistently higher by both excised roots (Section IV) and whole roots (Section VI) at the higher pH. Greater dissociation of carboxyl groups may only account for a small increase in uptake by roots as their active groups have a pKa of about 2.8 (Walker and Pitman 1976) and will be highly dissociated above pH 4.0. This was supported by the fact that calcium uptake during Phase I (adsorption) increased by only 7% with a pH increase from 4.0 to 4.2 (Moore *et al.* 1961b; Volz and Jacobson 1977) compared with a 20% increase for aluminium (mean three species) in the excised root study (Section IV.C.1.). Similar comparisons for a pH increase from 4.0 to 4.6, as used in the whole plant study (Section VI.C.3.), were 25% for calcium and 103% for aluminium (mean

three species). The decrease in mean net charge density per aluminium atom with increasing pH in the acid range (Hsu and Bates 1964; Hem 1968; Smith 1971) would lead to greater adsorption of aluminium and would have accounted for most of the higher uptake during Phase I. The formation of polymeric aluminophosphate with lower net charge at high pH (White *et al.* 1976) led to greater accumulation of acid extractable aluminium and phosphate in lucerne roots at pH 5.0 compared with pH 4.5 (White 1976). McLean (1976) suggested that this reaction appeared to involve adsorption of phosphate onto residual positively charged aluminium on the negative sites. He also indicated that in solution, the formation of insoluble aluminium hydroxide (pKsp 32.7) would proceed in favour of aluminium phosphate (pKsp 28-32).

EDX-analyses of the cell wall regions of roots indicated higher aluminium concentrations in the epidermis and cortex than stele. These roots had been desorbed in water hence the results are consistent with passive aluminium accumulation in free space of roots associated with cell walls as proposed by Clarkson (1967) and Clarkson and Sanderson (1969, 1971). Aluminium uptake during Phase I consisted of exchange-adsorption and appeared to be the dominant uptake process.

The consequence of the exchange of calcium from roots as a result of aluminium uptake during Phase I would appear to depend on the magnitude of this reaction. Plants contain considerably higher calcium levels than required for normal metabolic function to ameliorate against cation excess (Wallace *et al.* 1966) and it was not until 69-76% of the total calcium had been removed from beetroot storage tissue that membranes became leaky (Van Steveninck 1965). Garrard and Humphreys (1967) similarly demonstrated leakage of sucrose from corn scutellum slices in the absence of calcium. While this process

involves outward diffusion across membranes it would be reasonable to expect passive movement of aluminium into cells, particularly during equilibration with the external medium. The presence of aluminium in the protoplasm of cells (Waisel *et al.* 1970), largely in meristematic cells associated with the nucleus (Klimashevskii *et al.* 1972; Matsumoto *et al.* 1976; Keser *et al.* 1977; Naidoo *et al.* 1978), has been well documented.

Calcium occurs on cell membrane surfaces (Leggett and Gilbert 1967) and in addition to its role of neutralizing exchange sites in cell walls (Gillett and Lefebvre 1978), it appears to stabilize membranes (Christiansen and Foy 1979). The first signs of calcium deficiency start with membrane breakdown (Marinos 1962; Hecht-Buchholz 1979), a result recorded in the present study where lettuce roots became necrotic when grown in the presence of aluminium and at pH 4.0 (Section VI.C.1.). Loneragan *et al.* (1968) associated calcium deficiency with necrosis of roots, suggesting cell breakdown (Simon 1978). Calcium is also required to maintain selective ion absorption (Viets 1944; Epstein 1961) and this in addition to previous evidence suggests that aluminium, through its interaction with calcium in cell walls and membranes can enter cells via a passive process. Aluminium exchanged in excess of 70% of the calcium from excised roots of each species and where desorption was complete at the end of Phase I, additional aluminium uptake particularly by cabbage and lettuce during Phase II (Section IV.C.1.) may have represented passive movement across the plasmalemma.

The superficial location of polyvalent cations in roots allows them to control calcium entry into free space which reduces accessibility to the stele and transport to tops (Clarkson and Sanderson 1971). Aluminium would have a similar effect on other divalent and monovalent cations as evidenced by the general reduction in cation levels of roots and tops of cabbage, lettuce and kikuyu (Section VI.C.).

The presence of aluminium in the stele by EDX-analyses (Section V.C.2.1.) and in tops (Section VI.C.4.) and the non-metabolic nature of the accumulation phase by excised roots (Section IV.C.2.) confirmed that uptake during Phase II consisted of passive transport. There are several pathways available to account for radial aluminium transport to the stele which would bypass the barrier at the endodermis. The relatively uniform distribution of aluminium, particularly in xylem vessels, along the length of roots of cabbage, lettuce and kikuyu from EDX-analyses (Section V.C.2.1.) negated the need for a lateral root to provide a channel of entry to the cortex and stele (Rasmussen 1968). Aluminium was present in both the cortex and stele of the root tip of all species proximal to the zone of lateral root initiation. Dumbroff and Piersen (1971) suggested that lateral roots provide a transient break in the endodermis and allow mass flow of ions to the stele and were supported for calcium by maize roots (Ferguson and Clarkson 1975).

Apart from this process, calcium enters the stele of barley roots (Robards *et al.* 1973) and of *Cucurbita pepo* roots (Harrison-Murray and Clarkson 1973) only in the region of the primary endodermis. Robards *et al.* (1973) reported that the

Casparian strip in the primary endodermis presents a high resistance to apoplasmic calcium transport. Hence the only way in which calcium can move into the stele is by uptake through the plasmalemma of the endodermal cells at the outer tangential wall where it is exposed to the apoplast. When the suberin lamella has covered the whole inner surface (secondary state), this pathway for calcium transport across the endodermis is blocked. The asynchronous development of the endodermis gives the appearance of 'passage' cells adjacent to the protoxylem pole cells, although all cells eventually attain the same state and degree of wall thickening. Movement will continue as long as some 'passage' cells remain which lack suberin lamellae. Radial aluminium transport to the stele could follow a similar path to that of calcium, particularly as the former can readily exchange the latter and would account for the relatively uniform distribution of aluminium along roots particularly in xylem vessels.

The presence of aluminium in the protoplasm of cortical cells of all species (Section V.C.2.3.) suggests that the symplasm could provide a pathway for radial transport to the stele. This conclusion was supported by the presence of aluminium in the radial wall (and cytoplasm) of the endodermis. As discussed previously, the ability to exchange calcium and alter membrane selectivity and permeability would allow passive movement of aluminium into not only meristematic cells, but cortical cells as well.

An additional explanation which would account for transport to the stele and relatively uniform distribution in xylem vessels

along roots was provided by Henning (1975) who presented strong evidence that aluminium penetrated the boundary between the root apex and root cap of wheat cultivars and then, during a lethal treatment, moved into meristematic cells of the central cylinder. He concluded that differential species tolerance was related to differential accumulation of aluminium in meristematic cells which indicated that the plasmalemma played an important role in the control of tolerance. Klimashevskii *et al.* (1976) similarly concluded that disrupted membrane permeability caused greater accumulation of aluminium in sensitive pea cultivars.

One of the major effects of aluminium on plant growth is inhibition of root growth through its effect on cell division (Clarkson 1965). Aluminium accumulates in meristematic cells of the root apex largely associated with nuclei (Matsumoto *et al.* 1976a;Morimura *et al.* 1978). Clarkson and Sanderson (1969) showed that aluminium accumulation (Phase II) was only present for apical segments of roots and the evidence suggests that the meristematic zone of the root apex, because of the large concentration of nuclei in comparison with distal zones of the root, acts as a sink for passive aluminium accumulation. The movement of aluminium through the root tip as described by Henning (1975) may be the most important pathway for lateral aluminium transported to the stele.

The size of the aluminium uptake component during Phase II by excised roots (Section IV.C.1.) was related to the amount translocated to tops (Section VI.C.4.). This component was almost completely absent for kikuyu which translocated much less aluminium to tops than cabbage and lettuce. The two latter

species accumulated significant amounts of aluminium during Phase II. The size of this component was also related to species tolerance to both low pH and aluminium which removed most of the calcium and magnesium from roots (Section VI.C.5, 7). Kikuyu, whose roots contain low levels of endogenous calcium and high levels of endogenous magnesium, grew normally in the presence of aluminium and low pH. The evidence suggests that not only is exchange of calcium (and probably other cations, particularly magnesium (Epstein 1961; Van Steveninck 1965)) required for loss of membrane selectivity and permeability, but also the structure of the membrane as suggested by Henning (1975) and Klimashevskii *et al.* (1976) is important in controlling passive aluminium transport. Chlorella, which has similar calcium and magnesium requirements (Gerloff and Fishbeck 1969) to kikuyu and tolerates very high levels of aluminium (Foy and Gerloff 1972) suffered potassium loss when exposed to high concentrations of heavy metals (Fillipis 1978). Membrane leakage was strongly correlated with the strength of the metal-sulphydral bond in the cell walls and membranes.

Some cultivars of French bean (Foy *et al.* 1972), wheat and barley (Foy *et al.* 1967) appear to tolerate aluminium by exclusion at the plasmalemma. Aluminium tolerance through accumulation and inactivation in the protoplasm would not account for differential tolerance between cabbage, lettuce and kikuyu as this process is reflected in high concentrations in tops, the site of inactivation, where concentrations in excess of 1000 $\mu g\ g^{-1}$ have been recorded (Chenery and Sporne 1976).

Calcium application overcame leakage from calcium deficient tissue (Van Steveninck 1965) and restored ion selectivity (Epstein 1961) which suggests that these processes may have been involved in high calcium application reducing aluminium translocation to tops of cabbage, lettuce and kikuyu (Section VI.C.4.) and the lower aluminium levels in the protoplasm of some cortical and xylem parenchyma cells of roots (Section V.C.2.3.). This is consistent with calcium maintaining structural membrane integrity (Garrard and Humphreys 1967) and controlling the extent of aluminium uptake during Phase II. The fact that this result was not recorded by excised roots probably resulted from membrane damage by the high calcium chloride concentration used.

Aluminium bound to exchange sites as a result of uptake during Phase I precipitates phosphate (Clarkson 1967) and this reaction would have accounted for increased phosphate uptake by whole roots of cabbage and kikuyu (Section VI.C.11.). However, no evidence could be found for aluminium phosphate precipitation from EDX- analyses of these roots (Section V.C.2.1.). White (1976) also found aluminium phosphate precipitation in the free space of whole roots.

Aluminium uptake by roots is non-metabolic and consists of two phases. During Phase I, aluminium exchanges cations, particularly calcium and magnesium. The amount of aluminium adsorbed from an acid medium increases with the lowering of mean net charge density per aluminium atom as pH increases.

Calcium plays an important role in maintaining membrane selectivity and permeability which suggests that as a result of uptake during Phase I, aluminium moves across the plasmalemma and gains access to the stele. The size of the uptake component for Phase II was reflected in the amount of aluminium translocated to tops which in turn was related to the tolerance of cabbage, lettuce and kikuyu to aluminium. Differential response to calcium ions apparently controlled the extent to which aluminium could penetrate the plasmalemma of each species.

VIII. BIBLIOGRAPHY

VIII. BIBLIOGRAPHY

Adams, F., and Lund, Z.F. (1966). Effect of chemical activity of soil solution aluminium on cotton root penetration of acid subsoils. *Soil Sci.* 101, 193-8.

Ahmed, M. (1960). Aluminium toxicity of certain soils on the coast of British Guiana and problems of their agricultural utilization. *Trans. 7th int. Congr. Soil Sci.* 2, 161-70.

Aimi, R., and Murakami, T. (1964). Cell physiological studies on the effect of aluminium on growth of crop plants. *Bull. Natl. Inst. Agric. Sci. Ser. D.* 11, 331-96.

Ali, S.M.E. (1973). Influence of cations on aluminium toxicity in wheat (*Triticum aestivum*, Vill. Host). Ph.D. Thesis, Oregon State University.

Andrew, C.S., Johnson, A.D., and Sandland, R.L. (1973). Effect of aluminium on the growth and chemical composition of some tropical and temperate pasture legumes. *Aust. J. Agric. Res.* 24, 325-39.

Andrew, C.S., and Vandenberg, P.J. (1973). The influence of aluminium on phosphorus sorption by whole plants and excised roots of some pasture legumes. *Aust. J. Agric. Res.* 24, 341-51.

Armiger, W.H., Foy, C.D., Fleming, A.L., and Caldwell, B.E. (1968). Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminium. *Agron. J.* 60, 67-70.

Arnon, D.I., Fratzke, W.E., and Johnson, C.M. (1942). Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. *Plant Physiol.* 17, 515-24.

Arnon, D.I., and Johnson, C.M. (1942). Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. *Plant Physiol.* 17, 525-39.

Asher, C.J., and Loneragan, J.F. (1967). Response of plants to phosphate concentration in solution culture. I. Growth and phosphate content. *Soil Sci.* 103, 225-33.

Asher, C.J., Ozanne, P.G., and Loneragan, J.F. (1965). A method for controlling the ionic environment of plant roots. *Soil Sci.* 100, 149-56.

Awad, A.S., and Edwards, D.G. (1977). Reversal of adverse effects of heavy ammonium sulphate application on growth and nutrient status of kikuyu grass. *Plant Soil* 48, 169-83.

Awad, A.S., Edwards, D.G., and Milham, P.J. (1976). Effect of pH and phosphate on soluble soil aluminium and on growth and composition of kikuyu grass. *Plant Soil* 45, 531-42.

Bieleski, R.L. (1971). Enzyme changes in plants following changes in their mineral nutrition. Plant Anal. Fert. Probl. Abstr. 6th Int. Colloq. Tel Aviv, Israel, pp. 143-53.

Bilde, J. de. (1977). Effect of aluminium and calcium ions on root tips and phosphatase activity of calcicolous and siliceous ecotypes of *Silene nutans* L. (Caryophyllaceae). *Bull. Soc. Roy. Bot. Belg.* 110, 151-60.

Blue, W.G., and Dantzman, C.L. (1977). Soil chemistry and root development in acid soils. *Proc. Soil Crop Sci. Soc. Fla.* 36, 9-15.

Buttrose, M.S. (1978). Manganese and iron in globoid crystals of protein bodies from *Avena* and *Casuarina*. *Aust. J. Plant Physiol.* 5, 631-9.

Carter, O.G., and Lathwell, D.J. (1967). Effect of temperature on orthophosphate absorption by excised corn roots. *Plant Physiol.* 42, 1407-12.

Chamura, S. (1967). Relationship between crop tolerance of soil acidity and low soil pH. 15. Effects of aluminium. hydrogen ion, calcium, magnesium, nitrate and nitrite at varying concentrations on the growth of Italian ryegrass and milk vetch. *Proc. Crop Sci. Soc. Jpn.* 36, 361-8.

Chapman, H.D., and Pratt, P.F. (1961). "Methods of Analysis for Soils, Plants and Waters". (Uni. Calif., Div. Agric. Sci.).

Chenery, E.M., and Sporne, K.R. (1976). A note on the evolutionary status of aluminium accumulation among dicotyledons. *New Phytol.* 76, 551-4.

Chiasson, T.C. (1964). Effects of N, P, Ca and Mg treatments on yield of barley varieties grown on acid soils. *Can. J. Plant Sci.* 44, 525-30.

Chino, M., and Hidaka, H. (1977). Direct observation and X-ray microanalysis of frozen specimens of plant root in soil. *Soil Sci. Plant Nutr.* 23, 195-200.

Christian, G.D., and Feldman, F.J. (1970). "Atomic Absorption" Spectroscopy. Applications in Agriculture, Biology, and Medicine. (Wiley-Interscience, New York, London, Sydney, Toronto.)

Christiansen, M.N., and Foy, C.D. (1979). Fate and function of calcium in tissue. *Commun. Soil Sci. Plant Anal.* 10, 427-42.

Clark, R.B. (1975). Characterization of phosphatase in intact maize roots. *J. Agric. Food Chem.* 23, 458-60.

Clark, R.B. (1977). Effect of aluminium on growth and mineral elements of Al-tolerance and Al-intolerant corn. *Plant Soil* 47, 653-62.

Clark, R.B. (1978). Differential response of corn inbreds to calcium. *Commun. Soil Sci. Plant Anal.* 9, 729-44.

Clarkson, D.T. (1965). The effect of aluminium and other trivalent cations on cell division in the root apices of *Allium cepa*. *Ann. Bot.* (London) 29, 209-15.

Clarkson, D.T. (1966a). Aluminium tolerance in species within the genus *Agrostis* L. *J. Ecol.* 54, 167-78.

Clarkson, D.T. (1966b). Effect of aluminium on the uptake and metabolism of phosphorus by barley seedlings. *Plant Physiol.* 41, 165-72.

Clarkson, D.T. (1967). Interactions between aluminium and phosphorus on root surfaces and cell wall material. *Plant Soil* 27, 347-56.

Clarkson, D.T. (1969). Metabolic aspects of aluminium toxicity and some possible mechanisms for resistance. *Brit. Ecol. Soc. Symp.* 9, 381-97.

Clarkson, D.T., and Sanderson, J. (1969). The uptake of a polyvalent cation and its distribution in the root apices of *Allium cepa* and autoradiographic studies. *Planta* 89, 136-54.

Clarkson, D.T., and Sanderson, J. (1971). Inhibition of the uptake and long-distance transport of calcium by aluminium and other polyvalent cations. *J. Exp. Bot.* 22, 837-51.

Coleman, J.R. (1978). X-ray analysis of biological samples. *Scanning Electron Microscopy* II, 911-26.

Colliver, G.W., and Welch, L.F. (1970). Toxicity of preplant anhydrous ammonia to germination and early growth of corn. I. Laboratory studies. *Agron. J.* 62, 346-8.

Crooke, W.M. (1964). The measurement of cation-exchange capacity of plant roots. *Plant Soil* 21, 43-9.

Cutler, J.M., and Rains, D.W. (1974). Characterisation of cadmium uptake by plant tissue. *Plant Physiol.* 54, 67-71.

Dessureaux, L. (1969). Effect of aluminium on alfalfa seedlings. *Plant Soil* 30, 93-8.

Dodge, C.D., and Hiatt, A.J. (1972). Relationship of pH to ion uptake imbalance by varieties of wheat (*Triticum vulgare* L.). *Agron. J.* 64, 476-7.

Doss, B.D., and Lund, Z.F. (1975). Subsoil pH effects on growth and yield of cotton. *Agron. J.* 67, 193-6.

Drew, M.C., and Biddulph, O. (1971). Effect of metabolic inhibitors and temperature on uptake and translocation of ^{45}Ca and ^{42}K by intact plants. *Plant Physiol.* 48, 426-32.

Dumbroff, E.B., and Pierson, D.R. (1971). Probable sites for passive movement of ions across the endodermis. *Can. J. Bot.* 49, 35-8.

Edwards, D.G. (1968). Cation effects on phosphate absorption from solution by *Trifolium subterraneum*. *Aust. J. Biol. Sci.* 21, 1-11.

Edwards, D.G. (1970). Phosphate absorption and long-distance transport in wheat seedlings. *Aust. J. Biol. Sci.* 23, 255-64.

Edwards, J.H., and Horton, B.D. (1977). Aluminium-induced calcium deficiency in peach seedlings. *J. Am. Soc. Hort. Sci.* 102, 459-61.

Edwards, J.H., Horton, B.D., and Kirkpatrick, H. (1976). Aluminium toxicity symptoms in peach seedlings. *J. Am. Soc. Hort. Sci.* 101, 139-42.

Epstein, E. (1961). The essential role of calcium in selective cation transport by plant cells. *Plant Physiol.* 36, 437-44.

Epstein, E., Schmid, W.E., and Rains, D.W. (1963). Significance and technique of short-term experiments on solute absorption by barley roots. *Plant Cell Physiol.* 4, 79-84.

Fawzy, H., Overstreet, R., and Jacobson, L. (1954). Influence of hydrogen ion concentration on cation absorption by barley roots. *Plant Physiol.* 29, 234-7.

Ferguson,I.B., and Clarkson, D.T. (1975). Ion transport and endodermal suberization in the roots of *Zea mays*. *New Phytol.* 75, 69-79.

Ferguson, I.B., and Clarkson, D.T. (1976). Ion uptake in relation to the development of a root hypodermis. *New Phytol.* 77, 11-14.

Ferguson, J.F., and King, T. (1977). A model for aluminium phosphate precipitation. *J. Water Pollut. Control Fed.* 49, 646-58.

Filippis, L. F. de. (1979). The effect of heavy metal compounds on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol.* 92, 39-49.

Findlay, G.P., and Pallaghy, C.K. (1978). Potassium chloride in motor tissue of *Stylidium*. *Aust. J. Plant Physiol.* 5, 219-29.

Fleming, A.L. (1975). Characterization of acid phosphatase activity in wheat varieties differing in P efficiency. ASA Abstr. Ann. Meet., p.70.

Fleming, A.L. and Foy, C.D. (1968). Root structure reflects differential aluminium tolerance in wheat varieties. *Agron. J.* 60, 172-6.

Foy, C.D., Armiger, W.H., Briggle, L.W., and Reid ,D.A. (1965a). Differential aluminium tolerance of wheat and barley varieties in acid soils. *Agron. J.* 57, 413-7.

Foy, C. D., Armiger, W.H., Fleming, A.L., and Lewis, C.F. (1967a). Differential tolerance of cotton varieties to an acid soil high in exchangeable aluminium. *Agron. J.* 59, 415-18.

Foy, C.D. and Brown, J.C. (1963). Toxic factors in acid soils. I. Characterization of aluminium toxicity in cotton. *Proc. Soil Sci. Soc. Am.* 27, 403-7.

Foy, C.D., and Brown, J.C. (1964). Toxic factors in acid soils. II. Differential aluminium tolerance of plant species. *Proc. Soil Sci. Soc. Am.* 28, 27-32.

Foy, C.D., Burns, G.R., Brown, J.C., and Fleming, A.L. (1965b). Differential aluminium tolerance of two wheat varieties associated with plant induced pH changes around their roots. *Proc. Soil Sci. Soc. Am.* 29, 64-7.

Foy, C.D., Chaney, R.L., and White, M.C. (1978). The physiology of metal toxicity in plants. *Ann. Rev. Plant Physiol.* 29, 511-66.

Foy, C.D., Fleming, A.L., and Armiger, W.H. (1969). Aluminium tolerance of soybean varieties in relation to calcium nutrition. *Agron. J.* 61, 505-11.

Foy, C.D., Fleming, A.L., Burns, G.R., and Armiger, W.H. (1967b). Characterization of differential aluminium tolerance among varieties of wheat and barley. *Agron. J.* 31, 513-21.

Foy, C.D., Fleming, A.L., and Gerloff, G.C. (1972). Differential aluminium tolerance in two snapbean varieties. *Agron. J.* 64, 815- 8.

Foy, C.D., and Gerloff, G.C. (1972). Response of *Chlorella pyrenoidosa* to aluminium and low pH. *J. Phycol.* 8, 268-71.

Foy, C.D., Gerloff, G.C., and Gableman, W.H. (1973). Differential effects of aluminium on the vegetative growth of tomato cultivars in acid soil and nutrient solution. *J. Am. Soc. Hort. Sci.* 98, 427-32.

Foy, C.D., Orellana, R.G., Schwartz, J.W., and Fleming, A.L. (1974). Response of sunflower genotypes to aluminium in acid soil and nutrient solution. *Agron. J.* 66, 293-6.

Garrard, L.A., and Humphreys, T.E. (1967). The effect of divalent cations on the leakage of sucrose from corn scutellum slices. *Phytochemistry* 6, 1085-95.

Gerloff, G.C., and Fishbeck, K.A. (1969). Quantitative cation requirement of several green and blue algae. *J. Phycol.* 5, 109-14.

Gigon, A., and Rorison, I.H. (1972). The response of some ecologically distinct plant species to nitrate and to ammonium nitrogen. *J. Ecol.* 60, 93-102.

Gillett, C., and Lefebvre, J. (1978). Ionic diffusion through the *Nitella* cell wall in the presence of calcium. *J. Exp. Bot.* 29, 1155-9.

Greidanus, T., Peterson, L.A., Schrader, L.E., and Dana, M.S. (1972). Essentiality of ammonium for cranberry nutrition. *J. Am. Soc. Hort. Sci.* 97, 272-7.

Guerrier, G. (1978). Absorption d'aluminium par des racines de plantes entieres de feverole et de lupin jaune. *Plant Soil* 50, 135-44.

Gunatilaka, G.A., Asher, C.J., and Bell, L.C. (1977). Effects of solution aluminium concentration on plant growth. *Aust. Soc. Plant Physiol.* 18th General Meeting. p. 81.

Hagen, C.E., and Hopkins, H.T. (1955). Ionic species in orthophosphate absorption by barley roots. *Plant Physiol.* 30, 193-9.

Hara, T., Tanaka, T., Sonoda, Y., and Iwai, I. (1977). An interaction between magnesium and calcium in cabbage nutrition. *J. Japan Soc. Hort. Sci.* 46, 189-92.

Harrison-Murray, R.S., and Clarkson, D.T. (1973). Relationship between structural development and the absorption of ions by the root system of *Cucurbita pepo*. *Planta* 114, 1-16.

Havill, D.C., Lee, J.A., and Stewart, G.R. (1974). Nitrate utilization by species from acidic and calcareous soils. *New Phytol.* 73, 1221-31.

Hecht-Buchholz, C. (1979). Calcium deficiency and plant ultrastructure. *Commun. Soil Sci. Plant Anal.* 10, 67-81.

Helyar, K.R., and Anderson, A.J. (1971). Effects of lime on the growth of five species on aluminium toxicity, and on phosphorus availability. *Aust. J. Agric. Res.* 22, 707-21.

Hem. J.D. (1968). Aluminium species in water. *Adv. Chem. Series.* 73, 98-114.

Henning, S.J. (1975). Aluminium toxicity in the primary meristem of wheat roots. Ph.D. Thesis, Oregon State University.

Hiatt, A.J. (1967). Relationship of cell-sap pH to organic acid change during ion uptake. *Plant Physiol.* 42, 294-8.

Hiatt, A.J., and Lowe, R.H. (1967). Loss of organic acids, amino acids, K, and Cl from barley roots treated anaerobically and with metabolic inhibitors. *Plant Physiol.* 42, 1731-6.

Hoagland, D.R., and Arnon, D.I. (1950). The water culture method for growing plants without soil. Calif. Agr. Exp. Stat. Circ. 347.

Hoagland, D.R., and Broyer, T.C. (1940). Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. *Amer. J. Bot.* 27, 173-85.

Howeler, R.H., and Cadavid, L.F. (1976). Screening of rice cultivars for tolerance to Al-toxicity in nutrient solution as compared with a field screening method. *Agron. J.* 68, 551-5.

Hsu, P.H., and Bates, T.F. (1964). Fixation of hydroxy-aluminium polymers by vermiculite. *Proc. Soil Sci. Soc. Am.* 28, 763-9.

Hutchinson, F.E., and Hunter, A.S. (1970). Exchangeable aluminium levels in two soils as related to lime treatment and growth of six crop species. *Agron. J.* 62, 702-4.

Jackson, P.C., and Adams, H.R. (1963). Cation-anion balance during potassium and sodium absorption by barley roots. *J. Gen. Physiol.* 46, 369-86.

Jacobson, L., Hannapel, R.J., Moore, D.P., and Schaedle, M. (1961). Influence of calcium on selectivity of ion absorption. *Plant Physiol.* 36, 58-61.

Jacobson, L., Moore, D.P., and Hannapel, R.J. (1960). Role of calcium in absorption of monovalent cations. *Plant Physiol.* 35, 352-8.

Jacobson, L., Overstreet, R., Carlson, R.M., and Chastain, J.E. (1957). The effect of pH and temperature on the absorption of potassium and bromide by barley roots. *Plant Physiol.* 32, 658-62.

Jacobson, L., Overstreet, R., King, H.M., and Handley, R. (1950). A study of potassium absorption by barley roots. *Plant Physiol.* 25, 639-47.

Johnson, R.E., and Jackson, W.A. (1964). Calcium uptake and transport by wheat seedlings as affected by aluminium. *Proc. Soil Sci. Soc. Am.* 28, 381-6.

Jones, J.P., and Fox, R.L. (1978). Phosphorus nutrition of plants influenced by manganese and aluminium uptake from an oxisol. *Soil Sci.* 126, 230-6.

Jones, L.H. (1961). Aluminium uptake and toxicity in plants. *Plant Soil* 13, 297-310.

Kerridge, P.C. (1969). Aluminium toxicity in wheat (*Triticum aestivum* vill., Host). Ph.D. Thesis, Oregon State University.

Kerridge, P.C., Dawson, M.D., and Moore, D.P. (1971). Separation of degrees of aluminium tolerance in wheat. *Agron. J.* 63, 586-91.

Kerridge, P.C., and Kronstad, W.E. (1968). Evidence of genetic resistance to aluminium toxicity in wheat (*Triticum aestivum* vill. Host). *Agron. J.* 60, 710-11.

Keser, M., Benedict, F., Newbauer, F., Hutchinson, F.E., and Verrill, D.B. (1977). Differential aluminium tolerance of sugarbeet cultivars as evidenced by anatomical structure. *Agron. J.* 69, 347-50.

Kirkpatrick, H.C., Thompson, J.M., and Edwards, J.H. (1975). Effects of aluminium concentration on growth and chemical composition of peach seedlings. *Hort. Sci.* 10, 132-4.

Klimashevskii, E.L., and Bernatskaya, M.L. (1973). Activity of ATPase and acid phosphatase in growth zones of the roots of two varieties of peas having different sensitivity to Al-ion toxicity. *Sov. Plant Physiol.* 20, 201-4.

Klimashevskii, E.L., Markova, Y.A., Bernatzkaya, M.L., and Malysheva, A.S. (1972). Physiological response to aluminium toxicity in root zone of pea varieties. *Agrochimica* 26, 487-96.

Klimashevskii, E.L., Markova, Y.A., Zyabkina, S.M., Zinenko, G.K., Zolotukhin, G.E., and Pavolova, S.V. (1976). Aluminium absorption and localization in root tissues of different pea cultivars. *Fiziol. Biokhim. Kul't. Rast.* 8, 396-401.

Kotze, W.A.G. (1979). Ionic interaction in the uptake and transport of calcium by apple seedlings. *Commun. Soil Sci. Plant Anal.* 10, 115-27.

Kotze, W.A.G., Shear, C.B., and Faust, M. (1977). Effect of nitrogen source and aluminium in nutrient solution on the growth and mineral nutrition of apple and peach seedlings. *J. Am. Soc. Hort. Sci.* 102, 279-82.

Kumar, P.H. (1979). Effect of aluminium on the growth of *Areca triandra* Roxb. in sand culture. *Sci. Hortic.* 10, 183-5.

Lafever, H.N., and Campbell, L.G. (1978). Inheritance of aluminium tolerance in wheat. *Can. J. Genet. Cytol.* 20, 355-64.

Lane, S.D., and Martin, E.S. (1977). A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytol.* 79, 281-6.

Lance, J.C., and Pearson, R.W. (1969). Effect of low concentrations of aluminium on growth and water and nutrient uptake by cotton roots. *Proc. Soil Sci. Soc. Am.* 33, 95-8.

Leggett, J.E., and Epstein, E. (1956). Kinetics of sulphate absorption by barley roots. *Plant Physiol.* 31, 222-6.

Loneragan, J.F., Snowball, K., and Simmons, W.J. (1968). Response of plants to calcium concentration in solution culture. *Aust. J. Agric. Res.* 19, 845-57.

Lott, J.N.A., and Buttrose, M.S. (1977). Globoid in protein bodies of legume seed cotyledons. *Aust. J. Plant Physiol.* 5, 89-111.

Lott, J.N.A., Greenwood, J.S., Vollmer, C.M., and Buttrose, M.S. (1978). Energy-dispersive X-ray analysis of phosphorus, potassium, magnesium and calcium in globoid crystals in proteins from different regions of *Cucurbita maxima* embryos. *Plant Physiol.* 61, 984-8.

Loughman, B.C. (1966). The mechanism of absorption and utilization of phosphate by barley plants in relation to subsequent transport to the shoot. *New Phytol.* 65, 388-97.

Maas, E.V. (1969). Calcium uptake by excised maize roots and interactions with alkali cations. *Plant Physiol.* 44, 985-9.

Maas, E.V., Moore, D.P., and Mason, B.J. (1968). Manganese absorption by excised barley roots. *Plant Physiol.* 43, 527-30.

MacLean, A.A., and Chiasson, T.C. (1966). Differential performance of two barley varieties to varying aluminium concentrations. *Can. J. Soil Sci.* 46, 147-53.

MaCleod, L.B., and Jackson, L.P. (1965). Effect of concentration of the Al ion on root development and establishment of legume seedlings. *Can. J. Soil Sci.* 45, 221-34.

MaCleod, L.B., and Jackson, L.P. (1967). Water soluble and exchangeable aluminium in acid soils as affected by liming and fertilization. *Can. J. Soil Sci.* 47, 203-10.

McCormick, L.H., and Borden, F.Y. (1972). Phosphate fixation by aluminium in plant roots. *Proc. Soil Sci. Soc. Am.* 36, 779-802.

McCormick, L.H., and Borden, F.Y. (1974). The occurrence of aluminium phosphate precipitate in plant roots. *Proc. Soil Sci. Soc. Am.* 38, 931-4.

McElhannon, W.S., and Mills, H.A. (1978). Influence of percent NO_3^-/NH_4^+ on growth, N absorption, and assimilation by lima beans in solution culture. *Agron. J.* 70, 1027-32.

McLean, E.O. (1976). Chemistry of soil aluminium. *Commun. Soil Sci. Plant Anal.* 7, 619-36.

McLean, F.T., and Gilbert, B.E. (1927). The relative aluminium tolerance of crop plants. *Soil Sci.* 24, 163-75.

Marinos, N.G. (1962). Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. *Am. J. Bot.* 49, 834-41.

Matsumoto, H., and Hirasawa, E. (1979). Less involvement of phosphorus deficiency in the inhibition of root elongation of pea seedlings by aluminium. *Soil Sci. Plant Nutr.* 25, 93-101.

Matsumoto, H., Hirasawa, E., Morimura, S., and Takahashi, E. (1976b). Localization of aluminium in tea leaves. *Plant Cell Physiol.* 17, 627-31.

Matsumoto, H., Hirasawa, E., Torikai, H., and Takahashi, E. (1976a). Localization of absorbed aluminium in pea root and its binding to nucleic acids. *Plant Cell Physiol.* 17, 127-37.

Matsumoto, H., Morimura, S., and Takahashi, E. (1977). Less involvement of pectin in the precipitation of aluminium in pea root. *Plant Cell Physiol.* 18, 325-35.

Mathys, W. (1977). The role of malate, oxalate, and mustard oil glucosides in the evolution in zinc-resistance in herbage plants. *Physiol. Plant* 40, 130-6.

Medappa, K.C., and Dana, M.N. (1970). Tolerance of cranberry plants to Mn, Fe, and Al. *J. Am. Soc. Hort. Sci.* 95, 107-10.

Moore, D.P. (1974). Physiological effects of pH on roots. *In* 'The Plant Root and its Environment' (ed. E.W. Carson). pp. 135-51. (University Press: Virginia.)

Moore, D.P., Jacobson, L., and Overstreet, R. (1961b). Uptake of calcium by excised barley roots. *Plant Physiol.* 36, 53-7.

Moore, D.P., Overstreet, R., and Jacobson, L. (1961a). Uptake of magnesium and its interaction with calcium in excised barley roots. *Plant Physiol.* 36, 290-5.

Morimura, S., and Matsumoto, H. (1978). Effect of aluminium on some properties and template activity of purified pea DNA. *Plant Cell Physiol.* 19, 429-36.

Morimura, S., Takahashi, E., and Matsumoto, H. (1978). Association of aluminium with nuclei and inhibition of cell division in onion (*Allium cepa*) roots. *Z. Pflanzenphysiol.* 88, 395-401.

Mugwira, L.M., Elgawhary, S.M., and Patel, K.I. (1976). Differential tolerances of triticale, wheat, rye and barley to aluminium in nutrient solution. *Agron. J.* 68, 782-6.

Mugwira, L.M., Elgawhary, S.M., and Patel, S.V. (1978). Aluminium tolerance in triticale, wheat and rye as measured by root growth characteristics and aluminium concentration. *Plant Soil* 50, 681-90.

Mugwira, L.M., and Patel, S.V. (1977). Root zone pH changes and ion uptake imbalances by triticale, wheat and rye. *Agron. J.* 69, 719-22.

Mullette, K.J. (1975). Stimulation of growth in *Eucalyptus* to aluminium. *Plant Soil* 42, 495-9.

Mullette, K.J., Hannon, N.J., and Elliott, A.G.L. (1974). Insoluble phosphorus usage by *Eucalyptus*. *Plant Soil* 41, 199-205.

Munns, D.N. (1965a). Soil acidity and growth of a legume. I. Interactions of lime with nitrogen and phosphate on growth of *Medicago sativa* L. and *Trifolium subterraneum* L. *Aust. J. Agric. Res.* 16, 733-41.

Munns, D.N. (1965b). Soil acidity and the growth of a legume. II. Reactions of aluminium and phosphate in solution and effects of aluminium, phosphate, calcium and pH on *Medicago sativa* L. and *Trifolium subterraneum* L. in solution culture. *Aust. J. Agric. Res.* 16, 743-55.

Munns, D.N. (1965c). Soil acidity and the growth of a legume. III. Interactions of lime and phosphate in growth of *Medicago sativa* L. in relation to aluminium toxicity and phosphate fixation. *Aust. J. Agric. Res.* 16, 757-66.

Murphy, R.P. (1959). Some factors influencing cation uptake by excised roots of perennial ryegrass (*Lolium perenne*). *Plant Soil* 10, 242-9.

Naidoo, G., Stewart, J. Mc.D., and Lewis, R.J. (1978). Accumulation sites of Al in snapbean and cotton roots. *Agron. J.* 70, 489-92.

Nair, V.D., and Prenzel, J. (1978). Calculations of equilibrium concentration of mono- and polynuclear hydroxyaluminium species at different pH and total aluminium concentrations. *Z. Pflanzenernaehr.* 141, 741-51.

Neenan, M. (1960). The effects of soil acidity on the growth of cereals with particular reference to the differential reaction of varieties thereto. *Plant Soil* 12, 324-8.

Nielson, I.R., and Overstreet, R. (1955). A study of the role of hydrogen ion in the mechanism of potassium absorption by excised barley roots. *Plant Physiol.* 30, 303-9.

Nissen, P. (1977). Ion uptake in higher plants and KCl stimulation of plasmalemma adenosine triphosphatase: Comparison of models. *Physiol. Plant* 40, 205-14.

Osawa, T., and Ikeda, H. (1979). Heavy metal toxicities in vegetable crops. VII. The effect of potassium and calcium concentrations in the nutrient solution on zinc toxicities in vegetable crops. *J. Japan Soc. Hort. Sci.* 47, 485-91.

Oullette, G.J., and Dessureaux, L. (1958). Chemical composition of alfalfa as related to degree of tolerance to manganese and aluminium. *Can. J. Plant Sci.* 38, 206-14.

Pitman, M.G. (1970). Active H^+ efflux from cells of low salt barley roots during salt accumulation. *Plant Physiol.* 45, 787-90.

Presad, M. (1976). Nitrogen nutrition and yield of sugar cane as affected by N-serve. *Agron. J.* 68, 343-6.

Ragland, J.L., and Coleman, N.T. (1962). Influence of aluminium on phosphorus uptake by snapbean roots. *Proc. Soil Sci. Soc. Am.* 26, 88-90.

Rains, D.W., Schmid, W.E., and Epstein, E. (1964). Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. *Plant Physiol.* 39, 274-8.

Ramakrishnan, P.S. (1968). Nutritional requirements of the edaphic ecotypes in *Melilotus alba* Medic. 2. Aluminium and manganese. *New Phytol.* 67, 301-8.

Randall, P.J., and Vose, P.J. (1963). Effect of aluminium on uptake and translocation of phosphorus by perennial ryegrass. *Plant Physiol.* 38, 403-9.

Rasmussen, H.P. (1968). Entry and distribution of aluminium in *Zea mays:* Electron microprobe X-ray analysis. *Planta* 81, 28-37.

Reid, D.A., Jones, G.D., and Armiger, W.H. (1969). Differential aluminium tolerance of winter barley varieties and selections in associated greenhouse and field experiments. *Agron. J.* 61, 218-22.

Rhue, R.D. (1976). The time-concentration interaction of Al toxicity in wheat root meristems. Ph.D. Thesis, Oregon State University.

Rhue, R.D., and Grogan, C.O. (1977). Screening for Al-tolerance using different Ca and Mg concentrations. *Agron. J.* 69, 755-60.

Rhue, R.D,, Grogan, C.O., Stockmeyer, E.W., and Everett, H.L. (1978). Genetic control of aluminium tolerance in corn. *Crop Sci.* 18, 1063-7.

Robards, A.W., Jackson, S.M., Clarkson, D.T., and Sanderson, J. (1973). The structure of barley roots in relation to the transport of ions into the stele. *Protoplasma* 77, 291-311.

Robson, A.D., Edwards, D.G., and Loneragan, J.F. (1970). Calcium stimulation of phosphate absorption by annual legumes. *Aust. J. Agric. Res.* 21, 601-12.

Rorison, I.H. (1965). The effect of aluminium on the uptake and incorporation of phosphate by excised sanfoin roots. *New Phytol.* 64, 23-7.

Sampson, M., Clarkson, D.T., and Davies, D.D. (1965). DNA synthesis in aluminium treated roots of barley. *Science* 148, 1476-7.

Sampson, M., and Davies, D.D. (1966). Metabolically labile DNA in mitotic and non-mitotic cells of *Zea mays*. *Life Sci.* 5, 1239-47.

Siman, A., Cradock, F.W., Nicholls, P.J., and Kirton, H.C. (1971). Effects of calcium carbonate and ammonium sulphate on manganese toxicity in an acid soil. *Aust. J. Agric. Res.* 22, 201-14.

Simon, E.W. (1978). The symptoms of calcium deficiency in plants. *New Phytol.* 80, 1-15.

Sivasubramaniam, S., and Talibudeen, O. (1972). Effects of aluminium on the growth of tea (*Camellia sinensis*) and its uptake of potassium and phosphorus. *J. Sci. Food Agric.* 22, 325-9.

Smith, R.W. (1971). Relations among equilibrium and non-equilibrium aqueous species of aluminium hydroxy polymers. *Adv. Chem. Series* 106, 250-79.

Snedecor, G.W., and Cochran, W.G. (1967). 'Statistical Methods' (6th edition). pp. 114-5. (Iowa State University Press.)

Thawornwong, N., and Van Diest, A. (1974). Influences of high acidity and aluminium on the growth of lowland rice. *Plant Soil* 41, 141-59.

Totev, T. (1977). Effect of aluminium, manganese and iron on the fertility of light grey forest (pseudopodzolic) soil. 2. Effect of aluminium sulphate, manganese sulphate, ferric sulphate and lime on the growth and chemical composition of plants. *Pochvozn. Agrokhim.* 12, 29-44.

Townsend, J.R., and Blatt, C.R. (1966). Lowbush blueberry: Evidence for the absence of a nitrate reducing system. *Plant Soil* 25, 456-9.

Van Steveninck, F.R.M. (1965). The significance of calcium on the apparent permeability of cell membranes and the effects of substitution with other divalent ions. *Physiologia Plant.* 18, 54-69.

Van Steveninck, F.R.M., Ballment, B., Peters, P.D., and Hall, T.A. (1976). Ultrastructural localization of ions. II. X-ray analytical verification of silver precipitation products and distribution of chloride in mesophyll cells of barley seedlings. *Aust. J. Plant Physiol.* 3, 359-65.

Vickers, J.C., and Zak, J.M. (1978). Effects of pH, P, and Al on the growth and chemical composition of crown vetch (*Coronilla varia* L.). *Agron. J.* 70, 748-50.

Viets, F.G. (1944). Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. *Plant Physiol.* 19, 466-80.

Vogel, A.I. (1961). 'A Text Book of Quantitative Inorganic Analyses' (3rd edition). pp. 702-13. (Longmans: London.)

Volz, M.G., and Jacobson, L. (1977). Nature and magnitude of calcium uptake by excised roots of vetch and barley. *Plant Soil* 46, 79-91.

Waisel, Y., Hoffen, A., and Eshel, A. (1970). The localization of aluminium in the cortex cells of bean and barley roots by X-ray microanalysis. *Physiol. Plant* 23, 75-9.

Walker, N.A., and Pitman, M.G. (1976). Measurements of fluxes across membranes. *In* 'Transport in Plants. II. Part A. Cells' (Ed. U. Luttge and M.G. Pitman). pp. 93-124. (Springer-Verlag, Berlin, Heidelberg, New York.)

Wallace, A., Forlich, E., and Lunt, O.R. (1966). Calcium requirements of higher plants. *Nature* (London), 209, 634.

Wallihan, E.F. (1948). The influence of aluminium on the phosphorus nutrition of plants. *Am. J. Bot.* 35, 106-12.

Warren, K.S. (1962). Ammonia toxicity and pH. *Nature* (London) 195, 47-9.

Weast, R.C. (1973). 'Handbook of Chemistry and Physics' (54th edition). (The Chemical Rubber Company Press.)

White, R.E. (1973). Studies on mineral ion absorption by plants. II. The interaction between metabolic activity and the rate of phosphorus uptake. *Plant Soil* 38, 509-23.

White, R.E. (1976). Studies on the mineral ion absorption by plants. III. The interaction of aluminium phosphate and pH on the growth of *Medicago sativa*. *Plant Soil* 46, 195-208.

White, R.E., Tiffin, L.O., and Taylor, A.W. (1976). The existence of polymeric complexes in dilute solutions of aluminium and orthophosphate. *Plant Soil* 45, 521-9.

Wiltshire, G.H. (1973). Response of grasses to nitrogen source. *J. Appl. Ecol.* 10, 429-35.

Woolhouse, H.W. (1969). Differences in the properties of the acid phosphatases of plant roots and their significance in the evolution of edaphic ecotypes. *In* 'Ecological Aspects of the Mineral Nutrition of Plants' (ed. I.H. Rorison *et al.*). pp. 357-80. Symp. Brit. Ecol. Soc. no. 9. Sheffield, 1968. Oxford and Edinburgh. (Blackwell Scientific Publications.)

Wright, K.E. (1943). Internal precipitation of phosphorus in relation to aluminium toxicity. *Plant Physiol.* 18, 708-12.

Wright, K.E., and Donahue, B.A. (1953). Aluminium toxicity studies with radioactive phosphorus. *Plant Physiol.* 28, 674-80.

Wuytath, R., and Gillet, C. (1978). Nature des liaisons de l'ion calcium dans le paroi de *Nitella flexilis*. *Can. J. Bot.* 56, 1439-43.

Yeo, A.R., Kramer, D., and Lauchti, A. (1977a). Ion distribution in salt-stressed mature *Zea mays* roots in relation to ultrastructure and retention of sodium. *J. Exp. Bot.* 28, 17-29.

Yeo, A.R., Lauchli, A., and Kramer, D. (1977b). Ion measurement by X-ray microanalysis in unfixed, frozen, hydrated plant cells of species differing in salt tolerance. *Planta* 134, 35-8.

Zasoki, R.J., and Burau, R.G. (1977). A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. *Commun. Soil Sci. Plant Anal.* 8, 425-36.

IX. APPENDICES

Appendix I.1.

Aluminium uptake by excised cabbage roots ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$.

Data on which Figure IV.C.1.(i) is based.

Time (min)	Replicates	Treatment					
		25°C		1°C		25°C DNP	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	2651	2225	3277	1448	4483	2498
	2	2063	2361	2855	1355	4612	2750
10	1	2807	2740	2614	1891	5825	3181
	2	3067	2674	3317	1628	4607	3064
20	1	3098	3260	3973	2077	7507	3910
	2	3436	3190	4671	2241	8481	4351
40	1	4515	3415	3936	2264	9908	4861
	2	3948	3816	3448	2243	9373	4903
60	1	4563	3377	4507	2343	10260	6332
	2	4930	3166	5286	2569	9960	5973
80	1	4922	4099	4759	2834	12459	7506
	2	4864	3772	5392	2547	11695	8171
100	1	5296	4146	5440	2778	13195	9298
	2	6119	4187	5288	3012	12955	8780
120	1	5771	4579	7425	2872	14270	9262
	2	6306	4320	6099	2716	14467	11238
180	1	6888	4649	6644	3253	15839	11818
	2	7534	4800	6378	3068	16563	10995

Appendix I.2.

Aluminium uptake by excised lettuce roots (μg g^{-1} dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$.

Data on which Figure IV.C.1.(ii) is based.

Time (min)	Replicates	Treatment					
		25°C		1°C		25°C DNP	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	2012	1617	2023	1410	2940	2174
	2	1958	1678	1759	1394	2440	2324
10	1	2099	1828	2595	1680	3397	3113
	2	2360	1965	2596	1860	3407	3233
20	1	2477	2172	3049	2105	4440	4065
	2	2711	2162	2708	2119	4562	3930
40	1	3246	2905	3864	2803	6032	4388
	2	3666	2654	3408	2789	6182	4640
60	1	4048	2899	3895	3554	6192	5470
	2	3658	2664	4288	2919	6672	4826
80	1	4599	3576	4705	3284	7310	5917
	2	4519	3289	4745	4604	6960	6010
100	1	4407	3473	5313	3330	8143	6528
	2	4458	3534	4957	3150	6808	6152
120	1	5087	3285	5103	3770	8318	6871
	2	4861	4035	5584	3810	8729	6998
180	1	4873	4719	7008	4016	9449	7859
	2	5920	4223	5844	4532	10255	6313

Appendix I.3.

Aluminium uptake by excised kikuyu roots ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$.

Data on which Figure IV.C.1.(iii) is based.

Time (min)	Replicates	Treatment					
		25°C		1°C		25°C DNP	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	676	466	632	318	1742	673
	2	688	640	745	522	1346	663
10	1	1259	791	938	764	1625	971
	2	510	500	927	711	2021	695
20	1	887	704	1112	639	1768	644
	2	767	664	812	692	1595	1259
40	1	908	995	1198	934	4038	1117
	2	983	735	1176	481	1719	1028
60	1	742	747	1232	823	3070	1288
	2	1273	784	1336	692	2852	1099
80	1	1755	1178	1571	885	3337	1261
	2	1331	805	1315	969	3979	1394
100	1	1409	1110	1396	764	3821	1891
	2	1526	1133	1266	1077	3104	1387
120	1	1033	838	1191	946	4540	2107
	2	1491	1165	1493	979	4291	1674
180	1	909	1250	1294	1153	5009	2054
	2	1624	1151	1316	1043	5183	1891

Appendix I.4.

Aluminium uptake by Amberlite ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$ at 25^oC.

Date on which Figure IV.C.1.(iv) is based.

Time (min)	Replicates	pH 4.2	pH 4.0
5	1	335	217
	2	375	156
10	1	594	419
	2	616	330
20	1	923	519
	2	733	535
40	1	1489	937
	2	1233	733
60	1	1425	982
	2	1089	1192
80	1	1847	1100
	2	2281	1096
100	1	2600	934
	2	2164	874
120	1	2651	1491
	2	2189	1078
180	1	2660	1531
	2	1935	1184

Appendix I.5.

Aluminium uptake by excised roots of cabbage, lettuce and kikuyu (μg g^{-1} dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.6737M $CaCl_2$ at 25°C.

Data on which Figure IV.C.1.(v) is based.

Time (min)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	1591	1202	2051	2068	732	623
	2	1418	953	2197	1558	1064	622
10	1	2024	1629	3124	2544	1025	1066
	2	1850	1406	3218	2919	1147	879
20	1	2378	1703	3824	2945	1345	830
	2	2147	2253	4565	2549	1310	1016
40	1	3187	3016	4849	3982	1487	1217
	2	3194	2592	5150	4400	1328	1067
60	1	3852	3566	6557	5611	1430	1320
	2	3854	3497	5940	5233	1719	1137
80	1	4078	3650	7081	6251	1404	1138
	2	4361	3442	7752	6619	1765	1338
100	1	4534	3249	6429	6294	1653	1447
	2	4534	3264	6682	6028	1738	1415
120	1	4931	3748	7607	6427	1873	1435
	2	4395	3720	8014	6915	1761	1438
180	1	5304	4667	8990	7684	2262	2021
	2	5362	5753	7936	7283	2020	1875

Appendix I.6.

Aluminium uptake by Amberlite ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.6737M $CaCl_2$ at 25^oC.

Data on which Figure IV.C.1.(vi) is based.

Time (min)	Replicates	pH 4.2	pH 4.0
5	1	16	27
	2	20	57
10	1	53	50
	2	46	75
20	1	122	48
	2	108	62
40	1	216	115
	2	203	92
60	1	198	166
	2	166	148
80	1	247	149
	2	315	207
100	1	232	209
	2	246	187
120	1	274	213
	2	240	265
180	1	363	255
	2	360	216

Appendix I.7.

Calcium desorption from excised roots of cabbage, lettuce and kikuyu ($\mu g\ g^{-1}$ dry weight), time course of desorption by 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$ at 25°C.

Date on which Figure IV.C.1.(vii) is based.

Time (min)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	4825	2877	2368	2088	391	343
	2	4741	3056	2434	2046	447	334
10	1	4606	3230	2850	2405	541	428
	2	4978	3280	2769	2310	572	425
20	1	5168	3472	3060	2826	622	520
	2	5457	3738	3421	2929	657	540
40	1	5612	3914	3842	3412	648	613
	2	5204	3874	3617	3518	716	609
60	1	5730	3821	4213	3635	781	619
	2	5613	3815	4011	3688	764	625
80	1	5868	3954	4359	3827	771	668
	2	5749	3995	4155	3856	750	659
100	1	5810	4010	4562	3798	762	654
	2	5813	3944	4309	3843	769	598
120	1	5942	3933	4248	3990	793	657
	2	6070	3910	4344	8286	816	670
180	1	6201	3971	4723	4143	786	682
	2	6100	4077	4661	4071	811	643

Appendix I.8.

Calcium desorption from Amberlite ($\mu g\ g^{-1}$ dry weight), time course of desorption by 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$ at 25°C. Data on which Figure IV.C.1.(viii) is based.

Time (min)	Replicates	pH 4.2	pH 4.0
5	1	109	120
	2	126	116
10	1	51	84
	2	73	116
20	1	135	169
	2	160	168
40	1	166	182
	2	184	182
60	1	174	185
	2	181	177
80	1	189	194
	2	186	192
100	1	196	188
	2	197	181
120	1	187	194
	2	189	190
180	1	201	202
	2	199	206

Appendix I.9.

Calcium uptake by excised roots of cabbage, lettuce and kikuyu ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.6737M $CaCl_2$ at 25^oC.

Data on which Figure IV.C.1.(ix) is based.

Time (min)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
5	1	9769	8371	7894	9637	5593	3375
	2	10619	7573	7855	10842	3889	3974
10	1	13097	10017	10747	9922	6310	7660
	2	11663	9285	12680	11479	7376	8810
20	1	14841	13735	12596	17312	7296	7619
	2	14897	12314	18489	16486	7448	8927
40	1	15285	13881	23306	17178	7662	9727
	2	14959	14339	21000	18932	6850	13657
60	1	16932	14024	28586	22469	9468	9669
	2	16651	16976	23259	20066	9303	11660
80	1	16121	16897	27212	16497	8003	9250
	2	13274	15903	25547	16138	6818	10140
100	1	15113	4276	29491	18495	8280	11392
	2	14839	15324	28951	30589	8007	14535
120	1	15211	14517	32102	19832	7698	10353
	2	15746	15183	27008	21660	8755	12657
180	1	15989	17279	28476	20515	7157	13350
	2	15230	15841	24238	22199	7392	9465

Appendix I.10.

Calcium uptake by Amberlite ($\mu g\ g^{-1}$ dry weight), time course of uptake from 1.0mM $Al_2(SO_4)_3$, 0.6737M $CaCl_2$ at 25°C.

Data on which Figure IV.C.1.(x) is based.

Time (min)	Replicates	pH 4.2	pH 4.0
5	1	1623	1774
	2	1825	2029
10	1	2274	1996
	2	2212	2399
20	1	3525	3273
	2	3217	2600
40	1	4203	3067
	2	4128	2795
60	1	4103	2896
	2	3225	4367
80	1	4111	3356
	2	4034	3824
100	1	4246	3631
	2	4409	4222
120	1	4393	4034
	2	4103	4061
180	1	3963	3722
	2	4128	3246

Appendix I.11.

Aluminium uptake by excised roots of cabbage, lettuce and kikuyu ($\mu g\ g^{-1}$ dry weight), 0-60 min uptake period from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$, 1-50°C temperature range.

Date on which Figure IV.C.2.(i) is based.

Temp. (°C)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
1	1	4507	2761	3895	1680	1497	1391
	2	4310	2587	4287	2317	1707	1195
	3	5286	2677	4007	2581	1591	1623
10	1	4201	2837	3626	3274	1263	985
	2	3923	2758	4279	3221	1185	753
	3	4650	2905	3584	3538	1413	852
20	1	4714	3311	4082	3078	933	926
	2	5244	3333	4706	3123	1450	1075
	3	4934	3129	4188	3233	1206	884
30	1	5544	3485	4813	3914	1390	1062
	2	5366	3435	5156	4031	1675	1157
	3	5537	3674	4778	3652	1634	934
40	1	7291	7411	6901	6088	1397	937
	2	9108	7255	6504	5184	1373	981
	3	7765	6610	6795	5890	1308	749
50	1	11872	11193	15484	14826	3055	2447
	2	12222	11023	16393	12489	3289	2412
	3	13678	11114	17665	13983	3102	2392

Appendix I.12.

Aluminium uptake by excised roots of cabbage, lettuce and kikuyu ($\mu g\ g^{-1}$ dry weight), 60-120 min uptake period from 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$, 1-50°C temperature range.

Data on which Figure IV.C.2.(ii) is based.

Temp. (°C)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
1	1	1729	415	1102	633	61	18
	2	849	159	1441	825	41	27
	3	1391	146	1117	723	48	30
10	1	457	451	1280	93	350	22
	2	1211	604	1260	343	248	62
	3	1079	379	1419	212	200	80
20	1	767	923	699	1025	231	76
	2	752	589	916	446	503	33
	3	945	646	515	599	446	55
30	1	1282	531	1765	668	236	2
	2	1540	811	1627	921	466	9
	3	1041	837	1357	343	246	2
40	1	4580	2663	4509	3435	179	231
	2	4241	3642	4834	2800	265	250
	3	3922	4280	4656	2523	28	227
50	1	1684	268	4189	1234	1957	2987
	2	2121	474	3176	2467	4144	3018
	3	1794	75	3747	2817	3807	3010

Appendix I.13.

Aluminium desorption from excised roots of cabbage, lettuce and kikuyu ($\mu g\ g^{-1}$ dry weight), time course of desorption after 120 min absorption in 1.0mM $Al_2(SO_4)_3$, 0.5mM $CaSO_4$ at 25°C, 20 min rinse in deionized water at 1°C then in 22.5mM succinic-tartaric acids plus triethylamine, pH 4.5 at 1°C for periods up to 240 min.

Data on which Figure IV.C.3. is based.

Time (min)	Replicates	Treatment					
		Cabbage		Lettuce		Kikuyu	
		pH 4.2	pH 4.0	pH 4.2	pH 4.0	pH 4.2	pH 4.0
Endogenous	1	652	643	257	276	418	359
	2	692	613	222	294	412	379
	3	622	700	178	196	435	392
Desorption Water							
0	1	8030	5982	6954	5292	2229	1836
	2	7951	5518	6985	5677	2064	1884
20	1	7131	5595	6815	5176	1657	1170
	2	6314	5164	6812	5144	1486	1184
Organic Acid							
10	1	3086	2591	4302	2393	1029	793
	2	3546	2750	4351	2320	1194	816
30	1	2434	1999	3182	1494	981	907
	2	2837	2172	2888	1409	1041	900
60	1	2043	1792	2428	1017	998	950
	2	1834	1498	2461	1093	941	761
120	1	1389	1410	1661	829	749	667
	2	1393	1374	1748	786	726	707
180	1	1266	1286	1659	631	707	577
	2	1192	1059	1506	567	762	635
240	1	1089	1132	1426	492	577	445
	2	1183	1375	1408	519	708	598

Appendix II.1.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for cabbage ± Al (3) pH 4.0 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	3.57	0 55	0.88
Cortex	2.83	0.99	1.11
Endodermis	1.15	0.29	1.49
Protoxylem	0.51	0.17	1.36
Metaxylem	1.12	0.05	1.45
Xylem parenchyma	0.90	0.22	1.56
Phloem	0.81	0.18	1.55
Mean	1.56	0.35	1.34
$t_{0.05}\ S\bar{x}$	1.00	0.28	0.22
Mid			
Epidermis	1.41	1.24	0.96
Cortex	0.42	0.48	0.68
Endodermis	0.19	0.51	0.82
Protoxylem	0.88	0.15	0.77
Metaxylem	-0.24	0.06	0.51
Xylem parenchyma	0.62	0.23	1.03
Phloem	0.37	0.14	0.83
Mean	0.52	0.40	0.80
$t_{0.05}\ S\bar{x}$	0.20	0.35	0.39
Base			
Epidermis	1.13	0.77	0.24
Cortex	0.64	0.33	0.33
Endodermis	0.99	0.29	0.81
Protoxylem	0.95	0.21	0.67
Metaxylem	0.89	0.00	0.39
Xylem parenchyma	2.35	0.57	0.73
Phloem	1.90	0.22	0.79
Mean	1.26	0.34	0.57
$t_{0.05}\ S\bar{x}$	0.53	0.22	0.20

Appendix II.2.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for cabbage ± Al (1) pH 4.6 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.85	1.81	0.77
Cortex	1.10	0.60	0.55
Endodermis	-0.01	0.65	0.49
Protoxylem	0.19	0.23	1.76
Metaxylem	-0.16	0.07	0.81
Xylem parenchyma	-0.16	0.08	1.19
Phloem	-0.25	0.01	1.28
Mean	0.22	0.49	0.98
$t_{0.05}\ S\bar{x}$	0.46	0.54	0.39
Mid			
Epidermis	2.68	1.50	0.78
Cortex	1.44	0.73	0.65
Endodermis	0.32	-0.02	0.49
Protoxylem	0.32	0.00	0.95
Metaxylem	-0.25	-0.14	0.19
Xylem parenchyma	0.01	0.10	0.42
Phloem	-0.04	0.09	0.52
Mean	0.64	0.32	0.57
$t_{0.05}\ S\bar{x}$	2.37	0.22	0.21
Base			
Epidermis	0.71	0.97	0.82
Cortex	0.31	0.31	0.59
Endodermis	-0.11	0.13	0.84
Protoxylem	0.05	0.25	0.37
Metaxylem	-0.34	0.03	0.17
Xylem parenchyma	-0.16	0.01	0.80
Phloem	-0.10	0.20	0.52
Mean	0.05	0.27	0.59
$t_{0.05}\ S\bar{x}$	0.30	0.28	0.22

Appendix II.3.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for cabbage ± Al (1) pH 4.6 H Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.12	0.13	0.40
Cortex	-0.01	0.08	0.40
Endodermis	-0.11	0.13	0.45
Protoxylem	-0.18	-0.07	0.63
Metaxylem	0.11	0.08	0.92
Xylem parenchyma	0.10	0.00	0.73
Phloem	-0.05	0.05	0.72
Mean	0.00	0.06	0.61
$t_{0.05}\ S\bar{x}$	0.10	0.06	0.17
Mid			
Epidermis	0.46	0.93	0.54
Cortex	0.24	0.44	0.48
Endodermis	0.61	0.50	0.71
Protoxylem	0.08	0.35	0.80
Metaxylem	0.43	0.22	0.40
Xylem parenchyma	0.04	0.07	0.60
Phloem	0.29	0.21	0.69
Mean	0.31	0.39	0.60
$t_{0.05}\ S\bar{x}$	0.18	0.24	0.12
Base			
Epidermis	0.33	0.36	0.95
Cortex	0.11	0.17	0.09
Endodermis	-0.09	0.07	0.96
Protoxylem	1.36	0.18	0.94
Metaxylem	0.29	0.02	0.94
Xylem parenchyma	0.49	0.18	0.95
Phloem	1.98	0.28	1.05
Mean	0.64	0.18	0.84
$t_{0.05}\ S\bar{x}$	0.64	0.10	0.29

Appendix II.4.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for lettuce ± Al (3) pH 4.0 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.80	0.49	0.56
Cortex	0.79	0.29	0.92
Endodermis	0.09	0.05	0.96
Protoxylem	0.44	-0.04	0.71
Metaxylem	0.15	-0.10	0.91
Xylem parenchyma	0.23	-0.14	1.22
Phloem	0.25	-0.05	1.26
Mean	0.39	0.07	0.93
$t_{0.05}\ S\bar{x}$	0.25	0.20	0.21
Mid			
Epidermis	0.23	0.56	0.25
Cortex	0.70	0.90	0.67
Endodermis	0.37	0.64	0.73
Protoxylem	0.70	0.50	0.94
Metaxylem	0.32	0.29	1.00
Xylem parenchyma	0.59	0.91	1.18
Phloem	0.70	0.58	0.95
Mean	0.52	0.63	0.82
$t_{0.05}\ S\bar{x}$	0.17	0.19	0.26
Base			
Epidermis	0.23	0.23	0.06
Cortex	0.46	0.31	0.87
Endodermis	0.21	0.57	0.92
Protoxylem	-0.14	0.31	0.79
Metaxylem	-0.38	-0.49	0.24
Xylem parenchyma	0.18	0.24	0.99
Phloem	-0.08	-0.04	0.80
Mean	0.07	0.16	0.81
$t_{0.05}\ S\bar{x}$	0.24	0.29	0.31

Appendix II.5.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for lettuce ± Al (1) pH 4.6 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	1.16	1.06	0.44
Cortex	1.84	0.87	1.54
Endodermis	1.41	0.81	0.90
Protoxylem	1.63	0.76	1.08
Metaxylem	0.30	0.27	0.70
Xylem parenchyma	0.83	0.52	1.06
Phloem	1.03	0.58	1.04
Mean	1.19	0.70	0.97
$t_{0.05}\ S\bar{x}$	0.44	0.22	0.29
Mid			
Epidermis	2.42	1.05	0.85
Cortex	1.06	0.77	0.97
Endodermis	1.16	0.84	1.07
Protoxylem	0.12	0.24	1.07
Metaxylem	0.10	0.07	0.41
Xylem parenchyma	0.15	0.13	0.36
Phloem	0.14	0.25	1.17
Mean	0.74	0.48	0.84
$t_{0.05}\ S\bar{x}$	0.75	0.34	0.28
Base			
Epidermis	0.72	0.77	0.36
Cortex	0.86	1.03	1.22
Endodermis	0.28	0.41	1.19
Protoxylem	0.24	0.43	1.42
Metaxylem	-0.43	-0.35	0.67
Xylem parenchyma	0.14	0.32	1.41
Phloem	0.35	0.24	1.40
Mean	0.31	0.41	1.10
$t_{0.05}\ S\bar{x}$	0.36	0.37	0.36

Appendix II.6.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for lettuce ± Al (1) pH 4.6 H Ca from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.31	0.26	-0.29
Cortex	-0.20	-0.07	0.45
Endodermis	-0.37	-0.17	0.51
Protoxylem	0.06	-0.04	0.79
Metaxylem	0.15	0.04	0.66
Xylem parenchyma	0.00	0.00	0.99
Phloem	0.05	-0.02	0.73
Mean	0.00	0.00	0.55
$t_{0.05}\ S\bar{x}$	0.19	0.11	0.35
Mid			
Epidermis	1.46	0.60	0.79
Cortex	0.87	0.23	1.13
Endodermis	0.40	-0.06	0.29
Protoxylem	0.19	-0.06	0.43
Metaxylem	0.12	-0.08	0.26
Xylem parenchyma	0.38	0.12	0.44
Phloem	0.25	-0.15	0.57
Mean	0.52	0.09	0.56
$t_{0.05}\ S\bar{x}$	0.41	0.22	0.26
Base			
Epidermis	1.19	0.51	0.46
Cortex	0.15	0.17	0.38
Endodermis	-0.02	-0.06	0.52
Protoxylem	-0.25	-0.01	0.34
Metaxylem	-0.17	-0.05	0.28
Xylem parenchyma	-0.06	0.09	0.38
Phloem	-0.07	-0.03	0.59
Mean	0.11	0.09	0.42
$t_{0.05}\ S\bar{x}$	0.42	0.17	0.09

Appendix II.7.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for kikuyu ± Al (3) pH 4.0 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.05	0.13	0.52
Cortex	0.34	0.42	0.89
Endodermis	-0.08	0.09	0.97
Protoxylem	0.35	0.24	0.99
Metaxylem	0.24	0.15	1.13
Xylem parenchyma	0.12	0.12	1.08
Phloem	0.18	0.31	0.89
Mean	0.17	0.21	0.92
$t_{0.05}\ S\bar{x}$	0.13	0.10	0.17
Mid			
Epidermis	0.47	0.59	0.30
Cortex	0.70	0.79	0.54
Endodermis	0.13	0.68	0.47
Protoxylem	0.41	0.49	0.92
Metaxylem	0.45	0.49	1.07
Xylem parenchyma	0.23	0.45	0.99
Phloem	0.75	0.66	-0.12
Mean	0.45	0.59	0.60
$t_{0.05}\ S\bar{x}$	0.19	0.05	0.37
Base			
Epidermis	0.78	0.07	0.18
Cortex	1.24	0.48	-0.27
Endodermis	0.22	0.50	-0.19
Protoxylem	1.00	0.40	0.93
Metaxylem	0.46	0.42	0.75
Xylem parenchyma	0.26	0.36	1.00
Phloem	0.52	0.41	1.11
Mean	0.64	0.38	0.50
$t_{0.05}\ S\bar{x}$	0.33	0.12	0.50

Appendix II.8.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for kikuyu ± Al (1) pH 4.6 N Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.24	0.13	0.29
Cortex	0.69	0.35	0.99
Endodermis	0.14	0.12	0.97
Protoxylem	-0.03	0.03	0.80
Metaxylem	-0.13	0.15	0.95
Xylem parenchyma	-0.11	0.00	0.88
Phloem	0.10	0.09	1.05
Mean	0.13	0.12	0.85
$t_{0.05}\ S\bar{x}$	0.25	0.10	0.23
Mid			
Epidermis	1.08	0.35	0.23
Cortex	0.13	-0.21	0.20
Endodermis	0.35	0.26	0.39
Protoxylem	0.62	0.30	0.76
Metaxylem	0.29	0.10	0.62
Xylem parenchyma	0.09	0.30	0.69
Phloem	0.49	0.28	0.81
Mean	0.44	0.20	0.53
$t_{0.05}\ S\bar{x}$	0.29	0.17	0.22
Base			
Epidermis	-0.03	0.56	0.73
Cortex	0.14	0.57	0.55
Endodermis	-0.17	0.41	0.54
Protoxylem	-0.10	0.30	0.52
Metaxylem	0.64	0.27	0.78
Xylem parenchyma	0.61	0.25	0.51
Phloem	0.38	0.37	0.79
Mean	0.21	0.39	0.63
$t_{0.05}\ S\bar{x}$	0.29	0.11	0.11

Appendix II.9.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios for kikuyu ± Al (1) pH 4.6 H Ca, from EDX-analyses of freeze dried roots.

Tissue	Al	Al Si corrected	P
Tip			
Epidermis	0.94	0.86	0.49
Cortex	0.65	0.83	0.10
Endodermis	0.35	0.24	0.20
Protoxylem	0.97	0.34	0.20
Metaxylem	-0.10	-0.07	0.06
Xylem parenchyma	-0.19	-0.04	0.17
Phloem	0.29	-0.19	0.20
Mean	0.42	0.28	0.20
$t_{0.05}\ S\bar{x}$	0.40	0.16	0.12
Mid			
Epidermis	0.48	1.15	0.63
Cortex	0.20	0.72	0.86
Endodermis	0.31	0.56	1.11
Protoxylem	-0.22	-0.24	0.60
Metaxylem	-0.29	0.20	0.10
Xylem parenchyma	0.20	0.58	0.18
Phloem	-0.29	0.09	0.67
Mean	0.06	0.44	0.59
$t_{0.05}\ S\bar{x}$	0.27	0.39	0.30
Base			
Epidermis	1.23	1.63	0.70
Cortex	0.70	0.67	0.50
Endodermis	0.39	0.50	0.87
Protoxylem	-0.34	0.61	0.39
Metaxylem	0.48	0.90	1.21
Xylem parenchyma	0.60	0.57	1.18
Phloem	0.89	0.98	1.21
Mean	0.56	0.84	0.87
$t_{0.05}\ S\bar{x}$	0.43	0.33	0.30

Appendix II.10.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios and confidence limits for cabbage ± Al (1) pH 4.6 H Ca, mid root segment.

Data on which Table V.C.2.2.(i) is based.

Tissue	Peak to background ratio	Replicates										Mean	$t_{0.05}$ $S_{\bar{x}}$
Epidermis	Al	0.86	0.94	0.65	0.51	1.82	0.81	0.88	2.19	1.81	1.50	1.20	0.42
	Al Si corrected	1.16	1.46	1.09	1.10	1.64	0.93	1.35	2.04	1.93	1.29	1.40	0.26
Cortex	Al	1.41	1.48	0.79	0.81	0.98	1.05	0.96	0.68	1.07	1.18	1.04	0.19
	Al Si corrected	1.15	1.28	0.97	1.06	1.08	0.80	1.20	0.83	1.01	1.16	1.05	0.11
Endodermis	Al	0.07	-0.03	0.01	-0.07	0.10	-0.12	-0.15	-0.23	-0.20	0.23	-0.04	0.10
	Al Si corrected	0.60	0.56	0.49	0.37	0.53	0.40	0.52	0.43	0.21	0.58	0.47	0.09
Protoxylem	Al	0.12	0.20	0.43	0.36	0.31	0.48	0.32	0.33	0.35	0.08	0.30	0.09
	Al Si corrected	0.17	0.24	0.46	0.20	0.20	0.22	0.29	0.31	0.16	0.25	0.25	0.06
Metaxylem	Al	0.25	0.24	-0.05	0.07	0.02	0.11	0.00	-0.07	-0.02	-0.19	0.04	0.10
	Al Si corrected	0.19	0.21	0.15	0.34	0.57	0.29	0.32	0.27	0.26	0.25	0.29	0.08
Xylem parenchyma	Al	0.01	0.14	0.18	-0.02	0.09	0.03	0.18	0.08	0.08	0.26	0.10	0.06
	Al Si corrected	0.26	0.23	0.33	0.20	0.20	0.30	0.40	0.30	0.32	0.37	0.29	0.05
Phloem	Al	-0.01	0.07	0.18	0.04	0.15	0.08	0.10	0.16	0.10	0.16	0.10	0.04
	Al Si corrected	0.19	0.22	0.24	0.34	0.20	0.24	0.38	0.36	0.41	0.36	0.29	0.03

APPENDIX II.11.

Aluminium, phosphorus and silicon corrected aluminium peak to background ratios and confidence limits for lettuce ± Al (1) pH 4.6 N Ca, mid root segment.

Data on which Table V.C.2.2.(ii) is based.

Tissue	Peak to background ratio	Replicates										Mean	$t_{0.05}$ $S\bar{x}$
Epidermis	Al	1.44	0.97	1.12	2.31	1.98	1.44	1.29	1.31	1.19	1.18	1.42	0.30
	Al Si corrected	0.78	0.95	1.01	1.36	1.21	1.03	1.27	1.24	1.19	0,90	1.09	0.13
Cortex	Al	1.85	1.43	1.45	1.49	2.60	1.83	1.72	2.29	2.80	2.81	2.03	0.39
	Al Si corrected	1.46	1.43	1.25	1.17	2.03	1.53	1.18	1.14	2.06	1.66	1.49	0.24
Endodermis	Al	0.45	0.65	0.43	0.79	0.52	0.58	0.59	0.88	0.60	0.58	0.61	0.10
	Al Si corrected	0.33	0.53	0.30	0.52	0.58	0.45	0.45	0.63	0.58	0.41	0.48	0.08
Protoxylem	Al	0.63	0.68	0.65	0.81	0.74	0.78	0.71	0.59	0.49	0.50	0.66	0.08
	Al Si corrected	0.36	0.42	0.36	0.48	0.46	0.42	0.49	0.41	0.31	0.30	0.40	0.05
Metaxylem	Al	0.50	0.57	0.65	0.33	0.41	0.50	0.64	0.46	0.54	0.51	0.51	0.07
	Al Si corrected	0.32	0.33	0.36	0.20	0.22	0.27	0.40	0.34	0.22	0.37	0.30	0.05
Xylem parenchyma	Al	0.32	0.46	0.33	0.45	0.54	0.61	0.78	1.01	0.95	0.48	0.59	0.17
	Al Si corrected	0.19	0.09	0.09	0.12	0.20	0.14	0.29	0.51	0.36	0.29	0.23	0.10
Phloem	Al	0.31	0.35	0.38	0.62	0.35	0.41	-0.01	0.42	0.55	0.42	0.38	0.12
	Al Si corrected	0.21	0.26	0.21	0.13	0.19	0.15	-0.03	0.14	0.32	0.28	0.19	0.07

Appendix II.12.

Aluminium and silicon corrected aluminium peak to background ratios and confidence limits for the protoplasm of cortical and xylem parenchyma cells of cabbage, lettuce and kikuyu, ± Al (1) pH 4.6 N Ca, mid root segment. Data on which Tables V.C.2.3.(i) and V.C.2.3.(ii) are based.

Species	Protoplasm	Peak to background ratio	Replicates										Mean	$t_{0.05}$ $S_{\bar{x}}$
Cabbage	Cortex	Al	0.93	0.44	0.49	1.17	1.12	0.58	0.62	1.16	0.42	0.21	0.71	0.25
		Al Si corrected	0.24	0.32	0.31	0.32	0.53	0.30	0.50	0.23	0.27	0.34	0.34	0.07
	Xylem parenchyma	Al	0.06	-0.07	0.22	0.11	0.19	0.39	0.38	0.43	0.51	0.56	0.28	0.07
		Al Si corrected	-0.24	-0.08	-0.25	-0.30	-0.13	-0.21	-0.28	-0.21	-0.25	-0.48	-0.24	0.08
Lettuce	Cortex	Al	0.77	0.67	0.50	0.72	0.61	0.43	0.85	0.96	1.60	1.62	0.88	0.30
		Al Si corrected	0.77	0.67	0.43	0.52	0.64	0.52	0.41	0.57	0.11	0.08	0.47	0.16
	Xylem parenchyma	Al	0.26	0.08	0.07	0.02	-0.01	-0.07	-0.05	0.37	0.16	0.23	0.11	0.10
		Al Si corrected	0.12	0.17	0.11	0.05	0.19	0.02	0.09	0.27	0.09	0.43	0.15	0.09
Kikuyu	Cortex	Al	1.02	1.33	1.32	0.98	0.51	0.49	0.60	-0.03	0.95	0.66	0.78	0.30
		Al Si corrected	1.02	1.12	1.05	0.77	0.72	0.45	0.55	0.83	0.65	0.48	0.76	0.17
	Xylem parenchyma	Al	0.62	0.68	0.47	1.25	1.33	1.32	0.60	0.72	1.33	1.50	0.98	0.28
		Al Si corrected	0.31	0.30	0.20	0.42	0.29	0.43	0.40	0.32	0.70	0.48	0.39	0.10

Appendix II.13.

Aluminium and silicon corrected aluminium peak to background ratios and confidence limits for the protoplasm of cortical and xylem parenchyma cells of cabbage, lettuce and kikuyu, ± Al (1) pH 4.6 H Ca, mid root segment. Data on which Table V.C.2.3.(ii) is based.

Species	Protoplasm	Peak to background ratio	Replicates										Mean	$t_{0.05}$ $S_{\bar{x}}$
Cabbage	Cortex	Al	0.53	0.23	0.51	0.38	0.38	0.81	-0.19	0.27	-0.30	0.59	0.32	0.24
		Al Si corrected	0.43	0.41	0.23	0.34	0.41	0.55	-0.43	0.87	-0.51	0.75	0.31	0.32
	Xylem parenchyma	Al	-0.07	0.05	-0.16	-0.24	-0.16	-0.19	-0.17	0.04	0.18	0.15	-0.06	0.11
		Al Si corrected	-0.23	-0.17	-0.27	-0.28	-0.29	-0.30	-0.22	-0.25	-0.28	-0.21	-0.25	0.03
Lettuce	Cortex	Al	0.77	0.67	0.50	0.72	0.61	0.43	0.85	0.96	1.60	1.62	0.88	0.30
		Al Si corrected	0.77	0.67	0.43	0.52	0.64	0.52	0.41	0.57	0.11	0.08	0.47	0.16
	Xylem parenchyma	Al	0.26	0.08	0.07	0.02	-0.01	-0.07	-0.05	0.37	0.16	0.23	0.11	0.10
		Al Si corrected	0.12	0.17	0.11	0.05	0.19	0.02	0.09	0.27	0.09	0.43	0.15	0.09
Kikuyu	Cortex	Al	0.67	0.76	0.68	0.58	0.62	0.40	0.63	0.78	0.75	0.65	0.65	0.08
		Al Si corrected	0.92	0.76	0.73	0.74	0.74	0.44	0.73	1.07	0.88	0.83	0.78	0.12
	Xylem parenchyma	Al	-0.06	0.14	0.07	0.07	0.21	0.34	0.09	0.46	0.16	0.00	0.15	0.11
		Al Si corrected	0.12	0.37	0.24	0.24	0.32	0.26	0.14	0.24	0.18	0.32	0.24	0.06

Appendix III.I.

Whole plant data for cabbage grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.0.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub plot^{-1})		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.0508	1.9240	708	0	1910	11380	1151	5111	38728	70720	8058	5799	449	406
		2	0.0389	1.6676	643	0	2121	11624	1201	4965	38156	55907	7665	5579	447	400
		3	0.0370	1.7839	534	50	2275	11735	1250	4940	40288	67316	8094	6251	440	430
		4	0.0512	1.8615	702	0	1855	11850	1296	4678	38849	54751	8584	4616	348	401
	2	1	0.0375	1.7995	472	0	2011	10498	1123	4395	34471	53787	6921	5033	309	377
		2	0.0380	1.6696	599	40	1892	11042	1304	4502	33979	52835	6822	5029	335	388
		3	0.0212	1.6736	519	27	1997	10738	1042	4447	33731	55821	5227	5590	386	418
		4	0.0466	1.9800	567	6	1920	11807	1132	4712	35558	59728	7147	5294	417	428
	3	1	0.0344	1.6168	474	15	1956	10867	1234	4439	35959	56202	7070	5616	373	393
		2	0.0445	1.9290	533	53	1010	10625	1235	4646	37357	54781	7947	6433	413	343
		3	0.0468	1.4861	505	52	2130	12797	1413	5366	39517	60566	7530	5319	342	398
		4	0.0399	1.8286	499	38	2140	10765	1304	4426	42014	54933	7458	6015	552	421
		Total	0.4868	21.2200	6755	281	23217	135728	14685	56627	448607	697347	88523	66574	4811	4803
3	1	1	0.0449	1.4873	8179	558	1074	4487	841	2262	34574	38632	12925	6091	673	276
		2	0.0237	1.2560	8365	708	1205	4489	942	2698	32320	31520	10902	6158	634	205
		3	0.0326	1.5508	8119	775	1193	4734	852	2775	30797	37733	12506	6312	559	245
		4	0.0553	1.5658	8373	662	1252	4907	935	2839	37977	38115	13041	5984	360	227
	2	1	0.0324	1.5390	9518	407	1175	5136	837	2794	30998	38152	12588	5493	418	256
		2	0.0486	1.6339	9641	456	1074	5773	828	3147	29269	37589	11483	5412	320	252
		3	0.0318	1.6379	9661	639	1304	5210	854	2667	29612	39928	11601	6119	355	278
		4	0.0314	1.6895	9492	526	1147	5324	802	2732	30012	40818	13013	7146	360	292
	3	1	0.0173	1.4521	11818	366	1163	5466	927	2887	30136	36549	8684	5088	478	295
		2	0.0167	1.4939	10317	645	1023	4852	755	2588	27310	30881	10207	6770	497	277
		3	0.0207	1.6412	10113	518	954	4891	761	3137	31083	35528	12633	6271	563	286
		4	0.0266	1.4143	9669	607	1128	4988	781	2646	30968	29479	11830	5864	342	275
		Total	0.3820	18.3620	113265	6867	13692	60257	10115	33172	375056	434924	141413	72708	5559	3164

Appendix III.2.

Whole plant data for cabbage grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration (µg ml^{-1})	Replicates	Sub Plots	Dry Weight Yield (g sub plot^{-1})		Nutrient concentration (µg g^{-1} dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.0686	2.3129	530	0	2924	20871	2220	6245	64360	60457	7901	6806	451	538
		2	0.0742	2.1660	515	7	2643	20347	2062	6024	62798	61908	8127	6779	430	494
		3	0.0778	2.4191	698	41	2874	21458	2295	6674	63928	56625	8006	7146	422	516
		4	0.0528	2.2832	565	62	3176	20269	2505	6535	62313	58372	8057	6842	553	485
	2	1	0.1044	2.5060	905	52	2695	21529	2045	6326	67387	81714	8199	7207	488	559
		2	0.1227	2.6558	657	0	2726	22963	2020	6980	67475	59493	7688	7277	364	438
		3	0.0782	2.3466	719	60	2958	21751	1953	6895	69355	71674	7696	7023	473	563
		4	0.1055	2.3767	781	62	2862	21353	1960	6534	64246	76676	7894	6675	425	564
	3	1	0.0783	2.3161	656	16	3013	21058	2414	6457	57747	65623	7420	6894	446	523
		2	0.0801	2.3341	585	33	2615	20522	2350	6140	53190	64480	8037	6556	358	523
		3	0.0377	1.8770	498	26	4120	20037	2754	6490	53660	47824	8316	7234	508	485
		4	0.0560	2.0625	611	72	3378	20981	2477	6376	51571	69284	7223	6758	502	552
		Total	0.9363	27.6560	7720	431	35984	253139	27055	77676	738030	774130	94564	83197	5420	6240
1	1	1	0.0560	1.8221	17364	226	1914	12073	1081	4721	37430	56894	15168	7141	407	415
		2	0.0739	1.9631	19958	168	1748	12262	868	4941	36756	54150	16868	6688	291	413
		3	0.0607	1.6442	18573	303	1747	11922	983	4527	36544	68946	17017	7519	322	479
		4	0.0604	1.7821	18213	327	1730	12737	968	4916	34585	60675	15161	7200	341	446
	2	1	0.0691	1.7577	22373	246	1851	11725	939	4553	31935	66174	17345	7779	282	410
		2	0.0794	1.9656	19506	209	1885	12022	875	4684	34936	64489	16631	6852	270	449
		3	0.0635	1.8209	20802	248	1911	10357	812	3984	28750	59563	15683	6466	259	411
		4	0.0849	1.9648	22816	223	1641	11817	1004	4500	28830	61171	16752	6598	651	469
	3	1	0.0468	1.3843	15897	378	2278	11900	1319	4571	46607	61609	15911	7570	401	446
		2	0.0640	1.5541	15335	276	2078	12850	1210	4770	42673	54227	14637	6978	354	415
		3	0.0462	1.5488	18773	327	1968	13302	1011	5047	37285	59443	16607	7629	429	439
		4	0.0542	1.0873	9951	523	3035	12344	2083	4662	61588	55090	14456	7596	440	415
		Total	0.7591	20.2950	219561	3454	23786	145311	13153	55876	457919	722431	192236	86016	4447	5207

Appendix III.3.

Whole plant data for cabbage grown in nutrient solution, high calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1006	2.4929	390	0	5024	34248	895	2113	56400	76625	7483	7417	664	712
		2	0.1312	2.5742	496	0	4620	32550	1154	2168	75361	72291	9296	7181	444	733
		3	0.1415	2.2232	497	12	5465	32317	1104	1961	70566	73259	9506	6928	437	671
		4	0.1194	2.4192	594	0	4729	33572	1033	2070	64582	67384	8583	7292	393	650
	2	1	0.1007	2.3835	720	23	4542	34651	789	2042	51105	72194	7026	7850	432	657
		2	0.1376	2.4119	685	0	5342	33731	890	2127	66050	71566	8474	7119	394	662
		3	0.1101	2.2028	666	5	5470	34220	969	2029	68043	70593	8207	7165	362	698
		4	0.1039	2.2520	709	35	4918	34020	763	2093	46909	71163	6797	7273	343	687
	3	1	0.1056	2.1350	667	12	6318	36659	875	2051	68516	69039	8414	7395	447	737
		2	0.1308	2.4094	611	9	4710	35027	1038	2052	63672	72060	8742	7101	387	722
		3	0.1224	2.4256	571	37	4848	37259	813	2106	56530	70227	7011	7641	383	264
		4	0.1248	2.6486	686	6	5300	34970	1007	2024	67922	74023	8199	7441	437	827
		Total	1.4376	28.5790	7292	139	61286	413224	11330	24836	755656	860424	97738	87803	5123	8020
1	1	1	0.1722	2.1568	14339	57	5001	36115	657	1793	62460	71441	15729	6371	475	650
		2	0.1420	2.1001	13355	115	4341	34711	656	1928	68207	65189	15424	6570	391	586
		3	0.1527	2.1426	15113	49	4225	34249	660	1938	61410	71967	17481	7051	336	625
		4	0.1734	2.2099	13609	90	4860	34402	653	2059	57470	76247	16321	7123	338	673
	2	1	0.1413	2.0880	12034	83	5247	34518	716	1976	67628	74055	15751	7256	420	670
		2	0.1355	2.3692	11643	118	5340	34260	631	1771	66795	79130	14524	6376	382	697
		3	0.1483	2.3176	11975	107	4626	34000	537	1893	62454	78278	15195	6981	426	686
		4	0.1254	2.2170	12418	100	5253	34538	578	1941	60844	72520	14523	6853	383	667
	3	1	0.1594	2.3347	16385	91	3229	35788	547	2069	54550	65186	16807	7106	394	674
		2	0.1651	2.3898	14643	104	3241	35950	512	1805	60520	70389	16194	6604	309	725
		3	0.1536	2.2088	17678	115	3250	37638	500	1898	61029	63847	17212	6947	359	647
		4	0.1415	2.2936	16388	90	3100	35238	489	1854	65652	69471	17493	6965	309	653
		Total	1.8104	26.8280	169580	1119	51713	421407	7136	22925	749019	857720	192654	82203	4522	7953

Appendix III.4.

Whole plant data for lettuce grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.0.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.0384	0.3722	1063	47	2306	3635	1118	2695	28455	54329	7813	6355	2010	530
		2	0.0423	0.3614	986	102	2004	3573	1080	2988	25735	51845	7508	6578	2093	585
		3	0.0401	0.3678	1068	113	1897	3272	1117	2763	24611	53010	7469	6399	1736	580
		4	0.0470	0.3722	1060	145	2281	3267	1188	2924	30769	52718	8728	6778	1787	515
	2	1	0.0248	0.3212	655	14	2103	2865	1006	2918	12876	44118	6404	6211	858	349
		2	0.0161	0.3140	909	27	2295	3155	985	3111	13699	49155	3358	6901	740	370
		3	0.0434	0.4129	793	68	2478	3896	761	3215	8353	54856	5625	7072	648	635
		4	0.0330	0.4284	770	58	2503	3595	1052	3028	14286	51282	6384	6297	1187	607
	3	1	0.0262	0.3810	335	59	1842	3852	1063	3266	38462	39588	6041	7414	2589	446
		2	0.0252	0.3593	602	51	2025	3781	1108	3137	33755	52583	4866	6906	2138	444
		3	0.0365	0.4050	465	76	2229	4087	1214	3161	40000	57194	6240	7028	3404	613
		4	0.0421	0.4309	401	5	1993	4284	1232	3336	36946	62653	6675	7003	2607	608
		Total	0.4151	4.5263	9107	765	25956	43262	12924	36542	307947	623331	77111	80942	21797	6282
3	1	1	0.0306	0.2654	6711	509	1245	1778	1022	2333	19011	19692	6331	5474	1149	395
		2	0.0291	0.3029	6533	509	1442	1742	1147	2524	28226	22628	6016	5564	1136	402
		3	0.0230	0.3350	4153	410	1190	1745	986	2615	10695	20271	4395	5557	1015	519
		4	0.0310	0.3542	7022	354	1292	1833	1030	2642	14981	24864	6884	6111	1170	517
	2	1	0.0292	0.2784	7069	743	1606	1660	1029	2183	20080	18472	8076	5368	2076	490
		2	0.0247	0.3360	6866	552	1544	1921	1042	2563	17157	20253	7314	5703	1481	424
		3	0.0167	0.3085	6746	903	1452	1312	907	1725	16129	13974	6627	4948	1201	296
		4	0.0265	0.3111	6309	910	1239	1890	985	2309	15766	18834	6721	6061	1085	377
	3	1	0.0273	0.2745	6676	687	1445	1547	978	1919	15217	16859	7239	5027	1492	428
		2	0.0210	0.2815	6175	648	1497	1669	1048	2487	14970	19095	6906	6426	1381	484
		3	0.0231	0.3019	6707	825	1370	1775	964	2406	13298	19562	6135	6154	1391	601
		4	0.0265	0.3541	5949	679	1239	1774	1098	2892	15766	20186	6721	6388	854	882
		Total	0.3087	3.7035	76916	7729	16561	20646	12236	28598	201296	234690	79365	68781	15431	5815

Appendix III.5.

Whole plant data for lettuce grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1023	1.0220	606	28	1966	5769	1443	4853	75185	61629	8332	8293	1099	476
		2	0.0866	0.9400	647	3	1874	5750	1340	4275	77321	64795	8997	7922	1256	478
		3	0.1066	1.0512	629	93	1751	6062	1197	4292	71917	59556	9165	7675	1149	442
		4	0.1147	1.1514	618	72	1816	6080	1573	3648	61260	61784	9132	7411	1307	467
	2	1	0.0755	1.1005	329	31	2126	6395	1069	3827	73630	69034	8852	6851	1589	441
		2	0.0901	1.1911	504	26	2087	6608	1161	4011	71353	53861	9075	7209	1464	449
		3	0.0791	1.2071	442	43	2036	6806	1264	3859	66875	84206	8917	6898	1460	557
		4	0.0694	1.1039	506	65	2182	7212	973	4038	72855	63506	8558	6896	1427	440
	3	1	0.0760	1.0076	684	63	1916	6695	1119	3915	66286	71325	8289	6552	1158	428
		2	0.0769	1.0200	556	60	1814	6508	871	4228	63792	52454	7692	6760	1255	357
		3	0.0641	1.0083	504	41	1892	7040	848	4111	68944	80745	9298	6992	1182	450
		4	0.0779	1.0637	511	54	1890	7131	860	4183	69610	51629	8569	7216	1320	414
		Total	1.0192	12.8670	6536	579	23350	78056	13718	49240	839028	774524	104876	86675	15666	5399
1	1	1	0.0405	0.4858	6320	515	1415	1876	1175	1870	17587	22240	6038	4045	946	375
		2	0.0493	0.4418	6426	479	1213	1810	1232	2247	18288	22711	6051	3642	1009	300
		3	0.0738	0.4656	7176	520	1331	1926	1287	1989	21646	21726	7403	3298	1193	367
		4	0.0817	0.6292	6651	166	1299	1813	1268	2068	21069	22692	7167	3634	843	335
	2	1	0.0314	0.4758	9168	386	1053	1540	1084	2167	12350	26095	7920	3569	538	258
		2	0.0243	0.5400	9223	396	1106	1689	1011	2682	10654	27292	8066	4092	340	321
		3	0.0212	0.6038	7833	364	1176	1689	1078	2044	12392	23185	5703	3549	450	347
		4	0.0232	0.5841	7886	395	1064	1635	960	1919	11212	17302	6001	3669	553	294
	3	1	0.0290	0.4903	10366	663	1084	1615	903	2005	11121	22191	7970	4164	587	277
		2	0.0377	0.5282	10718	411	1067	1708	979	2076	13685	19573	8028	3655	558	504
		3	0.0329	0.6848	11893	599	892	1505	930	1920	11747	20039	8084	3645	412	356
		4	0.0244	0.5258	11302	491	950	1750	839	2121	10606	20667	7235	3855	570	358
		Total	0.4694	6.4552	104962	5385	13650	20556	12746	25108	172357	265713	85666	44817	7999	4092

Appendix III.6.

Whole plant data for lettuce grown in nutrient solution, high calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub plot^{-1})		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1205	1.1113	396	16	3570	14954	537	1254	48879	57720	10197	6855	962	560
		2	0.1150	0.9223	495	7	5038	13763	512	1307	52437	54803	9831	7464	846	500
		3	0.1213	0.9866	546	29	5584	13950	494	1203	58121	54176	9300	6715	832	478
		4	0.1326	1.0615	539	15	5222	12657	541	1156	49113	53778	10011	6444	786	451
	2	1	0.1269	1.0314	569	92	5403	13865	594	1215	50385	54518	10478	7231	794	498
		2	0.1283	1.0104	558	0	4754	13914	564	1195	59855	61940	9554	6620	755	557
		3	0.1208	1.1106	717	15	4278	13290	531	1216	51959	53650	9752	6662	587	530
		4	0.1118	0.9510	628	7	4933	13339	581	1177	56317	56676	9901	6225	737	507
	3	1	0.1009	0.9531	571	22	5177	13059	467	1412	63974	52269	8200	7423	596	422
		2	0.1219	1.1688	716	39	4574	13625	462	1248	52531	58110	9253	6706	581	470
		3	0.1175	1.0372	645	23	4725	13839	475	1421	47997	56943	10037	7421	604	503
		4	0.0970	0.9700	665	11	4870	12905	462	1222	63984	62799	9497	6319	660	498
		Total	1.4145	12.3140	7045	276	58128	163160	6220	15026	655532	677382	116011	82085	8740	5974
1	1	1	0.1481	0.5433	5094	229	3181	14365	709	1237	44431	48835	6684	4161	635	474
		2	0.1452	0.5554	5813	134	2986	12731	695	2219	45360	61479	6824	3872	726	496
		3	0.1627	0.5988	5156	187	3195	13115	643	1225	48235	48396	6801	3989	876	608
		4	0.1352	0.5690	4830	168	2935	12618	655	1165	45987	49617	6658	3620	811	652
	2	1	0.1361	0.4869	6576	357	3510	15261	784	1322	52381	53464	7303	4489	749	689
		2	0.1310	0.5326	6288	168	2766	13890	723	1192	44544	49422	6882	4073	606	593
		3	0.1487	0.5355	5842	358	2773	12519	694	1156	45118	47513	6982	3995	480	526
		4	0.1344	0.4770	6167	214	3238	13475	744	1164	50162	65957	7044	3890	562	609
	3	1	0.1314	0.5324	4953	257	2672	14603	624	1099	48382	54778	6681	3749	460	490
		2	0.1597	0.5827	5704	130	2941	12489	719	1125	50802	60994	6934	3771	516	581
		3	0.1226	0.4148	4773	447	2927	15243	631	1245	46696	70327	6249	4216	495	594
		4	0.1396	0.4721	5162	246	2496	14502	637	1211	44467	54447	6600	3908	486	499
		Total	1.6947	6.3005	66358	2895	35620	164811	8258	15360	566565	665229	81642	47733	7402	6811

Appendix III.7.

Whole plant data for kikuyu grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.0.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1495	0.7728	638	24	321	2787	5117	3512	59787	56112	6934	9721	352	296
		2	0.1325	0.7600	518	35	312	2645	4868	3664	62336	66699	7553	9837	528	335
		3	0.1306	0.7922	721	27	265	2498	5612	3389	58345	55382	7134	9759	319	309
		4	0.1412	0.8449	394	34	272	2768	5630	3386	57287	59412	7172	9789	463	340
	2	1	0.0966	1.0488	502	30	302	2890	5433	3691	50634	67556	7156	9283	271	379
		2	0.0965	0.9656	401	31	252	2817	4289	3708	37597	60588	5948	9583	282	370
		3	0.1017	0.9398	381	28	284	2536	5757	3430	49160	69403	6844	9513	241	514
		4	0.1017	0.9668	716	28	332	2453	5740	3122	51534	76162	7067	7763	270	460
	3	1	0.1212	0.6555	534	29	246	2687	5114	3513	58507	62805	6934	9577	379	323
		2	0.1704	0.9628	582	32	275	2749	5986	3597	53718	64435	7616	9794	323	295
		3	0.1386	0.8720	478	25	347	2581	4971	3466	66742	50891	7273	9592	397	312
		4	0.1432	0.8438	406	30	302	2665	5155	3375	59876	56425	7057	9775	415	312
		Total	1.5237	10.4250	6271	353	3510	32076	63672	41853	665523	745870	84688	113986	4240	4245
3	1	1	0.1124	0.9146	5429	246	177	1684	2417	2794	68420	58899	8402	10262	345	231
		2	0.1251	0.9392	5692	281	155	1576	2405	2836	65022	67360	8116	10254	387	251
		3	0.1175	0.9433	5580	295	166	1708	2496	2987	63978	56543	8431	10159	273	210
		4	0.1166	1.0407	5837	262	162	1692	2395	2975	67359	53845	8581	9935	524	241
	2	1	0.1193	0.7434	5811	254	161	1752	2399	2883	47286	54619	8454	10945	375	256
		2	0.1303	0.7673	5600	332	165	1612	2446	2981	60243	68094	8003	9173	328	261
		3	0.1185	0.7547	5483	220	160	1695	2407	2821	58255	57079	8085	9415	250	248
		4	0.1370	0.7937	5739	282	171	1595	2461	2907	63552	59509	8525	9350	311	368
	3	1	0.1170	0.9257	5616	301	159	1767	2378	2961	63379	71539	9280	8445	369	302
		2	0.1111	0.8939	5764	321	168	1673	2468	3001	61452	69747	8876	10113	230	405
		3	0.1100	0.9399	5431	268	164	1598	2447	2815	59264	63624	8891	7025	270	427
		4	0.1131	0.8388	5917	204	165	1605	2418	3085	60940	61908	8614	9956	332	503
		Total	1.4279	10.4950	67899	3266	1973	19957	29137	35046	739150	742766	102258	115032	3994	3703

Appendix III.8.

Whole plant data for kikuyu grown in nutrient solution, normal calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1478	0.8422	481	0	438	3436	9320	5153	53918	69184	5914	9596	384	504
		2	0.1621	0.8421	441	48	500	3387	9203	5334	40951	94921	6369	9968	277	510
		3	0.1672	0.8561	553	40	500	3259	9298	5163	39057	67167	6652	10046	267	475
		4	0.1494	0.9380	544	78	492	3674	9062	5296	41275	92097	8974	10236	257	582
	2	1	0.1671	0.9709	486	17	478	3312	8316	5273	40660	73394	6700	9727	292	477
		2	0.1764	0.9235	330	53	500	3572	8306	5713	41915	73281	6336	10239	235	530
		3	0.1573	0.8962	488	24	466	3695	8259	5109	40085	89929	6116	10319	241	613
		4	0.1892	0.7792	412	11	522	3697	12003	5385	41383	89927	6565	10090	249	580
	3	1	0.1344	0.7293	411	13	517	3633	12963	5399	40436	85429	6112	9829	301	562
		2	0.1723	0.9788	426	15	532	3275	8887	5527	40131	66562	5726	9815	346	396
		3	0.1336	0.8584	334	35	482	3599	11300	5347	41588	84170	6280	9844	337	436
		4	0.1516	0.9122	403	22	483	3265	11732	5297	39478	63562	6596	10084	301	407
		Total	1.9084	10.5270	5309	356	5910	41804	118649	63996	500877	949623	78340	119793	3487	6072
1	1	1	0.1762	0.8557	17060	114	82	2962	2505	4531	29104	85207	13123	9471	271	442
		2	0.1522	0.6267	15994	80	133	2571	3023	4099	36215	81912	12711	10003	256	465
		3	0.1440	0.7565	18558	90	81	2524	3206	4270	41500	75561	14168	14628	277	397
		4	0.1331	0.6622	15780	79	96	2675	2991	4516	39814	84407	12285	11844	213	437
	2	1	0.1416	0.7500	14898	176	100	2399	2948	4351	44548	62311	12375	10172	331	337
		2	0.1650	0.7616	17168	139	72	2459	2828	4294	41601	75183	13603	9535	222	363
		3	0.1588	0.8381	19558	131	65	2388	2683	3676	39512	75715	14476	8103	223	396
		4	0.1381	0.7543	17181	74	77	2332	2692	4108	40219	78144	13581	8937	257	390
	3	1	0.1539	0.7493	11363	65	73	2607	3141	3855	45614	82305	14925	10051	242	445
		2	0.1499	0.6786	17082	81	72	2832	2801	4291	41213	88906	13476	10384	241	520
		3	0.1355	0.7125	14857	192	101	2839	2946	3977	40873	83768	13019	9937	254	484
		4	0.1371	0.6796	17314	108	86	2770	2935	4150	40830	88459	13391	9364	238	465
		Total	1.7854	8.8251	196813	1329	1038	31358	34699	50118	481043	961878	161133	122429	3025	5141

Appendix III.9.

Whole plant data for kikuyu grown in nutrient solution, high calcium level at two levels of aluminium, pH 4.6.

Aluminium Concentration ($\mu g\ ml^{-1}$)	Replicates	Sub Plots	Dry Weight Yield (g sub $plot^{-1}$)		Nutrient concentration ($\mu g\ g^{-1}$ dry weight)											
					Al		Ca		Mg		K		P		Na	
			Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
0	1	1	0.1216	0.6236	454	31	1143	7326	7487	3391	46554	64467	6813	10441	305	411
		2	0.1348	0.6899	494	31	1119	6753	7221	3044	44662	58979	6780	9676	273	410
		3	0.1246	0.6891	558	32	1158	7169	7082	3166	43239	65870	6459	9878	314	426
		4	0.1135	0.5867	572	40	1216	7205	7399	3159	45416	85672	6929	9904	365	460
	2	1	0.1399	0.7420	526	18	1132	6587	7057	3640	43882	58975	6684	10366	352	388
		2	0.1342	0.7189	523	5	1094	6094	6625	3496	42910	65344	6136	9748	275	428
		3	0.1305	0.6243	754	25	1347	6336	6072	3314	43188	59572	6491	9156	267	412
		4	0.1374	0.7470	691	21	1154	6080	7115	2927	43768	81795	6812	8981	283	466
	3	1	0.1392	0.7031	539	15	1389	6607	6791	2567	45059	68584	6394	7524	279	381
		2	0.1552	0.7409	718	35	1208	6071	6428	3736	44392	54565	6597	9395	262	364
		3	0.1545	0.8073	689	13	1214	6532	6458	3629	43757	78007	6475	9356	250	424
		4	0.1685	0.8398	450	18	1193	5837	6828	3628	45363	61442	6618	9031	290	420
		Total	1.6539	8.5126	6968	284	14367	78597	82563	39697	532190	803272	79188	113456	3515	4990
1	1	1	0.1431	0.5898	21718	42	432	5918	4127	2942	50408	54794	17924	9928	287	344
		2	0.1753	0.6728	21356	55	389	5616	4334	2827	49634	72033	16947	9930	279	361
		3	0.1366	0.5143	20857	81	506	6641	3771	2675	50052	83182	16513	9666	255	423
		4	0.1571	0.5866	22756	37	441	6192	4142	2863	49849	67263	17502	9042	286	396
	2	1	0.1490	0.5780	20235	37	494	7097	3582	3037	50947	65674	16000	9585	247	399
		2	0.1535	0.5326	18527	29	551	8050	3677	3000	51082	56188	15247	9905	212	351
		3	0.1482	0.5846	21377	46	459	6306	3552	2687	48574	52633	15823	9561	234	377
		4	0.1594	0.6113	22024	45	425	7575	3269	3114	47447	62292	16739	9518	230	383
	3	1	0.1459	0.5419	18537	19	494	6511	3771	2687	52995	70328	15651	9937	252	392
		2	0.1370	0.5031	18804	34	493	8144	3779	2623	48932	66974	15483	10233	224	412
		3	0.1708	0.6635	17829	56	414	5363	3747	2925	47183	67684	15798	10000	190	410
		4	0.1696	0.7149	20328	52	417	6588	3929	3167	46763	82747	15918	9798	215	461
		Total	1.8455	7.0934	244348	533	5515	80001	45480	34547	593866	801792	195542	117103	2911	4709

Appendix III.10.

Analysis of variance from Appendix III.1, cabbage, normal calcium, pH 4.0.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.000458	0.000458	1.89ns
Experimental error	4	0.000967	0.000242	3.01ns
Sampling error	18	0.001448	0.000080	
Total	23	0.002873		
Al concentration roots				
Treatments	1	472682504	472682504	188.69**
Experimental error	4	10020196	2505049	16.63**
Sampling error	18	2711871	150660	
Total	23	485414571		
Ca concentration roots				
Treatments	1	3780234	3780234	106.23**
Experimental error	4	142341	35585	0.60ns
Sampling error	18	1068095	59339	
Total	23	4990671		
Mg concentration roots				
Treatments	1	870204	870204	59.29**
Experimental error	4	58709	14677	2.66ns
Sampling error	18	99321	5518	
Total	23	1028234		
K concentration roots				
Treatments	1	225406233	225406233	9.50*
Experimental error	4	94905306	23726326	6.51*
Sampling error	18	65579592	3643311	
Total	23	385891131		
P concentration roots				
Treatments	1	116556338	116556338	44.59**
Experimental error	4	10456725	2614181	2.77ns
Sampling error	18	16983327	943518	
Total	23	143996390		
Na concentration roots				
Treatments	1	23313	23313	1.11ns
Experimental error	4	84171	21043	2.94ns
Sampling error	18	129025	7168	
Total	23	236509		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.340459	0.340459	18.11*
Experimental error	4	0.075211	0.018803	1.04
Sampling error	18	0.324347	0.018019	
Total	23	0.740017		
Al concentration tops				
Treatments	1	1807308	1807308	107.34**
Experimental error	4	67350	16837	2.88ns
Sampling error	18	105310	5851	
Total	23	1979968		
Ca concentration tops				
Treatments	1	237327993	237327993	52739**
Experimental error	4	1800033	450008	1.66ns
Sampling error	18	4869089	270505	
Total	23	243997115		
Mg concentration tops				
Treatments	1	22922376	22922376	216.34**
Experimental error	4	423826	105956	1.50ns
Sampling error	18	1274446	70803	
Total	23	24620648		
K concentration tops				
Treatments	1	2869409622	2869409622	65.97**
Experimental error	4	173980153	43495038	2.45ns
Sampling error	18	319949758	17774987	
Total	23	3363339533		
P concentration tops				
Treatments	1	1567748	1567748	8.01*
Experimental error	4	783173	195793	0.60ns
Sampling error	18	5838791	324377	
Total	23	8189712		
Na concentration tops				
Treatments	1	111930	111930	87.24**
Experimental error	4	5132	1283	
Sampling error	18	9605	534	
Total	23	126667		

Appendix III.11.

Analysis of variance from Appendix III.2, cabbage, normal calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.001308	0.001308	1.13ns
Experimental error	4	0.004629	0.001157	6.39*
Sampling error	18	0.003261	0.000181	
Total	23	0.009198		
Al concentration roots				
Treatments	1	1869858720	1869858720	91.27**
Experimental error	4	81950694	20487674	7.22*
Sampling error	18	51107029	2839279	
Total	23	2002916443		
Ca concentration roots				
Treatments	1	6199634	6199634	19.56**
Experimental error	4	1267610	316903	2.62ns
Sampling error	18	2178870	121048	
Total	23	9646114		
Mg concentration roots				
Treatments	1	8052734	8052734	29.43**
Experimental error	4	1094529	273632	5.43*
Sampling error	18	906279	50349	
Total	23	10053542		
K concentration roots				
Treatments	1	3269257180	3269257180	14.70*
Experimental error	4	889682002	222420500	10.17*
Sampling error	18	393522950	21862386	
Total	23	4552462132		
P concentration roots				
Treatments	1	397492483	397492483	523.45**
Experimental error	4	3037483	759371	1.56ns
Sampling error	18	8772312	487351	
Total	23	409302278		
Na concentration roots				
Treatments	1	39447	39447	15.43*
Experimental error	4	10226	2556	0.30ns
Sampling error	18	155452	8636	
Total	23	205125		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	2.257680	2.257680	12.00*
Experimental error	4	0.752831	0.188209	7.30*
Sampling error	18	0.464228	0.025790	
Total	23	3.474739		
Al concentration tops				
Treatments	1	380772	380772	31.50**
Experimental error	4	48356	12089	3.76ns
Sampling error	18	57900	3217	
Total	23	487028		
Ca concentration tops				
Treatments	1	484453233	484453233	297.49**
Experimental error	4	6513898	1628474	4.59ns
Sampling error	18	6381652	354536	
Total	23	497348783		
Mg concentration tops				
Treatments	1	19801667	19801667	138.09**
Experimental error	4	573582	143395	2.27ns
Sampling error	18	1135695	63094	
Total	23	21510944		
K concentration tops				
Treatments	1	111366108	111366108	1.01ns
Experimental error	4	439822064	109955516	2.64ns
Sampling error	18	750189271	41677182	
Total	23	1301377443		
P concentration tops				
Treatments	1	331115	331115	2.12ns
Experimental error	4	623497	155874	1.25ns
Sampling error	18	2242594	124589	
Total	23	3197206		
Na concentration tops				
Treatments	1	44462	44462	145.40**
Experimental error	4	1223	306	0.25ns
Sampling error	18	21732	1207	
Total	23	67417		

Appendix III.12.

Analysis of variance from Appendix III.3, cabbage, high calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.005791	0.005791	18.74**
Experimental error	4	0.001236	0.000309	1.63ns
Sampling error	18	0.003421	0.000190	
Total	23	0.010448		
Al concentration roots				
Treatments	1	1097391456	1097391456	120.87**
Experimental error	4	36316348	9079087	23.79**
Sampling error	18	6869636	381646	
Total	23	1140577440		
Ca concentration roots				
Treatments	1	3818430	3818430	1.89ns
Experimental error	4	8071092	2017773	10.91*
Sampling error	18	3329573	184976	
Total	23	15219095		
Mg concentration roots				
Treatments	1	732902	732902	24.40**
Experimental error	4	120158	30039	4.54ns
Sampling error	18	119115	6617	
Total	23	972175		
K concentration roots				
Treatments	1	1835407	1835407	0.04ns
Experimental error	4	191761037	47940259	1.10ns
Sampling error	18	782130940	43451719	
Total	23	975727384		
P concentration roots				
Treatments	1	375376961	375376961	149.59**
Experimental error	4	10037613	2509403	4.19ns
Sampling error	18	10791574	599532	
Total	23	396206148		
Na concentration roots				
Treatments	1	15050	15050	2.05ns
Experimental error	4	29386	7347	1.84ns
Sampling error	18	71932	3996	
Total	23	116368		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.127706	0.127706	6.53ns
Experimental error	4	0.078191	0.019548	1.17ns
Sampling error	18	0.300398	0.016689	
Total	23	0.506295		
Al concentration tops				
Treatments	1	40017	40017	84.62**
Experimental error	4	1892	471	1.59ns
Sampling error	18	5364	298	
Total	23	47273		
Ca concentration tops				
Treatments	1	2790062	2790062	0.48ns
Experimental error	4	23254963	5813741	8.37*
Sampling error	18	12498366	694354	
Total	23	38543391		
Mg concentration tops				
Treatments	1	152163	152163	185.83**
Experimental error	4	3275	818	0.11ns
Sampling error	18	131906	7328	
Total	23	287344		
K concentration tops				
Treatments	1	304651	304651	0.01ns
Experimental error	4	157178472	39294618	3.86ns
Sampling error	18	183127655	10173758	
Total	23	340610778		
P concentration tops				
Treatments	1	1306667	1306667	46.17**
Experimental error	4	113196	28299	0.33ns
Sampling error	18	1566810	87045	
Total	23	2986673		
Na concentration tops				
Treatments	1	187	187	0.07ns
Experimental error	4	11373	2843	0.25ns
Sampling error	18	206424	11468	
Total	23	217984		

Appendix III.13.

Analysis of variance from Appendix III.4, lettuce, normal calcium, pH 4.0.

Source of variation	d.f.	Sum of squares	Mean Square	Variance ratio
Dry weight yield roots				
Treatments	1	0.000472	0.000472	4.85ns
Experimental error	4	0.000389	0.000097	2.18ns
Sampling error	18	0.000804	0.000045	
Total	23	0.001665		
Al concentration roots				
Treatments	1	191585853	191585853	497.51**
Experimental error	4	1540369	385092	1.15
Sampling error	18	6009623	333868	
Total	23	199135845		
Ca concentration roots				
Treatments	1	3677751	3677751	53.51**
Experimental error	4	274899	68725	2.73ns
Sampling error	18	453546	25197	
Total	23	4406196		
Mg concentration roots				
Treatments	1	19723	19723	0.77ns
Experimental error	4	103074	25768	4.06ns
Sampling error	18	114189	6344	
Total	23	236986		
K concentration roots				
Treatments	1	473934825	473934825	1.47ns
Experimental error	4	1291569613	322892403	23.35**
Sampling error	18	248883462	13826859	
Total	23	2014387900		
P concentration roots				
Treatments	1	211688	211688	0.05ns
Experimental error	4	16581614	4145403	5.17ns
Sampling error	18	14425222	801401	
Total	23	31218524		
Na concentration roots				
Treatments	1	1688582	1688582	0.97ns
Experimental error	4	6954956	1738739	16.15**
Sampling error	18	1937718	107651	
Total	23	10581256		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.028208	0.028208	57.42**
Experimental error	4	0.001965	0.000491	0.37ns
Sampling error	18	0.023797	0.001322	
Total	23	0.053970		
Al concentration tops				
Treatments	1	2020721	2020721	31.77**
Experimental error	4	254393	63598	8.72*
Sampling error	18	131300	7294	
Total	23	2406414		
Ca concentration tops				
Treatments	1	21311811	21311811	88.38**
Experimental error	4	964603	241151	3.69ns
Sampling error	18	1177760	65431	
Total	23	23454174		
Mg concentration tops				
Treatments	1	2629464	2629464	19.88*
Experimental error	4	529193	132298	2.30ns
Sampling error	18	1035684	57538	
Total	23	4194341		
K concentration tops				
Treatments	1	6293409453	6293409453	417.24**
Experimental error	4	60333220	15083305	0.68ns
Sampling error	18	399733141	22207397	
Total	23	6753475814		
P concentration tops				
Treatments	1	6162080	6162080	20.57*
Experimental error	4	1198104	299526	1.77ns
Sampling error	18	3052318	169573	
Total	23	10412502		
Na concentration tops				
Treatments	1	9087	9087	0.39ns
Experimental error	4	93627	23407	1.64ns
Sampling error	18	256969	14276	
Total	23	359683		

Appendix III.14.

Analysis of variance from Appendix III.5, lettuce, normal calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.012595	0.012595	10.20**
Experimental error	4	0.004939	0.001235	10.71*
Sampling error	18	0.002075	0.000115	
Total	23	0.019609		
Al concentration roots				
Treatments	1	403653228	403653228	40.83**
Experimental error	4	39543252	9885813	49.20**
Sampling error	18	3616506	200917	
Total	23	446812986		
Ca concentration roots				
Treatments	1	3920417	3920417	42.70**
Experimental error	4	367259	91815	16.83**
Sampling error	18	98210	5456	
Total	23	855886		
Mg concentration roots				
Treatments	1	39366	39366	0.24ns
Experimental error	4	654172	163543	14.63**
Sampling error	18	201265	11181	
Total	23	894803		
K concentration roots				
Treatments	1	18518759260	18518759260	347.05**
Experimental error	4	213444646	53361161	4.35ns
Sampling error	18	220908403	12272689	
Total	23	18953112309		
P concentration roots				
Treatments	1	15376004	15376004	17.77**
Experimental error	4	3461786	865446	1.81ns
Sampling error	18	8612525	478474	
Total	23	27450315		
Na concentration roots				
Treatments	1	2449287	2449287	11.39*
Experimental error	4	860266	215067	22.50**
Sampling error	18	172042	9558	
Total	23	3481595		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	1.712859	1.712859	156.51**
Experimental error	4	0.043776	0.010944	2.24ns
Sampling error	18	0.088065	0.004893	
Total	23	1.844700		
Al concentration tops				
Treatments	1	962402	962402	71.51**
Experimental error	4	53830	13458	1.84ns
Sampling error	18	131545	7308	
Total	23	1147777		
Ca concentration tops				
Treatments	1	137760417	137760417	247.85**
Experimental error	4	2223250	555812	12.90*
Sampling error	18	775487	43083	
Total	23	140759154		
Mg concentration tops				
Treatments	1	24264726	24264726	327.75**
Experimental error	4	296142	74035	1.06ns
Sampling error	18	1253222	69623	
Total	23	25814090		
K concentration tops				
Treatments	1	10787026405	10787026405	518.28**
Experimental error	4	83253211	20813303	0.32ns
Sampling error	18	1181854190	65658566	
Total	23	12052133806		
P concentration tops				
Treatments	1	73003840	73003840	129.59**
Experimental error	4	2253377	563344	7.23*
Sampling error	18	1401742	77875	
Total	23	76658959		
Na concentration tops				
Treatments	1	71177	71177	15.73*
Experimental error	4	18101	4525	1.63ns
Sampling error	18	50034	2780	
Total	23	139312		

Appendix III.15.

Analysis of variance from Appendix III.6, lettuce, high calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.003271	0.003271	18.71*
Experimental error	4	0.000700	0.000118	1.50ns
Sampling error	18	0.002101	0.000117	
Total	23	0.006072		
Al concentration roots				
Treatments	1	146584665	146584665	201.58**
Experimental error	4	2908770	727193	9.84*
Sampling error	18	1329782	73877	
Total	23	150823217		
Ca concentration roots				
Treatments	1	21108753	21108753	320.39**
Experimental error	4	263537	65884	0.31ns
Sampling error	18	3791756	210653	
Total	23	25164046		
Mg concentration roots				
Treatments	1	173060	173060	19.58*
Experimental error	4	35352	8838	9.34*
Sampling error	18	17025	946	
Total	23	225437		
K concentration roots				
Treatments	1	329796962	329796962	22.43**
Experimental error	4	58804275	14701069	0.69ns
Sampling error	18	383173386	21287410	
Total	23	771774623		
P concentration roots				
Treatments	1	49217840	49217840	132.85**
Experimental error	4	1481948	370487	2.18ns
Sampling error	18	3062786	170155	
Total	23	53762574		
Na concentration roots				
Treatments	1	74594	74594	1.09ns
Experimental error	4	272528	68132	10.43*
Sampling error	18	117566	6531	
Total	23	464688		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	1.506858	1.506858	559.70**
Experimental error	4	0.010769	0.002692	0.59ns
Sampling error	18	0.092987	0.004610	
Total	23	1.600614		
Al concentration tops				
Treatments	1	285798	285798	49.29**
Experimental error	4	23194	5799	1.14ns
Sampling error	18	91300	5072	
Total	23	400292		
Ca concentration tops				
Treatments	1	113575	113575	0.18ns
Experimental error	4	2473755	618439	0.81ns
Sampling error	18	13668488	759360	
Total	23	16255818		
Mg concentration tops				
Treatments	1	4648	4648	0.08ns
Experimental error	4	234816	58704	1.25ns
Sampling error	18	847378	47077	
Total	23	1086842		
K concentration tops				
Treatments	1	6153975	6153975	0.16ns
Experimental error	4	152633788	38158447	1.15ns
Sampling error	18	597635455	33201970	
Total	23	756423218		
P concentration tops				
Treatments	1	49169163	49169163	721.20**
Experimental error	4	272707	68177	0.50ns
Sampling error	18	2475392	137522	
Total	23	51917262		
Na concentration tops				
Treatments	1	29190	29190	8.61*
Experimental error	4	13563	3391	1.07ns
Sampling error	18	57139	3174	
Total	23	99892		

Appendix III.16.

Analysis of variance from Appendix III.7, kikuyu, normal calcium, pH 4.0.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.000382	0.000382	0.30ns
Experimental error	4	0.005072	0.001268	12.33*
Sampling error	18	0.001851	0.000103	
Total	23	0.007305		
Al concentration roots				
Treatments	1	158250433	158250433	37785*
Experimental error	4	16753	4188	0.17ns
Sampling error	18	431685	23982	
Total	23	158698871		
Ca concentration roots				
Treatments	1	98432	98432	181721**
Experimental error	4	2.16667	0.541667	0.00ns
Sampling error	18	11632	646	
Total	23	110066		
Mg concentration roots				
Treatments	1	49694426	49694426	19550000**
Experimental error	4	10.1667	2.5417	0.00ns
Sampling error	18	2516251	139792	
Total	23	52210687		
K concentration roots				
Treatments	1	225872297	225872297	1.60ns
Experimental error	4	564154146	141038537	6.38*
Sampling error	18	397947042	22108169	
Total	23	1187973485		
P concentration roots				
Treatments	1	12862704	12862704	34.05**
Experimental error	4	1510954	377738	3.52ns
Sampling error	18	1929224	107179	
Total	23	16302882		
Na concentration roots				
Treatments	1	2522	2522	0.16ns
Experimental error	4	63649	15912	3.29ns
Sampling error	18	86964	4831	
Total	23	153135		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.000205	0.000205	0.01ns
Experimental error	4	0.157503	0.039376	9.13*
Sampling error	18	0.077632	0.004313	
Total	23	0.235340		
Al concentration tops				
Treatments	1	353565	353565	95344**
Experimental error	4	14.8333	3.70833	0.00ns
Sampling error	18	16102	894	
Total	23	369682		
Ca concentration tops				
Treatments	1	6119590	6119590	325654**
Experimental error	4	75.1667	18.7917	0.00ns
Sampling error	18	248400	13800	
Total	23	6368065		
Mg concentration tops				
Treatments	1	1930635	1930635	635.60**
Experimental error	4	12150	3038	0.14ns
Sampling error	18	384289	21349	
Total	23	2327074		
K concentration tops				
Treatments	1	401451	401451	0.00ns
Experimental error	4	376689239	94172310	2.87ns
Sampling error	18	589754922	32764162	
Total	23	966845612		
P concentration tops				
Treatments	1	45588	45588	0.04ns
Experimental error	4	4628320	1157080	1.95ns
Sampling error	18	10663394	592411	
Total	23	15337302		
Na concentration tops				
Treatments	1	12240	12240	0.48ns
Experimental error	4	101557	25389	9.71ns
Sampling error	18	47086	2616	
Total	23	160883		

Appendix III.17.

Analysis of variance from Appendix III.8, kikuyu, normal calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.000630	0.000630	1.84ns
Experimental error	4	0.001370	0.000342	1.72ns
Sampling error	18	0.003591	0.000200	
Total	23	0.005591		
Al concentration roots				
Treatments	1	1528074251	1528074251	636.25**
Experimental error	4	9606705	2401676	1.12ns
Sampling error	18	38572715	2142929	
Total	23	1606253671		
Ca concentration roots				
Treatments	1	989016	989016	2297**
Experimental error	4	1722	431	0.82ns
Sampling error	18	9412	523	
Total	23	1000150		
Mg concentration roots				
Treatments	1	293650104	293650104	109.48**
Experimental error	4	10728556	2682139	2.48ns
Sampling error	18	19487430	1082635	
Total	23	313137534		
K concentration roots				
Treatments	1	16391148	16391148	0.67ns
Experimental error	4	97612317	24403079	1.65ns
Sampling error	18	265437132	14746507	
Total	23	379440597		
P concentration roots				
Treatments	1	285611702	285611702	526.33**
Experimental error	4	2170596	542649	0.78ns
Sampling error	18	12484193	693566	
Total	23	300266491		
Na concentration roots				
Treatments	1	8894	8894	3.70ns
Experimental error	4	9619	2405	1.76ns
Sampling error	18	24649	1369	
Total	23	43162		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.120672	0.120672	39.93**
Experimental error	4	0.012088	0.003022	0.54ns
Sampling error	18	0.100128	0.005563	
Total	23	0.232888		
Al concentration tops				
Treatments	1	39447	39447	39.70**
Experimental error	4	3975	994	0.89ns
Sampling error	18	20148	1119	
Total	23	63570		
Ca concentration tops				
Treatments	1	4546622	4546622	53.01**
Experimental error	4	343095	85774	3.30ns
Sampling error	18	467777	25988	
Total	23	4889717		
Mg concentration tops				
Treatments	1	8024954	8024954	128.93**
Experimental error	4	248966	62241	1.45ns
Sampling error	18	770172	42787	
Total	23	8795126		
K concentration tops				
Treatments	1	6257709	6257709	0.05ns
Experimental error	4	462076801	115519200	1.33ns
Sampling error	18	1561053873	86725215	
Total	23	2029388383		
P concentration tops				
Treatments	1	289521	289521	0.10ns
Experimental error	4	11093296	2773324	2.55ns
Sampling error	18	19601364	1088965	
Total	23	30984181		
Na concentration tops				
Treatments	1	36115	36115	3.29ns
Experimental error	4	43907	10977	4.71ns
Sampling error	18	41966	2331	
Total	23	121988		

Appendix III.18.

Analysis of variance from Appendix III.9, kikuyu, high calcium, pH 4.6.

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield roots				
Treatments	1	0.001530	0.000486	3.14ns
Experimental error	4	0.001946	0.000141	3.45ns
Sampling error	18	0.002540	0.004156	
Total	23	0.006016		
Al concentration roots				
Treatments	1	2347886017	2347886017	592.01**
Experimental error	4	15863859	3965965	5.75ns
Sampling error	18	12412313	689573	
Total	23	2376162249		
Ca concentration roots				
Treatments	1	3264913	3264913	600.04**
Experimental error	4	21765	5441	1.08ns
Sampling error	18	90763	5042	
Total	23	3377441		
Mg concentration roots				
Treatments	1	57297870	57297870	124.63**
Experimental error	4	1839034	459759	6.74*
Sampling error	18	1228056	68225	
Total	23	60364960		
K concentration roots				
Treatments	1	158497041	158497041	87.12**
Experimental error	4	7277511	1819378	0.78ns
Sampling error	18	42245526	2346974	
Total	23	208020078		
P concentration roots				
Treatments	1	564093888	564093888	418.41**
Experimental error	4	5392766	1348191	8.65*
Sampling error	18	2804540	155808	
Total	23	572291194		
Na concentration roots				
Treatments	1	15201	15201	5.47ns
Experimental error	4	11107	2777	3.80ns
Sampling error	18	13143	730	
Total	23	39451		

Source of variation	d.f.	Sum of squares	Mean square	Variance ratio
Dry weight yield tops				
Treatments	1	0.083922	0.083922	10.11*
Experimental error	4	0.033195	0.008299	2.00ns
Sampling error	18	0.074816	0.004156	
Total	23	0.191933		
Al concentration tops				
Treatments	1	2583	2583	9.20*
Experimental error	4	1123	281	1.80ns
Sampling error	18	2813	156	
Total	23	6519		
Ca concentration tops				
Treatments	1	82134	82134	0.07ns
Experimental error	4	4622517	1155629	3.01ns
Sampling error	18	6907236	383735	
Total	23	11611887		
Mg concentration tops				
Treatments	1	1105104	1105104	34.55**
Experimental error	4	127938	31985	0.36ns
Sampling error	18	1587182	88177	
Total	23	2820224		
K concentration tops				
Treatments	1	91267	91267	0.00ns
Experimental error	4	382794809	95698702	1.00ns
Sampling error	18	1730811147	96156175	
Total	23	2113697223		
P concentration tops				
Treatments	1	554192	554192	0.73ns
Experimental error	4	3033949	758487	2.99ns
Sampling error	18	4564778	253599	
Total	23	8152919		
Na concentration tops				
Treatments	1	3290	3290	2.10ns
Experimental error	4	6278	1570	1.88ns
Sampling error	18	15032	835	
Total	23	24600		

Appendix III.19.

Treatment means and L.S.D.'s from Appendix III.1, 4, 7, normal calcium, pH 4.0.

Variable	Unit	Aluminium concentration ($\mu g\ ml^{-1}$)		L.S.D.	
		0	3	0.05	0.01
Cabbage					
Dry weight yield roots	g sub plot^{-1}	0.0406	0.0318	0.0175	0.0254
Dry weight yield tops	"	1.7684	1.5301	0.1555	0.2258
Al roots	$\mu g\ g^{-1}$ dry weight	563	9439	1793	2605
Al tops	"	23	572	147	214
Ca roots	% dry weight	0.194	0.114	0.021	0.031
Ca tops	"	1.131	0.502	0.076	0.110
Mg roots	"	0.122	0.084	0.013	0.021
Mg tops	"	0.472	0.276	0.037	0.061
K roots	"	3.738	3.126	0.552	0.802
K tops	"	5.811	3.624	0.747	1.085
P roots	"	0.738	1.178	0.183	0.266
P tops	"	0.555	0.606	0.050	0.073
Na roots	"	0.040	0.046	0.016	0.027
Na tops	"	0.040	0.026	0.004	0.007
Lettuce					
Dry weight yield roots	g sub plot^{-1}	0.0346	0.0257	0.0111	0.0184
Dry weight yield tops	"	0.3772	0.3086	0.0250	0.0414
Al roots	$\mu g\ g^{-1}$ dry weight	759	6410	702	1165
Al tops	"	64	644	286	474
Ca roots	% dry weight	0.216	0.138	0.030	0.049
Ca tops	"	0.361	0.172	0.056	0.092
Mg roots	"	0.108	0.102	0.018	0.030
Mg tops	"	0.305	0.238	0.041	0.068
K roots	"	2.566	1.678	2.036	3.377
K tops	"	5.194	1.956	0.440	0.730
P roots	"	0.643	0.661	0.231	0.383
P tops	"	0.675	0.573	0.062	0.103
Na roots	"	0.182	0.129	0.149	0.248
Na tops	"	0.052	0.049	0.017	0.029
Kikuyu					
Dry weight yield roots	g sub plot^{-1}	0.1270	0.1190	0.0403	0.0668
Dry weight yield tops	"	0.8688	0.8747	0.2249	0.3729
Al roots	$\mu g\ g^{-1}$ dry weight	523	5658	73	122
Al tops	"	29	272	2	4
Ca roots	% dry weight	0.029	0.016	0	0
Ca tops	"	0.267	0.166	0.001	0.001
Mg roots	"	0.531	0.243	0	0
Mg tops	"	0.349	0.292	0.006	0.010
K roots	"	5.546	6.160	1.346	2.232
K tops	"	6.216	6.190	1.100	1.824
P roots	"	0.706	0.852	0.070	0.116
P tops	"	0.950	0.959	0.122	0.202
Na roots	"	0.035	0.033	0.014	0.024
Na tops	"	0.035	0.031	0.018	0.030

Appendix III.20.

Treatment means and L.S.D.'s from Appendix III.2, 5, 8, normal calcium, pH 4.6.

Variable	Unit	Aluminium concentration ($\mu g\ ml^{-1}$)		L.S.D.	
		0	1	0.05	0.01
Cabbage					
Dry weight yield roots	g sub plot^{-1}	0.0780	0.0633	0.0386	0.0640
Dry weight yield tops	"	2.3047	1.6913	0.4916	0.8154
Al roots	$\mu g\ g^{-1}$ dry weight	643	18297	5130	8508
Al tops	"	36	288	125	207
Ca roots	% dry weight	0.300	0.198	0.064	0.106
Ca tops	"	2.110	1.211	0.145	0.240
Mg roots	"	0.226	0.110	0.059	0.099
Mg tops	"	0.647	0.466	0.043	0.071
K roots	"	6.150	3.816	1.690	2.803
K tops	"	6.451	6.020	1.188	1.971
P roots	"	0.788	1.602	0.099	0.164
P tops	"	0.693	0.717	0.045	0.074
Na roots	"	0.045	0.037	0.006	0.010
Na tops	"	0.052	0.043	0.002	0.003
Lettuce					
Dry weight yield roots	g sub plot^{-1}	0.0849	0.0391	0.0397	0.0658
Dry weight yield tops	"	1.0722	0.5379	0.1185	0.1966
Al roots	$\mu g\ g^{-1}$ dry weight	545	8747	3564	5912
Al tops	"	48	449	132	218
Ca roots	% dry weight	0.195	0.114	0.034	0.057
Ca tops	"	0.651	0.171	0.084	0.140
Mg roots	"	0.114	0.106	0.046	0.076
Mg tops	"	0.410	0.209	0.031	0.051
K roots	"	6.992	1.436	0.828	1.373
K tops	"	6.545	2.214	0.517	0.857
P roots	"	0.874	0.714	0.105	0.175
P tops	"	0.722	0.374	0.085	0.141
Na roots	"	0.131	0.067	0.052	0.087
Na tops	"	0.045	0.034	0.008	0.013
Kikuyu					
Dry weight yield roots	g sub plot^{-1}	0.1590	0.1488	0.0211	0.0350
Dry weight yield tops	"	0.8772	0.7354	0.0622	0.1031
Al roots	$\mu g\ g^{-1}$ dry weight	442	16401	1757	2914
Al tops	"	30	111	36	59
Ca roots	% dry weight	0.049	0.009	0.002	0.004
Ca tops	"	0.348	0.261	0.033	0.055
Mg roots	"	0.989	0.289	0.186	0.308
Mg tops	"	0.533	0.418	0.028	0.047
K roots	"	4.174	4.009	0.560	0.929
K tops	"	7.914	8.016	1.218	2.020
P roots	"	0.653	1.343	0.084	0.139
P tops	"	0.998	1.020	0.189	0.313
Na roots	"	0.029	0.025	0.006	0.009
Na tops	"	0.051	0.043	0.012	0.020

Appendix III.21.

Treatment means and L.S.D.'s from Appendix III.3, 6, 9, high calcium, pH 4.6.

Variable	Unit	Aluminium concentration ($\mu g\ ml^{-1}$)		L.S.D.	
		0	1	0.05	0.01
Cabbage					
Dry weight yield roots	g sub $plot^{-1}$	0.1198	0.1509	0.0200	0.0331
Dry weight yield tops	"	2.3816	2.2357	0.1585	0.2629
Al roots	$\mu g\ g^{-1}$ dry weight	608	14132	3414	5663
Al tops	"	12	93	25	41
Ca roots	% dry weight	0.511	0.431	1610	2670
Ca tops	"	3.444	3.512	2732	4530
Mg roots	"	0.094	0.060	197	327
Mg tops	"	0.207	0.191	0.003	0.005
K roots	"	6.297	6.242	0.785	1.302
K tops	"	7.170	7.148	0.710	1.778
P roots	"	0.815	1.606	0.180	0.298
P tops	"	0.732	0.685	0.019	0.032
Na roots	"	0.043	0.038	0.010	0.016
Na tops	"	0.067	0.066	0.006	0.010
Lettuce					
Dry weight yield roots	g sub $plot^{-1}$	0.1179	0.1412	0.0150	0.0250
Dry weight yield tops	"	1.0262	0.5250	0.0589	0.0976
Al roots	$\mu g\ g^{-1}$ dry weight	587	5530	966	1602
Al tops	"	23	241	86	143
Ca roots	% dry weight	0.484	0.297	0.029	0.048
Ca tops	"	1.360	1.373	0.089	0.148
Mg roots	"	0.052	0.069	0.011	0.018
Mg tops	"	0.125	0.128	0.027	0.046
K roots	"	5.463	4.721	0.434	0.721
K tops	"	5.645	5.544	0.700	1.161
P roots	"	0.967	0.680	0.069	0.114
P tops	"	0.684	0.398	0.030	0.030
Na roots	"	0.073	0.062	0.030	0.049
Na tops	"	0.050	0.057	0.007	0.011
Kikuyu					
Dry weight yield roots	g sub $plot^{-1}$	0.1378	0.1538	0.0250	0.0414
Dry weight yield tops	"	0.7094	0.5911	0.1033	0.1713
Al roots	$\mu g\ g^{-1}$ dry weight	581	20362	2257	3743
Al tops	"	24	44	19	31
Ca roots	% dry weight	0.120	0.046	0.008	0.014
Ca tops	"	0.655	0.667	0.122	0.202
Mg roots	"	0.688	0.379	0.077	0.128
Mg tops	"	0.331	0.288	0.020	0.034
K roots	"	4.435	4.949	0.153	0.254
K tops	"	6.694	6.682	1.109	1.839
P roots	"	0.660	1.630	0.132	0.218
P tops	"	0.946	0.976	0.099	0.164
Na roots	"	0.029	0.024	0.006	0.010
Na tops	"	0.042	0.039	0.005	0.008

X. PUBLICATIONS

X. PUBLICATIONS

Huett, D.O., and Menary, R.C. (1979). Aluminium uptake by excised roots of cabbage, lettuce and kikuyu grass. *Aust. J. Plant Physiol.* (in press).

Huett, D.O., and Menary, R.C. (1980). Aluminium distribution in freeze-dried roots of cabbage, lettuce and kikuyu grass by energy dispersive X-ray analysis. *Aust. J. Plant Physiol.* (in press).