Effects of Acidic Precipitation on Calcium and Magnesium Uptake by *Pinus patula*.

Colleen Anne Carlson

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ABSTRACT

Acidified rain is thought to have the potential to affect the ability of plants to acquire nutrients. The effects of artificially acidified rain on calcium (Ca) and magnesium (Mg) uptake by *Pinus patula* were investigated in terms of changes in the Ca and Mg levels in the soil and changes in root growth and mycorrhizal colonization that might result from exposure to acidified precipitation. The uptake of these ions was also investigated in order to determine the possible effects of acid rain on the uptake process.

Soil analyses showed that the soil under investigation was acidic (pH in KCl of 4.64), with a high aluminium (Al^{3+}) content (1.12 cmol kg^{-1}) and low Ca and Mg contents (0.08 and 0.50 cmol kg^{-1} respectively). The Ca occurred predominantly in the top 100mm of the soil. Undisturbed soil cores were leached with simulated acid rain. To some cores calcium carbonate ($CaCO_3$) was added to determine the potential use of $CaCO_3$ as an ameliorative measure against any negative effects that acid rain may have. The rate of leaching of Mg^{2+} from the soil was found to increase, probably as a result of cation exchange reactions, as the acidity of the "rain" increased. This trend was not however statistically significant. The amount of Ca^{2+} lost in the leachate was undetectable. The leachate became increasingly acidic as the acidity of the "rain" was raised. The amounts of acid equivalents, Al^{3+}, H^+ and Mg^{2+} lost in the leachate were reduced when $CaCO_3$ was added to the soil. Over the time period investigated no significant changes in the soil composition could be detected, although the Al^{3+} and H^+ contents of the soil were greater when the "rain" was more acidic.

Soil that had been exposed to artificial rain of various acidities was placed in root ingrowth bags in the field. One season's root growth into this acidified soil indicated that root mass per unit length tended to be positively correlated with the Mg content of the soil. Root length and the degree of total

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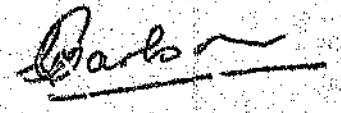
mycorrhizal colonization did not appear to be affected by any of the other soil parameters that were being monitored. This is probably a result of the range of soil conditions being too narrow. The mycorrhizal species composition changed in response to the various soil conditions. Of the seven species colonizing the roots, one species was found to be negatively affected by the Al^{3+} content of the soil, while another was more abundant when the pH of the soil was low.

The concentration profiles of Ca and Mg in the mycorrhizal-root rhizosphere were determined by sectioning this soil in 1mm sections. Magnesium was found to accumulate slightly at the root surface when the Mg concentrations in the soil were low. At higher soil Mg concentrations the rate of uptake increased, such that zones of depletion formed against the root surface. When Al^{3+} was added to the soil, Mg accumulated to a greater extent at the root surface. Consequently it is thought that at low Mg concentrations Al^{3+} inhibited the rate of Mg uptake, but as the Mg content of the soil increased, less inhibition by Al^{3+} occurred.

At low concentrations, Ca accumulated at the root surface, while at higher concentrations zones of depletion formed at the root surface. If Ca is actively taken up, inhibition of the uptake process could have resulted from the high Al^{3+} content in the soil with the inhibition being reduced at higher Ca concentrations. Conversely if Ca is actively extruded from the cells then the high Ca concentrations in the soil resulted in Ca being taken up a cation carrier not normally involved in Ca uptake.

Thus acid rain was found to have the potential to alter the uptake of Ca and Mg by decreasing the availability of these cations in the soil, altering the mycorrhizal species composition of the roots and, by increasing the Al^{3+} content of the soil, the rate of Ca and Mg uptake by the plant can be altered.

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



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CHAPTER 1 INTRODUCTION

Acid rain occurs as a result of air pollutants, principally sulphur and nitrogen oxides, being converted into acids through a series of atmospheric reactions. These acids can re-enter the terrestrial environment and may be detrimental to the living organisms and abiotic components of that environment.

Acidic precipitation has the potential to negatively affect plants in a number of different ways. It can cause damage to the protective surface structures of the plants, interfere with the functioning of guard cells, poison cells after it has diffused into the leaves, change the exudation processes or interfere with the reproduction of the plants. Acid rain can also increase the plants' susceptibility to other environmental stresses, disrupt symbiotic associations and cause nutrient stress as a result of acidification of the soil (Tamm and Cowling, 1977).

These potentially damaging effects have led to widespread concern regarding the viability and fitness of plants, particularly economically important plants, which are exposed to acidified rain.

One of the most consistent symptoms of acid rain damage is a decrease in the calcium (Ca) and magnesium (Mg) content of the foliar tissue (Hindawi *et al*, 1980; Godbold *et al*, 1988). In addition, acid rain generally increases the rate of nutrient leaching from the foliage (Evans, 1984b; Amthor, 1984). In the latter situation, the plant needs to absorb these ions at a faster rate so that it does not become nutrient stressed. If acid rain impairs the uptake of these ions, the observed nutrient deficiencies could result. The uptake of ions is dependent on both the attributes of the plant and the soil environment that the plant is growing in. As a result of the variability of these factors, plants may exhibit widely varying responses to acid rain.

A large amount of pollution is injected into the atmosphere on the Eastern Transvaal Highveld (ETH) as a result of urban and industrial activities. The former include domestic fires and motor vehicles, while the latter includes power stations, petrochemical plants, metallurgical industries, brickworks, foundries, fertilizer plants, saw mills, paper mills and chemical works. In addition, discard coal dumps and grassland burning contribute to the pollution levels (Tyson *et al*, 1988).

The climatology of the ETH is very unfavourable for the dispersion of the pollutants. These climatic conditions include high atmospheric stability, clear skies and low wind speeds. These conditions are generally associated with high pressure systems which prevail over the region (Tyson *et al*, 1988).

Some of the soils of the Eastern Transvaal are very old and highly weathered and thus have low Ca and Mg contents. They are described as being either strongly acidic (pH in water less than 5.2) or moderately acidic (pH between 5.2 and 6.0) (Tyson *et al*, 1988). The natural acidity of the soil is exacerbated by acid humus formation in pine plantations.

There is a shortage of indigenous timber that can be used industrially in Southern Africa - less than 0.25% of the land is natural evergreen forest. The indigenous forests in southern Africa are subject to careful control measures and only a trivial volume of high quality wood is extracted on a sustainable yield basis from forests in the Knysna-Humansdorp area (Anonymous, 1990). This timber is generally used for furniture (Scharfetter, 1987).

Although a small portion of timber is imported all the commercial timber used in South Africa comes from exotic plantations of pine, eucalyptus and wattle which have been established by both the government and private sectors. Over 1.12 million hectares of plantations have been established in South Africa (Scharfetter, 1987). At least half of these commercial forestry

resources are located in the ETH and along its periphery (Tyson et al, 1988).

Pine trees produce valuable softwood and are managed primarily for sawtimber, this being the biggest use of plantation timber in South Africa (Scharfetter, 1987). There are no indigenous members of the pine family in Southern Africa. The main pine species of the Eastern Transvaal region are *Pinus patula*, *Pinus taeda* and *Pinus elliotii* (Norskov-Lauritsen, 1963).

It has been projected that by the turn of the century, South Africa will be facing a timber shortage (Scharfetter, 1987). Effective utilization of the available timber is necessary to ensure that the timber demand is met in the future. In addition, factors which decrease yield and durability of the trees, such as insects, fungi, weathering and other pathogens, need to be identified and ways to overcome them sought. Since acid rain is thought to have the potential to decrease the growth and persistence of forests, and conifers in particular (Board, 1988), it has become necessary to determine what effects acid rain could have on the commercially grown trees in the ETH.

The aim of this investigation was to determine whether simulated acid rain, similar to that occurring on the ETH, would influence the amount of Ca and Mg taken up by one of the most extensively cultivated tree species of the region. A reduction in the uptake of these essential elements could cause a decline in the growth of these trees.

The plant used in this investigation was *Pinus patula* Schlecht. and Cham. It was first introduced to South Africa in 1907 from east-central Mexico. It is well adapted to the summer rainfall regions of southern and eastern Africa and has become a major species in softwood plantations (Morris, 1986). However it is now invading the natural forests in parts of the Eastern Transvaal and eastern Zimbabwe (Palgrave, 1977). It has a fast growth rate, is hardy to frost but sensitive to prolonged drought, fire and

hail (Grut, 1965).

Nutrient uptake is dependent on a number of factors. These are illustrated in Fig. 1.1 and include:

i) the ability of both the biotic and physical characteristics of the soil to supply the plant with nutrients, i.e. nutrient availability;

ii) the morphology and rate of growth of the roots;

iii) the nutrient absorption characteristics of the root system (Caassen and Barber, 1976).

Any one of these, if affected by acid rain, can decrease the rate of Ca and Mg uptake from the soil.

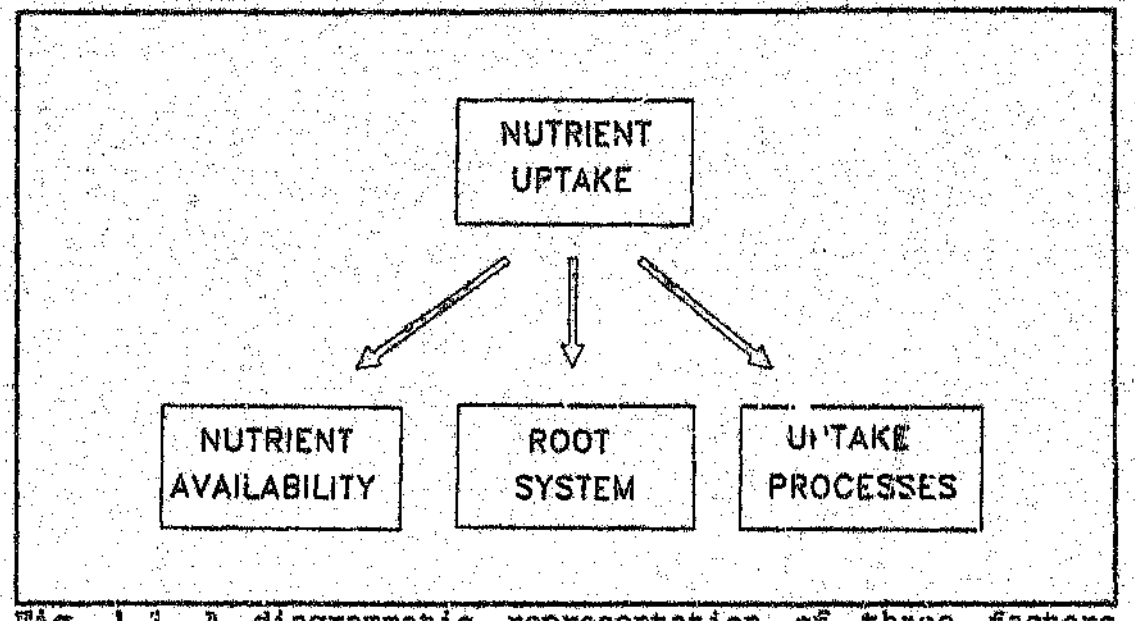

Fig. 1.1 A diagrammatic representation of three factors affecting nutrient acquisition.

In this study, three major experiments were undertaken in order to determine the possible effects of acid precipitation on Ca and Mg uptake by *P. patula*. These experiments investigated the three key issues in nutrient acquisition that are mentioned above. The following questions were addressed by each experiment:

Experiment 1.

i) Can acid rain alter the rate of Ca^{2+} and Mg^{2+} leaching from the soil?

ii) Can acid rain change the rate of soil acidification in terms of both the proton content of the soil and its aluminium content?

Experiment 2.

i) Can root growth, in terms of the rate of root extension and root mass, be affected by soil that has been exposed to acid rain?

ii) Can the degree of mycorrhizal colonization of roots growing into soil be altered if the soil has been exposed to acid rain?

Experiment 3.

Does acid rain have the ability to alter the uptake characteristics of the root for Ca and Mg?

These experiments are described and discussed in chapters 4, 5 and 6. The most important conclusions drawn from each experiment and their significance, are linked together and discussed in chapter 7.

CHAPTER 2 LITERATURE REVIEW

2.1 ACID RAIN

The phenomenon known as acid rain has always existed as a result of volcanic and thunderstorm activity. However industrialization during the last century has led to an increase in both the amount of acid rain occurring and in the area affected by the acidic precipitation.

2.1.1 Where the pollutants come from and how they re-enter the environment

Pollutants enter the atmosphere from a large number of sources. These sources include coal burning power stations, ore-smelting plants, motor vehicles, domestic coal stoves used for household cooking and heating, burning dumps of waste coal, burning underground coal seams and grassland burning. Atmospheric turbulences disperse the plumes of smoke, mixing the plume with the surrounding "clean" air which carries oxidants (Smith, 1988). This mixing of the plume can result in the primary pollutants (e.g. particulates, carbon monoxide (CO), hydrocarbons, carbon dioxide (CO_2), sulphur dioxide (SO_2) and nitrous oxides (NO_x)) being transformed, through a series of sunlight-induced reactions, into secondary pollutants. These secondary pollutants may affect the vegetation in a completely different way from the primary pollutants.

Both primary and secondary pollutants can re-enter the terrestrial environment by either dry or wet deposition. Dry deposition includes the absorption and adsorption of gases and the gravitational settling of fine aerosol and coarse dust particles. This manner of deposition occurs in all seasons throughout the year.

Alternatively pollutants return to the terrestrial environment through wet deposition, commonly referred to as acid rain. This involves the transfer of dissolved and suspended materials from the atmosphere to the land in raindrops, hail, dew, fog, snowflakes and other precipitation events including the interception

of cloud particles at high altitudes. Deposition in this manner occurs intermittently during the year, being dependent on precipitation events (Evans, 1984a).

2.1.2 Air pollution conditions on the Eastern Transvaal Highveld

The Eastern Transvaal Highveld (ETH) produces large amounts of pollution as a result of extensive industrial projects, as well as a wide range of domestic activities. The situation in the ETH is such that the bulk of the emissions originate from a very small area. Comparing a 30 000km^2 portion of the ETH (25°45' to 27° S and 28°30' to 30°30' E) with other areas of dense SO_2 emissions, it has been found that the emission densities of the ETH approximate some of the worst pollution conditions in the world (Tyson *et al*, 1988). The climatology of the ETH does not favour the dispersion of the pollutants (Tyson *et al*, 1988).

In 1984 the following emissions (tons per annum) were recorded (Tyson *et al*, 1988) for this area:

particulate	374 692
SO_2	1 038 556
NO_x	355 246
CO	339 574
hydrocarbons	276 503
CO_2	123 605 162

2.1.3 Composition of acid rain

The actual composition of acid rain is highly variable. Rain is naturally acidic, because water reacts with CO_2 in the air to produce weak carbonic acid with a pH of about 5.6, i.e. a proton (H^+) concentration of about 2.0×10^{-6} M (Clark and Thompson, 1989). Cations such as ammonium (NH_4^+) are also present in the precipitation. The positive charge carried by these cations is balanced by a variety of anions.

Acid rain contains increased sulphate (SO_4^{2-}) and nitrate (NO_3^-) concentrations. The molar ratio of SO_4^{2-} to NO_3^- in acidified

in acidified rain is between 3:1 and 1:1 (Patil *et al*, 1988). In a polluted environment, the negative charge, resulting from the increased amounts of SO_4^{2-} to NO_3^-, is balanced by an increase in the H^+ concentration, resulting in the pH of the precipitation decreasing below the norm.

The pH of the rain recorded in the ETH at the Sabie Forestry Research Centre is approximately 4.2 (Tyson *et al*, 1988). This is remarkably low compared to rain falling in areas relatively free from man-made pollution, where the mean pH is between 5.0 and 5.3.

2.1.4 Natural and anthropogenic sources of sulphur in the atmosphere

Large amounts of hydrogen sulphide (H_2S), liberated from the decay of organic matter and from biological SO_4^{2-} respiration, reach the atmosphere naturally. Hydrogen sulphide is highly toxic to humans, but it is rapidly oxidized to SO_2. A simplification of this reaction can be written:

$$H_2S + {}^3/_2O_2 \longrightarrow SO_2 + H_2O \qquad 2.1$$

Sulphur dioxide can also be released naturally into the atmosphere by sea spray, plankton and in certain areas, volcanic action. It has been approximated that half of the gaseous sulphur released into the atmosphere is from natural sources (Rodhe, 1989).

The principal source of oxidized sulphur entering the atmosphere from human activities is from the burning of coal. Coal that is used for energy purposes contains more than 2% sulphur, of which about half is present as pyrite (FeS_2) and the rest is of an organic nature. The burning of such materials readily produces SO_2 by reactions such as reaction 2.1 or by:

$$4FeS_2 + 11O_2 \longrightarrow 8SO_2 + 2Fe_2O_3 \qquad 2.2$$

(Kennedy, 1986). Sulphur dioxide can also enter the atmosphere from the smelting of sulphide metals and from other industrial processes (Evans, 1984b).

When the SO_2 reaches the atmosphere it reacts with moisture in the air. It is oxidized to sulphur trioxide and hence to sulphuric acid (H_2SO_4):

$$SO_2 + {}^1/_2O_2 + H_2O \longrightarrow H_2SO_4 \qquad 2.3$$

It is thought that a photochemical excitation is involved in this reaction (Kennedy, 1986).

In dry air the conversion of SO_2 to H_2SO_4 would require long periods of time. This can result in the SO_2 moving considerable distances from the source. This is often the case when power stations emit SO_2. Some SO_2 can also fall to the ground unconverted (Pearce, 1987). However when the SO_2 becomes incorporated into clouds or any other humid environment the reaction rate speeds up to approximately 0.1% conversion per hour (Kennedy, 1986). In addition, it has been suggested that tiny particles of metals, such as iron and manganese in the air, arising from industrial pollution, may act as catalysts for the reaction (Pearce, 1987). Thus, the rate of acidification increases under humid, polluted conditions. Gases, such as ozone and hydrogen peroxide, also appear to be critical in the acidification process.

2.1.5 Natural and anthropogenic sources of nitrogen in the atmosphere

Nitrogen oxides (NO_x) can also be responsible for an increase in the acidity of rain. Thunderstorm activity can produce NO_x naturally. Nitrogen becomes oxidized in the lightning discharge arc:

$$N_2 + O_2 \longrightarrow 2NO \qquad 2.4$$

This reaction can occur because the charge separation and voltage differences generated from the frictional processes of air masses, elevate the temperatures sufficiently to produce enough activation energy for the oxidation reaction (Kennedy, 1986). Although natural sources of NO_xs are much greater than the anthropogenic sources, the latter generally result in small regions having very high concentrations of NO_xs, while natural sources are more widely dispersed.

Nitric oxide (NO) is given off in the burning of fossil fuels in power stations and by motor vehicles. In unpolluted air, NO has a half life of a few days, however the particulates in polluted air act as reaction centres, rapidly converting NO to nitrogen dioxide (NO_2):

$$2NO + O_2 \longrightarrow 2NO_2 \qquad 2.5$$

Ozone can act as a catalyst for such a reaction:

$$NO + O_3 \longrightarrow NO_2 + O_2 \qquad 2.6$$

The NO_2 is itself oxidized and converted to nitric acid (HNO_3) (Pearce, 1987).

2.1.6 Other pollutants

In addition to the acids, (H_2SO_4 and HNO_3), that are being liberated into the atmosphere, large amounts of lead, zinc, copper, vanadium and cadmium are released as a result of industrial processes (Vogelmann, 1982). These heavy metals are toxic to plants and change the permeability of the cell membranes, which can change the rate and selectivity of metabolite exchange in plant cells. This would further aggravate any harmful affects that the acids may have on the plants.

2.2 DIRECT AND INDIRECT EFFECTS OF ACID RAIN ON VEGETATION

It has been speculated that acid deposition might be one of the causes for the observed decrease in the growth rate of trees in Northern Europe (Tamm, 1989). The reason for this is that the reduction in growth tends to correspond with large increases in atmospheric pollution and a drop in the pH of the precipitation. Experiments aimed at determining the effects of acid rain on vegetation, have often shown conflicting results. This may be the outcome of varying direct and indirect effects that the acid rain may have on the vegetation.

2.2.1 Fertilization effects of acid rain

Acid rain, with its increased nitrogen and sulphur concentrations, could act as a fertilizer on soils (Evans *et al*, 1981b; Abrahamsen, 1984; Tamm, 1989), since nitrogen and sulphur are

both essential elements in plant nutrition. This fertilization effect may be particularly important in natural soils, which do not receive the same degree of fertilization as agricultural soils.

Nitrogen is absorbed by the roots either as NO_3^- or as NH_4^+. Nitrate is not toxic to the plant and can accumulate to relatively high concentrations in the vacuoles of cells. The xylem transports most of the NO_3^- from the roots to the leaves where it is reduced to nitrite by the enzyme nitrate reductase, and then to NH_4^+ by nitrite reductase.

High NH_4^+ concentrations are toxic to most plants and thus it is rapidly translocated or assimilated. In general, acidic soils are inhibitory to nitrifying bacteria and therefore NH_4^+, rather than NO_3^-, is the predominant form of inorganic nitrogen in the soil solution (Vance and Griffith, 1990) and plants that are adapted to these acidic soils will tend to take up NH_4^+ in preference to NO_3^-. The NH_4^+ is incorporated into amino acids, and then into proteins, nucleic acids, lipids, as well as porphyrins (e.g. chlorophyll, cytochromes and phytochromes).

Sulphur is taken up principally as SO_4^{2-} by the roots, although SO_2 may be absorbed and assimilated by the leaves. Sulphur containing amino acids are cysteine, cystine and methionine (Noggle and Fritz, 1983).

Nitrogen deficiencies are common and fairly widespread in natural soils (Abrahamsen, 1984) hence the fertilization effect of acid rain may be beneficial to the vegetation. Sulphur deficiencies are quite common in agricultural areas, however they are rare in forests except in tropical and unglaciated areas in temperate zones (Abrahamsen, 1984). Since sulphur is not required in very large amounts for normal plant metabolism, it is thought that the atmospheric deposition of sulphur generally far exceeds the biological sulphur requirement (Reuss *et al*, 1987). The nutritional consequences of excessive SO_4^{2-} inputs are unclear, however

it is thought that excessive sulphur is unlikely to cause nutritional harm to the ecosystem. The uptake of SO_4^{2-} may even aid in solving charge balance problems in NH_4^+ utilizing forest ecosystems where the uptake of cationic nutrients is greater than the uptake of anionic nutrients (Evans *et al*, 1981b).

The fertilization effect of acid rain may be related to the fertility of the site. Tamm (1989) found that tree growth was affected in a positive manner on unfertilized plots, while the reverse was true for trees on fertilized plots. Hence, the effect of acid rain may be complexly related to stand fertility. This interaction could be the cause of inconsistent results obtained from simulation experiments investigating the effect of acid rain on vegetation. Morrison (1984) points out that the input of nitrogen from man-made sources, spread over a full year, may be insufficient to elicit any measurable response in terms of forest growth. However the long term effects may be a general enrichment of the forest system as a whole.

It has also been suggested that the recent increase in available nitrogen in the air and precipitation has resulted in the temperate forests of northern Europe becoming "over-saturated" with nitrogen (Nihlgård, 1985). This could lead to imbalances in the carbon to nitrogen ratios in the plant. Excess nitrogen can lead to hormonal imbalances and extremely fast growth with large cells having high volumes which would be more vulnerable to wind, drought and parasite attack. Reports indicate that fungal diseases, insect attack and algal growth on the leaf surfaces may be stimulated by excess nitrogen. In pine trees, an excess of nitrogen in the needles results in a decrease in their frost hardiness. In addition, excess nitrogen can interfere with winter hardening of buds and foliage (Pitelka and Raynal, 1989).

2.2.2 Symptoms of acid rain damage in conifers

The main symptoms of damage attributed to acid rain in conifers are a gradual discolouration and subsequent loss of needles occurring from the base to the top and from inner to outer

branches, premature growth reduction, branch dieback in the lower crown and formation of epicormic shoots as well as an extension of normal wet heartwood into the sapwood at the base of the tree. There may be damage to the fine root system as well (Blank, 1985). Analyses also show that there is often a calcium (Ca) and magnesium (Mg) deficiency in the foliage (Hindawi *et al*, 1980;, Godbold *et al*, 1988). The symptoms tend to vary with different plant species.

These symptoms attributed to acid rain could result in a decreased yield and/or quality of the plants, a lowering of the value of marketable plants due to visible damage and a decrease in their aesthetic value, as well as a decrease in the longterm growth of trees.

Local reports have indicated a decline in productivity of *P. patula* at Usutu, Swaziland (Tyson *et al*, 1988). In addition, for the past twenty years, chlorotic flecking and mottling of the needles of pine trees has been noted throughout South Africa (Tyson *et al*, 1988). These factors could be associated with acid rain damage.

2.2.3 Erosion of the cuticle

It has been proposed that acid rain has the potential to increase the rate of cuticle erosion (Evans, 1984a; Morrison, 1984). The cuticle performs a protective function preventing excessive transpiration, parasitic infections and the leaching of essential organic and inorganic nutrients from the surface of the plant.

It has been found that for some plants, e.g. the kidney bean and willow oak, simulated acid rain with a pH of 3.2 causes the submicroscopic structure of the superficial waxes, cutin and cuticular waxes to change and with time to erode (Evans, 1984a). Damage to the cuticle may increase to such an extent that necrotic spotting, "pits" or lesions result. These effects are reviewed by Morrison (1984). The damage to the cuticle was however not a consistent response to acid rain treatments. In

experiments where the cuticular waxes were not affected, the underlying surface cells tended to be damaged (Evans, 1984a).

2.2.4 Cellular damage

Cells throughout the entire plant can become damaged when the acidity of the precipitation increases. This has been illustrated by experiments performed by Ferenbaugh (1976) in which plants, exposed to simulated acid rain, contained smaller sized cells compared to their counterparts receiving rain of a normal pH. In addition the starch granules within the chloroplasts of acid-treated plants had starch grains of significantly smaller size than the controls. It has also been reported that low concentrations of aqueous SO_2 can cause structural injury to chloroplasts of *Pinus contorta* (Malhorta, 1976).

Visible leaf injury ascribed to acid rain ranges from chlorosis and necrotic spotting of leaves (Crittenden and Read, 1978) to galls and lesions (Evans et *al*, 1977; Morrison, 1984). It was hypothesized that these injuries may result from acidic substances diffusing into the foliar tissue through the stomata and poisoning the cells (Tamm and Cowling, 1977). The damage occurs because plants have limited means in the cytoplasm to neutralize the strong acids which would result from acidic substances entering the foliar tissue (Kennedy, 1986). However the degree of injury is generally not correlated with the age of the tissue (which is proportional to the density of stomata and trichomes on the tissue) which tends to indicate that th. injury is not due to acidic substances diffusing into the foliar tissue through the stomata (Evans et *al*, 1977).

Another way in which the acidity of the precipitation may affect the entire plant is through water uptake and usage by the plant. Water moves from the soil into the plant and then throughout the plant, so that every growing cell is surrounded by and impregnated with water. An increase in the acidity of the precipitation will cause a decrease in the pH of the water that is moving into the plant. Experiments have shown that barley seedlings counter-

act the additional acidity of simulated acid rain by actively pumping extra H^+ into their vacuoles, which results in a decrease in the pH of the latter (Wolfenden and Wellburn, 1986). This results in the cells having a relatively large buffering capacity. However, at some stage these excess H^+ ions need to be removed from the cells. The presence of strong acids in the microenvironment of the cell may make the release of the H^+ difficult (Nihlgård, 1985).

The build up of H^+ in the cells may interfere with cellular processes, in that localized changes in the electron or H^+ gradients close to the plasmalemma can result in changes in membrane function, even if the membrane permeability and/or integrity remained unchanged. Such changes in the H^+ could lead to disruptions in the production of ATP which is dependent on H^+ gradients across membranes (Evans, 1984b). The change in the pH of the cells could also affect enzyme reactions.

2.2.5 Effects of acid rain on plant metabolism

It has been found that in *Phaseolus vulgaris*, the diffusive resistance of the adaxial leaf surface exposed to rain of pH 2.7, was much lower compared with foliage exposed to rain of pH 5.7, however the abaxial leaf resistance remained unchanged (Evans et al, 1981a). Thus rain with a high acidity may interfere with normal functioning of guard cells, leading to a loss of control of stomata and thus changed rates of transpiration and gas exchange (Tamm and Cowling, 1977).

Acid rain has been found to affect both photosynthetic and respiratory processes. Hindawi et al, (1980) reported that acid mist resulted in a loss of chloroplast integrity and a reduction in the total chlorophyll content. This would tend to indicate that a reduction in the photosynthetic rate is likely. However, Ferenbaugh (1976) found an increase in the apparent photosynthetic rate of plants treated with acid rain, as well as an increase in the apparent respiratory rate. Hence, the net photosynthetic rate remained unchanged. In spite of the increase

in apparent photosynthetic rate, calculated with respect to oxygen evolution, there was a decrease in the sugar and starch content. This suggests that the process of phosphorylation is uncoupled as a result of the increased acidity of the precipitation. Hence, the acid rain may affect the vegetation by disturbing the normal metabolism of the plant.

Further evidence for the disruption of normal plant metabolism by acid rain can be deduced from the observation that acid-treated *Phaseolus vulgaris* can tend to be shorter and bushier than their counterparts which have not been exposed to rain of a low pH. The appearance of the acid-treated *P. vulgaris* was a result of shortened internodes and increased bud formation, which indicates possible interaction between the acid and auxins (Ferenbaugh, 1976).

Acid rain may also be able to alter the root:shoot ratio of plants (Troiano *et al*, 1982; Deans *et al*, 1990) as a result of a change in the carbon partitioning and would have important consequences for mycorrhizal associations.

2.2.6 Leaching of cations from foliage

An increase in acidity of the precipitation may accelerate the rate of leaching of substances out of the leaves, as a result of the erosion of the cuticle and damage to the underlying cells. It has been found that the rates of leaching of Ca^{2+}, Mg^{2+}, NO_3^- and SO_4^{2-} tend to be greater at low pHs (3.0 and 3.3) as opposed to the "normal" pH of rain of 5.6. However, the rates of leaching of other minerals, namely NH_4^+ and zinc remained constant at all pH levels (Evans, 1984b).

Mitterhuber *et al*, (1989) propose that, although acid fog can increase the rate of cation leaching from the twigs and needles of *Picea abies*, the plants should be able to replace, from the soil, the ions that were leached during breaks between the rain periods. This hypothesis will only hold if the nutrients are freely available and the uptake process is not retarded.

in apparent photosynthetic rate, calculated with respect to oxygen evolution, there was a decrease in the sugar and starch content. This suggests that the process of phosphorylation is uncoupled as a result of the increased acidity of the precipitation. Hence, the acid rain may affect the vegetation by disturbing the normal metabolism of the plant.

Further evidence for the disruption of normal plant metabolism by acid rain can be deduced from the observation that acid-treated *Phaseolus vulgaris* can tend to be shorter and bushier than their counterparts which have not been exposed to rain of a low pH. The appearance of the acid-treated *P. vulgaris* was a result of shortened internodes and increased bud formation, which indicates possible interaction between the acid and auxins (Ferenbaugh, 1976).

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Increased rates of leaching can decrease the rate of plant growth in that elevated rates of leaching would result in lowered concentrations of certain important nutrients within the leaf, which would alter normal metabolism. In addition, extra energy would be expended by the plant in absorbing and assimilating nutrients in order to replace those that were leached from the tissue. This extra energy expended in replacing nutrients is being diverted from growth processes, and hence the rate of plant growth would decrease. This could be one explanation for the observed decrease in plant growth as a result of acid rain treatments (Amthor, 1984).

Conversely, Mille[illegible]984) records no change in the rate of growth or the nutrition[illegible]alth of *Picea abies* which lost increasing amounts of Ca^{2+} and Mg^{2+} from the crowns as the pH of the artificially acidified rain decreased. This presumably indicates that the elements are readily reabsorbed by the roots and mycorrhizal hyphae with little or no negative effect on the trees.

2.2.7 Acid rain and ectomycorrhizas

If two organisms are intimately associated, as in the case of plants colon[illegible]ed by mycorrhizal fungi, the negative effect that acid rain may have on one of the organisms may have harmful effects on both organisms.

2.2.7.1 Ectomycorrhizal associations

Mycorrhizal fungi are found occurring naturally in soils throughout the world. These fungi form symbiotic relationships with the roots of plants. Nutrients are absorbed from the soil by the fungus and are released to the host. In return the mycorrhizal fungi obtain their organic nutritional requirements from the host. There are three types of mycorrhizal associations, these being ectomycorrhizal, endomycorrhizal and ectendomycorrhizal associations. These associations are defined on the basis of whether or not the fungal hyphae penetrate the root cortical cells of the host. The association occurring on the roots of pine trees is an ectomycorrhizal relationship because

the fungi do not penetrate the root cells of the host (Linderman, 1988). Ectomycorrhizas utilize simple carbohydrates and obtain these, as well as thiamine, other vitamins and organic growth factors, from the host. The fungi which form ectomycorrhizal associations are Basidiomycetes and Ascomycetes (Linderman, 1988).

In the ectomycorrhizal association a compact mantle of fungal hyphae form around the root. Hyphae extend from this mantle into the soil. These hyphae occur either individually or as fascicled strands and rhizomorphs (Fogel, 1980). The mantle can be inconspicuous or well developed and variously coloured (Bowen, 1984). The colour of the mantle of some species can change as the fungus ages (Egli and Kälin, 1989).

Hyphae also extend into the intercellular spaces of the cortex. This part of the association is known as the Hartig net. The exchange of material between fungus and host takes place in the Hartig net where there is extensive contact between the two organisms.

2.2.7.2 Colonization of the host

The mycorrhizal fungi, when not associated with the host, exist in the soil either as spores or as vegetative propagules in root fragments. It is thought that the propagules of mycorrhizas respond to the root exudates in such a manner that their hyphae grow and penetrate the epidermal tissue. The fungus then progresses both internally and externally along the root surface colonizing the host tissue (Linderman, 1988). The lifespan of the mycorrhizas varies quite considerably ranging from a couple of weeks to a maximum of two years (Egli and Kälin, 1989). The degree of mycorrhizal colonization is thought to be strongly influenced by environmental factors such as climate (specifically temperature and light), soil nutrient status, drought and waterlogging (Erland *et al*, 1990; McCool, 1988). In addition, human activities such as fertilization, liming and ploughing after clear cutting can also affect the symbiotic association

(Erland *et al*, 1990).

Colonization leads to changes in morphology and physiology of both the host and the fungus (Linderman, 1988). The root morphology of the host can change quite considerably when colonized by the mycorrhizas. Roots colonized by ectomycorrhizas, besides being surrounded by a thick fungal mantle, tend to loose their root hairs, branch extensively and increase their diameters compared to non-mycorrhizal roots. It is thought that some of the morphological changes in the root are caused by auxins produced by the fungus (Gerdermann, 1974).

2.2.7.3 Benefits conferred on the host by the mycorrhizal relationship

The presence of ectomycorrhizas enhances the host's uptake of immobile elements (eg. phosphorus, zinc, and copper) as well as the uptake of some mobile elements such as sulphur, Ca, potassium (K), iron, Mg, manganese, chlorine, bromine and nitrogen (Linderman, 1988). The significance of this role increases when considering natural soils, such as forest soils, which are not fertilized; in these soils the mycorrhizas ensure that their hosts receive an adequate nutrient supply. There are a number of ways that these increased uptake rates can be achieved.

The change in root morphology, shown by increased branching and thicker roots, caused by colonization can result in a larger absorptive surface area across which minerals can be absorbed (Linderman, 1988). In addition, due to the extensive network of fungal hyphae extending into the surrounding soil (Fogel, 1983), a greater volume of soil can be explored and nutrients absorbed over larger distances compared to non-mycorrhizal roots. Colonization is thought to prolong the life span of short roots in that the sheath absorbs nutrients for longer time periods than the root alone (Nye and Tinker, 1977).

It is thought that mycorrhizas absorb nutrients more rapidly per unit area than do non-mycorrhizal roots, i.e. the mycorrhizal

roots have a greater sink strength. However this has only been proven with short-term experiments in solution and thus may not apply to long-term growth under field conditions (Nye and Tinker, 1977). However, the fact that mycorrhizal fungi benefit the host even when the soil volume is limited and hyphal growth is restricted implies that the absorption and/or transport of nutrients by the fungi is more effective than by roots (Parke *et al*, 1983).

It is possible that mycorrhizas may increase the rate of nutrient absorption by absorbing nutrients in forms that would otherwise be unavailable to the root. It has been found that mycorrhizas can utilize inorganic, insoluble forms of phosphate which are otherwise unavailable to roots (Nye and Tinker, 1977; Lapeyrie, 1991). The manner in which the fungus might extract these compounds is by bringing them into solution by exuding a chelating substance or by changing the pH of the soil in contact with the hyphae. Organic phosphorous compounds could be utilized directly, or hydrolyzed by phosphatase enzymes on the hyphal surface (Nye and Tinker, 1977). Other nutrients could be absorbed in a similar way.

Besides benefitting from increased nutrient uptake, the host can profit from the mycorrhizal-host symbiotic relationship in a number of other ways. Ten month old pine seedlings colonized by mycorrhizas have been found to photosynthesize twice as rapidly as non-mycorrhizal seedlings and this was presumed to be as a result of the increased flow of nutrients to photosynthetically active leaves in mycorrhizal plants (Vogt *et al*, 1991).

Mycorrhizas have been found to increase water uptake and/or change the plant's physiology to reduce stress caused by soil drought (Parke et *al*, 1983). These authors propose a number of possible reasons why the host is less affected by water stress. Firstly the presence of mycorrhizas change the root morphology of the host such that the absorptive surface of the host is increased. In addition, the presence of the fungus may decrease

the resistance of water flow from the soil to the roots. Finally there is a potential for the fungal hyphae to penetrate smaller soil pores than the root hairs and thus obtain more water than the roots.

Mycorrhizas are thought to be able to increase the plant's tolerance of other soil stresses, such as high salt levels, toxicities associated with heavy metals or due to minor element imbalances, such as manganese toxicity (Linderman, 1988). However, other studies have indicated that the fungal sheath cannot prevent aluminium from entering the host tissue (Jentschke *et al*, 1991a). Consequently it is thought that the ability of the mycorrhiza to protect the host from heavy metal toxicities is dependent on the concentration of that metal in the soil.

There is evidence to suggest that ectomycorrhizas can protect root tissue from attacks by pathogens. Marx (1970) reports that the fungal sheath and Hartig net apparently form a mechanical barrier preventing the root tissue of pine roots from becoming infected by the highly infective vegetative mycelium and germtubes of the zoospores of *Phytophthora cinnamomi* Rands, a common root pathogen.

2.2.7.4 Effects of acid rain on mycorrhizas

It has been shown that mycorrhizas can protect the plant from air pollutants, such as ozone and SO_2 which usually inhibit root and shoot growth (Carney *et al*, 1978; Mahoney *et al*, 1985). The response of ectomycorrhizal species to the pH of the precipitation is highly variable and appears to be species specific: the colonization of roots by some species is stimulated by acid rain, other mycorrhizal species are not affected by the acidity of the rain, while some species are sensitive to acidity and are inhibited when the pH of the rain decreases (Dighton and Skeffington, 1987; Simmons and Kelly, 1989). Reich *et al*, (1985) found that the negative effects of acid rain were only evident if the pH of the rain was lower than the soil pH. Meier *et al*, (1989) has proposed that the effects of acid rain on mycorrhizal

formation may be a complex function of the soil type and acidity, the acidity and composition of the rain and the environmental tolerances of the species.

There are many ways in which the acid rain may affect this symbiotic relationship. Firstly, the mycorrhizas can be disturbed if the carbohydrate supply that they receive from the host is adversely altered (M°Cool, 1988). It has been reported that air pollutants can reduce the allocation of photosynthates to the roots (Carney *et al*, 1978), which would have negative influence on root metabolism. Acid rain may affect the amount of photosynthate allocated to the root by influencing photosynthesis through the uncoupling of phosphorylation or through the malfunctioning of the stomata, thus modifying the allocation of photosynthates to the fungus.

Mycorrhizas can also be directly affected by the low pH of the precipitation or by an increase in the acidification of the soil. It has been shown that increased acidity above the norm and the consequent increased solubility of certain metals can inhibit the germination of fungal spores and decrease the rate of mycorrhizal growth (Dighton and Skeffington, 1987).

If the nitrogen in the precipitation acts as a fertilizer to increase soil fertility, a reduction in the rate of mycorrhizal colonization may occur (Reich *et al*, 1985).

If the efficiency with which the mycorrhizas extract nutrients from the soil and release them to the host is altered then the host may suffer from nutrient deficiencies. Such a reduction in the efficiency of nutrient extraction may be caused by a decrease in extramatrical hyphal development. The host could even suffer from a decreased ability to fend-off soil pathogens if the mycorrhizas are damaged (Carney *et al*, 1978).

2.2.8 Effects of acid rain on soil microorganisms and plant pathogens

Other microorganisms in the soil associated with processes such as organic matter decomposition or nitrogen transformation may be affected by acid rain. The vegetation would consequently be affected by changes in the rate and efficiency of nutrient cycling in the ecosystem. Morrison (1984) reviews experiments which have investigated the relationship between acid rain and the soil organisms involved in litter decomposition and nitrogen cycling. The results indicate that acidified soil tended to show decreases in the fungal length and bacteria biomass, and had smaller rates of CO_2 evolution compared to soil and litter that was not acidified.

Evans *et al*, (1981b) point out that acid rain may require a number of years to change the soil pH, but that the microbial populations are able to adapt quite rapidly to the changing physical and chemical environment in which they occur. Hence, the soil microbes may not be negatively affected by decrease in the pH of the soil that might result from exposure to acid rain. Some fungi, e.g. *Aspergillus niger*, are involved in the solubilizing of minerals (Trudgill, 1977), if acid rain affects either the fungi or the process of solubilization, then the concentration of ions in the soil will be altered.

During the past few years the number of trees in Europe that are affected by fungal pathogens has increased markedly (Blank, 1985). It has been suggested that pathogens may play a secondary role in causing a decline in the forests as a consequence of the trees' lowered resistance or increased susceptibility to the pathogen. On the other hand, increases in the acidity of the precipitation and the pollution levels could change either the pathogen's virulence or the trees' susceptibility to the pathogenic infection. The effects of acidic precipitation on the organism may also vary with the nature of the pathogen involved (whether a fungus, bacterium, mycoplasma, virus, nematode, parasitic angiosperm or multiple pathogen complex) and with the

species, age and physiological status of the host (Tamm and Cowling, 1977).

2.2.9 The effect of acid rain on natural forest ecosystems

The above discussion has focused primarily on the effect of acid rain on individual plants. Cowling (1989) extends this discussion to speculate what effects acid rain may have on natural forest ecosystems. These effects include changes in competition and mortality, in patterns of succession, in species composition and in patterns of nutrient cycling. In addition the habitat quality for wildlife will be affected, as well as watershed functioning. These changes in the ecosystem may in turn affect species fitness and genetic diversity, which can have negative consequences for the survival of the species as a whole, as well as the continued normal functioning of the ecosystem. In addition the competition within the plant community or the herbivore population community might by altered as a consequence of acid rain, resulting in a simplification of the ecosystem (Evans *et al*, 1981b) and even destabilization of the ecosystem.

2.3 SOIL ACIDIFICATION

Experiments designed to determine whether acid rain has a negative effect on the vegetation have often provided conflicting results. In addition, the acidity of the simulated rain often had to be far greater than that of ambient rain to induce an effect (Amthor, 1984; Blank, 1985). Consequently, researchers have looked for another factor which affects plant growth that could be influenced by acid rain, such a factor is the soil. It is currently thought that acid rain might be compounding and accelerating the rate of natural soil acidification by destroying the soils natural buffering capacity (Blank, 1985). It has been observed that forest soils appear to be more sensitive to acidification than unforested lands (Falkengren-Grerup, 1989).

It is possible that acid rain might cause a build up in the H^+ concentration in the soil to such an extent that the uptake of

nutrients which depend on H^+ extrusion from the root will be negatively affected (Kennedy, 1986). Not only does soil acidification result in an increase in the H^+ content of the soil, but it is also associated with a loss of base cations, a reduction in the cation exchange capacity of the soil, the mobilization of aluminium (Al^{3+}) and mineral degradation (Falkengren-Grerup, 1986). These aspects will be discussed in more detail in the following sections.

2.3.1 Properties of the soil

The cation exchange capacity (CEC) is defined as the amount of cations adsorbed on soil-particle surfaces per unit mass of soil under chemically neutral conditions. This property of the soil is nearly constant and is independent of the species of cation. Clay minerals differ in specific surface area and in the number of exchange sites per unit area of particle surface thus the total CEC varies with different soil types (Hillel, 1982). The proportion of cations adsorbed to soil particles is dependent on the ionic charge of the adsorbed cation, their ionic size and the properties of the exchanger. In addition, the attraction of a cation to a negatively charged soil particle increases with increasing valency of the cation (Nye and Tinker, 1977).

Anions are generally repelled by the negatively charged clay and humic particles and so remain in the soil solution. Hence they are gradually leached from the soil by percolating water (Noggle and Fritz, 1983). Some anions may however, become adsorbed on the soil solids by specific and non-specific bonding which can be both reversible or irreversible (Nye and Tinker, 1977). These anions can then adsorb H^+, or other cations, from the soil solution (Patil *et al*, 1989).

Sulphate is geochemically less mobile than NO_3^-, this is thought to be as a result of SO_4^{2-} becoming adsorbed onto hydrous iron and aluminium sesquioxides. The adsorption of SO_4^{2-} to soil particles is an important acid-consuming process, in that hydroxide groups dissociate from the soils to be replaced by the SO_4^{2-}. The

hydroxide ions then combine with excess H^+ in the soil solution to produce water and thus decrease the acidity of the soil (Skeffington, 1987)

When SO_4^{2-} is adsorbed to the soil particles it creates an exchange site capable of retaining incoming H^+ and other cations. Thus SO_4^{2-} absorbing soils can be acidified by H_2SO_4 without an equivalent amount of cation leaching (Evans *et al*, 1981b). Older, more highly weathered soils tend to be richer in amorphous sesquioxides than relatively young forest soils (Reuss *et al*, 1987). Some soils of the ETH are very old and highly weathered and hence have the potential to retain SO_4^{2-}.

The number of SO_4^{2-} adsorption sites in the soil is finite. Consequently, as the sites are progressively filled, increasing amounts of SO_4^{2-} accompanied by cations would travel through the soil in the soil solution, resulting in cation leaching (Reuss *et al*, 1987).

Nitrate, on the other hand, is not strongly adsorbed by soil surfaces and hence tends to move through the soil profile accompanied by cations.

Soils can resemble weak acids in that clay minerals and organic macromolecules act as immobile anions (Krug and Frink, 1983). Thus the thermodynamic criteria for weak acids holds when applied to soils. For example, the addition of water dilutes the ion concentrations in the soil, thereby increasing the pH of the soil.

The percentage of cation exchange sites occupied by base cations (Ca^{2+}, Mg^{2+}, K^+ and sodium (Na^+)) is known as the percentage base saturation. The remaining sites occupied by H^+ and Al^{3+} are referred to as the exchangeable acidity. Soils with high base saturations tend to have higher pH's and be more fertile than those soils with low base saturations (Skeffington, 1987).

Soils generally possess large buffering capacities. Some of the

compounds responsible for this characteristic are inorganic and organic phosphates, organic carboxylate materials including fulvic and humic acids, phenolics, insoluble carbonates and bicarbonates and silicates. This capacity prevents sharp changes in pH on addition of acids or bases (Kennedy, 1936).

2.3.2 The soil as a nutrient supply

The nutritional requirements of plants are, with the exception of carbon, obtained from the soil. Only carbon, hydrogen and oxygen are not mineral elements. The mineral elements immediately available to the plants are present either in the soil solution which is in turn in equilibrium with the adsorbed ions, or they are adsorbed onto clay-humus particles. Most of ions assimilated by plants are taken up from the soil solution.

As the soil solution loses ions to the plant, the ions are replenished by desorption of exchangeable ions from the clay minerals and humus particles and from the breakdown of readily decomposable organic debris (Milthorpe and Moorby, 1986).

Mineral nutrients, in both ionic and complexed forms, move through the soil by convection (mass flow) and diffusion. The rate of ion movement is dependent on factors such as their concentration, the composition of the solid phase and the moisture content of the soil. Ions that interact only slightly with the solid phase of the soil generally reach the plant by mass flow in water that is either percolating through the soil and intercepts the roots, or is moving toward the root in order to satisfy the transpiration demands of the plant. Examples of ions transported in this way are chloride and NO_3^- ions. Minerals which interact with the solid phase, e.g. Ca^{2+} and Mg^{2+}, move through the soil much slower, generally, as a result of diffusion processes occurring within the soil.

If the rate of uptake of nutrients is less than the rate at which the ions are supplied to the root surface then the ions tend to accumulate at the root surface. This is though to occur for Ca^{2+}

and Mg^{2+} (Dunham and Nye, 1976; Marschner, 1986). On the other hand, the rate of uptake could be greater than the rate at which the ions can be supplied to the root surface. This results in a zone of depletion forming against the root surface. This has been found to occur in the case of phosphate (Tinker, 1990) and K^+ (Dunham and Nye, 1976). Depletion zones would tend to be more common and pronounced in natural and forest soils where fertilization is less intensive. In these situations root extension and exploration of new soils becomes an important factor in ensuring that the plants receive adequate nutrients.

2.3.3 Natural soil acidification processes

Soil acidity is determined by the activity of H^+ in the soil solution (soil pH) and the exchangeable acidity (i.e. exchangeable H^+ and Al^{3+} associated with the soil particles). There exists an equilibrium between the H^+ in the soil solution and the exchangeable acidity. It has been suggested that soil acidification should be defined in terms of a decrease in the base saturation, rather than as a decrease in the soil pH, due to the difficulty of defining soil pH in a meaningful manner (Skeffington, 1987). This problem is further compounded by the fact that soil pH can vary from day to day depending on the amount of soil water and its salt content (Larcher, 1980; Binns, 1988).

However, it has been pointed out that any changes to the soil system as a result of increased acidity of the rain, are likely to be manifest in the carrier agent, i.e. the soil solution (Bruggenwert *et al*, 1991). Thus it is acceptable to use the soil pH as the principal indicator of the soil status when discussing the effects of the addition of acid.

The acidification of the soil is a natural process that can, to a large extent, be attributed to microbial, plant and animal processes. Respiration which is carried out by all living organisms, is an important soil acidifying process. Living organisms utilize carbohydrates to obtain energy and in the process produce water and CO_2. The latter is liberated into the

soil and dissolves in water to form carbonic acid, which dissociates to form a H^+ and a bicarbonate ion. The dissociation of carbonic acid occurs only at a pH greater than 4.5. Hence the acidification of the soil as a result of respiration only occurs in soils that are only slightly acidic, neutral or alkaline (Skeffington, 1987).

Plants generally take up more basic components than acidic from the soil, consequently an increase in biomass acidifies the soil (Van Breemen, 1991). The uptake of cations usually involves H^+ extrusion in exchange for the basic cations, which results in the soil, particularly the rhizosphere, becoming naturally acidic (Haynes, 1990). In addition, H^+ are extruded against an electrochemical potential gradient in order to maintain the cytoplasmic pH between 6.0 and 7.0 which is generally the optimum for most enzymes (Bowling, 1976). Hence plant growth tends to acidify the soil. However, the effect of these acidifying processes is offset by the uptake of anions which results in bicarbonate ions being excreted into the rhizosphere thus making it less acid (Nye and Tinker, 1977).

In natural ecosystems the death of the plant or part of it and its subsequent decay will allow the elements to return to the soil. However, studies show that acid soils have low decomposition rates and so the recycling process is slower under these conditions (Skeffington, 1987). In addition, when trees are removed for timber purposes or crops harvested this process of recycling does not occur and the soils remain acidic and nutrient deficient. This is particularly significant when fast growing species are cultivated.

The secretion of soluble organic acids from plants and from the litter layer can through their dissociation supply H^+ to the soil.

$$HnA_{(aq)} \longrightarrow A^{n-}_{(aq)} + nH^+_{(aq)} \qquad 2.7$$

In addition, organic acids can act as mobile anions in the leaching process (Skeffington, 1987).

The oxidation of NH_4^+ to nitrite is an important natural acid forming process (Kennedy, 1986).

$$NH_4^+ + {}^3/_2O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \qquad 2.8$$

A similar process is the oxidation of sulphur compounds catalyzed by aerobic autotrophs. This reaction occurs at alkaline pHs. The sulphide in the soil is formed largely by the breakdown of cysteine and methionine in organic matter by microbes.

$$S^{2-} + {}^1/_2O_2 + 2H^+ \longrightarrow S + H_2O \qquad 2.9$$

The oxidation of elemental sulphur to SO_4^{2-} also produces H^+. The reactions can be performed by organisms such as *Thiobacillus thiooxidans* and *T. ferroxidans* which grow in a pH range of 1 to 7 (Kennedy, 1986).

$$2S + O_2 + OH^- \longrightarrow S_2O_3^{2-} + H^+ \qquad 2.10$$

$$S_2O_3^{2-} + 2O_2 + OH^- \longrightarrow 2SO_4^{2-} + H^+ \qquad 2.11$$

Other natural H^+ producing processes include the mineralization of anions (reaction 2.12), oxidations (reaction 2.13), reverse weathering (reaction 2.14) and the weathering of anionic components (reaction 2.15) (van Breemen *et al*, 1984).

$$nHO + Rn\text{-}A(org) \longrightarrow nR\text{-}OH + A^n{}_{(aq)} + nH^+{}_{(aq)} \qquad 2.12$$

$$Red_{(aq,e,g)} + mO_2 + nH_2O \longrightarrow Ox^{z-}{}_{(aq,s,g)} + nH^+{}_{(aq)} \qquad 2.13$$

$$M^{n+}{}_{(aq)} + {}^1/_2nH_2O \longrightarrow {}^1/_2nM_{2/n}O_{(s)} + nH^+{}_{(aq)} \qquad 2.14$$

$$NO_{(m+n)\,(s)} + mH_2O \longrightarrow NO^{2m-}{}_{(2m+n)\,(aq)} + 2mH^+{}_{(aq)} \qquad 2.15$$

2.3.4 Increased leaching of cations through the soil profile as a result of acid rain

The "mobile anion hypothesis" states that as solutions must be electrically neutral, cations cannot be leached from the soil unless there is an anion to accompany them (Skeffington, 1987). This hypothesis can be expanded to explain the effects of acid rain on the soil. The concentrations of SO_4^{2-} and NO_3^- in the acid rain cause the concentration of these ions in the soil solution to rise. Consequently there must be an equal concentration of cations in the solution. These are usually H^+ from the acid rain. However the H^+ in the soil may exchange with the base cations

Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} (Abrahamsen, 1984). These cations then become incorporated into the soil solution and can be leached from the soil.

The cations are transferred through the soil in the soil solution together with the anions. The vegetation is then forced to survive under nutrient deficient conditions. Morrison (1984) and Reuss *et al*, (1987) review some of the experiments which have shown that as the acidity of the input increases so too does the loss of cations increase. Experimental results show that this effect can persist for many years after the last acid application (Tamm, 1989) implying that the rate of mineral replenishment in the soil is much slower than the rate at which the ions are leached from the soil.

This theory of increased cation leaching as a result of acid rain is opposed by Krug and Frink (1983). Their argument is based on results of leaching experiments which showed that acid soils are actually less susceptible to acidification than soils that are less acidic, since acid soils retain base cations more efficiently. As a result the H^{+} from the acid rain remains in solution, accompanying the anion through the soil. Thus it was hypothesized that as soils become acidified, the effect of the acid rain on the soil diminishes.

Krug and Frink (1983) also point out that acidic soils are rich in humus. This tends to retard the movement of organic anions through the soil. The reason for this is that humic materials act as weak acids, and their solubility in water is a function of pH: the more acidic the water percolating through the soil, the less humic material is dissolved in the soil solution and the greater the accumulation of these materials in the soil. Sulphate ions, unlike the organic anions, do not react strongly with humic particles. Thus the flux of SO_4^{2-} from the acid rain through the soil would increase, but the rate of organic anion movement would decrease. As a result there is little or no measurable change in the pH of the soil. In this way the soil can be efficiently

buffered.

In addition, due to the constant changing of the earth's surface caused by erosion, tree throw, frost heaving and soil creep, new and less weathered mineral material is constantly being exposed. Krug and Frink feel that although the amounts of soil moved are small, the resistance to acidification is large.

2.3.5 Increased concentrations of aluminium as a result of acid rain

Aluminium is a common element in the soil and can be found in a number of forms, which include an insoluble form, forms associated with organic solutes or forms complexed with iron (Driscoll *et al*, 1985). However, if the H^+ concentration increases considerably, besides replacing basic cations adsorbed to the soil particles, the H^+ can react with the clay crystals, become adsorbed internally and simultaneously release Al^{3+} (McLean, 1982). Thus, the strong acids found in the acid rain cause the inorganic Al^{3+} to be released from the soil particles and transform it into a soluble form. The soluble Al^{3+} will tend to displace basic cations, such as Ca^{2+}, Mg^{2+}, Na^+ and K^+, from their negatively-charged exchange sites, since ions of a higher valency are preferentially adsorbed (Reuss *et al*, 1987). This process only happens when the pH is less than 5.5 (Van Breemen *et al*, 1984).

The soluble Al^{3+} is also capable of being absorbed by the plant (Vogelmann, 1982). Although there are reports that low concentrations of Al^{3+} can be beneficial to plants (Foy, 1974b), this element is generally regarded as a highly toxic metal. The chemical form exerting the toxic effect is thought to be the trivalent form or $Al(OH)_2^+$.

Mild toxicity effects of Al^{3+} are exhibited in a lack of vigour in root growth and fine root branching. The symptoms of Al^{3+} toxicity are very similar to those of phosphorus and Ca deficiencies. Roots injured by excess Al^{3+} appear stubby and spatulate

with brown root tips (Foy, 1974b).

There are many ways in which Al^{3+} is thought to affect root growth. Firstly, it has been found that Al^{3+} can inhibit the rate of phosphate uptake and assimilation by the roots (Cumming and Weinstein, 1990b; Abrahamsen, 1984; Foy, 1974b). The Al^{3+} complexes with phosphate which results in a precipitate forming. Thus excess Al^{3+}, either in the soil or within the cell, would result in a reduction in the concentration of soluble phosphate (Foy, 1974b). Changes in phosphate concentration in turn affect the energy status of the cell due to its association in the high energy ATP molecule. Such a reduction in the ATP levels in corn have been recorded in response to Al^{3+} (Pfeffer *et al*, 1986). Numerous cellular activities would be affected by a decrease in ATP concentrations.

Aluminium can inhibit hexose phosphorylation shown by:

$$ATP + C_6H_{12}O \longrightarrow ADP + C_6H_{12}O\text{-}P \qquad 2.16$$

Thus Al^{3+} acts to decrease the rate of ATP utilization in glucose phosphorylation which is an important initial step of respiration (Foy, 1974b).

Aluminium ions have been found to interfere with calmodulin-stimulated ATPase activity. It is thought to change the surface hydrophobicity and helix content of calmodulin, so altering its regulatory properties (Siegel and Haug, 1983; Slaski, 1989).

Aluminium has been found to decrease the rate of Ca uptake. It has been suggested that Al^{3+} might block, neutralize and reverse the negative charge in the pores on the cell walls and on the phospholipid and protein portions of the plasmamembrane. This would reduce the ability of such pores to bind Ca, resulting in a Ca deficiency within the plant (Clarkson and Sanderson, 1971; Asp and Berggren, 1990). It follows from this hypothesis that excess Al^{3+} would increase anion binding by the free space and hence total anion uptake.

In addition, Al^{3+} can displace cations from their exchange sites on the plasmalemma. This would reduce the availability of nutrients which can be taken up by the plant (Koslowsky and Boerner, 1989; Asp *et al*, 1988; Bengtsson *et al*, 1988). Since Ca plays such an important role in maintaining cell wall structure, the displacement of Ca^{2+} by Al^{3+} in the apoplast could result in a decrease in root elongation (Godbold *et al*, 1988).

Aluminium has also been reported to alter the properties of biological membranes, possibly by affecting the architecture of the membrane lipids (Miyasaka *et al*, 1989).

It has been suggested that Al^{3+} may affect cross-linking between adjacent protein molecules and may decrease the extensibility of cell walls by cross-linking pectins in the middle lamella (Foy, 1974b). This cross-linking of polymers could also increase the rigidity of the DNA double helix, thus decreasing the rate of cell division in roots. This inhibition of root growth is often regarded as a visual symptom of Al^{3+} toxicity (Slaski, 1989). Abnormalities in cell division, found to be caused by excess Al^{3+} include the formation of "sticky chromosomes" manifested by anaphase bridges, which result in binucleate cells in the meristematic regions of the root (Levan, 1945).

Both the H^+ and Al^{3+} are transported downwards in the soil water through the soil profile, thus decreasing the water quality (Falkengren-Grerup, 1989). This has further implications, not only for the vegetation in the ecosystem, but also for humans and animals living in the ecosystem.

2.3.6 Positive effects of acid rain on the soil

The acid rain could have an ameliorating affect on the soil, this being to mobilize an extra source of base cations. It is thought that if the soil solution was very acidic, it might increase the rate of weathering of the minerals in the soil. In this way a source of previously unobtainable elements would become available to the vegetation (Skeffington, 1987).

2.4 UPTAKE AND TRANSLOCATION OF MINERAL NUTRIENTS

Each new cell in a plant requires nutrients for its expansion and healthy functioning. Although between 50-60% of the nutrients may be transferred to the growing region from older senescing parts of the plant (Miller, 1984) an increase in plant size generally requires that the plant obtain the appropriate quantities of nutrients from an external source.

2.4.1 Uptake of nutrients

The nutrient concentrations of the cells of the root generally tend to be much greater than the concentrations of the media in which they grow. In addition, the major cations and anions are generally not in electrochemical equilibrium across the plasmalemma (Epstein, 1972). Since substances do not diffuse against an electrochemical potential gradient, most minerals must be absorbed by active transport. Additional evidence for the process being active is the finding that uptake is dependent on temperature and oxygen, and can be suppressed by inhibitors (Epstein, 1972). High correlations exist between the activities of membrane-bound ATPases and ion absorption rates (Leonard and Hodges, 1973) which suggest that ATPases mediate energy transfer to the ion transport system. The respiration energy required for nutrient uptake is supplied by photosynthate transported from the shoot to the roots (Barber, 1979).

It is thought that "carrier" molecules embedded in the plasmamembrane, play an important role in transporting ions from one side of the selectively-permeable membrane to the other. The carrier is thought to be a subunit of the membrane which binds an ion at the external surface. The carrier-ion complex then undergoes a spatial rearrangement so that the ion is brought to the inner side of the membrane. At this stage the carrier-ion complex breaks down and the ion is released into the cell (Epstein, 1972).

The carrier molecules are thought to operate in a manner similar to enzyme molecules being dependent on the substrate concentra-

tion, or in this case the nutrient concentration in the solution, until the carriers become saturated (Nye and Tinker, 1977). Thus the uptake of nutrients is expected to follow Michaelis-Menten type kinetics and the flux, F, of ions into a root cell can be described as follows:

$$F = F_{max}\, C_l \,/\, (\, Km + C_l) \qquad 2.18$$

where F_{max} is the maximum rate of transport of the ion into the cell when all the available carrier sites are loaded, Km is the Michaelis-Menten constant equal to the substrate concentration giving half the maximal transport rate and reflects the affinity of the carrier sites for the ion, and C_l is the concentration of the ion in the liquid phase (Marschner, 1986).

Carriers can operate in one of three systems. A uniport system involves carriers transporting ions in one direction, e.g. H^+ pumps. The operation of these pumps will generate an electrochemical potential gradient (Marschner, 1986). Antiport systems involve a direct stoichiometric 1:1 coupling of an efflux and an influx carrier. The uptake of NO_3^- in exchange for hydroxide ions is thought to operate in this manner (Pitman, 1976a). In symport systems two ions of opposite charge are transported in the same direction. For example $H_2PO_4^-$ is taken up simultaneously with H^+ (Haynes, 1990).

The ability of the root to be selective of the ions which are absorbed is essential for the normal functioning of plants. Selectivity involves binding the right cation to the right site, and is based on the ion concentration in the soil solution and on the affinity of the carrier for the ion. This affinity of the carrier for the ion is dependent on the charge of the ion, the size of the ion and the geometry of both the molecule and the carrier (Clarkson and Hanson, 1980).

The plasmalemma of the cortical cells is generally considered to be the site of nutrient absorption (Barber, 1979).

2.5 CALCIUM

Calcium is an essential macronutrient which performs a number of functions in the cell. Calcium is in relatively low concentrations within the cytoplasm of the cell the majority being bound to the cell wall, membrane surfaces and to the salts of organic acids and inorganic anions, as well as being stored in membranous organelles within the cell (Kauss, 1987). A high cytoplasmic Ca concentration would be disadvantageous in that the excess Ca might hamper the functioning of Mg, as well as lowering the activity of phosphate by forming the insoluble $(Ca)_3(PO_4)_2$. It has been hypothesized that Ca functions as a signal metabolite in conjunction with calmodulin and the low Ca concentrations in the cytoplasm allow it to fulfil this function (Dieter, 1984; Poovaiah and Reddy, 1987).

2.5.1 Calcium uptake

ATPase pumps situated in the plasmalemma, which are stimulated by Mg, are responsible for the efflux of Ca^{2+} from the cell. Calcium ions are also pumped out of the cytoplasm, against a potential gradient, into the vacuole, endoplasmic reticulum, mitochondria and in the shoots into the chloroplasts (Kubowicz *et al*, 1982; Poovaiah and Reddy, 1987). A Ca^{2+}/H^+ antiport system, driven by an ATPase, is thought to exist on the membrane fractions whereby Ca^{2+} is pumped against its concentration gradient in exchange for two H^+ (Rasi-Caldogno *et al*, 1987; Poovaiah and Reddy, 1987). Thus a low cytoplasmic Ca concentration is maintained.

The low physiological mobility of Ca is reflected in low rates of accumulation, cell-to-cell transport and phloem transport. Consequently, the uptake of Ca from the soil and its translocation to the xylem for transport throughout the plant is restricted to the apoplastic pathway. However, the endodermis tissue with its Casparian band hinders transport via this pathway, hence Ca uptake and translocation is restricted to the apical part of the root where the Casparian strip has not yet developed. The rate of uptake of Ca then decreases to a very low

rate near the base of the root (Barber, 1979). The lack of mobility of Ca is reflected in the observation that Ca tends to accumulate in older plant tissue and is present in high concentrations in the litter (Miller, 1984).

2.5.2 Functions of calcium

In plants most of the Ca is found in the apoplastic compartment complexed with the cell wall moieties and the plasmamembrane. Calcium is an essential agent in stabilizing the pectin-protein complexes of the middle lamella (Demarty *et al*, 1984). In addition, Ca in the apoplast is important in maintaining membrane integrity as well as protecting the membrane against the deleterious effects of low pH, salinity and toxic ions (Poovaiah and Reddy, 1987).

Inside the cell, Ca is able to regulate a number of target enzymes and act as a messenger translating stimuli into various responses. The reason why Ca is able to mediate responses lies in the fact that it is found in such low concentrations in the cytoplasm. Thus a small change in the absolute amount of Ca in the cytosol can result in a 10 - 100 fold increase in concentration but the ionic environment of the cell remains unperturbed (Poovaiah and Reddy, 1987).

Calcium also plays a limited role as an enzymatic cofactor. Certain hydrolases (e.g. some α-amylases, phospholipases and nucleases) require Ca for activity. Calcium however appears to have a structural role rather than a catalytic one as it binds to sites other than the catalytic site (Clarkson and Hanson, 1980) thus stabilizing those higher molecular aggregates associated with the enzyme. Calcium may also be required by macromolecular substrates, such as starch, as a bridging complex.

It is thought that Ca may even be able to regulate gene expression. An increase in cytoplasmic and nucleoplasmic concentrations of free Ca^{2+} could result in Ca binding directly to chromosomal proteins and thus changing gene transcription. On the other hand,

increased concentrations of Ca^{2+} could activate Ca^{2+}-calmodulin dependent enzymes such as protein kinases or phosphatases. These enzymes would phosphorylate or dephosphorylate either trans-acting elements to alter transcription, or proteins involved in gene translation (Schibeci and Martoncsi, 1980; Poovaiah and Reddy, 1987).

2.5.3 Calcium deficiencies

The visual symptoms associated with a Ca deficiency are marginal chlorosis of the leaves, leaves rolling and curling, petiole collapse, flower abscission, ovul collapse and poorly developed seeds (Foy, 1974a). In addition, there can be a breakdown of meristematic tissue in stems and roots resulting in the death of these regions in acute cases. Roots can become poorly developed, lacking fibre and even appearing gelatinous (Noggle and Fritz, 1983). The symptoms of Ca deficiency tend to appear first near the growing points of the stems and roots which is probably related to the fact that Ca becomes immobilized in older tissue.

In addition, Ca deficiencies have been reported to disturb cell division resulting in binucleate cells (Foy, 1974a). Thus cell division has low but specific Ca requirements.

2.6 MAGNESIUM

Magnesium is also an important macronutrient. The cytosol is dominated by this element, together with K. At least 70% of this Mg is freely soluble and it is thought that Mg must have the highest chemical activity of any divalent cation in the cyto-plasm. Although there is such a relatively high concentration of Mg within the cell, it is thought that Mg^{2+} enters the cell passively. The reason for this is that the plant cell has a negative electrical charge in relation to its outer medium, so there exists a consistent force which draws cations into the cell (Mengel, 1974).

2.6.1 Uptake of magnesium

Magnesium uptake by excised roots is generally equal to or greater than Ca absorption (Hiatt and Leggett, 1974). The ATPase pumps situated on the plasmalemma which are responsible for the efflux of Ca^{2+} from the cell, also transport some Mg^{2+} out of the cell, however, the small ionic radius of Mg^{2+} compared to Ca^{2+}, the high hydration energy of Mg^{2+} compared to Ca^{2+} and the fact that the pump is at least partially dependent on calmodulin (Poovaiah and Reddy, 1987; Kauss, 1987) ensures that more Ca^{2+} is exported from the cell and Mg^{2+} remains the dominant element in the cytosol.

2.6.2 Functions of magnesium

Magnesium plays a number of important roles in the plant cell. Many enzymatic reactions are promoted by Mg: these include enzymes involved in phosphate transfer (phosphatases, kinases and ATPases) and those involved in the transfer of carboxyl groups (carboxylases and decarboxylases) (Clarkson and Hanson, 1980), as well as membrane bound ATPases (Leonard and Hodges, 1973). Magnesium may also activate an enzyme by binding to the enzyme at a site distinct from the substrate site. Magnesium is also required for ribosome integrity and for the structural stability of nucleic acids and membranes (Clarkson and Hanson, 1980).

Magnesium is also a constituent of the chlorophyll molecule and as such is an essential factor in ensuring that photosynthesis can occur (Noggle and Fritz, 1983).

2.6.3 Magnesium deficiencies

The visible symptoms of a Mg deficiency are mottled and chlorotic leaves. Severely affected leaves may wilt and shed or may just abscise without first wilting. The leaves also tend to become brittle and necrosis often occurs (Noggle and Fritz, 1983).

CHAPTER 3 SITE DESCRIPTION

The site that was chosen was situated on Long Tom Pass, in the Eastern Transvaal, between Lydenburg and Sabie, at approximately 25°19'S and 30°37'E. The altitude of the site is between 1750 and 1800 m above sea level. The site falls in the summer rainfall area (obtaining the majority of rain between November and March), and receives roughly 800mm of rain per year. The average temperature of the area on the pass is about 13.3°C. The site was located on the eastern slope of the mountain and had a gentle to flat gradient.

The site was located in a *Pinus patula* stand (compartment 29B) which was planted in 1982. At the time of the field work conducted in this investigation the trees were 9 years old. This was the second rotation of *P. patula* on this land. The trees had been planted 2.4m apart, and had been pruned when they were five years old. At present there are approximately 886 stems per hectare. The mean annual increment (MAI) at maturity is expected to be 14.

This site was chosen for a number of reasons: (i) since most tree damage overseas has been found to occur at high altitudes, a forested site in the Eastern Transvaal at a high altitude was sought, (ii) since *Pinus patula* is one of the most intensely cultivated species of the forestry industry in this area the investigation should determine the affects of acid rain on this species first, and (iii) air pollution monitoring and other studies are being conducted in this area, thus the findings of this investigation can be easily integrated with the other work.

CHAPTER 4 THE EFFECTS OF SIMULATED ACID RAIN ON THE RATE OF SOIL ACIDIFICATION AND CATION LEACHING

4.1 INTRODUCTION

Most of the plant's nutritional requirements are obtained from the soil. Consequently the vegetation can be affected by changes in either the ionic composition of the soil or the availability of nutrients in the soil. Soil acidification is thought to be able to evoke such changes in the soil system and it has been hypothesized that acid rain has the potential to increase the rate of soil acidification.

The process and consequences soil acidification, both natural and as a result of acid rain, are discussed in detail in Sections 2.3.3 to 2.3.6. Most importantly, acidic precipitation is a source of protons (H^+) which, on entering the soil system, result in a number of changes to the system. The H^+ can exchange for basic cations which are adsorbed to the negatively charged soil particles. This exchange lowers the base saturation of the soil. The acid rain also provides mobile anions (sulphate (SO_4^{2-}) and nitrate (NO_3^-)) which transfer the displaced base cations through the soil profile. This removal of cations from the rooting zone can have a negative effect on the vegetation.

The increase in the H^+ content of the soil also results in the mobilization of aluminium (Al^{3+}) and other toxic metals. The reason for this is that the level of soluble Al^{3+} in the soil is dependent on the pH of the soil solution. When the pH of the soil solution drops, Al-containing soil minerals are dissolved. This releases both Al^{3+} and hydroxide ions (OH^-). The latter consumes H^+, thus making the reaction dependent on the pH of the soil solution (Singer and Munns, 1987). The effects that increased amounts of Al^{3+} in the soil can have on the plants are discussed in Section 2.3.5.

These results of the soil acidification process can affect the

vegetation directly or they can act together to exacerbate the effects of each other. Singer and Munns (1987) explain that H^+, Al^{3+} or manganese will be more detrimental to a plant if the calcium (Ca) content of the soil is at levels that, although low, would still be sufficient in neutral soil. In addition, the rate of soil acidification is thought to be dependent on the soil composition and its present acidity (Krug and Frink, 1983).

It is possible that soils can be acidified by the addition of sulphuric acid (H_2SO_4) without an equivalent amount of cation leaching. This occurs on sulphate (SO_4^{2-}) absorbing soils: the SO_4^{2-} which is absorbed creates exchange sites capable of retaining incoming H^+ (Evans *et al*, 1981b). These soils can absorb SO_4^{2-} without loosing basic cations in the leachate.

The aim of this investigation was to determine whether realistic dosages of artificially acidified rain can alter the rate of leaching of both acidic (H^+ and Al^{3+}) and basic (Ca^{2+} and magnesium (Mg^{2+})) cations from the soil (Fig. 4.1.1). This would indicate the potential that acid rain has to alter the chemical characteristics of the soil. In addition, the potential use of calcium carbonate ($CaCO_3$) as a means to ameliorate any acidification processes was investigated.

The leaching of ions from only the top 250mm was monitored because this is where the fine roots that are generally involved in nutrient uptake are found. If the loss of nutrients occurs within the fine root zone then this has important consequences for the plants because the loss of Ca^{2+} and Mg^{2+} from this soil could result in these nutrients becoming limiting for normal plant growth. However if the leaching losses that are thought to be attributable to acidic precipitation occur below the fine roots, i.e. generally below the top 250mm, this is of little consequence to the plant. Any changes in the concentration of Al^{3+} in this top 250mm of soil also needs to be determined in that this toxic element can affect root growth.

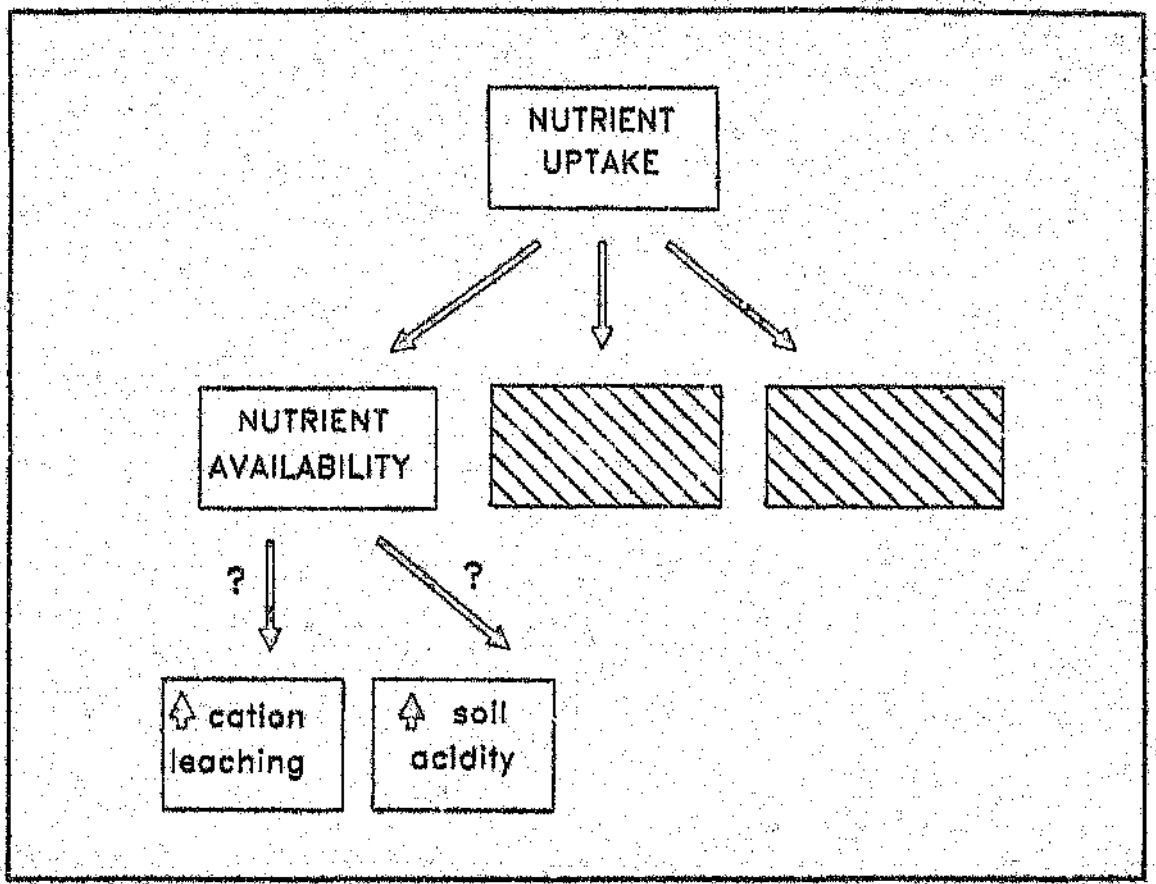

Fig. 4.1.1 A diagrammatic representation of the questions being addressed in this experiment.

4.2 MATERIALS AND METHODS

4.2.1 Method of collection of samples

Soil cores were collected in microlysimeters from the chosen site. A microlysimeter is a hollow stainless steel tube: 290mm long and 50mm diameter. The cores were taken between the trees, in the rows of the trees. Four cores were taken from around each tree at a distance of 0.7m from the tree stem.

The cores were extracted in the following manner. Firstly, the litter was cleared from the top of the soil, then a microlysimeter was knocked into the ground with a mallet. The microlysimeter was pulled out of the ground and a piece of filter paper (Whatman #42, 110mm diameter) was placed on the base of the tube and held in place with an elastic band. A piece of parafilm

was placed over the filter paper in order to protect it. The soil filled microlysimeters were then transported back to the University of the Witwatersrand.

4.2.2 Experimental design

The microlysimeters were set up according to Fig. 4.2.1, so that the simulated acid rain could leach through the soil cores. Filter paper was soaked in distilled water and the latter tested to determine what fraction of ions in the leachate had arrived there as a result of the presence of the filter paper. This fraction of ions from the filter paper was found to be negligible.

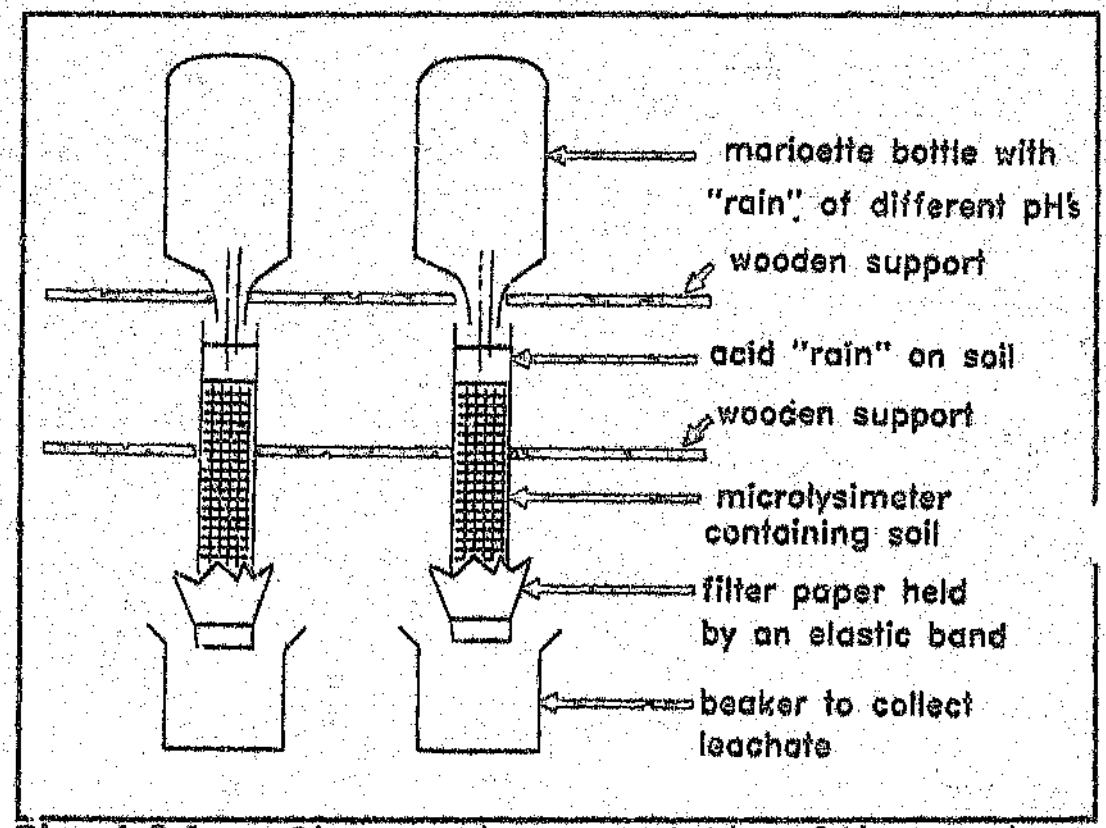

Fig. 4.2.1 Diagrammatic representation of the experimental design.

The acidity of the "rain" was varied in order to simulate different acid rain regimes. The artificial acid rain was made in the following manner. A stock solution of concentrated acid

was mixed so that the ratio of SO_4^{2-}:NO_3^- was 2:1. Other studies have determined that the ratio of SO_4^{2-}:NO_3^- in acid rain varies from between 1:1 and 3:1 in acid rain (Patil et al, 1989). Various amounts of this concentrated stock were added to distilled water to obtain the 4.22 pH value, which is the mean pH of the rain in Sabie. The H^+ concentration was calculated for this solution. A more acidic rain was obtained by doubling the H^+ concentration of the first solution and then setting the pH of this solution accordingly. The pH of this latter solution was 3.92. The control treatment had a pH of 5.60, which is the pH of rain that is in equilibrium with the CO_2 in the atmosphere when there is no pollution. Table 4.2.1 records the equivalent H^+ deposition rate of this artificial acid rain, as determined by titrating "rain" with sodium hydroxide (NaOH). It can be seen from Table 4.2.1 that the H^+ concentration of the solution with the lower pH 3.92) is not equal to an arithmetic doubling of the H^+ concentration of the solution with a pH of 4.22. This is because the H^+ concentration calculated by titration indicates the total amount of H^+ in solution i.e. the free H^+ as well as those associated with other ions, whereas the pH value indicates only the free, dissociated H^+ in the solution. Hence, in calculating the acidities of the different acid rain treatments in this experiment, the free H^+ concentration was doubled, but not the total H^+ concentration.

Each treatment had eight replicates.

Table 4.2.1 Mean deposition rate (± s.e.) of H^+ in artificial rain determined by titrating the "rain" with NaOH.

pH of "rain"	3.92	4.22	5.60
Equivalent H^+ deposition rate (kg ha^{-1} $year^{-1}$)	2.43 ± 0.16	1.66 ± 0.08	1.04 ± 0.04

The amount of rain that was intended to enter the tubes, simulated approximately 15 years of rain, where the rainfall was 1000 mm $year^{-1}$, (this is an approximation of the rainfall based on the

mean rainfall for the area). However, slow leaching rates due to a high degree of soil compaction, prevented this volume from entering all the cores.

An amount of 0.1g of $CaCO_3$ was added to some tubes in order to determine whether $CaCO_3$ could be used to ameliorate any changes that acidified rain may cause to the soil system. This amount of $CaCO_3$ was thought to be able to neutralize the incoming H^+ over the simulated 15 years of rain. This is approximately equivalent to a lime application of 500 kg h^{-1}.

Soil from five cores which were collected at the same time as those used in the leaching experiment, were analyzed at 50mm depth intervals in order to determine the composition of the soil at sampling. The following concentrations were determined: H^+ (or soil pH), Ca^{2+}, Mg^{2+}, potassium (K^+), Al^{3+} as well as the exchangeable acidity of the soil in those intervals.

At regular intervals through the experimental period the leachate was analyzed. The concentrations of the following ions were determined: H^+ (or pH of the leachate), Ca^{2+}, Mg^{2+}, Al^{3+} as well as the titratable acidity of the leachate. The cumulative loss of ions was recorded with respect to the volume of "rain" that had passed through the core for each acid treatment. A linear response was fitted using the SAS-Stats package, (SAS version 5.18). It was realised that the response was more likely to follow a Langmuir curve than a linear response, in that ions were not being supplied to the soil by weathering and decomposition and hence the rate of loss would probably decrease with time. This saturation point was not evident in the data that were collected and thus a linear response was fitted to the data.

At the end of the experiment, the soil was removed from the microlysimeters and analyzed to determine the pH of the soil, the exchangeable acidity, and the Al^{3+}, Mg^{2+}, Ca^{2+}, K^+ and Na^+ concentrations.

4.2.3 Methods of analysis

4.2.3.1 Determination of soil pH, exchangeable acidity and aluminium concentration

The soil pH and exchangeable acidity determinations are based on Buys (1980) and that of the aluminium determination on Thomas (1982). The soil was oven-dried and sieved through a 2mm sieve. A 5ml sample of this soil was scooped out and weighed. To this, 25ml 1M potassium chloride (KCl) was added and the mixture stirred for 10 minutes. The pH of the supernatant was read with a Beckman model 3500 digital pH meter. A further 25ml 1M KCl was added and the mixture stirred for another 10 minutes. The mixture was filtered, using Whatman #1, 90mm filter paper. A 10ml volume of filtrate was pipetted into an Erlenmeyer flask, to which 3 drops of phenolpthalien indicator were added. The sample was titrated with 0.001N NaOH using a magnetic stirrer until a faint pink endpoint was achieved. The milliequivalents (meq) KCl exchangeable acidity (EA) was calculated using the following equation:

$$EA = \frac{(V_{NaOH} - V_{blank})}{1000} \times N_{NaOH} \times \frac{V_{ext}}{V_{sub}} \times 10 \times \frac{1}{M_{soil}} \times 1000$$

where
- V_{NaOH} = volume of titrant used
- N_{NaOH} = normality of titrant (0.001N)
- V_{sub} = volume of subsample for titrating (10ml)
- V_{ext} = volume of extractant used (50ml)
- M_{soil} = mass of dry soil used (g)
- V_{blank} = volume of titrant used on 10ml of 1M KCl solution

After achieving the faint pink end point, 2ml 1N potassium fluoride were added to the solution, which was then titrated with 0.001N hydrochloric acid (HCl) until the pink colour disappeared. After 30 minutes additional HCl had to be added to obtain a clear endpoint. The aluminium concentration was calculated as follows:

$$\text{meq KCl exchangeable Al} = V_{HCl} \times N_{HCl} \times 100 \times \frac{V_{ext}}{V_{sub}} \times \frac{1}{M_{soil}}$$

where
- V_{HCl} = volume of titrant used
- N_{HCl} = normality of titrant (0.001N)

It has been assumed in this study that the valency of the aluminium ions is three, hence there are three equivalents for each Al molecule.

4.2.3.2 Determination of leachate pH

The pH of 10ml of leachate was measured with a Beckman model 3500 digital pH meter.

4.2.3.3 Determination of Ca^{2+}, Mg^{2+} and K^{+} in the soil

The cation extraction is based on Buys (1980). The cations were extracted from the soil in the following manner. A 5ml sample of oven-dried soil sieved through a 2mm sieve was scooped out, (and weighed), into a 100ml polyethylene centrifuge tube. To this 25ml 1M ammonium acetate (set at pH 7.0) was added and the tube was stoppered and shaken for 30 minutes. The mixture was then centrifuged for 10 minutes at 2500rpm in a Beckman model TJ 6 centrifuge, and the supernatant poured off. A further 25ml 1M ammonium acetate was added, the tube was again shaken for 30 minutes, then centrifuged for 10 minutes and the supernatant added to the previous extract. To 2ml of this sample, 10ml lanthanum solution (0.1% LaCl solution) and 8ml distilled water were added. The absorbances of the cations were read on a Varian Atomic Absorbance Spectrophotometer AA-275 (AAS) using the settings recorded in Table 4.2.2.

Table 4.2.2 Settings used for AAS in the determination of various cations.

Ion	Wavelength (nm)	Lampcurrent (mA)	Slit length (nm)
Ca^{2+}	422.7	3	0.2
Mg^{2+}	285.2	3	0.5
K^{+}	766.5	5	0.5
Na^{+}	589.0	5	0.2

4.2.3.4 Determination of Ca^{2+} and Mg^{2+} in the leachate

To 2ml of leachate sample, 10ml Lanthanum solution (0.1% LaCl solution) and 8ml distilled water were added. The absorbances of

the cations were read on the AAS.

4.2.3.5 Determination of Al in leachate

The method used was based on Thomas (1982). A 10ml sample of leachate was pipetted into an Erlenmeyer flask. To this 5 drops of phenolpthalien indicator were added. The solution was then titrated with 0.001M NaOH, until a faint pink endpoint was achieved. To this, 2ml 1N potassium fluoride were added, and the solution was titrated with 0.001N HCl until the pink colour disappeared. After 30 minutes additional HCl was added to achieve a clear endpoint. Aluminium concentration was calculated as follows:

meq KCl exchangeable Al per ml leachate = $V_{HCl} \times N_{HCl} \times 100 \times \frac{V_{ext}}{V_{sub}} \times \frac{1}{10}$

where V_{HCl} = volume of titrant used

N_{HCl} = normality of titrant (0.001N)

4.2.4 Statistical analysis of data

A homogeneity of slopes test was used to determine whether there was a significant difference in the rate of leaching of the various ions from the different treatments. One-way ANOVA was used to determine whether there was a significant difference in the amount of ions remaining in the soil after the different treatments. Both statistical analyses were performed on SAS/STAT release 6.03.

4.3 RESULTS

4.3.1 Initial soil analysis

The concentrations of various cations present in the top 250mm of soil was determined. The analysis was performed at 50mm intervals in order to ascertain the relative abundances of these ions through the top layers. Table 4.3.1 records the amounts of ions at the 50mm intervals in the surface layers of the soil. A diagrammatic representation of the relative abundances is shown in Fig. 4.3.1. The relationship between soil pH and the Al^{3+}

content of the soil is illustrated in Fig. 4.3.2. This is a power curve, a linear transformation of these data shown in the inset gives the following linear curve: log Al = 10.784 - 19.612log pH; r^2 = 0.986; F[1,23] = 1596.23; p < 0.0001.

Table 4.3.1 Initial ion concentrations present in the top 250mm of the soil at 50mm intervals through the soil core. Units are cmol (+) kg^{-1} ± s.e. except for pH (pH units ± s.e.) and percentage base saturation (B.S.) values (% ± s.e.). The final column shows the average concentration for the top 250mm.

depth / ion	0-50mm	50-100mm	100-150mm	150-200mm	200-250mm	Average
pH (in KCl)	4.23 ± 0.04	4.43 ± 0.04	4.59 ± 0.05	4.85 ± 0.04	5.07 ± 0.02	4.63
acid eq.	3.98 ± 0.72	2.09 ± 0.46	1.06 ± 0.29	0.30 ± 0.04	0.175 ± 0.01	1.52
Al^{3+} eq.	3.03 ± 0.43	1.45 ± 0.27	0.82 ± 0.21	0.20 ± 0.03	0.09 ± 0.01	1.12
Ca^{2+}	0.35 ± 0.12	0.06 ± 0.03	under range	under range	under range	0.08
Mg^{2+}	0.74 ± 0.06	0.71 ± 0.01	0.48 ± 0.05	0.34 ± 0.04	0.25 ± 0.03	0.50
K^{+}	1.93 ± 0.14	1.59 ± 0.07	1.15 ± 0.05	0.87 ± 0.04	0.70 ± 0.02	1.25
Na^{+}	0.08 ± 0.02	0.04 ± 0.004	0.01 ± 0.01	0.01 ± 0.003	0.02 ± 0.01	0.03
CEC	8.16 ± 1.14	5.19 ± 0.45	2.97 ± 0.25	1.77 ± 0.1[illegible]	1.20 ± 0.14	
% B.S.	7.09 ± 1.25	12.52 ± 2.05	23.11 ± 4.27	47.85 ± 3.63	74.52 ± 6.81	

4.3.2 Change in exchangeable acidity

Exchangeable acidity refers to the titratable acidity of the soil (or leachate), this being the total acidity of the soil as determined by titrating the extractable acid component of the soil with a strong base, namely NaOH. Thus this fraction includes H^{+} as well as other acidic cations such as Al^{3+}, and includes any organic acids that might be present. Any positive charge that can be neutralized with a base, in a typical acid-base reaction, is referred to as an acid equivalent.

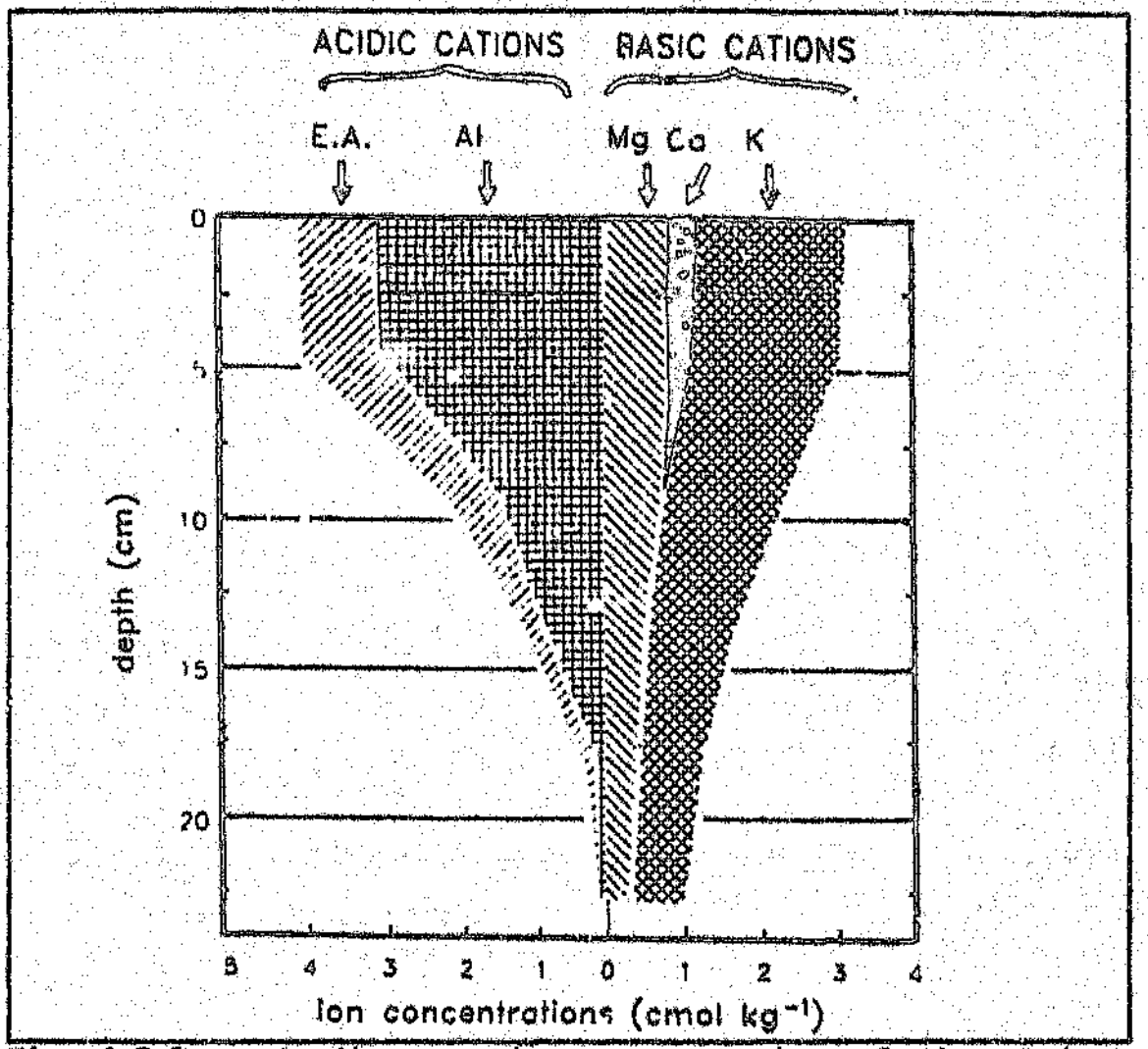

Fig. 4.3.1 A diagrammatic representation of the cations present in the top 250mm of soil, showing their relative proportions.

The most acidic "rain" (pH 3.92), resulted in the greatest amount of acid equivalents being leached from the soil (Fig. 4.3.3). There was no distinct difference in the cumulative loss of acid equivalents in response to acid "rain" of pH 5.60 and 4.22. The amount of acid equivalents lost when the "rain" had a pH of 3.92 was significantly different at the 95% confidence interval ($F[5,137] = 5.49$; $p < 0.0051$), compared with the amounts lost when the "rain" was less acidic. The addition of $CaCO_3$ further reduced the amounts of acid equivalents lost from the cores. Thus an increase in the acidity of the "rain" was found to increase the acidity of the leachate.

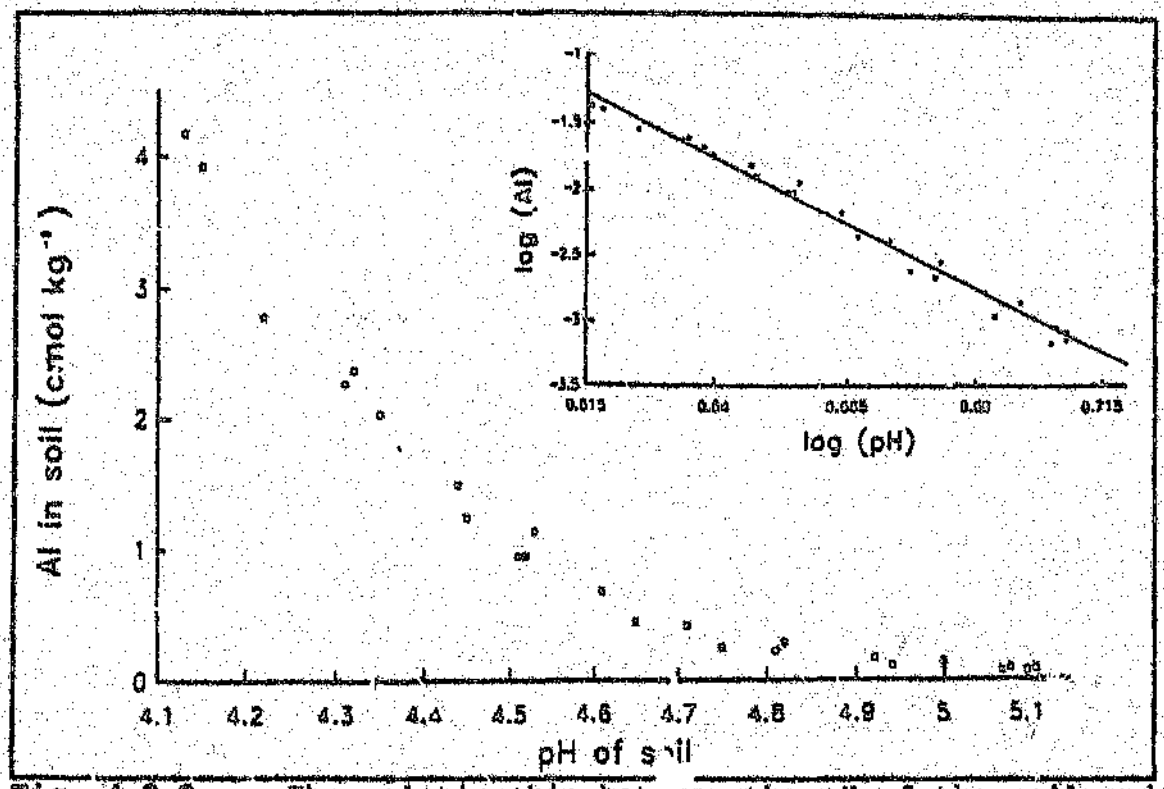

Fig. 4.3.2 The relationship between the pH of the soil and the Al^{3+} content of the soil, shows a power curve ($Al^{3+} = a\ (pH)^b$, where a and b are constants, b<0). The inset shows the linear transformation of these data.

The measurement of acid equivalents in the soil cores at the end of the leaching period did not however reflect this trend (Table 4.3.2), as no significant differences between the soil from the various treatments were found ($F[3,28] = 0.22$; n.s.).

4.3.3 Change in pH

It can be seen from Fig. 4.3.4 that as the acidity of the "rain" increased, the amount of H^+ found in the leachate also increased. The H^+ leached from the soil when the "rain" had a pH of 3.92 and 4.22 was significantly greater, at the 95% confidence interval, than when the "rain" had a pH of 5.60 ($F[5,139] = 7.97$; $p < 0.0005$). The addition of $CaCO_3$ further reduced the H^+ content of the leachate.

This variation in H^+ loss in response to the different acidic "rain" treatments was reflected in the soil data (Table 4.3.2), in that the soil that had been leached with "rain" of pH 5.60 had a higher pH (in KCl) than the soils leached with 'rain" of pH 4.22 or 3.92. These differences were not however significant at the 95% confidence interval ($F[3,28] = 1.43$; n.s.). The soil to which $CaCO_3$ had been added had the lowest pH, which was closest to the initial pH of the soil.

4.3.4 Change in exchangeable aluminium

The acidity of the rain did not appear to affect the rate of loss of acid equivalents arising from Al^{3+} (Fig. 4.3.5). The difference in rates of leaching were not significant at the 95% confidence interval in that the rates of loss were all very similar. However, the rate of loss of Al^{3+} from soil treated with "rain" of pH 4.22 and $CaCO_3$ was significantly lower than the rates of loss by "rain" of pH 3.92, pH 4.22 and pH 5.60, ($F[7;181] = 14.66$; $p < 0.001$).

The soil analysis indicates that the amount of exchangeable Al^{3+} was the least in the soil that had been leached with "rain" of pH 5.60. Although the soil leached with "rain" of pH 3.92 had a higher Al^{3+} content than the soil leached with "rain" of pH 4.22, it was still lower than the soil leached with "rain" of pH 4.22 when $CaCO_3$ had been added (Table 4.3.2). These trends were not however significant ($F[3,28] = 0.63$; n.s.).

4.3.5 Change in calcium

The amount of Ca^{2+} found in the leachate in response to various acidities of "rain" was indeterminable, except in the case when $CaCO_3$ had been added to the soil (Fig. 4.3.6). Similarly, the effect of the various acidities could not be measured in the soil (Table 4.3.2). This is due to the amounts of Ca^{2+} present in the soil being so low (Table 4.3.1), that the quantities that were found in the leachate were below the resolution of the AAS. The addition of $CaCO_3$ resulted in a significant increase in the amount of Ca^{2+} being lost in the leachate ($F[7;183] = 7.83$; $p <$

0.0001), as well as significantly greater amounts of Ca^{2+} being present in the soil at the end of the experiment (Table 4.3.2) ($F[3,28] = 5.14$; $p < 0.0059$).

4.3.6 Change in magnesium

The various acidities of the "rain" did not have a significant effect on the rate of leaching of Mg^{2+} ions from the soil ($F[5,139] = 0.91$; n.s.). However the most acidic "rain" (pH 3.92) did result in the most Mg^{2+} being lost (Fig. 4.3.7). The amounts of Mg^{2+} lost from the soil in response to the "rain" of pH 5.60 and 4.22 was similar. The presence of $CaCO_3$ significantly decreased the amount of Mg^{2+} leached from the soil at the 95% confidence interval ($F[7;183] = 5.09$; $p < 0.0021$).

Soil that was treated with "rain" of pH 5.60 and 4.22 with $CaCO_3$ had higher levels of Mg^{2+} compared with the soil leached with "rain" of pH 3,92 and 4.22 (Table 4.3.2). This trend was not significant at the 95% confidence interval ($F[3,28] = 0.39$; n.s.). In fact the amount of Mg^{2+} present in the soil leached with "rain" of pH 3.92 is greater than that in the soil leached with "rain" of pH 4.22.

4.3.7 Change in potassium

The loss of K^+ in the leachate was not monitored, however the soil analysis at the end of the experiment showed that K^+ had been lost from the soil leached with "rain" of pH 3.92. The K^+ levels in the soil leached with "rain" of pH 5.60 and 4.22 were greater than the initial values (Table 4.3.2). The difference in the amount of K^+ in the soil after being leached with "rain" of various acidities was not significantly different at the 95% confidence interval, ($F[3.26] = 1.85$; n.s.).

4.3.8 Change in sodium

The loss of Na^+ in the leachate was not monitored. The presence of Na^+ in the soil was small and varied quite considerably (Table 4.3.1). At the end of the leaching experiment no distinct trend in the amount of Na^+ present in the soil in response to the

different acid "rain" treatments could be observed.

Table 4.3.2 Ions (± s.e.) present in the soil after artificial rain of various acidities had been passed through the cores. Units are cmol kg^{-1} except for pH of the soil. Values within rows with the same superscript are not significantly different, while those with different superscripts are significantly different at the 95% confidence interval.

pH / ion	3.92	4.22	5.60	4.22 + $CaCO_3$
pH (in KCl)	4.70 ± 0.02 ^{a}	4.71 ± 0.02 ^{a}	4.77 ± 0.02 ^{a}	4.69 ± 0.05 ^{a}
exchangeable acid eq.	1.17 ± 0.09 ^{a}	1.17 ± 0.11 ^{a}	1.08 ± 0.11 ^{a}	1.27 ± 0.25 ^{a}
Al^{3+} eq.	0.91 ± 0.08 ^{a}	0.84 ± 0.08 ^{a}	0.80 ± 0.06 ^{a}	1.05 ± 0.22 ^{a}
Ca^{2+}	under range	under range	0.70 ± 0.17 ^{a}	0.80 ± 0.31 ^{b}
Mg^{2+}	0.49 ± 0.02 ^{a}	0.45 ± 0.06 ^{a}	0.51 ± 0.05 ^{a}	0.51 ± 0.03 ^{a}
K^+	1.60 ± 0.15 ^{a}	2.72 ± 0.60 ^{a}	2.67 ± 0.38 ^{a}	2.10 ± 0.13 ^{a}
Na^+	0.13 ± 0.01 ^{a}	0.010 ± 0.02 ^{a}	0.14 ± 0.03 ^{a}	0.11 ± 0.03 ^{b}

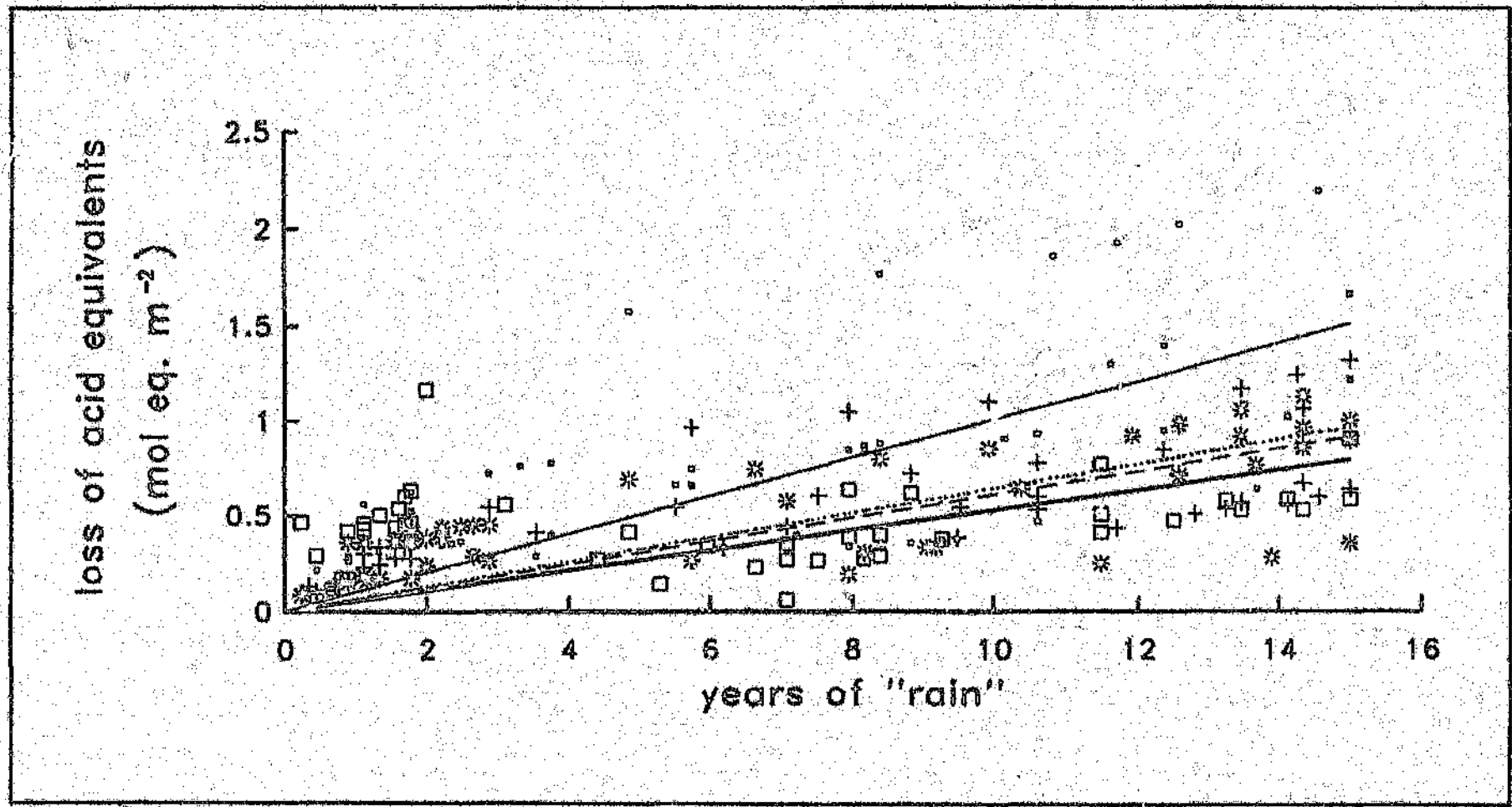

Fig. 4.3.3 Loss of acid equivalents from soil cores in the leachate after various simulated acid "rain" regimes. (Individual symbols are direct measurements from the cores; lines show the best fit regressions.)

Symbols are: ◦ pH 3.92; + pH 4.22; ✻ pH 5.60; □ pH 4.22 + $CaCO_3$
Equations for linear regressions through data points: —— pH 3.92: y=1.00E-1.x; r^2=0.813; p<0.0001; ..... pH 4.22: y=6.39E-2.x; r^2=0.858; p<0.0001; — — pH 5.60: y=6.05E-2.x; r^2=0.830; p<0.0001; —— pH 4.22+$CaCO_3$: y=5.24E-2.x; r^2=0.748; p<0.0001

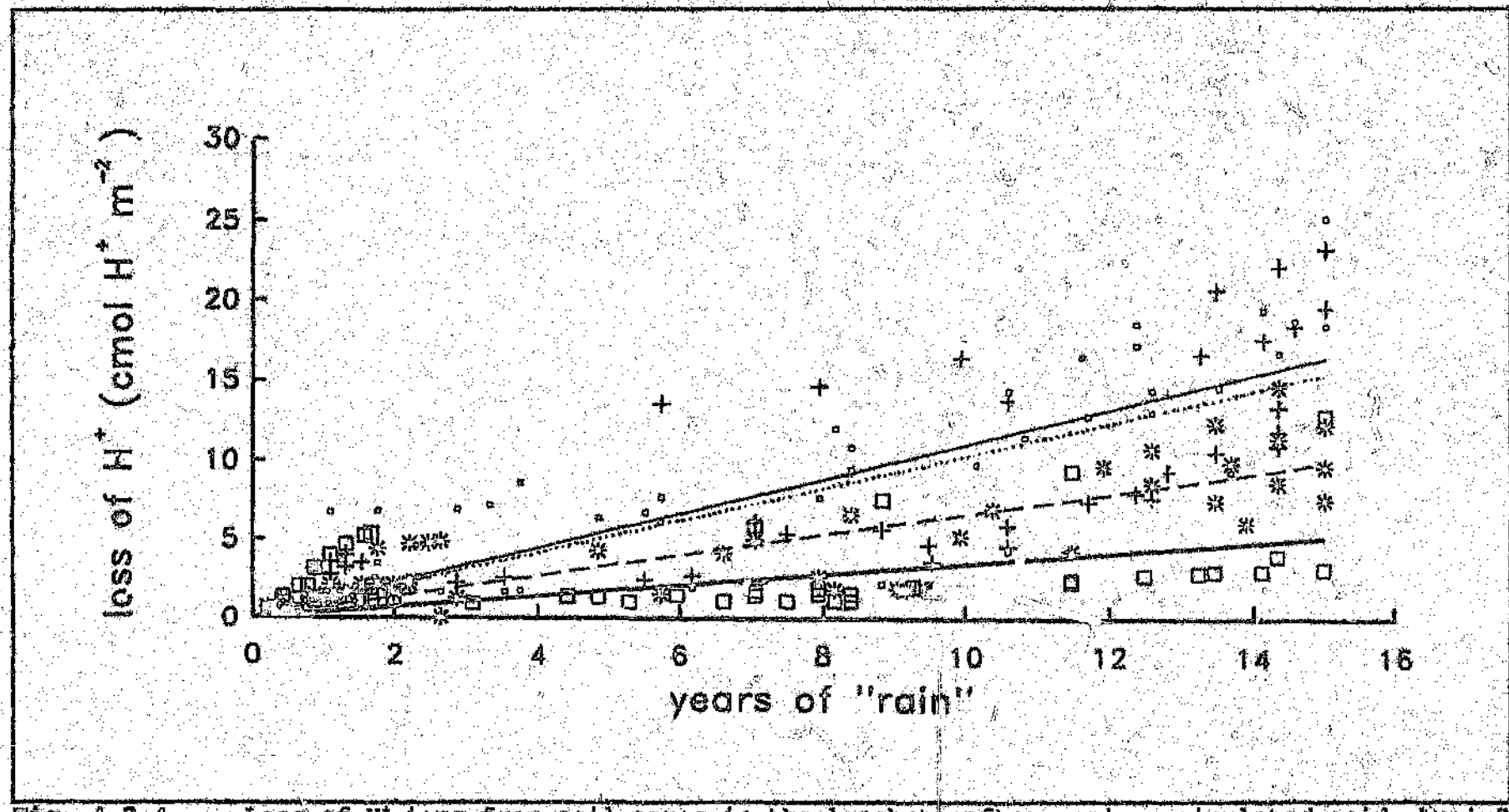

Fig. 4.3.4 Loss of H^+ ions from soil cores in the leachate after various simulated acid "rain" regimes. (Individual symbols indicate direct measurements from the cores; lines show best fit regressions.)

Symbols are: . pH 3.92; + pH 4.22; * pH 5.60; □ pH 4.22 + $CaCO_3$
Equations for linear regressions through data points: —— pH 3.92: $y=1.10.x$; $r^2=0.868$; $p<0.0001$; ····· pH 4.22: $y=1.03.x$; $r^2=0.856$; $p<0.0001$; — — pH 5.60: $y=0.659.x$; $r^2=0.877$; $p<0.0001$; —— pH 4.22+$CaCO_3$: $y=0.342.x$; $r^2=0.552$; $p<0.0001$

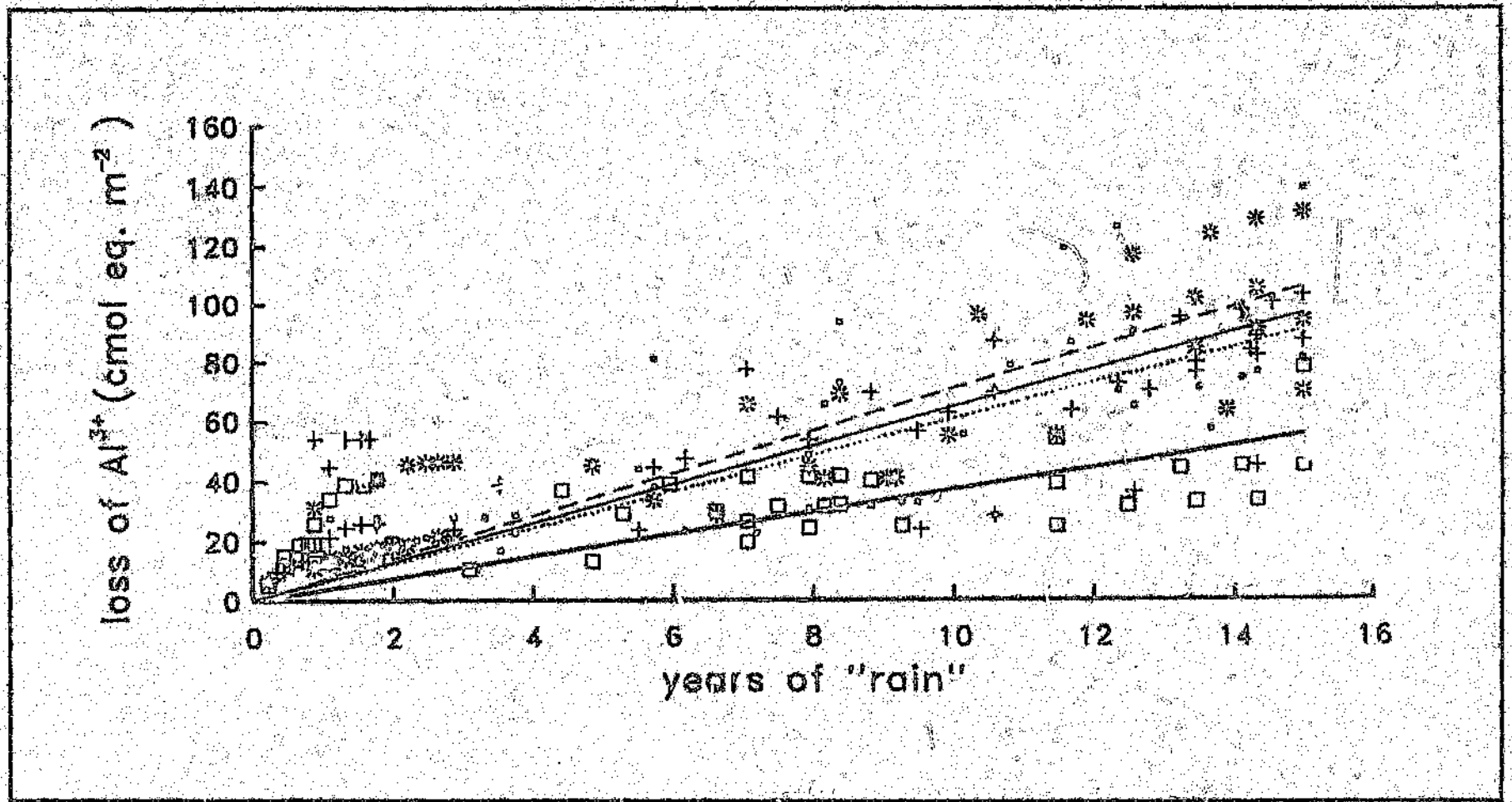

Fig. 4.3.5 Loss of Al^{3+} ions from soil cores in the leachate after various simulated acid "rain" regimes. (Individual symbols indicate direct measurements from the cores; lines show best fit regressions.)

Symbols are: ◦ pH 3.92; + pH 4.22; ✲ pH 5.60; □ pH 4.22 + $CaCO_3$
Equations for linear regressions through data points: —— pH 3.92: $y=6.43.x$; $r^2=0.884$; $p<0.0001$; ····· pH 4.22: $y=6.05.x$; $r^2=0.911$; $p<0.0001$; – – pH 5.60: $y=7.04.x$; $r^2=0.923$; $p<0.0001$; —— pH 4.22+$CaCO_3$: $y=3.68.x$; $r^2=0.787$; $p<0.0001$

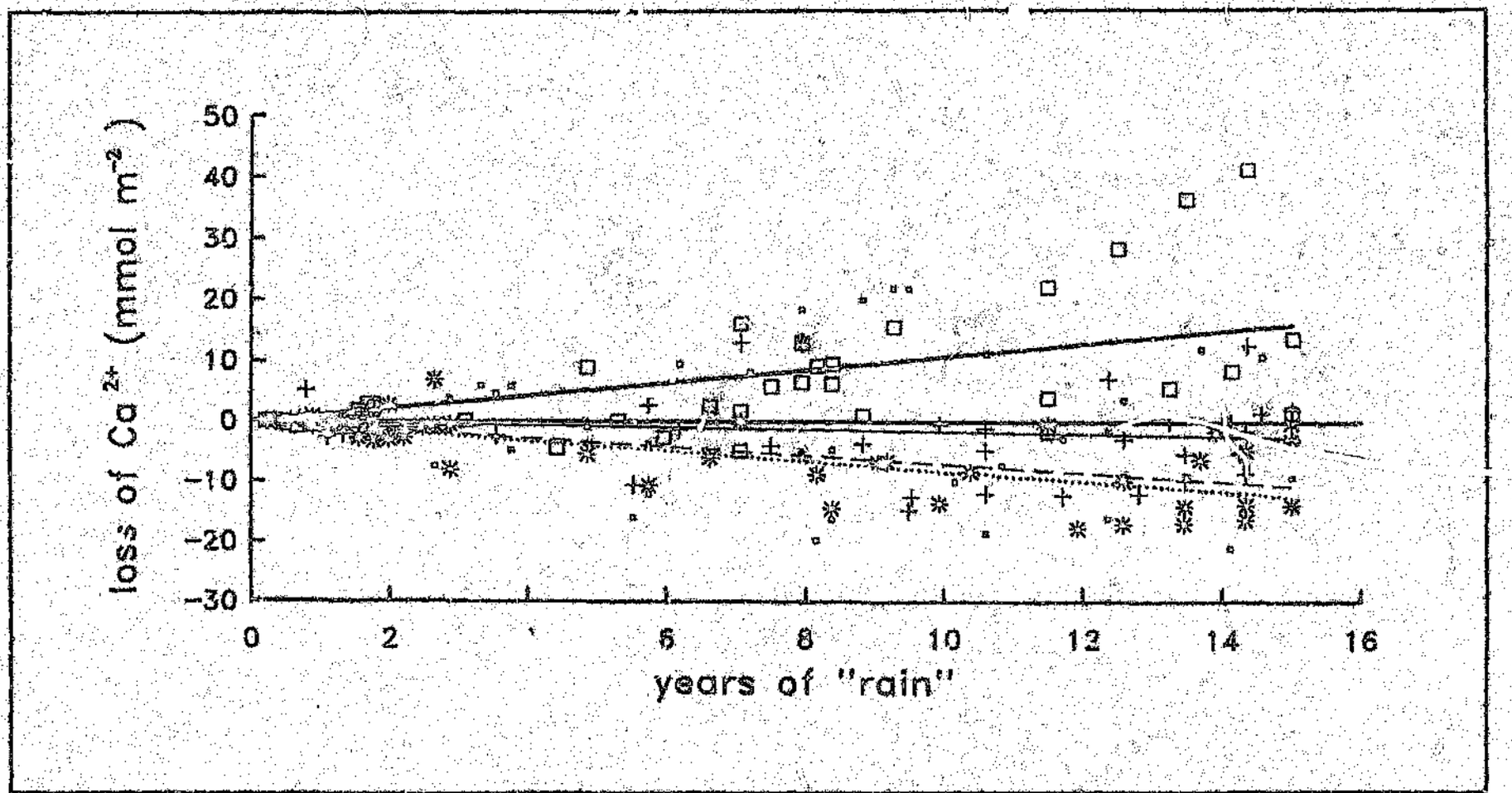

Fig. 4.3.6 Loss of Ca^{2+} ions from soil cores in the leachate after various simulated acid "rain" regimes. (Individual symbols indicate direct measurements from cores; lines show the best fit regressions.)

Symbols are: ◦ pH 3.92; + pH 4.22; ✳ pH 5.60; □ pH 4.22 + $CaCO_3$
Equations for linear regressions through data points: —— pH 3.92: $y=-0.178.x$; $r^2=0.023$; n.s; ····· pH 4.22: $y=-6.830.x$; $r^2=0.124$; $p<0.0153$; — — pH 5.60: $y=-0.717.x$; $r^2=0.571$; $p<0.0001$; ——— pH 4.22+$CaCO_3$: $y=1\ 07.x$; $r^2=0.518$; $p<0.0001$

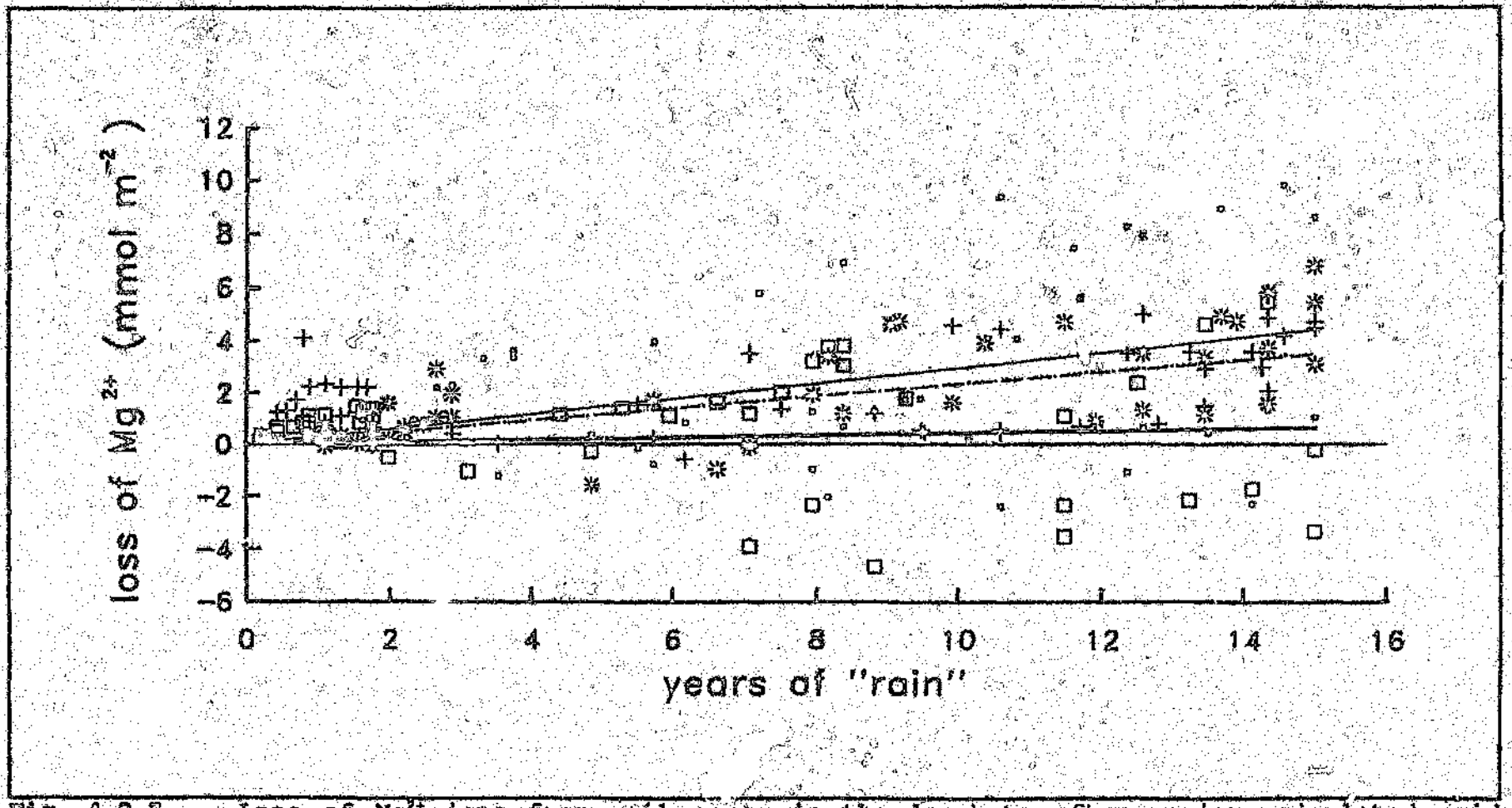

Fig. 4.3 7 Loss of Mg^{2+} ions from soil cores in the leachate after various simulated acid "rain" regimes. (Individual symbols indicate direct measurements from soil cores; lines show best fit regressions.)

Symbols are: ◦ pH 3.92; + pH 4.22; ✲ pH 5.60; □ pH 4.22 + $CaCO_3$
Equations for linear regressions through data points: —— pH 3.92: $y=0.295.x$; $r^2=0.426$; $p<0.0001$; ····· pH 4.22: $y=0.236.x$; $r^2=0.691$; $p<0.0001$; — — pH 5.60: $y=0.233.x$; $r^2=0.473$; $p<0.0001$; —— pH 4.22+C $y=0.043.x$; $r^2=0.023$; n.s.

4.4 DISCUSSION

4.4.1 Method

The method involved the use of undisturbed soil cores to determine the effects of leaching of acidified artificial rain on the soil, as apposed to soil cores that have been repacked after their extraction as has been done in similar experiments (Krug and Isaacson, 1984). It was felt that undisturbed cores gave a better representation of the soil system, in that the flow rates of the "rain" varied for different cores depending on the compaction of the soil in the core. This is similar to the way rain follows certain channels and courses in the soil, when such exist, and only seeps uniformly through the soil when it is homogeneously compressed. As a result, it is thought that when the water passes quickly through the cores few exchanges occur between ions in the soil solution and those on the soil particles, however, when the flow rate is slow, more exchange reactions occur. Consequently for the soil analysis at the end of the experiment which determined the change in soil composition as a result of the leaching, the amount of ions present in each core for each treatment was meaned, irrespective of the volume of "rain" that had passed through the core.

4.4.2 Initial analysis of top 250mm of soil

The initial soil analysis showed the soils to be naturally acidic with a low nutrient status (Table 4.3.1). The average pH (in KCl) of the soil in the top 250mm was 4.64 ± 0.06 (mean ± s.e.). Meiwes *et al*, (1986) points out that when the soil pH drops to below 5 chemical changes can occur to the soil which alter its capability to retain or release ions in the soil. This has important consequences for vegetation growing on this soil.

It is generally thought that in forest soils, Mg^{2+} is less readily available than Ca^{2+} (Lutz and Chandler, 1946). However this was not the situation in these soils: there was far less Ca^{2+} present compared with the amount of Mg^{2+} present. Below the first 100mm, Ca^{2+} concentrations were so low that they were below the resolution of the AAS (Table 4.3.1). It has been found that

forest soils which are acidic often tend to be deficient in Ca^{2+} (Lutz and Chandler, 1946).

Calcium is an essential macronutrient found in relatively low concentrations within the cytoplasm of plant cells, the bulk of it being stored either in membranous organelles, or being bound to the cell wall, membrane surfaces or the salts of organic acids and inorganic anions. It is known to play a number of important roles in normal cell functioning: these are discussed in detail in Section 2.5.2. Should the low Ca^{2+} concentrations in the soil result in low concentrations within the plant, this could affect the functioning of the cells which would have a negative effect on plant growth.

Low Ca^{2+} concentrations in the soil could have a negative effect on nutrient cycling. A disruption in the cycling of nutrients back into the soil could result in nutrient deficiencies which could be detrimental to the plant. The reason for this is that Ca^{2+} appears to be particularly influential in maintaining bacterial populations, which in turn are important in ensuring rapid decomposition of organic material. Lutz and Chandler (1946) record 10-20 times more bacteria in a calcareous soil than a noncalcareous soil. Hence a positive feedback would occur in that the low concentrations of Ca^{2+} would hinder the development of bacteria which would decrease decomposition rates and further limit Ca^{2+} in the soil. Nevertheless these authors conclude that an inadequate supply of Ca^{2+} in the soil is generally the result of a deficiency in the element rather than a low availability.

The acidity of the soil and the amounts of Al^{3+} present were relatively high. In the top 250mm the Al^{3+} content of the soil decreases as the depth and the pH of the soil increase (Table 4.3.1). The importance of this result lies in the finding that most of the Al^{3+} is found in the top layers where the soil is composed largely of organic, decomposing litter. The trend of increasing Al^{3+} content with decreasing pH is quite universal (Driscoll *et al*, 1985; Singer and Munns, 1987). However other

studies have found that Al^{3+} concentrations increase with soil depth (Jentschke *et al*, 1991b). In the soils that were examined the high acidity in the top layers which results from the acidic litter, is thought to be responsible for solubilizing Al^{3+}. Consequently as the acidity decreases with depth so too does the Al^{3+} content decrease.

Most of the Al^{3+} is present in the top 100mm where the Ca^{2+} is generally found. This could affect the uptake of Ca^{2+} by the plant in that Al^{3+} has been found to inhibit the uptake of Ca^{2+} by plants growing in acid culture solutions (pH 4.0-4.2) (Clarkson and Sanderson, 1971). It is thought that the Al^{3+} might neutralize the negative charge on the pores of the cell and thus prevent the movement of Ca^{2+} into the cells of the root.

Aluminium injury tends to occur more frequently when the molecular ratio of Ca:Al in the soil solution of forest soils is one or less (Abrahamsen, 1984). However Roelofs *et al*, (1985) in their work on *Pinus nigra* claim that the Ca:Al ratio can be as low as 0.2 before root damage is likely to occur. In the soil that was examined, the ratio of Ca:Al was approximately 0.22 (see Appendix 8.2 for calculation). This would imply that Al^{3+} injury to the roots can occur on these soils.

In addition it has been found that as the amount of Al^{3+} in the soil increases, the outward movement of Ca^{2+} across the plasmalemma decreases (Clarkson and Sanderson, 1971). Hence the amount of Ca^{2+} in the cells could accumulate, which in turn may have a negative affect on the plant because the functioning of Ca^{2+} as a signal metabolite is dependent on it being found in the cell at low concentrations (Poovaiah and Reddy, 1987). In addition, Ca^{2+} accumulation can inhibit the movement and transport of Mg^{2+} to the rest of the plant. Magnesium is required as an enzyme cofactor, for ribosome integrity and for chlorophyll synthesis. It also plays a role in maintaining the structural stability of nucleic acids and membranes. A high Ca^{2+} concentration in the cell can lower the activity of phosphate by forming the insoluble

compound $Ca_3(PO_4)_2$, thus inhibiting phosphate-based energy metabolism (Poovaiah and Reddy, 1987).

A number of parameters have been defined by Meiwes *et al*, (1986) which can be used to determine the stability and resilience of the ecosystem against soil acidification. If a system has a low resilience then a small stress, such as mild acid deposition, can cause significant changes to the ecosystem. The ratio of Ca:Al is a good indication of the resilience and stability of the soil. As increasing amounts of Ca^{2+} are leached from the soil and the Al^{3+} content of the soil increases this ratio decreases. In this study this ratio was determined to be 0.22 which is very low.

The pH of the soil can indicate its H^+ buffering capacity. The pH of the top 250mm of soil from the site on Long Tom Pass varied between 4.23 and 5.07. This, according to both Khanna and Ulrich (1984) and Meiwes *et al*, (1986), puts the soil into the "cation exchange buffer" range. In this category, during acidification H^+ become consumed in the clay lattices and in so doing release Al^{3+}. The base cations are exchanged for both the Al^{3+} and the incoming H^+, and are removed from the soil system. With time, most of the exchange sites become occupied by Al^{3+}. The ratio of exchangeable Al^{3+} to exchangeable cations is thought to vary in such soils from 0.2 to 0.9 (Khanna and Ulrich, 1984). As the amount of Al^{3+} on the exchange sites increases, this ratio will increase. In the soils that were investigated this ratio was determined to be 0.14 (see Appendix 8.2 for calculation). Hence a large proportion of the exchange sites in this soil are still occupied by cations other than Al^{3+}.

The cation exchange capacity (CEC) of the soil can also be used to describe the soils ability to buffer the acidification process. Generally CEC's have been found to vary from 2 to 60 meq $100g^{-1}$ depending on the humus and clay content of the soil (Brady, 1984). Factors which affect the CEC are the texture of the soil as finely textured soils tend to have a higher CEC than sandy soils. The organic matter content and the amount and kind

of clay present influence the CEC. A soil with a low CEC is thought to have a low buffering capacity and consequently a low resilience to acidification. The CEC of the soil examined was 3.857 meq $100g^{-1}$. According to Miewes *et al*, (1986) soils with a CEC of less than 0.5 meq $100g^{-1}$ are thought to have a very low resilience to acidification. This would mean that the soil that was examined does have some buffering capacity to protect it from further acidification. However, according to Morrison (1984) soils with a CEC of less than 6.2 meq $100g^{-1}$ are sensitive to acidification, which would make the soils from Long Tom Pass sensitive to acidification. Although conflicting, these suggestions indicate that the acidification process in the soil that was examined is not complete and although the soil can still buffer changes caused by acidification, this buffering capacity is limited and will require relatively little acid input to completely acidify the soil.

4.4.3 Soil acidification as a result of artificial acid rain deposition

Figure 4.3.4 shows that an increase in the acidity of the rain will increase the amount of H^+ lost in the leachate. This occurs because the H^+ inputs into the soil are so large that the neutralization of the acid input is incomplete. Only partial neutralization can occur as a result of a number of factors: firstly, a low concentration of soil-bound basic cations present in the soil would result in a low probability that these ions will be exchanged. Consequently only small amounts of H^+ will be removed from the solution.

Secondly, the dissolution kinetics of these cations might be very slow, or the retention time of water within the mineral soil may be exceedingly short (Van Breemen *et al*, 1984). Both of these latter factors are related to time, in that the water is passing through the soil pores so rapidly that the exchange reactions are not reaching equilibrium. When the exchange reactions do not near completion, the acidity of the simulated acid rain will not be neutralized.

All of these aspects are likely to have contributed to the leachate becoming acidified in the soil that was examined. The acidification of the leachate has important consequences to catchments in the area, since as the leachate becomes more acidic, the groundwater and runoff will acidify.

As the amount of H^+ entering the soil increases, the expected tendency is for the soil to acidify. Table 4.3.2 shows that soil leached with "rain" of pH 5.60 had a higher pH (in KCl) compared with the soils leached with "rain" of pH 3.92 and 4.22. However, there was little difference between these latter two treatments. The soil that had $CaCO_3$ added to it had the lowest pH, which was closest to the initial pH of the soil (Table 4.3.1). It is possible that the $CaCO_3$ served to preserve the initial status of the soil by neutralizing the acid before it entered the soil. The trends in the soil pH were not however significant. It can be speculated that if the time period were longer, these trends might become significant.

Other studies have indicated that soils with a higher pH acidified to a greater extent than soils that were already acid (Falkengren-Grerup, 1989; Schulze, 198). Krug and Frink (1983), suggest a reason for these results. If the base cation content of the soil is low, as was the situation in the soils that were examined in this study, then the ability of the H^+ to exchange with base cations is low due to the scarcity of the base cations. This results in the base cations being efficiently held. They also suggest that the humic materials which tend to accumulate in acidic soils, repel SO_4^{2-}. These two factors would result in the SO_4^{2-} moving through the soil with H^+ generally acting as the accompanying cation, with little change to the pH of the soil. This could explain the lack of response in the pH of the soil that was examined to the different acidities of the "rain".

Another possible reason why the changes in soil H^+ concentration are so small is that the pH scale, being logarithmic and at the lower end of the scale, requires large changes in concentration

before a change in pH is registered.

It is interesting to note that the pH of the soil is greater after the 15 years of simulated acid rain than before the treatments. It should be noted that this experiment monitored soil acidification in response to only one of the factors which can contribute to the acidity of the soil. Other natural inputs of acids such as the decomposition of litter and organic matter, respiration by living organisms and the uptake of ions by plants have been ignored. The cumulative effect of these other acidifying processes, together with the acid rain, would probably result in the soil becoming more acidic during the time period examined.

The total acidity, which includes all the acidic components (i.e. H^+, Al^{3+}, organic acids etc.) of the leachate, was significantly greater when the "rain" was most acidic (Fig. 4.3.3) compared with the amount lost when the pH of the "rain" was 5.60 and 4.22. There was very little difference between the latter treatments. The exchangeable acidity of the soil at the end of the leaching showed no distinct trend with treatment (Table 4.3.2). The soil leached with "rain" of pH 4.22 that had $CaCO_3$ added to it had the highest acid content which was the most similar to the average initial acid content of the soil (Table 4.3.1).

As with the pH of the soil, the exchangeable acidity is less after the 15 years of simulated acid rain than it was before. Again it can be speculated that natural inputs of acidity (mentioned above) together with the acid rain would result in an increase in the acidity of soil over the time period examined.

It is evident from these results that acid rain has the potential to alter the acidity of the soil. This can have consequences on the vegetation growing on these soils in that uptake of ions which are dependent on H^+ extrusion, e.g. NH_4^+, may be affected. If the pH gradient between the external medium and the cells of the root increases, the extrusion of H^+ from the root would be more difficult in that the gradient against which the H^+ are

being pumped would be much greater. Studies have indicated that the uptake of Ca^{2+} and other cations, can be markedly inhibited by H^+ in the pH range from 3 to 5 (Maas, 1969). Similarly the extrusion of excess H^+ produced in normal cell metabolism in the cell, might become difficult or be prevented if strong acids are present in the microenvironment of the cell and root (Nihlgård, 1985). The reason for this is that the gradient against which the H^+ are being pumped is greater and the rate of H^+ movement from the soil, down this gradient, into the root will increase, thus making the maintenance of the cytoplasmic pH more difficult. An accumulation of these ions in the cytoplasm might be toxic to the plant, and thus negatively affect the growth of the plant.

One of the most important sources of nutrients in the soil is from the decomposition of organic debris that reaches the forest floor by litter fall and from decaying dead roots and soil fauna. In most forest soils there is a rapid decomposition of organic matter which results in a rapid turnover of the nutrients in the soil. Studies which have compared the decomposition rates of plant material in acid soils with those in less acid soils, have generally shown that acid soils have lower decomposition rates (Skeffington, 1987). In addition coniferous vegetation produces acidic litter which is harder to decompose than less acidic litter (Gosz, 1984). It is thought that the low base content of the litter is responsible for this. The low base content results in proteins being complexed by polyphenols in the litter. These complexed proteins are more difficult to decompose.

Bacteria play an important role in the decomposition process and high soil acidities are generally unfavourable for bacterial populations (Lutz and Chandler, 1946). Hence an increase in soil acidity could have a negative effect on the cycling of nutrients in the soil. Fungi, unlike bacteria, are more abundant in acid soils (Lutz and Chandler, 1946). One of the functions of fungi is to decompose cellulose and related compounds. As a consequence, an increase in soil acidity might increase the rate of decomposition of cellulose and related compounds. However the

fats and waxes associated particularly with the cuticle, as well as hemicelluloses and lignins, would accumulate in the soil. The metabolites of fungal growth would increase the acidity of the soil still further (Gosz, 1984).

The acidity of the rain did not significantly influence the rate of leaching of acid equivalents arising from Al^{3+} in the soil as measured in the leachate (Fig. 4.3.5). This can be due to Al^{3+} in the soil being adsorbed to the soil particles more tightly than other cations as a result of its high valency.

On the other hand, Krug and Isaacson (1984) found that the leaching of Al^{3+} from acid, organic-rich soils in response to varying acidities of the rain increased together with the amount of organic matter in the leachate. The conclusion drawn from this finding is that the leaching of Al^{3+} is dependent on the solubility of the organic matter (Krug and Isaacson, 1984). With respect to the soils that were examined in the present study, if the organic matter was not soluble in the soil solution, this would explain the finding that the amount of Al^{3+} in the leachate had not varied in response to the acidity of the "rain".

The soil analysis shows that after 15 years of simulated rain, the least acid equivalents arising from Al^{3+} were present in the soil that had been leached with rain of pH 5.60, with increasing amounts present in soil leached with the more acidic rain (pH 4.22 and 3.92). This is probably due to the Al^{3+} becoming insoluble at pH's greater than 5.5 (Van Breemen *et al*, 1984). Hence some of the Al^{3+} in the cores leached with rain of pH 5.60 would have precipitated and not been extracted in the soil analysis. Although this trend was not significant - again, if the time period was increased, the differences might become significant.

The addition of $CaCO_3$ maintained a high Al^{3+} content in the soil (Table 4.3.2), probably as a result of the $CaCO_3$ neutralizing the acidity of the "rain" before it entered the soil, consequently

little Al^{3+} was leached out of the soil (Fig. 4.3.5). An increase in the Al^{3+} content of the soil could have a number of negative effects on plant growth (Levan, 1945; Clarkson and Sanderson, 1971; Abrahamsen, 1984; Nihlgård, 1985).

4.4.4 The effect of acid rain on major cations

From the initial soil analyses (Table 4.3.1) it was determined that there is very little Ca^{2+} present in the soil and in addition, practically all of the Ca^{2+} is found in the top 100mm of the soil. As a consequence the amount of Ca^{2+} in the leachate that was leached from the soil was practically indeterminable (Fig. 4.3.6), and the effect of varying the acidities of the rain could not be observed in the soil (Table 4.3.2).

The fact that the amount of Ca^{2+} in the top 250mm is so small and is found almost exclusively in the top 100mm, tends to indicate that mycorrhizas must be playing an essential role in ensuring that the plant receives an adequate supply of Ca^{2+}. Findings have indicated that mycorrhizas can assist the plant in acquiring mobile nutrients such as Ca^{2+} (Linderman, 1988). In addition, the uptake of Ca^{2+} has been found to be influenced by the amount of Al^{3+} present in the soil (Clarkson and Sanderson, 1971). Hence the mycorrhizae must be extremely efficient in extracting Ca^{2+} from the soil and in translocating it to the host. If the Al^{3+} in the soil tends to increase with increasing acidity of the rain (Table 4.3.2) the uptake of Ca^{2+} might become increasingly limited and more difficult.

Although the greatest amounts of Mg^{2+} were leached from the soil when the "rain" was most acidic, the amount of Mg^{2+} leached did not vary significantly in response to the different acidities (Fig. 4.3.7). These results were not expected in that other studies have shown that more Mg^{2+} tends to be leached when the solutions are more acidic (Krug and Isaacson, 1984; Nyegaard and Abrahamsen, 1991). There are a number of possible reasons why the amount of Mg^{2+} did not vary significantly with respect to the acid "rain" treatments.

Firstly, Krug and Frink (1983) propose that in acid soils where the proportion of basic to acidic cations is low, the rate at which cation exchange reactions occur is slow, consequently the basic cations are efficiently retained. It can be seen from Fig. 4.3.1 that such a situation may have occurred in the soil that was examined because the ratio of basic to acidic cations in the top 150mm is low. As a result of few cation exchange reactions occurring, the response of Mg^{2+} to the acid rain treatments, was not significant.

Another explanation for the lack of response of Mg^{2+} to the treatments is the possibility that the Mg^{2+} in the soil may be trapped in clay lattices and thus not exposed to cation exchange reactions. Clays can be divided into two broad categories depending on their structure: one type of clay does not have a silicon base and is composed of hydrous oxides of iron and aluminium, the other category is silicate clays which possess either silicon tetrahedra or aluminium octahedra which are joined together by sharing oxygen atoms in such a way so as to form sheets. As the silicate clays form - cations such as iron and Mg^{2+} can be isomorphically substituted for Al^{3+} in the sheet (similarly Al^{3+} can substitute for silicon) resulting in the clay particles having a negative charge (Singer and Munns, 1987). Figure 4.4.1 shows such a sheet-like structure of the clay. The incorporation of Mg^{2+} in these sheets would protect it from exchange reactions with the incoming H^{+} from the acid rain. (Singer and Munns, 1987).

Krug and Isaacson (1984) found that not only did the rate of leaching of Al^{3+} depend on the solubility of organic matter in acid, organic - rich soils, but the rate of leaching of Mg^{2+} was also dependent on this factor. Consequently, if the organic matter in the soils that were examined was not soluble in the soil solution, the loss of Mg^{2+} in the leachate, as with Al^{3+}, would not vary in response to the different treatments.

The soil analyses showed that large amounts of Mg^{2+} remained in

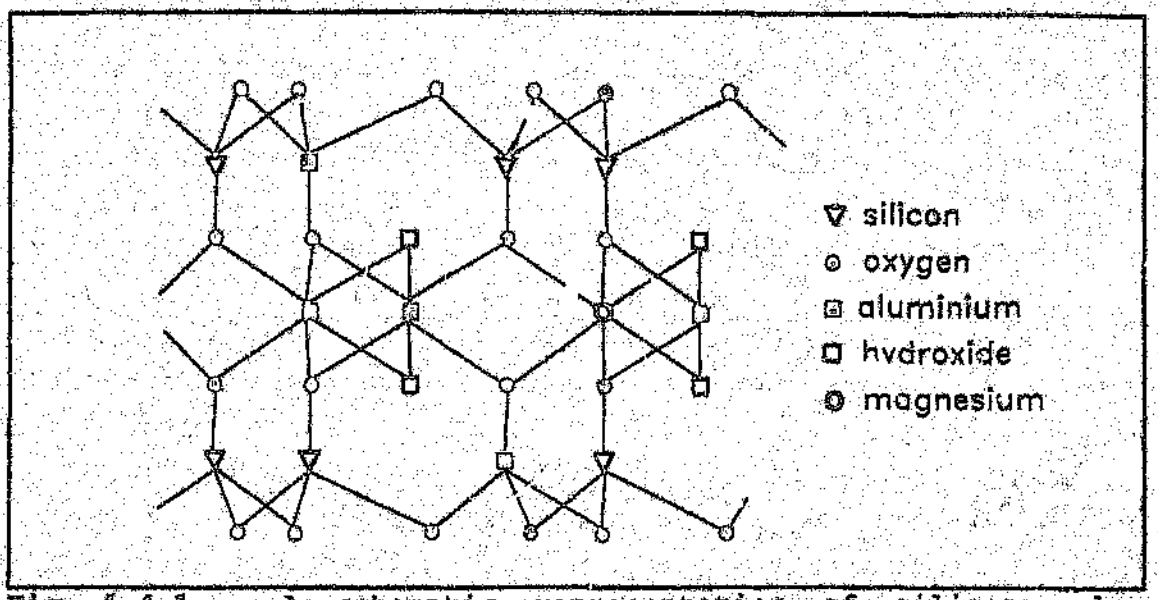

Fig. 4.4.1 A schematic representation of silicate clay showing the silicon and aluminium sheets which include magnesium.

the soil after the 15 years of simulated rain and that there were no significant responses to the different acid "rain" treatments. Other studies have shown that the response of soils to acid rain treatments have only been significant when the pH of the rain has been less than pH 4 (Krug and Isaacson, 1984). However the analysis of the leachate tends to indicate trends that will become significant if the experiment was continued for a longer period of time.

The amount of K^+ in the soil tended to increase during the leaching experiment (Tables 4.3.1 and 4.3.2). This increase cannot be explained although it is possible that the extra K^+ might have been leached from the steel cores themselves. At the end of the experiment the soil leached with the most acid "rain" contained the least K^+, while soil leached with "rain" of pH 4.22 and 5.60 contained the most K^+ (Table 4.3.2). The H^+ from the acid rain could have exchanged for K^+ on the soil particles while moving through the soil core. This would result in K^+ being lost to the plants in the top 250mm where most uptake occurs. Generally it is thought that Ca^{2+} and Mg^{2+} are lost due to

exchange reactions with the H^+ of the acid rain, because these two cations are as a rule the predominant cations in the soil solution (Nye and Tinker, 1977). However due to the large amounts of K^+ found in this soil it is likely that this cation could be lost in large quantities as a result of exchange reactions with the H^+ from the acid rain. In addition, K^+ is likely to be exchanged from the soil particles rather than Ca^{2+} or Mg^{2+} because the cations with the higher valency are more strongly attracted to the soil particles and hence are held more tightly to these particles. However other studies have shown that monovalent cations are less affected by the leaching solution, with the exception of K^+ from deeper soils (Krug and Isaacson, 1984).

As with K^+ there tended to be an increase in the amount of Na^+ present in the soil at the end of the leaching. The effect of acid rain on Na^+ loss could not be determined, because the amount of Na^+ in the soil was small and the loss of this cation from the soil was highly variable (Table 4.3.2). This variation could have resulted from high variation in the soil initially.

4.4.5 Liming as a means of ameliorating acidification

Species vary in their sensitivity to increases in the acidity of the soil, poorer nutrient conditions and increases in the concentrations of toxic cations such as Al^{3+}. Hence it is important to determine whether the effects of acid deposition can be ameliorated. One school of thought proposed by Krug and Frink (1983) is that a number of natural processes might decrease the effects of acidification. For example when the pH is below 5, aluminium oxides in the parent material may neutralize acid in water. Other natural buffering systems that commonly occur in the soil are neutralization of acidity by means of reactions with CO_3^{2-}, HCO_3^- iron oxides, as well as organic acids (Morrison, 1984).

These natural processes may not be sufficient to ameliorate the continual deposition of acids in the precipitation, consequently the potential of $CaCO_3$ to ameliorate any effects that acid rain

may have on the rate of soil acidification was investigated. The application of $CaCO_3$ resulted in a reduction in the rate of leaching of exchangeable acid equivalents, H^+, acid equivalents arising from Al^{3+}, as well as decreasing the rate of leaching of Mg^{2+} (Figs. 4.3.3, 4.3.4, 4.3.5 and 4.3.7 respectively). Thus the use of $CaCO_3$ to decrease the rate of acidification of the soil was effective.

4.5 CONCLUSIONS

The initial soil analysis showed the soil to be naturally acidic (pH in KCl 4.64), with a high Al^{3+} content (1.12 cmol kg^{-1}) and low Ca^{2+} and Mg^{2+} contents (0.08 and 0.50 cmol kg^{-1} respectively). The Ca^{2+} content of the soil was so low that below the top 100mm the concentration was below the resolution of the AAS.

When the soil cores were leached with artificial rain of various acidities, it was found that the leachate had a significantly higher H^+ content when the "rain" had a lower pH. Similarly the total amount of acid equivalents leached from the soil was greatest when the "rain" had a pH of 3.92. These results indicate that water percolating through the soil could become more acid if the precipitation acidifies. This could have important consequences for catchments in the area. There was however no significant change in the amount of Al^{3+} in the leachate with respect to the different acidities of the "rain".

The amounts of Ca^{2+} present in the leachate were below the resolution of the AAS, and consequently it was not possible to monitor the leaching of Ca^{2+} from the soil cores.

It was found that, although more Mg^{2+} was lost from the soil that was leached with "rain" having a pH of 3.92, the difference in amounts of Mg^{2+} lost in response to the "rain" of various acidities was not significant. The fact that the rate of leaching did not change with different acid "rain" regimes could be due to: (i) the low basic to acidic cation ratio resulting in few exchange reactions, (ii) a large portion of the Mg^{2+} in the soil

might be held in the clay lattices making exchange reactions difficult, or (iii) the low solubility of organic acids may result in low Mg^{2+} leaching rates. Over a longer period of time, the increasing amounts of acid in the precipitation may increase the amount of Mg^{2+} lost from the soil. This would reduce the availability of Mg^{2+} for the plants and since Mg^{2+} plays so many important roles in the plant, such a loss could be detrimental to the growth of the plant.

The response of Mg^{2+} to the acid "rain" treatments could not be detected in the amounts of Mg^{2+} present in the soil after it had been leached with "rain" of various acidities.

Although the soil that had been leached with "rain" of pH 3.92 had less K^{+} and more acids and Al^{3+} compared with soil that had been leached with "rain" of either pH 4.22 or 5.60, the soil analysis at the end of the experiment showed that the chemical composition of the soil did not appear to have responded to the different acid "rain" regimes in that none of these changes were significant.

The addition of $CaCO_3$ did ameliorate the acidification process in that it resulted in decreased amounts of exchangeable acid equivalents, H^{+}, Al^{3+} equivalents and Mg^{2+} being leached from the soil. Understandably, the application of $CaCO_3$ resulted in increasing amounts of Ca^{2+} being lost in the leachate.

CHAPTER 5 ROOT GROWTH AND MYCORRHIZAL COLONIZATION OF ROOTS GROWING INTO SOIL THAT HAD BEEN EXPOSED TO ARTIFICIAL ACID RAIN.

5.1 INTRODUCTION

One of the symptoms associated with forest decline in North America and Europe has been a loss of feeder root biomass (Schütt and Cowling,1985). This may result from either a decrease in root length or a reduction in root mass per unit length of root. If the loss of feeder root biomass is not accompanied by a simultaneous increase in nutrient uptake rates per unit length of root, the plant may become nutrient-stressed.

This damage to the root system of the trees may result from a change in the soil composition. Acid rain has the potential to generate changes in the soil composition by increasing the rate of soil acidification. (Chapter 4 discusses the effects that acid rain can have on the composition of the soil.) This chapter addresses the hypothesis that soil that has been acidified by acid rain, could have a negative influence on root growth, in terms of root length or mass per unit length of root, and/or the degree of mycorrhizal colonization occurring on the roots. Figure 5.1.1 illustrates the questions addressed in this chapter. These factors can alter the ability of the plant to extract nutrients from the soil and consequently affect the plant's growth and fitness.

The extension of plant roots into the bulk soil is an important physiological process in that it determines the volume of soil explored by the roots. Plants are only able to extract nutrients from the soil adjacent to the root surface, in the rhizosphere. With time, although ions move to the root surface by mass flow and diffusion, this soil can become nutrient deficient. In order for the plant to acquire sufficient nutrients, the roots of the plant need to extend into the bulk soil so that new nutrient reserves can be utilized.

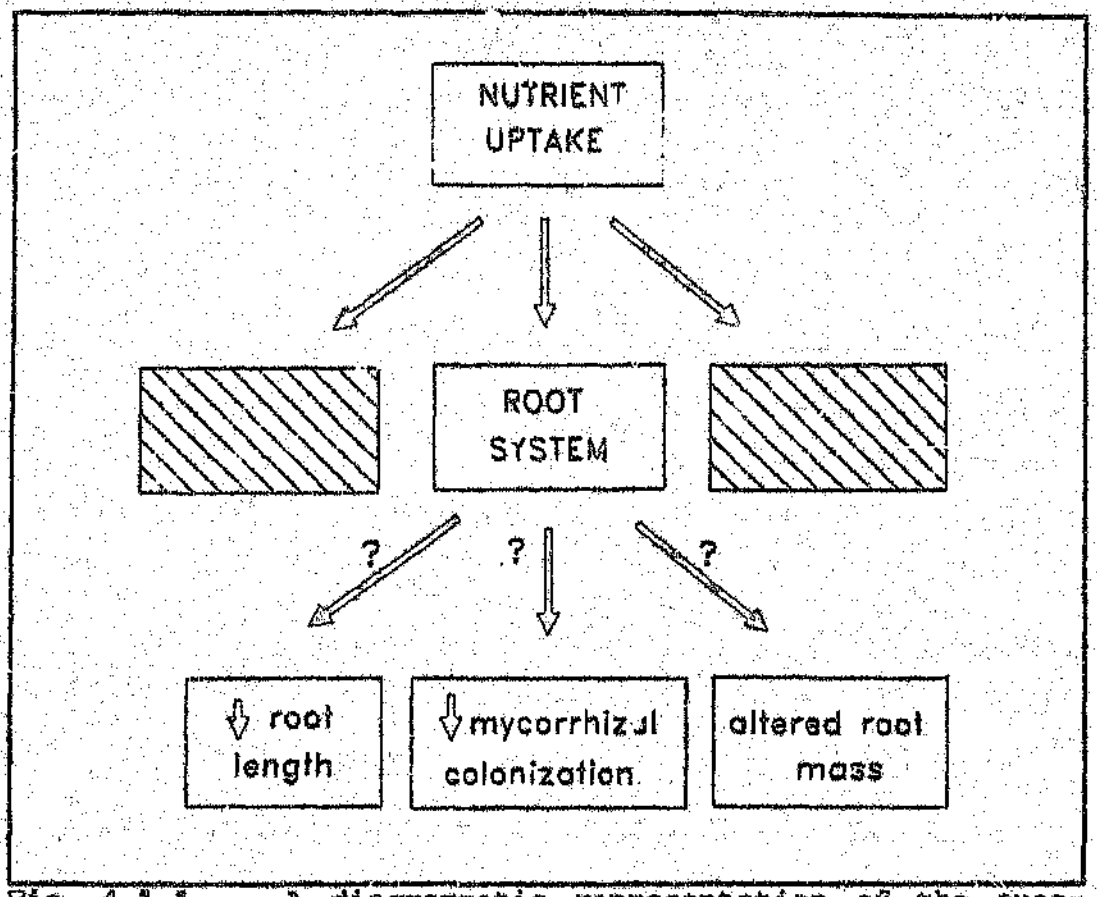

Fig. 4.1.1 A diagrammatic representation of the questions addressed in this chapter.

Factors which can affect root elongation include temperature, soil water potential, photosynthate supply, oxygen concentration, possibly growth substances, as well as the soil environment in terms of mechanical resistance and ions that are present in the soil (Drew, 1979; Milthorpe and Moorby, 1986). These factors, besides affecting the rate of root elongation, can determine the morphology of the root which can in turn influence nutrient uptake.

Changes in the mass per unit length of root gives an indication as to whether the morphology of the roots, such as the thickness of the roots, could be affected by acid rain. Variation in the morphology of the roots could be caused by changes in the anatomical structure of the root. Modifications in the anatomy of the roots could alter the pathway, and hence rate of movement,

of ions from the soil to the xylem. The mass per unit length of root can also give an indication of the vitality of the roots, in that lighter roots may be highly vacuolate and have large intercellular spaces. Roots of this nature would not respond well under either low soil water status or physical stresses.

Mycorrhizas have been found to confer a number of competitive advantages on their host. These are discussed in detail in Section 2.2.7. Most importantly, mycorrhizas assist their hosts in obtaining an adequate supply of nutrients particularly under low nutrient conditions (Tinker, 1984). There are many ways that this may be achieved. Firstly, the hyphae of the mycorrhizas extend further into the soil than root hairs, thus ensuring that a greater volume of soil is explored (Fogel, 1983). The hyphal network also increases the absorptive surface of the plant, as well as being able to penetrate smaller soil pores than root hairs. The possibility also exists that the fungi are more effective at absorbing and/or transporting and storing nutrients from the soil than the host's roots (Parke *et al*, 1983).

In this experiment, the length of roots growing into soil that had been exposed to various amounts and acidities of artificial acid rain, was determined. This would demonstrate whether soil that had been acidified by acid rain could affect root extension and, consequently, affect the acquisition of nutrients by the plant. The mass per unit length of the roots growing into this soil was also determined in order to ascertain whether the morphology of the root had been altered. The percentage of the root length that was colonized by mycorrhizas was determined because any negative effect that acid rain may have on the degree of mycorrhizal colonization can in turn affect the nutrient status of the plant.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of root ingrowth bags

The soil that had been exposed to artificially acidified "rain"

in the microlysimeters in the first experiment was removed from each of the stainless steel tubes and dried. Subsamples of this soil were analyzed, in order to determine the pH of the soil, the exchangeable acidity, as well as the aluminium (Al^{3+}), calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+}) content of the soil. (Chapter 4 contains the method and results of these analyses.)

The first experiment resulted in the soil from the cores being ameliorated to different degrees as a result of the varying volumes and acidities of the "rain" that had passed through the soil cores. (The volume of "rain" passing through the soil had varied as a result of differences in the compaction of the soil in the microlysimeters.) Consequently, a range of soil conditions had resulted as apposed to distinct treatments corresponding to the acidity of the "rain" that passed through the soil cores.

Root ingrowth bags were made out of a polypropylene mesh (2380PSE). They were 350mm long and had a diameter of 45mm. The mesh had an opening of 2.4mm. The root ingrowth bags were placed in the ground at the site in the forest on Long Tom Pass. The ground was cleared of litter, and for each bag, a soil core with a diameter 2mm greater than the bag was removed from the ground. A root ingrowth bag was then placed into each hole. Four bags were placed around each tree, in the rows of the trees, at a distance of 0.7m from the tree stem. Figure 4.2.1 illustrates the way the ingrowth bags were positioned in the rows of trees in the stand. The soil was put into the mesh bags, after which 100ml of distilled water was poured into the top of the bag.

After 7.5 months, which was approximately the duration of the growing season, the bags were removed from the ground. This was done by knocking a steel tube (with a 70mm diameter) into the ground around the bag. The bottom edge of the tube was sharpened so as to cleanly sever the roots and prevent them from being pulled out of the bag when the bag was removed from the ground.

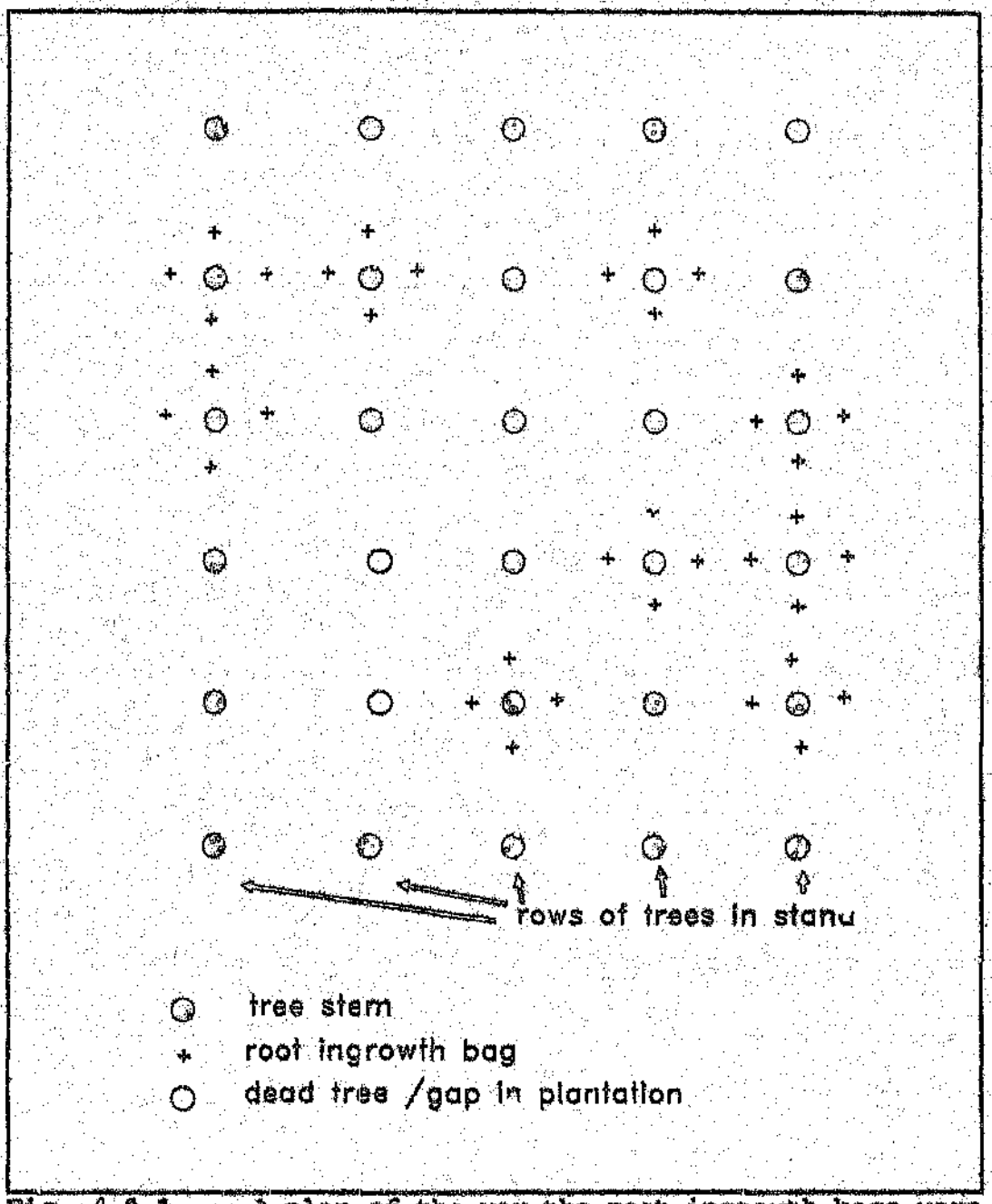

Fig. 4.2.1 A plan of the way the root ingrowth bags were placed in the ground in the plantation.

4.2.2 Removal of the roots from the soil

The roots that had grown into the bag were trimmed close to the surface of the bag. The contents of the bag were placed into a 1l erlenmeyer flask. A soil sample of 10ml was removed for analysis. Water (800ml) was added to the flask after which it was placed on a shaker for 30 minutes in order to dislodge the soil.

The large roots were then removed from the flask and the rest of the contents of the flask were placed in a root elutriator designed according to Smucker *et al*, (1982). The latter used water flow and compressed air to separate the organic matter from the soil. The organic matter was then caught on a 0.35mm mesh sieve.

In order to preserve the roots until the root data could be collected, the roots were placed in a 30% ethyl alcohol solution. It was determined that 30% ethyl alcohol did not cause shrinkage or distortion of the roots.

Before determining root length, root mass and degree of mycorrhizal colonization, the roots were sonicated for 30 minutes. This process removed some of the mucilage surrounding the roots as well as dislodging soil particles that were closely associated with the roots and the mycorrhizas.

5.2.3 Determination of root length

The method that was used to determine root length was the same as that proposed by Tennant (1975). The roots were placed in a shallow, flat bottomed white dish containing 30% alcohol and were teased out so that they lay across a 1x1 cm grid. The roots were viewed with the aid of a magnifying glass.

The length of the root was calculated by counting the number of times the roots intercepted one of the grids. A count of one was given if the root crossed a line or touched a line on the grid, while a count of two was given if the curved portion of root lay along the line. The following formula, from Tennant (1975), was then used to determine root length:

root length = (11/14) x number of intercepts

Root length, in this case, refers to that fraction of roots greater than 5mm in length. Some of the root tips (both mycorrhizal and non-mycorrhizal) broke off the roots in the elutriator. These broken tips generally had lengths less than

5mm. They were collected separately on a fine mesh and were found to form a constant fraction of the calculated root length - namely 45.55 ± 7.64% of the total root length. This fine fraction of broken tips was not counted for each core and hence the lengths are underestimates, by about 46%, of the total length.

5.2.4. Determination of degree of mycorrhizal colonization

The degree of mycorrhizal colonization was so high that the best way to quantify it was to calculate the percentage of root length that was mycorrhizal. The length of root that was colonized was determined using the same method as above however only mycorrhizal intercepts were recorded. The percentage of root that was colonized by mycorrhizas was calculated using the following formula:

$$\% \text{ mycorrhizal root} = \frac{\text{mycorrhizal root length}}{\text{total root length}} \times 100$$

In addition the presence, or absence, of various mycorrhizal species was noted. The different species were characterised on the basis of their colour and the branching pattern exhibited by the colonised roots. The colour of the fungus was used to differentiate species in spite of the fact that the colour of one mycorrhizal species may change during it's life (Egli and Kälin, 1989). The reason for the use of this parameter was that the duration of growth of the roots into the root ingrowth bags was only one season and was constant for all the bags, hence, it was thought that the age of all the mycorrhizas must be relatively constant and consequently the colour would hardly vary within one species.

5.2.5 Determination of root mass

The roots were oven dried and the dry mass of the roots which extended into the bags was determined for each of the bags. The dry root mass for each bag was divided by the root length for each bag in order to indicate the average root mass per unit length of root.

5.2.6 Data analysis

A principal components analysis (PCA) was performed using Canoco (Terbraak, 1988) in order to determine if any relationship existed between the soil characteristics and the amount of root elongation and mycorrhizal colonization that had occurred, as well as the mass of the roots per unit length. The data recording the presence or absence of the various mycorrhizal species on roots from the bags, was also subjected to PCA in relation to the soil characteristics.

Linear regressions were performed using SAS/STAT release 6.03.

5.3 RESULTS

The average length of root that grew into an equivalent of 1kg of soil in one growing season is listed in Table 5.3.1, as well as the dry mass per unit length of root and the percentage of the root that was colonized by mycorrhizas. These values are an average of all the ingrowth bags irrespective of the degree of acidification, and consequent ion concentrations, that had occurred in the soil of each of the bags.

Table 5.3.1 The average root length per kg soil, dry mass per unit root length, and percentage mycorrhizal colonization of the roots which grew into the root ingrowth bags, irrespective of soil composition, ($\bar{x}$ ± s.d.; n = 32).

	mean ± standard deviation
root length (m kg^{-1})	14.02 ± 5.60
root mass (g m^{-1})	0.12 ± 0.03
% mycorrhizal colonization	31.31 ± 7.51

The PCA analysis produces a biplot of environmental and plant characteristics which is a plot of both of these sets of characteristics on the first two axes that have been extracted by the analysis. The analysis groups the environmental characteristics with the plant characteristics that they possibly

influence and places characteristics which are negatively correlated to each other as far apart as possible. The length of the arrow joining the environmental characteristic point with the origin indicates the likelihood of the character contributing significantly to the regression. The eigenvalues of the analysis indicate how much variance has been accounted for by axis 1 and 2.

A biplot of the soil characteristics and the root characteristics was constructed using the output from the PCA analysis. The biplot (Fig. 5.3.1) indicated that a possible relationship existed between root mass and both the Ca and Mg contents of the soil, in that the PCA analysis grouped these factors. The eigenvalues of this analysis are recorded in Table 5.3.2.

Table 5.3.2 The eigenvalues of the PCA analysis of the soil characteristics and the root growth.

Axis:	Eigenvalue:
1	0.753
2	0.231
3	0.016

A plot of soil Ca content versus root mass showed that they were not correlated, however a similar plot of soil Mg content against root mass (Fig. 5.3.2) showed that Mg content of the soil tended to be positively correlated with the mass per unit length of root. This relationship was not significant, ($y = 8{,}320E\text{-}4x + 8{,}126E\text{-}4$; $r^2 = 0{,}103$; $n = 32$; $p < 0{,}0736$).

None of the other soil characteristics appeared to affect the rate of root growth, the root mass per unit length or the degree of total mycorrhizal colonization.

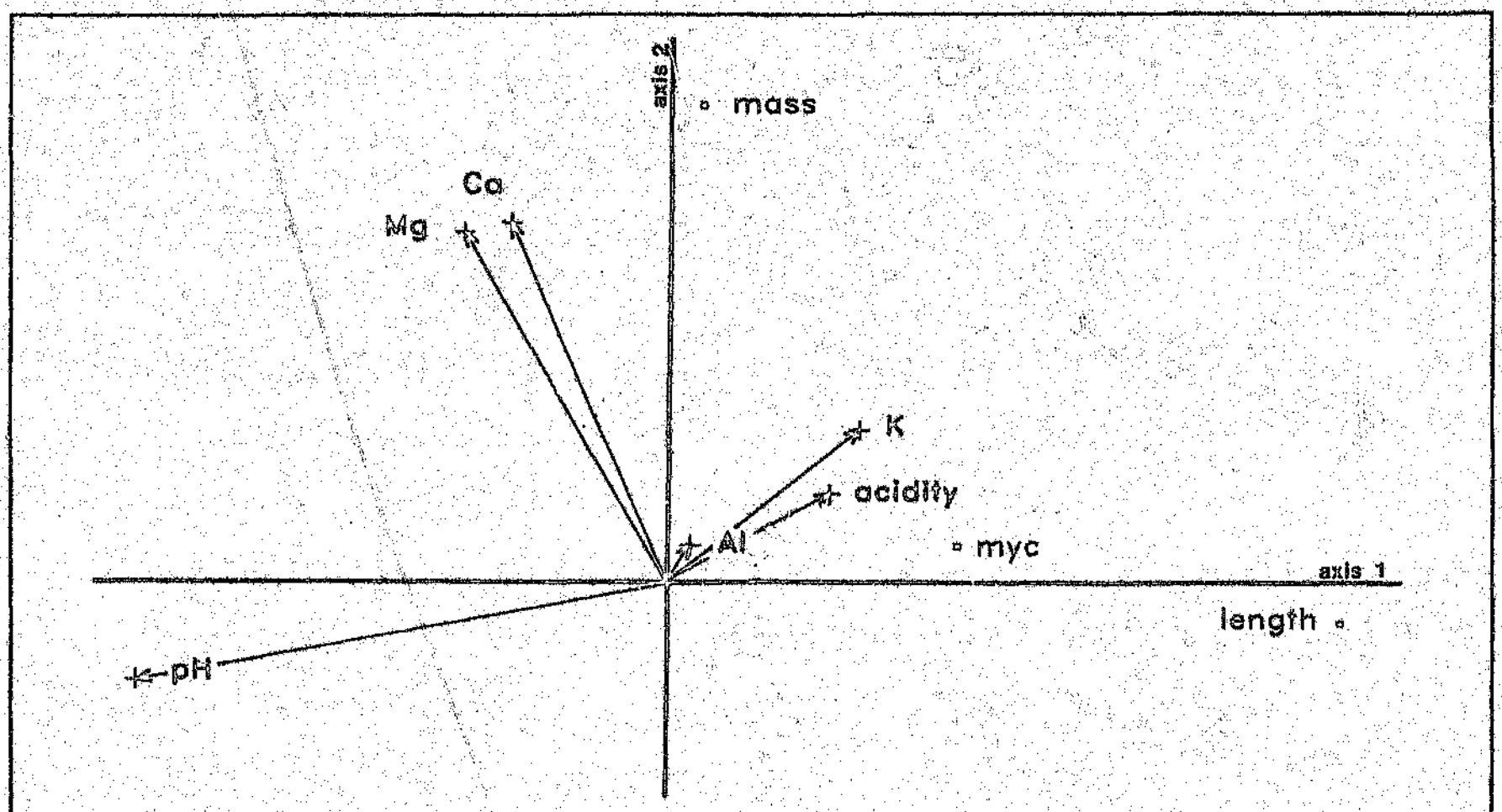

Fig. 5.3.1 Biplot of acidified soil characteristics and root characteristics of root growth into the soil ingrowth bags. (myc = total mycorrhizal length)

It should be noted that the amount of growth that occurred into the root ingrowth bags is not comparable with that of roots growing in the bulk soil. This is due to the fact that the bulk soil is much more compacted compared with the soil in the root ingrowth bags. In addition, there was presumably a difference in the water potentials of the two soils which would affect root growth. Hence, the amount of growth that occurred during the growing season into the bags cannot be used to estimate the rate of growth in the bulk soil, but is merely a parameter to compare the effects that acidified soil can have on root growth.

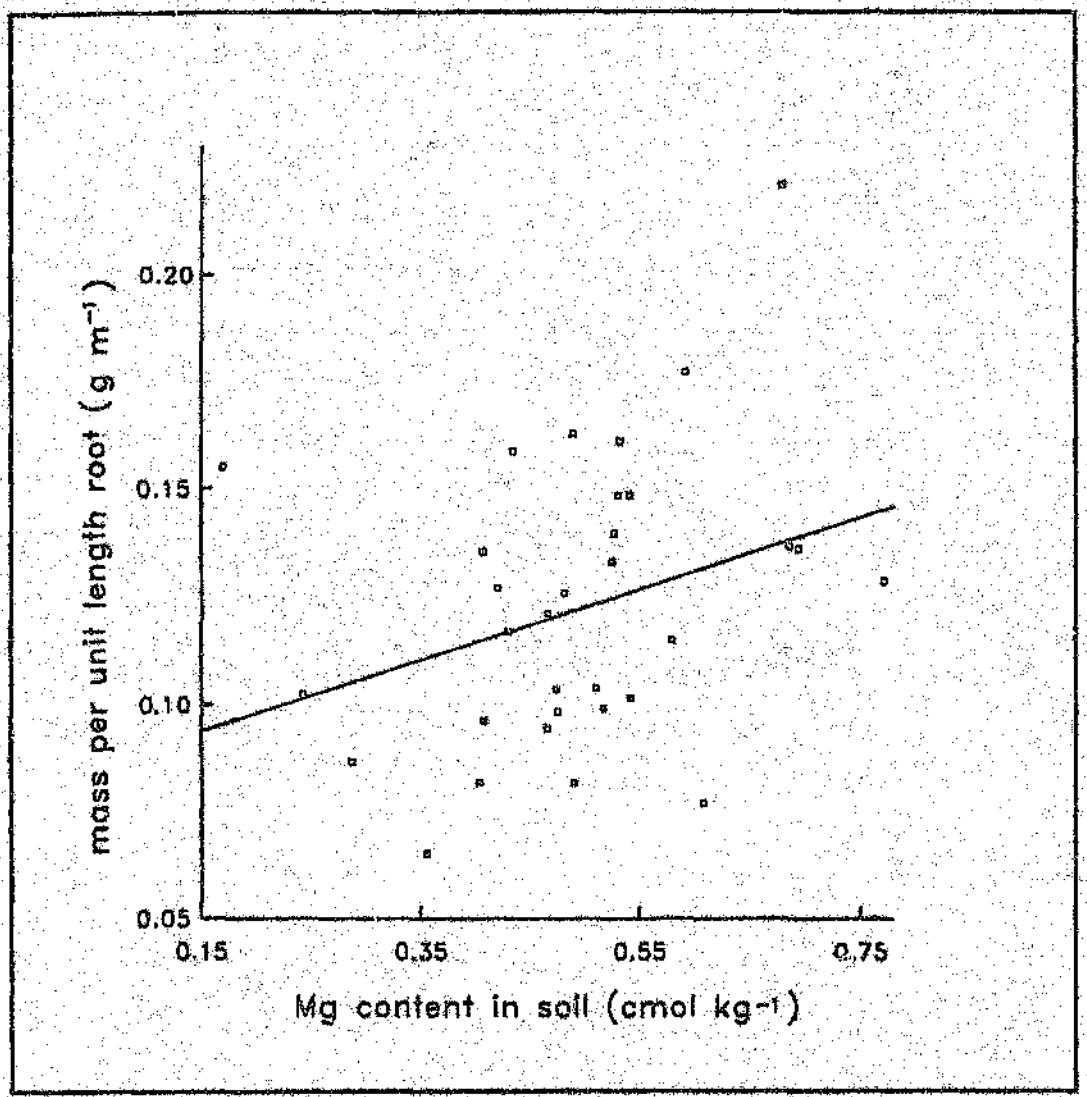

Fig. 5.3.2 The effect of soil Mg content on the mass (per unit length) of root. The line is the best linear fit (r^2 = 0.103, n = 32, p < 0.0736).

Seven different types of mycorrhizal species were found to colonize these roots. Plates 1 to 7 are a photographic record of the different types of mycorrhizas that were observed on the roots. The mycorrhizas were not characterized as there are no existing descriptions of mycorrhizas found in this area and their characterization would be a study on its own. Consequently they will be referred to as species 1, species 2, and so forth. Table 5.3.3 lists the surface morphological characteristics of the various types of mycorrhizas.

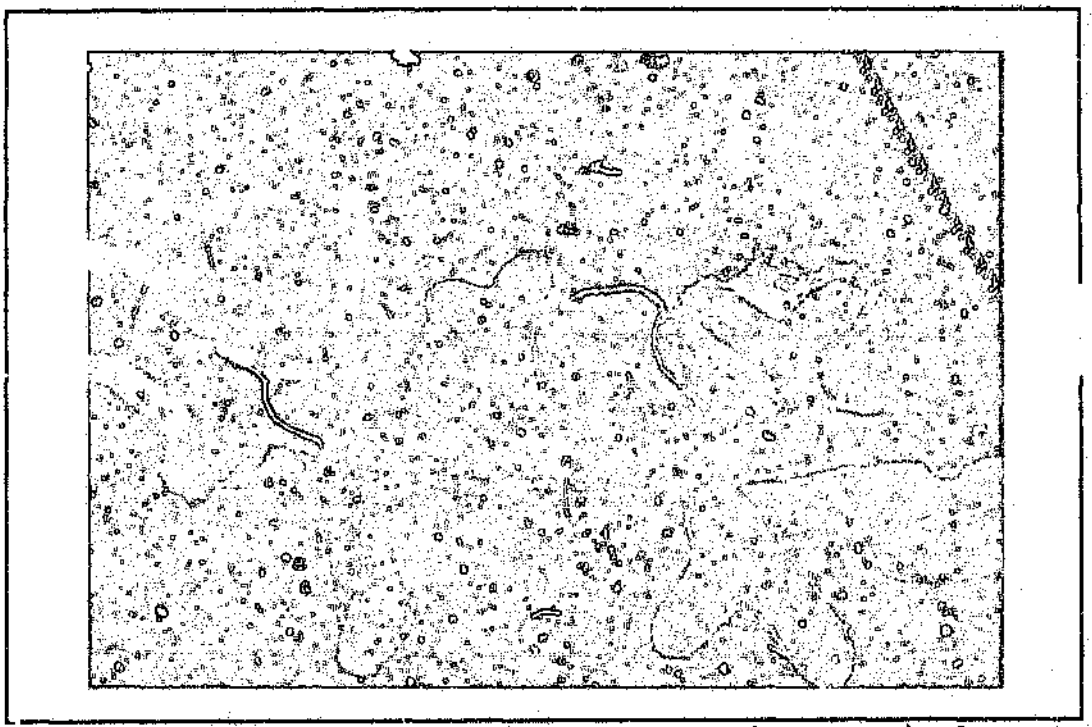

PLATE 1 Species 1 mycorrhizas - short, brown, simple

0.5mm

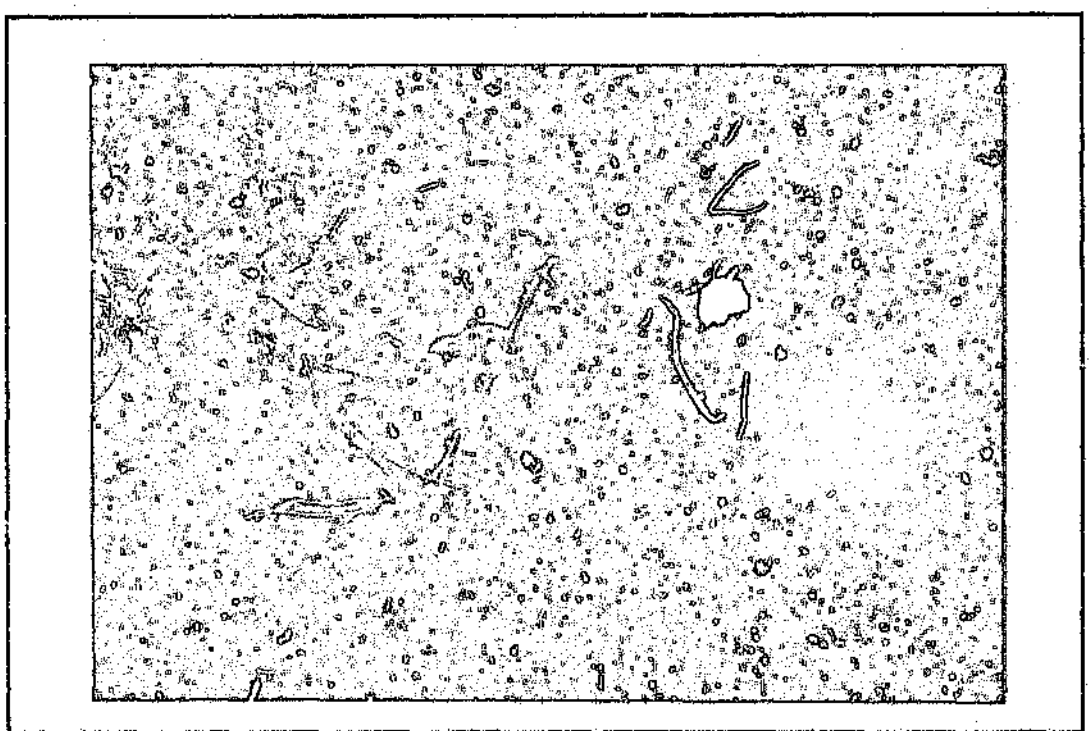

PLATE 2 Species 2 mycorrhizas - long, brown, simple

1mm

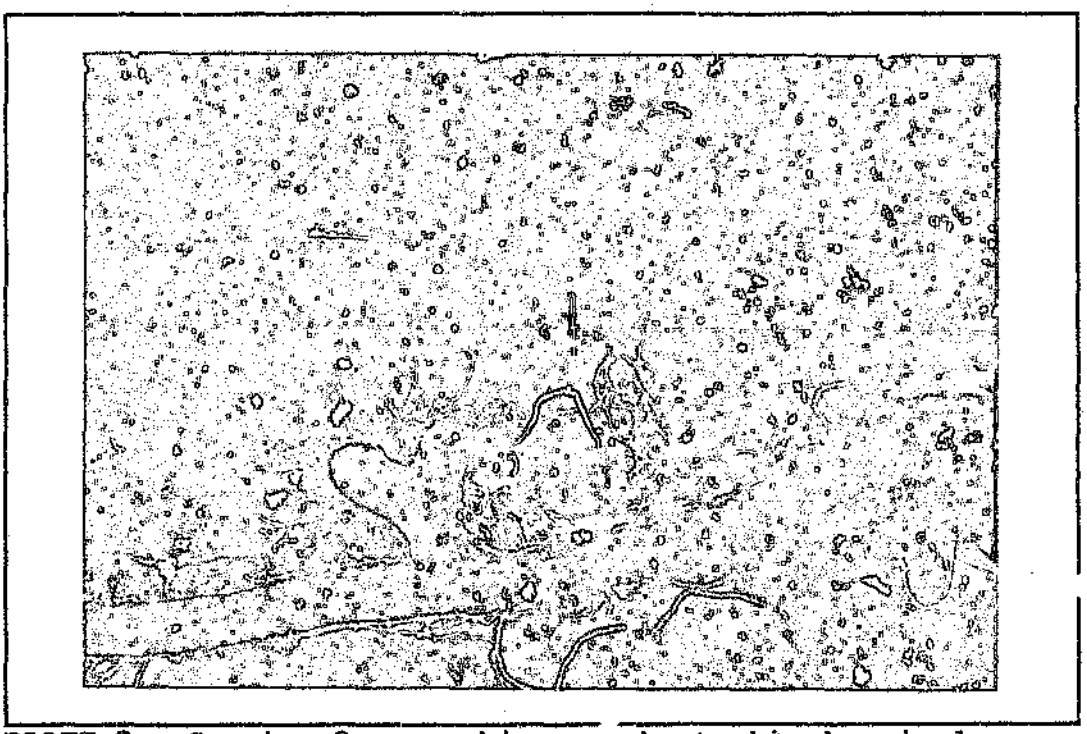

PLATE 3 Species 3 mycorrhizas - short, black, simple

0.5mm

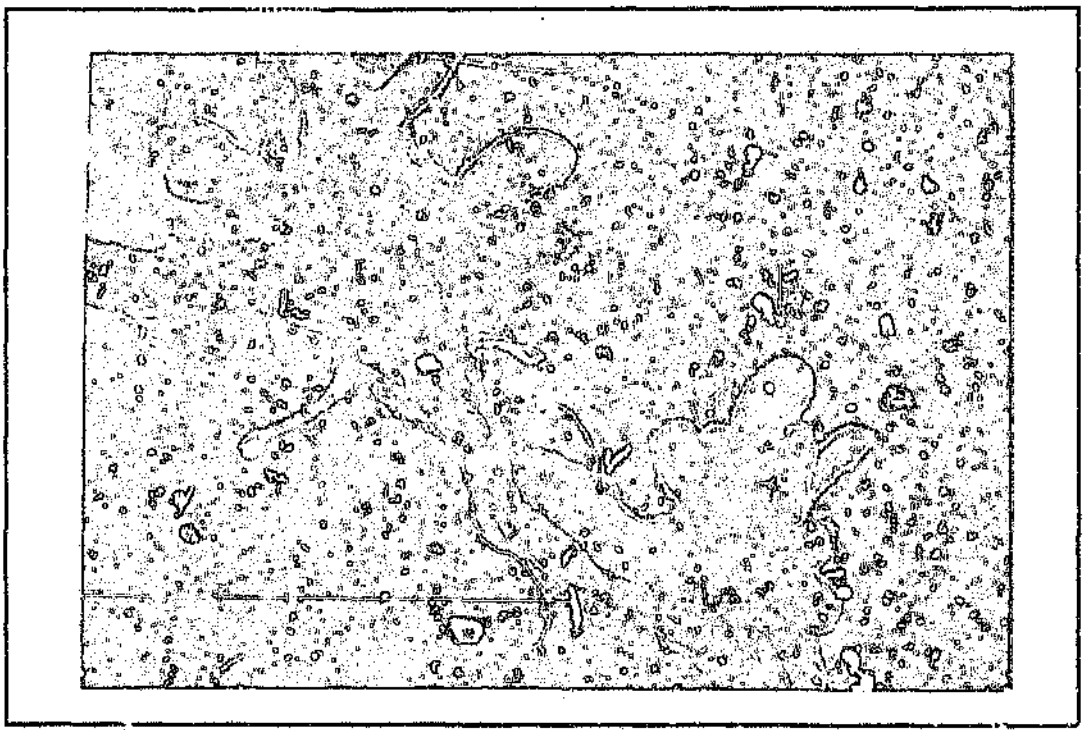

PLATE 4 Species 4 mycorrhizas - white, simple

0.5mm

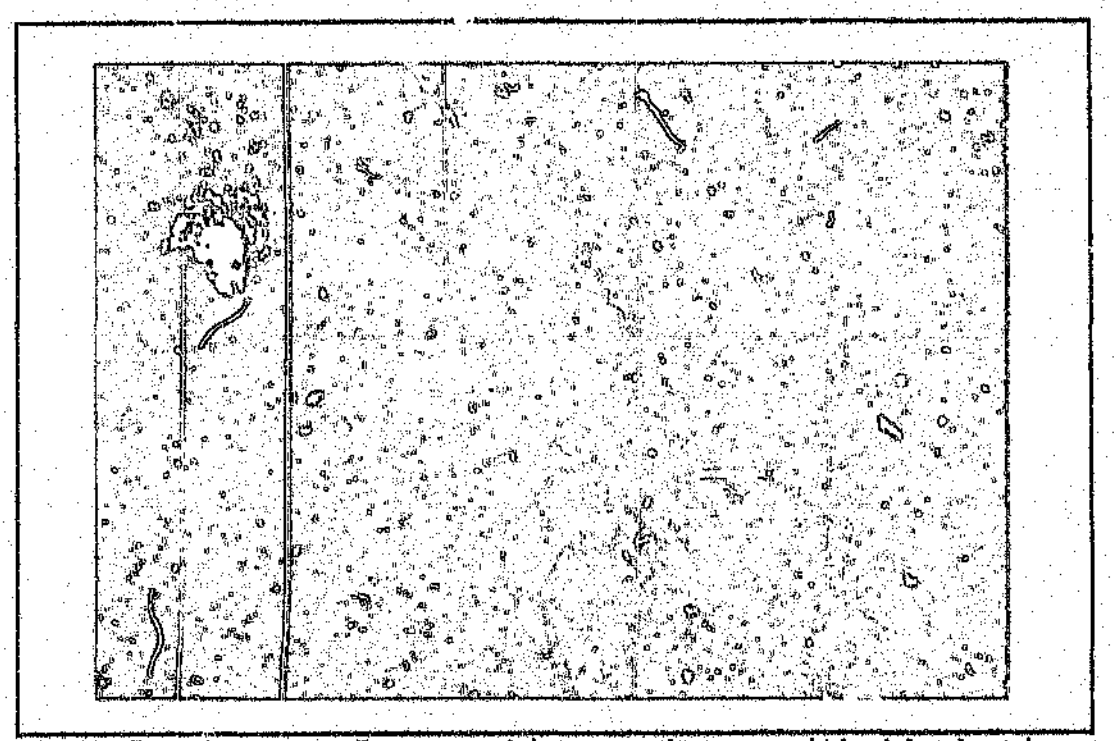

PLATE 5 Species 5 mycorrhizas - brown with black tips, simple

0.5mm

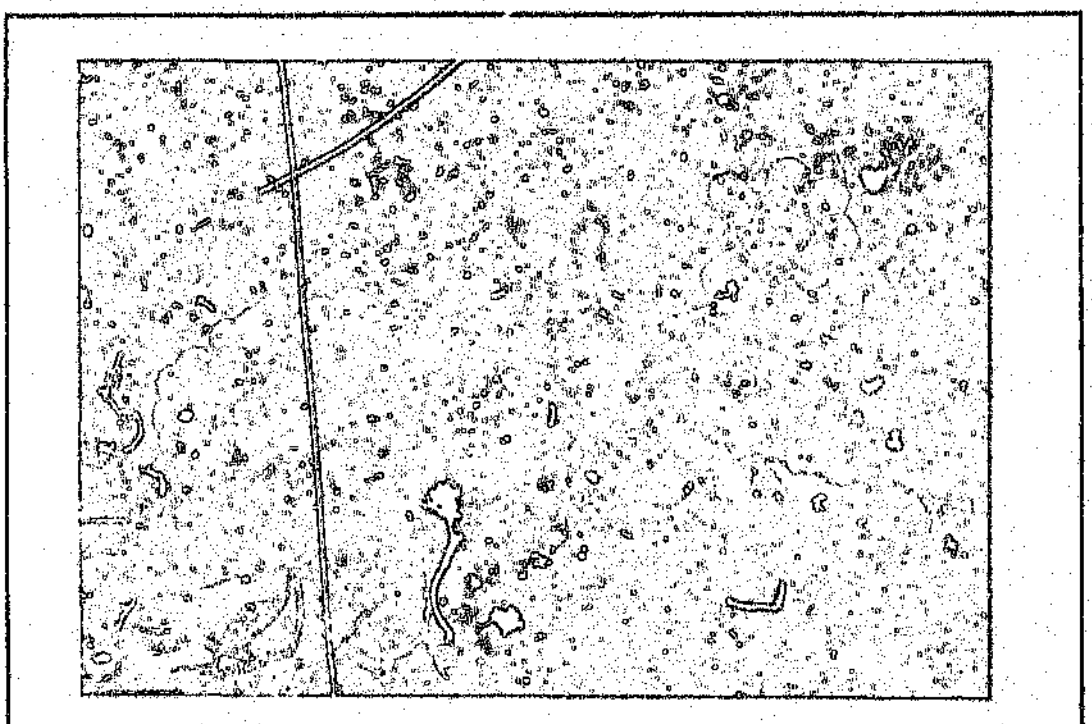

PLATE 6 Species 6 mycorrhizas - short, brown, coralloid

1mm

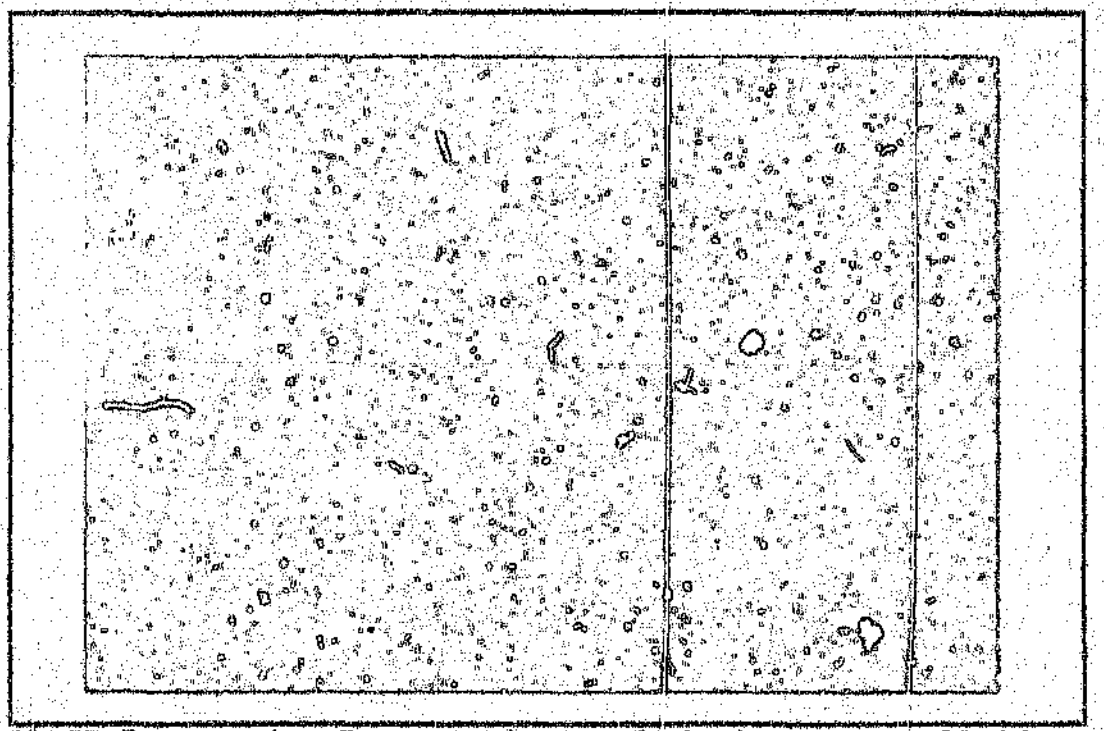

PLATE 7 Species 7 mycorrhizas - dark, brown, coralloid

0.5mm

Table 5.3.3 Description of the surface morphological characteristics of the mycorrhizae found colonizing the roots that had grown into the root ingrowth bags. (The standard deviations of the measurements were determined from a minimum of three determinations.)

Mycorrhizal species	Description
Species 1: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Either simple or dichotomously branched Light brown with the tip even lighter 1.232 ± 0.353 mm 0.454 ± 0.147 mm Although smooth, a dense mat of hyphae radiating from the surface
Species 2: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Long branches radiating from a common axis Light brown with the tip even lighter 1.343 ± 0.233 mm 0.223 ± 0.054 mm Smooth, hyphae present on surface, but sparse
Species 3: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Either simple or dichotomously branched Black with tips slightly darker 1.426 ± 0.129 mm 0.783 ± 0.462 mm Smooth with dense mat of hyphae emanating from the surface
Species 4: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Long branches emanating from a common axis White 1.715 ± 0.200 mm 0.304 ± 0.098 mm Rough with orange patches, hyphae appeared to be scarce
Species 5: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Either simple or dichotomously branched Light brown with a black tip 0.888 ± 0.114 mm 0.300 ± 0.037 mm Smooth with hyphae very dense on the surface
Species 6: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Coralloid - between 3 and an infinite number of species 1 grouped together originating from a common point Light brown with the tip even lighter 0.790 ± 0.074 mm 0.390 ± 0.026 mm Very smooth with very few hyphae
Species 7: *branching:* *colour:* *length of tips:* *width of tips:* *surface:*	 Coralloid - between 3 and an infinite number of species 3 grouped together originating from a common point Without microscope - black, using microscope - brown with black tips 1.980 ± 0.306 mm 0.200 ± 0.018 mm Smooth

The presence or absence of the different species colonizing the roots growing into each root ingrowth bag was recorded. A biplot of the soil characteristics and the presence or absence of the different mycorrhizal species was constructed from the results of the PCA analysis (Fig. 5.3.3). The eigenvalues of this plot are recorded in Table 5.3.4.

Table 5.3.4 The eigenvalues of the PCA analysis of the soil characteristics and the degree of mycorrhizal colonization that had occurred.

Axis:	Eigenvalue:
1	0.323
2	0.274
3	0.209
4	0.127

The biplot shows that species 4 is negatively correlated with the Al^{3+} content and the acidity of the soil as both the Al^{3+} content and the acidity of the soil are situated at opposite sides of the plot from species 4. Fig. 5.3.4 shows that when the Al^{3+} content of the soil increased, there was a decrease in the presence of this mycorrhizal species. The biplot also shows that species 5 is negatively correlated with the pH of the soil. The decrease in the presence of species 5 with respect to an increase in the soil pH is illustrated in Fig. 5.3.5. According to the analysis these are the only soil variables which contribute significantly to the regression.

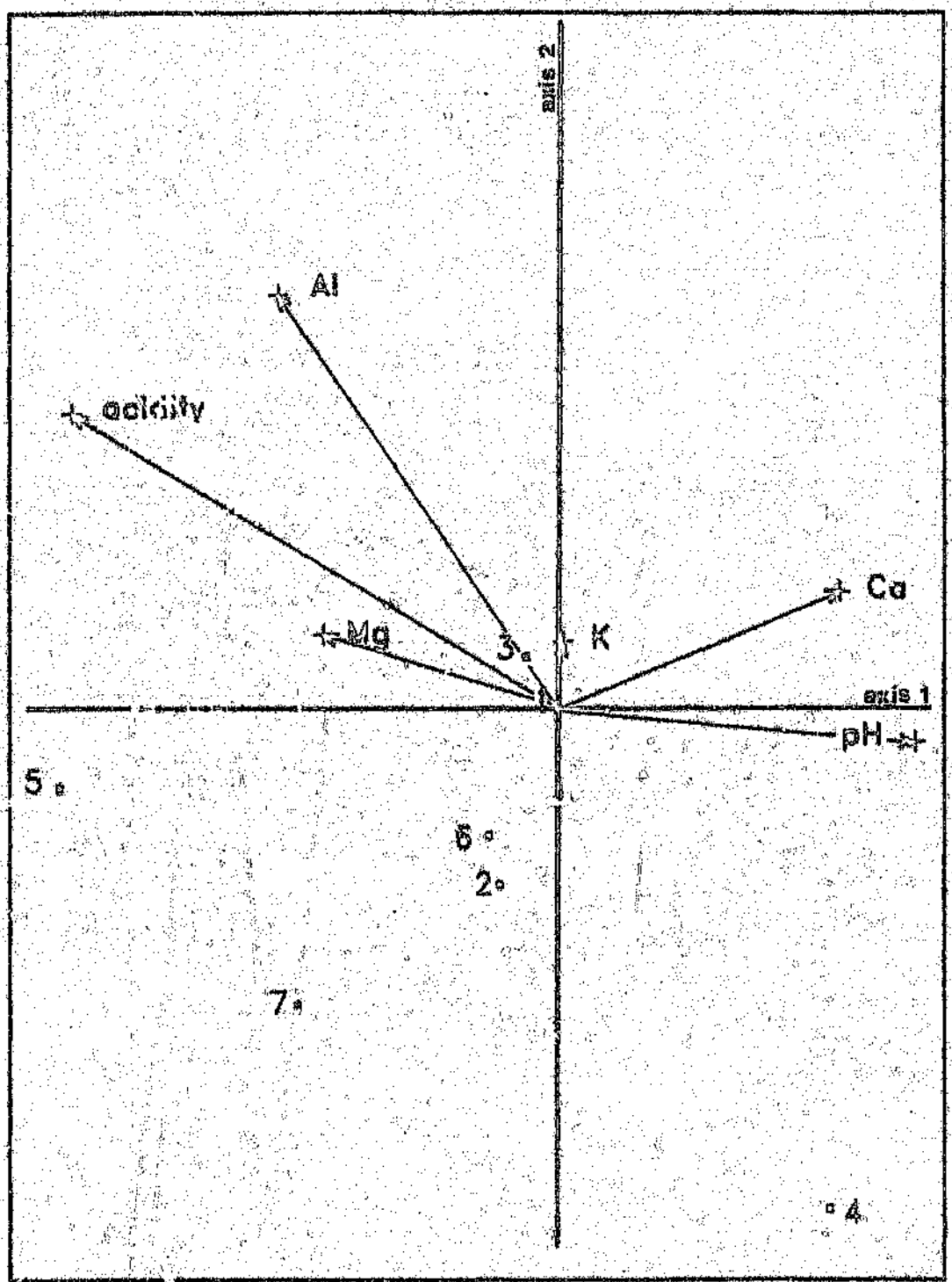

Fig. 5.3.3 Biplot of mycorrhizal species and soil characteristics.

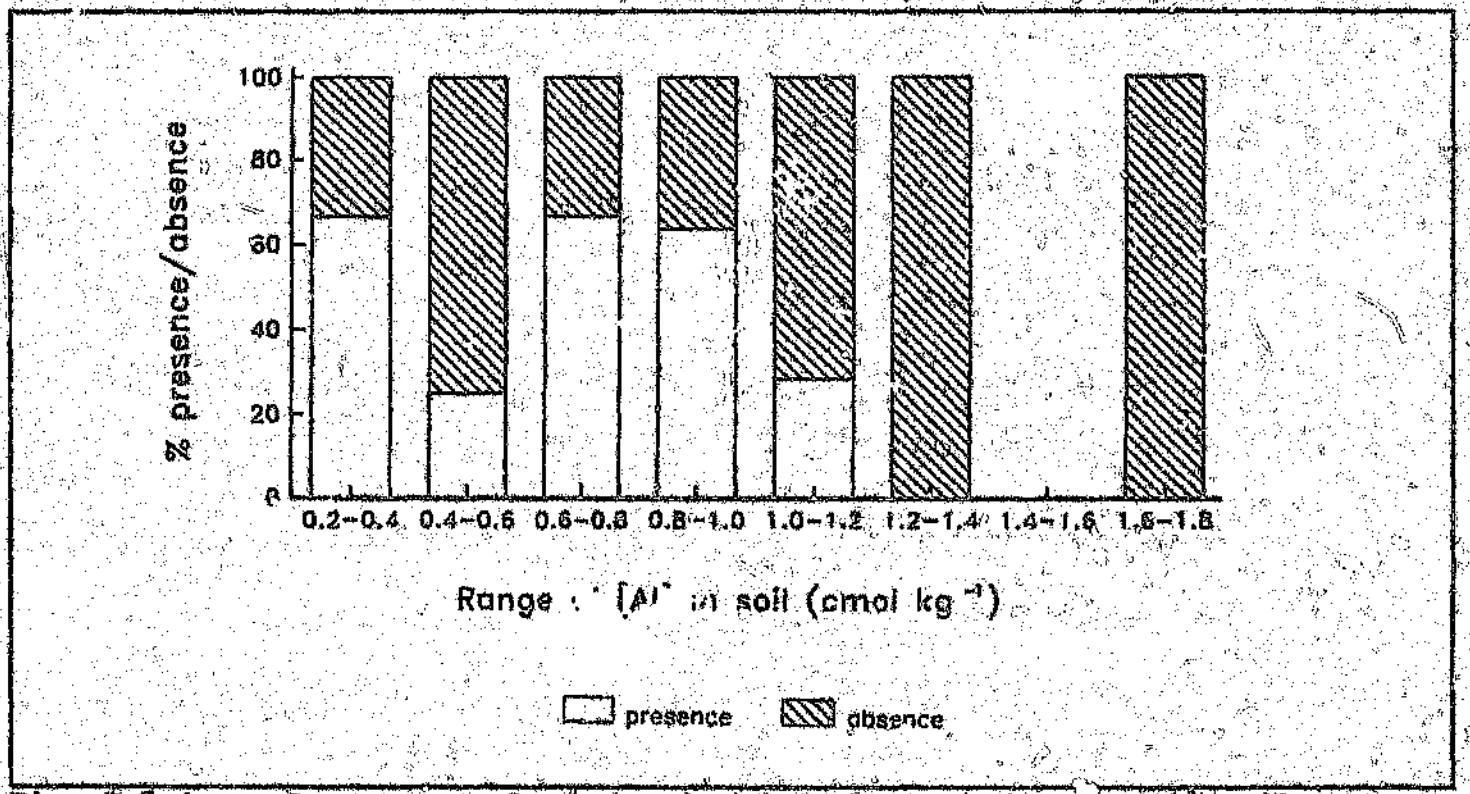

Fig. 5.3.4 Occurrence of species 4 with respect to changing Al^{3+} soil content.

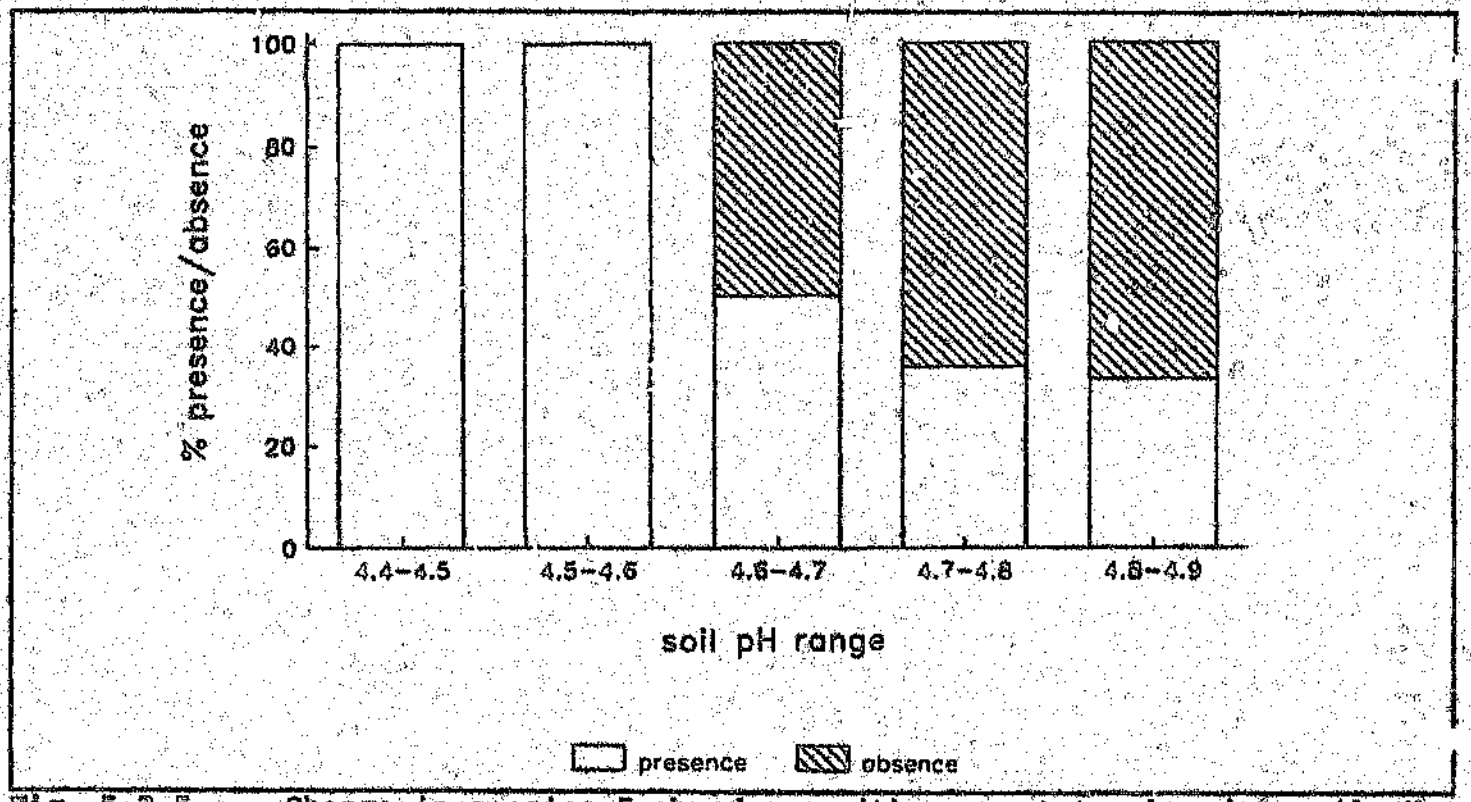

Fig. 5.3.5 Change in species 5 abundance with respect to changing soil pH.

5.4 DISCUSSION

5.4.1 Method

The use of root ingrowth bags is an efficient means of making comparisons of root growth into soil that has been adjusted. The reason for this is that the use of mature trees in the field reduces the likelihood that carbon assimilation rates, and consequently growth rates, between trees would vary markedly; this is in contrast to seedlings in a laboratory. In addition, the trees are all subject to the same nitrogen sources, iron salts etc. that are present in the natural conditions, compared with the pot grown seedlings in a laboratory.

5.4.2 Root length

The total length of root that grew into the soil exposed to artificial rain of different acidities, was found not to vary in response to the range of soil acidities that had resulted.

It is difficult to attempt to predict the impact that acidified soil may have on root growth. The reason for this is that it is impossible to separate the various responses of the root to the numerous changes that acid rain has on the soil.

Firstly acid rain can cause an increase in the H^+ content of the soil, (Chapter 4 discusses this aspect). An increase in H^+ concentration has been found to increase the extensibility of the cell walls (Rayle and Cleland, 1970). An increase in the rate of elongation of corn root segments occurred when solutions had a pH of 6.4 or less, with a maximum increase at pH 3.4 (Edwards and Scott, 1974). To explain this effect, it was proposed that the H^+ could exert a physical effect on the wall, such as the cleavage of acid-labile bonds which might result in the loosening of the cell wall. On the other hand, the effect might be a chemical one where the H^+ stimulates enzyme hydrolysis of cell wall polysaccharides allowing extension to occur (Edwards and Scott, 1974).

In a study investigating the effect of acid rain on root growth

of *Pinus sylvestris* seedlings, the acid rain had no effect on the length of fine roots (less than 1mm in diameter) but the coarse roots (greater than 1mm in diameter) tended to be longer in the acidified soil (Dighton and Skeffington, 1987). Simmons and Kelly (1989) found that rainfall pH had no effect on either coarse or fine root length.

On the other hand it has been reported that high H^+ concentrations (pH 4.0) can suppress root growth (Loneragan, 1979). The H^+ were thought to suppress cell division in the root meristems. Deans *et al*, (1990) found that acid rain significantly reduced the production of coarse roots of *Picea rubens* but that the greatest fine root production occurred when the acid mist had a pH of 3.0, compared with a pH of either 2.5 or 5.0.

Schrer (1987) found that there was a 36% decrease in the length of the primary roots of Pitch pine when watered with artificial acid rain of pH 3 compared with pH 4. After initiation, elongation of the lateral roots was also inhibited by rain of pH 3.

These conflicting reports concerning the response of roots to H^+ concentrations suggest a threshold effect for different root types and different plant species which appears to be related to both the buffering capacity of the soil environment and the plant's ability to change the acidity of its environment. If the plant is growing in an environment that is well buffered and the plant only mildly acidifies the rhizosphere, then an increase in the H^+ content of the soil as a result of acid rain may be advantageous to the plant. However, if the soil has a poor buffering capacity and the plant decreases the pH of the rhizosphere considerably, then a slight increase in the acidity of the soil as a result of acid rain may cause the pH of the soil to drop below a pH of 3.4, which will damage the cell membranes. This will influence both root elongation and nutrient acquisition.

It has been found that acid rain can cause inorganic Al^{3+} to be

released from the soil particles, thus transforming the Al^{3+} into a soluble form, (see Chapter 4). Aluminium is highly toxic and has been found to affect root growth negatively. Godbold *et al*, (1988) reported that the rate of root elongation of *Picea abies* seedlings, exposed to Al^{3+} at 800 or 1200 µmol dm^{-3}, was inhibited by 63-73%. This response is thought to result from the substitution of Ca^{2+} by Al^{3+} on the cell wall. A decrease in Ca^{2+} on the cell wall could alter both cell division and cell elongation in the roots because Ca plays such vital roles in these processes.

The presence of Al^{3+} can affect the branching of fine roots which results in the roots becoming "stubby and spatulate" (Foy, 1974b). Aluminium can inhibit root growth by binding to the DNA of root meristematic cells (Slaski, 1989), or by decreasing the extensibility of cell walls by cross linking the pectins in the middle lamella (Foy, 1974b). Hence one would expect root elongation to decrease with increasing Al^{3+} content as a result of increasing acidity in the rain. However the root biomass per unit length of root may increase as a result of soil acidification if the aluminium did increase the "stubbiness" of the roots.

The deposition of nitrogen in the acid rain can also affect root growth. It has been suggested that the energy that is utilized to convert the increasing amounts of nitrogen to protein can deplete the carbohydrate reserves thus reducing substrate availability for root growth (Dean *et al*, 1990).

The requirement of roots to have Ca^{2+} in their immediate environment in order to grow (Loneragan, 1979) is another means by which acid rain can affect the rate of root elongation. The reason for this is that acid rain can decrease the Ca^{2+} content in the soil as a result of increased cation leaching (discussed in Chapter 4). If the amount of Ca^{2+} in the soil should decrease below the critical concentration as a result of acid rain, this would have a negative effect on the rate of root elongation.

In this investigation the conflicting effects of increasing H^+,

Al^{3+} and nitrogen concentrations, as well as the very low concentrations of Ca^{2+} in the soil, might have resulted in there being little change in root elongation into soil that had been exposed to artificial rain of various acidities.

5.4.3 Root biomass

Root biomass production was not affected with respect to the acidity of the soil that was exposed to artificial acid rain. Artificial rain of pH 3 has been found to decrease the root dry weight of Pitch pine (Schrr, 1987). On the other hand, artificial rain of pH 3.8 was found to have no effect on either coarse or fine root biomass of loblolly pine (Simmons and Kelly, 1989). Increasing amounts of Al^{3+} have been found to significantly reduce the root dry weight of *Picea abies* seedlings.

In this investigation, the root biomass did change with respect to the Mg^{2+} content of the soil. There appeared to be an increase in biomass per unit length of root with respect to an increase in soil Mg^{2+} content (Fig. 5.3.2). This could be a result of increased activity in the cells of the root because Mg^{2+} is such an important cofactor of many enzymes. This trend was not however statistically significant.

The general lack of root response to the soil conditions could be due either to the range of conditions being too narrow to elicit a detectable response, or, as has been suggested by Simmons and Kelly (1989), be due to genetic control of root growth being greater than the treatments imposed. Should the latter be the case, then if the roots were exposed to the treatments for a couple of years instead of one growing season they might respond to the continued stress in a different manner.

5.4.4 Mycorrhizal colonization

The symbiotic relationship between plants and mycorrhizas confers a number of advantages on the host. Probably the most important of these is the acquisition of mineral nutrients by the fungus which are then made available to the host.

Reich *et al*, (1985) proposed that acid rain has the potential to affect this relationship in the following ways. Soil acidification or altered rhizosphere conditions can affect the growth, or infectivity of the mycorrhizas. Changes in carbohydrate production by the host or resource allocation of photosynthate to the root caused by acid rain could alter the level of mycorrhizal colonization. Nitrogen in the acid rain can act as a fertilizer which would change the amount of mycorrhiza infection that occurs (Schütt and Cowling, 1985). Ammonium has been found to reduce mycorrhizal colonization slightly, while nitrate had no effect on mycorrhizal development (Jentschke, 1991b).

In addition, the [illegible]dity of the soil, as well as the associated factors [illegible]anges in Al^{3+} concentrations, not only affect the rate [illegible] growth but also the germination potential of the fungal [illegible]Dighton and Skeffington, 1987). This could influence the [illegible] of mycorrhizal colonization that occurs.

It has also been suggested that acid rain could affect the equilibrium status that exists between deleterious microflora (which exist in all soils) and the mycorrhizas (Perrin and Estivalet, 1989). Such a change in the equilibrium balance could alter the survival of the mycorrhizas in the soil. Alternatively the ability of the mycorrhiza to successfully compete with other soil organisms for nutrients may be altered (Vogt *et al*, 1991).

It should also be noted that an increase in the H^+ concentration in the soil as a result of acid rain could enhance mycorrhizal formation because ectomycorrhizas are generally acidophilic (Shafer *et al*, 1985; Hung and Trappe, 1983). There may also be a difference in the optimal pH for mycelial growth and the optimal pH for mycorrhizal colonization (Erland and Söderström, 1990) so in order for the mycorrhizas to benefit the plant, the pH of the soil should be in such a range as to include both these optima.

This investigation showed that the percentage mycorrhizal colonizacion by all species did not vary with respect to the different soil compositions which resulted from exposure of the soil to acid rain. However, the species composition of the mycorrhizal communi., was responsive to the different soil compositions. In the present study seven different species of mycorrhizas could be distinguished colonizing the roots that had grown into the root ingrowth bags. These different types are illustrated in plates 1 to 7.

Other experiments show that the response of mycorrhizas to acid rain can be highly variable. Reich *et al*, (1985) showed that the percentage of roots infected by mycorrhizas decreased in a linear fashion with increasing acidity of the rain in mildly acidic soils (pH 6.3). However the response in strongly acidic soils (pH 4.1) was a quadratic relationship with a maximum mycorrhizal response occurring when the rain had a pH of 4. They suggested that the effect of the acidity on the mycorrhizas was highly dependent on the pH of the soil: when the rain was far more acidic that the soil, the effect of the acid rain on the mycorrhizas would be greatest.

It is more likely that the type of soil rather than the acidity of the soil is important in determining the degree of mycorrhizal infection with respect to the varying acidity of the rain. The reason for this is that the soil type will affect the buffering capacity of the soil, the ion content of the soil as well as many other aspects.

Shafer *et al*, (1985) also found that the response of mycorrhizas to simulated acid rain fitted a quadratic relationship. However in this case the percent ectomycorrhizal colonization was least when the rain had a pH of 4 and greatest at pH 2.4. Meier *et al*, (1989) found that the total number of ectomycorrhizal tips tended to increase with increasing acidity of rain. Simmons and Kelly (1989) found that when the simulated rain had a low pH, the degree of mycorrhizal colonization was greatest.

Although the percentage of the root length colonized by the different mycorrhizal species remained constant in this study, the individual types did respond differently to the change in soil composition as a result of the acid rain. Species 4 was found to be sensitive to the Al^{3+} content of the soil (Fig. 5.3.4), while species 5 was found to be sensitive to the pH of the soil (Fig. 5.3.5).

The variation in species response is similar to the findings of Meier *et al*, (1989); Simmons and Kelly (1989) and Dighton and Skeffington (1987) who all found that certain mycorrhizal species respond differently to the deposition of acidic rain. Similarly Erland and Söderström (1990) found that liming and the consequent change in soil pH influenced the degree of overall mycorrhizal infection as well as the relative frequencies of the different mycorrhizal species.

This variation in species response may be accounted for by differences in physiological attributes of the different species, thus enabling them to tolerate different soil acidities and ion concentrations.

The nutrient status of the trees may still be altered even though the overall degree of mycorrhizal infection has not been affected. The reason for this is that the change in the soil composition as a result of the acid rain, can modify the distance that the mycorrhizal hyphae extend into the bulk soil, as well as the ability of the hyphae to extract nutrients from the soil. Thus, although the percentage of mycorrhizal colonization is unchanged, the ability of the mycorrhizas to supply the host with adequate nutrients can still be affected.

The potentially adverse effect of acid rain on hyphal elongation can have another negative implication for plant growth. The rhizomorph tissue of fungi contains high concentrations of Ca^{2+}, nitrogen, phosphorus, sodium and zinc. Thus the fungal hyphae act as a sink for nutrients (Smith, 1980). It has also been

found that rhizomorph tissue is able to hold 99,9% of the elements measured against a leaching force equivalent to one years precipitation (Smith, 1980). Hence if the hyphal network is damaged as a result of acid rain, large amounts of nutrients could be lost as a result of leaching.

5.5 CONCLUSIONS

Soil that has been acidified with various amounts and acidities of artificial rain was found not to significantly affect root growth, dry root biomass per unit length or the degree of mycorrhizal colonization. There does however appear to be a positive relationship, although not significant, between root mass per unit length and the Mg^{2+} content of the soil. Since Mg^{2+} is a cofactor of many enzymes, an increase in the soil Mg^{2+} levels could result in increased biomass production.

The change in soil pH and acidity, as well as the changing concentrations of Al^{3+}, Ca^{2+}, Mg^{2+} and K^{+} in the soil, resulting from the application of artificial acid rain, was accompanied by a change in the mycorrhizal species composition present on the roots. This is in spite of the fact that the overall degree of mycorrhizal colonization was found not to vary with any of the soil parameters examined. One species was found to be negatively correlated with the Al^{3+} content of the soil, while another was negatively correlated with the pH of the soil. The change in species composition could have important consequences to the nutrient status of the trees.

CHAPTER 6 MAGNESIUM AND CALCIUM UPTAKE BY *PINUS PATULA*

6.1 INTRODUCTION

Plants require relatively fixed amounts of nutrients in order to grow. Although some nutrients may be transferred to the growing region from older senescing parts of the plant, an increase in plant size generally requires that the plant obtains the appropriate quantities of nutrients from an external source. The mineral elements immediately available to the plants are present in the soil solution, which is in turn in equilibrium with ions which are adsorbed onto the soil exchange surfaces.

Plants absorb ions only from the rhizosphere, i.e. the soil immediately adjacent to the root surface. As a result of the uptake process, as well as the presence of exudates and cells sloughed from the root, the soil in the rhizosphere has a different composition from the bulk soil.

It is generally thought that nutrient acquisition must be a predominantly active process (evidence for this assumption is discussed in Section 2.4.1). One possible exception to this generalization, pertinent to this study, is the uptake of calcium (Ca). Calcium is thought to enter the cells passively and be actively extruded from the cells (Clarkson and Hanson, 1980; Kubowicz *et al*, 1982; Poovaiah and Reddy, 1987; Haynes, 1990). Such a mechanism of passive Ca influx, and active efflux, is thought to occur in ectomycorrhizas as well (Lapeyrie and Bruchet, 1986).

Energy requiring "carrier" molecules, embedded in the plasma-membrane, are involved in the movement of ions from one side of a selectively permeable membrane to the other. Carriers and their functioning have been defined by Epstein (1972) as 'subunits of the membrane [which] bind the ion at the external face of the membrane, forming a carrier-ion complex. This complex then transverses the membrane, rotates within the membrane, or

undergoes some other spatial rearrangement within the membrane as a result of which, the ion is brought to the inner side of the membrane. Along with, or after, this change the carrier-ion complex breaks down as a result of a change in molecular configuration of the carrier so that the ion is released into the "inner" space'. The selectivity with which ions are taken up can be determined by differential affinities of active carrier sites for different ions (Epstein, 1976). The different affinities can result from conformational changes in the structure of the membrane or ion carriers (Maas *et al*, 1969). The term ion carrier is used in the same sense as ion pumps, i.e. as transporters of ions that utilize energy, as opposed to ion channels which allow for the passive movement of ions.

The kinetics of ion transport are usually described in terms of concepts that were developed in enzymology. This is as a result of a number of parallels between the two processes. Firstly, both processes undergo the formation of a transitory substrate-enzyme or substrate-carrier complex, and secondly, with graded substrate concentrations both show saturation kinetics. They differ in that generally enzymes bring about the chemical transformation of the substrate molecule, while carriers merely transport an ion across a membrane (Epstein, 1972).

Nutrient acquisition is dependent on the external ion concentration and energy supply, as well as the characteristics of the carrier. Examples of the latter factor include the inhibition, or stimulation, of the carrier by another molecule, or competition for the carrier site by another molecule or ion. These concepts are similar to those in enzymology when the rate of the enzyme reaction is altered as a result of the presence of a molecule other than the substrate molecule.

If acid rain can effect a change in the action of the ion carriers in the roots of *Pinus patula*, this could result in a decrease in the rate of ion uptake. This experiment aimed to investigate the uptake kinetics of magnesium (Mg) and Ca by *P.*

patula and to determine whether acid rain has the potential to affect the acquisition of these nutrients, (Fig. 6.1.1).

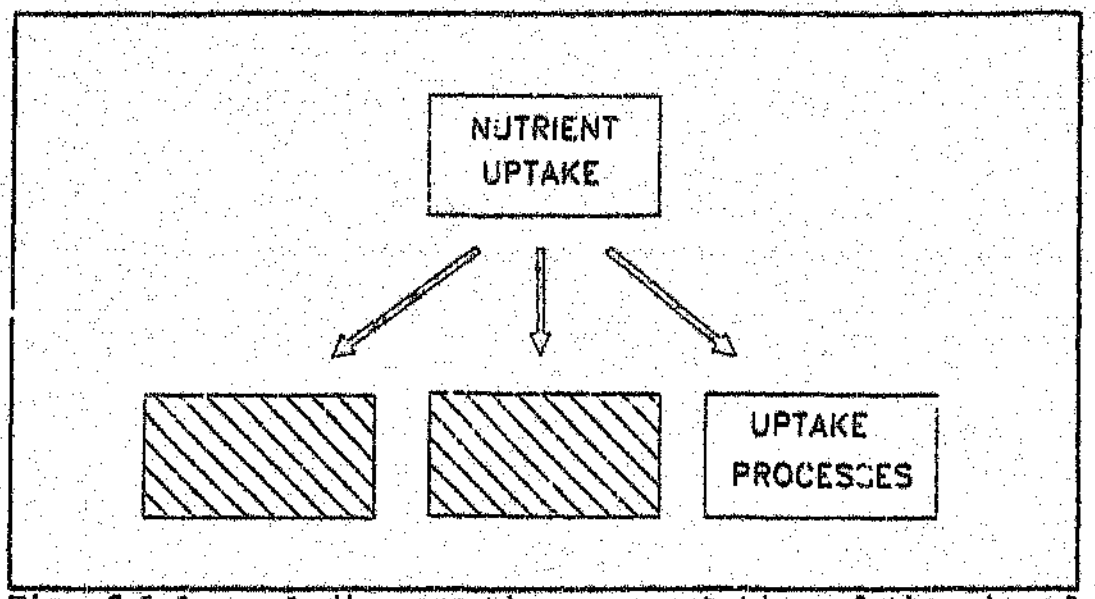

Fig. 6.1.1 A diagrammatic representation of the aim of this experiment in relation to the rest of the investigation.

The method that was used involved constructing a planar rhizosphere for seedlings. This rhizosphere which was paral l to the root surface, was then sectioned and analyzed, in order to determine the ion concentrations of the soil adjacent to the root surface and throughout the rhizosphere. By varying the concentrations of the ions in the bulk soil (referred to as treatments in this discussion), the uptake kinetics of the ions can be investigated. It should be noted that although the term "rhizosphere" is used, it is actually a "mycorrhizal-rhizosphere".

If the uptake of ions by plant roots is slower than the rate at which the ions are transported to the root surface then the ions will accumulate at the surface of the root. This has been found to occur in some soils for Ca and Mg (Dunham and Nye, 1976; Marschner, 1986). Conversely, the rate of ion uptake may be greater than the rate at which the ions are supplied to the root surface which results in the soil adjacent to the root surface

having a lower ionic concentration than the bulk soil. This has been shown to occur for potassium uptake (Dunham and Nye, 1976; Kuchenbuch and Jungk, 1982). Figure 6.1.2 illustrates these two scenarios.

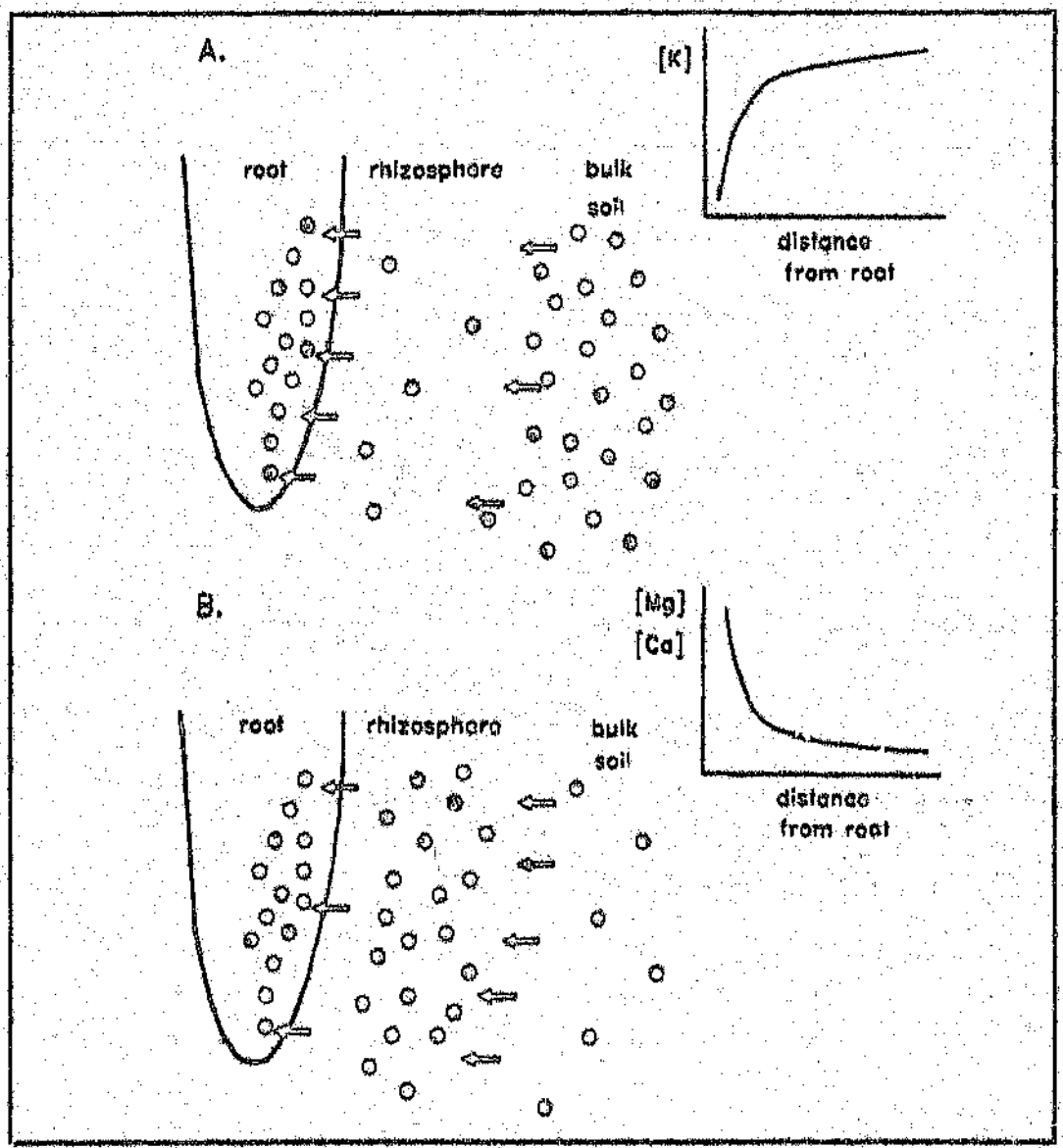

Fig. 6.1.2 Ion uptake can occur at a greater rate compared to the rate at which ions are transported to the root surface (A), or they can be taken up at slower than the rate at which they arrive at the root surface (B).

Figure 6.1.3, which shows a simplified box diagram of the process of nutrient uptake, helps to illustrate the processes involved in these two scenarios. It should be noted that R in the diagram

consists of a continuous series of compartments (R_1, R_2, R_3, ..., R_n) where R_1 is adjacent to the root surface and R_n has an ion concentration equalling that of B, the bulk soil. The rate at which ions are transferred from B to R is m and the ions move from R_2 to R_1 at rate m_1, from R_3 to R_2 at rate m_2, and so forth.

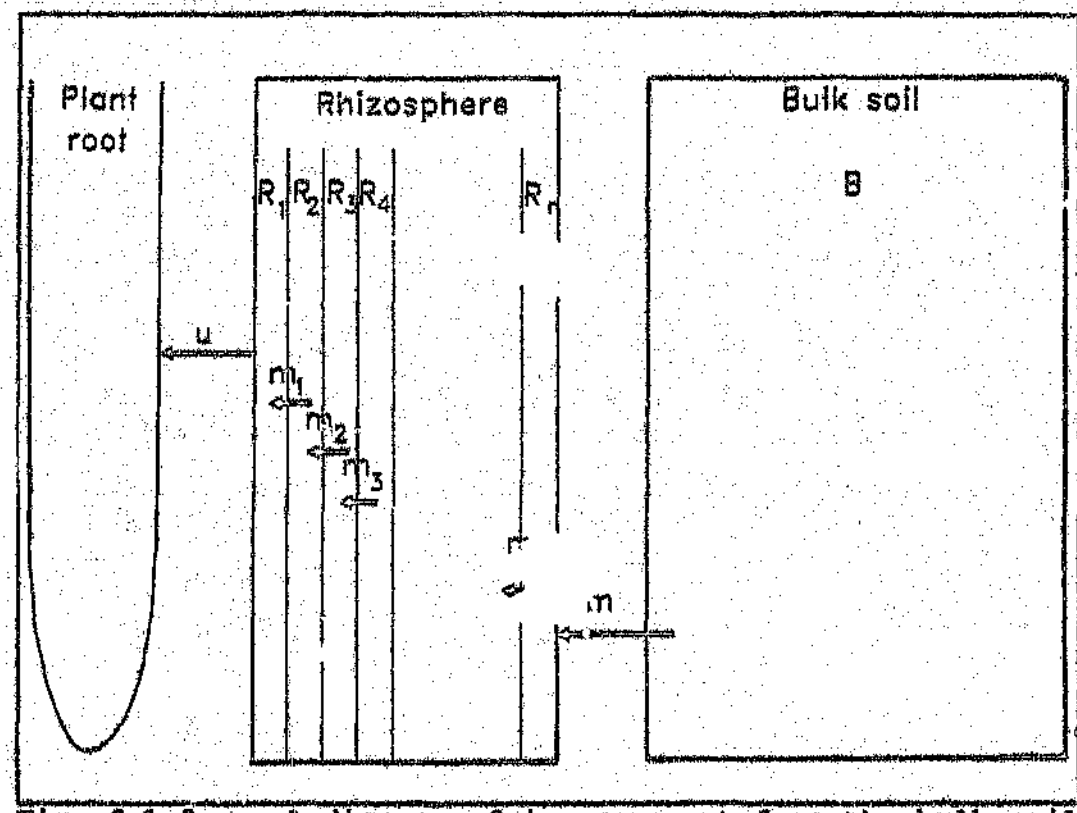

Fig. 6.1.3 A diagram of ion movement from the bulk soil to the rhizosphere, and through the rhizosphere to the root surface. (Symbols explained in text).

The factors which influence the uptake process but which were controlled or maintained at a constant value during this experiment so that their influence on the uptake processes that were being examined could be minimized, are mentioned in the ensuing description of the uptake process.

The rate at which ions are taken up by the plant roots is designated u, and is dependent on a number of factors. Firstly, the concentration of ions at the root surface, R_1, affects u. The reason for this is that as the ion concentration at R_1 increases,

so too will the number of filled carriers on the root surface increase. This occurs up to the point when all the carriers are full, after which any increase in ion concentration at R_s will have no effect on the rate of ion transport. When the carriers are full the saturation kinetics that are often observed in nutrient uptake studies would be obtained (Marschner, 1986).

Secondly, u can vary as a result of the characteristics of the carriers. For example, the presence of an inhibitory or stimulatory ion at the root surface can alter the rate of action of the carriers and hence the rate of ion uptake by the plant (Epstein, 1972). In addition, any ion that competes for the active site on the carrier, can also have a negative effect on u.

The metabolic energy requirement of the ion carrier needed to drive the nutrient uptake process can also influence u (Epstein 1972). If insufficient energy is available, u will decrease even if all the other factors controlling ion uptake are optimal. This factor was assumed not to vary in the experiment in this study, since all the treatments were exposed to the same environmental conditions.

The rate at which ions move from the bulk soil into the rhizosphere, where they are available to the plant, is denoted by m. It is actually the sum of two processes, namely diffusion and mass flow (Nye and Tinker, 1977). The relative importance of these two terms varies with respect to the soil type, soil water potential and the concentration of ions in the soil. In this experiment it was thought that mass flow was the more important, in that diffusion would take place very slowly compared with mass flow. The reason for this is that evaporation from the soil surface, as well as a water deficit resulting from transpiration would cause water to move through the soil relatively rapidly. In this way ions dissolved in the soil water are supplied to the roots.

Ion movement by mass flow from the bulk soil to the rhizosphere,

is dependent on both the concentration of ions in the bulk soil, since the adsorbed ions are in equilibrium with the soil solution, and on the rate of water movement to the root surface (Marschner, 1986). In this experiment, the rate of water movement was constant throughout the range of concentration values for B, so this component can be ignored. However the concentration of ions in B was made to vary during the experiment. Thus m can be thought to be a function of B, that is:

$$m = f(B) \qquad 6.1$$

The rate of movement of ions through the rhizosphere compartments occurs by mass flow. The rate of movement from R_2 to R_1 is designated m_1, while the rate of movement from R_3 to R_2 is m_2, and so forth. It should be noted that m occurs independently of u.

Any change in the concentration of ions in R is thus the difference between those transported from B at a rate m, and those taken up by R at a rate u, that is:

$$R = (m - u)t \qquad 6.2$$

where t is the time period involved.

When $u > m$, then a zone of depletion forms against the root surface, that is:

$$R_1 < R_2 < R_3 < \ldots < R_n \qquad 6.3$$

In this situation the rate of uptake by the plant would be dependent on the rate at which the nutrierts are transported to the root surface (Nye, 1977).

On the other hand, if $u < m$, then the ions tend to accumulate against the root surface, implying that:

$$R_1 > R_2 > R_3 > \ldots > R_n \qquad 6.4$$

In this case the rate of ion uptake is determined by the characteristics of the carriers on the root surface.

In many soils Ca and Mg tend to accumulate at the surface of the root as a result of the uptake of these ions being slower than the rate of supply (Dunham and Nye, 1976; Marschner, 1986).

However, since the soils being examined in this study are so nutrient deficient, (see Chapter 4), it was unknown whether these ions would accumulate, as is commonly found, or whether zones of depletion would form as a consequence of the low ion concentrations resulting in only small amounts of ions being transported to the root surface, resulting in the rate of uptake being greater than the rate of supply.

The concentration profiles of Mg and Ca in the rhizosphere give information about the uptake kinetics of these ions by *P. patula*. These profiles were used to determine the possible effects that acid rain could have on the uptake of Ca and Mg.

6.2 MATERIALS AND METHODS

6.2.1 Experimental design

The experimental design was based on that by Kuchenbuch and Jungk (1982) and Dunham and Nye (1976), but was modified so that the uptake of nutrients by a tree could be monitored.

Two *Pinus patula* seeds were planted in pots containing soil obtained from the site. If both seeds germinated, one seedling was weeded out. Mycorrhizal colonization occurred automatically as a result of fungal spores and hyphal fragments in the soil.

In order to compare the rate of uptake of mycorrhizal roots, as apposed to roots without mycorrhizal colonization, some seedlings were grown in sterilized soil. (The soil had been autoclaved twice during a six week period.) However these trees suffered from acute phosphorus deficiency (the needles on the trees turned a deep purple colour) and died when they were six months old. Inspection of the roots showed them to be completely free of mycorrhizas. The significance of this finding will be discussed in Chapter 7.

The seedlings were grown in the Phytotron, (day length: 14 hours light, 10 hours dark; temperature: 23°C, 18°C; relative humidity:

50%; radiant flux density: 200μmol m^{-2} s^{-1}).

The pots the trees grew in, had a 50mm diameter and were 70mm high with a 40μm aperture polyethylene mesh base (40 PSE). The seedlings were watered with Long Ashton Nutrient solution, (see Appendix 8.1). The experiments were conducted when the trees were 11 months old, at which stage the roots had formed a mat along the mesh base of the pot. This was the planar rhizosphere.

A diagrammatic representation of the experimental procedure is given in Fig. 6.2.1. The pots containing the trees were placed on top of a pot of sieved soil. (The soil had been passed through a 0.50mm sieve.) This pot had the same diameter as the pot containing the tree but was only 250mm high. The soil in the soil pots was adjusted to various ion concentrations in the following way. Soil that had been dried and sieved was placed in a polythene bag with a solution containing the appropriate ion concentration in order to give the soil a 40% moisture content. The bag was regularly shaken during a 10 day period in order to ensure that the contents of the bags were well mixed. After this time the soil was packed into the pots at a dry bulk density of 0.61g cm^{-3}.

When the tree-containing pot was placed onto the soil pot, the process of root growth into soil that had not previously been exposed to uptake processes by the plant was simulated. The two pots were placed onto a sand tray. The water content of the latter was maintained at a constant level in order to minimize the downward water movement in the soil pot. As a result of evaporation from the top of the pots and transpiration by the trees, water moved up through the soil pots, thus supplying the roots with water and nutrients. This simulated the movement of ions in solution to the root surface. Such movements of water in the soil occur as a result of precipitation events and gradients set up by water deficits at the root surface which arise from the uptake of water.

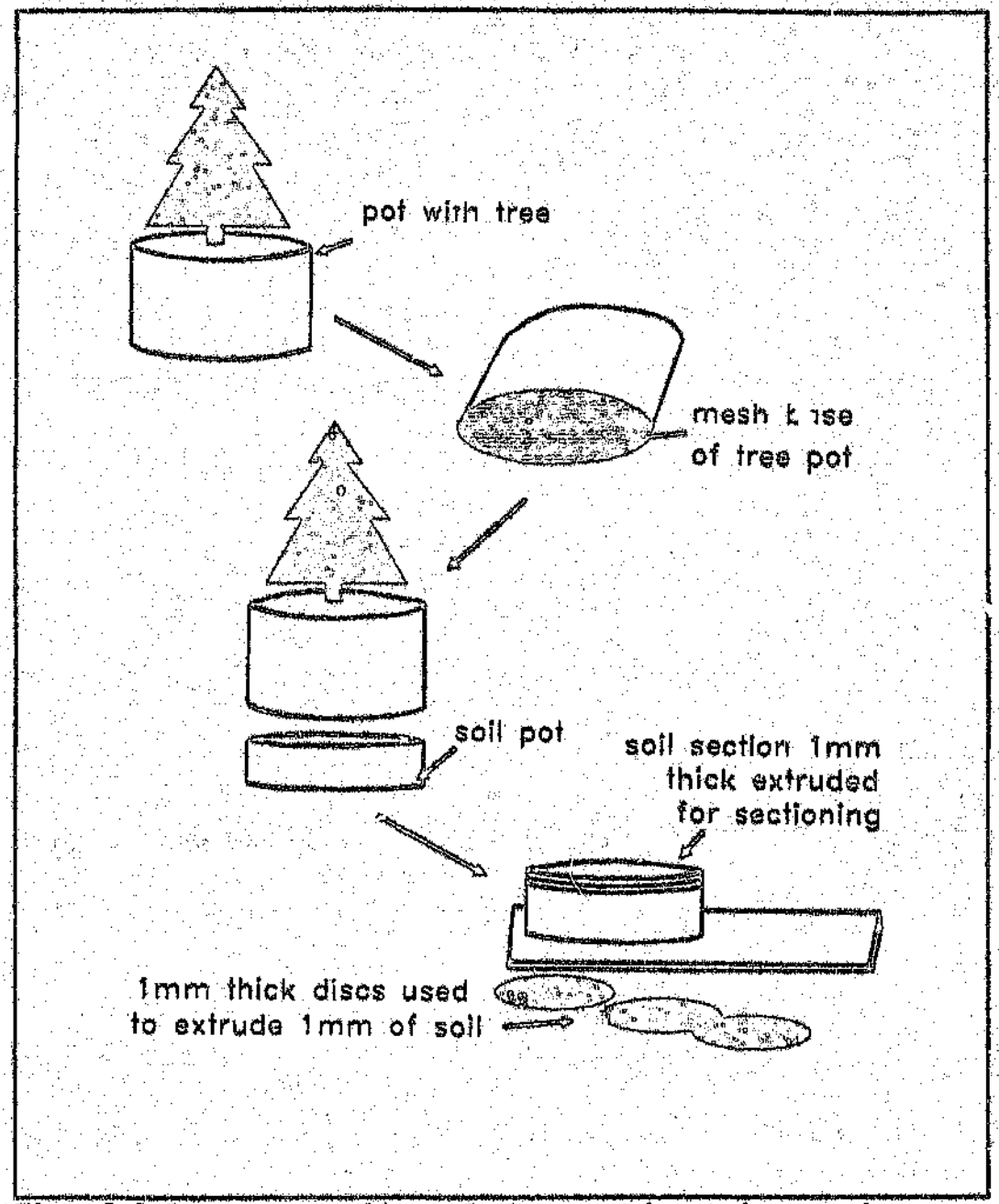

Fig. 6.2.1 An outline of the experimental procedure.

At the end of 15 days the pot with the tree was removed from the soil pot. The soil in the soil pot was sectioned in layers of 1mm thick. The sectioning was done by placing a 1mm thick disc under the soil in the pot and, by pushing the pot down, extruding 1mm of soil from the top of the pot. This 1mm soil section was scraped off and collected. The process was repeated by placing

another disc under the pot and extruding the next 1mm of soil. A total of 8 sections (ie. 8mm of soil) was obtained from each pot. The soil from each interval was then analyzed and the concentrations of the ion determined for each interval.

When the uptake of Mg^{2+} was determined, the soil was adjusted to approximately 1 times the original Mg^{2+} concentration (no adjustment); 1.5 times the original Mg^{2+} concentration; 2 times the original Mg^{2+} concentration; 3 times the original Mg^{2+} concentration and 5 times the original Mg^{2+} concentration. Magnesium sulphate was used to amend the soil. This was the greatest range of concentrations that could be used. If the concentration of Mg^{2+} in the bulk soil was greater than this, then the amount taken up was too small to be detectable against this large amount of Mg^{2+} in the bulk soil. These treatments are referred to as A, B, C, D and E respectively in the results.

To determine the effects of increased aluminium (Al^{3+}) concentrations on the uptake of Mg^{2+}, the Al^{3+} content of the soil was increased by 24.98 ± 0.24% by the addition of aluminium sulphate ($Al_2(SO_4)_3$).

When the uptake of Ca^{2+} was investigated, the soil was adjusted to approximately 1 times the original Ca^{2+} concentration (no adjustment); 2 times the original Ca^{2+} concentration; 5 times the original Ca^{2+} concentration; 10 times the original Ca^{2+} concentration and 15 times the original Ca^{2+} concentration. Calcium sulphate was used to amend the soil. These concentrations were chosen to obtain a wide range of concentrations in the bulk soil. For all of these conditions the amount taken up, relative to the amount of ion in the bulk soil, was large enough to be detectable under all these conditions. These increased concentrations are referred to as A, B, C, D, and E in the results and discussion.

There were 7 replicates for each treatment in each experiment.

6.2.2 Methods of soil analysis

The concentration of cations in the soil was determined using the following method based on that of Buys (1980). A mass of 1.2g of soil was weighed out and placed into a 100ml polyethylene centrifuge tube. To this 10ml 1M ammonium acetate was added. The stopper was placed on the tube and the tube was shaken for 30 minutes, after which the tube was centrifuged for 10 minutes at 2500 rpm in a Beckman model TJ 6 centrifuge. The supernatant was collected and 10ml 1M ammonium acetate added to the pellet. The stopper was replaced and the tube was shaken for a further 30 minutes. The tube was again centrifuged for 10 minutes at 2500 rpm and the supernatant was added to rest of the extract.

From this extract, a 2ml sample was taken to which 10ml of 0.1% lanthanum chloride and 8ml distilled water were added. The absorbances were read on an atomic absorbance spectrophotometer.

6.2.3 Data analysis

SAS/STAT release 6.03 was used to fit a rectangular hyperbola to the data of the ion uptake rate at the various ion concentrations in the bulk soil. The same computer package was used to perform a homogeneity of slopes test to determine whether the addition of Al^{3+} caused a significant change in the rate of Mg^{2+} uptake.

6.3 RESULTS

6.3.1 Magnesium concentrations in the rhizosphere

The amounts of Mg^{2+} present in the soil, as a function of the distance from the root surface, with respect to the different Mg^{2+} concentrations in the bulk soil are illustrated in Fig. 6.3.1. When no Mg^{2+} had been added to the soil, (Fig. 6.3.1A), the rate of Mg^{2+} uptake must have been approximately equal to the rate at which the ions were transferred to the root surface by means of mass flow. This is evident in that Mg^{2+} neither accumulated nor showed depletion shells at the root surface.

Figures 6.3.1B to E show that with increasing concentrations of

Mg^{2+} in the bulk soil, the amount of Mg^{2+} taken up, relative to the rate at which ions were supplied to the root surface by mass flow, must have increased. This is apparent from the zones of depletion which formed in the rhizosphere. These depletion shells are evident in the first 2 to 3mm of the rhizosphere.

6.3.2 Magnesium concentrations in the rhizosphere in the presence of increased aluminium

The uptake of Mg^{2+} in the presence of an increased amount of soil Al^{3+} was also investigated. An equivalent amount of 6.82mmol kg^{-1} Al in the form of $Al_2(SO_4)_3$ had been added to the soil. This increased the soluble exchangeable Al^{3+} content of the soil by 24.98 ± 8.24 % of the original amount present in the soil.

The uptake of Mg^{2+} by the trees at the various Mg^{2+} concentrations in the soil, as depicted by the concentration profiles in the rhizosphere, is illustrated in Fig. 6.3.2. By comparing Fig. 6.3.1C to E with Fig. 6.3.2C to E, it can be seen that the amount of Mg^{2+} taken up must have been depressed in the presence of increased Al^{3+} concentrations because the zones of depletion in Fig. 6.3.2, i.e. in the presence of increased Al^{3+}, are greatly reduced.

6.3.3 Calcium concentrations in the rhizosphere

The concentration profiles of Ca^{2+} in the rhizosphere are illustrated in Fig. 6.3.3. At low Ca^{2+} concentrations, similar to those found in the soil (Fig. 6.3.3A), the Ca^{2+} tended to accumulate at the surface of the root, while at high Ca^{2+} concentrations (Fig. 6.3.3D and E), the Ca^{2+} was taken up by the roots at a faster rate, which resulted in zones of depletion occurring at the root surface. The depletion shell extended for 3 to 4mm into the rhizosphere.

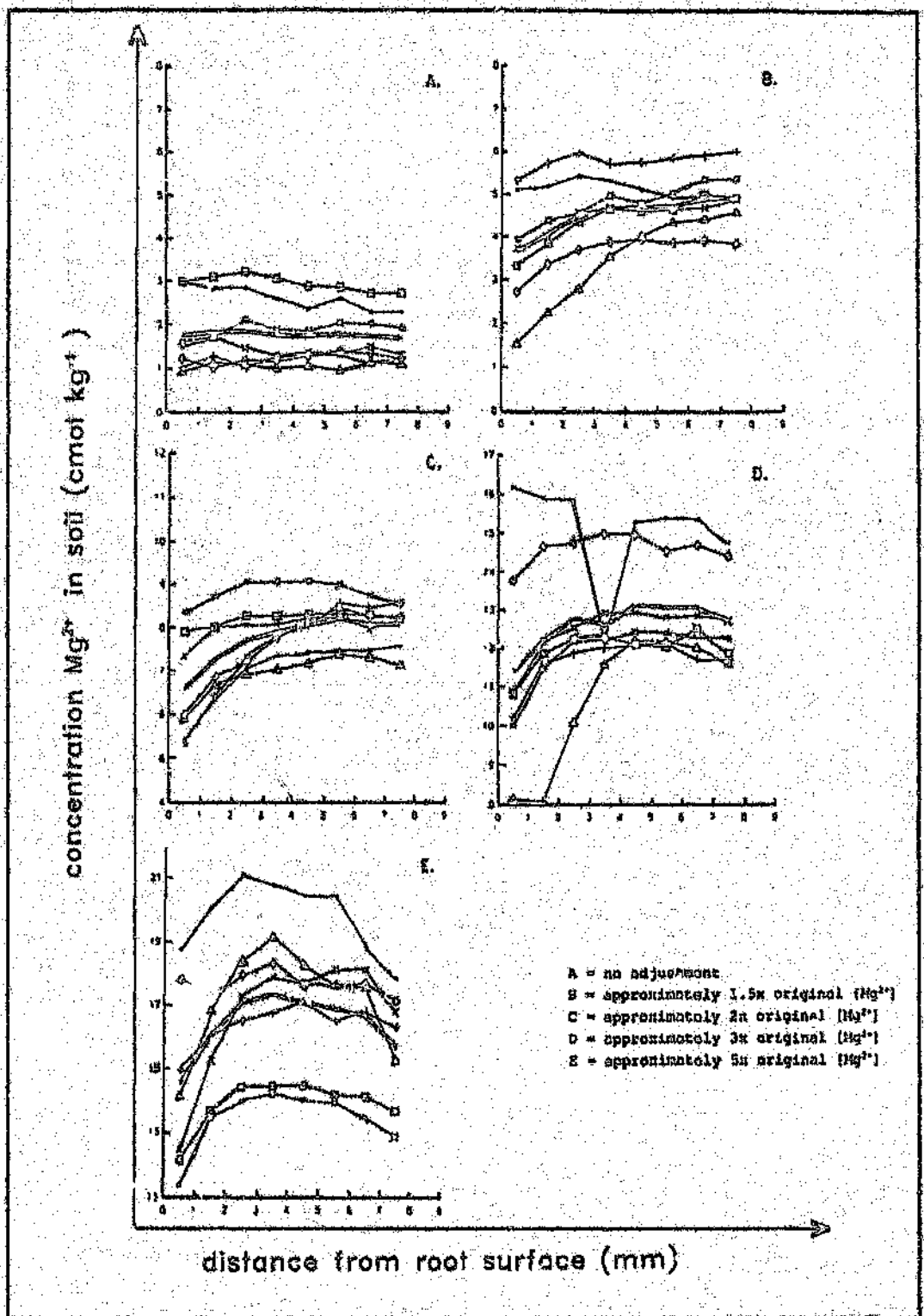

Fig. 6.3.1 Concentration profiles of Mg^{2+} in the rhizosphere when the concentration of Mg^{2+} had been varied. (Each curve represents the profile of one tree. The bold line indicates the mean for each treatment.)

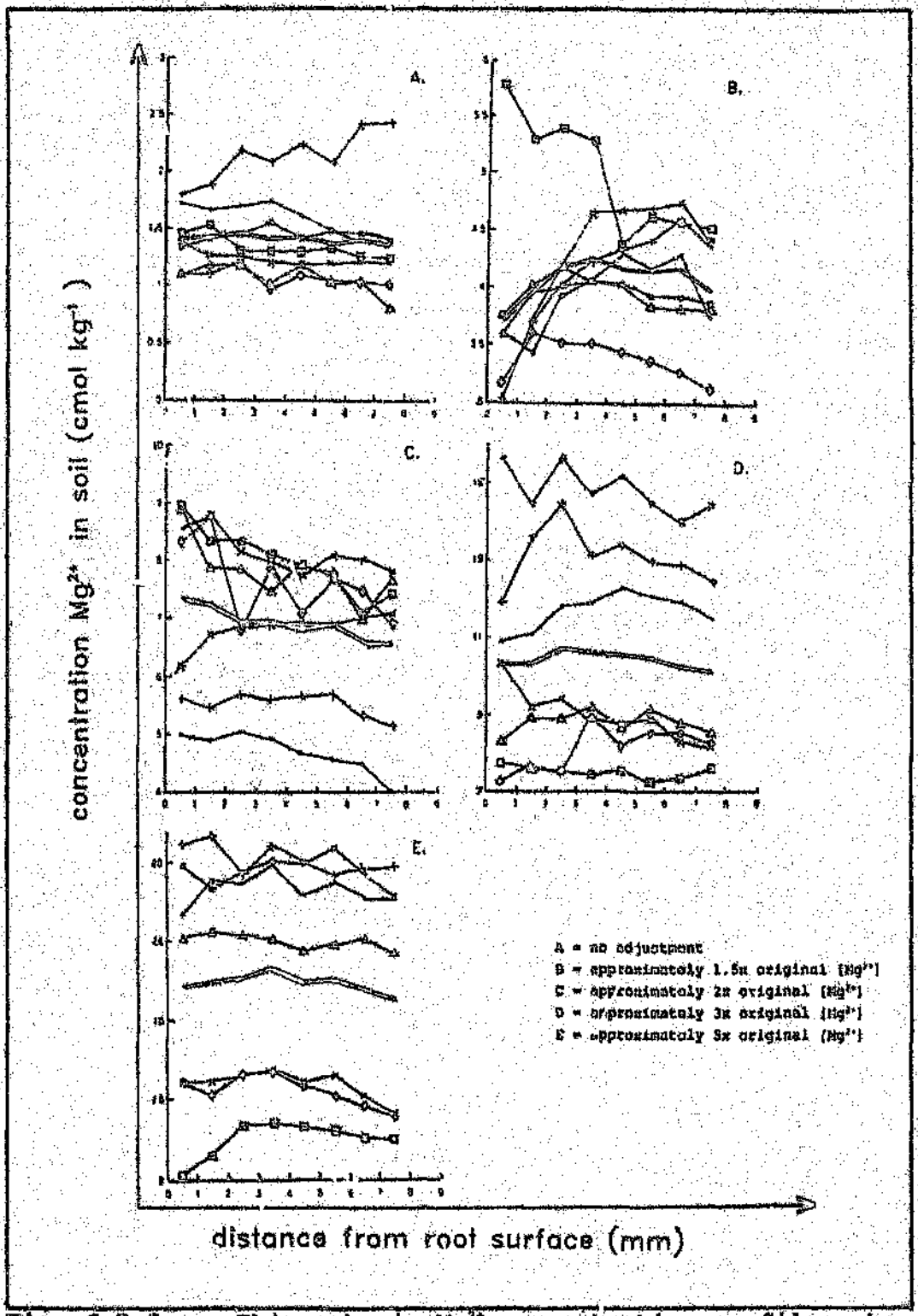

Fig. 6.3.2 Rhizospheric Mg^{2+} concentration profiles when the soil Al^{3+} content had been increased and the Mg^{2+} content was varied. (Each curve shows a profile for one tree. The bold line is the mean for each treatment.)

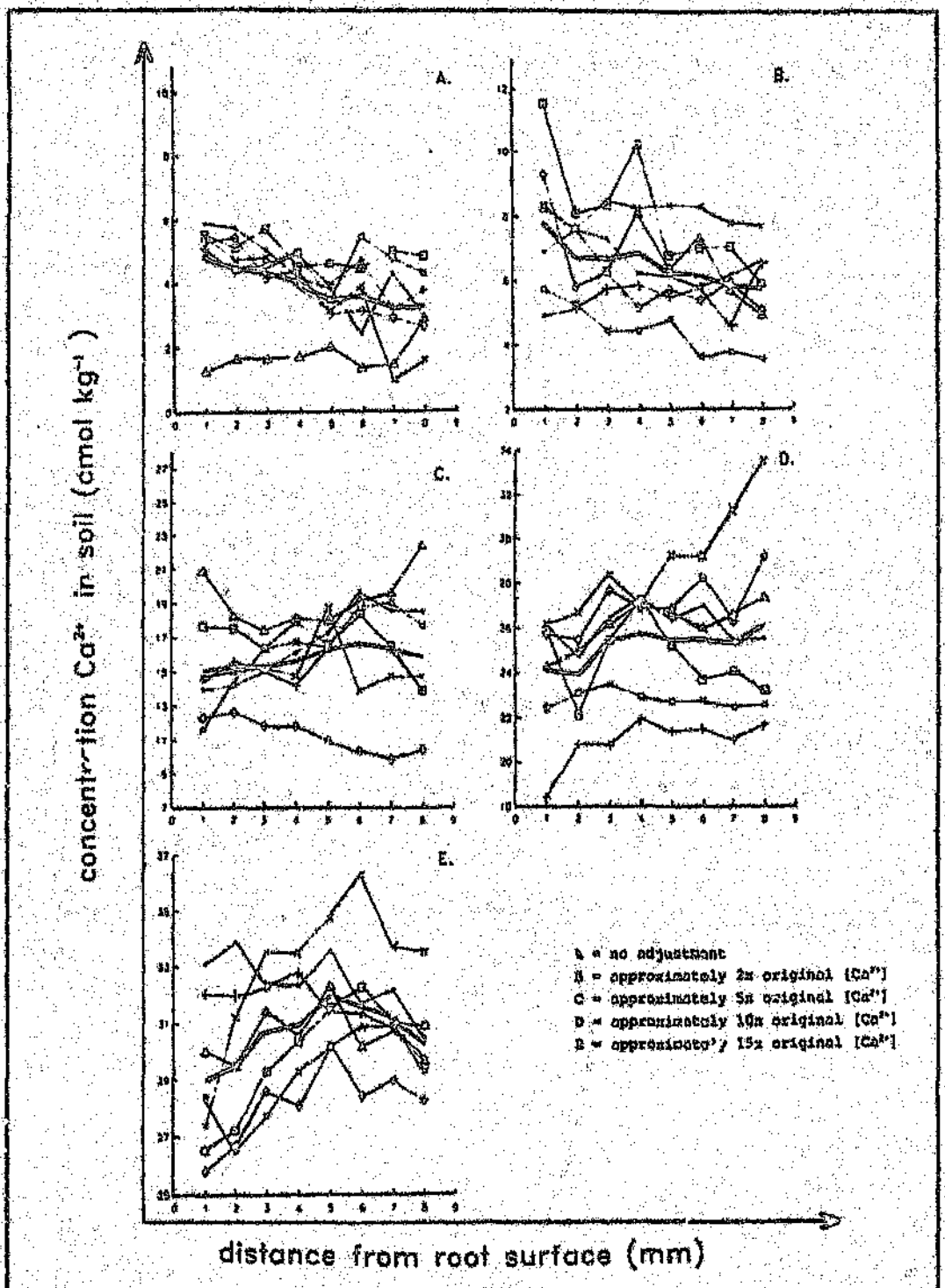

Fig. 6.3.3 Concentration profiles of Ca^{2+} in the rhizosphere when the concentration of Ca^{2+} had been varied. (Each curve represents the profile of one tree. The bold line indicates the mean for each treatment.)

6.3.3 Uptake rates

The rates of ion uptake were calculated for each plant in one of two ways depending on whether a zone of depletion had formed or whether the ion had accumulated at the root surface. These methods of calculation are described below.

A. When a zone of depletion in the rhizosphere had occurred, a peak in concentration in the rhizosphere generally formed: the ion concentration had increased as the distance away from the root surface had increased, until a peak was reached, after which, the ion concentration often decreased again. It was thought that this peak had resulted from two processes occurring simultaneously. These processes were i) the uptake process, resulting in a decrease in the ion concentrations, causing the depletion shell and ii) the process of ion transport to the root surface as a result of mass flow, which resulted in ions accumulating at the root surface. Thus, when these two processes occurred simultaneously, a peak in the ion concentration in the rhizosphere resulted. This is visualised in Fig. 6.3.4. The peak demarcated the edge of the rhizosphere, i.e. the maximum distance into the soil that had been adjusted by the presence of the plant roots.

When calculating the amount of ions that had been taken up, the amount of ions transported to the root surface by mass flow was assumed to be zero. This was due to the fact that the amount of ions transported to the root surface by mass flow could not be determined, because the rate of mass flow was dependent on the rates of transpiration and water evaporation from the top of the pot and these rates were unknown.

The amount of ions taken up was determined by extrapolating the peak onto the y axis so that the maximum amount of ions in the soil could be determined. The amount of ion taken up was then calculated by subtracting the amount of ion remaining in the soil, after the uptake process had reached equilibrium, from the maximum amount of ion that was calculated to be in the soil,

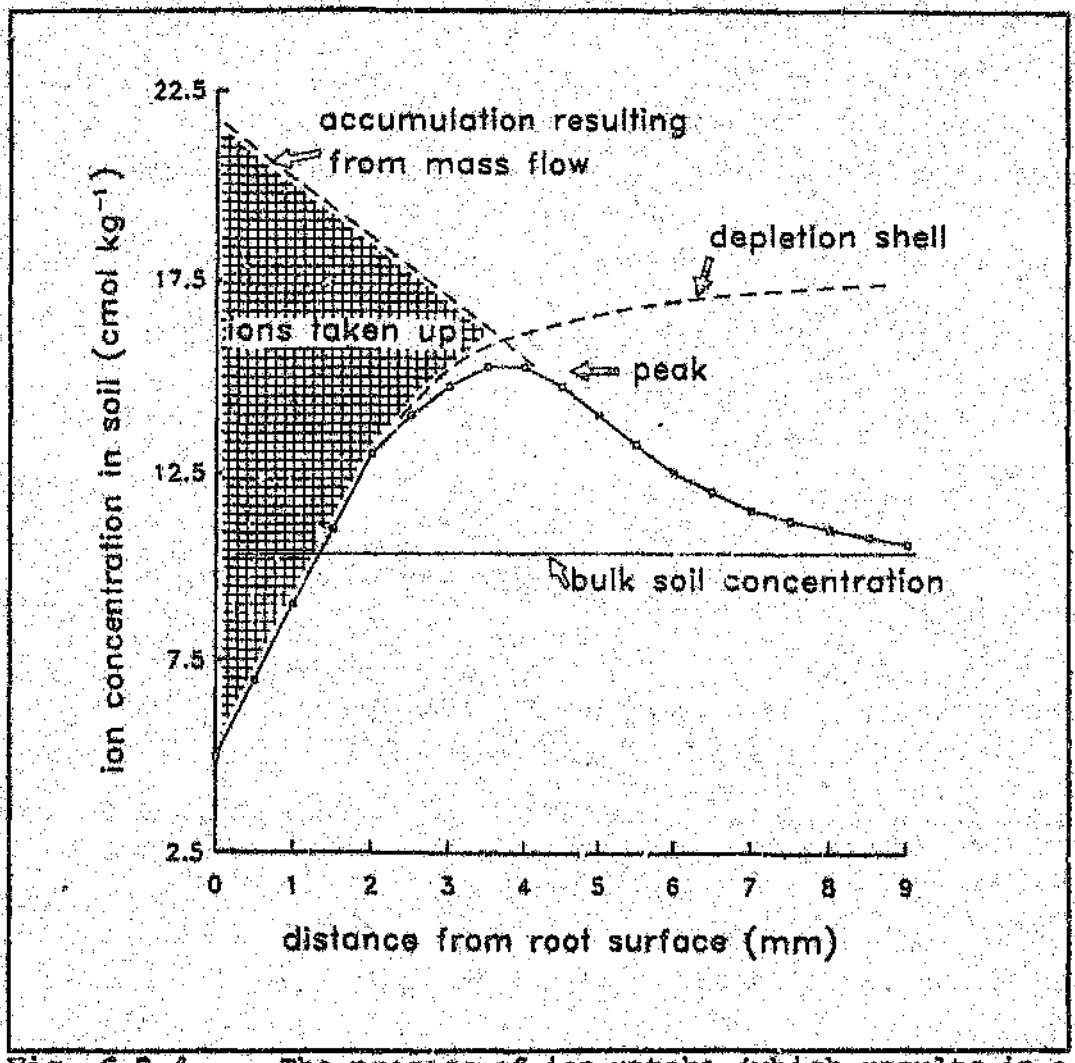

Fig. 6.3.4 The process of ion uptake (which results in a zone of depletion at the root surface) occurring simultaneously with mass flow (causing an accumulation of ions), results in a peak in the ion concentration in the rhizosphere.

(i.e. the concentration at the peak).

It should be noted that the amount that was calculated to be taken up is much smaller than the actual amount taken up. The reason for this is that the calculation does not take into account the amount of ions that were constantly transported to the root surface, by mass flow, throughout the time period. In order for a zone of depletion to be maintained, these ions were constantly being taken up.

B. When the ions accumulated at the root surface, portrayed by a decrease in ion concentration as the distance from the root surface increased, the amount taken up was calculated in a different manner. The ion concentration at the root surface was subtracted from the lowest ion concentration determined to be present in the rhizosphere. This gave rise to a negative value, which is smaller than the actual amount taken up because it does not take into account ions that might have been taken up at a slower rate than their arrival at the root surface. The sign merely indicates that the ions were accumulating at the root surface, rather than a negative uptake rate. In other words, the rate of uptake was less than the rate at which the ions were supplied to the root surface.

After determining the amount taken up, the uptake rate at th various concentrations over the fifteen day period, could be calculated.

The rates of Mg^{2+} and Ca^{2+} uptake, with respect to the concentration of that ion in the bulk soil, are illustrated in Fig. 6.3.5 and 6.3.7 respectively. The rate of Mg^{2+} uptake in the presence of an increased amount of Al^{3+} is shown in Fig. 6.3.6.

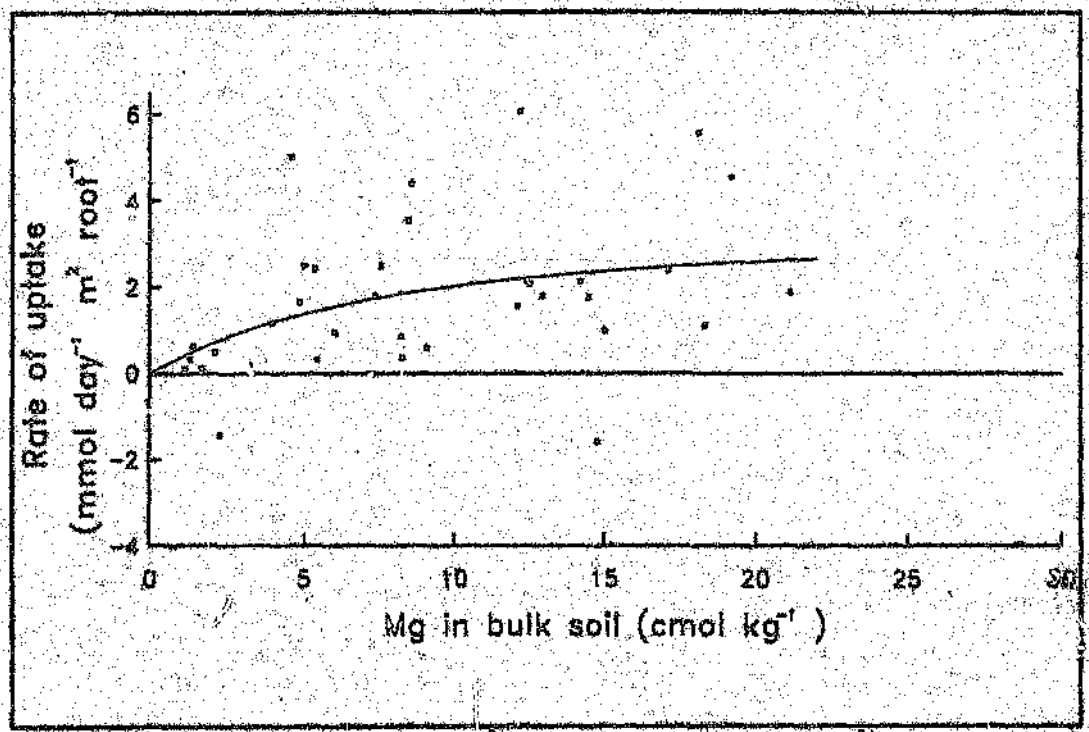

Fig. 6.3.5 Rates of Mg^{2+} uptake when the Mg^{2+} content of the bulk soil was varied. The curve shows a rectangular hyperbola that was fitted to the data (r^2 = 0.585).

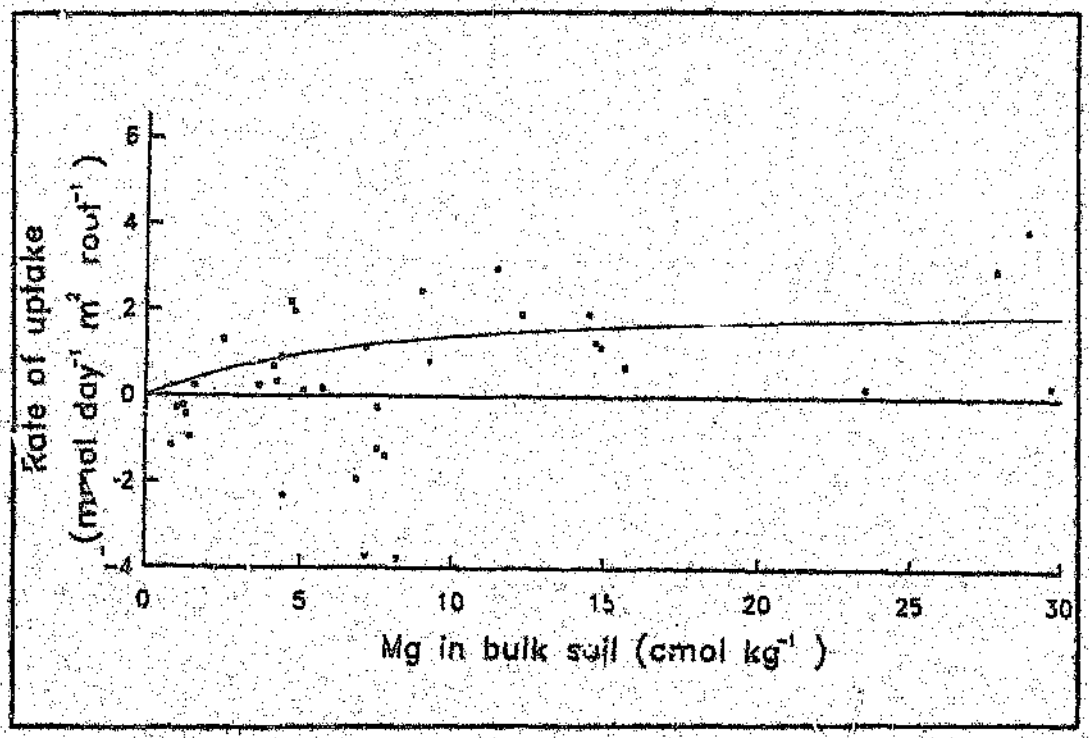

Fig. 6.3.6 Rates of Mg^{2+} uptake when the Al^{3+} in the soil was increased by 25% and the Mg^{2+} content was varied. The curve shows a rectangular hyperbola fitted to the positive data points (r^2 = 0.667).

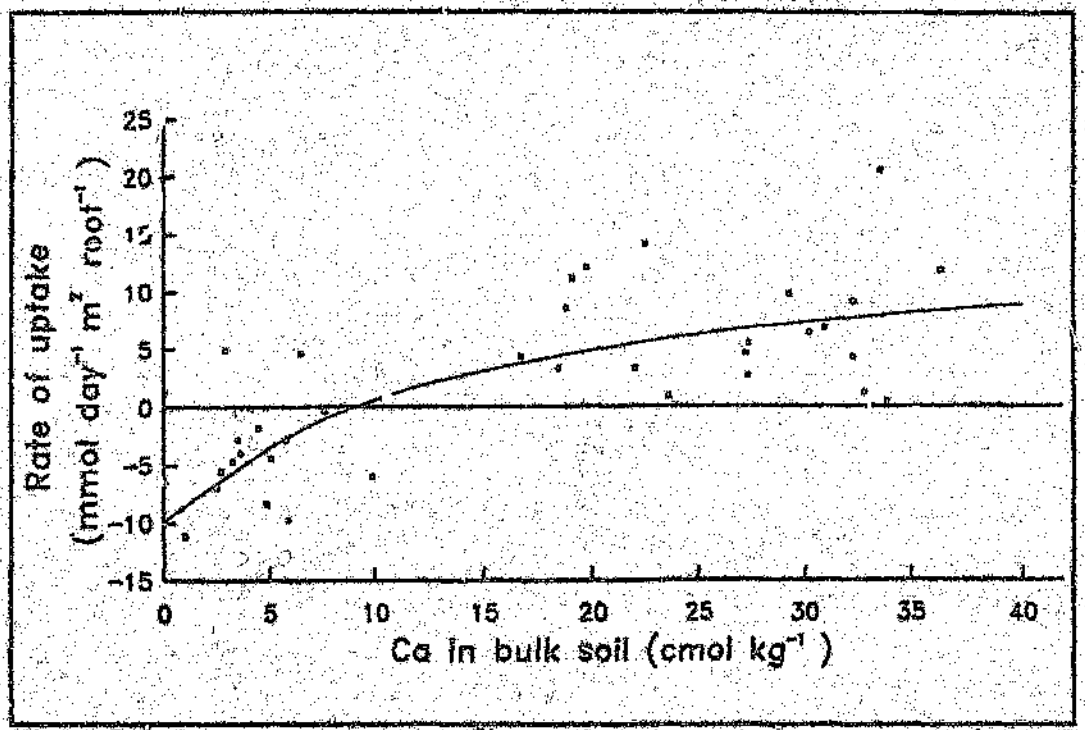

Fig. 6.3.7 Rates of Ca^{2+} uptake when the concentration of Ca^{2+} in the bulk soil was varied. The curve shows a rectangular hyperbola that was fitted to the data points (r^2 = 0.591).

Rectangular hyperbolas were fitted to all the data sets. The relevant equations and the calculated variables, r^2 values, F and p values are listed in Table 6.3.1. In determining the equation for Mg^{2+} uptake in the presence of increased Al^{3+}, the negative values were ignored in that they prevented the criteria for convergence from being met. The negative values were not however ignored when fitting the curve for Ca^{2+} because it was possible that active extrusion may be responsible for these values. As a result of the downward vertical shift of this graph, a constant was subtracted from the rectangular hyperbola.

Table 6.3.1 Summary of kinetic parameters for the uptake of Mg^{2+}, Mg^{2+} with increased concentrations of Al^{3+} and Ca^{2+} by 11 month old seedlings of *Pinus patula*. The constant is termed X in the third equation.

Ion	Equation for uptake rate (mmol m^{-2} day^{-1})	Vmax ± s.e. (mmol d^{-1})	Km ± s.e. (emol d^{-1})	constant ± s.e. (mmol d^{-1})	r^2	F / p>F
Mg^{2+}	$U = \frac{Vmax.C}{Km + C}$	3.494 ±1.720	6.935 ±8.359		0.585	23.28 / 0.01
Mg^{2+} + Al^{3+}	$U = \frac{Vmax.C}{Km + C}$	2.274 ±0.868	6.132 ±6.422		0.667	21.05 / 0.01
Ca^{2+}	$U = \frac{Vmax.C}{Km + C} - X$	24.516 ±4.779	13.338 ±14.596	9.894 ±4.823	0.591	15.14 / 0.01

The increased Al^{3+} content of the soil resulted in a suppression of the rate of Mg^{2+} uptake. This can be seen by comparing Figures 6.3.5 and 6.3.6. A linear transformation was performed on the uptake rates for Mg^{2+} in the presence of normal concentrations of Al^{3+} and increased amounts of Al^{3+}. (The negative values obtained for Mg^{2+} uptake in the presence of Al^{3+} were included in this test.) A homogeneity of slopes test using the transformed data showed that Al^{3+} significantly decreased the rate of Mg^{2+} uptake, ($F[3,66] = 24.86$, $p < 0.0001$).

6.4 DISCUSSION

6.4.1 Method

In the past most studies on nutrient uptake have been conducted using either plants growing in solution or excised roots, as opposed to whole plants growing in a soil environment. These methods are inadequate in that plants growing in solution do not take into account ion interactions with the soil particles. Soil-ion interactions effectively reduce the ion concentration in the soil (Pitman, 1976a) and thus should be taken into account.

When excised roots are used, ions which have been taken up can leak out through the cut and injured cells, which can give rise to underestimations of the amount taken up. In addition, the growth of the plant and ion transport to the shoot are important

processes which can alter ion uptake (Leggett and Gilbert, 1969), and are factors not taken into account when using excised roots.

In addition, the uptake rate at low concentrations in whole plants tends to be lower than those of excised roots (Pitman, 1976a). This is thought to be a result of the low concentrations impairing the growth of the whole plant, which in turn decreases the amount of ion taken up. The water content of the soil will change the rate of ion supply to the root surface, which will in turn affect the amount of ions taken up by the plant.

However many problems arise when examining the uptake of ions, by whole plants, from the soil. These difficulties include assessing the rate of uptake by roots that are continually extending into the soil. In addition the rhizosphere, from where the ions are extracted by the roots, extends only a few millimetres into the bulk soil and it is this small amount of soil that has to be analyzed to determine the degree of uptake that has occurred.

This experiment overcame these problems by creating a planar rhizosphere. This method increases the surface area of the rhizosphere so that the uptake of nutrients from the soil can easily be determined.

It was found that fungal hyphae often formed on the interface between the mesh and the soil. If these hyphae were not from the ectomycorrhizal fungi colonizing the pine roots, they could have been detrimental to the experiment in that the non-mycorrhizal fungi would be obtaining nutrients from the soil pot in a similar manner to the mycorrhizal fungi, which would result in an overlap of the zones of depletion.

In order to examine whether the fungal hyphae were from a member of the Basidiomycetes, the hyphae were stained with Cotton Blue in 1% Lactophenol. In addition to staining some of the hyphae from the bottom of the pots, soil from around the pine tree roots

that had ectomycorrhizas, were also stained. It was thought that if the hyphae contained clamp connections and septa then the fungus would probably be a Basidiomycete. (Ectomycorrhizas form largely from fungi which are members of the Basidiomycetes.)

Although clamp connections were rarely observed in both preparation, septa were observed. Rhizomorphs were also detected. These structures are generally associated with mycorrhizal Basidiomycetes. In addition the hyphae from under the pot were very similar in structure to the hyphae found in the soil from around the pine roots. Hence, it is thought that the fungal hyphae observed under the pots were probably hyphae from the ectomycorrhizas penetrating the mesh.

6.4.2 Magnesium concentrations in the rhizosphere

At low Mg^{2+} concentrations, (Fig. 6.3.1A), similar to those found in the soil at sampling, the uptake of Mg^{2+} tended to equal the rate at which the ions were transported to the root surface, resulting in neither accumulation nor depletion at the root surface. Thus in terms of Fig. 6.1.3, u was equal to m.

However, as Fig. 6.3.1B to E indicate, this trend was not consistent. As the Mg^{2+} concentration in the bulk soil, (B), increased, so the rate of uptake increased, such that zones of depletion formed at the root surface. Thus u became greater than m.

It was thought that if u had been greater than m, resulting in zones of depletion forming, then any increase in B would result in the amount of ions being transferred to R, as a result of m, increasing. An increase in u would result as the amount of ions at the root surface increases, until all the carriers become saturated, at this point u becomes constant. An increase in B would, by equation 6.1, result in an increase in m. Eventually since u is constant, but m is continually increasing, m will tend to be greater than u, resulting in ions accumulating on the root surface. Thus, the concentration profiles for Mg^{2+} uptake would

initially show zones of depletion, but as B increased, the Mg^{2+} would tend to accumulate at the root surface.

In other words, from equation 6.1, it can be seen that m is thought to be proportional to B. From equation 6.2, R will increase as m increases. This will result in u increasing as a result of more carriers being filled, until u is operating at a maximum velocity. Thus until saturation kinetics are achieved, u is proportional to B. After saturation, the degree of accumulation will be proportional to B.

However the finding that, in response to an increase in Mg^{2+} content of the bulk soil, the rate of uptake varied (initially being equal to the amount of Mg^{2+} arriving at the root surface, to relatively rapid which resulted in a zone of depletion forming), was not expected.

It was thought that if Mg^{2+} neither accumulated against the root surface nor a zone of depletion formed, (Fig. 6.3.1A), then u would have been equal to m. This according to Nye (1977), is indicative of the plant controlling the uptake of the ion, as opposed to the rate of soil transport restricting the uptake of the nutrients. Thus it was thought that the uptake process was in the transition between u being greater than m, to u being smaller than m, and thus, with any increase in B, the Mg^{2+} was expected to start accumulating at the root surface. However this did not happen. As B increased, u increased faster than m, resulting in zones of depletion forming (Fig. 6.3.1B to E).

The unexpected result that m was greater than u at very low concentrations, resulting in an accumulation, but as B increased u became greater than m, resulting in zones of depletion forming, tends to indicate that the increase in u with respect to B was not proportional. Rather, since u increased faster than m, as B increased, there might be an exponential relationship between B and u at low concentrations of B.

An initial exponential increase in u with respect to an increase in B will result in an uptake curve being sigmoidal rather than a rectangular hyperbola, that is usually recorded when determining uptake kinetics (Marschner, 1986). Figure 6.4.1 illustrates these curves with respect to the rate at which ions are transported to the root surface by mass flow. In this study, the variation in uptake rates by the trees makes it difficult to see such a trend in the data (Fig. 6.3.4). In particular, the standard error on the calculated Km values (Table 6.3.1) is very large as a result of the variation in uptake rates. A rectangular hyperbola which was fitted to the data only had an r^2 value of 0.585, which could indicate that a sigmoidal curve may fit the data. The low r^2 values that were obtained when fitting equations to these curves can also be due to the variation in uptake rates of the different trees in a soil environment. This variation between seedlings is a natural occurrence. The large standard errors that were obtained when fitting Michaelis-Menten type equations to these data sets (Table 6.3.1) should also be viewed in terms of the fact that these calculations are usually performed in enzyme studies where the experiments have been conducted under precise conditions. This carefully controlled experimental environment is difficult to emulate for nutrient uptake studies particularly for plants in a soil environment.

One can speculate that a sigmoidal curve could indicate that either there is substrate activation of the uptake process, i.e. the presence of increasing amounts of Mg^{2+} stimulated the uptake process resulting in increased rates of uptake which is possible since Mg^{2+} can stimulate ATPase activity (Leonard and Hodges, 1973), or that at low Mg^{2+} concentrations, the uptake process, u may have been inhibited. As the concentration of B increased this inhibition was overcome.

6.4.3 Magnesium uptake in the presence of increased aluminium

From Chapter 4 it can be seen that the soils that were examined in this study are acid (pH in KCl of 4.64) and have a high Al^{3+}

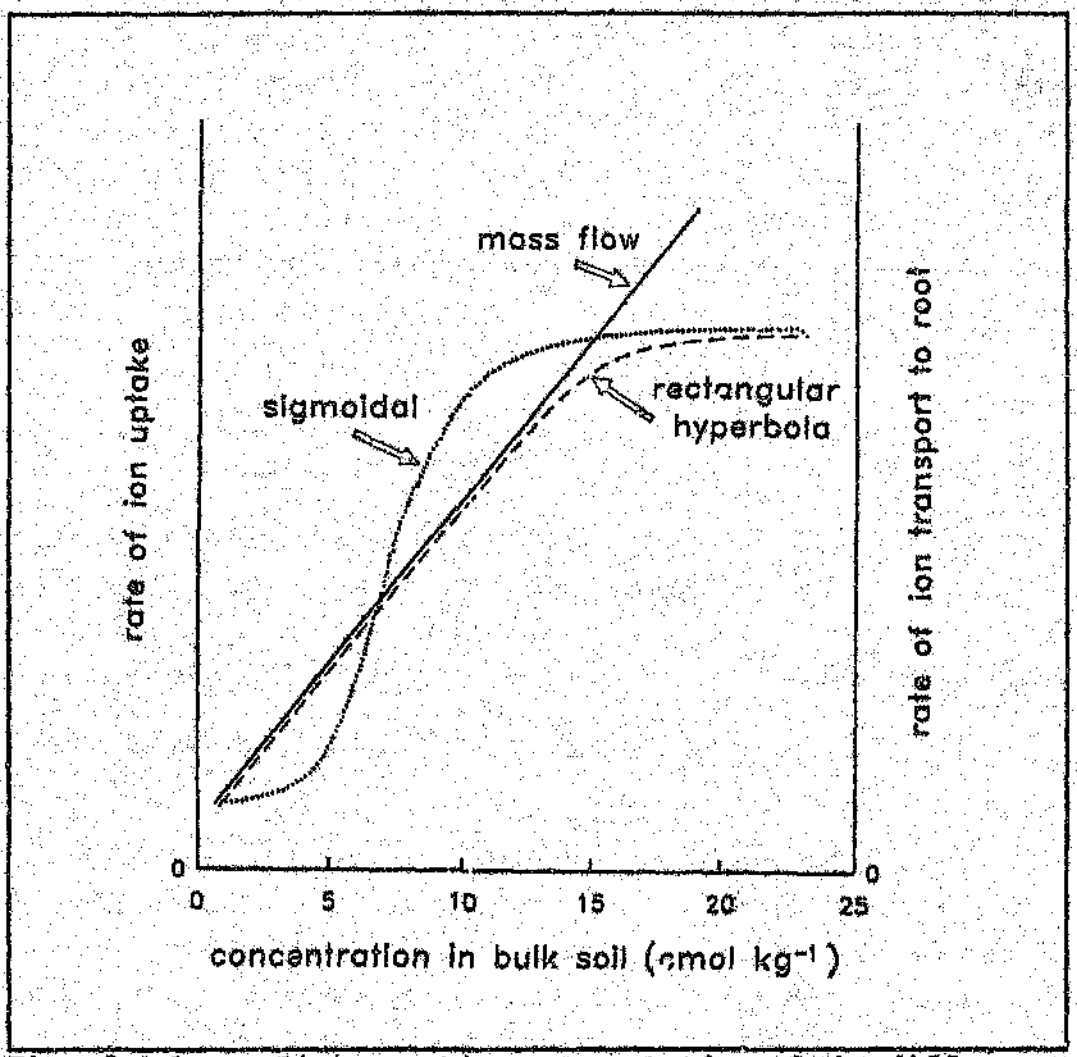

Fig. 6.4.1 Diagrammatic representation of the difference between a sigmoidal and a rectangular hyperbolic uptake curve in relation to the rate at which ions are transported to the root surface by mass flow.

content (1.12 cmol eq. kg^{-1}). In addition, acid rain can increase the soluble, exchangeable Al^{3+} content of the soil during the acidification process. In order to test the hypothesis that Al^{3+} could be responsible for the inhibition of Mg^{2+} uptake, the Al^{3+} content of the soil was increased and the uptake of Mg^{2+} investigated.

The results indicate that the uptake of Mg^{2+} in the presence of increased amounts of Al^{3+} was depressed (Fig. 6.3.5). The

rhizosphere concentration profiles indicate that Mg^{2+} tended to accumulate to a much greater extent at the root surface in the presence of increased Al^{3+}. This would tend to indicate that Al^{3+} could be responsible for an inhibition of Mg^{2+} uptake. The Vmax values that were determined when fitting equations to the uptake curves (Table 6.3.1) indicates that Al^{3+} inhibited the uptake of Mg^{2+} in that this parameter decreased when the uptake of Mg^{2+} was determined in the presence of Al^{3+}. A homogeneity of slopes test on linear transformation of the Mg^{2+} uptake rates in the presence of "normal" Al^{3+} concentrations and increased Al^{3+} concentrations showed that the suppression of Mg^{2+} uptake was significant.

Aluminium has been found to decrease the uptake of Ca^{2+} and Mg^{2+}, as well as other cations in numerous studies (Godbold *et al*, 1988; Koslowsky and Boerner, 1989; Rengel and Robinson, 1989; Asp and Berggren, 1990; Jentschke *et al*, 1991a). There are a number of ways that Al^{3+} could bring about this inhibition.

The excess protons (H^+) or Al^{3+} (resulting from an acidified system) at the root surface, could inhibit the functioning of the carrier. The rate of Mg^{2+} uptake has been found to increase markedly as the pH of the environment of excised roots increased from pH 3 to 5: above pH 5 the uptake was independent of the H^+ concentration (Maas and Ogata, 1971). As the concentration of Mg^{2+} increases, the ratios of Mg^{2+}/Al^{3+} or Mg^{2+}/H^+ increases, which results in less inhibition occurring.

Aluminium has been found to inhibit calmodulin-stimulated ATPase activity (Siegel and Haug, 1983), as well as the membrane linked Mg-stimulated ATPase (Rengel and Robinson, 1989). The latter ATPases are often involved in ion uptake, e.g. the uptake of K^+. Hence it is possible that the Al^{3+} can also affect the Mg^{2+} carrier, thus reducing the amount of Mg^{2+} taken up.

Aluminium can also reduce the amount of ATP present in the tissue, possibly by complexing with the ATP (Pfeffer *et al*, 1986). This could also decrease the rate of uptake in that the

rate of action of the cation carriers would be reduced as the ATP concentration decreases.

Aluminium has been found to alter the properties of biological membranes. This has led to the suggestion that Al^{3+} can affect the architecture of the membrane lipids (Miyasaka *et al*, 1989; Asp and Berggren, 1990). Such a change in the structure of the plasmalemma can disturb the structure and functioning of the carriers. This would reduce the rate of Mg^{2+} uptake.

In addition, if Al^{3+} binds to the negatively charged sites of the plasmamembrane, in place of Ca^{2+}, the permeability of the membrane can be changed. This may lead to uncontrollable leakage of ions across the membrane (Asp *et al*, 1988).

It has also been proposed that Al^{3+} can reduce the uptake of both Ca^{2+} and Mg^{2+} by occupying increasing numbers of the root cation exchange sites (Koslowsky and Boerner, 1989; Bengtsson *et al*, 1988; Asp *et al*, 1988; Jentschke *et al*, 1991a). Since Al^{3+} has a higher valency than both Ca^{2+} and Mg^{2+}, it would bind more tightly to the negative binding sites on the root surface. This would disturb the cation balance at the root surface (Bengtsson *et al*, 1988), and possibly decrease the rate of base cation uptake.

Although it has been found that the presence of mycorrhizas can, to some degree, prevent symptoms of Al^{3+} toxicity from developing (Cumming and Weinstein, 1990a; studies by Jentschke *et al* (1991a,b) show that the presence of a fungal sheath, resulting from the ectomycorrhizal colonization around the roots, did not prevent the Al^{3+} from reaching the cortex cell walls. Thus it is possible for Al^{3+} to affect the carriers directly as well as saturating the uptake sites.

Any such inhibition occurring at the surface of the *Pinus patula* roots would result in uptake kinetics similar to those which were observed.

Thus the results indicate that an increase in the Al^{3+} content of the soil can interfere with the uptake of Mg^{2+}. Consequently, acid rain has the potential to greatly reduce the Mg^{2+} content of plants growing in a nutrient deficient environment, such as the one examined in this study, because acid rain can increase the soluble Al^{3+} content of the soil.

In addition, since the velocity of Mg^{2+} uptake from soil with the ionic composition that is currently found in the plantation is far from maximum, any loss of Mg^{2+} from the soil system as a result of increased leaching in response to acid rain, can result in the uptake process occurring in conditions which are even further below saturation, where the effects of the Al^{3+} will become increasingly significant. This will result in the plants becoming increasingly nutrient stressed.

6.4.4 Calcium uptake

Figure 6.3.2 shows that the Ca^{2+} concentration profiles in the rhizosphere are similar to those observed for Mg^{2+} in Fig. 6.3.1. However as Fig. 6.3.2A and B indicate, Ca^{2+} accumulated at the root surface to a much greater extent than Mg^{2+}. As the concentration of Ca^{2+} increased in the bulk soil, the Ca^{2+} was taken up at a faster rate and, as a result, zones of depletion formed, (Fig. 6.3.2C to E). Due to the similarity in the concentration profiles of Ca^{2+} and Mg^{2+} in the rhizosphere, the uptake of Ca^{2+} was not investigated with respect to increased concentrations of Al^{3+} in the soil.

Similar "losses" or negative uptake rates have been observed in other studies, when the concentration of the Ca^{2+} in the external environment is low (Moore *et al*, 1961).

The rate of Ca^{2+} uptake with respect to the changing concentration of Ca^{2+} in the soil is illustrated in Fig. 6.3.6.

If Ca^{2+} is actively taken up by the roots, then the same explanation as given for Mg^{2+} applies. In addition, Al^{3+} will possibly

affect the uptake of Ca^{2+} in the same way as it affected Mg^{2+} uptake. Thus acid rain has the potential to decrease the rate of Ca^{2+} uptake and consequently can induce nutrient stress.

If, on the other hand, Ca^{2+} is not actively taken up, but enters the cell passively and is actively transported out of the cell then the results illustrated in Fig. 6.3.2 tend to indicate that the increase in Ca^{2+} concentration has interfered with the process of Ca^{2+} export from the cell. It has been hypothesized that there are a number of ATP pumps which remove Ca^{2+} from the cell. One such carrier is a nH^+/Ca^{2+} antiport system. The system would utilize the H^+ motive force built up by a H^+-pumping ATPase to drive Ca^{2+} transport. Another possible pump is a calmodulin-activated Ca^{2+} transport ATPase (Rasi-Caldogno *et al*, 1987). When the co ıtration of Ca^{2+} in the soil is relatively low then the pump acts in the normal way extruding Ca^{2+} from the cell in exchange for H^+. The extruded Ca^{2+} in addition to that transported to the root surface by mass flow, accumulates at the root surface. A rapid increase in the H^+ content of the soil, as a result of the acid rain, could however interfere with this process of active extrusion of Ca^{2+} from the cells. If a high H^+ concentration were present in the soil concurrently with a low Ca^{2+} concentration, a situation which could arise as a result of acid rain, then the rate of exchange of Ca^{2+} inside the cells for H^+ in the soil may increase. This could result in Ca^{2+} deficiencies in the plant tissue.

When the concentration of Ca^{2+} in the soil increases, and the concentration of Mg^{2+} remains low, then Ca^{2+} may be transported into the cell by means of another ion carrier not usually involved in Ca^{2+} uptake: such a carrier could be the Mg^{2+} transporter since Ca^{2+} and Mg^{2+} have the same valencies. This would result in a zone of depletion forming, similar to that which was obtained for Mg^{2+}.

Other studies have shown that Ca^{2+} can markedly depress the uptake of Mg^{2+} (Moore *et al*, 1961; Leggett and Gilbert, 1969).

Similarly increasing amounts of Mg^{2+} decrease the concentration of Ca^{2+} in the roots (Leggett and Gilbert, 1969), which gives credence to the above hypothesis that at high concentrations Ca^{2+} might be taken up on the Mg^{2+} transporter, in place of Mg^{2+}. However, it has also been hypothesized that Ca^{2+}, instead of competing for the same binding site, may change the structural configuration of the surface groups of the carrier such that their physical and/or chemical properties are altered, thus modifying the uptake of Mg^{2+} by the carrier (Moore *et al*, 1961; Maas *et al*, 1969 and Pitman, 1976b).

If the explanation that Ca^{2+} and Mg^{2+} compete for the binding site on the carrier holds, then these results show that liming with $CaCO_3$ without the simultaneous addition of Mg^{2+} could result in an imbalance in the nutrient uptake of the plants.

6.5 CONCLUSIONS

The uptake of Mg^{2+} resulted in neither accumulation nor zones of depletion forming at the root surface when the Mg^{2+} content of the soil was low, such as those conditions occurring in the soil at the time of sampling. This is a result of the rate of ion uptake being equal to the rate at which the ions were transported to the root surface. As the Mg^{2+} concentration in the soil increased, so too did the rate of Mg^{2+} uptake. The rate of uptake tended to increase faster than the rate at which the ions were supplied to the root surface which resulted in depletion zones forming at the root surface.

The initial slow rate of ion uptake relative to the rate at which ions were transported to the root surface was thought to be a result of either substrate activation of the uptake process by Mg^{2+} in the soil, or inhibition of the uptake process caused by the acidity of the soil or the high Al^{3+} content of the soil. The uptake of Mg^{2+} in the presence of increased amounts of Al^{3+} in the soil, (by almost 25%), was found to be significantly reduced. A number of mechanisms whereby the Al^{3+} could inhibit the uptake of

Mg^{2+} were proposed. The implication of the negative effect of Al^{3+} on the rate of Mg^{2+} uptake is that acid rain has the potential to decrease the rate of Mg^{2+} uptake by increasing the amount of Al^{3+} present in the soil.

The uptake of Ca^{2+} tended to follow a similar pattern, in that initially the Ca^{2+} accumulated at the root surface, but as the Ca^{2+} concentration increased, faster rates of uptake resulted in zones of depletion forming.

If Ca^{2+} is actively taken up, then the same explanations as postulated for Mg^{2+} uptake would hold for Ca^{2+}. In addition, Al^{3+} and acid rain are expected to have the same effect on the uptake of Ca^{2+} as occurred for Mg^{2+}. If Ca^{2+} passively enters the cells and is actively extruded, then it is thought that the increase in Ca^{2+} concentration in the bulk soil resulted in Ca^{2+} being transported into the roots on a carrier not usually associated with Ca^{2+} uptake. This could give rise to nutrient imbalances in the plant.

CHAPTER 7 SYNTHESIS AND CONCLUSIONS

The experiments undertaken in this investigation have shown that acid rain has the potential to increase the rate of acidification of the soil and, consequently, affect the nutrient status of the plants growing on this soil. It was shown that the leachate of the soil exposed to the most acidic 'rain" had the highest acid content and, after being leached with this "rain", the soil was inclined to have higher acid and aluminium (Al^{3+}) contents. Some of the mycorrhizal species colonizing the roots were sensitive to a decrease in the soil pH and an increase in the Al^{3+} content of the soil. The uptake of both calcium (Ca) and magnesium (Mg) from the soil were far below optimum and the uptake of Mg was shown to be affected by the Al^{3+} content of the soil. The implications of these findings have been examined in detail in the discussions of each experiment, however the results have greater implications when viewed in terms of each other. It is these implications which will now be considered.

The analysis of the soil at the time of sampling showed that the soil was acidic and had a low nutrient status. The morphology of the root system of plants growing in such soils must be such that the plant will be able to utilize the sparse nutrient reserves in the upper soil layers, in particular mycorrhizal colonization may be advantageous to plants growing on these soils because mycorrhizal roots are thought to be able to scavenge the available nutrients more efficiently from the soil than non-mycorrhizal roots (Linderman, 1988). Consequently the presence, and activity of the ectomycorrhizas on the *Pinus patula* roots could be essential in ensuring that the trees receive an adequate nutrient supply.

Pinus patula seedlings were grown in sterilized soil in order to compare the rates of Ca and Mg uptake by mycorrhizal tree roots with non-mycorrhizal tree roots. The seedlings growing in the sterilized soil all showed signs of phosphorus deficiency and died: this was in spite of the fact that they were watered with

a nutrient solution. It was concluded that the mycorrhizas not only assist the host in obtaining nutrients when the soil is nutrient poor, but that they are an integral part of the root system without which the plant cannot survive and factors which affect the mycorrhizas are considered to have the potential to affect the plant as a whole.

The leaching of the soil cores showed that there were no significant changes in the soil composition in response to the acid rain treatments over the time period examined. However although mathematically nonsignificant due to large amounts of variation in the soil system, these observed trends may be biologically significant in that the plants may respond to the observed trends. The lack of significant changes may also be a result of the time period being too short to elicit a response. This was compounded by the fact that if there were predefined water channels in the soil, the "rain" tended to pass along these paths, interacting only with the soil adjacent to these channels and having very little uniform contact with the entire soil core. Conversely, when no such channels existed in the soil core, the water took a long time to leach through the core, thus allowing only small volumes of "rain" to interact with the soil in these cores.

Although acid rain did not cause a significant increase in the rate of base cation leaching in response to an increase in the acidity of the "rain", the amount of Mg^{2+} lost when the "rain" had a lower pH was greatest. Over a longer period of time this trend could become significant. It should also be remembered that in the field situation, not all of the cations that are freed in the exchange will be lost in the drainage water as some will be taken up by the vegetation, thus aiding the growth of the plants. However the growth of the plants can be stimulated only for a short period because the amount of cations that can be liberated is finite and it is doubtful whether the rate of weathering of the soil particles can be fast enough to ensure that the amount of ions in the soil remains adequate.

As the ion concentrations in the soil decrease, there is likely to be an increase in competition among the plants to secure the nutrients which are now more scarce. This may result in changes in the root to shoot ratio. This is an important consideration for the timber industry which is trying to maximise above ground biomass.

Although there was no significant response in the amount of Al^{3+} lost in the leachate in response to the acidity of the "rain", there did appear to be increasing amounts of Al^{3+} in the soil as a result of the increasing acidity of the "rain". The effects that these increased concentrations of Al^{3+} may have on the plant are discussed in detail in Section 2.3.5. However it was shown that the presence of one of the seven mycorrhizal species colonizing the roots was sensitive to this increase in the Al^{3+} content of the soil. The presence of another of the mycorrhizal species was sensitive to the pH of the soil. Consequently, exposure of the soil to acid rain had the potential to alter the mycorrhizal species composition of the roots although it was not sufficient to significantly alter the chemical characteristics of the soil.

It should, however, be noted that it is not conclusive to assume that a decrease in a mycorrhizal species' presence is indicative of a negative response of that mycorrhizal species, and consequently the whole plant, to acidified soil. Further work needs to be done to determine whether the change in species composition will alter the efficiency with which the nutrient are extracted from the soil and transported to the host, and whether the new dominant species will have slower or faster growth rates, changed infection potential, greater carbon requirements and a different competitive ability compared with the old species composition. These are factors which will affect the mycorrhizal-host relationship and hence the nutrient status of the host.

The results of this study indicate that the mycorrhizal response

as more conspicuous than that of the plant (the latter being measured in terms of changes in root length and biomass) in response to changes in the soil characteristics as a result of exposure to acid rain. This could signify that the host plants may be less sensitive than the mycorrhizal fungi to the acidity of the rain. Thus changes in mycorrhizal species composition could be used as an indicator of acid rain damage to trees as the mycorrhizal response is more rapid than that of the root system.

The rates of uptake of both Mg^{2+} and Ca^{2+} at *in situ* Mg^{2+} and Ca^{2+} levels were far below maximum potentials and an increase in the concentrations of either element resulted in a definite increase in the rate of uptake of that element. The leaching of the soil cores showed that there is a trend for increasing amounts of Mg^{2+} and, although it could not be determined, presumably Ca^{2+}, to be lost in the leachate when the soil cores were leached with increasingly acidified "rain". Any decrease in the concentration of these ions in the soil will presumably decrease the rate of uptake still further. Such a decrease in the rate of nutrient acquisition could have a negative impact on the plants.

After the soil cores were leached with "rain" of various acidities, there tended to be more Al^{3+} present in the soil that was leached with the more acidic "rain". An increase in the Al^{3+} content of the soil was shown to decrease the rate of Mg^{2+} uptake. It was not possible to determine whether this inhibition occurred at the uptake sites on the root surface or whether the uptake of Mg^{2+} by the mycorrhizas was being suppressed since it was not possible to grow seedlings that were free from mycorrhizal infection. However, the findings indicate that the presence of increased Al^{3+} in the soil can result in an inhibition of Mg^{2+} uptake which will have a severe impact on the plant since Mg^{2+} has a number of important functions in plant metabolism, (these are discussed in Section 2.6.2).

The liming of soil cores did reduce the rate of cation leaching from the soil cores in response to artificial acid rain.

Understandably it also resulted in increasing amounts of Ca^{2+} being lost in the leachate. Once the Ca^{2+} moves below the rooting zone it is lost to the plant. If liming is used to ameliorate the effects of acid rain on the soil, it is thought that it might be better practise, but more labour intensive for the plantation owner, to lime more often with less lime, in an effort to prevent the loss of Ca^{2+}.

This study has shown that, although acid rain has the potential to increase the rate of soil acidification, this process of acidification takes place very slowly. Van Breemen (1991) has shown that a slow addition of acid can be relatively well buffered by weathering reactions. Such weathering process were not taken into account in this study but it should be noted that such processes occur under normal conditions in nature. Should the increase in acidification not be efficiently buffered, then the composition of the mycorrhizal species colonizing the root surface may be altered and the uptake of nutrients could be negatively affected. However this study has indicated that the effects of acid rain, over the time period that was examined, tend to be small and do not appear to have been capable of significantly reducing plant growth.

8.1 Long Ashton Nutrient Solution

A 50ml volume was taken from each of the stock solutions described below and placed in 5 litres of distilled water. This 5 litre solution was then used to water the trees.

Chemical:	Stock solution (gl^{-1}):
Solution 1	Micronutrients
$NaH_2PO_4.H_2O$	20.8
$MgSO_4.7H_2O$	36.9
$MnSO_4$ or $MnSO_4.H_2O$	0.223
$CuSO_4.5H_2O$	0.024
$ZnSO_4.7H_2O$	0.0296
H_3BO_3	0.186
$(NH_4)_6MO_7O_{24}.4H_2O$	0.0035
$CoSO_4.7H_2O$	0.0028
NaCl	0.585
Solution 2	
KNO_3	50.5
Solution 3	
$Ca(NO_3)_2$	82.0
Solution 4	
FeEDTA	3.0

8.2 Calculations

Ca : Al

$$= \frac{0.08 \text{ cmol kg}^{-1}}{0.37 \text{ cmol kg}^{-1}}$$

$$= 0.22$$

exchangeable Al : exchangeable cations

$$= \frac{0.37 \text{ cmol kg}^{-1}}{2.63 \text{ cmol kg}^{-1}}$$

$$= 0.14$$

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