# EFFECTS OF ALUMINIUM IONS ON UPTAKE OF CALCIUM, MAGNESIUM AND NITROGEN IN *BETULA PENDULA* SEEDLINGS GROWING AT HIGH AND LOW NUTRIENT SUPPLY RATES

ANDERS GÖRANSSON and TORIL DRABLØS ELDHUSET*
*Department of Ecology and Environmental Research, Swedish University of Agricultural Sciences, P.O. Box 7072, S-750 07 Uppsala, Sweden*

(Received 30 November 1992; accepted in final form 26 May, 1994)

**Abstract.** The effects of aluminium on plant nutrition in small birch plants (*Betula pendula* Roth) were investigated. By using relative addition rate ($R_A$, $g\ g^{-1}\ d^{-1}$) of nutrients as the growth-controlling variable, it was possible to grow the plants at very low external nutrient concentrations and to simulate plant requirements at two different fertility levels.

Before aluminium addition the plants were at steady-state relative growth rate, ($R_G$, $g\ g^{-1}\ d^{-1}$). The two addition rates were free access of nutrients with $R_G \approx 0.215\ d^{-1}$, or nutrient-limited, $R_A$ and $R_G = 0.10\ d^{-1}$.

Internal concentrations of calcium and magnesium decreased with increasing $Al^{3+}$ conncentration in the nutrient solution while nitrogen concentrations in the plants remained unchanged or increased. It was demonstrated in both nutrition treatments that calcium and magnesium decrease per se does not reduce plant growth and that uptake has to be considered in relation to plant requirement at different growth rates. The interpretation of the effects of aluminium on Ca and Mg uptake and plant biomass development suggested that processes other than disturbances in Ca and Mg uptake are the cause of the decrease in growth.

## 1. Introduction

Trees growing in acid forest soils are normally influenced by different potential stress factors to which they are more or less adapted, one of which is aluminium. Aluminium may reach relatively high concentrations in or on the roots, while internal leaf or needle concentrations are low. It is therefore likely that plants have mechanisms for aluminium exclusion in the root epidermis and cortex (see review by Foy *et al.*, 1978; Taylor, 1988; Bennet and Breen, 1991). Aluminium interactions with different nutrient elements are commonly reported, and decrease in cation uptake of forest tree species in the presence of aluminium, especially of calcium and magnesium is well documented (Jorns and Hecht-Buchholz, 1985; Asp *et al.*, 1988; Schaedle *et al.*, 1989; Lüttge and Clarkson, 1992). The decrease has been explained as caused by effects on negatively charged binding sites on the root surface (Wagatsuma, 1983; Godbold *et al.*, 1988a, b) or by effects on membrane proteins such as calmodulin (Siegel and Haug, 1983). Disturbances in phosphorus

* Present address: Section of Forest Ecology, Norwegian Forest Research Institute, Høgskoleveien 12, N-1432 Ås, Norway.

*Water, Air and Soil Pollution* **83:** 351–361, 1995.

uptake have also been proposed, and explained as aluminium-phosphorus precipitates in the epidermal and cortical regions of the root (McCormick and Borden, 1974; van Praag and Weissen. 1985; van Praag *et al.*, 1985).

In experiments with toxic substances, comparability between different studies is of great importance. In the case of aluminium toxicity, different responses to the same concentration within the same species have been described (e.g. McCormick and Steiner, 1978; Rost-Siebert, 1983; Hutchinson *et al.*, 1986). Several of the differences may be explained by the use of culture techniques in which control of growth prior to aluminium addition was neglected (see Ingestad, 1982; Ågren, 1985). The frequent use of comparatively high external nutrient concentrations may cause problem of interpretation as nutrients are liable to chemical interactions e.g. with aluminium. There may also be problems with studies in which nutrients are added in low, so-called ecologically relevant concentrations when growth and internal nutrient concentrations often decrease with time as a result of diminishing nutrition (Ingestad and Ågren, 1992). It is thus important to ensure non-varying experimental conditions with time when evaluating responses of plant growth to toxic elements. The addition technique used in the present investigation may be considered to model dynamic nutrient fluxes, and in the nutrient-limited treatment nitrogen was the growth-limiting nutrient element, as in most boreal forest ecosystems. The criteria and theory for steady-state growth is strictly defined mathematically and the reliability of the interpretation of the results depends on the ability to sustain steady-state nutrition (Ågren, 1985). When using relative addition rate as a growth-controlling variable, external nutrient concentrations may be very low, comparable to levels found in acid forest soils, and the plants have stable internal nutrient concentrations at steady-state growth, regardless of limitation level. Also, as demonstrated for steady-state growth, several tree species have similar nutrient requirement, e.g. *Alnus incana* and *Betula pendula* (Ingestad 1981), *Populus simonii* and *Paulownia tomentosa* (Jia and Ingestad, 1984), *Pinus contorta, P. sylvestris* and *Picea abies* (Ingestad and Kähr, 1985). Effects of aluminium on growth and nutrition of *Betula pendula* seedlings have been described elsewhere (Göransson and Eldhuset, 1987). The aim of the present paper is further to evaluate effects of aluminium on calcium, magnesium and nitrogen uptake in small *Betula pendula* plants, growing at two different relative addition rates of nutrients.

*Abbreviations*: *DW*, dry weight; *FW*, fresh weight; $R_A$, relative addition rate (g $g^{-1}$ $d^{-1}$); $R_G$, relative growth rate ($g$ $g^{-1}$ $d^{-1}$); $dn/dW_r$, uptake rate per unit root growth rate ($\mu$mol ($g$ root)$^{-1}$); $P_N$. nitrogen productivity ($g$ $DW$ ($g$ $N$)$^{-1}$ $d^{-1}$); $W_r$, root dry weight.

## 2. Materials and Methods

### 2.1. Germination and Pre-experiments

Seeds of *Betula pendula* Roth, collected from a maternal clone at Bogesund, Sweden, were germinated in Petri dishes on wet filter paper in low light conditions, approximately 20 $\mu$mol $m^{-2}$ $s^{-1}$. After 10 days, 200 seedlings were transferred to growth units with continuously recirculating nutrient solution, 5 litres, which was sprayed on the roots at a flow rate of 10 L $min^{-1}$. Conductivity and pH in the solution were measured automatically and adjusted by additions of small and exponentially increasing amounts of nutrients, normally once per hour to keep conductivity constant at $\approx$ 50 $\mu$S $cm^{-1}$, (CDM conductivity meter Radiometer, Copenhagen, Denmark) or pH at 3.8 (pHM 63 digital pH meter, GK 2501C combined pH electrodes, Radiometer, Copenhagen, Denmark). The stock nutrient solution was added from burettes into the recirculating solution and the contents by weight in relation to nitrogen (N = 100) were: 65 K, 13 P, 7 Ca, 8.5 Mg, 9 S, 0.7 Fe, 0.4 Mn, 0.2 B, 0.06 Zn, 0.03 Cu and 0.007 Mo. Total N content in the stock solution was 2.5 g $L^{-1}$ and the same composition of the nutrient solution was used in both pre-experiments and experiments. The accuracy of the conductivity and pH measurements was controlled once per day with reference electrodes. The pre-experiments, and experiments, were carried out in growth chambers (Weiss Technik, Giessen, Germany) with continuous illumination by 250 W lamps (Osram HQ-IR); photon flux density $\approx$ 300 $\mu$mol $m^{-2}$ $s^{-1}$, 400 to 700 nm, 24 hr $day^{-1}$; air and solution temperature 20 °C and vapour pressure deficit $\approx$ 0.6 MPa.

### 2.2. Experiments

After 10 to 14 days in the growth units, the fresh weight of each seedling was approximately 50 mg. Seedlings, as similar as possible in size, were selected and weighed in groups of 5 and thereafter put back into the growth units. Nutrients were added in free access by maintaining conductivity in the range 50 to 60 $\mu$S $cm^{-1}$ and pH of 3.8, or at a relative addition rate of 0.10 $day^{-1}$, corresponding to a nutrient limitation of approximately 50% of the maximum relative growth rate at a pH of 3.8. Prior to aluminium addition, 3 harvests were taken to check steady-state $R_G$ of the plants. The plants were harvested in groups at intervals of 3 days (free access) or 7 days (nutrient limitation) (Ingestad and Lund, 1979; Ågren, 1985). Aluminium was added immediately after the third harvest in different concentrations: 1, 3, 6 and 10 mM at free access and 1, 3, 6, 10 and 15 mM in nutrient-limited conditions. Following aluminium addition, three additional harvests were taken. If $R_G$ decreased because of aluminium, $R_A$ was lowered in order to maintain low nutrient solution concentrations and avoid chemical interactions between nutrients and aluminium.

At harvest, the roots were rinsed in distilled water and the plants were divided into leaf, stem and root fractions for determination of fresh and dry weight (65 °C

for two days). Plant parts were digested for two days in a 2.5:1 (v/v) mixture of nitric and perchloric acid and analysed for Ca, Mg and Al by plasma atomic emission spectrometry ICP-AES (Instrumentation Laboratory IL P-200, Andover, M.A, U.S.A). Total nitrogen was determined by a micro-Kjeldahl method using a flow injection analyser (Bifok FIA 05, Tecator, Höganäs, Sweden) with gas diffusion using phenol red as indicator (Svensson and Anfält, 1982). At the end of each experiment the culture solution was analysed for total (ICP-AES) and monomeric aluminium using a modification of the pyrocatechol method (Røyset, 1986). The laboratory is linked with the IUFRO intercalibration network, Forestry Commission of N.S.W., Australia, for calibration purposes.

### 2.3. Calculations and Statistics

By fitting plant fresh and dry weights ($W$), on different harvest days ($t$) to an exponential curve

$$W = W_0 e^{R_G t} \tag{1}$$

the average $R_G$ may be calculated (Ingestad, 1982). Uptake of any specified ion, e.g. Ca or Mg, at the growing root, the uptake rate per unit root growth rate, $dn/dW_r$, $\mu$mol ($g$ root biomass)$^{-1}$ was calculated as

$$\frac{dn}{dW_r} = \frac{n}{W}\frac{W}{W_r} \tag{2}$$

where $n$ is internal amount of Ca or Mg ($\mu$mol), $W$ is plant biomass and $W_r$ is root biomass (g) (Ingestad and Ågren, 1988). The specific effect of aluminium on calcium or magnesium uptake was calculated as per cent decrease in $dn/dW_r$ after aluminium addition (Göransson and Eldhuset, 1991).

The effect of a growth-limiting element may be expressed, e.g. as nitrogen productivity, $P_N$, ($g\ DW\ (g\ N)^{-1}\ d^{-1}$) and calculated as

$$P_N = \frac{R_G}{\frac{N}{W} - C_{N,\min}} \tag{3}$$

where $R_G$ is relative growth rate, $N$ is nitrogen concentration, $W$ is plant dry weight and $C_{N,\min}$ is the calculated nitrogen concentration at the intercept of the abscissa (cf. Ingestad and Ågren, 1992).

The statistical analyses were carried out by a F-test or by one-way ANOVA (Statgraphics, 1988). The pair-wise comparisons were done by the Least Significant Difference method at the confidence level 0.05.

## 3. Results

At steady state growth, before aluminium addition, the relative growth rate at free access was approximately 0.215 $d^{-1}$ and at nutrient limitation, approximately 0.105 $d^{-1}$, with a coefficient of determination, $r^2$, > 0.98 in each experiment.

TABLE I

Relative growth rates ($R_G$) and nitrogen productivities ($P_N$) in plants growing at free access of all nutrients or at nutrient limitation at different aluminium concentrations. The values are expressed as percentage of the values before aluminium addition. The number of experiments is denoted by $n$ and values followed by the same letter within each fraction are not significantly different, ($P$ = 0.05) for $R_G$ and (LSD = 0.05) for $P_N$

| $Al^{3+}$ | Free access | | | Nutrient limitation | | |
|---|---|---|---|---|---|---|
| mM | $R_G$ | $P_N$ | $n$ | $R_G$ | $P_N$ | $n$ |
| 0 | 100a | 100 | 6 | 100a | 100a | 7 |
| 1 | 95a | 93 | 3 | 93a | 64b | 2 |
| 3 | 88b | 77 | 1 | 46b | 24c | 1 |
| 6 | 61b | 62 | 1 | 38b | 26c | 1 |
| 10 | 70b | 102 | 1 | 30b | 28c | 2 |
| 15 | – | – | – | 26b | 23c | 1 |

Thus, the added amount of nutrients per unit of time was the growth-controlling parameter, according to theory (Ingestad and Ågren 1992). More than 85% of the added aluminium was recovered in the culture solution at the end of the experiments as monomeric aluminium. Decrease in plant $R_G$ ($P$ = 0.05) was recorded at an external aluminium concentration of 3 mM and decreased progressively at higher concentrations. The response of $R_G$ to increased aluminium concentration is summarised in Table I.

After aluminium additon, Ca and Mg concentrations decreased in plants growing at free access at the lowest aluminium concentration used (1 mM). The decrease was most pronounced for Ca, which decreased to approximately 51% of the value before the aluminium addition, while magnesium decreased to 56% (Figure 1). In the nutrient-limited plants, Ca was 88 and 98% at 1 and 3 mM aluminium, respectively, and decreased at higher concentrations, while Mg decreased at all aluminium concentrations. In Figure 1A and B, Ca and Mg concentrations in plants are plotted against $R_G$ in the different experiments, together with Ca and Mg before aluminium addition. The lines denote approximate birch requirement of Ca and Mg in experiments where $R_A$ of these elements was the growth controlling parameter (Ericsson, pers. com., Ericsson and Kähr 1995) in similar conditions to the aluminium experiments. In the same way, nitrogen concentration in plants are plotted against $R_G$ (Figure 2) and the line is derived from experiments where nitrogen was the growth-controlling parameter (Ingestad, 1981). After aluminium addition, internal nitrogen concentrations were unchanged or even increased, with

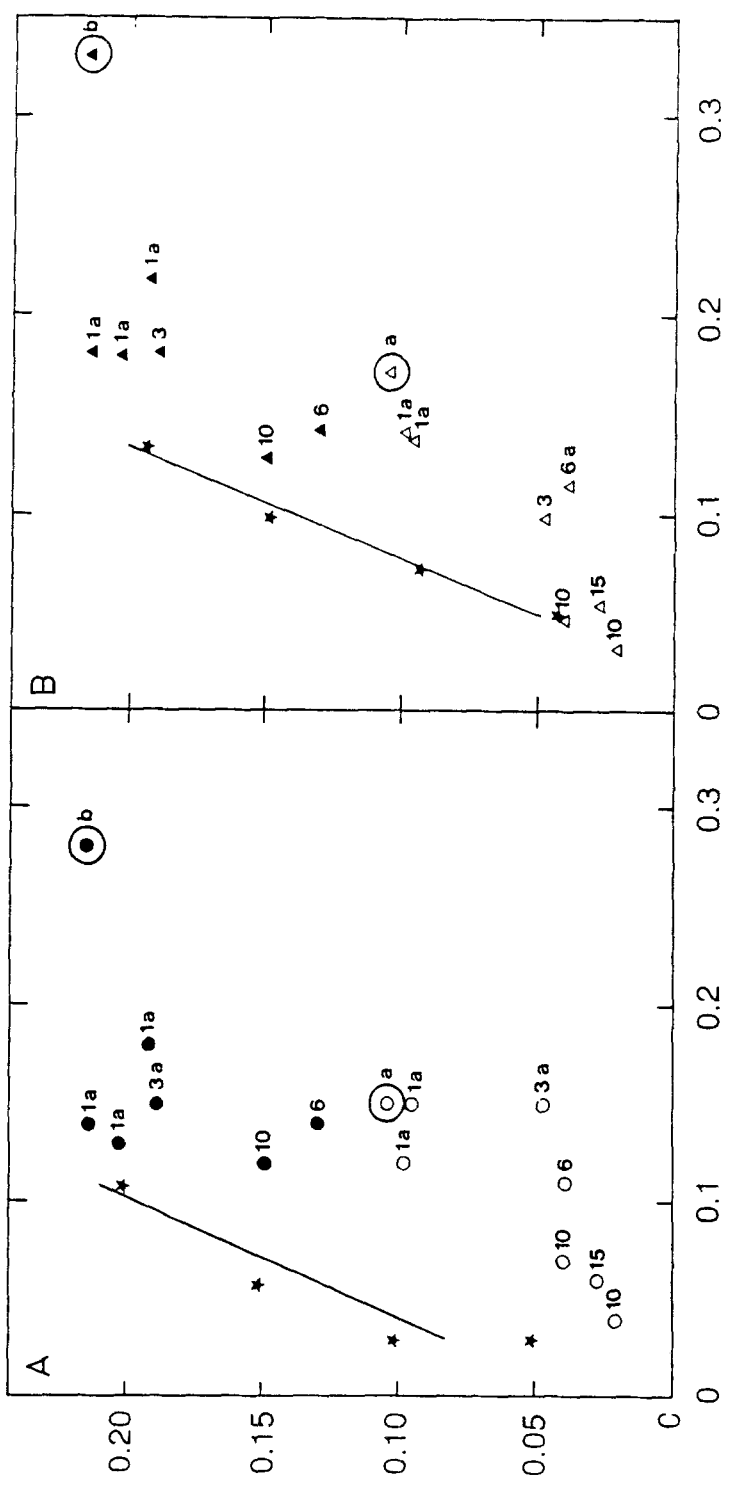


Fig. 1 Concentrations of calcium (○), Figure 1A and magnesium (△), Figure 1B in whole plants at aluminium concentrations of 1, 3, 6, 10, and 15 mM in the culture solution. The lines denote approximate Ca (Ericsson, pers. com.) and Mg (Ericsson and Kähr, 1995) concentrations when $R_A$ of Ca and Mg were the growth-controlling variable ($y = 0.034 + 1.59x$ with $r^2 = 0.85$ for Ca and $y = -0.376 + 1.797x$ with $r^2 = 0.98$ for Mg). The stars (★) show the data from which the lines were derived. For the aluminium experiments the filled symbols represent free access of all nutrients and open symbols nutrient-limited plants, both at a pH of 3.8. Encircled symbols represent mean Ca and Mg concentrations of the plants before aluminium addition. In each experiment analyses were made of bulk samples of two groups of 5 plants. The number of experiments is listed in Table I. The pre-aluminium values are mean concentrations for 12 groups of 5 plants (free access) and 14 groups of 5 plants (nutrient limitation). Symbols followed by different letters are significantly different (LSD = 0.05) from the approximate requirement of Ca or Mg when $R_A$ of these was the growth controlling variable, and from Ca or Mg means of the different aluminium treatments.

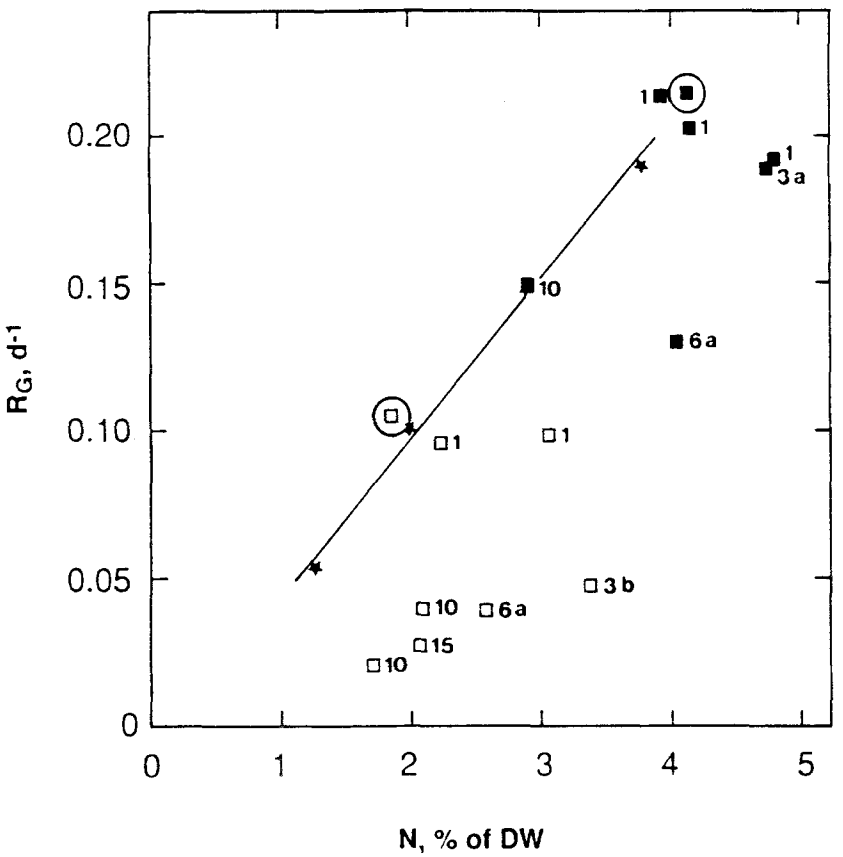


Fig. 2. Concentration of nitrogen (□) in whole plants at different aluminium concentrations in the culture solution. The line denotes nitrogen concentration in experiments where $R_A$ of nitrogen was the growth-controlling variable (redrawn from Ingestad 1981). The equation of the fitted line is $y = -0.011 + 0.054x$ with $r^2 = 0.995$. Symbols are as in Figure 1.

the most pronounced increases at external aluminium concentrations of 1 and 3 mM.

Calcium and magnesium uptake rates calculated per unit root growth rate decreased in the nutrient-limited treatments at aluminium concentrations higher than 3 mM (Ca) or 1 mM (Mg) (Figure 3). At free access, the uptake rates of calcium and magnesium decreased to ca 50 per cent of the values prior to the aluminium addition. The decrease was not correlated with external aluminium concentration ($P = 0.05$).

Before aluminium addition, nitrogen productivity was 5.5 or 6.4 *g DW* $(g\ N)^{-1}\ d^{-1}$ at free access and nutrient limitation, respectively. After aluminium addition, nitrogen productivity was unchanged at 1 mM in the free access treatment but decreased with increasing aluminium concentration in all other treatments (Table I).

## 4. Discussion

Decrease in calcium and magnesium uptake in the presence of aluminium is commonly reported and interpreted as determinental for plant growth. However, a decrease of non-growth-limiting nutrients, often occurring in excess of the quantitative requirement, should not per se imply growth disturbances or growth decrease. Ericsson and Ingestad (1988) demonstrated that internal concentration of phosphorous for constant maximal $R_G$ was 60% of what was found at free access of phosphorus. A similar pattern has been demonstrated for other elements. In experiments where $R_A$ of Ca or Mg was the growth-controlling variable, it was demonstrated that maximal $R_G$ for small birch plants grown in conditions similar to those in the present investigation occurred when internal concentrations were approximately 20% (Ca, Ericsson, pers. com.) and 50% (Mg, Ericsson and Kähr, 1995) of that at free access. It is therefore reasonable to assume that the decrease in internal Ca and Mg concentrations in the present study is not quantitatively large enough to influence growth in small birch plants (Figure 1A and B) at aluminium concentrations which are ecologically relevant in the rhizosphere of acid boreal forest soils.

Nutrient uptake may be calculated as uptake rate per unit root growth rate ($dn/dW_r$). In the free access treatments, $dn/dW_r$ of Ca and Mg decreased to approximately 50% of the rate prior to the aluminium addition, regardless of aluminium concentration. This is equivalent to that found in conifers at free access conditions (Göransson and Eldhuset, 1991). At nutrient limitation in birch, $dn/dW_r$ of Ca and Mg increased at 1 mM aluminium and then decreased with increasing aluminium concentration (Figure 3). Evidently the mechanisms for Ca and Mg uptake can function in the presence of aluminium concentrations as high as 1 mM for several days or weeks. Godbold *et al.* (1988b) and Schlegel *et al.* (1992) demonstrated that aluminium was located mainly in the root cortex and that losses of magnesium and calcium to a large extent took place in the apoplast, outside the suberised Casparian strip. The decrease in Ca and Mg at ecologically relevant aluminium concentrations, < 0.1 mM in the rhizosphere (Nilsson and Bergkvist, 1983; Berdén *et al.*, 1987), thus seems to be coupled to cations liable to exchange processes (Epstein, 1972; Hanson, 1984).

At nitrogen limitation, as in the nutrient-limited treatments, all nitrogen may be considered to be active in biomass production and there is no storage of excess nitrogen in the plant. The relationship between the internal nitrogen concentration and $R_G$, i.e. the slope of the regression line (Figure 2), has been defined as nitrogen productivity (Ingestad and Ågren, 1988) and may be regarded as a function of the ability of the plant to produce new biomass per nitrogen amount and time in the prevailing conditions. In the present investigation, the internal nitrogen concentration and hence nitrogen productivity, $P_N$, of the pre-aluminium plants was equal to that in experiments where $R_A$ of nitrogen was the growth-controlling variable (Figure 2). When internal nitrogen concentration increased, as in the nutrient-limited treatments at low aluminium concentrations, an increase in $R_G$

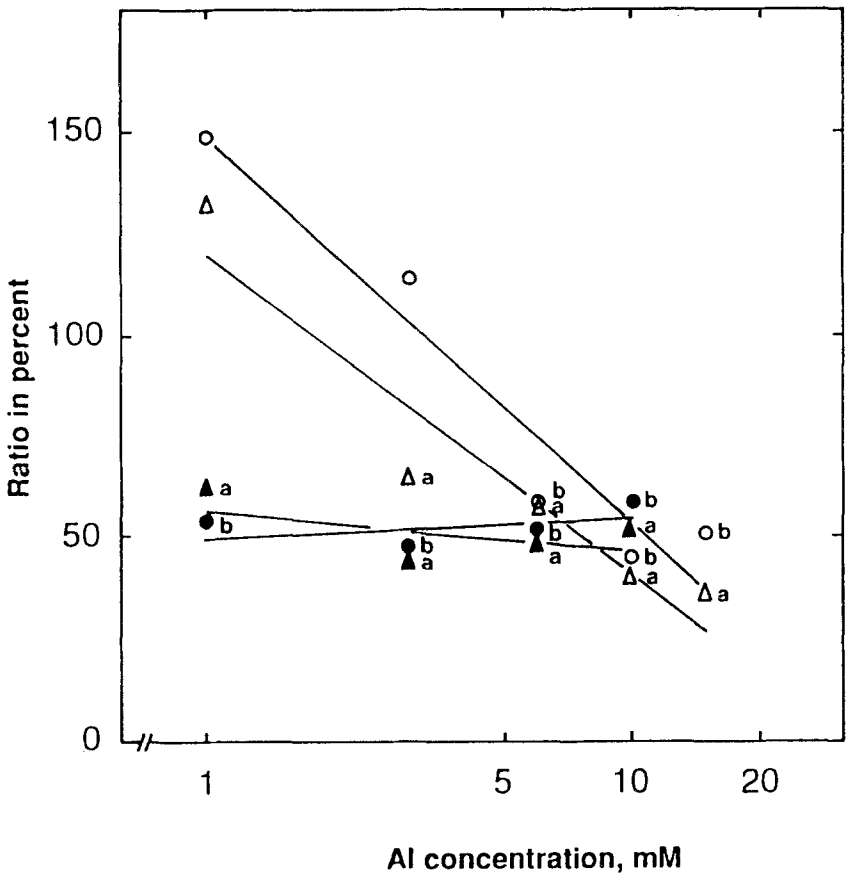


Fig. 3. Calcium (o) and magnesium (△) uptake rate per unit root growth rate, $dn/dW_r$, ($\mu$mol (root $DW)^{-1}$) at free access of all nutrients (filled symbols) and at nutrient limitation (open symbols) as percent of the pre-aluminium value. Analyses were made of bulk samples of two groups of 5 plants in each experiment. Symbols followed by different letters are significantly different (LSD = 0.05) from the value before aluminium addition.

would be expected. Instead, $R_G$ was virtually constant (Figure 2 and Table I) and $P_N$ decreased. The nutritional disorders had apparently no effect on plant growth at low aluminium concentrations (1 mM).

Plant responses to increased aluminium concentrations, i.e. tolerance and toxicity, are poorly understood (see reviews by Bennet and Breen, 1991; Lüttge and Clarkson, 1992). Increased concentrations of aluminium are known to influence DNA replication and to cause changes in root cell division (Clarkson, 1969). The visual toxic effect of aluminium on root development in the present investigation was a symptomatic stunted growth pattern with swollen, discoloured root tips. Those changes occurred at external concentrations of 3 to 6 mM and aluminium concentrations in roots exceeding approximately 4.5 mg $g\ DW^{-1}$ (Göransson and Eldhuset, 1987). Although not measured in the present investigation it may be suggested that chemical interactions, e.g. between aluminium and phosphate, may

occur in the cell nucleus at high aluminium concentrations, with effects on DNA replication.

## 5. Conclusions

Plant damage occurred at external concentrations which are high compared to those found in the rhizosphere of acid boreal forest soils. Although the primary objective of the present investigation was not to limit calcium and magnesium uptake, the plant response clearly demonstrates that uptake mechanisms for the nutrients are operating at aluminium concentrations which are high compared to those in the rhizosphere. Magnesium limitation, eventually a result of increasing acidification and leaching, seems to be a bigger hazard than Ca limitation, especially in relation to increased $Al^{3+}$. Decrease in plant Ca and Mg may be attributed to exchange processes on the root cell surfaces. Disturbances to Ca and Mg uptake may occur at low aluminium concentrations, although it is questionable if they are quantitatively large enough to affect growth. The present results favour the interpretation that growth damages occurred because of aluminium-induced changes in cell replication and root growth rather than disturbances to Ca and Mg uptake. It may be concluded from the present study that the decrease in calcium and magnesium has to be evaluated in quantitative terms in relation to plant requirement, not, as it often is, to arbitrary values before aluminium addition.

## Acknowledgements

The authors wish to thank Dr. T. Ericsson for providing us with data from Ca and Mg experiments. Thanks are due to Profs. S. Linder, T. Ingestad and G. I. Ågren for constructive discussions concerning the manuscript. Ms E. Arwidsson, A. Carlsson, A. Hovberg, Mr R. Reutlert and L. Östberg helped with technical assistance. Dr M. Kähr drew the figures. The investigation was supported by grants from the Agricultural Research Council of Norway, the Swedish Environmental Protection Board and the Swedish Council for Forestry and Agricultural Research.

## References

Ågren, G. I.: 1985, *Physiol. Plant.* **64**, 17.
Asp, H., Bengtsson, B. and Jensén, P.: 1988, *Plant and Soil* **111**, 127.
Bennet, R. J.. and Breen, C. M.: 1991, *Plant and Soil* **134**, 153.
Berdén, M. Nilsson, S. I., Rosén, K. and Tyler, G.: 1987, *National Swedish Environment Protection Board, Report,* **3291**, p. 54.
Clarkson, D. T.: 1969, in Rorison, I. H. (ed.), *Ecological Aspects of the Mineral Nutrition of Plants*, Blackwell, p. 381.
Epstein, E.: 1972, *Mineral Nutrition of Plants: Principles and Perspectives*, Wiley & Sons.
Ericsson, T. and Ingestad, T.: 1988, *Physiol. Plant.* **72**, 227.

Ericsson, T. and Kähr, M.: 1995. *Tree Physiol.* **15**, 85–93.
Foy, C. D., Chaney, R. L. and White, M. C.: 1978, *Ann. Rev. Plant. Physiol.* **29**, 511.
Godbold, D. L., Dictus, K. and Hüttermann, A.: 1988a, *Can. J. For. Res.* **18**, 1167.
Godbold, D. L., Fritz, E. and Hüttermann, A.: 1988b, *Proc. Natl. Acad. Sci.* **85**, 3888.
Göransson, A. and Eldhuset, T. D.: 1987, *Physiol. Plant.* **69**, 193.
Göransson, A. and Eldhuset, T. D.: 1991, *Trees* **5**, 136.
Hanson, J. B.: 1984, in Tinker, P. B. and Läuchli, A. (eds.), *Advances in Plant Nutrition*, Vol. **1**.
Hutchinson, T. C., Bozic, L. and Munoz-Vega, G.: 1986, *Water, Air, and Soil Pollut.* **31**, 283.
Ingestad, T.: 1981, *Physiol. Plant.* **52**, 454.
Ingestad, T.: 1982, *Plant Cell and Environ.* **5**, 443.
Ingestad, T. and Ågren, G. I.: 1988, *Physiol. Plant.* **72**, 450.
Ingestad, T. and Ågren, C. I.: 1992, *Physiol. Plant.* **84**, 177.
Ingestad, T. and Kähr, M.: 1985, *Physiol. Plant.* **65**, 109.
Ingestad, T. and Lund, A-B.: 1979, *Physiol. Plant.* **45**, 137.
Jia, H-J. and Ingestad, T.: 1984, *Physiol. Plant.* **62**, 117.
Jorns, C. A. and Hecht-Buchholz, C.: 1985, *Allgemeine Forstzeitschrift* **40**, 1248.
Lüttge, U. and Clarkson, D. T.: in Behnke, H.-D., Bochum, K. E., Kubitzki, K., Runge, M. and Ziegler, H. (eds.) *Progress in Botany* **53**, 63.
McCormick, L. H. and Borden, F. Y.: 1974, *Soil Sci. Soc. Amer. Proc.* **38**, 931.
McCormick, L. H. and Steiner, K. C.: 1978, *Forest Sci.* **24**, 565.
Nilsson, S. I. and Bergvist, B.: 1983, *Water, Air, and Soil Pollut.* **20**, 311.
Rost-Siebert, K.: 1983, *Allgemeine Forstzeitschrift* **38**, 686.
Røyset, O.: 1986, *Anal. Chim. Acta,* **185**, 75.
Schaedle, M., Thornton, F. C., Raynal, D. J. and Tepper, H. B.: 1989, *Tree Physiol.* **5**, 337.
Schlegel, H., Amundson, R. G. and Hüttermann, A.: 1992, *Can. J. For. Res.* **22**, 1132.
Siegel, N. and Haug, A.: 1983, *Physiol. Plant.* **59**, 285.
Statgraphics: 1988, 'Statistical Graphics System by Statistical Graphics Corporation', STSC Inc., Rockville, ML.
Svensson G. and Anfält, T.: 1982, *Clin. Chim. Acta* **119**, 7.
Taylor, G. J.: 1988, *Commun. in Soil Sci. Plant Anal.* **19**, 1179.
van Praag, H. J. and Weissen, F.: 1985, *Plant and Soil* **83**, 331.
van Praag, H. J., Weissen, F., Sougnez-Remy, S. and Carletti, G.: 1985, *Plant and Soil* **83**, 339.
Wagatsuma, T.: 1983, *Soil Sci. Plant Nutr.* **29**, 499.