

Effects of aluminum on growth, polyamine metabolism, and inorganic ions in suspension cultures of red spruce (*Picea rubens*)¹

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Abstract: The influence of age of red spruce (*Picea rubens* Sarg.) cell suspensions on aluminum (Al) effects was studied by adding AlCl₃ (0.2, 0.5, and 1.0 mM) to the media on each day of a 7-day culture period and analyzing for changes in total cell mass, polyamines, arginine decarboxylase activity, and inorganic ions after 24 h of incubation. The effective concentrations of monomeric Al in the medium were found to be approximately 0.09, 0.23, and 0.48 mM, respectively. All levels of Al inhibited growth and increased cellular putrescine levels and arginine decarboxylase activity. Spermidine and spermine levels were either slightly elevated or not affected by Al. Cellular content of Ca, Mg, Mn, and K decreased, while Al and P increased in Al-treated cells. Aluminum effects, in general, were dose dependent. Since the response to 24 h incubation with Al was generally independent of culture age, 3-day-old cultures were selected to further study short- and long-term (4 h to 11 days) effects of Al. Cellular putrescine levels remained elevated in the Al-treated cells for the entire duration of the experiment. There was a sharp dose-dependent increase in spermine levels by the 7th day of incubation. After the initial decrease, Al treatments for longer than 2 to 4 days did not cause consistent effects on Ca, Mn, and Mg levels. Potassium decreased and P and Al increased in a dose-dependent manner over the 11-day culture period.

Résumé : L'influence de l'âge des suspensions de cellules d'épinette rouge (*Picea rubens* Sarg.) sur les effets de l'aluminium (Al) a été étudiée en ajoutant 0,2, 0,5 et 1,0 mM de AlCl₃ au milieu à chaque jour durant une période de culture de 7 jours et en analysant les variations dans la masse totale des cellules, les polyamines, l'activité de l'arginine décarboxylase et les ions inorganiques après 24 h d'incubation. Les concentrations effectives de Al sous forme de monomère dans le milieu ont été évaluées à environ respectivement 0,09, 0,23 et 0,48 mM. Toutes les concentrations de Al ont inhibé la croissance et augmenté les niveaux de putrescine cellulaire et l'activité de l'arginine décarboxylase. Les concentrations de spermidine et de spermine étaient soit légèrement augmentés ou pas affectés par Al. Le contenu cellulaire de Ca, Mg, Mn et K a diminué tandis que celui de Al et P a augmenté dans les cellules traitées avec Al. En général, les effets de Al étaient dépendants de la dose. Puisque la réaction à 24 h d'incubation avec Al était généralement indépendante de l'âge des cultures, des cultures âgées de 3 jours furent sélectionnées pour poursuivre l'étude des effets à court et long terme (4 h à 11 jours) de Al. Les concentrations de putrescine cellulaire sont demeurées élevées dans les cellules traitées avec Al durant toute la durée de l'expérience. Il y a eu une forte augmentation dépendante de la dose dans la concentration de spermine après 7 jours d'incubation. Après la diminution initiale, les traitements avec Al dont la durée dépassait 2 à 4 jours n'ont pas eu d'effets consistants sur les concentrations de Ca, Mn et Mg. Le potassium a diminué et P et Al ont augmenté en lien avec la dose tout au long de la période de culture de 11 jours.

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Introduction

Although the phytotoxic effects of aluminum (Al) in acidic soils have been known for many decades (Hartwell and Pember 1918), it is only recently that Al has been implicated as a major cause of forest decline in the northeastern United States and parts of Europe (Ulrich et al. 1980; Godbold et al. 1988; Shortle and Smith 1988; McQuattie and Schier 1990). Aluminum affects the rates of cell division, DNA synthesis, needle biomass, root growth, seedling height, cellular inorganic ion content, etc. in red spruce

(*Picea rubens* Sarg.) and periwinkle (*Catharanthus roseus*) (Shortle and Smith 1988; McQuattie and Schier 1990; Schier et al. 1990; Minocha et al. 1992; Zhou et al. 1995). Among the physiological and molecular responses of cells to Al treatment are the changes in cellular content of polyamines, metabolism of malate, and induction of novel gene expression (Delhaize and Ryan 1995; Zhou et al. 1995). However, little is known about the primary site(s) of Al action and the chain of physiological and molecular events that eventually lead to inhibition of growth (Delhaize and Ryan 1995). It is, therefore, essential to study in more detail the changes in various physiological and biochemical processes associated with exposure of plants to Al solubilization using model experimental systems, e.g., in vitro grown cells.

The aliphatic polyamines spermidine and spermine, and their precursor putrescine, are ubiquitous in all cells. Polyamines are metabolically derived from amino acids and play an important role in the homeostatic regulation of cellular pH (Cohen 1971) and stabilization of plasma membranes (Slocum et al. 1984; Smith 1985). Recently, considerable attention has been paid to the study of changes in polyamine metabolism in plants subjected to various kinds of environmental stress. External stress can result in an increase or a decrease in cellular polyamines, depending upon the type of stress, the plant species, and the time of stress application (Zhou et al. 1995). Abiotic stress conditions such as low pH, SO₂, high salinity, osmotic shock, nutrient stress, low temperature (Flores 1991 and references therein), and Al (Minocha et al. 1992) all result in an increase in cellular putrescine levels within hours to days. In all cases except low temperature, this increase results from de novo synthesis and activation of arginine decarboxylase (ADC), one of the key regulatory enzymes responsible for putrescine biosynthesis (Flores 1991). Exposure to Al has also been shown to affect the levels of cellular inorganic ions in woody plants (McQuattie and Shier 1990; Minocha et al. 1992). There is evidence to suggest that polyamines show a reverse proportionality to ions such as Ca, Mg, Mn, and K in response to Al treatment (Minocha et al. 1992; Zhou et al. 1995).

Strong positive charges at cellular pH and extended conformational flexibility provide polyamines with certain unique properties for interaction with cell membranes and cellular macromolecules. A key distinction between the polyamines (organic cations) and the inorganic cations is that even if the latter (e.g. Ca²⁺ and Mg²⁺) undergo re compartmentalization in response to external stimuli, their cellular levels are ultimately derived from uptake across the biological membranes. In contrast, polyamines are synthesized within the cell, permitting adjustment of their cellular concentrations to meet physiological needs. Also, cellular polyamine levels can be regulated by conjugation, degradation, and sequestration via enzymatic means. Thus polyamine synthesis may play an important role in the survival of plants under stress (Galston 1989). In previous reports we demonstrated the effects of Al on cell division, synthesis of macromolecules, cellular levels of polyamines, and the activity of their biosynthetic enzymes using *Catharanthus roseus*, an ornamental woody plant (Minocha et al. 1992; Zhou et al. 1995). The present study

provides additional information on the effects of Al on the physiological responses of cell cultures of red spruce, a species of major economic value in the northeastern United States, and also one that is being affected by Al solubilization in the soil due to acidic deposition.

Materials and methods

Culture conditions

Embryogenic suspension cultures of red spruce (cell line RS61.1, 03-92) were obtained from the laboratory of Dr. Krystyna Klimaszewska, Petawawa National Forestry Institute, Chalk River, Ontario, Canada, and maintained in half-strength Litvay's medium (Litvay et al. 1981) as modified by Dr. Krystyna Klimaszewska (personal communication). The modifications include addition of 3.42 mM glutamine, 9.05 μM 2,4-D, 4.44 μM benzyladenine (BA), 1.0 g/L casein hydrolysate, and 2% sucrose instead of 3%. In addition, Fe-EDTA was replaced by 40 mg/L of a commercial plant product called Sequestrine containing 7% iron chelate (Plant Products Company Ltd., Brampton, Ontario L6T1, Canada³). The medium was adjusted to pH 5.7 before autoclaving. Cells were subcultured routinely at 7-day intervals by transferring 15 mL of cell suspension into 45 mL of fresh medium in 250-mL Erlenmeyer flasks. The flasks were incubated in the dark at 25 ± 2°C on a gyratory shaker at 120 rpm.

Experimental treatments

Seven-day-old cell suspensions from an appropriate number (5–9) of 250-mL flasks (containing 60-mL cell suspensions) were mixed with an equal volume of fresh medium. To obtain uniform distribution, the cells were constantly stirred at low speed with a magnetic stirrer during the period of transfer to experimental flasks. Ten milliliters of cell suspension was transferred to individual 50-mL experimental flasks containing 10 mL of fresh medium, thus providing a final cell density comparable to that of routine subcultures.

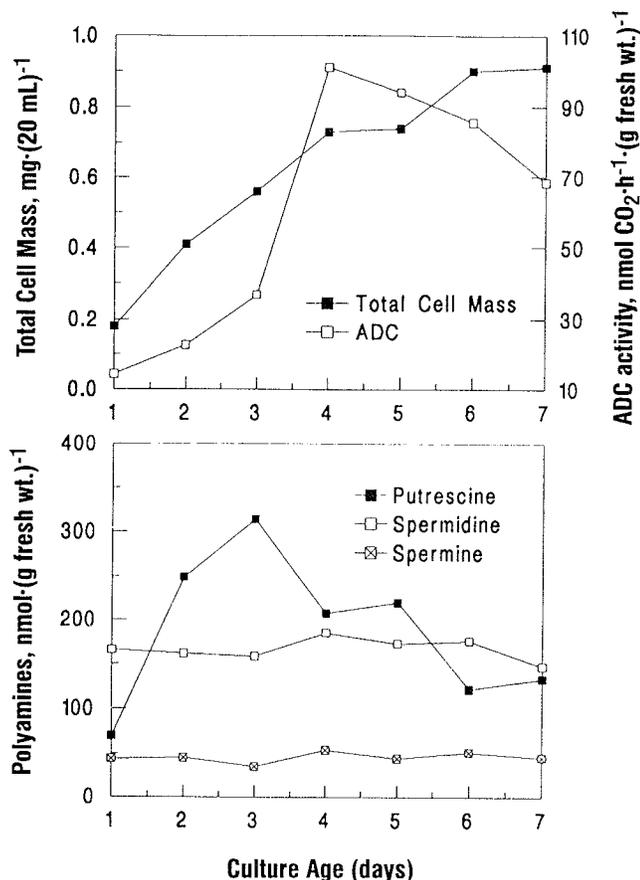
The Al levels (0.2, 0.5, and 1.0 mM of AlCl₃) used for this study were based upon preliminary studies on effects of Al on growth. To study Al speciation, Al was either added to the cell-free medium or to 3-day-old cultures at a pH of 4.2 or below. In either case, about 55% Al became insoluble. The precipitate was removed by centrifugation at 31 400 g for 20 min and the supernatant was analyzed for monomeric Al by the procedure of Driscoll (1984).

For testing the dependence of Al effects on age of these cultures, calculated amounts (final concentrations of 0.2, 0.5, and 1.0 mM) of filter-sterilized AlCl₃ (100 mM) were added to sets of nine 50-mL experimental flasks (3 replicates per treatment) each day at the same time for 1- to 7-day-old cultures. The cells from treated flasks and a set of control (3) flasks were collected for analysis after 24 h of incubation. For short- and long-term treatments (from 4 h to 11 days), AlCl₃ was added to 20 mL of 3-day-old cells maintained in 50-mL flasks and the flasks were kept on the shaker until the time of analysis.

The pH of the culture medium was not readjusted during the period of each experiment. By 24 h following subculture, the pH of the culture medium dropped to 4.2 ± 0.3 and stayed within this range for the duration of the experiments. On the day of subculture the pH of the medium was 5.0 ± 0.1 but the

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Fig. 1. Time courses of changes in total cell mass, cellular polyamines, and ADC activity during the 7-day growth period of red spruce suspension cultures. Values are means of three replicates.

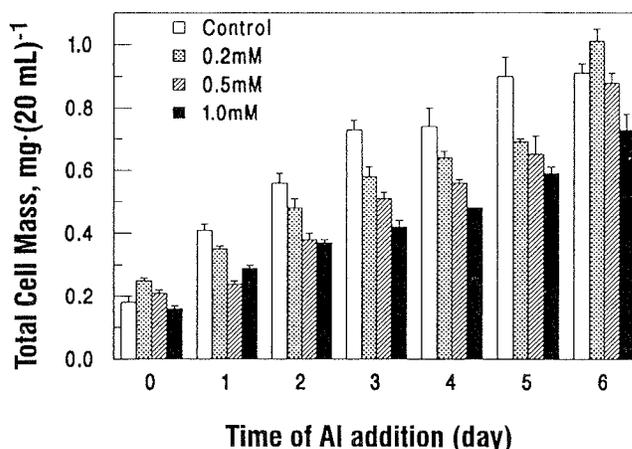


addition of Al itself changed the pH instantly to 4.55, 4.11, and 3.76 for 0.2, 0.5, 1.0 mM Al treatments, respectively. Thus, in the whole study the beginning of the culture was the only time when the pH was not lower than 4.5 before adding Al. At the end of the treatment period, cells were collected and analyzed for perchloric acid (PCA)-soluble fraction of cellular polyamines, their biosynthetic enzymes, and cellular inorganic ions using the methods described below. All treatments were run in triplicate and each experiment was repeated at least twice with most of them repeated three or four times. Although the actual polyamine numbers were variable in different experiments, replicate experiments showed similar trends. The data presented here are derived from a single representative experiment of each type.

Inorganic ion content

Cells were collected on Miracloth (Calbiochem-Behring Corp., La Jolla, Calif., U.S.A.) and washed twice with an equal volume of deionized distilled water. Cells (50 mg fresh wt.) were transferred to 12-mL test tubes, each containing 5 mL of 0.01 M HCl and subjected to three freeze-thaw cycles (Minocha et al. 1994). Extracts were either centrifuged at 18 000 g for 20 min at 4°C or filtered using a 45- μ m nylon syringe filter. The supernatant solutions were analyzed for inorganic ion content with a Beckman Spectrospan V ARL DCP (direct current plasma emission spectrometer, Beckman Instruments Inc., Fullerton, Calif., U.S.A.) using method 66-AE0029, 1986, of the U.S.

Fig. 2. Effects of AlCl₃ on total cell mass when Al was added to 0 to 6-day-old red spruce suspension cultures and the cells analyzed 24 h thereafter. Values are means \pm SE of three replicates.



Environmental Protection Agency. Appropriate samples were analyzed for monomeric inorganic Al at the U.S. Geological Survey laboratory in Albany, N.Y., using the method of Driscoll (1984).

Polyamine analysis

Subsamples of collected cells (200 mg) were placed in 1.5-mL microfuge tubes containing 0.8 mL of ice-cold 5% PCA. Polyamines were extracted by repeated (three times) freezing and thawing (Minocha et al. 1994). After the homogenate was centrifuged at 16 000 g for 10 min at 4°C, heptanediamine was added as an internal standard. Polyamines were dansylated and quantified by high-performance liquid chromatography according to the method of Minocha et al. (1990).

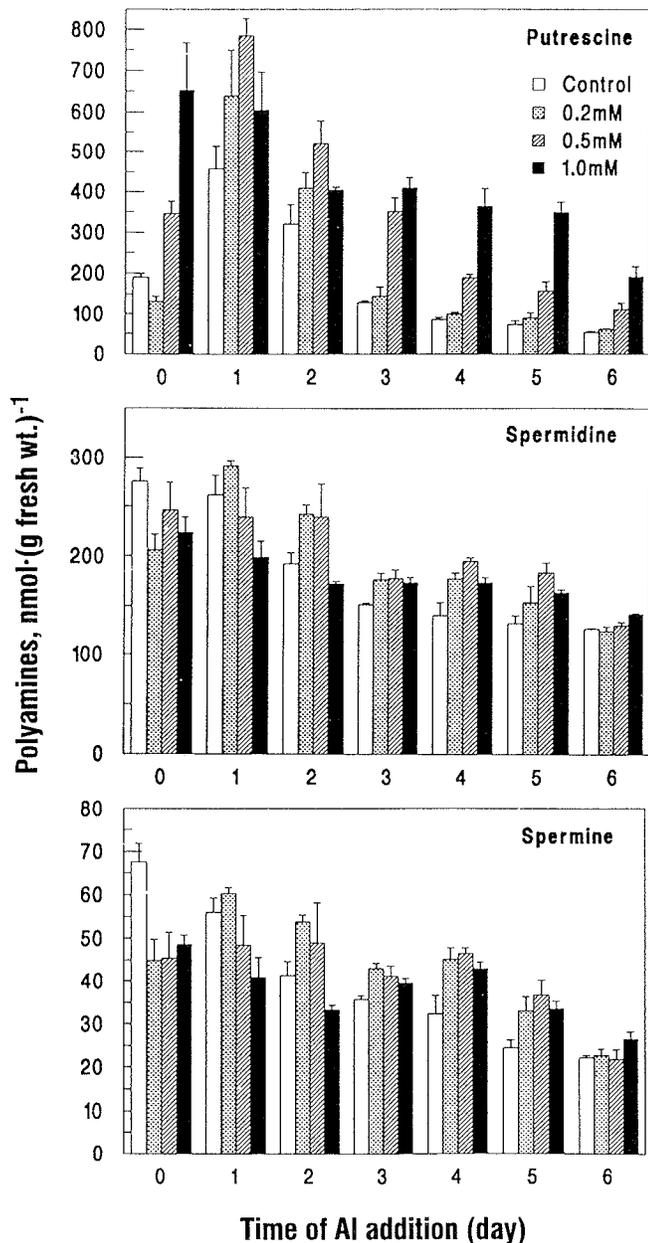
ODC and ADC assays

Cells were washed and subsamples (500 mg) collected as described above and homogenized in Tris extraction buffer (2 mL·(g fresh wt.)⁻¹) for 90 s at 20 000 rpm using a Brinkmann Polytron homogenizer. The Tris extraction buffer (TAB) for both ornithine decarboxylase (ODC) and ADC contained 50 mM Tris-HCl, pH 8.4, adjusted at 25°C, 50 μ M pyridoxal-5-phosphate, 0.1 mM Na₂-EDTA, and 5 mM dithiothreitol (DTT). The homogenates were centrifuged at 18 000 g for 20 min at 4°C, and the supernatant fractions were used for enzyme assays. ODC and ADC were assayed according to the procedure of Robie and Minocha (1989) using 1-¹⁴C-labeled ornithine and arginine, respectively.

Statistical analysis

Since the cultures used in this study were asynchronous and the initial cell numbers in control cultures changed with time through the duration of the experiments, the effects of Al treatment were statistically analyzed and compared only within a given Al incubation period. Data for each time period and each set of treatments were analyzed as a series of one-way analyses of variance (ANOVA) to determine whether statistically significant differences occurred among treatments and the control at a given time. The effect of culture age on Al toxicity was tested using mean values for each treatment (three replicates/treatment) expressed as percent of control for each age group and each experiment as a replicate for ANOVA. This conversion to percent of control compensated for changes in initial values

Fig. 3. Effects of AlCl_3 on cellular levels of polyamines when Al was added to 0 to 6-day-old red spruce suspension cultures and the cells analyzed 24 h thereafter. Values are means \pm SE of three replicates.



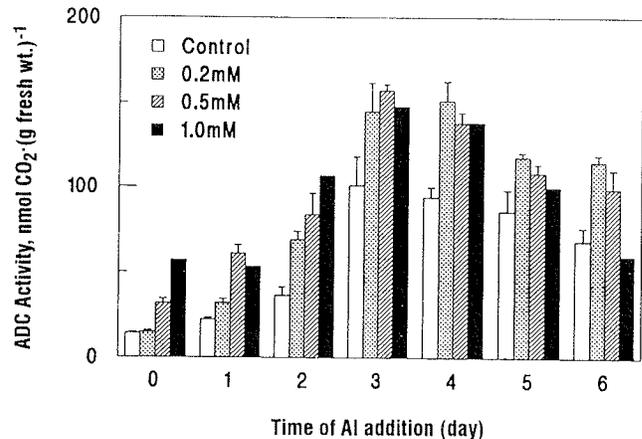
with time due to normal growth. When *F* values for one-way ANOVA were significant, differences in Al levels were tested by Tukey's multiple comparisons test. All analyses were performed with Systat for Windows, Version 5.0 (Systat Inc. Evanston, Ill.).

Results

Aluminum speciation

Addition of AlCl_3 to the cell cultures caused precipitation of about 55% of the added Al. The cell culture medium used for this study includes a plant product called Sequestrine, which contains 7% iron chelate. The remaining undefined

Fig. 4. Effects of AlCl_3 on ADC activity when Al was added to 0 to 6-day-old red spruce suspension cultures and the cells analyzed 24 h thereafter. Values are means \pm SE of three replicates.



component(s) of Sequestrine in half-strength Litvay's medium was largely responsible for the precipitation of Al (data not presented). All of the soluble Al was present in the monomeric form (Driscoll 1984) at final concentrations of approximately 0.09, 0.23, and 0.48 mM, respectively, for the 0.2, 0.5, and 1.0 mM AlCl_3 added to the cultures. More than 75% of this total monomeric Al was present as inorganic monomeric Al. The differences in P levels in the spent media collected from 3-day-old control and Al-treated cultures were not significant (data not presented).

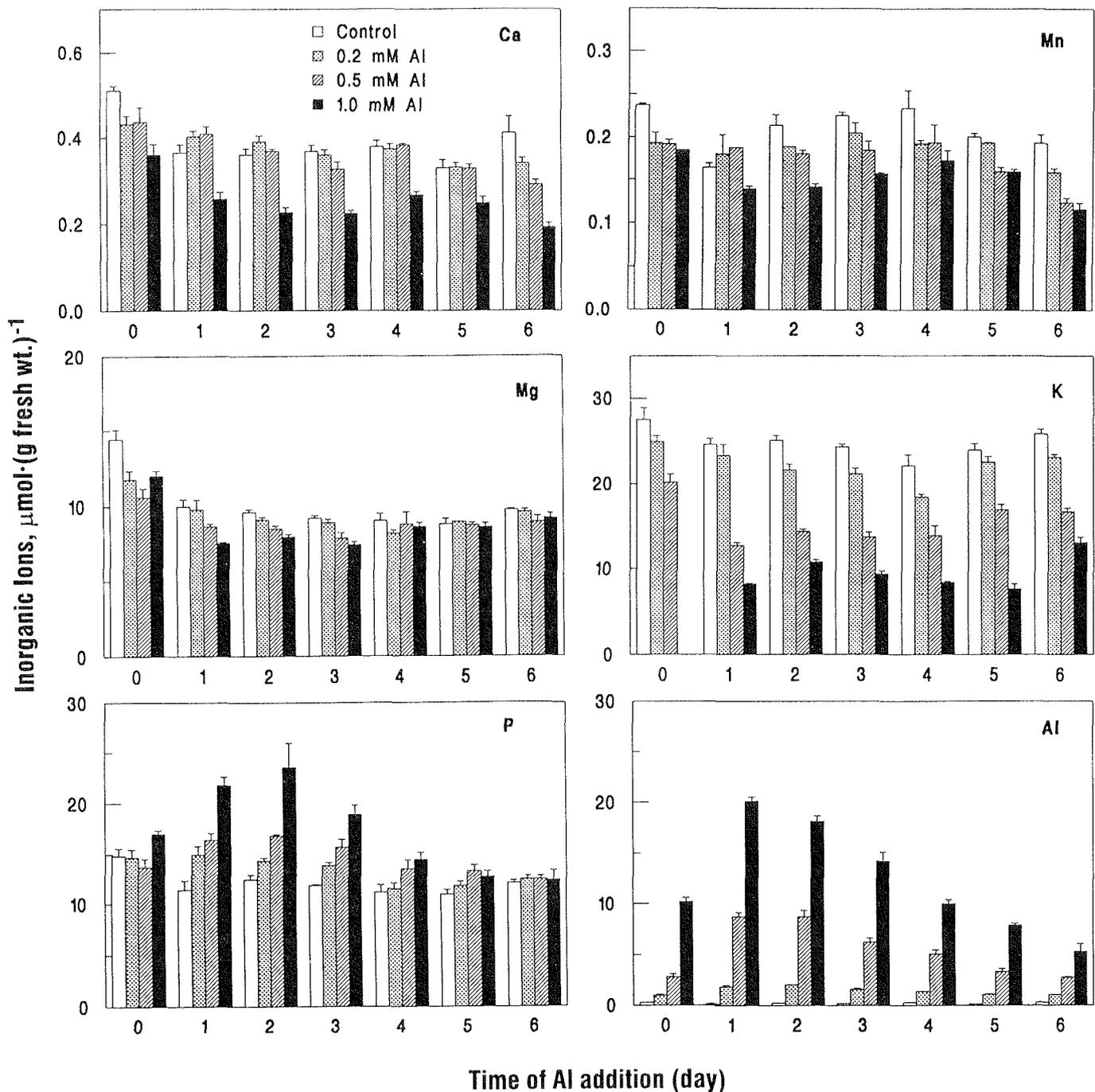
Time course of changes in total cell mass and polyamine metabolism in control cultures

The total cell mass doubled within 48 h after subculture, indicating a fast increase in cell size and (or) number. After day 2, the gain in total cell mass per culture was in the range of 20 to 40% per day up to day 7 (Fig. 1). The faster increase in cell mass coincided with the highest level of cellular putrescine between days 1 and 3. After this time, there was a gradual decrease in putrescine levels up to day 7 (Fig. 1). However, ADC enzyme activity slowly increased up to day 4 and stayed high. ODC activity was barely detectable in these cells. Neither spermidine nor spermine showed any consistent changes during the 7-day culture period. Whereas both putrescine and spermidine were present in reasonably high quantities (150–400 $\mu\text{mol}\cdot(\text{g fresh wt.})^{-1}$, spermine levels never exceeded 10% of the total PCA-soluble polyamine pool (Fig. 1).

Dependence of Al effects on cell age

With few exceptions, there was a dose-dependent inhibitory effect of Al on growth (as measured by change in total cell mass per culture) within 24 h, independent of the age at which Al was added to the culture. The inhibition by Al, however, was significantly higher for cultures that were 1–5 days old than for those that were 0 or 6 days old (Fig. 2). In the case of freshly subcultured cells and 6-day-old cells, 0.2 mM Al either had no effect or slightly promoted growth. Treatment with 0.5 and 1.0 mM Al caused a significant increase ($\alpha = 0.05$) in cellular levels of

Fig. 5. Effects of AlCl_3 on cellular inorganic ions when Al was added to 0 to 6-day-old red spruce suspension cultures and the cells analyzed 24 h thereafter. Values are means \pm SE of three replicates.



putrescine at all times except for 1 and 2 days (Fig. 3). This increase in putrescine was accompanied by an increase in ADC activity in these cells (Fig. 4). This effect was generally independent of culture age. With the exception of freshly subcultured cells, spermidine and spermine were either not affected or showed a slight increase in response to Al treatment (Fig. 3).

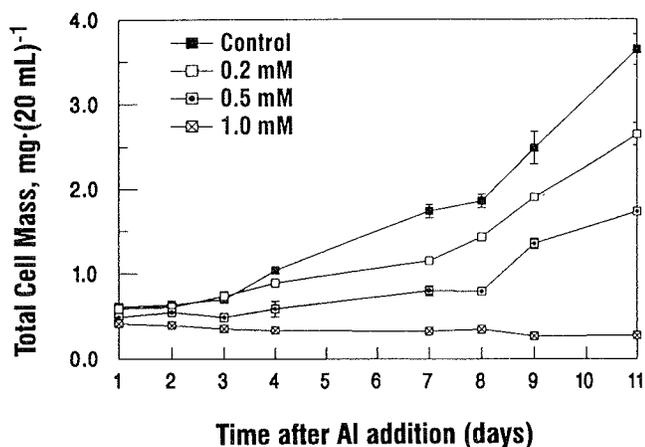
In general, cellular Ca was lowered by 1.0 mM Al treatment, Mg by 0.5 and 1.0 mM Al treatments, and Mn and K by all three Al treatments (Fig. 5). Both Al and P showed

an opposite trend. Maximum levels of Al and P were observed in 1 or 2-day-old cultures (Fig. 5). The above effects were observed throughout the 7 days of culture period with the exception of Mg and P, where little difference was seen at days 5 and 6.

Effects of short- and long-term (4-h to 11-day) incubation with Al

Once it was apparent that these cells were metabolically comparable between the culture age of 2 and 5 days and

Fig. 6. Effects of AlCl_3 on total cell mass when Al was added to 3-day-old red spruce suspension cultures and the cells analyzed at different times after Al addition. Values are means \pm SE of three replicates.



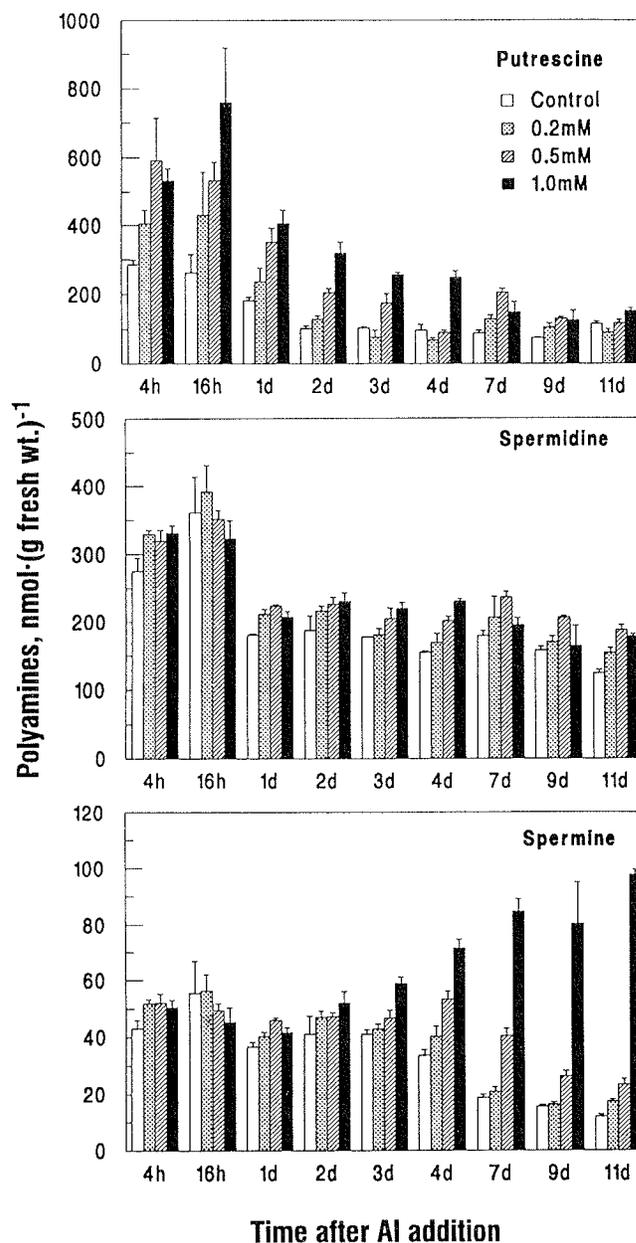
responded to Al in a similar manner, 3-day-old cultures were chosen for further studies. Our next objective was to see how early these effects of Al could be detected and what kind of long-term physiological effects Al treatments would have on these cells. The 3-day-old cultures were incubated with Al until the time of analysis. In cases where more than 4 days of incubation with Al were involved, the cells were not subcultured at their usual subculture interval (7 days of age); instead the cells continued to grow in the same flasks for up to 11 days.

As early as 1 day after the addition of Al, a significant effect on growth was observed at 1.0 mM Al concentration. Incubation with 0.2 mM Al, however, had no significant effect on growth for the first 3 days. Thereafter, there was a dose-dependent inhibition of growth that increased with increasing incubation time (Fig. 6).

Effects of Al treatments on polyamine metabolism could be seen as early as after 4 h of incubation (Fig. 7). In general, Al caused an elevation of cellular putrescine levels in these cells at all times. This effect was dose dependent for only up to 2 days of incubation. After 7 days of incubation (total culture age 10 days), only a small increase in putrescine was observed in response to Al. A maximum of a 3-fold increase was seen in putrescine with 1.0 mM Al after 2 days. Spermidine levels were either not affected or showed only a slight increase. This effect was not always dose dependent. With the exception of 4 and 16 h incubation, where results were variable in different experiments, Al addition always caused an elevation of cellular spermine levels. This effect was mostly dose dependent, and in contrast with changes in putrescine, the maximum increase in spermine was observed between 7 and 11 days.

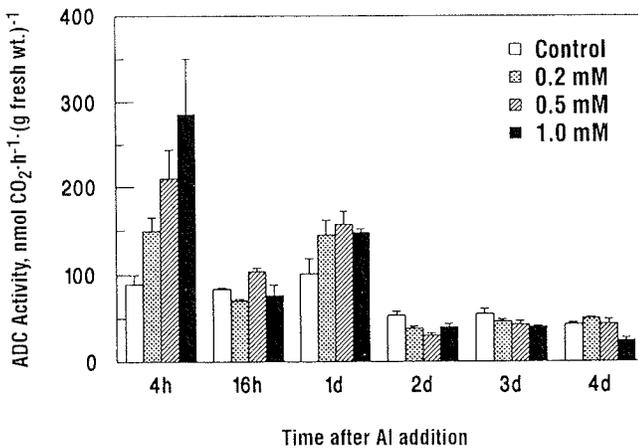
Changes in ADC activity were analyzed only up to 4 days of incubation and showed different trends from those observed for cellular putrescine (Fig. 8). An increase in ADC activity was seen only up to 24 h of incubation of 3-day-old cells with Al. After that period, ADC activity was slightly inhibited by all concentrations of Al.

Fig. 7. Effects of AlCl_3 on cellular polyamines when Al was added to 3-day-old red spruce suspension cultures and the cells analyzed at different times after Al addition. Values are means \pm SE of three replicates.



There was a dose-dependent increase in the cellular concentrations of Al and P at all times tested (Fig. 9). There was no change in cellular levels of Al with the time of incubation between 4 and 24 h when cultures were incubated with 0.2 mM Al. However, the final concentration of Al in 1.0 mM Al treated cells increased from 11.4 $\mu\text{mol}\cdot(\text{g fresh wt.})^{-1}$ 4 h after incubation to 23.94 $\mu\text{mol}\cdot(\text{g fresh wt.})^{-1}$ 24 h after incubation. Cellular K content showed a dose-dependent decrease during the first 7 days of the experiment. Consistent effects of Al on the cellular levels of Ca, Mn, and Mg were seen only for 2–4 days of incubation. Whereas K, Mn, and Mg decreased following

Fig. 8. Effects of AlCl_3 on ADC activity when Al was added to 3-day-old red spruce suspension cultures and the cells analyzed at different times after Al addition. Values are means \pm SE of three replicates.



treatment with 0.5 and 1.0 mM Al, Ca was significantly lowered only by 1.0 mM Al.

Discussion

Seedlings of red spruce and Norway spruce (*Picea abies* (L.) Karst.) have commonly been used for studying the effects of Al toxicity in forest trees. One-year-old red spruce seedlings grown in Al-containing nutrient solutions exhibited reduced root growth and increased root diameter (McQuattie and Schier 1990). A significant reduction of root biomass and foliar biomass was also reported with an increase in the levels of inorganic monomeric Al in the soils (Joslin and Wolfe 1988). A negative correlation between needle biomass and concentration of Al in the needles of red spruce was reported by Ohno et al. (1988). An inhibition of DNA synthesis and cell division was found to be associated with growth reduction in response to Al treatments in *in vitro* grown cells as well as intact plants of other species (Minocha et al. 1992; Ulrich and Clarkson 1992). The data presented here using suspension cultures of red spruce are consistent with the above reports on the inhibitory effects of Al on growth using intact plants.

The pattern of changes in cellular polyamines in response to physical, chemical, or biological stress varies not only with the species, cultivar, and tissue type, but also with the culture age (Flores 1991; Zhou et al. 1995). A variety of biological and chemical agents have been shown to induce an accumulation of cellular polyamines, especially putrescine, in several plant species (Dohmen et al. 1990; Santerre et al. 1990; Flores 1991). In most cases, the increased putrescine level is accompanied by an increase in ADC activity (Flores 1991). In this study also, treatment with Al resulted in an increase in cellular putrescine. This effect could be observed as early as after 4 h of incubation and was generally dose dependent after 2 days of incubation. In contrast with earlier reports with *C. roseus* (Zhou et al. 1995), the time of addition of Al, i.e., age of culture, had little effect on polyamine metabolism.

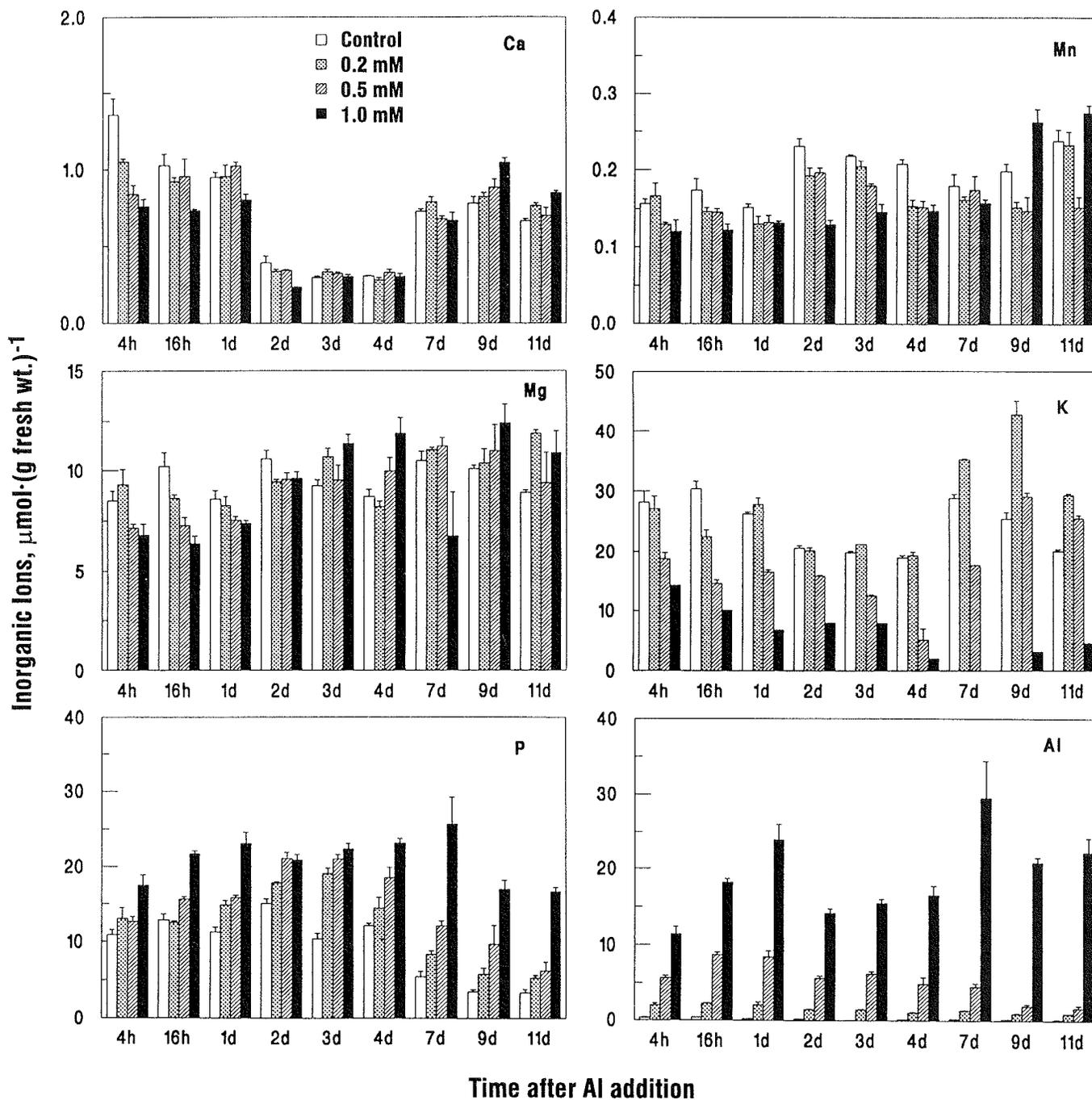
There is considerable variability in Al tolerance between species and even within species (Delhaize and Ryan 1995). The different responses observed for putrescine content during long-term incubation with Al for *C. roseus* (Minocha et al. 1992) and red spruce suspensions are probably due to their different sensitivities to Al. *Catharanthus roseus* showed an increase in putrescine content during the first 4 h, but an inhibition thereafter in response to Al treatment. In contrast, this stress response (increase in putrescine levels) lasted for several days in red spruce. Differences in the growth rates of cell cultures, i.e., *C. roseus* is faster growing than *P. rubens*, may also be partly responsible for this observed difference.

Based on data presented in Figs. 7 and 8, it can be argued that Al causes an increase in putrescine not only through increased ADC activity (4–24 h), but perhaps also through a reduction in putrescine catabolism. As the results show, ADC activity was actually lower in Al-treated cells after the first 24 h of incubation while putrescine levels remained high throughout this period. An alternative explanation may be that higher free putrescine levels are the result of its release from some covalently bound forms.

Aluminum is known to alter ion uptake and mineral composition in plants at relatively low concentrations. Aluminum often causes a reduction in Mg and Ca in needles, roots, or shoots under sand, water, or soil culture conditions in forest trees (Asp et al. 1988; Ohno et al. 1988; Schroder et al. 1988; Schier et al. 1990; Jentschke et al. 1991). Reports on the effects of Al on K do not show a consistent pattern. Whereas, in some cases, cellular K was inhibited by exposure to Al (Schier et al. 1990), in others there was no effect (Godbold et al. 1988; Ohno et al. 1988), and in yet others, Al caused an increase in cellular K (Cummings et al. 1985). Minocha et al. (1992) reported a decrease in Ca, Mg, Mn, and K accumulation in cells of *C. roseus* exposed to Al for only 4 h. This was coincident with an increase in putrescine in these cells. In the present study, where increased levels of putrescine persisted for much longer periods, decreased levels of these metal ions were also observed for a longer duration.

The comparison of P levels in the spent medium collected from 3-day-old control and Al-treated cultures showed no major differences, thus indicating that P was not limiting in Al-treated cultures, at least initially. The observed dose-dependent increase in P in the Al-treated cells is consistent with previous reports (Asp et al. 1988; Bengtsson et al. 1988; Minocha et al. 1992) and also suggests that P had not become limiting in the medium due to its precipitation. As discussed by Zhou et al. (1995), an interaction between Al and P may result in dual effects of Al on plants: (1) P may reduce Al toxicity by precipitating aluminum phosphate in the soil solution or on the root surfaces (McCormick and Borenden 1978) and (2) Al may cause P deficiency by its precipitation either on the cell surface or inside the cell (Robson and Pitman 1983). According to Hodson and Wilkins (1991), the plasma membrane acts as a barrier for Al to enter into the cytoplasm, thus a large proportion of Al measured as cellular component may actually represent that which is precipitated on the cell walls because of higher pH in the apoplast relative to the external solution. Recently, Lazof et al. (1994)

Fig. 9. Effects of AlCl₃ on cellular inorganic ions when Al was added to 3-day-old red spruce suspension cultures and the cells analyzed at different times after Al addition. Values are means ± SE of three replicates.



demonstrated the entry of Al into the symplast within 30 min of exposure of intact soybean root tips to Al, thus suggesting that a symplastic location for Al toxicity is possible. Whereas red spruce cells growing in the presence of 0.2 mM Al reached a quick equilibrium with the outside medium in terms of cellular Al concentration, those growing in 1.0 mM Al showed a steady increase in cellular Al levels with an increase in the time of incubation for up to 24 h. In comparison with *C. roseus*, where cells accumulated up to 5 $\mu\text{mol}\cdot(\text{g fresh wt.})^{-1}$ of Al within

24 h of incubation with 1.0 mM Al (Zhou et al. 1995), *P. rubens* cells showed a much higher cellular concentration of about 24 $\mu\text{mol}\cdot(\text{g fresh wt.})^{-1}$ at the same time.

The molar ratio of Al/Ca, rather than the absolute amounts of these ions, has been suggested to be critical in determining the level of Al toxicity (Schroder et al. 1988; Cronan and Grigal 1995). Several studies have reported that a molar ratio of greater than one for Al/Ca in the growth medium or in the root tips was correlated with an inhibition of growth (Schier 1985; Shortle and Smith

1988; Stienen and Bauch 1988; Kruger and Sucoff 1989; Schulze 1989). In the present study the molar ratio of Al/Ca was greater than one in the medium as well as in the cells. An inverse relationship between cellular putrescine and Ca was also observed in response to Al treatment. A similar response has been reported earlier (Minocha et al. 1992; Zhou et al. 1995). This observation supports the view that under stress conditions, putrescine (an organic cation) may substitute for Ca deficiency. A dose-dependent decrease observed in cellular K in response to Al may be analogous to the findings of Ryan et al. (1995), who observed an efflux of malate and K from root apices in wheat. It has been suggested that to maintain electroneutrality in the cells, malate must efflux from the cells along with equivalent efflux of some cations or influx of anions. The excreted malate detoxifies Al (by chelation) around the critical region of the root and thus protects the plant (Delhaize and Ryan 1995).

The consistency of our results (on the effects of Al on growth and inorganic ion uptake in *P. rubens*) with those obtained by others using seedlings of red spruce (Cronan and Grigal 1995) indicates that in vitro grown cell cultures are highly suitable for investigating the effects of Al on various biochemical pathways.

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