

# The effects of aluminum on *Picea rubens*: factorial experiments using sand culture

Stephen J. McCanny, William H. Hendershot,  
Martin J. Lechowicz, and Bill Shipley

**Abstract:** We used sand culture to examine the effects of aluminum ( $\text{Al}^{3+}$ ) on gas exchange and growth in 1+0 *Picea rubens* Sarg. seedlings. Growth solutions were prepared to match the elemental concentrations of the soil solutions in a declining *Abies balsamea* (L.) Mill. – *P. rubens* stand. In our first experiment, we used a four-way factorial design to examine the interactions between Al, pH, nutrients, and  $\text{CO}_2$ . All plants were nitrogen deficient. Photosynthetic rate and instantaneous water-use efficiency both declined at  $250 \mu\text{mol}\cdot\text{L}^{-1}$  Al, as did the root/shoot ratio of the seedlings. Plant weight, however, was not significantly affected by the 10-fold increase in Al solution concentration. These results were robust across a range of experimental conditions, suggesting that other aspects of soil acidification, such as  $\text{NO}_3$  fertilization in N deficient soils, could counteract the toxic effects of Al. In a second experiment, we examined the effects of subtoxic concentrations of Al at two levels of nutrient cation availability. Unlike in a previous study, cation fertilization affected neither gas exchange nor growth. While the decreases in root/shoot ratio and growth were expected at  $250 \mu\text{mol}\cdot\text{L}^{-1}$  Al, the peak in growth rates for intermediate concentrations of Al ( $25\text{--}100 \mu\text{mol}\cdot\text{L}^{-1}$ ) was not. The latter result was interpreted in light of the positive effects that low concentrations of Al are known to have on other species.

**Résumé :** Afin d'évaluer les effets de l'aluminium ( $\text{Al}^{3+}$ ) sur les échanges gazeux et la croissance de semis de *Picea rubens* Sarg. 1+0, nous avons utilisé une culture sur sable. Les solutions nutritives étaient préparées afin de correspondre aux concentrations en éléments de solutions du sol d'une forêt déperissante de *Abies balsamea* (L.) Mill. – *P. rubens*. Dans la première expérience, nous avons utilisé un dispositif factoriel à quatre paramètres afin d'évaluer les interactions entre Al, le pH, les nutriments et le  $\text{CO}_2$ . Toutes les plantes étaient déficientes en N. Le taux de photosynthèse et l'efficacité instantanée d'utilisation de l'eau ont diminué à  $250 \mu\text{mol}\cdot\text{L}^{-1}$  Al, aussi bien que le rapport racine/tige des semis. Toutefois, la masse des plants n'était pas affectée significativement par une augmentation de 10 fois des concentrations de Al dans les solutions. Ces résultats étaient valables pour une large gamme de conditions expérimentales, suggérant que d'autres aspects de l'acidification du sol, telle une fertilisation en  $\text{NO}_3$  dans les sols déficients, pourraient contrecarrer les effets toxiques de Al. Dans une seconde expérience, nous avons examiné les effets de concentrations sub-toxiques de Al à deux niveaux de disponibilité de cations nutritifs. Contrairement à ce qui avait été déterminé lors d'une étude précédente, la fertilisation en cations n'a affecté ni les échanges gazeux ni la croissance. Même si les diminutions du rapport racine/tige et de la croissance étaient prévisibles à  $250 \mu\text{mol}\cdot\text{L}^{-1}$  Al, le pic dans le taux de croissance pour les concentrations intermédiaires de Al ( $25\text{--}100 \mu\text{mol}\cdot\text{L}^{-1}$  Al) ne l'était pas. Ce dernier résultat a été interprété à la lumière des effets positifs connus de faibles concentrations de Al sur d'autres espèces.

[Traduit par la Rédaction]

Received January 17, 1994. Accepted August 2, 1994.

Stephen J. McCanny,<sup>1,2</sup> Martin J. Lechowicz, and Bill Shipley<sup>3</sup> Department of Biology, McGill University, 1205 Avenue Drive, Penfield, Montréal, QC H3A 1B1, Canada.

William H. Hendershot Department of Renewable Resources, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC H9X 1C0, Canada.

<sup>1</sup> Present address: Cultural and Natural Ecology, Professional and Technical Service Centre, Department of Canadian Heritage, 457 Main Street, Winnipeg, MB R3B 3E8, Canada.

<sup>2</sup> Author to whom all correspondence should be addressed.

<sup>3</sup> Present address: Département de biologie, Université de Sherbrooke, Sherbrooke, QC J1K 2R1, Canada.

## Introduction

The release of monomeric Al in acidified soils is a possible cause of forest decline in *Picea rubens* Sarg. Raynal et al. (1990) reviewed several studies that indicated a 20% reduction in seedling biomass for plants grown in solutions as dilute as  $250 \mu\text{mol}\cdot\text{L}^{-1}$  Al. Among forest tree species tested to date, this reflects a relatively high susceptibility to aluminum toxicity (Cronan and Goldstein 1989). Yet, Al rarely reaches these critical concentrations in forest soils (Cronan and Goldstein 1989; Miller et al. 1992). Here, we examine the effects of field concentrations of Al on seedling growth of *P. rubens*.

Aluminum effects, however, cannot be examined in isolation. Fossil fuel combustion, which is primarily responsible for acid deposition, leads to a number of positive and negative impacts on forest growth, including nutrient cation depletion, increased N deposition and elevated atmospheric  $\text{CO}_2$  concentrations.

Soil acidification tends to leach nutrient cations such as Ca and Mg from the soil column (Federer et al. 1989). There are strong indications that several forest decline events are related to nutrient cation deficiencies (Schulze 1989; Bondietti et al. 1990). Aluminum may play a role in these deficiencies, through its antagonism to Ca and Mg uptake (Schroeder et al. 1988; Goransson and Eldhuset 1991; Raynal et al. 1990). McLaughlin et al. (1991, 1993) proposed that low Ca availability results in a less efficient metabolism for *P. rubens*. They found that trees with lower foliar Ca concentrations respired larger proportions of the carbon fixed in photosynthesis. This trend held even when Ca was present at three to four times its threshold of sufficiency for growth (McLaughlin 1993). Such an unfavorable carbon balance could lead to long-term reductions in growth and susceptibility to the climatic events or disease and pest outbreaks that are often associated with forest decline (Johnson et al. 1989).

Acidic deposition also contains a strong component of nitric acid that acts as an N fertilizer (Johnson and Ball 1990). Several recent studies have shown increased growth or photosynthetic rate in *P. rubens* seedlings exposed to acid mist or simulated acid rain (Kohut et al. 1990; Lee et al. 1990; Thornton et al. 1992), an effect which is largely attributed to nitrates in the acid solutions. These inputs may lead to N saturation of a forest under certain circumstances (Johnson and Ball 1990) but the effect of increased inorganic N deposition is likely to be positive in most forests which remain deficient in N.

Increasing  $\text{CO}_2$  levels are also likely to have a positive effect on tree growth. Little is known about the capacity for an enriched atmosphere to alleviate symptoms of forest decline or Al toxicity (Johnson and Ball 1990). Several studies on N deficient trees have shown that the response to increased  $\text{CO}_2$  concentrations is independent of the response to N fertilization (Norby et al. 1986; Norby and O'Neill 1991). In this study, we test for a similar independence between the effects of Al and  $\text{CO}_2$ .

To examine these factors in different combinations, we conducted two growth chamber experiments in sand culture. The first examines the independence and relative magnitude of the effects of Al, nutrients, pH, and  $\text{CO}_2$  on the

photosynthesis and growth of *P. rubens*. The second focuses on the effects of subtoxic concentrations of Al and nutrient cations on respiration, photosynthesis, and growth. Together, these experiments help identify and separate the interactions that underlie *P. rubens* performance in complex and diverse natural environments.

## Methods

### Field study

To measure base-line concentrations of nutrients and Al for our sand culture solutions, we monitored soil solutions in a high-altitude *Abies balsamea* (L.) Mill. - *P. rubens* stand with an elevated mortality rate. Our study site was located at 900 m on Roundtop Mountain, Quebec, approximately 100 km southeast of Montréal. Precipitation at this altitude is  $124 \text{ cm}\cdot\text{year}^{-1}$  with an additional  $77 \text{ cm}\cdot\text{year}^{-1}$  in fog water deposition. In 1986, the precipitation had an average pH of 4.1 with  $46 \mu\text{mol}\cdot\text{L}^{-1}$  of  $\text{NO}_3$  and  $91 \mu\text{mol}\cdot\text{L}^{-1}$  of  $\text{SO}_4$ . The fog had a pH of 3.9 with  $73 \mu\text{mol}\cdot\text{L}^{-1}$  of  $\text{NO}_3$  and  $113 \mu\text{mol}\cdot\text{L}^{-1}$  of  $\text{SO}_4$  (Hendershot et al. 1992). Roundtop Mountain is part of the northern range of the Appalachian Mountains of North America and is composed of metamorphic rocks, specifically, phyllites of the West Sutton Formation (Slivitsky and St. Julien 1987). Soil solutions were collected in 10 ceramic-cup tension lysimeters at a depth of 10 cm below the top of the first mineral horizon. Solutions were sampled weekly from May 16 until October 22, 1990. Solution chemistry was analyzed as in Hendershot et al. (1992).

### Experimental conditions

*Picea rubens* seeds from eight eastern North American provenances were germinated in randomly ordered 110-mL IPL-45 containers. They were maintained for a period of one growing season under uniform conditions in the McGill University Phytotron. A computer-controlled glass house compartment was set to track outdoor conditions throughout the growing season. Seedlings were fertilized with "Plantprod" commercial fertilizer (20:20:20 N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O;  $3 \text{ g}\cdot\text{L}^{-1}$ ). After cold hardening, the seedlings were held just above  $0^\circ\text{C}$  in the compartment throughout the winter. They were transplanted into washed 16-mesh quartz sand, one per container. Each 3-L container measured 8 cm in diameter and 60 cm in depth. The plants were transferred in random order to four PGW36 Conviron growth chambers, where they were maintained under constant growth conditions and watered automatically with specific growth solutions (Shiple et al. 1992). The photosynthetic photon flux density at canopy height was  $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The chamber was set for a 15-h light period at  $23^\circ\text{C}$  and a 9-h dark period at  $15^\circ\text{C}$ . Relative humidity was held constant at 70%. Experimental concentrations of atmospheric  $\text{CO}_2$  were established within 2 days of transplanting. They were maintained within  $2 \mu\text{L}\cdot\text{L}^{-1}$  of the target value except for brief periods when the chamber doors were open.

Variability in the chemistry of growth solutions was minimized through the preparation of a concentrated stock solution for each treatment. We diluted these concentrates in 200-L polyethylene tanks, large enough to supply four growth chambers for 1 week. We adjusted pH by the addition

**Table 1.** Macronutrient composition of growth solutions simulating acidified soil in experiment 1.

Level	Weeks	Concentration ( $\mu\text{mol}\cdot\text{L}^{-1}$ )				
		NO <sub>3</sub>	NH <sub>4</sub>	K	Ca	Mg
Low	1–8	40	20	20	20	12
	9–15	15	5	10	20	12
High	1–8	120	60	60	60	36
	9–15	45	15	30	60	36

of several millilitres of 1.0 M H<sub>2</sub>SO<sub>4</sub> and added 5 mL of a micronutrient concentrate containing B, Mn, Fe, Cu, Zn, and Mo. During the first experiment, solutions were sampled each week for quality control of their contents. Departures of mean nutrient concentrations from the targeted values in Table 1 ranged from 0.3 to 5.0  $\mu\text{mol}\cdot\text{L}^{-1}$ , while the average coefficient of variation around these means was 5.2%.

Nutrient solutions were delivered to the plants using an automatic gravity siphon system (Shipley et al. 1992). Each plant received 25 mL of solution at approximately 70-min intervals. The average flow rate was 0.35 mL $\cdot\text{min}^{-1}$  for a total of 420 mL each day. There was a 4-h rest period during dark hours. Phosphorus was added separately to the system in the form of NaH<sub>2</sub>PO<sub>4</sub> to avoid precipitation with Al in the concentrated stock solutions.

### Experiment 1

This experiment consisted of a split-plot factorial design with two levels of the main plot factor (350 and 700  $\mu\text{L}\cdot\text{L}^{-1}$  CO<sub>2</sub>) and two levels for each of the split-plot variables: pH, aluminum, and nutrients. In each case, the lower level was chosen to reflect the base-line concentrations measured at our field site. The application of these three edaphic treatments was arranged in all possible combinations in a complete randomized block design, with 12 blocks of eight plants in each chamber.

The experiment was divided in two periods of 6–8 weeks with nutrient concentrations formulated to match a decline in soil-solution concentrations in the field. The lower nutrient level was chosen as the median concentration of the field data for a given period (Table 1). Inorganic P was not present in the soil solutions sampled in the field. Each week, we added 8.3 mL of 0.005 M NaH<sub>2</sub>PO<sub>4</sub> to each plant receiving the low-nutrient treatment and 25 mL/plant to those receiving the high-nutrient treatment. We chose the mean field pH (4.2) for the lower H<sup>+</sup> concentration and a relatively high field concentration of inorganic Al (25  $\mu\text{mol}\cdot\text{L}^{-1}$ ) as the lower Al<sup>3+</sup> level. High concentrations of nutrients and H<sup>+</sup> were three times greater than the low levels, while the high Al<sup>3+</sup> concentration was 10 times higher. This formulation yielded a high H<sup>+</sup> level (pH of 3.7) and a high Al<sup>3+</sup> concentration (250  $\mu\text{mol}\cdot\text{L}^{-1}$ ) at the toxicity threshold for the species.

We estimated the initial size of the seedlings 9–11 days after transplanting on April 29, 1991. We measured their total branch length and counted the number of buds. At 105–116 days after transplanting, the plants were har-

**Table 2.** Macronutrient concentrations of growth solutions used in experiment 2.

Level	Concentration ( $\mu\text{mol}\cdot\text{L}^{-1}$ )				
	NO <sub>3</sub>	NH <sub>4</sub>	K	Ca	Mg
Low	50	32	5	15	6
High	50	32	30	50	24

vested in random order. Harvest entailed gas-exchange measurements at the treatment concentration of CO<sub>2</sub> and the collection of needle, stem, and root tissue for dry weight determination and subsequent chemical analyses.

We estimated gas-exchange rates using a LI-COR 6200 (LI-COR Inc., Lincoln, Nebr.). Photosynthesis and transpiration were measured on the top 3–4 cm of each plant under growing conditions in a custom-made 0.5-L cuvette. Instantaneous water-use efficiency (WUE) was calculated as the number of millimoles of CO<sub>2</sub> fixed per mole of H<sub>2</sub>O transpired. We allowed enough time (30–75 s) for a 4  $\mu\text{L}\cdot\text{L}^{-1}$  drawdown of CO<sub>2</sub> within the cuvette before removing and harvesting the tissue within. We wore a mask with a tube leading out of the chamber while working at 350  $\mu\text{L}\cdot\text{L}^{-1}$  to avoid CO<sub>2</sub> buildup. The same objective was achieved in the 700  $\mu\text{L}\cdot\text{L}^{-1}$  chamber by leaving the door open. Mean cuvette concentrations of CO<sub>2</sub> were 406 and 694  $\mu\text{L}\cdot\text{L}^{-1}$ , respectively, for the low and high CO<sub>2</sub> treatments. Cuvette temperature and relative humidity averaged 26.7°C and 61.6% across treatments, with little variation. Vapor pressure tended to increase during the drawdown period with an average change of 1.2 mbar (1 mbar = 100 Pa) per observation.

The harvested tissue was dried and weighed separately to obtain the biomass of photosynthetic tissue in the cuvette for each photosynthesis measurement.

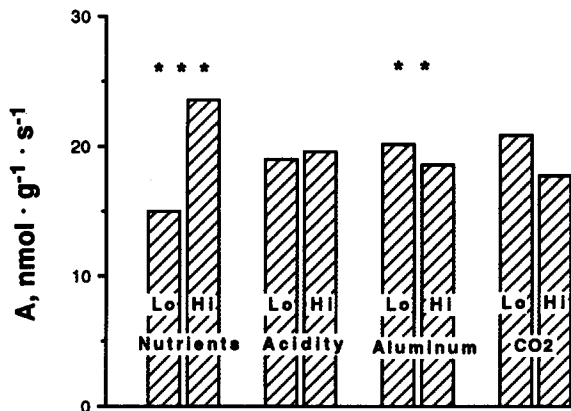
We harvested the remainder of each plant. These were dried at 70°C in a forced-air oven, separated into needles, stems, and roots, and then weighed. We ground needle tissue in a Wiley mill (40 mesh) and digested it in H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> for chemical analysis (Parkinson and Allen 1975). Foliar N concentration was estimated using flow injection analysis (TECATOR FIA 5020 analyzer), while Mg, Al, P, K, and Ca concentrations were determined through inductively coupled plasma spectrophotometry (Perkin-Elmer plasma 40 emission spectrophotometer).

We analyzed normalized response variables in analyses of covariance with blocking and a split-plot design. Total branch length and number of buds were included as initial size covariates in each analysis. This allowed us to remove any bias due to initially large seedlings in any treatment. We used type III sums of squares to handle the 2–25 missing values in the factorial design. There was a high probability of missing a significant CO<sub>2</sub> effect (type II error) because of the split-plot design with few degrees of freedom.

### Experiment 2

The experiment consisted of a factorial design with two levels of cations and four levels of Al. There were

**Fig. 1.** The main effects of the four factors on photosynthetic rate (A). Significant differences (for Figs. 1 and 2) between a pair of columns is represented by the following: \*\*\*,  $p < 0.0001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ .

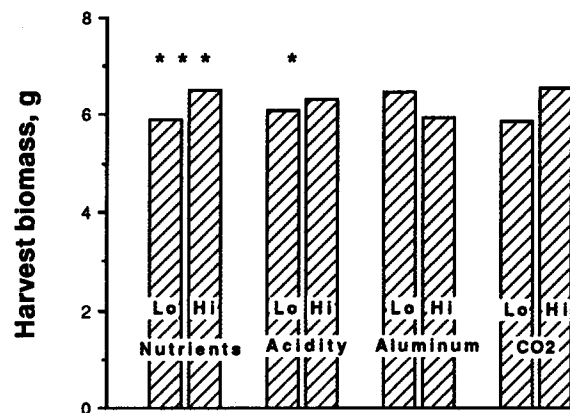


12 replicate blocks of these eight treatments. This time we did not lower the nutrient concentrations halfway through the experiment. The levels of Ca, Mg, and K were chosen to represent high and low concentrations as measured in the field (Table 2). Phosphorus was added as 8.3 mL/(plant·week) of a 0.015 M solution of  $\text{NaH}_2\text{PO}_4$ . Aluminum concentrations (5, 25, 100, and 250  $\mu\text{mol}\cdot\text{L}^{-1}$ ) were chosen to span the range of observed values and to include the previously observed toxicity threshold.

We estimated the initial height of the seedlings before transplanting. Only plants that were within one standard deviation of the mean height were used in the experiment. Thirty of these were harvested at the time of transplanting to determine the relationship between height and dry weight. Plants were grown for 4 months. At the end of this time, they were examined for their gas exchange properties and then immediately harvested. Photosynthesis was measured for 60 s on the top 3–4 cm of each plant. We then covered the cuvette with a double layered black felt bag. Following a 1-min equilibration period, dark respiration was measured for 60 s. Previous trials had shown that respiration rates did not change appreciably between 1 and 12 min after complete darkness. Cuvette temperature and relative humidity averaged 28°C and 49.8%, respectively. Adjustment of the flow of dry air into the cuvette allowed us to maintain vapor pressure roughly constant during the observations. The tissue within the cuvette was subsequently harvested and separated into needles and stems. Photosynthesis was expressed per gram dry weight of needles, while respiration was expressed per gram dry weight of needles and stem. We harvested the remainder of each plant. These were dried and weighed as in the first experiment. Needle tissues were combined by treatment and then analyzed as in the first experiment.

We examined normalized response variables in analyses of covariance with blocking. The volume of solution delivered to each plant during two replicate flushes was measured and estimates were used to calculate the total volume of solution delivered during the growing season. This variable was used as a covariate in the analyses to adjust for biases in the delivery system. The growth increment during

**Fig. 2.** Harvest biomass of *P. rubens* as affected by the four factors. Significant differences between a pair of columns is represented as in Fig. 1.



the experiment was calculated as the difference between the final harvest weight and the initial weight calculated from a binomial regression on plant height ( $r^2 = 0.50$ ).

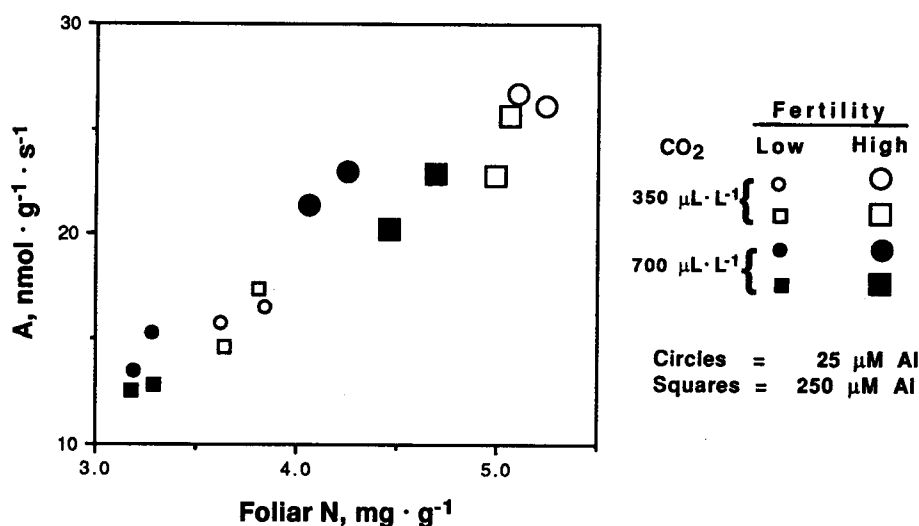
## Results

### Experiment 1

This experiment demonstrated that an increase in substrate Al concentration from 25 to 250  $\mu\text{mol}\cdot\text{L}^{-1}$  Al yields a negative effect that is robust across a range of growth conditions, including a strong fertility gradient (Figs. 1 and 2). Photosynthetic rates (A) were significantly lower for *P. rubens* grown at 250  $\mu\text{mol}\cdot\text{L}^{-1}$  Al (Table 3). This effect was independent of the fertilization, pH, and  $\text{CO}_2$  factors in the experiment (i.e., no significant interactions; Table 4, Fig. 1). Like photosynthesis, instantaneous water-use efficiency (WUE) declined by 7.9%. However, a proportional decline in harvest biomass was not significant (Table 3, Fig. 2). Neither water-use efficiency nor growth demonstrated an interaction between Al and the other factors. The relationship between foliar N concentration and photosynthesis was consistent across the full range of treatments (Fig. 3), indicating no effect of Al on N-use efficiency under these growth conditions.

Decreases in performance were accompanied by changes in foliar chemistry and biomass allocation. Apart from increasing the concentration of foliar Al, the application of a 250  $\mu\text{mol}\cdot\text{L}^{-1}$  solution of Al decreased the foliar concentrations of the divalent cations, Ca and Mg (Table 3). For Mg, this trend was more evident under acidic conditions ( $\text{H}^+ \times \text{Al}^{3+}$  interaction:  $F = 5.29$ ;  $\text{df} = 1, 328$ ;  $p < 0.05$ ). Concentrations of K increased in the foliage of Al-treated plants, especially under conditions of high fertility and high acidity (Table 3; nutrients  $\times$   $\text{Al}^{3+}$  interaction:  $F = 4.91$ ;  $\text{df} = 1, 328$ ;  $p < 0.05$ ;  $\text{H}^+ \times \text{Al}^{3+}$  interaction:  $F = 7.62$ ;  $\text{df} = 1, 328$ ;  $p < 0.01$ ). Foliar P concentrations were also enhanced by Al under acid conditions ( $\text{H}^+ \times \text{Al}^{3+}$  interaction:  $F = 6.9$ ;  $\text{df} = 1, 326$ ;  $p < 0.05$ ). Apart from its chemistry, the relative amount of foliage was altered for plants grown in high Al. The root/shoot ratio declined as a result of increased proportional investment in needles and decreased investment in roots (Table 3).

**Fig. 3.** Plot of treatment means of photosynthetic rate ( $A$ ) and foliar N concentration. Each point represents the mean value for 20–24 seedlings.



**Table 3.** Mean response of *Picea rubens* seedlings to a tenfold increase in Al concentration in their growth solutions in sand culture.

	<i>n</i>	Mean for low Al	Mean for high Al	% diff.	<i>F</i>	<i>p</i>
<b>Gas exchange</b>						
<i>A</i> (nmol CO <sub>2</sub> ·g <sup>-1</sup> ·s <sup>-1</sup> )	360	20.1	18.5	-7.9	14.2	0.0002
WUE (mmol/mol)	360	2.91	2.68	-7.9	11.1	0.001
<b>Biomass</b>						
Harvest weight (g)	383	6.47	5.93	-8.3	0.8	0.36
Needle allocation (%)	381	37.9	39.0	+2.9	6.7	0.01
Stem allocation (%)	381	20.2	19.9	-1.5	0.6	0.43
Root allocation (%)	381	41.9	41.1	-1.9	4.2	0.04
Root/shoot ratio (g·g <sup>-1</sup> )	381	0.74	0.71	-4.1	4.2	0.04
<b>Foliar chemistry</b>						
N (mg·g <sup>-1</sup> )	358	4.12	4.13	+0.2	0.5	0.49
P (mg·g <sup>-1</sup> )	357	1.31	1.30	-0.7	1.9	0.16
K (mg·g <sup>-1</sup> )	359	4.43	4.97	+12.2	16.2	0.0001
Ca (mg·g <sup>-1</sup> )	359	5.95	5.56	-6.6	5.3	0.02
Mg (mg·g <sup>-1</sup> )	359	1.24	1.18	-4.8	6.5	0.01
Al (mg·g <sup>-1</sup> )	359	0.08	0.14	+67.1	171.5	0.0001

### Experiment 2

Dark respiration was positively correlated with photosynthetic assimilation for the *P. rubens* seedlings in this experiment ( $r^2 = 0.30$ ). The Al and cation treatments did not appear to affect these metabolic rates nor the ratio of photosynthesis to respiration (Tables 5 and 6, Figs. 4a and 4b). Nonetheless, the results were consistent with experiment 1 in showing a significantly lower photosynthetic rate in the 250 μmol/L Al treatment than at 25 μmol/L Al when these two levels were analyzed as a separate data set (Fig. 4a;  $F = 4.76$ ;  $df = 1, 32$ ;  $p < 0.04$ ).

The growth increment of *P. rubens* during the 4-month experiment was significantly affected by Al but not by the nutrient cations (Fig. 4c; Table 6). Multiple regression yielded a significant quadratic term for Al with a negative sign ( $p < 0.01$ ). This indicates that growth was optimal

at intermediate Al levels, probably between 25 and 100 μmol·L<sup>-1</sup>. The root/shoot ratio declined with increasing Al concentration (Fig. 4d). This is the result of decreased allocation to root tissues at high Al levels (Table 6).

### Field versus phytotron

The foliar chemistry of seedlings grown in the phytotron did not match that of mature trees at our field site despite the exposure of their roots to similar nutrient concentrations (Table 7). Foliar N concentrations were consistently lower in the experimental plants, while Ca and Mg levels were higher. According to Swan's (1971) critical values, N was deficient in all groups of plants, particularly so in experiment 1. Needles from the field were at the threshold of sufficiency for Mg but above the threshold for the phytotron grown needles. In the experimental plants, foliar Al

**Table 4.** Split-plot analysis of covariance of the effects of Al, nutrients, pH, and CO<sub>2</sub> on the photosynthetic rate of *Picea rubens* seedlings in experiment 1, using initial seedling size estimates as covariates.

	df	Mean square	F	p
<b>Main plot factor</b>				
CO <sub>2</sub>	1	5.757	2.9	0.23
Main plot error	2	1.979		
<b>Covariates</b>				
Initial branch length	1	26.134	71.2	0.0001
Initial number of buds	1	2.471	6.7	0.001
<b>Spatial arrangement</b>				
Block	11	1.655	4.5	0.0001
<b>Split-plot factors</b>				
Al	1	5.197	14.2	0.0002
Nutrients	1	86.926	236.7	0.0001
pH	1	0.792	2.2	0.14
<b>Interactions</b>				
Al × nutrients	1	0.878	2.4	0.12
Al × pH	1	0.004	0.0	0.91
Al × CO <sub>2</sub>	1	0.516	1.4	0.24
Nut. × pH	1	0.638	1.7	0.19
Nut. × CO <sub>2</sub>	1	0.002	0.0	0.94
pH × CO <sub>2</sub>	1	0.084	0.23	0.63
Al × nutrients × pH	1	0.031	0.1	0.77
Al × nutrients × CO <sub>2</sub>	1	0.600	1.6	0.20
Al × pH × CO <sub>2</sub>	1	0.477	1.3	0.25
Nut. × pH × CO <sub>2</sub>	1	0.590	1.6	0.21
Al × nutrients × pH × CO <sub>2</sub>	1	0.952	2.6	0.11
Split-plot error	329	0.367		

NOTE: Photosynthetic rates were transformed to the square root of their values before analysis.

approached concentrations deemed to be toxic by Raynal et al. (1990).

## Discussion

### Applicability of results

Several indicators lead us to argue that our results have applicability beyond plants with critically low foliar N concentrations. There were no interactions between the fertilization and Al treatments for any of the variables except foliar K concentration. In other words, fertilization did little to interfere with the impact of Al on the physiology of *P. rubens*, even at very low N concentrations. The linear relationship between photosynthetic rate and foliar N for experiment 1 (Fig. 3) indicates that, within the range of our study, the former can be predicted through linear interpolation. The slope of this line is also consistent with that of Amundson et al. (1992) on *P. rubens* at higher N concentrations and photosynthetic rates, suggesting that extrapolation of our results is also possible. We expect that 250  $\mu\text{mol}\cdot\text{L}^{-1}$  Al will suppress the photosynthetic rates of plants with field concentrations of foliar N. This

**Table 5.** Analysis of covariance of the effects of Al and cations on the photosynthetic rate of *Picea rubens* seedlings in experiment 2, using nutrient solution volume and initial plant weight as covariates.

	df	Mean square	F	p
<b>Covariates</b>				
Nutrient volume	1	2.196	2.5	0.12
Initial weight	1	0.607	0.7	0.41
<b>Spatial arrangement</b>				
Block	11	1.170	1.3	0.23
<b>Factors and interaction</b>				
Cations	1	0.020	0.02	0.88
Al	3	1.490	1.68	0.18
Cations × Al	3	0.036	0.04	0.99
Error	74	0.885		

NOTE: Photosynthetic rates were transformed to the square root of their value before analysis.

suppression may occur at levels as low as 100  $\mu\text{mol}\cdot\text{L}^{-1}$  Al (Fig. 4a).

We highly recommend the use of sand culture for the separation of the various, partially correlated edaphic factors that affect plants in acid soils. However, future experiments should focus on the plant's perception of the nutrient regime (i.e., nutrient uptake or nutrient content) rather than on recreating the soil or soil solution in which the plant grows in the field. McLaughlin et al. (1993) were unable to reproduce the foliar nutrient concentrations of trees from the field, even when grown in the same soil. As in this study, they measured foliar Ca and Mg levels that were approximately twice those of trees grown in the field. In our effort to reflect field conditions we used NO<sub>3</sub> and NH<sub>4</sub> concentrations that were much more dilute than traditional growth solutions (Smith et al. 1983). Although our automatic gravity siphon system kept the roots bathed in solutions similar to those measured in the field, the N supply was clearly insufficient for plant uptake. It may be that our field sampling design failed to detect the vertical and horizontal variation of N concentrations in the field. However, exchangeable N pools are typically small compared with forest N uptake (Johnson 1992). This suggests that, like P, N is taken up from decaying litter without entering the soil solution.

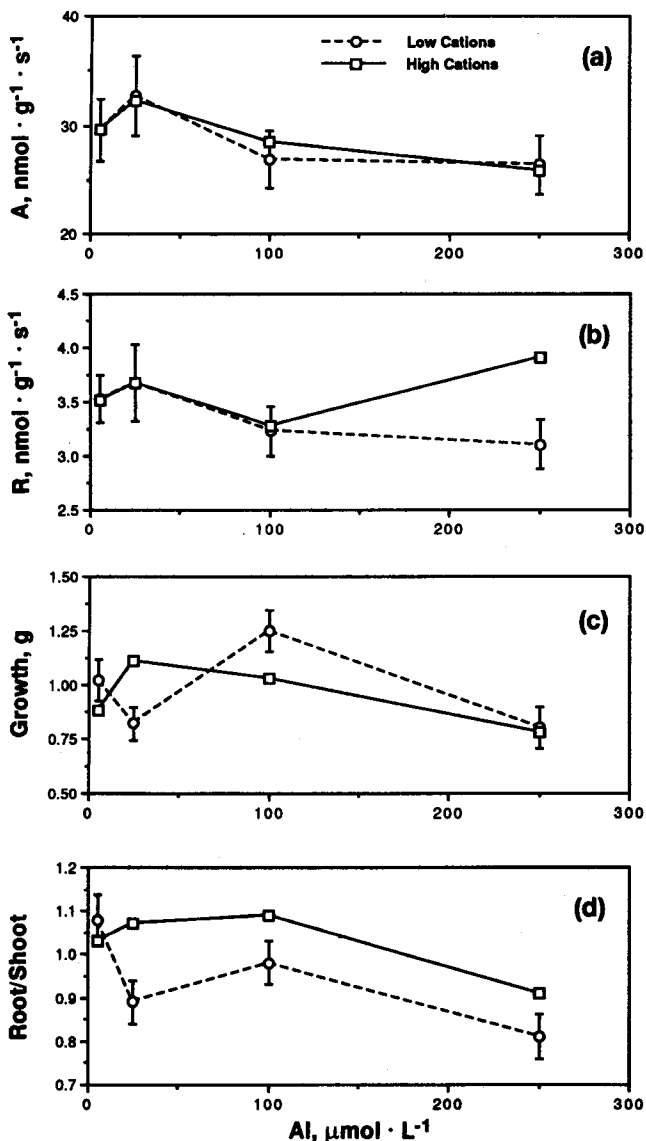
### Aluminum

Simulated acid rain studies (Lee et al. 1990; McLaughlin et al. 1993) and field exclosure experiments (Kohut et al. 1990; Thornton et al. 1992) have yielded variable results for the effects of acid precipitation on photosynthesis. This is partly the result of the lack of separation between the effects of Al release from the soil and NO<sub>3</sub> input from the precipitation. We have shown these effects to be independent, at least at low levels of N availability. Future deposition studies should quantify the relative importance of these two effects.

**Table 6.** Effects of Al and cations on gas exchange, growth, and carbon allocation in *Picea rubens* seedlings from experiment 2.

Variable	n	Mean	Al		Cations		Al × cations	
			F	p	F	p	F	p
Respiration (nmol·g <sup>-1</sup> ·s <sup>-1</sup> )	95	3.1	0.9	0.43	0.5	0.48	0.7	0.58
Photosynthesis/respiration ratio	95	8.5	1.1	0.37	0.9	0.35	0.6	0.60
Growth (g)	96	0.94	4.1	0.01	0.2	0.67	2.0	0.12
Needle allocation (%)	96	34.2	2.5	0.06	2.0	0.16	0.6	0.64
Stem allocation (%)	96	17.1	2.0	0.11	0.4	0.55	0.8	0.51
Root allocation (%)	96	48.7	3.7	0.01	1.8	0.17	1.3	0.29
Root/shoot ratio (g·g <sup>-1</sup> )	96	0.98	3.3	0.02	3.0	0.08	1.1	0.37

**Fig. 4.** Effects of Al and cations on (a) photosynthesis, (b) respiration, (c) growth, and (d) root/shoot ratio of seedlings in experiment 2. Error bars on the low-cation treatments represent  $\pm 2$  SE.



The suppression of photosynthesis and instantaneous water-use efficiency by 250  $\mu\text{mol}\cdot\text{L}^{-1}$  Al in *P. rubens* is more easily explained as the result of a decline in the root/shoot ratio than a change in foliar chemistry. Root inhibition is a common result of Al toxicity (Foy 1984; Thornton et al. 1987; Joslin and Wolfe 1988). It is likely to have a negative effect on nutrient supply to the leaves and subsequently on the rate of photosynthesis. Under the well-watered conditions of our experiments, the rate of transpiration is unlikely to decrease. Hence, the ratio of CO<sub>2</sub> uptake to H<sub>2</sub>O loss (or water use efficiency) declines. In the field, the effects of Al on photosynthesis may be more pronounced because of stomatal closure under variable moisture conditions.

Though we confirmed the tendency for Al to inhibit Ca and Mg uptake (Table 3; Raynal et al. 1990), the connection in this study between foliar cation concentrations and photosynthesis remains weak. Magnesium offers the simplest connection with photosynthesis, in being an essential component of chlorophyll. Zimmerman et al. (1988) provided evidence of the effect of low Mg concentrations on photosynthesis in field populations of *Picea abies* (L.) Karst. Yet, foliar concentrations of both Mg ( $r^2 = 0.04$ ) and Ca ( $r^2 = 0.18$ ) were not well correlated with photosynthetic rate for experiment 1.

McLaughlin et al. (1990) postulated a connection between cell membrane integrity at low foliar Ca levels and high rates of dark respiration. They argued that the loss of photosynthate from cell vacuoles resulted in increased respiration. In testing this idea, they found a positive relationship between foliar Ca and the efficiency of gas exchange (in this case, moles of carbon fixed per mole of carbon respired), even though their plants exceeded Swan's (1971) growth sufficiency limits for Ca (McLaughlin 1991, 1993). These experiments were performed in soil, making it difficult to estimate Al and Ca supply rates. Our experiment attempted to show the direct effects of Al and Ca amendments on gas exchange. In finding no effect on respiration, we place in doubt their interpretation of the response to an acidic deposition gradient. On the other hand, our foliar Ca levels were 2.0–3.0 mg·g<sup>-1</sup> greater than the already elevated levels in McLaughlin et al. (1993) and we may have reached the sufficiency level for this physiological response.

**Table 7.** Foliar nutrient concentrations ( $\text{mg}\cdot\text{g}^{-1}$ ) for mixed-age needle samples from mature *Picea rubens* trees at the Roundtop Mountain field site and for seedlings grown in the Phytotron with low concentrations of nutrients and Al in experiment 1 (Table 1) and experiment 2 (Table 2).

Element	Field	Exp. 1	Exp. 2	Critical concn.
N	12.0	3.6	6.5	13.0–16.0
P	1.0	1.1	2.7	1.4–1.8
K	4.7	4.0	4.8	3.0–4.0
Ca	3.0	5.8	5.6	0.8–1.2
Mg	0.7	1.3	1.2	0.6–0.8
Al	0.04	0.07	0.12	0.10–0.15

NOTE: Critical nutrient concentrations for sufficiency are from Swan (1971) and for Al toxicity are from Raynal et al. (1989).

### Other factors

The strong fertilization response in experiment 1 can be readily attributed to N effects in view of the critically low foliar concentrations of N and the linear relationship between foliar N and photosynthesis (Fig. 3).

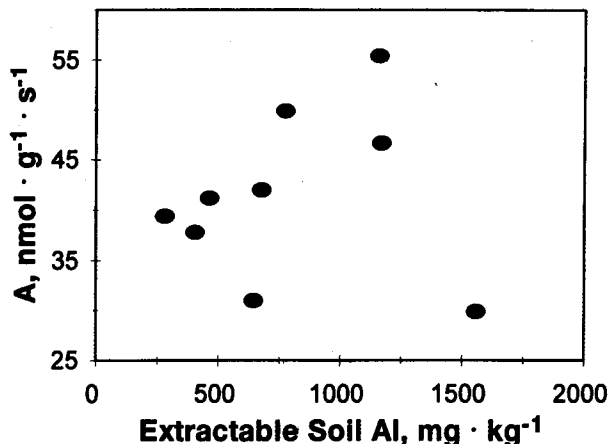
The responses to doubled  $\text{CO}_2$ , though proportionally larger than the effects of pH and Al, were not significant. The difficulty here is one of statistical power. The unit of replication for  $\text{CO}_2$  effects is the growth chamber. It is not valid to argue that plants in the same chamber represent replicates of a given  $\text{CO}_2$  level. There are a number of confounding chamber effects. The decline in photosynthetic rate at  $700\ \mu\text{L}\cdot\text{L}^{-1}$   $\text{CO}_2$  seems a little surprising but represents a known suppression phenomenon attributed to starch accumulation and end-product inhibition (Ehret and Joliffe 1985). The 9% increase in growth (Fig. 2) and the significant growth increase demonstrated in our earlier work (Shipley 1993) are within the range of previously reported responses of coniferous seedlings to elevated atmospheric concentrations of  $\text{CO}_2$  (Eamus and Jarvis 1989).

The positive effect of decreasing growth solution pH to 3.7 (Fig. 2) likely represents an artifact of sand culture. Plants grown in solutions with low pH had significantly higher foliar concentrations of P (results not shown). We hypothesize that Al precipitated the weekly dose of P to create a P nutrient pool within the sand column. Treatments with more acid growth solutions released this nutrient at a faster rate throughout the growing season. The higher concentration of foliar P in the high Al and low pH treatments (significant interaction term) would then represent the result of a larger pool of precipitated P where Al was abundant.

### Robustness of response

The independence of the Al response to pH, nutrients, and  $\text{CO}_2$  suggests that other factors related to acid precipitation may offset the toxic effects of Al. An enhanced  $\text{NO}_3$  supply, which is often concomitant with high Al levels in the field, will compensate for the effects of

**Fig. 5.** Scatterplot of photosynthetic rate ( $A$ ) in *P. rubens* saplings grown at different altitudes in the Great Smoky Mountains versus the extractable Al content of their soil. Adapted from McLaughlin et al. (1991).



Al on photosynthesis in N deficient soils (Fig. 3). Although  $\text{CO}_2$  concentrations close to  $700\ \mu\text{L}\cdot\text{L}^{-1}$  are not expected for another 50 years (Bolin 1986), this is well within the life-span of present-day seedlings. Trees in polluted environments may compensate for toxic Al in the soil with increased N and C supply rates.

### Unimodal response

The effects of Al on plant growth and metabolism were largely positive at low subtoxic levels in experiment 2. This is not an unusual response to low concentrations of Al. Foy (1984) gives examples of a variety of plant species, including *Eucalyptus* (Mullette 1975) and peach (*Prunus persica* (L.) Batsch; Edwards et al. 1976) trees, that benefit from the addition of Al. Because Al is not an essential element, this response has been explained by a variety of mechanisms, including the enhancement of Fe and P uptake, the alteration of growth regulators in roots, the prevention of Cu and Mn toxicities, and the control of invasive fungi (Foy 1984). The uptake of P does not appear to be the decisive factor in our results, because P was applied at three times the rate that was found to be sufficient in experiment 1 and the foliar P levels are well above critical concentrations (Table 7). We also found the same unimodal response to Al when the experiment was repeated at a lower P supply rate (results not shown).

This raises the possibility that Al exerts a positive influence on *P. rubens* growth at the concentrations it is found in the field. Toxic concentrations of Al are rarely encountered in acid soils (Cronan and Goldstein 1989). Miller et al. (1992) conclude that "if Al has played a role in the decline of red spruce at Whiteface Mountain, Al antagonism must operate at concentrations less than or equal to 60 or  $80\ \mu\text{mol}\ \text{L}^{-1}$  labile Al." We found growth to be optimal and root/shoot ratios little affected between 25 and  $100\ \mu\text{mol}\cdot\text{L}^{-1}$  Al (Figs. 4c and 4d). On the other hand, though the trend is not significant, it appears likely that the photosynthetic rate declines over this range (Fig. 4a). We do not have sufficient data on foliar chemistry from experiment 2 to comment on Al antagonism to Ca and

Mg at field concentrations. In sum, we were unable to detect strong effects of Al at the levels at which it is known to occur in declining *Abies balsamea* (L.) Mill. – *P. rubens* stands.

As we have aptly demonstrated for N in this study, the soil solution concentration of an element need not reflect its availability to the plant. *Picea rubens* may be more susceptible to Al in soil than in sand culture. Some of the beneficial aspects of moderate concentrations of Al, for example, its use as a fungicide, may not be as important in the field. Nonetheless, we believe that the enhanced response of *P. rubens* to intermediate concentrations of Al can be partially corroborated with field data. A re-examination of data from McLaughlin et al. (1991) indicates increasing photosynthetic rates with higher soil concentrations of Al except at the very highest concentration (Fig. 5).

## Conclusions

In our experimental system, Al did not inhibit growth in *P. rubens* nor cause a metabolic imbalance at field concentrations ( $<100 \mu\text{mol}\cdot\text{L}^{-1}$ ). It may even play a positive role. The toxic effects we found on photosynthesis and growth at  $250 \mu\text{mol}\cdot\text{L}^{-1}$  were slight and could potentially be counteracted by increases in nutrient or  $\text{CO}_2$  availability. We confirmed the antagonism of Al to Ca and Mg uptake but were unable to demonstrate its consequences for respiration, partly because of elevated foliar concentrations of these nutrients. Our work does not support the hypothesis that Al release in acidified soils plays a major role in the decline of *P. rubens* populations. The definitive test of this hypothesis would involve an experimental protocol where the concentrations of critical nutrients, especially Ca and Mg, can be reproduced from field concentrations in both the growth solution and the photosynthetic machinery.

## Acknowledgements

We thank Stéphane Dumont and H el ene Lalande for coordinating work in the growth facility and laboratory, respectively. They were ably assisted by Jennifer Hilbers, Rita Morbia, Catherine Leduc, and S ebastien Sauv e. Mark Romer and Claire Cooney provided assistance in the McGill University Phytotron. This research was supported by Strategic Grant STR 0045181 from the Natural Sciences and Engineering Research Council of Canada to W.H.H. and M.J.L.

## References

- Amundson, R.G., Hadley, J.L., Fincher, J., Fellows, S., and Alscher, R.G. 1992. Comparisons of seasonal changes in photosynthetic capacity, pigments and carbohydrates of healthy sapling and mature red spruce and of declining and healthy red spruce. *Can. J. For. Res.* **22**: 1605–1616.
- Bondietti, E.A., Momoshima, N., Shortle, W.C., and Smith, K. 1990. A historical perspective on changes in divalent cation availability to red spruce in relationship to acidic deposition. *Can. J. For. Res.* **20**: 1850–1858.
- Cronan, C.S., and Goldstein, R.A. 1989. ALBIOS: a comparison of aluminum biogeochemistry in forested watersheds exposed to acidic deposition. *In Advances in environmental science, acid precipitation series. Edited by D. Adriano and M. Havas.* Springer-Verlag, New York. pp. 113–135.
- Eamus, D., and Jarvis, P.G. 1989. The direct effects of increase in the global atmospheric  $\text{CO}_2$  concentration on natural and commercial temperate trees and forests. *Adv. Ecol. Res.* **19**: 1–55.
- Edwards, J.H., Horton, B.D., and Kirkpatrick, H.C. 1976. Aluminum toxicity symptoms in peach seedlings. *J. Am. Soc. Hortic. Sci.* **102**: 459–461.
- Ehret, D.L., and Jolliffe, P.A. 1985. Photosynthetic carbon dioxide exchange of bean plants grown at elevated carbon dioxide concentrations. *Can. J. Bot.* **63**: 2026–2030.
- Federer, C.A., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, R.S., and Smith, C.T. 1989. Long term depletion of calcium and other nutrients in eastern US forests. *Environ. Manage.* **13**: 593–601.
- Foy, C.D. 1984. Physiological effects of hydrogen, aluminum and manganese toxicities in acid soil. *In Soil acidity and liming. 2nd ed. Edited by F. Adams.* American Society of Agronomy, Madison, Wis.
- Goransson, A., and Eldhuset, T.D. 1991. Effects of aluminum on growth and nutrient uptake of small *Picea abies* and *Pinus sylvestris* plants. *Trees*, **5**: 136–142.
- Hendershot, W.H., Courchesne, F., and Schemenauer, R.S. 1992. Soil acidification along a topographic gradient on Roundtop Mountain, Quebec, Canada. *Water Air Soil Pollut.* **61**: 235–242.
- Johnson, A.H., Siccama, T.G., Silver, W.L., and Battles, J.J. 1989. Decline of red spruce in high-elevation forests of New York and New England. *In Advances in environmental science, acid precipitation series. Edited by D. Adriano and M. Havas.* Springer-Verlag, New York. pp. 85–112.
- Johnson, D.W. 1992. Nitrogen retention in forest soils. *J. Environ. Qual.* **21**: 1–12.
- Johnson, D.W., and Ball, J.T. 1990. Environmental pollution and impacts on soils and forests in North America. *Water Air Soil Pollut.* **54**: 3–20.
- Joslin, J.D., and Wolfe, M.H. 1988. Responses of red spruce seedlings to change in soil aluminum in six amended forest soil horizons. *Can. J. For. Res.* **18**: 1614–1623.
- Kohut, R.J., Laurence, J.A., Amundson, R.G., Raba, R.M., and Melkonian, J.J. 1990. Effects of ozone and acidic precipitation on the growth and photosynthesis of red spruce after two years of exposure. *Water Air Soil Pollut.* **51**: 277–286.
- Lee, W.S., Chevone, B.I., and Seiler, J.R. 1990. Growth response and drought susceptibility of red spruce seedlings exposed to simulated acidic rain and ozone. *For. Sci.* **36**: 265–275.
- McLaughlin, S.B., Andersen, C.P., Edwards, N.T., Roy, W.K., and Layton, P.A. 1990. Seasonal patterns of photosynthesis and respiration of red spruce

- saplings from two elevations in declining southern Appalachian stands. *Can. J. For. Res.* **20**: 485–495.
- McLaughlin, S.B., Andersen, C.P., Hanson, P.J., Tjoelker, M.G., and Roy, W.K. 1991. Increased dark respiration and calcium deficiency of red spruce in relation to acidic deposition at high-elevation southern Appalachian Mountain sites. *Can. J. For. Res.* **21**: 1234–1244.
- McLaughlin, S.B., Tjoelker, M.G., and Roy, W.K. 1993. Acid deposition alters red spruce physiology: laboratory studies support field observations. *Can. J. For. Res.* **23**: 380–386.
- Miller, E.K., Huntington, T.G., Johnson, A.H., and Friedland, A.J. 1992. Aluminum in soil solutions from a sub-alpine spruce–fir forest at Whiteface Mountain, New York. *J. Environ. Qual.* **21**: 345–352.
- Mullette, K.J. 1975. Stimulation of growth in eucalyptus due to aluminum. *Plant Soil*, **42**: 495–599.
- Norby, R.J., and O'Neill, E.G. 1991. Leaf area compensation and nutrient interactions in CO<sub>2</sub>-enriched seedlings of yellow-poplar (*Liriodendron tulipifera* L.). *New Phytol.* **117**: 515–528.
- Norby, R.J., O'Neill, E.G., and Luxmore, R.J. 1986. Effects of atmospheric CO<sub>2</sub> enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. *Plant Physiol.* **82**: 83–89.
- Parkinson, J.A., and Allen, S.E. 1975. Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological material. *Commun. Soil Sci. Plant Anal.* **6**: 1–15.
- Raynal, D.J., Joslin, J.D., Thornton, F.C., Schaedle, M., and Henderson, G.S. 1990. Sensitivity of tree seedlings to aluminum: III. Red spruce and loblolly pine. *J. Environ. Qual.* **19**: 180–187.
- Schroeder, W.H., Bauch, J., and Endeward, R. 1988. Microbeam analysis of Ca exchange and uptake in the fine roots of spruce: influence of pH and aluminum. *Trees*, **2**: 96–103.
- Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (*Picea abies*) forest. *Science (Washington, D.C.)*, **244**: 776–783.
- Shiple, B., Lechowicz, M., Hendershot, W.H., and Dumont, S. 1992. Interactive effects of nutrients, pH–Al and elevated CO<sub>2</sub> on the growth of red spruce (*Picea rubens* Sarg.) seedlings. *Water Air Soil Pollut.* **64**: 585–600.
- Slivitsky, A., and St. Julien, P. 1987. Compilation géologique de la région de l'Estrie-Beauce. Ministère de l'Énergie et des Ressources du Québec, Québec. Publ. MM 85-04.
- Smith, G.S., Johnston, C.M., and Cornforth, I.S. 1983. Comparison of nutrient solutions for growth of plants in sand culture. *New Phytol.* **94**: 537–548.
- Swan, H.S.D. 1971. Relationship between nutrient supply, growth, and nutrient concentrations in foliage of white and red spruce. Pulp and Paper Research Institute of Canada, Pointe-Claire, Que. Woodl. Rep. 34.
- Thornton, F.C., Schaedle, M., and Raynal, D.J. 1987. Effects of aluminum on red spruce seedlings in solution culture. *Environ. Exp. Bot.* **27**: 489–498.
- Thornton, F.C., Pier, P.A., and McDuffie, C. 1992. Red spruce response to ozone and cloudwater after three years exposure. *J. Environ. Qual.* **21**: 196–202.
- Zimmerman, R., Oren, R., Schulze, E.-D., and Werk, K.S. 1988. Performance of two *Picea abies* stands at different stages of decline. II. Photosynthesis and leaf conductance. *Oecologia*, **76**: 513–518.