INTERACTING EFFECTS OF NUTRIENTS, pH - Al AND ELEVATED CO_2 ON THE GROWTH OF RED SPRUCE (*PICEA RUBENS* SARG.) SEEDLINGS

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Abstract. A 4 mo growth chamber experiment was conducted to evaluate the presence and importance of interactions between nutrient supply, atmospheric CO_2 concentration, and four different combinations of pH - Al concentration on the growth, vitality, and tissue element concentrations of 1-yr-old red spruce seedlings. Solution chemistry was chosen to simulate soil conditions at a red spruce die-back site at Roundtop Mountain (Quebec) that has high acid loadings. CO_2 levels were chosen to simulate ambient levels and those expected in the next century. All three experimental factors affected growth and all factors except CO_2 affected the visual symptoms of die-back. There was an important interaction between nutrient levels and the different pH - Al combinations, indicating that the response of red spruce to various pH and Al concentrations changes with soil fertility. The positive growth response to enriched CO_2 was not sufficient to offset the negative effects of the acid rain induced stresses. A principal component analysis showed that multivariate functions of foliar element concentrations could clearly distinguish plants from different experimental regimes.

1. Introduction

The closely related phenomena of forest dieback and forest decline of red spruce (*Picea rubens* Sarg.) have become important conservation and management concerns in North America (Johnson and Siccama, 1983; Adams *et al.*, 1985; McLaughlin, 1985; Johnson and McLaughlin, 1986; McLaughlin *et al.*, 1987). The dieback of red spruce/balsam fir forests is particularly striking. Tritton and Siccama (1990) found that red spruce/balsam fir forests had the highest proportion of sanding dead trees in the northeastern United States with from 11 to 44% of the trees, on a basal area basis, being dead.

The ecological and economical implications of this phenomenon require remedial action. Such action is hampered by our ignorance of the relative importance of the large number of factors implicated in the dieback of red spruce. Possible factors include O_3 damage (Fincher *et al.*, 1989; Amundson *et al.*, 1991), insect pests, adverse

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meteorological conditions, competition (LeBlanc, 1990), poor forest management, marginal soil conditions, acid mist (Fowler *et al.*, 1989; Deans *et al.*, 1990; Cape *et al.*, 1991) and toxic levels of soil Al concentration (Johnson and Siccama, 1983; McLaughlin, 1985; McLaughlin *et al.*, 1987; Raynal *et al.*, 1990). To complicate matters further, synergistic interactions between these potential factors are possible but are relatively uninvestigated (McLaughlin *et al.*, 1987; Seiler and Paganelli, 1987; Geballe *et al.*, 1990).

The direct effects of acid precipitation on foliage do not appear to explain the decreased growth or health of forest trees (Reich *et al.*, 19986; Taylor *et al.*, 1986; Seiler and Paganelli, 1987; Deans *et al.*, 1990; Geballe *et al.*, 1990) although prolonged acute acid mist may reduce frost hardiness of red spruce (Fowler *et al.*, 1989; Cape *et al.* 1991). On the other hand, acid rain may have a number of indirect effects through interactions with the soil environment. It is well known that soil acidity affects the bioavailability of soil elements, especially Al. Elevated Al in the soil solution has been suggested as a possible result of acid rain that acts either directly at toxic levels to limit root development (Joslin and Wolfe, 1988) or through selective inhibition on nutrient uptake at sub-toxic levels that results in nutrient imbalances (Schroeder *et al.*, 1988). As well, acid precipitation may increase the leaching of available nutrients from the rooting zone and decrease soil fertility (Urlich *et al.*, 1980). Hendershot (1991) has found that fertilizing sugar maple (*Acer saccharum*) forests could offset the visual symptoms of forest dieback. In Germany forest fertilization has been successfully used to limit dieback in coniferous forests (Huettl and Wisnieski, 1987; Huttermann, 1985).

In deciding the management responses to current losses of red spruce one should also consider the forecasted doubling of atmospheric CO_2 within a century (Barcastou *et al.*, 1985; Gammon *et al.*, 1985) along with the concomitant 'greenhouse' effect. Since elevated CO_2 is known to stimulate plant growth (Kramer and Sionit, 1987) the increased CO_2 could potentially offset the negative effects of acid precipitation. Circumstantial evidence suggests that enhanced forest productivity may already be occurring. LaMarche *et al.*, (1984) claimed that increased tree ring widths in subalpine conifers could be due to increased CO_2 levels, although this claim has been challenged (Kienast and Luxmoore, 1988; Graumlich, 1991). Graham *et al.*, (1990) extrapolated from 3-D atmospheric tracer model studies to suggest that the increased annual variations of atmospheric CO_2 carbon, especially in northern latitudes, is due to an increased growth rate of temperate and boreal forests.

A comprehensive evaluation of the interactions of the possible factors implicated in forest dieback, in a form relevant to forest trees in nature and throughout their ontogeny, will require a combination of field and laboratory studies as well as large-scale comparative surveys. Before embarking on such expensive and difficult large-scale studies however, it is only prudent to determine whether the potential for such interactions exists under controlled conditions. It is in this context that we report the results of a 4 mo experiment designed to evaluate the interactions between nutrient concentration, four different combinations of solution pH – Al concentration, and atmospheric CO_2 concentration on the growth and vitality of 1-yr-old red spruce seedlings grown in sand culture.

2. Methods

The experiment was conducted from 8 August until 30 November 1990 at the McGill University Phytotron. Four PGW3 6 Conviron growth chambers were used, two chambers maintained at an ambient CO_2 concentration (350 μmol L^{-1}) and two chambers at the projected CO_2 concentration one century from now (700 μmol L^{-1}). CO_2 levels were constantly monitored and controlled during the experiment. The uniformity and performance of these chambers has recently been reported in Potvin *et al.*, (1990).

The two remaining factors in the experiment – nutrient supply rate at two levels and four different pH – Al solution combinations, each with a unique pH and Al concentration – were arranged in a complete randomized block design within each of the four chambers. There were twelve blocks per chamber and each block consisted of eight 1-yr-old spruce seedlings. Thus, each of the eight seedlings in a block received a unique combination of nutrients, pH and Al. The blocking within chambers and the replication of chambers allowed us to more accurately determine treatment responses (Potvin *et al.*, 1990).

2.1. ENVIRONMENTAL CONDITIONS

The four chambers were programmed to simulate a typical late June evvironment in southern Quebec, Canada, as summarized in Table I. Relative humidity was constant at 70% throughout the experiment. Each environmental parameter was constantly monitored and controlled by computer.

Nutrient solutions were formulated to reproduce the chemistry of soil solutions collected in a declining red spruce forest located at 850 m on Roundtop Mountain, Quebec, Canada (Hendershot *et al.*, 1991). Roundtop Mountain is part of the northern range of the Appalachian Mountains of North America. The 'low' level

TABLE I

Environmental conditions in the growth chambers during the experiment

Time	Photon Flux Density[1] (μmol m^{-2} s^{-1})	Temperature (°C)[2]
05:00 to 05:30	650	15 to 23
05:30 to 20:30	1200	23
20:30 to 21:00	650	23 to 15
21:00 to 05:00	0	15

[1] 15 cm from lights.

[2] temperature ranges represent smoothly changing values within the indicated time period.

TABLE II

Mean values of element concentrations (μM) and pH used in the eight solutions represented in the experiment

pH – Al combinations				Nutrient concentrations					
	pH	Al		Ca	Mg	NH_4	K	NO_3	PO_4
1	4.0	82	low	24	13	7	13	38	5
2	4.0	681	high	58	30	31	37	117	20
3	4.5	24							
4	4.5	125							

of the nutrient treatment (Table II) falls within the range of values measured in soil solutions at the die-back site. The ‘high’ nutrient level, simulating fertilization, was approximately 3 times the ‘low’ value.

Solutions were prepared using de-ionized water and commercially-available, reagent-grade chemicals. The two solutions with more acidic pH (Table II) reproduce soil pH at the dieback site at Roundtop Mountain (Henderson *et al.*, 1991). The two solutions with less acidic pH have acidity levels similar to soils from healthy red spruce sites and represent the effect of liming on soil pH. The two Al levels were chosen such that the total dissolved Al would be in equilibrium with alunite at the ‘high’ Al level and undersaturated at the ‘low’ Al level. The low Al level in the more acidic pH solution was calculated to give the same activity of toxic Al^{3+} as the high Al level in the less acidic pH solution. The four different combinations of solution pH and inorganic Al concentration (Table II) fall within values measured in the field at Roundtop Mountain at various soil depths and also span the ranges reported at other sites in Europe and North America (Cronan *et al.*, 1989).

2.2. PLANTING

One-year-old bare root red spruce seedlings were obtained from the Ministère de l’Energie et Ressources du Québec (Berthierville, Québec, Project 70I16A, zone 05) in late May 1990. The seedlings were planted from 23 to 25 May, one per container, in washed 16 mesh quartz sand. Each container was a clear plexiglass cylinder 8 cm in diameter and 60 cm in depth, covered with a black plastic sheath. The plants were then grown outside for 8 weeks under a 50% shade cloth. Each plant was watered daily and received a ‘Plantprod’ commercial evergreen fertilizer (30:10:10 N:P:K plus micronutrients) twice a week at one tenth the recommended concentration. Plants were transferred into the growth chambers, following a random order, from 16 to 20 July 1990. The plants continued to receive the same watering and fertilizer schedule, described above, until 7 August. The experiment started on 8 August and the plants began receiving the appropriate nutrient solutions.

2.3. NUTRIENT DELIVERY SYSTEM

The nutrient solutions were delivered to the plants using a modified version of the nutrient delivery system of Millet and Zaccai (1989). A peristaltic pump, with a flow rate of 4 mL min^{-1}, continuously delivered the relevant solution to a holding container. The holding container had a gravity siphon that spontaneously emptied the holding container when the solution level reached 300 mL. The solution, after leaving the holding container, emptied into a plexiglass manifold which split the solution volume into twelve delivery tubes; each delivery tube received 25 mL of solution and led directly to the proper plant. Thus, each plant received 25 mL of the relevant solution each 1.2 hr during the 4 mo experiment.

2.4. INDEPENDENT VARIABLES

Since the seedlings varied in their initial size, we measured their total above ground length (i.e. vertical height plus branch lengths) during 13-15 August. This first measure served as a covariate in subsequent statistical analyses. At the end of the experiment two independent assessments of the vitality of each plant were obtained, based on a visual ranking, according the criteria given in Table III. Plants were then harvested, dried at 80 °C in a forced draft air oven, and then separated into needles, stem and root biomass.

After weighing, the roots, stems and needles were separately bulked for the same treatment in each chamber and ground in a Wiley mill. These bulked tissue samples were digested in H_2SO_4–H_2O_2 (Robarge and Fernandez, 1986). The digests were analyzed for N and P using automatic colorimetric procedures (Anonymous, 1977), Ca, Mg, and K were measured by atomic absorption spectrophotometry and Al by the manual colorimetric procedures of Wilson (1984).

2.5. STATISTICAL ANALYSIS

The experiment, described above, was a split-plot design. The effects of the experimental treatments on red spruce growth were tested using a split-plot analysis of covariance, using the initial branch plus stem lengths and the blocking factor as covariates. The CO_2 term existed at the whole-plot level and its significance was tested using the mean square attributed to chamber effects nested within the CO_2 effect. All other effects existed at the split-plot level and were tested using the error mean square of the model (Hicks, 1973). Biomass was transformed to its natural logarithm to obtain homoscedacity of variance and a normal distribution

TABLE III

Visual criteria used to rank the vitality of red spruce seedlings

Score	New stem and needle growth	Chlorosis
1	yes	no
2	no	no
3	no	yes

within each treatment level. Because of effects not related to the experimental factors (see Results) 56 plants died during the experiment; these plants were removed from all subsequent analyses. The analysis of covariance was therefore unbalanced and we used the General Linear Models procedure of SAS (SAS, 1988) with type III sums of squares for the analysis; all effects were considered fixed. Means associated with particular factors were calculated using the Least Square Means procedure of SAS.

Data on the visual assessment of plant vitality, obtained by ranking each plant on a scale of 1 to 3 (Table III), were analyzed by fitting a series of ordinal logistic regressions using the CATMOD and procedure of SAS.

The data on tissue element concentrations were summarized using a principal components analysis (PROC PRINCOMP of SAS) based on the tissue element correlation matrix. The selection of eigenvectors representing significant proportions of the total variance in tissue element variation was based on Anderson's chi-squared test criterion (Anderson, 1958).

3. Results

3.1. EFFECTS ON RED SPRUCE GROWTH

Table IV summarizes the results of the split-plot ANOVA based on the natural logarithms of the final dry weight of the plants. There were significant ($p<0.05$) main effects for each of the three experimental treatments. There was also a significant interaction between the nutrient treatment and the four different pH – Al combinations. A total of 56 plants died during the experiment, mostly within a week of the commencement of the experiment; an inspection of their roots showed no new growth and the deaths were attributed to transplant shock.

TABLE IV

Split-plot analysis of variance of ln transformated final dry weights of one-year-old red spruce seedlings; significant terms are shown in bold. CO_2=carbon dioxide, N=nutrient levels, pH-Al=solution acidity – Al concentration, covariate=ln (total initial height), Chamber (CO_2)=chambers nested within CO_2 levels

Source	df	Type III Mean Squares (probability)
I. Plot level		
CO_2	1	1.38(0.046)
Chamber (CO_2)	2	0.07 (plot-level error term)
II. Split-plot level		
covariate	1	31.60 (<0.0001)
block	11	0.10(0.20)
N	1	2.28 (<0.0001)
pH-Al	3	1.11 (<0.001)
CO_2xN	1	0.10 (0.24)
CO_2xpH-Al	3	0.08 (0.36)
NxpH-Al	3	0.28 (0.01)
CO_2xNxpH-Al	3	0.06 (0.49)
replication	297	0.07 (split-plot level error term)

Increasing the CO_2 level from 350 to 700 μmol L^{-1} increased the average mass of the red spruce plants (CO_2 term in Table IV) by 0.06 g. this increase in mass was dependent on neither nutrient supply nor on the pH – Al solution, since CO_2 did not display any significant interactions with these terms. Increasing the nutrient concentrations from values typical of the dieback site at Roundtop Mountain to concentrations three times higher (Table II) increased the average mass of red spruce plants by 0.08 g (Nutrient term in Table IV). The effect of nutrient supply rate was dependent on the type of pH – Al combinations that the plants experienced, as shown by the significant interaction term in Table IV.

Figure 1 illustrates the nature of the statistical interaction between nutrient supply rates and the four pH – Al combinations. There were only small differences in the effects of the four pH – Al combinations when the nutrient concentrations were low. At higher nutrient concentrations however, the four different pH – Al combinations produced quite different effects on the growth of the plants. The greatest growth depression was caused by a combination of acidic (pH=4.0) solution with a high Al concentration (681 μmol L^{-1}); this combination largely negated

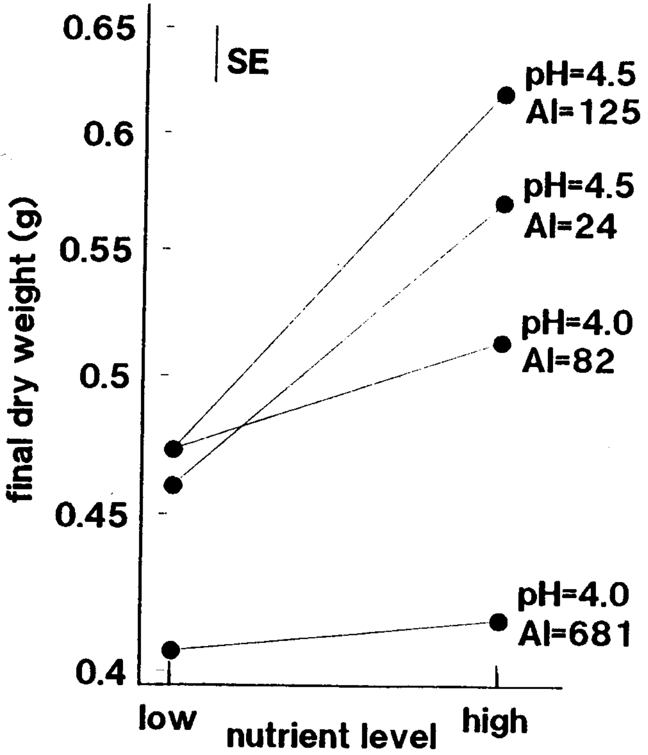

Fig. 1. The least squares means of the final dry weight of 1-yr-old red spruce seedlings (g) grown at low and high nutrient levels and four combinations of pH and solution Al concentrations. The range of 1 standard error is shown in the upper right.

any advantage of the high nutrient concentrations. The least growth depression was observed in the more neutral (pH=4.5) solution. It is not possible to statistically separate the effects of pH from those of Al with our experimental design, but Figure 1 suggests that both pH and Al affected the growth response due to nutrient enrichment.

3.2. EFFECTS ON VISUAL ASSESSMENTS OF VITALITY

Table V summarizes the distribution of visual rankings of plant vitality among the eight different types of solutions, based on evidence of new needle production and needle chlorosis. A parallel lines logistic regression for the ordinal scores was fit using the CATMOD procedure of SAS (1988), but the assumption of parallel response of the four levels of scores to the experimental treatments was rejected ($p < 0.05$). We therefore fit a series of logistic regressions to binary responses, created by pooling different scores.

We first modeled the effects of the experimental treatments on a binary response by contrasting those plants that both exhibited new stem and needle growth and showed no evidence of chlorosis (score=1) to those plants that did not exhibit new growth and that may also have exhibited various levels of chlorosis (scores 2 and 3). This binary classification therefore contrasted those plants showing no visual signs associated with forest dieback to those exhibiting at least some visual signs indicative of various stages of forest dieback, i.e. reduced growth or needle chlorosis. The resulting analysis showed that both decreased nutrient concentrations and the different pH – Al combinations significantly increased the proportion of the red spruce plants that exhibited some visual evidence associated with forest dieback. No evidence for an effect of CO_2 levels was found. Two of the pH – Al combinations at the low nutrient level had no plants without some visual evidence of dieback and this prevented a test for an interaction between nutrient levels and the pH – Al combinations (SAS, 1988).

TABLE V

The number of red spruce seedlings having different vitality rankings among the eight different solutions; Al concentrations in $\mu mol\ L^{-1}$. The two CO_2 levels are combined, since this factor had no effect of the vitality rankings

Solution profile			Red Seedling vitality ranking			
Nutrient	pH	Al	Green & growing	Green & not growing	Chlorotic	Dead
low	4.0	82	0	12	25	11
low	4.0	681	0	5	37	6
low	4.5	24	3	18	25	2
low	4.5	125	2	8	32	6
high	4.0	82	22	15	7	4
high	4.0	681	7	9	22	10
high	4.5	24	22	15	5	6
high	4.5	125	22	3	12	11

We then created a second binary response variable by contrasting those plants that exhibited no (score=1) or very slight (score=2) visual signs of dieback, i.e. plants without any needle chlorosis, against those plants that exhibited obvious (score=3) visual signs of needle chlorosis. A binary regression on this response showed significant main effects of nutrient concentration and on the pH - Al combinations but no significant interactions, nor was CO_2 level found to be significant. Table V summarizes the number of plants in each category among the experimental treatments.

3.3. TISSUE NUTRIENT CONCENTRATIONS

Appendix 1 gives the means of needle, stem and root tissue element concentrations of N, P, K, Ca, Mg, Mn and Al, of plants subjected to each of the eight different combinations of nutrients, pH and Al and to 350 or 700 μmol L^{-1} CO_2. There were a number of clear correlations between the concentrations of different elements in all three biomass fractions, but these correlations were mainly within three separate groups of elements: N-P-K, Ca-Mg-Mn, and Al.

The significant principal components reflected these three groups. The first four principal components accounted for significant proportions of the total variance in each of the three biomass fractions but in each case most of the variance was summarized in the first two principal components (Table VI).

In the needle tissues, the first principal component represents the major trend (47% of the variance) of increasing concentrations of all elements except Al. The second principal component represents the subordinate trend (31% of the variance) contrasting N, P, K, Al with Ca, Mg, Mn. The minor trend, represented in the third principal component, represents increasing concentrations of Al in the needles. A principal component analysis of the root tissue element concentrations produced the same results (Table VI). A principal component analysis of the stem tissue

TABLE VI

The first three principal components (P1. P2, P3) of correlation matrices of tissue element concentrations in needle, stem and root tissues of one-year-old red spruce seedlings grown in different nutrient, pH, Al and CO_2 conditions. Values are the coefficients associated with each element concentration in a given principal component

element	needles			stem			roots		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
N	0.39	0.43	0.01	–0.08	0.56	0.04	0.38	–0.48	–0.10
P	0.33	0.48	–0.04	0.13	0.53	–0.18	0.40	–0.46	–0.05
K	0.44	0.34	–0.26	0.23	0.53	0.06	0.50	–0.16	–0.03
Ca	0.42	–0.32	0.40	0.49	–0.21	–0.08	0.39	0.43	0.02
Mg	0.43	–0.29	0.18	0.50	0.17	0.45	0.45	0.29	0.07
Mn	0.40	–0.38	0.06	0.48	–0.22	0.39	0.26	0.51	–0.39
Al	–0.17	0.36	0.86	–0.46	0.05	0.78	0.15	0.10	0.91
% variance	46.6	31.0	11.7	44.0	39.6	7.5	51.3	24.4	14.8

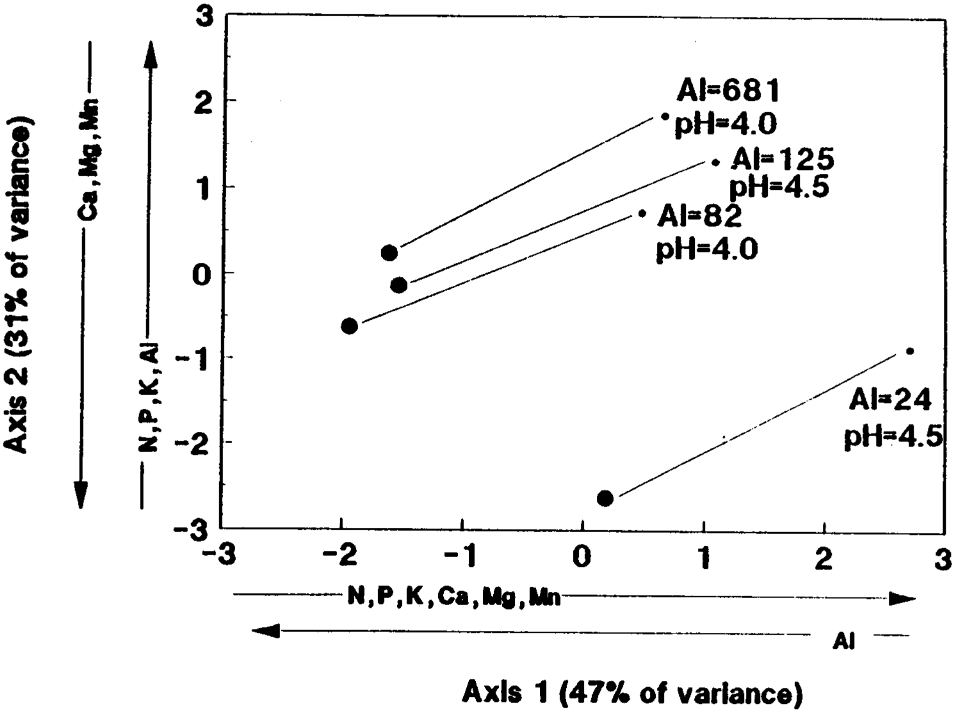

Fig. 2. Shown are the projections of the mean foliar element concentrations of 1-yr-old red spruce seedlings, grown at high (large circles) and low (small circles) levels of nutrient supply and four different combinations of solution pH and Al concentration on their first two principal components. The arrows indicate the directions on which each foliar element loads on each axis, as given in Table VI.

element concentrations also produced the same general results except that N did not exhibit any systematic trend along the first principal component.

A projection of the mean needle tissue element concentrations on the first two principal components reveals the effects of the different experimental treatments on these element concentrations (Figure 2). In general, low nutrient concentrations, low CO_2, or acidic solutions all resulted in increased needle concentrations of all elements except Al and therefore was associated with high scores on the first principal component. The second principal component is associated with treatments that subjected the plants to more acidic solutions and/or to increased availability to Al and represents increased concentrations of N, P, K, and Al relative to Ca, Mg, and Mn. Thus, the treatment having the highest Al concentration in the most acidic solution scored highest on this second axis while the treatment having the lowest Al in the more neutral solution scored lowest on this second axis. The third principal component was associated with increased Al concentrations in solution, which were reflected in higher foliar concentrations.

4. Discussion

Field surveys are capable of documenting when and where tree dieback occurs, and of identifying environmental variables that co-vary with the incidence of dieback. They are not capable of determining how, or whether, manipulation of these correlated environmental variables will affect the incidence of dieback. Such information can only be obtained through experimental manipulation relative to controls. Growth chamber, greenhouse or small-scale field experiments concerning forest dieback are capable of identifying which, among a chosen set of variables, will cause the symptoms of dieback in a given experimental setting. Unfortunately, such experiments are always simplifications of the field environment, and is never straightforward to extrapolate from such experimental results to the field. Clearly, the most relevant experiments would be those that are conducted at spatial and temporal scales relevant to the phenomenon of forest dieback, but such experiments are not practical. One solution, given such constraints, is to rely on field studies to identify correlated variables of forest die-back and to determine realistic ranges of such variables from field measurements, and then test their causal efficacy in controlled experiments that reproduce such realistic environmental ranges. This is the strategy used in this research.

There were three main goals in this project: first, to determine the relative importance of the experimental factors, and their interactions, to red spruce dieback; second, to determine wheter one proposed solution, forest fertilization (Huettl and Wisniewski, 1987; Hendershot, 1991) is likely to produce positive results; and third, to determine wheter the proposed increase in atmospheric CO_2 could be expected to reduce the incidence of red spruce decline during the next century.

The solution chemistry used in this experiment was based on the measured soil solution chemistry of a die-back stand of red spruce at Roundtop Mountain, Quebec (Hendershot *et al.*, 1991). The soils along the topographic gradient of this mountain are all derived from *in situ* weathering of phyllite bedrock (Hendershot *et al.*, 1991), and the similarity in the mineralogy at all sites on the mountain is an indication of the homogeneity of the parent material. This suggests that differences in soil chemistry are due to environmental conditions rather than lithologic heterogeneity. Due to fog interception by the forest canopy, the amount of time in the clouds is a major factor determining the amount and chemistry of precipitation reaching the soil at this site (Shemenauer *et al.*, 1988). Both the fog and the direct precipitation at the high elevation sites at Roundtop Mountain are quite acidic and the topographic gradient of acid deposition is directly correlated with measured changes in soil chemistry (Hendershot *et al.*, 1991). Furthermore, the changes in soil chemistry cannot be easily attributed to vegetational change down the mountain, since sites at different elevations within a given vegetation type follow the same general trends. These results imply that the low nutrient levels, acidic soil solution and elevated Al that were observed at the red spruce die-back site, and that were reproduced in our growth chamber experiment, are related to acidic deposition.

Nutrient levels and the solution pH - Al combinations all had strong and highly significant effects on the growth of the red spruce seedlings, although effect of enriched CO_2 was only marginal. Each of the experimental factors, except CO_2, also affected the visual symptoms of dieback. However, the presence of strong interactions in the ANOVA (Table IV) shows that the effect of any of the factors, except CO_2, was dependent on the levels of the other factors. This interdependence may explain the large variations among studies in the observed range of Al toxidity concentrations, as reviewed by Raynal *et al.* (1990).

The most dramatic effect, shown by all of our response variables, was due to fertilization, i.e. to the increase in nutrient supply to levels three times higher than those measured at the dieback site, although the effects of the four pH - Al combinations were not negligible (Figure 1). This increased nutrient level represents conditions in a fertilized site.

When Al concentrations were low and when solution pH was less acidic, increased nutrient levels had large positive effects on both growth and vitality of the seedlings. In contrast, as Al concentrations increased and as pH became more acidic the increased nutrient levels produced negligible improvements (Figure 1). These results suggest that forest fertilization of acidic sites, without considering both the concentration of inorganic Al in the soil and the level of soil acidity, would be of little practical use.

The analysis of tissue element concentrations was particularly interesting. The symptoms of dieback – poor growth and needle chlorosis – can potentially be caused by many different environmental stresses. The principal component analysis of the needle element concentrations shows that one can differentiate between poor growth and needle chlorosis due to low nutrient supply from the same symptoms due to solution acidity and high levels of inorganic Al.

The clearest interpretation of the needle tissue concentrations can be obtained by considering both the first and second principal component axes together. The higher the score that a plant receives on the first axis, the lower the nutrient status of the solution. The higher the score that a receives on the second axis, the lower the availability of inorganic Al (i.e. more neutral solution or lower the Al concentration).

Many studies have tried to relate foliar element concentrations to soil acidity and soil Al concentrations, but there are few consistent trends between studies. For instance, the Ca:Al ratio has been suggested as a diagnostic tool in relation to Al toxicity but in the studies reviewed by Sucoff *et al.* (1990), toxic Al effects were sometimes detected in plants with Ca:Al ratios from 0.4 to 40.7 whereas no toxic Al effects were sometimes detected in plants with Ca:Al ratios from 0.07 to 6.9. The foliar Ca:Al ratios in our study were only partially associated with decreased growth or increased needle chlorosis and ranged from 8.09 to 25.66. McLaughlin *et al.* (1987) found that the foliar chemistry of natural stands of red spruce in high elevation (acidified) sites had reduced levels of Ca and Mg relative to N and also increased levels of Al. These trends are reflected in our study since the second principal component axis, reflecting the Al/pH effects, consisted of a contrast between N, P, K and Ca, Mg, Mn. As well foliar Al concentrations were higher in plants subjected to high concentrations of inorganic Al. The pattern of foliar elements suggests that Al was producing its effects through a disruption of the normal ratios of nutrient uptake (Foy *et al.*, 1978).

Based on the evidence to date, it seems unlikely that any simple foliar ratio will be a useful predictor of plant vitality under acidification. However, the multivariate functions of foliar chemistry obtained in our study were able to clearly distinguish between nutrient, pH and Al stress. Our principal components can probably not be applied to adult red spruce under natural conditions, but the similarities between our results and those of McLaughlin *et al.* (1987) suggest that a similar multivariate approach, based on a more representative foliar analysis, may have diagnostic value.

The effect of increasing CO_2 concentration to levels predicated for the next century did increase growth, as expected (Kramer and Sionit, 1987), and this increase was independent of nutrient status or of pH - Al combinations. However, the increased CO_2 resulted in only an average of 0.06 g increase in biomass and this was not enough to compensate for the deleterious effects observed in the more acidic or more concentrated Al solutions. If significant interactions with CO_2 do exist, they may only be expressed in longer term experiments or under field conditions.

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APPENDIX I

Mean tissue element concentrations of N, P, K, Ca, Mg, Mn and Al in needle, stem and root tissues of 1-year-old red spruce seedlings subjected to high and low levelsl of nutrients supply, pH, Al concentration and CO_2. Element concentrations are in $\mu g\ g^{-1}$ and the solution Al concentrations are in $\mu mol\ L^{-1}$

Solution			Tissues						
Nutrient	pH	Al	N	P	K	Ca	Mg	Mn	Al
Needle tissues, 350 $\mu mol\ L^{-1}$ CO_2									
low	4.0	82	13.68	2.29	6.46	7.10	0.94	0.09	0.43
low	4.0	681	15.43	2.96	6.85	6.39	0.79	0.07	0.79
low	4.5	24	14.81	2.67	7.19	8.21	1.46	0.13	0.32
low	4.5	125	13.43	2.92	6.66	7.60	0.94	0.09	0.54
high	4.5	82	10.09	1.49	3.53	6.06	0.78	0.06	0.51
high	4.5	681	11.14	1.60	4.43	6.11	0.94	0.05	0.62
high	4.5	24	9.70	1.30	3.63	7.88	1.13	0.11	0.37
high	4.5	125	10.80	1.94	4.00	7.21	0.81	0.08	0.66
Needle tissues, 700 $\mu mol\ L^{-1}$ CO_2									
low	4.0	82	12.25	2.42	5.51	6.27	0.79	0.08	0.45
low	4.0	681	13.08	2.55	5.94	6.96	0.85	0.09	0.47
low	4.5	24	11.70	1.74	6.65	7.83	1.22	0.13	0.30
low	4.5	125	12.08	3.05	7.02	6.39	0.80	0.08	0.42
high	4.5	82	8.77	1.37	3.17	6.26	0.79	0.07	0.47
high	4.5	681	9.11	1.71	4.12	6.26	0.82	0.05	0.72
high	4.5	24	8.35	1.38	3.80	8.32	1.00	0.15	0.29
high	4.5	125	8.38	1.80	3.21	5.91	0.67	0.07	0.54
Stem tissues, 350 $\mu mol\ L^{-1}$ CO_2									
low	4.0	82	10.50	2.54	6.47	3.00	1.03	0.07	0.15
low	4.0	681	10.38	1.28	5.10	2.26	0.73	0.05	0.32
low	4.5	24	10.41	2.27	7.48	3.09	1.60	0.09	0.19
low	4.5	125	8.77	1.16	5.31	2.94	0.93	0.06	0.15
high	4.0	82	7.62	0.73	3.09	3.29	0.76	0.07	0.23
high	4.0	681	9.00	1.01	3.14	2.44	0.66	0.06	0.40
high	4.5	24	6.13	0.62	3.29	3.58	1.15	0.10	0.20
high	4.5	125	7.08	0.82	3.38	3.07	0.82	0.07	0.23
Stem tissues, 700 $\mu mol\ L^{-1}$ CO_2									
low	4.0	82	10.88	1.81	5.23	3.06	0.96	0.06	0.16
low	4.0	681	10.92	1.08	4.62	2.76	0.74	0.06	0.26
low	4.5	24	9.27	1.15	5.96	3.45	1.46	0.09	0.12
low	4.5	125	10.49	1.86	6.26	2.90	0.95	0.06	0.16
high	4.0	82	6.68	0.81	2.74	2.99	0.73	0.07	0.16
high	4.0	681	8.45	0.85	2.90	2.24	0.65	0.05	0.30
high	4.5	24	6.40	0.84	3.61	3.48	1.15	0.10	0.14
high	4.5	125	7.90	0.88	3.37	3.04	0.85	0.08	0.16

Appendix I (Continued)

Solution			Tissues						
Nutrient	pH	Al	N	P	K	Ca	Mg	Mn	Al
Root tissues, 350 µmol L^{-1} CO_2									
low	4.0	82	12.26	2.49	8.94	2.49	0.66	0.07	3.00
low	4.0	681	13.75	2.98	7.55	1.96	0.37	0.05	2.24
low	4.5	24	12.46	2.79	9.93	3.09	1.41	0.09	1.57
low	4.5	125	11.71	2.87	8.39	2.74	0.73	0.06	1.89
high	4.0	82	8.79	1.40	4.56	2.43	0.43	0.06	2.59
high	4.0	681	10.05	1.60	4.08	2.01	0.35	0.06	2.86
high	4.5	24	8.97	1.11	5.15	2.67	0.74	0.08	1.90
high	4.5	125	9.10	1.51	4.63	2.42	0.49	0.06	2.27
Root tissues, 700 µmol L^{-1} CO_2									
low	4.0	82	11.67	2.34	7.29	2.20	0.58	0.05	2.05
low	4.0	681	11.16	2.36	6.07	1.82	0.38	0.05	2.66
low	4.5	24	10.89	2.49	8.75	2.80	1.26	0.07	1.69
low	4.5	125	11.89	2.33	8.65	2.44	0.65	0.05	2.11
high	4.0	82	8.98	1.39	4.54	2.18	0.41	0.06	2.48
high	4.0	681	8.83	1.53	3.71	1.61	0.26	0.06	3.09
high	4.5	24	7.72	1.26	5.63	2.49	0.71	0.08	1.67
high	4.5	125	7.40	1.41	4.32	2.03	0.48	0.05	1.70