

THE EFFECTS OF DOUBLING ANNUAL N AND S DEPOSITION ON FOLIAGE AND SOIL CHEMISTRY  
AND GROWTH OF JAPANESE LARCH (*LARIX LEPTOLEPIS* SIEB. AND ZUCC.) IN  
NORTH CENTRAL WEST VIRGINIA

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**Abstract:** Atmospheric deposition has been recognized as a significant environmental problem for several decades, but its impact on forest ecosystems in North America remains controversial. In an effort to further elucidate the impacts of atmospheric deposition to forested watersheds in the Mid-Appalachian region, several related watershed studies have been initiated by the U.S. Forest Service in Parsons, WV over the past decade. One of the experiments involved doubling the annual ambient rate of N and S deposition by aerial application of ammonium sulfate to Watershed 9 which had previously been converted from low grade hardwoods to Japanese larch. We report here on the impacts of this treatment on soil and foliar chemistry, and height and diameter growth of the Japanese larch. Foliar samples were collected from treated and control trees and analyzed for Al, Ca, K, Mg, Mn, N, P and S. Soil sample analysis included Al, C, Ca, K, Mg, Mn, N and S. Treated soil had significantly greater exchangeable Al and Mn and significantly lower exchangeable Ca and Mg ( $\alpha \leq 0.05$ ) compared to control soil. Treated larch had significantly higher ( $\alpha \leq 0.05$ ) foliar N, Mn and Al concentrations and significantly lower ( $\alpha \leq 0.05$ ) P and Mg concentrations. Height and diameter growth of treated larch were significantly ( $\alpha \leq 0.05$ ) less. The reasons for these growth differences are as yet unclear; however, preliminary analysis indicated significant correlations with selected soil chemical variables, some of which have changed in response to the treatment.

## INTRODUCTION

The debate continues over the effects of anthropogenic nitrogen and sulfur deposition on forest health, resulting in a shift in research emphasis away from direct impacts and towards predisposing and/or inciting stresses. The forest health perspective has broadened from the issue of acid deposition to include effects on forest soils, tree nutrition, accelerated soil base cation leaching and metals toxicity (Tomlinson and Tomlinson 1990). Nutrient budgets for forests, such as those on Walker Branch Watershed, Tennessee, and Bear Brook Watershed, Maine (Johnson and others 1985; Kahl and others 1993) have been studied carefully and the impacts of atmospheric deposition on the cycling of nutrients in these forest ecosystems documented.

Ulrich and Matzner (1986) characterized soil acidification by base cation loss from the soil and acidic cation accumulation. Selective cation exchange leads to accelerated leaching of exchangeable cations (VanMiegroet and Cole 1984). Federer and others (1989) reported that calcium was being leached from some eastern forest soils. For forest soils already low in base cations, a further decrease in these nutrients associated with acidic deposition leaching may be critical to plant growth.

Documented changes in soil characteristics due to acidic inputs under field conditions are few (David and others 1990). Elevated acidic loadings have been shown to increase cation leaching (including  $Al^{3+}$ ), decrease soil pH and adversely affect biological processes (Fernandez and Rustad 1990). The loss of cations is dictated by the amounts of

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anions in solution, and concentrations of strong acids, such as  $H_2SO_4$  and  $HNO_3$ . Increased soil acidity also increases Al, Mn and Fe solubility (Johnson and Cole 1980). Soil solution electroneutrality is maintained, following base cation losses, by these potentially-phytotoxic elements. Thus, soil chemistry changes may influence vegetation adversely.

The objective of this study was to quantify soil and foliar chemical changes and the response of Japanese larch to inputs of strong acids as a part of a catchment-scale acidification project. The results reported in this paper relate the growth and nutritional status of larch trees that have received increased deposition of N and S to the changes in soil chemistry resulting from this treatment.

## METHODS

### Study Site

Watershed 9 is an 11.6-ha catchment located in the unglaciated Allegheny Plateau of north central West Virginia. Kochenderfer and Helvey (1989) provided a general description of the watershed and a history of past land use. Minimum and maximum elevations on the watershed are 744 and 878 m, respectively. The watershed has a southerly aspect, with a 25% average slope. The predominant soil is Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrept) weathered from the Hampshire formation. Agriculture was the primary land use until the early 1930s, when the watershed was abandoned and allowed to naturally revegetate with low grade hardwoods. The U.S. Forest Service obtained the land in 1939. A mechanical site preparation study was conducted on the watershed beginning in 1983, in which all vegetation was removed except for an undisturbed 1.42-ha buffer zone along the stream. Site preparation included root raking and windrowing brush. In the spring of 1984, the watershed (excluding the buffer zone) was planted with 2-0 stock Japanese larch at 1.8 by 1.8 m spacing.

### Experimental Treatments

Prior to treatment, six 30.5 by 30.5 m control plots were randomly located in the watershed and permanently marked. To simulate inputs of double the ambient rates of N and S deposition, granular ammonium sulfate fertilizer (21-0-0-24, proportions of N, P, K and S) was applied to watershed 9 by helicopter beginning in April 1987. The entire watershed including the buffer zone but excluding the control plots received three ammonium sulfate treatment applications per year through August 1993 (for a total of 18 applications prior to the August 1992 sampling and 20 applications prior to the August 1993 sampling) in an attempt to mimic seasonal deposition patterns. Determination of treatment amounts were based on historical throughfall deposition in the area, accounting for seasonal variation. Double the deposition rate for the January-April period was applied in March, double the rate for the May-August period was applied in July, and double the September-December rate was applied in November. The rates corresponded to 8.1 kg S ha<sup>-1</sup> and 7.1 kg N ha<sup>-1</sup> for applications in March and November, and 24.4 kg S ha<sup>-1</sup> and 21.3 kg N ha<sup>-1</sup> for the July applications (Adams and others 1993).

### Study Design

Access to the control plots on the watershed was restricted by dense vegetation; consequently, only one control plot was selected for study. Other control plots could not have been studied without severe disturbance to the plots. A northeastern control plot was selected, and a 25 by 25-m plot was surveyed and marked within the 30.5 by 30.5-m control plot to allow a treated buffer zone. One adjacent treated area of the same dimensions was surveyed and marked along the same contour 10 m east of the control plot. Four transect lines along a north-south orientation were selected at 5-m intervals for both the control plot and the treated plot (Figure 1). Starting at the southern boundary line, 6 Japanese larch trees were selected and brass tagged at 5 m intervals in a northern direction along each transect line. Three additional larch trees were tagged between transect lines 1 and 2 and between lines 3 and 4. The total of 30 sample trees per plot represents approximately 45% of the total number of larch trees on each plot.

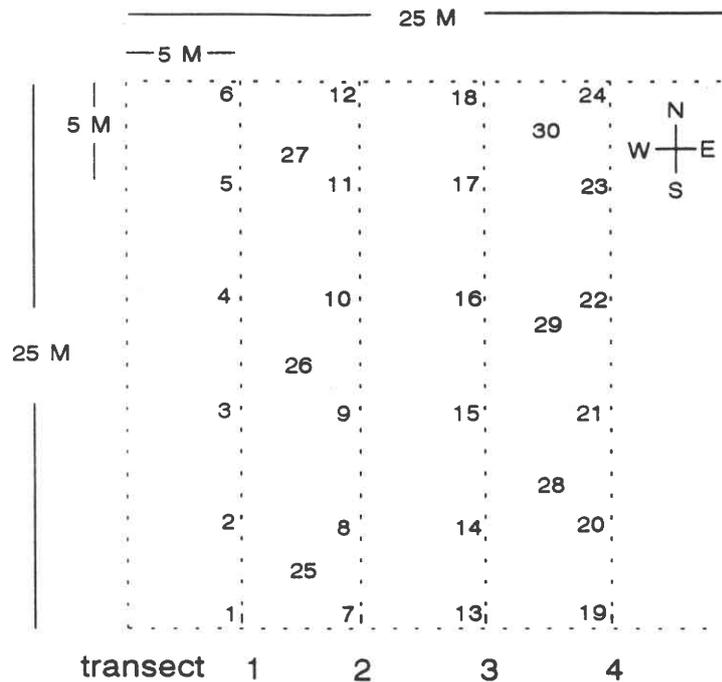


Figure 1. Transect and sampling design.

#### Sampling and Measurements

In August of 1992 and 1993, a telescoping pole was used to measure tree height to the nearest 0.1 cm and a caliper was used to measure tree diameter to the nearest 0.1 cm on all sample control and treated trees. A foliar sample for chemical analysis also was obtained from each of these trees. All needle whorls from a breast-high lateral shoot immediately below the terminal shoot were removed. Samples were handled with vinyl gloves, placed in paper bags, and kept cool until delivery for analysis (within 24 hours). Foliar analysis was performed by the Agricultural Analytical Services Laboratory, The Pennsylvania State University. Elemental analysis included Al, Ca, K, Mg, Mn, and P by inductively coupled plasma emission spectroscopy (Dahlquist and Knoll 1978), Kjehdahl N (Isaac and Johnson 1976) and sulfur (Huang and Schulte 1985).

In August 1992, 6 control and 6 treated soil pits (approximately 20 by 20 cm) were hand excavated near the sample larch trees along control transect 1 and treated transect 4. In August 1993, small pits were hand excavated by each sample larch tree on transect lines 1 and 4 for both the control and treated plots (12 pits per plot total). Soil samples were collected in the 0-2 cm layer (O horizon) and in the 2-10 cm layer (A horizon). These depths were chosen to represent the effective rooting horizons (Kochenderfer 1973). Samples were placed in paper bags and transported to the Environmental Resources Research Soil Chemistry Laboratory at The Pennsylvania State University. All analysis was conducted on air-dried soil sieved to 2 mm. Soil pH (1993 only) was determined in water with soil-to-solution ratios of 1:1 for both horizons (Black 1964). The base cations Ca, K, and Mg (K 1993 only) and metal cations Al and Mn (Mn 1993 only) were determined by atomic absorption spectrophotometry following 0.01 M SrCl<sub>2</sub> extraction and preparation as outlined by Joslin and Wolfe (1989). Soil carbon (Schulte 1991), NO<sub>3</sub>-N (Isaac and Johnson 1976) and SO<sub>4</sub>-S (Black 1964) 1992 analysis were performed by Agricultural Analytical Laboratory. In 1993, total C and N were analyzed by combustion using an organic elemental analyzer and for total S by combustion using the organic elemental analyzer with an electron capture detector (Baccanti and others 1993; Carlo Erba Instruments 1990) at the Timber and Watershed Laboratory, Parsons, WV.

## Data Analysis

Statistical analysis followed a Model I two-treatment ANOVA for a fixed treatment. The SAS statistical package was used for data analysis, incorporating t-tests for mean comparisons (SAS Institute 1985). All significant differences are reported at  $\alpha \leq 0.05$ . Correlations using soil and tissue variables were performed using means for a particular treatment, soil horizon and chemical element. Quality assurance/control for all analysis included field duplicates, analytical duplicates, standard reference materials and blanks. Generally, duplicate analyses had relative standard deviation values below 10 percent.

## RESULTS AND DISCUSSION

### Larch Height and Diameter

Larch height and diameter growth were significantly greater for the control trees compared with treated trees for both 1992 and 1993 (Figure 2). Positive growth on both control and treated plots was evident, in that height and diameter measurements were greater in 1993 than 1992. However, control larch exhibited significantly greater height and diameter growth than treated larch in both years. Data for larch height growth on the entire watershed also indicate significantly greater height growth on the control plots compared to larch receiving the acidifying treatment (personal commun., J. Kochenderfer, U.S. Forest Service).

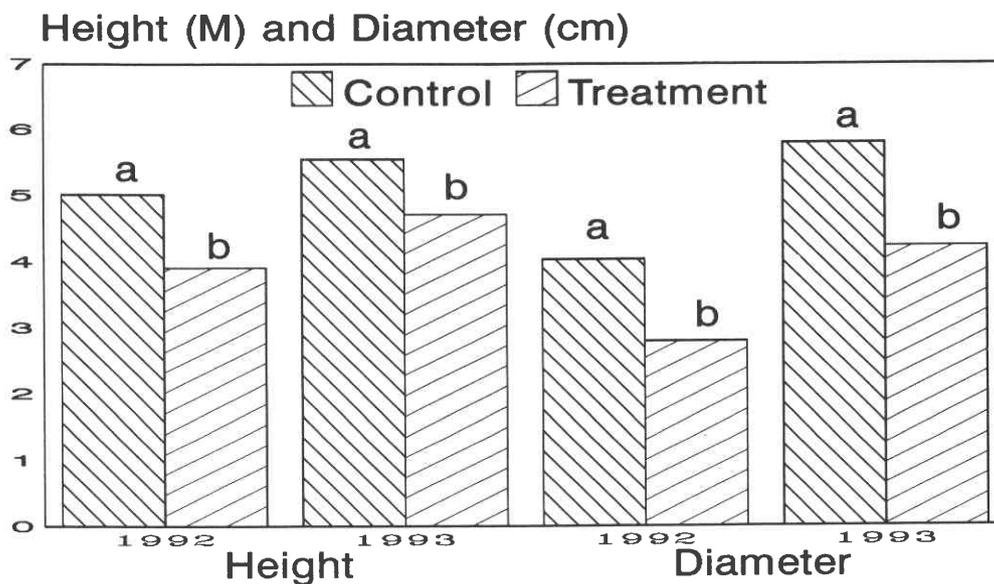


Figure 2. Larch height and diameter comparisons between control and treated plots; ( $\alpha \leq 0.05$ ).

### Soil Chemistry: Treatment Response (Within Horizon Comparisons)

Control and treated soil data comparisons were made for 1992 and 1993 by sample depth (O horizon or A horizon). In 1992, treated soil from the O and A horizon had greater values of  $\text{NO}_3\text{-N}$  and C compared to soil from controls. The A horizon differences were statistically significant. Treated soil from the A horizon had significantly greater  $\text{SO}_4\text{-S}$  than the control (Table 1).

Table 1. Soil SO<sub>4</sub>-S, NO<sub>3</sub>-N and C comparisons between control and treated samples collected in August 1992.

|           | O HORIZON                     |                           |          | A HORIZON                     |                           |          |
|-----------|-------------------------------|---------------------------|----------|-------------------------------|---------------------------|----------|
|           | SO <sub>4</sub> -S<br>(mg/kg) | NO <sub>3</sub> -N<br>(%) | C<br>(%) | SO <sub>4</sub> -S<br>(mg/kg) | NO <sub>3</sub> -N<br>(%) | C<br>(%) |
| Control   | 148.83a                       | 0.965a                    | 16.9a    | 29.50a                        | 0.113a                    | 1.9a     |
| Treatment | 131.33a                       | 1.108a                    | 18.6a    | 121.60b                       | 0.155b                    | 4.1b     |

different letters within a soil parameter indicate significant difference at  $\alpha \leq 0.05$

n=6

According to Stevenson (1986), net mineralization leading to an increase in mineral N levels occurs at C:N ratios below 20. For the 1992 soil data, the O-horizon C:N ratio was 17:1 and the A-horizon C:N ratio was 15:1, in both the control and the treated soil. Control O- and A-horizon total C and N were positively correlated with one another (O horizon:  $r=+0.89$ ,  $p < 0.05$ , A horizon:  $r=+0.97$ ,  $p < 0.01$ ), as was treatment O-horizon C and N ( $r=+0.96$ ,  $p < 0.05$ ). Mineralization of N under these C:N ratios may be enhanced with N addition. Elevated humus layer N and increased organic matter in the Germany's Solling forest has been attributed to litter decomposition retardation, due to declining microbial activity under increasing acidic inputs (Robarge and Johnson 1992). The potential for N saturation is indicated by the low C:N ratios (Kahl and others 1993). The possibility of N saturation and the increase in N and S in treated soils provided the potential for SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub><sup>-</sup>-induced leaching and increased soil acidification. In 1993, soil pH was significantly lower in the treated soil (O-horizon pH 4.47, A-horizon pH 4.91) compared to control (O-horizon pH 4.98, A-horizon pH 5.38), indicating increased soil acidification in the treated soil.

Exchangeable Al values in the treated soils were significantly greater and exchangeable Ca and Mg values significantly lower compared to control, for both years and at both depths (Table 2). In 1993, Mn was found to be significantly greater in the treated soil at both depths. Robarge and Johnson (1992) described increased solubility and mobility of Al as a potential effect of increased N and S inputs to forest soils. Soil acidification also can induce the loss of exchangeable cations such as Ca and Mg and increase exchangeable Al and other metals such as Mn (Schlegel and others 1992). Because the growth of larch is not stimulated by N and the Ca demand of larch foliage is relatively high, N-induced acidification and consequent Ca leaching may be contributing to the observed growth reduction of the treatment trees (Tyrrell and Boerner 1987; VanGoor 1953).

Table 2. Chemical comparisons (0.01 M SrCl<sub>2</sub> exchangeable) between control and treated soil sampled in August of 1992 and 1993.

|           | O HORIZON |        |            |        |        |       | A HORIZON |        |            |        |       |       |
|-----------|-----------|--------|------------|--------|--------|-------|-----------|--------|------------|--------|-------|-------|
|           | Al        | Ca     | K<br>-mg/L | Mg     | Mn     | pH    | Al        | Ca     | K<br>-mg/L | Mg     | Mn    | pH    |
| 1992      | -----     | -----  | -----      | -----  | -----  | ----- | -----     | -----  | -----      | -----  | ----- | ----- |
| Control   | 2.63a     | 175.5a | NA         | 22.48a | NA     | NA    | 2.02a     | 170.7a | NA         | 12.29a | NA    | NA    |
| Treatment | 6.21b     | 104.0b | NA         | 13.04b | NA     | NA    | 17.23b    | 30.8b  | NA         | 3.96b  | NA    | NA    |
| 1993      |           |        |            |        |        |       |           |        |            |        |       |       |
| Control   | 4.11a     | 160.8a | 13.45a     | 18.08a | 9.07a  | 4.98a | 1.54a     | 123.5a | 5.89a      | 8.33a  | 0.87a | 5.38a |
| Treatment | 12.95b    | 96.5b  | 21.63b     | 10.18b | 21.41b | 4.47b | 10.66b    | 65.3b  | 5.14a      | 3.64b  | 1.76b | 4.91b |

different letters within a soil parameter indicate significant difference at  $\alpha < 0.05$

1992 n = 6

1993 n = 12

NA = not analyzed

## Soil Chemistry: Within Horizon Observations

Control and treated soil N was significantly greater in the O horizon than the A horizon in 1992 and in 1993. Sulfur showed a similar, non-significant trend in 1992. Analysis of S for 1993 is ongoing. For the control soil sampled in 1992 and 1993, Mg was significantly greater in the O horizon than the A horizon. In 1993, O-horizon Al, K, Mn and N were all significantly greater than A-horizon levels, while pH was significantly lower in the O horizon. These differences may be due to greater leaching losses in the A horizon or to greater nutrient inputs (needle-fall) in the O horizon or both.

For the treated plots in 1992 and in 1993, O-horizon Mg was also significantly greater than A-horizon Mg. This may be a reflection of initial, pretreatment soil status, as the control O horizon also had significantly greater Mg than the A horizon. Therefore, although the control and treated soil comparisons showed that the treated soil had lost more Mg in both horizons compared to the control, the losses may be more severe in the A horizon as a result of its inherently lower Mg status compared to the O horizon.

There were no significant differences between control and treated soils for Mg in the O horizon between 1992 values and 1993 values, although both treatments had a trend of decreasing Mg values. However, for the control A horizon, the Mg level in 1993 was significantly less than that for 1992, and a similar trend (non-significant) was observed in the treated plots.

In 1992, treated plot Al and Ca levels were significantly greater in the O compared to the A horizon, but these differences were not significant in 1993. In 1993 the O horizon had significantly greater K and Mn and significantly lower pH compared to the A horizon.

Overall, there are more nutrients found in the O horizon compared to the A horizon on both the control and the treated plots. Carbon and N were also greater in the O horizon. These differences may be due to greater leaching losses in the mineral horizon or to greater nutrient inputs (needle-fall) in the O horizon. Larch allocate a small proportion of total annual C gain below ground (Gower and Richards 1990). Fine roots growing in the nutrient-rich O horizon are also exposed to elevated Al levels in the treated soil. Joslin and Wolfe (1989) showed root branching sensitivity in northern red oak (*Quercus rubra* L.) to Al in acidification treatments. Larch, which has been planted extensively on harsh sites, would not be expected to be very sensitive to Al toxicity; however, it is possible that Al toxicity may have decreased nutrient uptake and contributed to decreased growth.

## Foliar Chemistry: Treatment Response

Foliar samples from the larch on the treated plot collected in 1992 and 1993 had significantly greater Mn and Al levels and significantly lower P and Mg levels compared to the control (Figure 3). Foliar K values were not significantly different between control and treatment larch. The treated larch had significantly greater foliar N (23000 mg/kg) compared to the control (21300 mg/kg) in 1993. Also, the 1993 values of treated larch for Mn, N, P and S were significantly greater than its 1992 values (1993 values: Mn=3443.8 mg/kg, N=23030 mg/kg, P=1330 mg/kg, S=2120 mg/kg; 1992 values: Mn=2427.2 mg/kg, N=20960 mg/kg, P=1040 mg/kg, S=1860 mg/kg). Lower foliar P in treated larch may be due to the formation of an Al-PO<sub>4</sub> precipitate, possibly contributing to lower P availability (McCormick and Borden 1974). VanGoor (1953) reported the existence of a N/P antagonism in Japanese larch. The lower the availability of soil P the more quickly this antagonism develops with added N. VanGoor (1953) reported that the optimum range of N/P in needles was 4 to 5. The ratios in this study were 17 and 15 for the treated and control larch, respectively. Both soil and foliar Mn were elevated in the treated larch compared to the control. Studies with agronomic crops have shown that Mn can become elevated in acidic soils and reach toxic levels, impairing Ca and Mg uptake, disturbing physiological processes and reducing growth (Elamin and Wilcox 1986; Smith and others 1983). A greenhouse study designed to evaluate larch growth under elevated soil Mn found no height or diameter depressions in soils with Mn levels ranging from 12.8 to 150 mg/L and foliar Mn from 1655 to 5550 mg/kg (Unpublished data, C. Pickens, The Pennsylvania State University).

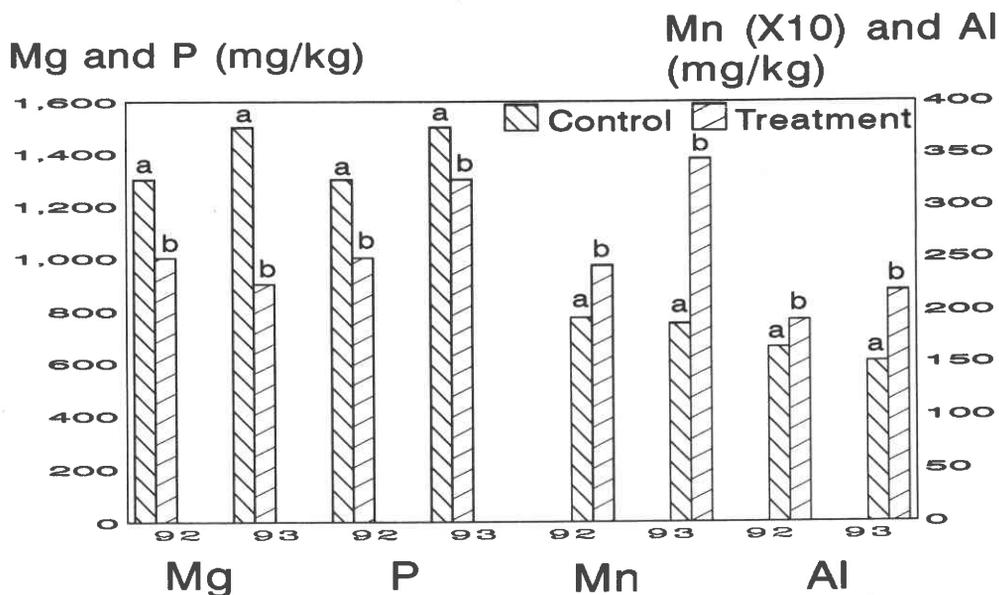


Figure 3. Foliar chemical comparisons between control and treated larch.

The patterns of foliar Al, Mg, Mn, N and S in the treated larch foliage compared to the control were somewhat similar to the patterns observed for soils. A general trend was that the treated plot had lower foliar and soil Mg levels, while Al, Mn, N and S were greater in treated soils and foliage compared to control values. The link between increased deposition of strong acids and an increase in soil Al has been made (Ulrich and others 1980), as has the antagonistic relationship between Al and the uptake and transport of Ca and Mg (Joslin and others 1988). Prior farming on watershed 9 included the addition of burned lime (personal comm., J. Kochenderfer, U.S. Forest Service) One possible explanation for the lack of Ca response (leaching) to acidic inputs may be due to these Ca additions, which still provide adequate Ca. However, Mg levels appear to be affected by the acidification treatment, with Mg levels reduced in the treated soil and foliage, possibly as a consequence of Mg leaching losses with mobile  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  ions. Magnesium content of sandstone derived soils is low relative to Ca and, thus, it might be expected that the available pool of Mg might be affected before that of Ca. Magnesium deficiencies have been reported in declining Norway spruce (*Picea abies* L.) in Europe (Tomlinson and Tomlinson 1990) and in the eastern US (Ke and Skelly 1994), and Tomlinson and Tomlinson (1990) attributed these deficiencies to excessive leaching as a consequence of acidic deposition. Bergmann (1992) gives an adequate Mg range for European larch (*Larix decidua* Mill.) of 1200-3000 mg/kg. The treated plot larch had less than 1200 mg/kg of Mg in both 1992 and 1993 suggesting a possible deficiency.

#### Foliar and Soil Chemistry Correlations

Foliar Mg consistently correlated with a number of soil chemistry parameters. In the treated samples, foliar Mg was positively correlated with A-horizon Ca ( $r = +0.86$ ,  $p < 0.05$ ) and Mg ( $r = +0.82$ ,  $p < 0.05$ ) (1992) and O-horizon Ca ( $r = +0.58$ ,  $p < 0.05$ ) (1993). In 1992, soil values for treated A-horizon Ca and Mg were correlated positively with larch height (Ca:  $r = +0.83$ ,  $p < 0.05$ ; Mg:  $r = +0.84$ ,  $p < 0.05$ ) and diameter (Ca:  $r = +0.91$ ,  $p = 0.01$ ; Mg:  $r = +0.83$ ,  $p < 0.05$ ). Negative correlations were observed between O-horizon N and larch height ( $r = -0.81$ ,  $p < 0.05$ ) and diameter ( $r = -0.84$ ,  $p < 0.05$ ), and A-horizon Al and diameter ( $r = -0.81$ ,  $p < 0.05$ ). In 1993, A-horizon N again was correlated negatively with diameter measurements ( $r = -0.81$ ,  $p < 0.05$ ). In 1993, foliar Mg in treated larch were correlated positively to both height ( $r = +0.60$ ,  $p < 0.05$ ) and diameter ( $r = +0.67$ ,  $p < 0.05$ ). Negative correlations existed between foliar Mg and O-horizon C in 1992 ( $r = -0.97$ ,  $p < 0.01$ ) and 1993 ( $r = -0.63$ ,  $p < 0.05$ ), and between foliar Mg

and O-horizon N ( $r = -0.97$ ,  $p < 0.01$ ) and A-horizon S ( $r = -0.81$ ,  $p < 0.05$ ) in 1992, and A-horizon Al ( $r = -0.70$ ,  $p < 0.01$ ) in 1993.

Based on correlations with foliar and soil chemistry, Mg appears to be the most important variable potentially influencing larch growth. The negative correlations between larch growth parameters and soil N support the hypothesis that larch has a low N requirement and responds adversely to increased N (Leyton 1957). Treated soil Ca and Mg were correlated positively with growth; deficiencies of these nutrients under acidification stress may be detrimental to larch growth. Heenan and Campbell (1981) demonstrated that increasing Mn supply reduced Mg uptake in soybean, and that Mn reduced Mg leaf concentrations by reducing the rate of Mg absorption. It also has been reported that a liberal supply of Ca can counteract Mn toxicity (Ouellette and Dessureaux 1985). Plant available Mn was elevated in the treated soil, and such an increase along with a decrease in exchangeable Mg may have interfered with larch growth. The negative correlations between larch growth and soil Al also may implicate Al in the observed growth reduction.

### SUMMARY AND CONCLUSIONS

After 18 (1992) -20 (1993) treatments (total applications) with ammonium sulfate fertilizer over 5 yrs/8 mos (1992) and 6 yrs/8 mos (1993), the larch trees growing on watershed 9 have reduced height and diameter growth compared to control trees. Soil chemistry revealed greater levels of Al, C, Mn, N and S and lower levels of Ca, Mg and pH compared to control soils. Significantly greater foliar N in the treated larch indicates greater N availability on the treated areas. Earlier studies have shown high N/P ratios to be detrimental to larch growth, but the N/P ratios for foliage in control and treated larch were high and well above the reported optimums. Treated larch height and diameter were correlated positively with foliar Mg. Foliar Mg was reduced significantly on the treated plot. Reduced Mg availability also may have contributed to reduced larch growth, since foliar Mg concentrations of treated larch were less than adequate. It appears most likely that a change in Mg supply coupled with increases in available Al and Mn have contributed to nutrient deficiencies and imbalances and impaired growth of treated larch.

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