Effects of mist acidity and ambient ozone removal on montane red spruce

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Summary Recent forest studies have established that high-elevation (> 900 m) populations of red spruce (Picea rubens Sarg.) in the northeastern USA are declining. Because it has been suggested that changes in air quality are responsible for the decline, we examined the effects of acidic mists and ozone on several biochemical and growth parameters in mature montane red spruce. We used branch-sized environmental chambers to introduce mists of controlled composition and exclude ambient clouds and ozone from individual branches within a tree. Mists consisting of distilled water increased the end-of-season pigment concentration and shoot length of enclosed branches relative to ambient or artificial mists. Needle and twig weights and starch concentrations were not significantly altered by the acidic mist treatments. Removal of ambient ozone had no apparent effect on the variables measured.

Keywords: acid precipitation, artificial mist, chlorophyll loss, Picea rubens.

Introduction

Red spruce (Picea rubens Sarg.) is a long-lived conifer found throughout the Appalachian Mountains and the Canadian Maritimes. Recent widespread mortality of montane red spruce populations (Siccama et al. 1982, Scott et al. 1984, Battles et al. 1988, Craig and Friedland 1991) has prompted speculation that the decline is caused or exacerbated by the presence of acidic cloud-water and precipitation (Siccama et al. 1982). This idea is reinforced by both the pattern of acid deposition in regions experiencing decline (Johnson and Siccama 1983) and the dramatic increase in mortality of forests above cloud-base (Battles et al. 1988). Severe decline is largely restricted to trees above cloud-base, suggesting a direct effect of acidic cloud-water. In addition, several experiments have shown adverse effects of the presence of simulated or natural acidic cloud-water, including necrotic flecking (Jacobson et al. 1990), reduced foliar nutrient concentrations, increased respiration (McLaughlin et al. 1990, McLaughlin et al. 1991) and decreased cold tolerance (Fowler et al. 1989, DeHayes and Hawley 1991, Vann et al. 1992).

At Whiteface Mountain, NY, clouds extend as low as 1000 m for 15% of the year (Mohnen 1989), and studies of cap-cloud chemistry have found an average summertime pH of 3.5, arising primarily from sulfate ions. Additionally, ozone concentrations at these elevations average 47 ppb daily during the growing season and do not show the diurnal pattern typical of lower elevations (Mohnen 1989). Vann (1993) found that needles on upper surfaces of branches in high-elevation trees became increasingly chlorotic throughout the growing season, whereas shaded needles maintained their pigment concentrations, suggesting the possible involvement of oxidative agents such as ozone that promote photobleaching (Guderian et al. 1985).

Several groups have examined the effects of ozone and acid mist, singly or in combination, on red spruce seedlings or saplings in controlled environmental chambers at low elevations (Percy 1986, Johnson et al. 1988, Fowler et al. 1989, Fincher et al. 1990, Jacobson et al. 1990, Lee et al. 1990, Percy et al. 1990, Thornton et al. 1990, Patton et al. 1991, Wolfenden et al. 1991). The results from these experiments are somewhat mixed, although most studies showed adverse effects of simulated acid cloud-water. However, it is difficult to extrapolate low-elevation studies on seedlings to mature trees in high-elevation forests. To test the hypothesis that ambient air quality is a contributor to spruce decline, we installed environmental exclusion chambers (Vann and Johnson 1988, Vann and Johnson 1995) on branches of mature red spruce trees growing under natural field conditions in a high-elevation forest and modified the air quality of the chamber environment.

Materials and methods

Site description

The experimental site was located at an elevation of 1170 m on a ridge (Mt. Esther) on the northwest face of Whiteface Mountain, NY. The site is subject to high winds and frequent storms and is immersed in cloud for approximately 20–25% of the growing season (May–October). The site is in an old-growth forest where 70% of the mature red spruce are dead or severely declining.
Tree and branch selection

Trees at the site were classified as “declining” or “healthy” based on: visual assessments of needle loss, radial increment growth determined from cores taken at breast height, and ultramicroscopic evaluation of cell structure in needles collected in August 1986. All selected trees were dominant or codominant trees, had emergent crowns and were 75+ years old. Four trees at the site were assigned to the “healthy” class and were used in an experiment conducted in 1988. These asymptomatic trees exhibited no apparent needle loss (i.e., < 10%), no unusual decrease in radial growth increment within the past twenty years, and less than 30% of the cells contained ultrastructural anomalies suggesting injury. Four declining trees at the site were selected in 1989. These trees had needle losses in excess of 25%, and radial increment growth showed a decrease over the past 20 years similar to that seen throughout the northern range of red spruce (Johnson et al. 1988). Other declining trees at the site showed subcellular abnormalities in approximately 40% of the cells. Winter injury was severe during the winter of 1988–1989; accordingly, we attempted to select trees with similar amounts of damage.

Five experimental branches from each tree were selected based on: position on the northwesterly aspect of an exposed portion of the upper crown (whorls 7–13), branch age, visual estimates of 1987–1988 needle color (subsequently checked by chlorophyll measurements), and similar and minimal amounts of winter injury (for the 1989 experiment).

Treatments

Experiments were conducted in 1988 and 1989. Healthy trees were used in the 1988 experiment, and declining trees were used in the 1989 experiment. One branch on each tree in each experiment was left outside the chamber and used as a reference to determine chamber effects (designated NC). In each experiment, all treatments were randomly assigned among the branches of a tree.

1988 Experiment Each tree in the 1988 experiment included a control in which ambient air and clouds were drawn through the chamber (AA) plus three treatments: CF = charcoal-filtered air (cloud-water and charcoal-reactive gases removed), NM = ambient air with cloud-water removed by a graded series of Teflon mesh filters, and DF = same as CF, but with deionized water mist added to the chamber during cloud immersion. Because of the nature of the exclusion process, the NM and CF treatments did not contain mists. As a result, the conditions within the NM and CF treatment chambers more closely simulated the environment at low elevations, below cloud-base. The treatments began on June 27–July 2, 1988 and were terminated on September 30, 1988.

1989 Experiment Each tree in the 1989 experiment included four treatments and a control in which ambient air and clouds were drawn through the chamber (AA). The treatments comprised all combinations of charcoal-filtered and ambient air with deionized and synthetic acid mists in a 2 × 2 factorial design: SA = synthetic acid mist + ambient air, SF = synthetic acid mist + filtered air, DA = deionized mist + ambient air, and DF = deionized mist + filtered air. The experiment began July 15, 1989 and was terminated on September 20, 1989.

Sampling

Shoot samples were collected at intervals for determinations of pigment and starch concentrations, shoot growth and weight, and needle and twig numbers. Samples consisting of terminal shoots comprising 2 years of growth were cut from unshaded lateral branches exclusive of the three apical whorls. To control for intra-branch variability, two to three samples were removed every sampling period from each branch used in the study. Samples were cut from the branch through ports in the rear of the chamber, bagged and frozen in liquid nitrogen. At the end of each sampling, the collection was packed in dry ice and shipped to the laboratory overnight. Samples were stored at −80 °C until processed. In all cases, sampling removed < 10% of the total available plant tissue in these age classes.

Eight sample collections were taken for the 1988 experiment. The first sampling occurred in June 1988, before bud break; subsequently, collections were taken every 2 weeks through July and August, and then on September 30 when the treatments were terminated. Collections were also made after the treatments were terminated on December 20, 1988 and in April 1989. In the 1989 experiment, bud break occurred between June 27 and July 1, 1989. Shoots were sampled four times during the season, before chamber installation (July 6) and removal (September 25), and twice during the experimental period (August 2 and September 1).

Biochemical analysis

Samples were separated into twigs and needles by year class. Individual tissue samples were then divided into two portions, one of which was weighed, oven-dried at 65 °C and re-weighed to determine water content. The remaining portion was ground in liquid nitrogen, and two replicate 50-mg subsamples were each re-ground in solvent with a shear grinder (Ultra-Turrax T-25, IKA Works, Cincinnati, OH) and extracted twice with 5 ml of methanol/chloroform/water (12/3/5) saturated with magnesium carbonate. Subsamples were centrifuged at 3000 g (model TJ-6, Beckman Instruments Inc., Palo Alto, CA). The pellet was dried and analyzed for starch, and a 1.5 ml aliquot of the supernatant was used for pigment analysis.

Chlorophylls and carotenoids were determined spectrophotometrically at 649, 665 and 478 nm (model DU-50, Beckman Instruments Inc.). Extinction coefficients were determined using purified chlorophyll a and b and mixed carotenoids (Sigma Chemical Co., St. Louis, MO). The value for carotenoids represents mixed carotenoids and xanthophylls. The formulae used were as follows:

\[
\text{Chlorophyll a} = 20.44 \times A_{665} - 9.29 \times A_{649}, \\
\text{Chlorophyll b} = 31.34 \times A_{649} - 10.37 \times A_{665}, \\
\text{Carotenoids} = \frac{A_{478}}{0.09221} + 6.941 \times A_{665} - 23.07 \times A_{649}.
\]
Starch was analyzed according to a modification of the method of On-Lee and Setter (1985). The pellet was resuspended in 0.1 M acetate buffer, pH 4.5, and hydrolyzed with 15 mg ml⁻¹ amylglucosidase (Boehringer-Mannheim Biochemicals, Indianapolis, IN) for 24 h at 37 °C. The hydrolysate was then analyzed for glucose by a commercial glucose oxidase method (Sigma Chemical Co.).

An initial measurement of current-year shoot length was made to confirm that the branches were at a similar phenological stage. The final measurement was made in October 1988. Shoot length, determined as the distance from the point of shoot emergence to the base of the terminal bud, was measured with digital calipers (Mitutoyo, Japan) on in situ shoots. All shoots that were enclosed within chambers and shoots on the distal meter of unenclosed branches were measured.

Statistical analysis

In the 1988 experiment, the trees chosen represented a non-random selection of trees within this particular forest (survivors in a population of declining trees); consequently, the data were analyzed by a Model I ANOVA with trees (blocks) and treatments as fixed effects. Because of environmental differences between chambers providing mist treatments (AA and DF) and chambers without mist treatments (NM and CF), these groups were also analyzed separately using post-hoc procedures (Sokal and Rohlf 1981). In September 1988, ambient temperatures dropped below 5 °C on several occasions. Because the chamber temperatures did not always decline to ambient temperatures during these periods, we also tested September mean temperature as a covariate in the ANOVA.

In 1989, the statistical analysis was confined to the four manipulated treatments, because the synthetic acid mist treatments were not strictly comparable with the AA treatment (see discussion below). The experimental design was a 2 x 2 factorial randomized complete block. In the analysis, “Tree” was used as the random block variable, with “Mist” and “Filtered” as fixed effects. In both experiments, we tested the hypothesis that acid mist or ozone would result in a decrease in the measured variable; the test is therefore one-tailed (Sokal and Rohlf 1981, pp 225-226). Shoot length data were log-transformed before statistical calculations. Analyses of variance were performed with the GLM procedure of SAS (SAS Institute, Cary, NC).

Chamber construction

The chamber design and construction has been described by Vann and Johnson (1995). Briefly, chambers enclosed the distal meter of a branch and were half-ovoid in shape with a volume of about 85 l. This minimized the wind profile and reduced the amount of unmixed air space. The proximal end consisted of an oval polyethylene backplate through which the branch passed. Backplates had access ports for sampling and supported instruments to monitor environmental conditions within the chamber. Custom fitted PFA Teflon bags (made from 50 mm thick film) covered a frame of Teflon-coated fiberglass tubes. Air was drawn through the chambers at 300 l min⁻¹ by axial vane fans mounted on the backplate, assisted by fans located in the filters. Air flow was distributed by a plenum tube made of PFA Teflon. Air inside the chambers was mixed by two fans displacing 280 l min⁻¹.

Filter systems were built inside polyethylene containers connected to the chambers by 3-m long Teflon-lined silicone rubber tubes. Insects, rain and cloud-water were excluded with graded layers of PFA Teflon mesh. In treatments requiring charcoal filtration, the filter unit included 100 mm of activated charcoal mesh. Measurements of ozone inside and outside the chambers were taken on 20 dates in August–September 1988 with a portable instrument. Ozone concentrations in CF and DF treatments averaged < 20% of ambient (range 5–40%), with the upper values occurring when the charcoal became accidentally wetted. Ozone concentrations for the AA and NM treatments averaged 91% of ambient (range 82–102%). Ambient ozone concentrations ranged from 22 to 47 ppm (Vann and Johnson 1995).

Artificial mist was generated from deionized water or simulated acid rain using custom designed mist generators built around ultrasonic transducers using Teflon- and platinum-coated components to minimize chemical contamination of the mist. Ultrasonic mist generation was used because it produces droplet distributions similar to natural mists (A. Lazrus, personal communication). The misters were operated manually during periods when trees were immersed in clouds, or on overcast days. Operating problems in 1988 reduced mist generation to about 50% of the total natural immersion time. In 1989, total exposure time was 50 h, or about 20% of the total time that the site was above natural cloud base. Mist water content was about three times that of natural clouds. The composition of the artificial mist was based on analysis of clouds on Whiteface Mountain in 1987 (Miller et al. 1993). The ion concentrations used were (in mmol l⁻¹): Ca 10.0, K 3.0, Mg 3.0, Na 4.0, NH₄ 228, NO₃ 127, SO₄ 230, Cl 10.1, and H 302 (adjusted to pH 3.5).

Temperatures were measured with copper-constantan thermocouples fitted with electronic ice-point simulators. Data were recorded as hourly averages plus min/max values with dataloggers. During August and September, 1988, average daily temperatures inside the chambers were 1.7 °C warmer than ambient, and peak hourly averages in full sun at midday were several degrees warmer. In 1989, chamber temperatures averaged about 1 °C warmer than ambient temperatures as a result of changes in chamber design.

Results

1988 Experiment

During the 1988 growing season, peak ozone values occurred in mid-July, with values above 100 ppb for 18% of the experimental period (Figure 1). Based on daily weather records, the site was immersed in cloud for 23% of the experimental period. The average pH of these clouds was about 3.5 (Mohnen 1989).

Because the patterns of response of chlorophylls a and b were similar to that of total chlorophyll (sum of chlorophylls a and b), only the latter value is reported here. In 1-year-old
(1987) needles, chlorophyll concentration increased after bud break (Figure 2A) and continued to increase until mid-August, when a plateau was attained. In autumn, chlorophyll concentration again increased before declining through late winter. A similar seasonal pattern for chlorophyll has been reported for Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Lewandowska and Jarvis 1977). By late August, 1-year-old needles on unchambered branches (NC) had significantly lower mean chlorophyll concentrations than 1-year-old needles in the chambered treatments (*P* ≤ 0.10). Needles in the DF treatment attained a higher chlorophyll concentration earlier in the season than needles in the other treatments and maintained higher values throughout the experimental period. By April 1989, chlorophyll concentrations were low in all treatments and highly variable as a result of freezing injuries.

By early to mid-August, chlorophyll concentrations of current-year (1988) needles were similar to those of 1-year-old needles (Figure 2B). By early August, needles on chambered branches had significantly higher mean chlorophyll concentrations than needles on unchambered branches (*P* ≤ 0.05); this pattern persisted, although the difference was not significant in the winter-injured needles sampled in April 1989. Chlorophyll concentrations of current-year needles in the chambered treatments were not significantly different from one another until September 30 when the mean chlorophyll concentration of DF-treated needles was significantly higher than that of needles in the other chambered treatments (Tables 1A and 1D). Because chamber temperatures varied with location in the canopy, the September 30 data were also analyzed using the mean September chamber temperature as a covariate (Table 1C). Although temperature did not have a significant effect on chlorophyll concentration, it did account for some of the variation between treatments, increasing the overall significance of the model.

Needles on branches collected in April 1989 had lower chlorophyll concentrations than needles on comparable branches collected in the previous year as a result of winter injuries. When the April values were separated according to whether branches were visibly injured, it was evident that the decrease in chlorophyll concentration was due to the injured branches. The uninjured branches had chlorophyll concentrations similar to those of branches collected the previous September (Figure 3).

Carotenoid concentration was closely correlated with total chlorophyll concentration throughout the year (*R*² = 0.75, *P* < 0.0001, *n* = 659; all samples collected in 1988). Because carotenoids break down when exposed to ozone (A.R. Wellburn, personal communication), we expected to see declines in carotenoid concentration of needles in the AA-, NM- and NC-treated branches. However there were no significant effects of the AA, NM and NC treatments on mean carotenoid concentration of current-year needles. The chamber effect caused an increase in carotenoid concentration of current-year needles, and by September 30, significantly higher carotenoid concentrations (*P* ≤ 0.05) were observed in the DF treatment compared with the other treatments (Figure 4).

Starch concentrations showed a clear seasonal trend, with starch being mobilized in older tissues at bud break (Figure 5) and continuing to decline throughout the remainder of the season. Pre-bud break starch concentrations in 1-year-old tissues were typically four times higher than the subsequent peak concentration.
values for both current-year and 1-year-old needles. Starch concentrations of current-year needles increased to a maximum during late July, subsequently declining toward autumn. By late September, foliar starch concentrations were negligible in the declining trees. No significant effect of treatment on starch concentrations of any tissues was found.

Shoot extension in conifers is largely dependent on the previous season’s stored carbohydrates (Kozlowski and Winget 1964, Rangnekar and Forward 1972) and early-season photosynthetic activity by the previous year’s needles (Little 1970, Loach and Little 1973). Consequently, shoot length might be expected to respond primarily to conditions existing before chamber installation. However, shoot elongation continued for some time after installation and carbohydrate mobilization from older tissues occurred through a period of elevated ozone concentration in early July. Shoots grown in chambers were all significantly \( P \leq 0.05 \) longer than those from NC branches (Figure 6). Within the chambers, shoots from both CF and DF treatments were longer than those from AA and NM treatments. The differences were small, as most shoots on all branches tended to be short (lognormally distributed). The greatest differences occurred in the longest shoots, typically apical and subapical shoots. When mean shoot length

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>SE</th>
<th>( t )-test(^1)</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>1.18</td>
<td>0.055</td>
<td>a</td>
</tr>
<tr>
<td>Dry</td>
<td>1.30</td>
<td>0.049</td>
<td>ab</td>
</tr>
<tr>
<td>Filtered</td>
<td>1.42</td>
<td>0.049</td>
<td>b</td>
</tr>
<tr>
<td>Mistered</td>
<td>1.66</td>
<td>0.056</td>
<td>c</td>
</tr>
</tbody>
</table>

Source df SS MS \( F \)-Value \( P > F \)

| Model | 15 | 1.437 | 0.096 | 3.40 | * |
| Error | 15 | 0.423 | 0.028 |
| Corrected total | 30 | 1.861 |
| Tree | 3 | 0.5056 \(^2\) | 0.1686 | 5.97 | ** |
| Treatment | 3 | 0.4239 \(^2\) | 0.1413 | 5.01 | ** |
| Interaction | 9 | 0.4508 \(^2\) | 0.0501 | 1.78 | ns |

Chambers with mist (AA and DF)

Tree | 3 | 0.2791 | 0.0930 | 2.60 | ns |
Treatment | 1 | 0.2911 | 0.2911 | 8.14 | * |
Interaction | 3 | 0.2478 | 0.0826 | 2.31 | ns |

Chambers without mist (CF and NM)

Tree | 3 | 0.4189 | 0.1396 | 6.47 | ns |
Treatment | 1 | 0.0577 | 0.0577 | 2.67 | ns |
Interaction | 3 | 0.0401 | 0.0134 | 0.62 | ns |

\( \text{Means followed by different letters are significantly different at } P = 0.05.\)
\( ^2 \text{Sums of squares are Type III.}\)
\( ^3 \text{\( * = \) significant at } P < 0.05; \text{ \( ** = \) } P < 0.01; \text{ \( ns = \) not significant } (P > 0.05).\)

Figure 3. Needles formed in 1988 and collected in April 1989, after a winter injury event: I = visibly winter-injured branches (reddened needles) in all treatments. Uninjured (green) needles shown by treatment. Abbreviations as in Figure 2. Bars represent ± SE.

Figure 4. Mean carotenoid concentration (fresh weight basis) of 1-year-old needles (A) and current-year needles (B) for the 1988 experiment. Treatments: NC = no chamber ((circle), AA = ambient air + mist (triangle), NM = ambient air + no mist (triangle), CF = charcoal-filtered air (square), and DF = deionized water mist + charcoal-filtered air (diamond). Bars represent ± SE.
was tested, differences were highly significant among trees ($P \leq 0.01$); the interaction was also significant. When this occurred, the correct $F$-ratio was determined by dividing fixed effects SS by the interaction MS (Underwood 1981). Differences among treatments were marginally significant ($P \leq 0.10$). No correlations were found between average shoot length and within-chamber environmental variables, suggesting that the trend in shoot length was a response to the treatment.

1989 Experiment

The chambers treated with simulated acidic mists were exposed to acidic mists of constant composition, whereas the AA treatments received natural mists, whose acidity varied (Mohnen 1989). Consequently, the overall exposure to acids was probably greater in the SA and SF treatments than in the AA treatment. As a result, the statistical analyses were restricted to those chambers whose environments we manipulated.

The chlorophyll concentration of needles that expanded in 1989 increased 100–150% during the first 18 days of exposure, after which the chlorophyll concentration of needles on unchambered branches reached a plateau (Figure 7B). Chlorophyll in needles on chambered branches continued to increase throughout August, with the shoots in DF and DA treatments developing the highest values. By the end of the season, mean total chlorophyll concentrations of needles in treatments receiving deionized water mists were significantly higher than those in acidic mists (Figure 7C). Charcoal-filtration increased chlorophyll values slightly, but not significantly (Table 2). Chlorophyll values of needles in the AA chambers were higher than those in the SA and SF chambers, in concert with their lower exposure to acidic mists. Carotenoid concentration followed the same pattern, and was closely correlated with chlorophyll concentration (data not shown). No significant effects of treatment were found for chlorophyll concentrations in 1-year-old (1988) needles (Figure 7A).

All age classes of needles showed a significant increase in chlorophyll concentration in response to the presence of the chamber ($P < 0.05$). However, using the chamber parameters in the test of response variables had no effect on the analysis.
Seasonal starch values followed the same trends as in the 1988 experiment (Figure 8). Starch was mobilized from older needles during bud break and continued to decline throughout the season. In current-year needles, starch accumulated as the new needles became self-sufficient and declined toward the end of the season as it was mobilized into other parts of the tree; by September, the starch concentration reached zero. We found no significant differences in starch concentration between treatments or due to chamber presence.

As a result of the winter injury event, the selection of branches for the 1989 experiment was limited. Although we chose branches with relatively little visible injury, the loss of tissues had an effect on subsequent growth of the 1989 shoots (Vann et al. 1992). As a result, shoot length was not analyzed for these treatments.

Individual trees responded differently to the treatments. Although this response appeared to be significant, the differing histories of individual trees introduce the possibility of restriction error (Anderson and McLean 1974); consequently, there was no estimable $F$-value. The value shown in Table 2 assumes no restriction error and must be interpreted with caution.

Other variables measured, including percent water content for twigs and needles of both age classes, twig and needle weight in either age class, and needle number per twig, were not significantly different between treatments and no trends were evident. Also, there was no chamber effect.

### Discussion

We hypothesized that one or more atmospheric components produced cellular injuries requiring repair, thus reducing carbon available for growth. Removal of these stresses was expected to increase tissue starch concentrations, dry weights and shoot growth. In addition, the exclusion of oxidative chemicals was expected to mitigate the observed summer decline in chlorophyll. The trees used in 1988 all appeared to be healthy and represent survivors of the phenomena responsible for the region-wide decline in red spruce. As a consequence, the response of these trees may represent a minimal effect of changes in air quality. The chlorophyll concentrations of the 1989 declining trees were lower than those of nondeclining trees. These declining trees might have been unable to respond to any amelioration of stresses on single branches, as a result of the demands for photosynthate in other parts of the tree, a disturbance of hormonal balance or irreversible processes attendant upon senescence.

The mist treatments were not directly comparable to natural cloud immersion. Although the short exposure to simulated mists was offset in part by a higher mist density, there was also a significant chamber effect as a result of slightly elevated temperatures and higher, more constant humidities (Vann and Johnson 1995). The chamber effect resulted in higher pigment concentrations in branches in chambered treatments than in unchambered (NC) treatments.

Because the 1988 NM and CF treatments did not include mists, the treatments approximate conditions at low-elevation sites, and thus may not fully reflect the response of trees in clouds. The difference between the NM and CF treatments represents the effect of ozone removal (as well as other dry-phase oxidants) in the absence of cloud-water. Charcoal filtration of the air did not result in a significant difference in pigment concentrations in either year. It is possible that the chamber effect masked ozone-mediated photobleaching losses.

The absence of any charcoal-filtration treatment effect on starch concentration suggests that ambient air quality does not have a direct effect on carbon balance and is not directly responsible for the decline in radial increment growth (Adams et al. 1985, Johnson et al. 1988). The mid-July 1988 starch values for 1-year-old needles on unchambered branches were significantly higher than those of chambered branches, suggesting a lag in starch mobilization. This occurred during a period of high ambient ozone (about 80 ppb) and may be associated with ozone-induced membrane damage or inactivation of transport mechanisms. High concentrations of ozone for short periods of time have been shown to reduce carbohydrate concentration (Kozlowski and Constantinadou 1986). Continuous exposure to 40–60 ppb ozone may result in sublethal injuries that are repaired at a cost to other carbon sinks.

### Table 2. ANOVA for chlorophyll concentrations of 1989 needles sampled on September 25, 1989 (model: $R^2 = 0.915, c.v. = 8.786$).

<table>
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<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$-value$^1$</th>
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<tr>
<td>Model</td>
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<td>1.0408</td>
<td>0.0694</td>
<td>11.53**</td>
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<tr>
<td>Error</td>
<td>16</td>
<td>0.0963</td>
<td>0.0060</td>
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<tr>
<td>Corrected total</td>
<td>31</td>
<td>1.1370</td>
<td></td>
<td></td>
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<tr>
<td>Tree</td>
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<td>0.3960</td>
<td>5.210</td>
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<tr>
<td>Mist</td>
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<td>0.2831</td>
<td>0.0398</td>
<td>7.116a</td>
</tr>
<tr>
<td>Filtered</td>
<td>1</td>
<td>0.0109</td>
<td>0.0360</td>
<td>0.302</td>
</tr>
<tr>
<td>Tree × mist</td>
<td>3</td>
<td>0.1194</td>
<td>0.1048</td>
<td>0.380</td>
</tr>
<tr>
<td>Tree × filtered</td>
<td>3</td>
<td>0.1081</td>
<td>0.1048</td>
<td>0.344</td>
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<tr>
<td>Mist × filtered</td>
<td>1</td>
<td>0.0058</td>
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<td>0.055</td>
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<tr>
<td>Tree × mist × filtered</td>
<td>3</td>
<td>0.3143</td>
<td>0.0060</td>
<td>17.49**</td>
</tr>
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</table>

$^1$ Sums of squares are Type III.

$^a = $ significant at $P < 0.05$; ** = $P < 0.01$. 

![Figure 8. Starch concentrations of 1-year-old needles (A) and current-year needles (B) harvested in 1989. Symbols as in Figure 1C. Bars represent ± SE.](image-url)
The lack of any charcoal-filtration treatment effects on the starch concentration of needles may indicate that either the variation observed in chlorophyll concentration did not result in differences in photosynthetic production, or there was a demand for carbon for repair of pollutant-induced injuries in other tissues. Thornton et al. (1990) found that removing cloud-water and ozone from seedlings in field chambers resulted in lower respiration rates in older foliage. Thus, higher respiration rates in older or injured foliage may consume most of the carbon produced by younger needles, resulting in no measurable difference in starch concentration. If this occurred in the 1989 experiment, we would have expected to find low starch concentrations in all tissues examined, whereas starch concentrations were 3–4 times higher than those found in healthy trees. Electron micrographs of chloroplasts from declining trees show large starch grains, even in dying cells (D.R. Vann, unpublished data). It is possible that transport mechanisms in declining trees are not functioning properly, resulting in the accumulation of starch. If so, the starch concentration may represent the maximum storage capacity of the chloroplasts, a factor that is independent of treatment.

In both years, needles exposed to ambient or synthetic acidic mists had significantly lower pigment concentrations at the end of the growing season than needles not exposed to acidic mist. Pigment concentrations remained essentially constant during the growing season until August, then, with the exception of NC branches, increased substantially in September. A similar pattern was reported by Lewandowska and Jarvis (1977) for unpolluted Sitka spruce trees. At the end of the growing season, winter hardening events are the dominant physiological processes. These events include a substantial increase in membrane quantity, accompanied by increases in chloroplast number (Senser et al. 1975, Singh et al. 1975, Senser and Beck 1984). Differences in chlorophyll concentration between treatments at this time of year may be associated with differences in cold hardness.

In April 1989, needles from the 1988 experiment showed substantial decreases in chlorophyll concentration. The decrease was caused by a severe incident of winter injury, in which 40–98% of the 1988 needles were killed in nearly all trees examined (D.R. Vann, unpublished data). The 1987 needles did not show visible symptoms of injury (reddened needles), but lost similar amounts of chlorophyll as the 1988 needles. It is possible that nonlethal, nonvisible cellular injuries occurred in these needles. However, both 1-year-old and current-year needles on branches that were not exposed to ambient acidic mists had higher chlorophyll concentrations than needles on branches exposed to acidic mists. As a result of the high variability in extent of winter injury, the effect was not significant but suggests that exposure to acidic mists may compromise the physiological status of the tree prior to bud break.

In conclusion, the study failed to establish a clear effect of ambient ozone, and the possibility of long-term effects was not eliminated. The experimental trees did respond to a single season of exposure to deionized water mists, suggesting that the presence of acid mists represents a chronic abiotic stress in these montane forests. For trees at the edge of their physiological range, this additional stress may contribute to growth loss and mortality, particularly if increased sensitivity to winter injuries results from exposure to acidic mists.

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References


