RESISTANCE OF THE CARIBOU LICHEN Cladina stellaris (Opiz.) BRODO TO GROWTH REDUCTION BY SIMULATED ACIDIC RAIN

MARTIN J. LECHOWICZ

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montréal, Québec, H3A 1B1, Canada

(Received April 14, 1986; revised October, 1986)

Abstract. Natural mats of C. stellaris growing in the subarctic lichen woodlands of northern Québec were treated in a randomized complete block design with solutions of simulated rain at pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.6. These solutions were acidified by addition of mixtures of sulfuric and nitric acids to give both 2:1 and 6:1 equivalent ratios of SO$_4^-$:NO$_3^-$. After two years of acidification there was no significant effect of either pH or SO$_4^-$:NO$_3^-$ ratio on the growth of C. stellaris, but thallus discoloration was evident below pH 3.5. After three years of acidification marginally significant (p = 0.05) but erratic depression of growth occurred under the 6:1 but not the 2:1 acidification regime, especially at pH 4.5 or less. Acid precipitation therefore only very gradually impairs the growth of C. stellaris, and the deleterious effects of acidification may be partially offset by nitrogen enrichment when precipitation is relatively rich in nitrate compared to sulfate ions.

1. Introduction

Various important metabolic activities are often, although not always, impaired when a lichen thallus is wetted by precipitation more acidic than pH 5.6. The photosynthetic activity of Cladina stellaris was reduced after wetting by even moderately acidic solutions (Lechowicz, 1982). At pH 4.0 photosynthetic capacity was lowered 27% from normal levels and the lichen took 14% longer to recover from dormancy after being wetted. Similarly, Bailey and Larson (1982) found that the net photosynthetic rate of Umbilicaria mammulata collected in February, when stored reserves are at their lowest in northern lichens (Holopainen, 1982), was typically as much as 50% lower when wetted by solutions of pH 2.0 compared to pH 6.0; intermediate levels of depression of net photosynthesis were also apparent after wetting at pH 4.0. In contrast neither the net photosynthetic nor respiratory responses of this Umbilicaria were affected by the pH of the wetting solutions for samples collected in August (Bailey and Larson, 1982) when lichen reserves are highest (Holopainen, 1982). Sigal and Johnston (1986a, b) reported impairment of photosynthesis in Lobaria pulmonaria, Pseudoparmelia caperata, and Umbilicaria mammulata exposed to simulated rain of pH 3.3 and lower, but found no effect on the photosynthesis of Usnea cf sulfusca exposed to the same treatments. In both Cladina rangiferina and Lobaria pulmonaria the activity of phosphatase, an enzyme involved in lichen mineral nutrition, actually increased as pH dropped from 8.9 to 2.2 (Lane and Puckett, 1979). Conversely N fixation in Peltigera aphthosa and P. polydactyla was reduced 80% by simulated acid rain of pH 4, and completely inhibited at pH 2 (Fritz-Sheridan, 1985). Sigal and Johnston (1986b) found that simulated acidic rain of pH 2.6 reduced N fixation in Lobaria pulmonaria. Denison et al. (1977) reported similar
short-term losses of N-fixation capacity under acidification in Lobaria pulmonaria and L. oregana, and showed that recovery of fixation capacity could occur after wetting at pH 4 but not pH 2. Overall the deleterious effects on C and N metabolism suggest that acidic precipitation might reduce the net annual productivity of lichens (Lechowicz, 1982; Gilbert, 1980). To test this supposition the effects of chronic acidic precipitation on the growth of Cladina stellaris, a lichen which is a dominant component of many boreal and subarctic ecosystems throughout the northern hemisphere (Ahti, 1977), were monitored in a series of simulated acidic rain plots established in a subarctic lichen woodland.

2. Study Area

The experiments were conducted in a previously undisturbed lichen woodland near Schefferville, Québec at 54°48' N, 66°55' W and at an altitude of about 540 m. This subarctic site represents a fully mature lichen woodland community typified by an open, almost park-like aspect and a relatively species-poor plant cover. The trees, mostly black spruce (Picea mariana) with some white spruce (P. glauca) and larch (Larix laricina), are widely spaced. From increment core data, the largest trees are in excess of 125 yr old (Lucarotti, 1981). The sparse shrub layer is predominantly dwarf birch (Betula glandulosa), blueberry (Vaccinium angustifolium), and Labrador tea (Ledum groenlandicum). A 8 cm thick mat of the caribou lichen, Cladina stellaris, carpets 73% of the ground surface. Similar lichen woodlands are common both around Schefferville (Rencz and Auclair, 1978) and throughout the Canadian boreal and subarctic regions (Kershaw, 1977; Ahti, 1977; Carroll and Bliss, 1982; Clayden and Bouchard, 1983).

3. Experimental Design and Procedures

The basic experiment consisted of weekly waterings of the Cladina stellaris mat in this lichen woodland community with simulated rain solutions ranging from pH 2.5 through pH 5.6. The composition of the simulated rain solution in mg L⁻¹ was 0.22 Ca, 0.06 Mg, 0.12 Na, 0.08 K, 0.22 ammonium, 0.53 sulfate, 0.74 nitrate, 0.42 Cl, 0.002 H, and 0.13 bicarbonate mg L⁻¹ (Shriner 1978, Table 11-7). The rain solution was acidified with either 2 : 1 or 6 : 1 µequ ratios of sulfate : nitrate chosen to reflect the range of strong acid compositions most likely for precipitation events in northeastern North America (Munger and Eisenreich, 1983; Gorham et al., 1984). The two ratios also provide data on the possibility that any negative effects of acidification may be countered by N enrichment of the lichen. The watering treatments began in summer 1981 and continued through 1983 with between 8 to 10 treatments per snow-free season. At each watering the equivalent of 2 mm of rain was applied to each plot as a fine mist; this is just sufficient to saturate the lichen mat. Care was taken to prevent wind drift between adjacent plots during watering. In this paper the mean annual growth rates of Cladina stellaris are compared after two and three summers of treatments using samples collected in September 1982 and 1983.
The treatments were applied in a randomized complete block design (Cochrane and Cox, 1957) involving 16 treatment plots in each of five blocks. The individual treatment plots were 1 × 1 m with 0.5 m buffer strips between them and at the periphery of the block. Each block of 16 plots was a 6.5 × 6.5 m square located between the trees in the lichen woodland. Twelve of the treatments in each block were watered with simulated rain solutions acidified with either 2 : 1 or 6 : 1 ratios of sulfate : nitrate to pH 2.5, 3.0, 3.5, 4.0, 4.5 or 5.0. Ambient rainfall in the vicinity of our study site averaged about pH 5 during this period with occasional storm events approaching pH 4 (Lewis and Hrebenyk 1979; Drake 1980; US/Canada Work Group 1981). In addition, two unwatered plots were used as controls, and two plots were watered with simulated rain solution with pH 5.6.

The mean annual growth rate of lichens such as Cladina stellaris can be readily monitored since only one whorl of branches is produced each year (Andreev, 1954). An individual branch whorl continues to grow for about 9 yr after its inception, (Lechowicz, 1983). The six youngest whorls comprise 18% of the living thallus biomass and account for 50% of the potential photosynthetic activity; The mean annual biomass increment based on the youngest six branch whorls was used to compare lichen response to the acidification treatments. These metabolically active tissues should serve as especially sensitive indicators of any effects of acidic precipitation on growth. The dry biomass of one 6 yr growing tip was measured in each lichen sample using a Cahn model G2 electrobalance accurate to 0.01 mg; four samples were taken in each treatment plot in 1982, and eight in 1983. Growth increments are expressed as an average over the six year period in mg (dry biomass) yr⁻¹.

In a natural lichen woodland, it is impossible to assure that all plots have the same growth rates prior to treatment. Fortunately, analysis of covariance can be used to statistically correct for any pretreatment differences that would confound the analysis of responses to the acidification treatments (Huitema, 1980). This analysis essentially compares the growth responses to the various treatments after adjustment for any growth differences among plots before the experimental waterings were begun. Samples collected in June 1981 were measured as described above to determine the pretreatment growth rates used as a covariate in these analyses. All growth data were log transformed before analysis. Comparisons of particular treatments were made using preplanned contrasts rather than multiple comparison tests (Freund and Littell, 1981). All analyses were done using the ANOVA procedure in the 82.3 release of the Statistical Analysis System (SAS Institute, 1982).

4. Results

There were no significant differences among the overall growth responses of Cladina stellaris treated for two summers with simulated acidic rain solutions (Figure 1, Table I). The pH 5.6 watering treatment resulted in higher growth rates but these were not quite significantly different from the unwatered controls (p = 0.11); the biomass of the youngest six branch whorls of Cladina stellaris increased an average of 2.8 mg yr⁻¹ compared to only 1.8 mg yr⁻¹ in unwatered controls. There were no significant differ-
Fig. 1. Growth increments for *Cladina stellaris* after two summers under different levels of simulated acidic rain treatment. The means are adjusted by analysis of covariance to account for any pretreatment differences in growth. The figure shows the least square means of 5 blocked replicates with their standard errors.

TABLE I
Analysis of covariance for the 1982 log mean growth increment

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>4</td>
<td>0.2177</td>
<td>0.0544</td>
<td>8.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>15</td>
<td>0.0922</td>
<td>0.0061</td>
<td>0.97</td>
<td>0.494</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>0.0026</td>
<td>0.0026</td>
<td>0.41</td>
<td>0.524</td>
</tr>
<tr>
<td>Error</td>
<td>59</td>
<td>0.3726</td>
<td>0.0063</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0.6850</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ences in growth among lichens watered with 2 : 1 vs 6 : 1 microequivalent ratios of sulfate : nitrate (*p* = 0.43). There is, however, some indication that growth may be reduced in the pH 2.5 (*p* = 0.14) and pH 3.0 (*p* = 0.09) 6 : 1 ratio treatments compared to the pH 5.6 treatment. By the end of the summer discoloration of the thallus was apparent in the plots receiving the more acidic treatments.

After three summers of treatment the overall differences in growth rate among plots were nearly significant (Table II), but they were no strong trends in growth rate related
TABLE II
Analysis of covariance for the 1983 log mean growth increment

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>4</td>
<td>0.3460</td>
<td>0.0865</td>
<td>34.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>15</td>
<td>0.0566</td>
<td>0.0038</td>
<td>1.50</td>
<td>0.133</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>0.0031</td>
<td>0.0031</td>
<td>1.25</td>
<td>0.261</td>
</tr>
<tr>
<td>Error</td>
<td>59</td>
<td>0.1479</td>
<td>0.0025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0.5536</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Log annual biomass increment: 1983 least square means

Fig. 2. Growth increments of Cladina stellaris after three summers under different levels of simulated acidic rain treatment. The means are adjusted by analysis of covariance to account for any pretreatment differences in growth. The figure shows the least square means of 5 blocked replicates with their standard errors.

to increasing acidity (Figure 2). Depressions in growth for all the acidic treatments regardless of sulphate : nitrate ratio compared to simulated rain of pH 5.6 did approach significance \((p = 0.08)\). Particular contrasts like that for pH 3.5 vs pH 5.6 showed significant reductions in growth under the acid treatment \((p = 0.04)\), but these cannot readily be interpreted as indicative of a general growth depression under all acid
conditions. For example, growth at either pH 2.5 \( (p = 0.30) \) or pH 3.0 \( (p = 0.23) \) did not differ significantly from that at pH 5.6. Although the growth responses to acidification by the 2:1 and 6:1 ratios of sulfate: nitrate did not differ significantly \( (p = 0.32) \), the 6:1 treatments significantly reduced the growth of *Cladina stellaris* \( (p = 0.05) \). While the effects of acidification were erratic (Figure 2), there appears to be a general depression of growth in lichens exposed to pH 4.5 or less when \( \text{H}_2\text{SO}_4 \) contributes predominantly to the acidity of the simulated rains. In the 2:1 acidification treatments, which were proportionately richer in N, growth did not differ significantly from that at pH 5.6 \( (p = 0.20) \). The watering treatments themselves did not alter lichen growth rates since the growth of unwatered controls and plots receiving simulated rain solutions of pH 5.6 did not differ significantly \( (p = 0.49) \). After three years, bleaching of lichen thalli was seen in all plots watered with solutions below pH 3.5.

5. Discussion

Lichens are unusually sensitive to a variety of environmental pollutants, often in sufficiently low concentrations that they can be used as indicators of chronic pollution levels (Richardson and Nieboer, 1981). Air pollutants like \( \text{SO}_2 \) and \( \text{F}^- \) which form acids when absorbed by a wet lichen have especially deleterious effects on lichen growth processes and on lichen survival around point sources (Skye, 1979; Richardson and Nieboer, 1981). It is therefore surprising that experimental treatments with \( \text{HNO}_3 \) and \( \text{H}_2\text{SO}_4 \) in simulated rain solutions have not significantly affected the growth of *Cladina stellaris* in subarctic Québec. This widespread and dominant lichen in many northern ecosystems (Ahti, 1977) appears to be resistant to at least short term exposures to even extremely acidic precipitation.

The possibility remains that effects on growth are gradual enough to fall below the detection limits set by our experimental methods and design. The likelihood of discerning any small effects on growth will increase with continued exposure to acidification because as treatment continues more of the sampled 6-yr segment will have been formed under the experimental regime. At present, a small portion of the sampled thallus segment grew prior to treatment. The increased significance of treatment effects after three versus two summers suggests that acidification does generally reduce net annual production in *Cladina stellaris*. It is also possible that continued acidification treatment would eventually overcome any resistance mechanisms that may be preventing acidification effects on growth.

Acknowledgments

I thank Jim Schaefer, Anne Bruneau, and Lisa Loring for their assistance in both the field and laboratory. Doug Barr provided logistic support through the McGill Subarctic Research Station. Continued support and advice from Kathy Fischer and Keith Puckett have been appreciated. The Canadian Wildlife Service of Environment Canada and the Natural Sciences and Engineering Research Council of Canada funded the work.
References


