THE EFFECTS OF SIMULATED ACID-PRECIPITATION ON PHOTOSYNTHESIS IN THE CARIBOU LICHEN

Cladina stellaris (Opiz) Brodo

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Abstract. The photosynthetic capacity of the caribou lichen, Cladina stellaris, was impaired when the lichen was wetted by artificial acid precipitation. When wetted by a solution having a pH = 4.0 and a sulfate concentration = 10.00 mg l\(^{-1}\), the maximal photosynthetic capacity was lowered 27% from normal levels. Moreover, the lichen which is dormant when dry, took 14% longer after wetting to attain even this reduced rate of photosynthesis. The possibility that lichen growth rates may be reduced by acid precipitation with serious implications for many northern ecosystems is discussed.

1. Introduction

The extreme sensitivity of many lichens to atmospheric pollutants such as SO\(_2\) and NO\(_2\) is well known and has been reviewed by Nash (1976) and by Skye (1979). After 3 weeks exposure under field conditions to concentrations of gaseous SO\(_2\) no higher than 250 ppb, photosynthesis in the lichen Cladina stellaris was reduced 40% (Moser et al., 1980). Respiration rates of Usnea hirta, on epiphytic lichen on ponderosa pine, were reduced about 80% by exposure to 94 ppb SO\(_2\) in the field (Eversman, 1978). The disruption of these critical physiological processes will lead to a decrease in the annual growth rate of lichens as ambient SO\(_2\) levels increase. Taking advantage of the resultant inability of some species to survive at even moderate levels of ambient SO\(_2\), biologists have used lichens as bioindicators for mapping pollution zones around urban and industrial sites (Hawskworth, 1973). Most such studies have reported 'normal' lichen communities at relatively short distances (dozens of km) from a point source of atmospheric pollutants.

Only recently has any threat to lichens from gaseous pollutants in areas more remote from urban and industrial sites been perceived (Puckett, 1979; Gilbert, 1980). The further oxidation of S and N combustion products during long range atmospheric transport results in both dry and wet deposition of strong acids (Dovland and Semb, 1980; Fowler, 1980). The deleterious effects of this acid deposition, even in wilderness areas far from any human activity, is increasingly apparent (Drablos and Tollan, 1980; Overrein et al., 1980). Considering their known sensitivity to SO\(_2\) and NO\(_2\), the effects of acid deposition on lichens have surprisingly not been studied. The irony of this lack of research is heightened because the metabolic nature of lichens leaves them especially vulnerable to acid precipitation.

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Unlike most higher plants, lichens are able to survive extended periods of drought at tissue water contents only a few percent of their dry weight (Blum, 1973). In this dry condition lichen metabolism is dormant; respiration occurs at only minuscule rates and photosynthesis not at all (Cowan et al., 1979). Upon wetting of the lichen, its metabolism is rapidly reactivated (Bewley and Krochko, 1981) and the physiological processes controlling growth proceed at rates dependent on ambient conditions of light, temperature, and moisture (Lechowicz, 1981a). Lichens, however, have no specialized structures for the uptake or conservation of water. Thus these periods of metabolic activity are episodic and strongly dependent on the local precipitation and evaporative regimes. A dormant lichen may, for example, be reactivated by a morning rain shower, and dry to dormancy level water content by midafternoon of the same day. During such an episode of metabolic activity, the respiratory losses associated with recovery from dormancy must, on average, be offset by photosynthetic gains if the lichen is to grow and survive in that habitat (Lechowicz, 1981a, b). Any decline in the photosynthetic capacity after wetting by acid rather than normal precipitation will thus have serious implications for lichen growth and survival. Neither the time course of photosynthetic recovery nor its sensitivity to wetting of the lichen by acid rather than normal precipitation, however, has been studied. The purpose of this paper is to describe these critical physiological responses for the caribou lichen Cladina stellaris — an abundant and ecologically important species of circumboreal distribution.

2. Methods

Air-dry Cladina stellaris was collected in August 1980 in an open spruce-lichen woodland near Schefferville in northern Quebec, Canada (see Figure 1; also Renz and Auclair, 1978). The collections were flown to Montreal and stored in dry condition at −20 °C until used in an experiment; Larson (1978) has shown that such frozen storage for up to 3.5 yr had no significant effect on the gas exchange metabolism of the arctic lichen Alectoria ochroleuca. Schefferville is the site of a large iron ore mine but processing of the ore occurs elsewhere. Except for the localized disruption of the mining activity itself, the region is representative of the pristine lichen woodlands which occupy much of the Canadian subarctic. Both Lewis and Hrebienyk (1979) and Drake (1980) report recent data on precipitation chemistry for Schefferville suggesting only minor impact of acid precipitation in the area; precipitation pH was typically only slightly below 5. This is in accord with data from the few CANSEP network stations in northern Quebec and Labrador (U.S./Canada Work Group, 1981). The C. stellaris used in these experiments has not been selected for resistance to atmospheric pollutants and can provide insight into the potential impact of acid precipitation on lichens in remote regions.

A single podetium of Cladina stellaris was used in each experiment. The podetium represents a natural individual unit in the intricately branched and interwoven mat of Cladina stellaris (see Figure 1). Yarronton (1975) describes the demographic characteristics of C. stellaris podetia. All podetia used in these experiments were in the mature growth phase (Andreev, 1954). Only the living, actively growing tissues from the upper
Fig. 1. Aspect of the lichen woodland in the vicinity of Schefferville in northern Quebec, Canada. The ground is covered by extensive mats of the caribou lichen *Cladina stellaris*. The inset shows a typical podetium of *C. stellaris* like those used in the experiments reported here; the scale on the inset has mm divisions.
part of the podetium were used. Senescent tissues, recognized by their duller, more gray color, were removed. Each experiment consisted of three essential steps: (1) wetting of a dry, dormant podetium, (2) an interval of incubation of the wetted sample, and (3) radiometric assay using $^{14}$CO$_2$ of the lichens' photosynthetic capacity after the incubation interval.

In preliminary experiments lichens were wetted by 15 min immersion in distilled water; this is sufficient time to fully hydrate C. stellaris (Blum, 1973). In subsequent experiments an artificial rain solution (Shriner, 1978) was used to wet the lichens instead of distilled water. This artificial rain contained trace amounts of Ca$^{++}$, Mg$^{++}$, Na$^+$, K$^+$, NH$_4^+$, SO$_4^{--}$, NO$_3^{--}$ and Cl$^-$ representative of unpolluted rain. To examine the effects of pH of the wetting solution on lichen metabolism, this artificial rain which had pH 5.6 was acidified by addition of HCl to achieve solutions of pH 2.5 and 4.0. Comparable solutions were also prepared with sulfate concentrations of 10 mg l$^{-1}$ to independently assess possible effects of sulfate ion, an important component of acid precipitation especially in remote regions (U.S./Canada Work Group, 1981). After wetting, the C. stellaris was incubated in the dark at room temperature until assayed for photosynthetic activity.

After a fixed interval of up to 400 min from initial wetting, a podetium of C. stellaris was removed from the incubation chamber and reimmersed in the wetting solution for 1 min. The sample was centrifuged for 45 s at a standard speed to remove loosely adherent water that can impede gas exchange (Lange, 1980; Snellgar et al., 1981). The lichen was sealed into a cylindrical, plexiglas chamber and exposed to a light intensity of 2000 μEinsteins·m$^{-2}$·s$^{-1}$. This irradiance is sufficient to achieve maximal photosynthesis in C. stellaris (Lechowicz, 1978). Water was circulated through the double wall of the chamber to maintain the lichen tissue temperature at 20 °C. After exactly 1 min in the light, radioactive air with a CO$_2$ concentration of 358 μl l$^{-1}$ and a $^{14}$CO$_2$ specific activity of 1.38 mCi mmol$^{-1}$ was released to the chamber at a flow rate of 0.34 l m$^{-1}$ for exactly one additional minute. This flow rate provided a turnover time of 40 s for the air in the exposure chamber. A squirrel cage fan built in to the chamber assured uniform exposure of the entire lichen to the radioactive air. Immediately after the 1 min exposure to radioactive air, the chamber was flushed with normal air at a flow rate of 3.0 l m$^{-1}$. After 1 min the lichen was removed and immersed in liquid nitrogen to stop metabolic activity and then killed by rapid drying in a 100 °C oven. This protocol, adapted from the methods of Tieszen et al. (1974), provides an estimate of photosynthesis unconfounded by respiratory gas exchange.

The dried lichen was ground to a fine powder in a mortar and pestle to obtain a homogeneous sample representative of the whole podetium. A weighed subsample of the ground, labelled lichen was catalytically combusted in an Intertechique Oxymat to oxidize all organic material to CO$_2$ and H$_2$O. The released CO$_2$ was trapped directly in a scintillation cocktail containing phenylethylamine and counted on a Beckmann Model LS-200 Scintillation Counter. The samples were quench corrected by the internal standard channels-ratio method (Wang et al., 1975). Calibration standards of known activity were oxidized and counted with each set of samples to maintain a check on
machine function. The photosynthetic rate was calculated as mg CO\textsubscript{2} g\textsuperscript{-1} (oven dry weight) h\textsuperscript{-1} (see Tieszen \textit{et al.}, for calculation details).

Between 4 and 7 replicate photosynthetic rates were determined by this method for every combination of the following conditions: (1) pH of 5.6, 4.0, and 2.5, (2) sulfate concentrations of 0.53 and 10.00 mg l\textsuperscript{-1}, and (3) 15, 45, 75, 105, 135, 165, 196, 255, 315, and 375 min since initial wetting. These data were analyzed by least-squares multiple regression (Draper and Smith, 1966) using the Statistical Analysis System computer routines (Barr \textit{et al.}, 1976).

3. Results

The general nature of the recovery of photosynthetic capacity in \textit{Cladina stellaris} after wetting of the dormant lichen by distilled water appears in Figure 2. Photosynthetic rates on the order of 40\% of photosynthetic capacity are attained in as little as 30 min after the dormant lichen is wetted. Maximal photosynthetic capacity is attained about 3 to 4 h after wetting. After the maximum photosynthetic rate is attained there may be a slow decline in photosynthesis but this possible trend is confounded by the high variance between replicate podetia.

The photosynthetic time course for \textit{C. stellaris} wetted by an artificial rain solution at pH 5.6, however, also shows a slow photosynthetic decline after maximal photosynthetic capacity is attained (Fig. 3A). By 30 min after wetting 45\% of the maximal photosynthetic capacity is attained. At pH 5.6, representative of normal rain, the maximal photosynthetic rate of 0.44 mg CO\textsubscript{2} g\textsuperscript{-1} h\textsuperscript{-1} is attained at just over 4 h after wetting.

![Graph](image_url)

Fig. 2. The time course of photosynthesis of the caribou lichen \textit{Cladina stellaris} after wetting by immersion in distilled water. Each point represents the photosynthetic rate determined radiometrically for a single podetion. Tissue temperature was about 20 °C and tissue water content was near saturation.
These results and the contour graphs in Figure 3 are derived from the fitted regression equation:

$$P = 0.00040492 \cdot TH - 0.00003162 \cdot TS - 0.00000014 \cdot T^2 H^2 + 0.13409764$$

where $P$ is the photosynthetic rate in mg CO$_2$ g$^{-1}$ h$^{-1}$, $T$ is time since wetting in min, $H$ is the

$$[SO_4^{2-}] = 0.53 \text{ mg} \cdot l^{-1}$$

**A**

![Diagram A](image)

**B**

$$[SO_4^{2-}] = 10.00 \text{ mg} \cdot l^{-1}$$

![Diagram B](image)

Fig. 3. The time course of photosynthesis of the caribou lichen *Cladina stellaris* after wetting by immersion in normal and simulated acid rain solutions. The contours of photosynthetic rate, mg CO$_2$ g$^{-1}$ h$^{-1}$, appear as a function of time since wetting and the pH of the wetting solution. In interpreting this graph it may be helpful to recognize the analogy between these photosynthetic contours and the altitudinal contours on a topographic map. The closer together are two photosynthetic contours, the more rapidly photosynthetic rate is changing with changes in pH and time. The pH of artificial rain solutions was adjusted with HCl at two sulfate concentrations: 0.53 mg l$^{-1}$ representative of normal rain (Figure 3A) and 10.00 mg l$^{-1}$ representative of polluted rain (Figure 3B). Tissue temperature was about 20 °C and tissue water content was near saturation.
pH of the wetting solution, and $S$ is the sulfate concentration in mg l$^{-1}$. The regression is highly significant as are all its individual terms ($p < 0.0001$). The high replicate variance apparent in Figure 2 is reflected here in an $r = 0.57$ but the residuals show no trend. The fitted model is a sound predictor of the general photosynthetic time course after wetting.

When wetted by artificial acid rain low in sulfate (Figure 3A) *C. stellaris* recovers photosynthetic capacity more slowly than when wetted by normal rain. For example, at pH 4.0 30 min after wetting, photosynthesis is 43% of maximum and the maximal rate is attained only after 6.0 h. When wetted by artificial acid rain high in sulfate (Figure 3B) the maximal rate of photosynthesis attained is depressed. At pH 4.0 a maximum rate of only 0.32 mg CO$_2$ g$^{-1}$ h$^{-1}$ is attained after 4.9 h. This is 27% less than the maximal rate achieved after wetting by normal rain and it takes 14% longer after wetting to attain. This depression of photosynthetic capacity occurs in the presence of high sulfate concentration even at the normal pH of 5.6. Acid rain high in sulfate will lengthen the time necessary to recover full photosynthetic capacity and depress the maximal rate of photosynthesis achieved.

4. Discussion

The data presented here for the caribou lichen *Cladina stellaris* show that acid precipitation has deleterious effects on lichen photosynthesis. *Cladina stellaris* wetted by artificial acid rain recovers from dormancy more slowly than when wetted by normal rain. When wetted by artificial acid rain high in sulfate concentration not only is photosynthetic recovery slower but the maximal rate of photosynthesis attained is lower. These trends are qualitatively clear but any precise quantitative prediction of the photosynthetic time course after wetting is confounded by the high variance evident between replicates.

This high replicate variance most likely arises from two sources: (1) differences in the relative amounts of vital and senescent tissue included in a sample, and (2) differences in the rates of recovery due to differences in the respiratory substrate available to fuel the recovery process. Both possible explanations arise from poorly understood aspects of lichen biology.

Our knowledge of the temporal dynamics of growth in the caribou lichens is only little extended from Andreev's (1954) early summary. The mature podetia used in the experiments here are growing at the tip and dying at the base; each year a single new whorl of branches is produced. Growth of the annual whorl continues for up to 8 yr after its initiation (Andreev, 1954; Kärenlampi, 1970). Thus about the upper 6 cm of the podetium of *Cladina stellaris* includes the most vital tissues (Figure 1). This region of active growth, distinguished by color from older, senescent tissues toward the podetial base, was used in these experiments. If some senescent tissue is inadvertently included in the sample our weight-based estimate of photosynthesis will be spuriously low. Furthermore Nash et al.'s (1980) data suggest that there is an increasing gradient in photosynthetic capacity upward within even the living tissues. Thus inadvertent inclusion of unusually low amounts of older living tissue could raise observed photosynthetic rates. It is necessary to define the tissue age-dependence of photosynthesis within a podetium of *Cladina*
stellaris. Then a uniform standard including a fixed number of annual branch whorls could be used to delimit the podetial samples. This improvement in the experimental protocol should remove part of the high replicate variance.

The remainder of the variance will likely be reduced by inclusion of another term in the statistical model: the total polyol content of the sampled podetium. The substantial polyol or sugar alcohol pool in the lichen tissue is utilized as respiratory substrate during recovery from dormancy (Farrar 1976a, b). The rate of recovery after wetting may be proportionate to the available polyol concentration. The high variance in the time course of photosynthetic recovery would then be partly due to combining individuals differing in polyol concentration, and therefore in rates of recovery, as replicates for a particular interval after wetting. Consideration of polyol concentration as well as time since wetting should result in more precise prediction of photosynthetic recovery.

Despite the relatively high variance in the present data, wetting by acid precipitation lengthens the time required for photosynthetic recovery and can depress the rate of photosynthesis attained. Photosynthesis is the only source of the carbon and energy essential for lichen growth. Both phenomena will thus reduce the growth and annual productivity of lichens unless offset by correlated reductions in respiratory rates. Growth rates in the caribou lichens, under natural conditions, range from only 3 to 6 mm yr\(^{-1}\) (Andreev, 1954; Scotter, 1963; Pegau, 1968; Ouzilleau and Payette, 1975). Any reduction of these already very low growth rates by acid precipitation will decrease the survival of lichens in northern ecosystems.

The lichens play a substantial role in many northern ecosystems. The caribou lichens, especially C. stellaris, constitute a major winter food resource for caribou and reindeer (Bergerud, 1977). Hanson et al. (1975), for example, determined that a free-roaming female caribou in northern Alaska ingested 5 kg dry weight of lichens per day during January through April. Rouse (1976) has shown that the extensive mats of C. stellaris in subarctic regions control the regional water balance through their mulching effect on the soil surface. Lichen mats, by ameliorating soil surface temperatures and reducing evaporative stress, influence establishment of vascular plant seedlings (Kershaw, 1977, 1978). Through release of organic acids, lichens can inhibit the growth of vascular plant competitors (Rundel, 1978). Finally, nitrogen-fixing lichens can improve the nutritional status of northern ecosystems (Crittenden and Kershaw, 1978). Considering the depression of photosynthetic capacity in lichens wetted by acid precipitation, it is therefore essential that the threat of long term reductions in lichen biomass in northern ecosystems because of acid precipitation receive further study.

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