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Review article

Lichens as Bioindicators of Sulfur Dioxide

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Abstract

Lichens are excellent indicators of sulfur dioxide levels in the lower trophosphere. With the advent of the industrial revolution, sulfur dioxide levels increased steadily throughout western Europe until legislation in the 1960's and 1970's resulted in reductions in recent decades. Parallel with these developments was a major impoverishment of the region's lichen flora, particularly in city centers and industrial regions. But as conditions ameliorated, reinvasion of many lichen species has occurred. Concomitantly, *Lecanora conizaeoides*, a species with an apparent major sulfur requirement, has declined in abundance in recent decades. Similar trends have been observed along SO_2 gradients in other parts of the world. Although field situations can be complex with a variety of factors, including other air pollutants, influencing lichen community dynamics, a wide range of experimental investigations support the assertion that lichens do respond to sulfur dioxide. When moist, lichens can be a major sink for SO_2 because of the high solubility of SO_2 in water. Approximately 70% of the SO_2 absorbed by lichens can subsequently be leached as sulfate, but the retained 30%, present primarily as bisulfite and/or sulfite, can readily become toxic when accumulated to sufficient levels. Under chronic exposures, a variety of physiological parameters (photosynthesis, respiration, nitrogen fixation, retention of electrolytes, etc.) may decline and, under acute exposures, death may result. Retained sulfur can be reduced metabolically to

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H_2S and released, but the magnitude of H_2S release is relatively small. Most biochemical investigations emphasize the effects of SO_2 in terms of either acidification or oxidation potential due to the formation of free radicals. Observed differential sensitivity to SO_2 among different lichen species can be partly understood in relation to the parallel, differential magnitude of antioxidants, such as glutathione or ascorbate, occurring in those lichens. Furthermore, differential activity of antioxidant enzymes, such as superoxide dismutase and peroxidases, in removing radicals apparently is also important in explaining the parallel differential sensitivity among lichen species.

Keywords: Lichens, SO_2, photosynthesis, electrolyte leakage, acidification, antioxidants

1. Introduction

Lichens are photosynthetic autotrophs and are a symbiotic association between a fungus (usually an ascomycete) and one or more photosynthetic partners (a green alga and/or cyanobacterium). They occur widely on all continents as epiphytes on bark or wood, epiphylls on leaves in the tropics, epilithic organisms on rocks, cryptoendoliths within rocks, or on soil or mosses (Nash, 1996a).

One of the major successes in the use of lichens as bioindicators of air pollution has been with sulfur dioxide (Nieboer et al., 1976; Seaward, 1993), which was initially recognized as an air pollutant in the early 1900's. Previously air pollution had, of course, been recognized, but it was principally associated with particulates in the air, such as those generated by coal burning. During the 1800's coal was used extensively for home heating as well as industrial activities and the air of virtually every major northern city was filled with particulates, especially during the winter season. Some forms of coal (and other fuel products) have particularly high levels of sulfur, and its oxidation leads to the formation of sulfur dioxide, one of the major gases associated with acid rain. In fact, sulfur dioxide has an average atmospheric residency time of only 12 hours because its high solubility in water leads to its being trapped in water vapor aerosols and rapid conversion to sulfuric acid.

The disappearance of lichens from urban areas was noted as early as the mid-1800's (Hawksworth, 1971), and was widely attributed to air pollution. In fact not all lichens disappeared, as differential sensitivity among species was readily evident if one examined patterns along a transect from the city center to the country side. A few species were relatively city tolerant (e.g. *Lecanora muralis* and *L. dispersa*) and one species (*Lecanora conizaeoides*) may have evolved in response to high SO_2 (possibly near fumaroles on Iceland [Seaward, personal communication], as there are no British collection records of it prior to

the mid-1800's. By the mid-1900's that species had become perhaps the most abundant lichen in northern Europe (Richardson, 1974).

Lichen distribution patterns in relation to SO_2 gradients

Field evidence that lichens have been responding to sulfur dioxide has now been obtained from throughout the world (Aarrestad and Aamlid, 1999; Aguiar et al., 1998; Chen et al., 1989; Eversman, 1978; Freedman et al., 1990; Hamada et al., 1995; McCune, 1988; Nimis et al., 1990; Piö't and Lisicka, 1985; Søchting and Johnsen, 1978; Sugiyama et al., 1976; Taylor and Bell, 1983; Türk and Christ, 1980; Will-Wolf, 1980). For example, Skye (1968) elegantly demonstrated how bark of trees became acidified and lost its buffering capacity with proximity to the city center (in his case a detailed investigation of Stockholm). This caused a shift in lichen epiphytic species composition to those that are more acidophilic. But probably the most convincing field data came from England, where a grid of sulfur dioxide monitoring stations was established throughout the country in the mid-1900's. Because SO_2 measurements across the grid covered a wide range of atmospheric SO_2 levels, it was possible to examine patterns in epiphyte community composition on adjacent trees and infer relative SO_2 sensitivities based on those patterns across sites. Hawksworth and Rose (1970) did exactly such an investigation. On the basis of differential changes in the epiphytic lichen communities, they established two 10-point scales (one for basic bark and the other for acidic bark) relating long-term SO_2 measurements to changes in the epiphytic lichen communities. Their scale essentially provided an elaborate hypothesis about putative differential sensitivity to SO_2 among different lichen species. Although initial experimental investigations involving SO_2 fumigations did not strongly support the scale (e.g. Baddley, 1973), subsequent SO_2 experiments have, in fact, strongly supported the scale (Türk et al., 1974; Nash, 1988, etc.).

Although some of the changes noted in the scale may well have been related to factors other than sulfur dioxide (e.g. Kostner and Lange, 1986), the general trends observed by Hawksworth and Rose have proved to be quite robust. The most dramatic evidence in support of this assertion has been the widespread reinvasion of lichens as trophospheric SO_2 levels have recently decreased throughout England. Recognition of the detrimental effects of SO_2 had led to legislation limiting SO_2 emissions and implementation of that legislation. The result was a dramatic decrease in measured SO_2 levels, starting in the early 1970's (Laxen and Thompson, 1987; Micallef and Colls, 1999). Concomitantly, many of the putatively sensitive species have reinvaded urban areas (Henderson-Sellers and Seaward, 1979; Rose and Hawksworth, 1981; Hawksworth and McManus, 1989, 1992; Bates et al., 1990; Boreham, 1992;

Gilbert, 1992; Seaward, 1997). Similar trends are occurring elsewhere, such as Paris (Seaward and Letrouit-Galinou, 1991) and Munich (Kandler, 1987), although in some cases reinvasions are limited to nitrogen tolerant species due to continued high levels of nitrate and ammonium deposition levels (e.g. The Netherlands – van Dobben and De Bakker, 1996, 1998; van Dobben and ter Braak, 1999). In parallel with these reinvasions, the abundance of *Lecanora conizaeoides* has declined dramatically (Bates et al., 2001). That *Lecanora* is the lichen species judged to be most tolerant of SO_2 in Hawksworth's and Rose's original scale and inferred to be a sulfur-requiring species. Similar trends in the latter species' distribution are occurring in Germany as well (Wirth, 1993).

Types of experimental investigations

When investigating variables potentially important in controlling variation observed in the field, it is also important to conduct investigations in the laboratory under conditions where these variables can be regulated. Thus, the use of lichens as bioindicators of sulfur dioxide has gained much validity from a plethora of experimental investigations involving exposure to controlled levels of SO_2. However, experimental conditions have varied considerably and one needs to critically evaluate the degree to which they are equivalent. Initial investigations used closed containers (e.g. Pearson and Skye, 1965) where only the initial concentration was known. The uptake of the gas by the lichens and/or the walls of the container was unknown, and thus the exposure treatment remained undefined. A second approach involved exposing the lichens to aqueous solutions of SO_2, which are prepared by bubbling gaseous SO_2 through water and weighing the resultant solution (e.g. Puckett et al., 1974). The method is intuitively attractive in so far as physiologically active lichens have aqueous solutions in their protoplasts, and much pioneering research has been accomplished with this technique. Nieboer et al. (1977) have proposed a method for calibrating aqueous solutions with gas phase concentrations and this may be appropriate for defining initial conditions. However, the physiochemical behavior of SO_2 in aqueous solutions may result in a decline in SO_2 levels with time (Richardson and Nieboer, 1983), and consequently the degree to which such experiments can be equivalent to longer-term gaseous experiments, where the lichen is continuously exposed to a constant SO_2 concentration, remains a question. Probably more important is the fact that almost all lichens are terrestrial organisms, and as such typically exist in a gaseous microenvironment. Their behavior in an aqueous medium may well be altered. The third approach involves a flow-through gas exchange system, in which SO_2 concentrations are maintained at a constant level. The system works best if the fumigated lichens are maintained at controlled temperatures and at

nearly constant water contents by adjusting the air-flow's dew point to keep the relative humidity high (Türk et al., 1974; Türk and Wirth, 1974). If one monitors SO_2 concentrations at both the entrance and exit to the exposure chambers, then one can calculate SO_2 uptake by the lichens (e.g. Gries et al., 1997b).

In the field it is possible to conduct small scale SO_2 fumigations, simply by slowly bleeding SO_2 from a tank through a tube with multiple small orifices, as was done by Moser et al. (1980) across a lichen mat in the arctic tundra. After monitoring periodically both the SO_2 concentrations across the top of the lichen mats and the photosynthetic capacity of dominant lichens across the growing season, these authors demonstrated a pattern of decreasing SO_2 injury with increasing distance from the exposure system. The dose-response relationships established therefore closely corresponded to long-term, theoretical predictions of Richardson and Nieboer (1983) for the same species. Subsequent measurements at the site demonstrated no apparent recovery three years later (Moser et al., 1983).

Sulfur in lichens and SO_2 uptake dynamics

Most sulfur in lichens apparently originates from atmospheric sources. For example, investigations around sour gas processing plants in Alberta by Case and Krouse (1980) established that the atmospheric SO_2 from the plants had a unique ^{34}S signature. The same pattern was found in the surrounding lichens; however, the vascular plants of that region had a very different sulfur signature consistent with the inference that the soil was their principal source of sulfur. Furthermore, lichens may differentially take up sulfate from dry and wet deposition to canopies in relatively unpolluted areas (Knops et al., 1996). Consequently, low levels of SO_2 may actually enhance the sulfur budget of lichens, and not be detrimental. Evidence is now accumulating that the toxitolerant lichen, *Lecanora conizaeoides*, may well have a higher sulfur requirement than other lichens (Bates et al., 2001; Hauck et al., 2001).

Because of the high solubility of SO_2 in water, any moist lichen may be a major sink for SO_2, even when not metabolically active (Gries et al., 1997b). Initially, absorption of SO_2 leads to higher total sulfur levels in lichen thalli (O'Hare and Williams, 1975). A number of field investigations have also demonstrated that total sulfur levels increase with length of exposure of lichen transplants (Richardson and Nieboer, 1983). However, the overall relationship between SO_2 exposure and total sulfur (or sulfate) concentrations in lichens is more complex, because severely injured specimens lose their ability to retain sulfur (Galun and Ronen, 1988; Häffner et al., 2001), a fact probably related to increased membrane permeability.

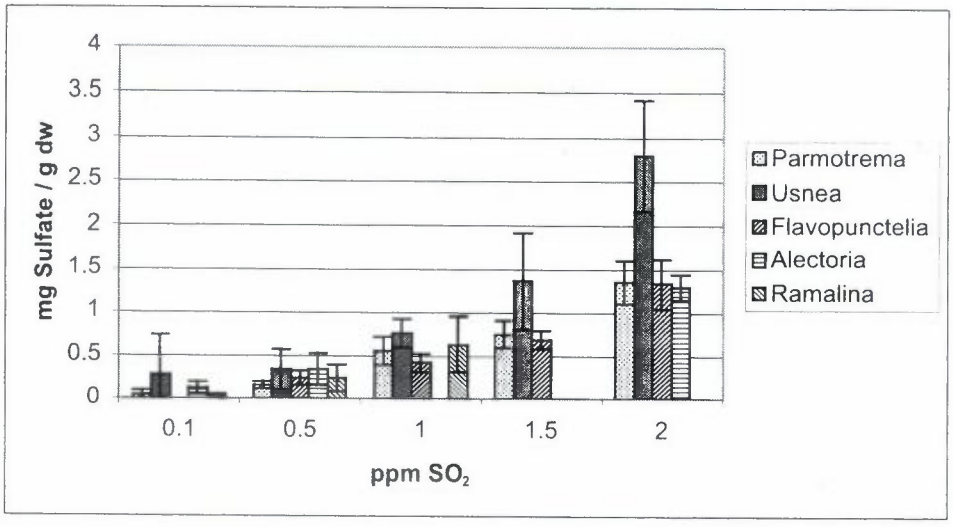

Figure 1. Leachable sulfate as a function of different SO_2 concentrations for 6-h fumigations for five lichen species (*Alectoria sarmentosa, Flavoparmelia praesignis, Parmotrema hypotropum, Ramalina menziesii* and *Usnea hirta*).

A fundamental property of lichens is that they are prominent examples of poikilohydric organisms whose water status varies passively with microenvironmental conditions (Nash, 1996a). Under 'air-dried' conditions, water content often declines to 10–20%. In contrast, moist lichens may readily obtain water contents of 200–300% in green algal-containing lichens and upwards to 2000% in gelatinous cyanobacterial-containing lichens (Lange, 2000). Thus, it is widely assumed that lichens must be moist to be sensitive to SO_2 (Ferry and Baddeley, 1976; Grace et al., 1985b; Richardson and Nieboer, 1983; Wirth, 1987). However, Coxson (1988) has experimentally demonstrated that some injury can occur when air-dried lichens are fumigated, a fact that may reflect the residual water present in such lichens and the fact that some SO_2 absorption occurs under such conditions. The fact that cyanolichens frequently occur in moist habitats and that they achieve higher water contents, may well explain why they are generally absent from regions with high SO_2 loadings.

Under aqueous conditions, SO_2 rapidly forms the strong acid, H_2SO_4, which dissociates into H^+ and HSO_3^- (bisulfite) ions at pH's near neutrality (Nieboer et al., 1984). Many physiological effects due to SO_2 exposure are attributed to the resulting acidity (Türk and Wirth, 1975; Rennenberg and Polle, 1994). In

addition, the bisulfite anion is also recognized as being quite toxic, and some experiments have been run with aqueous bisulfite solutions as a surrogate for SO_2 fumigations (Hill, 1971; Hällgren and Huss, 1975; Silberstein et al., 1996a,b). Cells gradually oxidize bisulfite to sulfate, which is much less toxic. Across a wide range of SO_2 concentrations and using five different lichen species, we have shown that approximately 70% of the SO_2 taken up can subsequently be leached as sulfate (Fig. 1). Understanding how the lichens deal with the remaining 30% (see biochemical section below) then becomes the critical issue in understanding known differential sensitivity to SO_2 among lichens.

For experiments of constant duration with moist lichens, SO_2 uptake directly varies with SO_2 concentration (Gries et al., 1997b). Furthermore, if lichens dry relatively rapidly during experimental SO_2 fumigations, it may appear that responses are only a function of fumigation concentration and are independent of fumigation duration (Huebert et al., 1985). However, if lichen water content is maintained at relatively constant levels, then both length of exposure and SO_2 concentration contribute to measured response, as was established from the dose-response investigations of Sanz et al. (1992). The idea of SO_2 dose received by a lichen being a function of both duration and concentration is an implicit assumption of the threshold extrapolations proposed by Richardson and Nieboer (1983). Remarkably, calculated thresholds based on laboratory experiments with aqueous SO_2 correspond quite closely to estimates based on field investigations, such as those taken from Moser et al. (1980) for *Cladonia rangiferina* (Richardson and Nieboer, 1983; see also Grace et al., 1985b and Winner et al., 1988). Furthermore, wind speed is also an important variable. Greater uptake will occur at higher wind speeds (Grace et al., 1985a), if the SO_2 concentration is constant and the lichen does not dry appreciably. Higher wind speeds reduce the relatively stable boundary layer over the lichen surface, reducing the resistance to the inward flux of SO_2.

Physiological response variables employed to measure effect of SO_2

A wide range of response variables (Nieboer et al., 1976; Richardson and Nieboer, 1983; Fields, 1988; Galun and Ronen, 1988; Geebelen and Hoffmann, 2001) have been employed to measure physiological response of lichens, both to field conditions (*in situ* or in transplants) or in laboratory experiments where concentrations of SO_2 are controlled. Initial work focused on chlorophyll degradation (Pearson and Skye, 1965; Rao and Leblanc, 1966; Nash, 1973; Gries et al., 1995; Balaguer and Manrique, 1995), which requires moderately high SO_2 fumigation levels. With improvements in techniques to measure CO_2 gas exchange, measurements shifted to dark respiration (Baddeley et al., 1973;

Marsh and Nash, 1979; Gordy and Hendrix, 1982) or net (or gross) photosynthesis (Türk et al., 1974; Punz, 1979; Huebert et al., 1985; Puckett et al., 1974; Türk and Christ, 1980). In general, these investigations have established differential sensitivity among species that closely match inferred sensitivity of the species based on field investigations (Nash, 1988), even though field sites are often subjected to a mixture of pollutants (von Arb et al., 1990; Garty et al., 1997). With aqueous SO_2 experiments, SO_2 response may be modified by the presence of metal ions: decreased in the case of Cu, unaffected by Pb and increased by Mg, Zn and Ca (Richardson et al., 1979). Results of gas exchange measurements during SO_2 fumigations have been corroborated by using chlorophyll fluorescence techniques (Calatayud et al., 1996; Gries et al., 1995; Deltoro et al., 1999), that allow inferences about effects on photosystems I & II. One aspect of the lichen symbiosis is the partial transfer of carbohydrates from the photobiont (alga or cyanobacterium) to the fungus as an immediate result of the dark reactions of photosynthesis (Richardson et al., 1968). Fields and St. Clair (1984) and Nieboer et al. (1976) have shown that carbohydrate transfer is often decreased following short-term exposure to SO_2.

Inside cells, potassium is the dominant inorganic ion. Puckett et al. (1977) and Tomassini et al. (1977) demonstrated that a major efflux of K^+ occurred in response to aqueous SO_2 exposure. With increasing SO_2 concentrations, a biphasic loss of K^+ was observed and the results were interpreted as reflecting an increase in permeability of the cells. The magnitude of K^+ release due to SO_2 depended in part on co-occurrence of other ions, increasing in the case of Cu and Pb, unaffected by Mg and Ca and decreasing in the case of Sr, Ni or Zn (Nieboer et al., 1979). Quantification of K^+ in small concentrations requires accurate analytical techniques (e.g. atomic absorption spectrometry or equivalent) and similar results may be obtained by measuring total conductivity with a simple meter in a lichen-rinsed solution (Pearson and Henriksson, 1981). Conductivity measurements are often used in field investigations as well (Garty et al., 1993; Alebic-Juretic and Arko-Pijevac, 1989).

Approximately 10% of the lichen species contain cyanobacteria, either as the main photobiont or secondarily in specialized structures called cephalodia (Nash, 1996b). Many cyanolichens are capable of nitrogen fixation, and nitrogen fixation rate, as measured indirectly by acetylene reduction activity (ARA), has also been used as a response variable. Under aqueous conditions, Hällgren and Huss (1975) found that nitrogen fixation reduction occurred at lower SO_2 concentrations than corresponding photosynthetic measurements (^{14}C uptake) in *Stereocaulon paschale*. Reductions in ARA due to SO_2 exposure have also been reported by Sheridan (1979) and Henriksson and Pearson (1981), but their results are of limited value because of the techniques employed (Richardson and Nieboer, 1983). Although it is often suggested that nitrogen

fixation is more sensitive than the parameters discussed above (Fields, 1988; Richardson and Nieboer, 1983), additional data are certainly necessary to assess the generality of the assertion.

Reproduction in lichens is also inhibited by SO_2. Reduction in the production of apothecia in the lichenized ascomycetes (98% of the species belong to the ascomycetes) has frequently been observed in polluted areas (LeBlanc and De Sloover, 1970; Bedeneau, 1982), and ascospore germination may also be inhibited by SO_2 (Belandria et al., 1989). Even asexual means of propagation, such as the germination of soredia (Margot, 1973) may be inhibited by SO_2.

Ultrastructure and biochemical responses: towards a mechanistic understanding

Holopainen and Kärenlampi (1984) have documented ultrastructural changes in two lichens exposed to a range of SO_2 concentrations (0.05 to 1.0 ppm). In the alga, initial injury included swelling and deformation of the mitochondria, stretching of the chloroplast envelopes and deformation of pyrenoglobuli. As injury progressed (particularly at the higher concentrations and with longer exposures), the thylakoids stretched and the pyrenoids, chloroplast stroma, nucleus and mitochondria all degenerated. Injury to the fungus primarily occurred at higher concentrations and included swelling of mitochondria and vesiculation of the mesosome-like organelles. These patterns of injury have been largely confirmed by subsequent investigations (Eversman and Sigal, 1987; Holopainen and Kauppi, 1989; Sharma et al., 1982; Plakunova and Plakunova, 1987) in other species. The initial injury symptoms documented by Holopainen and Kärenlampi (1984) were also observed in field-collected lichens from central Finland (Holopainen, 1983).

The pattern of ultrastructural injuries corresponds well to biochemical and physiological responses observed in lichens exposed to SO_2. For example, Malhotra and Khan (1983) found reductions in protein and lipid biosynthesis and CO_2 fixation in *Evernia mesomorpha* exposed to 0.34 and 0.1 ppm SO_2, concentrations that correspond well to mid-range concentrations employed by Holopainen and Kärenlampi. Because lipids and proteins are essential constituents of cell membranes, these types of changes may well be associated with the observed ultrastructural changes. Similar effects on lichen lipids and fatty acids were found by Bychek-Guschina et al. (1999). Changes to the mitochondria would certainly affect respiration and changes to the thylakoids would affect photosynthesis. The most severe ultrastructural effects probably correspond to situations where a major reduction in photosynthesis, increase electrolyte leakage and destruction of chlorophylls occur (see earlier sections).

One of the primary effects of SO_2 absorption is cellular acidification (Pfanz et al., 1987; Renneberg and Polle, 1994). At neutral pH (7.0) SO_2 in water

dissociates approximately 50% to bisulfite (HSO_3^-) and 50% to sulfite (SO_3^-), but at pH 4.0 sulfite dominates, and this accounts in part for the greater toxicity under lower pHs due to the greater oxidizing power of sulfite (Nieboer et al., 1976). To a degree, lichen species may vary in their ability to oxidize sulfite. For example, Miszalski and Niewiadomska (1993) found that a SO_2 tolerant species is a more efficient oxidizer than less tolerant species. Acidification has consequences for many enzyme reactions, as activity is frequently pH dependent. For example, Ziegler (1977) found that sulfite inhibited RuBisCO activity in *Pseudevernia furfuracea*. Furthermore, with acidification, carboxylation efficiency is reduced (Price and Long, 1989) and the electron transport system is inhibited (Chen et al., 1992). All of these factors would readily reduce photosynthesis. At the whole plant level, the effect of SO_2 on inhibition of growth of *Trebouxia*, the most common lichen alga, is much greater at pH 4.0 than pH 5.0 (Marti, 1983). Likewise, lichen net photosynthesis is dramatically reduced at lower pHs (Puckett et al., 1973; Türk and Wirth, 1975). The ameliorating effect of Ca^{2+} in reducing the effect of SO_2 on photosynthesis (Richardson et al., 1979) relates to the ion's ability to reduce the degree of acidification. Likewise, when considering concrete substrates, the occurrence of some otherwise sensitive lichen species in areas with higher SO_2 levels (Gilbert, 1970) can be understood in terms of the buffering effect of the substrate. In contrast, poorly buffered tree bark supports few if any lichens (Skye, 1968).

One possible mechanism for detoxifying excessive sulfur is enhanced sulfur metabolism. One pathway in the assimilation of SO_2 leads to the formation of sulfite and its reduction to the gas H_2S, as mediated by the enzyme sulfite reductase (Romagni et al., 1997). This process results in dissipation of part of the sulfur loading and is thought to take place in the thylakoids (Rennenberg, 1984). Unfumigated lichens are known to release H_2S (Gries et al., 1994), but at very low concentrations (0.01–0.04 pmol g dw^{-1} s^{-1}). Fumigations with low levels of SO_2 results in increased H_2S emissions that persisted to a reduced degree following cessation of the fumigation, and differences among species in the amount released was evident (Gries et al., 1997a). Furthermore, species varied in their ability to release more H_2S at higher SO_2 fumigation levels. Overall, the magnitude of H_2S release was relatively small (0.4–2.5%) compared to the inferred amount of absorbed SO_2 that was not leached as sulfate. Other trace sulfur gases are known, carbonyl sulfide (COS), CH_3SH, dimethyl sulfide (DMS), CS_2, and lichens are known to assimilate COS and release DMS and sporadically release small quantities of the other gases (Gries et al., 1994), but the magnitude of release is also very small (same order of magnitude as H_2S). Thus, the release of trace sulfur gases in lichens is a relatively unimportant mechanism to reduce excess sulfur loading. Alternatively, sulfur metabolism could be enhanced by increasing the activity

of the enzymes, ATP sulfurylase or cysteine synthase (Stulen and De Kok, 1993), but these enzymes have not yet been investigated in lichens.

Recent work on SO_2 injury mechanisms in plants have focused on the production of free radicals, particularly those occurring in the chloroplast due to sulfite accumulation (Rennenberg and Polle, 1994). Under unfumigated conditions, photolysis leads to the formation of the superoxide radical, which is normally scavenged by superoxide dismutase (SOD). Hydrogen peroxide is the product of the SOD mediated reaction, and it can be removed by glutathione, ascorbate, or peroxidases. Sulfite can alter the conformation of enzymes involved in these last two steps (Alscher, 1984). Under chronic SO_2 fumigations of pea cultivars, activity of SOD increased gradually over days in a SO_2 resistant cultivar, apparently in response to higher superoxide radical formation (Madamachani and Alscher, 1991). At high concentrations, sulfite can be photooxidized by the superoxide radical of free oxygen, leading to the formation of hydroxyl radicals that disrupt the lipid fraction of the chloroplast membranes (Peiser and Yang, 1985). Such effects correspond well to the types of injury observed by Holopainen (1984) in lichens. For lichens suffering from acute injury, similar effects would likely be manifest in chlorophyll degradation.

There is at least partial evidence that most of the processes discussed in the last paragraph occur in lichens as well. Modenesi (1993) provides evidence that free radicals are generated in lichens as a response to SO_2 and Köck et al. (1985) demonstrated that a different form of SOD is formed in *Trebouxia* in response to sulfite treatments. Using a variety of physiological and biochemical techniques, Silberstein et al. (1996a) carefully documented that *Xanthoria parietina* was SO_2-tolerant and *Ramalina duriaei* was SO_2-sensitive. Subsequently, they showed that parietin, the orange secondary product present in the upper layer of *Xanthoria* but absent in *Ramalina*, could act as a potential antioxidant (Silberstein et al., 1996b). Furthermore, they demonstrated a loss in SOD activity in the sensitive species following treatment with bisulfite, a result that parallels observations following SO_2 fumigations with another sensitive lichen, *Evernia prunastri* (Deltoro et al., 1999). Deltoro et al. (1999) also found stimulation of SOD in a tolerant species, *Ramalina farinacea*, as did Kong et al. (1999) for a different species. But Thomas (1999) essentially found no change in SOD activity with shorter term fumigations of several species. With increasing bisulfite treatment, Silberstein et al. (1996b) found a reduction of peroxidase activity in sensitive species, but an increase in peroxidase activity in tolerant species. Deltoro et al. (1999) found the same pattern (but the differences were not always significant) for peroxidase, ascorbic peroxidase and catalase following SO_2 fumigation. Thus, there is some evidence that tolerant species can more effectively deal with oxygen radical formation than sensitive species.

The SH groups in glutathione (GSH) give it antioxidant properties, and, consequently, GSH may well play a role in protecting tolerant species against excess sulfur (De Kok and Stulen, 1993). It is probably the most abundant thiol in plants, and is well known to change in pool size during the initial stages of rewetting dry lichens (Kranner and Grill, 1994). Silberstein et al. (1996b) demonstrated some initial increase in the glutathione pool following bisulfite treatment, and Kong et al. (1999) demonstrated a major increase in glutathione with increasing SO_2 fumigations of *Xanthoparmelia mexicana*. Although Romagni et al. (1998) did not confirm such a response with SO_2 fumigations, they did demonstrate that a SO_2-tolerant lichen, *Pseudevernia intensa*, had an order of magnitude larger pool size of glutathione than other more sensitive species. The enzyme, glutathione reductase (GR), controls the regeneration of GSH from GSSG (glutathione disulfide). Deltoro et al. (1999) reported that fumigation with high SO_2 concentrations resulted in stimulation of GR activity in their tolerant species, but Thomas et al. (1997) did not find a significant change in glutathione reductase activity with SO_2 fumigations of other lichen species. Thomas did, however, find higher rates of GR activity in green algal lichens than in a cyanolichen species. Thus, SO_2-sensitive cyanolichens may not be able to generate as much of the antioxidant GSH as green algal containing species.

2. Conclusions

Clearly the case for using lichens as bioindicators of sulfur dioxide is very strong. In areas with high atmospheric levels of SO_2, most lichen species have disappeared. Experimental fumigations of lichens with SO_2 have demonstrated a wide range of detrimental effects that parallel observations on injured specimens in the field. Furthermore, as SO_2 levels have decreased in recent decades, partial recovery of the lichen flora in many regions of Europe has occurred. Consequently, the use of lichens can provide a powerful tool in predicting potential effects of projected SO_2 levels, as is required under the U.S. Clean Air Act, as long as one recognizes that many changes in lichen communities are due to factors other than air pollution.

Although the experimental evidence for lichens responding to SO_2 is quite strong, additional experiments would certainly be appropriate. For example, the relative sensitivity of the fungal partner in the lichen symbiosis remains a question because responses of the photobiont are more commonly measured. In addition, further information concerning the mechanisms behind differential sensitivity among different lichen species to SO_2 is desirable. Only a few of the enzymes involved in sulfur metabolism have been investigated in lichens. Even for the enzymes that have been investigated, we have relatively little

information on the time course dynamics between length of exposure and response.

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