Predictability of the combined effects of sulphur dioxide and nitrate on the green-algal lichen *Ramalina farinacea*

Luis Balaguer, Esteban Manrique, and Carmen Ascaso

Abstract: The interactive effects of SO$_2$ and NO$_3^-$ on the green-algal lichen *Ramalina farinacea* (L.) Ach. were investigated using the degree of chlorophyll phaeophytization and ultrastructural damage to the *Trebouxia* photosynthetic thallus. Plants were subjected to several factorial design experiments in which they were exposed to mean SO$_2$ concentrations ranging from 118 to 140 mmol·mol$^{-1}$ plus or minus aqueous solutions of NO$_3^-$ at either 50 or 1000 mmol·L$^{-1}$ for 6–14 days. Interactive effects of SO$_2$ and NO$_3^-$ were synergistic when the effects of each individual pollutant were slight. However, this pattern reversed to additive effects, and sequentially to antagonistic effects as the detrimental impact of SO$_2$ alone became more severe. Despite this transition, neutralization of the damaging impact of SO$_2$ by simultaneous treatment with NO$_3^-$ was not observed. The results show that interactions between SO$_2$ and NO$_3^-$ were variable, but predictable on the basis of the expected effects following exposure to each pollutant alone. The coincidence of this empirical pattern with those observed in previous studies suggests involvement of unspecific causes. The wider ecological significance of interactions between SO$_2$ and NO$_3^-$ is discussed in the light of other findings with respect to the effects of air pollution on lichens in the field.

Key words: air pollution; sulphur dioxide; nitrate; interactive effects; lichens; pigments; ultrastructure.

Résumé : Les auteurs ont examiné les effets interactifs du SO$_2$ et du NO$_3^-$ sur un lichen à algue verte, le *Ramalina farinacea* (L.) Ach., en utilisant le degré de phaeophytisation de la chlorophylle et les dommages ultrastructuraux chez le photobionte *Trebouxia*. Ils ont soumis les thalles à plusieurs expériences en disposition factorielle en les exposant à des teneurs moyennes en SO$_2$ allant de 118 à 140 mmol·mol$^{-1}$, avec ou sans solutions aqueuses de NO$_3^-$ à 50 ou 1000 mmol·L$^{-1}$ pendant 6 à 14 jours. Les effets interactifs de SO$_2$ et du NO$_3^-$ sont synergiques quand les effets de chaque polluant individuellement sont légers. Cependant, ce patron renverse aux effets additifs et successivement aux effets antagonistes, à mesure que l’effet mort du SO$_2$ seul devient plus sévère. En dépit de cette transition, la neutralisation de l’impact destructeur du SO$_2$ par un traitement simultané avec le NO$_3^-$ ne se présente pas. Les résultats montrent que les interactions entre le SO$_2$ et le NO$_3^-$ sont variables, mais prévisibles sur la base des effets observés isolément. La concordance de ce patron empirique avec ceux observés dans des études précédentes suggère l’implication de causes non-spécifiques. Les auteurs discutent la signification écologique éventuelle des interactions entre le SO$_2$ et le NO$_3^-$ à la lumière d’autres constatations relatives aux effets de la pollution atmosphérique sur les lichens en conditions naturelles.

Mots clés : pollution de l’air, dioxyde de soufre, nitrate, effets interactifs, lichens, pigments, ultrastructure.

Introduction

Long-range transport of air pollutants has heightened international concern and prompted the development of strategies and methods for reducing emissions and assessing potential impact on natural ecosystems (Bull 1991). Proposed emission-control policies are currently based on present knowledge of the critical loads or levels above which direct adverse effects on receptors, such as vegetation, may occur (United Nations Economic Commission for Europe 1988). However, as has been demonstrated in studies on vascular plants, the values of toxicity thresholds for each individual pollutant are strongly dependent on the joint action of different chemicals, which coexist and interact in polluted atmospheres (Leifeld and Ormsdor 1984). Even though there is a wealth of knowledge on the effects of individual gaseous pollutants, few studies relate to the impact of mixtures of pollutants (Bull 1991).

The simultaneous occurrence of pollutants such as sulphur dioxide (SO$_2$) and nitrogen oxides (NO$_x$, NO) which share the same combustion sources is to be expected. Furthermore, in polluted atmospheres, exposure to SO$_2$ is shown to enhance nitrogen deposition to the surface of vegetation, through reciprocal increases in their solubilities (McLeod...
et al. 1992). This SO2 effect on nitrogen deposition may be of particular relevance to polychaetous organisms such as lichens (Bates et al. 1996).

Considering the heterogeneous group of nitrogenous pollutants, current studies on their effect in combination with either SO2 or NO2- on lichens is not entirely new, but has also yielded contrasting results (Hyvärinen et al. 1993). It has been reported that nitrogenous pollutants ameliorate (Kytöviita and Crittenden 1994) or neutralize (Kauppi 1980; Kauppi and Mikkonen 1980; Kauppi and Halonen 1992) the damaging effect of SO2-2, or even maintain their fertilizing effect in combination with this pollutant (Scott et al. 1989). Such a fertilizing effect is consistent with the fact that NO3- and NH4+ are probably the main sources of nitrogen for most lichens (Nash 1996). However, it has been also reported that the damaging effect of H2SO4 was either not ameliorated by simultaneous NO2- addition, or even exacerbated in the presence of NH4+ (Hallingback and Kelner 1992). Exposure to SO2 interferes with the metabolic pathway of NO2- assimilation and consequently the mixture SO2 and NO1, NO3- or NO2- may exhibit synergistic deleterious effects on autotrophs (Welburn et al. 1981). In this context, the role of NO3- in green-algal lichens could change dramatically from a nutrient to a phytotoxic agent in the presence of SO2 (Balaguer and Martínez 1991). Evidence of synergistic effects of SO2 together with NO3- (Balaguer and Martínez 1991) completes a full range of potential interactions between nitrogenous pollutants and SO2 on lichens.

In view of the lack of a general agreement among studies of interactive effects of SO2 or SO2-2 and nitrogenous pollutants on lichens, it is relevant to consider that vascular plants also display variable effects ranging from antagonistic to synergistic responses to the same pollutant mixtures (Heagle and Johnston 1979). It has been suggested that the direction and intensity of the interaction depend on the dose and quality of the single pollutant (Lejohn and Ornato 1984). Consequently, lichen response to SO2 exposure, which has been widely used as a reliable indicator of SO2 toxicity in field studies, may actually be significantly altered by simultaneous exposure to NO3- and, as observed in vascular plants, the direction and extent of this interaction could vary from an amelioration to an enhancement of the damaging effects of SO2. In agreement with such a variable pattern of response, Balaguer and Martínez (1995) recently indicated that the action of NO3- on SO2 toxicity on lichens was not explained by a linear model.

In the present study, we argue that even when simultaneous NO3- supply can either ameliorate or promote the deleterious effects of SO2, the divergence from an additive pattern can be predicted from the impacts produced by exposure to each individual pollutant. The response of Ramsdellina farinacea (L.) Ach. to chronic exposure to SO2 singly or in combination with NO3- during several factorial design experiments was assessed by determining the phaeophytinization quotient of chlorophyll a (PQa), a quantitative indicator of chlorophyll degradation (Ronen and Galun 1984; Martínez et al. 1989). The extent to which this estimate of injury was accompanied by changes in photobiont ultrastructure was also examined.

Materials and methods

Samples of R. farinacea were collected in natural forests of Quercus fageta and Quercus pyrenaica in Selas (Guadalajara) and San Pedro de los Monteros (Toledo), situated in "clean air" regions in central Spain.

Thalli were maintained in separate chambers in a glasshouse illuminated by natural daylight in Audabella, Terrasa, Spain, a region in which R. farinacea flourish. Relative humidity and temperature conditions in the chambers tracked those registered externally, and mean values ranged from 60 to 100% and from 7 to 20°C, respectively (for a more detailed description of ambient conditions see Balaguer and Martínez 1995). During six independent experiments, thalli were exposed to factorial design experiments to two SO2 treatments (charcoal-filtered air and elevated SO2), NO3- levels were maintained at values considered to be intermediate between good and poor air quality (125 μg m-3; 1-hourly average concentrations; Quality of Urban Air Review Group 1995) and two NO3- regimes (spray of distilled water, and NO3- enriched; NaNO3 was supplied to the water spray at 5 mg L-1 NO3-; ) concentration observed in rainfall from polluted areas; United Kingdom Review Group on Acid Rain 1987). In all experiments thalli were watered once a day, every day of the week. In one of the experiments, in addition to realistic NO3- levels, a high-NO3 treatment (1000 μg L-1) was also included. Those thalli which were daily sprayed with distilled water and maintained in charcoal-filtered air were taken as controls. The length of chronic SO2 treatments ranged from 6 to 14 days to assay different doses of pollutant, but treatment duration did not exceed 15 days to avoid a significant decline in control thalli. Concentrations of SO2 in the centre of each chamber were monitored, logged and adjusted as described elsewhere (Balaguer and Martínez 1991). Experimental treatments and duration of individual experiments are shown in Table 1.

Chlorophyll phaeophytinization was estimated as previously described (Barnes et al. 1992). Briefly, thallus fragments were washed in carbonate-saturated acetone and extracted with dimethyl-sulphoxide, and the optical densities of the extracts were measured at 415 and 435 nm. The ratio between these values provides an estimate of the phaeophytinization quotient (PQa), i.e., the ratio between chlorophyll a and phaeophytin a (Ronen and Galun 1984; Martínez et al. 1989). Pollutant effects were assessed using the percent decrease in PQa with respect to the corresponding mean control thallus.

Samples selected for the ultrastructural study of the photobiont were fixed, dehydrated, and embedded in Spur's resin as described by Ascor et al. (1988), within 2 days after termination of the experimental treatment. Ultrathin sections were stained with lead citrate and observed using a Philips EM 300 electron microscope.

Data relating to the decline in PQa were subjected to a two-way analysis of variance (ANOVA) to determine the significance of the main factors (SO2 and NO3-) and their interaction. Assumptions of normality and homoscedasticity were tested using the Kolmogorov—Smirnov test and Cochran's C test, respectively. Where necessary, data were logarithm transformed prior to ANOVA. Pairwise comparisons were performed using the least significant difference (LSD) calculated at p < 0.05.

Reciprocal effects of NO3- and SO2 were summarized in a response-surface model, as previously proposed for the interaction between SO2 and HF (McCune 1984). In the present study, a nonadditive effect was defined as the divergence from an additive pattern and was calculated by subtracting the effects observed following single exposures from the effect produced by both pollutants applied in combination. A response-surface model was generated by plotting the nonadditive effect of the pollutant mixture as a function of the decrease in PQa resulting from single exposure to SO2 or NO3-.
Fig. 1. Electron micrographs of <i>Trebouxiella</i> from <i>Ramalina farinacea</i>. (A) Photobiont cell submitted to control treatment for 14 days. (B) Close occurrence of algal cells displaying contrasting degrees of injury after fumigation with 122 nmol·mol<sup>−1</sup> SO<sub>2</sub> and daily spray of distilled water. (C, D) Photobiont cell showing moderate symptoms of SO<sub>2</sub>-induced damage with severely affected mitochondria (arrowheads; 122 nmol·mol<sup>−1</sup> SO<sub>2</sub> and daily spray of distilled water). (E) Plasma lysed cells in thalli exposed to 122 nmol·mol<sup>−1</sup> SO<sub>2</sub> and daily spray of 50 μmol·L<sup>−1</sup> aqueous NO<sub>3</sub> −. (F) Appearance of electron-opaque bodies (arrowheads) after treatment with 50 μmol·L<sup>−1</sup> aqueous NO<sub>3</sub> − for 14 days in charcoal-filtered air. Scale bars = 1 μm.

Table 1. Effect of single and simultaneous exposure to SO<sub>2</sub> and NO<sub>3</sub> − on chlorophyll phaeophytinization in thalli of <i>Ramalina farinacea</i> (L.) Ach.

<table>
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<tr>
<th>Pollutant concentration</th>
<th>Exposure duration (days)</th>
<th>Percentage of PQa decrease&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nonadditive effect&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>SO&lt;sub&gt;2&lt;/sub&gt; (nmol·mol&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>NO&lt;sub&gt;3&lt;/sub&gt; − (μmol·L&lt;sup&gt;−1&lt;/sup&gt;)</td>
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<sup>a</sup>Values represent the mean quotient for 5–10 independent measurements of separate thalli. Asterisks indicate a significant difference from the controls (one-way ANOVA followed by LSD, <i>p</i> < 0.05).

<sup>b</sup>Values were obtained by subtracting the addition of PQa decreases caused by single exposures from those observed following simultaneous exposure to both pollutants. More or less than additive effects correspond to positive or negative values, respectively. Dagger indicates a significant interaction between SO<sub>2</sub> and NO<sub>3</sub> − either synergistic or antagonistic according to a two-way ANOVA (<i>p</i> < 0.05).

Results

Control thalli, wetted with distilled water and maintained in charcoal-filtered air, displayed a decline of mean PQa values over the period of the experiments which was always less than 15% and not statistically significant.

Exposure to elevated SO<sub>2</sub> always resulted in mean values of PQa lower than controls (Table 1), and effects on PQa were paralleled by severe alterations in normal photobiont ultrastructure (Fig. 1A). Early stages of damage (PQa decrease from control values < 5%) were characterized by the close coexistence of algal cells with contrasting degrees of injury (Fig. 1B). In initial stages of damage, swollen and curled thylakoids resulted in wider interthylakoidal spaces and led to a granulated appearance of the stroma in the absence of any apparent change in the shape of the chloroplasts (Fig. 1C). At this stage, mitochondria presented severe symptoms of injury such as degeneration of cristae and mitochondrial matrix as well as the appearance of concentric, myelin-like figures (Fig. 1D).

When the percent PQa decrease was greater than 17% most of the photobiont cells appeared plasma lysed. There was a complete loss of organelle integrity, and photobiont cells appeared to consist of amorphous matrices in which only lipid bodies and starch granules were recognizable (Fig. 1E).

Daily supply of NO<sub>3</sub> − did not produce any significant variation in PQa at any of the tested doses (Table 1). Consistent with the lack of change in PQa, no qualitative changes were observed in the ultrastructure of the algal cells (Fig. 1F).

However, electron-opaque bodies occasionally appeared in the cytoplasm of thalli treated with NO<sub>3</sub> − applied singly or in combination with SO<sub>2</sub> (Fig. 1F).

Simultaneous treatment with NO<sub>3</sub> − and SO<sub>2</sub> resulted in a variable response which ranged from more than additive to less than additive effects. Nonadditive effects of the mixture varied from approximately +20%, a significant synergism (<i>p</i> < 0.05), to approximately –10%, a significant antagonism (<i>p</i> < 0.05; Table 1). These values were more highly correlated with the degree of injury caused by SO<sub>2</sub> singly than to the pollutant average concentration or the dose (log(SO<sub>2</sub> dose), <i>r</i> = −0.85, <i>p</i> < 0.03; log(SO<sub>2</sub> concentration), <i>r</i> = −0.28, <i>p</i> < 0.39; log(SO<sub>2</sub> dose), <i>r</i> = −0.02, <i>p</i> < 0.98). Nonadditive effects were not significantly correlated with the concentration, dose, or effect on PQa of NO<sub>3</sub> − individually (<i>p</i> > 0.60).

A response surface was interpolated to the distances from an additive pattern using the percent PQa decrease induced by exposure to each pollutant alone as independent variables in an effort to search for a spatial pattern in the variation of the interaction between both pollutants (Fig. 2). This response-surface model revealed a region where NO<sub>3</sub> − levels that would have produced a slight PQa increase if supplied alone paradoxically promoted the damaging effect of slightly toxic SO<sub>2</sub> levels. Conversely, antagonistic or less than additive effects were observed when injury was severe following single exposure to SO<sub>2</sub>. It is relevant that despite this ameliorating effect of NO<sub>3</sub> − in the mixture it was never sufficient to neutralize the damaging effect of SO<sub>2</sub> at any of the doses of pollutants tested. Nonadditive effects followed a clear-cut continuous gradient from synergism to antagonism, with a boundary line roughly positioned between 13 and 21% PQa decrease after exposure to SO<sub>2</sub> singly.
Fig. 2. Divergence from an additive pattern is shown with reference to the effect of exposure to SO$_2$ on the apparent effect of NO$_3^-$, measured in both cases as percent PQa decrease from control values. In this response surface, regions of exposure in which joint action of both pollutants resulted in greater than additive, additive, or less than additive effects are denoted by positive, zero, or negative values, respectively. Each data point ($\bullet$) represents the mean for 5 – 10 independent measurements of separate thalli per treatment.

![Graph showing SO$_2$-induced PQa decrease (%) vs. NO$_3^-$-induced PQa decrease (%).]

(Fig. 2). Ultrastructural symptoms produced by simultaneous exposure to both pollutants did not differ qualitatively from those described above, induced by single treatments.

**Discussion**

The close relationship observed in the present study between qualitative changes in cell ultrastructure of the photobiont and chlorophyll degradation supports the use of variations in PQa as a quantitative estimation of SO$_2$-induced damage. At a slight degree of chlorophyll phaeophytinization, algal cells already displayed marked ultrastructural changes, which were particularly evident in the case of mitochondria. The sequence of symptoms of ultrastructural injury following exposure to SO$_2$ or NO$_3^-$ was consistent with that previously described by Hoekstra (1986).

The results of the present investigation indicate that the mere knowledge that NO$_3^-$ deposition and SO$_2$ coexist in polluted areas is not sufficient to predict the pattern of their interactive effect (i.e., synergistic, antagonistic, or additive). The response of *R. farinacea* after simultaneous exposure to mixtures of NO$_3^-$ and SO$_2$ ranged from synergistic to antagonistic. The variation in the pattern of response has been described as a variable response in previous studies on the interaction of SO$_2$ with other pollutants (Heagle and Johnston 1979). However, the present study indicates that response does not vary erratically. Its divergence from additive effects is related to the degree of injury that SO$_2$ would have caused if supplied alone, in the absence of any other pollutant. Thus, when slight damage was produced by single exposure to SO$_2$ the corresponding effect of the same dose of this pollutant in combination with added NO$_3^-$ resulted in greater than additive effects. On the other hand, antagonistic interactions were observed when NO$_3^-$ was supplied with SO$_2$ doses that, alone, induced severe degrees of injury.

In contrast to what has been hypothesized elsewhere (Kauppi 1980), slight SO$_2$ effects were not nullified but enhanced by simultaneous exposure to NO$_3^-$, and they were ameliorated but not neutralized only when severe effects induced by SO$_2$ were expected.

The observed response pattern is consistent with the general model proposed by Lefohn and Ortnroth (1984) for interactive effects of SO$_2$, NO$_3^-$, and O$_3$ on crops. These authors indicated that synergistic effects of gas mixtures are observed when pollutant doses are below or at the threshold for individual injury responses. As doses increase, damage may be additive, and finally, at relatively high doses antagonistic effects develop. The simplest and most general model to summarize and interpret this variable response is a two-dimensional response surface, previously proposed by McCune (1984) for the joint action of SO$_2$ and HF on vascular plants. It is striking that the response surface which depicted interactions between SO$_2$ and HF (McCune 1984) broadly overlaps with that interpolated in the present study for the response of *R. farinacea* to the combination of SO$_2$ and NO$_3^-$ (Fig. 2).

In contrast to the model described by Lefohn and Ornroth (1984), the response-surface model described in the present study considers the effects induced by each pollutant individually as independent variables, instead of pollutant doses. Although a highly significant relationship between SO$_2$ dose and effect on PQa has been previously observed (Balagué and Manrique 1995), the variation in the dependent variable not explained by the SO$_2$ dose amends its suitability for describing the divergence of the lichen response from an additive effect of the pollutant mixture. Even when environmental conditions in the chamber or thallus status would partly explain large differences in the lichen response to similar treatments (Balagué and Manrique 1995), direct effects of these variables on the direction of the pollutant interaction seem unlikely to occur considering the high correlation between the effect induced by SO$_2$ alone and the nonadditive effect of the mixture.

The results of the present study suggest that, although lichen responses to pollutant mixtures may differ from those caused by each pollutant individually, interactive effects can be estimated from the damaging effect of each pollutant alone. Thus, the values predicted by models based on effects on lichens caused by exposure to either SO$_2$ alone (Grace et al. 1985) or NO$_3^-$ alone (Kauppi 1980; Blum et al. 1989) would be suitable inputs for a response surface designed to estimate the expected interactive effects of simultaneous exposure to both pollutants. From this alternative point of view, apparently contradictory observations such as the potential role of nitrogen-rich pollution as mitigator (Scott et al. 1989; Kauppi and Halonen 1992; Kyovii and Citterdien 1994) versus enhancer (Balagué and Manrique 1991) of the damaging effect of SO$_2$ could be interpreted as extreme phenomena of the same underlying pattern of interaction.
Fig. 3. Nonadditive effect of a varied set of pollutant mixtures was significantly correlated \((r = -0.72, p < 0.00001)\) with the effect induced by the most damaging pollutant of the mixture when applied alone, regardless of the species of autotroph studied or the physiological indicator measured. The effect of the most damaging pollutant alone was calculated as percent reduction from mean control values. In addition to the data of the present study (●), the figure shows data derived from previous studies on the interactive effects of \(\text{SO}_2\) and \(\text{NO}_2\) on growth of several grasses (♀: TANNER and MAURY 1978; \(\text{SO}_3\) and \(\text{O}_3\) on either photosynthetic rates of green-algal lichens (♀: DAVIES and SIGAL 1987) or foliage injury in soybean plants (♀: HEAGLE and JOHNSON 1979); \(\text{H}_2\text{SO}_4\) and \(\text{HNO}_3\) on nitrogenase activity of lichenized cyanobacteria (♀: KRYZKOS and CRINDERDA 1994); heavy metals on nitrate uptake of free-living cyanobacteria (♀: RAI and RAIZADAD 1989); and \(\text{SO}_2\) and \(\text{NO}_2\) or \(\text{O}_3\) on photosynthesis performance in chloroplasts of spinach (♀: SUGAHARA 1984).

![Graph showing nonadditive effect of pollutant mixtures](image)

**Single effect of the most damaging pollutant**

As mentioned earlier, the variable response of \(R.\) farinacea to \(\text{SO}_2\) and \(\text{NO}_2\) observed in the present study displays the same pattern as those previously described for the interactive effects of \(\text{SO}_2\), \(\text{NO}_2\), and \(\text{O}_3\) (HEAGLE and JOHNSON 1979; LEFON and ORINOD 1984) and \(\text{SO}_2\) and \(\text{HF}\) (MCCUNE 1984) on vascular plants. It has been proposed that MCCUNE’s response-surface model may be applicable not only for predicting interactions among those pollutants but also for \(\text{SO}_2\) and \(\text{NO}_2\) and \(\text{O}_3\) and heavy metals, and even that all such interactions are chemically interrelated (RUCHEKES 1984). Using heterogeneous data which relate to the interactive effects of binary combinations of \(\text{SO}_2\), \(\text{H}_2\text{SO}_4\), \(\text{HNO}_3\), \(\text{NO}_3^-\), \(\text{NO}_2\), \(\text{O}_3\), \(\text{N}_i\), \(\text{Cr}\), and \(\text{Pb}\) on diverse physiological processes, ranging from nitrogenase activity to visible foliage injury in a wide range of autotrophs, from cyanobacteria to angiosperms and including lichens containing either cyanobacteria or green algae, it is conspicuous that nonadditive effects are highly correlated \((r = -0.72, p < 0.00001)\) with the individual impact of the most damaging pollutant in the mixture (Fig. 3).

Although the mechanisms are still not known, such a generalized response depicts an empirical pattern which suggests the participation of specific common causes, such as the convergence of these interactions in the production of free radicals. These highly reactive molecular species arise as a secondary consequence of exposure to oxidative stresses (ASCHER and AMER 1987; WILHELM 1990), and their involvement in toxicity mechanisms of \(\text{SO}_2\) has also been reported in lichens (MOUAT 1993; RICHARDSON and NIEBOER 1983).

The results of the present study could have several implications in the interpretation of data derived from field studies. First the fact that lichen-impoverished zones have been reported to exceed the area where levels of \(\text{SO}_2\) are above the toxicity threshold (NASH and SIGAL 1981) might be associated with the greater than additive effect expected when the most damaging component is below or at its threshold of toxicity in a mixture of pollutants. Synergistic interactions could also explain the more damaging effect of \(\text{NO}_2\) observed on lichens in field studies than that following controlled fumigations (BLUM et al. 1989). A complex pattern of interaction, such as that displayed by \(R.\) farinacea in response to \(\text{SO}_2\) and \(\text{NO}_2\) in the present study, could explain the decline of \(R.\) murrifoliis in areas where \(\text{SO}_2\) levels are low and \(\text{NO}_2\) dry deposition is important but does not reach a potential \(\text{NO}_2\) toxicity threshold (BOONE et al. 1991). This pattern of interaction would support the codeposition of nitrogenous pollutants and \(\text{SO}_2\) in triggering lichen responses at annual mean concentrations of \(\text{SO}_2\) below the theoretical toxicity threshold of the pollutant (BATES et al. 1996). Finally, the response-surface model proposed in the present study would elucidate, to some extent, the causes of impeded lichen recolonization (BATES et al. 1990; MCCUNE 1993) in a changing air pollution scenario where atmospheric \(\text{SO}_2\) concentrations are declining in parallel with increasing levels of nitrogen oxides (BATES et al. 1990; SLOVIK et al. 1996).

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