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Dose–response relationships for SO_2 fumigations in the lichens *Evernia prunastri* (L.) Ach. and *Ramalina fraxinea* (L.) Ach.

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SUMMARY

Sulphur dioxide fumigation of the lichens *Evernia prunastri* (L.) Ach and *Ramalina fraxinea* (L.) Ach, whose thallus water content was held at 100–120% throughout each experiment, resulted in changes in net photosynthesis, dark respiration and chlorophyll content in relation to both concentration and duration of exposure. Net photosynthesis was the most sensitive response variable. Significant reduction in chlorophyll content was found when no recovery in net photosynthesis occurred after two weeks. A reduction in dark respiration was only found at high SO_2 concentrations. *Evernia prunastri* was affected by lower concentrations and shorter exposure times than *Ramalina fraxinea* and the data for both species showed dose–response relationships.

Key words: Lichens, sulphur dioxide, dose–response relationships, photosynthesis, respiration, chlorophylls.

INTRODUCTION

Lichens are recognized as excellent bioindicators of sulphur dioxide air pollution (Richardson & Nieboer, 1983; Nash & Wirth, 1988). Even though many fumigation studies have been conducted at higher SO_2 levels than occur typically in the field (Fields, 1988), results on differential sensitivity among species from controlled fumigation studies have correlated well with sensitivities based on field studies (Nash, 1988). With the decline in ambient SO_2 levels in the United Kingdom in recent years lichens are reinvading urban areas (Rose & Hawksworth, 1981; Seaward, 1982).

Lichenological literature has not often been used in air quality criteria documents because much of the data was not amenable to dose–response analysis (Sigal, 1988). In principle, equal experimental doses of an air pollutant, where dose is the product of concentration and exposure time, should yield approximately the same response (e.g. decline in photosynthesis). However, Huebert, l'Hirondelle & Addison (1985) suggest that duration of exposure, an important component used in the calculation of dose, is unimportant and this inference may also be drawn from other literature (Fields & St Clair, 1984*a*, *b*). We suspect that this inference is incorrect and results from the way in which these experiments were conducted.

Lichens are poikilohydric organisms, whose water content varies passively with environmental conditions (Lange, 1969). In contrast to homoiohydric vascular plants, it is very difficult to maintain the water status of a poikilohydric organism exposed to an air stream during fumigation experiments (O. L. Lange, personal communication and unpublished observations). Net photosynthesis and dark respiration, two frequently used response variables, vary markedly as a function of lichen water status (Lange, 1969). In the absence of water status control, it is difficult to separate changes in gas exchange due to fumigation treatment from those due to changes in water status. In the extreme situation, where a lichen dries during the fumigation, responses to the fumigation might well appear to be independent of exposure time because response to the fumigation treatment would only occur in the initial part of the experiment.

The objective of this study was to re-examine dose–response relationships for SO_2 using an experimental system where it was possible to maintain lichen thallus water content at nearly constant levels for the entire fumigation period. This was achieved with an open flow-through system coupled to a state-of-the-art humidity control system, which provided 98% relative humidity without the formation of liquid water (see 'Materials and Methods'). Water contents of the thalli were maintained at values near

optimal for SO_2 uptake. Net photosynthesis, dark respiration and chlorophyll breakdown were measured since these variables are known to respond to different concentrations of SO_2 (Fields, 1988).

MATERIALS AND METHODS

Materials

Two fruticose species of lichens were used: *Ramalina fraxinea* (L.) Ach., collected in Río Curueño, León, Spain (42° 40′ N, 01° 45′ E) in June 1990, and *Evernia prunastri* (L.) Ach., collected in San Pablo de los Montes, Madrid, Spain (39° 35′ N, 00°30′ E) in September 1990. Both species grow on the bark of a deciduous oak (*Quercus pyrenaica* (L.) Willd.). Both of these areas occur at the lower end of SO_2 gradients within Spain and have a high diversity of lichen species that are not obviously modified morphologically by anthropogenic influences (MOPU, 1986). After collection the material was air-dried and stored at −20 °C in the dark [a standard storage treatment procedure, that has no demonstrable effect on green-algal containing lichens from regions with a Mediterranean climate (Nash *et al.*, 1987), used to prevent physiological deterioration that occurs after several weeks of storage at *c.* 20 °C] until it was flown (within 24 h) to Arizona State University (Tempe, Arizona, USA), where it was stored in an environmental chamber at 200–250 μmol m^{-2} s^{-1} (PAR) and 12 h light/dark photoperiod at 20/15 °C temperatures respectively. The experiments were conducted within 4 weeks of collection. To simulate natural conditions of dewfall, the material was moistened daily by spraying with distilled water in the morning.

Gas exchange measurements

The responses of net photosynthesis and dark respiration to fumigations with different exposure times and SO_2 concentrations were measured using the discrete sampling technique of infrared gas analysis (Larson & Kershaw, 1975) following the procedures described by Matthes-Sears (1985). A 2 cm^3 sample of gas was drawn from each 250 ml glass exposure chamber containing the lichen before and after 8- to 12-minute incubation periods, during which the lichen was sealed airtight in the chamber. The gas samples were injected into a carrier gas stream flowing through an infrared gas analyzer (ADC 225). Photosynthesis was measured at 175 μmol m^{-2} s^{-1} (PAR) and dark respiration in black chambers. The change of CO_2 concentration was measured in the differential mode and recorded with a Perkin-Elmer 024 recorder (Matthes-Sears & Nash, 1986). Calibration curves were generated daily using the method of Clegg, Sullivan & Eastin (1978) and fitted as straight lines. Thallus water content was determined gravimetrically immediately after each exposure. All lichen samples were dried for two days at room temperature (25 °C). Because repeated gas exchange measurements were made on the same lichen samples (see below), water content values are expressed as a percentage of air dry weight (% d. wt), as oven drying would have injured the samples. Separate measurements showed a linear correlation ($r > 0{\cdot}9$) between air dried weights (two days) and oven dried weights (24 h, 100 °C) in both species.

All net photosynthesis and dark respiration measurements were performed in a temperature-controlled environmental chamber (Moore Environmental Systems model 135 DMLH 050) at 15 °C. To eliminate the effects of resaturation respiration (Farrar & Smith, 1976) the samples were moistened with distilled water 2 h before making each measurement of net photosynthesis and dark respiration.

The response curve of net photosynthesis to thallus water content was obtained for both species under the same experimental conditions, and optimum water contents were determined (100–120 %) prior to running the fumigation experiments.

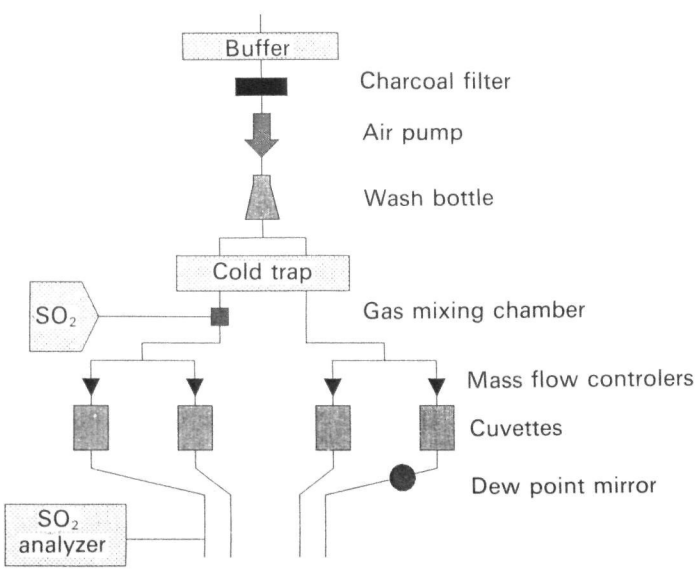

Figure 1. Diagram of the fumigation system.

Fumigation system

The fumigation system is illustrated in Figure 1. To reduce potential problems with SO_2 absorption and desorption, all the connections in contact with SO_2 were Teflon-coated or made of stainless steel. To provide a constant CO_2 concentration the air stream was initially drawn through a large buffer (50 gal carboy). To remove background levels of air pollutants (if present) from the air stream, an activated charcoal filter was placed immediately downstream from the air pump. The air stream was then pumped through a wash bottle filled with distilled water at room temperature (*c.* 25 °C) for moistening. In a cold trap (MGK1 Walz) the humidity was regulated to a dew point of 15 °C (±0·1 °C), which resulted in a relative humidity of 98 % (±1 %) in the cuvettes.

This was controlled by a Dew Point Mirror Measuring System (Walz MTS1). The fumigations were carried out at 15·0 ± 0·1 °C air temperature in the dark using black cuvettes (internal volume 500 ml) lined internally with Teflon and submerged in a water bath (Forma Scientific Model 2425) for temperature control. The flow rate was maintained at 0·5 l min^{-1} with a calibrated mass flow controller (Matheson Multiple Flow Controller Model 8274). SO_2 in nitrogen from a gas cylinder was introduced into the air stream through a needle and the concentration (±5 %) was controlled with a gas regulator and a micrometering valve (Nash, 1973). The concentration of SO_2 was monitored using an empty fumigation chamber, by means of an electroconductimetric SO_2 analyer (Scientific Industries Model 67).

Fumigation experiments

Fumigations were conducted with 0·1, 0·25, 0·5, 0·9 and 1·5 ppm SO_2 (respectively *c.* 260, 650, 1300, 2340 and 3900 $\mu g\ m^{-3}$). For each concentration, separate fumigations were made for 1, 2, 4 and 6 h. Three replicate experiments were run for each combination of concentration and duration. Prior to fumigation two approximately equal amounts of air-dry material (*c.* 0·75 g of *Ramalina fraxinea* and 0·35 g of *Evernia prunastri*) were moistened to *c.* 100 % water content and net photosynthesis was measured. If essentially the same net photosynthesis rates were found in both samples, the material was placed in control and fumigation chambers respectively. Periodically, parallel control samples were selected in this manner and when replicate (see below) responses were measured, no significant differences (*t* test) were found. Samples with different net photosynthesis values were discarded and new material tested. At the termination of each fumigation, the material (control and fumigated) was taken from each chamber and divided into 3 parts of equal wet weight. For these subsets, net photosynthesis and dark respiration were determined at optimal water content at 0, 2, 24 and 48 h, and at 2 weeks following the end of each fumigation. Between measurements the samples were returned to the same growth chamber used for pretreatments (see above).

Chlorophyll content measurements

To determine pigment status, about 30 mg of each air-dried sample were extracted in 10 ml of dimethyl sulphoxide (DMSO) following the method of Ronen & Galun (1984). The method is more efficient and reproducible than grinding in DMSO or 90 % acetone (Burnison, 1980). Absorption values were measured with a Bausch & Lomb Spectronic 1001 spectrophotometer. The concentrations of the chlorophylls were determined according to the equations established by Lichtenthaler (1987) for 80 % acetone, since the pigments have almost the same absorption spectra between 600 and 700 nm in both solvents (Shoaf & Lium, 1976; Hiscox & Israelslam, 1979; Ronen & Galun, 1984; Jagels *et al.*, 1989). Also the ratio OD435/OD415, used to measure chlorophyll a/phaeophytin a, proposed by Ronen & Galun (1984) as a parameter for chlorophyll degradation, was determined.

Statistical analysis

Because significant differences were evident among the controls (Figs 2 & 3), probably due to non-homogeneous distribution of algal cells within the lichen material, statistical analyses were limited to paired *t* tests (Steel & Torrie, 1960) applied to each control ($n = 3$) and fumigated ($n = 3$) pair ($P < 0.05$) for all the parameters considered (net photosynthesis, dark respiration, chlorophyll content, 435/415 o.d. ratio).

RESULTS

Both SO_2 concentration and exposure time had significant effects on net photosynthesis of *E. prunastri* (Fig. 2). Significant reductions in net photosynthesis were found after 4 or 6 h exposures to 0·1 ppm SO_2 concentration, but these were temporary, recovery occurring within 2 h (Table 1). In contrast, net photosynthesis of *E. prunastri* decreased significantly ($P < 0.05$) after a 6 h exposure to 0·25 ppm (Fig. 2) and no recovery was evident even after two weeks (Table 1). Furthermore, net photosynthesis was reduced to essentially zero after 6 h of exposure to 0·5 ppm SO_2 (Fig. 2). Also at higher

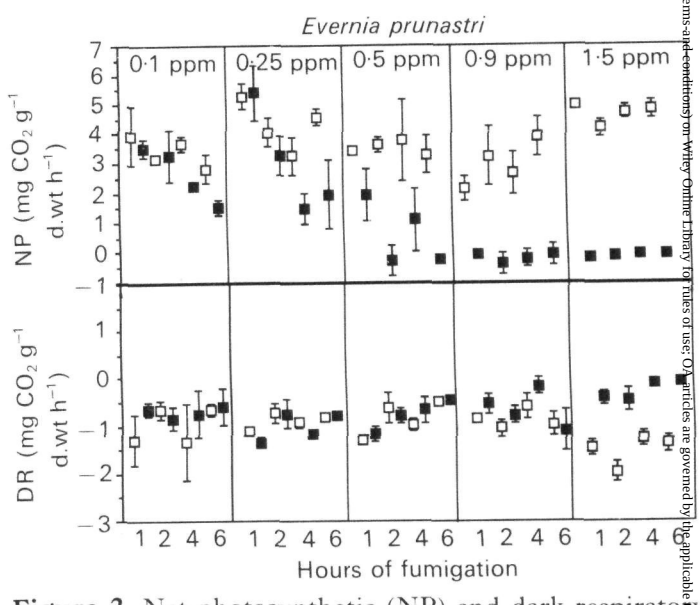

Figure 2. Net photosynthetic (NP) and dark respiratory (DR) responses of *Evernia prunastri* to different SO_2 concentrations and exposure times. Means ($n = 3$) ± 1 SE are plotted for paired observations (□, control samples; ■, fumigated samples) for each concentration by fumigation time combination.

Table 1. *Recovery of net photosynthesis and dark respiration (fumigated samples compared to control by* t *test* $P < 0.05$*) in* Evernia prunastri. *Numbers in the table are defined as: 0 = no effect of fumigation; 1 = significant effect immediately after fumigation, but recovery (e.g. no significant difference between fumigated and control) 2 h later; 2 = recovery beginning 24 h after fumigation; 3 = recovery 48 h after fumigation; 4 = recovery two weeks after fumigation; 5 = no recovery was found, net photosynthesis and dark respiration still significantly different in fumigated and control samples after two weeks*

Time exposure (h)	SO_2 concentration (ppm)				
	0·1	0·25	0·5	0·9	1·5
	Net photosynthesis				
1	0	0	1	2	5
2	0	0	1	5	5
4	1	2	2	5	5
6	1	5	5	5	5
	Dark respiration				
1	0	0	0	0	5
2	0	0	0	0	5
4	0	0	0	0	5
6	0	0	0	0	5

concentrations (0·9 and 1·5 ppm) significant differences between control and fumigated samples were found consistently after all exposures (Fig. 2). After two weeks there was recovery in samples exposed to 1, 2 and 4 h at 0·25 or 0·5 ppm SO_2 and 1 h at 0·9 ppm SO_2 (Table 1).

For *E. prunastri*, dark respiration was not as sensitive as net photosynthesis to SO_2 (Fig. 2). Dark respiration decreased significantly only after very high SO_2 exposures (Fig. 2, 1·5 ppm) and no recovery was found after 2 weeks (Table 1).

For *E. prunastri*, chlorophyll degradation (Table 3) was not as sensitive a response variable as net photosynthesis, but it was more sensitive than dark respiration. In general significant differences in chlorophyll degradation between control and fumigated samples were found whenever there was no recovery in net photosynthesis after two weeks. One exception was found at 0·25 ppm for 6 h where no significant decrease in chlorophylls occurred (Table 3). At high SO_2 concentration (1·5 ppm) chlorophyll destruction was almost complete. The 435/415 o.d. ratios showed the same results as the chlorophyll content measurements.

For *R. fraxinea* both SO_2 concentration and exposure time affected net photosynthesis (Fig. 3), but its degree of sensitivity did not equal that found in *E. prunastri* (Fig. 2). The lowest concentration after which a significant ($P < 0.05$) decrease in net photosynthesis was found initially was 0·5 ppm SO_2 after 6 h of exposure (Fig. 3). Exposure to 0·9 or

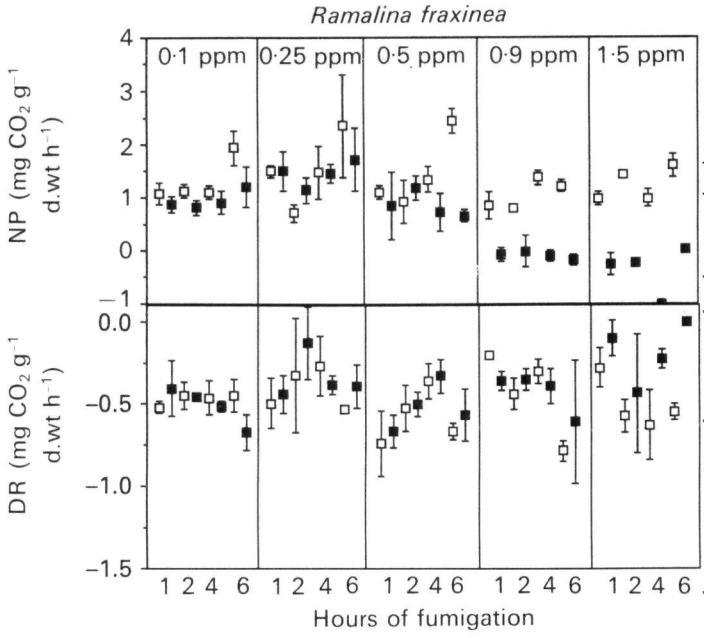

Figure 3. Net photosynthetic (NP) and dark respiratory (DR) responses of *Ramalina fraxinea* to different SO_2 concentrations and exposure times. Means ($n = 3$) ± 1 SE are plotted for paired observations (□, control samples; ■, fumigated samples) for each concentration by fumigation time combination.

Table 2. *Recovery of net photosynthesis and dark respiration (fumigated samples compared to controls by the* t *test* $P < 0.05$*) in* Ramalina fraxinea. *Numbers in the table are defined as: 0 = no effect of fumigation; 1 = significant effect immediately after fumigation, but recovery (e.g. no significant difference between fumigated and control) 2 h later; 2 = recovery beginning 24 h after fumigation; 3 = recovery 48 h after fumigation; 4 = recovery two weeks after fumigation; 5 = no recovery was found, net photosynthesis and dark respiration still significantly different in fumigated and control samples after two weeks*

Time exposure (h)	SO_2 concentration (ppm)				
	0·1	0·25	0·5	0·9	1·5
	Net photosynthesis				
1	0	0	0	4	5
2	0	0	0	5	5
4	0	0	0	5	5
6	0	0	5	5	5
	Dark respiration				
1	0	0	0	0	0
2	0	0	0	0	4
4	0	0	0	0	5
6	0	0	0	0	5

1·5 ppm SO_2 consistently resulted in a significant reduction in net photosynthesis. In general, *R. fraxinea* did not exhibit as much ability to recover from an initial depression in net photosynthesis as *E. prunastri*. Only after exposure to 0·9 ppm SO_2 for 1 h was recovery found after 2 weeks (Table 2).

Dark respiration of *R. fraxinea* decreased (e.g.

Table 3. *Chlorophyll content means* ($\mu g\ mg^{-1}$) of Evernia prunastri *and* Ramalina fraxinea. *Numbers in brackets = standard deviation; c = control; f = fumigated. * Significant* ($P < 0.05$) *reduction in fumigated mean compared to the control mean*

Time of exposure (h)		SO_2 concentration (ppm)				
		0·1	0·25	0·5	0·9	1·5
		Evernia prunastri				
1	c	2·19 (0·53)	2·13 (0·31)	2·79 (0·45)	1·08 (0·23)	1·59 (0·03)
	f	1·55 (0·25)	2·45 (0·13)	2·67 (0·38)	1·11 (0·13)	0·12 (0·03)*
2	c	1·40 (0·16)	2·79 (0·32)	1·43 (0·13)	3·43 (0·44)	1·52 (0·04)
	f	1·48 (0·47)	3·05 (0·12)	1·28 (0·22)	2·58 (0·33)*	0·18 (0·05)*
4	c	1·56 (0·13)	2·25 (0·34)	2·73 (0·11)	1·33 (0·26)	1·86 (0·12)
	f	1·31 (0·36)	2·39 (0·40)	2·33 (0·39)	0·29 (0·16)*	0·20 (0·05)*
6	c	1·24 (0·18)	2·59 (0·27)	1·48 (0·09)	2·94 (0·25)	1·35 (0·05)
	f	1·18 (0·11)	2·13 (0·34)	1·13 (0·11)*	1·95 (0·16)*	0·24 (0·05)*
		Ramalina fraxinea				
1	c	1·75 (0·15)	1·43 (0·24)	1·48 (0·35)	0·67 (0·09)	1·16 (0·29)
	f	1·49 (0·20)	1·42 (0·18)	1·15 (0·33)	0·69 (0·05)	0·67 (0·31)*
2	c	1·39 (0·16)	1·56 (0·23)	1·04 (0·07)	0·98 (0·09)	1·67 (0·16)
	f	1·21 (0·05)	1·31 (0·16)	0·95 (0·08)	0·67 (0·41)	0·21 (0·13)*
4	c	0·88 (0·09)	1·24 (0·44)	0·75 (0·06)	0·78 (0·25)	1·11 (0·24)
	f	1·06 (0·29)	1·23 (0·19)	0·82 (0·16)	0·30 (0·05)*	0·26 (0·05)*
6	c	0·98 (0·13)	1·41 (0·38)	1·26 (0·12)	1·38 (0·16)	1·52 (0·18)
	f	0·85 (0·16)	1·43 (0·05)	0·56 (0·21)*	0·28 (0·10)*	0·23 (0·10)*

lower absolute values) significantly only after exposure to 1·5 ppm SO_2 for 4 or 6 h. Recovery was found only in samples which had been fumigated for 2 h (Table 2).

As with *E. prunastri*, chlorophyll degradation (Table 3) was a more sensitive response parameter to SO_2 in *R. fraxinea* than dark respiration, but it was less sensitive than net photosynthesis. Significant chlorophyll reduction (Table 3) was found in *R. fraxinea* when no recovery in net photosynthesis occurred (Table 2). Only the material exposed to 0·9 ppm for 2 h did not show significant chlorophyll degradation (Table 3) when net photosynthesis did not recover. High SO_2 concentration (1·5 ppm) caused almost a total degradation of chlorophylls.

DISCUSSION

High concentrations of, and long exposure times to, SO_2 resulted in reduced photosynthetic rates, chlorophyll destruction and reduced respiratory rates in both species. Of the three response parameters, net photosynthesis was clearly the most sensitive and this is consistent with results reported earlier (Fields, 1988). Temporary responses in net photosynthesis at 0·1 ppm for *E. prunastri* and at 0·5 ppm for *R. fraxinea* were observed. Since the mycobiont is mainly responsible for the measured dark respiration, we infer that it is more resistant to the effects of SO_2 than the phycobiont, as is also suggested by Holopainen & Kärenlampi (1984).

The degree of sensitivity found in *E. prunastri* is very similar to that found in the closely related *Evernia mesomorpha*, which is known to respond to concentrations as low as 0·085 ppm (Huebert *et al.* 1985). Indeed, these two species exhibit significant responses to SO_2 fumigations at concentrations lower than any other species thus far tested (Fields, 1988). Nevertheless, these concentrations may seem high relative to the correlations made in field work with lower mean concentrations (e.g. Hawksworth & Rose, 1970). Such field correlations utilize long-term, ambient SO_2 means calculated over months (or even years). The occurrence of significant correlations of such SO_2 values with lichen community variation does not mean that the community patterns are caused by such low SO_2 levels. These ambient SO_2 data sets must include a wide range of SO_2 values and it may well be that the apparent responses among the lichen communities are caused by episodes of shorter duration but with relatively high SO_2. Although it may be difficult to reconcile fully the two approaches, it is germane that significant correlations exist for differential sensitivities among species based on both field and short-term experimental studies (Nash, 1988).

It is clear that *E. prunastri* is more sensitive to SO_2 than *R. fraxinea*. The reason for this was not specifically investigated. However, differences in the thallus anatomy of the two species may be related to differences in resistance to SO_2 diffusion to the algal layer. The cortex of *R. fraxinea* is considerably thicker and denser and consequently should offer a greater barrier to diffusion. In addition, the lower cortex of *E. prunastri* is discontinuous due to the presence of loosely organized soredia, and consi-

quently SO_2 may also reach the algal cells via the lower cortex surface more easily than in *Ramalina fraxinea*.

On the basis of experiments at 1·6 ppm SO_2 for 14 h, Türk, Wirth & Lange (1974) found that *E. prunastri* was extremely sensitive to SO_2 compared with 11 other common European species. More recently Balaguer & Manrique (1991) conducted a long-term experiment (38 days with periodic drying) that included *Evernia prunastri* and a different *Ramalina* (*R. farinacea*) species. *Evernia* was more sensitive to SO_2 than the *Ramalina*. On the basis of field data, the relative sensitivity of our two experimental species to SO_2 is not well established. In part this may be because they grow frequently on different substrates. In England *Ramalina fraxinea* is more common on eutrophicated bark and on such substrates it is reported to be very sensitive to SO_2 relative to other eutrophic lichen species (Hawksworth & Rose, 1970).

Water relations are of paramount importance in determining SO_2 uptake by lichens (Grace, Gillespie & Puckett, 1985) and, as a consequence, on the effect of SO_2. Dry lichens absorb far less SO_2 than moist ones and are known to be resistant to SO_2 fumigations (Coxson, 1988). These observations may well explain some of the differences in the literature, particularly with respect to effects of length of exposure to SO_2. Any lichen exposed to a light source will be subject to drying even in high humidities (Türk *et al.*, 1974). As a consequence SO_2 absorption characteristics will not be uniform across fumigation periods. Low humidities used in some experiments, such as those of Showman (1972), Nash (1973) or Beekley & Hoffman (1981), probably led to the complete drying of the thalli. Thus, it is not surprising that responses in some studies appear to be independent of exposure time (Fields & St Clair, 1984*a*, *b*; Huebert *et al.*, 1985).

An anticipated outcome of air pollution studies is a clear exhibition of dose–response relationships. Thus, fumigations for a long period at a low pollutant concentration should have the same effect as a shorter fumigation at a higher concentration where the dose (concentration × time) is equal for each fumigation. Because some workers (Fields & St Clair, 1984*a*, *b*; Huebert *et al.*, 1985) have concluded that duration of exposure is unimportant for inducing effects in lichens, dose–response analyses have not been widely applied to lichenological data (Sigal, 1988). Examination of the net photosynthesis data, particularly for *E. prunastri*, illustrates that dose is a meaningful concept for our data. For example, initial significant depression of net photosynthesis in *E. prunastri* occurs at 0·1 ppm for 4 h (dose = 0·4 ppm-h), at 0·25 ppm for 4 h (dose = 1·0 ppm-h) and at 0·5 ppm for 1 h (dose = 0·5 ppm-h). Similarly, permanent reduction in net photosynthesis in *E. prunastri* occurred at 0·5 ppm for 2 h (dose = 1·0 ppm-h) and at 0·9 ppm for 1 h (dose = 0·9 ppm-h). For *R. fraxinea* permanent reduction in net photosynthesis occurred at 0·5 ppm for 6 h (dose = 3·0 ppm-h), 0·9 ppm at 2 h (dose = 1·8 ppm-h), and at 1·5 ppm for 1 h (1·5 ppm-h). Overall, these responses correspond to relatively narrow ranges of dose.

The use of dose–response calculations needs to be made within appropriate ranges. For example, at higher concentrations it is inappropriate to calculate such values because the lichen is essentially dead (e.g. chlorophyll is degraded and net photosynthesis is 0 or less). Under such circumstances, increasing the concentration or increasing the length of exposure will not increase the response.

Re-examination of the data of Huebert *et al.* (1985) reveals that their claim that the response to SO_2 is independent of length of fumigation can only partly be supported. Their initial gas exchange response data are consistent with the claim (Huebert *et al.*, 1985, Table 1), because similar responses were found across a range of exposure times. However, response to an air pollutant may be measured in several ways. For example, whether a particular dose leads to a temporary or more permanent response is important. They also examined net photosynthetic recovery (Huebert *et al.*, 1985, Table 2). The maximum dose at which recovery still occurred appears to have been approximately 0·35 ppm-h (0·355 ppm-h for 1 h at 0·355 ppm; and 0·340 ppm-h for 4 h at 0·085 ppm). Thus, with respect to a threshhold (*c.* 0·35 ppm-h) above which permanent net photosynthetic injury occurs, *Evernia mesomorpha* does indeed exhibit dose–response characteristics.

If thallus water content is well controlled during fumigations with other poikilohydric organisms, we suggest that dose–response relationships will become more readily apparent in future research.

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