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Air Pollution Damage to Cell Membranes in Lichens III. Field Experiments

By

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Summary

PEARSON L. C. & RODGERS G. A. 1982. Air pollution damage to cell membranes in lichens. III. Field experiments. — Phyton (Austria) 22 (2): 329—337. — English with German summary.

Downwind from field furnaces burning sulfur at the rate of 300 g/hr, SO_2 levels varied from 0.52 ppm at 10 m to 0.23 at 40 m in an open area where fumes could readily disperse but were considerably higher in an area where air movement was chanelled along a swale. The conductivity of water in which pieces of lichens were immersed following exposure to fumes from the field burners increased slightly in liches collected upwind from the burners or over 100 meters downwind, but increased considerably in specimens collected 15—30 m downwind. Leakage of electrolytes, as measured by increase in conductivity of water in which lichen pieces were immersed, was significantly higher downwind from burners in an open area than from those in a densely forested area.

Zusammenfassung

PEARSON L. C. & RODGERS G. A. 1982 Schädigung von Zellmembranen in Flechten. III Feldversuche. — Phyton (Austria) 22 (2): 329—337. — Englisch mit deutscher Zusammenfassung.

Windabwärts von im Freien aufgestellten Öfen, in denen 300 g.h^{-1} Schwefel verbrannt wurde, variierten die SO_2-Gehalte der Luft zwischen 0,52 ppm in

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10 m Entfernung bis 0,23 ppm in 40 m; wenn sich die Abgase nicht frei ausbreiten konnten, sondern durch ein Tal kanalisiert wurden, traten wesentlich höhere SO_2-Werte auf. Flechtenstücke, die windaufwärts von den Brennern oder in über 100 m Entfernung windabwärts exponiert worden waren, erhöhten nach kurzem Eintauchen in Wasser dessen Leitfähigkeit nur wenig, Proben aus 15—30 m Entfernung windabwärts erhöhten die Leitfähigkeit deutlich. Windabwärts von den Brennern war der durch die Erhöhung der Leitfähigkeit nach Immergierung der Flechten faßbare Elektrolytaustritt im offenen Gelände signifikant höher als im dichten Wald.

(Editor transl.)

Introduction

Air pollution is reported to affect photosynthesis, cellular respiration, and nitrogen fixation in plants, and to dissolve the cytoplasm of some kinds of cells (Pearson & Skye 1965; Darley & *al.* 1966, Ferry & *al.* 1973, Evans & Miller 1975, Hällgren & Huss 1975, Henriksson & Pearson 1981). There is also evidence that it affects amino acid synthesis (Pearson 1973), and that it damages cell membranes (Pearson & Henriksson 1981, Pearson 1981). Lichens, because of their sensitivity to air pollution and their active yearround metabolism, have been valuable research organisms for this type of study.

In previous studies of the effects of air pollution on cell membranes, lichens were collected from a number of localities in western Europe and pieces of the thalli were immersed in deionized distilled water for 3—10 minutes. Lichens collected near urban centers, where air pollution was bad, caused a considerable increase in the conductivity of the water, but lichens collected in rural areas far removed from pollution centers did not (Pearson 1981). Lichens collected from rural areas and grown for several weeks in flasks containing sulfur dioxide also caused the conductivity to increase (Pearson & Henriksson 1981).

In the present study, we set up small sulfur burners in the field to further study the effects of air pollution on lichens. Our objectives were (1) to observe the effects of sulfur dioxide on membrane integrity in lichens under field conditions, and (2) to compare the effects of different terrain and different vegetational types on dispersal of sulfur dioxide.

Material and Methods

The study was conducted near Vårdsätra, 10 km southeast of Uppsala, in a forest owned by the University. At four sites, sulfur was burned on days when the wind was out of the south or southwest. Following ten days of fumigation over a three week period of time, specimens of *Hypogymnia physodes* (L.) Nyl. were taken to the laboratory and tested for membrane leakage of electrolytes. Samples of *Usnea ceratina* (Ach.) Nyl., a species much more sensitive to air pollution than *H. physodes* and almost completely absent from forests in the industrialized Uppsala—Stockholm area, were collected in northern Sweden, and these were tied to trees at one of the sites (Station no. 3), fumigated, and tested for leakage. Other species of lichens found in the area, e. g., *Evernia prunastri* (L.) Ach. and *Peltigera canina* (L.) Willd., were not sufficiently abundant at the four fumigation sites to be used in the field tests.

Two of the sites were rather open, the other two densely forested. The first site, Station no. 1, was about 8 km southeast of the university botanical garden; the second site was about a km southwest of the first, and the others were about 1 km and 2 km southwest of the second.

Station no.1 was an open, flat area extending 120 meters northward from a small *Pinus sylvestris* L. grove to a forest of *Pinus*, *Picea*, and *Betula*. Small bushes of *Juniperus communis* L. were scattered over the area.

Station no. 2 was a flat, forested area dominated by *Pinus sylvestris*, *Picea abies* L., and *Betula verrucosa* L. with considerable underbrush. In the spring, this area was quite boggy.

Station no. 3 was in rough terrain with older pines and little underbrush. A swale with numerous outcroppings of granite and with steep sides about two meters high extended northward 50 meters from the site chosen for the burner.

Station no. 4 was in a swampy, rather open area covered with low brush and heath, and with scattered birch trees, many of which were dead.

Approximately 9.2 kg of sulfur was burned in each of the burners over the three-week period at a rate of about 300 $g.h^{-1}$. No sulfur was burned unless the wind was from the south or southwest at a velocity of 1 m/sec or more. Wind velocity ranged, on days sulfur was burned, from 1 to 10 m/sec. Wind velocity was invariably greatest at station 1 and almost as great at station 4.

About 20 samples of air were tested for SO_2 content using the Thorin spectrophotometric method described by Brosset & Ferm (1975). Sampling equipment consisted of test-tubes of hydrogen peroxide through which air was bubbled, a portable battery-driven pump, an automobile storage battery, a gas flowmeter, and a titration spectrophotometer. The battery-pump-flowmeter system was set up in the field at various distances downwind from the sulfur burners. Sulfur dioxide in the air was oxidized to sulfate as it bubbled through the peroxide and as such could be stored for several days before testing, if necessary. The sampling runs averaged two to four hours; before titrating, the sulfate solution was sometimes concentrated by boiling away some of the water so that the samples were of uniform volume when titrated. Wind velocity and temperature were measured several times during each run. The sulfate was titrated with barium perchlorate in the laboratory, and the amount of perchlorate used was converted to micrograms sulfur dioxide equivalent by means of graphs made specially for each titration series. Because of the difficulty in titrating accurately very small amounts of sulfate, all of the samples prepared with less than 7 liters of air were excluded from the final analysis.

Sulfur dioxide concentration was calculated from the formula S = 60 V/MT where S is the concentration of SO_2 in μg per liter air, V is the titration value express in μg SO_2 equivalent, M is the meter reading in liters of air per hour, and T is the time in minutes the sampling took. At standard temperature and pressure, 2.857 μg SO_2 per liter equals one part per million (ppm) of the gas. In Table 1, all measurements have been converted to ppm.

Membrane integrity was evaluated by measuring the conductivity of water in which pieces of lichens had been immersed for two or three minutes as previously described (SIMON 1974, PEARSON 1981, PEARSON & HENRIKSSON 1981). After immersion, the lichens were placed in a desiccator until dry and then weighed; the resistance of the water was measured with a Metrohm Herisau E-382 conductivity meter. Analysis of variance with partition of treatment means squares into a regression component and a residual component was performed as described by FEDERER (1955).

Results and Conclusions

Several samples of air at two of the sites were analyzed for SO_2 content (Table 1). Measurements were much more erratic at station no. 3, where

Table 1

Sulfur dioxide content of air at Stations 1 and 3 based on 15 samples collected between March 25 and April 6 while sulfur burners were in operation. Each sample, with one exception, contained 25 to 85 liters of air; samples with less than 7 liters of air are not included in the table

Site	Distance Downwind from Burner (m)	Range in SO_2 Content of Air (ppm) Lowest	Highest
1	40	.028	.231
1	28	.084	.245
1	10	.420	.525
3	28	.102	1.116

the terrain was very rough, than at no. 1 which was flat and open. At no. 3, the wind was funnelled up the swale with little chance to diffuse left or right because of the rocky sides; at no. 1, the fumes could disperse much more freely to the east and west, and the sulfur dioxide concentrations were not as high. Other samples taken at station 3 indicated values well above 0.5 ppm at 16 m downwind, about 0.5 ppm at 100 m downwind, and well below 0.5 ppm in the protected area below a 3 m high cliff at 45 m downwind; these values are not included in the table because of the small amount of air in the samples. A 24-hour sample of laboratory air, taken on a windless day when pollution levels in the botanical garden were undoubtedly rather high, indicated an SO_2 level of 0.036 ppm.

Table 2 summarizes over 50 individual conductivity measurements gathered after at least 4 kg of sulfur had been burned at each of the four stations. Conductivity of the deionized distilled water averaged 1.8 $\mu S.m^{-1}$, but after bits of lichen had been immersed in the water its conductivity increased to 2.2 to 20.4 depending on the size of the piece and the degree of pollution injury. In general, conductivity decreased with increasing distance from the burners; this tendency was more pronounced at stations 2 and 4, where the terrain was flat, than at station 3 where the terrain was very rough and irregular. At station 4, the correlation between distance from the burner and conductivity of the water was statistically significant with a reduction of 0.54 $\mu S.m^{-1}$ per mg *Hypogymnia* tissue per ml water for every 10 meters increase in distance from the burner. At stations 2 and 3, the decrease in conductivity was 0.1—0.3 $\mu S.m^{-1}$ for every 10 meters for *Hypogymnia physodes* and 3.1 for *Usnea ceratina*. In calculating regression and correlation coefficients, upwind samples were arbitrarily equated to 130 m downwind (10 m more than the maximum distance sampled at station 2).

At station 3, the lichens at 46 m downwind were protected by an outcropping of granite just upwind from them, while the lichens at 16 m were on trees growing on top of a large outcropping. At station no. 1, there were no trees, other than small juniper bushes, nearer the burner than 95 m downwind. A specimen of *H. physodes* found on a juniper 37 m downwind tested 2.0, or about the same as the upwind controls; however, this specimen was found very close to the ground where fumes were less likely to be present and if present more likely to be neutralized by reactions with the soil.

In Table 3, the data from stations 2, 3, and 4 in Table 2 have been combined into five distance groups. At stations 3 and 4, the lichens growing adjacent to the burners leaked fewer electrolytes than those somewhat farther away. At station 4, the lichens on the tree adjacent to the burner were obviously dead; adjacent lichens at station 3 were somewhat bleached and may have also been dead. Under field conditions, dead lichens would soon have their electrolytes leached from the cells by fog, dew, and precipitation; this may account for the relatively low conductivity values in these lichens.

In combining the data from the three stations to produce Table 3, the 1 to 3 specimens nearest the burner which showed distinct evidence of severe injury, including discoloration and bleaching, were labeled "adjacent". The next pair of specimens at each station was labeled "close" and the pair farthest from the burner was labeled "far". The specimens between "close" and "far" were lumped together under the label "moderate". Ignoring the "adjacent" collection, the correlation between distance from the source of

Table 2

Leakage of electrolytes from *Hypogymnia physodes* and *Usnea ceratina* as measured by the conductivity of water in which bits of thallus were immersed following exposure for 5 to 10 days to SO_2 fumes from sulfur burners in field experiments, Vårdsätra, Uppland, Sweden. Sulfur was burned on days when the wind was from the south or southwest. Each value in the table is the average conductivity, in $\mu S . m^{-1}$ (mg tissue/ml H_2O), of two or more specimens collected from the same tree or adjacent trees at the indicated distance downwind from the burners

STATION 4 (marshy; scattered trees)		STATION 2 (wet; dense forest)		STATION 1 (flat, open; no trees)		STATION 3 (upland forest; hilly)		
H. physodes		*H. physodes*		*H. physodes*		*H. physodes*, *U. ceratina*		
3 m	10.3 (dead)	10 m	8.6	1 m	4.1	1 m	6.1	
		20 m	5.9			6 m	5.2	20.4
						11 m	4.5	
15 m	16.3	30 m	8.5			16 m	9.8	
25 m	11.0	40 m	5.8			21 m	5.5	
35 m	12.5	50 m	7.7	37 m	2.0	26 m	5.0	
50 m	11.3	60 m	7.4			31 m	7.4	9.1
55 m	8.6	80 m	7.3			36 m	5.5	
80 m	9.9					41 m	7.0	
90 m	8.3	120 m	6.7	118 m	5.8	46 m	3.9	8.3
UPWIND (controls)								
10 m	6.0	10 m	6.1	7 m	2.1	10 m	3.9	
				15 m	2.9	20 m	4.5	
r = −.76 *)		r = −.39		r = .02		r = −.35		r = −.87
b = −.054		b = −.010		b = 0		b = −.032		b = −.312
EXPOSURE TO POLLUTION								
23 days; 9.2 kg sulfur burned		23 days; 9.2 kg sulfur burned		23 days; 9.2 kg sulfur burned		5 days; 4.3 kg sulfur burned		

*) Statistically significant at P = .05 level

pollution and the average conductivity was statistically significant ($r = -.91$) with 83% of the variation in leakage accounted for by the distance from the pollution source. On the average, conductivity decreased in *H. physodes* 0.51 $\mu S.m^{-1}$ for every 10 meter increase in distance from the sulfur burners.

For the same amount of sulfur burned, injury to lichen membranes as estimated from conductivity data, was significantly less at station 2, where the forest was dense, than at station 4, where trees were scattered. Conductivity from the upwind controls was the same for the two stations, but at the greater distances downwind, differences were statistically significant ($P<0.01$). Presumably, sulfur dioxide was adsorbed on the vegetation and converted into harmless sulfate ions. If so, the planting of trees in urban areas where sulfur dioxide is a serious pollutant could help to reduce the level of pollution harmful to animals, including man, and to lichens and other plants.

Table 3

Average leakage of ions through membranes following exposure to sulfur dioxide fumes at three stations with analysis of variance of leakage data. Combined data from Table 2

Distance Grouping	Distance from Source Range	Average	Appearance of lichens Following exposure	Conductivity ($\mu S.m^{-1}$)
Adjacent	1—20 m	8 m	Thalli dead or severely injured	7.6
Close	15—30 m	20 m	Alive but obviously injured	11.5
Moderate	20—80 m	45 m	Alive; some thalli injured	7.9
Far	46—120 m	85 m	Alive and healthy	6.3
Upwind		—10 m	Unaffected	5.4

Source of variation	df	ms (variance)
Among stations	2	26.24 **)
Among distance groupings	4	16.45 *)
Adjacent vs Others	1	.09
Regression, ignoring Adjacent	1	59.80 **)
Residual	2	2.96
Error	8	2.57

*) Significant at 5% level of Probability
**) Significant at 1% level of Probability

The possibility that the increase in conductivity in the water in which the lichens from polluted areas were immersed was due to particulate matter adsorbed on the lichen surface must be considered. In laboratory experiments (PEARSON & HENRIKSSON 1981), a high correlation between level of concentration of SO_2 or HF and conductivity of water in which lichens had been immersed was observed even though pure gas was used and no particulates were present. Also, water in which lichens were vigorously rinsed for three seconds (PEARSON 1981) showed very little increase in conductivity. These observations indicate that the increased conductivity was due to electrolyte leakage and not simply a washing of adsorbed ions from the thallus into the water used for the conductivity tests.

Differences in cell membrane injury, as measured by increase in the conductivity of deionized distilled water in which bits of lichen thallus have been dipped, are noticeable after less than two weeks of fumigation, long before any visible morphological abnormalities become apparent. Because of this sensitivity, and also the ease with which the test is conducted, we recommend the cell membrane integrity test to evaluate degree of air pollution.

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