EFFECT OF DIFFERENT LEVELS OF AIR POLLUTION ON PHOTOSYNTHETIC ACTIVITY OF SOME LICHENS

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ABSTRACT

Four lichen species: *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Parmelia saxatilis*, and *Platismatia glauca* were collected from two sites (S. Poland) with a different air pollution level: "Kamienica valley" (less polluted) and "Kopa" (more polluted). The thalli were compared with respect to their: net photosynthetic rate (P_N), fluorescence parameters (F_v/F_m , F_m , F_m/F_0), chlorophyll *a+b* content, and phaeophytinization quotient ($O.D.435/O.D.415$). P_N intensity, chlorophyll *a+b* and $O.D.435/O.D.415$ were reduced only in *P. furfuracea* collected from Kopa, which is in agreement with the Hawksworth-Rose scale of sensitivity of lichens to air pollution. Fluorescence parameters were significantly lowered in all lichens coming from the more polluted site (except of F_v/F_m and F_m/F_0 in *P. saxatilis*). Parameters based on chlorophyll fluorescence measurements enable to reveal the very early signs of decreased photosynthetic capacity of the thalli, caused by air pollution, before changes in the other photosynthetic parameters become measurable.

KEY WORDS: air pollution, chlorophyll fluorescence, lichens, photosynthesis.

INTRODUCTION

Lichens are organisms very sensitive to air pollution. Their sensitivity to many toxic agents such as: SO_2 (Puckett et al. 1973, Misztalski and Mydlarz 1988, Holopainen and Kauppi 1989, Sanz et al. 1992), HSO_3^- (Hill 1971, Puckett et al. 1973, Misztalski and Niewiadomska 1993), SO_2 and nitrate (Balaguer and Manrique 1991), several metals and low pH (Garty and Delarea 1991, Garty et al. 1992) has been extensively studied. In their natural environment they are often subjected to a mixture of pollutants (toxic gases, metals, acid rain) and it is difficult to identify the component primarily responsible for injury. The Hawksworth-Rose distribution scale is based on mean values of SO_2 during winter (Hawksworth and Rose 1970) and has been adapted for the Polish flora by Kiszka (1992). Metabolic activity within the lichen is the greatest in the algal layer and this is the area most severely damaged by air pollution (Richardson and Nieboer 1981, Richardson 1988, Misztalski and Mydlarz 1988, Sanz et al. 1992, Treshow 1989). As generalized by Lechowicz (1982), maximizing photosynthesis means maximizing individual fitness.

In this paper we evaluated the impact of air pollution on photosynthetic abilities of four lichen species, differing in sensitivity to air pollution, from two sites characterized by

various levels of air pollution (SO_2 , NO_x , and dust). The following parameters have been determined: gas exchange, photoinhibition of PSII, (indicators of activity *in vivo*), chlorophyll *a+b* content, and phaeophytinization quotient (characterizing the pigment status of the thalli).

MATERIALS AND METHODS

Four species of foliose lichens – *Hypogymnia physodes* (L.) Nyl., *Pseudevernia furfuracea* (L.) Zopf., *Parmelia saxatilis* (L.) Ach. i *Platismatia glauca* (L.) W. Culb & C. Culb – were collected in a mixed forest, from the sites known as Kamienica valley and Kopa in the Gorczański National Park (S. Poland). Pollution at these sites is determined by means of technical monitoring data (Monitoring Techniczny 1994) through twenty-four hours average values of winter sulphur deposition of 11.2 and 14.6 mg SO_2 m⁻² in Kamienica valley and Kopa respectively; nitrogen deposition of 0.06 and 0.15 mg NO m⁻²; deposition of dust 2.1 and 3.1 mg m⁻².

The collected thalli were air dried and kept at 4°C, in the dark. Before measurements of P_N and chlorophyll fluorescence the samples of thalli, about 1 g f.wt., were placed for 24 h on wet Whatman paper in closed Petri dishes, at room temperature. After such treatment the thalli reached the water

content of about $1.10 \pm 0.15 \text{ mg mg}^{-1} \text{ d.wt.}$ This hydration procedure was sufficient to obtain a high level of photosynthesis and maximum values of chlorophyll fluorescence, which is in agreement with data obtained by Jensen and Feige (1991) and Manrique et al. (1993).

Net photosynthesis (P_N) was measured immediately after transfer from wet Whatman paper using an infrared gas analyser (ADC, England) in the open system, equipped with PLC2 leaf chamber of 0.137 l volume and 250 ml min^{-1} air flow. Measurements were performed at ambient CO_2 level, 25°C , at an irradiance of $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD.

Measurements of fast fluorescence kinetics were made using a Plant Stress Meter (BioMonitor S.C.I, Sweden), at room temperature, after 15 min. dark adaptation of the thalli. Samples were then illuminated with an actinic light of $450 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 2 sec. Fluorescence emissions were monitored from the upper surface of the thalli. The following parameters were monitored: F_m – maximal fluorescence, F_v/F_m – the ratio of maximal to variable fluorescence, and F_0 – minimal fluorescence.

Chlorophyll $a+b$ and phaeophytin a contents were determined, as described by Barnes et al. (1992), after 6-times repeated washing of the thalli with 100% acetone saturated with carbonate, followed by 40 min. extraction in DMSO containing PVP.

Statistical analysis was done using Student's t-test.

RESULTS

A significant decrease in net photosynthesis was found in the thalli of *Pseudevernia furfuracea*, collected from Kopa, in comparison with the thalli collected from Kamienica valley (Fig. 1). A tendency for decreased P_N was observed in *Hypogymnia physodes* and *Parmelia saxatilis*, but not in *Platismatia glauca*.

The thalli of *P. furfuracea* collected from Kopa were characterized by a significantly decreased content of chlorophyll $a+b$ in comparison with that of Kamienica valley (Fig. 2). A similar tendency was measured in *H. physodes* and *P. saxatilis*. On the other hand, in *P. glauca* a significant increase in

Fig. 1. Net photosynthetic rate (P_N) of lichens collected from sites with different air pollution level ("Kamienica valley" and "Kopa"); $n = 6$. * indicates the relative significance from "Kamienica valley" when $p < 0.05$.

the content of chlorophyll $a+b$ was recorder in the thalli collected from this site.

The phaeophytinization quotient, measured as the reduction in the ratio of O.D.435/O.D.415 (Table 1), was significantly decreased in the thalli of *P. furfuracea* from Kopa (by 16% in comparison with the value of Kamienica valley), while an opposite reaction was found in the thalli of *P. glauca* (increase by 26%). *H. physodes* and *P. saxatilis* were characterized by a slight tendency to a decreased value of this ratio.

The fluorescence parameters: F_v/F_m (the ratio of maximal to variable fluorescence), F_m (maximal fluorescence), and F_m/F_0 (the ratio of maximal to minimal fluorescence) were lowered in all tested lichen collected from Kopa, in comparison with the values of Kamienica valley (Table 2).

DISCUSSION

The chosen lichen species belong to the following classes of lichen distribution with respect to air pollution, according to the 10-point scale of Kiszka (1992). *H. physodes* and *P. saxatilis* (moderately tolerant) belong to 4, *P. glauca* 5, and *P. furfuracea* 6 (the most sensitive of the chosen species). *H. physodes*, was the first to reveal a significantly decreased rate of P_N in Kopa in comparison with Kamienica valley (Fig. 1), suggesting a slight deterioration of photosynthetic capacity at this site. This observation is in agreement with the distribution scale.

The chlorophyll loss, with subsequent bleaching or discoloration of the thalli, is one of the clearest symptoms of damage to lichens caused by air pollution (Gilbert 1973, Ronen and Galun 1984, Garty et al. 1992, Modenesi 1993). However, a decrease in net photosynthesis should appear earlier than a reduction in chlorophyll pigments (Showman 1972, Nash 1973, Fabiszewski et al. 1987, Sanz et al. 1992). In the present study, a significant decrease in the content of chlorophyll $a+b$ was noted only in *H. physodes*, collected from Kopa, in comparison with Kamienica valley (Fig. 2). This suggests that the reduction of P_N in *H. physodes* is due to the breakdown of chlorophyll pigments. In the other lichen species studied, the changes were not so pronounced, and

Fig. 2. The content of chlorophyll $a+b$ in the lichen thalli collected from sites with different air pollution level ("Kamienica valley" and "Kopa"); $n = 6$. ** indicates the relative significance from "Kamienica valley" when $p < 0.01$, * – when $p < 0.05$.

TABLE 1. The ratio of chlorophyll *a* to phaeophytin *a* (O.D.435/O.D.415) in lichen thalli from sites with different air pollution level ("Kamienica valley" and "Kopa"). Values are the means $\pm$ SD (n=6). ** indicates the relative significance from "Kamienica valley" at $p < 0.01$

	<i>Hypogymnia physodes</i>	<i>Pseudevernia furfuraceae</i>	<i>Parmelia saxatilis</i>	<i>Platismatia glauca</i>
Kamienica valley	0.87 $\pm$ 0.01	1.19 $\pm$ 0.06	0.92 $\pm$ 0.04	0.91 $\pm$ 0.05
Kopa	0.83 $\pm$ 0.04	1.00 $\pm$ 0.07 (**)	0.90 $\pm$ 0.08	1.15 $\pm$ 0.08 (**)

TABLE 2. Parameters of fluorescence in lichens collected from sites with different air pollution level ("Kamienica valley" and "Kopa"). F_m – maximum fluorescence, F_0 – minimal fluorescence F_v – variable fluorescence ($F_m - F_0$). Values are the means $\pm$ SD (n=9). ** indicates the relative significance from "Kamienica valley" at $p < 0.01$, * – at $p < 0.05$

	<i>Hypogymnia physodes</i>			<i>Pseudevernia furfuraceae</i>			<i>Parmelia saxatilis</i>			<i>Platismatia glauca</i>		
	F_v/F_m	F_m	F_m/F_0	F_v/F_m	F_m	F_m/F_0	F_v/F_m	F_m	F_m/F_0	F_v/F_m	F_m	F_m/F_0
Kamienica valley	0.653 $\pm$ 0.027	0.765 $\pm$ 0.141	2.88 $\pm$ 0.25	0.628 $\pm$ 0.05	0.593 $\pm$ 0.175	2.74 $\pm$ 0.33	0.650 $\pm$ 0.024	0.657 $\pm$ 0.125	2.87 $\pm$ 0.21	0.651 $\pm$ 0.024	0.800 $\pm$ 0.179	2.87 $\pm$ 0.21
Kopa	0.579 $\pm$ 0.037	0.364 $\pm$ 0.108	2.39 $\pm$ 0.21	0.574 $\pm$ 0.044	0.392 $\pm$ 0.117	2.42 $\pm$ 0.30	0.622 $\pm$ 0.045	0.505 $\pm$ 0.124	2.67 $\pm$ 0.31	0.606 $\pm$ 0.035	0.592 $\pm$ 0.197	2.58 $\pm$ 0.23
	(**)	(**)	(**)	(**)	(**)	(*)		(**)		(**)	(**)	(*)

only a slight tendency to decreased P_N and chlorophyll content was found in the thalli of *H. physodes* and *P. saxatilis* collected from Kopa.

An opposite effect was observed in *P. glauca* from Kopa. The increased chlorophyll content was measured in thalli from the more polluted site (Fig. 2). This suggests a stimulative effect of some pollutants, especially NO_x , or a beneficial influence of the microhabitat. Such a stimulative effect has been reported earlier for epiphytic lichens as the result of local shelter or nutrient flushing on some parts of the tree trunk (Gilbert 1973, Treshow 1989), or pollution with NO_3^- (Balaguer and Manrique 1991).

The ratio of chlorophyll *a* to phaeophytin *a*, measured by O.D.435/O.D.415, has been used to indicate acid-induced chlorophyll degradation (Ronen and Galun 1984). A decrease in this ratio caused by pollution with SO_2 and NO_x has been documented (Balaguer and Manrique 1991). In the present study a significant reduction in the ratio O.D.435/O.D.415 has been measured only in *H. physodes* collected from Kopa (Table 1). We may conclude that chlorophyll degradation in the thalli of *H. physodes* from Kopa could result from the acidic compounds of air pollution.

It has been shown that various environmental stresses directly and indirectly affect the functioning of PSII (Öquist 1987, Krause 1988). During illumination of leaves the rate of energy dissipation from the light-harvesting antenna of PSII is increased and this has been shown to be protective against irreversible photoinhibition. The ratio of F_v/F_m is a useful parameter of PSII photoinhibitory damage in higher plants and in lichens (Björkman and Demmig 1987, Jensen and Feige 1991, Manrique et al. 1993). The ratio of F_v/F_m and the value of F_m

were lowered in all the tested lichens from Kopa in comparison with the thalli from the Kamienica valley (Table 2), thus, showing a partial photoinhibition of the thalli from Kopa. The ratio of F_m/F_0 was also lowered, being indicative, according to Jensen and Feige (1991), for the decreased vitality of lichens from Kopa (except of *P. saxatilis*).

In conclusion, this work shows that methods based on measurements of chlorophyll fluorescence enable to reveal the very early signs of decreased photosynthetic capacity, resulting from air pollution, before changes of the other photosynthetic parameters become measurable. This allows to determine more precisely the deterioration of local environment, than it is possible by distribution studies. This conclusion coincides well with the recently obtained results (Branquinho et al. 1997), showing that fluorescence parameters are good indicators of lichen sensitivity to metal pollution.

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WPLYW ZRÓŻNICOWANEGO POZIOMU ZANIECZYSZCZEŃ NA AKTYWNOŚĆ FOTOSYNTETYCZNĄ U KILKU GATUNKÓW POROSTÓW

STRESZCZENIE

Badania aktywności fotosyntetycznej przeprowadzono na czterech gatunkach porostów: *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Parmelia saxatilis* i *Platismatia glauca* pochodzących z 2 stanowisk, na terenie Gorczańskiego Parku Narodowego, różniących się poziomem zanieczyszczeń – „Dol. Kamienicy” (stanowisko charakteryzujące się niskim poziomem zanieczyszczeń) i „Kopa” (stanowisko bardziej zanieczyszczone). Fotosynteza netto (P_N), poziom chlorofilu *a+b* oraz stosunek chlorofilu do feofityny (O.D.435/O.D.415) wykazywały istotny spadek w plechach pochodzących ze stanowiska bardziej zanieczyszczonego tylko u *Pseudevernia furfuracea*. Parametry opisujące fluorescencję chlorofilu (F_v/F_m, F_m, F_m/F₀) wykazywały istotny spadek u wszystkich badanych gatunków porostów pochodzących z terenu Kopy (z wyj. F_v/F_m i F_m/F₀ u *P. saxatilis*). Parametry oparte na fluorescencji chlorofilu są najbardziej czułym, z badanych wskaźników, wskazującym na pogarszającą się kondycję porostów wraz ze wzrostem poziomu zanieczyszczeń.

SŁOWA KLUCZOWE: fluorescencja chlorofilu, fotosynteza, porosty, zanieczyszczenia atmosferyczne.