The Effect of UV-B Radiation on the Antioxidant System in the *Peltigera aphthosa* and *Peltigera rufescens* Lichens

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Abstract. Ultraviolet (UV) radiation is the short wavelength region of the solar spectrum. The high-energy photons of UV-B (280–315 nm) are potentially dangerous for all living organisms. The effect of UV-B radiation on lichens has not been studied sufficiently. We conducted a comparative study of the effects of the long-term (10 d) exposure to the environmentally realistic dose of UV-B radiation on the accumulation of lipid peroxidation products (TBARS), $\text{H}_2\text{O}_2$ content, superoxide dismutase (SOD) activity, and respiration rate in *Peltigera aphthosa* from the forest community and *Peltigera rufescens* from the open spaces of floodplain meadow. The $\text{H}_2\text{O}_2$ content and the SOD activity were found to increase in the thalli of *P. rufescens*. The TBARS content in the UV-B treated thalli of *P. rufescens* did not differ from the control thalli and was 2.5 times higher than in *P. aphthosa*. In *P. aphthosa* thalli, SOD activity did not change after UV-B exposure, and TBARS content increased by 33% with an increase in the total UV-B dose. Both lichens exhibited an increase in the alternative respiratory pathway (AP) activity and a decrease in the ratio of the main (cytochrome) pathway to the energy low efficient AP. The AP involvement was more pronounced in *P. aphthosa*. The results of our study indicate the species-specific response in lichens and differences in their resistance to oxidative stress, which were due to adaptation to the light conditions in the typical habitats of these species.

Keywords: *Peltigera aphthosa*, *Peltigera rufescens*, lichens, UV-B radiation, lipid peroxidation, hydrogen peroxide, superoxide dismutase, alternative respiratory pathway, resistance.

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Аннотация. Ультрафиолетовая (УФ) радиация относится к коротковолновой части солнечного спектра. Высокоэнергетические фотоны УФ-излучения (280–315 нм) потенциально опасны для всех живых клеток. Реакция лишайников на УФ-излучение исследована недостаточно. Мы провели сравнительное изучение влияния длительного действия экологически обоснованной дозы УФ-В-радиации на накопление продуктов перекисного окисления липидов (ПОЛ), содержание Н₂О₂, активность супероксиддисмутазы (СОД) и дыхание талломов Peltigera aphthosa из лесного сообщества и Peltigera rufescens, обитающей на хорошо инсолируемых участках пойменного луга. Выявили повышение содержания Н₂О₂ и активности СОД в талломах P. rufescens, экспонированных к УФ-В. Содержание продуктов ПОЛ в импактных талломах P. rufescens не отличалось от контроля и было в 2,5 раза выше по сравнению с P. aphthosa. Уровень активности СОД в талломах P. aphthosa не изменился, но содержание продуктов ПОЛ возрастило на 33 % с увеличением суммарной дозы УФ-В. У обоих видов лишайников отмечали повышение активности альтернативного пути (АП) дыхания, что приводило к изменению соотношения основного (цитохромного) и энергетически малоэффективного АП. Вовлечение АП было сильнее выражено у P. aphthosa. Результаты исследования свидетельствуют о видовой специфике реакции лишайников на воздействие УФ-В-излучения, а также указывают на различия в их устойчивости к окислительному стрессу, обусловленные приуроченностью видов к местообитаниям с разным режимом освещенности.

Ключевые слова: Peltigera aphthosa, Peltigera rufescens, лишайники, УФ-В-радиация, перекисное окисление липидов, пероксид водорода, супероксиддисмутаза, альтернативный путь дыхания, устойчивость.

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Introduction

Lichens are a stable self-regulating association of heterotrophic mycobionts with photoautotrophic green algae and/or cyanoprokaryotes. The mycobiont accounts for over 90% of the thallus biomass and most of the respiration (Palmqvist et al., 2008). The symbiotic nature and poikilohydric properties of lichens ensure that these organisms have high resistance to adverse environmental stresses (Kranner et al., 2005). Due to this, lichens occupy various stressful terrestrial habitats, including deserts, highlands, Arctic and Antarctic regions, and high stress microhabitats in less stressful habitats.

Of the sun ultraviolet (UV) radiation spectrum, only near-UV-A radiation (315–400 nm) and a small portion of UV-B photons (280–315 nm) reach the Earth's surface. The high-energy UV-B photons can damage biologically important macromolecules and induce oxidative stress in the cells of living organisms (Frohnmeyer, Staiger, 2003). Adaptive (architecture modification, synthesis of a range of secondary metabolites) and nonspecific harmful (damage to DNA, proteins and membranes, inhibition of photosynthesis and growth, accumulation of reactive oxygen species (ROS)) effects of UV-B radiation on plants have been well-documented (Caldwell et al., 1995, 2003; Jenkins, 2009). Fewer data are available on the effects of UV-B on lichens. High doses of UV-B radiation are known to induce programmed cell death (Ünal, Uyanikgil, 2011) and suppress the growth of lichen thalli (Chowdhury et al., 2017). Different secondary lichen compounds synthesized by the mycobiont play an important role in protecting the lichen photobiont against excessive visible light and UV radiation (Nguyen et al., 2013). UV-B induces the synthesis of parietin and melanins in the thalli of some lichen species (Solhaug et al., 2003; Nybakken et al., 2004; Solhaug, Gauslaa, 2012; Mafole et al., 2019). These compounds are synthesized in the thallus upper cortex cells and absorb or reflect UV (A+B) rays, thereby shielding the underlying layers of cells.

Most lichen species in the Komi Republic grow in forest communities. The boreal species often prefer shaded and moist habitats. In the forest, lichens are rarely exposed to the direct sunlight. Such lichens can serve as a convenient model for studying the adverse effects of UV-B radiation. For comparison, lichens from the open habitats, which are exposed constantly to UV radiation, are of great interest for studying protective mechanisms against the UV-B impact. We hypothesized that these species differ considerably in their antioxidant system activity and protective reactions to oxidative stress. To test this idea, we conducted a comparative study of the UV-B effects on the activity of the key antioxidant enzyme – superoxide dismutase (SOD), the $H_2O_2$ content, and the level of lipid peroxidation in Peltigera aphthosa (L.) Willd. and Peltigera rufescens (Weiss) Humb. These lichen species grow in different types of habitats. The effect of UV-B on the respiration rate and the ratio of the cytochrome pathway to the alternative respiratory pathway was studied also to estimate changes in lichen metabolism.
Materials and methods

Lichens

*P. aphthosa* is a foliose lichen with a circumpolar distribution. It is found in the Arctic, boreal, and temperate zones. The lichen grows on moss, soil, and plant debris in shaded and moist sites (Thomson, 1984). The main photobiont of *P. aphthosa* is the green algae of the *Pseudococcomyxa* genus. The cephalodia on the thallus surface contain cyanoprokaryotes of the genus *Nostoc*.

*P. rufescens* is a foliose cyanolichen. Its thallus contains cyanobacteria of the *Nostoc* genus. This species has a polyzonal distribution and grows in the temperate and boreal latitudes of the Northern hemisphere and in South America and Australia, in fully sunlit habitats, on the open sites in the fields and roadsides (Brodo et al., 2001).

Sampling sites

The study was conducted in the summer of 2018. *P. aphthosa* thalli were collected in a pine-dominated forest mixed with spruce and deciduous trees, in shaded and moist places. The thalli of *P. rufescens* were collected in open, well-lit areas of meadows adjacent to the river Vym floodplain. The meadow soil was a well-drained sod-layered sandy loam.

Lichen samples were collected at midday, during clear sunny weather. Simultaneously with sampling, the photosynthetic active radiation (PAR) intensity, air temperature, and relative humidity were measured using a portable weather station (Data Logger LI-1400, U.S.A.). The intensity of near-UV (UV-A and UV-B) radiation was determined using a UV radiometer (TKA-PKM 12, Russia). The thalli were cleaned to remove substrate residues and transported to the laboratory, where air-dry thalli were stored in the dark at 4 °C.

Experiment design

Before starting the UV radiation treatment, the thalli were moistened and kept for 3 d under fluorescent lamps (Philips TL-D Aquarelle) at 80 μmol m⁻² s⁻¹ (10/14 h photoperiod, 25 °C). After that, the thalli were exposed to UV from lamps (LER40, Russia) for 10 d (2 h d⁻¹). The peak emission spectrum of the lamp was 315 nm and the UV-B irradiation intensity was 2 W m⁻². Consequently, the thalli of lichens received about 14 kJ d⁻¹ of the UV-B light. That was an environmentally realistic dose, corresponding to the daily dose of UV-B radiation that reaches open ground in this region on a sunny summer day.

The effect of UV-B treatment on thalli was analyzed after 1, 3, and 10 d, which corresponded to the total dose of UV-B radiation of 14, 43, and 144 kJ, respectively. The control thalli were not treated with UV radiation.

Biochemical analyses

The level of lipid peroxidation was estimated according to the method of Heath and Packer (1968) by assaying the thiobarbituric acid reactive substances (TBARS). The concentration of H₂O₂ was measured using the method of Bellincampi et al. (2000). The activity of SOD was determined by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Beauchamp, Fridovich, 1971). The protein content was determined according to the method of Bradford (1976) with bovine serum albumin taken as the standard.

The respiration rate (\(V_{\text{total}}\)) was determined by performing the polarographic measurement of \(O_2\) uptake using the Oxytherm System Clark electrode (Hansatech Instruments, U.K.). The marginal regions of the lichen thalli were used. The cytochrome (\(V_{\text{cyt}}\)) and alternative respiratory capacities (\(V_{\text{alt}}\)) and their ratio were estimated using specific inhibitors (Bahr, Bonner, 1973). The inhibitors – 6 mM benzhydroxamic acid (Lancaster, U.K.) and 2 mM KCN (Sigma Aldrich,
St. Louis, MO, U.S.A.) – were added successively after measuring the total O\textsubscript{2} uptake rate.

\textit{Statistical analysis}

The results are presented as means with standard errors (SE) of n (n = 3–8) for the control and for samples exposed to each UV-B dose. After checking for normal distribution of variables (Shapiro-Wilk's test), data were analyzed using one-way ANOVA followed by post hoc multiple range testing (Duncan's test, \( P < 0.05 \)). Tests were conducted using Statistica 10.0 software (StatSoft. Inc., U.S.A.).

\textbf{Results}

\textit{Microclimatic conditions at the sample sites}

The habitats of the lichens studied in the present work differed in the amount and quality of light (Table 1). In sunny weather, \textit{P. rufescens} thalli in the meadow were exposed to PAR intensity that was 5 times higher and UV (A+B) intensity that was one order of magnitude higher compared to \textit{P. aphthosa} in the forest. In the meadow, the air warmed up to an average of 31 °C, while in the forest, the air temperature was 10 °C lower.

\textit{Level of lipid peroxidation}

The widely used thiobarbituric acid-reactive-substances (TBARS) assay measures free malondialdehyde (MDA). MDA is largely the product of peroxidation of fatty acids with more than two double bonds. We found that the content of TBARS in the control thalli of \textit{P. rufescens} was 3 times greater than in \textit{P. aphthosa} (Table 2) indicating that UV-B exposure increased fatty acid peroxidation in a species-specific way. After 3 d, the TBARS content in \textit{P. aphthosa} increased by 33 % compared with the control and did not change after that, until the end of the

| Parameter                     | Meadow       | Forest      |
|-------------------------------|--------------|-------------|
| PAR, \( \mu \text{mol photons (m}^{-2}\text{s}^{-1}) \) | 1461 ± 42    | 311 ± 18    |
| Intensity of UV (A+B) radiation, \( \text{W m}^{-2} \) | 26.9 ± 0.5   | 1.4 ± 0.1   |
| \( T_{\text{air}}, ^\circ\text{C} \) | 31.1 ± 0.3   | 21.2 ± 0.4  |
| RH, %                         | 35.7 ± 2.3   | 40.3 ± 1.4  |

| Total dose of UV-B, kJ (day) | \textit{Peltigera aphthosa} | \textit{Peltigera rufescens} |
|------------------------------|-----------------------------|-----------------------------|
|                              | TBARS, \( \text{nmol g}^{-1} \text{DW} \) | \( \text{H}_2\text{O}_2, \text{µmol g}^{-1} \text{DW} \) | TBARS, \( \text{nmol g}^{-1} \text{DW} \) | \( \text{H}_2\text{O}_2, \text{µmol g}^{-1} \text{DW} \) |
| Control                      | 150.0 ± 3.2\textsuperscript{a} | 41.4 ± 3.0\textsuperscript{a} | 464.7 ± 9.2\textsuperscript{a} | 36.0 ± 1.4\textsuperscript{c} |
| 14 (1)                       | 143.7 ± 3.3\textsuperscript{a} | 40.5 ± 1.1\textsuperscript{b} | 487.5 ± 9.3\textsuperscript{a} | 42.3 ± 1.8\textsuperscript{b} |
| 43 (3)                       | 196.0 ± 7.0\textsuperscript{b} | 50.7 ± 1.3\textsuperscript{b} | 540.6 ± 21.8\textsuperscript{d} | 48.5 ± 1.5\textsuperscript{b} |
| 144 (10)                     | 196.2 ± 6.2\textsuperscript{b} | 30.3 ±0.5\textsuperscript{b} | 473.9 ± 3.9\textsuperscript{a} | 43.7 ± 1.8\textsuperscript{b} |

Lipid peroxidation products (TBARS) and \( \text{H}_2\text{O}_2 \) content are presented as means ± SE (n = 3–4). Significant differences between values depending on total UV-B dose are indicated by different superscript letters (one-way ANOVA, Duncan test, \( P < 0.05 \)). DW – dry weight.
experiment. By contrast, in P. rufescens, after 3 d, the TBARS content was only 16 % higher than in the control but did not differ from the control after 10 d.

Hydrogen peroxide content

The lichen species did not significantly differ in the hydrogen peroxide levels (Table 2). The maximum accumulation of H$_2$O$_2$ (48–50 µmol g$^{-1}$ DW) in the thalli of both lichen species occurred after 3 d, and it was 20–25 % higher than in the control. The H$_2$O$_2$ content decreased by 40 % in P. aphthosa and did not change in P. rufescens after 10 d of UV-B exposure.

Superoxide dismutase activity

The control thalli of P. aphthosa and P. rufescens showed similar levels of SOD activity (Table 3). The UV-B treatment did not affect the SOD activity in P. aphthosa thalli. We observed a significant increase in the SOD activity in P. rufescens thalli after 3 and 10 days of the UV-B exposure. After 10 d of UV-B treatment, the enzyme activity in the thalli of P. rufescens was 35 % higher than in the control.

Respiration

Total O$_2$ uptake rate in the control and UV-treated thalli of P. rufescens was more than 1.5 times higher than in P. aphthosa (Table 4). UV-B radiation significantly affected the total O$_2$ uptake rate and the proportions of the activities of the respiratory pathways. A decrease in total O$_2$ uptake rate and in cytochrome pathway (CP) activity was observed immediately after 1 d in both species. The alternative pathway (AP) capacity increased and the CP/AP ratio decreased by 2.5 times compared to the control samples. After 3 d, total O$_2$ uptake increased, but the CP/AP

### Table 3. Superoxide dismutase activity (units mg$^{-1}$ protein) in the thalli exposed to UV-B radiation

| Total dose of UV-B, kJ (day) | Peltigera aphthosa | Peltigera rufescens |
|-----------------------------|--------------------|--------------------|
| Control                     | 12.2 ± 0.3$^a$     | 11.1 ± 0.3$^a$     |
| 14 (1)                      | 11.9 ± 0.2$^a$     | 11.0 ± 0.2$^a$     |
| 43 (3)                      | 12.0 ± 0.2$^a$     | 12.8 ± 0.3$^b$     |
| 144 (10)                    | 11.8 ± 0.1$^a$     | 17.3 ± 0.5$^c$     |

Enzyme activity is presented as means ± SE (n = 3–4). Other designations as in Table 2.

### Table 4. Influence of UV-B radiation on the total respiration rate and the activities of the cytochrome and alternative respiratory pathways in lichen thalli (nmol O$_2$ g$^{-1}$ DW min$^{-1}$)

| Total dose of UV-B, kJ (day) | Peltigera aphthosa | Peltigera rufescens |
|-----------------------------|--------------------|--------------------|
|                             | $V_{total}$        | $V_{cyt}/V_{alt}$  | $V_{total}$        | $V_{cyt}/V_{alt}$  |
| Control                     | 939 ± 39$^b$       | 3.6 ± 0.7$^b$      | 1306 ± 56$^a$      | 3.3 ± 0.5$^b$      |
| 14 (1)                      | 740 ± 34$^a$       | 1.3 ± 0.2$^{ab}$   | 1153 ± 7$^b$       | 1.5 ± 0.2$^a$      |
| 43 (3)                      | 1042 ± 39$^b$      | 1.3 ± 0.3$^{ab}$   | 1343 ± 51$^a$      | 1.4 ± 0.1$^a$      |
| 144 (10)                    | 660 ± 47$^a$       | 0.9 ± 0.1$^a$      | 1069 ± 73$^b$      | 1.3 ± 0.1$^a$      |

O$_2$ uptake rate ($V_{total}$), cytochrome and alternative respiratory pathway activities ratio ($V_{cyt}/V_{alt}$) are presented as means ± SE (n = 5–15). Other designations as in Table 2.
ratio did not change. After 10 d, total O\textsubscript{2} uptake rate was 35 % lower than after 3 d and 30 % lower than in the control thalli of *P. aphthosa*. The decrease in *P. rufescens* respiration rate was less pronounced. The value of CP/AP ratio remained low in both species.

In the control thalli of both species, the proportion of CP accounted for more than 60 % of the total respiration rate (Figure). The contribution of AP to total O\textsubscript{2} uptake was only 20 %. Increased involvement of the low energy effective alternative pathway and a decrease in the main (cytochrome) pathway contribution to the total respiration were observed in UV-B treated thalli. The CP contribution decreased on average by 1.4 times and the AP contribution doubled after 10 d of exposure to UV-B radiation. The contributions of AP to the total respiration of *P. aphthosa* and *P. rufescens* thalli were 44 % and 35 %, respectively.

![Figure](image.png)

**Figure.** Effect of UV-B radiation on the relative contribution of each respiratory pathway to total respiration in the lichens *A) Peltigera aphthosa* and *B) Peltigera rufescens*. The contributions of the cytochrome (black) and alternative pathways (grey) and residual respiration (white) to total respiration are shown. Different letters in each column indicate significant differences between the control and different UV-B doses (one-way ANOVA, Duncan test, \( P < 0.05 \)). 0 – Control samples without UV-B exposure. 14, 43, 144 – The total dose of UV-B radiation received by thalli on the first, third and tenth days of exposure, respectively.
Discussion

The aim of this work was to obtain information on the effects of UV-B radiation on the metabolism of *P. aphthosa* and *P. rufescens*. In nature, the lichen thalli grow at different levels of insolation and temperature (Table 1). High insolation, especially the high-energy short-wavelength photons, increases the generation of ROS, which causes oxidative stress in living cells. The effect of high-energy photons on lichens depends on their state. It is believed that ROS can attack membrane lipids, proteins, and other biologically significant molecules in the wet lichens. The dry lichens are inactive and resistant to extreme conditions. They retained their vital functions even after exposure to open space conditions (De Vera et al., 2008; Sánchez et al., 2014). However, *Cladonia arbuscula* ssp. *mitis* (Sandst.) Ruoss was sensitive to UV-B irradiation in the air-dried state and was not able to completely repair the DNA damage (Buffoni Hall et al., 2003). Furthermore, temperature and light intensity played a role in the capacity of the lichen to self-repair the damage.

Our results show that the two lichens studied here exhibited different physiological responses to an environmentally realistic dose of UV-B. We found that *P. rufescens* thalli had a higher TBARS content but accumulated considerably smaller amounts of lipid peroxidation products under UV-B treatment compared to *P. aphthosa* (Table 2). This is most likely due to the adaptation of *P. rufescens* to brighter light and higher temperature conditions in its microhabitat. However, interestingly, an increase in H$_2$O$_2$ content in *P. rufescens* thalli was observed shortly after exposure to UV-B light while in *P. aphthosa*, the H$_2$O$_2$ content increased significantly only after 3 d, when the total UV-B dose was around 40 kJ.

The control thalli of both species did not differ in SOD activity. SOD is highly efficient in catalytic removal of the superoxide radical (O$_2^-•$). SOD catalyzes the dismutation of O$_2^-•$ to H$_2$O$_2$ and O$_2$. H$_2$O$_2$ is a relatively stable compound, which regulates many cell processes. We did not find a direct relationship between changes in the SOD activity and the content of H$_2$O$_2$ following UV-B exposure. The UV-B treatment had no effect on SOD activity in *P. aphthosa* thalli. Rehydration of desiccated lichens did not cause any response or decrease in SOD activity (Weissman et al., 2005). A «burst-like» formation of ROS and, in particular, the superoxide radical, was observed in lichens after a long desiccation period (Beckett et al., 2003). Probably, any upregulation of SOD during rehydration was suppressed due to inactivation by high ROS concentrations in thalli. The increase in TBARS content in *P. aphthosa* thalli was probably a result of the accumulation of ROS. Lichens can contain from six to ten Fe-, Cu/Zn-, and Mn-SOD isoforms (Schlee et al., 1995; Weissman et al., 2005). UV could have different effects on the activity of different SOD isoforms, which resulted in the absence of the pronounced effect on the total SOD activity. A significant increase in the SOD activity in *P. rufescens* thalli was observed after 3 d of the treatment. SOD activity was maximal after 10 d of exposure, when the total UV-B dose reached 144 kJ, indicating that UV-B increased the capacity of this species to dismutate O$_2^-•$ into more stable H$_2$O$_2$. A more rapid conversion of O$_2^-•$ to H$_2$O$_2$ can contribute to a more successful defense of cells from the most active ROS. At the same time, an increase in the H$_2$O$_2$ content also contributes to the development of oxidative stress, and future work needs to focus on the effect of UV-B on the enzymes, such as catalase, that metabolize H$_2$O$_2$.

Respiration is a crucial process that provides all living organisms with energy and metabolites for growth and cellular maintenance. We found that UV-B treatment had a significant effect on the total respiration rate and CP/AP
ratio in the thalli of both lichen species studied here (Table 4). The activity and proportion of AP in total respiration increased, whereas the contribution of CP decreased (Figure), suggesting that the UV-B treatment activated the energy dissipation processes. The effect of UV-B on the respiratory pathway ratio was more pronounced in *P. aphthosa* than in *P. rufescens*. We noted the same reaction to UV-B in *Cladonia stellaris* (Opiz) Pouzar & Vezda (Shelyakin et al., 2018). The change in the ratio of the respiratory pathways in UV-B treated lichens is probably a common event. The activation of AP may prevent an excessive reduction in the mitochondrial electron transport chain and the development of oxidative stress in cells. The role of the alternative respiratory pathway as a component of the ROS-scavenging system in lichens has already been discussed by other researchers (Beckett et al., 2008). Since the fungal component accounts for more than 90% of the lichen thalli, we assume that the mycobiont is the main cause of the changes in the ratios of the respiratory pathways found here. The question of the contribution of mycobiont and different types of photobiont to the total respiration, peroxide accumulation, and the activity of antioxidant enzymes in lichens needs further investigation.

**Conclusion**

The results demonstrate that the response to UV-B radiation of the antioxidant system and respiration of lichens is species-specific. *P. rufescens*, growing in the open habitat, accumulates greater amounts of products of lipid oxidation and has a higher rate of respiration than *P. aphthosa*, the species from the more shaded forest habitat. This may indicate that *P. rufescens* thalli experience a higher oxidative stress under normal field conditions. Exposure to UV-B of *P. rufescens* thalli increased SOD activity, while in *P. aphthosa*, UV radiation did not affect SOD activity but increased lipid peroxidation. Thus, the tolerance to oxidative stress of the two species is different, probably because of the dissimilarities in the temperature and light conditions in typical habitats of these species. However, in both species, particularly in *P. aphthosa*, UV-B increased the activity and contribution of energy dissipative alternative respiratory pathway. Such changes in respiration are likely a universal response of lichens to the oxidative stress.

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