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Iron and phosphate uptake in epiphytic and saxicolous lichens differing in their pH requirements

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ABSTRACT

The hypothesis is tested that pH-dependent Fe and P uptake influence the preference of epiphytic and saxicolous lichens for certain ranges of ambient pH. Five species from acidic substrata (*Hypogymnia physodes*, *Parmeliopsis ambigua*, and *Platismatia glauca*) or covering the range from weakly acidic to alkaline substrata (*Lecanora muralis* and *Phaeophyscia orbicularis*) were exposed to solutions of $FeCl_2$, $FeCl_3$, or KH_2PO_4 at pH 3 and 8 in the laboratory. Avoidance of alkaline substrata is explainable by low Fe^{3+} uptake at pH 8 in the case of *H. physodes* and the inability for net P uptake and membrane damage in *P. ambigua* at this pH. Preference for acidic substrata in *Pl. glauca*, however, is neither related to Fe nor P uptake. Efficient Fe^{3+} and P uptake at pH 8 explains the tolerance of *L. muralis* and *Ph. orbicularis* to alkaline conditions. Intracellular accumulation of Fe^{2+} in probably toxic amounts at pH 3 in *Ph. orbicularis* is correlated with the absence of this lichen from strongly acidic substrata. Avoidance of acidic sites by *L. muralis* is not attributable to Fe or P uptake. In summary, the results suggest that pH-dependent Fe and P uptake characteristics are involved in the determination of pH preferences of epiphytic and saxicolous lichens, but are not the only relevant factor.

1. Introduction

Lichen vegetation is strongly influenced by the pH of the substratum, but mechanisms causing these pH preferences are little understood. Recently, Paul et al. (2009) showed that the calcifuge–calcicole behavior of two terricolous lichens, *Cladonia furcata* subsp. *furcata* and *C. rangiformis*, was caused by the pH dependency of Fe and P uptake. This parallels with the causes of calcifuge–calcicole behavior in vascular plants (Tyler, 1996; Zohlen and Tyler, 2000). Parallels between lichens and vascular plants are limited, though, as mineral uptake in lichens is not restricted to the substratum, but also takes place from atmospheric sources (Nash, 2008). Furthermore, pH dependence in lichens is not only found in species growing on acidic or calcareous soil, but also in saxicolous and epiphytic taxa. While many lichen species are strongly specific for either bark (and wood) or rock, others have only a preference for either type of substratum. Thus, there is a significant overlap between epiphytic and saxicolous species (Purvis et al., 1992; Wirth, 1995). Lichen species regularly occurring both on soil and bark or soil and rock are much rare, though.

Epiphytes differ from terricolous and strictly saxicolous taxa by the lack of calcifuge or calcicole species in the strict sense, as alkaline bark or wood substrata are largely absent (Wirth, 1995). Even bark impregnated by alkaline dust is usually slightly acidic or, if at all, neutral (Gilbert, 1976; Van Herk, 2001; Marmor and Randlane, 2007). In the acidic range, considerable variation of bark pH is found. In areas with significant acidic air pollution, pH values between 3 and 4 are common and even bark of pH < 3 is occasionally found, especially in conifers (Gauslaa and Holien, 1998; Hauck et al., 2001; Schmull and Hauck, 2003). Many broad-leaved tree species (Gauslaa, 1995; LaGreca and Stutzman, 2006) or conifers in unpolluted areas (Hauck and Spribille, 2005; Hauck and Javkhlan, 2009) have bark pH values between 4 and 5.5. Some genera of broad-leaved trees, including *Acer*, *Populus*, and *Ulmus*, harbor species with bark of pH values up to 7 or 7.5 (Wirth, 1995; Mežaka and Znotiņa, 2006). Most epiphytic lichens are strongly bound to certain pH intervals within the range from around 2.5–7.5 (Wirth, 1995; Gauslaa and Holien, 1998).

Published approaches to explain the preferences of epiphytic or saxicolous lichens for certain pH ranges involve the hydrophobicity of the thallus surface and the content of lichen secondary metabolites. Surface hydrophobicity is applicable to explain the tolerance of lichens to acidic solution deriving from the atmosphere or dissolved from the substratum (Shirtcliffe et al., 2006; Hauck et al., 2008). Hydrophobicity is a pure avoidance strategy preventing lichens from being soaked with solutions of pH values below the plasmatic tolerance of the lichens. Lichen substances exert more sophisticated effects on the formation of pH preferences, as they interact biochemically with the acidity of water films covering the

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lichen thalli. Lichen substances can act as protonophores limiting the acidity tolerance of lichens (Abo-Khatwa et al., 1996; Hauck and Jürgens, 2008). Moreover, pH-dependent metal binding (Takani et al., 2002) is apparently involved in the metal homeostasis of lichens (Hauck, 2008; Hauck et al., 2009a) and is thereby responsible for interrelations between ambient pH and the availability of nutrients or toxic metals (Hauck et al., 2009b). In the present paper, laboratory experiments with five lichen species were carried out to test the hypothesis that pH dependence of Fe and P uptake is not only responsible for the calcifuge–calcicole behavior of terricolous lichens, but also contributes to the determination of pH preferences in epiphytic and saxicolous lichens. Verification of this hypothesis for epiphytes would imply that Fe and P uptake would determine the preference for much narrower pH intervals than suggested by calcifuge–calcicole behavior of terricolous or saxicolous lichens.

2. Materials and methods

2.1. Study species

Five foliose lichens of the most common type of the lichen symbiosis, i.e. symbioses of lichen-forming ascomycetes of the order Lecanorales and unicellular green algae of the genus *Trebouxia* (Friedl and Büdel, 2008), were selected for the experiments. *Hypogymnia physodes* (L.) Nyl., *Parmeliopsis ambigua* (Wulfen) Nyl., and *Platismatia glauca* (L.) W. Culb. & C. Culb. are markedly acidophytic epiphytes, more rarely also growing on siliceous rock or, in the case of *H. physodes*, even sandy soil (Purvis et al., 1992; Wirth, 1995; Hauck, 1996). *Lecanora muralis* (Schreb.) Rabenh. and *Phaeophyscia orbicularis* (Necker) Moberg prefer slightly acidic to alkaline environments, including calcareous rock, artificial lime-containing substrata, like concrete, or more rarely nutrient-rich siliceous rock (Purvis et al., 1992; Cezanne et al., 2008). *Ph. orbicularis* is also regularly found on nutrient-rich slightly acidic to neutral bark of broad-leaved trees, whereas *L. muralis* only rarely occurs at the base of broad-leaved trees or wood exposed to alkaline dust (Purvis et al., 1992; Wirth, 1995; Hauck, 1996). Lichen samples for the experiments were collected in a radius of 60 km from Göttingen, northern Germany. *H. physodes* and *Pl. glauca* were sampled on *Quercus*, whereas *L. muralis* derived from a wall of variegated sandstone (Buntsandstein), *P. ambigua* from *Picea abies*, and *Ph. orbicularis* from a concrete wall. Particles of the substratum were carefully removed from the lichen thalli in the laboratory with a scalpel.

2.2. Experimental

Lichens were stored in air-dry condition at a temperature of 18 °C in the dark before the experiment. Freezing is not harmful to dry lichen thalli and is routinely applied in our work group for storing lichens prior to experiments. After thawing, thallus pieces of c. 2 cm^2 were put on Petri dishes with moist cellulose filters. Particles of the substratum and contaminant plant material were carefully removed. Five thallus pieces were put on each Petri dish, and five of these dishes served as replicates. The Petri dishes were stored for 2 days at 80% relative humidity, a day temperature (for 13 h daily) of 13 °C during a photon flux of 30 $\mu mol\ m^{-2}\ s^{-1}$, and a night temperature of 10 °C in the growth chamber for acclimatization. Lichen samples were then exposed for 1 h to 30 ml of salt solution or to a control of deionized water on a shaker. Metal solutions included $FeCl_2$, $FeCl_3$, and KH_2PO_4 adjusted with HCl and NaOH to pH 3 or 8. The activities of the Fe^{2+}, Fe^{3+} or $H_2PO_4^-$ ions in the solutions amounted to 2 mM. Calculations of activities were carried out with the software PHREEQC 2.0 (U.S. Geological Survey, Reston, Virginia, USA). Following the Henderson–Hasselbalch equation, the ratio of $H_2PO_4^-$ to PO_4^{3-} amounted to 13,490 at pH 3, whereas the PO_4^{3-} ion amounted at pH 8 with a ratio to $H_2PO_4^-$ of 7.4. Throughout the paper, we refer to $H_2PO_4^-$ and PO_4^{3-} as phosphate or P irrespective of the degree of dissociation.

Viability of lichen thalli was controlled by measuring the chlorophyll fluorescence yield (Φ_2) of the light-adapted samples at photosystem II prior to and after the exposure to the test solutions with a PAM-2100 chlorophyll fluorometer (Walz, Effeltrich, Germany). Two measurements were made per replicate. Φ_2 stayed invariably in a range between 0.64 and 0.69, irrespective of the treatment or the point in time, and the values are, therefore, not displayed in Section 3.

After the exposure to iron, phosphate or water, extra- and intracellular cations were sequentially extracted (Brown and Brown, 1991; Hauck et al., 2002). For this purpose, samples were shaken twice with 30 ml of deionized water to remove free apoplastic ions. These water samples were not analyzed. Metal ions bound to hydroxylic or carboxylic exchange sites of the cell wall were exchanged by shaking samples twice each for 20 min with 30 ml 20 mM $NiCl_2$. The two $NiCl_2$ solutions per sample were filtered with ash-free filters (Blue Ribbon Filters, Whatman-Schleicher & Schuell, Dassel, Germany) and pooled for analysis. Afterwards, samples were dried at 105 °C, homogenized, and digested with 65% HNO_3 in order to determine the intracellular ions. Total concentrations of Fe, P and additionally K, Ca, and Mg in the $NiCl_2$ solutions and acid digests were determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES, Spectraflame, Spectro Analytical Instruments, Fitchburg, Massachusetts, USA). Cross-analyzed plant material was used as an internal standard. The measuring error inherent to the ICP-AES amounted to <1% for Fe, <2% for P and <3% for K, Ca, and Mg. A quantitative release of phosphate from the apoplast by the Cl^- ions of the $NiCl_2$ solution was not expected despite the high concentration of the $NiCl_2$ solution, because Cl^- is a weak competitor for potential cationic binding sites in the apoplast. Therefore, P concentrations of $NiCl_2$ and HNO_3 fractions were summed to determine the total P concentration.

2.3. Statistics

All data are given as arithmetic means ± standard error and were tested for normal distribution with the Shapiro-Wilk test. The effect of the Fe or P treatment and the pH on element concentrations in the lichen thalli was quantified using two-way analysis of variance (ANOVA). Statistical analyses were calculated with SAS 6.04 software (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Iron uptake

The acidophytic epiphyte *H. physodes* readily took up Fe^{2+} at pH 3 and 8 as well as Fe^{3+} at pH 3, whereas intracellular Fe^{3+} uptake was low at pH 8 (Tables 1 and 2). Extracellular Fe^{3+} adsorption was also limited at pH 8 (Tables 3 and 4). The two other acidophytes, *P. ambigua* and *Pl. glauca*, principally took up Fe^{2+} and Fe^{3+} at both pH values, but uptake of Fe^{2+} at pH 8 and Fe^{3+} at pH 3 significantly exceeded that of Fe^{2+} at pH 3 and Fe^{3+} at pH 8 (Tables 1 and 2). Further, these two species were characterized by (1) extracellular adsorption of significant amounts of Fe^{2+} and Fe^{3+} at pH 3, (2) reduced intracellular K concentrations due to Fe^{3+} uptake at pH 8, and (3) strong losses of extracellular Ca in both Fe treatments at pH 3 (Tables 1–4). *Pl. glauca* differed from *P. ambigua* by adsorbing more Fe^{2+} at pH 8 in the apoplast and by reduced intracellular Mg concentrations due to Fe^{3+} uptake at pH 8.

In the slightly acidic to alkaline substrata preferring *L. muralis*, Fe was readily taken up intracellularly, but poorly adsorbed extracellu-