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Investigations of Thiols in Lichens

By

I. KRANNER[1)], H. GUTTENBERGER[1)], D. GRILL[1)], M. DELEFANT[1)] & R. TÜRK[2)]

K e y w o r d s : Glutathione, altitude, seasonal variations.

S u m m a r y

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Glutathione (γ-glutamyl-cysteinyl-glycine) was the most abundant low molecular weight thiol in lichens and only traces of cysteine and (γ-glutamyl-cysteine) were found. For lichens growing on trees, it was shown that within one species the amount of glutathione was independent of the bark substrate. Lichens showed seasonal variations in glutathione content with a minimum in spring and autumn. Glutathione content also varied with the altitude: lichens collected from 1400 m above sea level contained the highest content.

Introduction

Many lichens show the remarkable ability to survive total desiccation. After rehydration, they are able to start their metabolic processes rapidly. The possibility to survive under drought conditions requires protection of proteins against denaturation. Thiols may play an important role during frost and drought stress (LEVITT 1980, DANIEL & GAFF 1980, STUIVER & al. 1988) by preventing irreversible formation of disulfide bridges of proteins. Additionally, oxidized glutathione is thought to mediate the drought stress induced inhibition of protein synthesis (FAHEY & al. 1980, DHINDSA 1987, 1991). We have investigated the content of low molecular weight thiols in lichens collected from different tree species and from different altitudes in order to obtain insight into thiol metabolism of these poicilohydric organisms.

[1)] Institute of Plant Physiology, Univ. Graz, Schubertstraße 51, A-8010 Graz.

[2)] Institute of Plant Physiology, Univ. Salzburg, Hellbrunnerstraße 34, A-5020 Salzburg.

Materials & Methods

Samples of *Pseudevernia furfuracea* (L.) Zopf, *Hypogymnia physodes* (L.) Nyl., *Cetraria islandica* (L.) Ach. and *Usnea filipendula* Stirton were collected from Salzstiegel, Austria. After air drying for some days they were stored at -25 °C. Samples were frozen in liquid nitrogen and pulverized with mortar and pestle. Low molecular weight thiols were separated and quantified by reversed phase HPLC after derivatisation with monobromobimane according to SCHUPP & RENNENBERG 1988. To estimate the proportion of algae and fungi, pulverized samples of *Usnea filipendula* were fractionated by using sieves with different porosity (0.5, 0.2, 0.1 and 0.05 mm) as described by GUTTENBERGER & al. 1989. The fraction > 0.5 mm contained 100 % hyphes, the fraction < 0.05 mm contained 100 % algae; all other fractions contained a mixture of both.

Results and Discussion

There were only slight differences in thiol content of the collected lichen species. Even though in some samples traces of cysteine and γ-glutamyl-cysteine were present, glutathione was the most abundant thiol in all lichen species, its content ranged from 420 to 620 nmol g dry weight^{-1}. *Cetraria islandica*, a terrestrial species, also contained 400 nmol g dry weight^{-1} glutathione (data not presented). For lichens growing on trees, it was shown that within one species the amount of glutathione was independent of the bark substrate (Fig. 1).

By fractionated sieving of powdered *Usnea filipendula* samples we tried to estimate the share of low molecular weight thiols of both algae and fungi separately. On a dry weight basis 1 g fungi contained a 15-fold higher content of glutathione than 1 g algae. (Fig. 2).

In order to investigate seasonal variations, we measured the glutathione content of *Pseudevernia furfuracea*, harvested from *Picea abies*, monthly. Glutathione content of *Pseudevernia* showed a minimum in spring and in autumn (Fig. 3), which is different from seasonal variations in glutathione content in spruce needles (*Picea abies*). The latter showed high glutathione concentrations during winter and early spring and low concentrations in summer (ESTERBAUER & GRILL 1978). They suggested that glutathione and glutathione reductase may play an important role for the winter hardiness of the tree.

In *Pseudevernia furfuracea* the glutathione content varied at different altitudes. The highest content was measured in samples collected at 1400 m above sea level (a.s.l.) (Fig 4). At lower and higher altitudes than 1400 m a.s.l. substantial lower glutathione contents were present. The relation between altitude and thiol content was observed for lichens collected from different locations all over Austria (GUTTENBERGER & al. 1991). The altitude dependency of the thiol content in the poicilohydric lichens is similar to that found in homoiohydric spruces (GRILL & al. 1988). The high glutathione content of lichens at 1400 m a.s.l. might be induced as a response to multiple stress, which may occur at this altitude.

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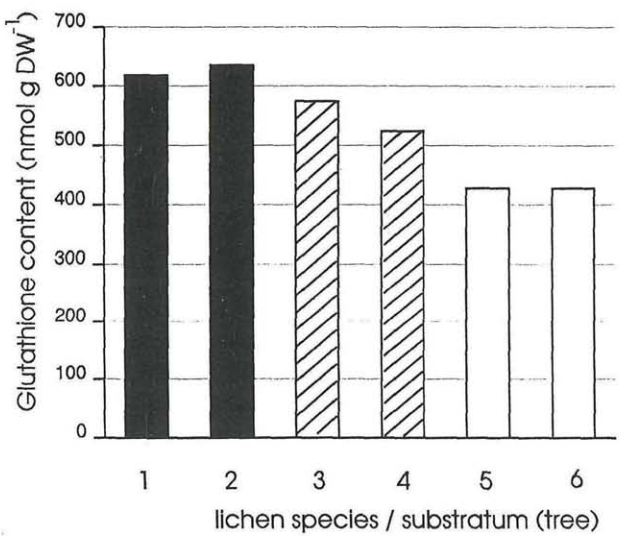

Fig. 1. Glutathione content of three lichen species, growing on *Acer pseudoplatanus* (1,3,5) and *Picea abies* (2,4,6), 1300 m a.s.l. Black: *Usnea filipendula* (1,2), hatched: *Hypogymnia physodes* (3,4) and white: *Pseudevernia furfuracea* (5,6). All values are means of three experiments. The standard deviation ranged between 12 and 17 % of mean.

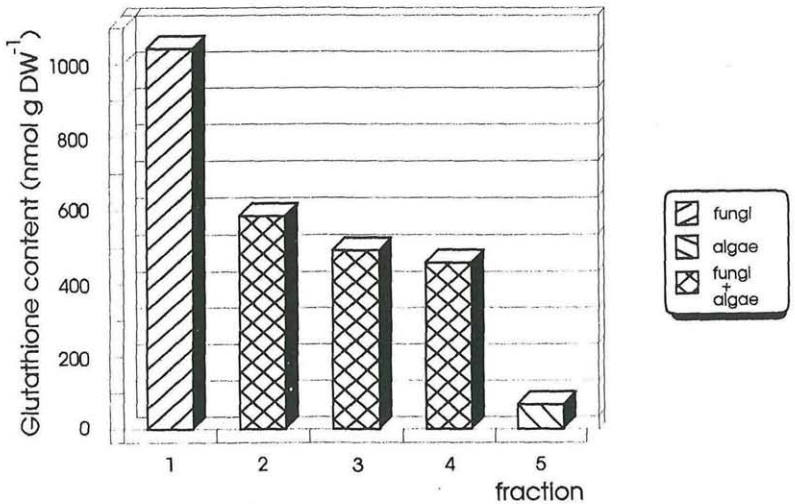

Fig. 2. Glutathione content of algae and fungi of fractionated *Usnea filipendula*, harvested from *Larix decidua*, 1500 m a.s.l. The glutathione content of each fraction is shown in nmol g dry weight^{-1}. On a dry weight basis, fraction 1 made up 26 % of the whole lichen and contained only fungi. The fractions between 2 and 4 contained a mixture of algae and fungi with decreasing percentage of fungi. Fraction 2 made up 40 %, fraction 3 21 % and fraction 4 12 % of the intact lichen. Fraction 5 contained only algae and made up 1.6 % of the intact lichen. All values are means of three experiments. The standard deviations ranged between 7 and 13 % of mean.

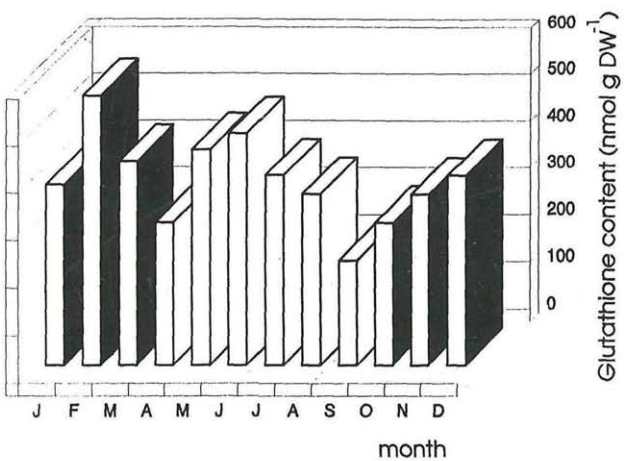

Fig. 3. Seasonal variations of glutathione content in *Pseudevernia furfuracea*, growing on *Picea abies*. Black: winter season, white: summer season. All values are means of three experiments and the standard deviations ranged between 20 and 25 % of mean.

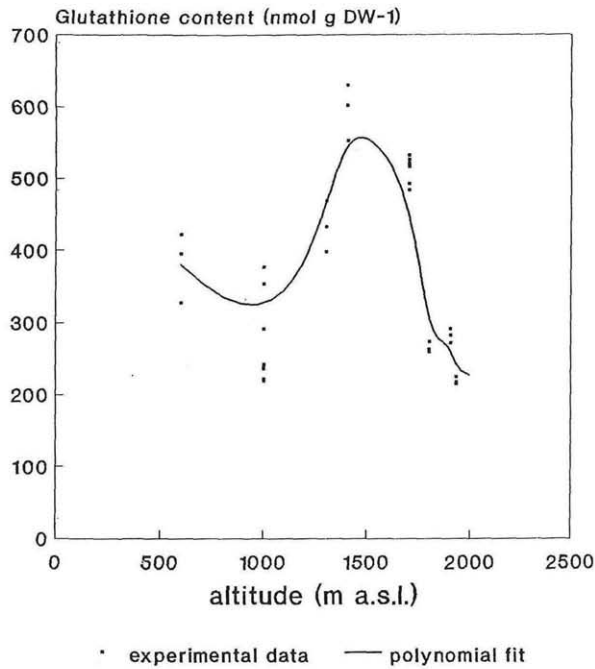

Fig. 4. Glutathione content of *Pseudevernia furfuracea*, growing on *Picea abies*, at various altitudes.