Phyton (Austria)	**Vol. 27**	Fasc. 2	311–317	30. 11. 1987

Further Investigations on the Thiol Content of Norway Spruce Needles

By

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Received April 2, 1987

Key words: Thiols, glutathione, light conditions, needle age, spruce, *Picea abies*.

Summary

GRILL D., PFEIFHOFER H. & ESTERBAUER H. 1987. Further investigations on the thiol content of Norway spruce needles. – Phyton (Austria) 27 (2): 311–317. – English with German summary.

In needles of Norway spruce (*Picea abies* [L.] KARST.) the content of water soluble thiols depends significantly on light conditions. Light exposed needles contain about 50% more thiols than shadow needles of the same tree. Needles from the top of a tree have about 30% more thiols than parts below. In each case glutathione is with 95% the main component of the thiols. The altering daily light conditions do not produce a daily rhythm of the thiol content. Obviously the plants can regulate short lasting influences. Spruces, growing under comparable ecological conditions, show only a small amplitude in the fluctuations of their thiol content. The amount of water soluble SH-compounds depends also on the needle age. The increase from the first to the fifth needle class is about 60% and then the value-continues in the further needle classes (related to the fresh weight). If the SH-content is related to dry weight the thiol increase continues however. Glutathione makes up more than 95% of the water soluble thiols in these needles too.

Zusammenfassung

GRILL D., PFEIFHOFER H. & ESTERBAUER H. 1987. Weitere Untersuchungen über den Thiolgehalt von Fichtennadeln. – Phyton (Austria) 27 (2): 311–317. – Englisch mit deutscher Zusammenfassung.

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Der Gehalt an wasserlöslichen Thiolen in Nadeln der Fichte (*Picea abies* [L.] KARST.) ist signifikant von den Lichtverhältnissen abhängig. Lichtexponierte Nadeln enthalten ca. 50% mehr Thiole als Schattennadeln des gleichen Baumes, Nadeln der Wipfelregion sind um ca. 30% thiolreicher als die aus tieferliegenden Kronenregionen. Stets stellt Glutathion mit 95% die Hauptkomponente an Thiolen dar. Die mit den Tageszeiten wechselnden Lichtverhältnisse rufen keinen Tagesrhythmus des Thiolgehaltes hervor. Kurzfristigen Streß vermögen die Pflanzen offenbar auszugleichen. Fichten zeigen unter vergleichbaren ökologischen Bedingungen nur geringe Schwankungen des Thiolgehaltes. Vom ersten zum fünften Nadeljahrgang steigen die auf das Frischgewicht bezogenen Thiolgehalte um ca. 60% an und bleiben weiterhin konstant. Auch bei Bezug auf das Trockengewicht ergibt sich ein Anstieg. Auch in diesen Nadeln macht Glutathion 95% der wasserlöslichen Thiole aus.

Introduction

The water-soluble thiol (-SH) content of needles of spruce (*Picea abies* [L.] KARST.) has been shown to vary seasonally. It is as much as three times higher in winter than in summer (GRILL & ESTERBAUER 1973 a, b, ESTERBAUER & GRILL 1978). Glutathione makes up more than 95% of the water-soluble -SH content, cysteine the rest. Only for a short time during flushing is the cysteine level considerably elevated. Both glutathione and cysteine are important in metabolism RACKER 1954, KROHNE-EHRLICH & UNTUCHT-GRAU 1976, (ESTERBAUER 1976, GRILL & al. 1980 RENNENBERG 1982, 1984), and they have been considered to have a protective and restorative function under conditions of stress to the plant (LEVITT 1980). Though many previous studies have been conducted under defined conditions, the situation of the individual plant has not been considered sufficiently.

Material and Methods

Plant samples were taken from spruces chosen according to the question under consideration: exposition, light conditions, age and the like. Exact descriptions of the individual trees are given with the results. Thiol content was measured by a previously described method (GRILL & ESTERBAUER 1973 a, GRILL & al. 1979). The methodic error ranges between ± 1% and ± 2.5%.

The column-chromatographic separation of cysteine and glutathione was done by a modification of a previously described method (GRILL & ESTERBAUER 1973a). The plant symples were homogenized immediately in 30 ml DTNB (Ellman reagent: 18 mg DTNB (2.2′ dinitro- 5.5′ dithiodibenzoic acid and 45 mg L (+)- ascorbic acid) dissolved in 30 ml 0.2 M sodium phosphate buffer, pH = 7.0). After filtration (602 h, S & S), 20 ml were applied to the equilibrated column (sodium phosphate buffer 0.05 M; column: DEAE Sephadex A-25). A NaCl gradient of 0.0 M–0.5 M was used for eluation. 10-ml fractions (colorless) were mixed with 0.5 M thioglycolate (1 M thioglycolic acid and KOH so that pH = 7.0). Fractions containing thiols turned yellow. Spectrophotometric measurements were done at 412 nm; a colorless fraction was used as a reference. Before measurement, it was advisable to make sure that the pH of the fractions was 7.0. As described here the method is simpler than the original procedure, and practically nothing is lost by oxidation.

A large amount of needles cannot be processed immediately and must be conserved. If spruce needles are stored in a cool (+12° C) and dark place, the change in -SH content after 1 day is within the methodic error. For longer storage the needles can be frozen for several weeks. In our study, the cut-off needles were put into smallest possible phials (plastic or glass) and shock frozen in liquid nitrogen. The phials were then sealed with a plastic plug and stored at −30° C. Variations in the -SH content lay within the methodic error. It is important that the specimens, once thawed, are processed as quickly as possible.

Results and Discussion

The influence of light on the -SH content of spruce needles was studied in sets of eight approximately 20-year-old spruces of the same afforestation. Needle samples were taken in September from trees whose midcrowns were shaded by closely neighbouring trees; these needles were considered shaded. The south sides of the same trees formed the edge of the forest and were thus exposed to the sun. Needles from the southern aspect of these trees were considered light-exposed (NAPP-ZINN 1966, SCHMIDT-VOGT 1986).

A clear difference was found between the -SH content of the two needle types. The average value of the shaded needles (0.332 ± 0.037 μmol/g fresh weight; 0.828 ± 0.096 μmol/g dry wt) was less than that of the light-exposed needles (0.488 ± 0.06 μmol/g fresh wt; 1.190 ± 0.128 μmol/g dry wt). The difference, in relation to fresh weight and dry weight, was 47% and 49%, respectively.

Studies in other years showed similar patterns (KÖLLY 1984). According to CELEDIN (unpublished data), the observed differences in -SH content between shaded and sun exposed needles agreed in relation to fresh weight (47.0% ± 10.3%), dry weight (41.1% ± 11.6%), and protein content (where the differences were almost as high). This is because the nitrogen content per gram dry weight of the sun exposed and shaded needles, which is used for calculating the protein content, differed in the same range (43.7% ± 11.9%). The fresh weight/dry weight ratio of sun exposed and shaded needles differed by less than 5%, but the difference of the thiol content was approximately 40%; thus the differing -SH values cannot be explained by differences of needle morphology or reference weights. Nutrition can also be ruled out, since the needles came from the same tree. The cause of the different -SH content of the shaded and axposed needles probably lies in the different photosyntheses of the two needle types. For the reduction of sulfate in chloroplasts, ATP is supplied for the synthesis of PAPS (3′-Phosphoadenosine – 5′-phosphosulfate) and electrons are supplied for the reduction of ferredoxin by the light reactions of photosynthesis (SCHIFF 1983). The difference in photosynthesis probably also caused the variation of -SH content as a function of height-of-insertion. We examined the needles from the southern aspect of an approximately 51-years-old, free-standing spruce in March. Needle samples were taken at 1.0–1.5 M intervals from the top of the felled tree down. Thiol content decreased from the top down (Table 1). No differences of the glutathione/cysteine ratio were found in exposed or shaded needles, or in needles from different heights. Except at flushing, glutathione made up more than 95% of water-soluble thiols. We also tried to establish whether the influence of light on -SH content is a circadian pattern or a long-term effect. On a sunny day in July, first and second-year needle samples were taken from the southern, exposed aspect of a 30-year-old spruce at 3-hour intervals between 4 o'clock and 1 o'clock the next day. Light intensity was up to 100,000 Lx. A circadian rhythm of -SH content was not found in either needle-year; fluctuations were within the methodic error.

This was unexpected, in light of the earlier results. The plant seems to be able to keep its -SH content stable in the short term. Changes in light intensity, to which the plant is constantly exposed, would otherwise cause large fluctuations in sulfate reduction. The resulting variations in the thiol/disulfide ratio could probably not be tolerated by the plant, since essential metabolic functions are extremely sensitive to even small variations in -SH concentration. Thus a mechanism for the exact regulation of the -SH content of the cells is essential to the survival of the plant in an ever changing environment. Such a mechanism might be the release of H_2S that has been described by several authors (RENNENBERG 1984, KINRAIDE & STALEY 1985, DEKOK & al. 1986).

Table 1

Thiol content of needles from a 51-year-old spruce according to height of insertion.

whorl (from the top down, 1.0–1.5 m intervals)	-SH content µmol/g fresh weight	-SH content µmol/g dry weight
2	0.665	1.191
4	0.591	1.142
6	0.573	1.113
8	0.485	0.971
10	0.537	1.092
12	0.520	1.107

The dependence of -SH content on the light exposure of the needles is apparently both a long-term metabolic adaptation and subject to short-term regulation. The physiologic consequences of an elevated -SH content cannot be evaluated here, but PFEIFHOFER & al. 1986 have found a dependence of respiration on -SH content and ambient temperature.

This regulatory mechanism is also evident when one measures the variability of water-soluble thiols in a group of similar trees. We examined 15 spruces of the same afforestation. The trees were approximately 25-years-old, standing together, at the same elevation, and all at the edge of the forest. Epidemic phytopathogens were neither found in the wood nor in the needles. Because of the reasons mentioned above, the needles were taken from the sun-exposed side of the top of the crown in October. Their -SH content was 0.382 ± 0.03 µmol/g fresh wt (± 8% variability; range, 0.323–0.430 µmol). In relation to dry weight, the average value was 0.965 ± 0.06 µmol (± 6.4% variability; range, 0.874–1.047 µmol). Hence the values in relation to dry weight showed a some-what lower variability coefficient (8.0% vs. 6.4%) and a smaller difference between minimum and maximum values (30% vs. 20%) than in relation to fresh weight. In both cases, however, it is clear that plants from comparable ecologic conditions have very similar water-soluble thiol levels. The glutathione/cysteine ratio is also the same. Despite the larger differences within the samples, the -SH content varies only within a certain range, even between different tree species (GRILL & al. 1979). Age also influences the -SH content of spruce needles. We examined needles from the crown of an approximately 80-year-old tree in January; all were light-exposed. Tab. 2 shows the age dependence of -SH content. It increased from the first to the fifth needle-year-from 0.246 to 0.410 µmol/g fresh wt. Levels stayed similarly high in the sixth to

Table 2

Thiol content of different needle-years in January.

needle class	-SH content µmol/g fresh weight	-SH content µmol/g dry weight
1	0.246	0.602
2	0.302	0.777
3	0.362	0.814
4	0.364	0.787
5	0.410	0.820
6	0.408	0.842
7	0.396	0.887
8	0.400	0.900

eighth needle-years – 60% above those in young needles. Studies at other times of year have shown a similar increase in -SH content with age (KÖLLY 1984), but the difference between the youngest and oldest needle-years was 40%–50% (cf. GRILL & ESTERBAUER 1973 b). In opposite relating the values to dry weight a further increase of thiol content is to be seen however. Such an increase of thiols is a result of changes in an aging metabolism. However, glutathione makes up over 96% of water-soluble thiols at all ages; cysteine is present only in small amounts. Though other reports have indicated that the regulation of sulfur metabolism can change with age (RENNENBERG 1984), nothing is known of evergreens. According to other authors (RENNENBERG 1982, 1984, DEKOK & al. 1986) glutathione is only a depot of reduced organic sulfur – cysteine is the active form. By releasing H_2S, plants can regulate their -SH content so as not to disturb the SH/SS ratio. Our results indicate that glutathione has more than a depot function. In certain metabolic conditions, such as age or environmental factors, glutathione may be able to protect the plant against stress (LEVITT 1980). In any case, an excess of thiols – as occurs when excess sulfur is available (e. g. SO_2) – has a detrimental effect on plant metabolism (GRILL & al. 1982; RENNENBERG 1984).

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