Phyton (Austria) Special issue: "Plant Physiology"	**Vol. 39**	Fasc. 3	(171)-(174)	30. 11. 1999

Are Winter Adaptive Changes in Glutathione and Chlorophyll Contents in Spruce Needles Triggered by Low Temperatures or Short Days?

By

Karin HERBINGER[1)], Michael TAUSZ[1)] & Dieter GRILL[1)]

Key words: *Picea abies*, seasonal variations, winter adaptation, oxidative stress, glutathione, chlorophyll.

Summary

HERBINGER K., TAUSZ M. & GRILL D. 1999. Are winter adaptive changes in glutathione and chlorophyll contents in spruce needles triggered by low temperatures or short days? - Phyton (Horn, Austria) 39 (3): (171) - (174).

Concentrations of glutathione and chlorophyll a and b in spruce needles undergo seasonal variations in the field. Most prominent changes including increases in glutathione contents take place in autumn when winter adaptation begins. Since in the field studies the factors low temperature and light period length cannot be separated, the present experiment was conducted in climate chambers. In August young trees of *Picea abies* (L.) Karst. were transferred to climate chambers conditions: One experimental variant with control temperature and short days and the second with control day length and low temperature. The conditions of the control chambers were outdoor conditions in August. Low temperature caused an increase in glutathione and decreases in chlorophyll a and b. On the contrary, short days induced a decrease in glutathione and increases in chlorophylls. The results indicate that the factor temperature might be more import for winter adaptations than the factor day length.

Introduction

Contents of glutathione and chlorophyll a and b undergo pronounced seasonal rhythms in spruce needles (*Picea abies* (L.) Karst.) at field stands. A maximum of glutathione concentrations (ESTERBAUER & GRILL 1978) and a chlorophyll reduction (KÖSTNER & al. 1989) were observed in autumn when temperature decreases and days become shorter. GRILL & al. 1987 ascribed

[1)] Institut für Pflanzenphysiologie, Universität Graz, Schubertstraße 51, A-8010 Graz, Austria.

autumnal changes of glutathione to the decrease in temperature. However, decrease in both temperatures and light period lengths always coincide at Middle European field plots in fall.

The present experiment was conducted to find out which of these two environmental factors induce changes of glutathione and chlorophyll a and b similar to those observed in the field.

Materials and Methods

Plant material

Four year-old trees (*Picea abies* (L.) Karst., one provenance from about 600m a.s.l. were grown in the botanical garden in Graz prior to the experiments.

Experimental conditions

Experiments were conducted in Heraeus, Vötsch HPS 1500 (cold temperature treatment) and Vötsch VB 5221/1 (short day length treatment) growth chambers. Experiment began in August 1997. Control conditions were like outdoor conditions at the beginning of the experiment: day/night temperature 20/15°C, relative humidity day/night 70/80%, light period day/night 14/8 hours with one hour dusk and one hour dawn. Light intensity was between 300 and 400 μmol m^{-2} s^{-1} (PAR) at canopy height. Two experimental variants were chosen: 1. Short days: climate chamber with control conditions except 8 hours light period. 2. Low temperature: climate chamber with control conditions except 4°C at day and night. Exposure time was 7 weeks after one week adaptation.

Sample preparation

Current year's spruce needles were collected in the light, frozen in liquid nitrogen and kept at –70°C until lyophilizing. The lyophilized needles were powdered in a dismembrator. The dry powder was stored in humidity proof plastic vials at –25°C before it was subjected to HPLC analysis.

Biochemical analyses

Glutathione in the reduced and oxidized form were analysed according to KRANNER & GRILL 1993 and chlorophyll a and b according to PFEIFHOFER 1989.

Statistics

Statistical evaluations were done with the help of Statistica Software package. 8 variants per experimental variant and control, respectively, were used. Differences between variants and controls were calculated by Mann-Whitney U test. Decision rule: $P \geq 0.05$ non-significant (no symbol), $P<0.05$ *, $P<0.005$ ***.

Results and Discussion

Concentrations of reduced glutathione showed a decrease in short days-treatment and an increase in low temperature-treatment compared to the respective controls (Fig. 1). The concentration and proportion of oxidized was not significantly affected neither by short light periods nor by low temperature. In the field, the amount of reduced glutathione usually increases from September to March, i. e. with the beginning of shorter days and lower temperature (ESTERBAUER & GRILL 1978). Experimental data by TAO & al. 1998 ascribed this increase to cold (not freezing) temperature, but in that work the day length did not affect glutathione concentrations. The increase of glutathione in low temperature might be explained by an increased need for radical scavenging capacities in a situation of higher probability of production of toxic oxygen species. Though the affinity of glutathione towards oxygen radicals is not very high, it plays a crucial part in the regeneration of ascorbate (POLLE & RENNENBERG 1994). Since in plant cells the major proportion of toxic oxygen is produced in the photosynthetic apparatus (ELSTNER & OSSWALD 1994), short light periods may even counteract this danger. Consequently, levels of cellular glutathione might be decreased without harmful effects.

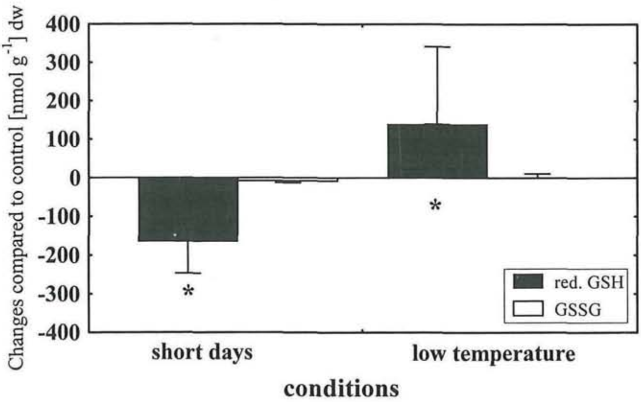

Fig. 1. Changes of reduced glutathione (GSH) and oxidized glutathione (GSSG) contents compared to control in current year's spruce needles. The control needles of short days-treatment contained 793 ± 173 nmol g^{-1} dry weight (dw) of GSH and 41 ± 9 nmol g^{-1} dry weight of GSSG. The control needles of low temperature-treatment contained 426 ± 90 nmol g^{-1} dry weight of GSH and 35 ± 12 nmol g^{-1} dry weight of GSSG.

Both chlorophyll a and b contents increased significantly upon short day treatment, but decreased upon low temperature treatment (Fig. 2). Field study results are not uniform in this respect: KÖSTNER & al. 1990 did not find much variation of chlorophyll contents in the time period in question. Since in plant cells radical production is mainly driven by excess energy absorption, this might be avoided by degrading the absorbing pigments. In shorter days, but at optimal temperature, an increased capacity for light absorption due to higher chlorophyll contents might be useful.

In summary, the effects of low temperature-treatment resembled the changes the investigated biochemical components undergo in the field during winter adaptation. Higher concentrations of glutathione and lower chlorophyll contents might contribute to the protection from oxidative stress conditions. The short days-treatment produced effects which were contradicting field results. These effects might be governed by a need for an improvement of photosynthetic production rather than by requirements for stress avoidance.

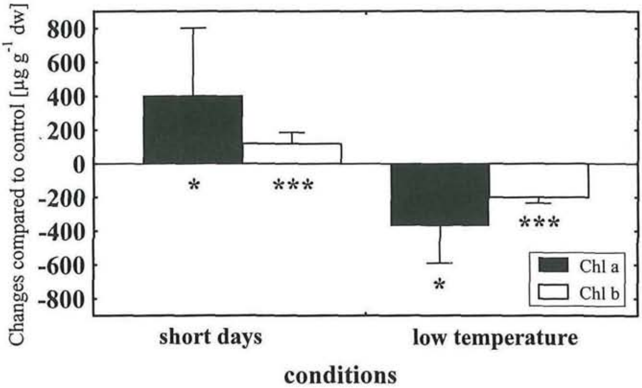

Fig. 2. Changes of chlorophyll a and b contents compared to control in current year's spruce needles. The control needles of short days-treatment contained 673 ± 240 µg g^{-1} dry weight of chlorophyll a and 264 ± 52 µg g^{-1} dry weight of chlorophyll b. The control needles of low temperature-treatment contained 1378 ± 364 µg g^{-1} dry weight of chlorophyll a (Chl a) and 528 ± 107 µg g^{-1} dry weight of chlorophyll b (Chl b).

References

ELSTNER E.F. & OSSWALD W. 1994. Mechanisms of oxygen activation during plant stress.- Proceedings of the Royal Society Edinburgh 192B: 131-154.

ESTERBAUER H. & GRILL D. 1978. Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*. – Plant Physiol. 61: 119-121.

GRILL D., PFEIFHOFER H. & TSCHULIK A. 1987. Untersuchungen über die jahreszeitlichen Schwankungen von Nadelinhaltsstoffen unter Berücksichtigung von Frosthärtefaktoren. – Phyton 27: 221-240.

KÖSTNER B., CZYGAN F.-C. & LANGE O.L. 1990. An analysis of needle yellowing in healthy and chlorotic Norway spruce (*Picea abies*) in a forest decline area of the Fichtelgebirge (N.E. Bavaria). – Trees 4: 55-67.

KRANNER I. & GRILL D. 1993. Content of low-molecular-weight thiols during the imbibition of pea seed. - Physiol. Plant. 88: 557-562.

PFEIFHOFER H. W. 1989. Evidence of chlorophyll b and lack of lutein in *Neottia nidus-avis* plastids. – Biochem. Physiol. Pflanzen 184: 55-61.

POLLE A. & RENNENBERG H. 1994. Photooxidative stress in trees. – In: FOYER C. H. & MULLINEAUX P. M. (Eds.) Causes of photooxidative stress and amelioration of defense systems in plants, pp. 199-218. – CRC Press, Boca Raton.

TAO D.-L., ÖQUIST G. & WINGSLE G. 1998. Active oxygen scavengers during cold acclimation of Scots pine seedlings in relation to freezing tolerance. – Cryobiology 37: 38-45.