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Seasonal Variations of Glutathione Reductase and Glutathione in Leaves and Leaf Buds of Beech Trees (*Fagus sylvatica* L.)

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Summary

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Glutathione content and glutathione reductase activity were investigated in leaves and leaf buds of beech trees. Glutathione reductase activity showed periodic seasonal fluctuations and was high in leaf buds in winter and in mature leaves in summer. Leaves as well as buds in different developmental stages contained not less than 250 nmol glutathione g fresh weight^{-1}. The role of glutathione and glutathione reductase in cold acclimation is discussed.

Introduction

Glutathione is an important storage and transport form for reduced sulfur in many plant species (RENNENBERG 1982). In addition, glutathione has been implicated in cellular defence mechanisms against herbicides, oxidants and other environmental stresses (SMITH & al. 1990). For these functions it is crucial that glutathione is present in its reduced form. This can be achieved by glutathione reductase (EC 1.4.6.2) which uses NADPH for reduction of oxidized glutathione.

Field studies with leaves of evergreens have demonstrated that both

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glutathione and glutathione reductase show seasonal fluctuations with higher levels in winter than in summer (ESTERBAUER & GRILL 1978, HAUSLADEN & al. 1990, POLLE & RENNENBERG 1992, SCHUPP & RENNENBERG 1992). It has been suggested that enhanced glutathione content and glutathione reductase activity may play a role for winter hardiness (ESTERBAUER & GRILL 1978). However, to date the hypothesis that glutathione and glutathione reductase activity are important for adaptation to low temperature is a controversial subject. Statistical analysis revealed only a low correlation between glutathione content and air temperature ($r = 0.55$, SCHUPP & RENNENBERG 1992). Besides, spinach leaves with an increased glutathione content did not acquire increased freezing tolerance (DE KOK & al. 1981) and pea leaves contained less glutathione reductase activity in winter than in summer (GILLHAM & DODGE 1987). In stems of field-grown dogwood no increase in glutathione was observed in late fall, although freezing tolerance increased (GUY & al. 1984). In the present communication seasonal fluctuations of glutathione reductase activity and glutathione content were investigated in leaves and leaf buds of beech trees (*Fagus sylvatica*, L.). The significance of the observed changes with respect to changes in temperature and pollutant concentrations is discussed.

Materials and Methods

The investigation was performed with leaves and leaf buds obtained from two mature beech trees grown at valley level (735 m above sea level) close to the Fraunhofer Institute at Garmisch-Partenkirchen. The site has been described previously (POLLE & al. 1992). Leaves or buds were harvested at about 9.00 am and transported to the laboratory within 10 min. For the analysis of glutathione reductase fresh leaves and leaf buds were extracted and assayed immediately (POLLE & al. 1990). This was necessary, because storage of extracts or leaves at - 20 or - 80° C resulted in a loss of glutathione reductase activity in the range of 50 to 80 % as compared to freshly analyzed material.

For analysis of glutathione plant material was frozen in liquid nitrogen and stored at - 80° C. Glutathione was extracted as described previously (SCHUPP & RENNENBERG 1988) and analyzed with the cyclic assay according to GRIFFITH 1980. Recovery of glutathione during the extraction of plant material was 104 ± 14 % ($n = 18$).

Results and Discussion

In leaf buds in winter and during the emergence of new leaves in spring the glutathione concentration ranged from 400 to 600 nmol g fresh weight^{-1} (Fig. 1a). In fall mature leaves contained as much glutathione as buds in winter. In contrast, spruce needles of trees grown at the same site as the beech trees used in the present investigation, contained in fall only half of their winter level of glutathione (SCHUPP & RENNENBERG 1992). This observation suggests differences in the utilization of glutathione in beech and spruce. Differences between these two species have also been found in the thiol composition of xylem saps which contained predominantly cysteine in beech, but glutathione in spruce (SCHUPP & al. 1991, SCHUPP & al. 1992).

Senescent beech leaves which contained only 5 % of the chlorophyll content present in leaves in summer (data not shown) were sampled from trees at the end of October. These leaves still contained 30 to 50 % of the glutathione content of that of leaves in summer (Fig. 1a). Even though part of senescent beech leaves are not shed in fall, apparently a significant portion of reduced sulfur is lost during senescence.

Glutathione reductase activity showed periodic seasonal changes (Fig. 1b). The activity was low in young buds in the vegetation period (August/September) and increased in the cold season. When leaves emerged in the following spring glutathione reductase activity declined. In mid-summer glutathione reductase activity in beech leaves increased strongly, which is in contrast with spruce needles where its activity remained low (ESTERBAUER & GRILL 1978). The maxima in glutathione reductase activity were observed in beech leaves during the months in which peak concentrations of ozone of 0.1 $\mu l\ l^{-1}$ occurred frequently (SLADKOVIC, personal communication) and in which mean ozone concentrations were also the highest (Table 1). The concentrations of SO_2 and NO_x were generally low (Table 1). Neither glutathione reductase activity nor glutathione content correlated with changes in mean temperature (Table 1). From this result we can not conclude whether or not changes in glutathione reductase activity or glutathione content have a significance for adaptation to low temperatures, but apparently other environmental or physiological factors have also profound effects on both components.

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Table 1. Seasonal variations in climatic conditions and pollutant levels at the Fraunhofer Institut, Garmisch-Partenkirchen. Sum of total precipitation (Pre) and mean daily temperature (T), global radiation (Rad), ozone (O_3), sulfur dioxide (SO_2), and nitrogen oxides (NO_x) are given for a 3 months period.

Season	T (°C)	Rad ($Wm^{-2}h^{-1}$)	Pre (mm)	O_3	SO_2	NO_x
					($nl\ l^{-1}$)	
NOV-JAN	- 1.9	40	99	11.9	3.3	17.2
FEB-APR	+ 4.7	114	282	28.0	2.1	7.3
MAY-JUL	+13.4	213	442	37.1	0.9	4.2
AUG-OCT	+12.1	138	378	24.5	0.7	4.3

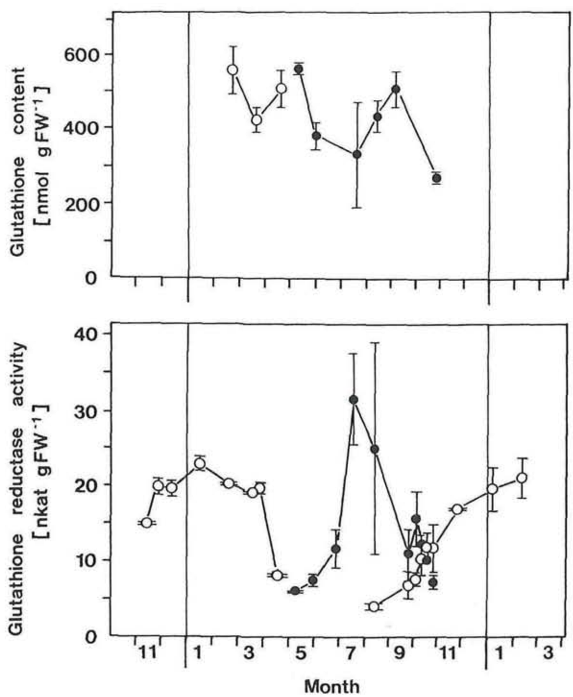

Fig. 1. Seasonal variations of glutathione (a) and glutathione reductase (b) in leaves and leaf buds of beech trees. The data represent the mean of 3 determinations with 2 trees (± SD). Open circles = buds, closed circles = leaves.

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