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Low Molecular Weight Thiols and Chromosomal Aberrations in *Picea omorika* upon Exposure to Two Concentrations of H_2S

By

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Summary

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Three years old spruce trees (*Picea omorika*) were exposed to 100 and 225 nl l^{-1} H_2S for three weeks. Contents of glutathione and cysteine in needles and fine roots as well as the number of chromosomal aberrations and the mitotic index in the root tips were determined. H_2S exposure resulted in a substantial increase of the cyst(e)ine (up to 7-fold) and total glutathione content (up to 3-fold) of the needles, whereas in fine roots there was solely and increase in the glutathione content (2-fold) at 225 nl l^{-1}. Glutathione was predominantly present in its reduced form, also upon H_2S exposure. The number of chromosomal aberrations in the root tips increased significantly upon H_2S, it was 2-fold higher than that in control roots. However, the mitotic index was not affected by H_2S .

Introduction

The sulfurous gases SO_2 and H_2S play important roles as phytotoxic air pollutants. In contrast to the great many of literature dealing with SO_2, there is a substantial lack of investigations regarding the effect of H_2S on trees (DE KOK & al. 1989, RENNENBERG & HERSCHBACH 1995), especially studies which are aimed at the effects on the roots of trees are scarce.

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In herbaceaous plants, atmospheric H_2S is directly metabolized in foliar tissue, resulting in increased levels of both cysteine and glutathione, and of γ-glutamyl-cysteine in the dark (DE KOK 1990, DE KOK & al. 1998). Part of the metabolized sulfur may be transported as glutathione from the shoot to the roots via the phloem. However, the level of its accumulation in the roots was generally lower than that in shoots (DE KOK 1990, RENNENBERG & LAMOUREUX 1990, DE KOK & al. 1997, 1998, RENNENBERG & HERSCHBACH 1995, HERSCHBACH & al. 1995).

In recent studies, it has been observed that there was an increased number of chromosomal aberrations in the root tip meristems of trees from canopies exposed to high levels of air pollutants, like ozone or SO_2 (MÜLLER & al. 1998, WONISCH & al. 1998).

In the present study the impact of atmospheric H_2S on low molecular weight thiols in shoots and roots and the number of chromosomal aberrations in meristems of conifer trees were determined.

Materials and Methods

Plant material

Three years old spruce trees (*Picea omorika* [Pancic] Pyrkyne) from Boomkwekerij Zundert B. V., Meirseweg 45, 4881 MJ Zundert, The Netherlands. Plants were kept in the greenhouse prior to the experiment.

Fumigation conditions

The fumigation (21 days) was conducted at the University of Groningen (September 1995). In addition to control, low level (100 nl l^{-1}) and high level H_2S (225 nl l^{-1}) were applied. Plants were fumigated in 150 l cylindrical stainless steel cabinets with polycarbonate tops. Pressurized H_2S diluted with nitrogen was injected into the incoming air stream at the desired concentrations by ASM electronic mass flow controllers (Bilthoven, The Netherlands). Air exchange rate in the cabinets was 50 l min^{-1} and the air inside the cabinets was circulated by a ventilator (with an air movement capacity of 20 l s^{-1}) to reduce the boundary layer surrounding the leaves. The air temperature was 19 ± 2 °C and the relative humidity amounted to 40 ± 10 %. Photoperiod was 14 h day^{-1} and the light intensity at plant height was 200-300 µmol m^{-2} s^{-1} (PAR).

Sampling

Needles of the previos year's flush and fine roots (<1mm diameter) of n=6 trees per treatment were sampled.

Biochemical analyses

Fine roots and needles were immersed in liquid nitrogen and lyophilized. The lyophilized material was powderized in a dismembrator, and the lyophilized material subjected to an adapted determination procedure based on the method given in KRANNER & GRILL 1993.

Chromosomal analyses on the root tip meristems were performed according to MÜLLER & al. 1991, 1998.

Statistics

Differences between treatments and control were evaluated using the Mann-Whitney U-test. Calculations were performed with the help of Statistica (StatSoft, Tulsa, OK, USA) software package.

Results and Discussion

A three week exposure of *Picea omorika* to 100 and 225 nl l^{-1} H_2S had no effects on growth of the trees (tree height, root and shoot weight) and no visible injury symptoms were observed.

Both H_2S levels resulted in increased concentrations of the thiols cysteine and glutathione in needles. Exposure to 100 and 225 nl l^{-1} H_2S resulted in a 3-fold and 7-fold increase in the cysteine content, and in a 2.5-fold and 3.5-fold increase in the glutathione content of the needles, respectively (Table 1). The redox state of glutathione was unaffected upon H_2S exposure and about 11 % of the glutathione was present in the oxidized form. These findings are comparable to data of herbaceous plants upon H_2S impact (DE KOK & al. 1989, 1997, HERSCHBACH & al. 1995).

Table 1. Low molecular weight thiols in needles and fine roots and chromosomal aberrations in root tip meristems of *Picea omorika* fumigated for 21 days with two concentrations of H_2S. Significance for differences compared to control: *P<0.05, **P<0.01. The data represent the mean of six measurements (± SD).

	Thiols in needles [nmol g^{-1}dw]		Thiols in fine roots [nmol g^{-1}dw]		Chromosomal aberrations
	Total GSH	Total Cys	Total GSH	Total Cys	[%]
Control	463 ± 69	33 ± 16	396 ± 150	29 ± 25	4.7 ± 1.0
100 nl.l^{-1}	1228 ± 32**	94 ± 23*	627 ± 34	39 ± 2	6.9 ± 0.3*
225 nl.l^{-1}	1629 ± 44*	213 ± 183*	705 ± 136*	37 ± 12	7.5 ± 0.3*

Exposure to 225 nl l^{-1} H_2S (but not 100 nl l^{-1}) resulted in a 1.8-fold increase in the glutathione content of the fine roots, whereas cysteine contents remained unaffected by H_2S exposure (Table 1). Similar to the observation in needles, glutathione was predominantly present in the reduced form; only 15 % was present as oxidized glutathione. The accumulation of glutathione in the roots upon high H_2S treatment may be a reflection of a surplus of reduced sulfur in the needles, which is transported from the shoots to the roots via the phloem (DE KOK & al. 1997). This corresponds with studies on herbaceous plants, where translocations of reduced sulfur in form of glutathione to the roots has been documented (RENNENBERG & LAMOUREUX 1990, HERSCHBACH & al. 1995). Alternatively, it may be assumed that glutathione was produced in roots and transport to the shoots was limited due to reduced sulfur surplus in the needles derived from the metabolized H_2S. This assumption would coincide with investigations of RENNENBERG & HERSCHBACH 1995, who did not find evidence for basipetal transport of glutathione via the phloem in spruce trees.

The number of chromosomal aberrations in the root meristematic cells increased significantly upon exposure to 100 and 225 nl l^{-1} H_2S (Table 1). Effects of H_2S on the chromosomal structure have not been investigated so far, but studies on spruce trees, which were fumigated with different SO_2 levels, showed similar results (MÜLLER & al. 1998). Even though this indicated structural damages of the

chromosomes, the mitotic index remained unaffected by both H_2S treatments. The rate of cell division was stable at 8 to 10 %. To what extent there is a relation between enhanced levels of glutathione and the development of chromosomal aberrations upon H_2S exposure needs further to be evaluated.

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