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Occurrence of heavy metal binding phytochelatins in plants growing in a mining refuse area

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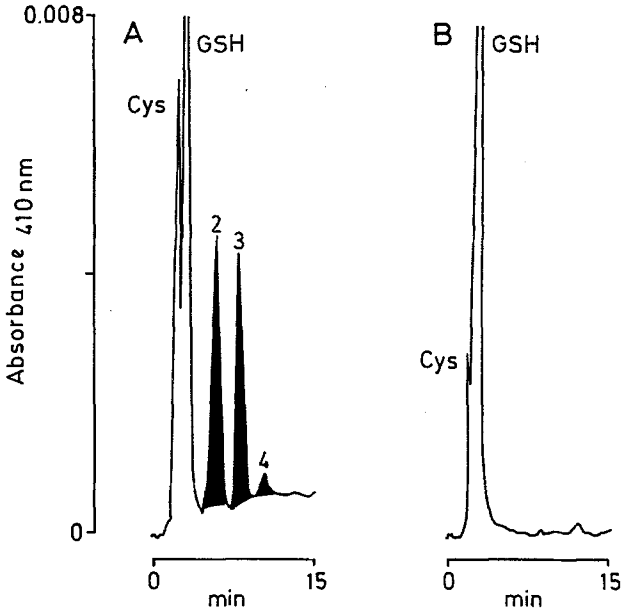
Summary. Phytochelatins were identified in roots of heavy metal-sensitive *Acer pseudoplatanus* and -resistant *Silene cucubalus* plants grown in zinc-rich soil of a mine dump. Both plants, when collected from a metal-uncontaminated stand, located nearby, revealed no phytochelatins. Thus, we concluded that metal-binding phytochelatins are specifically induced in plants of heavy metal enriched ecosystems.
Key words. Phytochelatin; heavy metal; detoxification.

Plants are to a certain degree tolerant to heavy metals. The mechanism by which plants could detoxify these ions has recently been elaborated[1,2]. The detoxifying function is assigned to small, cysteine-rich peptides capable of binding heavy metal ions via thiolate coordination; molecules we have named phytochelatins. An analogous mechanism for metal sequestration is provided in animals and certain fungi by the low molecular weight protein metallothionein[3]. Phytochelatins are induced upon exposure of plant suspension cultures to a wide range of heavy metals. The general structure of phytochelatins is $[\gamma\text{-Glu-Cys}]_n-\text{Gly}$ ($n = 2-11$), and they are inducible in the whole plant kingdom ranging from the phylogenetically simple algae[4] to the highly advanced orchids[2]. An exception is found in the order *Fabales* where the carboxyl-terminal Gly of phytochelatins is replaced by β-alanine, which leads to the homologous series of homo-phytochelatins[5]. The chemical structure of phytochelatins induced in plant cell cultures upon heavy metal stress has independently been verified[6]. Up to now, however, the occurrence of heavy metal induced phytochelatins has not been reported in plants grown in the ecosystem. In order to test the ecological relevance of phytochelatins as a heavy metal detoxification system of plants we investigated plants growing on heavy metal contaminated soil. For this purpose we chose a mining refuse area, located at the Rauschberg (N 47° 44'30"; E 12° 43'28") near Inzell in Upper Bavaria, a site of former zinc and lead workings derelict for over 160 years and colonized by a range of plant species. The heavy metal sensitive *Acer pseudoplatanus* and the metal-resistant *Silene cucubalus* were collected in mid July 1987. Control plants of the same two species growing on uncontaminated forest soil about 100 m northwest of the mine dump were also taken. The soil of the mining area yielded 28 g total Zn/kg dried soil, while the forest soil as a control contained only 0.30 g/kg measured by atomic absorption spectrometry. The root system of *Acer* plants yielded a content of 0.92% Zn, in leaves only 0.02% Zn was found. In contrast control plants grown on forest soil yielded 0.01% Zn in the root and 0.004% of the metal in the leaf tissue (all data on dry weight basis). The root system of the *Acer* plants was subsequently assayed for phytochelatins using our established method of analysis[1,2]. The figure shows the HPLC-profile of the root extract of the *Acer* collected from the mining refuse. Besides cysteine and glutathione the typical peaks of phytochelatins with two, three and four (γ-Glu-Cys) units are visible. The roots of *Acer* plants grown in uncontaminated forest soil were devoid of phytochelatins. The roots of *Silene* from zinc-rich soil showed basically the same phytochelatin pattern as the *Acer* plant. Again, in control plants no heavy metal chelating peptides were detected.



Assay for phytochelatin in extracts of roots of *Acer pseudoplatanus* grown (*A*) in a zinc-enriched mine dump, and (*B*) in a soil, located nearby, of low heavy metal content. The analysis was performed as described[2, 8]. Briefly, roots were frozen in liquid nitrogen and ground with a mortar and pestle. The plant material was suspended in alkaline borohydride solution and homogenized by sonification. After centrifugation the supernatant was deproteinized by acid precipitation. The cleared extract was separated by HPLC with sulfhydryl-specific detection by means of post column derivatization with Ellman reagent[9]. The first peaks represent cysteine (Cys) and glutathione (GSH), not fully resolved, followed by the individual phytochelatins. Phytochelatins are marked by shaded peaks and signified by the index number n of the primary structure $(\gamma\text{-Glu-Cys})_n$-Gly.

Comparison of metal-sensitive *Acer pseudoplatanus* with metal-tolerant *Silene cucubalus* grown on the same mine dump.

	Zinc level (mg Zn/g d. wt)		Phytochelatin level (µMol Glu-Cys-units/g d. wt)	
	Root	Leaf	Root	Leaf
Acer pseudoplatanus	9.2	0.23	0.90	<0.008
Silene cucubalus	2.2	0.42	0.06	<0.008

There was, however, an interesting difference (table). The *Acer* plants produced substantial amounts of phytochelatins (0.9 µmole γ-Glu-Cys units per g (d. wt) root tissue) while the metal-tolerant *Silene* plant yielded a distinctly lower level of phytochelatins (0.06 µmoles γ-Glu-Cys) even in relation to the zinc content of the root system, which was about 4 times higher in *Acer*. In both extracts the glutathione content was comparable (ca 0.9 µmole/g d. wt). This finding could indicate that plants adapted to heavy metals have evolved additional mechanisms of tolerance[7] besides complexation of metal ions by phytochelatins. The fact that root tissue contains a much higher concentration of heavy metals as well as of phytochelatins than the leaf tissue points to the fact that metals are obviously immobilized to a far greater extent at the site of metal uptake. The question whether phytochelatin-metal complexes can be transported at all in plants is currently under investigation.

This study, however, demonstrates that plants grown on soils containing considerable amounts of heavy metals do contain phytochelatins.

This is the first report of the natural occurrence of phytochelatins in the ecosystem and emphasizes the role of phytochelatins in the detoxification of heavy metals in plants.

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Reproductive effort of short shoots in silver birch (*Betula pendula* Roth)

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Summary. The degree of autonomy was estimated for generative short shoots of silver birch by defoliating them after leaf flush. Defoliation reduced both final length (4%) and weight (18%) of female inflorescences. Short shoots are thus reproductively semi-autonomous modules of silver birch.

Key words. Modularity; reproduction; birch; short shoot.

The modular structure of higher plants is a basic theoretical foundation of modern plant population biology[2,3]. Trees, for example, develop as a result of the births and deaths of their iterated structural elements, such as buds, shoots and branches[4,5]. The term 'module' originally referred to developmental products of single apical meristems[5,6], but it has since been used more loosely to denote all repeated units of 'multicellular structure, normally arranged in a branch system'[3]. Such morphological modules, however, may not always correspond to any appropriate physiological units. If modules are thus re-defined on a physiological basis, basic functional consequences of modular organization may become more apparent[7-10].

Functional modularity has two basic ecological consequences: 1) Functionally, modules can be defined as partially self-maintaining, repetitive, and multicellular parts of physically coherent 'structural individuals'[10]. 2) Reproductive effort in modular organisms is divided among a number of modular units which assimilate at least a part of the resources required for their own maintenance, growth and reproduction[7]. Consequently, the total reproductive output of functional modules in trees should depend both on their own photosynthetic activity, and on resources imported from other parts of the tree[11]; and the relative importance of these internal and external components of reproductive effort may be used to estimate the independence of modular units in their reproduction[12].

In the present study, we tested how the artificial defoliation of separate short shoots influences female catkins in the silver birch (*Betula pendula* Roth). Several (usually 2–3) leaves and a female catkin emerge from generative short shoot buds of the silver birch in early May[11]. Since reproductive organs do not generally have a positive net photosynthesis[13], we assume that the leaves of generative short shoots are the main sources for the internal component of reproductive effort that supports the growth of female catkins. We therefore studied how the removal of these leaves affects the final length and weight of female catkins on separate short shoots.

Materials and methods. The study was carried out at the Archipelago Research Institute of the University of Turku in SW Finland (60° 14′ N, 21° 59′ E). Eleven birch trees (*B. pendula*) were used which were, on average, 10.0 m in height (SD = 4.2 m) and 15.5 cm in diameter at 1.30 m above the ground (SD = 8.3 cm).

At the beginning of the experiment (12–15 May, 1986), 20–40 generative short shoots were selected from each tree. Short shoots were selected in pairs so that control and defoliated short shoots were located at similar positions on the