Phyton (Horn, Austria) Special issue: "Bioindication ..."	Vol. 36	Fasc. 3	(57)-(60)	15.09.96

Cytokinins in Norway Spruce Seedlings and Forest Soil Pollution

By

HOJKA KRAIGHER[1] & DAVID E. HANKE[2]

Key words: Isopentenyladenine-type cytokinins, Norway spruce seedlings, bioindication, model system.

Summary

KRAIGHER H. & HANKE D.E. 1996. Cytokinins in Norway spruce seedlings and forest soil pollution. - Phyton (Horn, Ausrtia) 36 (3): (57) - (60).

Cytokinins can correlate with pollution damage in spruce trees and also with different strains and species of their mycorrhizal symbionts. In our study, Norway spruce seedlings were grown in vitro on sterilized or nonsterile soil substrates from two differently polluted forest research plots. Cytokinins in needles were analysed by a combined HPLC-ELISA method. Differences were found predominantly in the isopentenyladenine-types of cytokinins. These were elevated in seedlings, grown on polluted soils in comparison to those grown on soils from the unpolluted plot, as well as in seedlings grown on nonsterile substrates in comparison to those grown on sterile soil substrates. A possible explanation might be a change of metabolism in the roots due to pollution stress or/and a change in the mycorrhizosphere organisms. Norway spruce seedlings as tester organisms for soil pollution are discussed.

Introduction

Several forest decline symptoms can reflect disturbances in hormonal balances of forest trees. In this view cytokinins can enhance the resistance of plants to various forms of stress, reduce the dominance of the apical bud, delay senescence of plant parts and stimulate the development of chloroplasts (KAMINEK 1992). The cytokinin content in needles of mature spruce trees was shown to correlate with different degrees of damage (VON SCHWARTZENBERG & HAHN 1991); and can also be affected by different species and strains of mycorrhizal fungi (KRAIGHER & al. 1993), while the range of the fungal types can vary according to pollution (ARNOLDS 1988). In our study a model system was developed, in which

[1] Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia
[2] Department of Plant Sciences, Downing Street, Cambridge CB2 3EA, U.K.

cytokinin contents in needles of Norway spruce seedlings grown in vitro on two differently polluted forest soil substrates were analysed in order to contribute to the physiological characteristics of Norway spruce, already employed for bioindication.

Materials and Methods

Two differently polluted forest research plots (U-unpolluted, P-polluted) of the Slovenian Forestry Institute in the emission zone of the Šoštanj Thermal Power Plant (TPP) were chosen for this study. The two plots (850 m/asl, distric cambisol, *Luzulo - Fagetum*, predominant tree species *Picea abies*, ca 80 - 100 years old) are comparable regarding soil characteristics (pH_{INKCl} 3.8 (P) & 3.5 (U), total C to total N ratio 21 (P), 23 (U), available K 18 & 19 mg/100 g soil, available P in traces) but polluted differently by the emissions from the TPP, as indicated by the total S% 0.073 (P) & 0.060 (U) and by the lichenological studies (BATIČ & KRALJ 1990).

For cytokinin analyses, Norway spruce seedlings were grown in test tubes from surface sterilized seeds on sterilized (S) or nonsterile (N) sieved soil substrates, for two to three weeks. The needles were ground in liquid nitrogen, extracted in ethanol on ice, purified through Sep-paks, evaporated to ca 1 ml and purified additionally by a polyvinylpyrrolidone (PVP) purification step. The centrifuged cytokinins were fractionated by C_{18}-bonded silica reverse-phase HPLC (modified after TURNBULL & HANKE 1985, KRAIGHER & al. 1991), using a gradient of buffer, pH and methanol concentration, at a flow rate of 1 ml per minute. The protocol is shown in Tab. 1.

Tab. 1. Protocol for HPLC-fractionation of cytokinins.

time (min)	solvent A%	solvent B%
0	90	10
20	60	40
30	40	60
35	40	60

Solvent A was 10% methanol in deionized water, with 4.0 ml acetic acid added per 1000 ml, pH adjusted with TEA to 3.35; solvent B was 80% methanol with 5.6 ml acetic acid added per 1000 ml, pH 3.66. The column was cleaned with 100% methanol and initialized with solvent A.

One-minute fractions were collected, evaporated and assayed with three different ELISA-tests (for Zeatin- (Z), Isopentenyladenine- (iP) and Dihydrozeatin- (DZ) types, modified after STRNAD & al. 1992 and KRAIGHER & al. 1993. Four forms could be detected in each ELISA test: the Ribotides, 9-Glucosides, free bases and Ribosides. [^{3}H]-Isopentenyladenosine-Diol (^{3}H-Diol) was added to the ground needles as an internal standard for estimations of recovery. The presence of Isopentenyladenosine-ribotides (iPMP) was checked by an alkaline-phosphatase dephosphorylation step, repeated fractionation and ELISA-test on the Riboside-fractions. The recoveries of ^{3}H-Diol ranged from 56 to 89% and of iPMP from 36 to 84%. After extraction, roots of seedlings were used for morphological and mycorrhizal observations.

Results and Discussion

The differences in cytokinin contents in needles of Norway spruce seedlings, grown on the two sieved soil substrates (sterilized or nonsterile), were greatest in the iP-types of cytokinins (Fig. 1).

Statistically significant differences in the levels of iP9G, iP and iPA were found in seedlings grown on nonsterile substrates (PN, UN) from the two plots. The variability of data was low in needles of seedlings grown on sterile substrates (PS, US). For these the differences were statistically significant for iPMP and for total

iP-cytokinins (iP-types at 25±4 (PS), 85±26 (PU), 9±7 (US), 70±53 (UN) (all in pmol/g fr.wt.)). Therefore, the presence of microorganisms in nonsterile sieved soil substrates (PN, UN) could have an influence on both the variability of results and on the absolute concentrations of cytokinins detected in needles, in comparison to those from sterilized substrates.

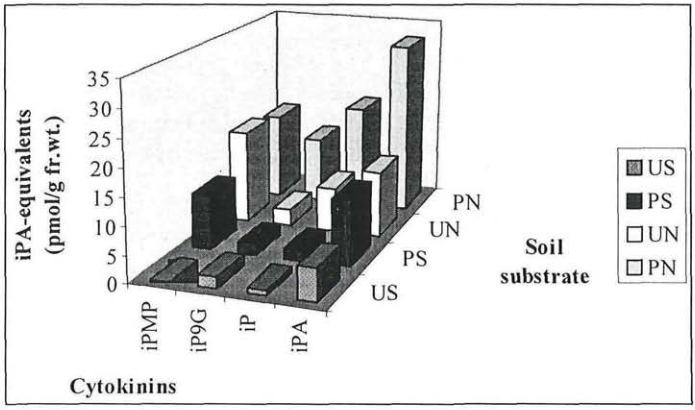

Fig. 1. Isopentenyladenine (iP) - cytokinins in needles of spruce seedlings, grown on sterile (S; 2 replicates) or nonsterile (N; 7 replicates) soil substrates from the unpolluted (U) and the polluted (P) plot. iPMP = iP-ribotide, iP9G = iP - 9-glucoside, iPA = isopentenyladenosine.

On roots of seedlings, grown in sterilized substrates, poor root growth and root meristematic abortions were observed, while the types of ectomycorrhizae occurring in the samples were at too an early stage to be determined unequivocally.

In the model system used, the only difference in growth conditions of the four sets of seedlings was in soil substrate, therefore also the different cytokinin levels in the needles would have been influenced predominantly by signals from roots. Cytokinins in xylem sap are supposed to act as indicators of changes in the root environment (DAVIES & al. 1987). The predominant forms of cytokinin in the xylem sap were reported to be Z-type, therefore they are also the predominant form synthesized in roots (TORREY 1976); while iP-type cytokinins were detected in phloem exudates, therefore they could be synthesized in shoots (GRAYLING & HANKE 1992, BERNIER & al. 1990).

In our conditions the effect of soil substrates on metabolism in young roots has possibly slowed down phloem transport which could be reflected in an accumulation of iP-type cytokinins in needles of seedlings grown on sterile substrates from the polluted plot. Therefore the shift in cytokinin contents could be explained as an accumulation of iP-type cytokinins in needles due to the lower sink for assimilates and could occur even before the inhibition of root growth. This is further supported by the comparably higher levels of Z-type cytokinins and lower iP-type cytokinins in needles of spruce seedlings, grown on nonpolluted sterile substrates (as the 'normal' relation for cytokinins in the shoots).

In nonsterile soil substrates, the mycorrhizosphere organisms are supposedly the predominant factor influencing cytokinin concentrations in seedlings. Their contribution to cytokinin contents in soil extracts can be significant, since these can

reach physiologically active concentrations (VAN STADEN & DIMALLA 1976). The concentrations of cytokinins in needles could be influenced by a shift in species and strain diversity in polluted soil substrates or by a difference in cytokinin production by a single fungal species under the influences of changes in growth conditions (KAMPERT & STRZELCZYK 1984, KRAIGHER & al 1991).

In conclusion young spruce seedlings were used as 'tester' organisms, with the aim of showing a physiological response to a certain experimental condition, here a difference in the soil substrate. The seedling response was measurable and showed significant differences in two differently polluted soil substrates. The system should be extended to a range of different soil substrates in order to apply it to elucidate better the existing physiological characteristics of Norway spruce, already employed for bioindication.

Acknowledgements

The study was supported by the Ministry for Science and Technology of the Republic of Slovenia, the ALIS Link No.250 through The British Council & TEMPUS JEP 04667.

References

ARNOLDS E. 1988. The changing macromycete flora of the Netherlands. - Trans.Br.Myc.Soc. 90: 391-406.

BATIČ F. & KRALJ T. 1989. Bioindikacija onesnaženosti zraka z epifitsko lišajsko vegetacijo pri inventurah propadanja gozdov. - Zbornik gozd. in les. 34: 51-70.

BERNIER G., LEJEUNE P., JACQMARD A. & KINET J.-M. 1990. Cytokinins in flower initiation. - In: PHARIS R.P. & ROOD S.B. (eds.), Plant growth substances 1988, pp. 486-491. - Springer-Verlag, Berlin.

DAVIES W.J., METCALFE J.C., SCHURR U., TAYLOR G. & ZHANG J. 1987. Hormones as chemical signals involved in root to shoot communication of effects of changes in the soil environment. - In: HOAD G.V. & al. (eds.), Hormone action in plant development - a critical appraisal, pp. 201-216. - Butterworths, London.

GRAYLING A. & HANKE D.E. 1992. Cytokinins in exudates from leaves and roots of red *Perilla*.- Phytochemistry 31: 1863-1868.

KAMINEK M. 1992. Progress in cytokinin research. - Tibtech 10: 159-164.

KAMPERT M. & STRZELCZYK E. 1984. Effect of pH on production of cytokinin-like substances by bacteria isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus silvestris* L.). - Acta Microbiologica Polonica 33: 77-85.

KRAIGHER H., GRAYLING A., WANG T.L. & HANKE D.E. 1991. Cytokinin production by two ectomycorrhizal fungi in liquid culture. - Phytochemistry 30: 2249-2254.

--, STRNAD M., HANKE D.E. & BATIČ F. 1993. Cytokiningehalte von Fichtennadeln (*Picea abies* (L.) Karst) nach Inokulation mit zwei Stämmen des Mykorrhizapilzes *Thelephora terrestris* (Ehrh.). - Forstw. Cbl. 112: 107-111.

STRNAD M., PETERS W., BECK E. & KAMÍNEK M. 1992. Immunodetection and identification of N^6-(*o*-hydroxybenzylamino)purine as a naturally occurring cytokinin in *Populus x canadensis* Moench cv *Robusta* leaves. - Plant Physiol. 99: 74-80.

TORREY J. G. 1976. Root hormones and plant growth. - Annu. Rev. Plant Physiol. 27: 435-459.

TURNBULL C. G. N. & HANKE D. E. 1985. The control of bud dormancy in potato tubers. Measurement of the seasonal pattern of changing concentrations of zeatin-cytokinins. - Planta 165: 366-376.

VAN STADEN J. & DIMALLA G.G. 1976. Cytokinins from soils. - Planta 130: 85-87.

VON SCHWARTZENBERG K. & HAHN H. 1991. The cytokinin content in needles of Norway spruce (*Picea abies* (L.) Karst) with different degrees of damage. - J. Plant Physiol. 139: 218-223.