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Altering N Nutrition Changes the Cytokinin Content of Leaves of Woody Plants

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Summary

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Nitrogen-starved plants of *Protea aurea* respond to a N-supplement by an increase in the activation by *trans*-hydroxylation of precursor types to zeatin cytokinins, leading to the accumulation of greater total cytokinin content in leaves of plants grown with a moderate supply of N. *Picea sitchensis* trees in the experimental ecosystem of SHEPPARD & CROSSLEY 2000 subject to simulated wet neutral N deposition showed the same sequence of responses in cytokinins, albeit over a longer timescale. *Picea sitchensis* seedlings did not respond to N supplements by increases in active cytokinins unless associated with an ectomycorrhizal fungus, in this case *Thelephora terrestris*.

Introduction

Plant growth substances provide information on changes in the environment in the light of which development is adjusted to counter or exploit the change. Abscisic acid levels are linked to the availability of water, ethylene to changes in temperature and pressure, and gibberellin to light. On which environmental parameter do cytokinins provide information? Our previous work with herbaceous crop plants showed that cytokinins can increase to active levels in response to chilling (TURNBULL & HANKE 1985), and photoperiodic induction by short days (GRAYLING & HANKE 1992). We consider that these are derived responses, acquired during domestication in fertilised soil, by selective modification of a primal link between growth-limiting levels of mineral nutrient and a low endogenous content of active cytokinins. Such a primal link is suggested by the fact that the most widespread features of the low cytokinin phenotype: accelerated onset of leaf senescence and

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increased stringency of shoot apical dominance, are core components of the classical strategy for sustaining growth of the terminal shoot on poor soil by efficient internal re-cycling of nutrients. Also, a direct relationship between the supply of nitrate and the content of endogenous cytokinins in leaves was reported by HORGAN & WAREING 1980 for *Betula pendula*, a tree species able to acclimate to low availability of mineral nutrients.

We report preliminary results from two woody species, *Protea aurea* and *Picea sitchensis*, which extend our knowledge of the link between nitrogen nutrition and cytokinin biochemistry in woody plant species.

Materials and Methods

Protea aurea seeds were obtained from Silverhill Seeds, Kenilworth, RSA, and grown in 15cm pots on peat/acid-washed sand mixtures augmented with diluted Hoagland's solution, in an unheated greenhouse. *Picea sitchensis* needles were sampled from the experimental ecosystem of SHEPPARD & CROSSLEY 2000. Cultures of *Thelephora terrestris* were a kind gift from Dr. Hojka KRAIGHER, Slovenian Forestry Institute.

Tissues were extracted (TURNBULL & HANKE 1985) and extracts analysed for cytokinins by HPLC-ELISA (KRAIGHER & al. 1991).

Results and Discussion

Protea aurea seedlings were grown for 24 months in one of two regimes. 'Nitrogen starved' plants were given 0.1 x Hoaglands solution initially and watered with reverse-osmosis distilled water thereafter. 'Nitrogen supplied' plants differed only in that 200ml of 0.2mM NH_4NO_3 was supplied at monthly intervals. Leaf samples were taken just before plants were watered with 200ml 2mM NH_4NO_3 or 200ml water (t=0). A further set of samples were taken 72h later (t=72h). The results are presented in the Table 1.

The long term consequence of supplementation with mineral nitrogen in this species is that the content of cytokinin in the leaves was 5 times greater than that of N-starved plants. The increase involved all the different types of cytokinin. This result confirms that there is a direct relationship between availability of mineral nitrogen and leaf content of cytokinins in *Protea aurea*, a woody plant which persists in soils chronically impoverished in minerals by efficient internal re-cycling of minerals from senescing older leaves to terminal shoots.

In the short term, when a moderate N supplement was given to N-starved plants, 3 days later there was no change in the total leaf cytokinin content, but a decrease in the level of inactive precursor types and a compensatory increase in active types. The *trans*-hydroxylation ratio, a rough estimate of the activation process, shifted from 1 to approx. 40. This response was not shown by N-starved plants given a water supplement, or by plants grown on a moderate supply of N and given the same N supplement (Table 1). We propose that the short term increase in *trans*-hydroxylation detected here in response to an increase in the availability of mineral nitrogen reveals the mechanism for driving the progressive accumulation of cytokinins in plants on fertile soils, an accumulation that relaxes shoot apical dominance and prolongs the life of older leaves leading to a different pattern of vegetative development.

Table 1. Content of cytokinins (pmol riboside equivalents g-^{1}FW) in leaves of *Protea aurea*. Each value is the sum of the contents of freebase, riboside, ribotide and 9Nglucoside.

The *trans*-hydroxylation ratio is total zeatin types plus total dihydrozeatin types divided by the total isopentenyladenine types, and is a measure of the extent to which inactive precursor cytokinins have been converted by *trans*-hydroxylation to the biologically active zeatin types. The reduction ratio is total dihydrozeatin types divided by the total zeatin types, and is a measure of the extent to which active zeatin types have been converted by reduction to the biologically less active dihydrozeatin types.

	N-starved plants				N-supplied plants	
	N-added		Water control		N-added	
Cytokinin type	before addition	after addition	before addition	after addition	before addition	after addition
isopentenyladenine	80	16	14	38	255	111
zeatin	23	37	48	66	290	123
dihydrozeatin	39	154	57	44	230	33
trans-hydroxylation ratio	2	37	6	3	1	1
reduction ratio	2	5	1	0.7	0.3	0.2

One of the reasons for choosing *Protea aurea* is that it is a member of a family of woody plants adapted to survive on nutrient poor sites without assistance from mycorrhizal fungi. Contributions to the cytokinin economy of plants by hormone made by the mycorrhizal symbiont are a complicating factor (KRAIGHER & al. 1993, KRAIGHER & HANKE 1995). There is no record of whether the seedlings of *Betula pendula* used by HORGAN & WAREING 1980 were mycorrhizal or not.

Recently, we have been monitoring the cytokinin content of leaves of *Picea sitchensis* trees subject to simulated wet N deposition in the experimental ecosystem developed and described by SHEPPARD & CROSSLEY 2000. Our first samples, taken in December 1996, six months after spraying began, showed no effect of any treatment on total cytokinin content either of the current year's needles or of the previous year's. There were, however, treatment-induced increases in the *trans*-hydroxylation ratio for neutral nitrogen (N) and single dose acid nitrogen with sulphate (NS) treatments, and the increases were more marked for the previous year's needles. With time, a high hydroxylation ratio (value of 8 as opposed to around 1 in controls) was only sustained in needles subject to the neutral N treatment. From December 1997, one year later, the total content of all active cytokinins in needles subject to neutral N had increased 5 fold, reaching levels 5x higher than those of all other treatments, which have remained essentially constant. This large disparity in cytokinin content has been maintained subsequently.

The similarity with changes in cytokinins in *Protea aurea* following N supplementation is striking: an upward shift in *trans*-hydroxylation followed by progressive accumulation of active cytokinins. The time scale of events was different, probably reflecting the huge difference in size between these plants. The Sitka spruce trees have not yet shown a commensurate difference in phenotype in consequence of the difference in cytokinin content, but then this species is not specially adapted to N-impoverished sites and growth is checked in low N conditions.

We have carried out preliminary experiments to test whether mycorrhizal fungi contribute to N-induced increases in leaf cytokinin content. Surface sterilised seed of *Picea sitchensis* was germinated in sterilised soil, and at 3 months half the seedlings were implanted close to older *Picea sitchensis* already in mycorrhizal association with *Thelephora terrestris*. At 2y, both sets of plants, one set in sterile culture and the other exposed to *Thelephora terrestris*, were given a range of N-supplements as NH_4NO_3 solution supplied to the roots. Twelve hours later, needles were analysed for cytokinins. Significant increases in zeatin types directly related to the amount of N added were detected in needles from plants grown in the presence of fungus, but not for plants in sterile culture. We conclude that association with an ectomycorrhizal fungus contributes to N-induced changes in leaf cytokinin. This finding is consistent with previous work in which the higher cytokinin content of needles of Norway spruce seedlings grown in soil from polluted sites, compared to that of seedlings in non-polluted soil, was not seen if the soil was sterilised (KRAIGHER & HANKE 1995). In mycorrhizal species, only the fungus is in intimate contact with the soil solution and apparently takes over the role of relaying to the shoot information on the N-status of the soil, as cytokinin, to which shoot tissues may be programmed to respond so as to exploit a surfeit or to survive a deficit.

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