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Cytokinins in the perennial herb *Urtica dioica* L. as influenced by its nitrogen status

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Abstract. The effect of nitrogen on the cytokinin relations of *Urtica dioica*, the stinging nettle, has been investigated. The plants were grown in quartz sand and nutrient solutions providing levels of nitrate ranging from 1 to 22 mM. Nitrogen supply did not affect biomass production within the range of 3–15 mM NO_3^-. However, the shoot: root ratio of biomass was significantly higher at 15 mM (standard plants) than at 3 mM (low-nitrogen plants) nitrate supply. The cytokinin patterns of the roots, stems and adult, as well as meristematic leaves of plants grown at these two levels of nitrate supply, were determined by means of high-performance liquid chromatography (HPLC) and immunoassays. Enzyme-linked immunosorbent assays (ELISAs) for zeatin riboside, dihydrozeatin riboside, isopentenyladenosine, benzyladenosine and *o*-hydroxybenzyladenosine enabled the quantification of 17 cytokinins, 13 of which were found in the various tissues of *Urtica*. *trans*-Zeatin and its conjugates were the predominant cytokinins in all examined samples. While the free base *trans*-zeatin and its O-glucoside were the major cytokinins in adult leaves, *trans*-zeatin riboside was prominent in the other tissues of at least the standard plants. Glucosides of the *trans*-zeatin-type cytokinins were present only in lower amounts. However, considerable amounts of a compound, tentatively identified as *cis*-zeatin riboside-O-glucoside, were found, particularly in roots and meristematic leaves. Comparatively high amounts of *trans*-zeatin nucleotide as well as isopentenyladenosine phosphate were also demonstrated in these tissues. Analysis of the root-pressure exudates similarly showed *trans*-zeatin riboside and, at a lower concentration, *trans*-zeatin to be the only substantial components. In the low-nitrogen plants, shortage of nitrogen was manifest only in the roots; the nitrogen contents of the shoots did not respond to the nitrogen supply. Likewise, the total content of cytokinins in the shoots of the low-nitrogen plants equaled that of the standard-plant shoots, while it was lower by about 25% in the roots of the low-nitrogen plants. In the latter, the amounts of cytokinins exuded via the root-pressure fluid were also approximately 25% lower. Since the levels of only the *trans*-zeatin cytokinins in the roots showed a linear correlation with the shoot-to-root ratios, these cytokinins may play an important role in biomass partitioning in *Urtica dioica*.

Key words: Cytokinin (tissue distribution, N-dependence) – Nitrogen status – *Urtica* (cytokinin, nitrogen) – Xylem sap (cytokinin content)

Introduction

Zeatin (Z), dihydrozeatin (DHZ) and isopentenyladenine (2iP) and their ribosides N- and O-glucosides are the predominant cytokinins in plants (Koshimizu and Iwamura 1986). Although two biosynthetic routes (Horgan 1992; Klämbt 1992), as well as several cytokinin metabolizing and -degrading enzymes, have been identified, knowledge of the biochemical and physiological roles of the variety of natural cytokinins is still fragmentary. In addition to the fact that cytokinins are rapidly interconverted (Nishinari and Syono 1980, 1986; Laloue and Pethe 1982; Fußeder and Ziegler 1988; Fußeder et al. 1989; Mok et al. 1992), cytokinin metabolism of individual plant tissues is superimposed upon and thereby influenced by long-distance transport processes. The root is considered to be the principal cytokinin producer of the plant (Horgan 1992), while the shoot is dependent on the import of these phytohormones via the transpiration stream (Letham and Palni 1983). Nevertheless, it cannot be excluded that a reflux of cytokinins from the shoot to the root via the phloem takes place (Weiler and Ziegler 1981), or that cytokinins are synthesized in the meristems

Abbreviations: DHZ = dihydrozeatin; ELISA = enzyme-linked immunosorbent assay; –G = glucoside; HPLC = high-performance liquid chromatography; 2iP = isopentenyladenine; 2iPA = isopentenyladenosine; –N = nucleotide (ribotide), –OG = O-glucoside; –R = riboside; S/R = shoot-to-root (ratio); Z = zeatin

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of the shoot (Chen et al. 1985). Because there is insufficient knowledge of cellular cytokinin metabolism which is influenced by the continuous depletion or replenishment of pools, a cytokinin balance of a whole plant has not yet been presented.

External factors such as water stress (Itai and Vaadia 1971), hypoxia (Neumann et al. 1990) and especially nitrogen supply (Sattelmacher and Marschner 1978; Salama and Wareing 1979; Kuiper 1988; Kuiper et al. 1989) exert a considerable influence on the cytokinin level of the plant. In particular, Kuiper et al. (1989) have provided evidence to support the hypothesis "that a decrease in mineral nutrition decreases the cytokinin levels in shoot and root tissue and ... will decrease the relative growth rate and lower ... the shoot to root ratios." Their data resulted from short-term variations of the nutrient supply to *Plantago major*, whereupon the levels of zeatin and zeatin riboside (Z + ZR) in the shoots and roots were estimated.

In the present paper we address the above-cited hypothesis by comparing the cytokinin patterns of *Urtica dioica* L. plants which were grown either under conditions of slight nitrogen shortage or at optimal nitrate supply. The cytokinin patterns of the roots and of the shoots were related to the cytokinin composition of the respective xylem fluids as represented by root-pressure exudates. Use was made of immunological assays (Weiler 1986) combined with HPLC, methods which have improved cytokinin analysis by rendering it independent of questionable biotests.

A preliminary report on the cytokinins of *Urtica* has been published elsewhere (Wagner and Beck 1992).

Materials and methods

Plant material. Urtica diocia L., the stinging nettle, was grown from seeds that had been collected from a natural stand. Seedlings were transferred to modified 1-l Kick-Brauckmann pots possessing a drainage outlet 3 cm above the bottom. The pots were filled with quartz sand and continuously percolated at a rate of approx. 1 $l \cdot d^{-1}$ with Knop's nutrient solution (Hoagland 1948) or the modifications thereof shown in Table 1. The plants were grown in a temperature-controlled greenhouse at 20–22° C. Illumination in the greenhouse was supplemented by Osram (München, FRG) Power Star HQL TS 25D W/NAL lamps to yield a photon fluence rate of 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the surface of the pots. To maintain the nettles in the vegetative state, the daylength was limited to 10 h.

Table 1. Composition of the nutrient solutions (mM) employed for growing *Urtica dioica* in sand culture. One ml of Hoaglands A–Z micronutrient solution was added to each 1-l-solution. Nutrient solution No. IV is Knop's solution

Nutrient solution	I	II	III	IV	V
NO_3^-	1	3	9	15	22
SO_4^{2-}	2	2	2	2	2
PO_4^{3-}	3	3	3	3	3
Cl^-	1	1	0	0	0
K^+	4	4	4	4	4
Mg^{2+}	1	1	2	3	4
Ca^{2+}	1.5	2.5	4	6	8.5

Plants were harvested when about 75 cm high, when formation of rhizomes had already commenced. Shoots were harvested in the morning and separated into fractions containing (i) the shoot apex bearing the very young light-green and still-meristematic leaves, (ii) the adult leaves and (iii) the stalks. After weighing, the material was immediately frozen in liquid N_2, lyophilized and kept at −20° C. Roots were used for collection of root-pressure exudate prior to being harvested and frozen as above. The initial 50 µl of root-pressure exudate were discarded and samples of 0.5–0.75 ml were then collected for 1 h.

The pots were then flooded with water, whereupon the roots could be extricated from the sand. The roots (plus rhizome initials) were then treated in the same manner as the other fractions.

Statistics. The corresponding fractions of four plants were combined to yield one sample each. The data shown in this paper represent the means ± SD of four of such samples (altogether 16 plants).

Extraction and fractionation of cytokinins. In principle the method of Palmer et al. (1981a) was followed. Freeze-dried plant material equivalent to 10 g fresh weight was powdered in liquid N_2 with mortar and pestle and extracted three times with 100 ml of 80% methanol at 4° C for 1 h. The combined extracts were concentrated in vacuo and the pH was adjusted to 3.1 with 1 M acetic acid. The resulting suspension was frozen and thawed and cleared by centrifugation (45 min at $12000 \cdot g$).

The supernatant was subjected to cation-exchange chromatography on a column of 25 g of P 23 cellulose (Serva, Heidelberg, FRG) that had been adjusted to pH 3.1 with 36.3 mM acetic acid. Cytokinin nucleotides and other non-cationic compounds were eluted using 350 ml of this solvent. Subsequently the cytokinin bases, ribosides and glucosides were eluted with 2 M ammonium hydroxide. Both fractions were concentrated in vacuo to 10 ml. After adjusting the pH to 9.3 with 1 M ethanolamine, the nucleotides were dephosphorylated with 10 units of alkaline phosphatase (12 h at 30° C). The resulting ribosides were purified by cation-exchange chromatography as described above. Prior to HPLC, both the former nucleotide and the original base, riboside and glucoside fractions were filtered through disposable C_{18}-columns (6 ml, Bakerbond spe; Baker, Groß-Gerau, FRG). After washing the loaded columns with 10 ml of 10% methanol, the cytokinins were eluted with 35 ml of 50% methanol. The solvent was evaporated in vacuo and the residues were dissolved in 1 ml of distilled water.

Principle of cytokinin analysis. The pre-purified fractions were subjected to HPLC by which about 20 cytokinins could be separated (Fig. 1). Because of a relatively high content of UV-absorbing contaminant, the cytokinins could not be quantified from the records of the UV monitor. Therefore HPLC was combined with enzyme-linked immunosorbent assay (ELISA). Aliquot volumes of all fractions of the HPLC run were analyzed with antibodies against isopentenyladenosine (2iPA), zeatin riboside (ZR) and dihydrozeatin riboside (DHZR; Fußeder et al. 1988). Another aliquot volume of the fractions was deglucosylated with β-glucosidase and reanalyzed using the ELISAs. The resulting immuno-plots (Fußeder et al. 1988) could be further quantified using the cross-reactivities of the standard substances with the three standard antibodies (Table 2). To quantify unequivocally those cytokinins for which a particular ELISA was not available, use was made of the cross-reactivities of the substances in the various ELISAs. Only those sets of data were used where the patterns of cross-reactivities were in agreement with those of authentic test substances. Analysis of the individual fractions of the HPLC run as described above also allowed the quantification of cytokinin pairs such as zeatin-O-glucoside (ZOG)/dihydrozeatin-9-glucoside (DHZ9G) and DHZR/*cis*-ZR, which could not be satisfactorily separated by HPLC.

Details of analysis. For HPLC the cytokinins were separated on an analytical column (250 mm long, 4.6 mm i.d.) of Hypersil octadecyl-reversed phase material (pore width 5 µm; Shandon, Runcorn, UK)

in sequence with a precolumn (30 mm long, 4.6 mm i.d.) of the same material. Elution was performed with a gradient of acetonitrile as the non-polar phase (B) in 5 mM triethanolammonium bicarbonate (adjusted with acetic acid to pH 6) as the polar phase (A). For the gradient the following protocol was used (% B in A each case): 0–12 min 10%; 12–13 min 10–11.5%; 13–26 min 11.5%; 26–55 min 11.5–28%; 55–80 min 28%. The flow rate was 1 ml · min^{-1}. The absorbance of the effluent was monitored at 265 nm. Fractions of 1 ml volume were collected and evaporated to dryness at room temperature.

For ELISA the HPLC fractions were dissolved in 0.1 M sodium-acetate buffer (pH 5.8). The preparation of the immunogens and enzyme tracers, the production of the antibodies and the procedures of the immunoassays have been described in detail by Fußeder et al. (1988) and Strnad et al. (1992), respectively. The specificity of the antibodies is shown in Table 2. The lower limits of the reliable determinations were in the range of 0.01–0.2 pmol per assay. Aliquot portions of the HPLC fractions were also treated with 0.2 units of β-glucosidase (Sigma G-4511; 12 h at 35° C; Wang et al. 1977) prior to analysis by ELISA. The antibodies against benzyladenosine and *o*-hydroxybenzyladenosine were a kind gift from Dr. M. Strnad (Institute of Experimental Botany, Czech Academy of Sciences, Olomouc, CSFR); their specificities have been described elsewhere (Strnad et al. 1992).

Standardization and evaluation of the analytical procedure. The entire procedure was monitored with an internal standard composed of the free bases, ribosides and glucosides of Z, DHZ and 2iPA added to an aliquot portion of an extract of *Urtica* leaves and determining the recoveries. In all cases, more than 90% of the internal standards were recovered. The recovery of the nucleotides, as determined by the same method, was between 85 and 95%.

The reliability of the ELISAs was examined according to Pengelly (1986) by using samples to which increasing amounts of internal standards had been added. To further rule out errors resulting from impurities with high affinity to the antibody, the dilution method of Crozier et al. (1986) was also employed. None of these tests indicated inaccuracies due to non-cytokinin compounds in the extracts of the various tissues of *U. dioica*.

Chemicals. Zeatin, ZR, DHZ, DHZR, 2iP, 2iPA and benzyladenine were bought from Sigma (München, FRG). The O-glucosides of Z (ZOG), ZR (ZROG), DHZ (DHZOG) and DHZR (DHZROG), zeatin-9-glucoside (Z9G), dihydrozeatin-9-glucoside (DHZ9G), zeatin nucleotide (ZN), dihydrozeatin nucleotide (DHZN) and isopentenyladenine nucleotide (2iPN) were obtained from Apex Organics, Oxford, UK). Benzyladenosine, *o*-hydroxybenzyladenine and *o*-hydroxybenzyladenosine were a gift from Dr. M. Strnad (see above) and *cis*-ZR was kindly provided by Dr. K. Conrad (University of Greifswald, FRG). For the preparation of standard solutions the molar absorption coefficients were taken from the literature (Daly and Christensen 1956; Kissman and Weis 1956; Fasman 1975) or obtained from Apex Organics. The absorption coefficient E (ε_λ cm^2 · mol^{-1}) of DHZ (17800 at 269 nm) was obtained as a personal communication from Sigma, while that of DHZR was determined, using the purified substance, to be 21000 at 268 nm.

Results

Growth of Urtica dioica at different levels of N-supply. Biomass production of *U. dioica* plants which were grown at levels of nitrogen supply ranging from 1 to 22 mM nitrate showed an optimum-type response with a broad maximum between 3 and 15 mM (Fig. 2A). Biomass distribution between the shoots and roots of these plants was consistently in favour of the shoots. The shoot-to-root (S/R) ratio was lowest at 1 mM nitrate supply, increased at higher levels and reached a plateau at 15 mM nitrate (Fig. 2B). Thus, the S/R ratio could be shifted without affecting the biomass production within the range 3–15 mM nitrate supply. The nitrogen content of the shoot, as expressed by the C/N ratio, was independent of the N-supply. However, the roots responded to higher levels of nitrate supply by accumulating more nitrogen (lower C/N ratio, Fig. 2C). In order to reveal a possible interrelation between the distribution of the biomass and the cytokinin patterns of the individual organs of *U. dioica*, nettles grown at 3 (low nitrogen) and 15 mM nitrate were selected for cytokinin analysis. These plants showed similar biomass production but different S/R ratios. From an ecological viewpoint the plants with the highest above-ground biomass show optimal performance. Therefore, the plants grown at 15 mM nitrate were considered to represent standards.

Table 2. Reactivities ("cross-reactivities") of the antibodies against DHZR, ZR and 2iPA with various cytokinin standards. The intensity of the reaction in the ELISA with the immediate antigen was set at 100%

Cytokinin	Antibody against		
	DHZR	ZR	2iPA
trans-ZR	0.58	100.00	0.21
cis-ZR	8.12	0.85	1.82
Z	0.17	44.50	0.08
DHZR	100.00	2.77	0.19
DHZ	34.25	2.27	0.03
2iP	0.8	0.28	58.94
2iPA	1.14	0.28	100.00
Kinetin	2.11	0.03	0.36
Z9G	2.57	87.30	1.43
DHZ9G	76.10	5.35	0.14
Benzyladenosine	4.11	0.92	12.13
ZN	2.42	64.36	0.25
DHZN	58.54	4.28	0.19
2iPN	1.08	0.28	61.38
ZROG	0.02	0.25	0.002
Z7G	0.022	0.201	0.006

Cytokinin patterns of Urtica diocia plants at optimal nitrate supply. The cytokinin patterns of roots (plus rhizome initials), stems, adult leaves and very young and still-meristematic leaves of the plants are shown on a fresh-weight basis in Fig. 3. Cytokinins of the Z type were dominant in all tissues, amounting to more than 80% of the total cytokinin content. The bulk of the Z conjugates was of the *trans*-Z type. However, a *cis*-Z derivative was also present in considerable amounts, especially so in roots and meristematic leaves. The compound was tentatively identified as *cis*-ZROG from the retention time of the deglucosylated product during HPLC (53 min) and from the coincidence of the cross-reactions with DHZR-, ZR- and 2iPA-antibodies. *Cis*-ZR, the limit of detection of which was 2.1 pmol · (g FW)$^{-1}$, could not be detected in any of the examined tissues. Except in the fully developed adult leaves, *trans*-ZR was present in large excess in comparison with the other *trans*-Z conjugates. Except again for the adult leaves and in contrast to the case with *cis*-ZROG, the O-glucosides of *trans*-Z and *trans*-ZR did not accumulate to any extent. However, another compound with a very short retention time in HPLC reacted

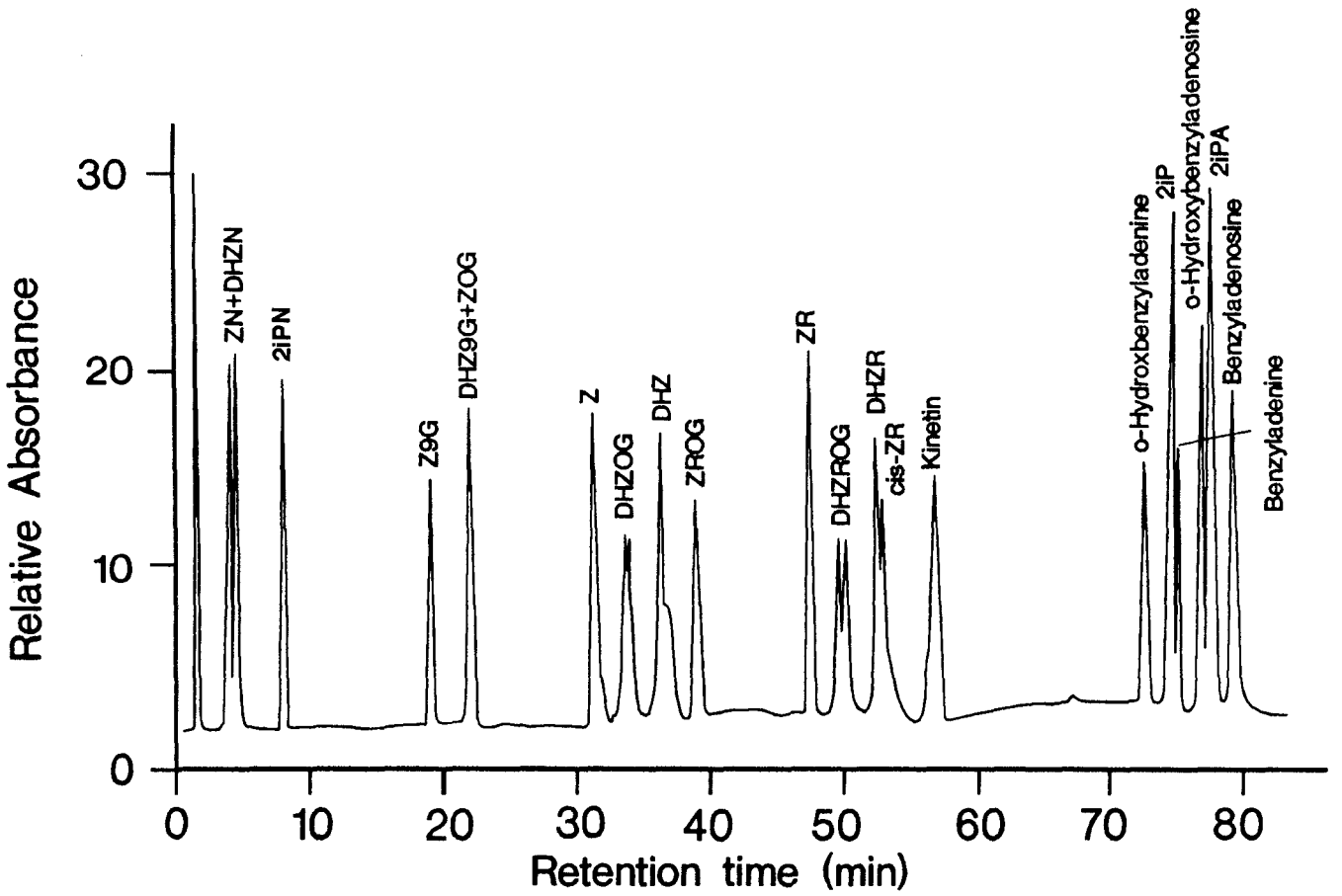

Fig. 1. Separation of cytokinin standards by HPLC on a reversed-phase octadecyl silica column (250 mm long, 4.6 mm i.d.), eluted with a gradient of acetonitrile in 5 mM triethanolammonium bicarbonate. The absorbance of the eluate was monitored at 265 nm. A standard of *cis*-ZROG was not available. The compound tentatively identified as *cis*-ZROG eluted with a retention time of 44 min

strongly with the ZR- but not with the DHZR- and 2iPA-antibodies. Neither deglucosylation nor dephosphorylation resulted in a shift of the retention time or in an altered immunoreaction pattern of this substance. This compound was tentatively addressed as *trans*-Z7G (Horgan and Kramers 1979). Although quantification was not possible, the intensity of the reaction with the ZR-antibody indicated relatively high amounts of this compound to be present in roots, stems and adult leaves. The free base *trans*-Z was present in all examined tissues; except in those of the adult leaves its concentration were less than one-tenth of those of the total *trans*-Z-type cytokinins. The second most important cytokinin in the meristematic leaves was *trans*-ZN, but it was also present in significant amounts in the roots and stems.

In adult leaves the total content of cytokinins was much lower than in the other tissues, and *trans*-ZR and *trans*-ZN, which were otherwise dominant, represented only minor fractions. The free base Z and its O-glucoside were the major adult-leaf cytokinins, followed by 2iPA which was also present in the other tissues of *Urtica*. The amounts of other 2iP-type cytokinins were virtually negligible, except perhaps that of 2iPN in the meristematic leaves. The amounts of DHZ-type cytokinins were also insignificant. One representative of the aromatic cytokinins, namely *o*-hydroxybenzyladenosine, was detected, but only in root samples and at a very low level (0.37 pmol · (g FW)$^{-1}$).

In the root-pressure exudates which were collected during the first hour of the daily light period, eight cytokinins could be demonstrated and quantified. However, only two of these were found in notable concentrations, namely *trans*-ZR and *trans*-Z (Fig. 4). The former was by far the dominant species. Again, more than 90% of the cytokinins identified in the root-pressure exudates were representatives of the *trans*-Z type. Neither *cis*-Z conjugates nor any type of glucoside were detected in the xylem fluid.

The influence of a low nitrate supply to the plant on the cytokinin patterns. In the following the cytokinin patterns of the low nitrogen plants grown at 3 mM nitrate and showing a relatively low S/R ratio (2.9 ± 0.3) are compared with those of the standard plants ($S/R = 4.1 \pm 0.2$) grown at 15 mM NO_3^-. In the adult leaves the level of the nitrogen supply did not significantly affect the cytokinin pattern. In the meristematic leaves and in the stems of the low-nitrogen plants the contents of *trans*-ZR were considerably lower than in the corresponding organs of the standard plants. However, these differences in the *trans*-

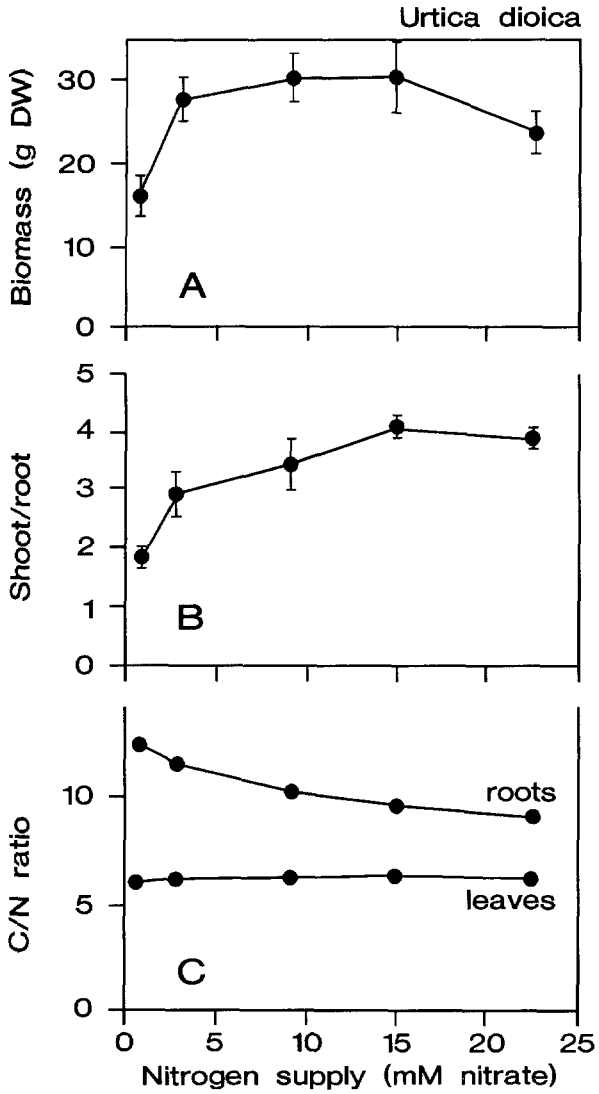

Fig. 2. Influence of the level of nitrogen supply on the total plant biomass (**A**), on the shoot-to-root ratio of biomass (**B**) and on the C/N ratios (**C**) of three-month-old *Urtica dioica* plants, grown at five different concentrations of nitrate in the nutrient solution. Data are means ± SD ($n = 4$)

ZR content were completely compensated for by larger pools of the nucleotides of *trans*-ZR and, to a smaller extent, of 2iPA. The contents of the tentative *cis*-ZROG were substantially lower in the meristematic leaves, but not in the roots and stems of the low-nitrogen plants.

Both the ribosides and the nucleotides were present in significantly lower amounts in the roots of low-nitrogen plants than in the roots of the standard plants. The cytokinin patterns and concentrations of the root-pressure exudates were very similar for both types of plant (Fig. 4). However, the production rate of the low-nitrogen roots was about 40% lower than that of the standard roots when the amounts of exudate were related to the root masses. In addition, the rates of exudation from the roots of the low-nitrogen plants were 25% lower than those from the roots of the standards.

A plot of the cytokinin contents of the roots versus the S/R ratios of the low-nitrogen and standard plants

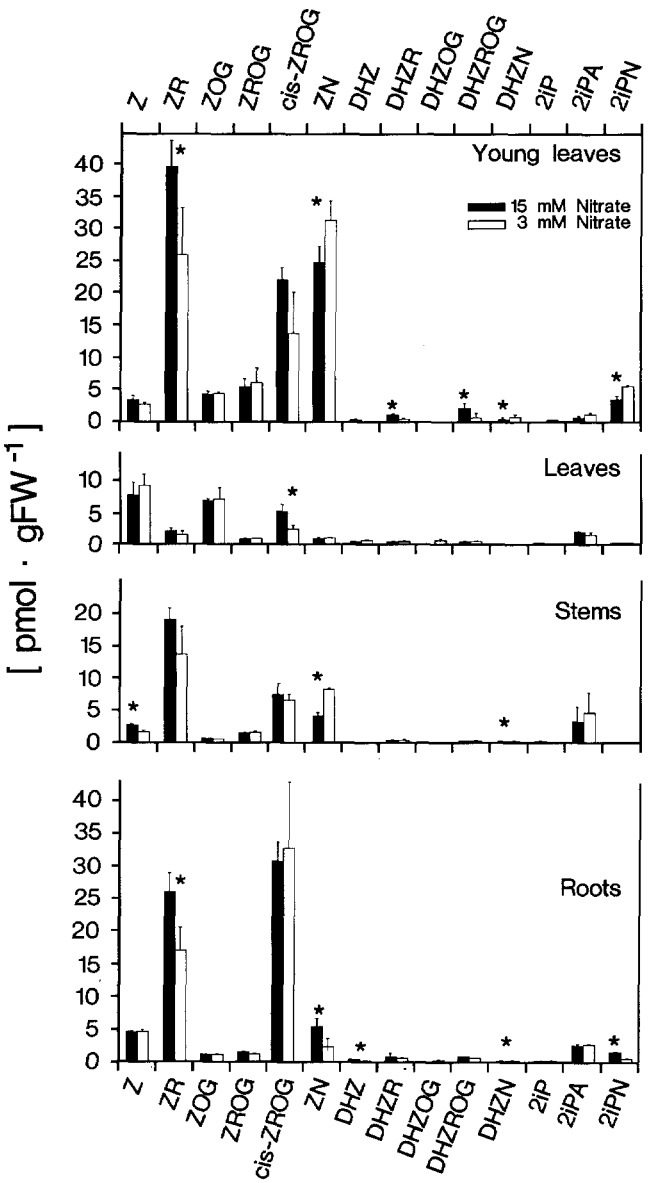

Fig. 3. Cytokinin patterns of various organs of non-flowering plants of *Urtica dioica*. The columns represent mean values of four samples, each of which was prepared from corresponding material of four plants. The *bars* indicate ± SD. *Solid columns*, plants grown at 15 mM nitrate; *open columns*, plants grown at 3 mM NO_3^-. * = statistical error < 5%

Table 3. Export of cytokinins from the roots of *Urtica diocia* via the root-pressure exudate in relation to the nitrogen supply to the plants during growth

	Nitrate concentration in the nutrient solution (mM)	
	15	3
FW of the root system (g)	45.9	54.0
Rate of exudation (ml · h^{-1})	0.5–0.75	0.4–0.5
Cytokinin export per g FW (pmol · h^{-1})	0.41	0.24
Export of total cytokinins by the whole root (pmol · h^{-1})	18.9	13.1

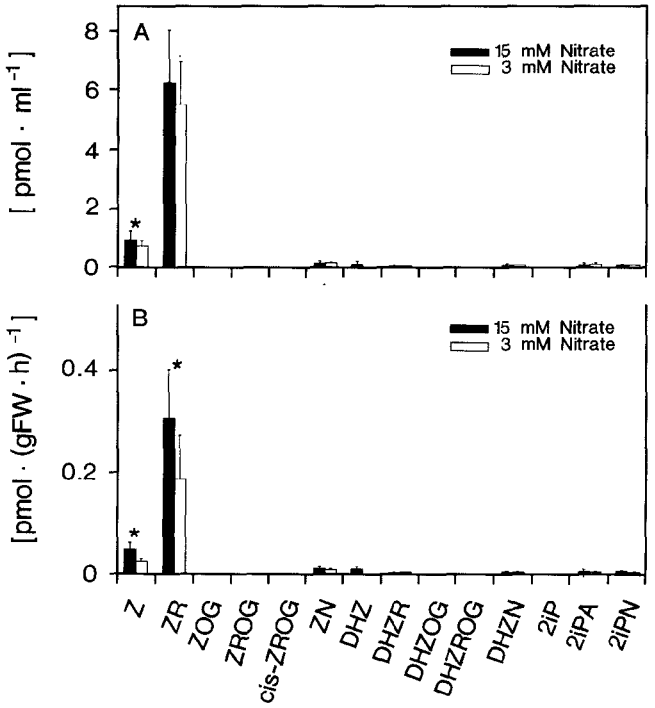

Fig. 4. Cytokinin pattern of the root-pressure exudates collected during the first hour of the daily illumination period from freshly cut stumps of *U. dioica* stumps. The columns represent mean values of four samples for each of which exudate was collected from four stumps. The *bars* indicate ± SD. *Solid columns*, plants grown at 15 mM nitrate; *open columns*, plants grown at 3 mM NO_3^-

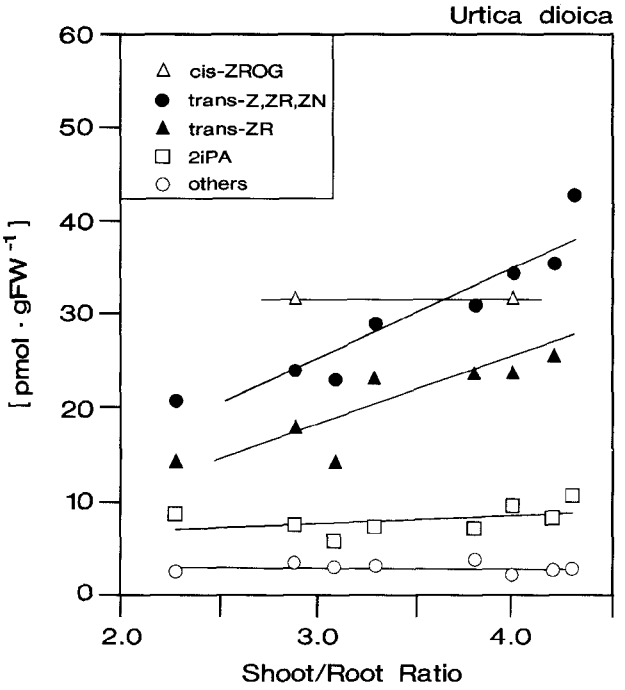

Fig. 5. Correlation of the ratio of shoot:root biomass with root cytokinin content of three-month-old *Urtica dioica* plants

showed a linear relationship. However, only the species of the *trans*-Z family were responsible for this correlation, as the contents of all other cytokinins showed no dependence on S/R (Fig. 5).

Discussion

The cytokinin pattern of Urtica diocia. Because of the demanding analytical procedures involved, comparative studies of the cytokinin patterns of different organs of a plant have not previously been undertaken. A detailed comparison of the cytokinin complement of the various tissues of *Urtica* with that of another species is therefore not practicable. However, the predominant cytokinins have been identified for a number of plant species. In the hop plant, for example, *cis*- and *trans*-ZR are the major cytokinins (Watanabe et al. 1978, 1982) and the same holds for rice (Yoshida et al. 1971; Yoshida and Oritani 1972). Whilst these findings are in agreement with the prevalence of Z-type cytokinins in *Urtica*, DHZ and its conjugates apparently predominate in representatives of the Leguminosae family (Palmer et al. 1981b; Jameson et al. 1987; Griggs et al. 1989).

Since it is well known that the free bases, ribosides and ribotides of the N^6-isoprenyl cytokinins are readily interconvertible by enzymes of adenine metabolism, as well as by those acting on the side chain (Laloue and Pethe 1982; Stuchbury and Burch 1987), the relevance of establishing and comparing cytokinin patterns might be generally questioned. Indeed, the establishment of such patterns in order to deduce certain physiological effects is not very meaningful, since the physiological role of the various classes of cytokinins is still unknown. However, if it is accepted that cytokinins in general are chemical messages which are produced mainly by the root (Letham and Palni 1983), the patterns of such compounds could provide information regarding the root-to-shoot communication of a plant. For that purpose the cytokinin composition of the xylem fluid, by which the root signal enters the shoot, was included in the present study. Only two cytokinins were identified in the root-pressure exudate of *Urtica* in quantities adequate to be considered as root signals, namely Z and, in particular, ZR. The concentrations of even these two compounds in the exudate (Fig. 4) were about fivefold lower than those reported for the xylem saps of other herbaceous plants (Davey and van Staden 1976; Salama and Wareing 1979; Heindl et al. 1982; Palmer and Wong 1985; Upadhyaya et al. 1991). Consequently, the traces of the other six cytokinins in the root-pressure exudate of *Urtica* (Fig. 4) may represent impurities rather than xylem-borne cytokinins.

Zeatin riboside has been reported to be an important translocation form of cytokinins in the xylem (Letham 1978), which is in agreement with the data reported here. However, many other cytokinins, such as *cis*-Z, *cis*-ZR and *trans*-ZN (Murofushi et al. 1983) or the whole 2iP-series (Salama and Wareing 1979; Cahill et al. 1986; Hautala et al. 1986; Hall et al. 1987) and even 0-glucosides (Palmer and Wong 1985) have been identified more or less tentatively in xylem exudates of various herbaceous species. In an earlier communication, we reported the occurrence of significant quantities of Z, ZN, 2iP, 2iPA and 2iPN in the root-pressure exudate of nettles which had been grown under the natural (long day) light regime (Fußeder et al. 1988). Flowering had already been induced in these nettles, while it had not in the plants reported on here: maintenance of the short-

day nettles for an additional month did not result in the development of inflorescences. Comparison of the cytokinin patterns of the xylem fluid of induced and non-induced *Urtica* plants actually demonstrates that the search for a special cytokinin form for long-distance transport may be physiologically less relevant than comparison of the concurrent cytokinin patterns of tissues and the xylem sap.

In the present investigation, O-glucosides were identified in the various tissues of *Urtica*, but not in the root-pressure exudate. They are known to be storage products (Letham et al. 1976; Koshimizu and Iwamura 1986) which are sequestered into the vacuole (Fußeder and Ziegler 1988; Fußeder et al. 1989). Consequently, the O-glucosides must have been produced in situ either from imported precursors or from precursors produced in the individual tissues.

While the dominance of ZR in roots, in the root-pressure exudate, and in stems and meristematic leaves is in good correspondence, the relevance of the nucleotides, in particular of 2iPN and ZN is less obvious. The nucleotide of 2iP is the first compound to be formed along the pathway from AMP and isopentenyl-pyrophosphate to the variety of cytokinins (Koshimizu and Iwamura 1986). Thus its occurrence in the roots, the main site of cytokinin synthesis, is not surprising. However, the comparatively high content of 2iPN in meristematic leaves (Fig. 3) may also reflect de-novo synthesis of cytokinins in these organs (Chen et al. 1985).

Adult leaves, supplied with the same xylem sap as the meristematic leaves, exhibit a different mode of cytokinin metabolism, namely deribosylation of ZR (by adenosine nucleosidase; Stuchbury and Burch 1987) and O-glucosylation of the free base Z. A substantial decline in the level of ZR in favour of the free base and ZOG upon leaf maturation has also been observed with leaves of *Capsicum annuum* (Ulvskov et al. 1992).

Influence of the nitrogen status on the cytokinin patterns. A direct response of the contents (or activities, when determined with a bioassay) of cytokinins to the nitrogen status of a plant has been reported for several annual or perennial species (Wagner and Michael 1971; Sattelmacher and Marschner 1978; Salama and Wareing 1979; Darral and Wareing 1981; Kuiper et al. 1988, 1989), and Kuiper et al. (1988) have shown with *Plantago* that cytokinins can control the distribution of biomass between the shoot and root. This also applies to *U. dioica* grown at low and optimal nitrate concentrations, even within that range of nitrate concentrations at which nitrogen supply only affects the S/R ratio, but not yet the biomass production (a detailed study of this phenomenon is being published elsewhere: Fetene et al. 1993). A clear picture of the correlation between the nitrogen status of *Urtica* and the cytokinin patterns of the plant emerges when the tentative *cis*-ZROG, the physiological significance and origin of which is yet doubtful, is not taken into account. The total contents of all of the other cytokinins in the various organs of the shoots were similar in low-nitrogen and standard plants, and thus an influence of the nitrogen status on the cytokinin level could be seen only for the roots. The observation that the nitrogen content of the shoot was not affected by the level of the nitrate supply, whereas that of the root clearly responded to it (Fig. 2C), merits attention. Since the S/R ratio also correlated with the amount of *trans*-Z cytokinins in the roots (Fig. 5), an interrelationship between the nitrogen status of the roots, the root content of *trans*-zeatin cytokinins, and the biomass distribution between roots and shoots is apparent. A lower rate of cytokinin export from the roots of the low-nitrogen plants as suggested by the calculation presented in Table 3, indicates that the shoots of the low-nitrogen plants receive less ZR and Z from their roots than do the shoots of the standards. Nevertheless, it is presumably oversimplistic to attribute the cytokinin content of the shoot exclusively to export from the roots. This is indicated by the lower contents of ZR, but corresponding higher levels of the nucleotides, in the meristematic leaves and stems of the low-nitrogen plants. As mentioned above, de-novo synthesis in situ may contribute to the cytokinin content of meristematic tissues. Whether the increased levels of nucleotides in the growing tissues of the shoots of the low-nitrogen plants resulted from an enhanced rate of de-novo synthesis or from phosphorylation of the imported ribosides is beyond the evidence of our data.

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