Opinion articles

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Regulation of shoot/root ratio by cytokinins from roots in *Urtica dioica*: Opinion

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Abstract

According to current knowledge, cytokinins are predominantly root-born phytohormones which are transported into the shoot by the transpiration stream. In the "hormone message concept" they are considered the root signals, which mediate the flux of the photosynthates to the various sinks of the plant. In this review, experiments are assessed, in which changes of the shoot to root ratio of biomass, caused by different levels of nitrogen supply to a model plant, *Urtica dioica*, could be traced to the natural cytokinin relations of the plant. Disturbance of the internal cytokinin balance of the plant resulted in a disproportionate distribution of the assimilates in favour of the cytokinin-enriched shoot. Inspite of some shortcomings of the "hormone message concept", the presented work corroborates the significance of root-sourced cytokinins in the regulation of biomass partitioning between shoot and root.

Abbreviations: S/R ratio – shoot:root ratio of biomass (dry matter), Z – zeatin, ZR – zeatin riboside, OG – O-glucoside.

Background

The growth pattern of higher plants, especially in the pre-reproductive state manifests an economic principle (Bloom et al., 1985; Van der Werf et al., 1993) which is illustrated by the partitioning of the photosynthetically produced biomass between roots and shoot in order to effect optimal utilisation of all available resources. Under water or nutrient shortage, relatively more biomass is allocated to the root system, resulting in an increase of the absorbing plant organ. Under sufficient supply of soil-born resources, biomass is, in contrast, predominantly directed to the shoot. This increases photosynthetically active leaf area thereby enhancing growth of plant biomass. Hence, a plant's ratio of shoot to root biomass is not fixed but may vary within species-specific limits in response to environmental factors (McMichael and Quisenberry, 1991).

What do we know about the mechanisms by which external factors, e.g., nutrient or water supply, anaerobiosis, or salinity control growth of the shoot and of the root? A comprehensive review of the whole body of experimental data and conclusions published until 1992 on this topic, has been presented by Jackson (1993) in which he critically assessed the generally adopted concept that the root regulates the distribution of biomass between the shoot and the root. In this paper, Jackson put forward criteria for useful experimental strategies and results for implicating hormonal control in developmental phenomena. In what follows, several more recent sets of data will be reviewed which largely meet these demands. The quoted experiments address the classical question of how nutrients, in particular the nitrogen supply, influences the growth pattern of a plant.

The extent, to which nutrients are supplied to a plant determines its nutrient status (which shall be addressed here only by the principle categories "low, adequate,

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excessive"). Since the sites where growth of the shoot and the root is being effected are the opposite poles of the plant, the involvement of signals in the regulation of biomass partitioning is one reasonable assumption. Another explanation which is derived from a common trait of human behaviour is: "First come, first served" which is a condensed paraphrase of the so-called nutrient control theory (Jackson, 1993; Wilson, 1988). The root system, usually having immediate access to the soil solution, should therefore be the plant organ which is best supplied with soil-born nutrients. In case of a low overall nutrient status of a plant, the root thus can still maintain growth while growth processes at the shoot apex would already suffer from the shortage. This in turn would result in a decrease of the S/R ratio. In the case of those macronutrients which do not undergo biochemical changes like oxidation or reduction, i.e., with Mg, Ca, K and phosphate, this mechanism cannot be ruled out and has therefore been favoured by several investigators (Thornley, 1972; Wilson, 1988). In the case of nitrate or sulfate, however, the presumption of "first served" by the root is less probable, since in many plant species the reduction of these ions to the amino- and thiol groups, respectively, takes place predominantly in the leaves (Gebauer et al., 1988; Marschner, 1986) which would have the primary access to the nutrient. Nevertheless, changes of the R/S ratio are particularly pronounced in response to a plant's nitrogen status, and the relative increase of the root system under nitrogen shortage has been termed "N-etiolement of the root" (Ericsson, 1981). Especially in this case, the involvement of signals is reasonable and the role of the root as sensor of the nutrient status and as producer of the signal(s) is conceivable. Various plant constituents have been discussed to act as such signals, e.g. the main carbon transport metabolite itself, sucrose, (Farrar, 1996; Munns and Cramer, 1996; Van der Werf and Nagel, 1996) or phytohormones, in particular those which affect cytokinesis and cell elongation, the typical reactions of meristematic tissues. Cytokinins and auxin are promising candidates in that respect. Both are required for thriving the cell division cycle at the entry of the S- and mitotic phases, respectively (John et al., 1993). In addition, auxin is the classical phytohormone for cell elongation. According to common knowledge, cytokinins are produced in meristems predominantly in the root, (e.g. Letham and Palni, 1983), while auxin is a typical hormone of the shoot. Both types of phytohormones can migrate from cell to cell but are also phloem-mobile. More important, cytokinins are regular components of the xylem fluid, by which they are transported from the root to the shoot (Letham and Palni, 1983).

A "hormonal message hypothesis" for the regulation by phytohormones of biomass partitioning between shoot and root would therefore comprise the perception by the root of the nutrient (or water) supply to the plant (Kuiper et al., 1989), the translation of the nutrient or water situation into a root-born signal, e.g. a cytokinin, the spreading of this signal over the shoot by the transpiration stream and the direct or indirect control of the cell division activity and cell elongation in the meristematic tissues by the phytohormone(s).

For a balanced view of root to shoot relations and the relative rates of growth it is essential to consider source-sink relations (Geiger, 1987; Geiger and Fondy, 1991). The S/R ratio of biomass is merely an outcome of a continuous distribution of photosynthates between the meristems of the roots and the shoot, which both are, by definition, meristematic (Farrar, 1993a) i.e. utilisation sinks (Ho, 1988). Up to the present, the biochemical mechanisms controlling the assimilate fluxes to such sinks are still a matter of debate. Sink strength, interpreted as the capacity for biomass uptake by a growing tissue could be one of the crucial and therefore controlled factors. However, what determines sink strength and how is it controlled?

In several detailed studies, a feedback limitation of photosynthesis (e.g. Wareing et al., 1968; however, see the comment by Farrar, 1993b) has been envisaged since increases in (photosynthetic) source efficiency are seen after reducing the source capacity by removal or darkening of part of the source leaves. Hence, at least in these cases, sink strength does not comply with the supply of assimilates but rather is a matter of how much of the accumulated photosynthates can be utilised by a growing tissue. In this sense phloem unloading may be compared with opening of a valve to meet the demands of the sink. In our hypothesis, the root signal does not directly operate the valve, but controls the sink activity which finally generates the demand (Möller and Beck, 1992). This view of the sink strength is in accordance with the practical experience of a minimum factor (Marschner, 1986), e.g. shortage of a nutrient, water or oxygen, high salinity, high or low temperature which may give rise to a root signal (e.g. abscisic acid or cytokinins), and thus may inhibit growth inspite of a high potential photosynthetic efficacy.

If the realisation of the signal's message is connected with its turnover, the effectivity of the root signal depends on the fluxes, i.e. on the daily export from the root into the shoot. If the same compound controls also the sink activity of the roots, its actual concentration should be critical which results from the rates of biosynthesis minus those of export and turnover.

The described "hormonal message concept" is certainly oversimplified because it considers only one type and origin of signal, the root-born cytokinins. It further neglects the potential recycling of hormones, e.g. from the source leaves via the phloem into the sinks. However, it provides a kind of directrix for experimental examinations.

Experimental strategies

In many cases a change in the proportion of assimilates incorporated into the roots is connected with an over-all growth limitation on the plant, effected by the shortage of a soil-born factor. However, other factors, such as shading of the lower by the upper leaves can also impair the photosynthetic gain of the whole plant under otherwise optimal conditions. Slightly reduced leaf size, e.g., when caused by a shortage of nutrients, could result in a better illumination of the lower leaves and thus increase their photosynthetic performance. Combinations of environmental factors exist, under which the total biomass production of a plant is not far from maximum due to such mutual compensation of shortcomings of individual parameters. In such near-maximal range of growth, the regulatory machinery which is responsible for the adjustment of the biomass partitioning between root and shoot, can be investigated independently of serious growth limitations (Beck, 1994). Nitrogen shortage is the most convenient method for changing the partitioning of assimilates in a controlled way. Nitrogen supply to the plant is also known to affect the cytokinin content of the plant tissue (for review, see Jackson, 1993). If a causal connection between the cytokinin balance of the plant and the R/S ratio is to be investigated meaningfully, it is essential that the developmental stage of the plant and the growth conditions are carefully maintained. Transitions, such as from preflowering to flowering change sink relations and the cytokinin budget, and must be avoided.

In the experiments reviewed here, stinging nettles (*Urtica dioica* L.) were used as a model system which were grown for 3–6 months in quartz sand. The root-bed was continuously percolated with nutrient solutions, containing very low (1 m*M*), low (3 m*M*), adequate (15 m*M*) and excessive (22 m*M*) nitrogen, respectively. Short day conditions (10 h light, 14 h darkness) were consistently applied to maintain the plants in the preflowering stage. Under these conditions the stems sustain a more or less constant number of leaves, usually 12 to 14 leaf pairs by shedding old leaves when new ones emerge at the apex. Total biomass production, the S/R-ratios and the specific nitrogen content of the plants, measured after 3 months of controlled growth, are shown in Figure 1.

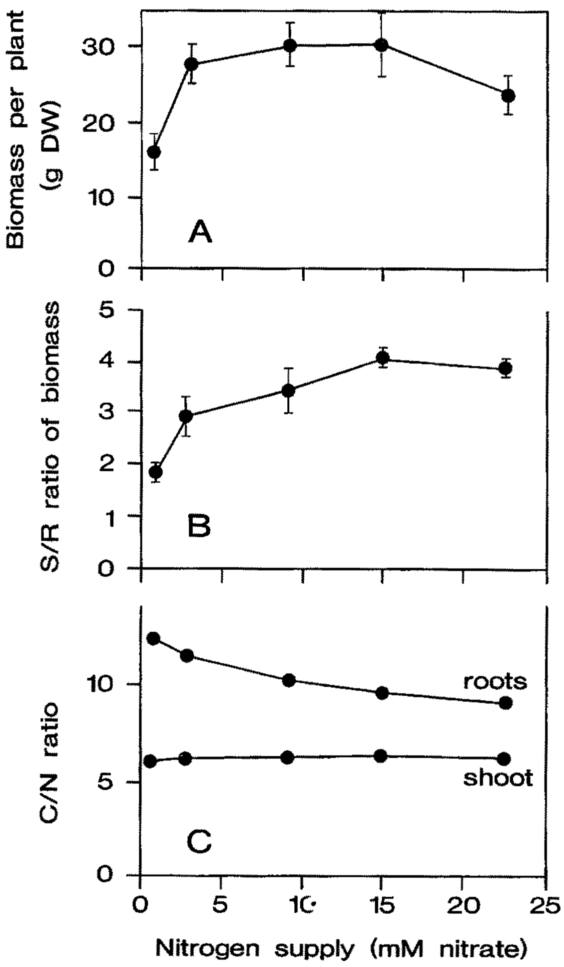

Figure 1. Influence of the level of continuous nitrate supply on (A) the total plant biomass, (B) the shoot to root ratio of biomass, and (C) on the C/N ratios of the shoot and the root, respectively, of three-month-old *Urtica dioica* plants. Data are means ± SD ($n = 4$). (From Beck and Wagner, 1994, modified).

Examination of sink-source relations

Measurement of growth curves of shoots and roots immediately yields the S/R ratios which at steady state growth should be constant. However, this parameter is a derived character, an outcome, and not a biological or metabolic process. When searching for a control mechanism, we must first analyse a plant's internal sink-source relations. An experimental design by which the S/R ratio can be resolved into partial processes requires quantification of the contribution of each source leaf to the total biomass production and the examination of the partitioning of each of the individual shares.

In our experiments, the source-sink relations were analysed for the 12 leaf pairs maintained by the stinging nettles. One leaf of each pair was enclosed in a cuvette and exposed for 3 h to an atmosphere containing 350 ppm $^{14}CO_2$ instead of $^{12}CO_2$ under otherwise unchanged environmental conditions. In this way the natural source-sink relations were maintained and after the usual nocturnal dark phase the export and allocation of the assimilates of each investigated leaf could be determined. Since the vegetatively growing nettles sustained only two major sink regions (the shoot apex and the root system), the radiocarbon recovered from the various plant parts is attributable to one of these sinks. Only ^{14}C in the internode below the examined leaf, due to the vascular architecture, may migrate to both sinks (Fetene et al., unpublished results) and therefore cannot be properly appointed. Neglecting the ^{14}C-content of that internode, two portions of radiocarbon could be formed - above and below the treated leaf - and attributed to the shoot and root sink, respectively, and the same procedure can be applied to each leaf. The ratio of shoot-bound ^{14}C to root-bound ^{14}C was termed relative sink strength (of the shoot). It is characteristic of the respective leaf (pair) and changes in accordance with a change of the R/S ratio (Fetene et al., 1993). These experiments showed that only the youngest two leaf pairs of the nettle represented pure sinks. Leaf pairs # 3 and 4 (as counted from the apex) imported assimilates from source leaves, but simultaneously exported a minor portion of their own photosynthates. The fifth leaf pair and the older leaves were pure source leaves which typically did not incorporate any ^{14}C exported from other leaves. In general, the pattern of assimilate distribution from the individual leaves confirmed Kursanov's (1984) observation that each sink attracts assimilates predominantly from the closest source. However, the relative sink strengths of the apex and of the root system, as measured at the individual leaf pairs, are adjustable by altering environmental factors such as nutrient supply to the plant. Their effects may largely override those of the sink-source distance: In the low nitrogen *Urtica* plants, the fifth leaf pair feeds predominantly the root system, while at an adequate N-supply these leaves export almost their total carbon gain to the shoot apex (Fetene et al., 1993).

Probing the concept of "first come, first served"

The explanation "first come, first served" for the "N-etiolement of the root" could be examined by comparing the effect of ammonium with that of nitrate on the biomass distribution. In the stinging nettle, as in many plants, nitrate is predominantly assimilated in the leaves (Rosnitschek-Schimmel, 1983, 1985). The enzymic equipment for ammonium assimilation, however, is also active in the roots, as evidenced by the patterns of nitrogenous compounds, especially of amino acids, in the xylem sap. In accordance with the principle of "first come, first served" the N-etiolement of the root should be more pronounced when ammonium is supplied, than with nitrate as nitrogen source because the useful assimilated form of N is available first to the roots, not to the shoot. However, such effect could not be observed (Table 1). Analysis of the nitrogenous compounds in the xylem sap revealed low concentrations of nitrate and amino acids when nitrate was the nitrogen source, and low concentrations of ammonium but high concentrations of amino acids when NH_4^+ was supplied (Rosnitschek-Schimmel, 1985). Ammonium assimilation requires and attracts carbon skeletons (Platt et al., 1977), but in the case of *Urtica* the resulting nitrogenous compounds were again exported into the shoot, rather than utilized for disproportionate growth of the root. In summary, evidence for the hypothesis of "first come, first served" could not be produced.

Control of biomass partitioning by cytokinins: Correspondence of the N- and the cytokinin status

Cytokinins represent a class of phytohormones which comprises a great variety of compounds and a high diversity of effects (Letham and Palni, 1983). In addition, most of the cytokinins are considered as biochemically interconvertible which severely complicates analysis and investigation of their mode of action. Today the individual cytokinins are separated, identified and quantified either by GC-MS or by HPLC combined with immunoassays.

Table 1. Biomass distribution between roots, stems and leaves of approximately 6 months old *Urtica dioica* plants, grown in quartz sand which was continuously percolated with nutrient solution with the following nitrogen contents: 3 m*M* nitrate, or 3 m*M* ammonium sulfate, 22 m*M* nitrate or 22 m*M* ammonium sulfate. The data are mean values from a sample of 5 plants (from Rosnitschek-Schimmel, 1982, modified)

Nitrogen source	3 m*M* NO_3^-	3 m*M* NH_4^+	22 m*M* NO_3^-	22 m*M* NH_4^+
% Biomass in roots	64	56	33	30
% Biomass in stems	17	26	34	30
% Biomass in leaves	19	17	33	40
Biomass of roots (g dry matter)	39.3	19.3	17.2	11.7
Biomass of shoots (g dry matter)	21.3	15.3	32.6	24.0

In the experiments with *Urtica*, the latter technique was used. Each fraction obtained by HPLC was routinely examined with 4 antibodies (against isopentenyladenosin, *trans*-ZR, dihydrozeatin riboside and hydroxybenzyladenosine), and the whole procedure was performed with the original and the dephosphorylated and deglycosylated fractions, respectively. Only those data were considered reliable where the cross-reactivities with at least three different antibodies gave similar results. The quantitative cytokinin spectra of the roots, stems, adult and meristematic leaves (including the entire shoot tip), and of the xylem fluid were identified (Wagner and Beck, 1993).

On the (assumed) basis of a close relation of N-status with the cytokinin pattern (for review of numerous papers see Jackson, 1993), they represent the "cytokinin status" of these plants.

In all organs of *Urtica*, trans-zeatin-type[1] cytokinins were dominant, representing more than 90% of the total cytokinin budget. Except the fully expanded source leaves, where the free base zeatin and its O-glucoside were the major cytokinins, *trans*-zeatinriboside was in large excess over other zeatin conjugates. On the other hand, expanding leaves contained a high concentration of *trans*-zeatin nucleotide and a relatively large amount of isopentenyladenosine monophosphate. Both substances are considered the first cytokinin-type compounds in the biosynthetic pathway from 5′-adenosine monophosphate to the great variety of cytokinin species (Koshimizu and Iwamura, 1986; McGaw, 1988). The high concentrations of the nucleotides in the young leaves (and the shoot tip) raises the question of hormone autonomy of the meristems (Chen et al., 1985). The xylem fluid contains only *trans*-zeatin riboside and small amounts of the free base (Figure 2) and therefore may not be the direct source of the cytokinin nucleotides in the meristematic tissue. Add to this that the bulk of the transpiration takes place in the fully expanded leaves and that only a minor portion of the xylem fluid is transpired by the young leaves. Therefore the relatively high amounts of nucleotides in the young leaves must have been produced in situ, either by de novo-synthesis or by phosphorylation of imported ribosides.

The total content of cytokinins in meristematic and adult leaves, and in the stem of *Urtica* did not show any effect of the nitrogen status of the plants. Only the roots and the root pressure exudate of low nitrogen plants contained significantly less cytokinins than those of plants given adequate N. This finding is not surprising because the root system was the only plant part whose N-status responded to the level of nitrogen supply (Figure 1). Thus, with respect to the total cytokinin content, a positive correlation between the N- and the cytokinin status can be attested for all examined fractions of the plants[2].

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[1] *Cis*-zeatin has also been identified, mainly as *cis*-zeatinriboside-O-glucoside (Figure 2); however, *cis*-zeatin is not known to produce cytokinin effects. Another cytokinin conjugate, tentatively identified as *trans*-zeatin-7-glucoside was also present, mainly in adult leaves and roots. It is considered as inactivation derivative of *trans*-zeatin-type cytokinins. None of these compounds were components of xylem fluid.

[2] It should be emphasized that a comparison of total cytokinin contents (concentration) is only meaningful because cytokinin species known as storage compounds, such as the O-glucosides were detected in significant proportions only in the source leaves, and presumed inactivation products, such as the 7-glucoside were not taken into consideration. Therefore the only cytokinins compared were those with physiological activity.

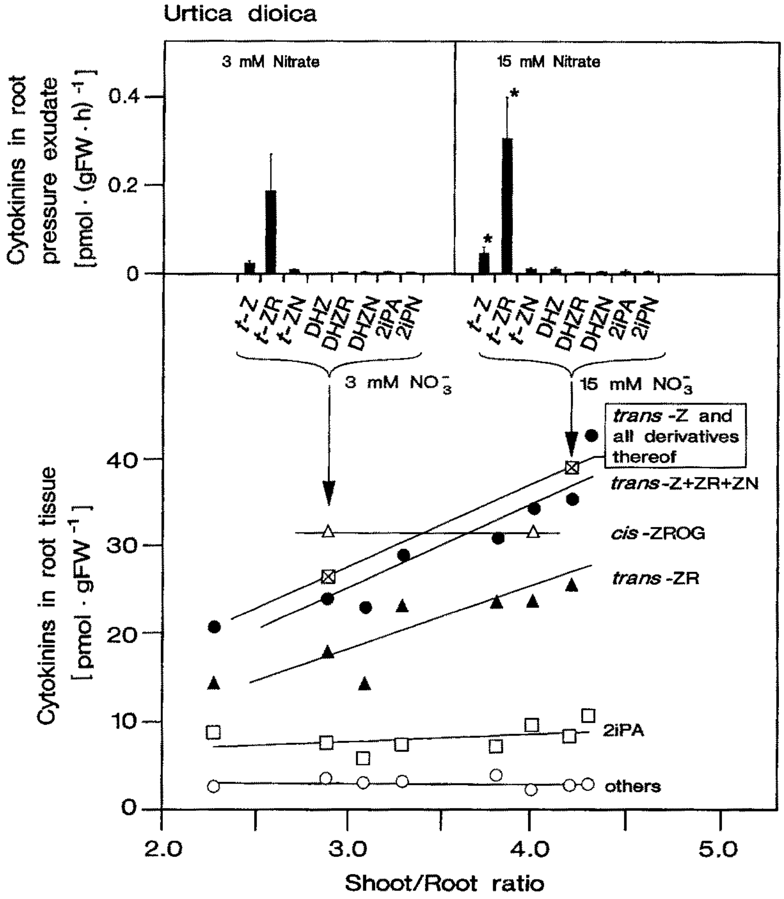

Figure 2. Correlation of the S/R ratios and the cytokinin contents of the roots of three-month-old *Urtica dioica* plants. Insert: Cytokinin patterns of the root pressure exudates collected during the first hour of the daily illumination period from freshly cut stumps of nettles grown at 3 m*M* (S/R ratio = 2.8) and 15 m*M* (S/R ratio = 4.2) nitrate, respectively (from Wagner and Beck 1993, modified).

Table 2. Cytokinin balance of *Urtica dioica* plants of low (3 m*M*) and adequate (15 m*M*) N-status. Four plants and four xylem fluid collections were combined to form one sample each. The contents of cytokinins in the roots are shown in Figure 2. (Data from Wagner and Beck, 1993; Beck, 1994)

Nitrate concentration of the nutrient solution	3 m*M*	15 m*M*
Nitrogen status of the root (C/N ratio given in Figure 1)	Low	Adequate
Root biomass: (g FW)	54.0	45.9
Shoot to root ratio of biomass (see Figure 1)	2.9	4.1
Total cytokinin content of the shoot (pmol)	2696	3116
Total cytokinin content of the root (pmol)	1718	2097
Daily cytokinin export from the root to the shoot (pmol day^{-1})	829	1204

Control of biomass partitioning by cytokinins: Correlation of the S/R ratio and the daily cytokinin synthesis and export by the root

A plot of the cytokinin content of the root system versus the S/R ratio of the nettles shows a positive correlation with respect to the species of the *trans*-zeatin family (Figure 2). However, with respect to cytokinin as a signal, its content in the root is of less importance than the daily cytokinin flux from the root to the shoot. As emphasised by Jackson (1993), the concentrations of

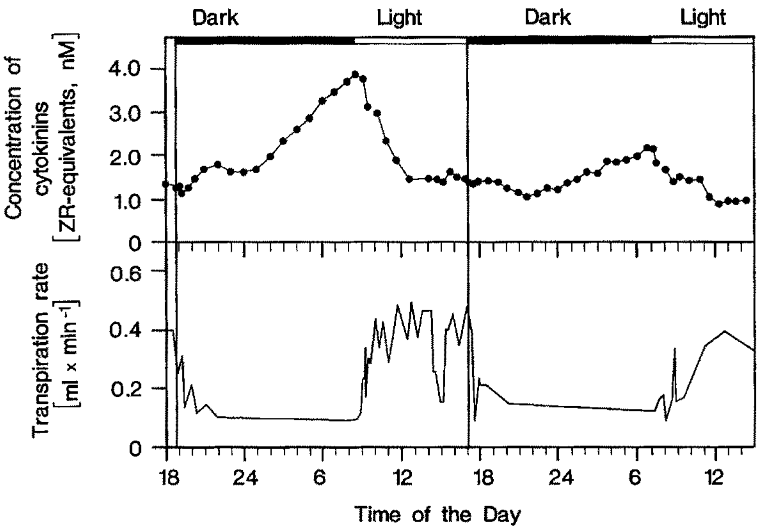

Figure 3. Daily course of the cytokinin concentration in the xylem fluid of a transpiring *Urtica dioica* plant. Xylem fluid was collected from a cut petiole in the middle of the stem. Transpiration was monitored with a balance onto which the plant, mounted in the root pressure chamber, was placed (from Beck and Wagner 1994, modified).

cytokinins in the root pressure exudate do not reflect the concentrations in the actual xylem fluid, the volume of which should by far exceed that of the root exudate. The correlation between both volumes is unknown, as is the root's capacity for cytokinin synthesis. Growing nettles in the root pressure chamber described by Passioura and Munns (1984), allowed an artificial increase of the root pressure exudate up to the rate of the natural transpiration stream (which was determined with the same plants before cutting the stems). Expectedly, cytokinin concentrations in the root pressure exudate decreased with increasing exudation rates. The total amounts of exported cytokinin approached a maximum of 90 pmol h^{-1} at flow rates of 15 mL h^{-1} and higher (maximal transpiration rate: 30 mL h^{-1}, see Figure 3) and thus should reflect the maximal rate of cytokinin biosynthesis (Beck and Wagner, 1994). However, the volume of the transpiration stream is not constant in the course of the day (Figure 3). Transpiration was very low during the night and if cytokinin synthesis and excretion into the xylem take place at a relatively constant rate throughout the day, the concentration in the actual xylem fluid must be high in the morning and should decrease in the course of the day. Samples of the actual xylem fluid could be collected from a petiole after carefully pressurizing the pot in the root pressure chamber. Figure 3 shows the daily course of the cytokinin concentration in the actual xylem fluid which is in good agreement with the above expectation. From such data the actual daily cytokinin flux from the root into the shoot could be calculated and related to the total cytokinin contents of the shoots and the roots of nettles of a low and an adequate N-status, respectively. Table 2 illustrates the lower over-all cytokinin status of the low-nitrogen plants[3]. The data further show that the daily cytokinin gain by the shoot positively correlates with the S/R ratio. A lower cytokinin input into the shoot should result in a lower sink activity or relative sink strength of the shoot apex. This idea is corroborated by the above mentioned finding that in the low nitrogen plants the fifth leaf pair almost exclusively feeds the root system while in plants with an adequate N-status the assimilates of this leaf pair are almost completely incorporated into the apical sink of the shoot.

Control of biomass partitioning by cytokinins: Direct evidence

The results presented in the preceeding paragraphs provide suggestive correlations, but little direct evidence for the control of biomass partitioning by cytokinins. To overcome this deficiency, an experiment was performed in which the cytokinin input in the shoot was artificially increased and the relative sink strengths

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[3] Which is different from the cytokinin "concentration", i.e. the content per gram fresh or dry weight.

Table 3. Source-sink relations of three-month-old *Urtica dioica* plants upon artificially increased flux of the natural cytokinin, zeatin riboside, from the roots to the shoots. Due to experimental limitations the portions of the cut root tips of the total root system varied, and as a consequence the amounts of the absorbed zeatin riboside did not exactly correspond to the supplied concentrations of the ZR-solutions. The distribution ratio between shoot and roots (^{14}C allocated to the shoot apex/^{14}C allocated to the roots) of photosynthates exported from the 6th leaf (as counted from the apex) was used as measure of the relative strengths of both sinks. (Beck, 1994; Data from Fetene and Beck, 1993)

Concentration [*M*] of the applied ZR solution	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
ZR (nmol) taken up by the plant		0.24	5.0	56	40	400
Net CO_2 uptake rate (μmol m^{-2} s^{-1}) of 6th leaf	2.0	1.9	2.8	2.2	2.3	2.1
Respiration (% loss of ^{14}C) from the whole plant	0.7	5.3	2.7	2.7	5.2	1.9
Exported carbon by the ^{14}C-fed 6th leaf as % of the total fixed ^{14}C	35.1	19.2	28.7	19.0	19.0	26.2
Relative sink strength (shoot sink/root sink)	0.018	20	4	20	250	>250

of roots and shoots were determined with the ^{14}C-method (Fetene and Beck, 1993). A small portion of the root system of a nettle was excavated, and immersed in a solution of zeatin riboside, the major xylem-mobile form of cytokinins in *Urtica*. The root tips were then cut and the plant was allowed to take up the cytokinin for 6 hours prior to exposing individual leaves to $^{14}CO_2$. Several concentrations of zeatin riboside were applied. Results are shown for the sixth leaf pair which under normal growth conditions (15 m*M* nitrate in the nutrient solution) exclusively feeds the root system (Table 3).The additional cytokinin-flow via the stumps of a few roots must have bypassed the bulk of the otherwise undisturbed root system. Consequently, the natural cytokinin relations of *Urtica*, as described above (i.e. approximately 1.2 nmol zeatin riboside imported by the shoot per day) were changed in favour of the shoot in a range of between 20% and up to 33,300%. The extra zeatin riboside did not greatly affect photosynthesis, respiration and carbon export from the source leaf. However, already a slight increase by 20%, and the more so a multiple enhancement of the cytokinin import into the shoot resulted in a complete reversal of the direction of the carbon movement. In the control plants, 50% of the exported ^{14}C and 98% of the radiocarbon which could be attributed to one of the major sinks migrated towards the roots. In all ZR-treated plants, however, the shoot apex was the by far dominant sink. Identical relations were found when leaves of the 4th pair were allowed to fix $^{14}CO_2$. These results provide direct evidence that cytokinins represent a powerful root signal for the allocation of recently produced photosynthates. With respect to the control of a plant's biomass partitioning by its N-status, the involvement of the hormonal signal has thus been established, corroborating the "hormone message concept" (Jackson, 1993).

Conclusions

In summarizing the outcome of the work with the model system of vegetatively growing *Urtica dioica*, several conclusions can be made:

1. Cytokinins have been shown to fit the requirements for a root signal acting as hormonal message for shoot growth. However, contributions by other phytohormones to the control of the S/R ratio cannot be ruled out, especially under stress, e.g. by drought (Munns and Cramer, 1996). Moreover, the impact of hormone cycling between xylem and phloem (Taylor et al., 1990), particularly in source leaves (Taylor et al., 1990; Beck and Liegl, unpublished results) requires more attention[4].
2. With regard to the different kinds of hormonal message mentioned by Jackson (1993) the communication of the root with the shoot by cytokinins should be considered a positive message, as the intensity of the signal is positively correlated with the S/R ratio.
3. The suspicion of Trewavas (1991) that any regulatory information carried by hormone traffic between root and shoot would be destroyed by "noise" from environmental irregularities appears to be unfounded. Even small changes in the partitioning of biomass were correlated with significant changes in the cytokinin flux from the root into the shoot and therefore substantial quenching or even destruction of this kind of signal by other environmental influences is unlikely, except those which override the effect of N-status.
4. It is probable that cytokinins have their molecular action in the meristems of the shoot. Likewise, cytokinins should also stimulate or maintain the meristematic activity in the root system. The total cytokinin content of low nitrogen plants is significantly smaller than that of plants of an adequate N-status, and this holds also for the daily cytokinin input into the shoot from roots. However, there is an obvious inconsistency of the results with respect to concentrations of cytokinins in the tissue and the S/R ratio: On the one hand we can appreciate the notion of the root as the presumptive master control station since it is the only organ whose cytokinin content (in the sense of a concentration) corresponds to the N-status of the plant[5]. But, how can we explain that a lower cytokinin "concentration" in the root results in a relatively or even absolute enhanced root growth as indicated by the S/R ratio? The easiest explanation which, however, still needs to be proven for *Urtica* is, that optimal growth of the root requires lower cytokinin concentrations than that of the shoot (Bertell and Eliasson, 1992; Stenlid, 1982). As a consequence, additional assumptions, perhaps including other hormones (Bertell and Eliasson, 1992) are required to explain the different sensitivity for phytohormones of the various tissues of a plant (Trewavas, 1986).
5. Experimental proof of the "hormonal message concept" is not yet complete and several black boxes remain. One major open question is the mechanism, by which the N-supply to the root or its N-status is translated into cytokinin status. Comparison of the quantitative levels of nitrogenous compounds in the root tissue with the much smaller concentrations of the cytokinins suggests regulatory effects by the levels of nitrogenous compounds, such as amino acids, on the biosynthetic pathway to the cytokinins. However, the enzymes of this pathway are not yet well known, and this holds in particular for their regulatory properties. Another possibility is, that independently of the plant's N-status, the nitrate or ammonium concentration in the nutrient solution is sensed by the roots, perhaps by the uptake systems, and is immediately translated into a cytokinin-mediated message to the shoot. However, the mechanism explaining this is even less well-understood than that linking N-status with cytokinin accumulation.

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[4] In *Urtica*, a significant contribution of the phloem to the cytokinins transported via the xylem fluid from roots to shoot appears to be less likely: The qualitative pattern of cytokinins in the root pressure exudate which was collected several hours after cutting the stem, was very similar to that of xylem sap collected via a cut petiole in the middle of the shoot of an otherwise intact plant (Beck and Wagner, 1994).

[5] In Kuiper's (Kuiper et al., 1988, 1989) experiments with *Plantago major* ssp. *pleiosperma* a significant decrease of the cytokinin content of both shoot and root was observed upon lowering the nitrate concentration of the nutrient solution from 2.5 mM to 25 or 50 μM, i.e. subjecting the plants to strong nitrogen deficit. Under these conditions growth was markedly inhibited, whereas in the experiments with *Urtica*, the nitrogen regime was selected to shift the S/R ratio but not to decrease biomass production.

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