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Simultaneous measurement of water flow velocity and solute transport in xylem and phloem of adult plants of *Ricinus communis* over a daily time course by nuclear magnetic resonance spectrometry

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ABSTRACT

A new method for simultaneously quantifying rates of flow in xylem and phloem using the FLASH imaging capabilities of nuclear magnetic resonance (NMR) spectrometry was applied in this study. The method has a time resolution of up to 4 min (for the xylem) and was used to measure the velocity of flows in phloem and xylem for periods of several hours to days. For the first time, diurnal time course measurements of flow velocities and apparent volume flows in phloem and xylem in the hypocotyl of 40-d-old *Ricinus communis* L were obtained. Additional data on gas exchange and the chemical composition of leaves, xylem and phloem sap were used to assess the role of leaves as sinks for xylem sap and sources for phloem. The velocity in the phloem (0·250 ± 0·004 mm s^{-1}) was constant over a full day and not notably affected by the light/dark cycle. Sucrose was loaded into the phloem and transported at night, owing to degradation of starch accumulated during the day. Concentrations of solutes in the phloem were generally less during the night than during the day but varied little within either the day or night. In contrast to the phloem, flow velocities in the xylem were about 1·6-fold higher in the light (0·401 ± 0·004 mm s^{-1}) than in the dark (0·255 ± 0·003 mm s^{-1}) and volume flow varied commensurately. Larger delays were observed in changes to xylem flow velocity with variation in light than in gas exchange. The relative rates of solute transport during day and night were estimated on the basis of relative flow and solute concentrations in xylem and phloem. In general, changes in relative flow rates were compensated for by changes in solute concentration during the daily light/dark cycle. However, the major solutes (K^+, NO_3^-) varied appreciably in relative concentrations. Hence the regulation of loading into transport systems seems to be more important to the overall process of solute transport than do changes in mass flow. Due to transport behaviour, the chemical composition of leaves varied during the day only with regard to starch and soluble carbohydrates.

Key-words: chemical composition; fast nuclear magnetic resonance imaging., long-distance transport; phloem flow; xylem flow

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INTRODUCTION

Characterization of long-distance transport systems is fundamental to understanding the physiology of higher plants. In the xylem and phloem, water and solutes are transported by a mass flow. Transported solutes vary widely in composition (e.g. inorganic ions taken up from the environment by the roots, organic compounds produced by plant metabolism) and, in the phloem in particular, photo-assimilates are transported as energy in chemical form. For a number of physiological questions, knowledge of transport velocities and rates of transport are of basic interest. Additionally, the rate of response of transport in xylem and phloem to rapid changes in environmental conditions (such as light, temperature, humidity) is integral to many aspects of plant metabolism but poorly understood owing to lack of quantitative methods. Current knowledge of the 'driving forces' for xylem and phloem transport of water and solutes is still based on gradients in hydrostatic pressure and water potential for the xylem (but see Benkert *et al*. 1995 and Thürmer *et al*. 1999), and chemical potential (gradients in concentrations of photo-assimilates, the pressure-flow theory of translocation, Münch 1930) for the phloem. Gas exchange of water vapour, CO_2 and O_2 and associated processes are central to the regulation of long-distance transport in plants. Both phloem and xylem systems are reasonably complex and susceptible to manipulation. For these reasons, it has remained difficult to investigate the long-distance transport systems of plants *in situ*.

Heat-based methods have been used to trace movement of xylem sap and can yield reasonably accurate measures

of flow and velocity (see Smith & Allen 1996 for a review). Nonetheless, some comparisons of methods for quantifying xylem flow (e.g. Köstner *et al*. 1996), have produced unsatisfactory results (Cohen & Li 1996). Dyes and radioactive tracers have been used to investigate phloem transport (see Eschrich & Eschrich 1989; Patrick 1997; Knoblauch & van Bel 1998; Oparka & Turgeon 1999). However, the extreme sensitivity of the phloem structure to physical manipulation or wounding (Knoblauch & van Bel 1998; Oparka & Turgeon 1999) has made reliable quantification of flow and velocity difficult. Further problems arise owing to the much lower cross-sectional area in phloem than in xylem and the (assumed) lower rates of flow of liquid in phloem. For example, modelling of water flows in whole plants revealed that in a period of 9 d, only 1% water was transported basipetally in the phloem at the hypocotyl compared to the transpiration stream in the xylem (Jeschke *et al*. 1996). Tanner & Beevers (1990) argued that the relative amount of phloem-recycled xylem-water depends on transpiration.

Köckenberger *et al*. (1997) recently applied a non-invasive nuclear magnetic resonance (NMR) technique to measure water flow in *Ricinus*. They showed that previous methods for measuring water flow in phloem affected the flow rate. However, they used young, poorly transpiring and photosynthetically inactive plants. In addition, measurement periods were longer than 4 h – longer than desirable when we seek to investigate physiological responses to rapidly changing environmental variables such as light that may change within minutes. Herein we report studies of flow velocity and apparent volume flow in phloem and xylem measured simultaneously by fast NMR FLASH imaging (Rokitta, Zimmermann & Haase 1999a) that can be applied in combination with gas exchange measurements (Rokitta *et al*. 1999b). We examined flows in the hypocotyl; the part of phloem involved in transport (van Bel 1993) as opposed to loading and unloading zones. Additionally, photosynthesis and transpiration were measured since they provided much of the 'driving force' for mass flow, as well as the chemical composition of transport fluids. This combination of methods was used to quantify, for the first time, the effect of light regime on long-distance transport systems simultaneously.

MATERIALS AND METHODS

Plant cultivation

Ricinus communis L. plantlets were cultivated as described by Peuke, Hartung & Jeschke (1994). Modifications were made as dictated by the requirements of the NMR instrument. Seeds were germinated in vermiculite moistened with 0·5 mM $CaSO_4$. After 10–12 d in the dark to induce etiolated hypocotyls, uniform seedlings were each transferred to 1 dm^3 pots (9 cm diameter) that fitted within the bore of the NMR magnet. The pots were filled with quartz sand, and the plants were supplied daily with a nutrient solution in excess, that contained 4 mM nitrate as N source (Peuke *et al*. 1994).

The plants were cultivated for 35–40 d in a greenhouse (15–25 °C; 45–70% relative humidity) with an artificial 16 h photoperiod provided by mercury-vapour lamps (Osram HQL 400; Osram, Munich, Germany) (300–500 μmol photons m^{-2} s^{-1}).

Light microscopy

Cross-sections of 40-d-old *Ricinus* hypocotyls were prepared using a cryomicrotome (Cryostat H 500 M; Microm, Walldorf, Germany). Sections of 10–15 μm were transferred to glass slides, embedded with glycerol/water (1 : 1; v/v) and examined by an Axiolplane microscope (Zeiss, Oberkochen, Germany) using bright field illumination or fluorescence excitation at 365 nm (Zeiss filter set number 01).

NMR measurements

The NMR pulse sequence is separated in two parts: (a) flow encoding of the whole sample (the part of the stem inside the RF coil), obtained with a so-called phase-contrast method, and (b) spatial resolution, achieved by a FLASH imaging sequence. Phase-contrast flow encoding works in the following manner. The experiments are carried out at a magnetic field strength of 7 T with the protons spinning in the sample precessing around the external magnetic field at a resonance frequency of 300 MHz. This frequency depends linearly on the field strength. For flow encoding, a pulsed magnetic field gradient (duration of approx. 1 ms) is applied along the flow direction. The gradient means that the field strength of this additional magnetic field varies linearly with position. This also leads to a different resonance frequency at every position along the flow direction. After a rather long time of 200 ms, an inverse gradient pulse is applied which turns back all spins to a net phase shift of zero with respect to the base frequency. However, if proton spins have moved between the two gradient pulses they do not see the same target as when an inverse additional magnetic field is applied and thay therefore have a net phase shift which is not zero. This phase shift is proportional to the flow velocity and it is converted into signal intensity and is spatially resolved by FLASH imaging. To increase the accuracy of the measurement, eight images with different gradient strength are acquired. In a pixel by pixel fit of the signal intensity to the gradient strength, the flow velocity is determined in absolute units. A more mathematical description is given in Rokitta *et al*. (1999a). The method has an intrinsic lower limit of approx. 0·1 mm s^{-1}. Velocities lower than this limit will be rejected by the fitting procedure and appear as zero in the flow map. The flow velocities given here are average values over the xylem or phloem cross-sectional area, respectively, which is selected by hand. For calculation of these averages only non-zero values are taken into account. This leads to a slight over-estimation of

the average velocities. Volume flow is calculated as flow velocity times conducting area. Summation over all pixels leads to the total volume flow. As the vessels can be smaller than a single image pixel, we do not know the conducting area exactly. Signal intensity is a measure of the amount of flowing water in the pixel but cannot be used for absolute quantification. Therefore, we only give relative values for the volume flow which are normalized to the maximum value of each series. In this case summation over the region of interest (xylem or phloem) includes zero values to take into account that some pixels might drop below the lower limit during a long-term measurement. If we could quantify in absolute units this would lead to an underestimation of the volume flow (see also Rokitta *et al*. 1999b).

The NMR experiments were conducted using a horizontal bore magnet (7T Bruker BIOSPEC 70/20; Bruker, Karlsruhe, Germany). The temporal resolution was 7 min for the phloem and 4 min for the xylem. The spatial resolution is in the range of 75×300 μm in the plane and the minimum detectable velocity was approx. 0·1 mm s^{-1}. More detailed information about the NMR technique is given in Rokitta *et al*. (1999a).

Gas exchange measurements

The purpose-built NMR probe-head was sealed air-tight during the NMR experiments in order to monitor transpiration and assimilation rate simultaneously. A circulating gas system was used in conjunction with a commercial gas exchange system (Walz, Effeltrich, Germany) to monitor CO_2 and water vapour concentrations. Plants were illuminated through the front window of the probehead with an irradiance of about 500 μmol m^{-2} s^{-1}. Temperature and relative humidity were kept at about 25 °C and 50%, respectively. Additional information about the data handling is given in Rokitta *et al*. (1999b).

Harvesting and analysis

For harvesting, plants were divided in roots, stems, petioles, and leaf laminae. The leaves were carefully washed with water before chemical analysis. Xylem and phloem sap were collected from the hypocotyl, the site of NMR measurements. All procedures for harvesting of plant material, extraction, collecting xylem and phloem saps, and chemical determinations were recently described in detail (Peuke & Jeschke 1993; Peuke *et al*. 1994). Amino acids in the transport fluids were determined by use of an amino acid analyser (Aminosäureanalysator Biotronik Co., Maintal, Germany). Cations were measured by atomic absorption spectrometry (FMD 3; Carl Zeiss, Oberkochen, Germany) and anions by anion chromatography (Anionenchromatograph, Biotronik Co.).

The elemental composition of the dry matter was analysed using an ICP spectrometer (JY 70 plus; ISA, Instrument S.A. division, Jobin-Yvon, Longjumeau, France) and carbon and nitrogen were analysed using a CHN analyser (CHN-O-Rapid; Heräus, Hanau, Germany). For the determination of carbohydrates, dry matter was extracted with water and after centrifugation the pellet was incubated with amyloglucosidase. Soluble carbohydrates were estimated in the water extract and starch after enzymatic digestion by the method of Roe (1955).

Statistics

The NMR experiments were repeated six times with different plants. Determinations of ion contents of plant parts were based on three plants from three independent cultivation procedures and each plant part was analysed individually. Each analysis consisted of two replicates of extraction and two or three independent measurements per extraction. In the case of transport sap, 36 samples of the xylem and 27 samples of the phloem were analysed.

All statistical calculations were carried out using the statistical software system SAS® release 6·12 (SAS/ETS 1993). The procedure TTEST was used to compare flow parameters in dark and light. Analysis of variance (ANOVA) was used, with the help of the procedure GLM, to test for differences among experiments. ANOVA and *t*-tests were used, although it is acknowledged that assumptions of independence of data obtained within experiments may not have been fulfilled. A non-parametric test on the basis of Wilcoxon scores was calculated with the procedure NPAR1WAY.

The daily time courses of the different parameters were investigated by the analysis of time series – a procedure that overcomes problems of independence within single experiments. With this method it is possible to evaluate whether there is a significant relationship between input and output series, the intensity of influence and whether there is a delay and, if so, its duration. These calculations were made with the help of the procedure ARIMA of SAS including the instructions, assumptions, and criteria of this software (SAS/ETS 1993). This procedure of analysis is described in more detail by Busch, Brechtel & Urfer (1994).

RESULTS

Growth, macroscopic and microscopic observations

The plants used in the NMR experiments reached a biomass of 25–35 g fresh mass, a shoot : root ratio of 0·85–1·14 and had four to six leaves with a leaf area of 240–280 cm^2, after 35–40 d of growth under the described cultivation conditions. The diameter of the hypocotyl, the site of measurement, was between 5·1 and 6·1 mm.

Using a microscopic analysis of a cross-section of the hypocotyl, the distance between the axis surface and the outer limit of the phloem was assessed to be about 250–300 μm (Fig. 1). The phloem ring itself was 100–150 μm thick, and was separated from the xylem by the cambium ring of about the same dimension. The secondary xylem

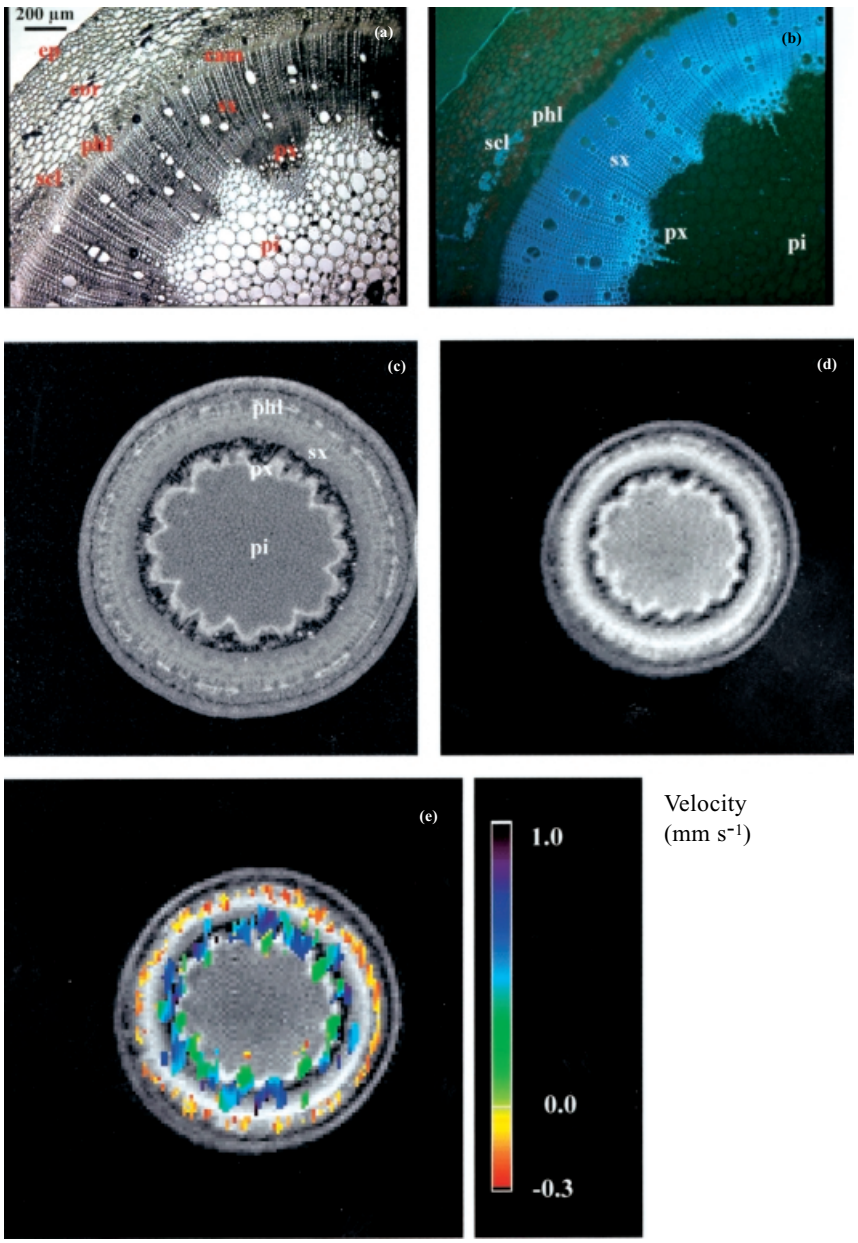

Figure 1. Cross-sections through the hypocotyl of 40-d-old *Ricinus communis* L. (a) Light microscopy with bright field illumination; (b) UV-microscopy with fluorescence excitation at 365 nm; (c) spin echo image by NMR (echo time = 15 ms; repetition time = 0·5 s; slice thickness = 0·6 mm; field of view = 8 mm × 8 mm; nominal in-plane resolution = 31 μm × 31 μm); (d) FLASH image (echo time = 4·3 ms; repetition time = 11·5 ms; slice thickness = 3 mm; field of view = 6 mm × 6 mm; nominal in-plane resolution = 47 μm × 47 μm^2); (e) flow velocity map (matrix = 128 × 32 zero-filled to 128 × 128; slice thickness = 4 mm; field of view = 10 mm × 10 mm reduced to 6 mm × 6 mm; measuring time = 7 min; nominal in plane resolution = 78 μm × 313 μm). Abbreviations: ep, epidermis; cor, cortex; scl, sclerenchyma; phl, phloem; cam, cambium; sx, secondary xylem; px, primary xylem; pi, pith.

ring had a thickness of 300 ± 100 μm. In the pith the initial seven to eight bundles could be observed. Cells in the phloem had a diameter of about 30 μm. The vessels in the primary xylem were about 50–60 μm and in the secondary xylem 80–100 μm.

Flow velocity in xylem and phloem during daily time course

NMR was used to quantify flow velocity simultaneously in phloem and xylem over a daily time course with good resolution (up to 4–7 min). Six experiments were done and all yielded similar results with respect to light (Figs 2 and 3). In both xylem and phloem, transport took place over the whole day. In the light, the velocity of xylem sap was clearly higher than that of phloem sap, whereas in the dark both velocities were similar.

The velocity of phloem sap was independent of the light regime (Fig. 2a–f) and mean values for each experiment were significantly different with the exception of experiments 5 and 6 (Table 1). The mean velocity of phloem sap in the light was 0·243 ± 0·003 mm s^{-1} and in the dark was 0·255 ± 0·004 mm s^{-1}. The average value from all experiments was 0·250 ± 0·004 mm s^{-1}. Although, significant effects of light on phloem velocity were found in single experiments, the effects were mostly small (maximum factor 1·12) and sometimes opposite (Table 1). ANOVA/*t*-test and Wilcoxon scores resulted in similar *P*-values. Only a *t*-test of the pooled data suggested any significant difference between rates of flow in the dark and in the light.

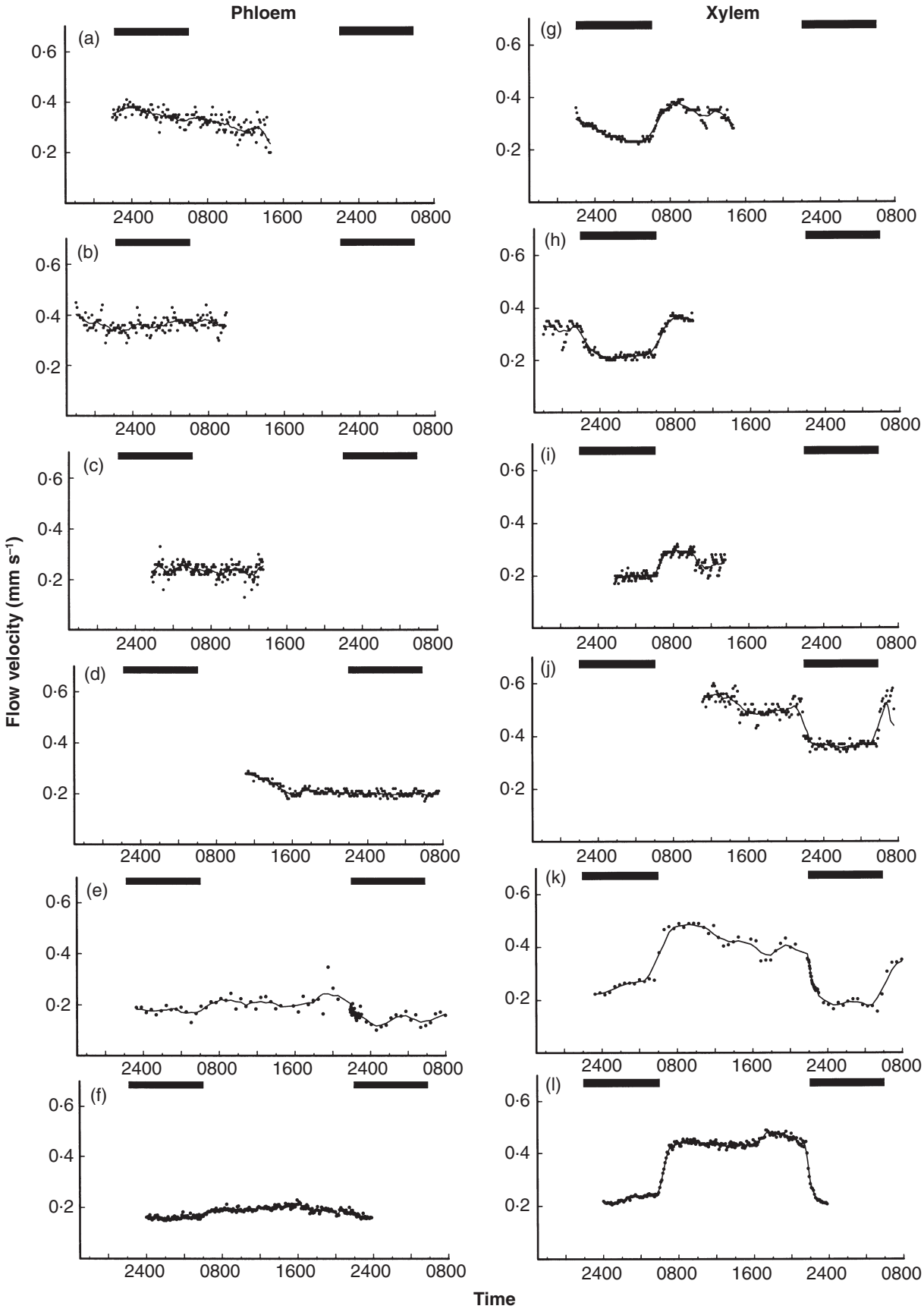

Figure 2. Flow velocities in the phloem (experiment 1–6: a–f) and in the xylem (experiment 1–6: g–l) in the hypocotyl of 40-d-old *Ricinus communis* during daytime measured by fast NMR micro-imaging. The symbols show the raw data and the lines the moving average (n = 15). The dark period is indicated by black boxes.

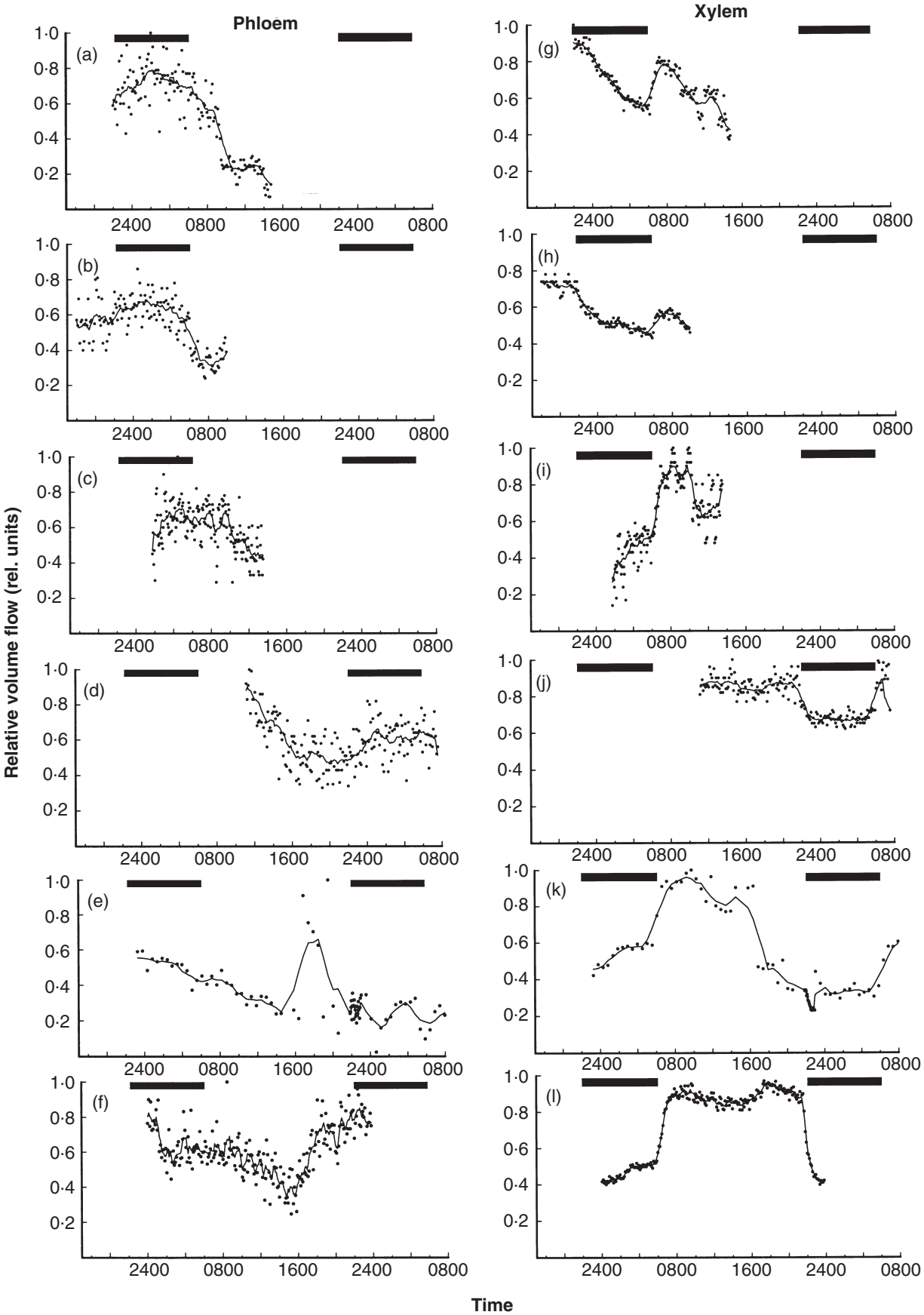

Figure 3. Relative volume flow in the phloem (experiment 1–6: a–f) and in the xylem (experiment 1–6: g–l) in the hypocotyl of 40-d-old *Ricinus communis* during daytime measured by fast NMR micro-imaging. The symbols show the raw data and the lines the moving average ($n = 15$). The dark period is indicated by black boxes.

The flow velocity in the xylem showed a clear positive response to light (Fig. 2g–l) and was generally about 1·6-fold higher in the light (0·401 ± 0·004 mm s^{-1}) than in the dark (0·255 ± 0·003 mm s^{-1}). ANOVA showed that light had a significant effect on xylem velocity in each experiment and that the response varied significantly among experiments (Table 1). In experiment 4 only the rates in the light (0·365 mm $s^{-1)}$ and dark (0·520 mm s^{-1}) were remarkably higher than in the other three experiments.

The product of velocity and the cross-sectional area of conducting tissue provides an index of relative volume flow. In the phloem and the xylem, flow data were standardized (related to the maximum value within each experiment) and expressed in relative units (as 'apparent volume flow'). The effect of light on the apparent phloem flow was not clear (Fig. 3a–f). In four of six experiments, apparent flows were increased in the dark, but in the two other experiments, flow was reduced. Considering all the experiments, the relative volume flow in the phloem increased once the light was switched off by a factor of 1·14.

In contrast, the relative flow in the xylem was 30% less in the dark (Fig. 3g–l) and only in experiment 1 was this effect not significant (Table 1). These observations corresponded with the observed xylem velocities.

Gas exchange measurements during daily time course

As was to be expected, transpiration and CO_2-exchange were strongly affected by the light conditions (Fig. 4). In the light, transpiration was more than twice that in the dark. Equipment limitations prevented the recording of the full transpiration curve in experiment 4.

During the dark period, CO_2 was released (negative values, respiration) and in the light period CO_2 was taken up (positive values, assimilation). Assimilation appeared to respond more quickly to changes in light than transpiration.

If experiments were initiated during the light period (experiments 2 and 4), it took several hours for rates of assimilation and transpiration to stabilize.

Time-series analysis

A time-series analysis of the effect of light on sap velocities and apparent volume flows in xylem or phloem (output series) was undertaken to further investigate the significance of the observed changes. According to the necessary assumptions and criteria of this method the following model fitted best to the data (for more details of the procedure see Busch *et al*. 1994), varying only in the period of delay:

$$(1-B^1)\ln(\ldots) = \mu \pm SE + (N^1 \pm SE \times B{*}{*}(1)) \times L + (1 + M^{1,1} \pm SE \times B^1) \times a_t,$$

where B is the backshift operator; d is the differencing with period 1 (d times); Y_t is the time series; μ is the intercept of the differenced series (trend component); L is the light; $M^{1,1}$ (ma1,1) is the moving average factor; a_t is white noise; and N^1 (num1) is the numerator factor of the input light.

The estimated factors for the time-series transfer function of each transport parameter per experiment are given in Table 2. The intercept (μ) and the moving average factor (M^1/ma 1) describe the internal mathematical structure of the time model. Interpretation is not simple, and depends on the coding of the input series (0 = dark, 1 = light) and is only of moderate biological interest. The numeric factor (N^1/num 1) gives the amount and direction of influence of light on the measured parameters (in the actual case logarithm of velocity and flow). The time lag (nlag) gives the time period at which the cross-correlation function first became significant. The time lag is not part of the equation and can be seen only in the output of the software. Together with the time period of the measurements, the delay can be calculated.

Only in the first and third experiment was a significant cross-correlation between light and phloem velocity found. The calculated delay was 76 min in the first experiment and 73 min in the second. Relative phloem flow was unaffected by light.

In contrast to the phloem, time-series analysis of xylem velocity and relative flow showed significant cross-correlation. Only in the third experiment was there no dependency on light (and additionally in experiment two for relative xylem flow). A delay in response of xylem velocity of 34 min was observed in the first two experiments. No delay was detected (with respect to a time resolution of 7–32 min) for all other significant functions. The numerical factors were generally larger for the xylem parameters than for the phloem. In experiments 4 and 5, there was a strong effect of light on the xylem flow.

Time-series analysis of the gas exchange measurements always revealed significant cross-correlation functions. Due to technical difficulties in experiments 1, 3, and 6 no gas exchange measurements were made. Transpiration exhibited a delay of 1–3 min in response to changes in photon flux density, whereas CO_2-assimilation was immediately affected.

A direct comparison between transpiration/CO_2-assimilation (input series) and transport parameters (output series) was not possible, as the time periods were different.

Chemical composition of xylem and phloem saps during daytime course

The concentrations of most measured solutes in the phloem were higher in the light period than in the dark (Fig. 5). The changes were significant for glutamate (dark concentration of 70% that in the light), phosphorus (88%), Mg (82%), Ca (47%), and sucrose (88%). In contrast, concentrations of potassium and glutamine, two of the most abundant solutes, remained nearly unchanged over the entire time course.

In the xylem sap, nitrate and potassium were the major solutes (Fig. 6). There was a tendency for concentrations to

Table 1. Velocities and apparent volume flows in the xylem and the phloem of 40-d-old *Ricinus communis* L. measured by NMR in six independent experiments/plants.

	Experiment 1 (n = 147)	Experiment 2 (n = 143)	Experiment 3 (n = 174)	Experiment 4 (n = 178)	Experiment 5 (n = 83)	Experiment 6 (n = 202)	Overall (n = 927)
Phloem velocity (mm s^{-1})							
Light	0·308 ± 0·006	0·367 ± 0·004	0·235 ± 0·003	0·224 ± 0·003	0·204 ± 0·006	0·193 ± 0·001	0·243 ± 0·003
Dark	0·345 ± 0·004	0·364 ± 0·003	0·240 ± 0·003	0·199 ± 0·001	0·161 ± 0·003	0·161 ± 0·001	0·255 ± 0·004
Factor dark/light	1·12	0·99	1·02	0·89	0·79	0·83	1·05
ANOVA signif.	***	NS	NS	***	***	***	*
Differences in exp.	A	B	C	D	E	E	–
Wilcoxon scores	***	NS	NS	***	***	***	NS
Phloem flow (rel. units)							
Light	0·387 ± 0·026	0·434 ± 0·019	0·571 ± 0·016	0·604 ± 0·016	0·375 ± 0·034	0·582 ± 0·012	0·524 ± 0·009
Dark	0·641 ± 0·023	0·666 ± 0·012	0·586 ± 0·012	0·574 ± 0·013	0·323 ± 0·020	0·682 ± 0·014	0·596 ± 0·008
Factor dark/light	1·66	1·53	1·03	0·95	0·86	1·17	1·14
ANOVA signif.	***	***	NS	NS	NS	***	***
Differences in exp.	A	A/B	B	B/C	D	C	–
Wilcoxon scores	***	***	NS	NS	NS	***	***
Xylem velocity (mm s^{-1})							
Light	0·336 ± 0·004	0·334 ± 0·005	0·261 ± 0·004	0·520 ± 0·004	0·412 ± 0·009	0·435 ± 0·003	0·401 ± 0·004
Dark	0·264 ± 0·003	0·234 ± 0·004	0·203 ± 0·002	0·365 ± 0·002	0·240 ± 0·006	0·229 ± 0·002	0·255 ± 0·003
Factor dark/light	0·79	0·70	0·78	0·70	0·58	0·53	0·64
ANOVA signif.	***	***	***	***	***	***	***
Differences in exp.	A	B	C	D	E	E	–
Wilcoxon scores	***	***	***	***	***	***	***
Xylem flow (rel. units)							
Light	0·633 ± 0·016	0·595 ± 0·012	0·751 ± 0·016	0·869 ± 0·006	0·682 ± 0·041	0·870 ± 0·006	0·773 ± 0·007
Dark	0·668 ± 0·017	0·570 ± 0·014	0·479 ± 0·011	0·671 ± 0·005	0·359 ± 0·018	0·468 ± 0·005	0·548 ± 0·007
Factor dark/light	1·06	0·96	0·64	0·77	0·53	0·54	0·71
ANOVA signif.	NS	NS	***	***	***	***	***
Differences in exp.	A	B	A/B	C	D	A	–
Wilcoxon scores	NS	**	***	***	***	***	***

Means, standard errors (±) and the number of points measured (n) for each experiment are shown. The factor of the average values dark/light and the significance (***, $P \leq 0{\cdot}0001$; **, $0{\cdot}0001 < P \leq 0{\cdot}001$; *, $0{\cdot}001 < P \leq 0{\cdot}05$; NS, not significant) of the light effect in ANOVA (experiments 1–6) or t-test (overall) as well as the linear rank statistics based on Wilcoxon scores are also given. Differences between the experiments are indicated by letters.

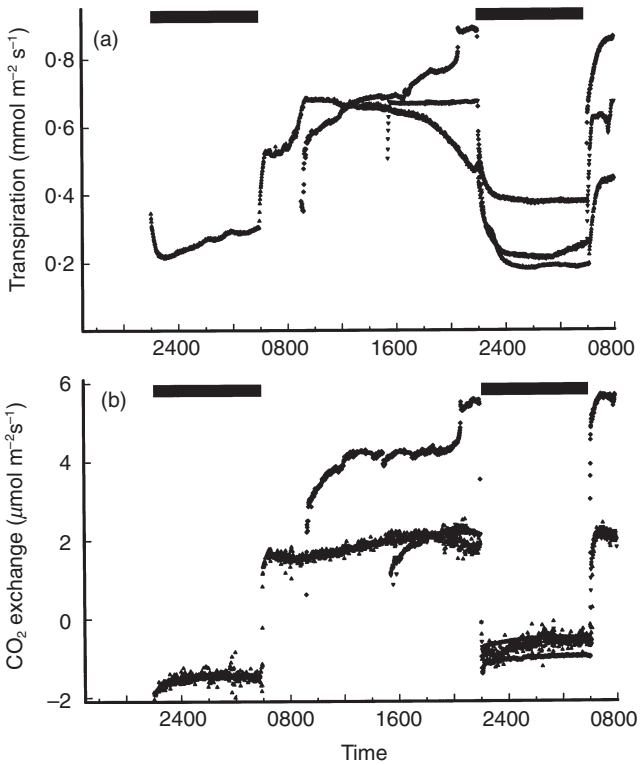

Figure 4. Transpiration and CO_2-exchange rates in whole plants of 40-d-old *Ricinus communis* during the NMR experiments 2 (▼), 4 (◆), and 5 (▲; compare Figs 2 and 3) over a daily time course. The dark period is indicated by black boxes. Due to equipment limitation the transpiration curve of experiment 4 was cut off.

increase during the dark (potassium increased 20%, phosphorus increased 90%). Only sulphur and nitrate were at lower concentrations during the night. Concentrations of most solutes peaked at the time of onset of light.

Chemical composition of leaves during daily time course

Starch accumulated during the light period, and was degraded in the dark (Fig. 7a). Furthermore, the concentration of soluble carbohydrates in leaf dry matter was significantly higher in the light than in the dark. In contrast to starch concentrations, sucrose concentrations were more or less constant within the light or dark period, respectively. Although the absolute difference was small (1%) the concentration of C was significantly less in the light, as was the concentration of S. For other elements, there were few changes in concentration over the daily time course.

DISCUSSION

Flow velocity in xylem and phloem during the daytime course

The applied technique allows us to quantify simultaneously the mass flow of water in the xylem as well as in the phloem in the hypocotyl over a daily time course with good time resolution. Due to the demands of the NMR equipment and the limited space therein, the plants used in this technique must be small. On the other hand, the spatial resolution of NMR imaging is low in comparison with light microscopy and the size of the single image pixel (for imaging, nominal 'in-plane' resolution = 31 μm × 31 μm, or for flow measurements, a minimum 47 μm × 188 μm in-plane) is in the range of thickness of the phloem.

Sap was transported in both phloem and xylem systems, throughout the day. However, there was only a pronounced effect of light in the xylem. Significantly higher velocities were measured in xylem in the light than in the dark. Additionally, there was a strong cross-correlation between the light regime and the xylem velocity or flow-time series. In the light, velocity was increased by a factor of about 1·6 and correspondingly, the volume flow was increased. These changes corresponded to changes in transpiration – not surprising as larger loss of water from leaves must be compensated by a larger supply via the xylem. Huber (1956), using an early heat-tracing method, found larger water-flow velocities during the day than during the night. In a comparison of modern heat-tracer methods, similar results were obtained with differences in absolute values depending upon the system of measurement (Köstner *et al*. 1996). In the present investigations there were consistent discrepancies between relative flow in xylem and transpiration. Transpiration was more than doubled in the light compared with an increase of 1·6 for xylem parameters.

We determined no clear trend in transport parameters for the phloem with variation in the daily light regime. Flow velocities were more or less constant and only in experiments 1 and 3 were there significant cross-correlation between light and phloem velocity. These correlations had a large delay (73–76 min) but a low numerical factor. The apparent volume flow was increased in the dark in four of the six experiments but the reverse effect was observed in the remaining two experiments. Poor spatial resolution may account for these variations. Time-series analysis suggested no significant cross-correlation and it is concluded that there is – surprisingly – no effect of light on the transport velocity and relative flow in phloem over a daily light/dark cycle.

The primary literature and a number of botanical compendia list a wide range of velocities of flow in xylem and phloem. Strasburger (1983) suggested that xylem flows of about 0·30 mm s^{-1} in conifers and 17 mm s^{-1} in deciduous trees might be less than those found in vines (maximum at noon) of 40 mm s^{-1}. For the phloem, values are typically less and between 0·14 mm s^{-1} and 0·28 mm s^{-1}. Mohr & Schopfer (1985) and Marschner (1995) both mentioned a range of 0·05–280 mm s^{-1} for both xylem and phloem. Thus while it is widely accepted that the velocity in the phloem is less than in the xylem, there is considerable variation. Köckenberger *et al*. (1997) applied an NMR technique to very young *Ricinus* seedlings (6 d old) and found that the average velocity in the phloem (0·58 mm s^{-1}) was somewhat higher than that (0·47 mm s^{-1}) in the xylem. However, these experiments with plants in the early stage of development

Table 2. Estimates, time lags and variances calculated for the model of transfer function of the light time series (input series) and velocity or apparent flow in phloem or xylem or gasexchange measurements times series (for the model see Results/time series analysis). The models were calculated by the procedure ARIMA of SAS® release 6·12 and selected by the minimum of Akaike's information criterion and standard deviation. The data were transformed by logarithm to achieve stabilization of the variances. For further details see the Material and Methods section

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
Time period (min)	6·88	6·71	4·07	7·58	31·5	6·80
Phloem velocity:μ	0·0049 ± 0·0039	0·0009 ± 0·0024	0·0010 ± 0·0021	0·0018 ± 0·0013	–0·0061 ± 0·0007	–0·0000 ± 0·0008
ma 1	0·5793 ± 0·0895	0·6270 ± 0·0683	0·7583 ± 0·0501	0·6881 ± 0·0556	1·000 ± 0·0599	0·7323 ± 0·0474
num 1	0·0014 ± 0·0516	0·0053 ± 0·0336	0·02 . . . ± 0·0312	0·0404 ± 0·0198	0·2458 ± 0·0548	0·0673 ± 0·0210
∂^2	0·0199	0·0055	0·0129	0·0024	0·0238	0·0019
nlag/*P*-value	11/0·001	–/n.s.cc.	18/0·007	–/n.s.cc.	–/n.s.cc.	–/n.s.cc.
Phloem flow: μ	–0·0121 ± 0·0077	–0·0035 ± 0·0041	0·0001 ± 0·0039	–0·0023 ± 0·0028	–0·0209 ± 0·0031	0·0000 ± 0·0028
ma 1	0·5785 ± 0·0682	0·6855 ± 0·0635	0·7328 ± 0·0523	0·8116 ± 0·0444	1·000 ± 0·0672	0·7603 ± 0·0452
num 1	0·0025 ± 0·1031	–0·0894 ± 0·0880	–0·0292 ± 0·0538	0·0783 ± 0·0565	0·0883 ± 0·1144	0·0174 ± 0·0755
∂^2	0·0475	0·0236	–	0·0362	0·2348	0·0282
nlag/*P*-value	–/n.s.cc.	–/n.s.cc.	–/n.s.cc.	–/n.s.cc.	–/n.s.cc.	–/n.s.cc.
Xylem velocity: μ	–0·0019 ± 0·0027	–0·0010 ± 0·0030	0·0004 ± 0·0028	–0·0000 ± 0·0015	0·0031 ± 0·0119	–0·0002 ± 0·0022
ma 1	0·1098 ± 0·0837	0·3133 ± 0·0819	0·4414 ± 0·0691	0·6015 ± 0·0636	–0·0462 ± 0·1368	–0·0056 ± 0·0707
num 1	0·0309 ± 0·0211	0·0789 ± 0·0282	0·1096 ± 0·0240	0·2569 ± 0·0202	0·2724 ± 0·0536	0·0814 ± 0·0228
∂^2	0·0013	0·0029	0·0043	0·0024	0·0079	0·0010
nlag/*P*-value	5/0·014	0/0·002	–/n.s.cc.	0/0·000	0/0·000	0/0·000
Xylem flow: μ	–0·0064 ± 0·0038	–0·0058 ± 0·0020	0·0060 ± 0·0070	0·0003 ± 0·0012	0·0004 ± 0·0136	
0·00002 ± 0·0023						
ma 1	0·2573 ± 0·0812	0·5229 ± 0·0741	0·511 ± 0·0659	0·7389 ± 0·0534	0·2544 ± 0·1277	0·1425 ± 0·0701
num 1	0·0042 ± 0·0348	0·0609 ± 0·0258	0·2627 ± 0·0655	0·2379 ± 0·0207	0·2956 ± 0·0794	0·1094 ± 0·0277
∂^2	0·0038	0·0026	0·0347	0·0037	0·0199	0·0015
nlag/*P*-value	0/0·049	–/n.s.cc.	–/n.s.cc.	0/0·007	0/0·041	0/0·000
Time period (min)	1·13	1·02	1·14	1·11	1·13	n.m.
Transpiration: μ	n.c	0·0001 ± 0·0002	n.c.	0·0003 ± 0·0004	0·0001 ± 0·0001	–
ma 1	–	–0·3430 ± 0·0265	–	–0·4636 ± 0·0253	0·1767 ± 0·0234	–
num 1	–	0·0041 ± 0·0026	–	–0·0422 ± 0·0042	0·0216 ± 0·0025	–
∂^2	–	0·00003	–	0·00009	0·00002	–
nlag/*P*-value	–	2/0·000	–	1/0·000	0/0·000	–
Assimilation: μ	–	0·0009 ± 0·0035	–	0·0036 ± 0·0082	0·0009 ± 0·0005	–
ma 1	–	–0·0896 ± 0·0281	–	–0·4735 ± 0·0251	0·8233 ± 0·0159	–
num 1	–	0·0033 ± 0·0572	–	–0·0984 ± 0·0858	2·1714 ± 0·0536	–
∂^2	–	0·0132	–	0·0379	0·0178	–
nlag/*P*-value	–	1/0·000	–	0/0·000	0/0·000	–

μ, intercept; ma 1, moving average factor; num 1, numeric factor (effect of light on the output series); ∂^2, variance of the model; nlag, time lag at which the cross-correlation becomes significant ($P = 0·05$); n.s.cc., no significant cross-correlation; n.m., not measured; n.c., not calculated.

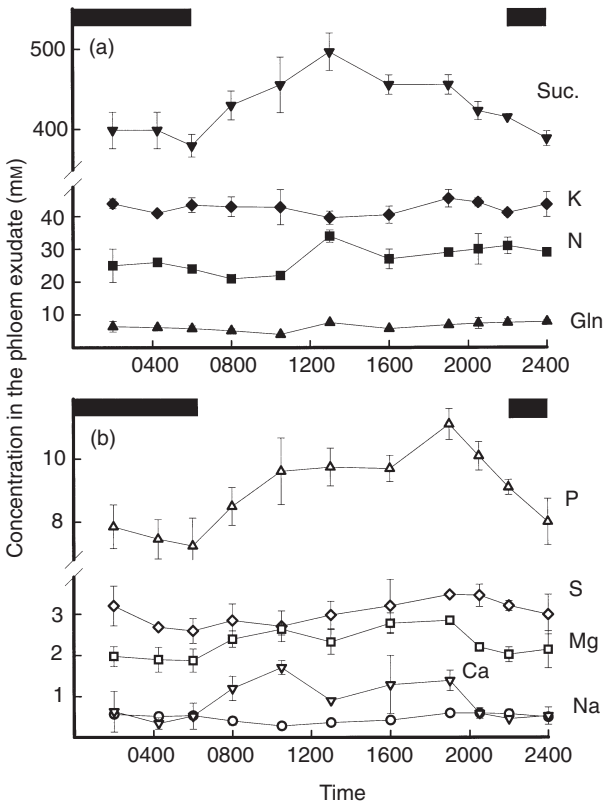

Figure 5. Chemical composition of phloem bleeding sap in 35–45-d-old *Ricinus communis* collected from the hypocotyl during daytime. (a) Sucrose (▼), total nitrogen (■), glutamine (▲), potassium (◆); (b) sodium (○), calcium (▽), magnesium (□), phosphorus (△), sulphur (◇). The dark period is indicated by horizontal black boxes, standard deviations by vertical error bars.

are of limited value as the plants had no transpiring leaves and endosperm was still the major source of carbohydrates and nutrients for growth. This may explain the high rates of flow in the phloem.

The present data for the phloem demonstrate that transport in the phloem is possible without concomitant photosynthetic activity. Sharkey & Pate (1976) found earlier that phloem bleeding varied little over a daily cycle. According to the pressure flow theory of Münch (1930), the difference in osmotic potential between source and sink tissues generates flow in the phloem that is sustained by loading and unloading of the phloem; processes which must also function in the dark (Eschrich & Eschrich 1989). During the light period, photo-assimilates were stored in the form of starch, whereas the concentration of soluble carbohydrates remained more or less constant (see Fig. 7). In the dark, starch was continuously degraded resulting in a reduced, but still steady, concentration of soluble carbohydrate in the leaves. This process presumably maintained the loading of the phloem in the dark. Interestingly, concentrations of soluble sugars in the leaves corresponded to those in the phloem. The sugar export from leaves may be directly related to the current concentration of sugars in leaves (Sharkey & Pate 1976). On the basis of recent experiments the following three compounds represent the major solutes in the phloem sap of *Ricinus*: sucrose 73%, potassium 13%, and amino acids 9%. Organic acids, are present in the form of malate 2% and others are present only in traces. In the present experiment potassium was not affected, but sucrose and amino acids decreased in the night. Therefore, it is very likely that the osmopotential of the phloem decreased in the dark. Additionally the availability of water for phloem loading was higher at night, as the transpiration was reduced. These results support the idea that leaves grow at night (Prantl 1874; Schmundt *et al*. 1998). In conclusion the lower concentration of solutes in the phloem must be compensated for by higher availability of water for phloem loading in the dark to maintain the same flow in the phloem over the whole day.

The lack of variation in rates of phloem transport was surprising in view of the major role of photosynthesis in producing soluble sugars. This transport behaviour is possibly related to the structure of the phloem. Constant transport will maintain pressure within the phloem, a condition required to maintain phloem structure as there is no supporting tissue. A further putative argument for night-time

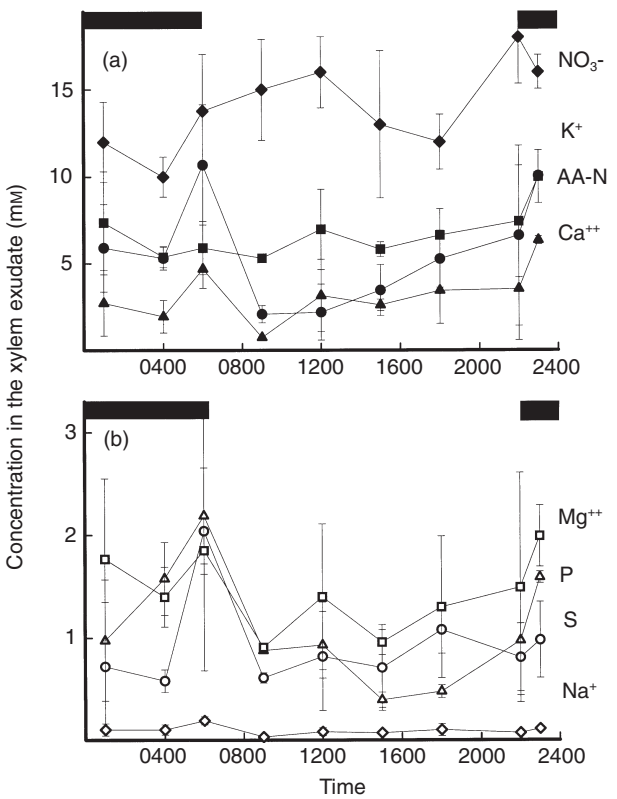

Figure 6. Chemical composition of xylem root pressure saps in 35–45-d-old *Ricinus communis* collected from the hypocotyl during daytime. (a) Amino acid nitrogen (●), potassium (■), calcium (▲), nitrate (◆); (b) sulphur (○), magnesium (□), phosphorus (▽), sodium (◇). The dark period is indicated by horizontal black boxes, standard deviations by vertical error bars.

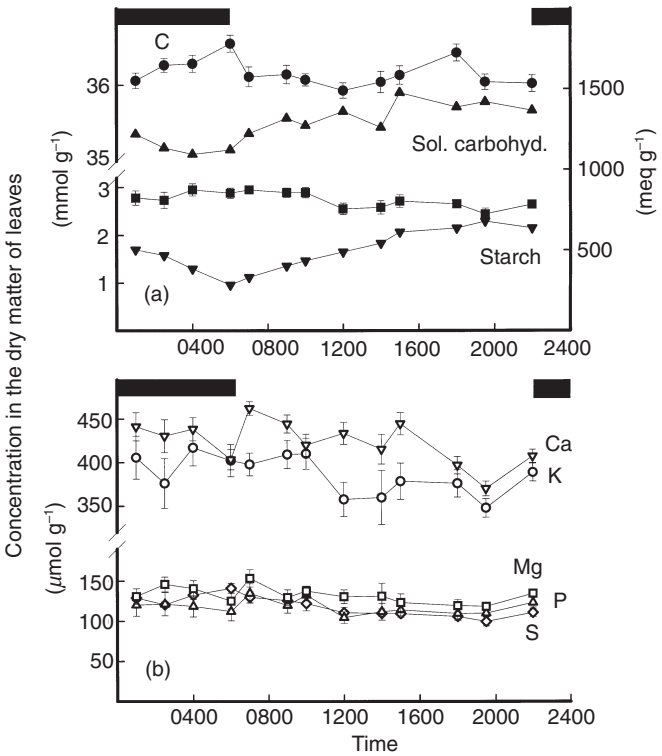

Figure 7. Chemical composition of leaf dry matter in 35–45-d-old *Ricinus communis* during daytime. (a) carbon (●), nitrogen (■), starch (▼), soluble carbohydrates (▲); (b) potassium (○), calcium (△), magnesium (□), phosphorus (▽), sulphur (◇). The dark period is indicated by horizontal black boxes, standard deviations by vertical error bars.

transport is that the phloem transport system may be unable to cope with the amounts of carbohydrate synthesized during the day.

The major feature of xylem transport was the delay in response to changes in light intensity. Wegner & Zimmermann (1998) and Thürmer *et al*. (1999) showed that changes in xylem pressure were only observed several minutes after changing illumination but we know of few other studies that may shed light on our observations.

Effects of changes in mass flow due to the light regime on solute transport in xylem and phloem

On the basis of relative volume flow and the concentration of solutes in xylem and phloem, a rough estimation of relative transport over a daily time course can be made. Our results suggest considerable daily fluctuations in concentrations of solutes in phloem and xylem. These results are in general agreement with earlier investigations for phloem (in *Lupinus*, Sharkey & Pate 1976; Hocking *et al*. 1978), xylem (Schurr & Schulze 1995), as well as for the chemical composition of the leaves (Sharkey & Pate 1976).

During the night, changes in relative volume flow in the phloem (increased by a factor of 1·14) were compensated for by lower solute concentrations (reduced by a factor of 0·80). The combination of both observations result in the outcome that solute transport in the phloem is more or less constant over the whole day. In contrast the relative flow in the xylem was reduced in the dark (by about 30%) and generally the solute concentrations increased. These effects will again help to 'balance' solute transport over a 24 h period. In general, and in both transport systems, changes in relative flow were compensated for by changes in solute concentrations. Nonetheless, there were major variations in composition of the solute load. For example, nitrate (and potassium as a counter-ion) transport in the xylem was far slower during the night than during the day. We can conclude that loading of solutes into transport systems has a larger impact on fluctuations in daily transport than the effects of flow. This conclusion must be viewed in the context of the hypothesis that transport and distribution of elements are independent of transpiration (Shaner & Boyer 1976; Tanner & Beevers 1990).

The macro-element composition of leaves was not affected by xylem import or phloem export. Only the concentrations of starch and soluble carbohydrates were correlated to daily transport.

CONCLUSIONS

With the help of fast NMR imaging, it is possible to observe non-destructively the water flow velocity and apparent relative flow in phloem and xylem simultaneously in adult plants with high time resolution. This is a tool that can be used to investigate changes in long-distance transport in response to fast environmental changes without destructive manipulations. In the present experiments it was used to monitor the effects of the light regime on water flows over the time course of a day. The observed effects of light did not change water flow in the phloem very much, whereas light significantly increased velocity and apparent flow in the xylem. Solute transport was mainly affected by the solute concentration (probably via loading), as there was a general tendency that effects on flow were compensated for by changes in concentrations.

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