Quantification of water transport in plants with NMR imaging

T.W.J. Scheenen, D. van Dusschoten¹, P.A. de Jager and H. Van As²

Department of Biomolecular Sciences, Laboratory of Molecular Physics, Wageningen University, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands

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Abstract

A new nuclear magnetic resonance imaging (NMRi) method is described to calculate the characteristics of water transport in plant stems. Here, dynamic NMRi is used as a non-invasive technique to record the distribution of displacements of protons for each pixel in the NMR image. Using the NMR-signal of the stationary water in a reference tube for calibration, the following characteristics can be calculated per pixel without advance knowledge of the flow-profile in that pixel: the amount of stationary water, the amount of flowing water, the cross-sectional area of flow, the average linear flow velocity of the flowing water, and the volume flow. The accuracy of the method is demonstrated with a stem segment of a chrysanthemum flower by comparing the volume flow, measured with NMR, with the actual volumetric uptake, measured with a balance. NMR measurements corresponded to the balance uptake measurements with an rms error of 0.11 mg s⁻¹ in a range of 0 to 1.8 mg s⁻¹. Local changes in flow characteristics of individual voxels of a sample (e.g. intact plant) can be studied as a function of time and of any conceivable changes the sample experiences on a time-scale, longer than the measurement time of a complete set of pixel-propagators (17 min).

Key words: Water transport, flow quantification, xylem, NMR imaging, propagator.

Introduction

One of the key tools in the continuing debate about mechanisms of long-distance water transport in plants (Passioura, 1991; Zimmermann et al., 1994; Canny, 1995; Milburn, 1996; Tyree, 1997) is the ability to measure the water transport directly, non-invasively and spatially resolved. To validate recent theories (Holbrook and Zwieniecki, 1999; Tyree et al., 1999) in fundamental issues about the occurrence and refilling of embolized xylem vessels one needs a technique to measure the volume flow, the linear flow velocity and the cross-sectional area of flow in the xylem vessels of an intact plant.

The heat pulse velocity technique (Swanson, 1975) and derived techniques, that use heat as a tracer, can be useful in measuring water uptake by trees or plants non-destructively, although heater probes and temperature sensors have to be inserted into the stem. Moreover, no information about the exact positions of flow within the measured stem can be obtained and heterogeneities in the stem tissues cause problems in calibrating the technique (Cohen and Fuchs, 1989). Other techniques to assess sap flow in stems include the injection of radioactive tracers into the xylem and the use of porometers to measure leaf transpiration of a representative part of a plant to estimate the total transpiration, resembling total water uptake.

In the last ten years or so, Nuclear Magnetic Resonance (NMR) has proven to be a useful non-invasive technique to measure flow in plants in vivo (Callaghan et al., 1994; Kuchenbrod et al., 1996; Reinders et al., 1988; Schaafsma et al., 1992; Van As et al., 1994). One of the NMR imaging techniques, dynamic NMR microscopy (Callaghan et al., 1988), provides direct information on the distribution of all spin displacements within a pixel of an NMR image using predefined labelling times. To accomplish this, dynamic NMR microscopy combines standard imaging with Pulsed Field Gradient (PFG) NMR. Since flow in xylem vessels can change rapidly,

¹ Present address: Max Planck Institut für Polymerforschung, Ackermannweg 10, D-55128, Mainz, Germany.
² To whom correspondence should be addressed, Fax: + 31 317 482725. E-mail: Henk.VanAs@water.mf.wau.nl

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methods to visualize flow and measure changes in flow should be faster than the time it takes for these changes to occur. Dynamic NMR experiments normally are time-consuming (Callaghan et al., 1988; Köckenberger et al., 1997), but recently, new methods have been developed to decrease the measurement time drastically (Kuchenbrod et al., 1996; Rokitta et al., 1999a; Xing et al., 1995; Scheenen et al., 2000).

The measurement time can be decreased by not recording the complete distribution of displacements of spins (the so-called propagator) for every pixel (Kuchenbrod et al., 1998; Rokitta et al., 1999a, b). This approach can be used if the displacement distribution of the spins is known in advance. If so, the need to record the complete propagator disappears, less flow encoding steps are required and the NMR-signal as a function of the flow encoding steps can be fitted to a model function. However, in plant tissue, and probably in any non-artificial sample, the distribution of displacements of spins in a pixel is unknown and can vary with time.

The reason why in (plant) tissue the use of a model function is essentially wrong is 3-fold. Firstly, it is not possible to choose the pixel size (e.g. 100 × 100 × 2500 μm) and position of an image in such a way that one pixel always contains no more than one complete vessel. Vessels differ in size and are often grouped, so pixels will generally contain more than one vessel (at low spatial resolution) or only part of one vessel (at high spatial resolution or with large vessels). Secondly, it is assumed that on the time-scale of flow encoding (Δ) spins do not move perpendicular to the flow direction. Diffusion of spins from one position on the flow profile to the other within the flow encoding time, changes the shape of the displacement distribution drastically (Tallarek et al., 2000). The third reason is that vessels in biological tissues are often not perfectly circular and have a rough surface, which can influence the displacement distribution.

In conclusion, the best way to quantify flow is to avoid any model for flow within a pixel. This can be done by recording the propagator, which is the distribution of displacements within a certain flow encoding time, and by extracting flow-characterizing parameters from these propagators that are essentially independent of any flow profile. The flow-sensitive PFG Turbo Spin Echo (TSE) technique (Scheenen et al., 2000) produces these propagators for every pixel in an image in a physiologically relevant measurement time. Here a method is presented to extract several important variables from the pixel-propagators without reverting to any model or assumption for the flow profile. These variables include the volume flow, the linear flow velocity and the cross-sectional area of the flowing fluid of the pixel. Using a reference tube to calibrate the NMR-signal, absolute values can be calculated for the volume flow and the cross-sectional area of the flowing fluid, rather than normalizing the volume flow to the maximum volume flow observed (Rokitta et al., 1999b). In principle, the method can be used for any set of pixel-propagators of any sample. In this work stem segments of chrysanthemum (Dendranthema × grandiflorum Tzelev cv. Cassa) were used because the volume flow through the stem segment could easily be controlled, and did not depend on environmental conditions as relative humidity, light intensity and temperature.

Theory

Principles of NMR imaging

1H-nuclei possess a quantum mechanical property called spin, which, when placed in a magnetic field, processes around the magnetic field vector at a frequency (the Larmor frequency) that depends on the magnetic field strength (Farrar and Becker, 1971). The small, thermally equilibrated difference between spins processing along the magnetic field vector (spin up) and spins processing in an opposite direction to the magnetic field vector (spin down) causes a net magnetization vector of the spins along the magnetic field axis. This net magnetization vector can be manipulated by disturbing the equilibrium of the nuclear spin system with radio frequency (RF) pulses, produced with an RF coil around the protons in the magnetic field. Any magnetization in the plane perpendicular to the magnetic field after an RF pulse induces an RF signal in the coil around the sample. The magnetization vector will return to its initial size and direction with two typical time constants: the spin–spin relaxation time (T2) characterizes the loss of magnetization in the plane perpendicular to the magnetic field (which is the loss of the NMR-signal), whereas the return of magnetization along the magnetic field is characterized by the spin-lattice relaxation time (T1). Since the NMR-signal represents the magnetization in the plane perpendicular to the magnetic field axis, it is also a vector with an amplitude and phase, proceeding at the Larmor frequency. Linear magnetic field gradients, superimposed on the static magnetic field, generate a position-dependent Larmor frequency: different frequencies of the NMR-signal can be processed (by a Fourier transform) into an NMR image, which is a map of the proton (usually water) density or an NMR-specific parameter for every position in a sample.

Probing movement of protons

The NMR-signal for every position in a sample can be made sensitive for the displacement of the protons in a certain direction by use of two pulsed magnetic field gradients (PFGs) with spacing Δ. Here the influence is
explained of two kinds of displacements, diffusion and laminar flow, of spins on the NMR-signal and the shape of the distribution of displacements (propagator) deduced from the signal as a function of PFG amplitude. The theory concerning the propagator formalism (Callaghan et al., 1988) and the fast acquisition of complete pixel-propagators (Scheenen et al., 2000) has already been extensively described elsewhere.

If an ensemble of molecules exhibits self-diffusion due to the Brownian motion, the PFGs will attenuate the amplitude of the NMR-signal of the protons of the molecules, while the phase of the NMR-signal will remain unaffected. A Fourier transform of the signal into \( P(R, \Delta) \), the probability distribution of displacements \( R \) in the direction of the PFGs within the labelling time \( \Delta \), results in a Gaussian shape (Fig. 1) as described by:

\[
P(R, \Delta) = A \exp\left(-\frac{(R - p)^2}{2\sigma^2}\right)
\]

with \( A \) as amplitude and \( p \) as mean displacement (\( p=0 \) for stationary water) of the water protons. The characteristic width of the Gaussian \( \sigma \) can be used to calculate the self-diffusion coefficient \( D \) of the molecules:

\[
D = \frac{\sigma^2}{2(\Delta - \delta/3)}
\]

where \( \delta \) is the duration of each of the two PFGs.

Suppose one observes slowly flowing water inside a cylinder as an example of displacements of molecules due to flow. The water in the cylinder can be divided into thin circular layers that glide past one another. The water adjacent to the wall of the cylinder is essentially stationary. As one moves away from the wall to the centre of the cylinder the flow velocity increases to a maximum in the centre. If viscous drag forces dominate the inertial forces of the fluid, the flow profile through the cylinder has a parabolic shape and is called a laminar flow profile. In the hypothetical case that the fluid does not exhibit a Brownian motion, only the phase of the NMR-signal of these circular layers is shifted without modulation of the signal amplitude as a function of PFG amplitude. Figure 2a depicts a laminar flow profile through a cylinder and its propagator in the direction of flow, in the absence of self-diffusion. The propagator consists of a distribution of displacements \( R \) from zero to maximum displacement \( R_{\text{max}} \) within \( \Delta \) and is a boxcar function:

\[
P(R, \Delta) = C \quad \text{for} \quad 0 \leq R \leq R_{\text{max}}
\]

\[
P(R, \Delta) = 0 \quad \text{for} \quad R < 0 \quad \text{and} \quad R > R_{\text{max}}
\]

where \( C \) is a constant and the integral \( \sum_{\Delta} P(R, \Delta) = 1 \).

Different layers in the cylinder coincide with different parts of the propagator, as shown by the arrows in Fig. 2a. If the circular layers in the cylinder are thought to be infinitesimally thin, the corresponding part in the propagator will be a spike at the correct displacement.

Usually, the fluid in the cylinder also exhibits self-diffusion, which combines the phase-shift of the NMR-signal due to flow, with the amplitude attenuation of the signal due to diffusion. The shape of the corresponding propagator changes: every thin circular layer in the cylinder now has a Gaussian shape instead of a spike.
(cf. Fig. 1). All these layers together form a propagator as shown in Fig. 2b: the boxcar function is broadened by a Gaussian. If an observed ensemble of spins contains more than one cylinder with flowing water, the propagator will be the sum of several boxcar functions, broadened by diffusion. If only a part of one cylinder with flowing water is observed, the propagator will be only a part of this broadened boxcar function.

Materials and methods

NMR image analysis

The PFG-TSE (turbo spin echo) pulse sequence (Fig. 3) has been described elsewhere (Scheenen et al., 2000). The amplitude and phase modulation of the NMR-signal as a function of the displacement encoding steps can be Fourier transformed to obtain the propagator. Generally, the pixel-propagator within a slice through a plant stem has a Gaussian shape, since it contains only stationary, diffusing water (cf. Fig. 1). However, some of the pixels in the stem are in regions where active xylem or phloem vessels are present, so the corresponding propagators will show displacements originating from flowing sap.

Propagators representing both stationary and flowing water can be analysed in the following way. The half, which doesn’t display flow, is fitted to a half-Gaussian. Subsequently the complete Gaussian (including the other half) is subtracted from the propagator. The remaining part of the propagator $P_c(R, \Delta)$ represents the flowing water. This propagator is calibrated into $P_c(R, \Delta)$ by dividing it with the integral $I_{ref}$ of a propagator of a pixel in a reference tube that is filled with doped water:

$$P_c(R, \Delta) = \frac{P_c(R, \Delta)}{I_{ref}}$$  

Relating the calibrated propagator $P_c(R, \Delta)$ to the experimentally known surface $A_{ref}$ of one pixel the cross-sectional area of flow $A$ of the flowing water can be calculated for every pixel:

$$A = \sum_{R=0}^{R_{max}} P_c(R, \Delta) A_{ref}$$  

The first moment of the calibrated propagator represents the volume flow $Q$ of the corresponding pixel and can be calculated by adding the propagator intensities multiplied by the displacement values and relating it to $A_{ref}$ and $\Delta$:

$$Q = \frac{R_{max}}{\Delta} \sum_{R=0}^{R_{max}} (P_c(R, \Delta) R) A_{ref}$$  

The average linear flow velocity $\bar{v}$ is the volume flow divided by the cross-sectional area of flow (strictly the calibration is not necessary for $\bar{v}$):

$$\bar{v} = \frac{Q}{A} = \sum_{R=0}^{R_{max}} \frac{P_c(R, \Delta) R}{\sum_{R=0}^{R_{max}} P_c(R, \Delta) \Delta}$$  

A few assumptions are made here: within one pixel water does not flow in two directions and the loss of NMR-signal within the time between excitation and detection of the first echo (te1, Fig. 3) is comparable for the reference tube and the water in the xylem.

The spectrometer

The NMR spectrometer consists of an SMIS console (SMIS Ltd., Guildford, Surrey, UK), operating at 30.7 MHz, an electromagnet with a 10 cm air gap (Bruker, Karlsruhe, Germany) generating the magnetic field of 0.72 T and an external $^{19}$F lock unit (SMIS) stabilizing the magnetic field. The magnet is equipped with a custom-engineered gradient probe (Doty Scientific Inc., Columbia, South Carolina, USA) with a 45 mm (i.d.) cylindrical central bore, accessible from both ends. The stem segments of cut flowers were measured in a custom-made vase (Fig. 4) that can be inserted in the gradient probe. A solenoid radio frequency (RF) coil (12 mm inner diameter), wrapped around the shallow part of the vase, transmits the NMR-pulses and receives the signal.

Plant material

Chrysanthemum (Dendranthema × grandiflorum Tzvelev cv. Cassa) plants were grown in a greenhouse at Wageningen University, in plastic pots (14 cm diameter) with a commercial potting soil. The average temperature in the greenhouse was 18 °C. The plant had a photoperiod of 16 h until the plant had formed 15–17 leaves longer than 0.5 cm (3–4 weeks), followed

Fig. 3. Pulsed field gradient turbo spin echo pulse sequence. The two large pulsed field gradients in the flow direction with duration $\delta$ and spacing $\Delta$ are stepped to acquire flow information.

Fig. 4. Draft of the set-up to control the water uptake of stem segments (after van Ieperen et al., 2000).
by an 8 h photoperiod until harvest. The photoperiods were
lengthened by high-pressure sodium lamps or shortened by
black screens when necessary. Flowering stems at commercial
maturity and stem segments were cut off underwater with razor
blades to ensure that no air entered the xylem vessels of the
stem.

**Set-up to control water uptake of stem segments**

A method has been described to control the water uptake of
stem segments (van Ieperen et al., 2000). This method is used
here, since it enables a very precise control of the water flow
level through a stem segment, it measures water uptake directly
(not by way of transpiration) and it is straightforward to
implement. The method will be summarized briefly here. The
water level in the vase is controlled with a communicating vessel
on a precision balance: the uptake of water by a cut flower or
stem segment is measured with the balance (LC3201D, Sartorius
AG, Goettingen, Germany) by sampling the weight decrease
every 20 s. The uptake of water by a stem segment is controlled
with underpressure: the top of the stem segment is connected
with water-filled silica tubing to a vessel of which the under-
pressure is controlled with a pump (Fig. 4). The pump (505DI
Watson-Marlow Limited, Falmouth, UK), the vacuum sensor
(DVR5, Vacuubrand, Gmbh & Co, Wertheim, Germany) and
the balance were connected to a computer to automate under
pressure control and uptake measurements.

**Results**

The stem segment of a chrysanthemum flower in the
set-up was 25 cm long, measured at 40 cm from the roots,
10 cm above the cut surface. The NMR measurements
show a distribution of displacements of all protons for
every pixel in an image (Fig. 5). Figure 5a displays
a transverse image of the stem segment at $g=0$: this is
a standard TSE image that displays the proton density
for every pixel (Scheenen et al., 2000). The striking feature
of the image is that it displays only a single, relatively
thin, ring. As a reference, Fig. 5b is a photograph of a
transverse section through a chrysanthemum stem: the
spongy tissue with large dead parenchyma cells in the
middle of the stem hardly contains any water, so it does
not give a detectable NMR-signal. The outer ring of the
stem contains all the functional tissue, including xylem,
cambium, phloem, supporting tissues, and epidermis.
Pixels in the xylem region of the stem segment can display
stationary and flowing water. The propagator of a
particular pixel (100×100×2500 μm) in that region
(Fig. 5c) reveals a peak, centred at a displacement of
0 μm, representing stationary water, with a large asym-
metrical shoulder with positive displacements, represent-
ing flowing water. The propagator was calibrated with the
averaged intensity of nine pixels in the reference tube
(equation 4). The dotted line in Fig. 5c is the result of a fit
to the left half of the calibrated propagator using a
Gaussian function. After subtraction of this Gaussian
from the propagator, the asymmetrical flowing part of the
propagator remains. This part is plotted in Fig. 5c below
the complete propagator. The integral of the flowing part
of the calibrated propagator represents the fraction of the
corresponding pixel through which water flows, relative
to a pixel in the reference tube. This fraction can be
recalculated into the cross-sectional area of flow within
the pixel in mm$^2$ (equation 5), knowing the surface $A_{ref}$ of

![Fig. 5. (a) A TSE image of a chrysanthemum stem segment, perpendicular to the stem axis. (b) A photograph of a transverse section through
a chrysanthemum stem. (c) The calibrated propagator of a pixel in the xylem region of the stem (solid line) with the Gaussian fit to the left half of the
propagator (dotted line). The bottom panel shows the difference between the original calibrated propagator and the fit on the same scale. The crosses in
the solid lines indicate the individual data points. Parameters: resolution 100×100×2500 μm, tel 25.0 ms, 32 PFG steps, Δ 19.4 ms, δ 2.5 ms, PFG$_{max}$
0.36 T/m, repetition time 1 s, number of averages 4, total measurement time 17 min.](image)
Fig. 6. Images of the calculated flow characteristics of the stem segment: (a–e) respectively, water content, amount of stationary water, cross-sectional area of flow, linear flow velocity, and volume flow. The grey scale bar relates intensities to quantitative values. Water content and the amount of stationary water are expressed as fractions, relative to the mean water content of a pixel in the reference tube (= 1 unit) and can easily be recalculated into a volume or a surface. (f) The areas that show flow superimposed on an enlarged image of the water content (cf. a).

A pixel inside the reference tube, and assuming that the anatomy of a stem segment does not change along the axis of the stem within the slice thickness (2500 μm). Apart from the cross-sectional area of flow the volume flow and linear flow velocity can be calculated for every pixel with equations 6 and 7. Knowing these flow characteristics for each pixel one can construct images with the characteristics: total amount of water, total amount of stationary water, cross-sectional area of flow, linear flow velocity, and volume flow (Fig. 6a–e, respectively). Figure 6f indicates the regions of flow (cf. Fig. 6e) superimposed on the image of the water content of the stem segment (cf. Fig. 6a).

The total volume flow through the stem segment was monitored with the precision balance and could easily be changed by varying the underpressure of the vessel that was connected to the top of the stem segment. Stepwise decreasing the pressure differences over the stem segment in a range of 47 to 0 kPa resulted in uptake values from 1.8 to 0 mg s⁻¹; a physiologically sensible range for chrysanthemum (Fig. 7). Small negative uptake values were the result of a small overpressure of remaining water in the silica tubing on top of the stem segment, pushing water backwards through the xylem. After about 2 h the uptake, which was constant in the first three pressure steps, decreased with (maximum) 7% during one pressure step: the hydraulic resistance of the stem segment increased slowly with time. This effect is even more evident when pressure steps 1 and 5 or 2 and 6 are compared: at the same pressure difference the uptake in the measurements later in time has decreased. However, the increase in hydraulic resistance of the stem segment is of little importance for a comparison of two ways to measure water transport in a single stem and the measurement time (17 min) for the dynamic NMR imaging experiment is short enough to have a constant uptake during one measurement.

The NMR measurements are represented by triangular markers in Fig. 7 and were calculated by adding all pixels of the NMR image of the volume flow that had intensities larger than a manually set threshold value (~2/3 of peak noise level) with at least one neighbouring pixel that also exceeded the threshold value. Except for the first two pressure steps, the NMR flow values correspond within an error of 0.10 mg s⁻¹ with the actual flow that was measured with the balance. In the first two steps, the difference is 0.16 and 0.20 mg s⁻¹, respectively. The overall rms error of all points is 0.11 mg s⁻¹. For negative uptake values, the positive halves of the propagators were used for the Gaussian fit and the negative halves were used to calculate the volume flow.

Discussion and conclusions
A pixel-propagator from the xylem region of a chrysanthemum stem is not simply the sum of a Gaussian peak at zero displacement and a broadened boxcar function. This becomes especially clear when the
Gaussian fit is subtracted from the propagator. Due to the large pixel size (100 × 100 × 2500 μm) a propagator from a pixel in the xylem region will always represent multiple xylem vessels and accompanying cells, since xylem vessels in *Dendranthema grandiflorum* Tzelev cv. Cassa have diameters in a range from 10–40 μm (IJ Nijssen et al., unpublished results). Therefore, the shape of a single pixel propagator is not known *a priori* and the use of a model function for the modulated NMR-signal to calculate flow data is clearly not correct. However, one can accurately calculate the volume flow (Tsai et al., 1999), by way of the first moment of the propagator by differentiating the modulated NMR-signal at $g=0$ (first moment theorem of Fourier transforms (Bracewell, 1965)). If not just the volume flow, but also the linear flow velocity and the cross-sectional area of flow are of interest the only accurate solution, i.e. not assuming any flow-profile, as far as we know is the method described above (see Materials and methods).

The fit of the stationary water to the half-Gaussian function is, in principle, only validated if the stationary water can diffuse without restrictions. This means that on the time-scale ($Δ$) of labelling the bulk of the molecules should not reach any walls or membranes. In practice, the bulk of the water-molecules resides in vacuoles and moves 9 μm (rms value $σ$ from equation 2) with the instrumental settings used here ($Δ-δ/3=18.6$ ms) and $D=2.0 \times 10^{-9}$ m$^2$ s$^{-1}$ (free water at 20 °C). Even if displacements of 9 μm were already restricted by the membrane of the vacuoles of the cells surrounding the vessels, the effect on the shape of the stationary water part of the propagator would be small: the Gaussian fit would still remove the stationary water part quite effectively.

One other issue to be mentioned here is the fact that flowing water has intensity at zero displacement in the propagator (cf. Fig. 2). This intensity originates from water near the walls of the vessel or tube in which it is flowing. In the method presented here no intensity is left at zero displacement after subtraction of the Gaussian fit from the propagator (Fig. 5c). This is not a problem in calculating the volume flow of such a propagator, since any intensity at zero displacement is multiplied by the zero value of the displacement axis (equation 6). However, loss of intensity at zero displacement increases the linear flow velocity and decreases the cross-sectional area of flow (equation 7 and equation 5): water at the walls of vessels, which does not appear as flowing water, is indeed part of flowing water. Regions at the vessel walls are in fact part of the cross-sectional area of flow of that vessel. As a solution to this problem the instrumental settings of an experiment can be chosen in such a way that within $Δ$ water can diffuse from the vessel walls into the regions where flow is present. Recently, the shape of a propagator of a laminar flow profile as a function of $Δ$ has been published (Tallarek et al., 2000) showing a decreasing intensity at zero displacement with increasing $Δ$. The rms displacement due to diffusion within $Δ$ combined with the velocity gradient of the flowing water near the vessel wall (depending on the vessel diameter and the volume flow through the vessel) determine the actual intensity of the propagator at zero displacement.

The comparison between the results obtained with the balance and with NMR, as presented in Fig. 7, show the accuracy of the quantification method. An agreement was found between both uptake measurements with a rms error of 0.11 mg s$^{-1}$, not by using an unclear constant to
correlate NMR results to the actual (‘balance’) volume flow or by normalizing the volume flow to the maximum volume flow observed (Rokitta et al., 1999b), but by calibrating NMR-signal intensities to the averaged signal intensity of a reference tube. If, for certain studies, the labelling time $\Delta$ between the two PFGs is increased (increasing also tl1), problems may arise from using a reference tube with water for calibration. The decay of the NMR-signal, characterized by the relaxation time $T_2$, varies in different tissues of the sample. Suppose the $T_2$ of the water in the reference tube (‘doped’ with paramagnetic ions to decrease $T_2$) differs substantially from the $T_2$ of the water in the xylem vessels and the time from signal excitation to first detection (tl1 in Fig. 3) is of considerable size compared to the $T_2$ values of the water in the tube and/or in the xylem vessels. The signal intensity of the water in the tube and in the xylem at the moment of detection will now be weighted with their different $T_2$ values and in the calibration this extra $T_2$ weight has to be considered. In these results the $T_2$ values of the water in the reference tube and in the xylem vessels were comparable (around 100 ms) and large compared to the first echo time (maximum 27 ms), so these difficulties were not experienced. If need be, it is possible to record pixel-propagators in combination with a $T_2$ experiment to link a $T_2$ value to every point of a pixel-propagator, though imaging time will be longer.

For in vivo applications of the described method in intact plants one assumption stated earlier has to be evaluated carefully: there should be no bidirectional flow within one pixel. In other words, the resolution of the NMR image has to be high enough to discriminate between xylem and phloem tissue. In a pixel-propagator one can immediately see if both xylem and phloem flow are present: the propagator will show intensities at positive and negative displacements beyond the rms displacement of diffusing stationary water. Flow quantification is only hampered if the linear flow velocities in the two directions in the same slice are of comparable size. In that case, one might consider using the rms displacement from stationary water of neighbouring pixels to get rid of the stationary water in the pixel with bidirectional flow, after which positive and negative displacements can be evaluated separately. For full-grown intact plants active xylem and phloem areas are usually more than 100 $\mu$m apart, which means the resolution used here would be high enough to avoid bidirectional flow within one pixel. Increasing the spatial resolution of the images would increase measurement time drastically, a problem that might be solved by measuring at a larger magnetic field strength although examples of decreasing image quality with increasing magnetic field strength have been reported (Donker et al., 1996) for biological tissues (especially plant tissues).

In summary, it can be stated that relevant and accurate information about water transport can be acquired non-invasively with the method presented here. The information is relevant to the debate about long-distance transport in plants, and accurate since it does not need a model for flow to calculate flow characteristics. The method is demonstrated with chrysanthemum stem segments, but can easily be used on intact plants (Scheenen et al., 2000) or any other system that fits within the NMR-imager. Since the overall uptake of NMR and balance measurements match, the local information of every pixel can be studied individually: the set-up for the chrysanthemum stem segments is being used as a model to investigate the restoration of original flow profiles and of hydraulic conductance for chrysanthemum flowers after cutting.

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