NONINVASIVE MEASUREMENT OF PLANT WATER FLOW BY NUCLEAR MAGNETIC RESONANCE

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ABSTRACT Water flow through the stem of an intact cucumber plant has been measured by using pulsed NMR. This method yields the linear flow velocity of the sapstream, found to be proportional to the loss of weight due to evaporation. The presence of a large excess of stationary water (for cucumber 95% of the total water content) does not interfere with the detection of a small amount of flowing water, due to cancellation of the NMR signal of stationary water. This makes the method particulary suitable for application to biological systems with a high stationary water content.

INTRODUCTION

The sapstream velocity in the plant stem can be used as an indicator of the rate of transpiration and has been studied by several methods (1). Most of these methods do not measure linear flow velocity or detect the movement of water itself. In contrast to many of these methods, NMR measurement of liquid flow (2) is nondestructive as well as noninvasive. When applying existing NMR methods to biological objects, flowing as well as stationary water gives rise to a signal. Both phases can in principle be distinguished by applying a proper combination of pulse sequence, magnetic-field gradient, and mode of detection (3). Here, we report a novel use of this method for noninvasive measurements of water flow in the xylem vessels of the stem of an intact cucumber plant.

The main advantage of the method is that flowing and stationary water can be effectively discriminated, i.e., a large excess of stationary tissue water does not interfere with the flow measurements. As is evident from its linear relationship with the loss of weight due to evaporation, the flow velocity determined by the present method is a measure for the actual average sapstream velocity.

By applying a slightly different pulse sequence, the water content of the plant tissue can be measured also (4). We believe that the pulsed NMR method, presented here, opens new paths to the study of plant water relations, e.g., the effect of various physiological conditions on the rate of water transport and its relation to the tissue water content.

PRINCIPLES

Linear flow velocity of liquids containing nuclei with nonzero spin can be measured by NMR when that liquid moves in a magnetic-field gradient (5). During this motion, the nuclei "see" a continuously changing magnetic field and therefore change their Larmor precession frequency resulting in a time-dependent phase shift with respect to stationary nuclei (6, 7). On the other hand, the measurement of volumetric flow rate makes use of the amplitude of the NMR signal (2).

The method makes use of a series of equidistant radio frequency (rf) pulses, which are applied to the sample placed in a static magnetic field, $\mathbf{B}_0//z$, and a magnetic-field gradient, $\mathbf{G}$, in the direction of flow (3) (Fig. 1 *A*). The response of the net magnetization vector $\mathbf{M}$, resulting from all nuclear spins, to an rf pulse is described in an $\{x', y', z'\}$ axis system, rotating about $z' = z$ with the average Larmor precession frequency (8). The rf pulses are applied along x'; $\mathbf{M}$ is detected along the same axis. The rf pulses have a width and amplitude such that $\mathbf{M}$ is rotated over a pulse angel of π radians around the x' axis for the nuclei in the center of the coil ($y = 0$, Fig. 1 *B*). For nuclei experiencing π pulses only, $\mathbf{M}$ is either parallel to $+z'$ or $-z'$ and no component develops in the x', y' plane. However, when the sample extends beyond the rf coil (e.g., in the case of plant stems) (Fig. 1 A), the π pulses are spatially inhomogeneous, resulting in a gradual decrease of the pulse angle from π to 0 with increasing distance from the center of the coil along $+y$ and $-y$. As shown in Fig. 1 *B*, at $|y| = a$, the pulse angle α has decreased to $\pi/2$, resulting in an y' component of $\mathbf{M}$. Due to the presence of the field gradient $\mathbf{G}//y$, $M_{y'}$ of the spins at $y = +a$ and $y = -a$ precess in opposite directions with respect to the rotating frame $\{x', y', z'\}$. Therefore, no net component of $\mathbf{M}$ is generated along x' in the case of stationary spins. Flow, however, introduces a net $M_{x'}$, since spins at $y = -a$ are moving into the coil, whereas spins at $y = +a$ are leaving and are lost for detection. Although the pulse angle outside the coil varies between 0 and π (Fig. 1 *B*), the generation of the NMR signals can be satisfactorily described by only considering those regions where $\alpha = \pi/2$.

A typical shape of the signals of flowing water obtained by the NMR method is shown in Fig. 2 *A*. During a pulse sequence the signal $S(t)$ progressively develops in real

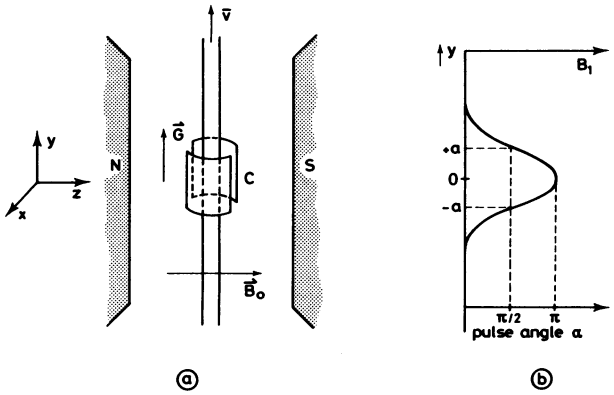

FIGURE 1 (*A*) Schematic diagram of a plant stem placed in a static magnetic field, B_o. The stem is surrounded by a Helmholtz type rf coil *C*. A linear magnetic field gradient, *G*, is imposed on B_o along *y*, the direction of flow, resulting in $B_z(y) = B_o + yG$. *x*, *y*, and *z* are the axes of a laboratory coordinate system. (*B*) Spatial distribution of the rf field, B_1, along the *y*-axis. The distribution of the pulse angle α is also shown; $\alpha = \gamma B_1 t_p$, where γ is the gyromagnetic ratio and t_p is the pulse duration.

time. Without further proof, we apply the results obtained for flow in glass capillaries (see reference 3 for further details) to determine the linear flow velocity of the sapstream in cucumber. From t_{max}, the time at which the NMR signal reaches a maximum (see Fig. 2 *A*), the average linear flow velocity, $\bar{v}$ (e.g., in mm · s^{-1}), is obtained making use of (3)

$$\bar{v} = C/t_{max}, \quad (1)$$

where *C* is a calibration constant (determination described in next section) depending on *G*, the time τ between the rf pulses, the coil dimensions, and the flow profile.

EXPERIMENTAL

Plant Material

Measurements were carried out on an intact cucumber plant (*Cucumis-sativus L.*) potted in a plastic container and placed on a top balance. The plant was illuminated using two 400-W Na lamps at ~2 m above soil surface. The NMR rf coil surrounded the plant stem at ~0.5 m above the soil surface.

NMR Flow Measurements

A 15-MHz ^{1}H single-coil pulsed NMR spectrometer equipped with a 17-cm electromagnet was used, details of which are described elsewhere (3). A hinged Helmholtz-type rf coil, allowing measurements on intact plants, was used with a length of 11 mm and a diameter of 10 mm. A total of 4,096 π pulses were applied, with pulse period τ = 1.6 ms. Each data point represents the integrated signal over a period of 25.6 ms. The results reported here were obtained from reproducible, single scan experiments. The static magnetic field, B_o, was adjusted to resonance so that no signal was observed from stationary water.

The linear flow velocity, $\bar{v}$, was calculated via Eq. 1. The calibration constant, *C*, was obtained using a glass capillary with known internal cross section, *A*, measuring t_{max}, and calculating $\bar{v}$ using $\bar{v} = Q/A$; *Q* is the volumetric flow rate, representing the easily measured volume of water flowing through the capillary per unit of time.

Balance Transpiration Flow Rate Measurements

The transpiration rate of the plant was measured on an electrical differential top balance model PE-11 in combination with model BE-13; Mettler Instrument Corp., Hightstown, NJ. To prevent evaporation of water from the soil, its surface was covered with aluminum foil.

Cross-sectional Area

The cross-sectional area of the flow conducting elements was calculated from microscopic measurements assuming the vessels to be unobstructed capillaries in the radial section, taken from that part of the stem that was positioned in the NMR rf coil.

RESULTS AND DISCUSSION

Fig. 2 *A* presents experimental results for flowing water in the stem of an intact cucumber plant. Figs. 2 *A*1 and *A*2 represent an experiment, where water was added to the soil, resulting in an increase of $\bar{v}$ = 9.9 ± 0.8 mm · s^{-1} to 14.3 ± 1.1 mm · s^{-1}. The effect of adding water to the soil is detected after a few seconds; when sufficient water is available, $\bar{v}$ approaches a maximum of 28 ± 2.2 mm · s^{-1}, under the given experimental conditions. The performance of the method can be judged from Fig. 2 *B*. After interrupting the supply of water to the measuring region by slicing the stem, typically <0.5% of a 20-fold excess of stationary tissue water is observed.

To calculate $\bar{v}$ from t_{max} via Eq. 1, the calibration constant, *C*, has been determined using a glass capillary (see Experimental section). A plant stem has a distribution of vessel radii *R*, however, which would predict a distribution of $\bar{v}$ and, consequently, a spread of t_{max} values and a

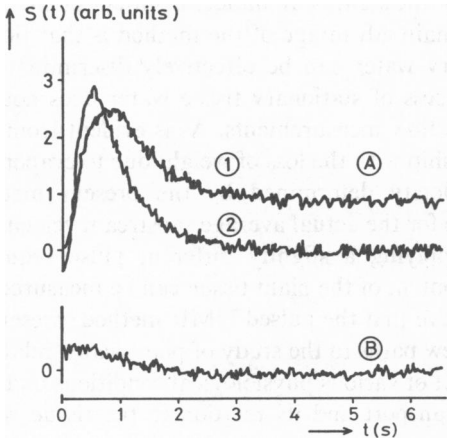

FIGURE 2 Single scan 15-MHz ^{1}H NMR signal amplitude, $S(t)$ (arbitrary units) vs. time for water flowing in the stem of an intact cucumber, obtained by the $(\pi - \tau)_n$ pulse sequence (τ = 1.6 ms). At t = 0 the first π pulse has been applied. G = 13 ± 2G(gauss)/m. (*A*) Signal of flowing water during illumination of the plant with two Na lamps (400 W each) at 1-m mean distance from the leaves, zero air velocity, ambient temperature 23°C. (*1*) $\bar{v}$ = 9.9 ± 0.8 mm/s, (*2*) $\bar{v}$ = 14.3 ± 1.1 mm/s, before and after adding water to the soil, respectively. (*B*) Signal 15 min after cutting off the stem closely below the rf coil; all other conditions identical to those in *A*.

broadening of the signals. Therefore, one may wonder whether the calibration using one capillary is justified. Flow in that capillary (diameter ~1 mm) is laminar for the relevant range of flow velocities ($v \leqq 30$ mm · s^{-1}). This is also what is expected for flow in plant stems (vessel diameter <1 mm, and $\bar{v} < 20$ mm · s^{-1}). Assuming that the pressure gradient is equal for all vessels in the measuring region, it can be easily shown that for laminar flow, $\bar{v}$ is proportional to R^2 and thus t_{max} increases with decreasing R as R^{-2}. At the same time, the maximum amplitude of the signal decreases roughly as $\exp(-t_{max}/T_2)$, where T_2 is the spin-spin relaxation time of the flowing water. In addition, the amount of flowing water in a vessel is proportional to R^2, resulting in a rapid reduction of the signal amplitude for decreasing R. The net result of these combined effects is that the NMR signal almost exclusively originates from water flowing in vessels with the largest diameter. The volume flow rate, Q, can be shown to be proportional to R^4 in the case of laminar flow, and the above-mentioned effect is even stronger. Indeed, we have observed (see Fig. 2 A) that the NMR signals of flowing water in plant stems are hardly broadened with respect to those from flowing water in a single capillary. As a further confirmation that mainly vessels with a radius close or equal to the maximum value contribute to the NMR signal, we found that Eq. 1 was obeyed for water flowing under hydrostatic pressure through an excised segment of cucumber. In conclusion, the aforementioned calibration by a glass capillary is very unlikely to introduce significant errors in measuring flow in plant stems.

Fig. 3 illustrates the linear relationship between $\bar{v}$ as measured by NMR and the experimental volume flow rate, Q_B, using the weight balance for cucumber. The slope of $\bar{v}$ vs. Q_B yields an effective total cross-sectional area for flow of $\simeq 0.49$ mm^2. On the other hand, microscopically determined total cross section of the xylem vessels yields 0.92 ± 0.04 mm^2. It is not surprising that the two values are considerably different, in view of the possible presence of nonconducting vessels (9–11), and the above mentioned distribution of R.

Flow could not be measured by NMR in a number of plants that typically have a lower value of R for the largest vessels. In these plants the value of $\bar{v}$ and/or the amount of flowing water may be too low to be detected by the method. Moreover, the T_2 value of water in a vessel is likely to depend on its radius R, just as in typical biological systems, where T_2 becomes progressively shorter as the cell dimensions decrease (12).

Cucumber, gherkin, and tomato have large diameter vessels, and the T_2 of the flowing water is expected to be relatively long. This is probably the main reason why flow measurements in these plants have been successful. In plants with smaller vessels, T_2 probably becomes too short to observe a maximum in the NMR signal (3). The limitations of the method due to spin-spin relaxation can be relaxed by decreasing the time-interval between rf

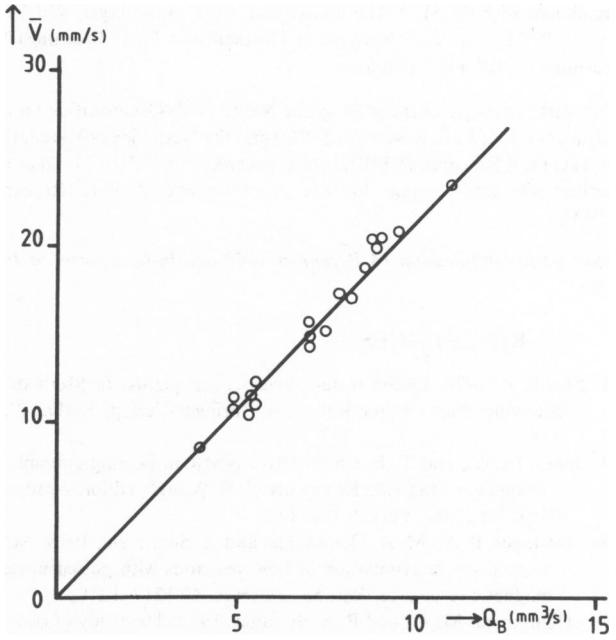

FIGURE 3 Plot of $\bar{v}$ obtained by the NMR method vs. Q_B as measured with the weight balance method for an intact cucumber plant. The values of $\bar{v}$ have been obtained via Eq. 1 and are corrected for the actual value of the relaxation times T_1 and T_2 (13). The flow rate has been varied by varying the light intensity incident on the leaves and adding water to the soil.

pulses. Under our present instrumental conditions, the NMR method permits measurement of water flow with $\bar{v} \geqq 5$ mm · s^{-1} in cucumber xylem vessels with $R \geqq 30$ μm and a total flowing volume of water $\geqq 5$ μl.

The above-mentioned results demonstrate that the pulsed NMR method, as compared with standard methods, is an accurate and powerful technique to study flow in plants and its dependence on physiological conditions. In an earlier report (4) the effect of plant water content on the value of T_2 of the stationary tissue water was discussed. The combination of T_2 and flow measurement in a plant stem opens a novel approach for the nondestructive analysis of the plant water balance, with an unusually short (4–12 s) time resolution (13).

The application of NMR to flow is not restricted to plants, but has recently also been successfully applied to measure blood flow in human fingers (14). Due to the dependence of the signal shape on the value of T_2 in blood, which in turn is determined by its oxygen content and, for small blood vessels ($R < \sim 0.5$ mm), by R, this method is also useful in monitoring blood oxygenation and observing defects in the blood circulation system. Finally, by combining this NMR method with the "sensitive line" imaging method (15), spatially resolved flow patterns were measured (H. van As, J. M. Kleijn, P. A. de Jager, and T. J. Schaafsma, unpublished results).

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