

APPLICATION OF NMR TO WATER FLOW AND -BALANCE IN PLANTS

H. van As, T.J. Schaafsma and J. Blaakmeer
Department of Molecular Physics
Agricultural University
De Dreijen 11
6703 BC Wageningen
The Netherlands

Abstract

Water flow and water potential have been measured non-invasively in the stem of an intact gherkin plant in a single, pulsed NMR experiment under various physiological conditions.

Flow can be measured directly by NMR, whereas the water content and water potential has been monitored by the NMR spin-spin relaxation time T_2 . Based on our experimental results, we suggest that a portable pulsed NMR spectrometer may serve as a sensor in greenhouse climate control.

1. Introduction

Control of the greenhouse climate can be partially based on the measurement of plant processes associated with the plant water balance. Mutually coupled uptake, transport, storage and transpiration contribute to this balance. Sap stream velocity in the stem and tissue water content are important parameters describing the water balance and can be measured by several methods (Slavik, 1974), many of which are invasive, however. None detects the flow and water content simultaneously. By contrast, proton Nuclear Magnetic Resonance (NMR) can do just that (Van As et al., 1980; Van As and Schaafsma, 1984).

In this paper we report measurement of flow and water potential by NMR in the stem of an intact gherkin plant. Our results demonstrate that the water content (P_w) of plant tissue - and thus the water potential (ψ_w) - can be monitored by the NMR spin-spin relaxation time T_2 . The effects of various physiological conditions on the rate of water transport, tissue water content and the relationship between both quantities are presented.

2. Materials and Methods

2.1. NMR flow measurements

Water flow in the transport vessels in plant stems have been measured non-invasively by pulsed NMR, using the so-called repetitive pulse (RP) method (Van As and Schaafsma, 1984). Measurements of this type yield the mean linear flow velocity (v , $\text{mm}\cdot\text{s}^{-1}$) and the volume flow rate (Q , $\text{mm}^3\cdot\text{s}^{-1}$), without interference of the NMR signal of stationary fluid, such as non-flowing water in the tissue surrounding the transport vessels. In this contribution, we focus on the mean linear flow velocity, which has been obtained from the time at which the NMR signals of flowing fluid reaches its maximum (Van As and Schaafsma, 1984).

2.2. NMR T_2 measurements

The spin-spin relaxation time T_2 was measured by the Carr-

Purcell-Meiboom-Gill (CPMG) method (Farrar and Becker, 1971). The CPMG T_2 decay was measured by sampling the height of the echoes. The echo decays have been analysed using a non-linear least squares fit (Provencher, 1976), after rejecting the first two datapoints before analysis. Invariably, a non-exponential T_2 decay was observed. Least squares fitting has been carried out to a maximum of four exponentials. The best solution contains the sum of three exponentials. From these results we have calculated a mean effective relaxation rate \bar{R}_2 , defined by

$$\bar{R}_2 = \sum_{i=1}^3 P_i T_{2i}^{-1} \quad (1)$$

where P_i represents the fraction of component i .

2.3. Apparatus

Flow and T_2 measurements in intact plants were performed on a 20 MHz (^1H) single-coil pulsed NMR spectrometer, equipped with a 17 cm electromagnet, a Bruker Aspect 2000 computer, a Bruker Z17C pulse programmer and modified Bruker Minispec pc20 transmitter/receiver electronics. The T_2 measurements for the determination of the relationship between \bar{R}_2 , water content and water potential have been performed on a Bruker Minispec p20 (20 MHz, ^1H). In all measurements the time interval between the 180° r.f. pulses was 1.6 ms. For T_2 measurements a train of 1920 180° r.f. pulses was applied. For flow measurements this number was 4096, resulting in 256 datapoint by integrating the signal over a period of 16 r.f. pulses.

2.4. Relationship between \bar{R}_2 , P_w and ψ_w

The relationship between \bar{R}_2 , water content and water potential has been measured in cucumber stem segments of + 1 cm length. Firstly, the full turgor weight was determined after a 4 hrs incubation in demi-water. The water potential ψ_w in the stem segments was adjusted subsequently by incubation (40 hrs, until equilibrium) of the segments in aqueous poly-ethylene glycol (PEG, $M = 20\ 000$) solutions over a range of concentrations. Values of ψ_w of the PEG solutions were determined by using a vapor pressure osmometer. For each incubated segment T_2 as well as the water content was determined, the latter by weighing the segments before and after drying at 100°C during 48 hrs.

3. Results

3.1. Relationship between \bar{R}_2 , P_w and ψ_w

The results of these measurements are shown in figures 1 and 2. We note, that a comparison of the water content of adjacent stem segments incubated in PEG solutions of various concentrations, reveals that the PEG molecules hardly penetrate into the plant tissue. We believe therefore, that the water potential of the tissue in the segments, adjusted by incubation in PEG solutions, reliably reflects the water potential of plant tissue *in vivo*.

3.2. Effects of external conditions of the plant on flow and water potential in the stem

The effect of leaf illumination on \bar{v} and \bar{R}_2 has been measured simultaneously in the stem of an intact gherkin plant (pot plant), 0.5

m above the soil surface (figure 3). The relationship between \bar{R}_2 and ψ_w , given in figure 2, has been used to relate both quantities for the intact gherkin. Under the actual environmental conditions a maximum linear flow velocity of 13 mm.s^{-1} was found. After turning off the lamps, \bar{v} decreases from 13 mm.s^{-1} to $\sim 7 \text{ mm.s}^{-1}$ over a period of ~ 30 min. The decrease in \bar{v} is due to closure of the stomata. As suggested by Slatyer (1967) at that moment the upward movement of water to the leaves, due to mild water stress in the leaves and stem, begins to exceed the rate of loss due to evaporation, and the differences in ψ_w between soil, root and leaves decrease. As a result, the leaf apoplast and -cells and the stem cells are rehydrated, resulting in an increase in water potential, as reflected by the decrease of \bar{R}_2 . After turning on illumination, \bar{v} and \bar{R}_2 increase again and approach about the same value as at the beginning of the experiment.

The effect of a decrease in soil water potential on \bar{v} and \bar{R}_2 has also been measured: five days after adding water to the soil \bar{v} had decreased from 17.5 mm.s^{-1} to $\sim 5 \text{ mm.s}^{-1}$. Simultaneously, \bar{R}_2 had increased from 3.5 s^{-1} to $\sim 8 \text{ s}^{-1}$, corresponding to a change in ψ_w from -6 to -11 to -12 bar. This situation corresponds to a severe dehydration, as was observed by wilting of the leaves.

4. Discussion

As has been demonstrated above, combined pulsed NMR measurements of flow and \bar{R}_2 can provide useful parameters describing the plant water balance and its dependence on environmental conditions. Flow has been measured directly, whereas the stem water content and water potential were obtained *via* \bar{R}_2 . In various tissues and materials a positive correlation has been found between T_2 and water content. Recently, Fullerton et al. (1982) demonstrated that

$$R_2 \equiv \frac{1}{T_2} \propto P_w^{-1} \quad (2)$$

for protons which are in fast exchange with each other. This condition is introduced artificially by treating the data according to equation (1).

In figure 3 \bar{R}_2 and ψ_w have been related, based on the results presented in figure 2. The $\psi_w = 0$ position on the ψ_w axis with respect to the \bar{R}_2 depends somewhat on the age of the plant and the conditions of growing up. Preliminary results indicate that the shape of the curve relating \bar{R}_2 and ψ_w is insensitive to these differences. This results in an uncertainty of $\sim 0.25 \text{ s}^{-1}$ in the $\psi_w = 0$ position with respect to the \bar{R}_2 axis. The relationship between \bar{R}_2 , P_w and ψ_w for different types of plant tissue is currently investigated in our laboratory.

It is convenient to consider the plant water pathways in terms of electrical analogies (Sheriff, 1974). Using this concept, one can design NMR experiments as described above on the water balance in the plant stem just above the root system, which yield information on the effects of the environmental conditions on the water balance of the root- and shoot system separately when in addition to the NMR flow and \bar{R}_2 measurements the soil and air water potential are known.

Based on the results presented here, we suggest that the pulsed NMR spectrometer can serve as a sensor in controlling greenhouse climate. Up till now, the heavy equipment (200 kg magnet system) has prevented

application of NMR measurement to plants in a greenhouse. For that purpose, a portable apparatus is now being developed.

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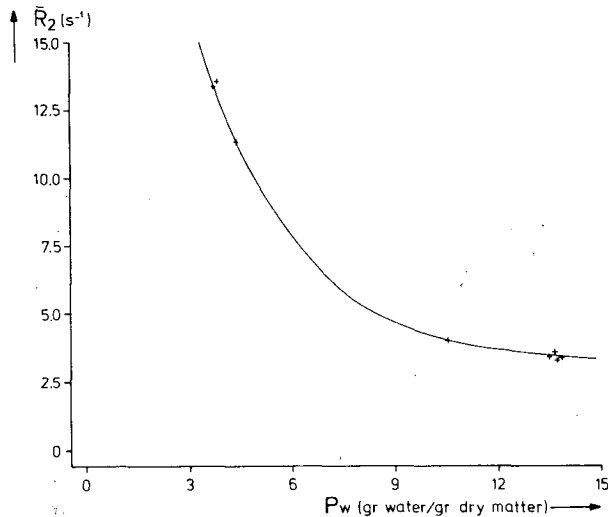


Figure 1 -Effective spin-spin relaxation rate (\bar{R}_2) versus water content (P_w) for stem segments of Cucumber (water culture plant).

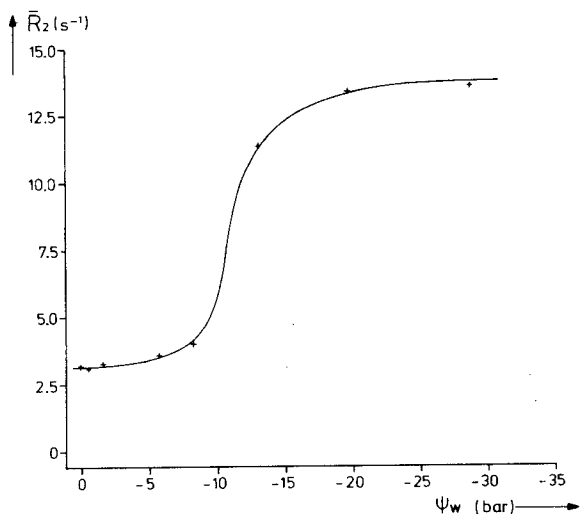


Figure 2 - Effective spin-spin relaxation rate (\bar{R}_2) versus the water potential (ψ_w) in stem segments of Cucumber plant.

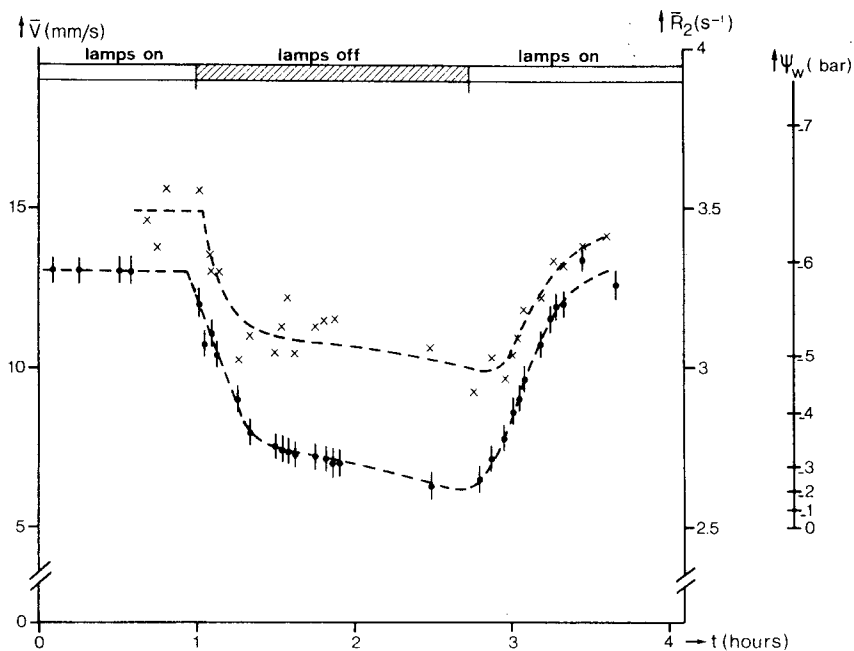


Figure 3 - Effect of leaf illumination intensity on \bar{v} and \bar{R}_2 measured in a stem-section of 1 cm of an intact gherkin plant (pot plant), at 0.5 m above the soil surface.