Potential of (portable) NMR and MRI for plant phenotyping

Henk Van As

WAGENINGEN UR
For quality of life

NMR/MRI and plant phenotyping

- Non-invasive NMR and MRI sensors to measure plant performance and uncover genetic determinants of enhanced agronomic traits.
- A number of approaches and parameters are available from such sensors to study complex plant physiological traits such as growth, biomass accumulation and yield throughout the plant (crop) growing season.
- Both below-ground (3D root architecture), above-ground (stem, leaves, water status, growth, fruit development) and transport traits can be studied.

Suggested NMR/MRI accessible traits

Table 1. Conceptual overview showing how better understanding or quantification of specific below-ground, above-ground or transport traits will can contribute to improve light-use-efficiency (LUE), water-use-efficiency (WUE) or nitrogen-use-efficiency (NUE)

	Phenotype	Method/approach	Improvement of
Above ground	Energy conversion in light reaction	Fluorescence, imaging spectroscopy	LUE
	3-D canopy structure	Stereo imaging	LUE
Transport/allocation	Sap flow	MRI, velocity radiotracers	WUE, NUE
	Plant water status	NMR	WUE
Below ground	Root architecture	2-D rhizotrons, 3-D MRI	NUE, WUE

Rascher et al, 2011, Functional Plant Biol. 38

There are some more possibilities ….

WAGENINGEN UR
For quality of life

(TD-)NMR and MRI

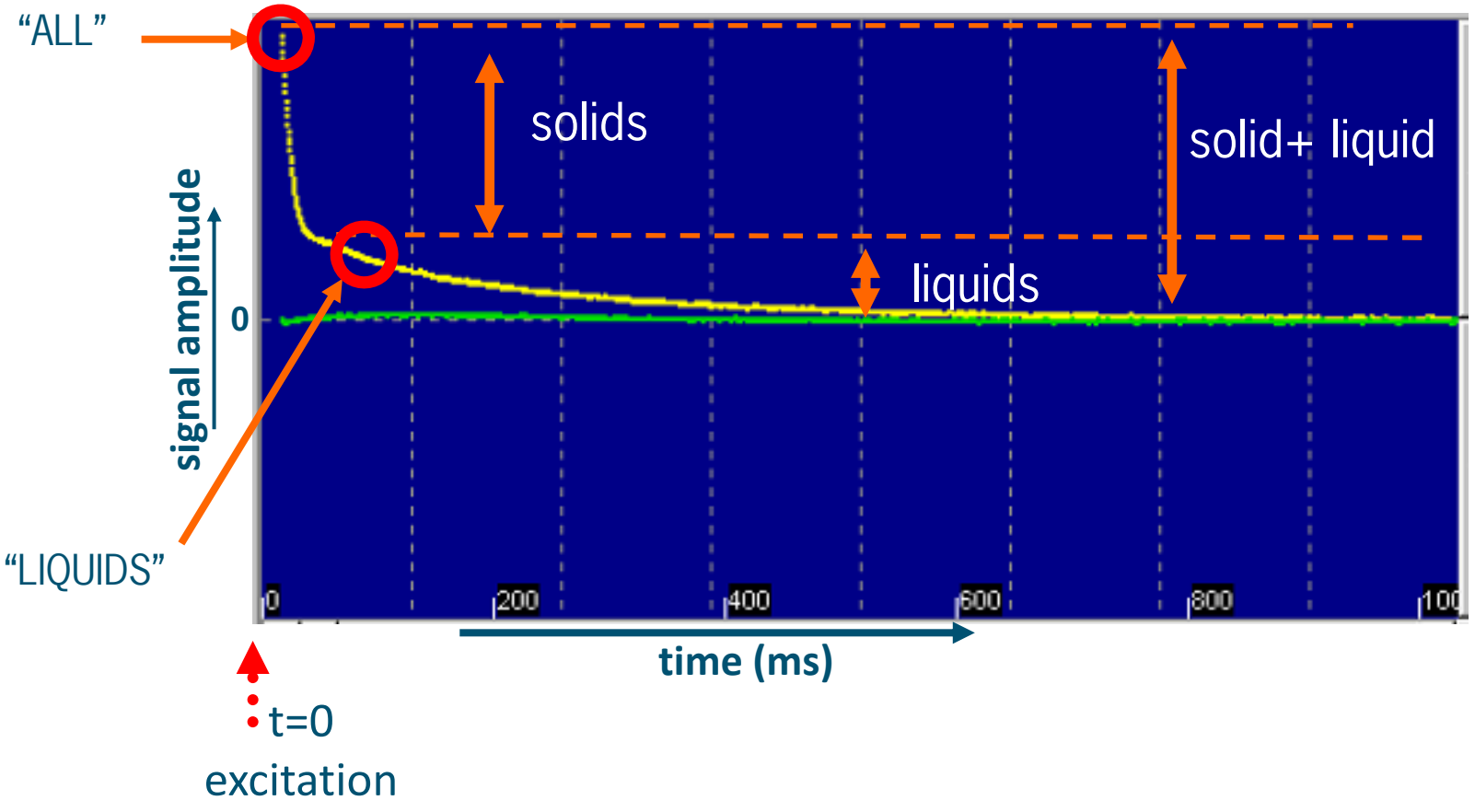
- NMR: non-spatially resolved, fast, detailed information, relatively cheap.

  Problem: tissue heterogeneity

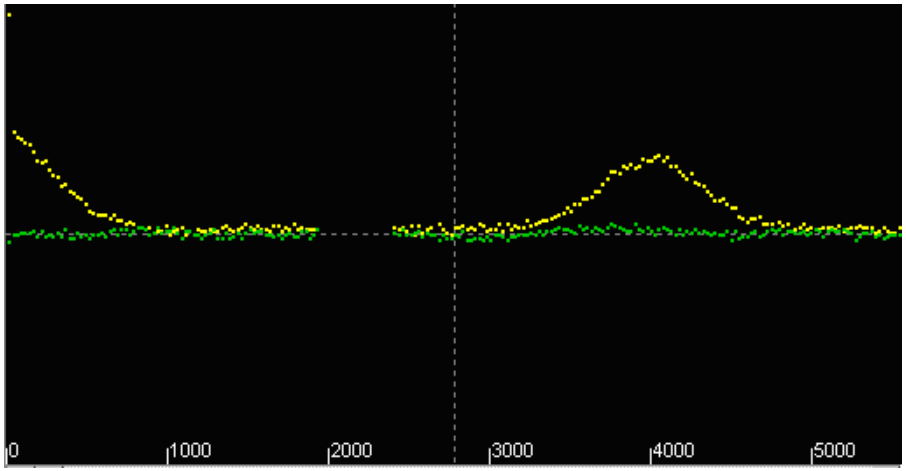
- MRI: spatially resolved, longer measurement times, less detailed information on cell level.

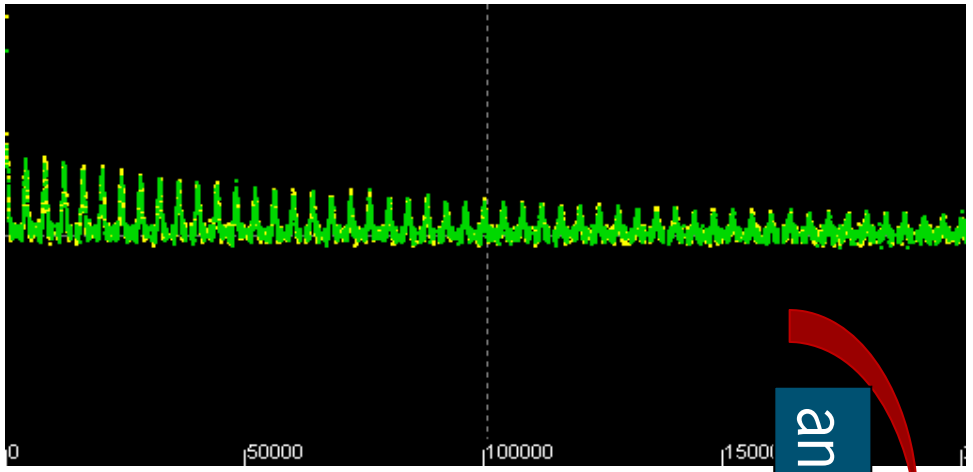
  Problem: expensive dedicated equipment. But ….

The (TD-)NMR signal



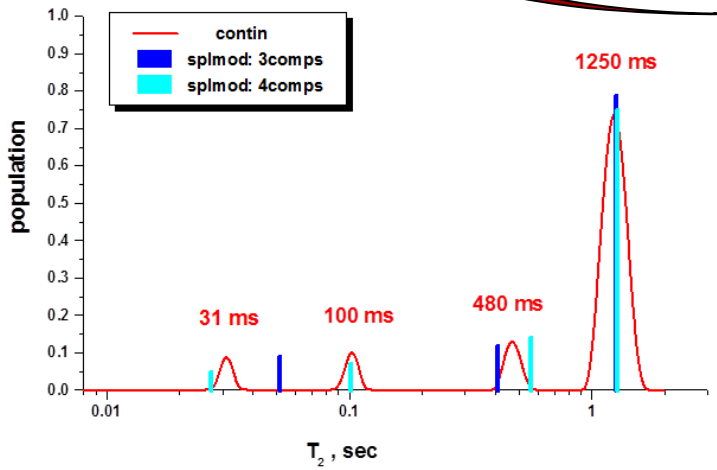
TD NMR: dynamics of water ^{1}H's





One → multi echoes

analysis



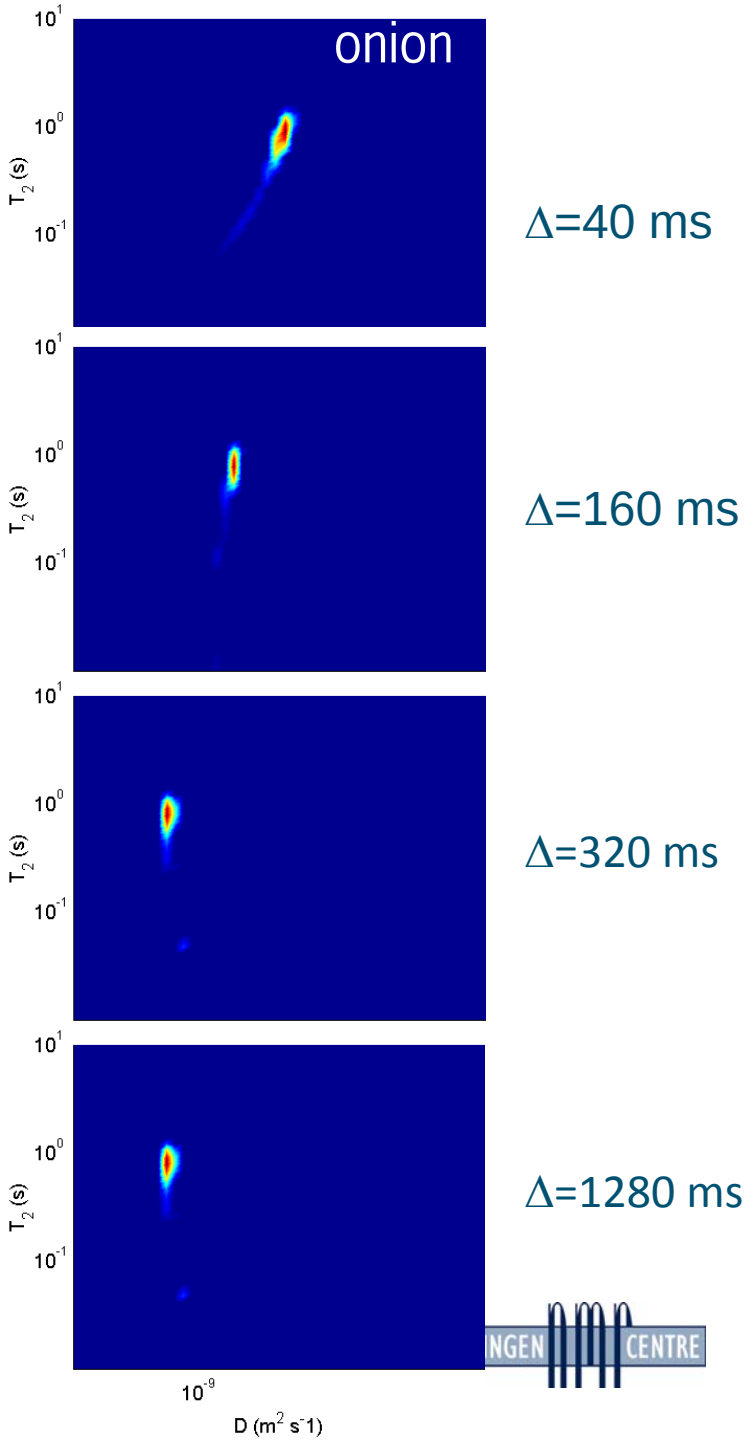
Sum of exponentials

T2 distribution (CONTIN)

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

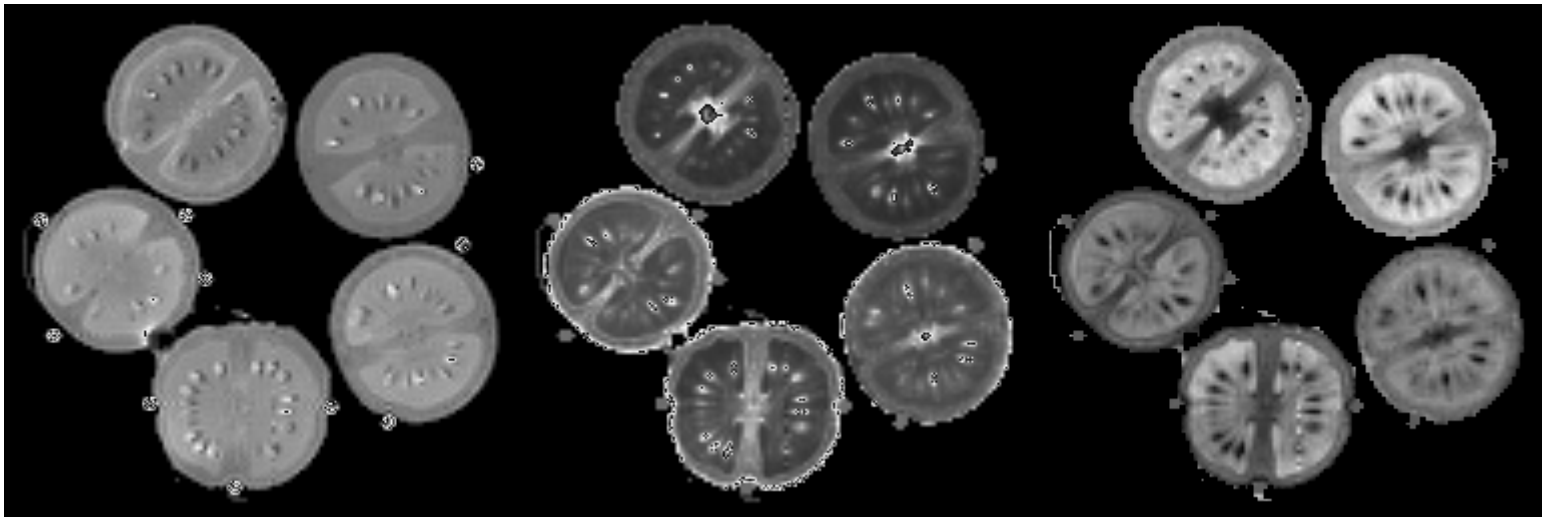
More details: use diffusion of water molecules to characterize free diffusion path length, combine with T2



WAGENINGEN UR
For quality of life

Effect of membrane permeability on T2

Amplitude R_2 (s^{-1}) T_2 (s)=1/R_2



x35

0.0 Amplitude 8.5

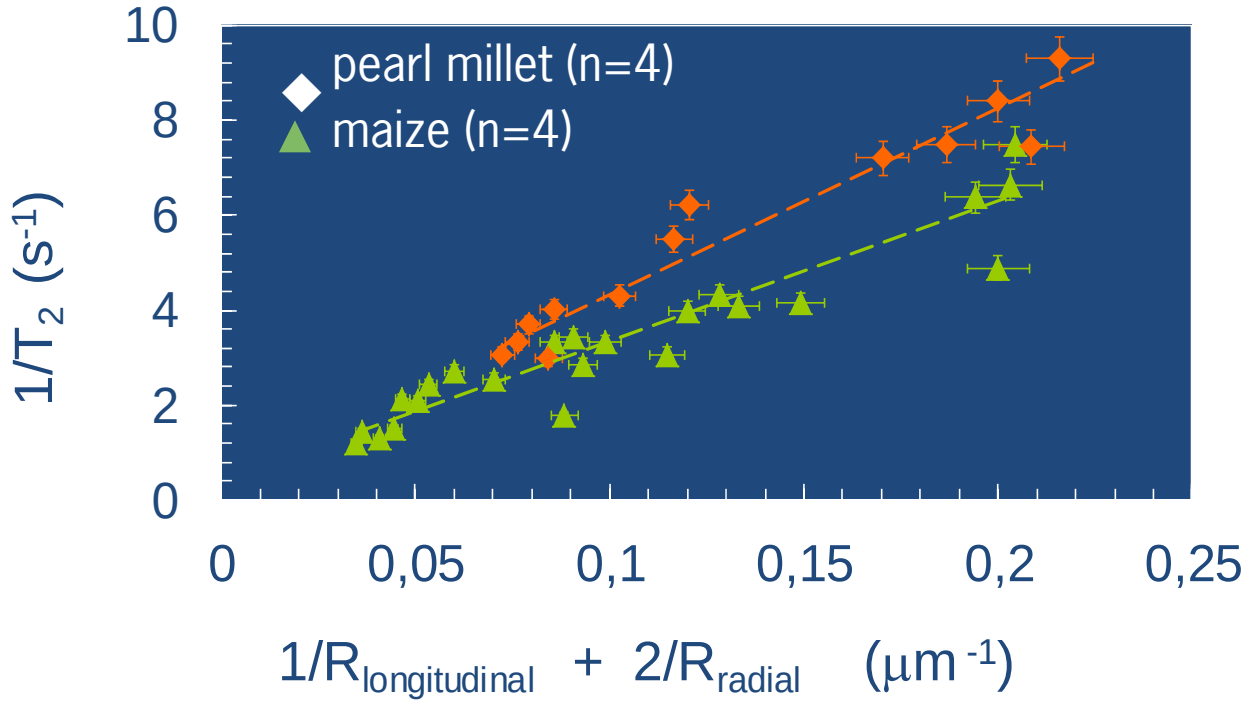
0.0 T_2 (s) 1.4

WAGENINGEN UR
For quality of life

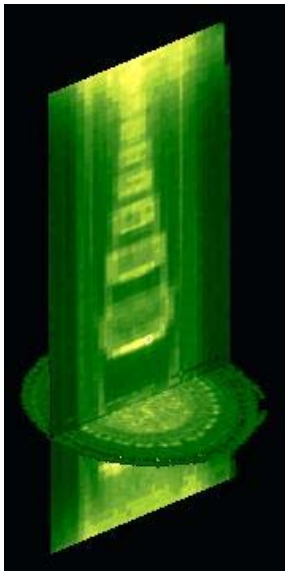
WAGENINGEN CENTRE

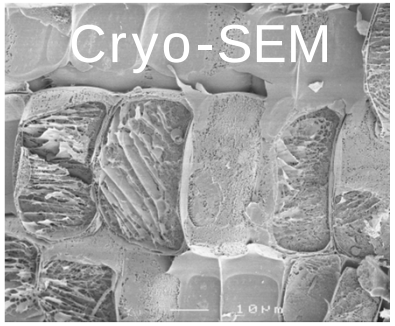
Longest T2, cell (vacuole) size and membrane permeability (H)

- $1/T_{2,obs} = H(1/R_x + 1/R_y + 1/R_z) + 1/T_{2,bulk}$



MRI



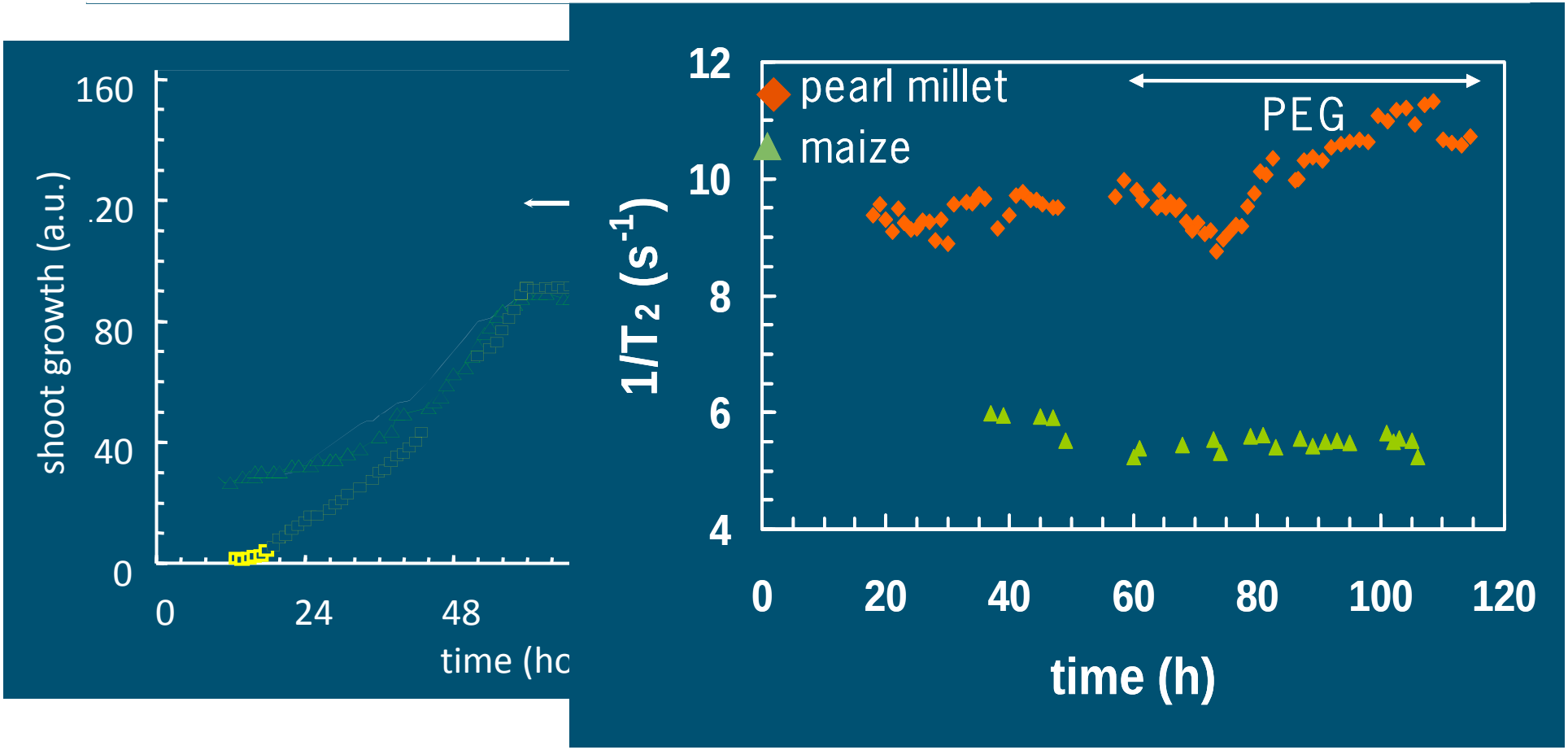


L. van der Weerd *et al.*, Plant, Cell and Environment 2002

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Stem (apex) growth and T_2 during osmotic stress: maize and millet

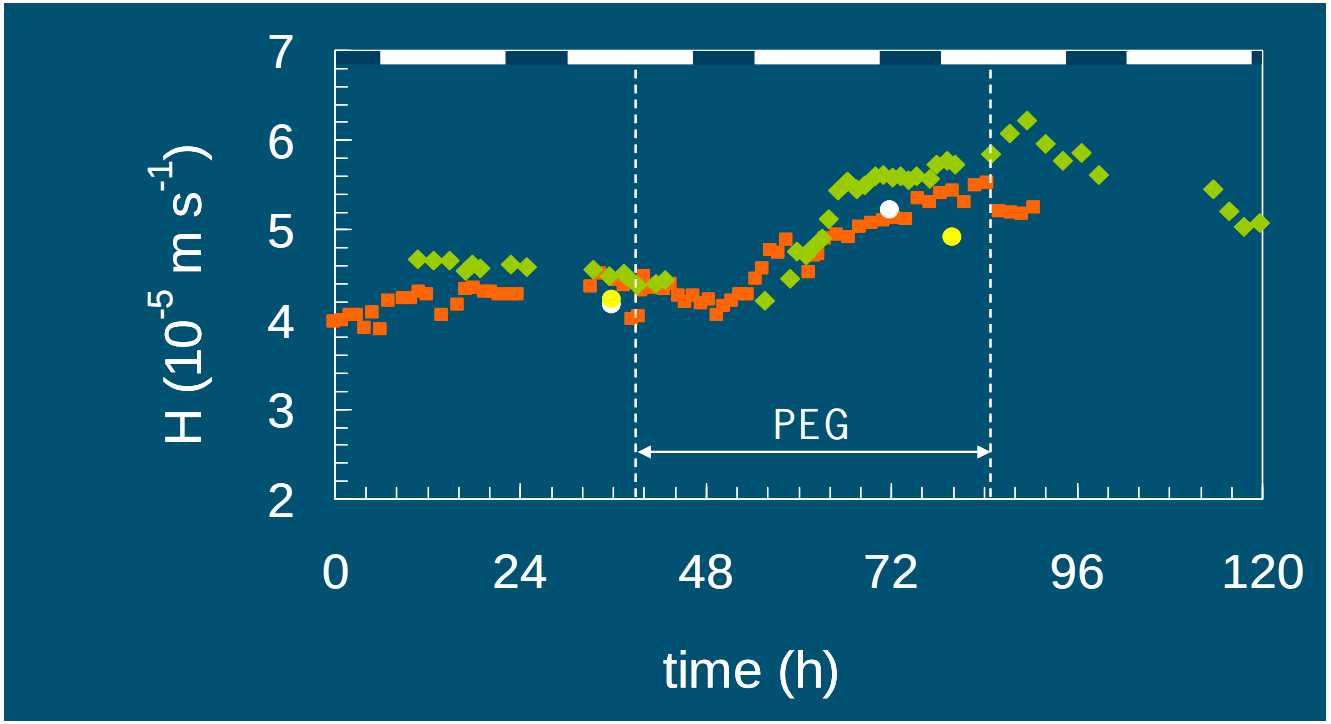


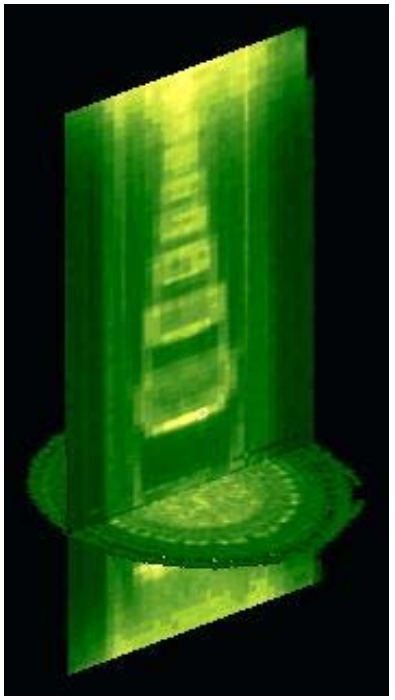
van der Weerd *et al.,* Plant, Cell and Environment 2002

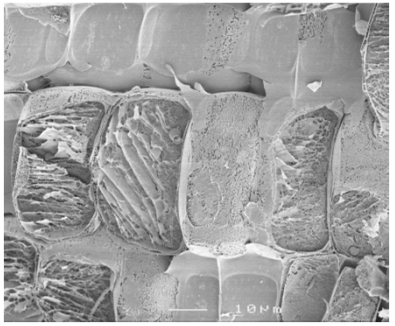
WAGENINGEN UR
For quality of life

Tonoplast membrane permeability in millet during osmotic stress:

- $1/T_{2,obs} = H\,(S/V) + 1/T_{2,bulk}$







Increase in H may facilitate water redistribution between tissues

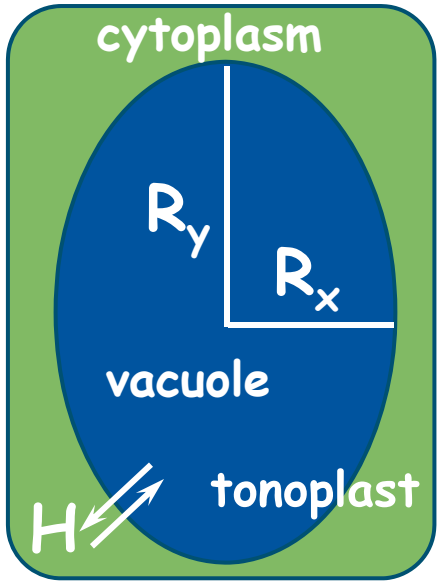
aquaporins?

van der Weerd *et al.*, Plant, Cell and Environment 2002

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

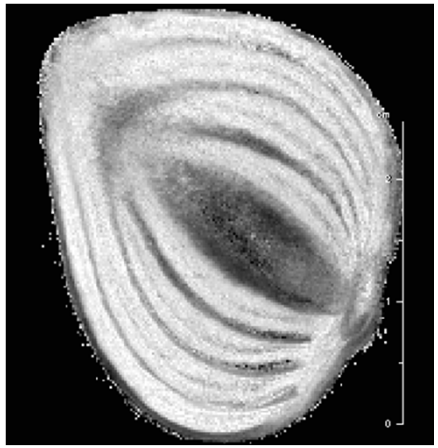
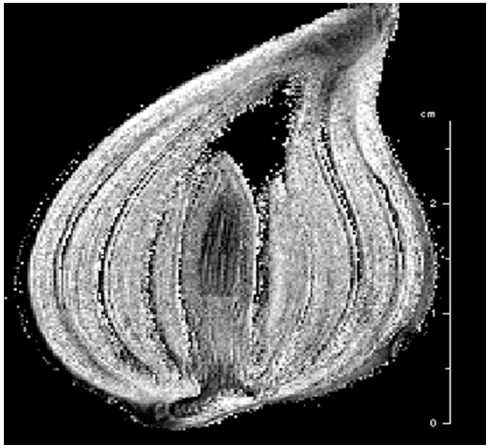
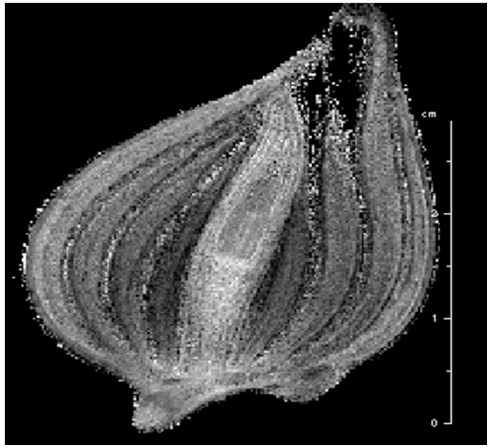
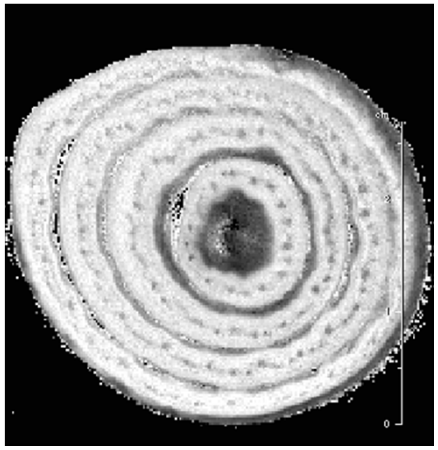
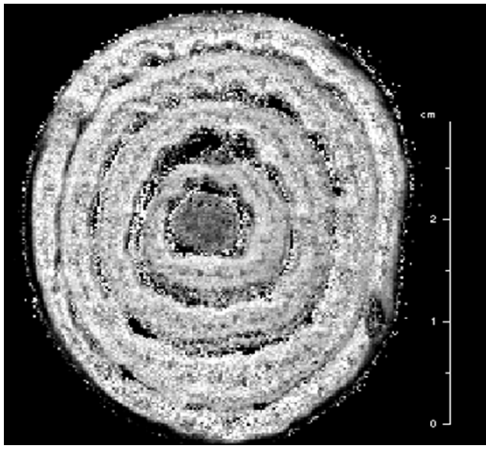
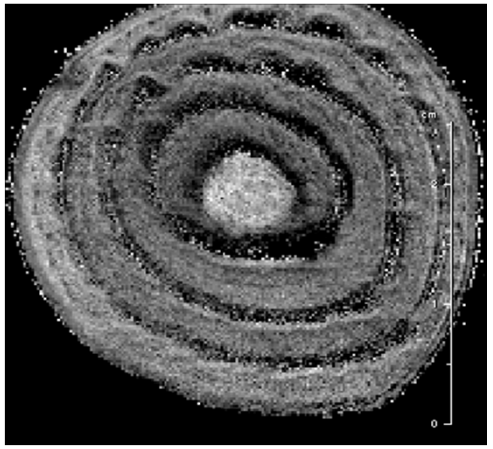
T2, diffusion and permeability



- Relaxation: $1/T_{2,obs} = H(S/V) + 1/T_{2,bulk}$
  - Can we get information about S/V?
- Yes! Diffusion as a function of diffusion time:
  - Restricted due to finite compartment size
  - Short diffusion distance:

    $\mathbf{D_{app}\ (=ADC) = D_0 - S/V\ C\ D_0^{1/2}\ \Delta^{1/2}}$
  - **Long Δ time-scale: (tissue) permeability**
- Can we become independent of S/V? Yes: T1/T2

Conversion of starch: interaction between mobile and solid protons

T = 0 **8 weeks** **8 weeks**

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Transport traits: especially appealing

- Xylem: Simple proportionality (modified Darcy's law) to compare (woody) plants by xylem specific conductivity, K_s (length normalized) is rate of sap flow per cross-sectional area at a given pressure gradient:

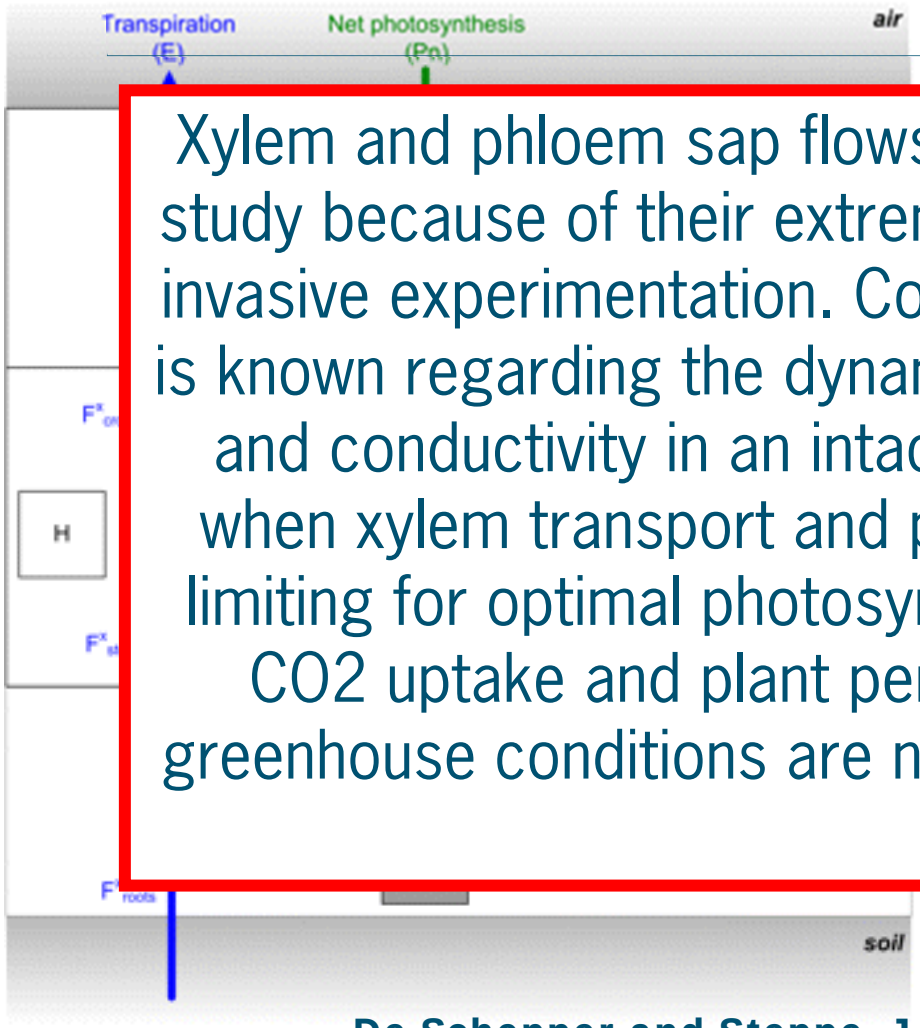
$$K_s/(LA/XA) \propto VPD.g_s.Ht/(\Psi_s - \Psi_L)$$

- Phloem: transport between source (leaves) and sinks (roots, fruits, growing tissue,..). Can it limit optimal photosynthesis?

  Loading from sucrose/starch.

  Can we benefit from optimized unloading?

Integrated model: photosynthesis, water and sugar

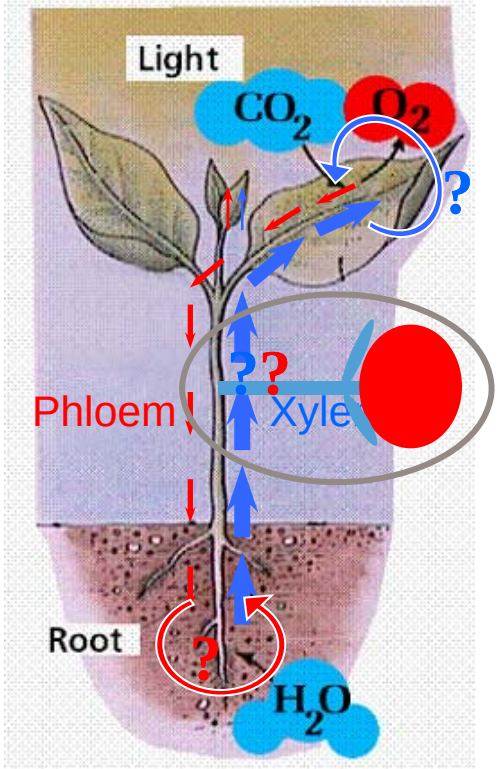


Xylem and phloem sap flows are difficult to study because of their extreme sensitivity to invasive experimentation. Consequently little is known regarding the dynamics of sap flow and conductivity in an intact plant. If and when xylem transport and phloem can be limiting for optimal photosynthetic activity, CO2 uptake and plant performance in greenhouse conditions are not yet resolved.

$$\Psi^X_{roots} = k_{soil}\Psi^X_{soil}$$

$$C^S_{crown} = C_0 \quad \Rightarrow L = V_{max,L}\frac{C_0}{K_{M,L}+C_0} \qquad \textit{(assumption (v))}$$

$$C^S_{roots} = C_0 \quad \Rightarrow M^S_{roots} = \frac{W^S_{roots}}{\rho_w}C_0 \qquad \textit{(assumption (v))}$$



(mass balance sugar)

$$C^S_{crown} = C_0 \quad \Rightarrow M^S_{crown} = \frac{W^S_{crown}}{\rho_w}C_0 \qquad \textit{(assumption (v))}$$

$$-f^{X\to S}_{crown} = f^{X\to Pc}_{crown} - F^{Pc}_{crown}$$

$$\Rightarrow \Psi^S_{crown} = P^X_{crown} - R^{X\to S}_{crown}\left(\frac{P^X_{crown}-\Psi^{Pc}_{crown}}{R^{X\to Pc}_{crown}} - \frac{P^{Pc}_{crown}-P^{Pc}_{stem}}{R^{Pc}}\right)$$

$$\Rightarrow P^S_{crown} = \Psi^S_{crown} + \frac{R\,T}{MM_{sucrose}}C_0 \qquad \textit{(mass balance water)}$$

if ($f^{X\to S}_{crown} = f^{S\to Pc}_{crown}$ and $f^{X\to Pc}_{crown} + f^{S\to Pc}_{crown} = F^{Pc}_{crown}$ and $f^{S\to Pc}_{crown} > 0$)

then "start model simulation"

else "$f^{X\to Pc}_{roots} = I + 0.00001$"

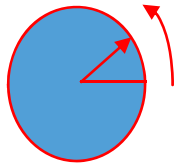
De Schepper and Steppe, J Exp Bot. 2010

WAGENINGEN UR
For quality of life

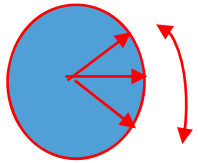
Dynamic or displacement imaging

P(r|r',τ), conditional displacement probability

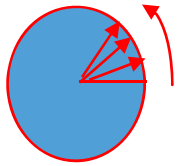
displacement Δr + gradient G
=>
frequency or phase shift $\Delta\theta$



diffusion, perfusion $\Delta\theta$ random,
=> no net phase shift



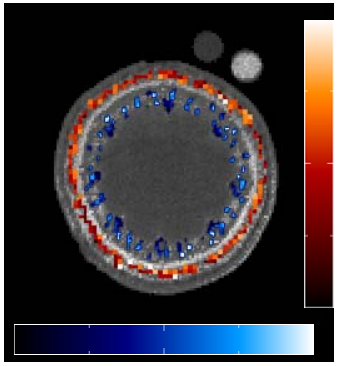
flow: $\Delta\theta$ equal for all spins
=> net phase shift

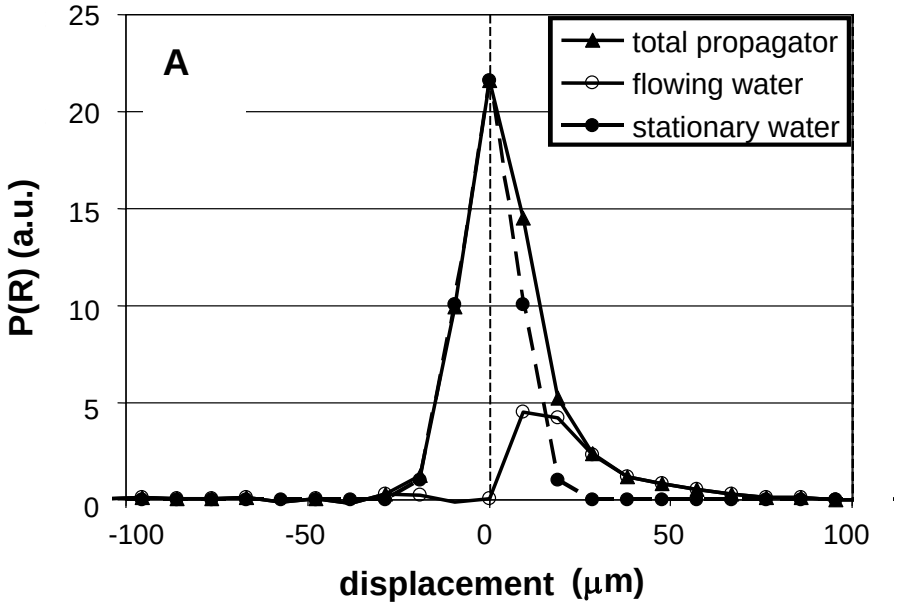


detect signal as function of G =FT=>
distribution of displacements Δr

Propagator and flow characteristics

For every pixel in the image or selected regions!





Volume-flow

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

Average velocity

$$v = \frac{\sum_{R=0}^{R=R\max} P_n(R,\Delta)\cdot R}{\sum_{R=0}^{R=R\max} P_n(R,\Delta)\cdot \Delta}$$

$$FCA = \sum_{R=0}^{R=R\max} P_n(R,\Delta)\cdot A_{ref}\,(=Q/v)$$

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

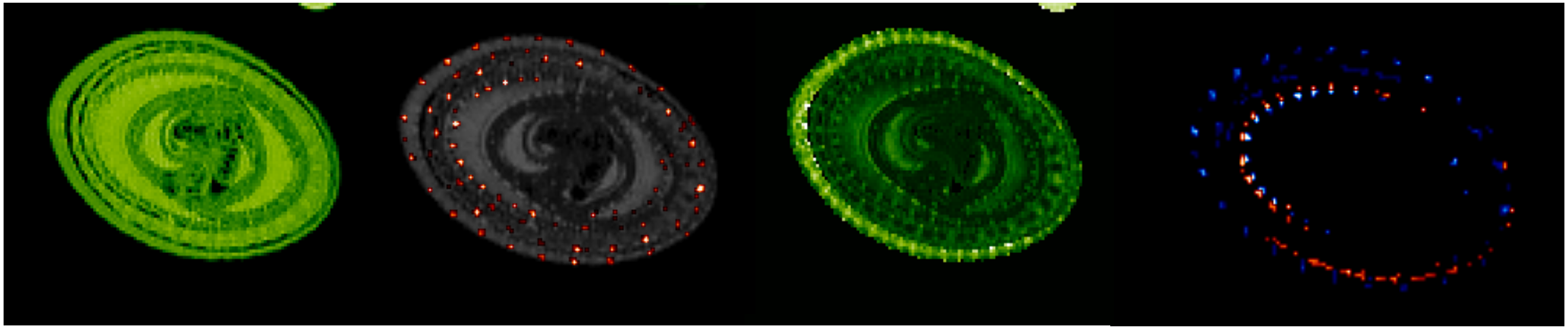
Flow: xylem and phloem in Maize

Amount of water

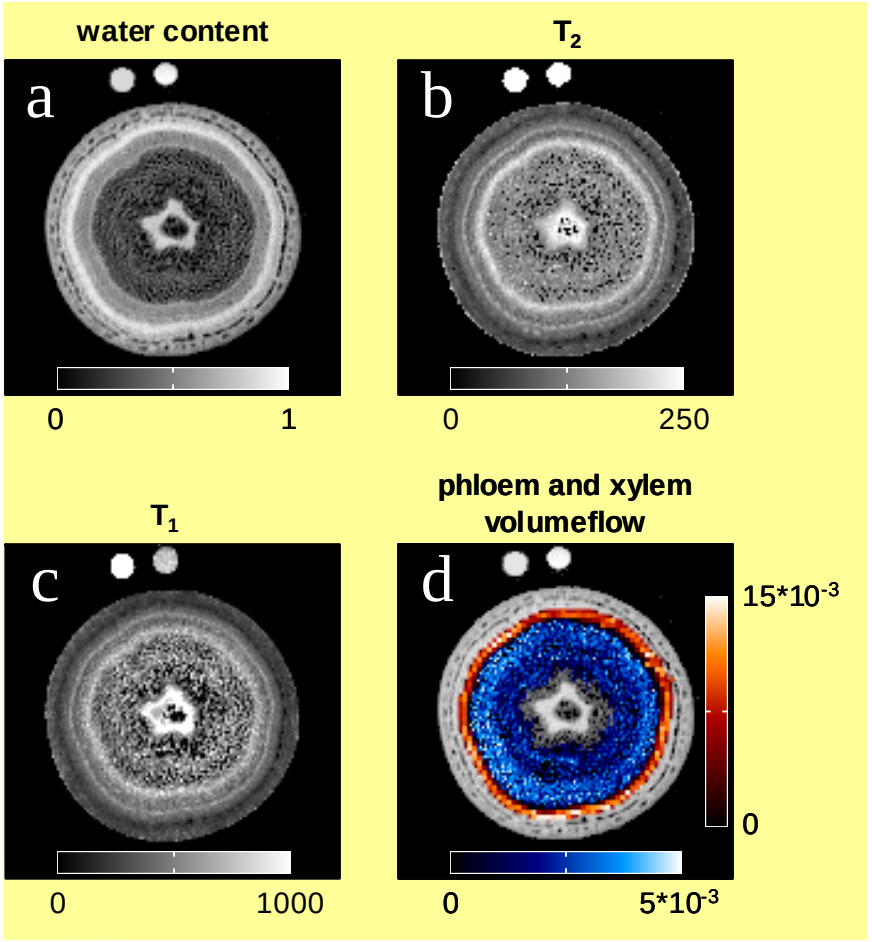
flow: xylem (day)

T2

flow: xylem and phloem (night)



NMR/MRI: axial and radial transport and water content in storage pools

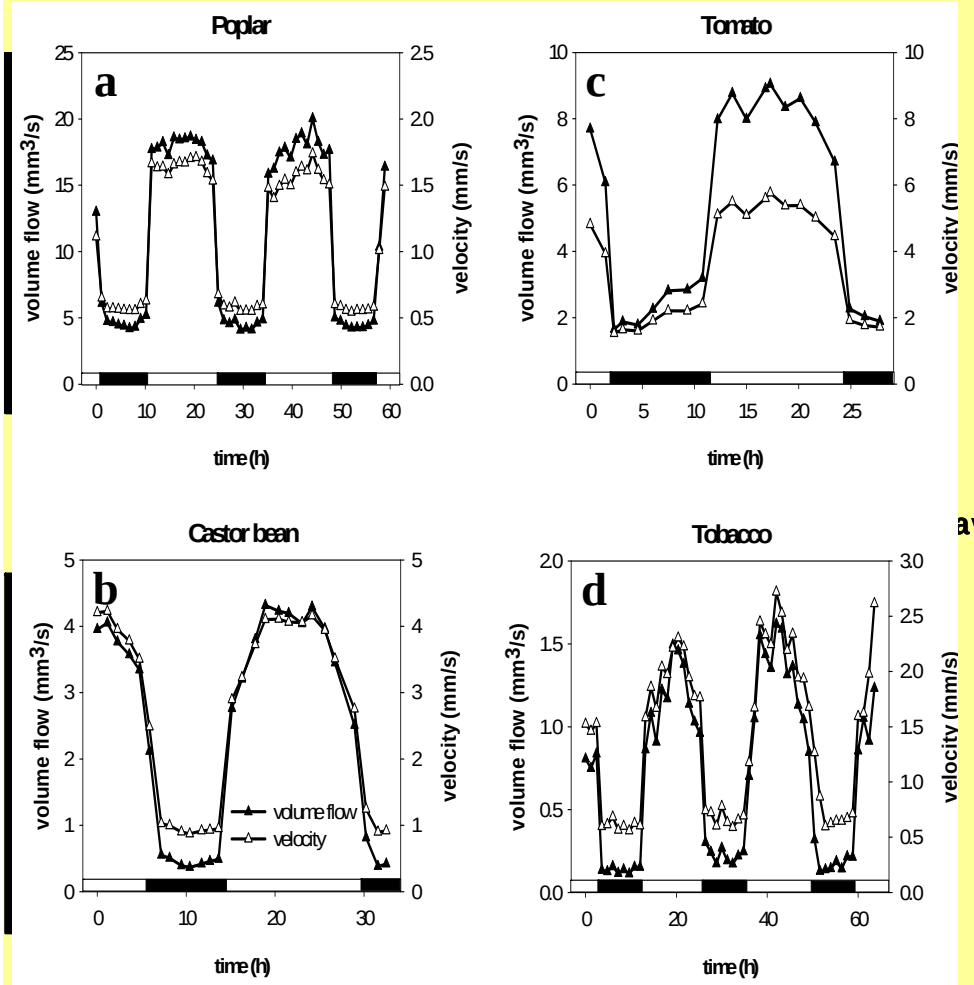


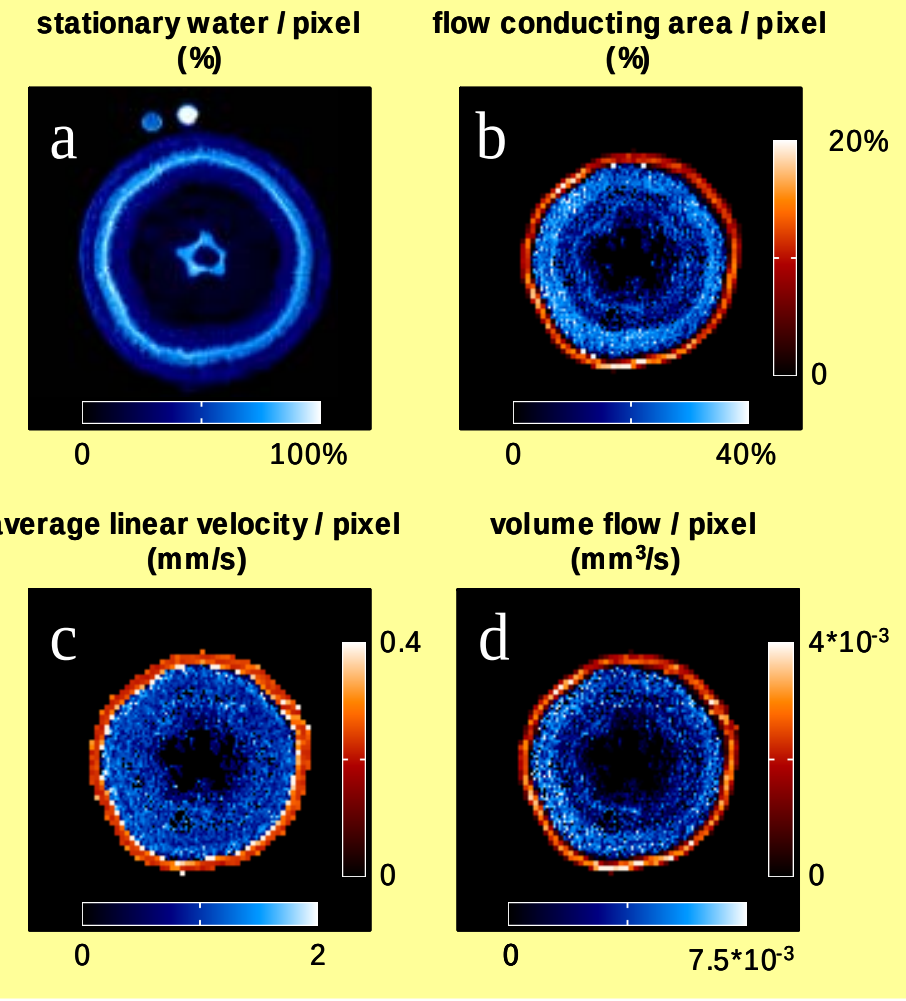
Resolution: 80 - 100 μm
Velocities:
100 μm/s – tens of cm/s

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Dynamics in xylem and phloem flow





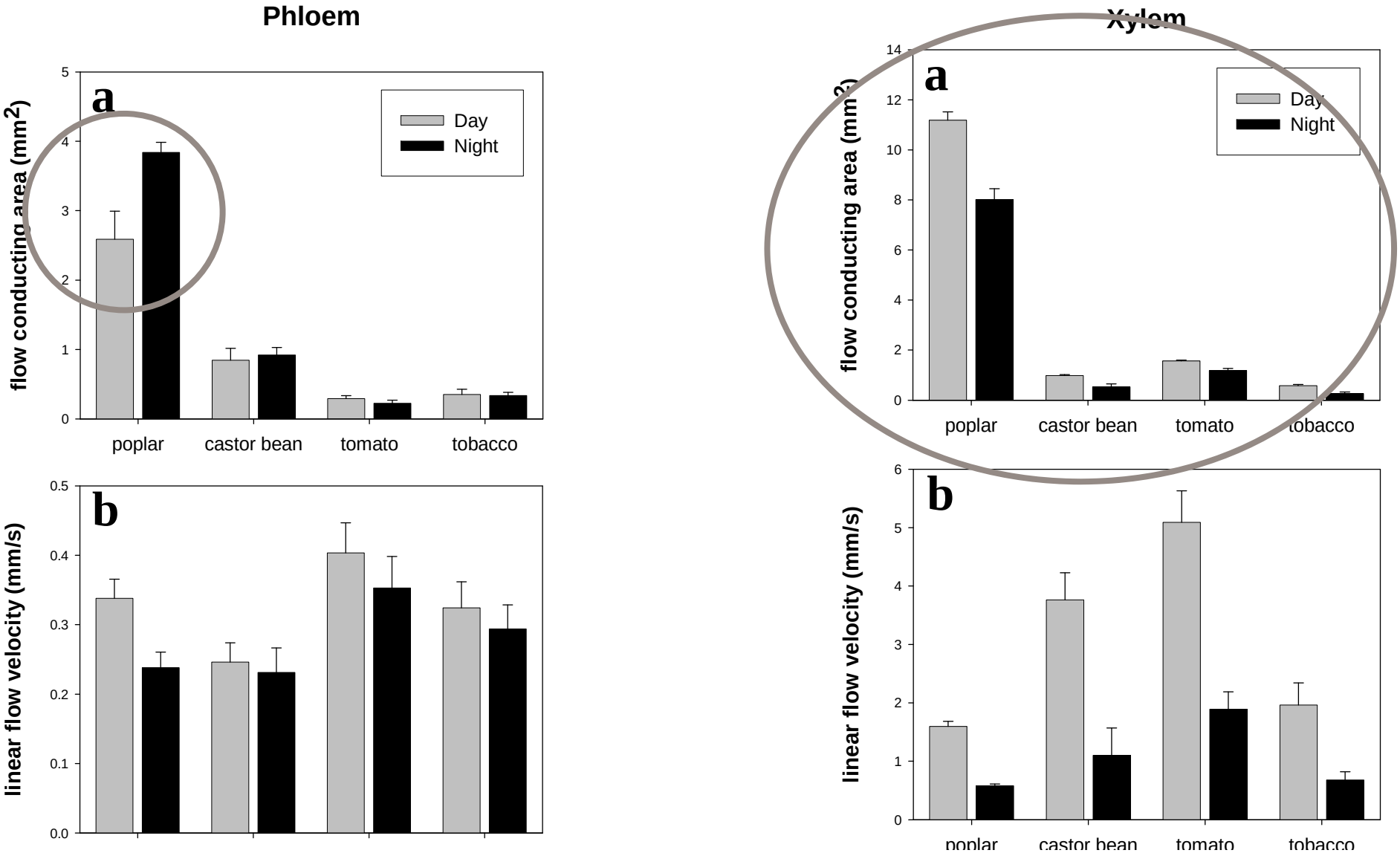
(Windt *et al*, PC&E 2006)

WAGENINGEN UR
For quality of life

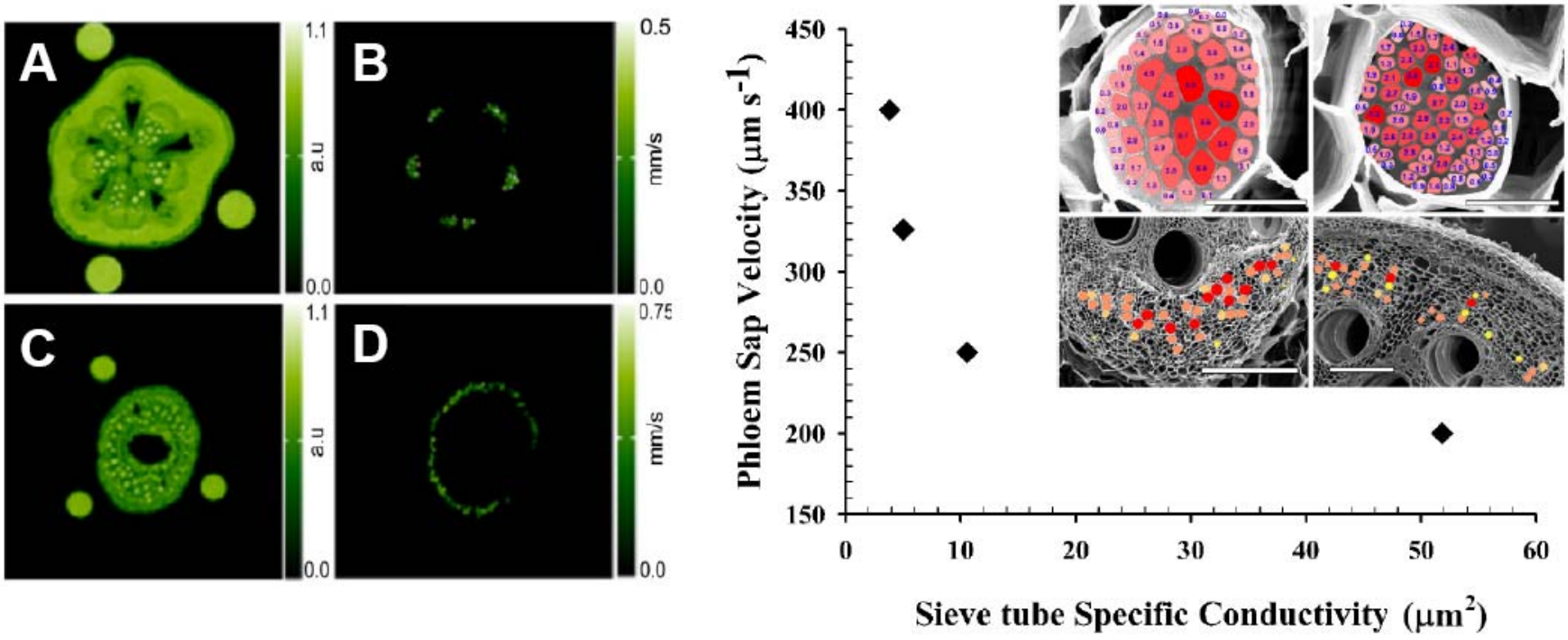
WAGENINGEN CENTRE

Dynamics in flow conducting area / conductance!?

(Windt et al, PC&E 2006)

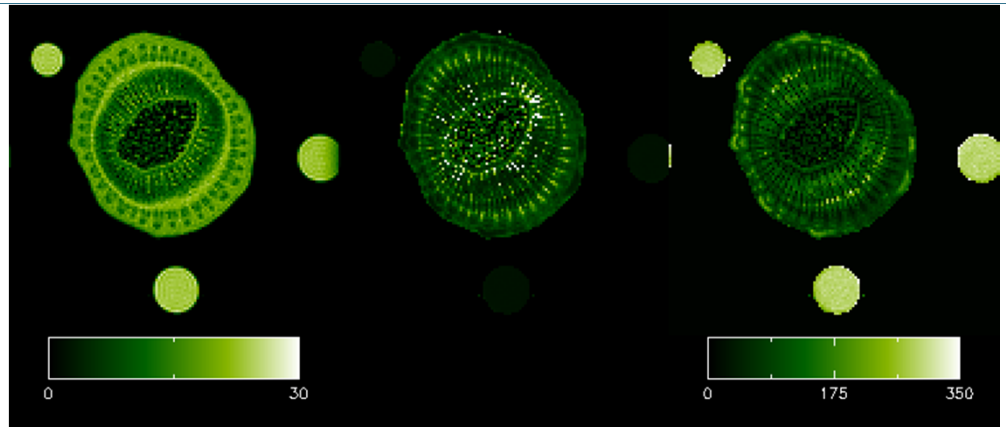


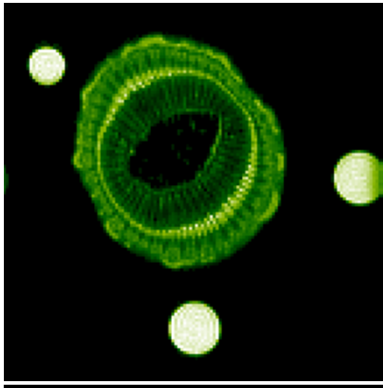
Phloem flow velocity can not simply be explained by conductivity and / or laminar flow?

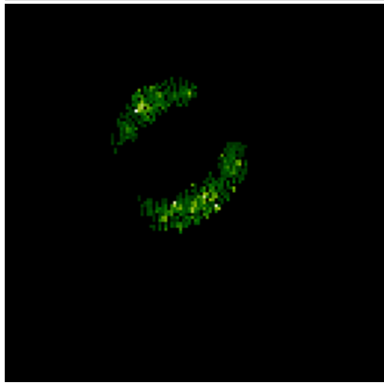


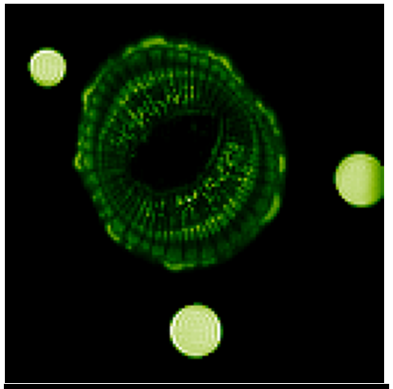
Mullendore *et al*, The Plant Cell 2010

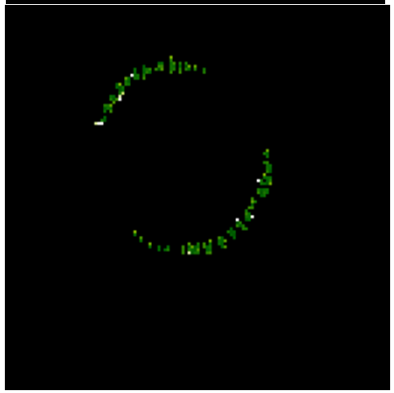
Grape: bi-directional









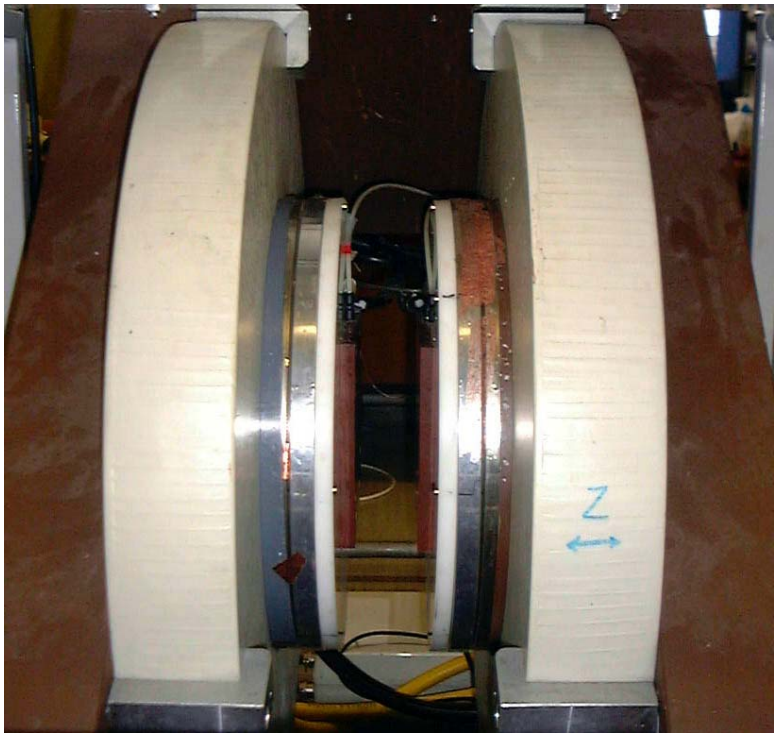


- Phloem has been observed in both directions:
  Upward and downward
  (different plants)

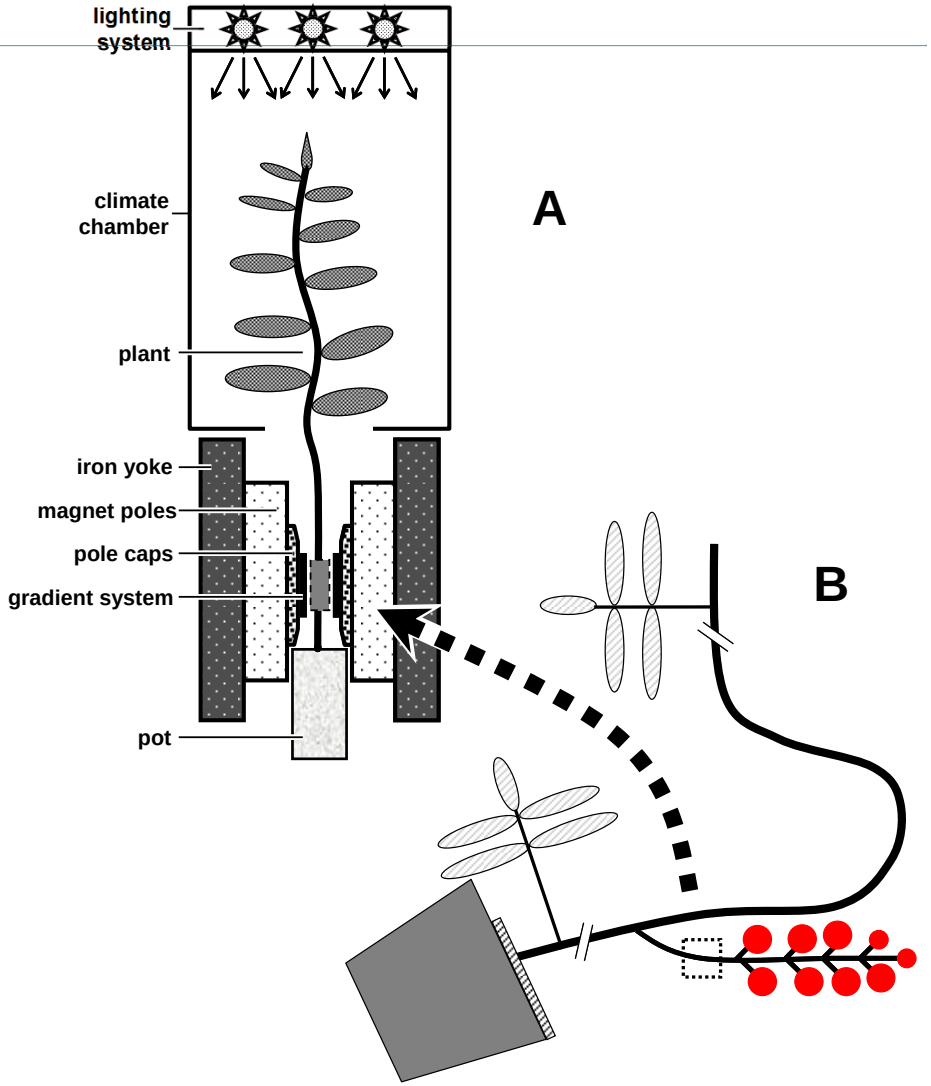
WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

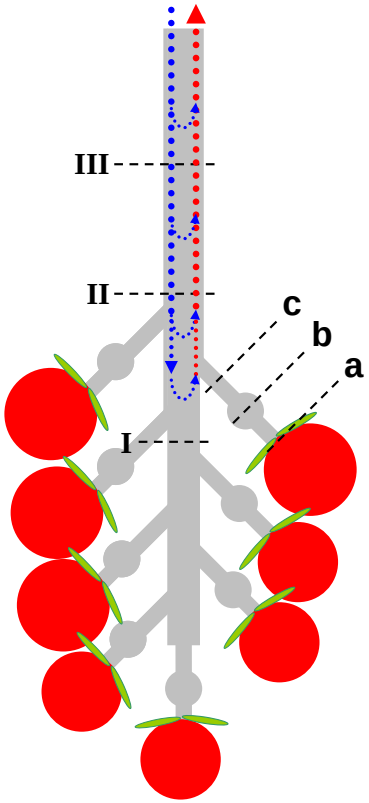
Imaging stem-truss stalk connection

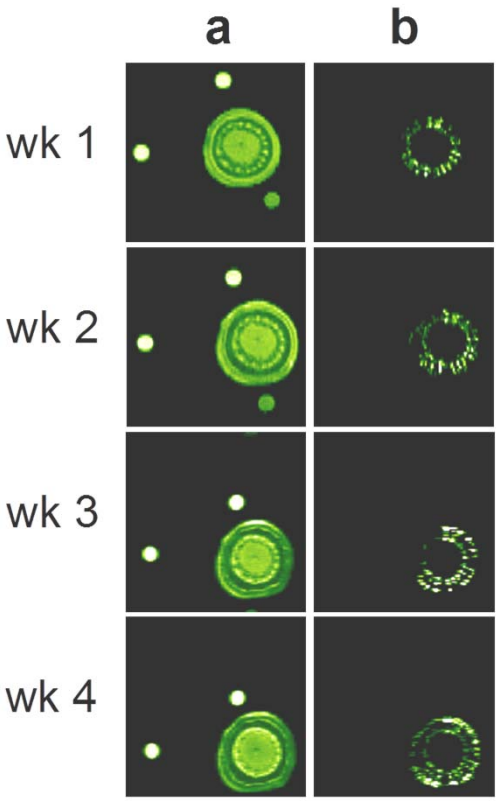


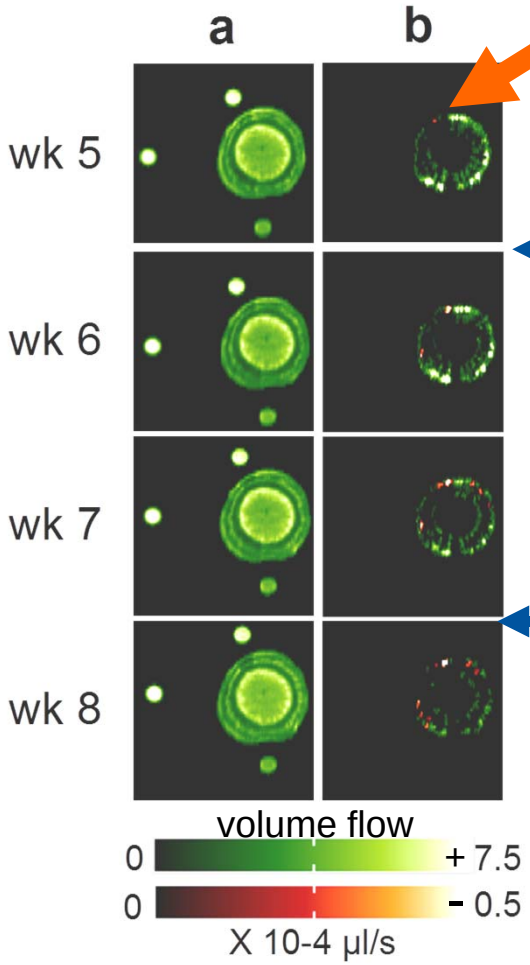
electromagnet and open gradient system



Flow in truss stalk tomato during 8 weeks of truss growth: Ratio [xylem]/[phloem] is >3:1and not 1:9!!!







back flow

5 fruits red, one green remaining

last fruit red

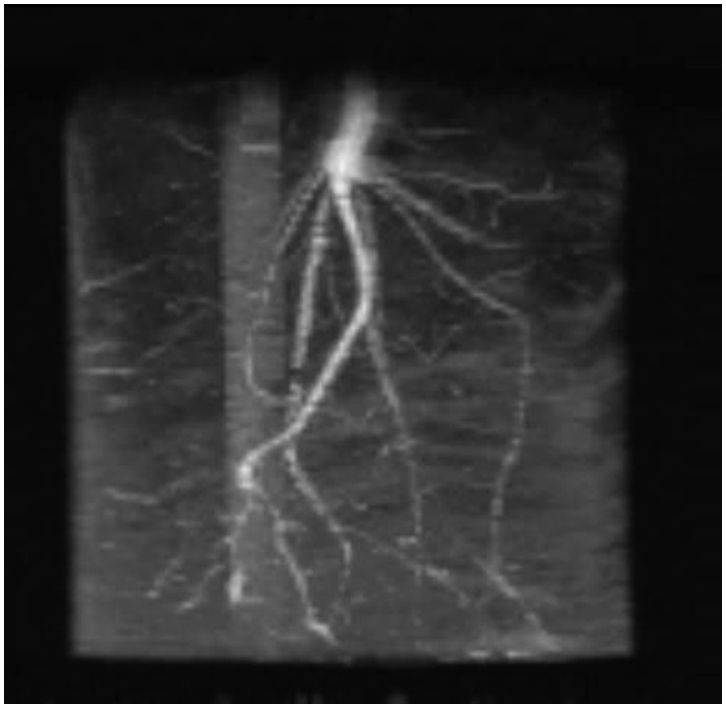
Windt *et al*, Plant Physiol 2009

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Below ground: 3D root anatomy and water uptake

Maximum intensity projection



- 3T-MRI, Fast Spin Echo
- 128 x 128 x 128 pixels (FOV 10 cm -> ~ 0.8 x 0.8 x 0.8 mm^3 per pixel)
- Total 3D experiment time 51 min 12 s

WAGENINGEN UR
For quality of life

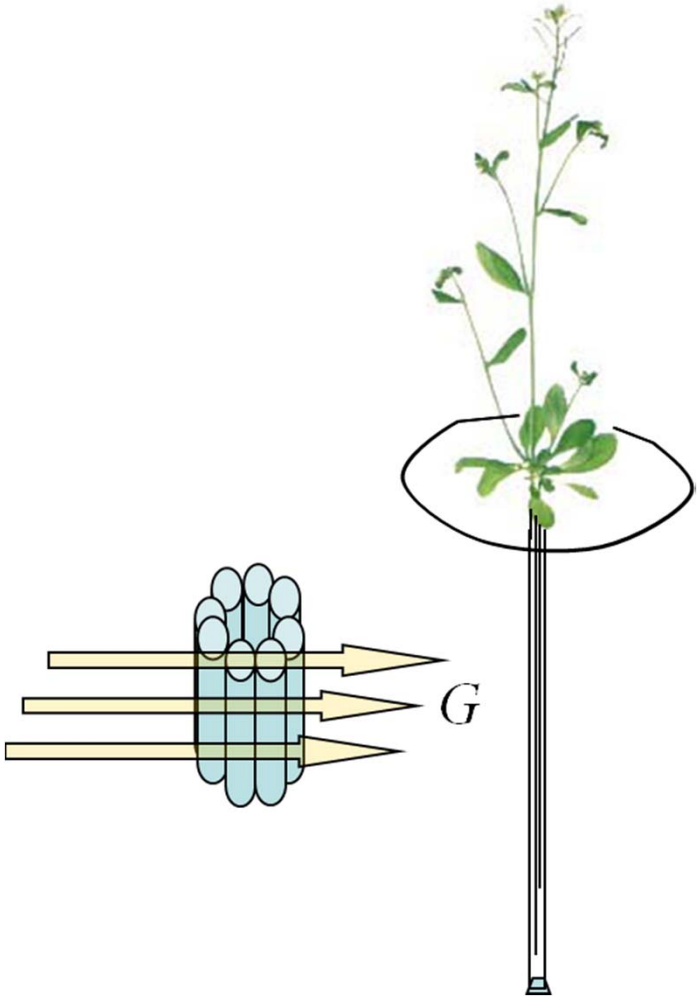
WAGENINGEN CENTRE

Root transport: e.g. ecotypes in relation to aquaporin expression

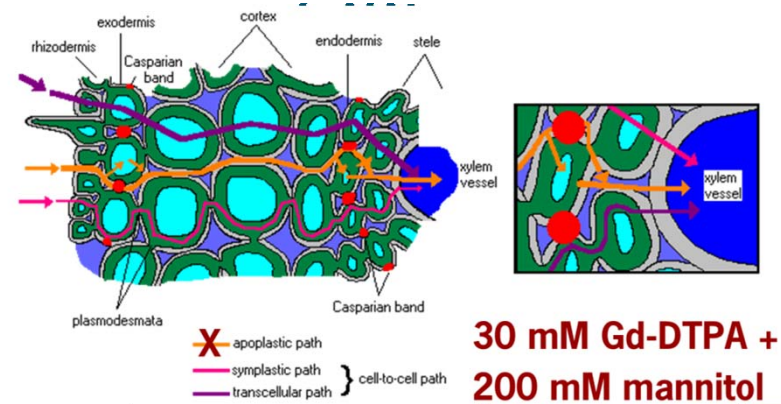
- *Arabidopsis thaliana*

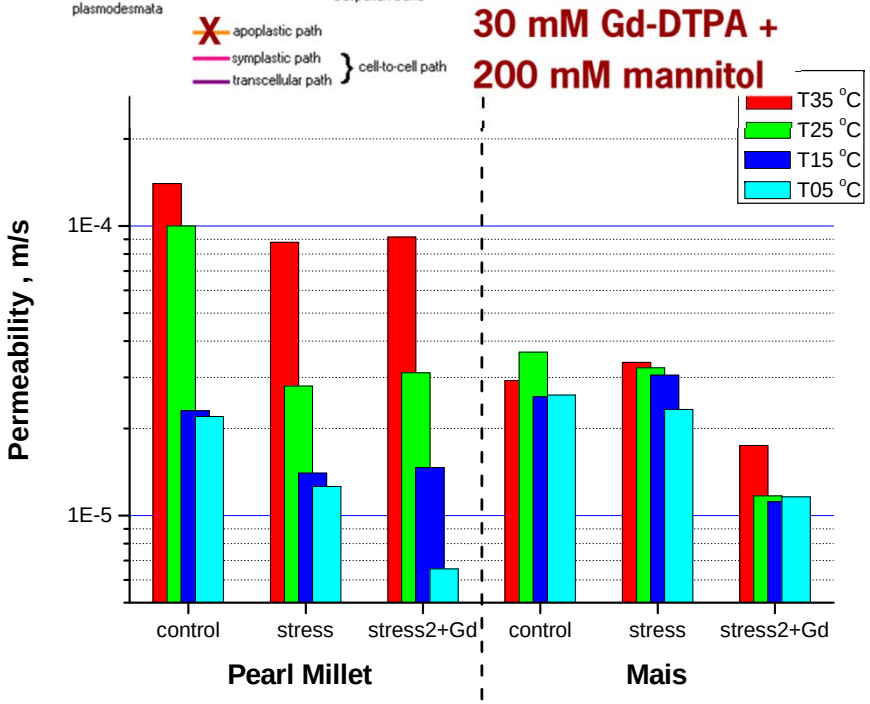
  ecotype: *Columbia*;

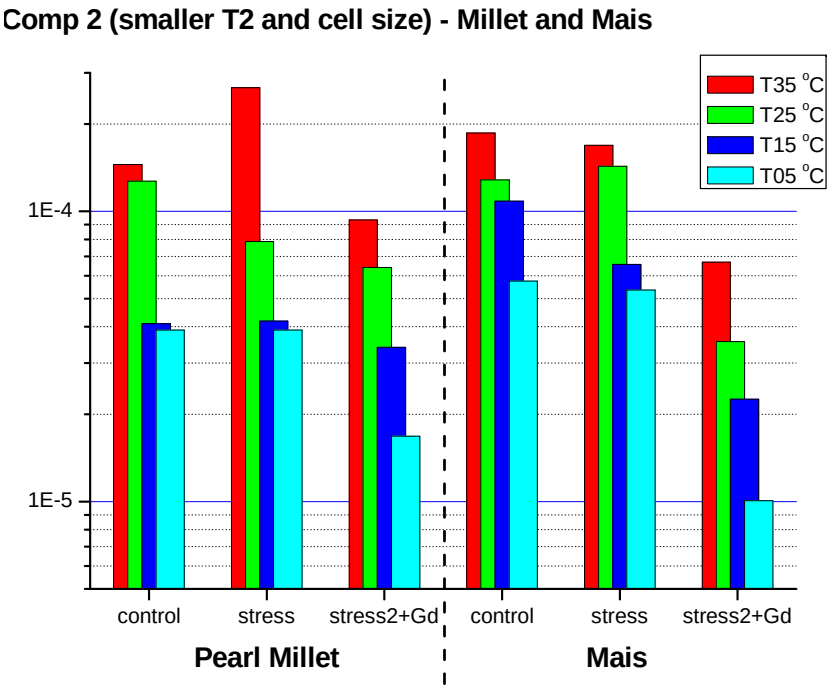
  age: 4~6 weeks.



Transport in roots: pathway and tissue







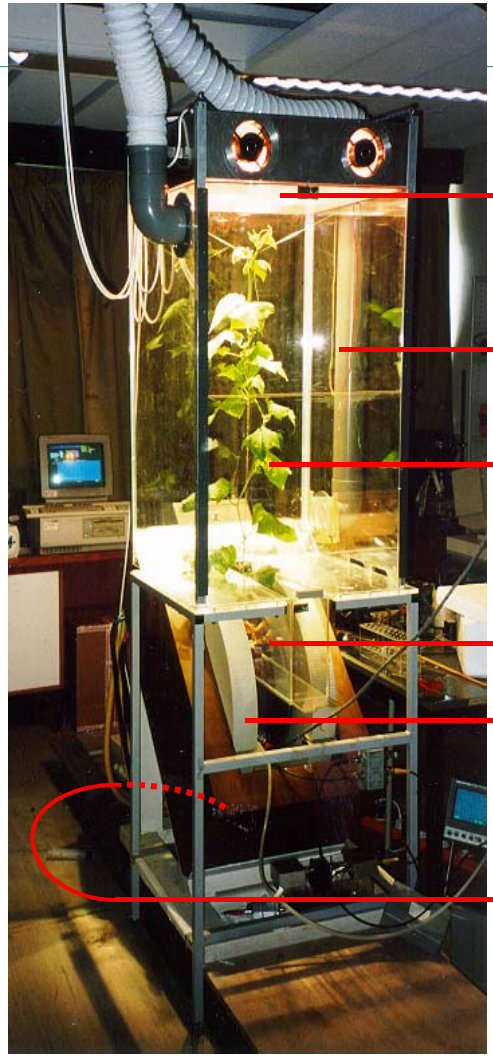
NMR / MRI and phenotyping

- Anatomy: leaf thickness, 3D root structure, thickness of different tissues, cell size, vessel diameter, compartments,...
- Internal parameters: water content in different tissues, transport and flow conducting area (xylem and phloem; sink-source connection), membrane or tissue permeability, starch content (leaves, beans), ...
- Static versus dynamics (growth and adaptation processes and rate)

MRI

- Lab bound, but … starting to become (trans)portable

Dedicated Plant MRI systems and climate control: 0.7 and 3T



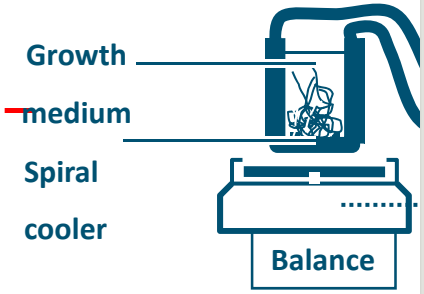
lights

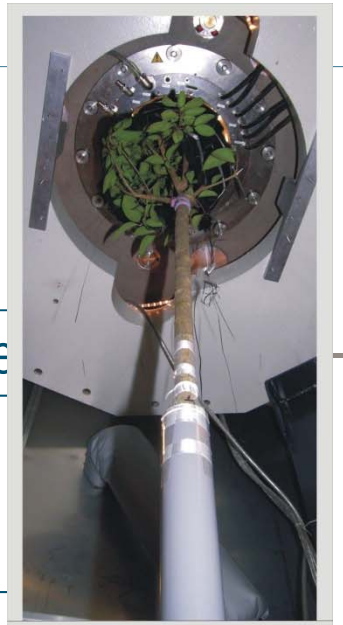
climate chambe

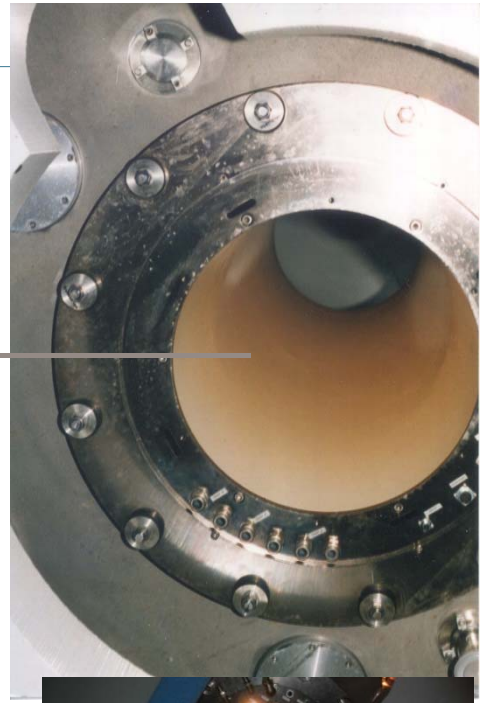
plant

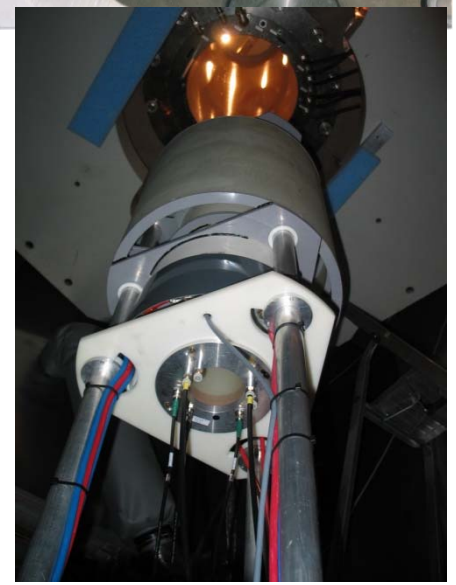
gradient probe

electromagnet



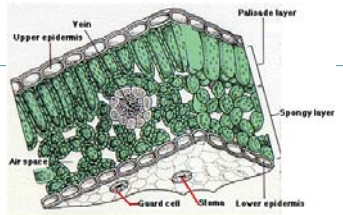


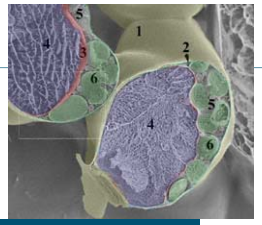


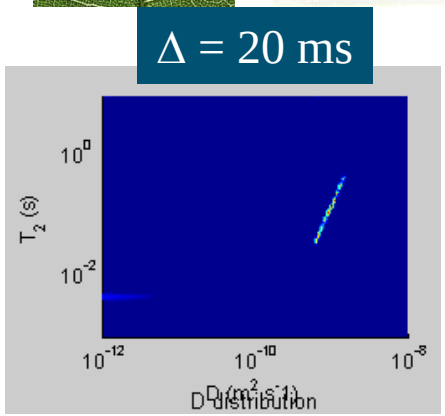


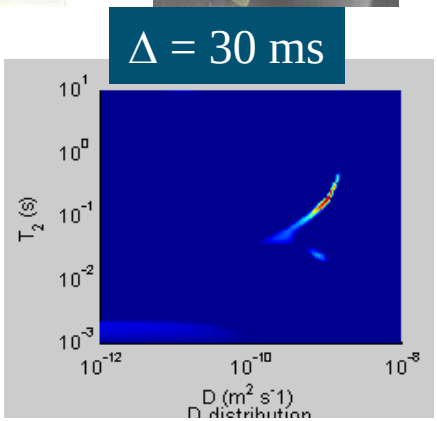
WAGENINGEN UR
For quality of life

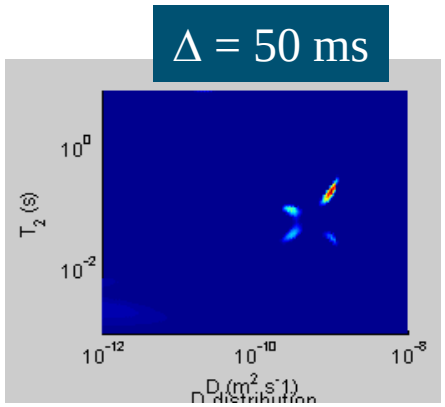
Chloroplasts, photosynthesis and single-sided portable NMR

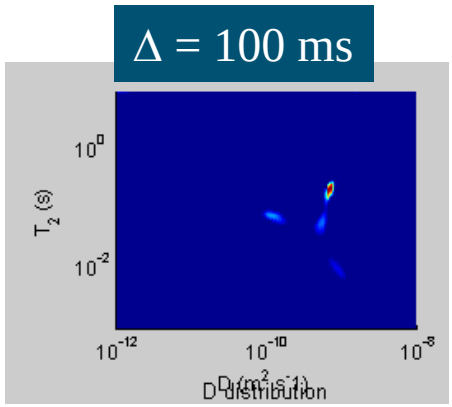


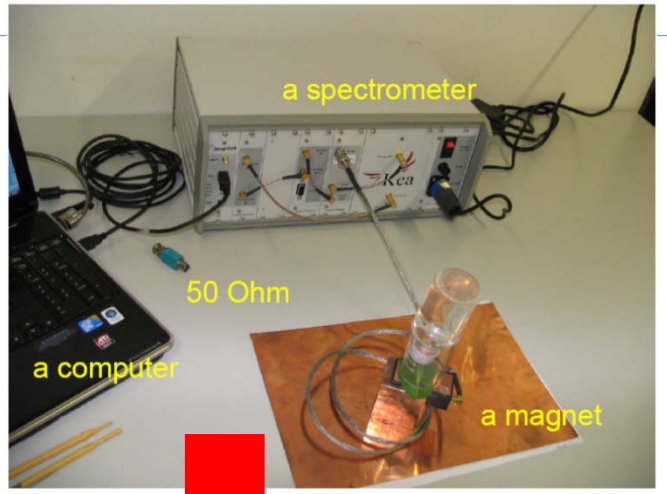


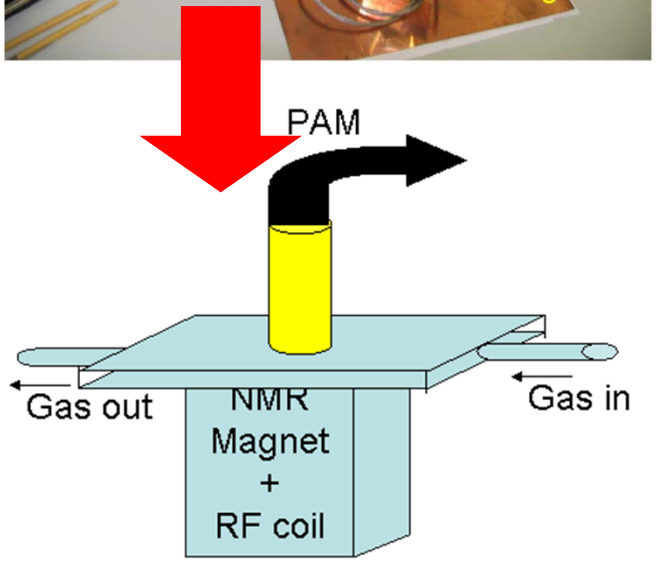












WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Rascher et al Func Plant Biol 2011

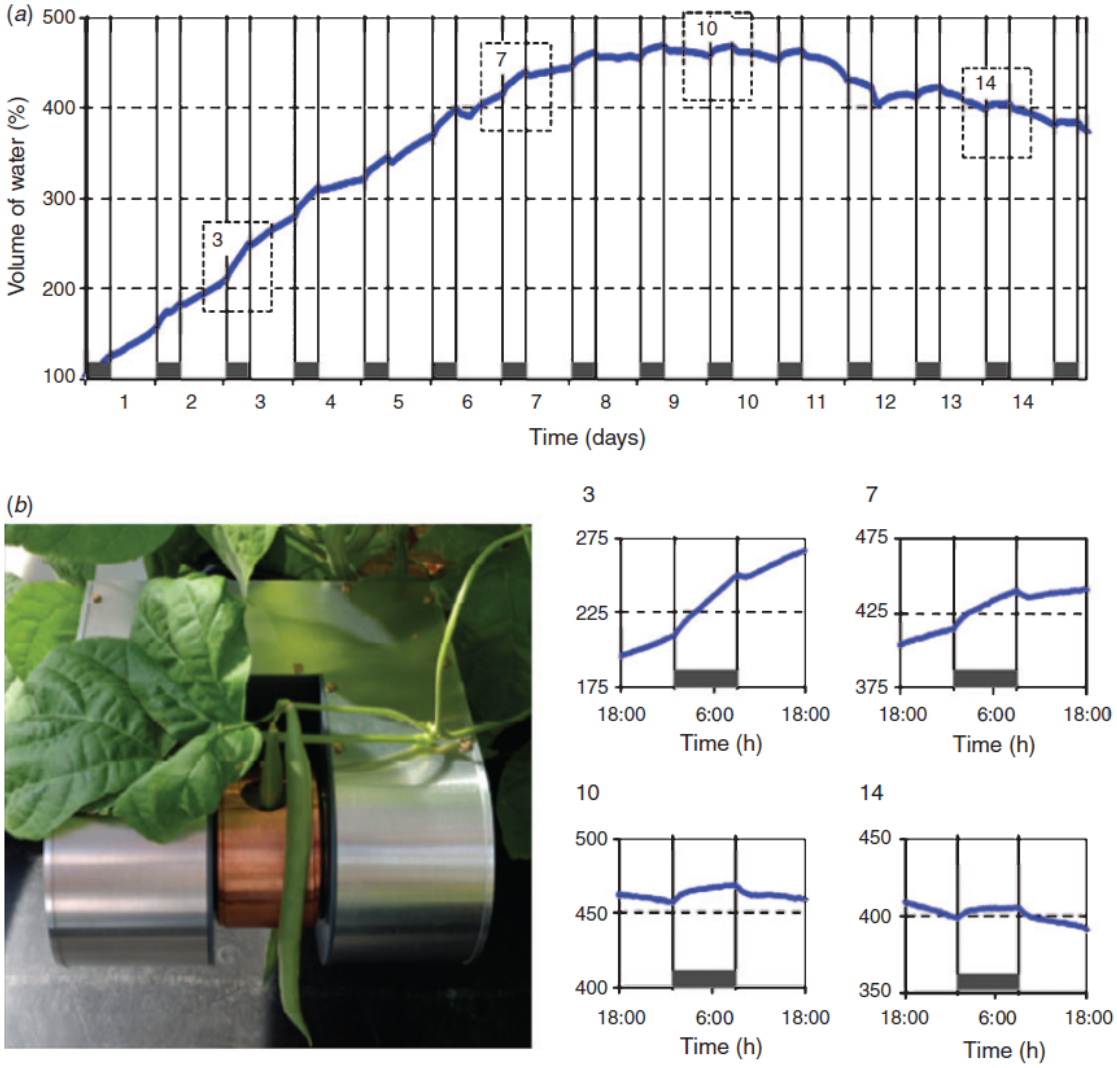


Fig. 4. (*a*) Quantifying shoot transport by portable nuclear magnetic resonance (NMR). Growth of a bean pod during a period of 2 weeks. Shown is the relative change in the volume of water in a 15 mm section in the middle of the bean pod, as placed within the coil of a custom built portable NMR scanner. The bean pod was 7 days old at the start of the measurement. Four day-night-day transitions are shown in detail, at night number 3, 7, 10 and 14 (indicated with dashed lines in the main graph). The night periods are indicated with dark grey bars. (*b*) Image of the C-shaped NMR magnet is shown, fitted with a sample holder containing a bean pod. During pod growth the environmental conditions were as follows (climate chamber): day 23°C, RH 65%, 300 µE, 16 h; night 23°C, 65% RH, 8 h. NMR hardware and settings: custom built portable C-shaped magnet, 10.35 MHz, air gap 30 mm, temperature kept constant at 25°C ± 0.1°C by means of an electronically controlled heater. The magnet was insulated to protect it against sudden temperature changes. For the RF coil a solenoid with an inner diameter of 15 mm and 13 windings was used. Magnet and coil were used in conjunction with a Magritek Kea 2 spectrometer with integrated RF amplifier. For each cycle a CPMG type measurement was run, with a repetition time of 7.5 s, 3000 echoes, eight complex points per echo, 32 averages and a spectral width of 100 KHz; total scan time for each point was 4 min.

WAGENINGEN UR
For quality of life

Mobile MRI: Kimura *et al*, 2011

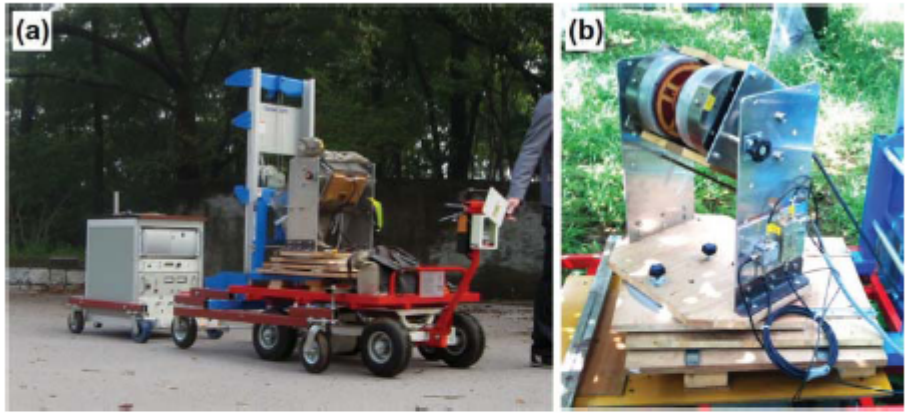
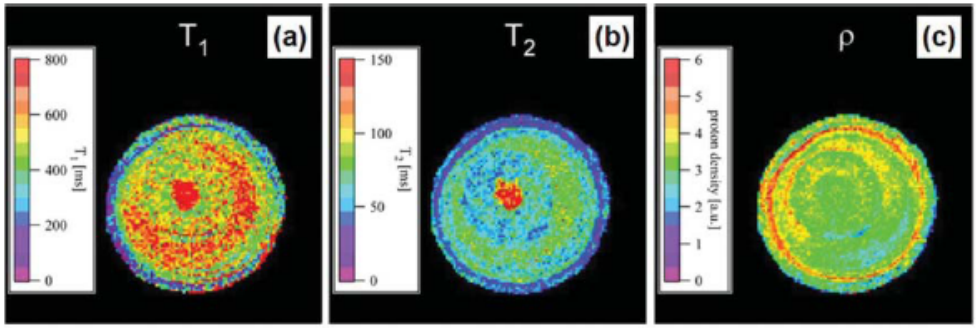


FIG. 1. (Color online) (a) Overview of the electrically motorized mobile MRI system using a permanent magnet. (b) Close view of the permanent magnet with two rotation axes and two horizontally sliding tables. The rotation axes are vertical and horizontal axes.



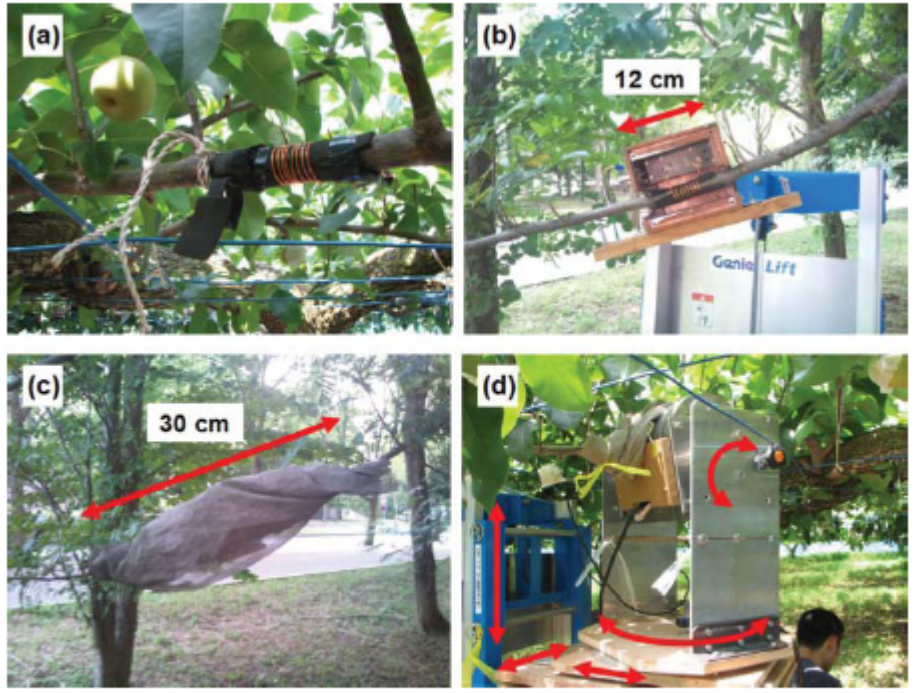
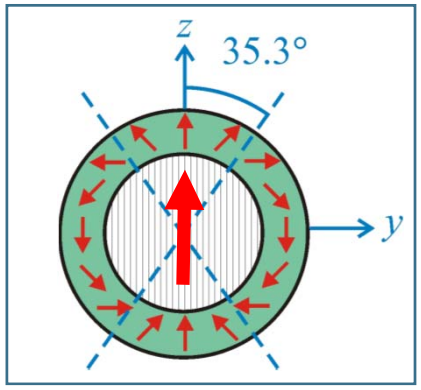


FIG. 2. (Color online) (a) RF coil wound around a branch of a pear tree. (b) Open view of the RF probe. (c) Electromagnetically shielded RF probe with a shielding cloth wound over the RF probe. (d) Setup of the RF probe in the gap of the permanent magnet. The magnet has two rotation axes and three translational mechanisms.

Halbach Tree scanner

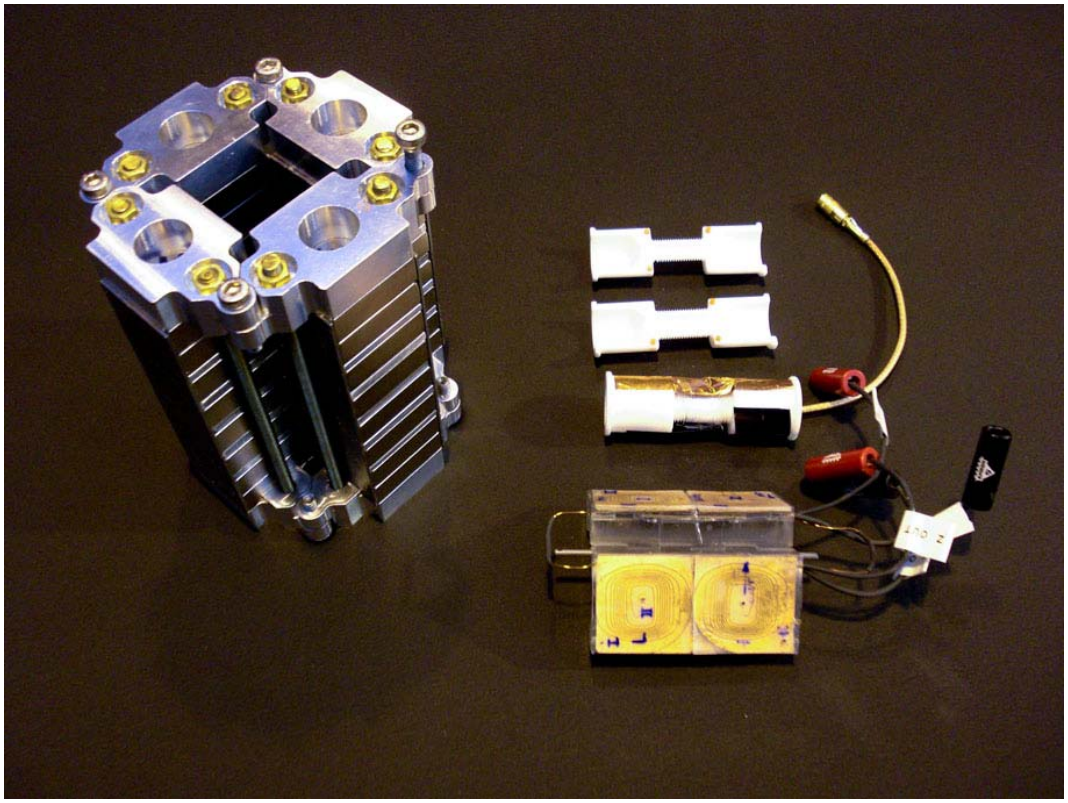


FZJuelich: Blumler et al.

WAGENINGEN UR
For quality of life

WAGENINGEN CENTRE

Hinged Halbach (NMR-CUFF) and U-shape magnet



Windt *et al*, JMR 2011
Rascher *et al*, 2012

WAGENINGEN UR
For quality of life

WAGENINGEN NMR CENTRE

Tree Hugger

- An open access transportable 1.1 MHz ^{1}H MRI system for the *in situ* analysis of living trees in the forest. Access up to 21 cm. Magnet: 55 kg.

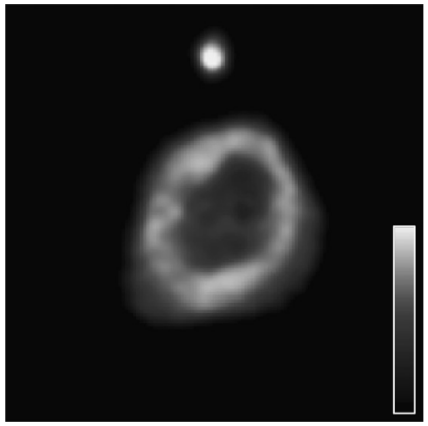


Fig. 3. Cross section image of the bird cherry tree, recorded September 2011. The white dot towards the top of the image is the reference tube of water.

Jones *et al*, JMR 218: 133-40 (2012)

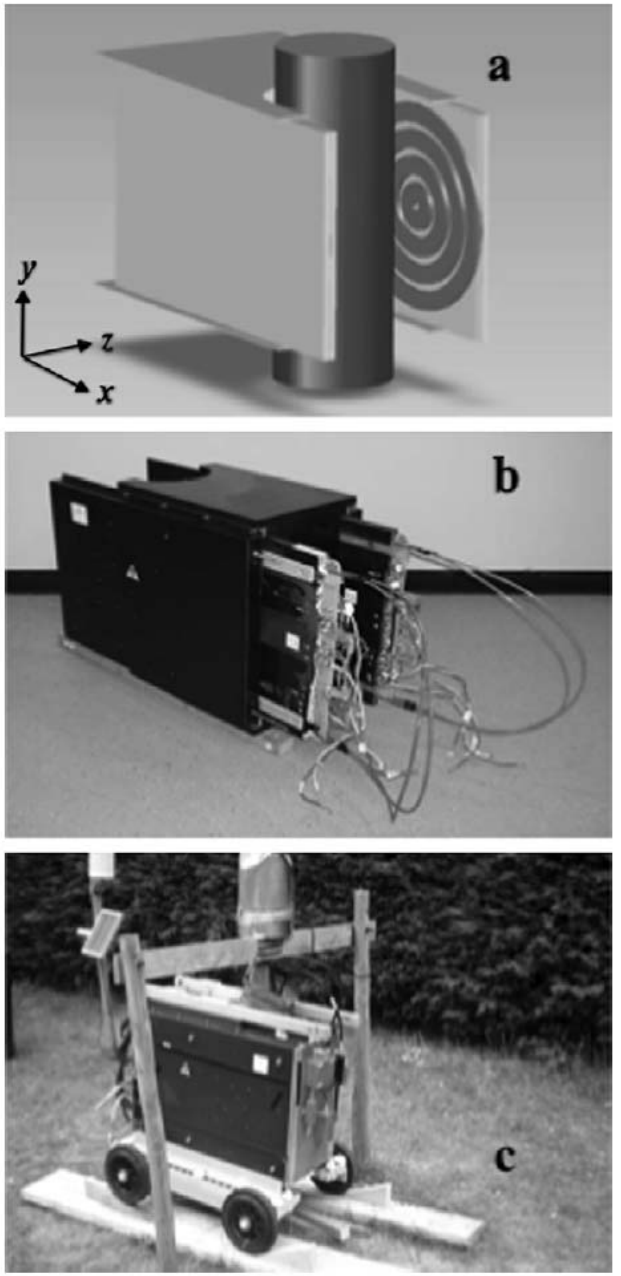


Fig. 1. a: Schematic of magnet design showing open access frame and concentric ring design (4 of 5 rings shown) of magnet with a mock tree between the poles; b: magnet as built with the gradient plates pulled half way out and c: magnet around a tree at Forest Research, Farnham, UK.

WAGENINGEN UR
For quality of life

Conclusions

- Large number of parameters, some quite unique, is available by both NMR and MRI.
- The only systematic application (to my best knowledge) is MRI of 3D root anatomy (24 plants/day, can be increased to max 50 plants per day)
- There is a strong potential, but protocols and hardware have to be further developed
- This can only be realized via pilots combining NMR/MRIsts and plant scientists

Acknowledgements:

Carel Windt

Natalia Homan

Frank Vergeldt

Edo Gerkema, John Philippi

Alena Prusova

Louise van der Weerd, Tom Scheenen, Timur Sibgatullin

NWO, LNV, WUR, SENTER, EU

WAGENINGEN UR
For quality of life

Space for partner logo's

(place a white shape behind the logo's to hide this text and border)