Water relations of *Robinia pseudoacacia*:

Do vessels cavitate and refill every day/night or are R-shaped vulnerability curves wrong?

Authors

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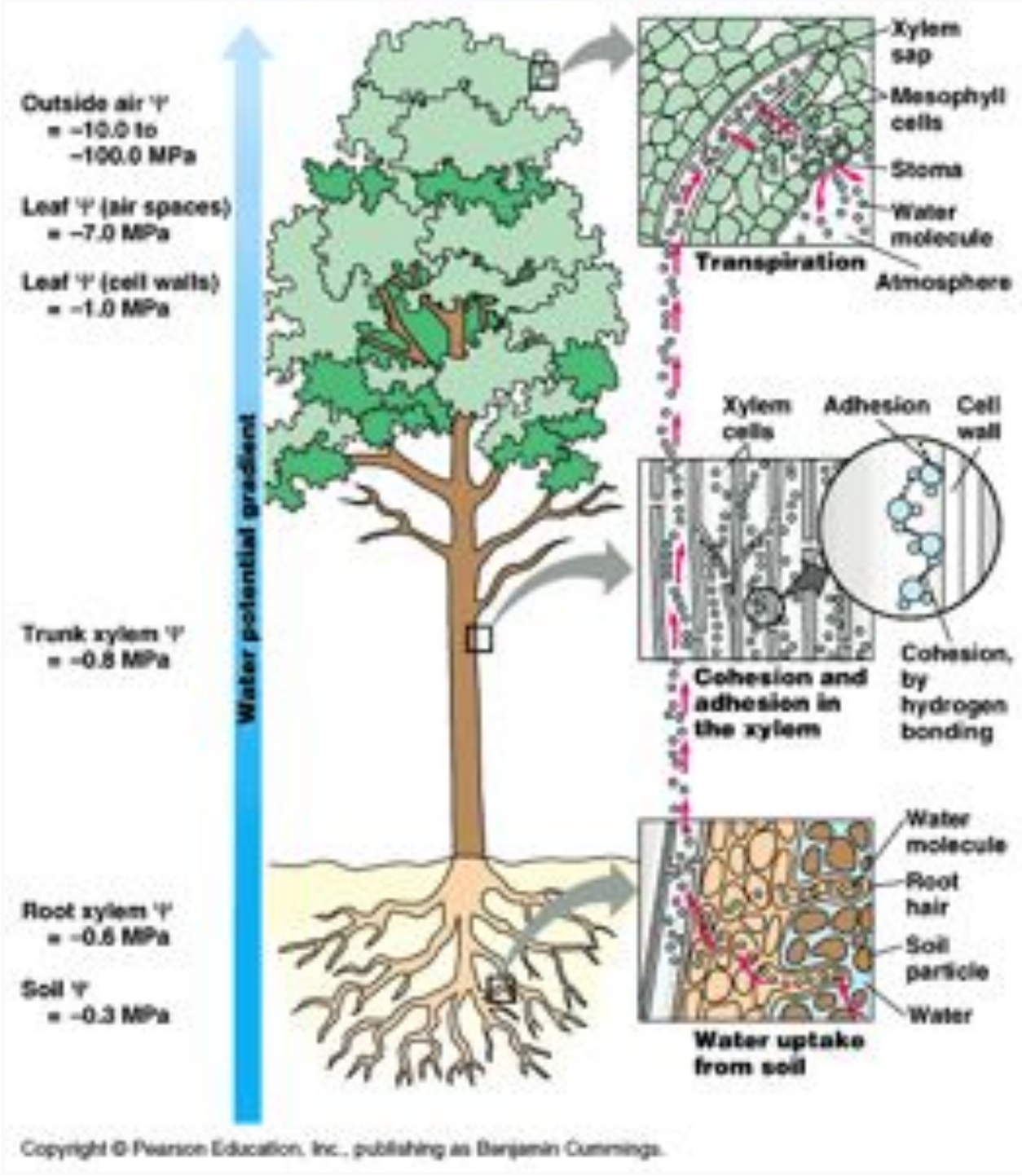
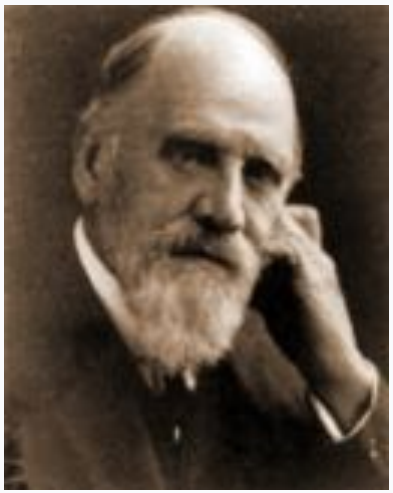
Preservation of the water conducting system of plants is important:

- Water transport is an essential part of photosynthesis (~600 H_2O's evaporate for every CO_2 fixed).
- Hydraulic efficiency correlates with CO_2 fixation rate.
- Water stress will cause embolism and cavitation that will reduce hydraulic efficiency.
- Refilling of embolized vessels with water is important in the species that can refill, because refilling vessels requires less carbon than growing replacement vessels.



Röntgen 1892
Cohesion-Tension Theory by HH Dixon 1896

Francis Darwin, President Royal Society, listens



Cohesion Tension Theory

+

Soil Plant Continuum model

Predicts that: $T\downarrow x = R\downarrow x\, F\downarrow x - \Psi\downarrow soil = R\downarrow x\, Ag\downarrow l$ $\Psi X - \Psi\downarrow soil$

- Xylem tension (T_x) will increase with increasing water flow, F_x, thru plants
- Water under tension is meta-stable (unstable) and prone to breakdown resulting in…
- Cavitation & embolism (air bubbles in vessels)…
- Which reduces the ability of stems to conduct water (reduces hydraulic conductivity) and plant responds by reducing g_l

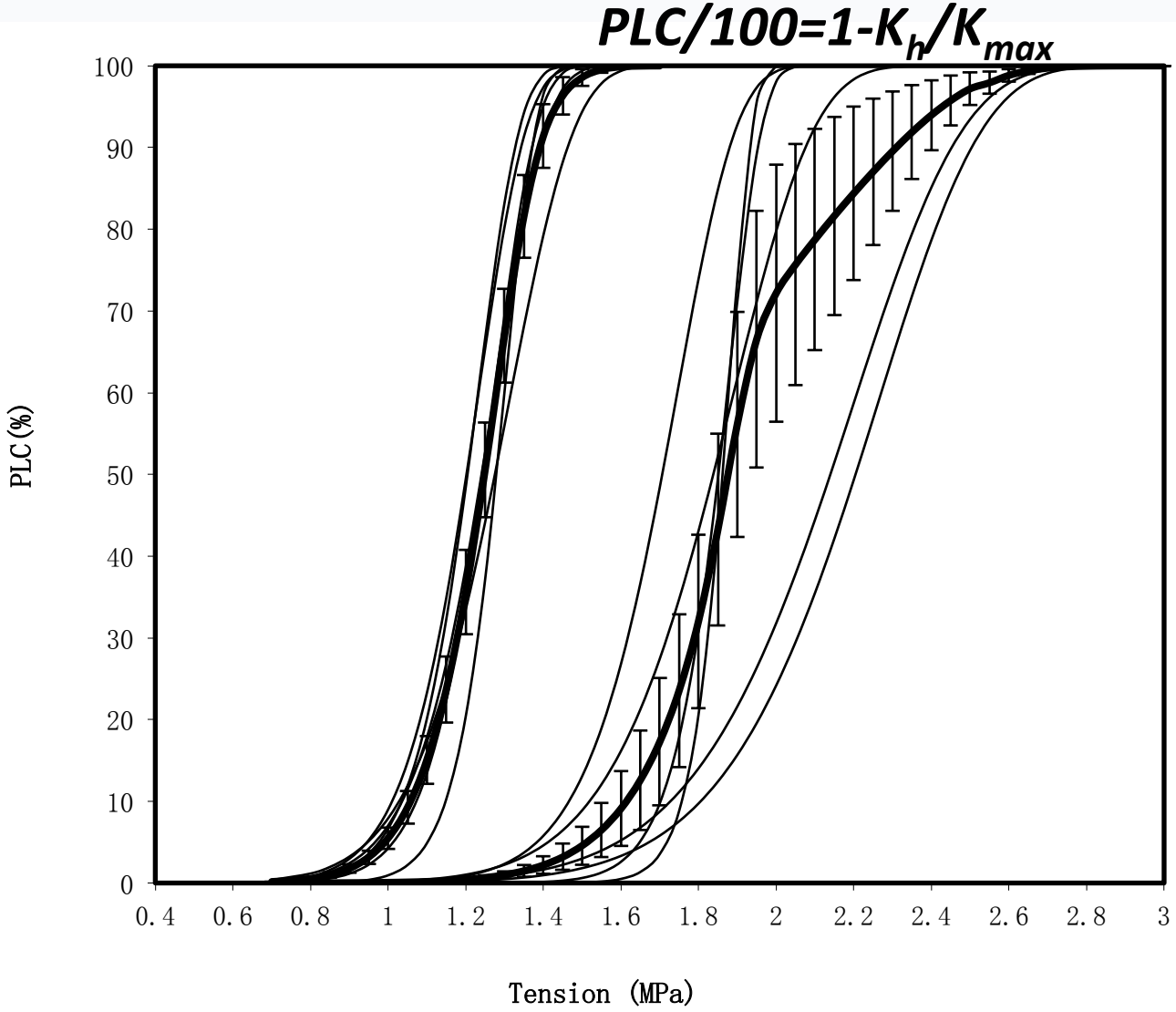
Vulnerability curves (VCs) measure the resistance to cavitation

Measured by 3 methods:

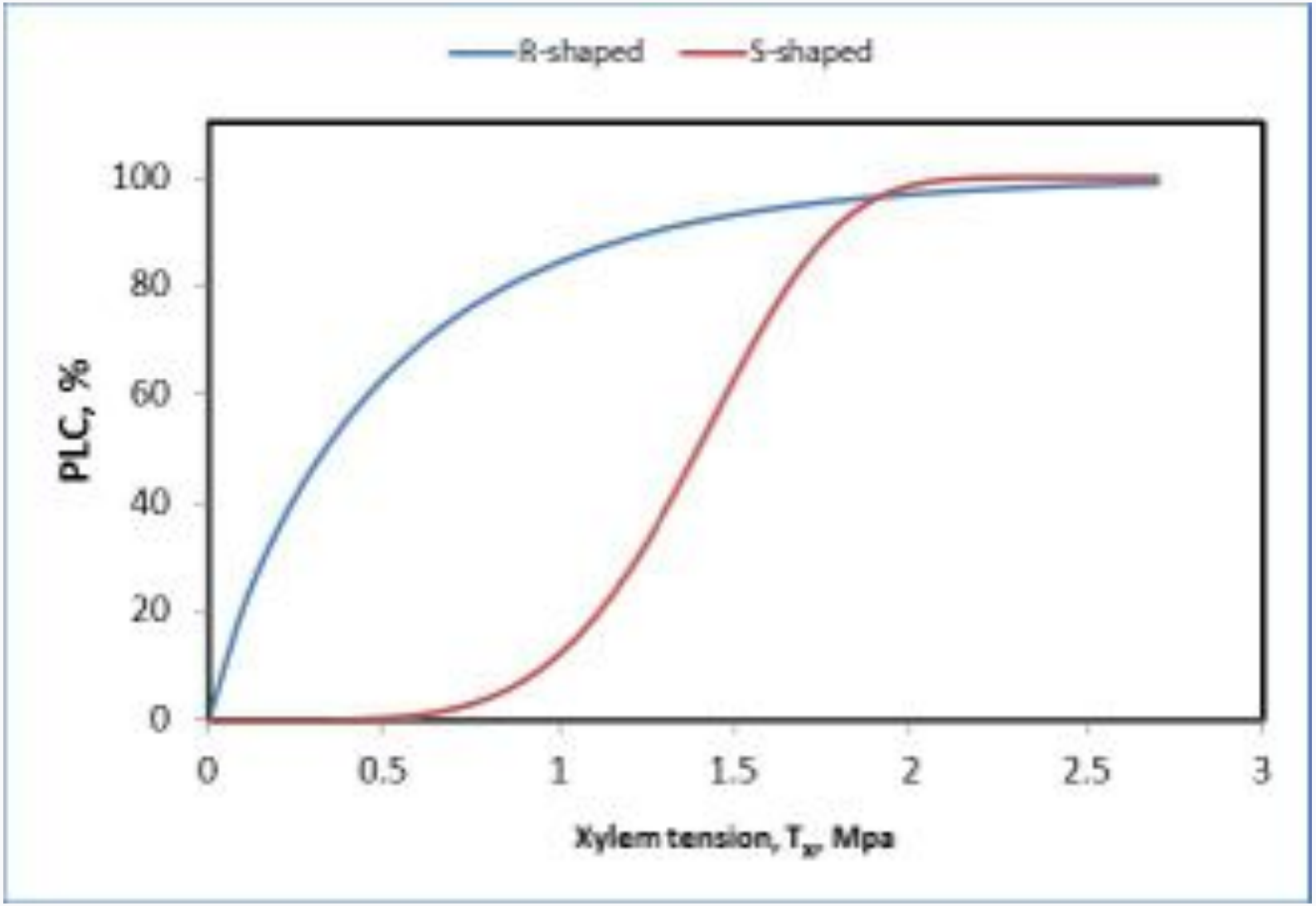
- Bench top dehydration (oldest 'gold standard') but slow!
- Air injection method (faster but sometimes prone to errors).
- Centrifugation method (fastest but also prone to errors).

Examples of S-shapped VCs

Dr. CAI Jing
NWAFU



Examples of VCs S- vs R-shaped
PLC = loss of hydraulic conductivity



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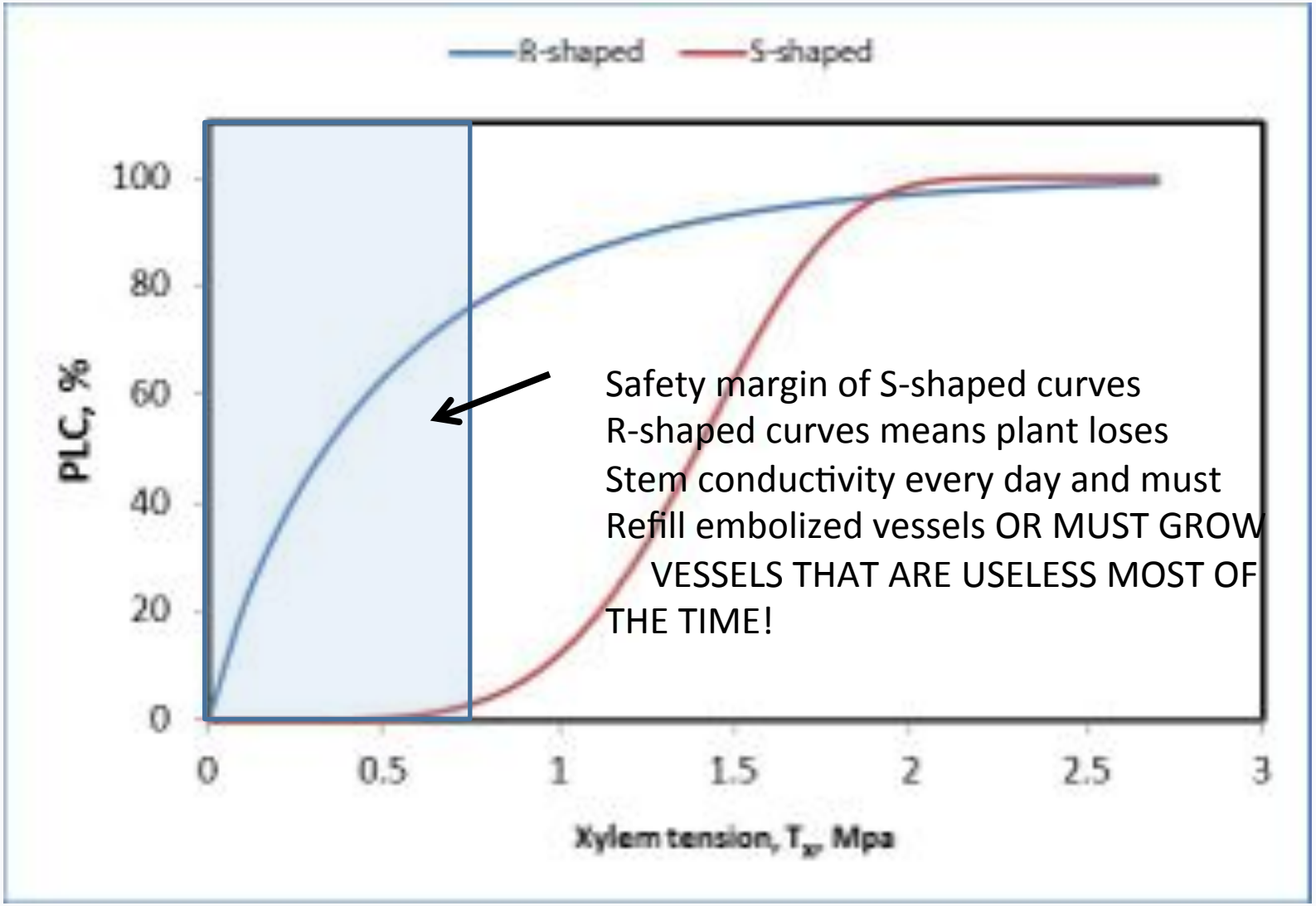


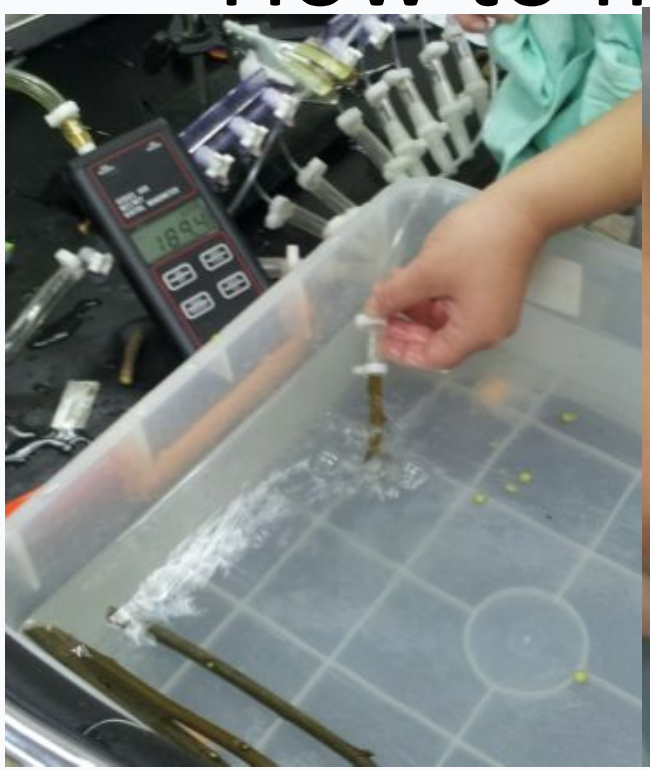
Refilling ability is rare but reported for

- *Vitis* (grape many labs including Harvard)
- *Laurus* (University of Triest, Italy)
- *Acer & Fraxinus* (Harvard)
- *Quercus* (Univ. Utah)
- *Robinia* (NWAFU) and other species.

There has been a lot of debate in recent years!

- Harvard has reported method problems in determining when stems embolize & refill
- INRA (France) & Univ Utah have reported problems of Centrifuge methods when measuring stems with long vessels (longer than ½ sample length used in Centrifuge). **SO**
- PURPOSE OF THIS PAPER IS TO RE-EXAMINE EVIDENCE FOR *Robinia*

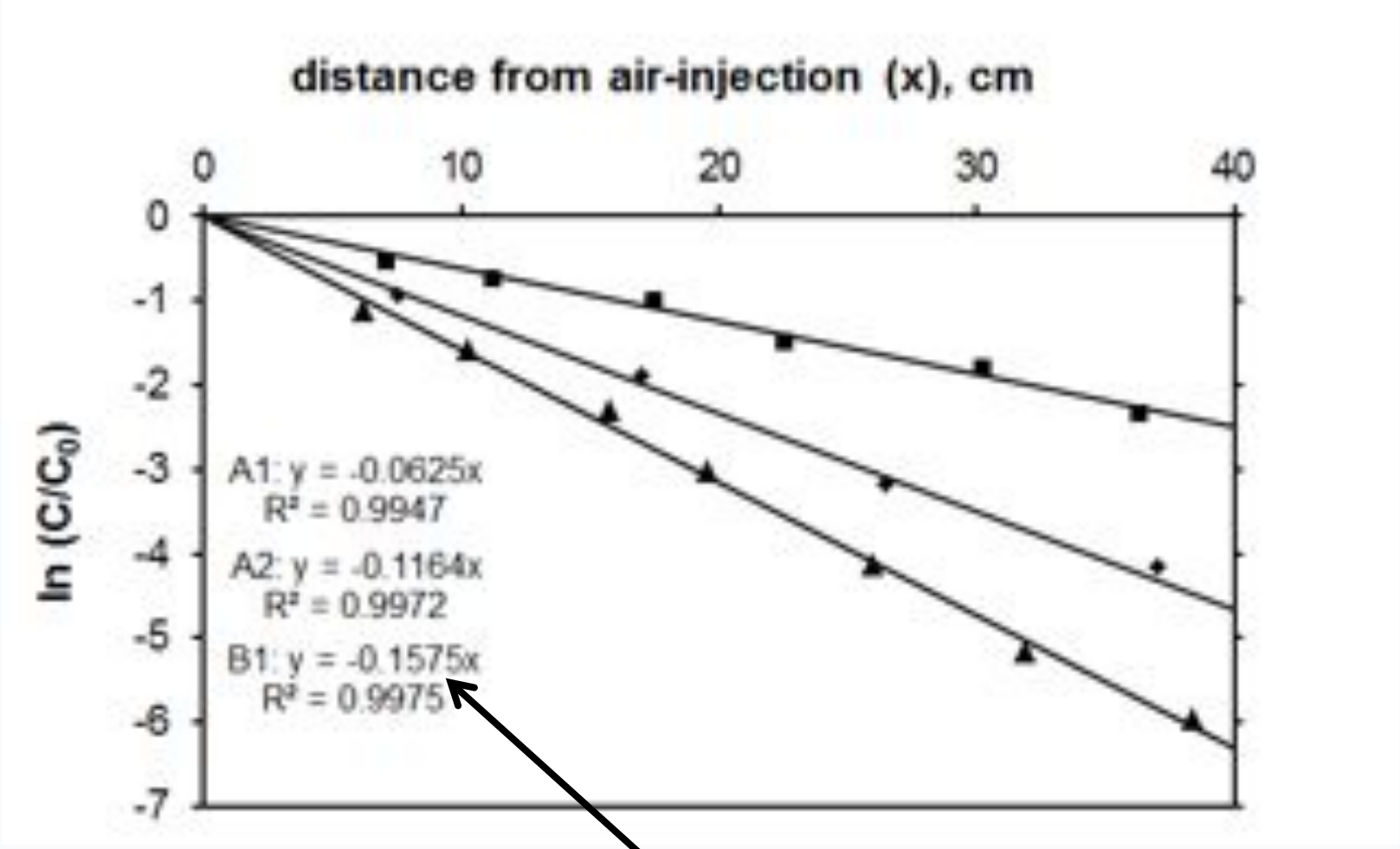
How to measure vessel length



Vessels are often “short” to prevent air from passing between them! Water passes through pit membranes between vessels but air bubbles cannot. So vessel length is measured by cutting stems progressively shorter (cutting open more vessels) and measuring air flow thru cut-open vessels.

Theory of analysis

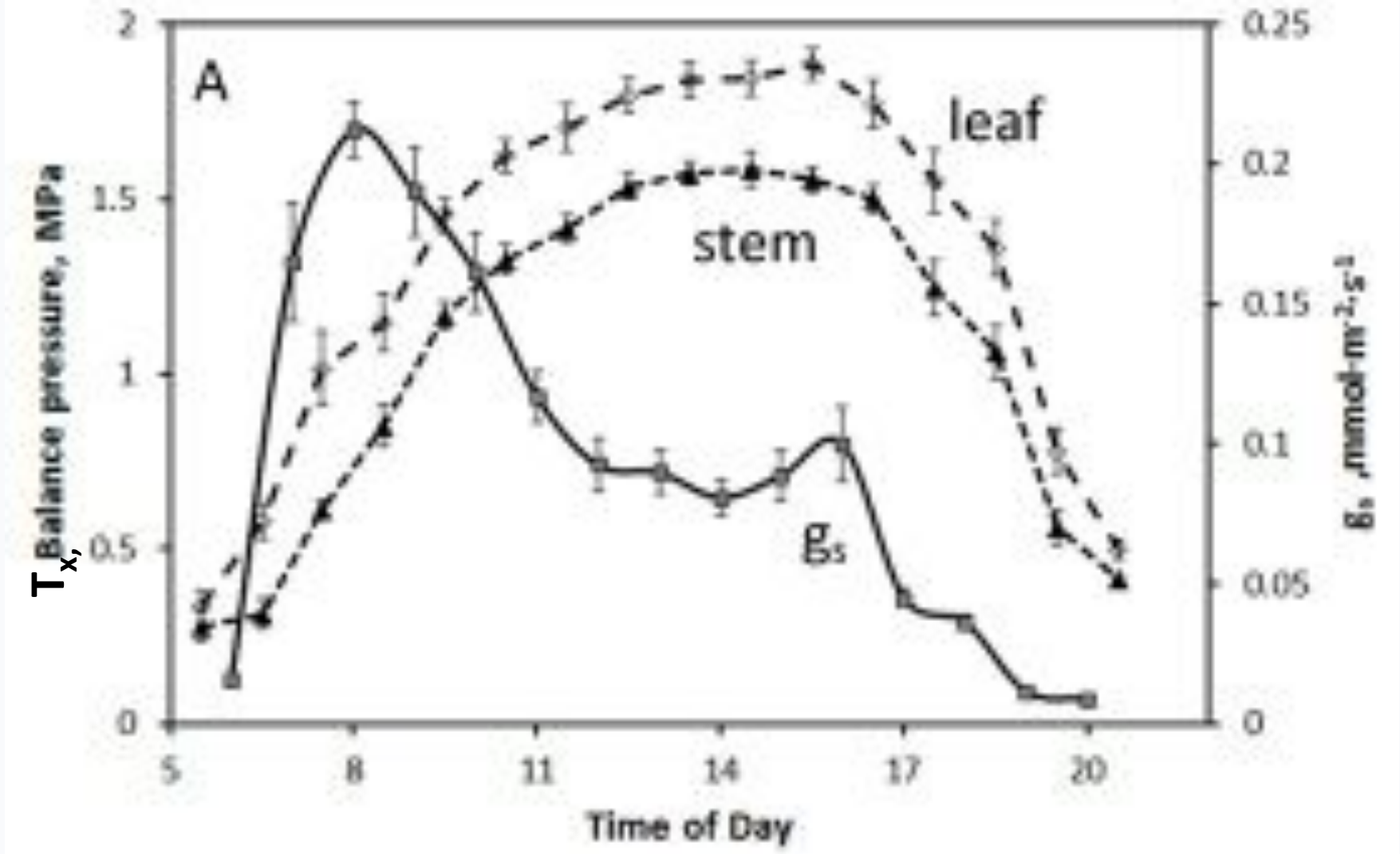
Cohen, Bennink & Tyree 2003 **Journal of Experimental Botany, 54(389):1889-1897**



Mean vessel length = -2/slope

***Robinia* mean = 20 cm. Range of means 5 to 30 between stems**

Daily water relations
xylem tension, T_x & stomatal conductance



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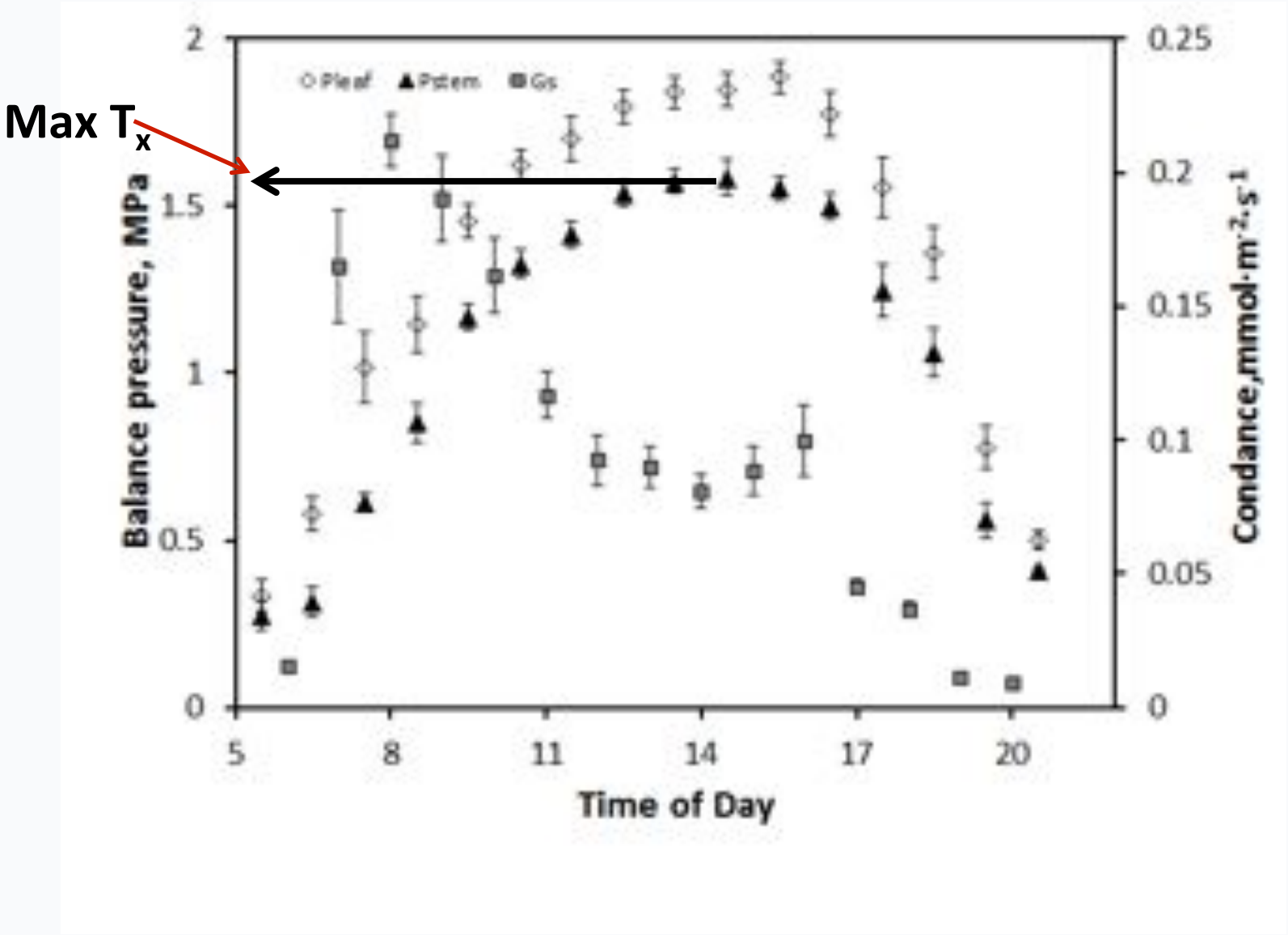
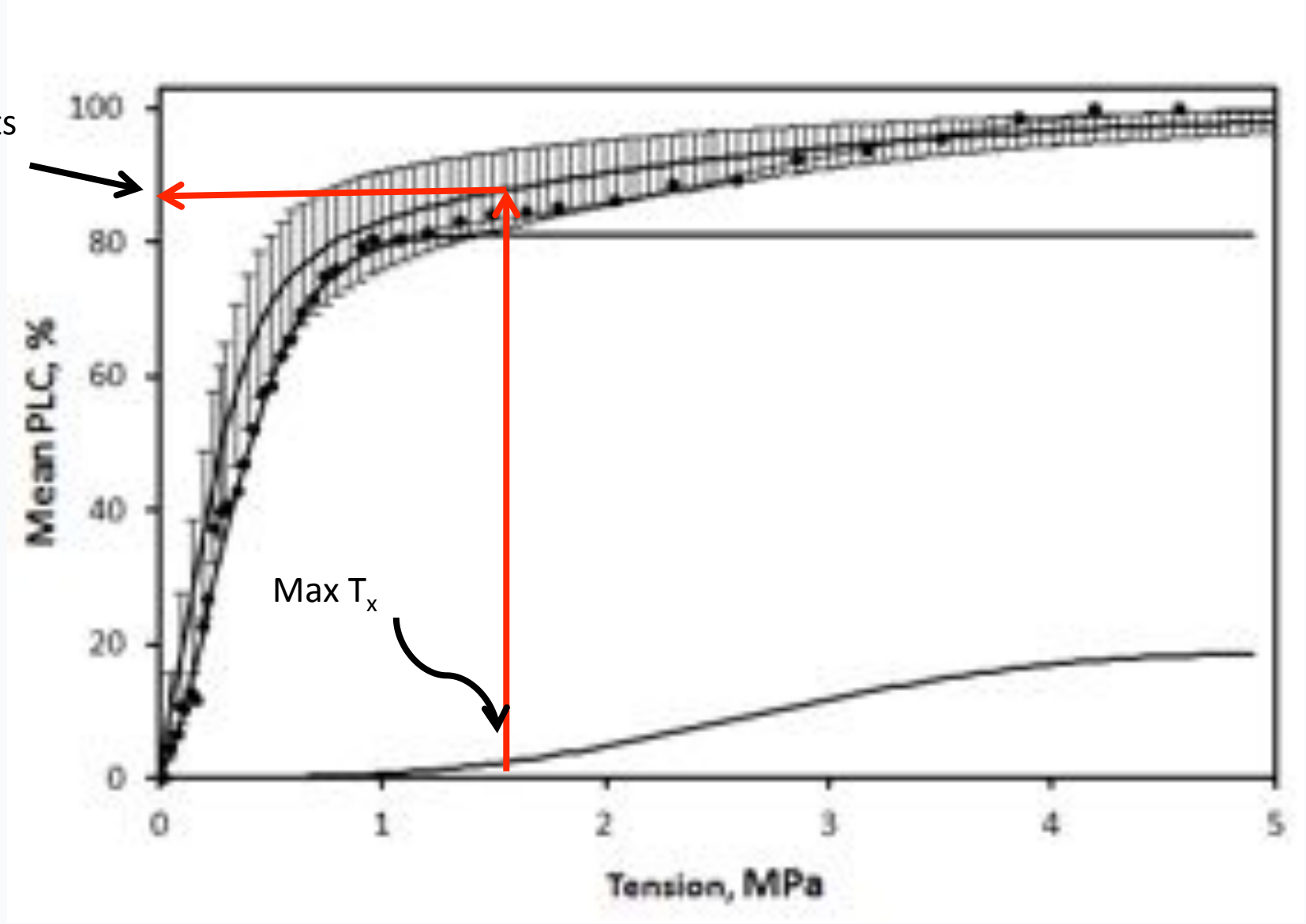


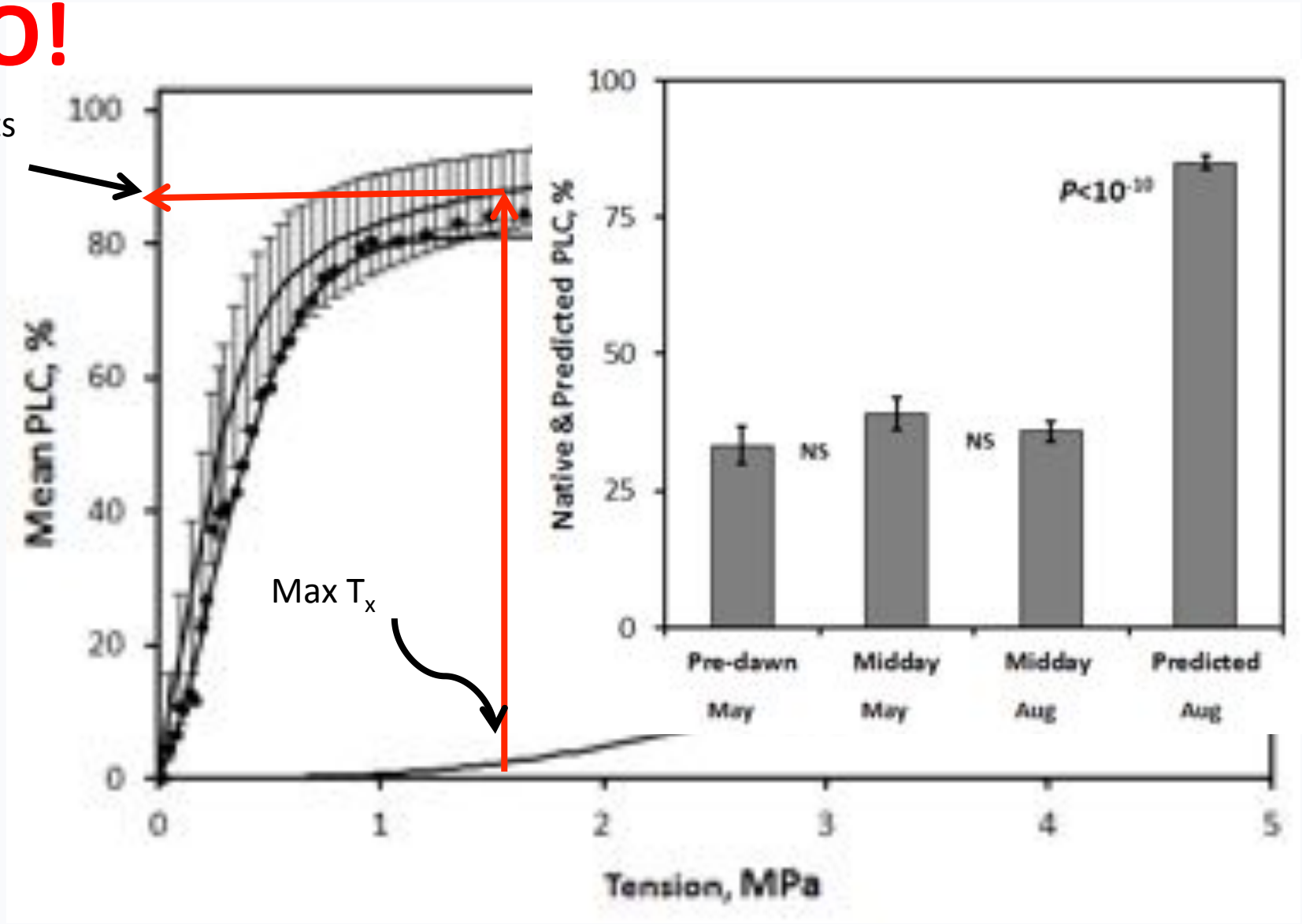
Robinia VC measured in centrifuge



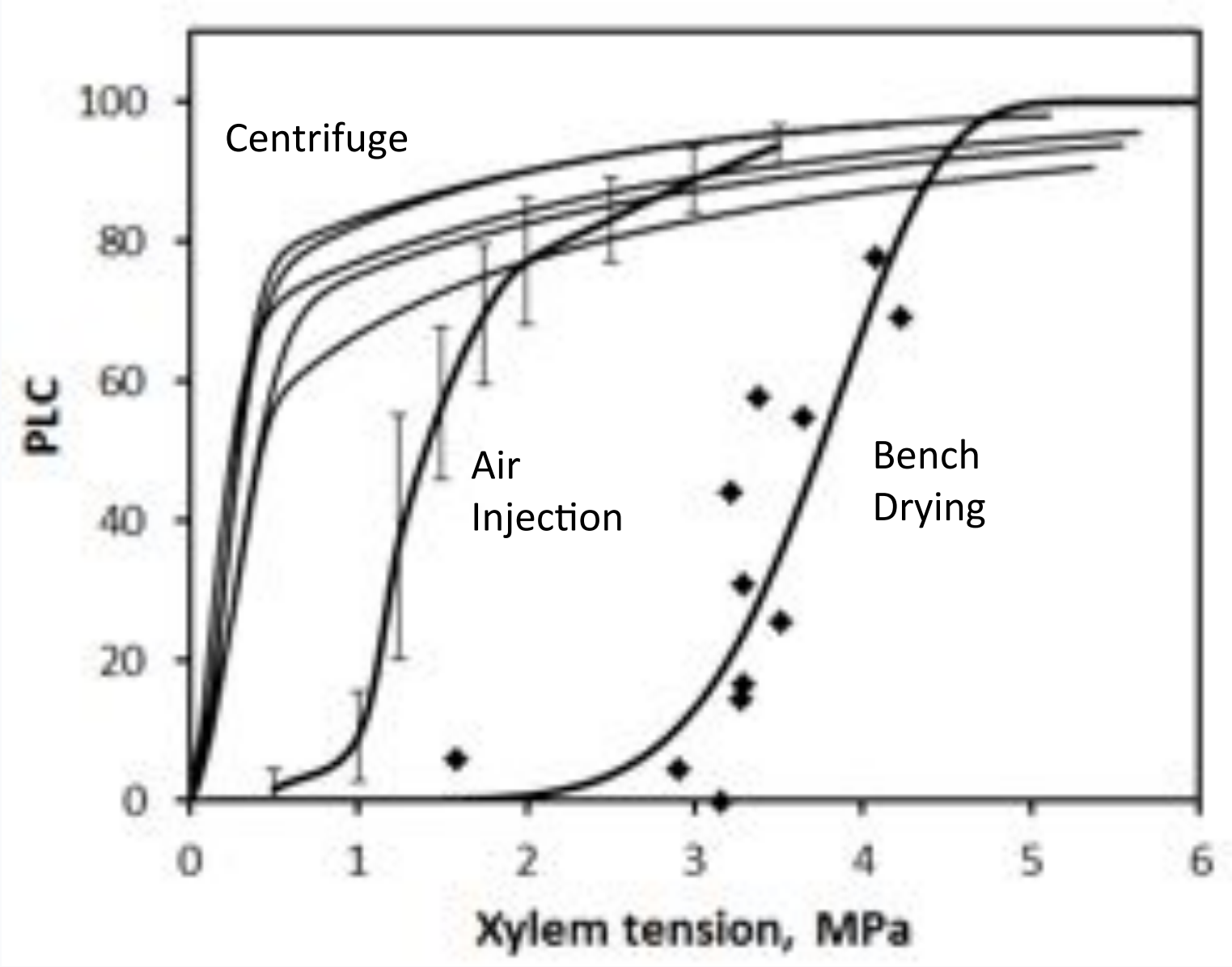
VC measured in centrifuge

NO!

Predicts Daily PLC?



What do other methods yield for VCs?



Air injection method known to have artifact caused by vessel length

Ennajeh…Cochard et al 2011 *Trees*, *25*(4), 705-710

When vessel longer than injection chamber then S-curve shifted to left.

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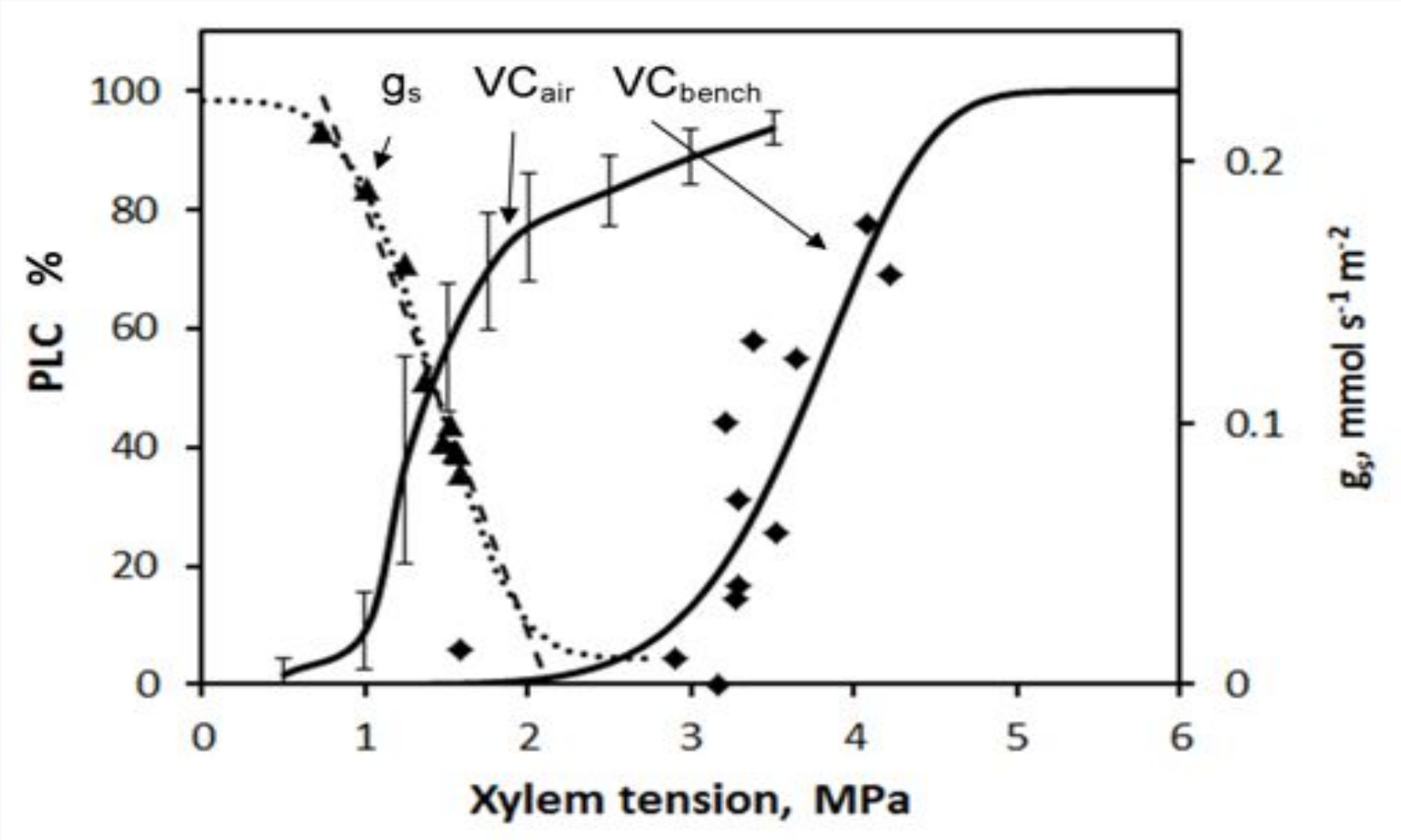
What other reasons for rejecting the air injection method are there?

Stomatal regulation and the conservative plant

$$T_x = R_x F_x - \psi_{soil} = R_x Ag_l X - \psi_{soil}$$

$$g_l = g_s + g_{cuticle}$$



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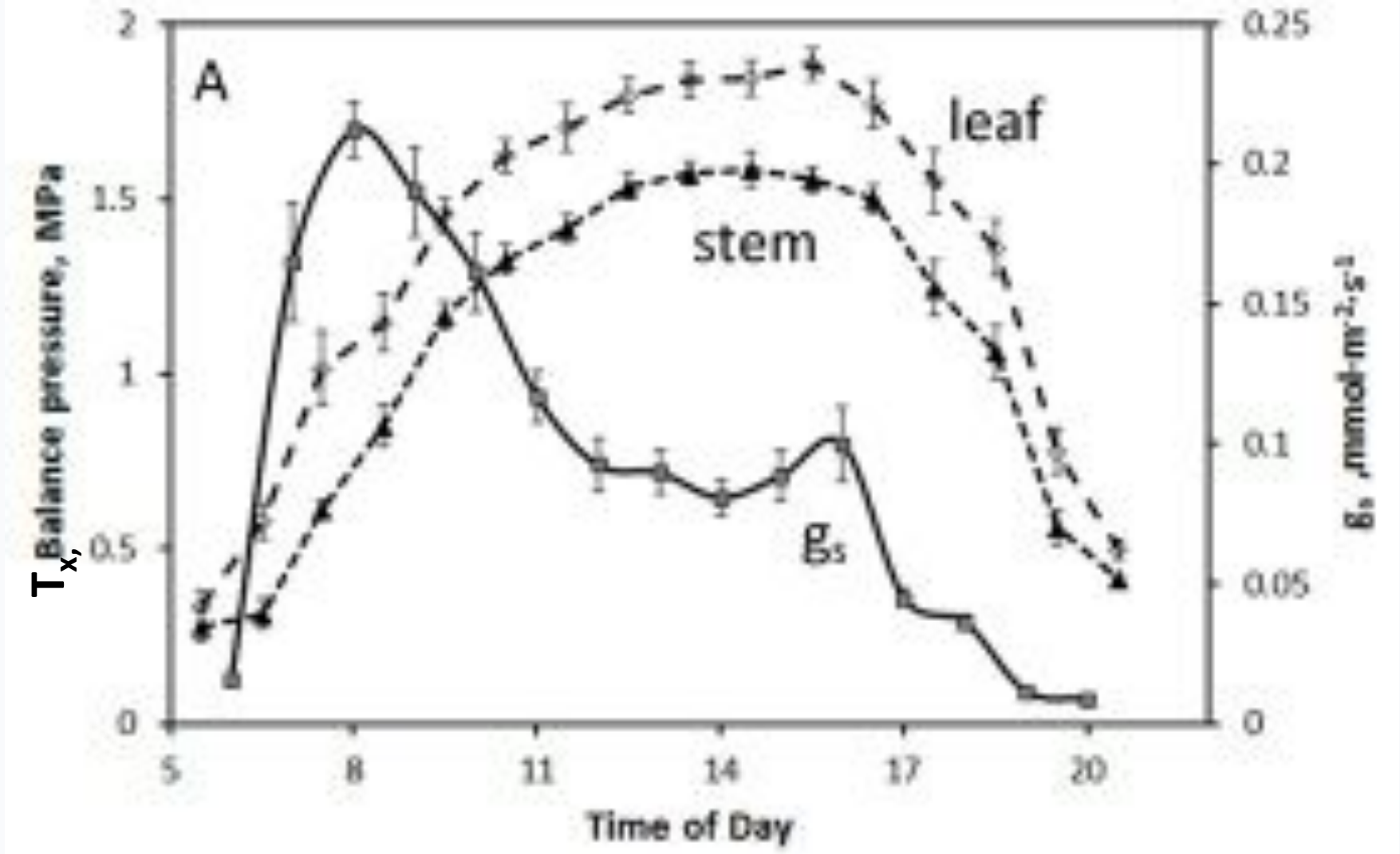

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Cochard Cavitron

modern way of detecting cavitation

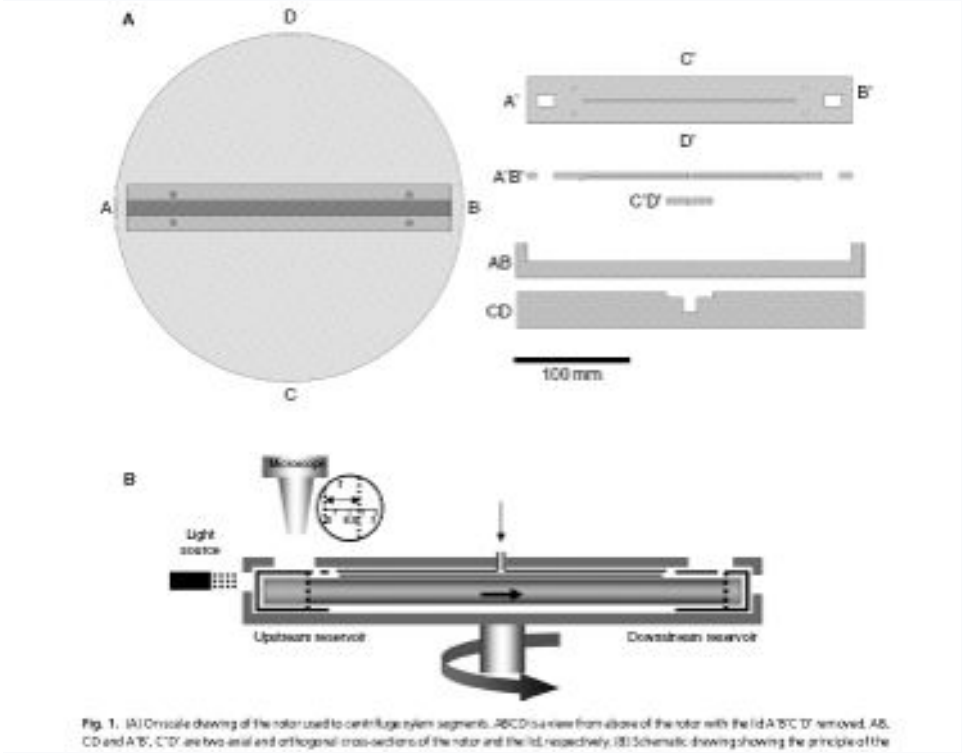

Fig. 1. (A) Cross-sectional drawing of the rotor used to centrifuge xylem segments. ABCD is a view from above of the rotor with the lid A'B'C'D' removed. AB, CD and A'B', C'D' are two axial and orthogonal cross-sections of the rotor and the lid, respectively. (B) Schematic drawing showing the principle of the

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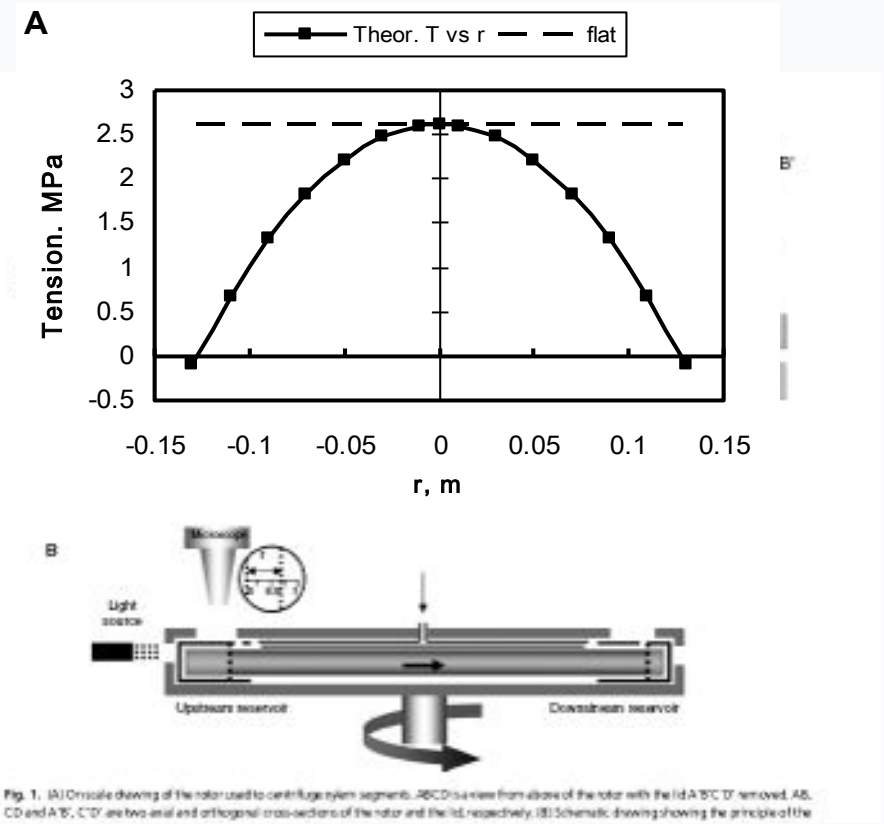

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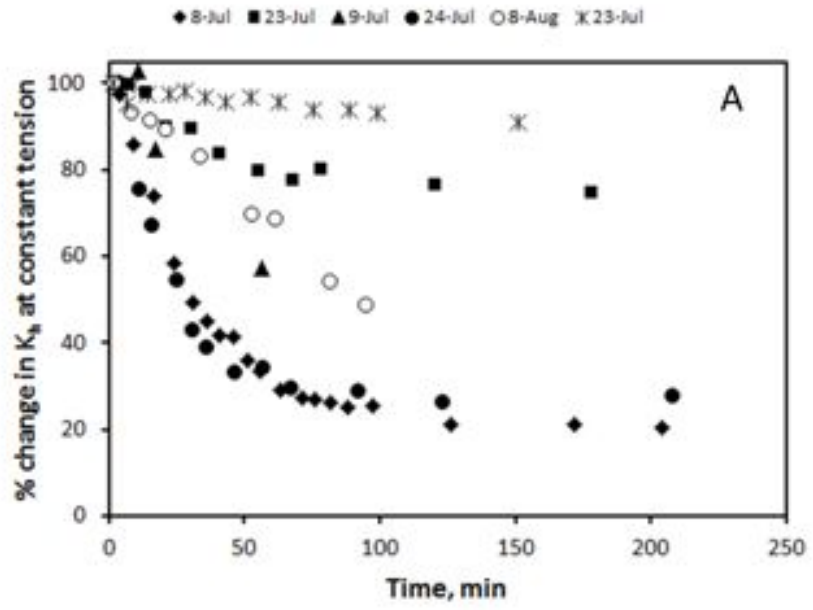
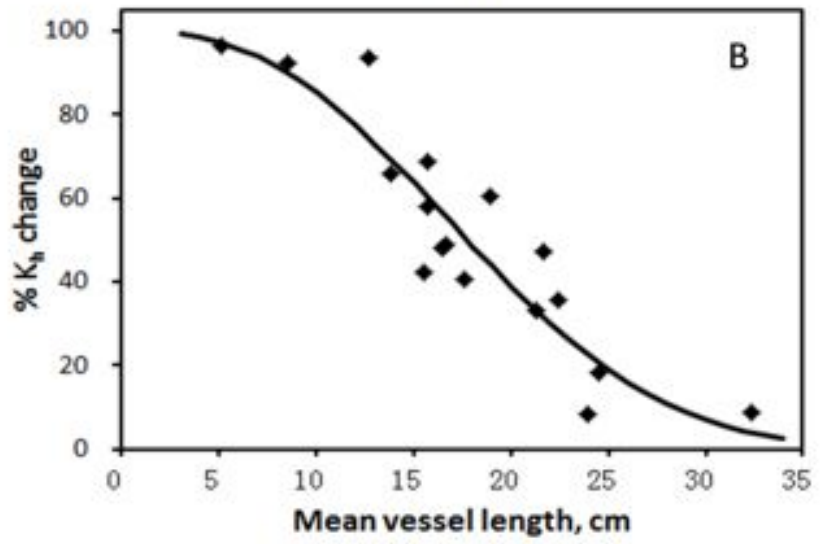
But what is happening in the centrifuge to cause embolism too soon?

HYPOTHESIS: Maybe due to bubble created during water injection into cuvette?

- In a centrifuge the most tension is near the axis of rotation with very small tension at the ends
- If bubbles injected into stems with short vessels then bubbles are trapped near the base of the stem where they will not expand
- But in a stem with long vessels the bubbles will travel toward the center and expand to block flow and cause loss of conductivity
- THE TEST: SO EVEN IF WE DO REPEATED MEASUREMENTS AT LOW TENSION THE AMOUNT OF EMBOLISM SHOULD INCREASE WITH TIME

TEST OF HYPOTHESIS



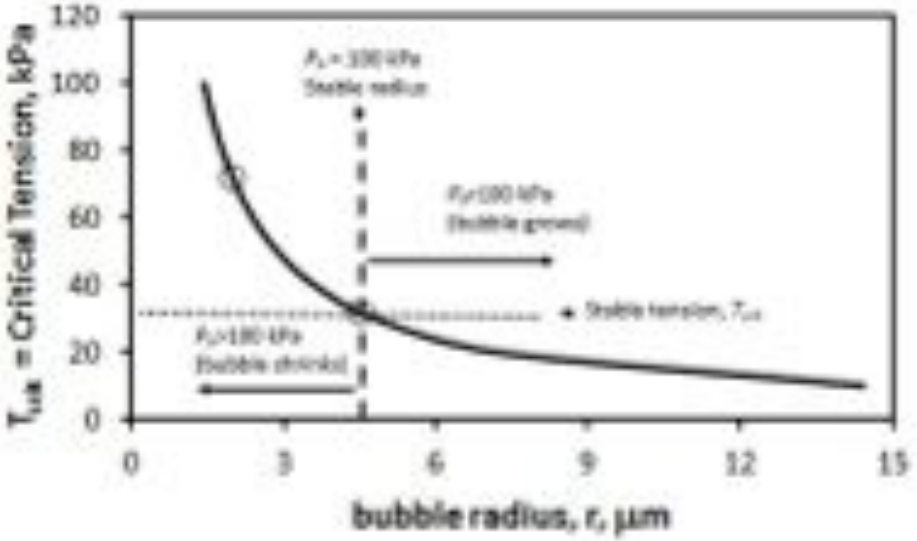


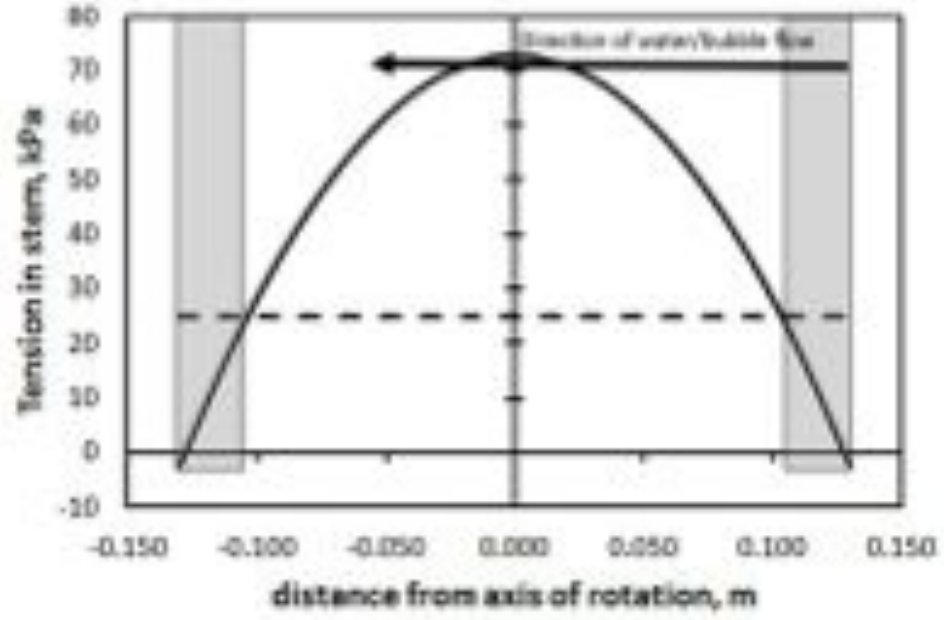
SHOWS THAT LOSS OF HYRAULIC CONDUCTIVITY IS A FUNCTION OF TIME AT LOW TENSION 0.03 MPa

SHOWS THAT LOSS OF CONDUCTIVITY IS MORE IN 4 HR WHEN VESSEL LENGTH IS MORE

How big are the bubbles?

$$2\tau/r = (P_b - P_w) = T_{crit}$$





τ = surface tension of of water/air interface

From Henry's law:

- When bubble pressure P_b=100 it is in equilibrium with air dissolved in water
- When P_b<100 it will grow in size
- When P_b > 100 it will shrink.

Conclusions

- R-shaped curves are caused by bubbles drawn into vessels in a centrifuge and loss of conductivity occurs without increases in tension.
- R-shaped curves are inconsistent with known water relations of *Robinia*
- *Robinia* is conservative; stomates close as T_x increases to lower transpiration and to prevent excessive embolism.
- Contrary to earlier reports, there is no evidence of diurnal cycles of embolism during the day and refilling at night.