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Survivorship in potted *Populus deltoides* x *Populus nigra* hybrids in response to gradual soil water depletion

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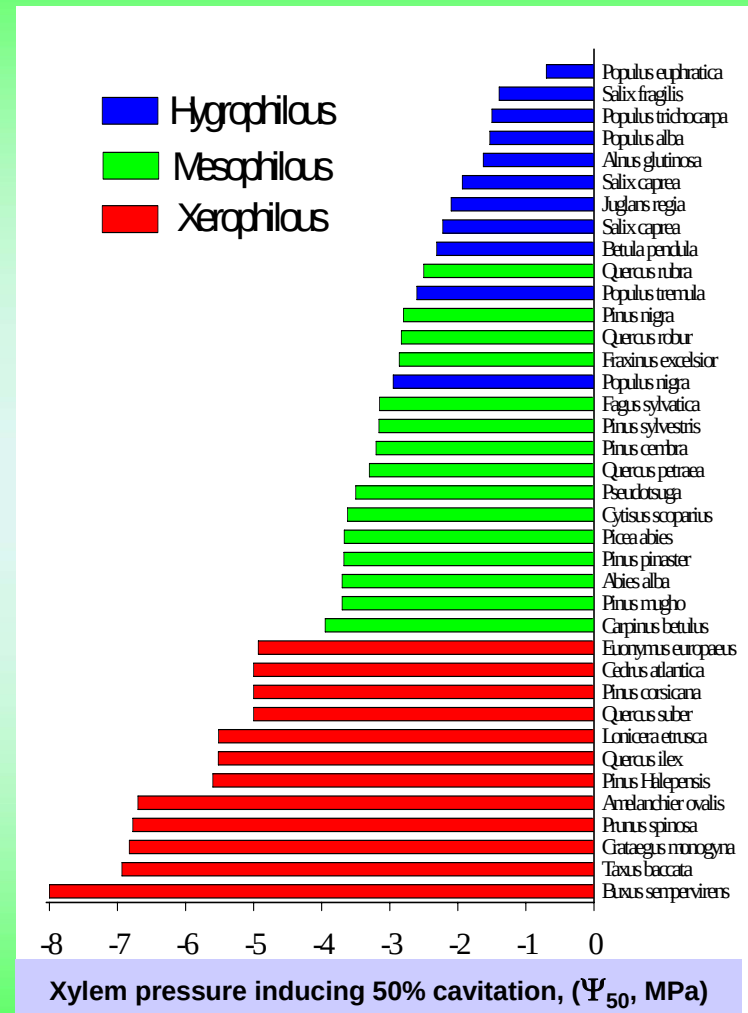
³Université d'Orléans, UFR-Faculté des Sciences, UPRES EA 1207 Laboratoire de Biologie des Ligneux et des Grandes Cultures (LBLGC), BP 6759, F-45067, France

⁴INRA, USC2030 Arbres et Réponses aux Contraintes Hydrique et Environnementales (ARCHE), BP 6759, F-45067, France

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Introduction

- ❖ Recent climate projections → increase in frequency and duration of intense summer droughts (Badeau et al., 2005; Salinger et al., 2005; IPCC, 2007).
- ❖ Studies on drought-induced plant mortality and survivorship have rekindled interest in foresters and scientists' communities.
- ❖ Ψ_{50} (a proxy of cavitation resistance) was regarded as a bio-sensing device (gauge) for drought sensitivity detection in trees (Cochard et al. 2005)
- ❖ Ψ_{50} was reported to vary consistently with the reputed habitat of tree species (Hacke et al. 2000; Pockman and Sperry 2000; Brodribb and Cochard 2009)
- ❖ But the effectiveness of Ψ_{50} as a gauge is not demonstrated yet!



Objectives

The objectives of this study were to:

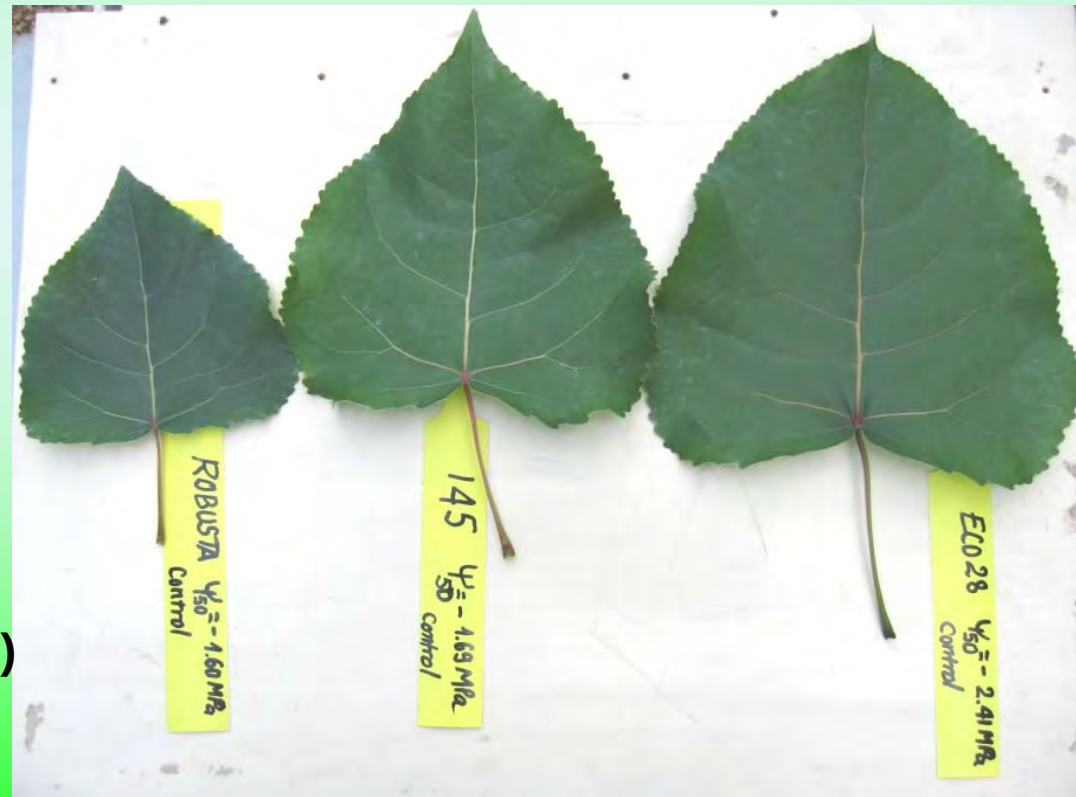
- ❖ test and interpret the differences in response to protracted summer drought of the chosen unrelated poplar hybrids mainly **if the hybrid with the lowest Ψ_{50} was the most resistant to drought-induced cavitation,**
- ❖ link cutting's leaf water potential and their hydraulic features to volumetric soil moisture content,
- ❖ check for whether xylem dysfunction leads to plant mortality.

Material and methods

Populus deltoides Bartr. ex Marsh x *Populus nigra* L.
unrelated hybrids

- Eco 28 ($\Psi_{50} = -2.41$ MPa)
- I45-51 ($\Psi_{50} = -1.69$ MPa)
- Robusta ($\Psi_{50} = -1.60$ MPa)

(Fichot et al., 2009; Fichot et al., 2010)



Growth conditions



- 3x50 current year cuttings of *Populus deltoides* x *Populus nigra* hybrids fed in 20-liter pot each
- 3x8 harvested sprouts per week
- Relative radiation: 80% of full sunlight
- Temperature: 15 - 30°C
- Relative humidity: 40-70%
- Daily drip irrigation for control plants and water shortage for the others.

- *Time domain reflectometer*
(Soil moisture content)



- *Pressure chamber*
(Leaf and xylem water potential)



- *Xylem Embolism Meter*
(Xylem native steady-state embolism)



- *Cavitron*
(Water potential inducing 50 % loss of conductance)



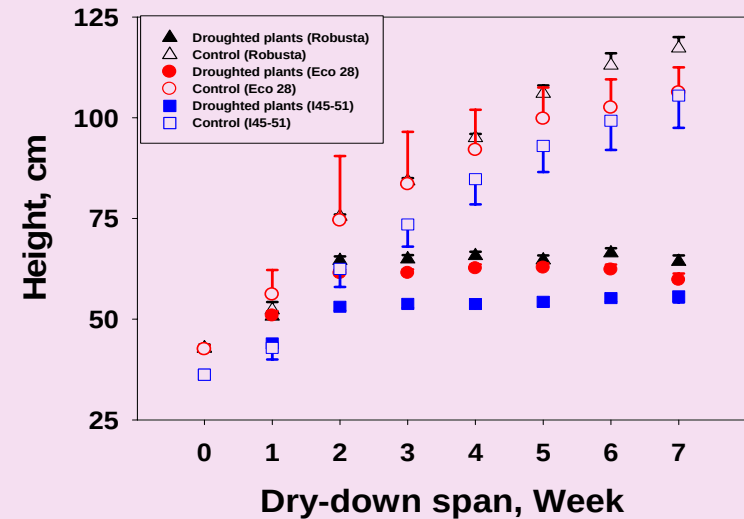
- *Microcalorimeter*
(Bud respiration rates)



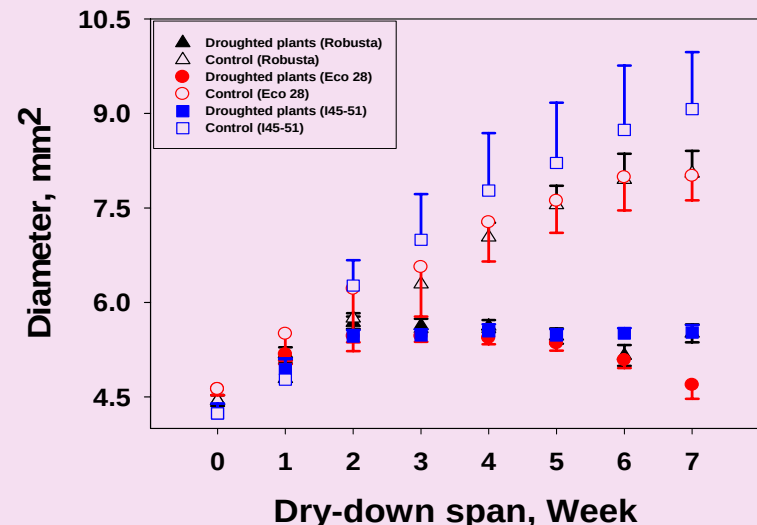
Results

- It comes as an evidence that water shortage inhibits growth in plant size
- Growth in height tended to be the lowest in I45-51 but the highest in diameter for control plants while for drought-treated plants, Robusta tended to display the highest growth in height but did not differ in diameter from others.

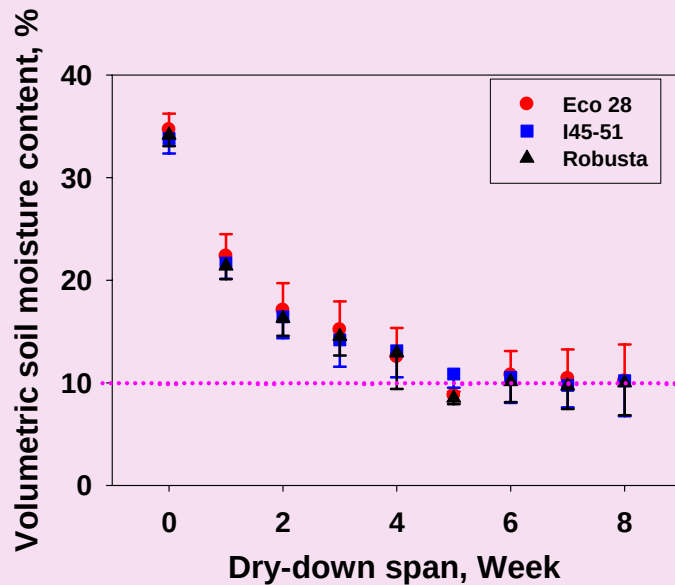
Growth in height for three Poplar hybrids versus dry-down span



Growth in diameter for three Poplar hybrids versus dry-down span

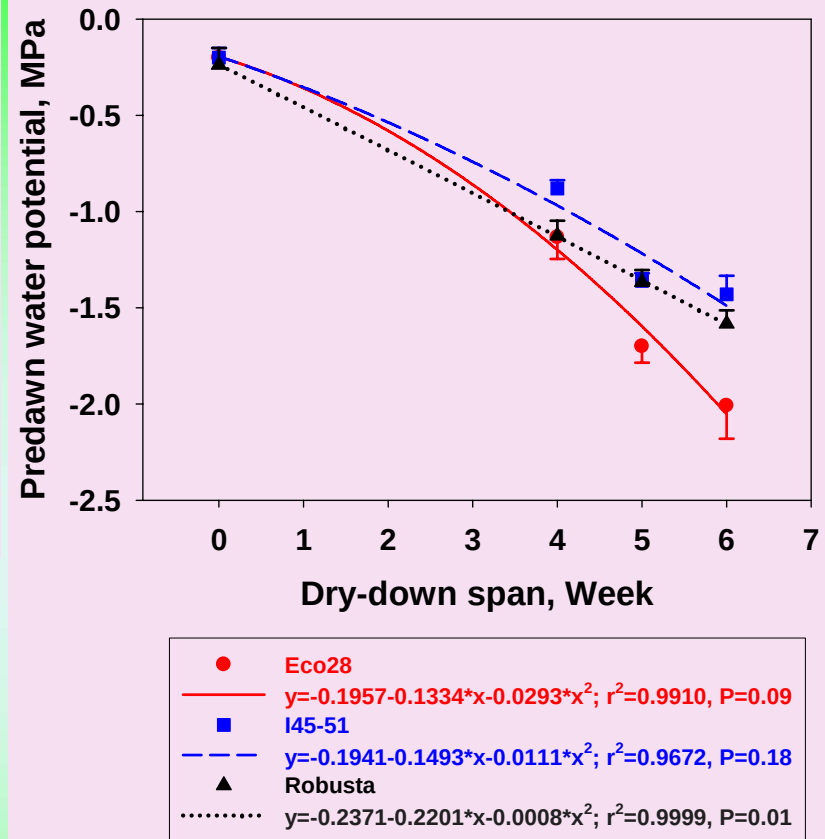


Dynamic of volumetric soil moisture content within the pots of the Poplar hybrids versus dry-down span



- Steep drop in volumetric soil moisture content (VSMC): more than 50% loss over 2 weeks
- 10% of maximum VSMC threshold appeared to be critical.

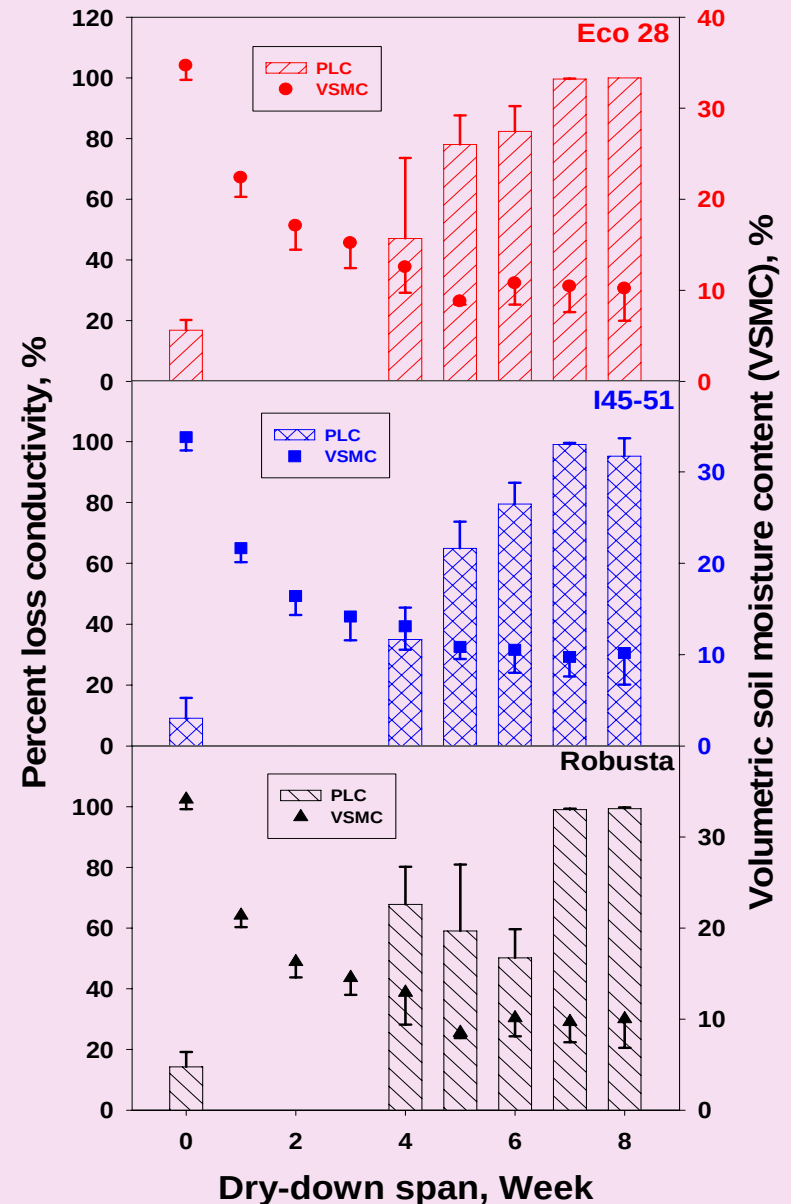
Variation in predawn leaf water potential of Poplar hybrids versus dry-down span



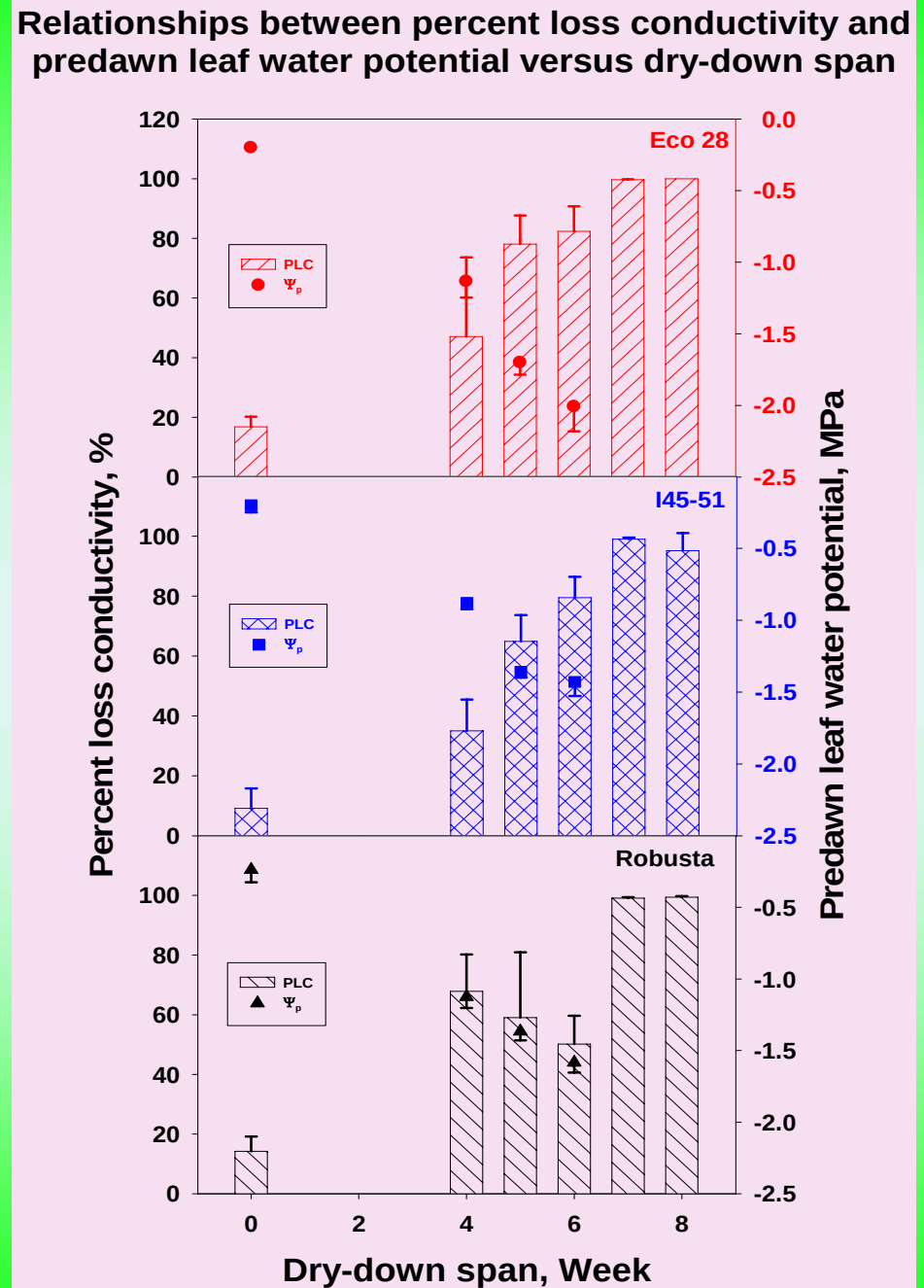
- Fast drop in Ψ_p for Eco28 in comparison with the other 2 hybrids.

Percent loss conductivity (PLC) increased sigmoid-like along with decreasing in volumetric soil moisture content for all three hybrids

Relationships between percent loss conductivity and volumetric soil moisture content versus dry-down span

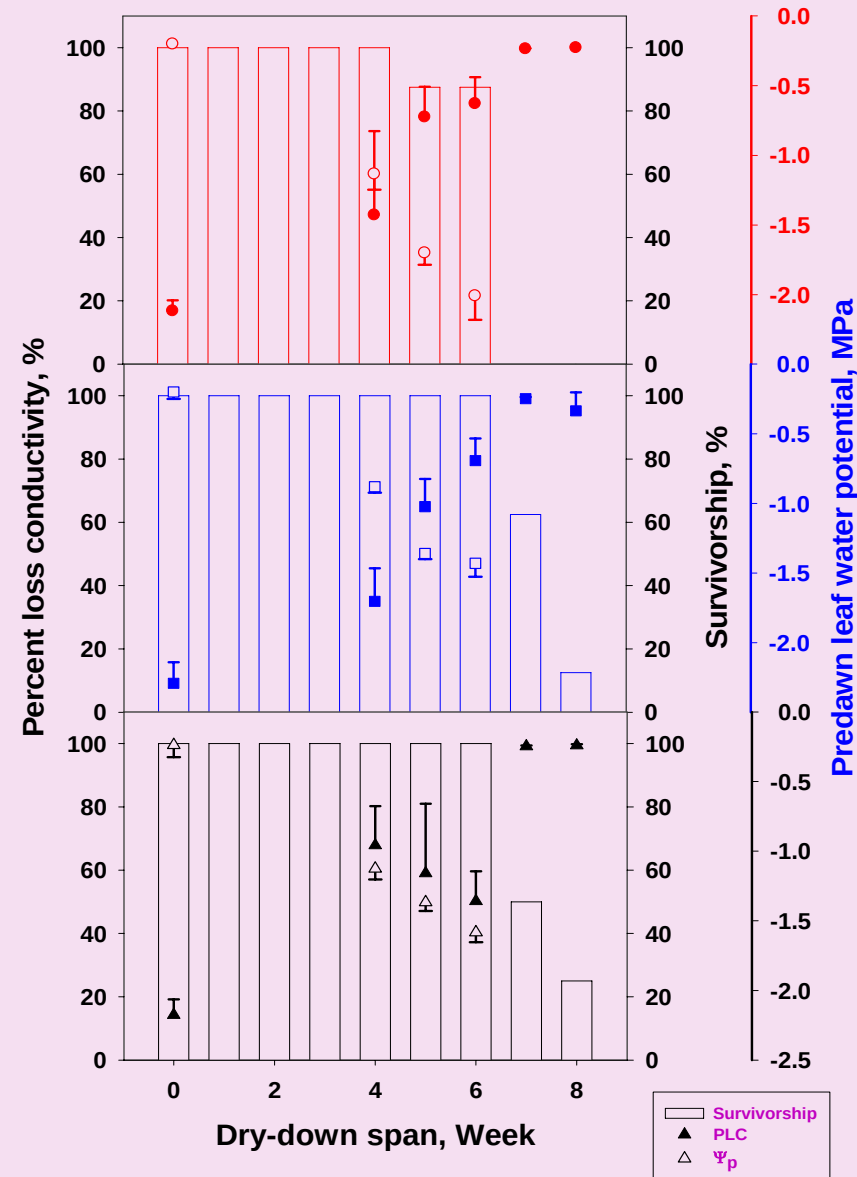


Over time, percent loss conductivity increased while predawn leaf water potential dropped for all 3 hybrids.



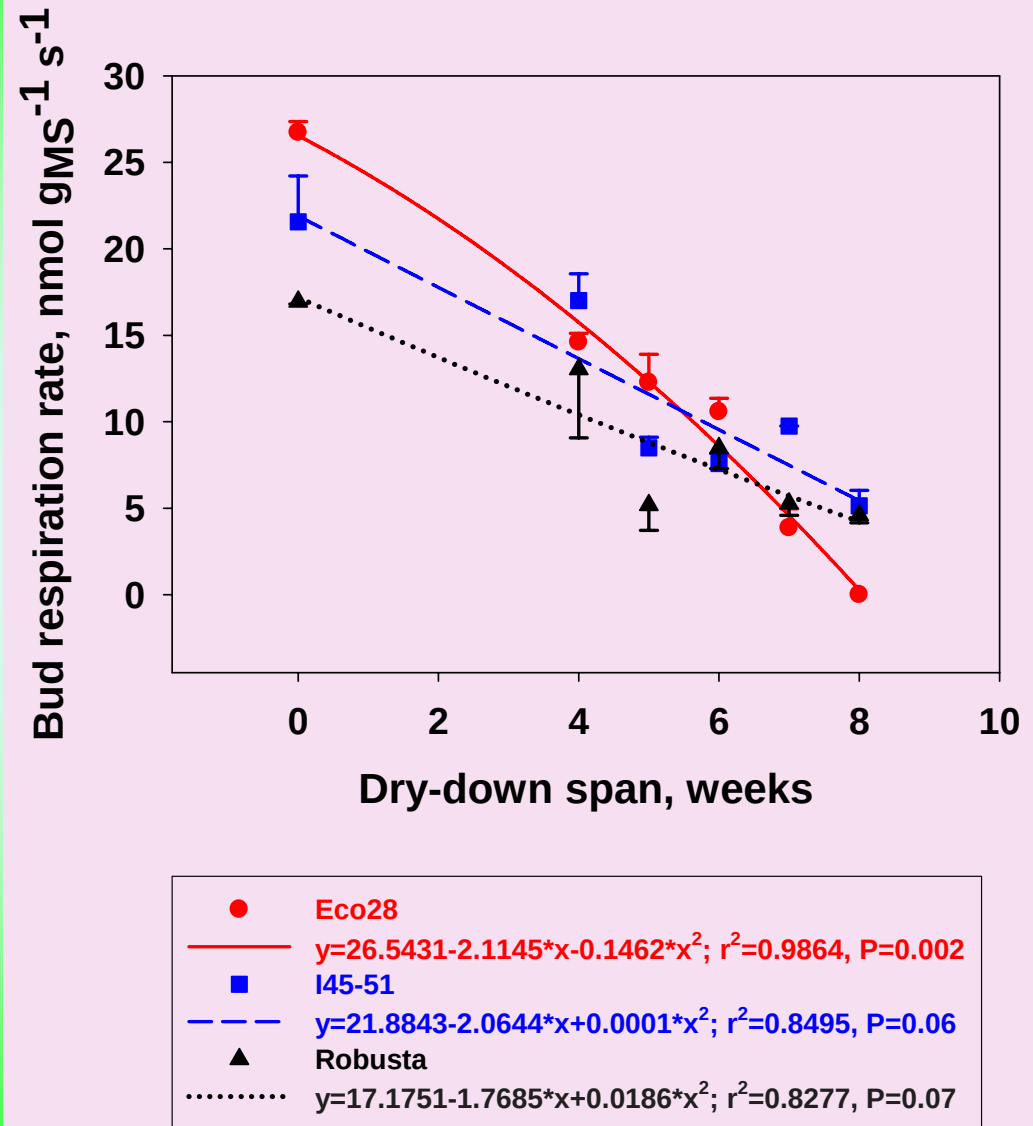
- Since drought inception mortality occurred in 5 weeks in Eco 28 hybrids but 2 weeks later in the 2 others
- None of Eco 28 individuals survived 7 weeks after drought inception roughly when Ψ_p got below -1.0 MPa
- Therefore, survivorship was threatened the most in Eco 28 hybrids

Relationships between percent loss conductivity and survivorship of Poplar hybrids versus dry-down span



- Bud respiration rate for Eco28 control sprouts was the highest in comparison with others
- The respiration rate dropped to nil only in Eco28 hybrids by week 8.

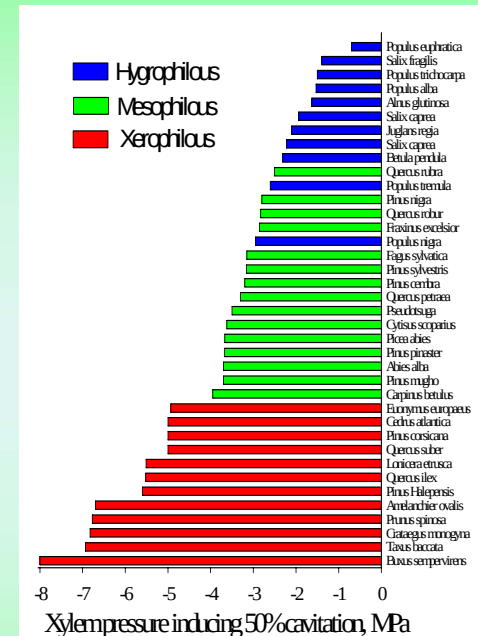
Bud respiration rate in 3 Poplar hybrids versus dry-down span



Conclusion

Sprouts of Eco28 were the most sensitive to the gradual soil moisture depletion whatever the morphological or the physiological parameter we considered. Therefore, we concluded that using Ψ_{50} as a gauge to stand for drought resistance in Poplar hybrids does not hold.

However, we still believe that Ψ_{50} is relevant enough to sort out samples of different species ... regarding the displayed picture.



Perspectives

❖ Check for the consistency of our findings

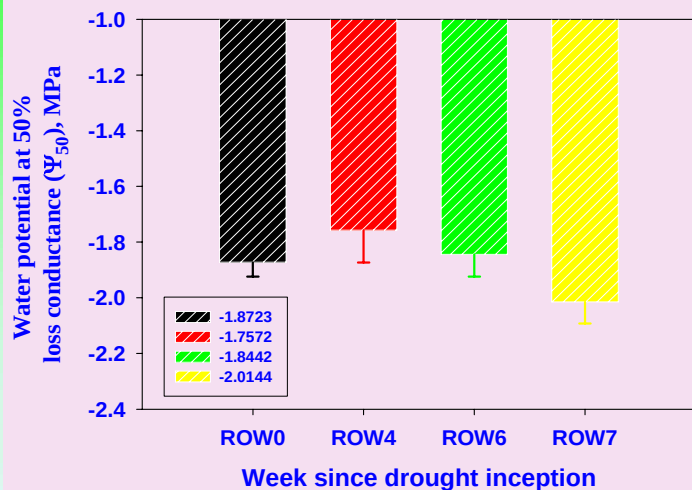
❖ Look into drought-induced acclimation in hydraulic features of newly produced shoots after the release from water shortage.

**Many thanks for
your attention**

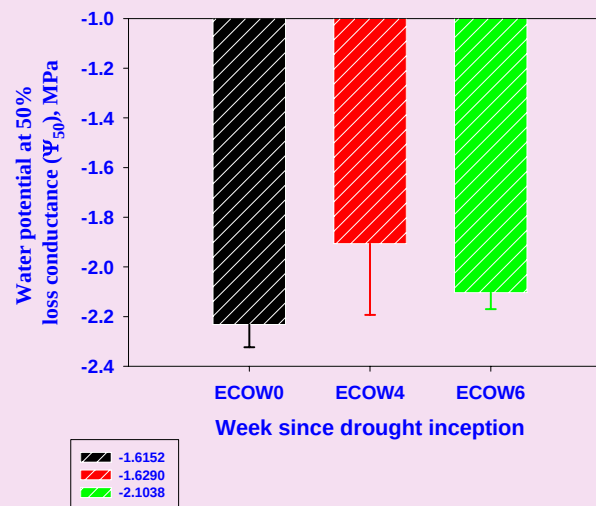
and

**Our acknowledgements to
C. Bodet, C. Serre, P. Conchon,
P. Chaleil and A. Faure
for their field assistance!**

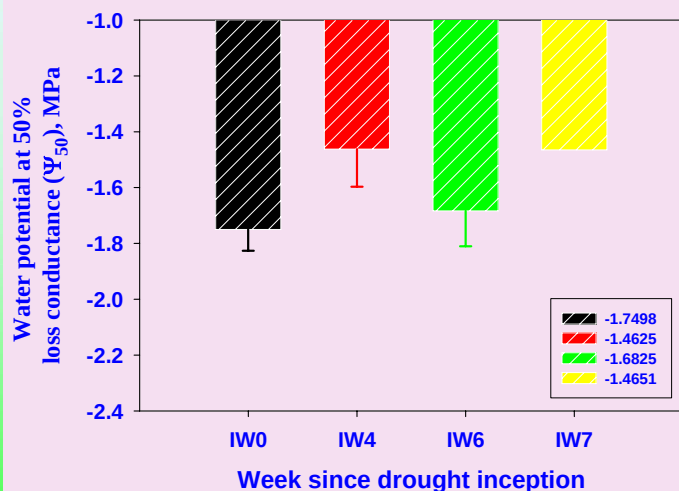
**Delayed-effects of drought spells
on newly released sprouts of Robusta plants**



**Delayed-effects of drought spells
on newly released sprouts of Eco 28 plants**



**Delayed-effects of drought spells
on newly released sprouts of I45-51 plants**



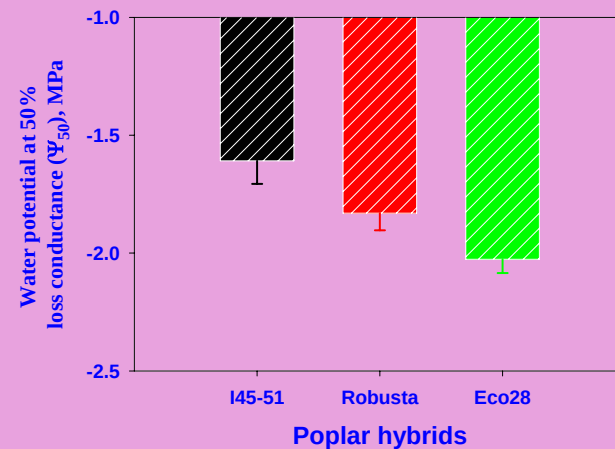
Eco 28 (Ψ_{50} = -2.41 MPa)

I45-51 (Ψ_{50} = -1.69 MPa)

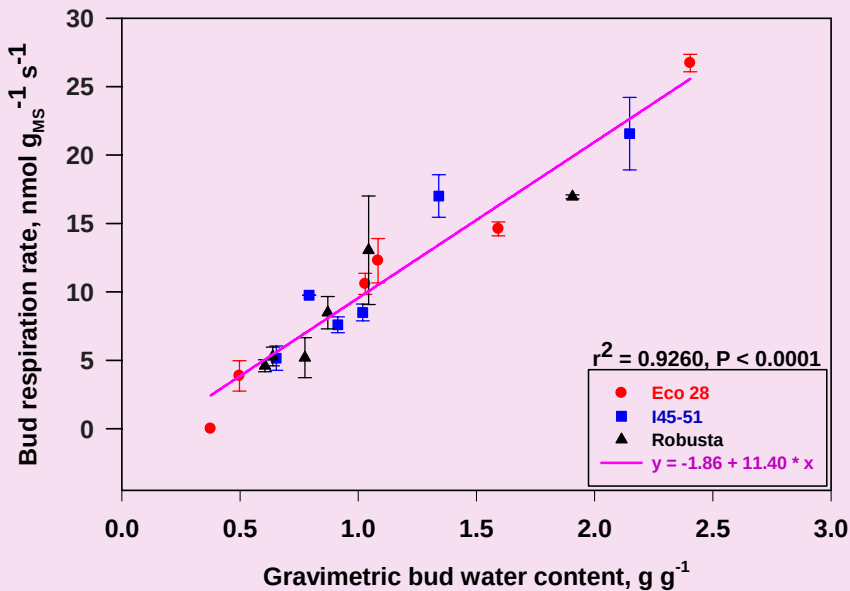
Robusta (Ψ_{50} = -1.60 MPa)

(Fichot et al., 2009
Fichot et al., 2010)

**Water potential at 50% loss conductance (Ψ_{50})
of newly released shoots (control) of the 3 poplar hybrids**

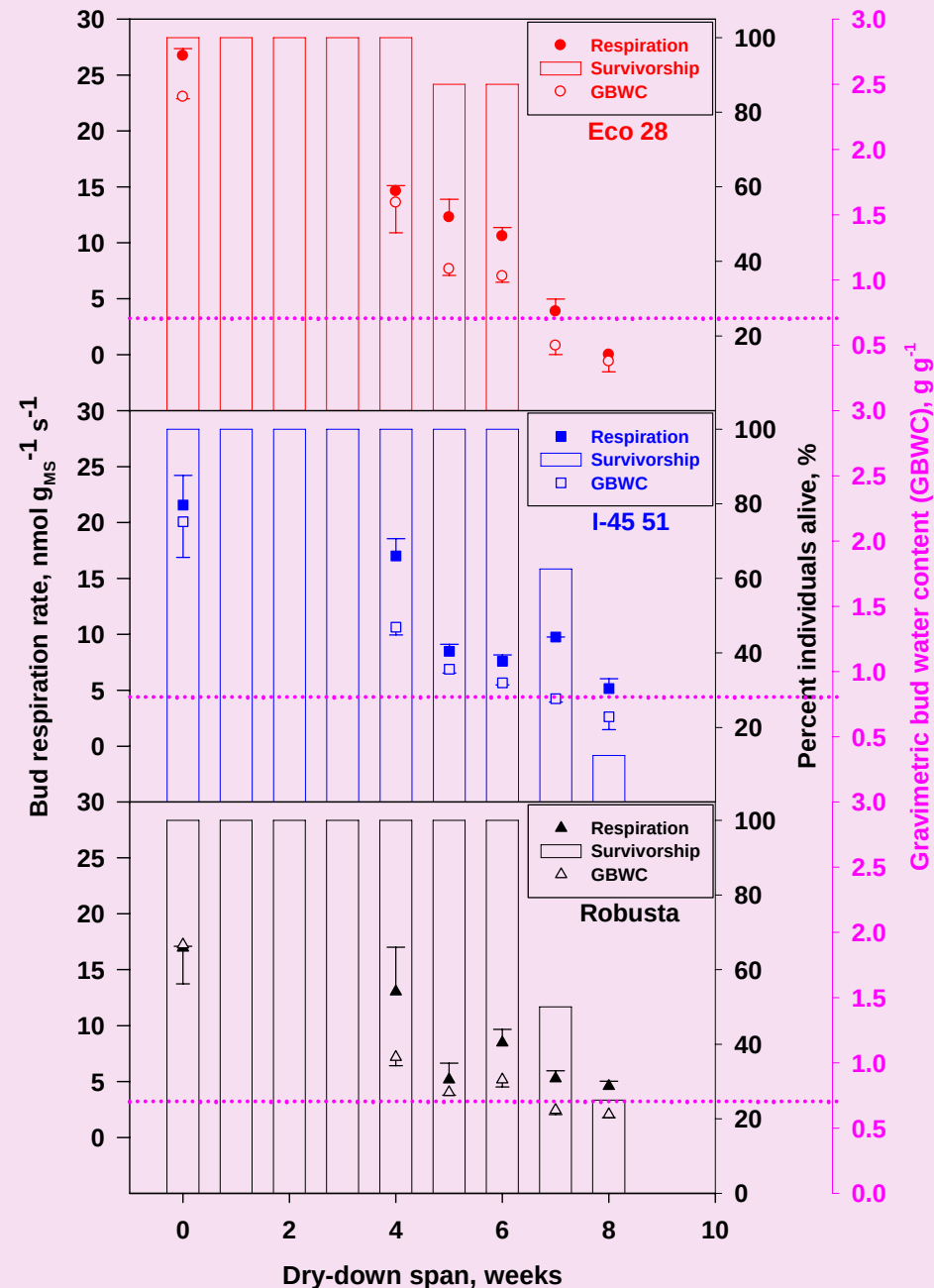


Relationships between bud respiration rate and gravimetric bud water content



Gravimetric bud water content or bud respiration rates can stand for gauges of plant mortality.

Relationships between bud respiration rate, gravimetric bud water content and survivorship in Poplar hybrids versus dry-down span



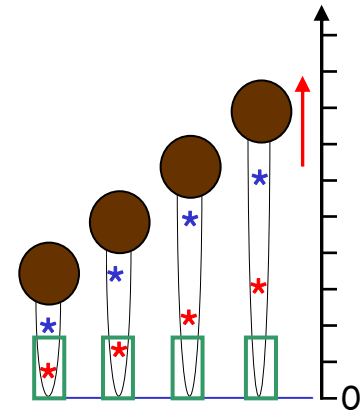
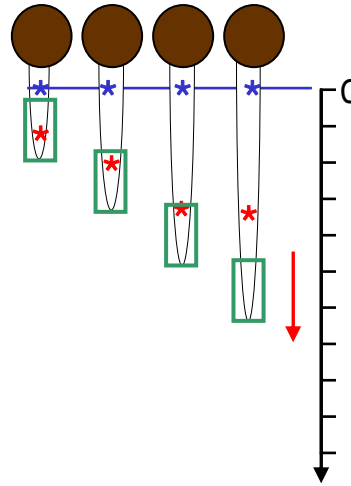
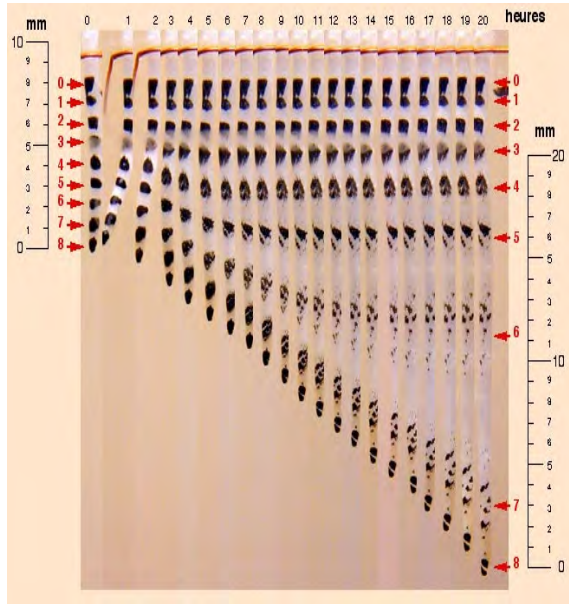
Aquaporin *TIP1* expression and its regulation in the growing root apex under two levels of osmotic stress

*Rémy Merret, Irène Hummel, Bruno Moulia, David Cohen
et Marie-Béatrice Bogeat-Triboulot*

*UMR EEF INRA-UHP, Nancy
UMR PIAF INRA-UBP, Clermont-Ferrand*



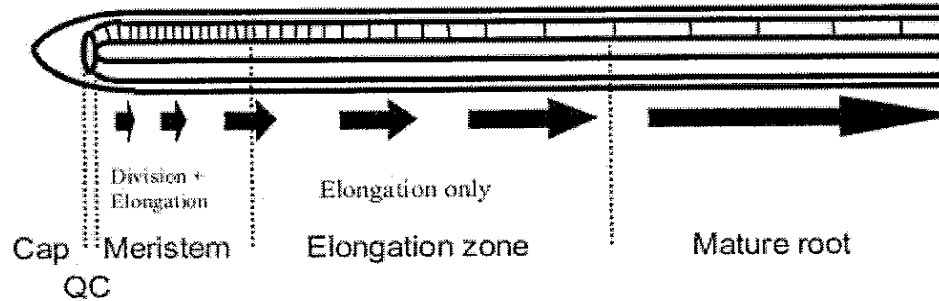
Referential change



Referential change :

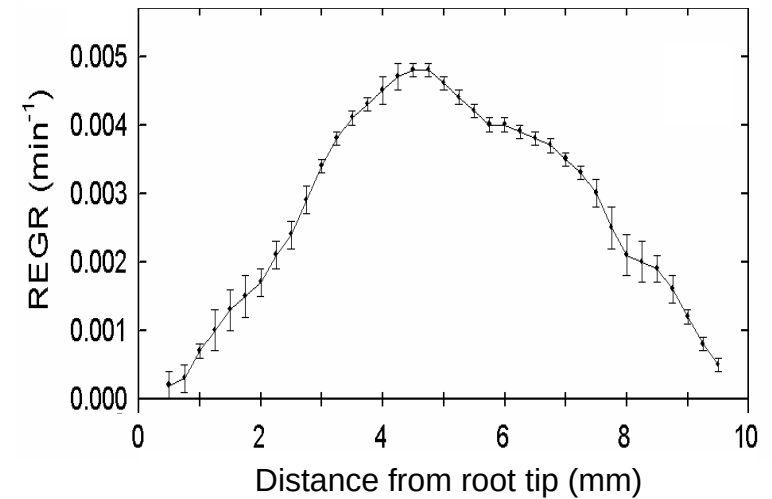
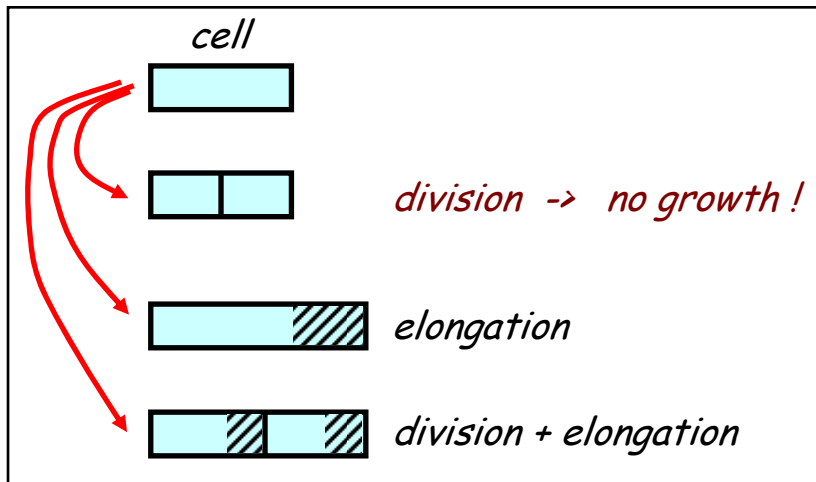
The root apex is a constant structure in which elements are continuously renewed

Growth = division + cell expansion



Beemster et al, 2002

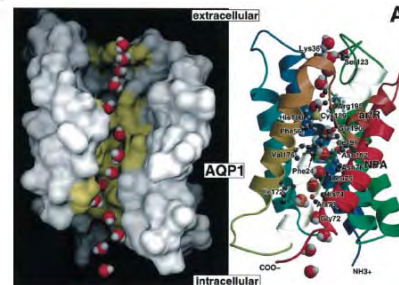
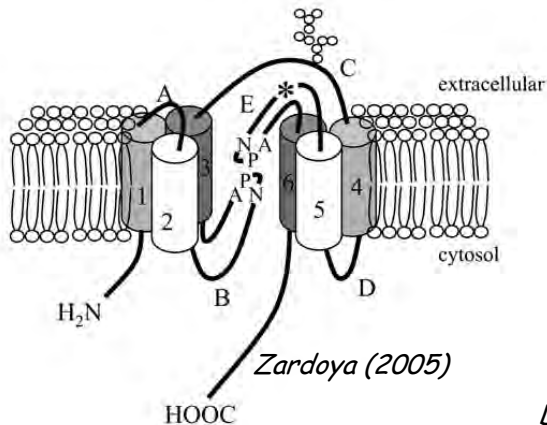
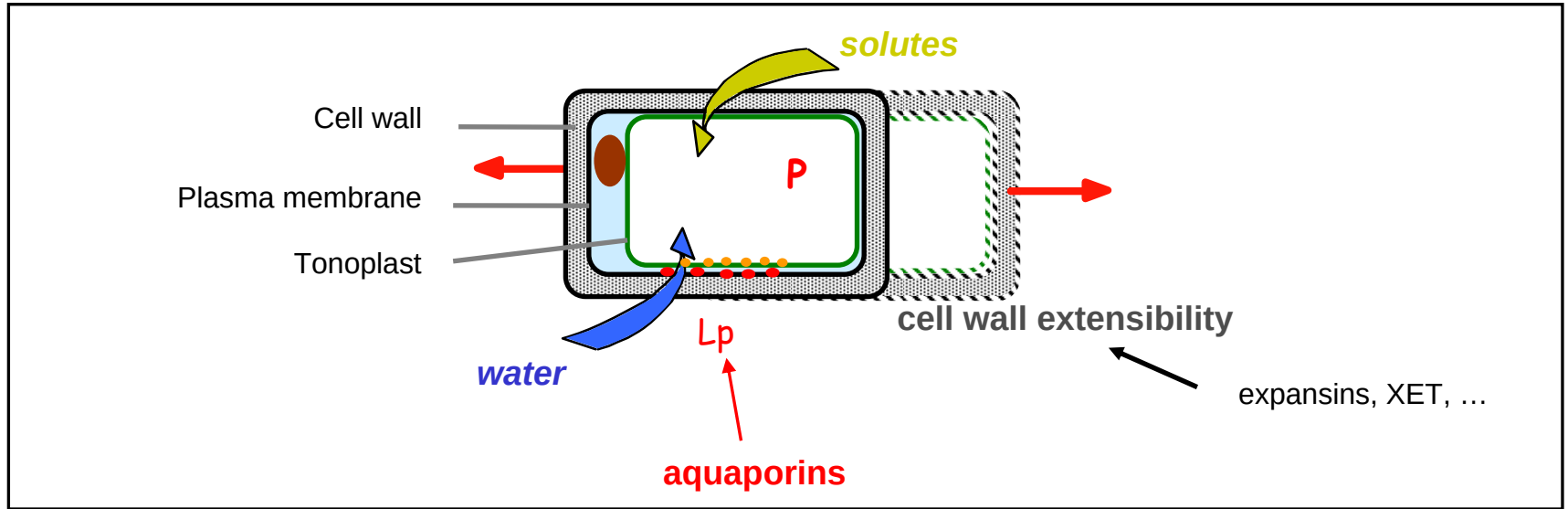
$$\text{Growth} = V_{\text{cell production}} \times L_{\text{mature}}$$



Control of cell expansion

Biophysical model of cell expansion (Lockhart, 1965)

- Motor : turgor pressure
- Controls : Cell wall extensibility & membrane hydraulic conductivity (L_p)

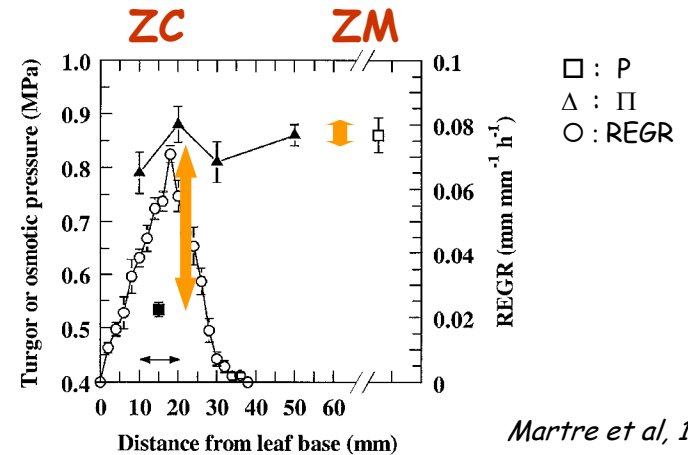


Aquaporin family : two main classes

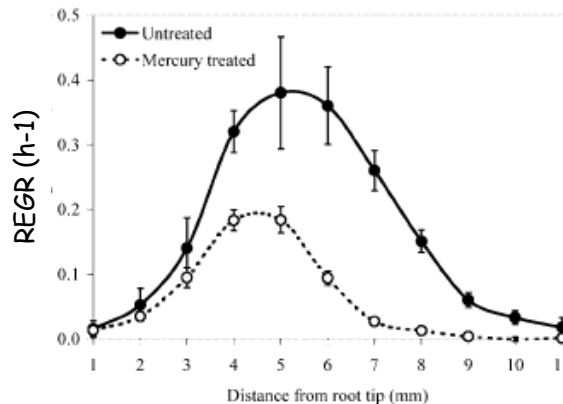
- PIP : Plasma membrane intrinsic protein
- TIP : Tonoplast intrinsic protein
- + NIP, SIP, XIP ...

Hydraulic limitation of cell expansion ?

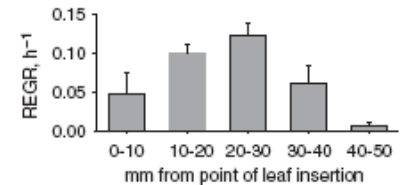
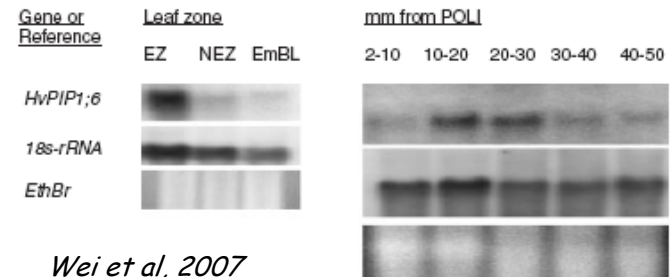
- Boyer versus Cosgrove
- estimation from the magnitude of Growth Induced/Sustaining Water Potential Gradient
- about 3-4 bars in leaves and hypocotyls
- never found in roots ...



Involvement of Aquaporins ...



(Hukin et al, 2002)



Context and aims

↪ $Lp_{\text{tonoplast}} \gg Lp_{\text{plasma membrane}}$ but $Lp_{\text{tonoplast}}$ also important for rapid water balance between cytoplasm and vacuole

↪ Aims of the study :

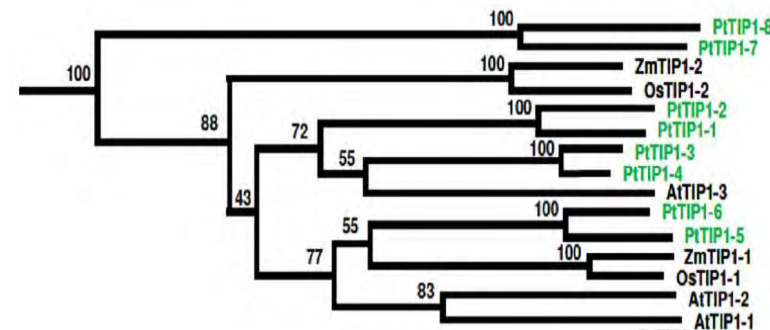
- Which *TIP1s* are expressed in the Poplar root growth zone?
- How are the expression affected by osmotic stress?
- On the basis of transcript accumulation and their changes, can we detect a link between cell expansion and the expression of some *TIPs*?
- What can we learn from the regulation of *TIPs* expression?

Pt : *Populus trichocarpa*

Os : *Oryza sativa*

At : *Arabidopsis thaliana*

Zm : *Zea mays*



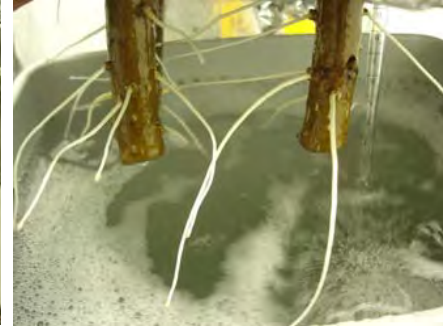
Gupta and Sankaramakrishnan, 2009

8 *TIP1s* in *Populus trichocarpa* genome
4 paralog pairs (gene duplication)

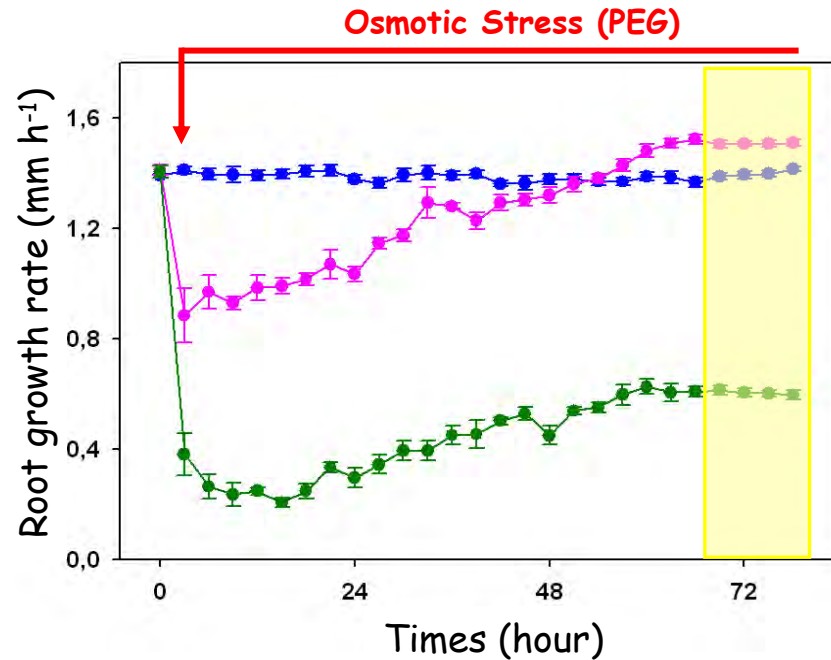
Root growth under osmotic stress



- Cuttings of *Populus deltoides* x *nigra* cv Soligo (15 cm)
- Hydroponics : Hoagland $\frac{1}{2}$ + phosphates
- Controlled environment (21°C, 70 % relative humidity, 16h light regime)

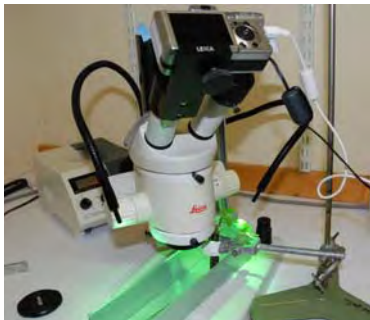
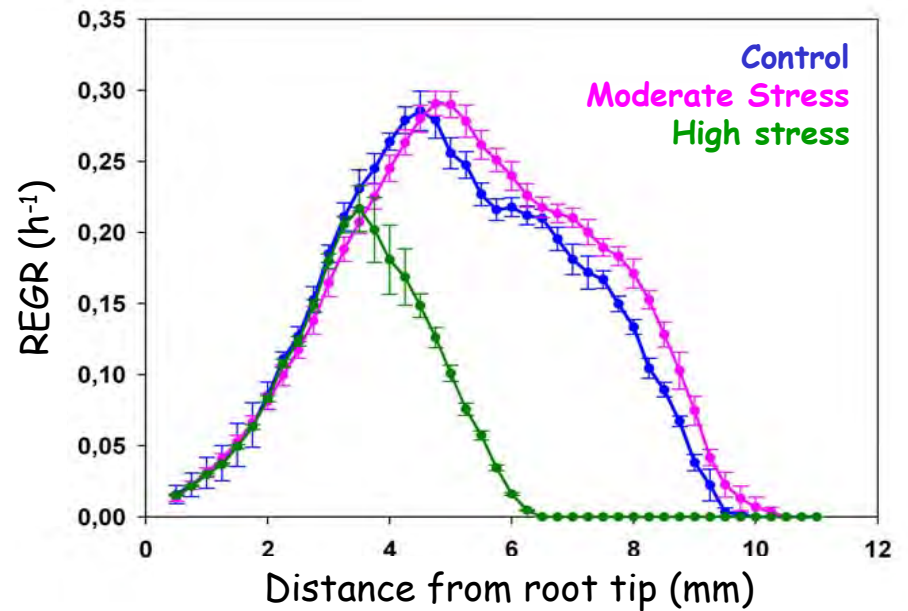
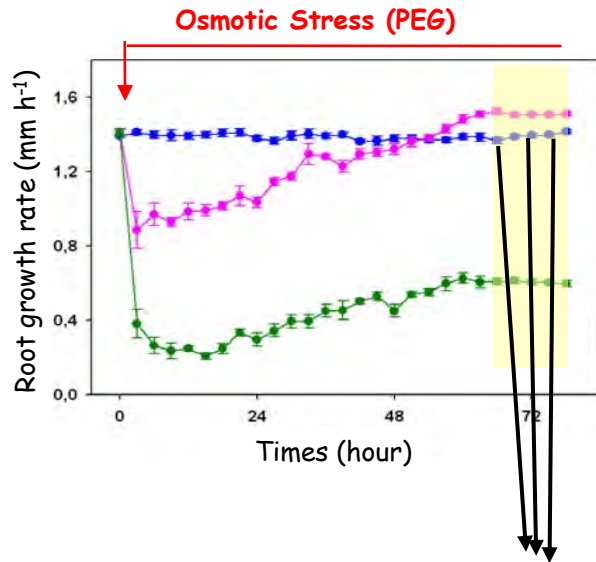


Control
100g/L PEG (90 mosmol/kg)
200g/L PEG (260 mosmol/kg)

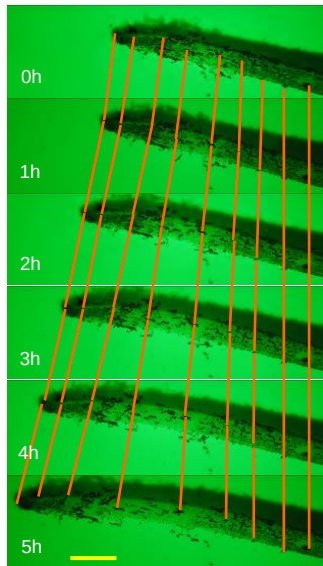


3 contrasted steady growth rates

REGR in the root apex

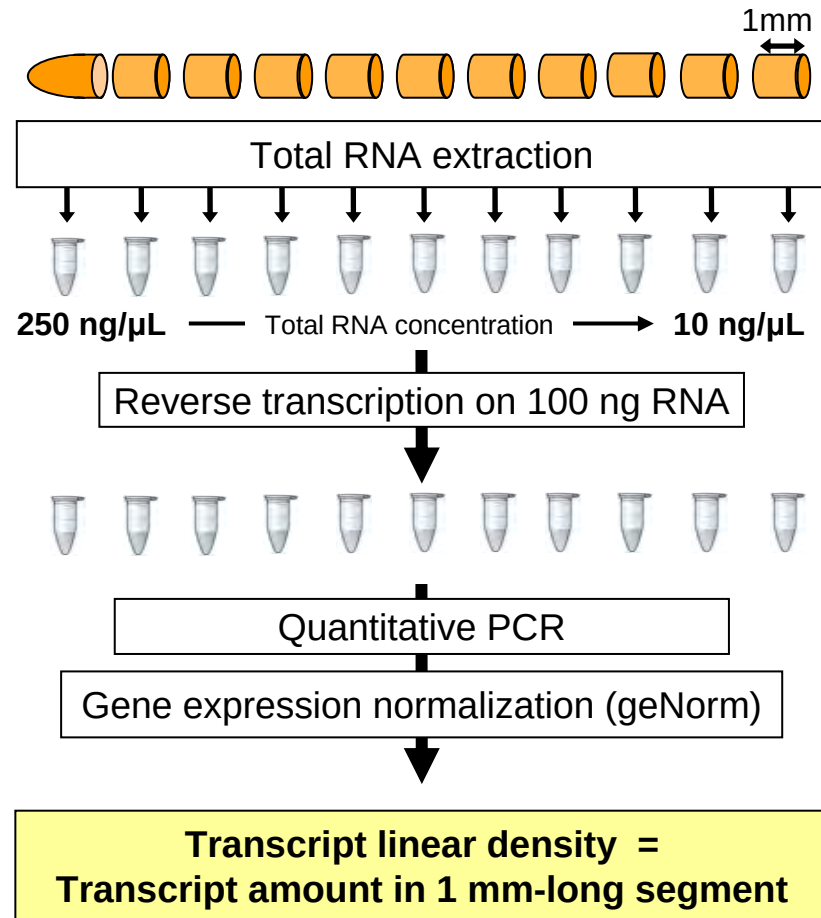


Carbon particles labelling
+
Kineroot
(Basu et al, 2007)



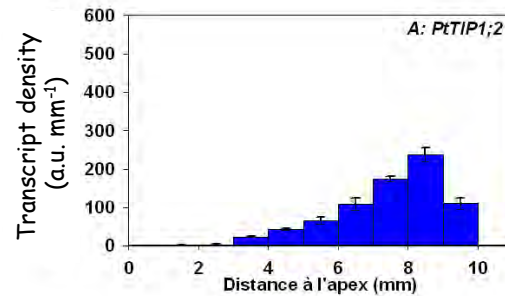
- similar REGR in [0.5-3.5 mm]
- high growth rate $\Leftrightarrow$ long growth zone
- low growth rate $\Leftrightarrow$ short growth zone

Transcript density quantification



TIP1s transcript density profiles

PtTIP1;1 :
reference gene
(geNorm)



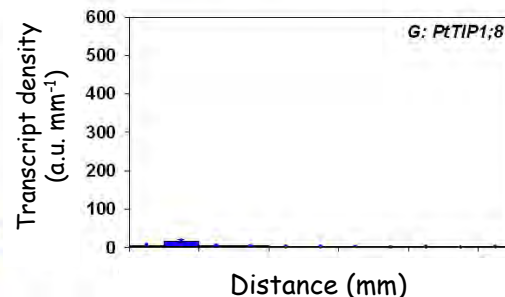
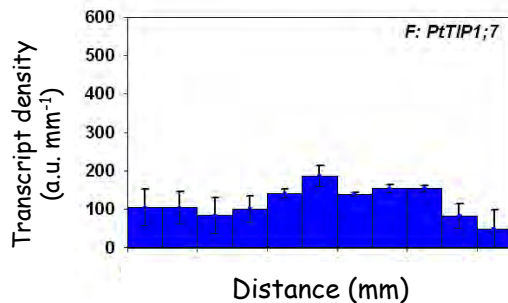
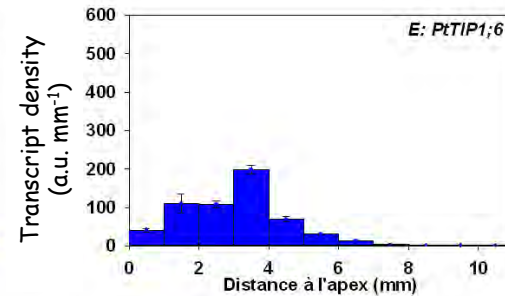
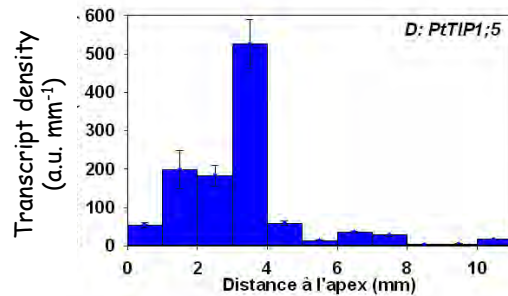
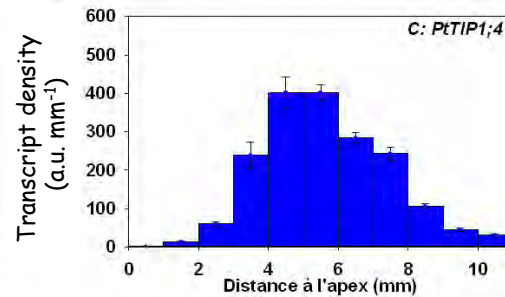
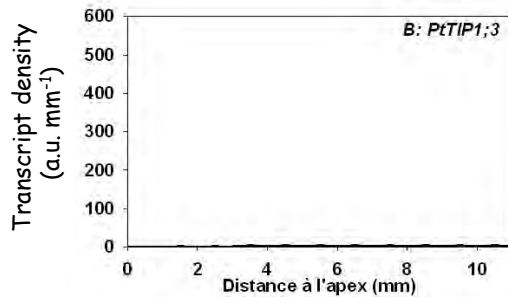
Mean $\pm$ s.e.m. (n=3)

Among 14 analysed genes,
TIP1;1 : the most stable gene
across segments and treatments

-> reference gene

TIP1;3 and *TIP1;8* very weakly accumulated

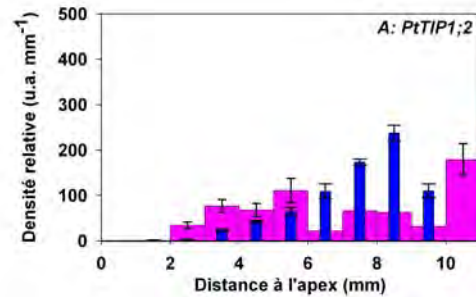
5 others : distinct accumulation patterns



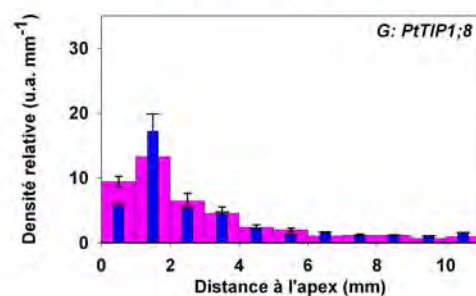
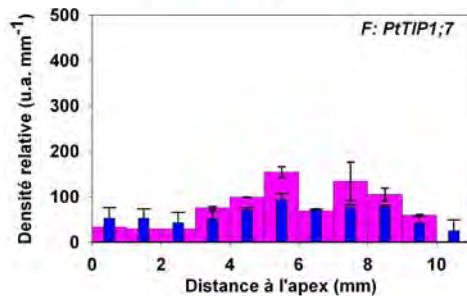
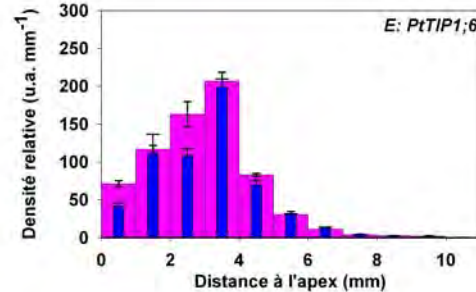
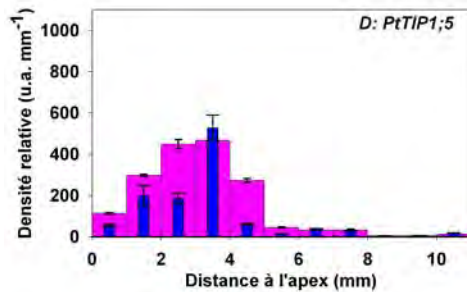
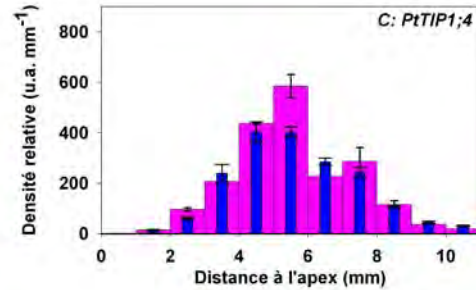
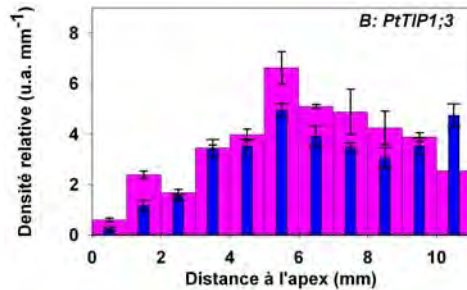
Effect of moderate osmotic stress

Control

Moderate stress



Mean $\pm$ s.e.m. (n=3)

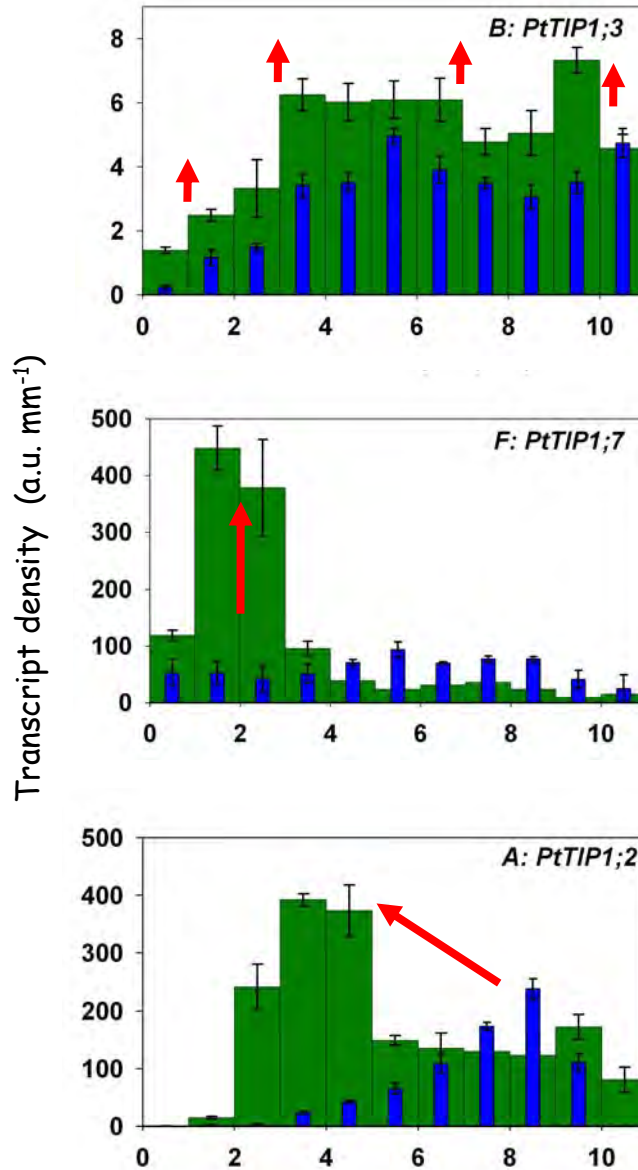


Low impact of moderate stress on *TIP1s* accumulation patterns (except *PtTIP1;2*)

... as on REGR profile

Effect of high osmotic stress

Control
High stress



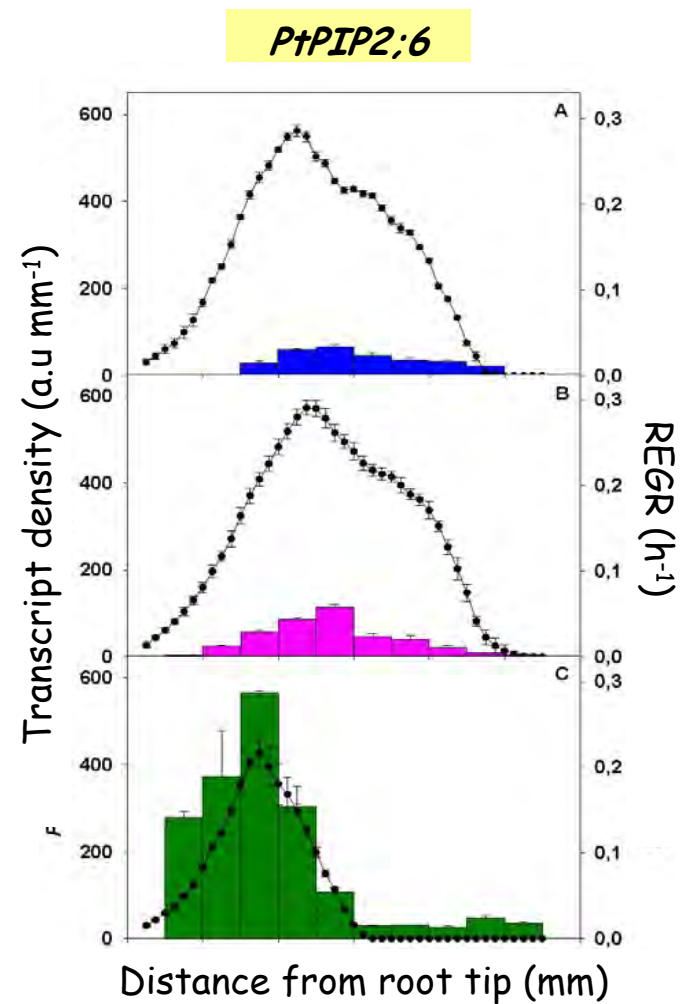
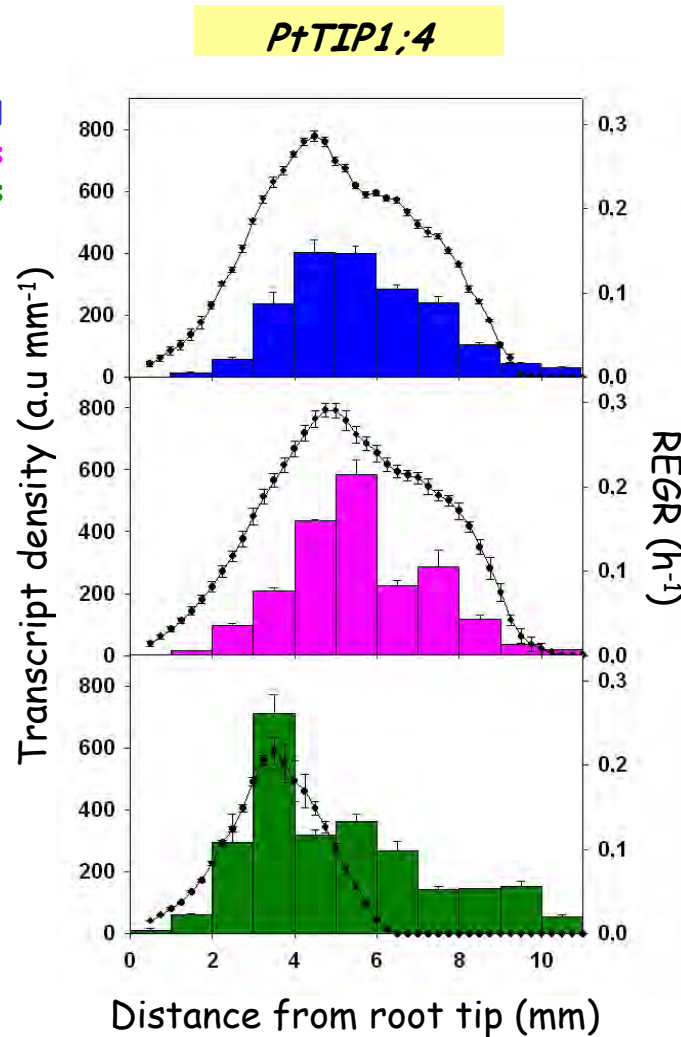
Mean $\pm$ s.e.m. (n=3)

High stress:

TIP1s accumulation patterns strongly and differently affected

Expression and REGR profiles

Control
Moderate Stress
High stress



Changes of REGR profile are accompanied by similar changes in *TIP1;4* accumulation patterns

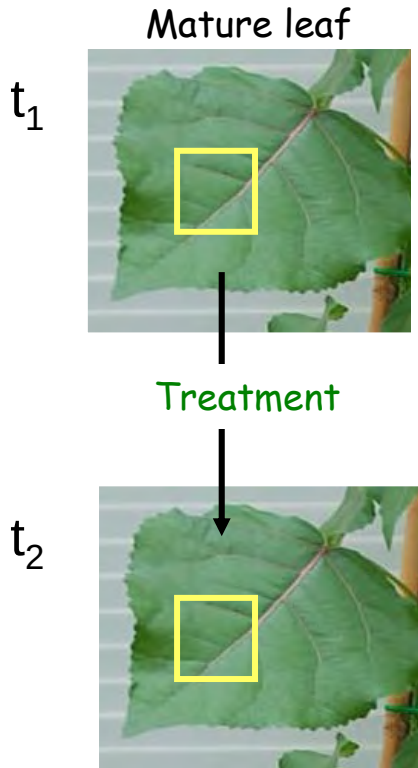
Transcript is not a proxy of functional protein ... but a change of expression is costly :

- > **change of transcript density has a sense in terms of maintenance/increase in protein level**

↳ for the 3 growth states, REGR and TIP1;4 patterns overlap
-> clue for implication of TIP1;4 in cell expansion

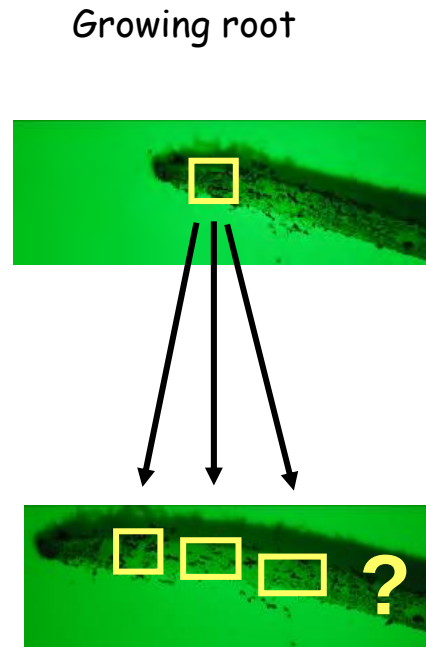
↳ What about regulation of its expression?

Regulation of gene expression in a growing organ?



Mature organ :

Gene regulation =
temporal variation of
transcript density
 $\delta\rho/\delta t$

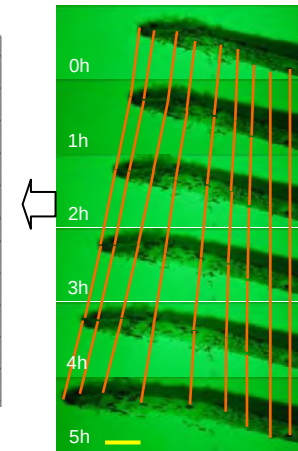
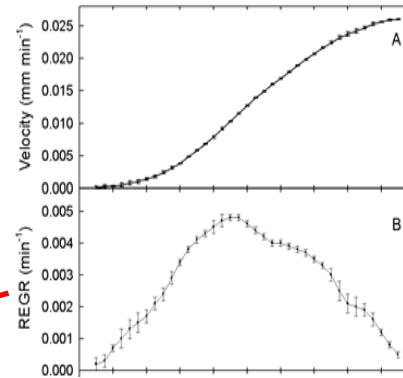
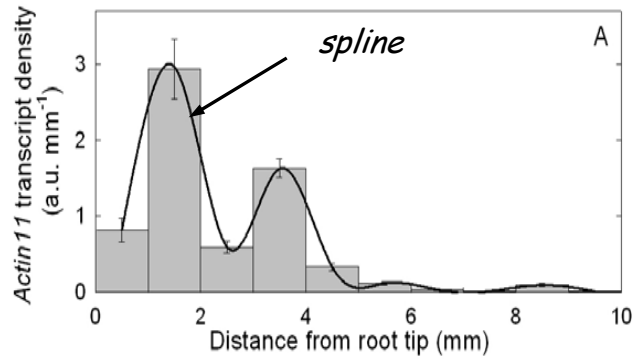


In a growing organ, it is necessary
to take into account
cells movement and their expansion.

$$D_{(x)} = \underbrace{\frac{\partial \rho}{\partial t}_{(x)}}_{\text{Temporal variation}} + \underbrace{v \frac{\partial \rho}{\partial x}_{(x)}}_{\text{Convection}} + \underbrace{\rho \frac{\partial v}{\partial x}_{(x)}}_{\text{Dilution}}$$

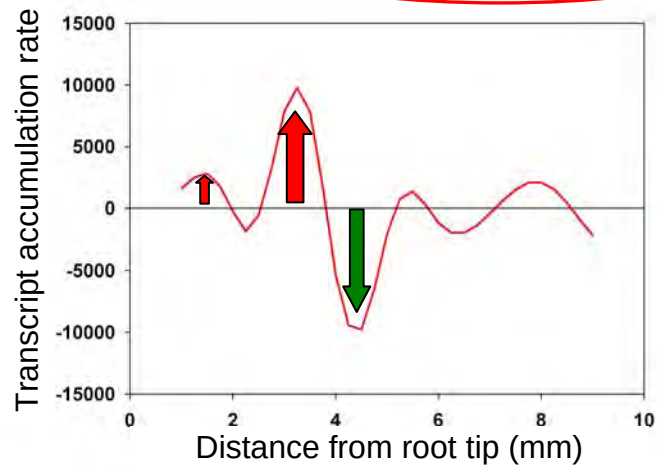
Material derivative

The **continuity equation** (issued from a fluid mechanics formalism) gives access to the **material derivative of transcript density**, i.e., the **regulation of gene expression**



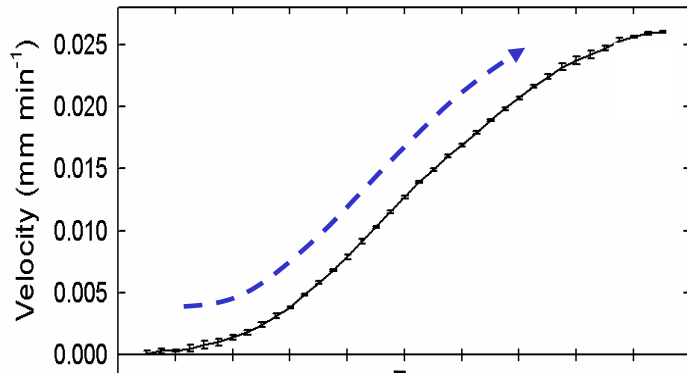
Continuity equation

$$D_{(x)} = \frac{\partial \rho}{\partial t_{(x)}} + v \frac{\partial \rho}{\partial x_{(x)}} + \rho \frac{\partial v}{\partial x_{(x)}}$$



**Spatial pattern of
local transcript accumulation rate
at a given time point**

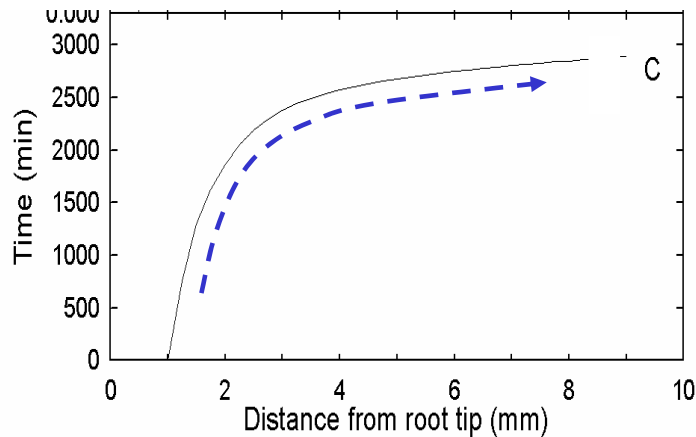
Steady state and growth trajectory



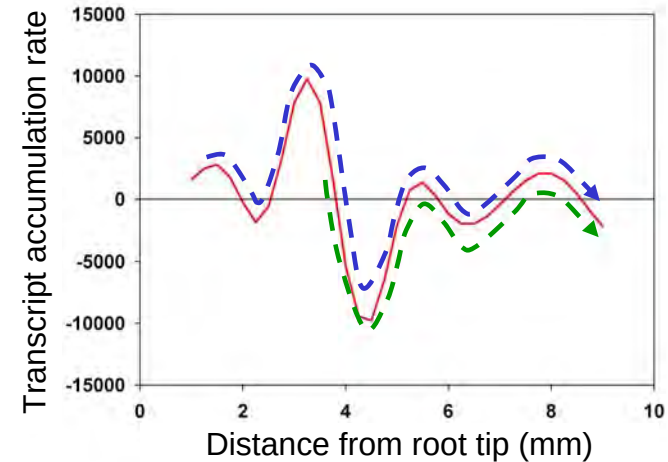
If steady state :
integration



Growth trajectory : $D = f(\text{Time})$

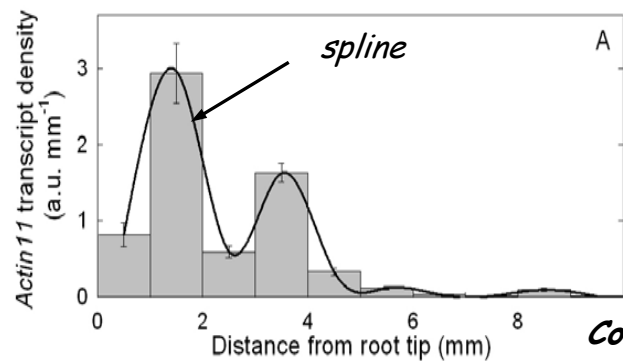


These eulerian patterns are valid for any particle
for the "steady-state" time window

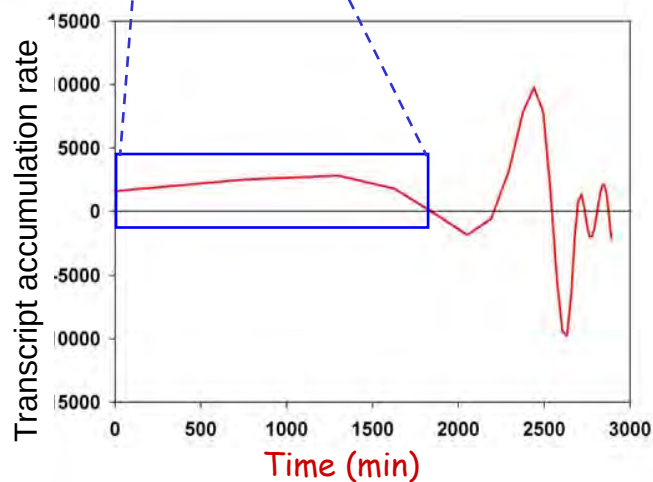
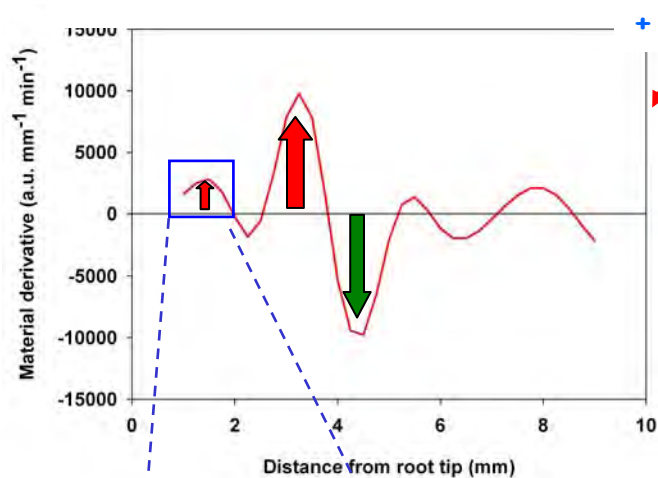
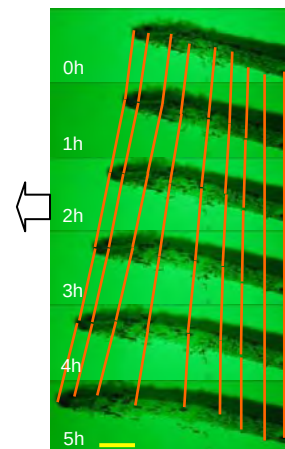
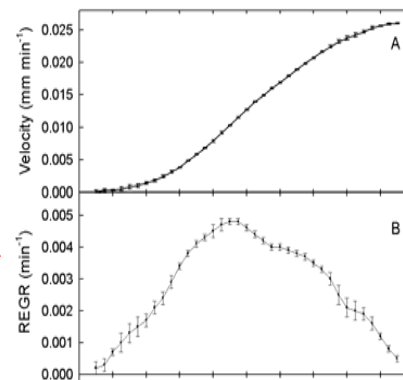


**Spatial pattern of
transcript accumulation rate
in a 'moving particle'**

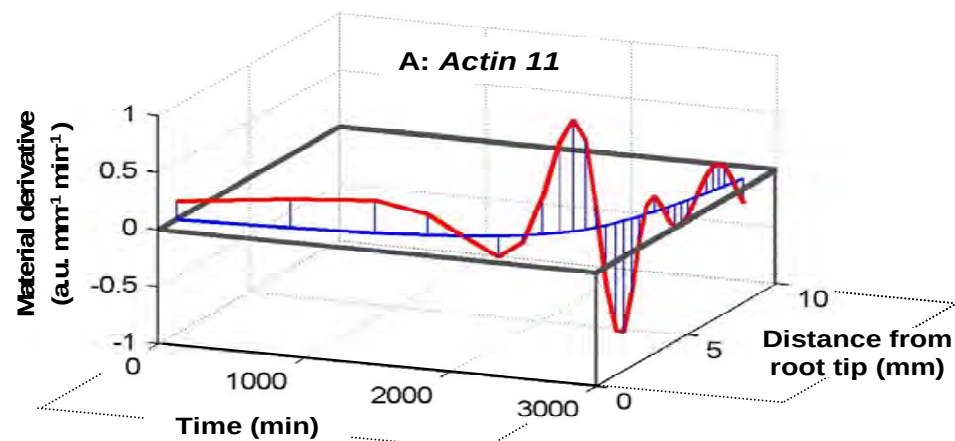
spatial coordinates -> temporal coordinates



Continuity equation

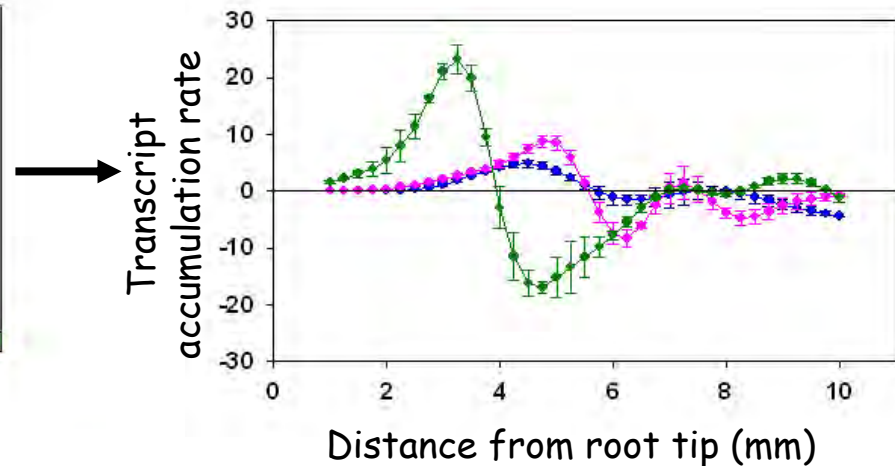
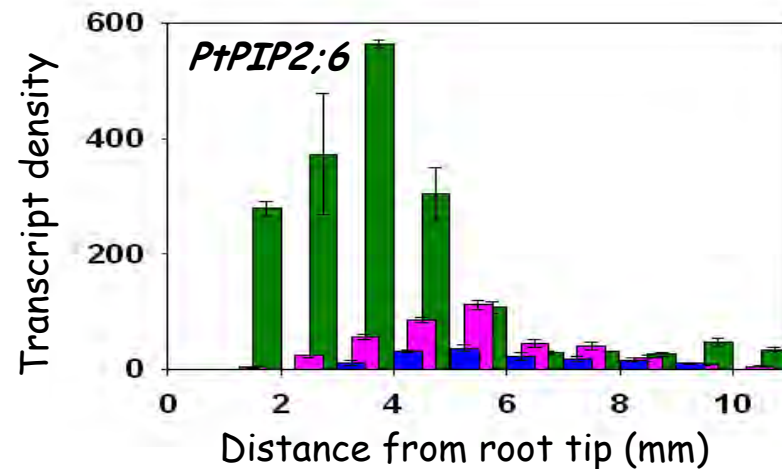
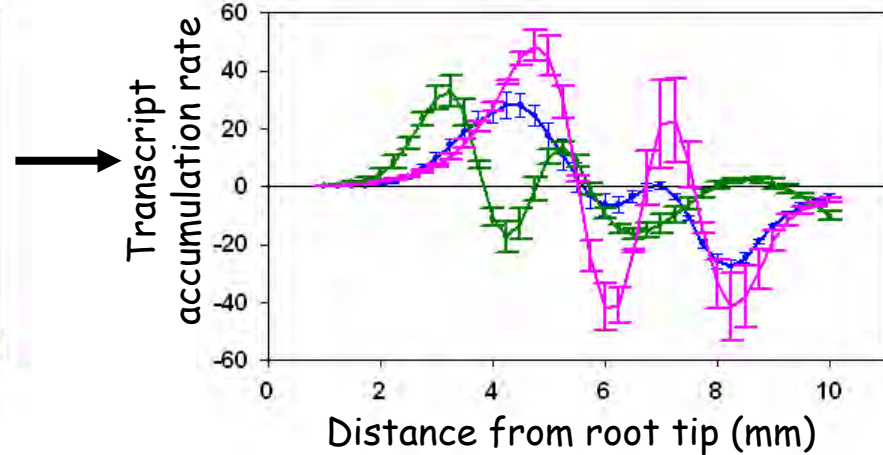
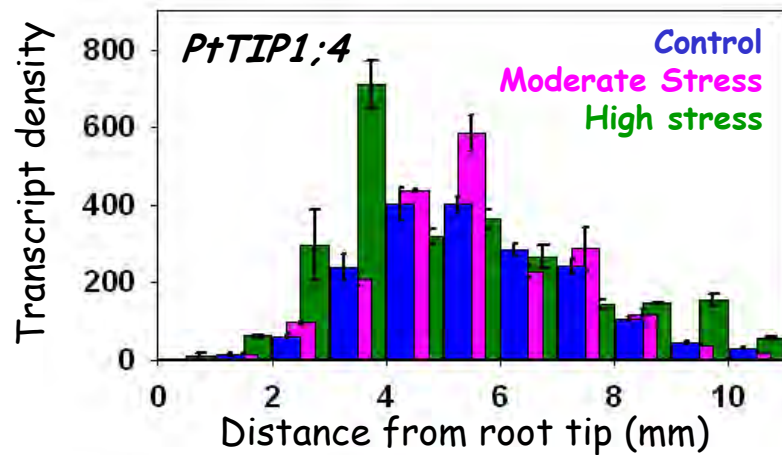


Spatio-temporal description of regulation of gene expression in a 'moving particule'



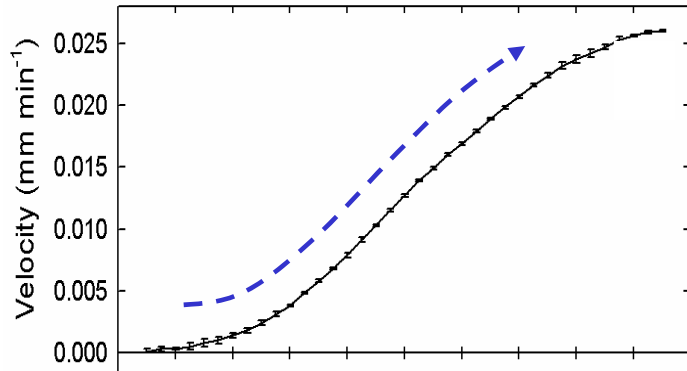
Merret R. et al (2010)

Regulation of *PtTIP1;4* and *PIP2;6* expression



PtTIP1;4 under high stress : high expression without higher induction
PtTIP2;6 under high stress : strong induction

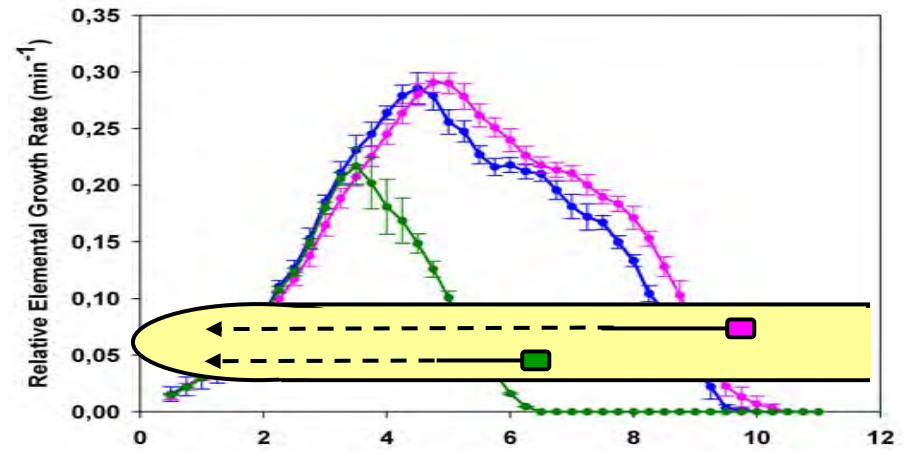
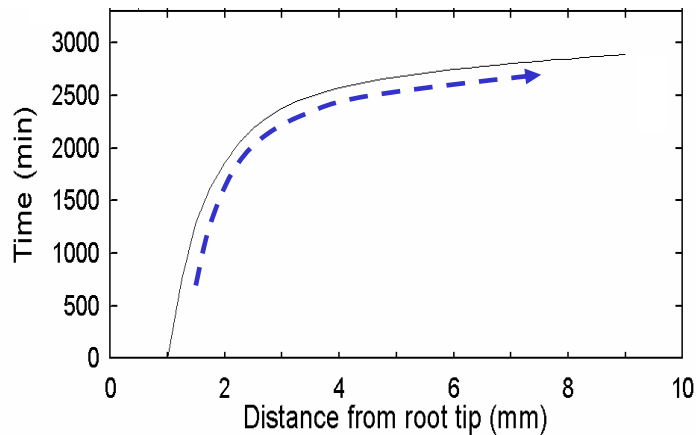
Reverse trajectory



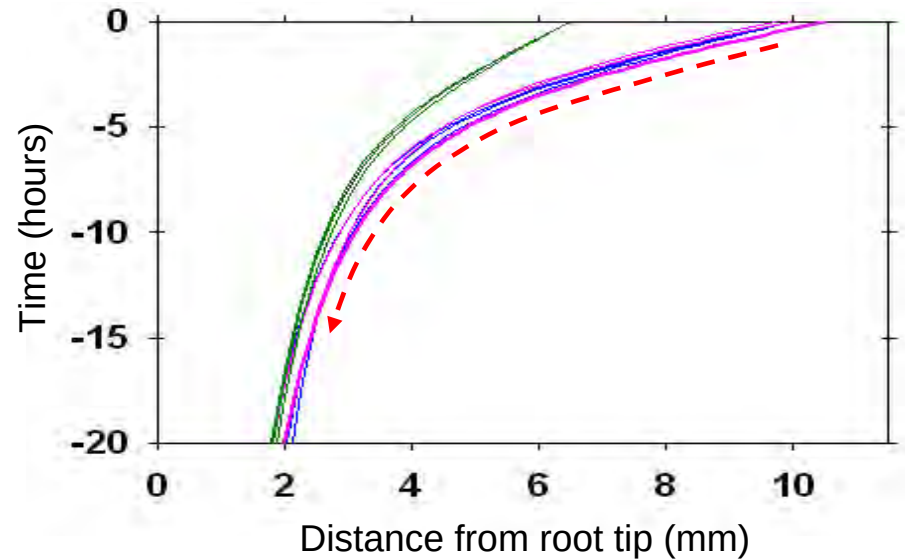
If steady state : integration



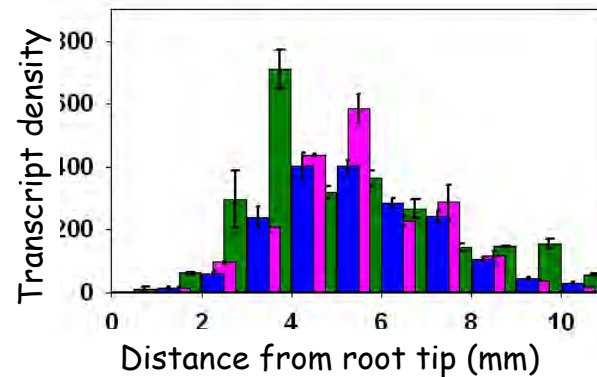
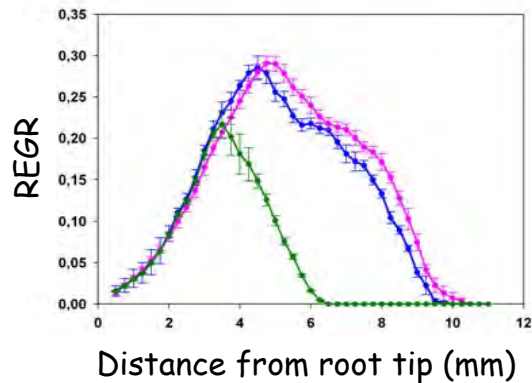
Growth trajectory : $D = f(\text{Time})$



Growth trajectories of the cells just finishing
their expansion for the preceding 20h



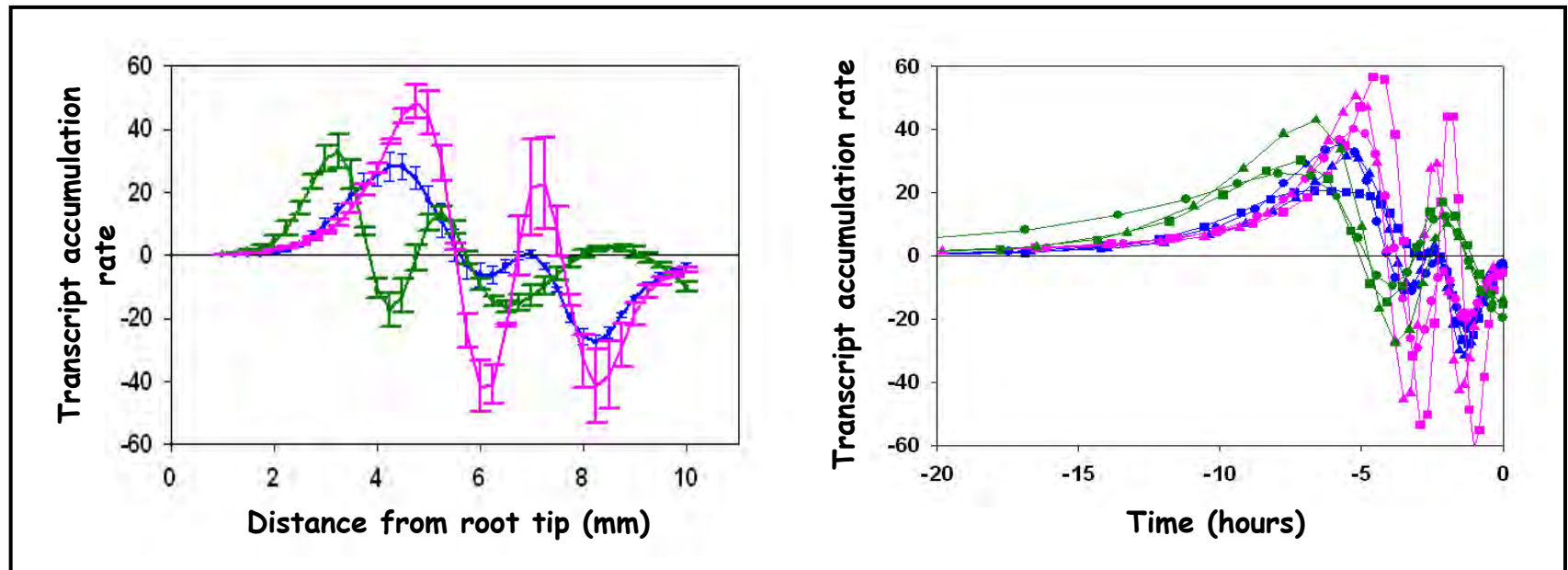
Spatial and temporal patterns of regulation of TIP1;4 expression



Control

100g/L PEG (90 mosmol/kg)

200g/L PEG (260 mosmol/kg)



Contrasting spatial patterns but similar temporal patterns
The regulation of TIP1;4 expression seems to be temporally governed

- ↪ analyse the regulation patterns of other *TIP1s*
- ↪ immunolocation of aquaporins
- ↪ use the framework to analyse the molecular control of synchrony between cell division and cell elongation

Hydraulic safety of *Pinus pinaster* needles

Katline Charra-Vaskou,
Régis Burlett, Sylvain Delzon,
Stefan Mayr

6th april 2011

Xylem colloquium, Nancy

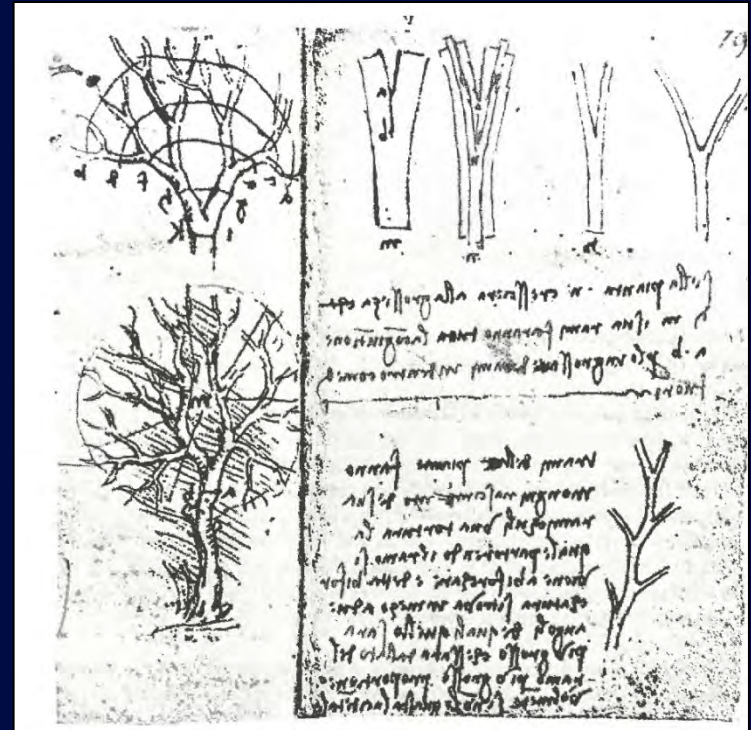
**Institute of Botany,
Innsbruck University,
Austria**

**UMR BIOGECO,
Bordeaux 1 university,
France**



- 🌲 Soil-plant-atmosphere continuum
- 🌲 Efficiency of the plant hydraulic system
 - ↳ Roots
 - ↳ Axes
 - ↳ Leaves
- 🌲 Plant hydraulic resistance:
 - ↳ Water potential in the plant
 - ↳ Water availability for tissues
- 🌲 Aim:

Analysis of vulnerability to drought-induced loss of conductivity in needles of *Pinus pinaster*



Leonardo da Vinci



Material:

Pinus pinaster

Twigs and needles

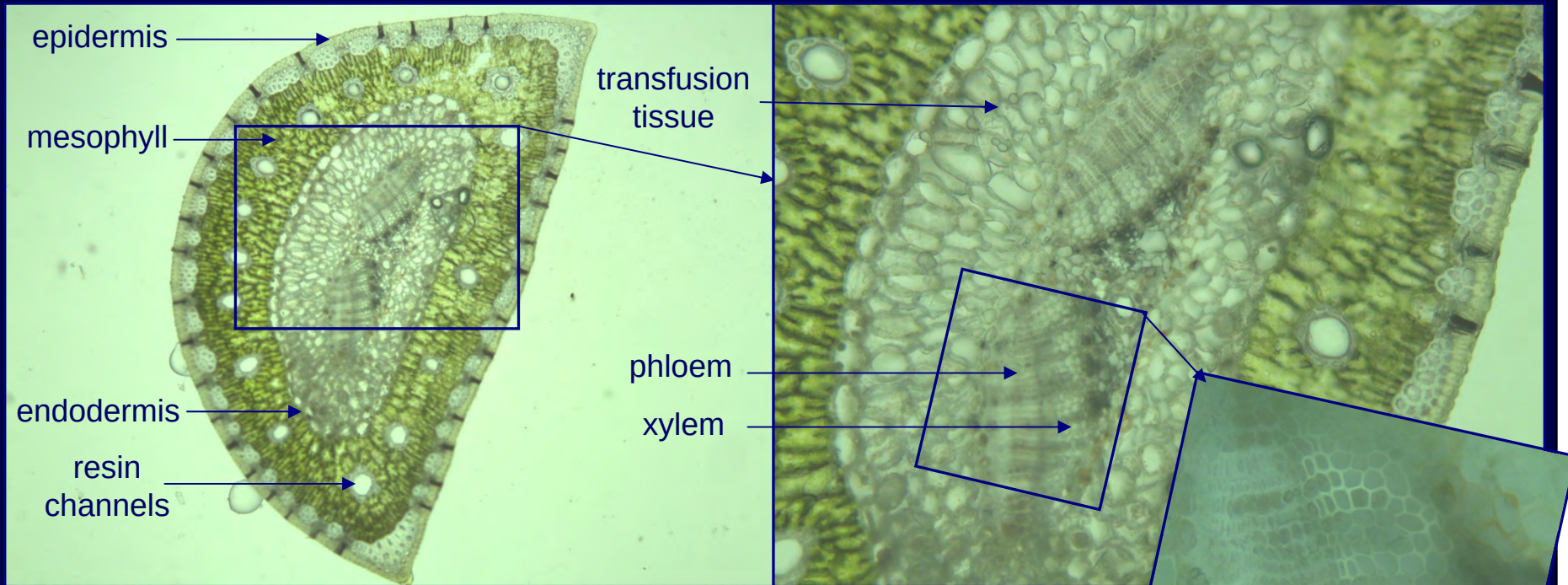


Methods:

1. Cavitron
2. Rehydration kinetics method
3. Ultrasonic acoustic emission analysis



Needle hydraulic conductivity



Cross section of *Pinus pinaster* needle

🌲 Xylem: conductivity is influenced by tracheid diameter, tracheid length and the number of pit connections

🌲 Extra-vascular pathway: transfusion tissue, mesophyll

phloem
xylem

🌲 *Pinus pinaster*

↳ needles

mean length: 14 to 18 cm

↳ twigs

🌲 Campus of Talence, Bordeaux



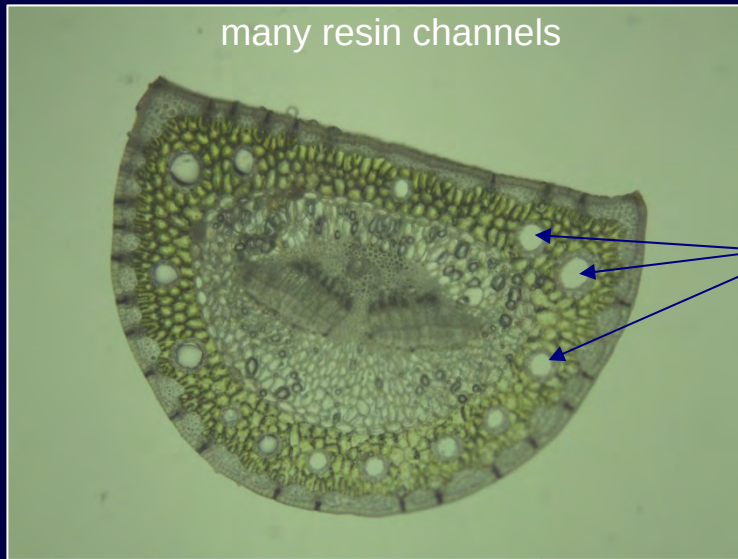
🌲 Needle preparation:

↳ Branches harvested the day before

↳ Cut under water

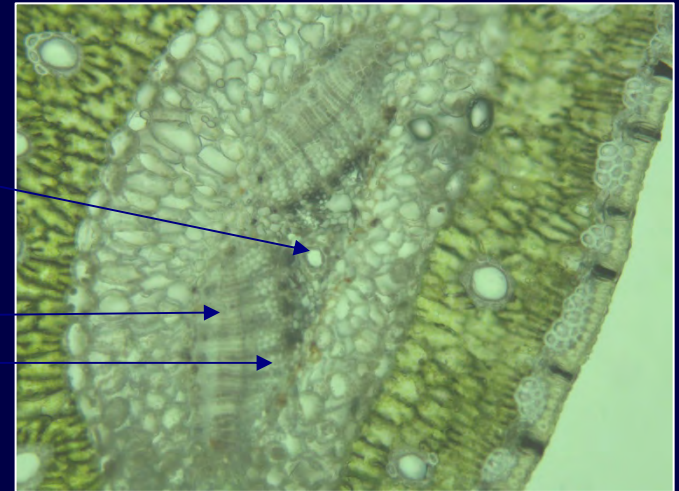
↳ Rehydrated in refrigerator with plastic bag

Cavitron



resin
channel

phloem
xylem



To avoid emptying of resin channels:

- Needles immersed 1 hour in cold water (5 – 7°C)
- Needles cut 2 times (15 minutes interval)
 - ↳ Resin channels blocked
 - ↳ Resin does not block xylem



Cavitron

Preparation for cavitron measurements:

- 🌲 Cooled centrifuge and rotor (5 to 7°C)
- 🌲 Needle length (after cutting) : 14,5 cm
- 🌲 20 needles inserted in water reservoir

Cavitron measurements:

- 🌲 Procedure according to Cochard et al., 2005
- 🌲 Temperature cavitron at 5 to 7°C during the measurement
- 🌲 Use of software “Cavisoft” (Regis Burlett, Biogeco)
- 🌲 Problem: Measurements take long time (3 to 4h cavitron measurement per sample)

Acoustic

2 well hydrated twigs

8 sensor

4 sensors per twig

2 Sensors on
STEMS

6 Sensors on
NEEDLES



Acoustic

While acoustic emission measurement were made, branches were slowly dehydrating (bench dehydration) and water potentials were measured every 6 to 12 hours (Scholander apparatus).

stems used for UAE
measurements



Needles used for water
potential measurements

Needles used for UAE
measurements

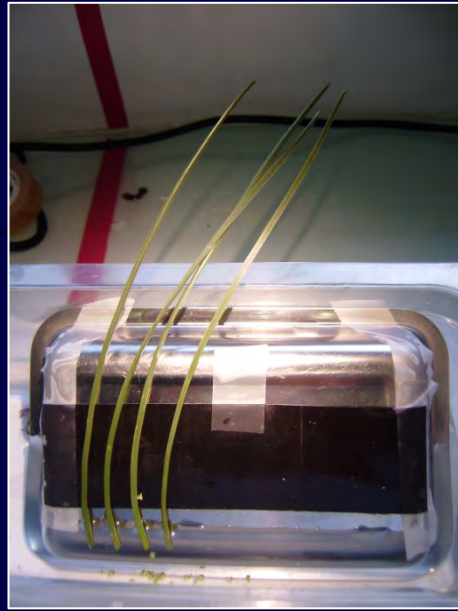


Rehydration kinetics measurements

- 6 well hydrated branches were slowly dehydrated (bench dehydration) from -0,3 to -3 MPa
- Before measurements, branches were bagged and put in the refrigerator to ensure homogeneous Ψ among needles
- Many times during dehydration of branches:
 - Measurements according to “Brodribb and Holbrook, 2003)
 - 2 needles: initial water potential (Scholander apparatus)
 - 4 needles: rehydration measure and final water potential

Rehydration kinetics measurements

Measurement
of initial water
potential (Ψ_0)



Time « t » in
distilled water

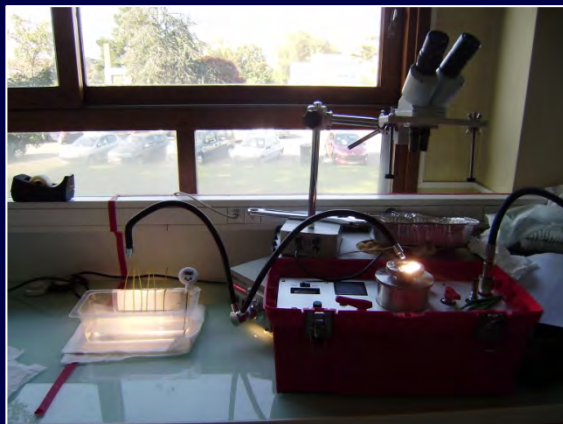
Measurement
of final water
potential (Ψ_f)

Capacitance C_N
[mol·m⁻²·MPa⁻¹]



Conductance K_N
[mmol·m⁻²·s⁻¹·MPa⁻¹]

$$K_N = C_N * \ln (\Psi_0 / \Psi_f) / t$$



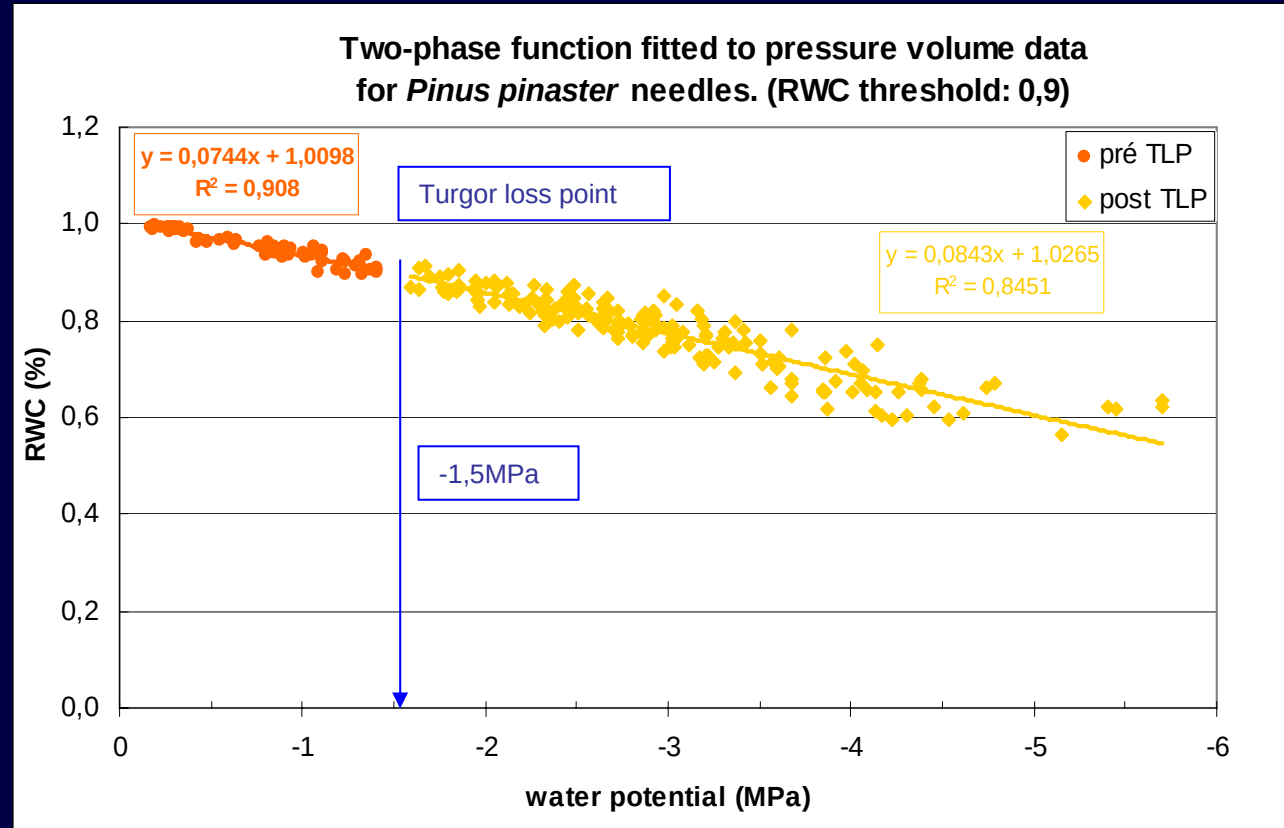
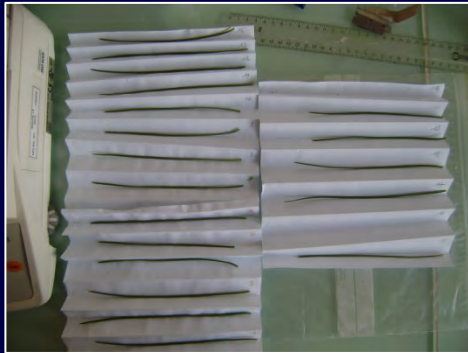
Rehydration kinetics measurements



Needle capacitance C_N :

$$C_N = \Delta RWC / \Delta \Psi * (DW/LA) * (WW/DW) / M$$

DW: leaf dry weight (g); **LA**: leaf area (m²); **WW**: mass of leaf water at 100% RWC (g); **M**: molar mass of water (g.mol⁻¹)

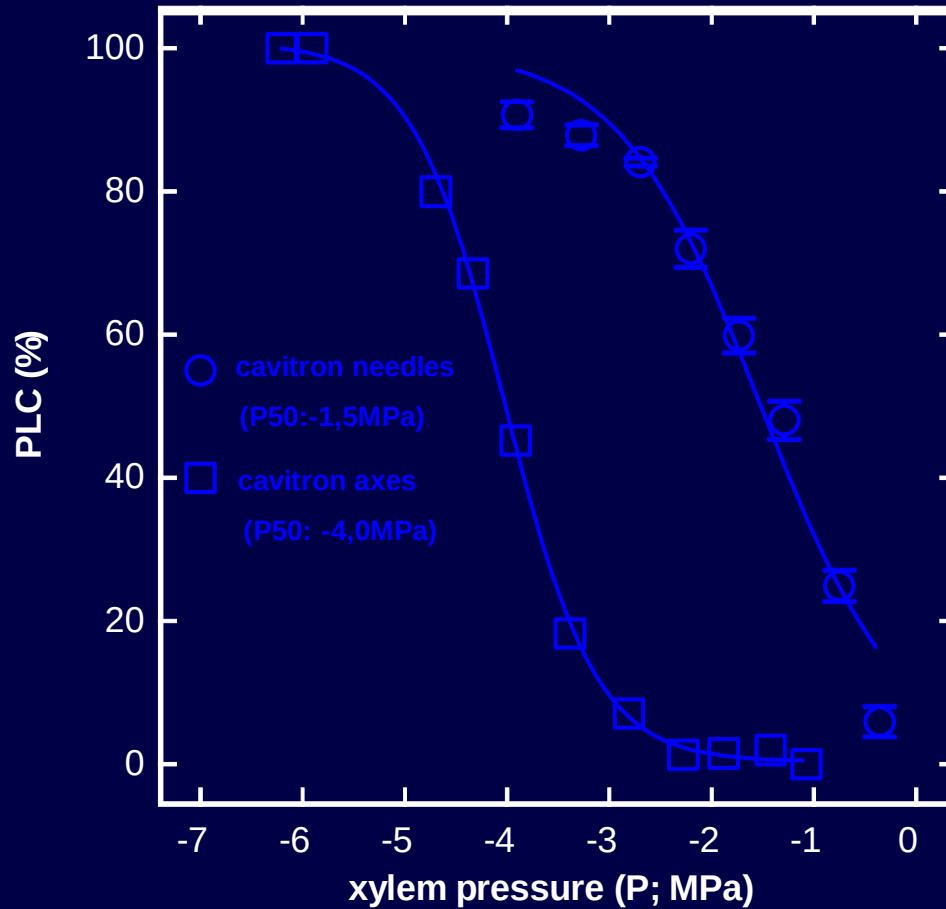


Needle conductance

$$K_N = C_N * \ln (\Psi_0 / \Psi_f) / t$$

Cavitron measurements

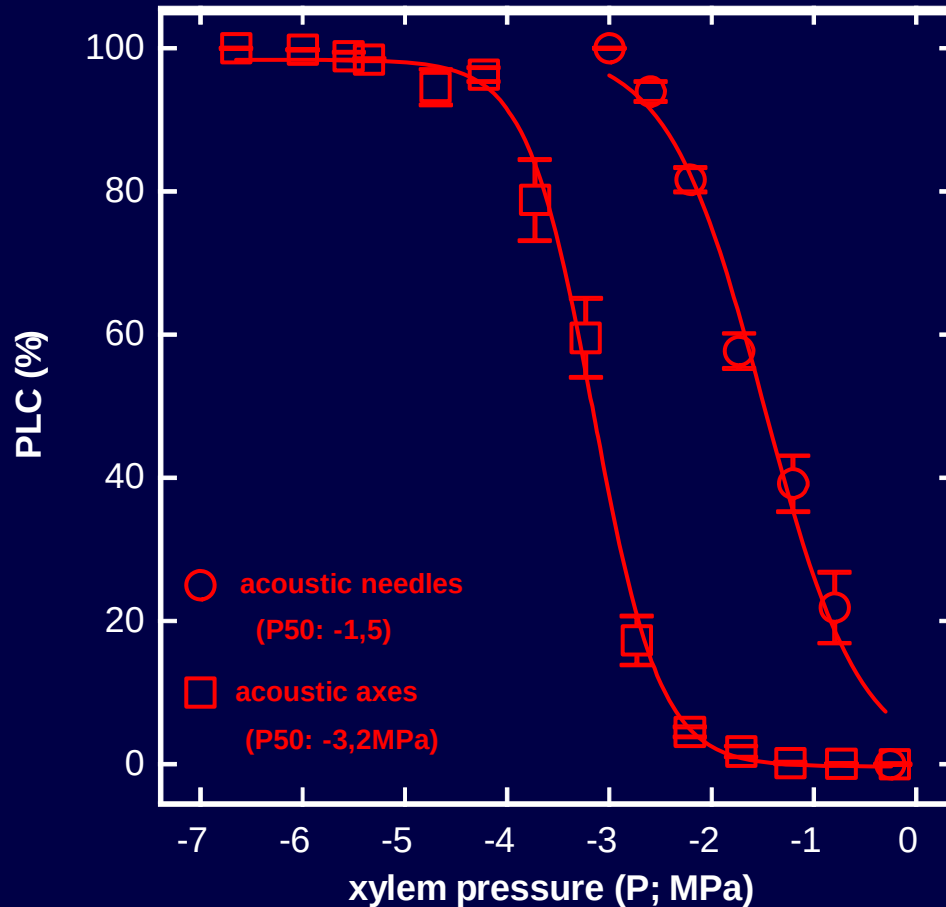
Vulnerability curves of *Pinus pinaster* needles and twigs



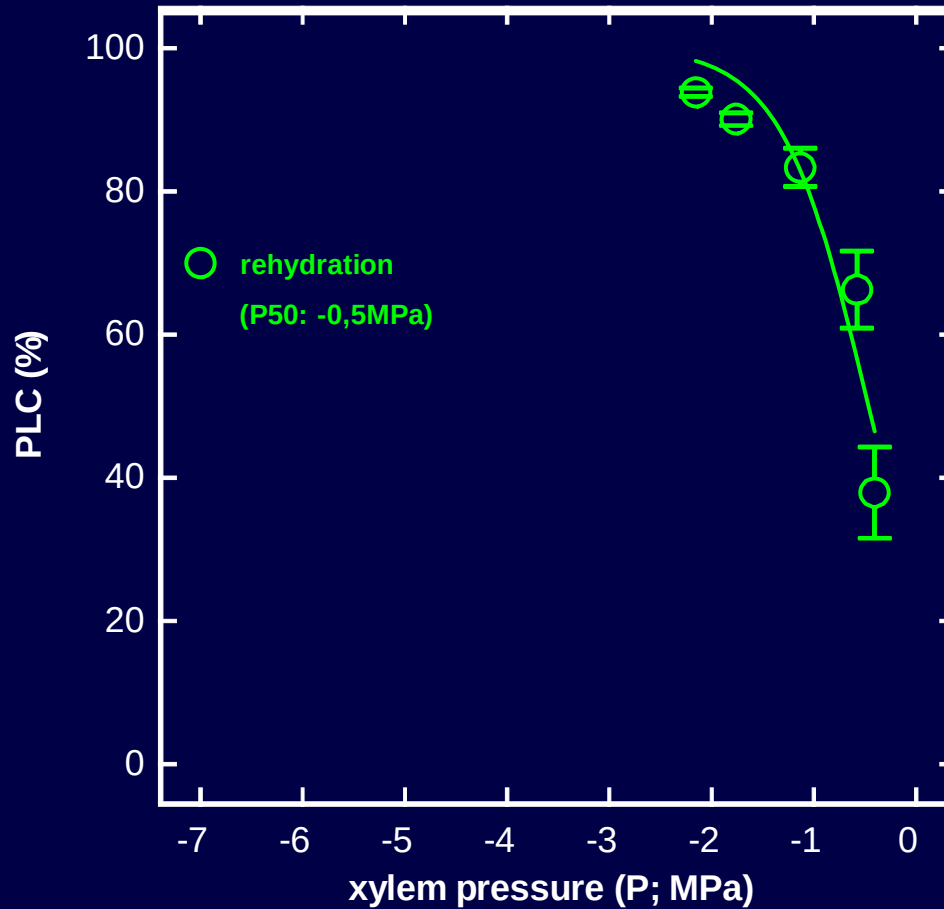
 Ψ_{50} needles: **-1,5 MPa**

 Ψ_{50} twigs: **-4,0 MPa**

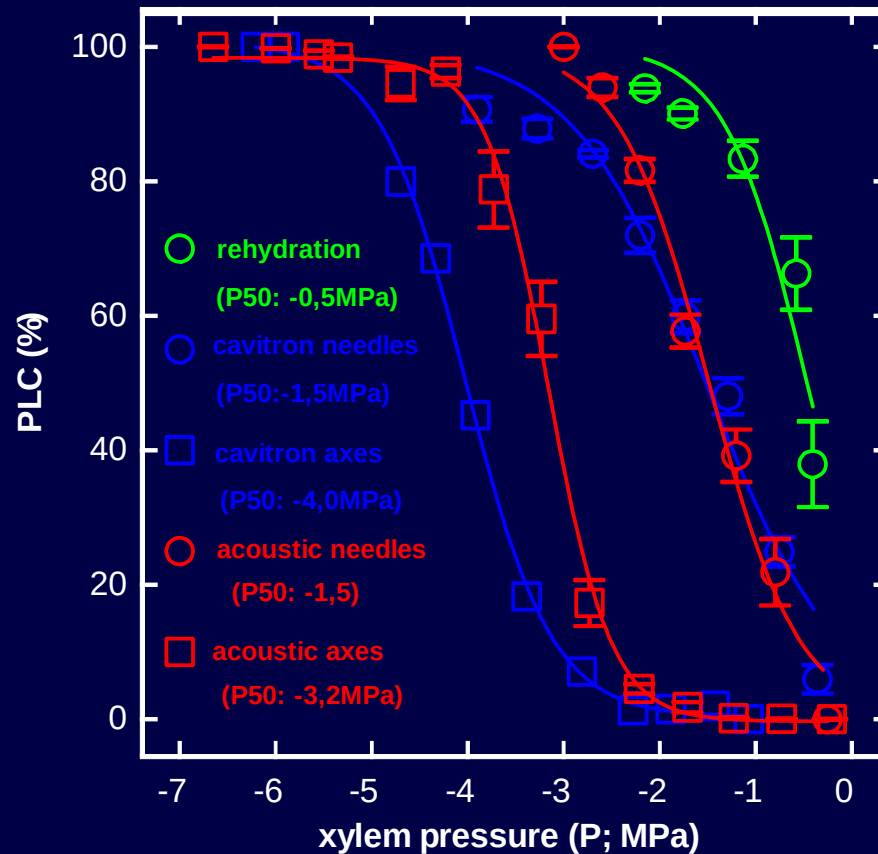
Acoustic measurements

Vulnerability curves of *Pinus pinaster* needles and twigs Ψ_{50} needles : -1,5 MPa Ψ_{50} twigs : -3,2 MPa

Rehydration kinetics measurements

Vulnerability curve of *Pinus pinaster* needles Ψ_{50} needles: -0,5 MPa

Pinus pinaster needles vulnerability



Ψ_{50} needles

-0,5MPa (rehydration)

-1,5 MPa (cavitrion)

-1,5 MPa (acoustic)

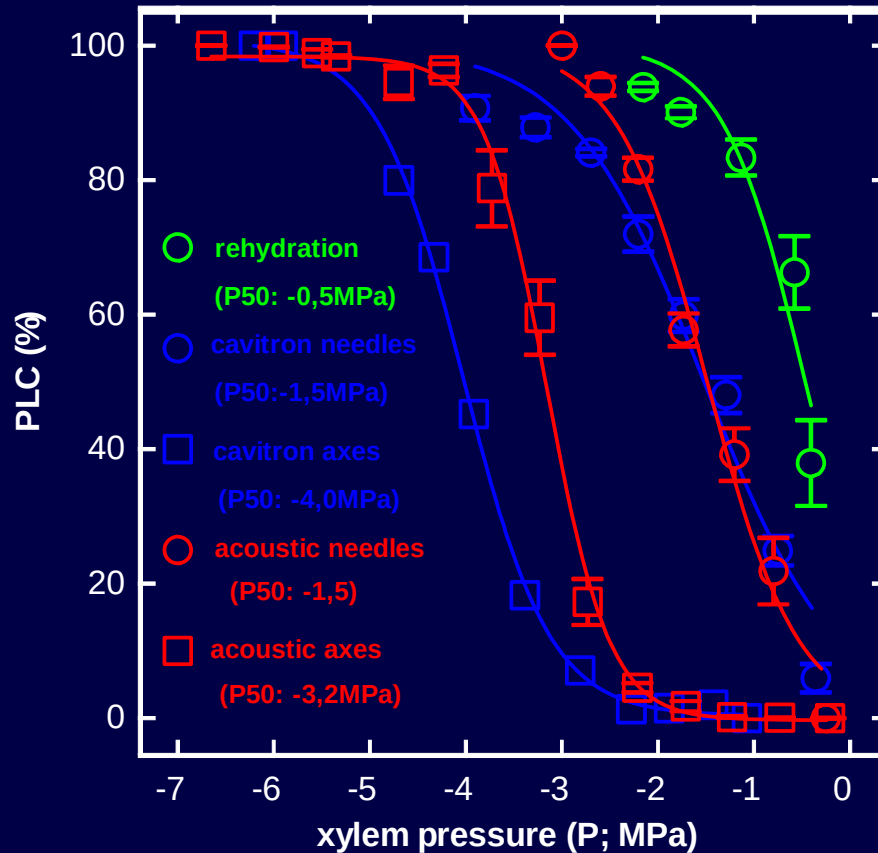


Quite similar results between cavitrion and acoustic



High Ψ_{50} with rehydration kinetics method

Pinus pinaster vulnerability



Ψ_{50} needles

-0,5MPa (rehydration)

-1,5 MPa (cavitrion)

-1,5 MPa (acoustic)

Ψ_{50} branches

-4,0 MPa (cavitrion)

-3,2 MPa (acoustic)



Quite similar results between cavitrion and acoustic

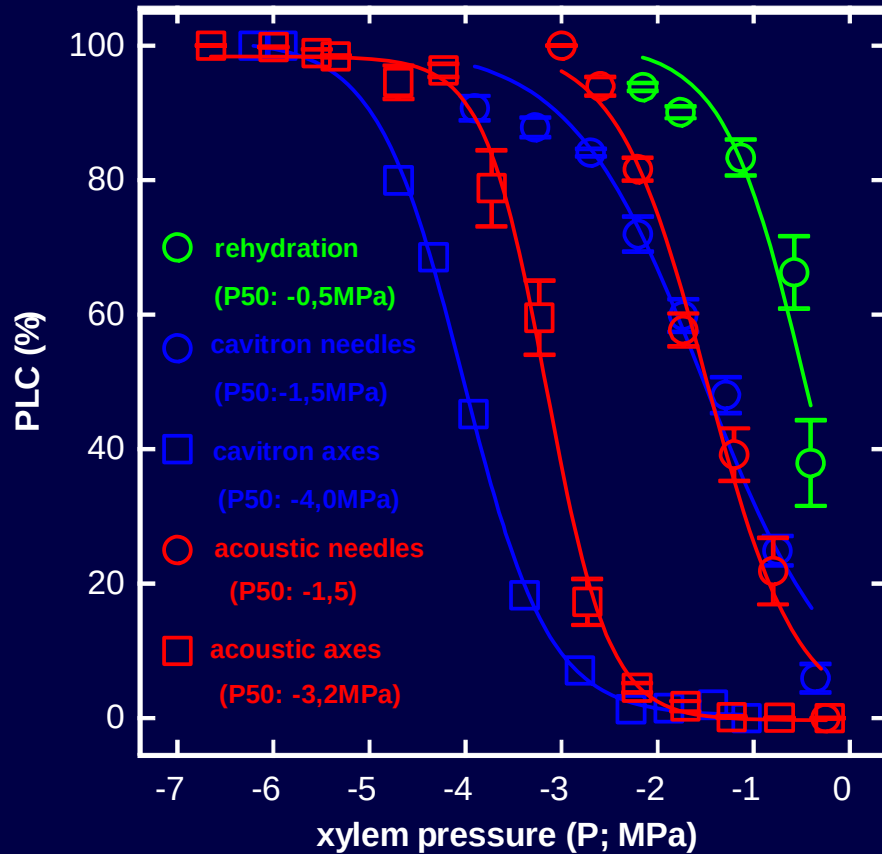


High Ψ_{50} with rehydration method



Loss of conductivity in needles occurs before cavitation in branches

Pinus pinaster vulnerability



Overall high Ψ_{50} in needles



Methodical problems?



Vulnerability of needles:



cavitation?



Collapse? (fast recovery)



Something else?

Ψ_{50}	needles	twigs
acoustic	-1,5 MPa	-3,2 MPa
cavitron	-1,5 MPa	-4,0 MPa
rehydration	-0,5MPa	

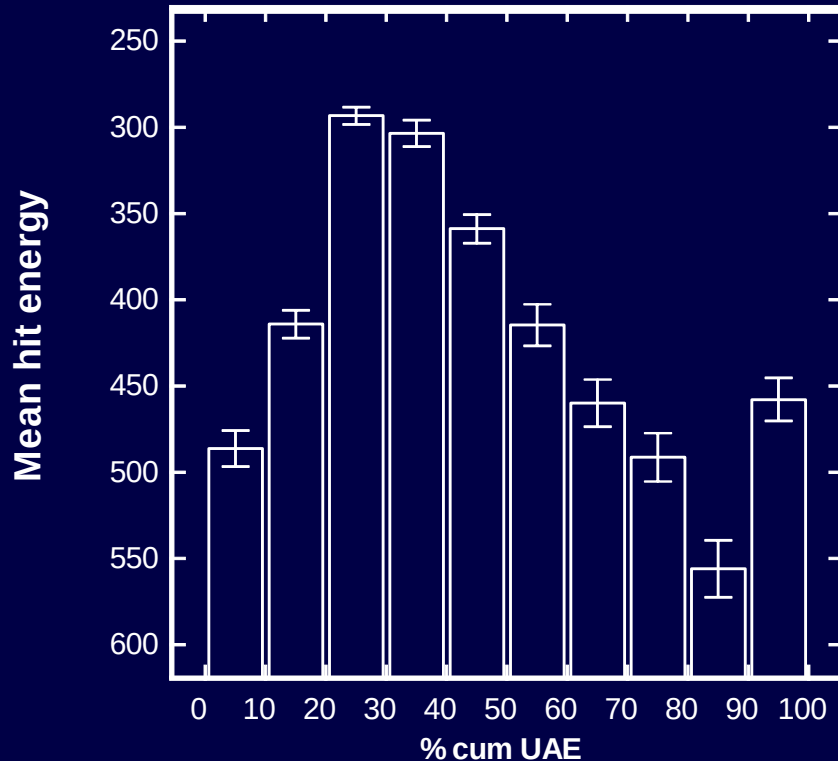


Is the needle capacitance related to Ψ_{50} in needles?

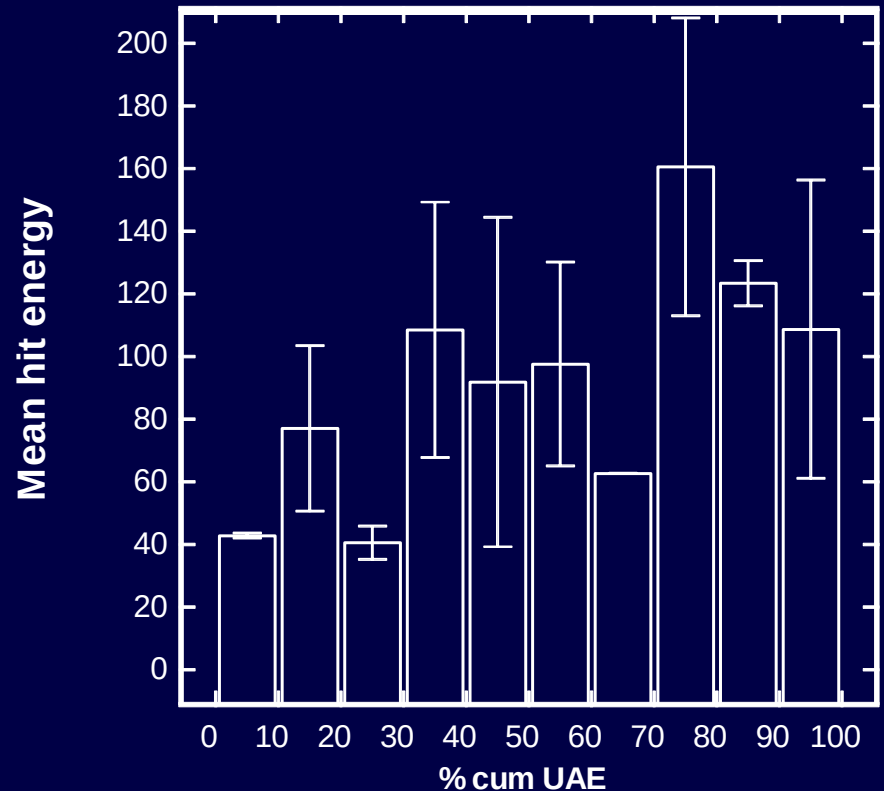
Cavitation ou collapse?

hit energy from twigs and needles during dehydration
(acoustic measurements)

Twigs



Needles



Most energy between 20 to 40
% of cum UAE



“cavitation pattern” in twigs



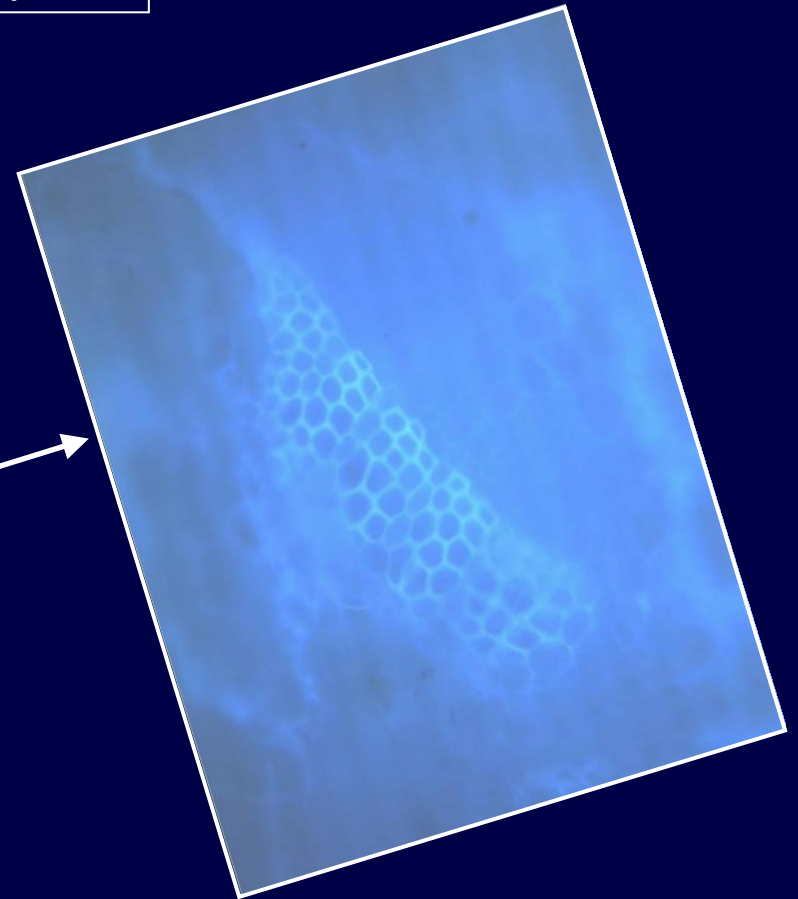
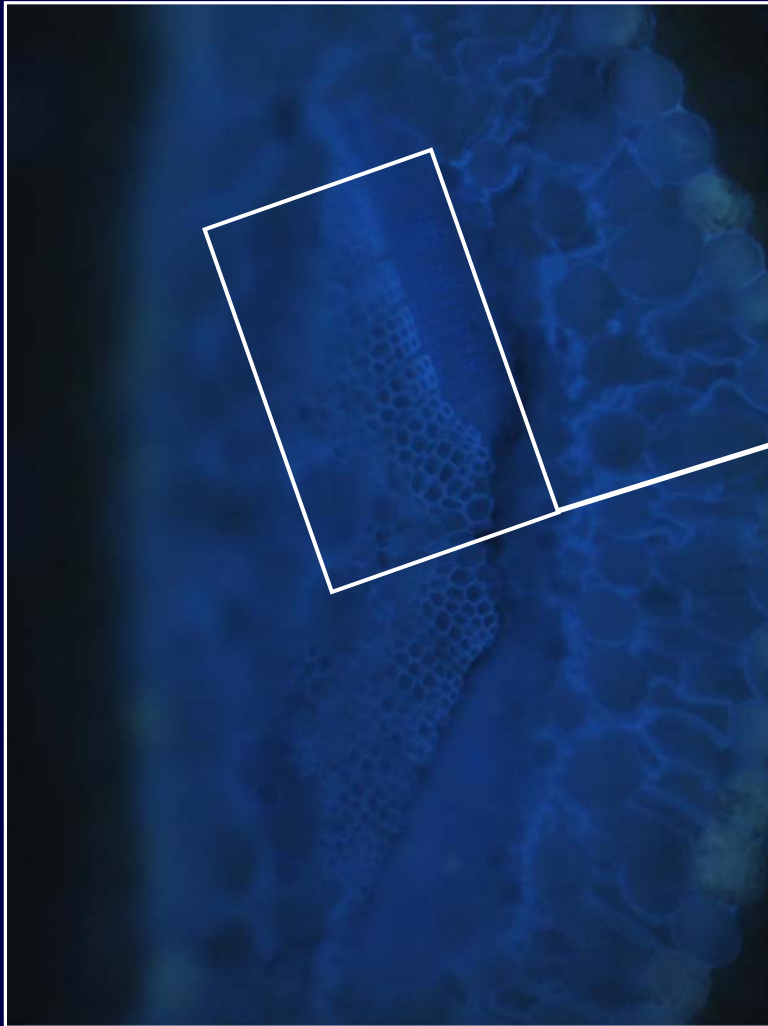
Less energy than in twigs



Complex pattern

Cavitation ou collapse?

Epifluorescence technique on frozen samples

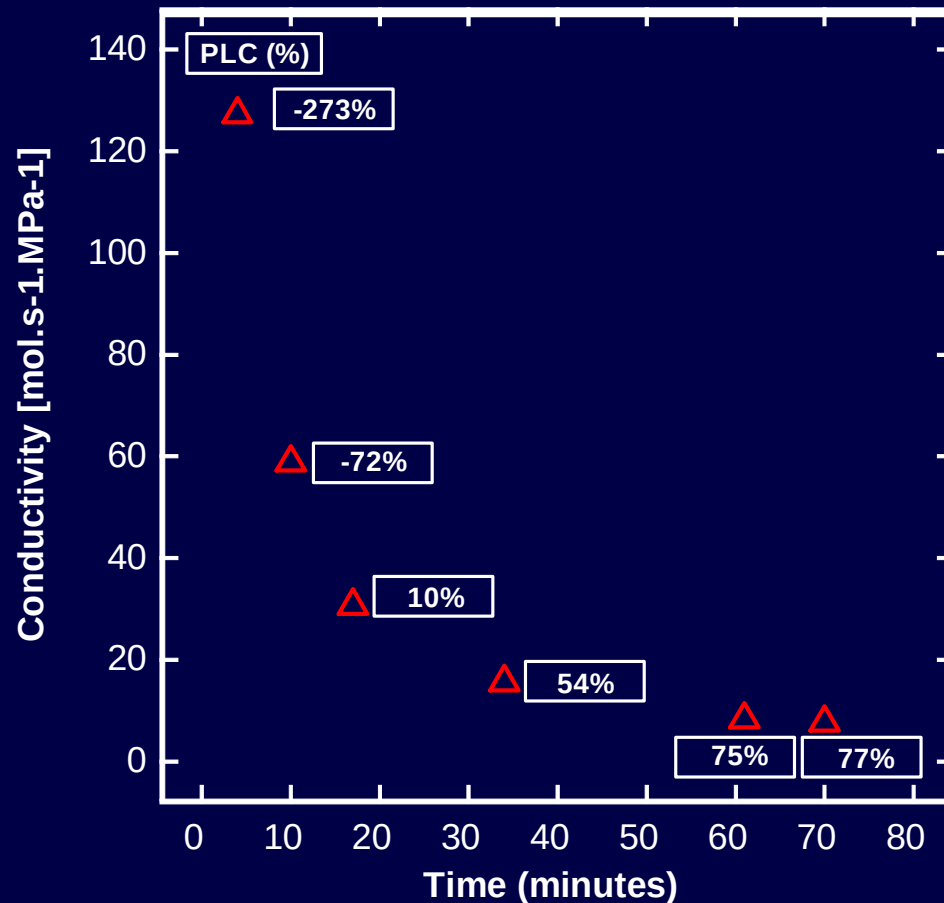


On fresh to dried needles:

No observed collapse

Capacitance

Time to recovery the loss of conductivity reached at -3,9MPa
at -0,4MPa with cavitron



Ψ max: -3,91MPa
(80% PLC)

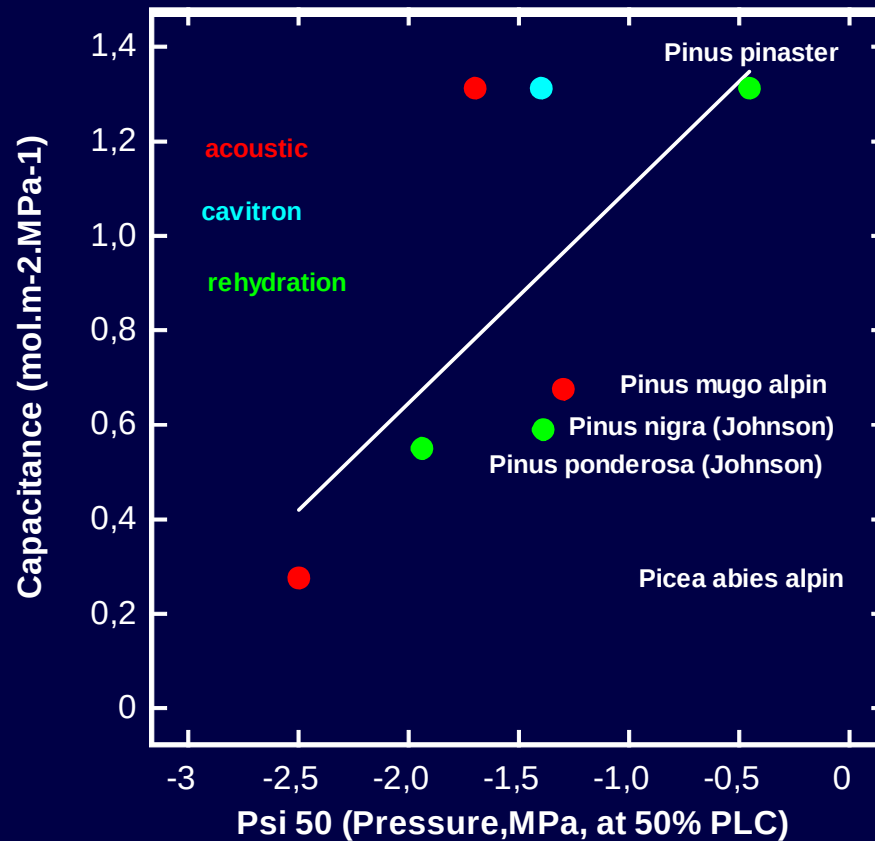
Ψ measure: -0,43MPa



High capacitance in *Pinus pinaster* needles

Capacitance

Needles capacitance VS needles vulnerability (P50)



 Linear relation between Ψ_{50} and capacitance

 More vulnerable needles have a bigger capacitance

 *Pinus nigra* and *Pinus ponderosa* of „Johnson, 2009,PCE“

Acknowledgments:



Fonds zur Förderung der Wissenschaftlichen
Forschung



University of Innsbruck and Bordeaux
Institute of Botany and UMR Biogeoco



Jean-Baptiste Lamy, Yann Guengant



Thanks !



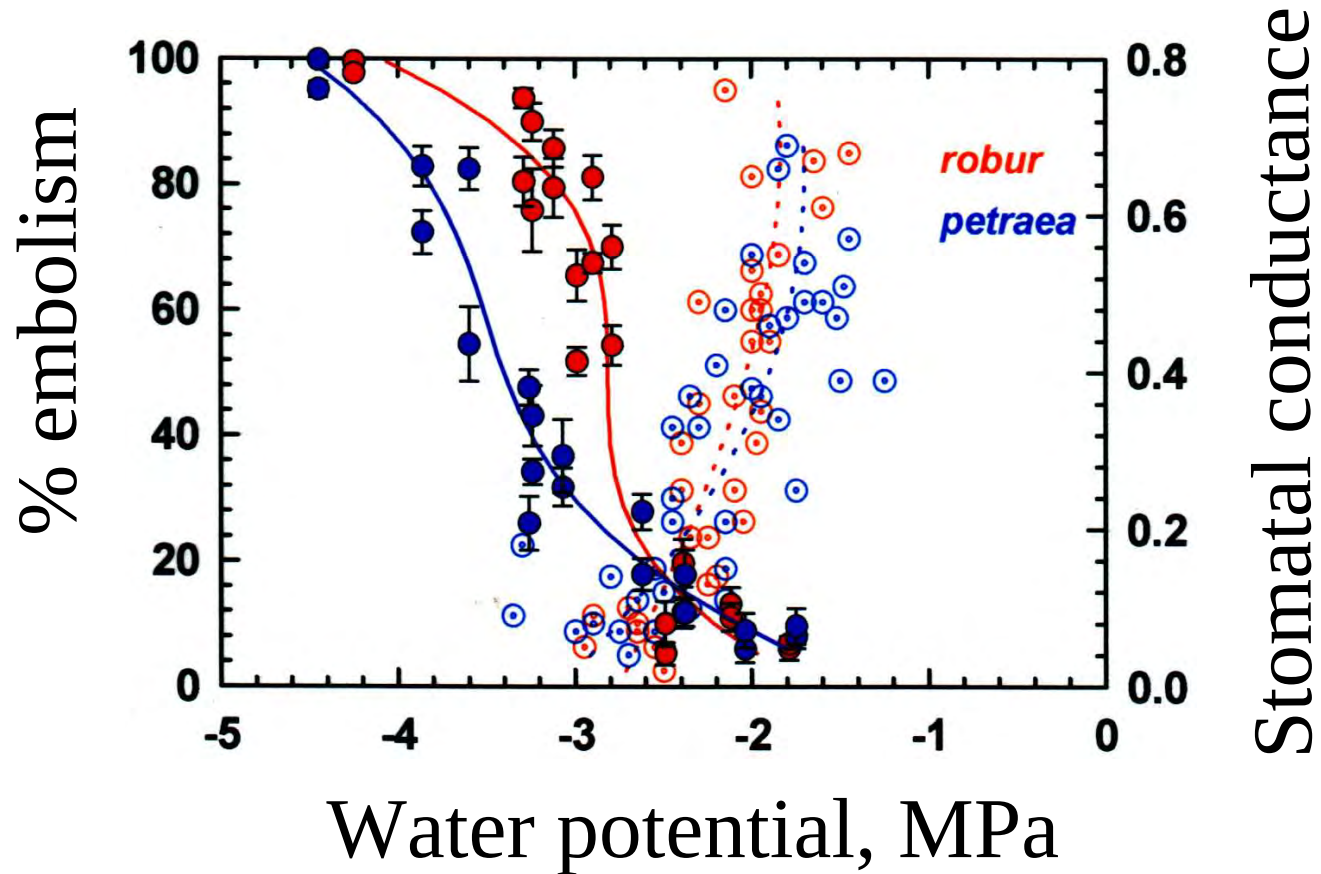
Miraculous xylem refilling in plants

Hervé Cochard

UMR PIAF

INRA, Clermont-Ferrand, France

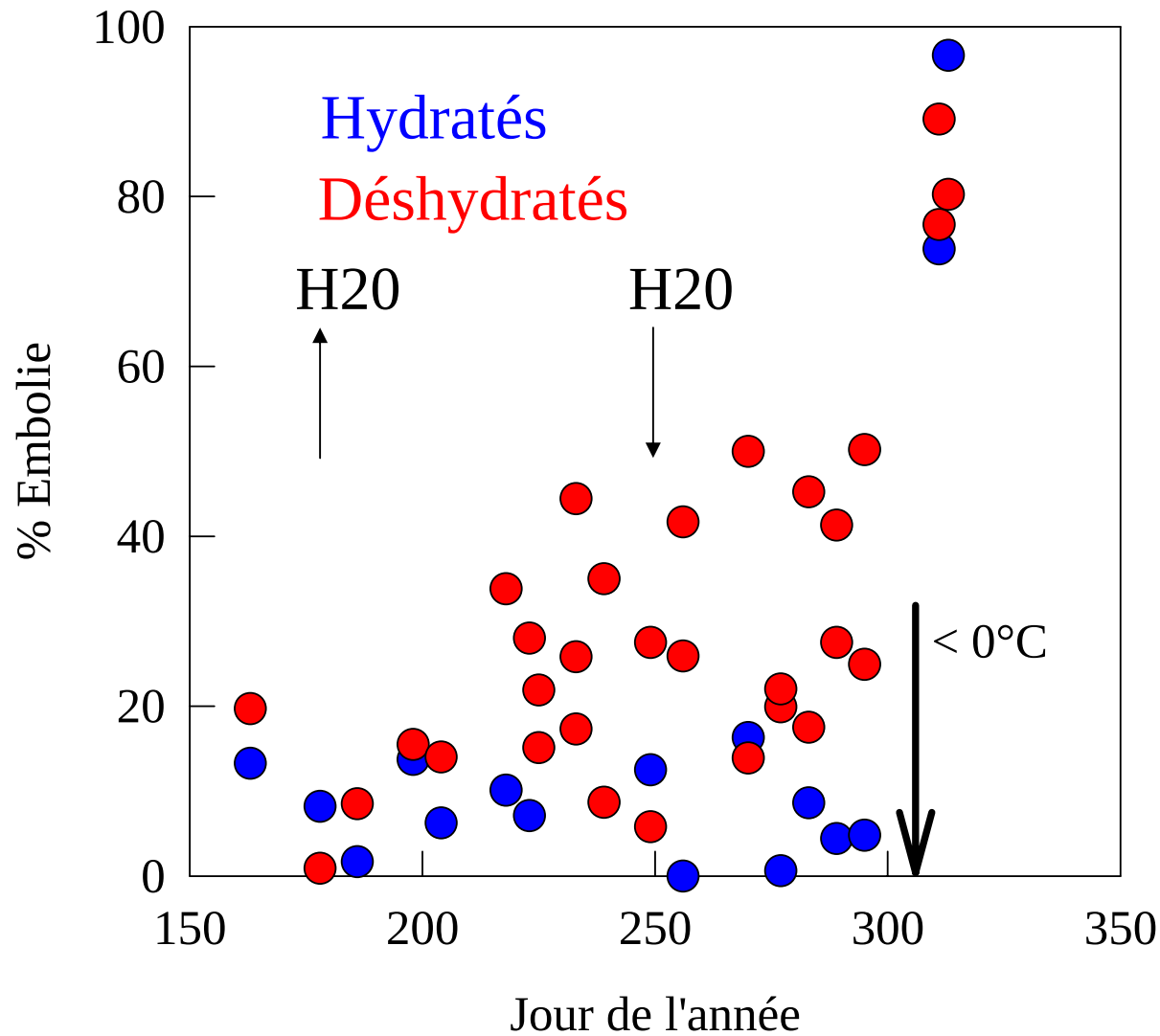
Previous paradigms



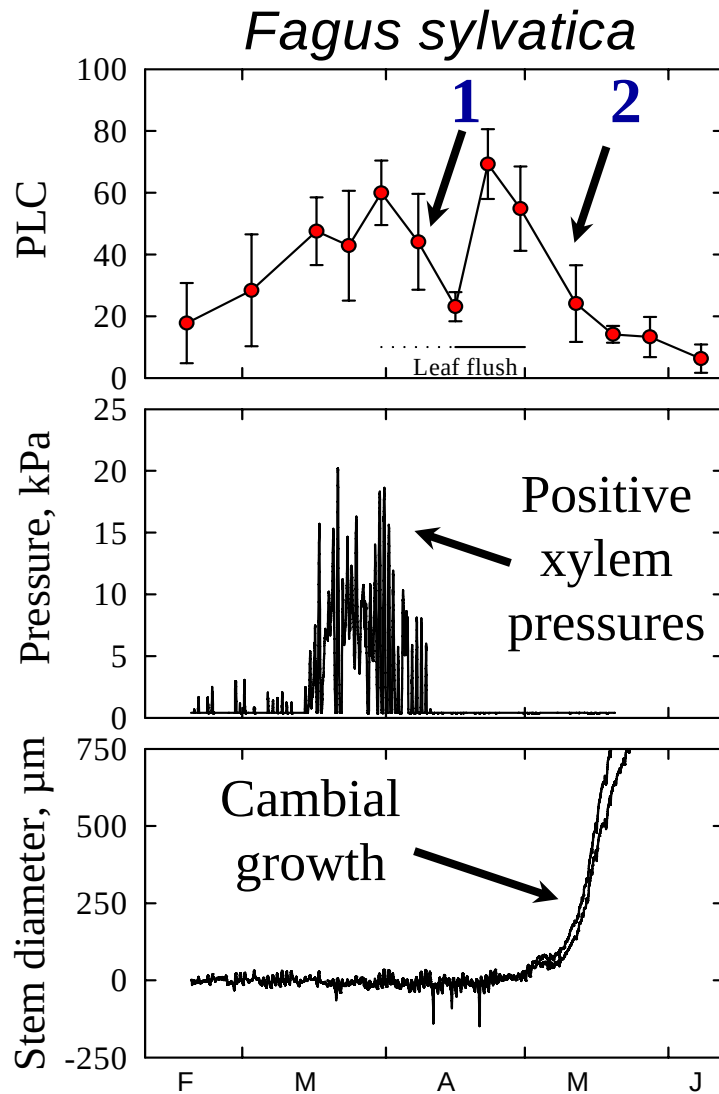
Plants operate near the point of xylem dysfunction

Stomatal closure prevents xylem embolism

Previous paradigms



How plants were able to recover from xylem embolism?



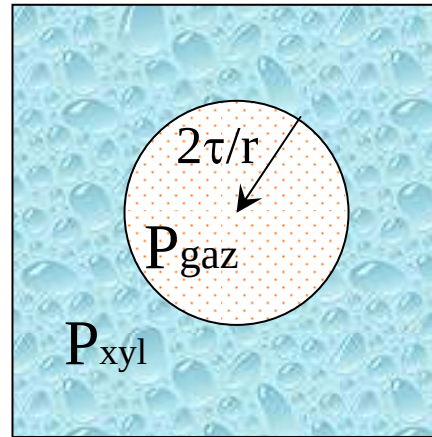
1- Xylem refilling

2- Xylem recovery

Physics of xylem refilling

(Yang and Tyree 1992)

Air bubble at
atmospheric
pressure (P_{gaz})



Xylem sap
saturated with air
at P_{xyl} pressure

For the bubble to collapse:

$$\mathbf{P_{xyl} > P_{gaz} - 2\tau/r}$$

If $r = 30 \mu\text{m}$ $P_{\text{xyl}} > -5\text{kPa}$



No transpiration + root pressure to
compensate gravitational forces

Can xylem vessels refill if $P_{\text{xyl}} < - 2\tau/r$?

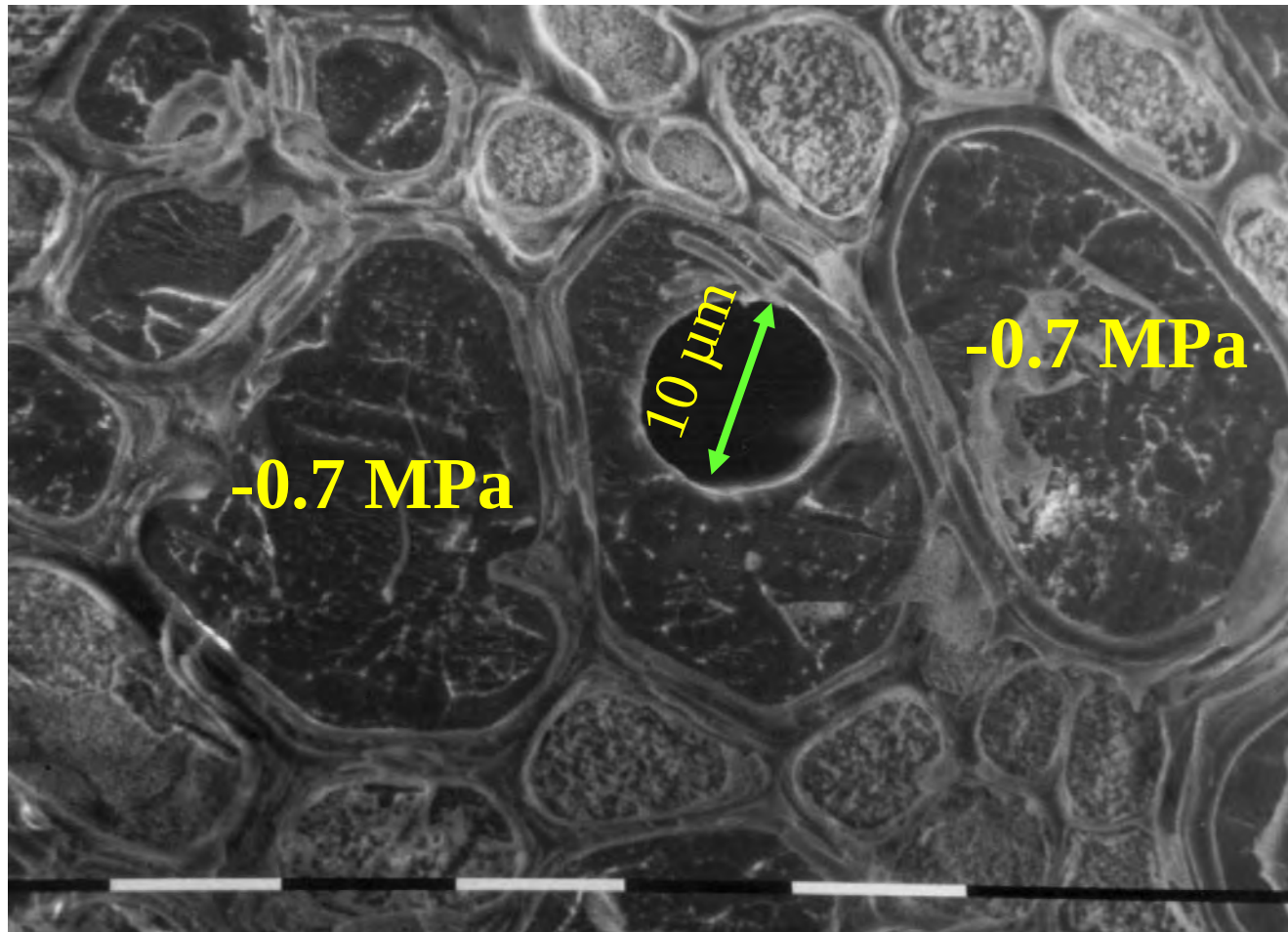
Many reports in the literature of refilling
when $P_{\text{water}} < - 2\tau/r$ and transpiration is high

Cryo-SEM : virtually all the studies

Acoustic technique : many reports

Hydraulic technique : more and more reports

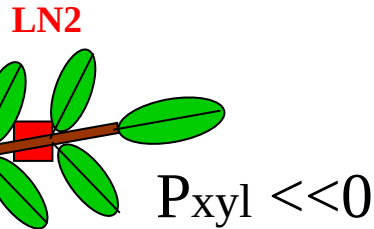
Evidence from the Cryo-SEM technique



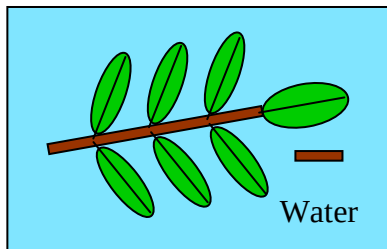
Refilling while $P_{\text{xyl}} < 2\tau/r$?

Diurnal trends of embolism in walnut petiole (Cochard et al 2000)

Cryo-SEM ●



**Cryo-SEM
+ $P_x = 0$** ●

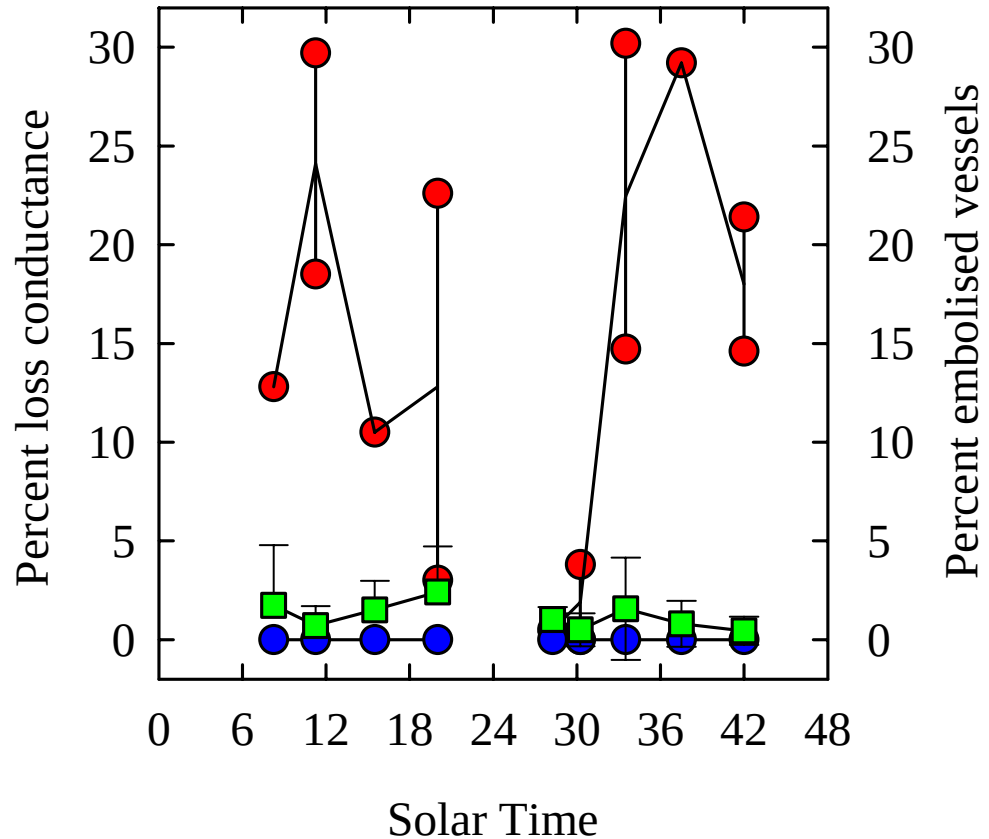


$P_{xyl} = 0$

Hydraulic ■



Freezing artifact when $P_{xyl} < 0$



Evidence for xylem refilling while $P_{\text{xyl}} < - 2\tau/r$?

Vitis (Salleo et al 1989, Brodersen et al 2010, Zufferey et al 2011)

Laurus (Salleo et al 1996, Tyree et al 1999, etc...)

Pinus (Edwards et al 1994)

Zea (McCully et al 1998)

Fraxinus (Zwieniecki et al 1998)

Acer (Zwieniecki et al 1998)

Picea (Zwieniecki et al 1998)

Helianthus (Nardini et al 2008)

Oryza (Stillet et al 2005)

Populus (Secchi et al 2010)

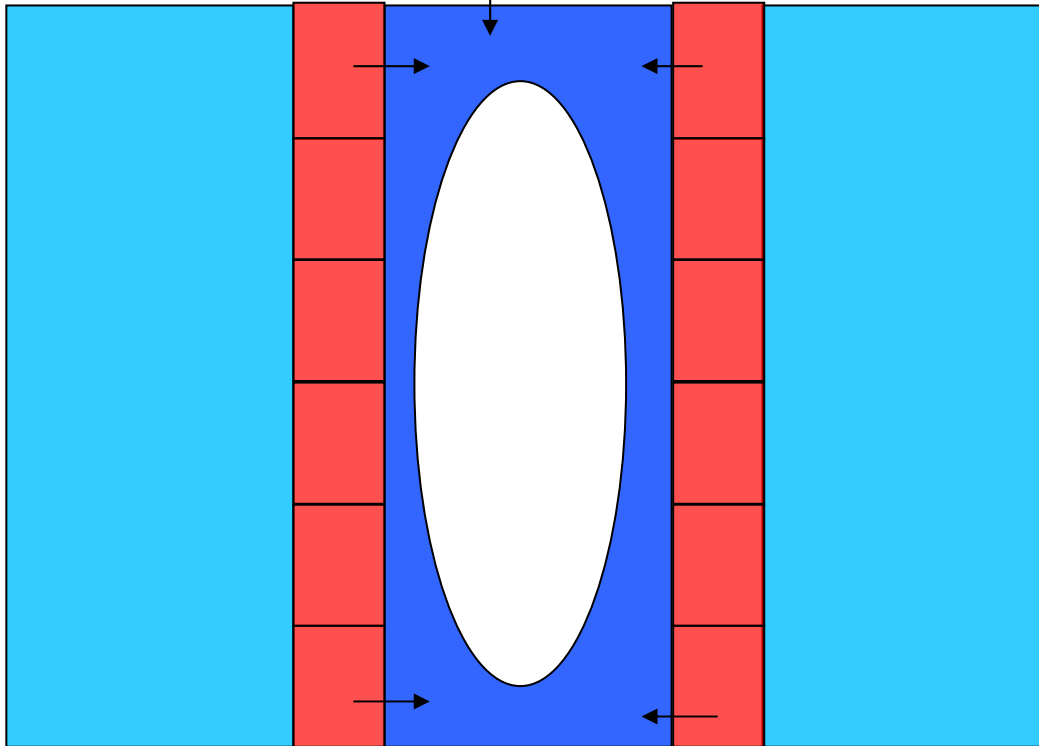
...

What is this novel mechanism?

“Do we need a new paradigm?” (Tyree et al 1999)

“Do we need a miracle?” (Holbrook and Zwieniecki 1999)

$$P_{\text{xyl}} > P_{\text{gaz}} - 2\tau/r$$



Hydraulic isolation ?

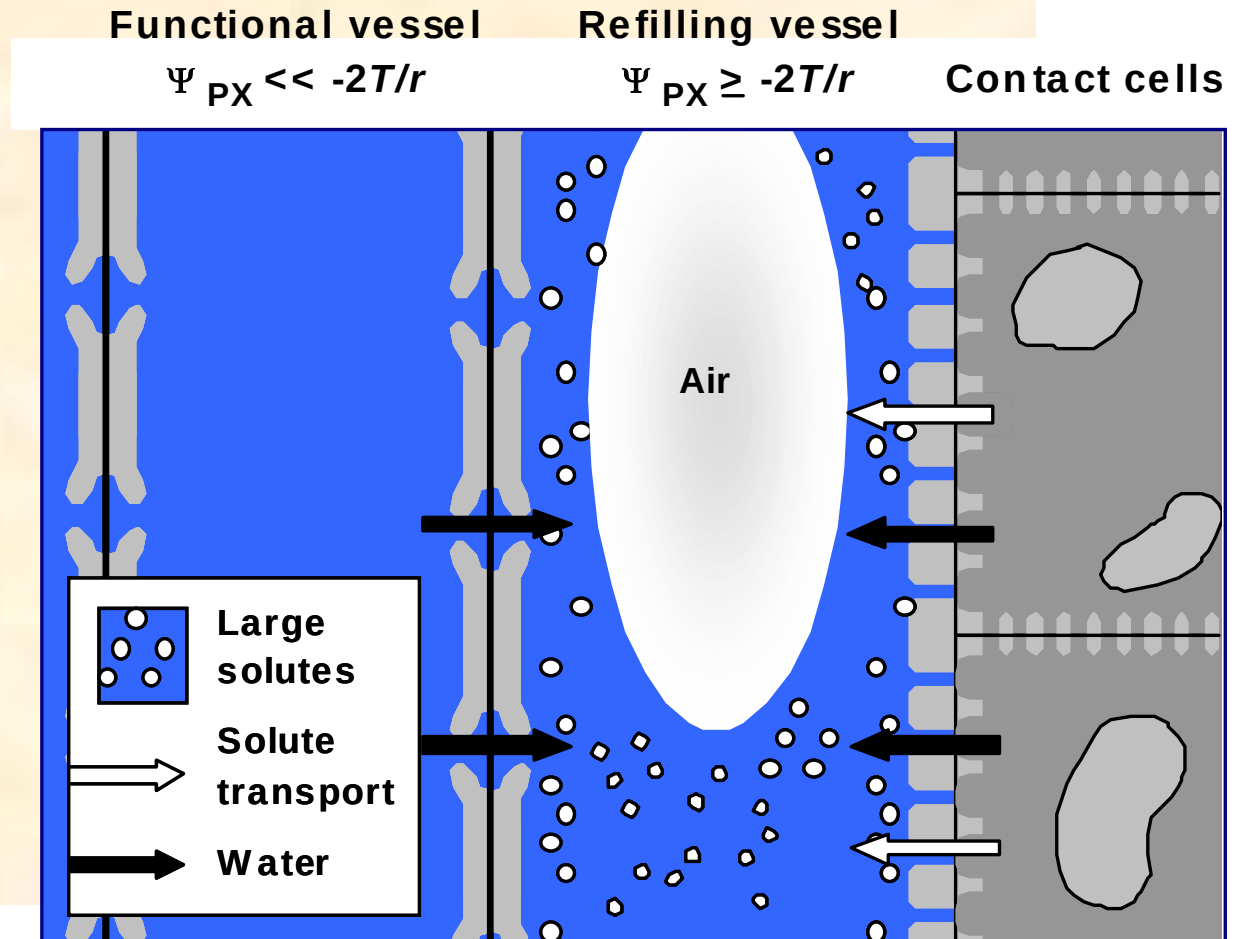
Driving force for water flow ?

Hypotheses for a “novel” refilling mechanism

The “large organic solute” hypothesis (Hacke & Sperry 2003)

Large organic solute
flow into the vessel
causing a water flow
and the refilling

Pit membrane acts as
an osmotic membrane

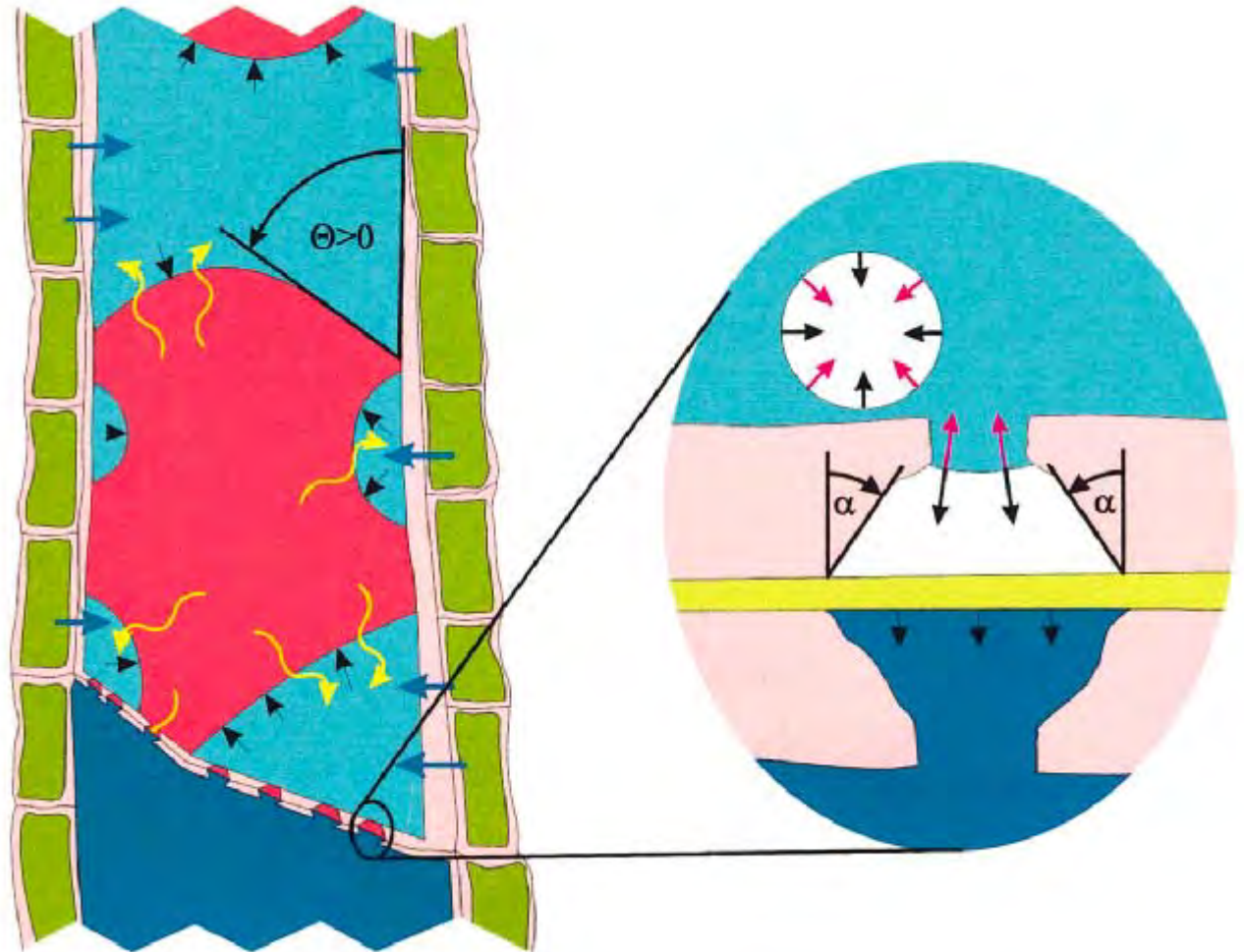


Hypotheses for a “novel” refilling mechanism

The “pit valve” hypothesis (Holbrook & Zwieniecki 1999)

Solute flow into the
vessel causing a water
flow and the refilling

Embolised vessels
have to be
hydraulically isolated



More insights into this “novel” refilling mechanism

Phloeme is important (girdling exp) Salleo et al 2003, Bucchi et al 2003

Starch and sucrose is implicated in the mechanism : Salleo et al 2006

Requires energy (Proton pump) Salleo et al 2004

Aquaporins are implicated Sakr et al 2003, Secchi & Zwieniecki 2010

Sensing and triggering the refilling

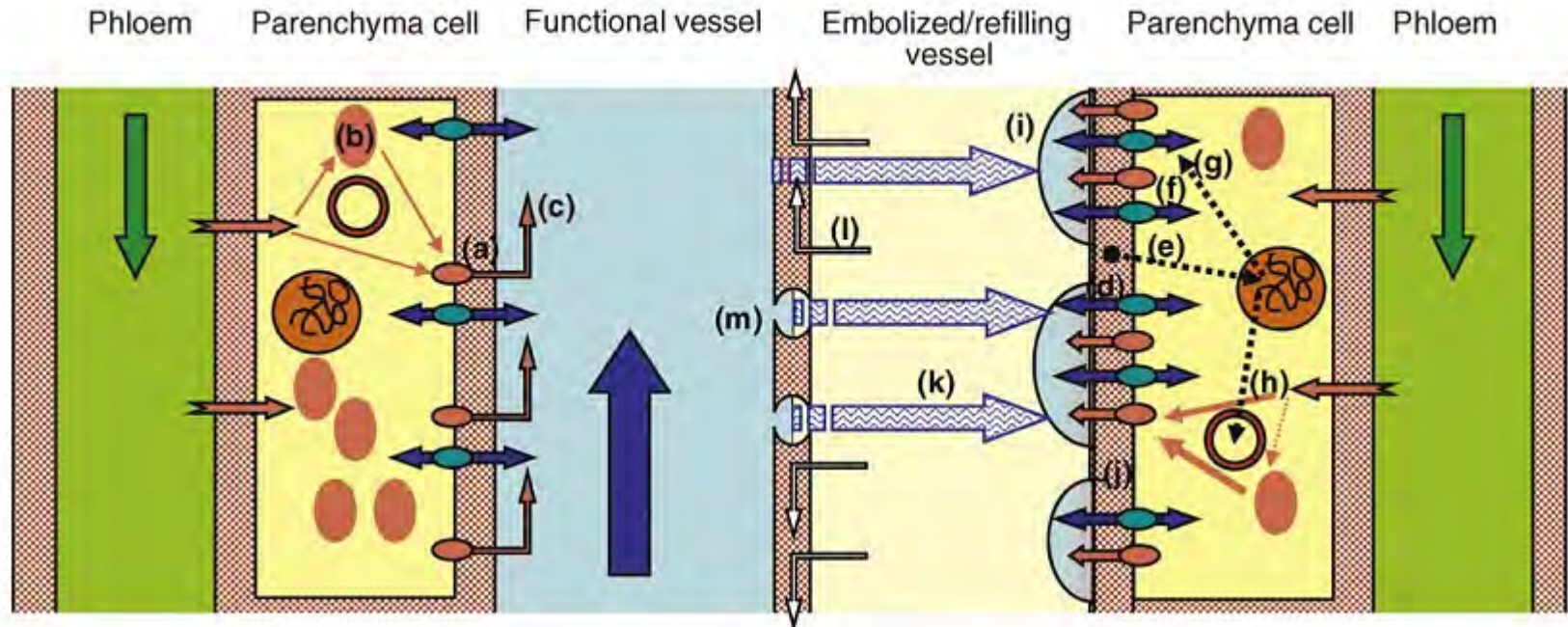
- Wall vibration Salleo et al 2008

- Chemical sensing (Zwieniecki and Holbrook 2009)

- Simulations Vesela et al 2003 Gas convection not diffusion

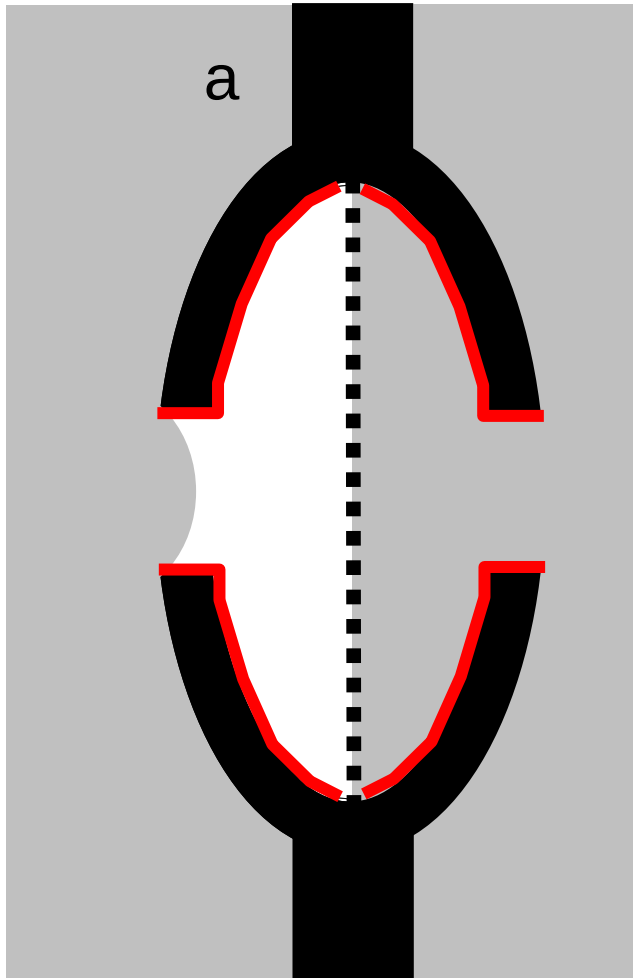
- Water condensation instead of liquid water flow (Zwieniecki and Holbrook 2009)

- New technologies (RMN, Tomography)

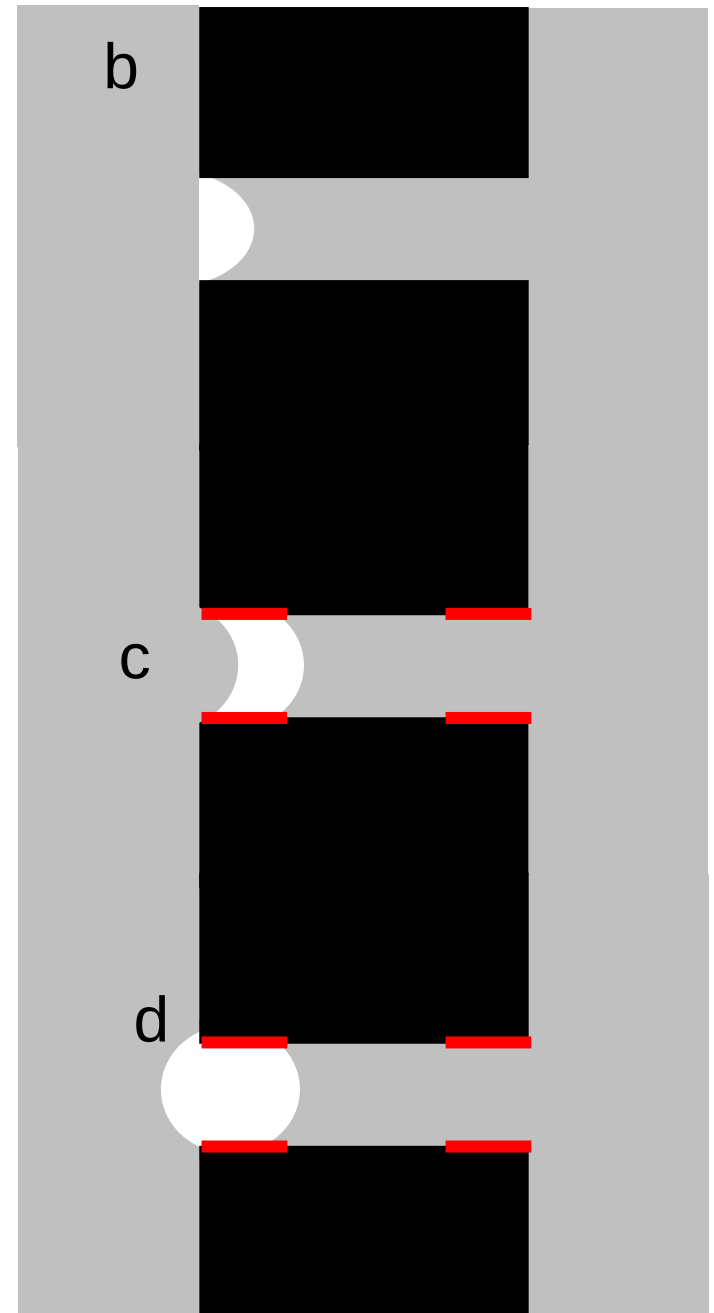


Key:

- | | | |
|--|--|--|
|  Nucleus |  Sugar metabolic activity |  Liquid water flow |
|  Starch |  Sugar flow |  Water vapor flow |
|  Membrane sugar transporter |  Membrane water channel |  Air transport path |
|  Signaling paths | a-m Major points of interest (see figure legend for details) | |



— Hydrophobic layer (lignin?)



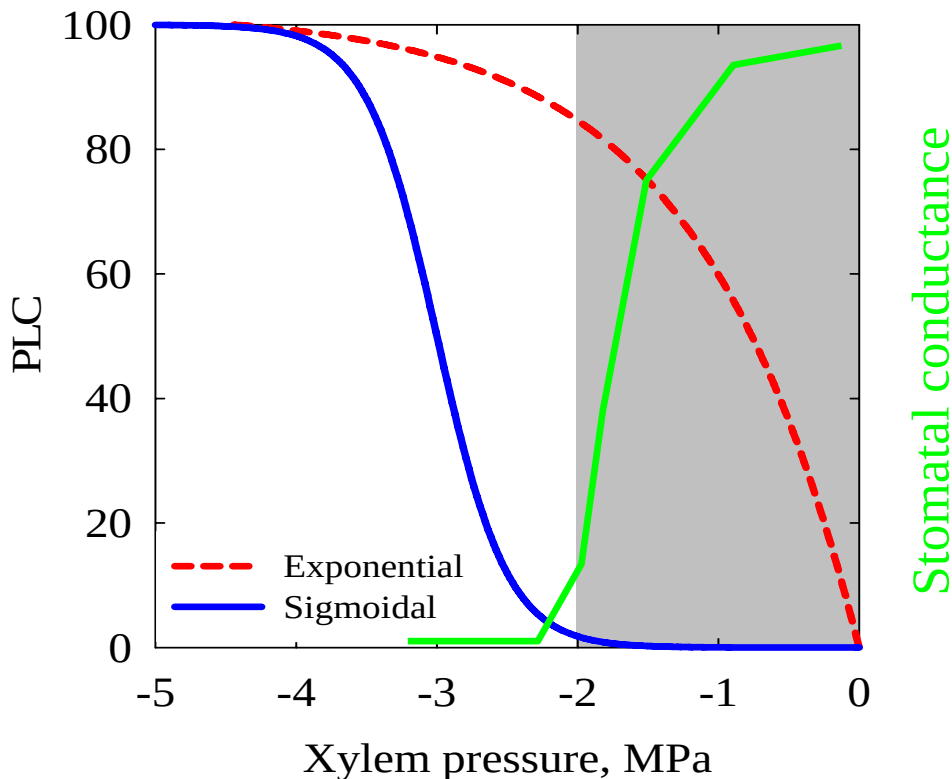
Conclusions

‘Miraculous’ refilling seems to exist

Less and less miraculous

More explicitly included in our experiments

New paradigms



How generic?
Mechanisms ?
Functional benefit ?
(cost vs gain)

What's new at Bordeaux?



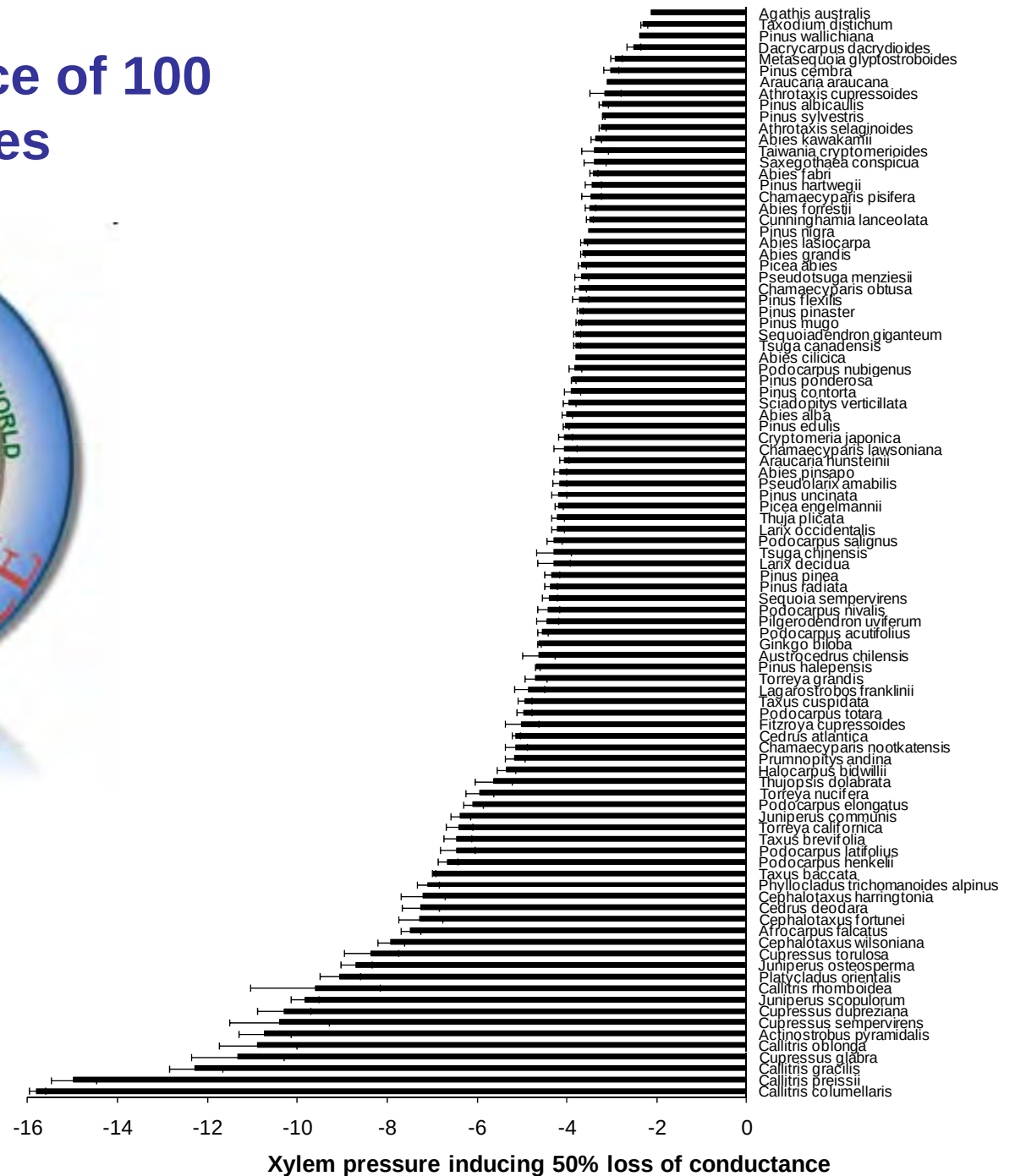
Taxonomic diversity of conifers and species measured for cavitation resistance

	Genera	species	Sampled species	Remaining sp.	sampled genera
Araucariaceae	3	41	3	38	1
Cephalotaxaceae	1	11	3	8	1
Cupressaceae	30	133	32	101	13
Phyllocladaceae	1	4	1	3	1
Pinaceae	11	228	34	194	7
Podocarpaceae	18	186	10	176	0
Sciadopityaceae	1	1	1	0	1
Taxaceae	5	23	6	17	2
Total	70	627	90	537	26

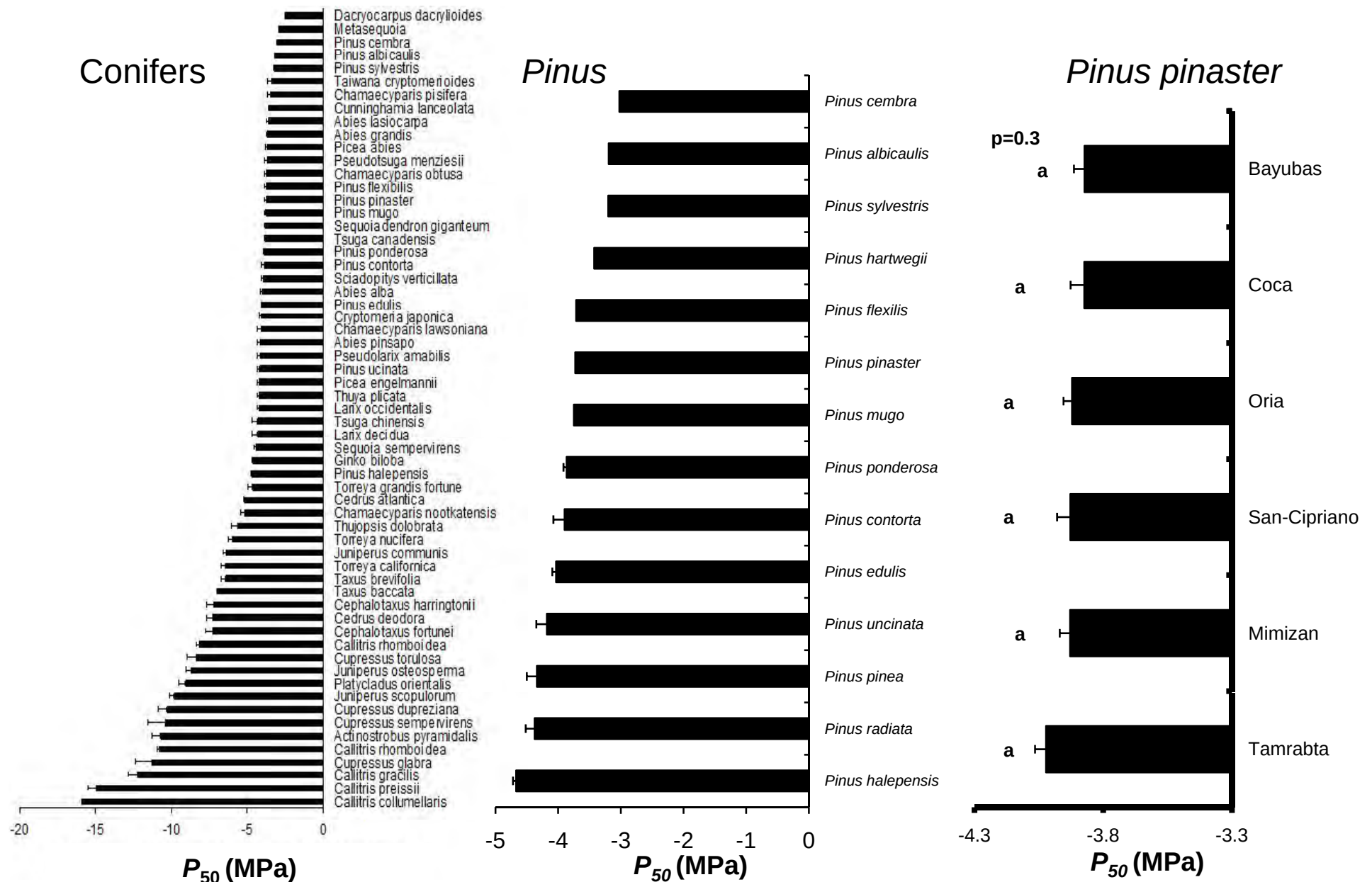
Cavitation resistance of 100 conifer species



High variability of P50
from 2.1 (*Agathis*)
to 16 MPa (*Callitris collu*)



Different levels of variation in cavitation resistance



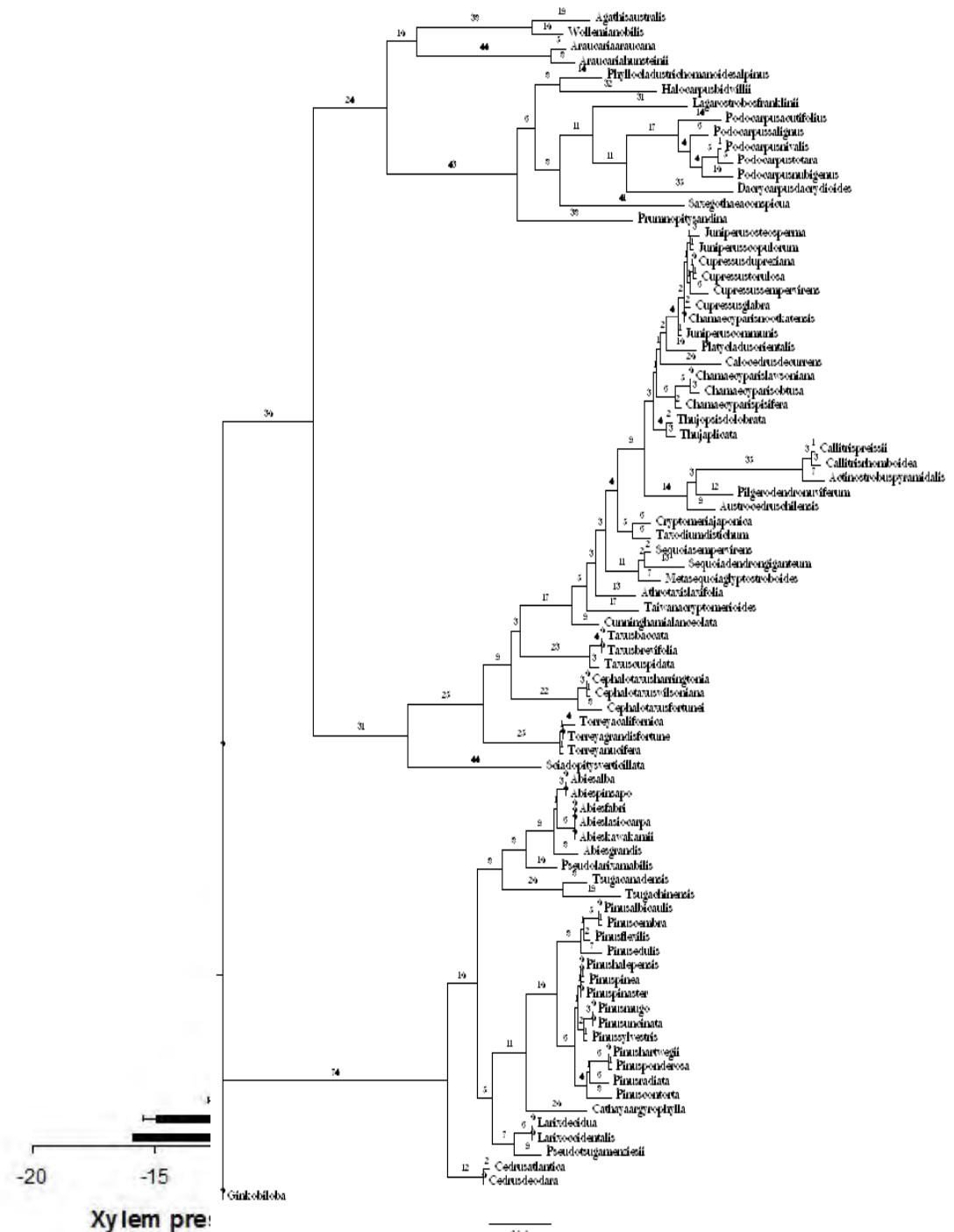
Delzon et al., PCE, 2010

Lamy et al., submitted, PLoS ONE

Objectives

1. Extend the existing database for P50 and other traits (wood density, anatomy)
2. Construct a phylogeny of conifers, using online sequence databases (NCBI)
3. Test for evolutionary convergence versus evolutionary conservatism

Correlate P50 with other measured traits.



Height-related effects on cavitation resistance in maritime pine trees

Sylvain Delzon, Mélanie Lucas, Régis Burlett, Hervé Cochard



Eucalyptus regnans measured at 132.6 m in 1872
near Watts river, Victoria, Australia

It has long been believed that senescence is an inevitable consequence of ageing in all plants and animals.

old animal



old trees



How to test the hydraulic limitation hypothesis ?

$$F = K_L * \Delta\Psi$$

• **small tree**
.....

VPD = 3kPa

$g_s=100$

$F=0.7$

$\Psi_{Lmin} = -4$

$\Delta\Psi = -3.5$

$\Psi_{soil} = -0.5$

$K_L=0.2$



• **tall tree**
.....

VPD = 3kPa

$F=0.35$

$\Psi_{Lmin} = -7.5$

$\Delta\Psi = -7.0$

$\Psi_{soil} = -0.5$

$K_L=0.1$

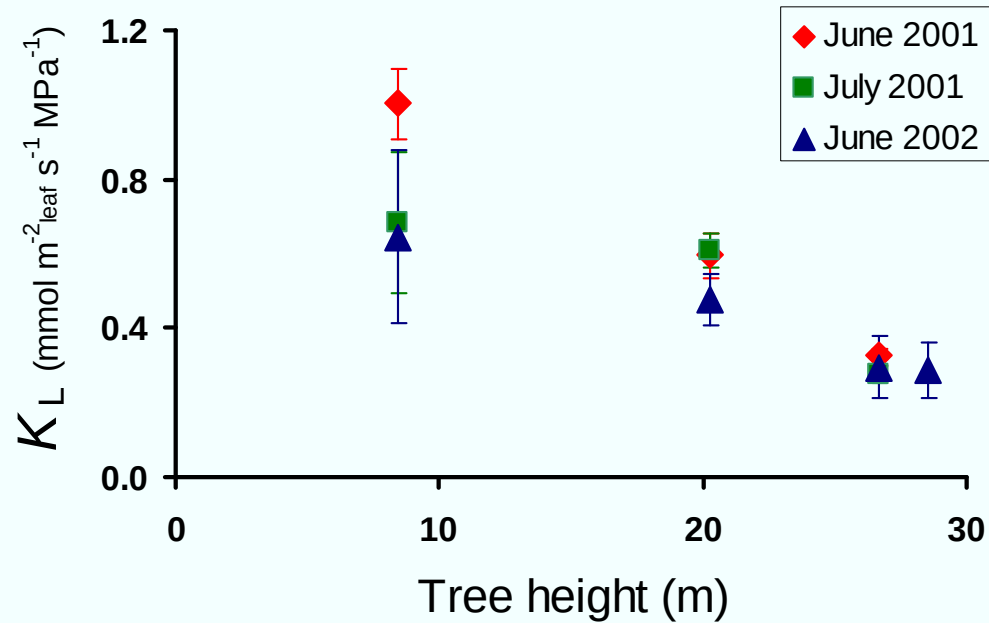


homeostasis

Transpiration
decrease

$g_s=50$

Leaf-specific hydraulic conductance



Leaf-specific hydraulic conductance (K_L) versus tree height

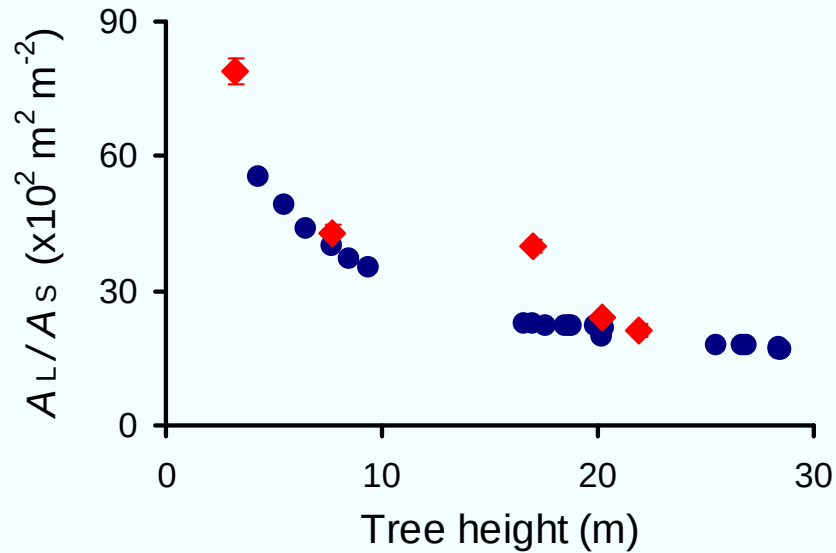


Substantial decrease in hydraulic conductance with increasing tree height

Which hydraulic compensation mechanisms occur?

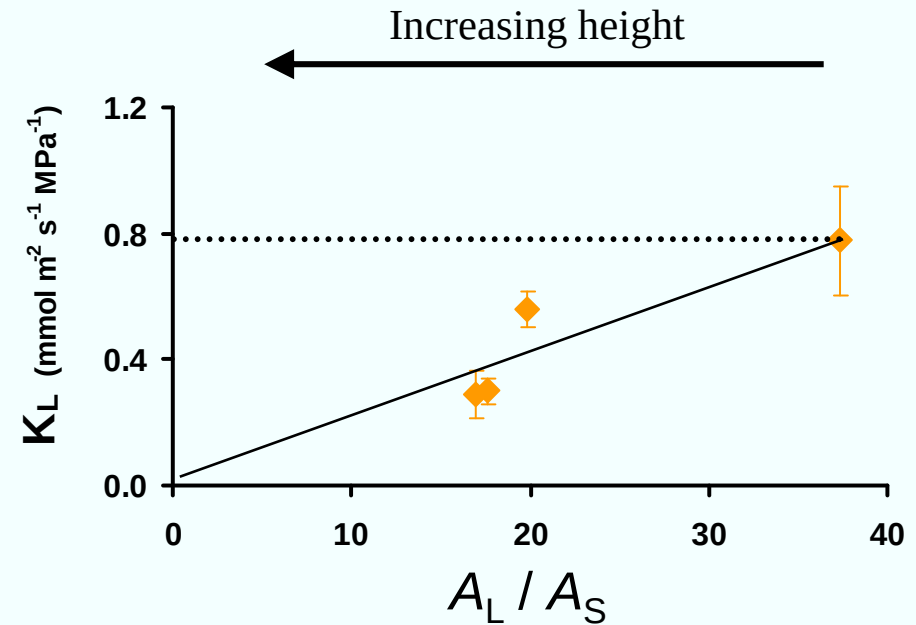
- ✓ hydraulic adjustment: decrease in leaf to sapwood ratio ($A_L:A_S$)
- ✓ increase in soil to leaf water potential gradient (decrease in minimum leaf water potential (Ψ_m))
- ✓ production of xylem tracheids with increased permeability (higher sapwood-specific hydraulic conductivity (k_S))
- ✓ increased water storage in the stem

Compensation mechanisms



Leaf / sapwood area ratio versus tree height

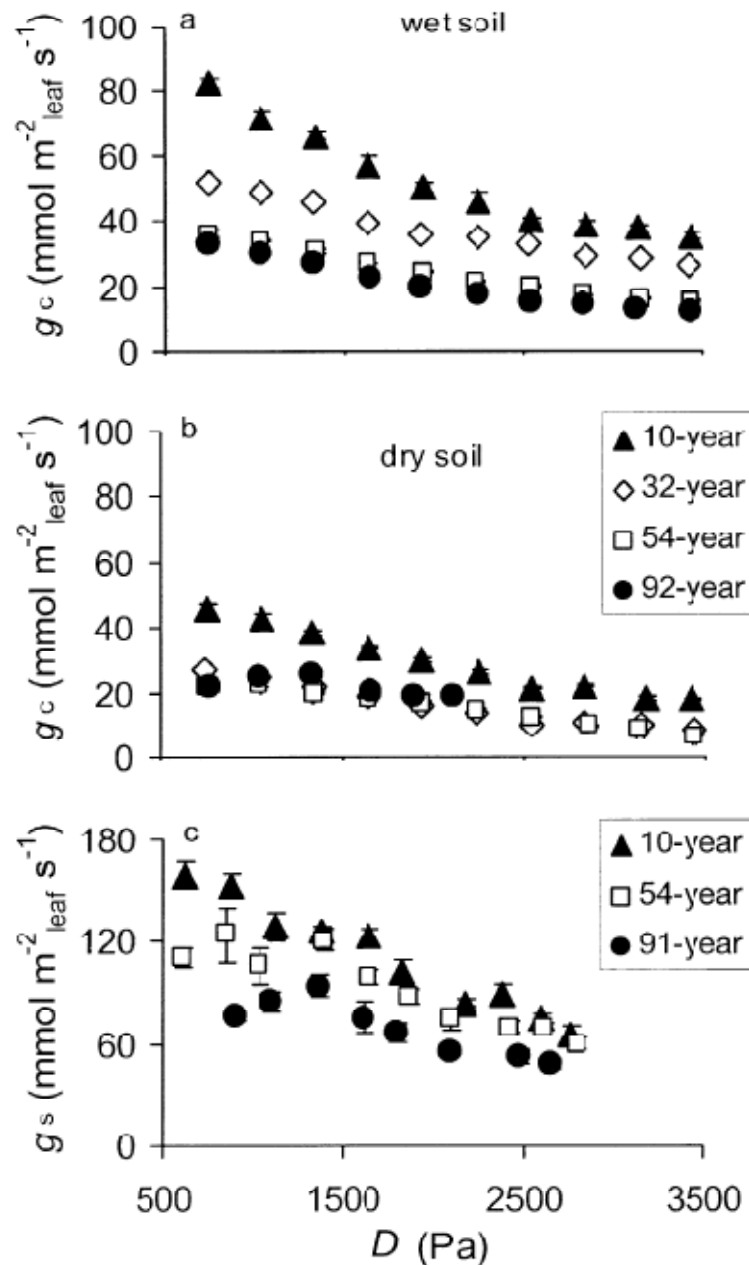
➡ Hydraulic compensation
(decrease in $A_L : A_S$)



Leaf-specific hydraulic conductance versus leaf / sapwood area ratio

➡ Compensation is incomplete

Consequence for stomatal conductance



Stomatal conductance versus air vapor pressure deficit (VPD)

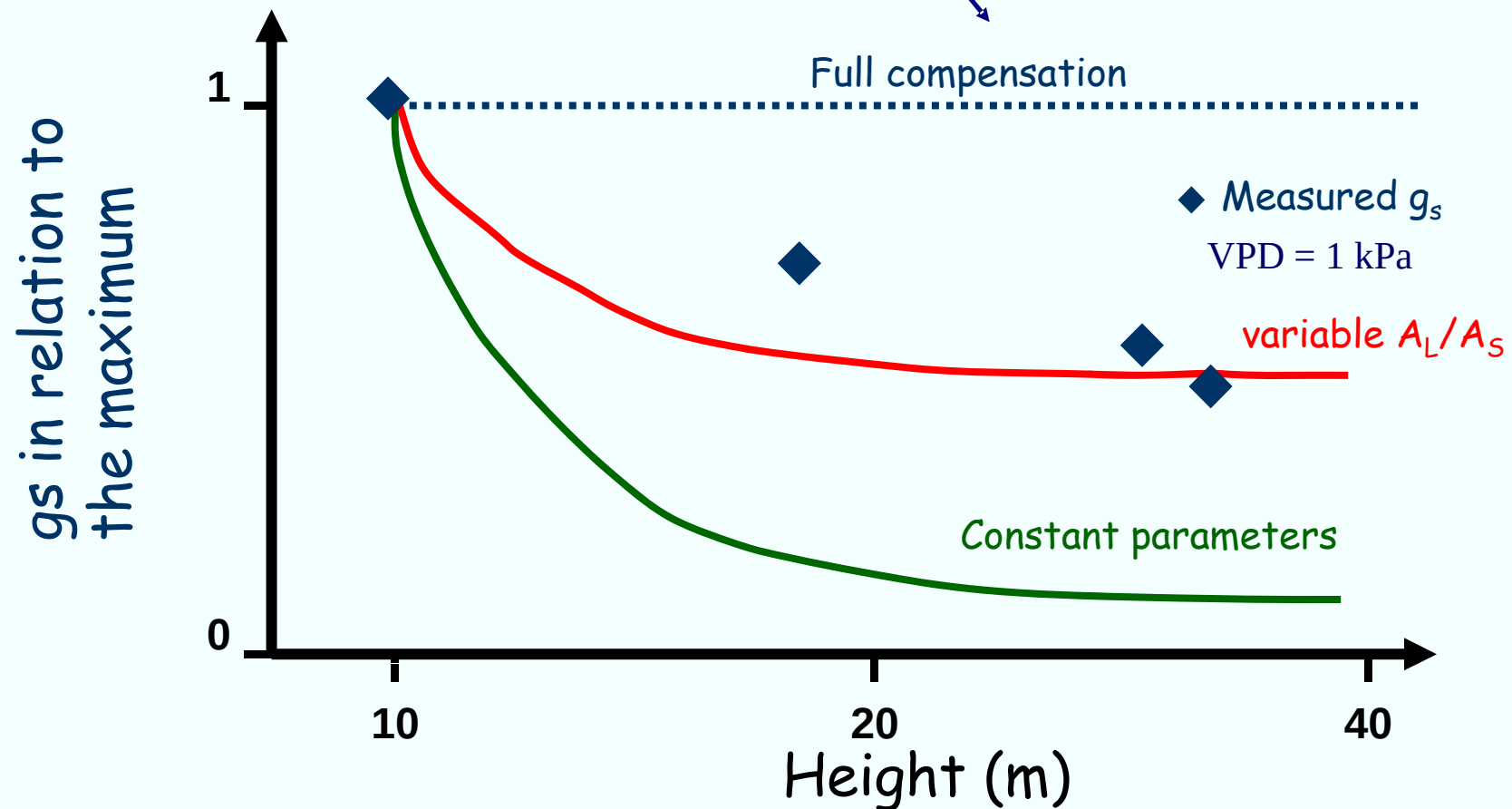
- Stomatal closure allows to maintain minimum water potential above the critical threshold in tall trees
- Decrease in stomatal conductance induces both transpiration and assimilation declines

Delzon et al. 2004 PCE

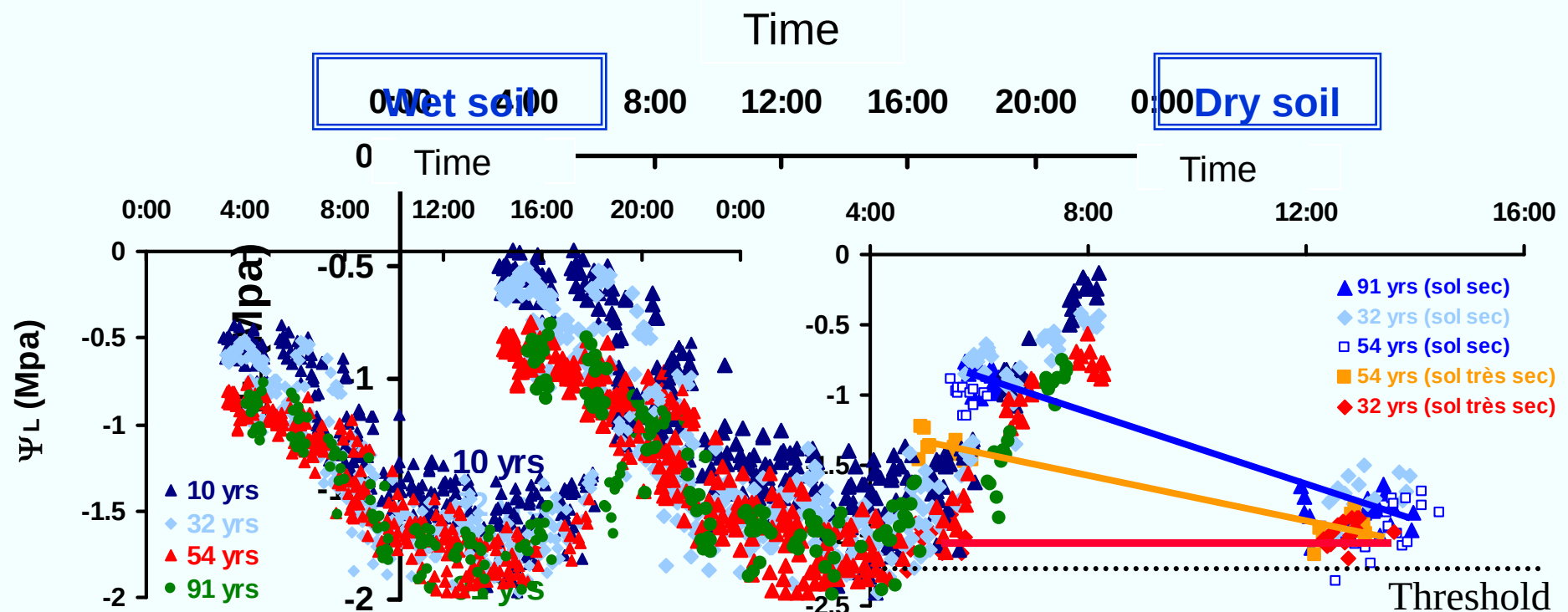
Summing up

$$g_s = \frac{1}{h} \frac{1}{A_L / A_S} \frac{\Delta \Psi}{VPD} K_s$$

Annotations: $\Delta \Psi$ is circled in green with "cst ?" above it; K_s is circled in red with "cst ?" above it; A_L / A_S is circled in blue with an arrow pointing to the graph.



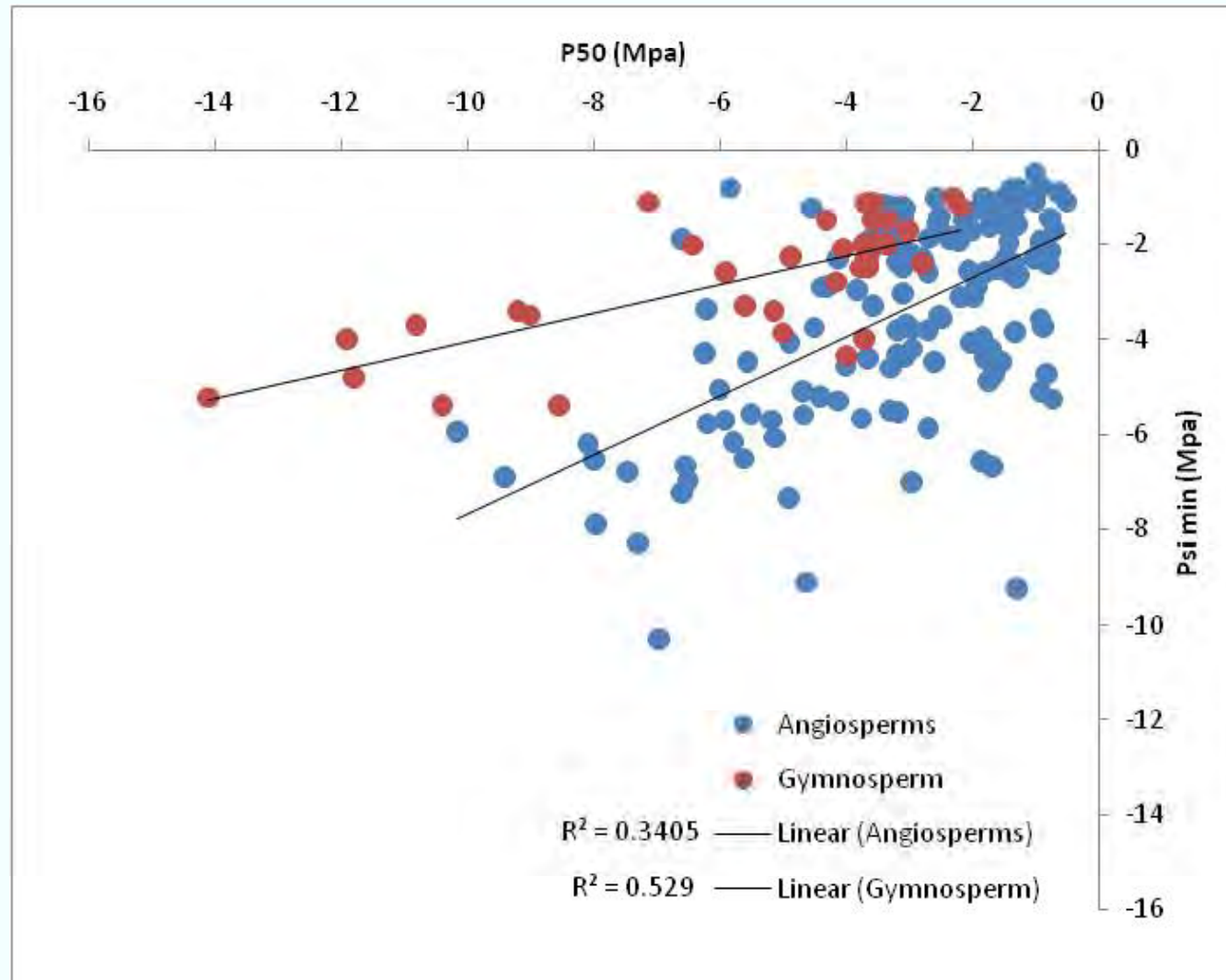
Homeostasis in maritime pine tree ?



Needle water potential measurements carried out across the chronosequence

➡ Threshold values about -2 MPa

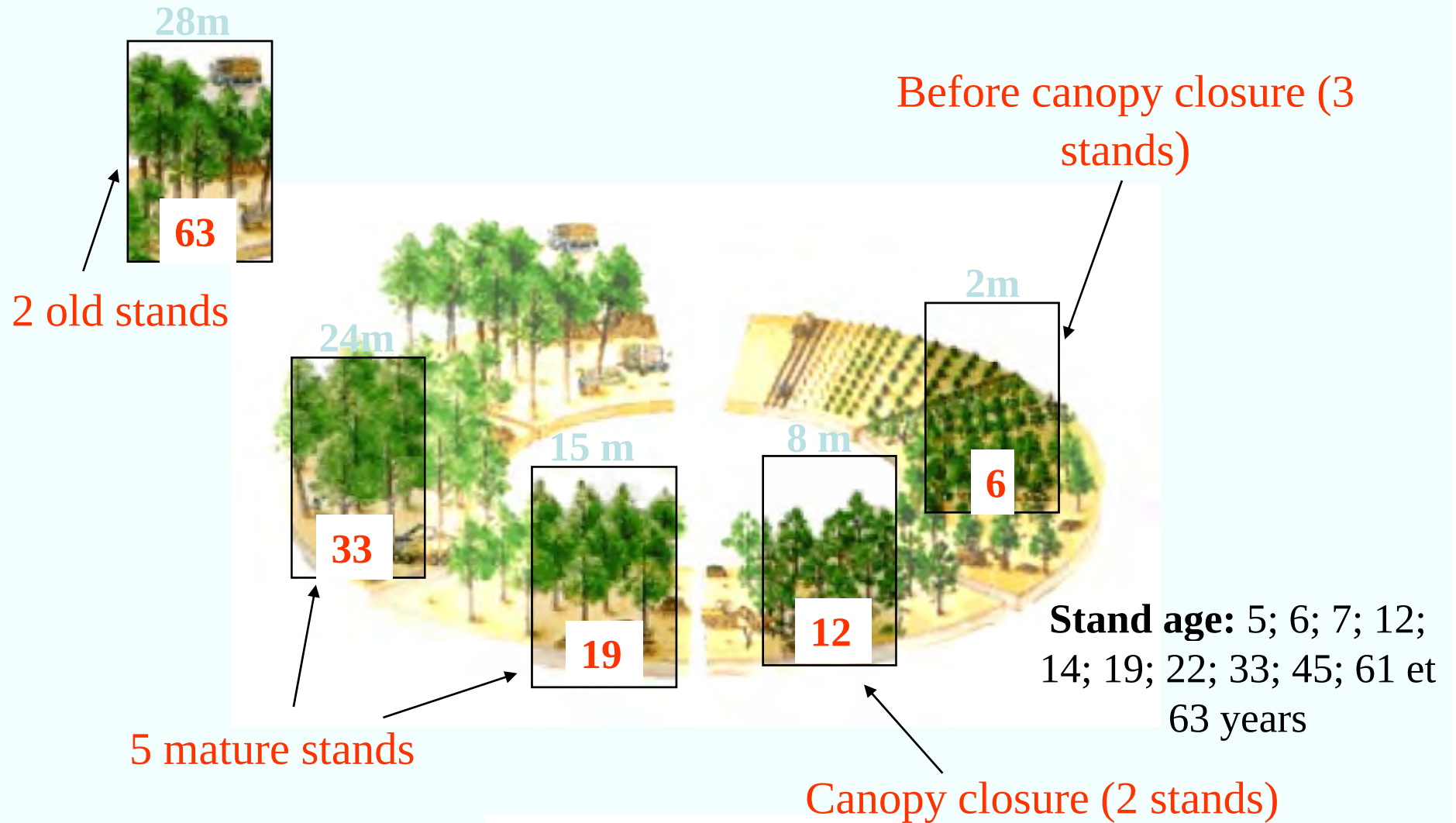
Relationship between cavitation resistance and minimum water potential



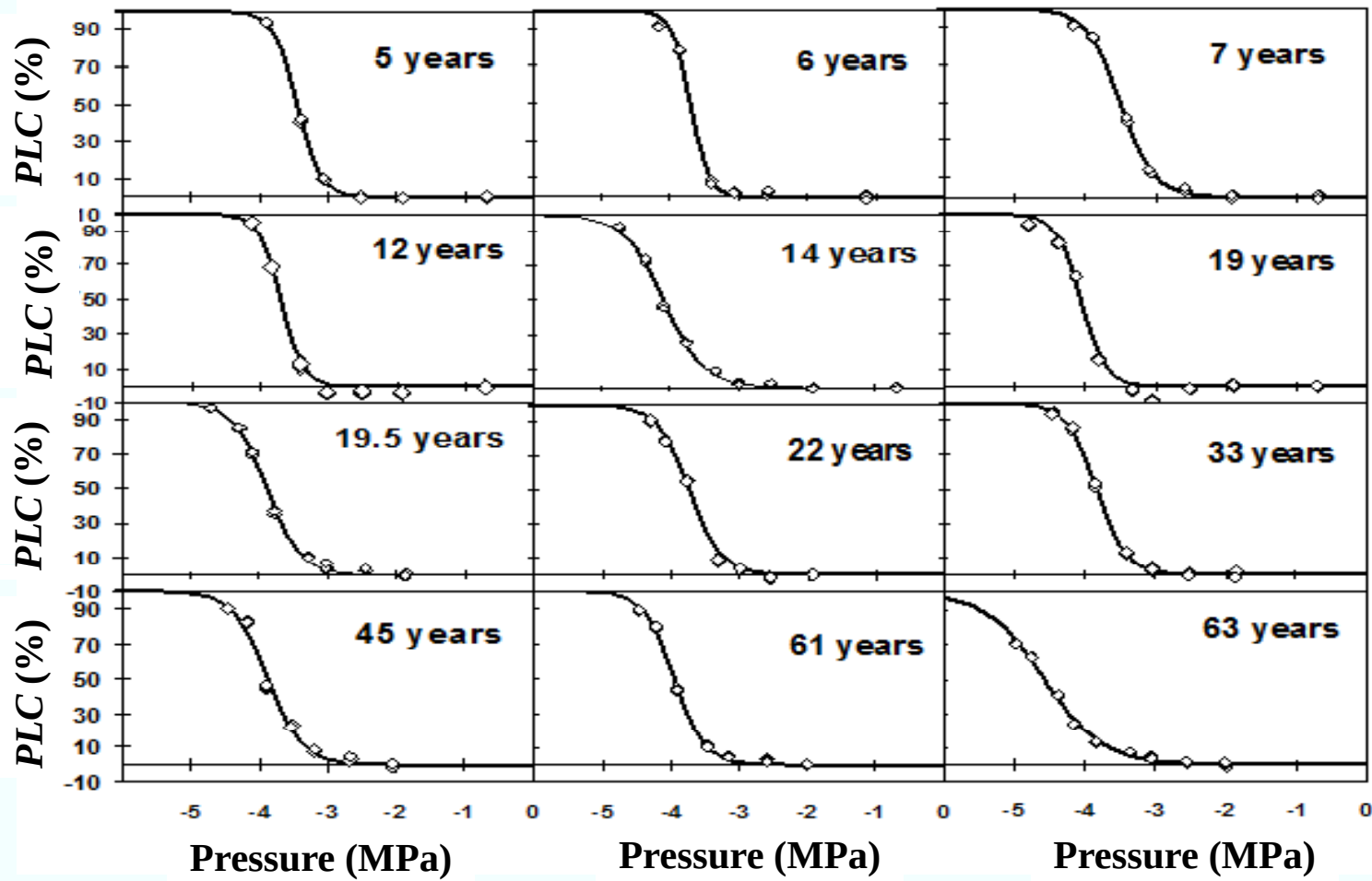
Do cavitation resistance (safety) and specific hydraulic conductivity (efficiency) remain constant with increasing tree height?

Maritime pine chronosequence

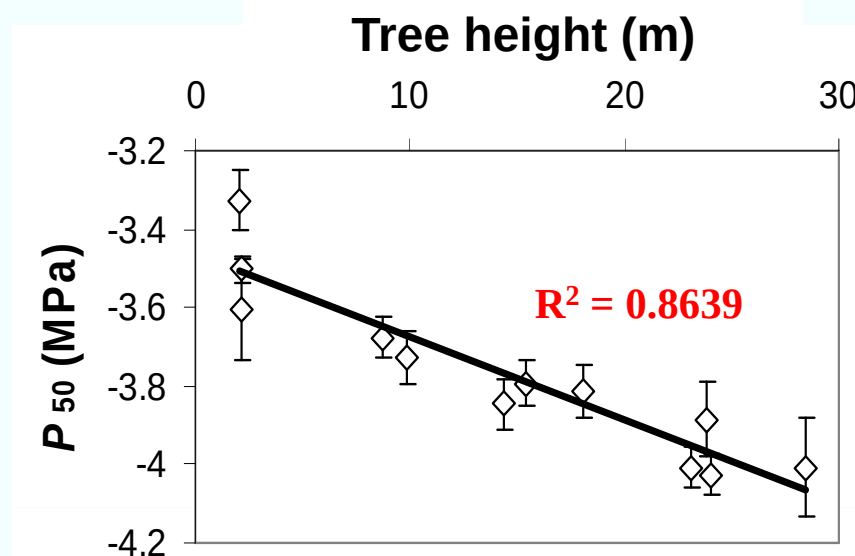
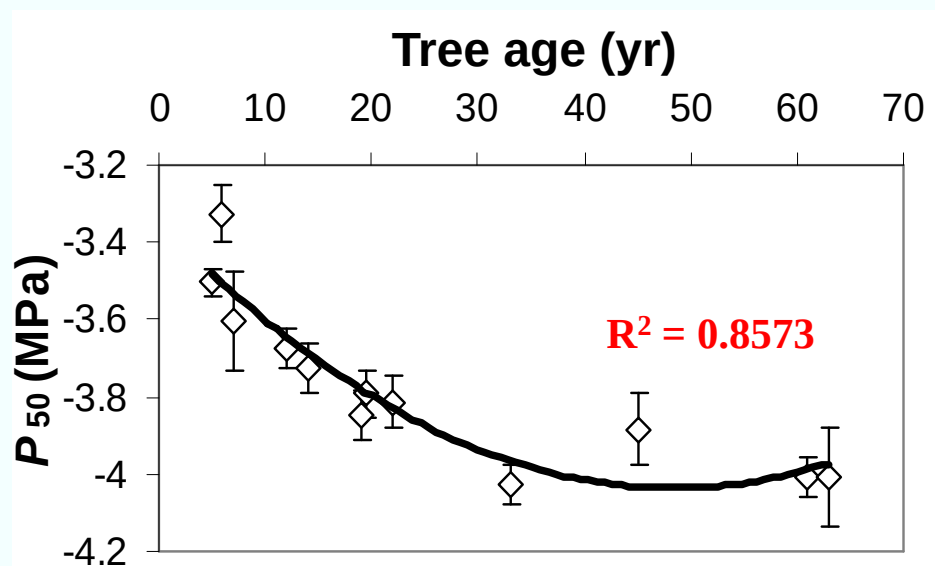
12 even-aged stands



Vulnerability curves



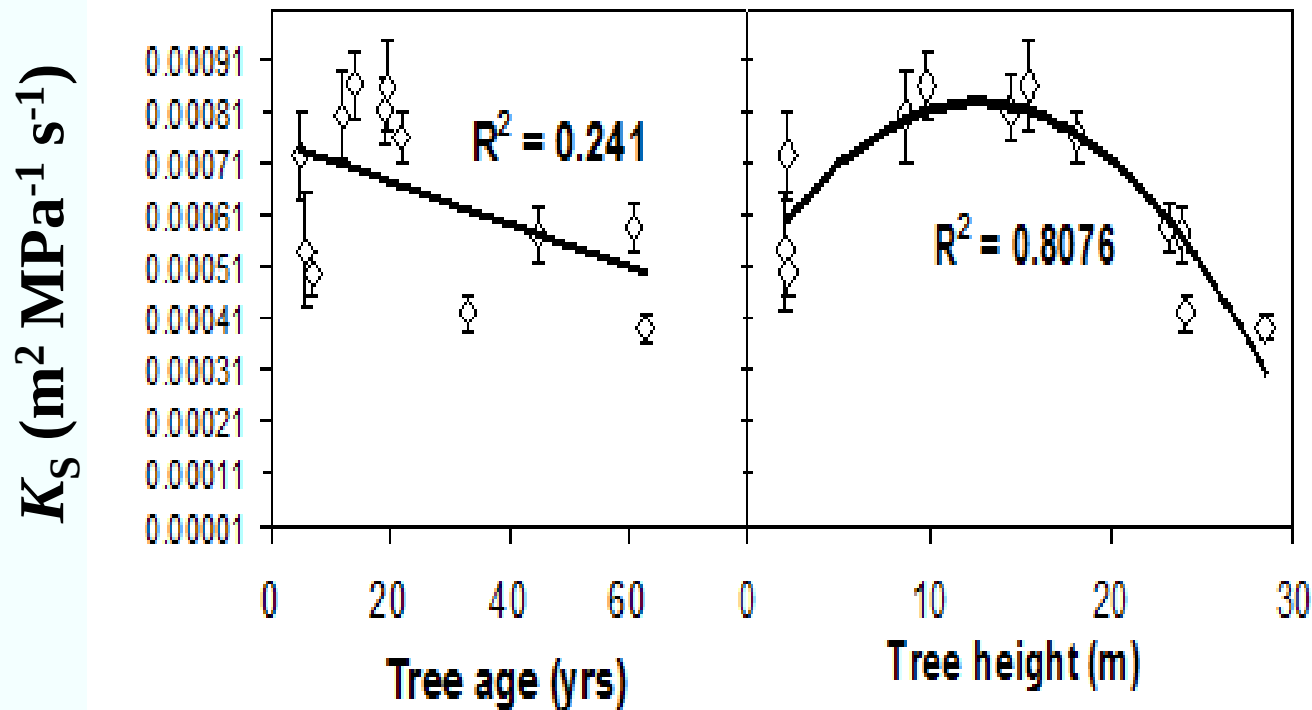
Evolution of cavitation resistance



Significant linear trend according to tree height

Tall trees are more cavitation resistant by 0.6 MPa

Evolution of xylem-specific hydraulic conductivity



k_s reaches an optimum at 15 m height and then decreases as trees grow taller

Hydraulic compensation mechanisms

✓ **SAFETY: YES**

As cavitation resistance increased with increasing tree height, tall trees could reach lower minimum water potential, thus increasing soil to leaf water potential gradient

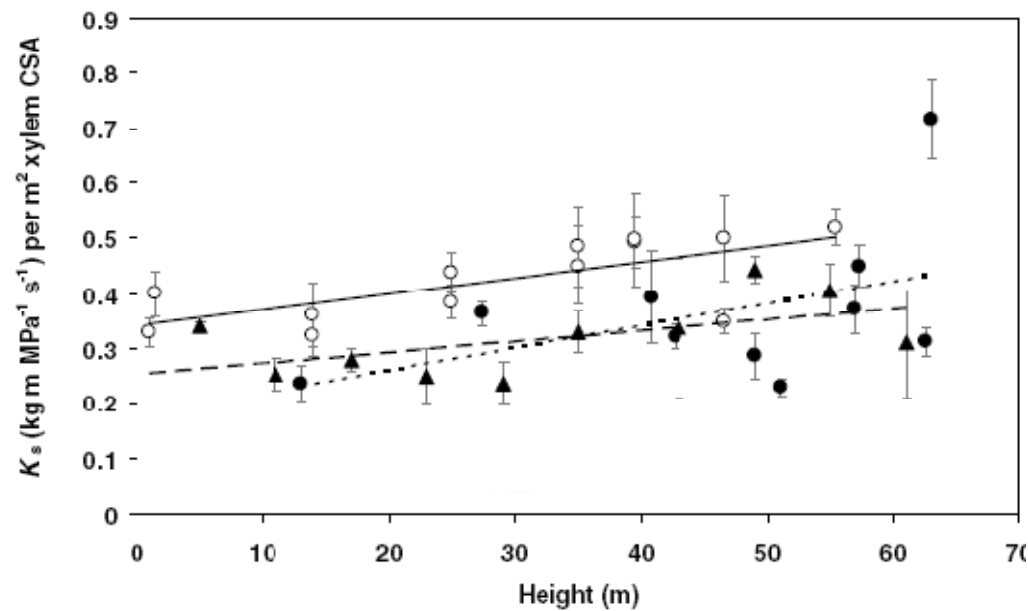
✓ **EFFICIENCY: NO**

Tall trees did not produce more efficient xylem and had even lower xylem specific hydraulic conductivity.

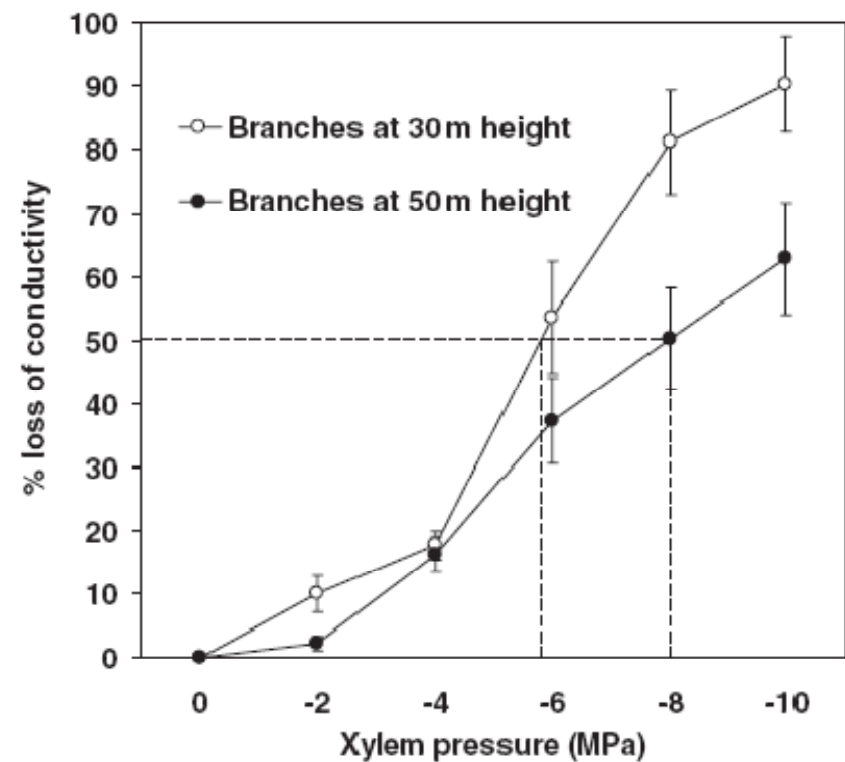
✓ Hydraulic adjustments that enhanced the ability to cope with vertical gradients of increasing xylem tension were attained at the expense of reduced water transport capacity and efficiency

Height-related effect within tree crown

Efficiency

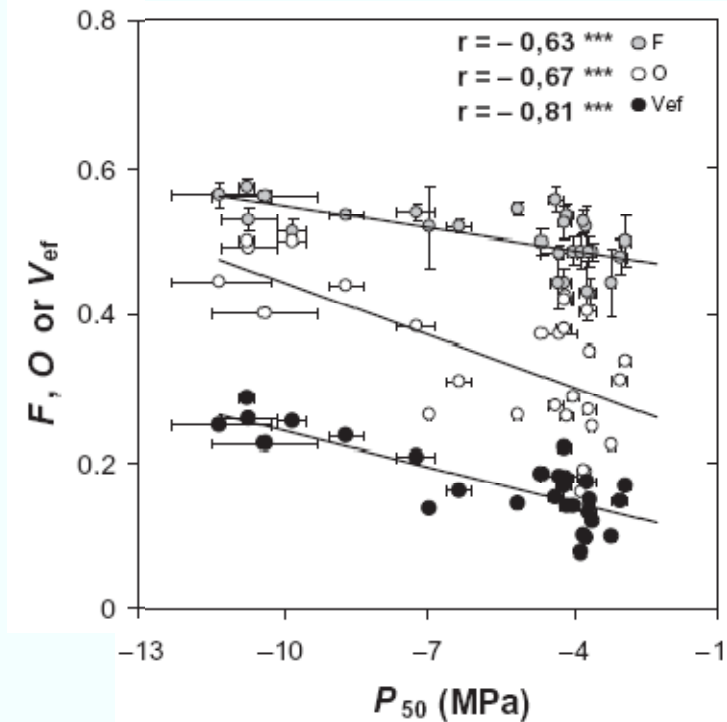


Safety



No trade-off in *Sequoia sempervirens*

Perspectives: xylem anatomy

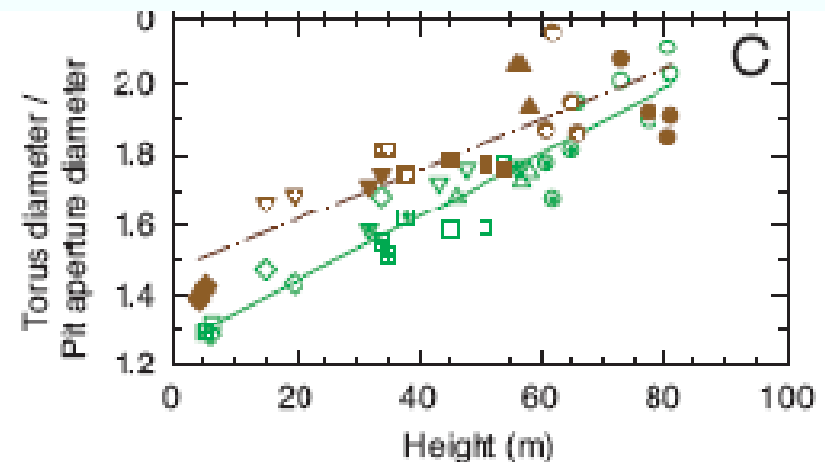


Pit membrane properties (margo flexibility, torus overlap and valve effect) highly correlated with P_{50}

Delzon et al. 2010 PCE

Torus overlap increased in Douglas-fir trees along a height gradient of 85 m

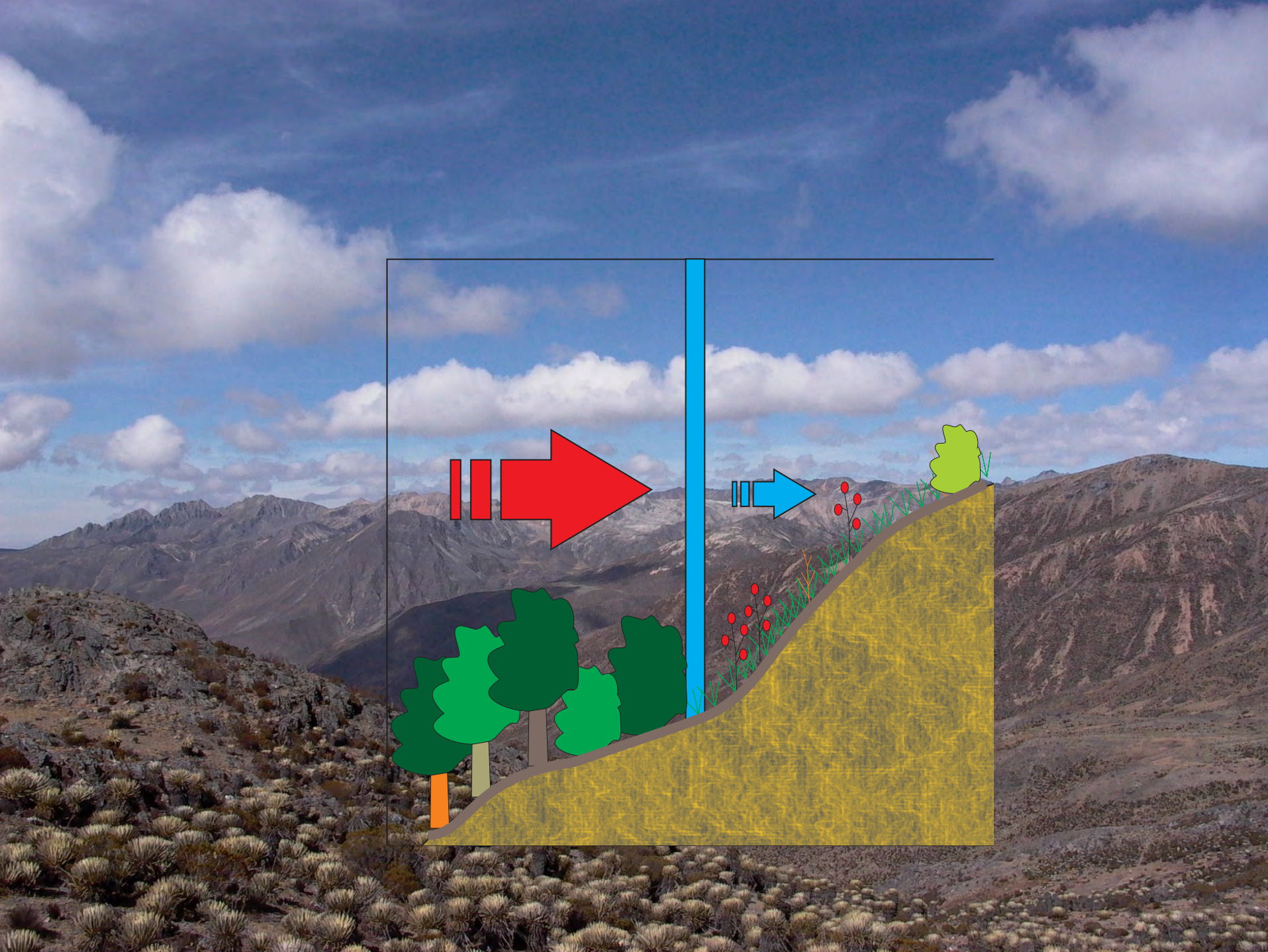
Domec et al. 2008 PNAS



Relations hydriques dans des arbres en treeline des Andes vénézuéliennes.

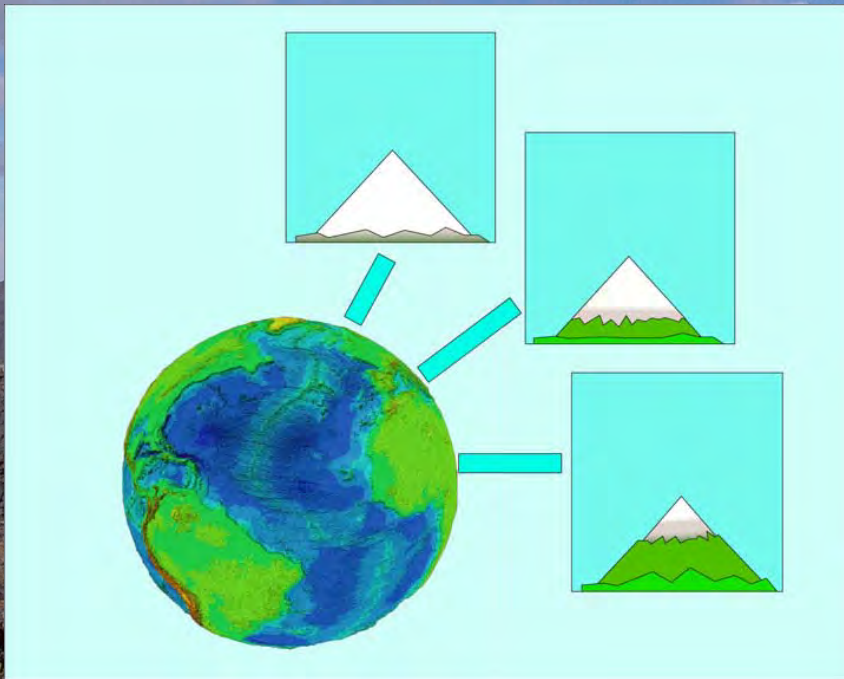
Dulhoste Raphaël
Rada Fermin





General Hypothesis

Climatic stress

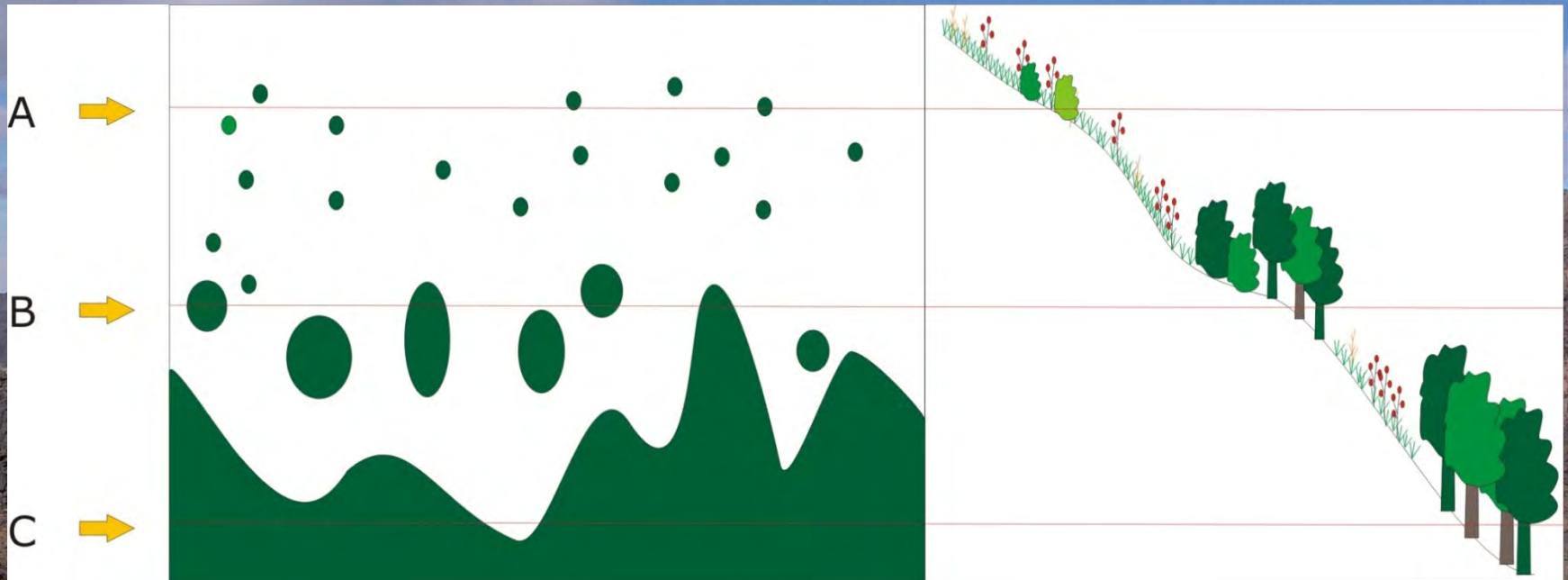


Disturbance





Treeline Structure



Our case

Tropical
Treeline

Freezing
Temperatures

Water
Deficit

Low
Temperature

High
Radiation

Treeline
Hypothesis

Tropical Mountains

Leuschner, 2000 .



Tropical Mountains

Daily water deficit
High VPD



Tropical Mountains

Seasonal deficit

- Decreased rainfall (december to march)

Erika Rodriguez

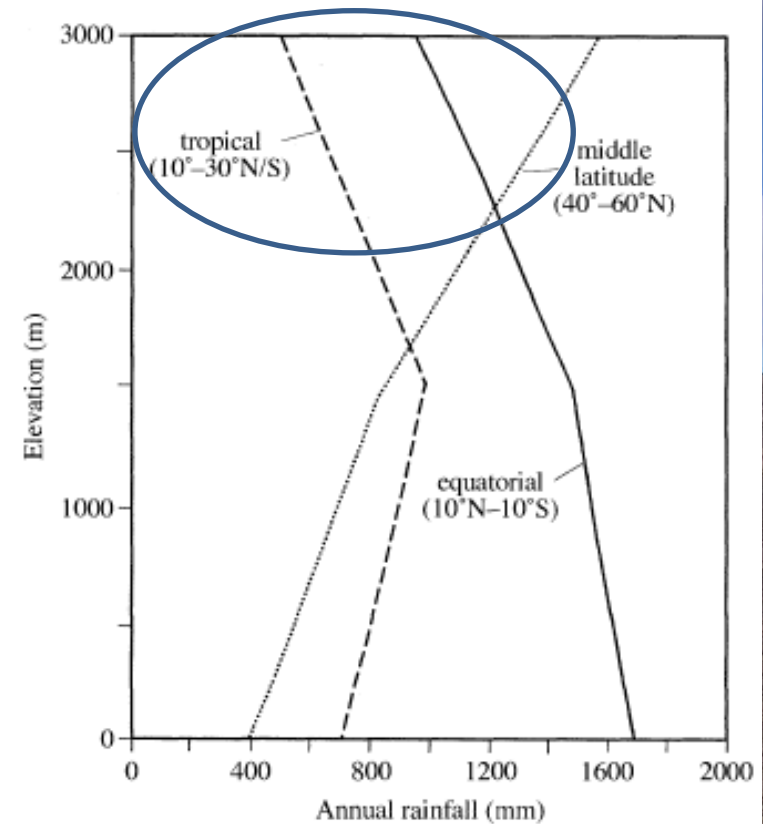


FIG. 5. Change in annual rainfall with elevation in mountains of the equatorial, tropical, and middle-latitude zones based on long-term observations at 1300 weather stations (after Lauscher 1976).

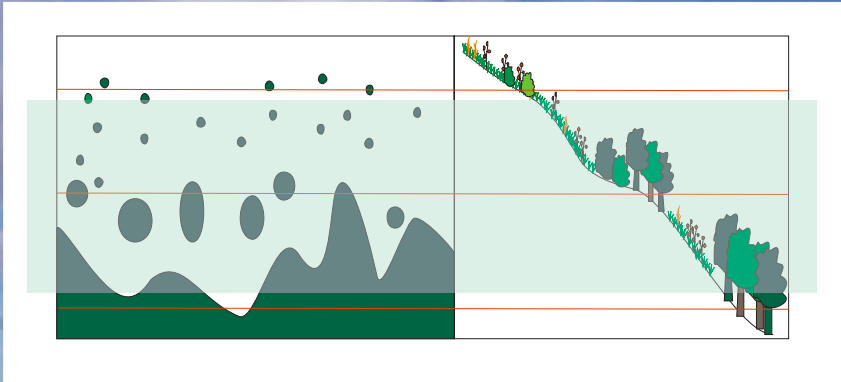
HYPOTHESIS

- Species adapted to higher altitude in the ecotone present mechanisms to improve their water status under conditions of greater deficit.

OBJETIVOS

- Determine the minimum water potential of three species of cloud forest-páramo ecotone in adult individuals in the field.
- Identify their various components of water potential.
- Characterize the behavior of the response of stomata to leaf water potential in these species, adult leaves.

Material & methods



3200

2800

Species

Altitude (m)

Diplostephium venezuelense

3200, 3000

Miconia jahnii

3150, 3000

Libanothamnus neriifolius

3150, 3000, 2800



SPECIES

- *Diplostephium venezuelense*
(3200 m, 3000 m)



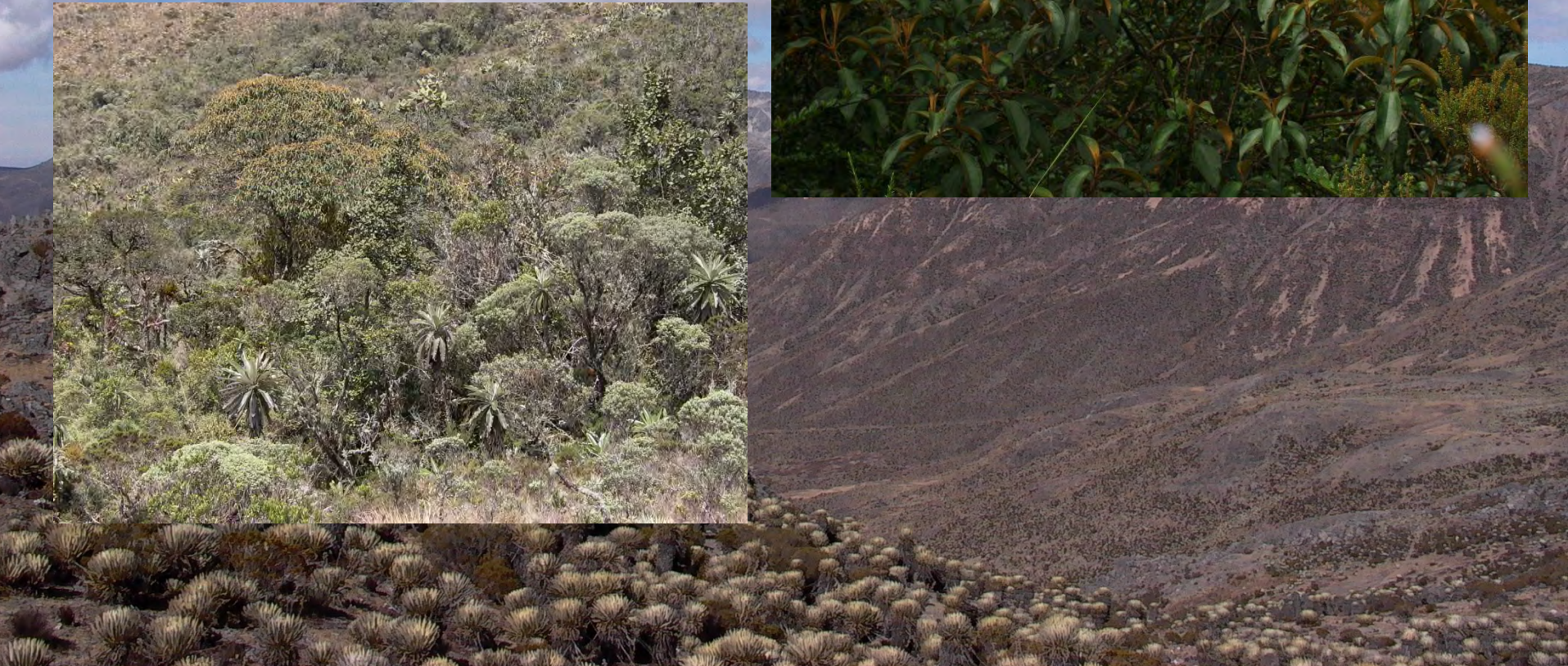
SPECIES

- *Libanothamnus neriifolius*
(3150, 3000, 2800)

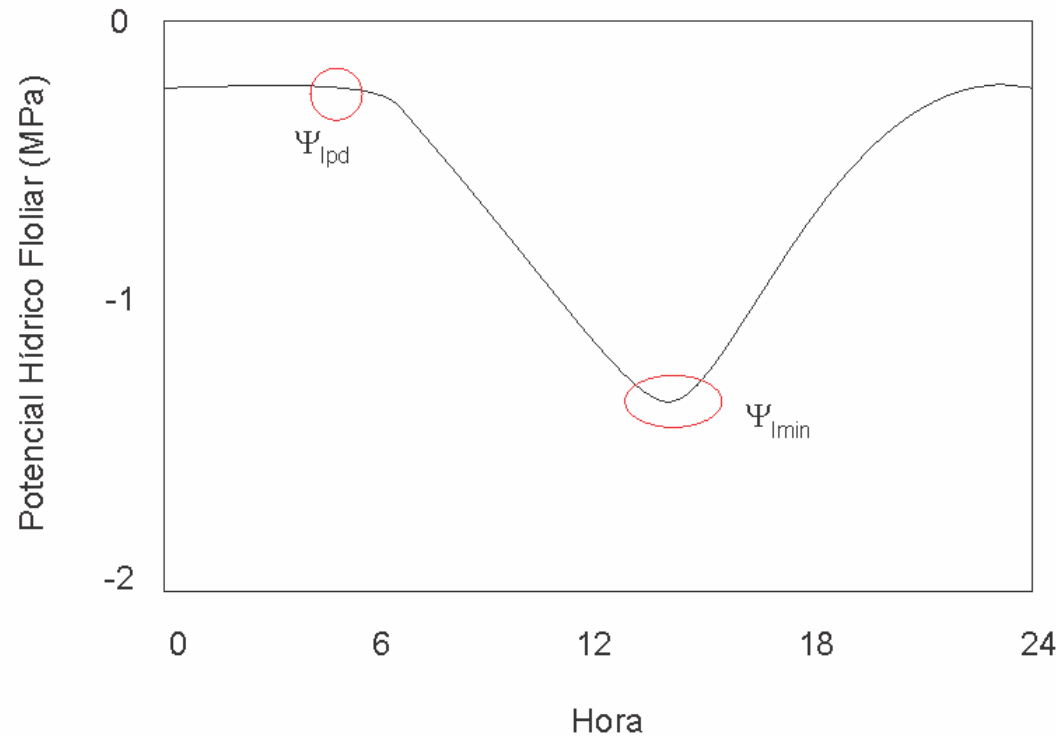


SPECIES

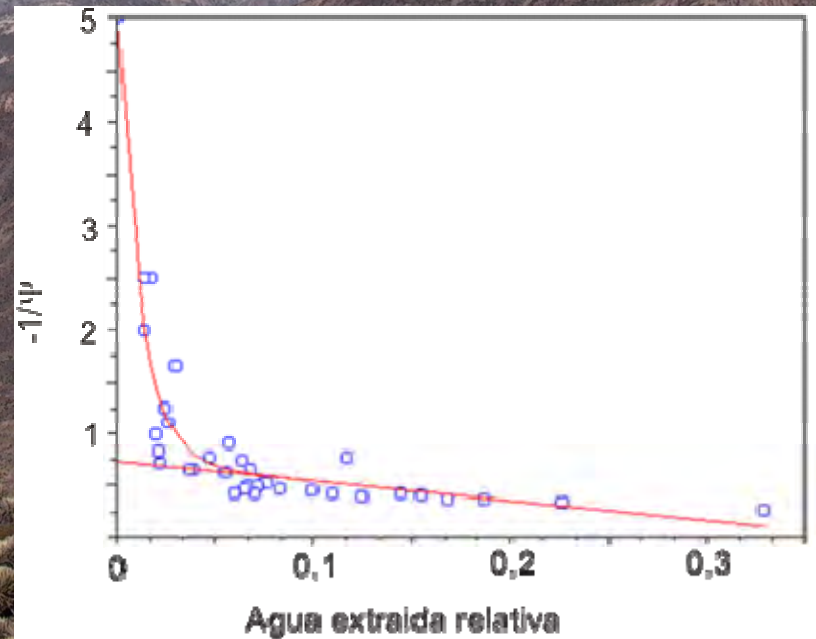
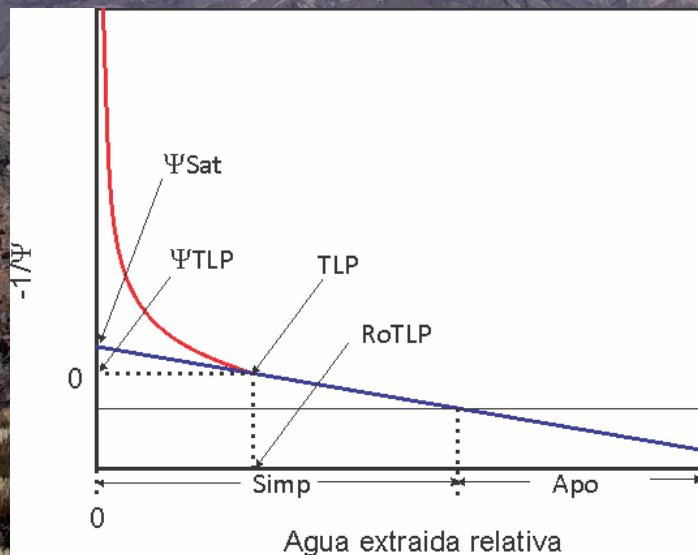
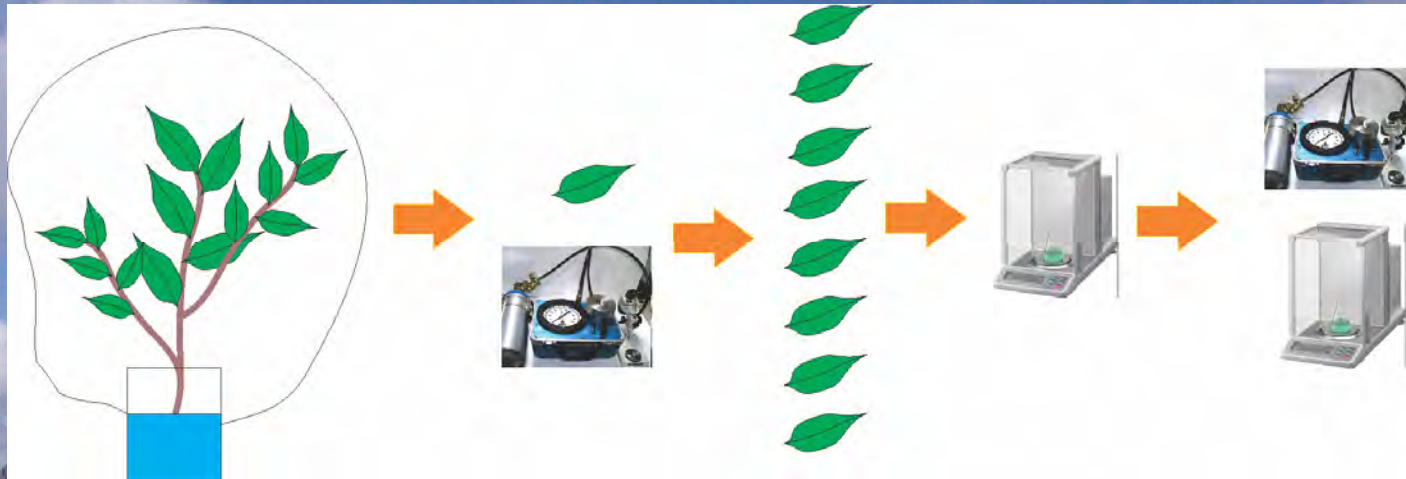
- *Miconia jahnii*
(3150, 3000)



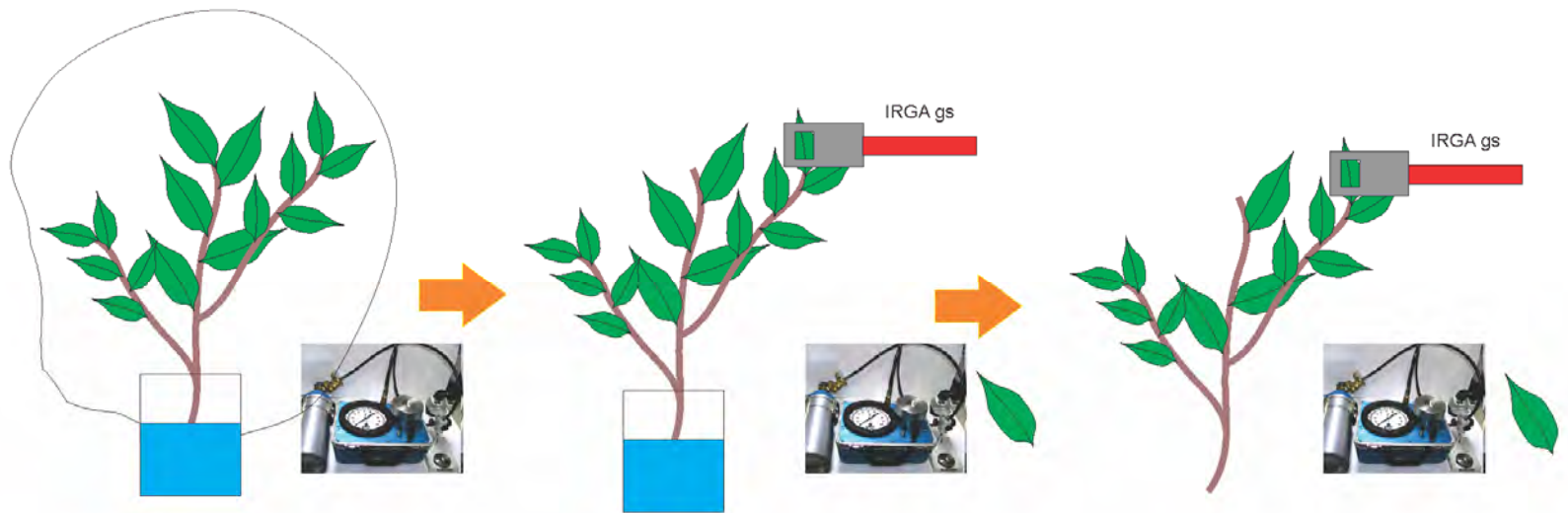
Minimum (Ψ_{lmin}) and predawn(Ψ_{lpd}) leaf water potential .



Ψ components



Curve $g_s - \Psi_l$



Results & Discussion

Field Ψ_l

Species	Altitude	Ψ_{lmin} (Mpa)	Ψ_{pd} (Mpa)
<i>Diplostephium venezuelense</i>	3200	-1,25 (0,2)	
	3000	-1,15 (0,3)	-0,20 (0,1)
<i>Libanothamnus neriifolius</i>	2800	-0,90 (0,3)	
	3000	-1,05 (0,3)	-0,20 (0,1)
	3150	-1,20 (0,3)	
<i>Miconia jahnii</i>	3000	-1,10 (0,4)	-0,30 (0,1)
	3150	-1,20 (0,3)	

Results & Discussion

Species	Altitude	Ψ_{lmin} (Mpa)	Ψ_{pd} (Mpa)
<i>Diplostephium venezuelense</i>	3400*	-1,50 (0,2)	-1,10 (0,2)
	3200	-1,25 (0,2)	
	3000	-1,15 (0,3)	-0,20 (0,1)
<i>Libanothamnus neriifolius</i>	2800	-0,90 (0,3)	
	3000	-1,05 (0,3)	-0,20 (0,1)
	3150	-1,20 (0,3)	
<i>Miconia jahnii</i>	3000	-1,10 (0,4)	-0,30 (0,1)
	3150	-1,20 (0,3)	

Field Ψ_l

Ψ , Component

	Alt	Saison	$Ro_{t\bar{p}}$	$\Psi_{t\bar{p}}$	Ψ_{sat}	A_{simp}	ε_{max}
<i>Diplostephium venezuelense</i>	3000	Wet	0,07 (0,003)	-0,91 (0,04)	-0,70 (0,04)	0,31 (0,01)	11,31* (0,19)
			0,19 (0,012)	-1,06 (0,01)	-0,75 (0,02)	0,40 (0,01)	8,25* (0,56)
	3200	Wet	0,13 (0,009)	-1,00 (0,06)	-0,81 (0,08)	0,57 (0,05)	15,66* (2,35)
			0,22 (0,012)	-1,47 (0,02)	-1,14 (0,01)	0,64 (0,02)	6,73* (0,30)
							-

Ψ , Component

	Alt	Saison	$Ro_{t\bar{p}}$	$\Psi_{t\bar{p}}$	Ψ_{sat}	A_{simp}	ε_{max}
<i>Diplostephium venezuelense</i>	3000	Wet	0,07 (0,003)	-0,91 (0,04)	-0,70 (0,04)	0,31 (0,01)	11,31* (0,19)
		Dry	0,19 (0,012)	-1,06 (0,01)	-0,75 (0,02)	0,40 (0,01)	8,25* (0,56)
	3200	Wet	0,13 (0,009)	-1,00 (0,06)	-0,81 (0,08)	0,57 (0,05)	15,66* (2,35)
		Dry	0,22 (0,012)	-1,47 (0,02)	-1,14 (0,01)	0,64 (0,02)	6,73* (0,30)
	3400 ^a	Wet	0,13 (0,013)	-1,17* (0,26)	-0,88* (0,23)	0,57 (0,09)	12,52 (2,01)
		Dry	0,12 (0,038)	-1,81* (0,11)	-1,68* (0,13)	0,59 (0,05)	11,42 (3,81)

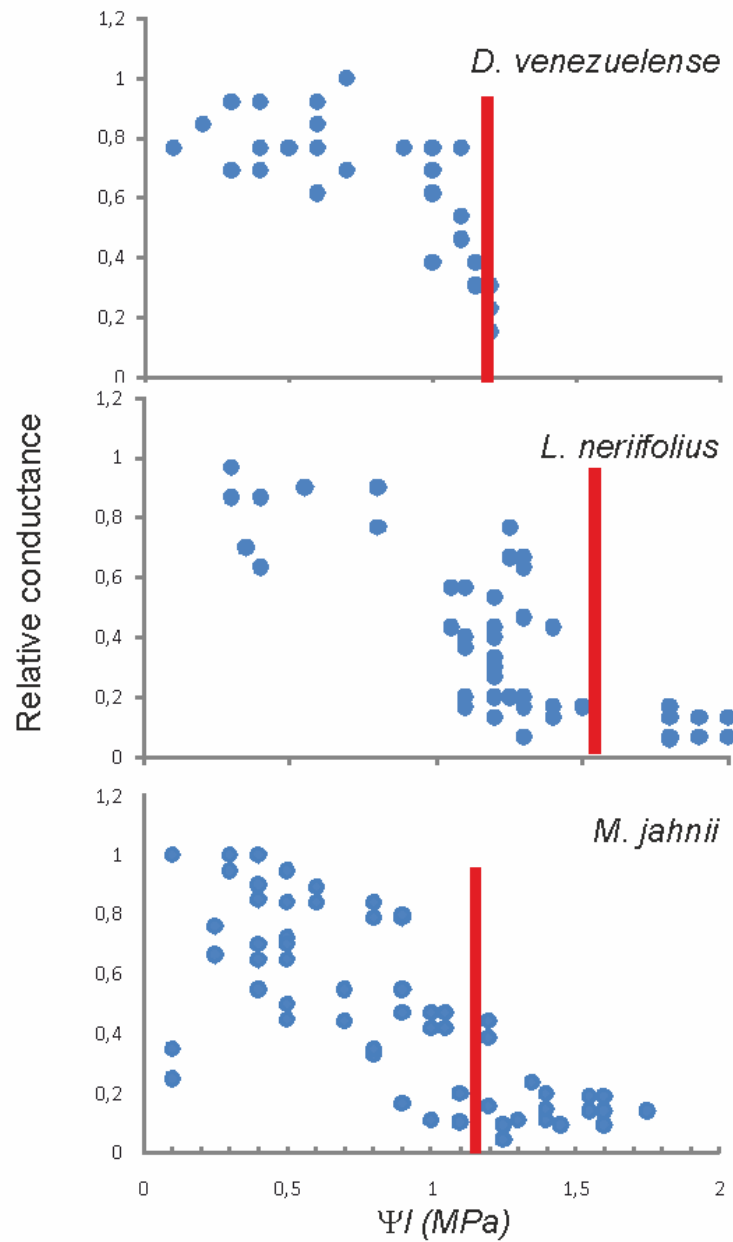
Ψ , Component

	Alt	Saison	Ro_{tlp}	Ψ_{tlp}	Ψ_{sat}	A_{simp}	ε_{max}
<i>Libanothamnus neriifolius</i>	2800	Wet	0,06 (0,007)	-1,13 (0,27)	-1,01 (0,10)	0,60 (0,06)	20,22* (1,87)
			0,08 (0,017)	-1,77 (0,18)	-1,44 (0,24)	0,41 (0,06)	12,80* (2,26)
	3000	Wet	0,09 (0,042)	-1,36 (0,11)	-0,93 (0,09)	0,31 (0,04)	19,16* (2,72)
			0,15 (0,003)	-1,52 (0,08)	-1,23 (0,14)	0,60 (0,09)	5,51* (3,08)
	3150	Wet	0,08 (0,018)	-1,60 (0,09)	-1,25 (0,05)	0,38 (0,06)	19,20* (4,29)
			0,13 (0,023)	-2,04 (0,04)	-1,64 (0,11)	0,60 (0,04)	10,53* (0,80)

Ψ , Component

	Alt	Saison	$Ro_{t\bar{p}}$	$\Psi_{t\bar{p}}$	Ψ_{sat}	A_{simp}	ε_{max}
<i>Miconia jahnnei</i>	3000	Wet	0,09 (0,015)	-1,06 (0,07)	-0,70 (0,13)	0,37 (0,12)	14,05* (3,61)
		Dry	0,21 (0,012)	-1,85 (0,06)	-1,08 (0,13)	0,47 (0,15)	11,51* (0,52)
	3150	Wet	0,07 (0,011)	-1,12 (0,13)	-0,96 (0,11)	0,48 (0,13)	26,28* (1,85)
		Dry	0,16 (0,021)	-1,53 (0,12)	-1,25 (0,17)	0,48 (0,14)	20,44* (1,87)

gs vs Ψ_l .



Discussion





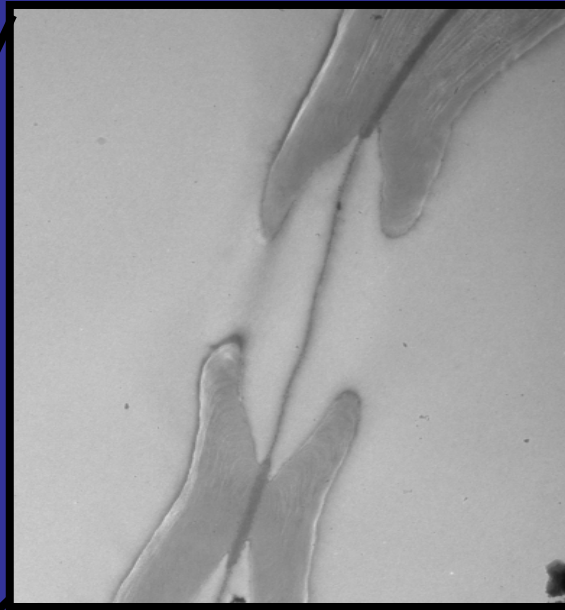
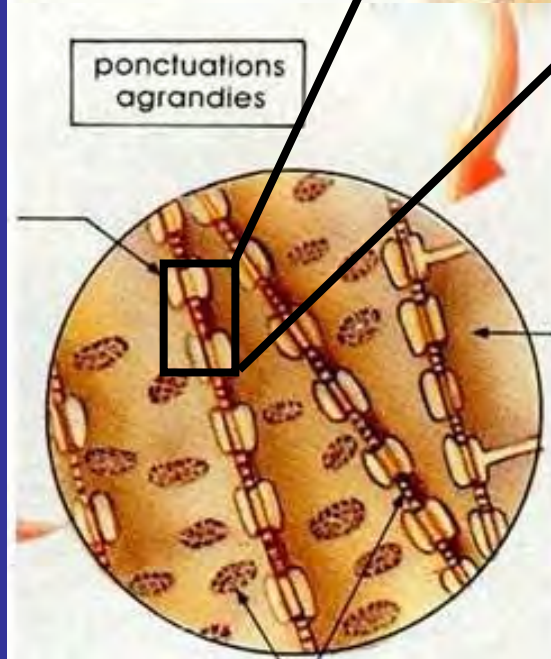
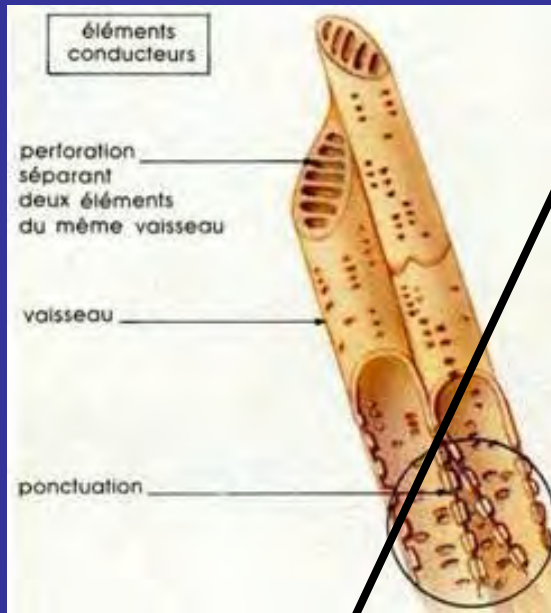
Merci



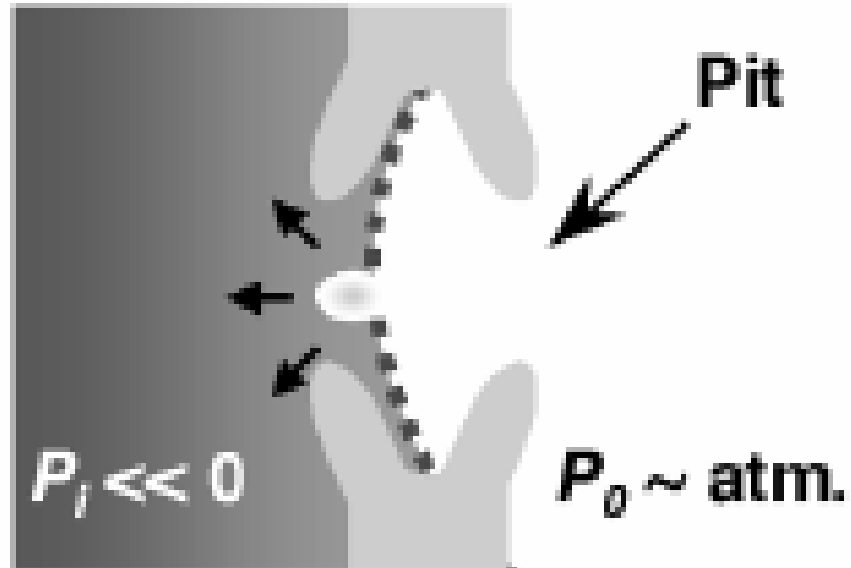
Calcium in Vulnerability to Cavitation (VC)

S. Herbette, A. Tixier, H. Awad, E. Mellerowicz, H. Cochard.

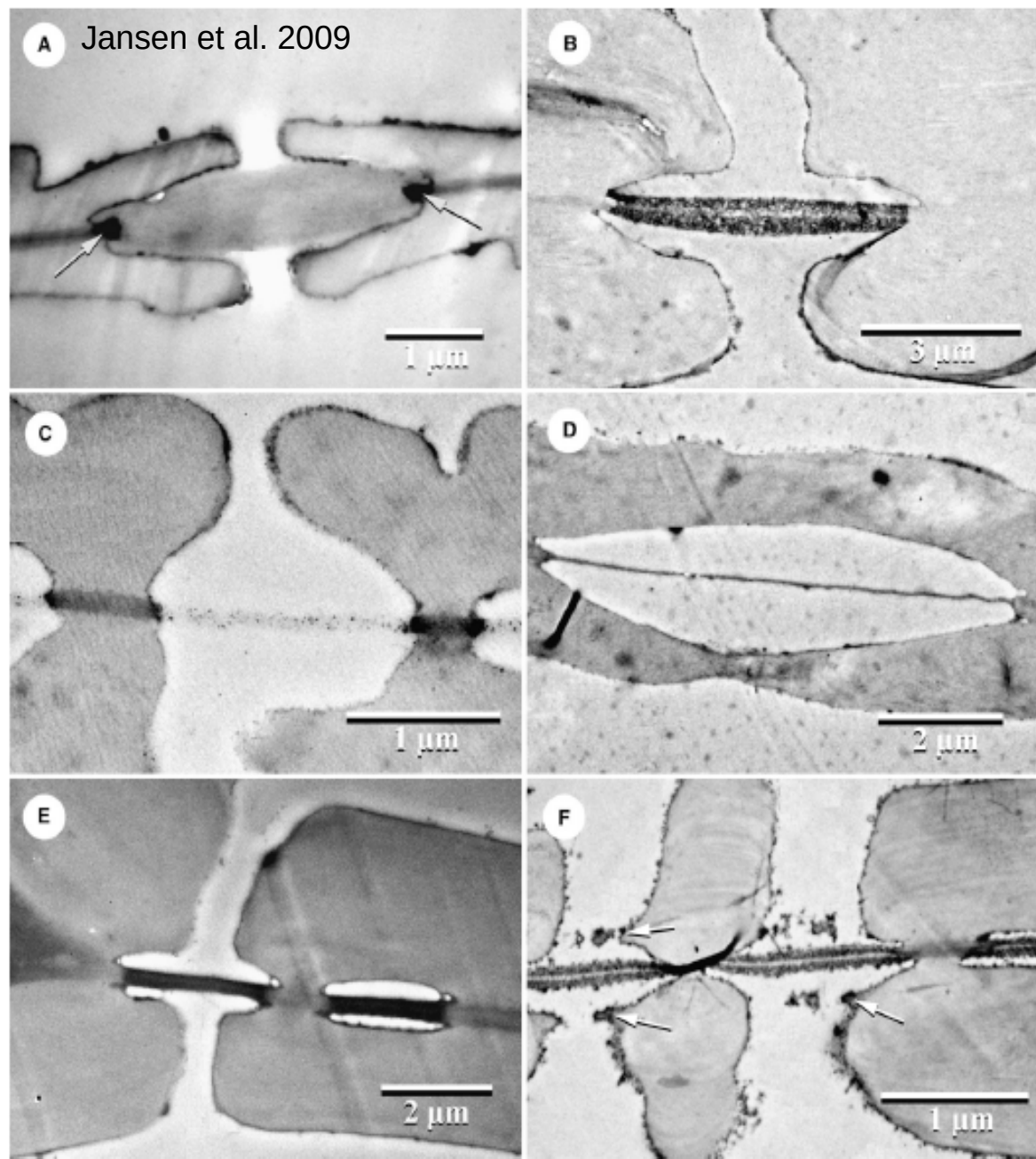
Mechanism of cavitation: air seeding hypothesis



Populus tremula x alba

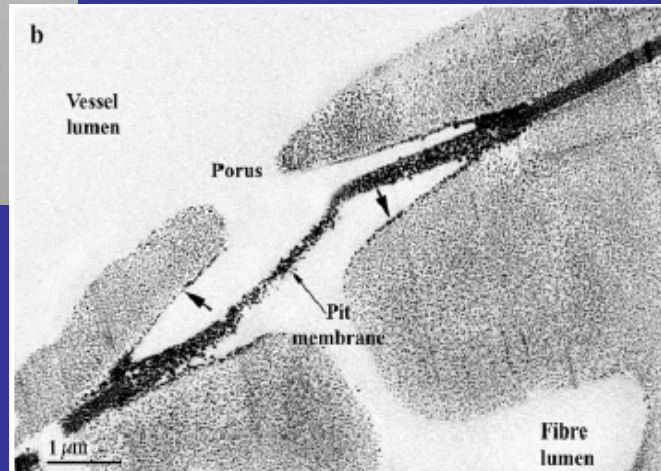
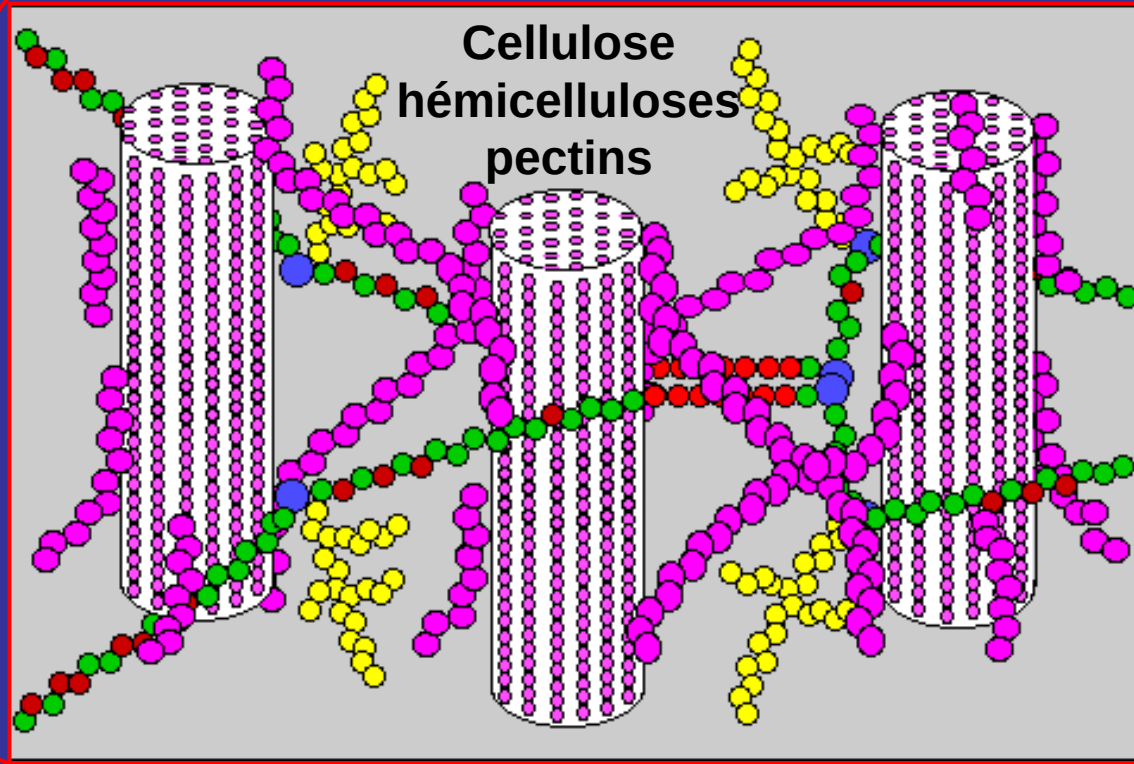
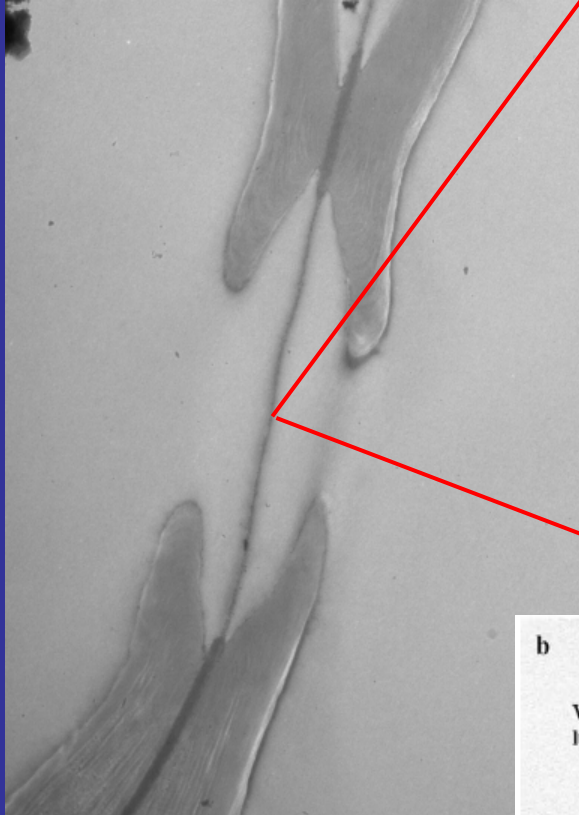


Pit membrane composition



Great variations
between species

Pit membrane composition



and lignins ?

Lignins stained using KMnO_4 in pit membrane from beech latewood (Fromm et al., 2003)

pit membrane composition: what about the hemicelluloses?

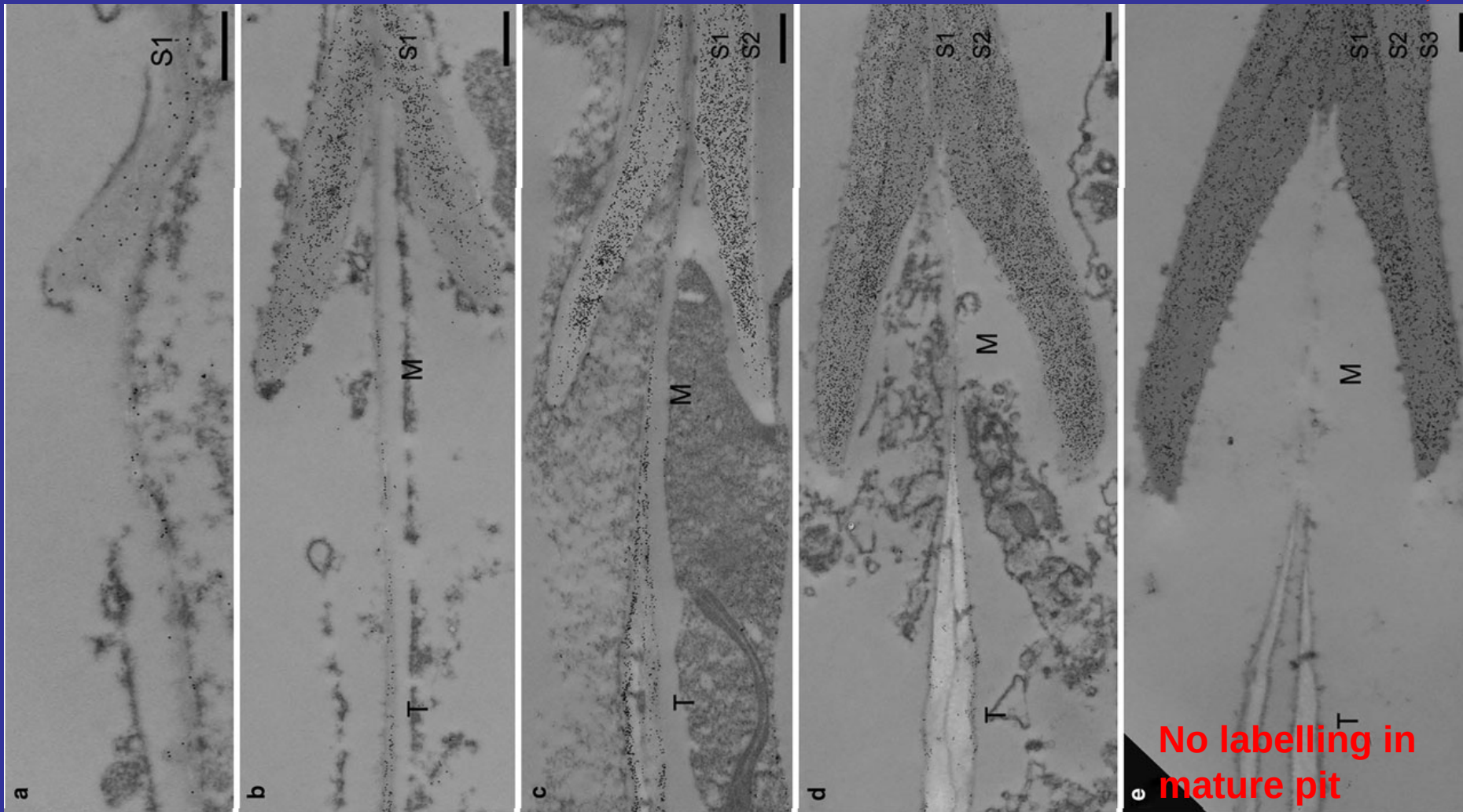
Immunolabelling of **mannanes**
(O-Acetyl-galactoglucomannans)

Cryptomeiria japonica

Early formation
stage of the pit

Pit differentiation

Mature pit

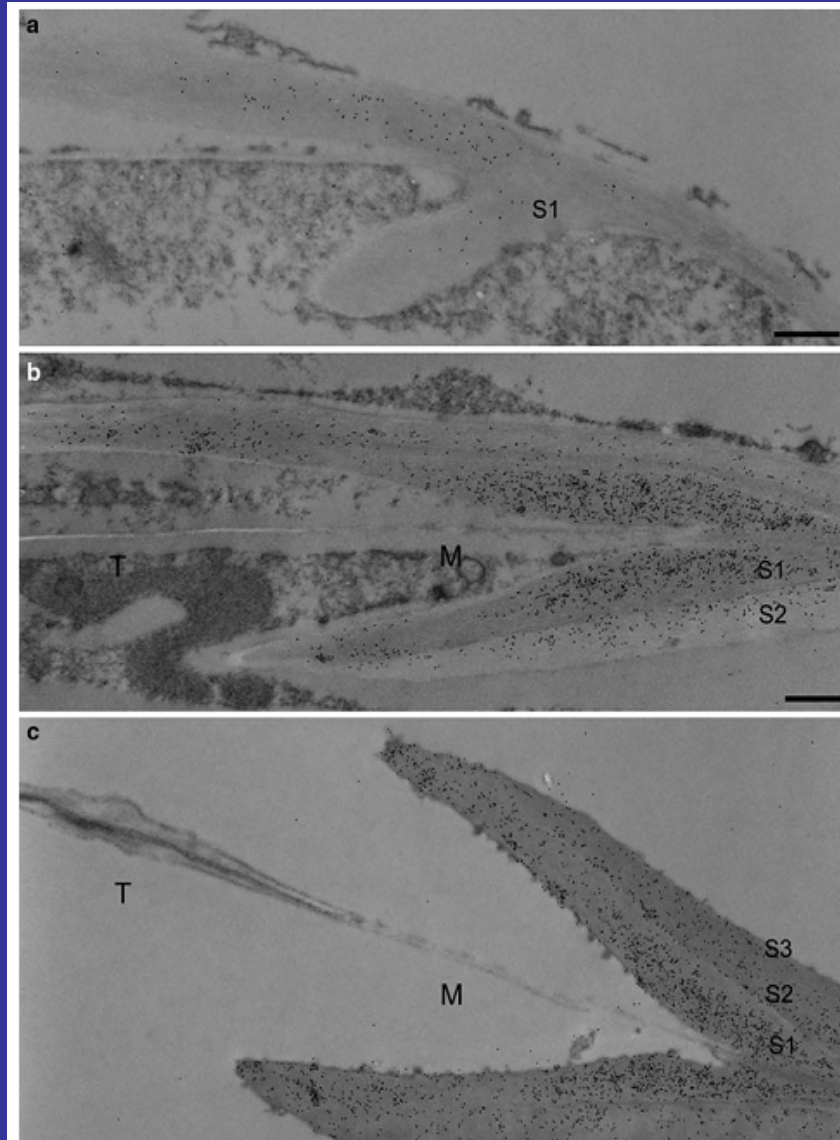


pit membrane composition: what about the hemicelluloses?

Early formation
stage of the pit

Pit differentiation

Mature pit



Immunolabelling of xylanes
(Arabino-4-O-methylglucuronoxylans)

Kim et al., 2011

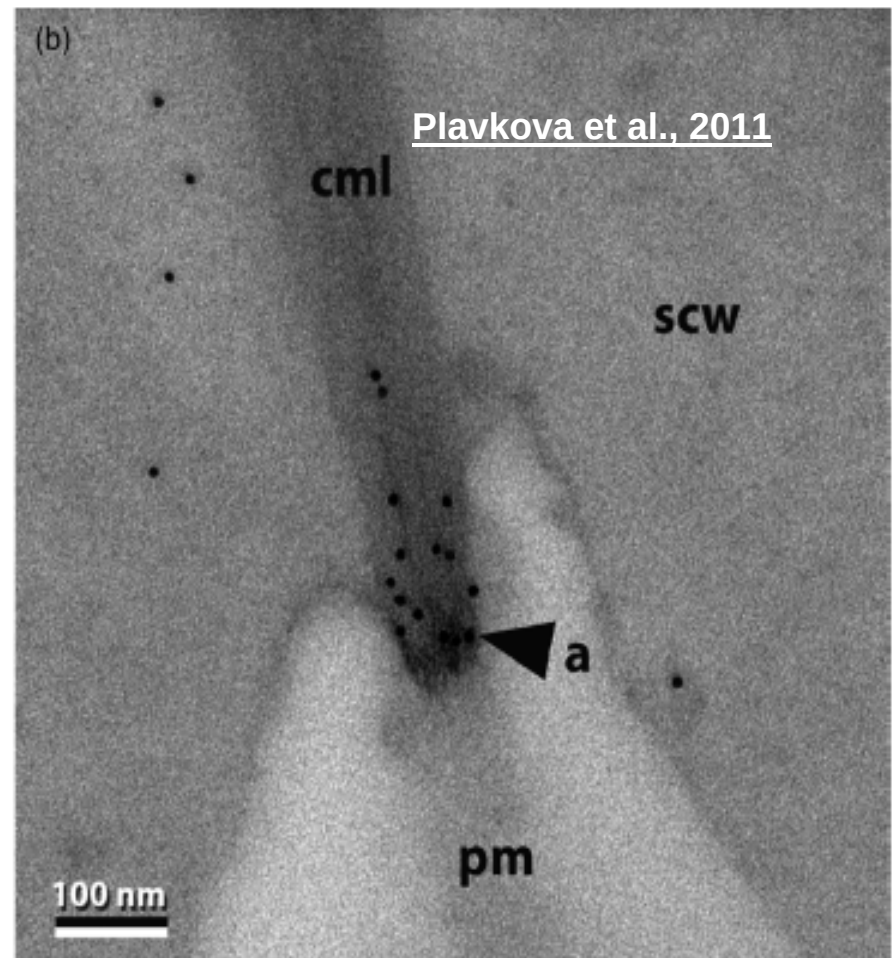
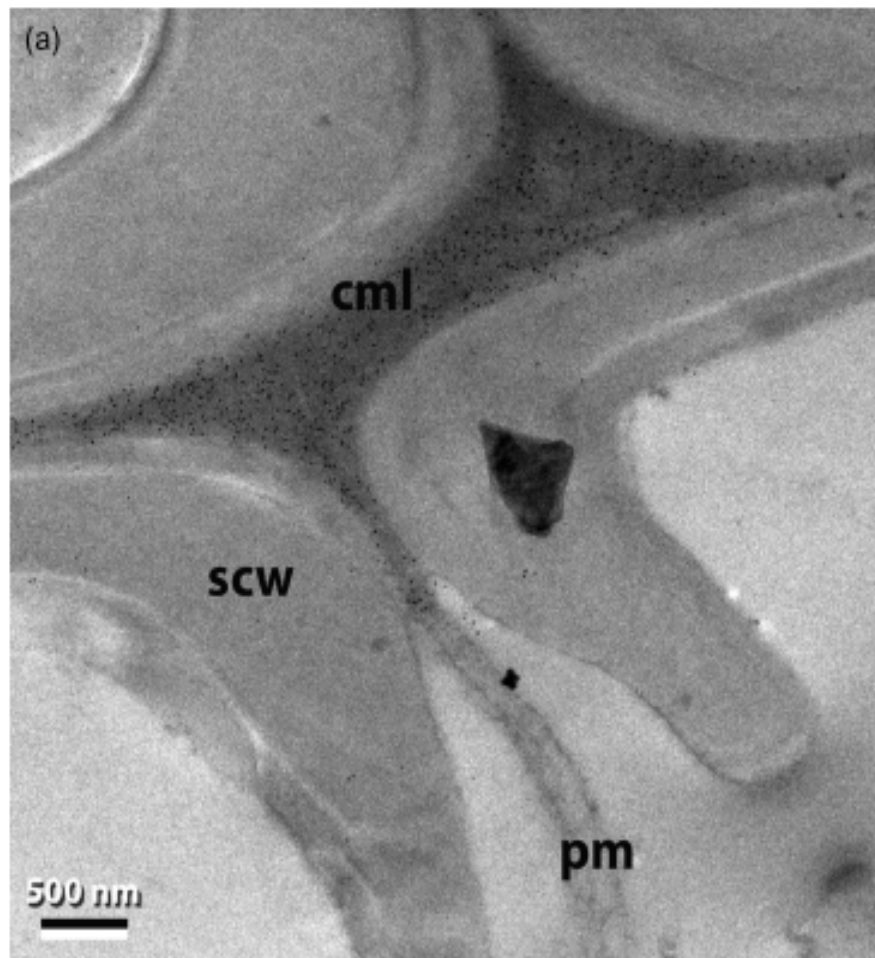
pit membrane composition: are there pectins ?

Immunolabelling of homogalacturonans (HG) domains of pectins.

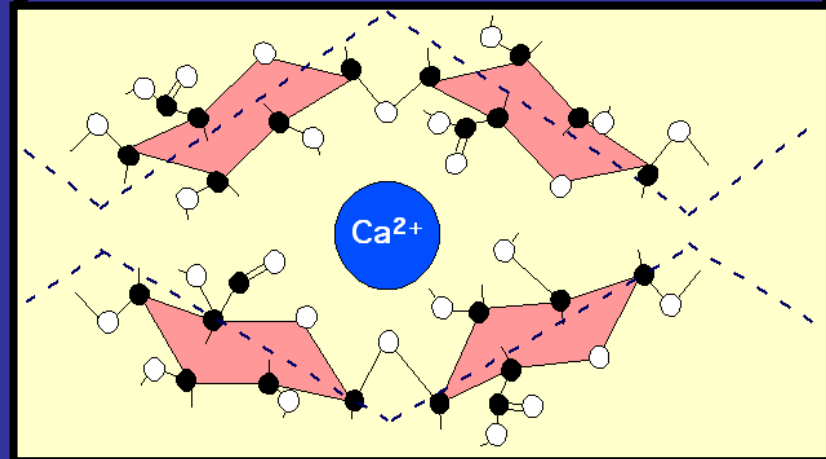
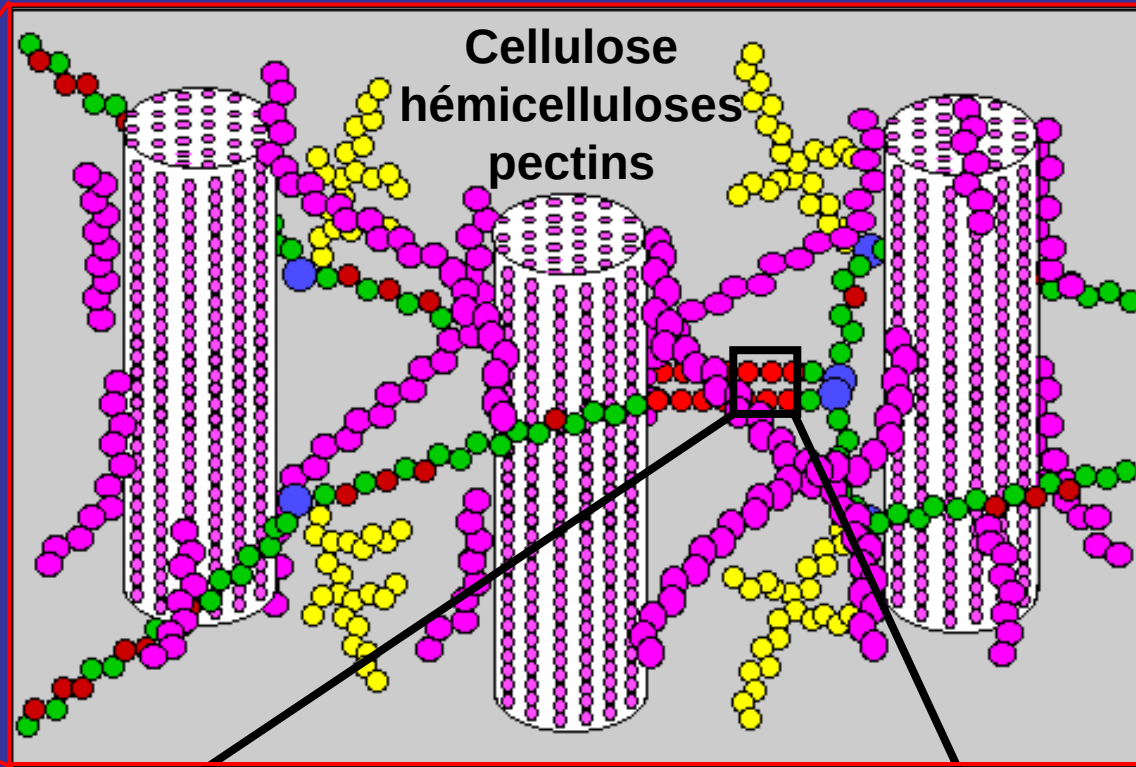
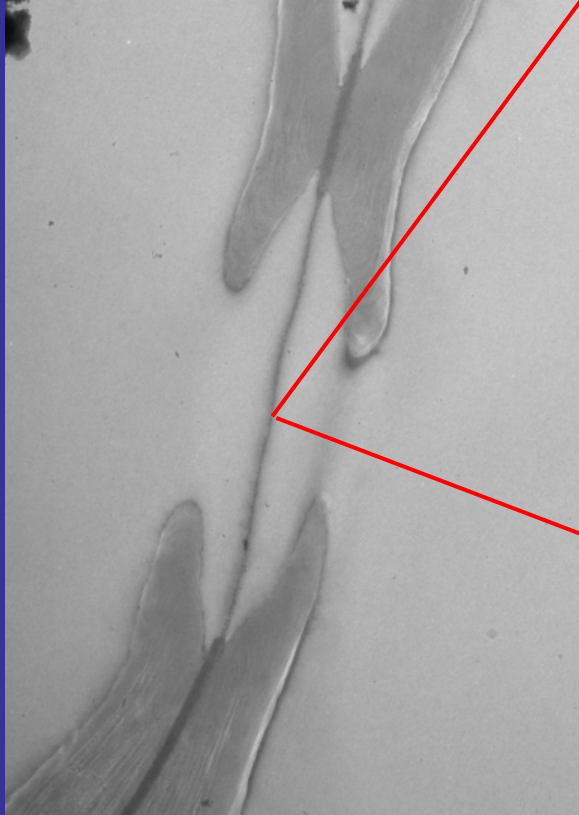
JIM7 recognize highly methylated HG

JIM5 recognize low methylated HG

Populus trichocarpa x deltoides



Pit membrane composition



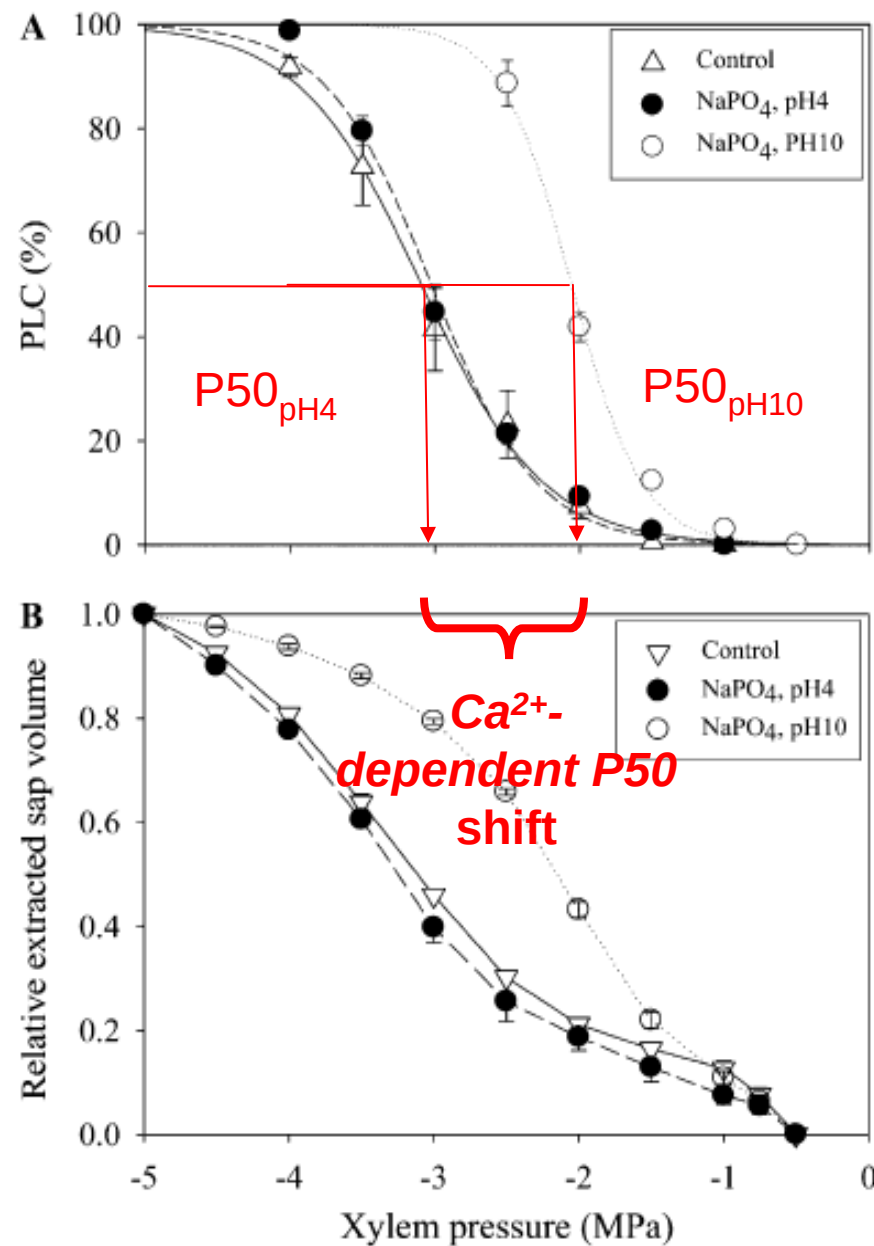
Calcium precipitation or chelation in the xylem

And effect on the VC



Perfused Solutions	P_{50} MPa
Control	$-3.09^a, \alpha (\pm 0.03)$
Water	$-3.11^a, \alpha (\pm 0.06)$
NaPO ₄ , pH 4	$-2.99^{ab}, \alpha (\pm 0.13)$
NaPO ₄ , pH 10	$-2.03^d (\pm 0.08)$
EGTA	$-2.58^{bc} (\pm 0.14)$
Oxalic acid, pH 2	$-2.33^{cd} (\pm 0.13)$
Oxalic acid, pH 5	$-2.76^{abc} (\pm 0.19)$
Tris-HCl, pH 6	$-3.06^a (\pm 0.01)$
Tris-HCl, pH 8	$-3.00^a (\pm 0.02)$
CaCl ₂ , 10 mM	$-3.03^a (\pm 0.11)$
NaPO ₄ , pH 10 → Tris-HCl, pH 10	$-2.26^b (\pm 0.38)$
NaPO ₄ , pH 10 → Tris-HCl, pH 10 + CaCl ₂	$-3.06^a (\pm 0.14)$

Calcium precipitation or chelation in the xylem And effect on the VC

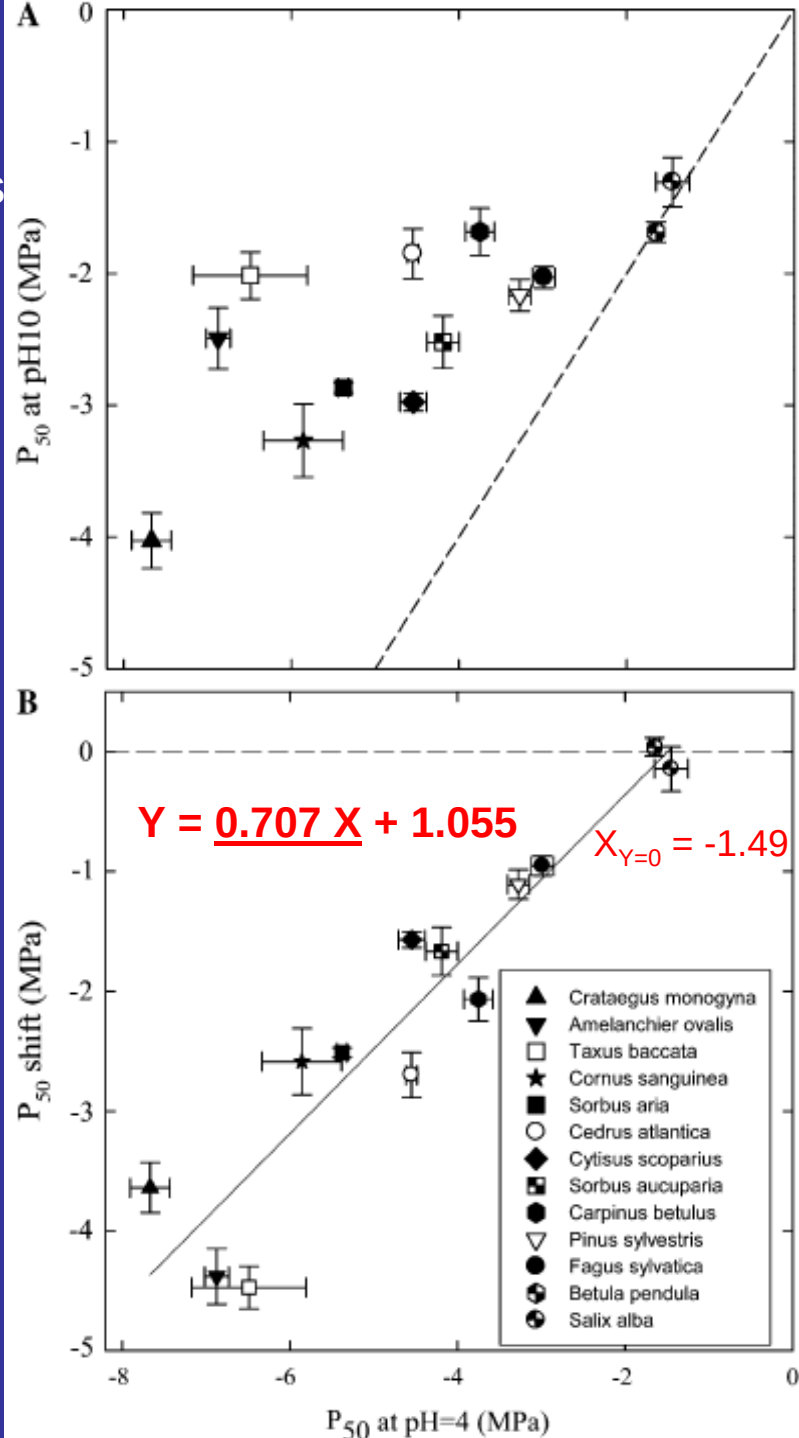
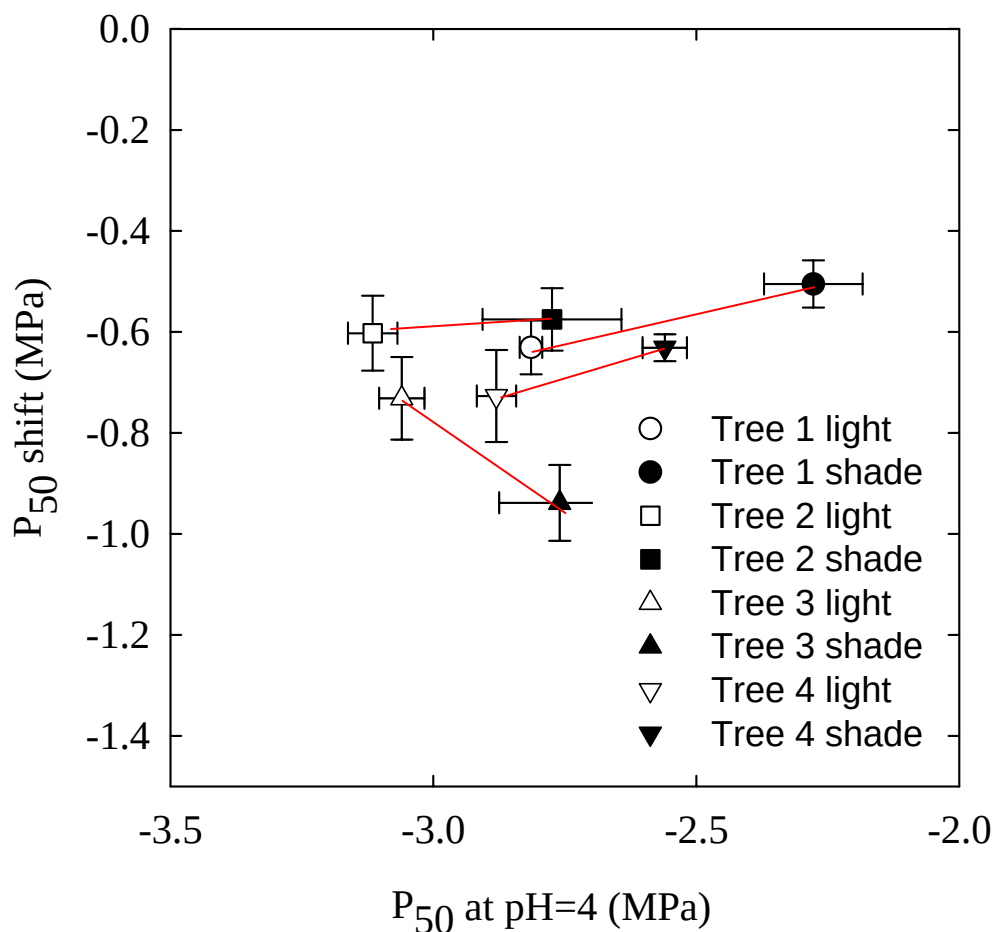


Calcium and variability of VC

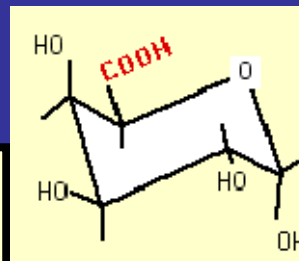
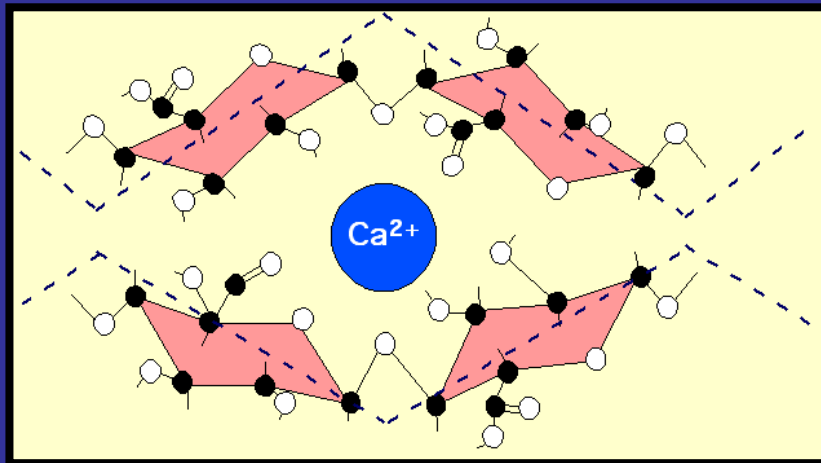
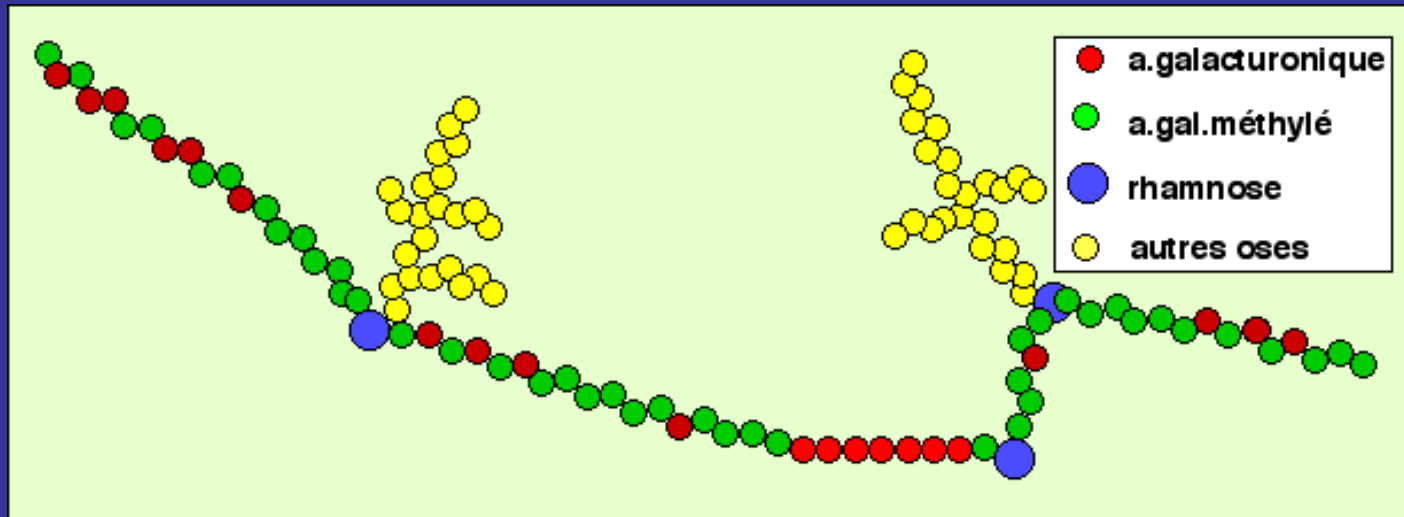


...between species

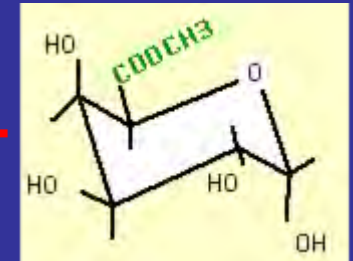
...within species



Genetic control of the role of Calcium in VC



PME *



* PME : Pectine methyl esterase

Genetic control of the role of Calcium in VC

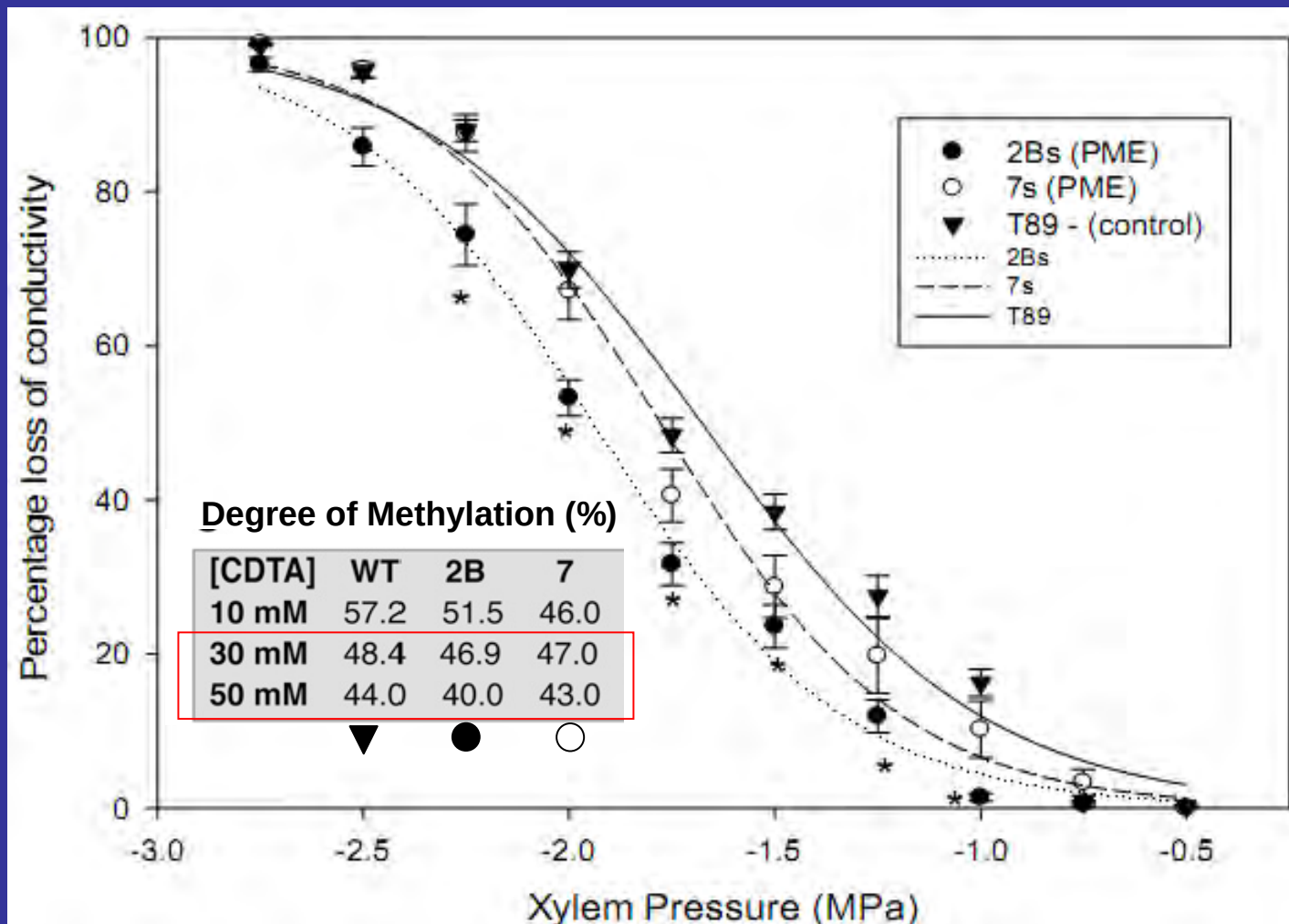
Pectin Methyl Esterase Inhibits Intrusive and Symplastic Cell Growth in Developing Wood Cells of *Populus*^{1,2[W][OA]}

Anna Siedlecka, Susanne Wiklund, Marie-Amélie Péronne, Fabienne Micheli³, Joanna Leśniewska⁴, Ingmar Sethson, Ulf Edlund, Luc Richard, Björn Sundberg*, and Ewa J. Mellerowicz



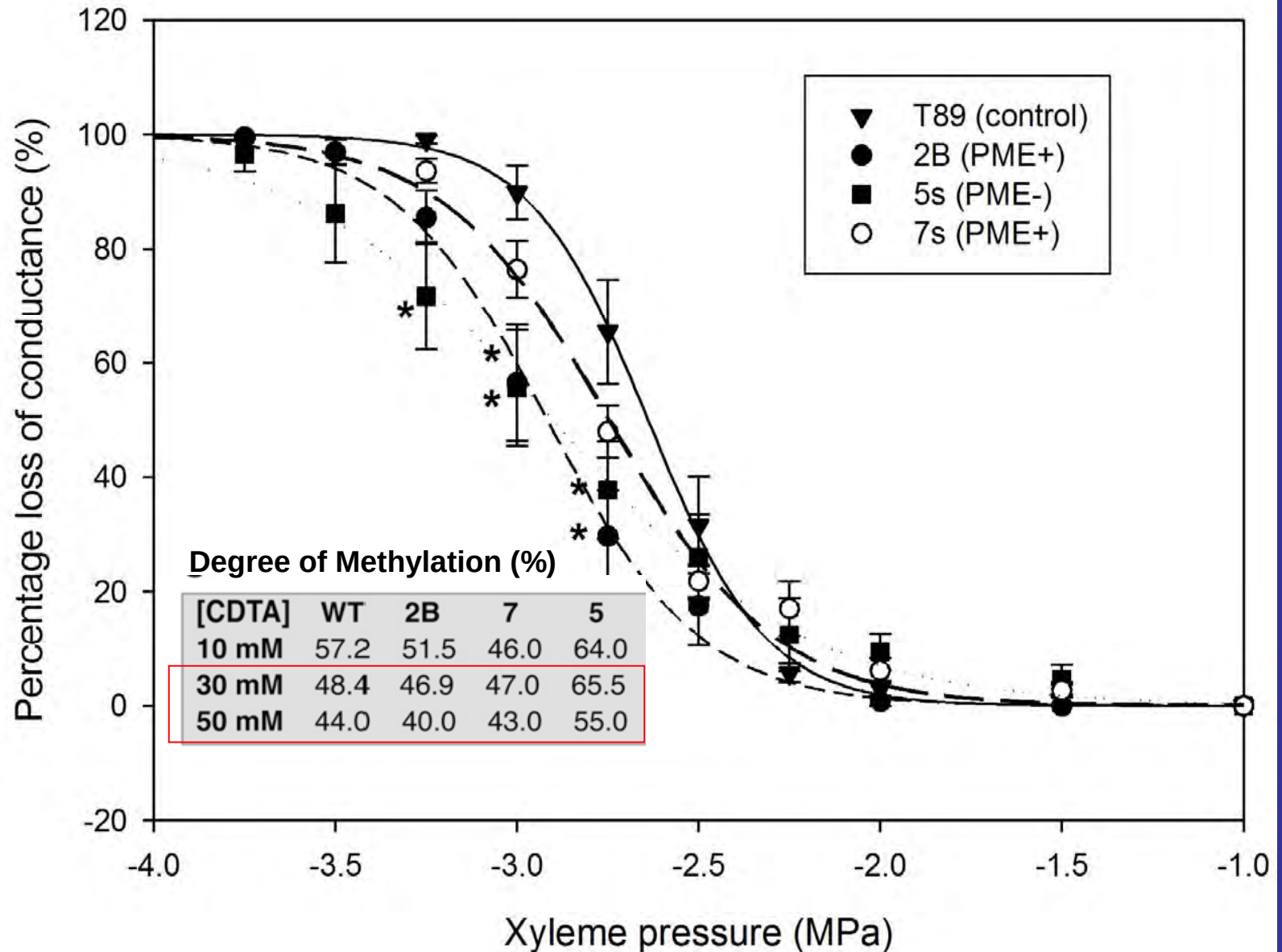
	T89 (control)	7s (PME+)	2Bs (PME++)
Ψ_p , MPa ($\pm$ S.D.)	-0.55 ($\pm$ 0.053)	-0.53 ($\pm$ 0.047)	-0.54 ($\pm$ 0.048)
Ψ_m , MPa ($\pm$ S.D.)	-1.22 ($\pm$ 0.087)	-1.23 ($\pm$ 0.13)	-1.19 ($\pm$ 0.094)
Height, m ($\pm$ S.D.)	1.24 ($\pm$ 0.12)	1.29 ($\pm$ 0.055)	1.25 ($\pm$ 0.13)
Conductivity, ($\pm$ S.D.)	1.96 ($\pm$ 0.47)	2.08 ($\pm$ 0.32)	2.34 ($\pm$ 0.33)
Embolism rate, % ($\pm$ S.D.)	34.3 ($\pm$ 15.3)	28.1 ($\pm$ 22.5)	24.0 ($\pm$ 13.3)

Effect of PME overexpression in VC



Increase in PME causes a decrease in VC

Effect of PME overexpression in VC



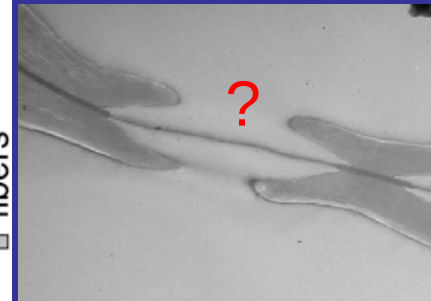
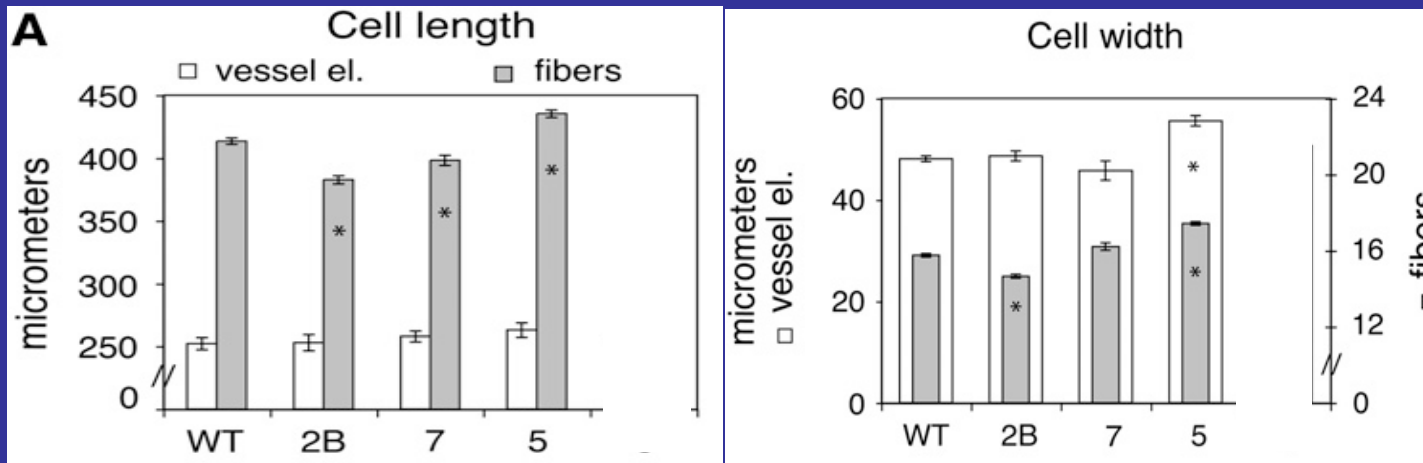
Increase or decrease in PME causes a decrease in VC

Conclusions :

- ✓ Xylem Calcium is responsible for a major part of the between species variability for VC.
- ✓ It would not be involved in the phenotypic plasticity within species
- ✓ PME over- and under-expression increases the VC

Perspectives :

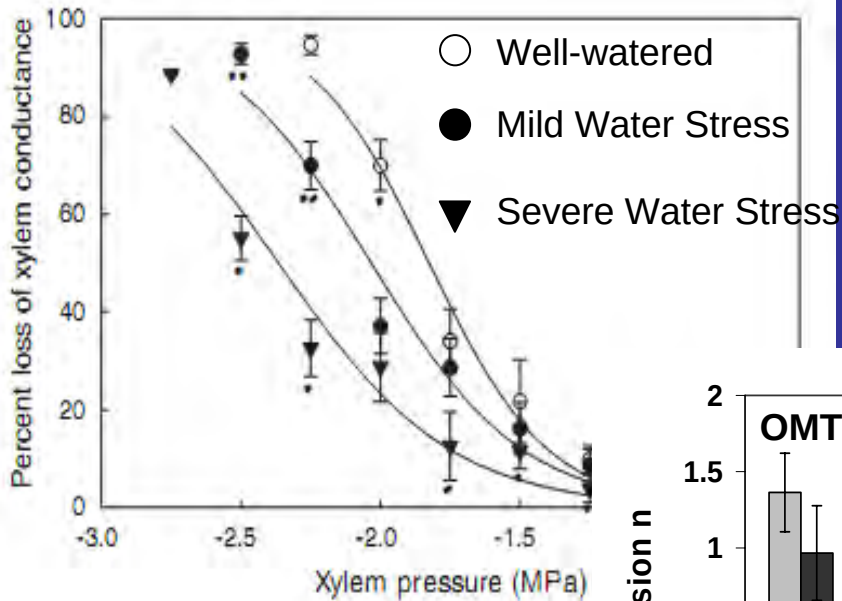
-Investigate the xylem structure and pit membrane structure and composition in poplars over- or under-expressing PME.



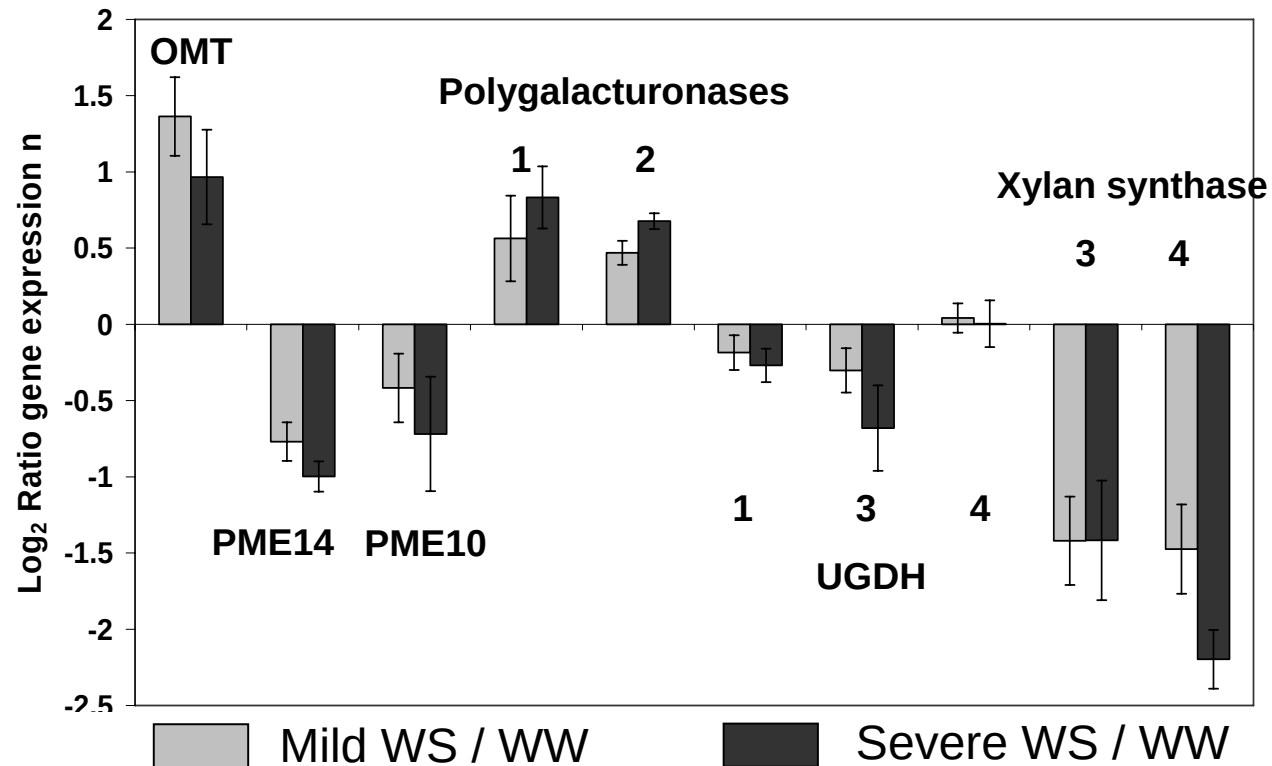


Thanks for your attention

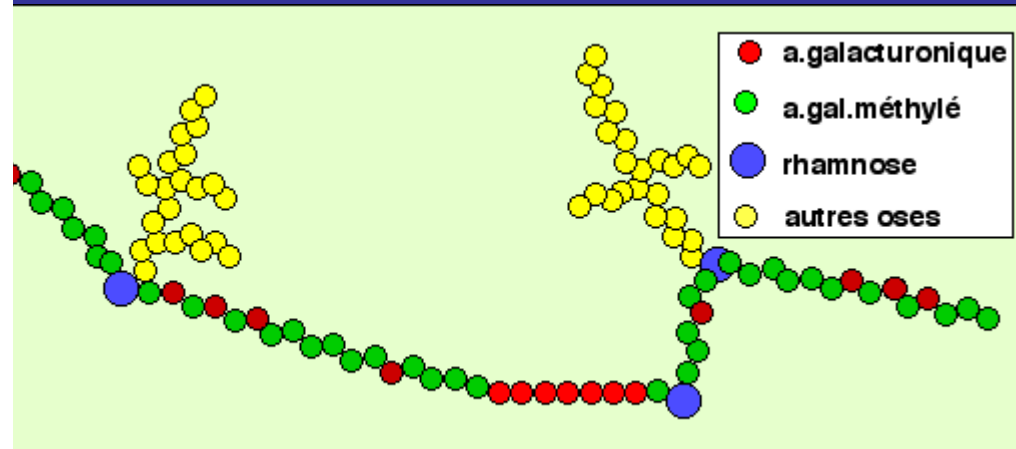
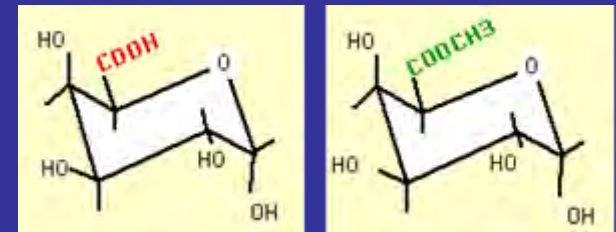
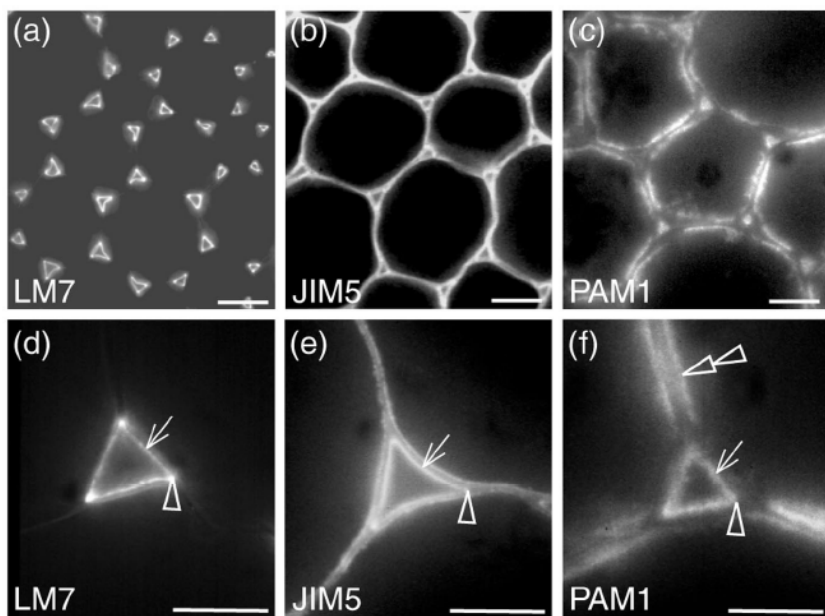
The contribution of gene expression studies on searching the genetic control of VC ?

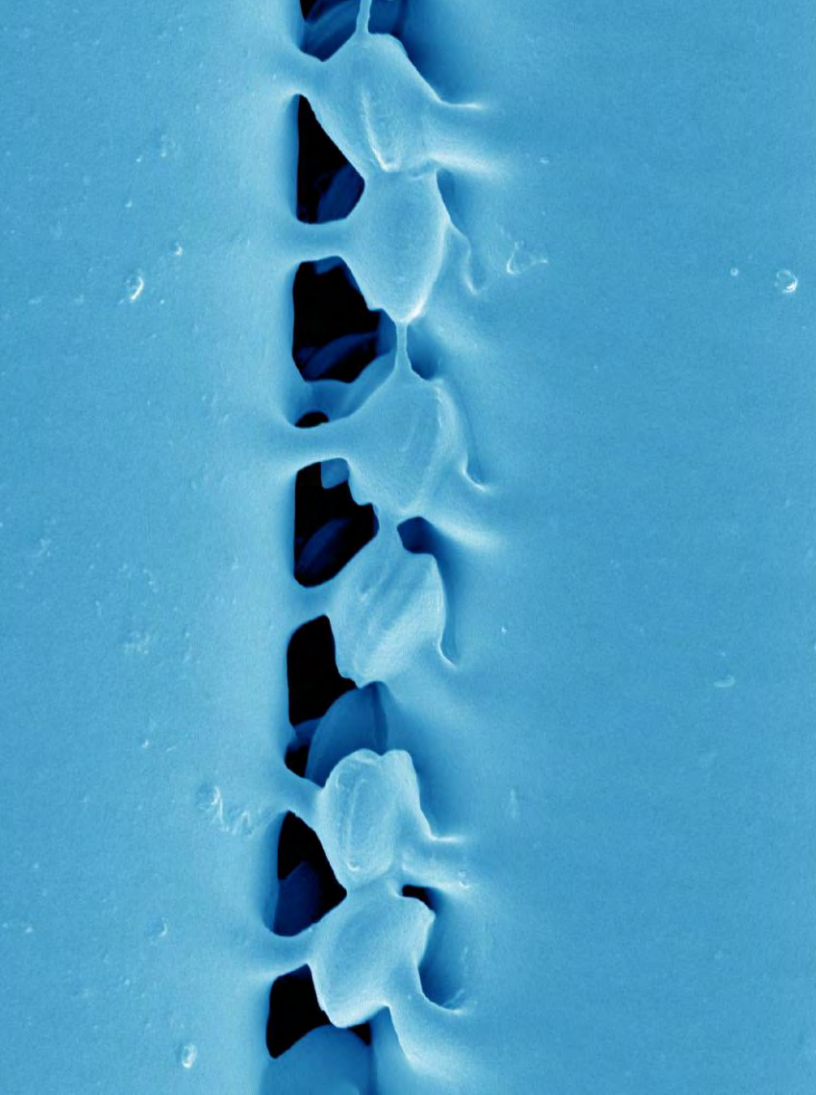


The Classical investigations of gene expression are not suitable to identify gene of the VC.



(Willats et al. 2001)





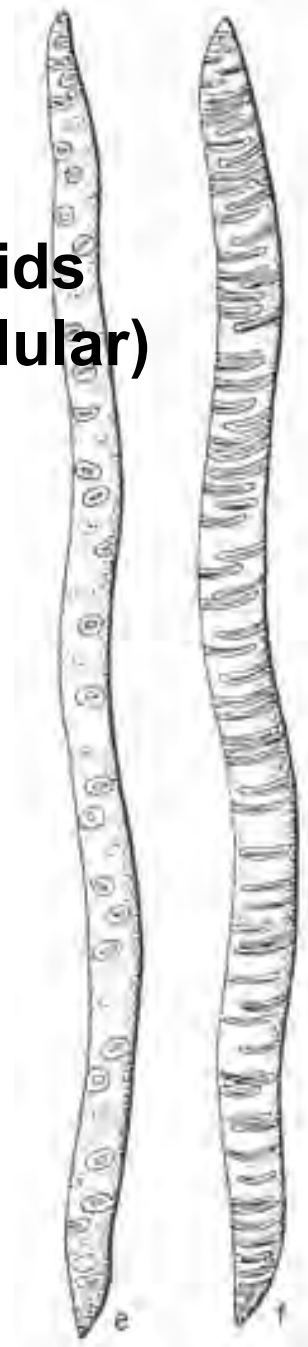
La structure et la fonction des ponctuations aréolées dans le réseau hydraulique de plantes

Steven Jansen

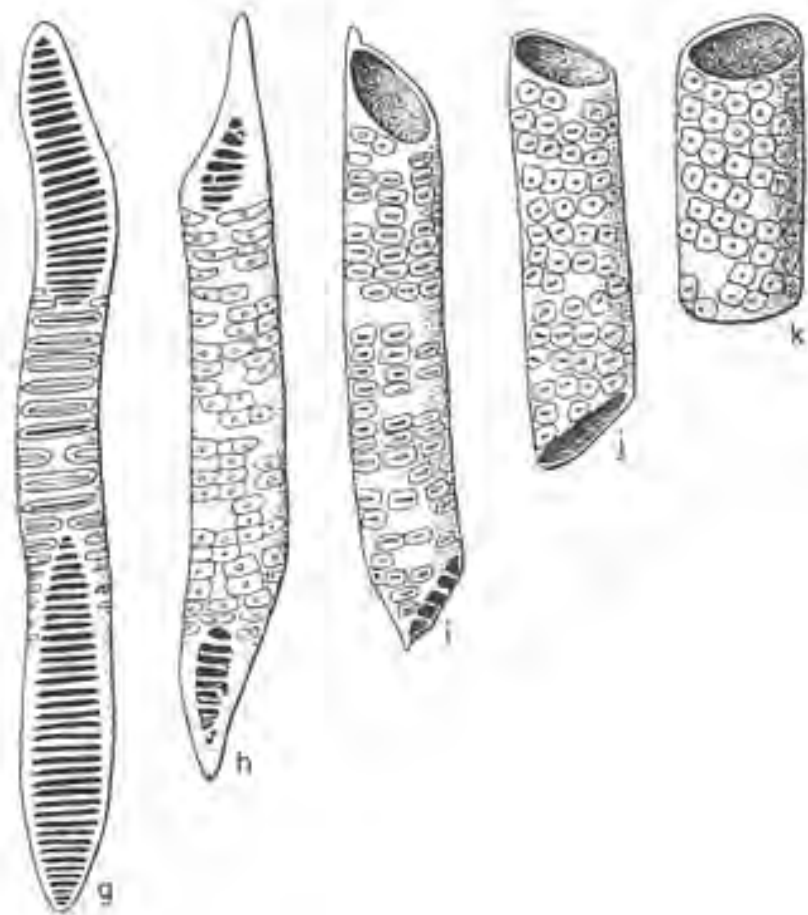
Institute for Systematic Botany and Ecology



**Tracheids
(unicellular)**



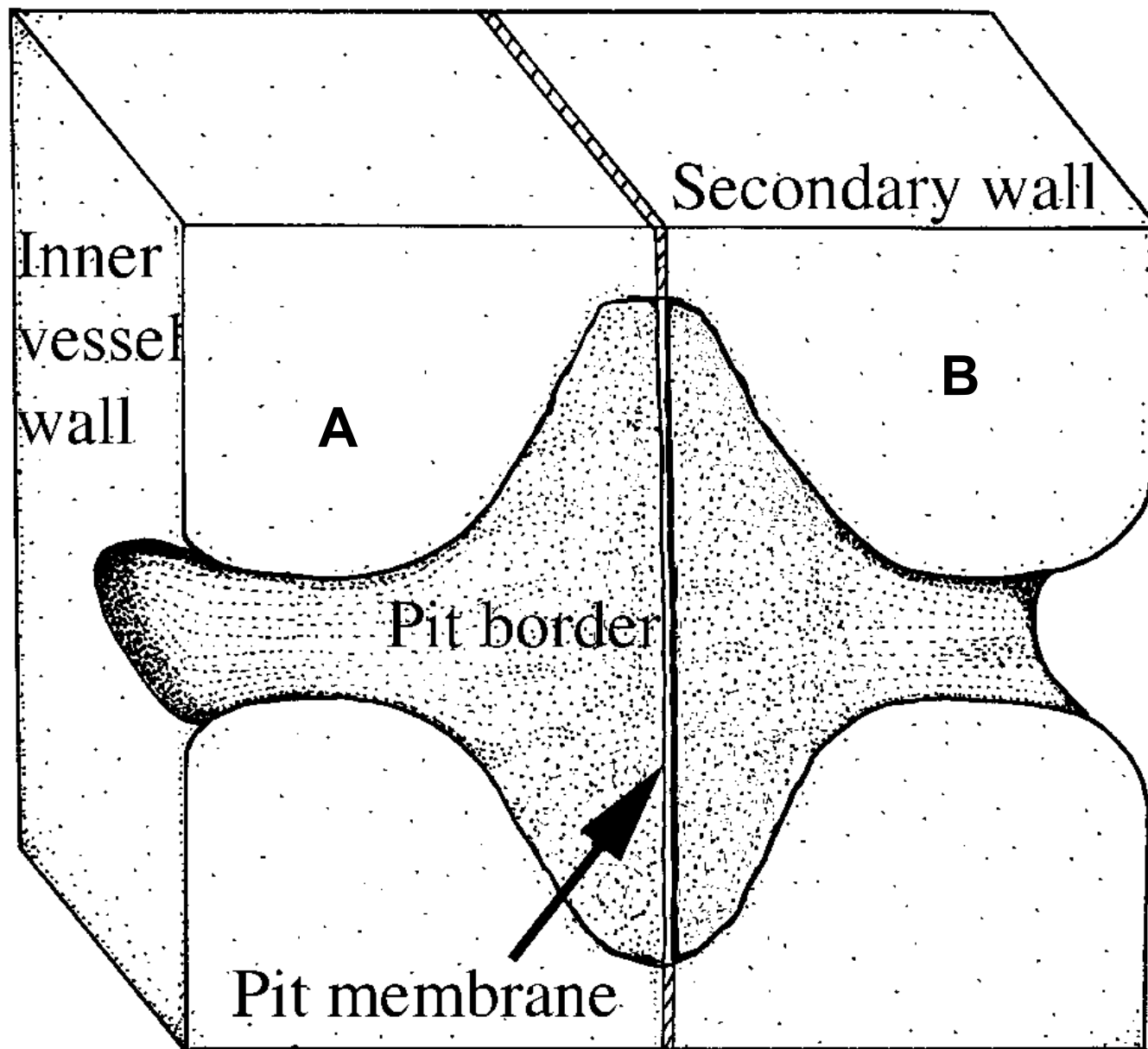
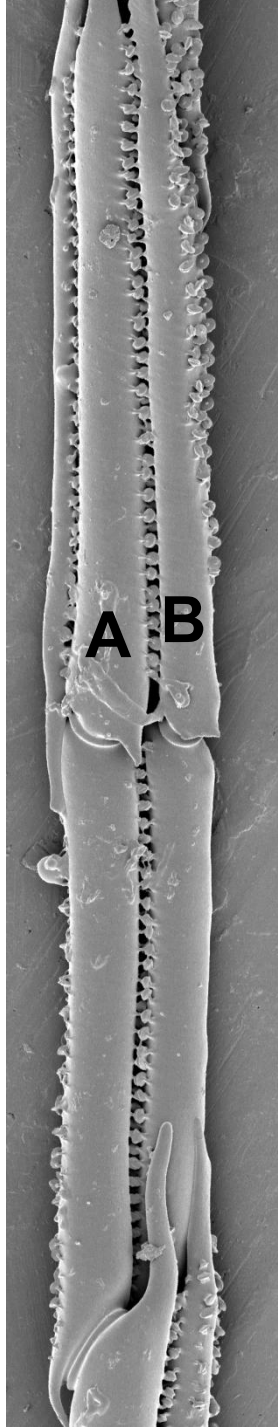
**Vessel elements with a
scalariform - simple
perforation plate**



**Vessels
(multicellular)**



*Meryta
tenuifolia*





(a)

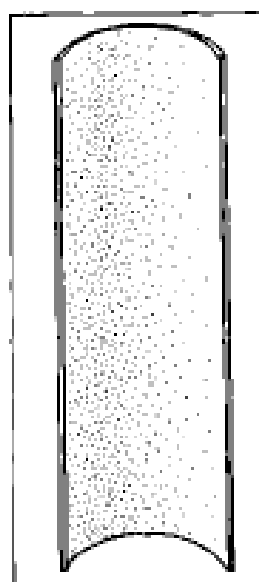


(d)

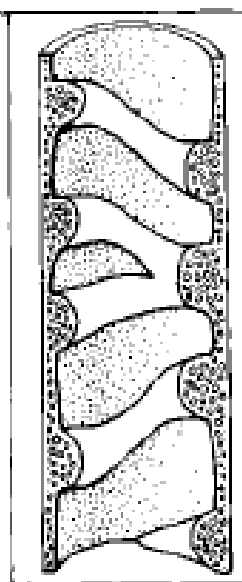


(g)

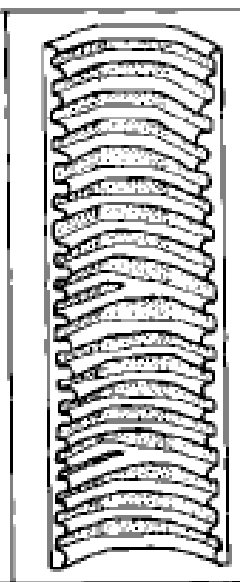
a



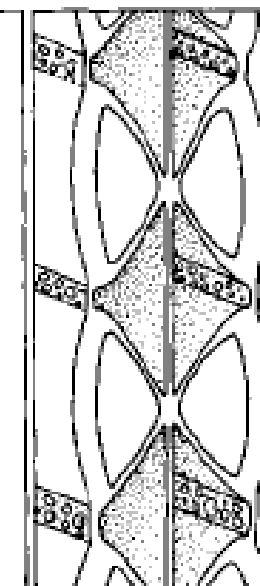
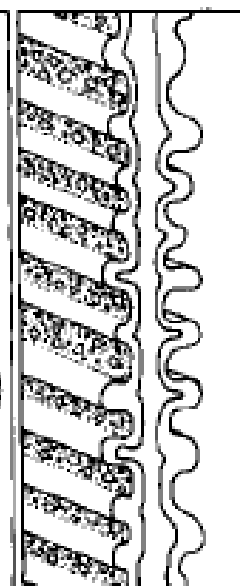
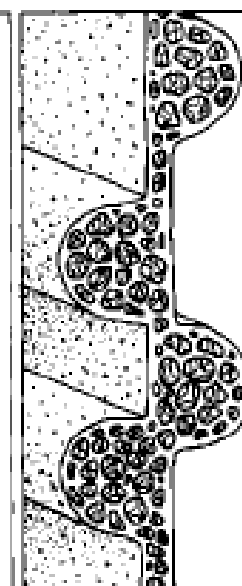
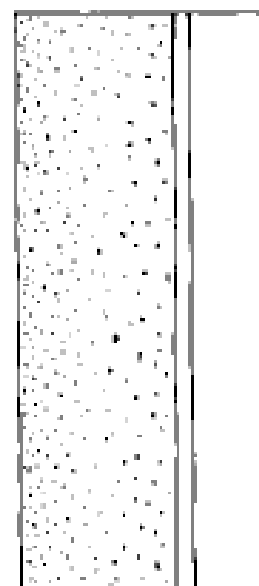
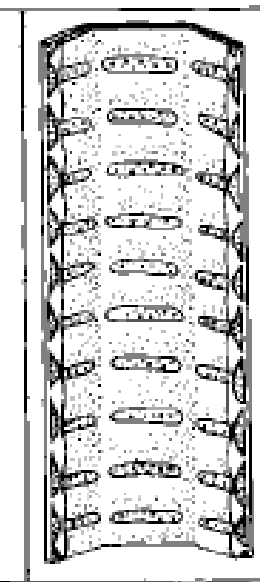
b



c

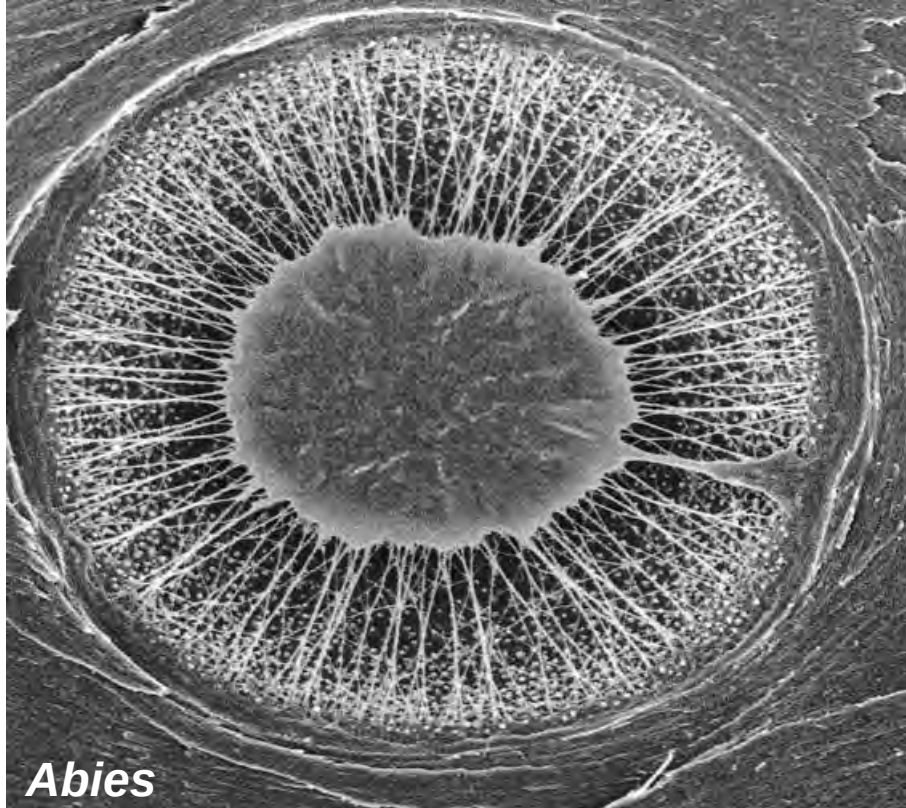


d

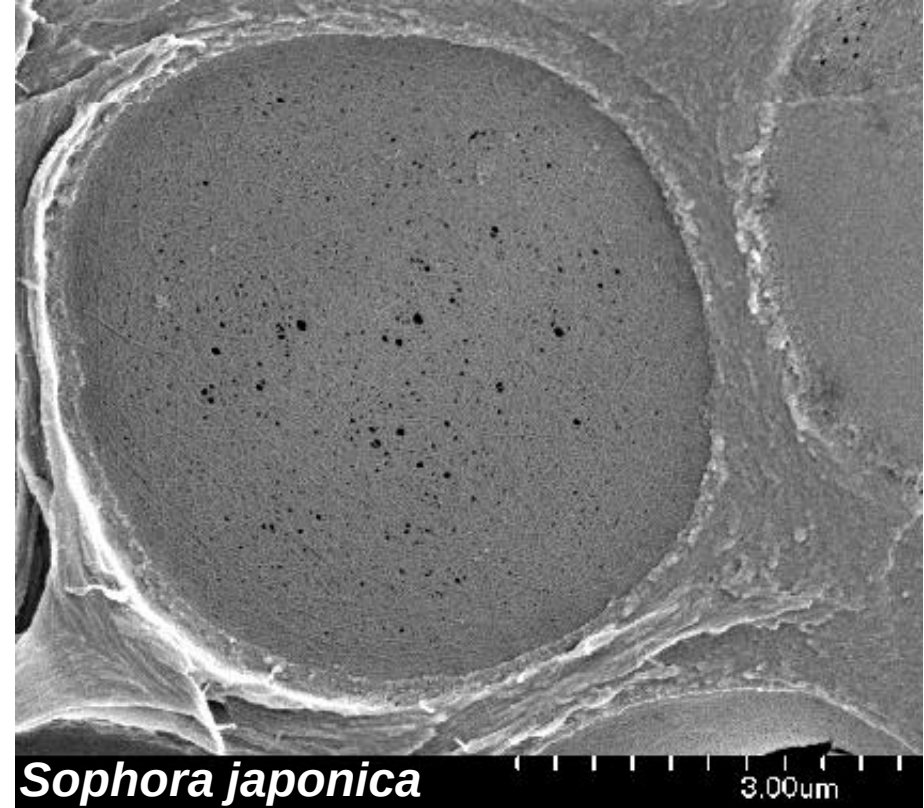


From: Kenrick & Crane (1997); Friedman & Cook (2000); Pittermann (2010)

Pit membranes

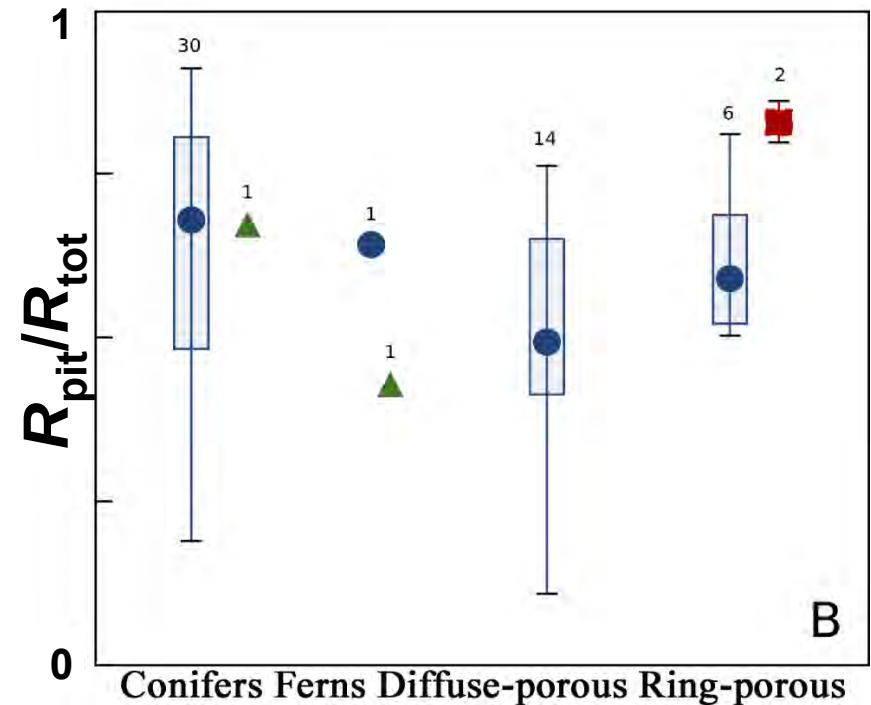
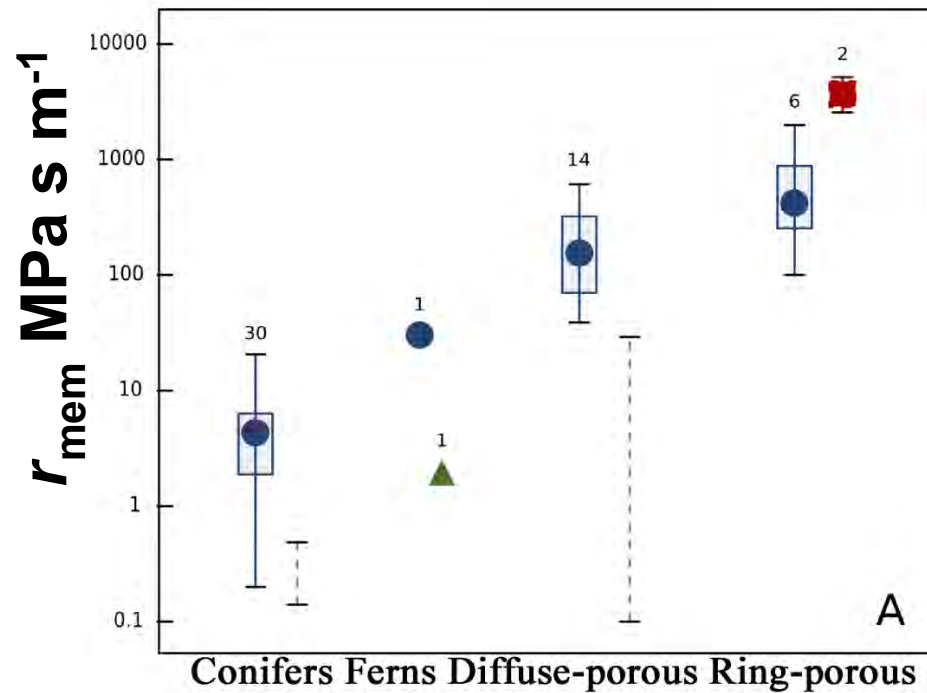


**Gymnosperms –
torus-margo**

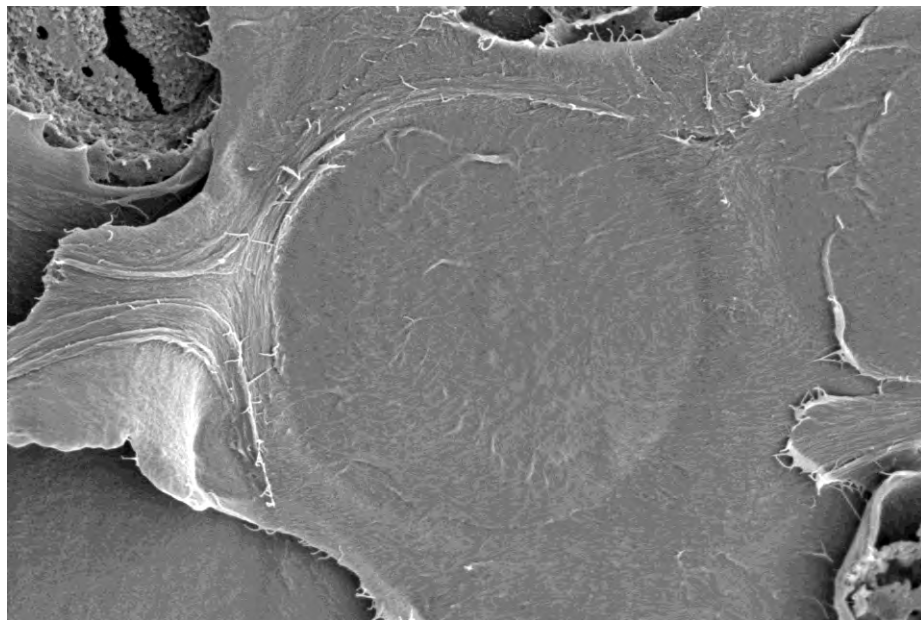


**Angiosperms –
homogeneous pit membrane**

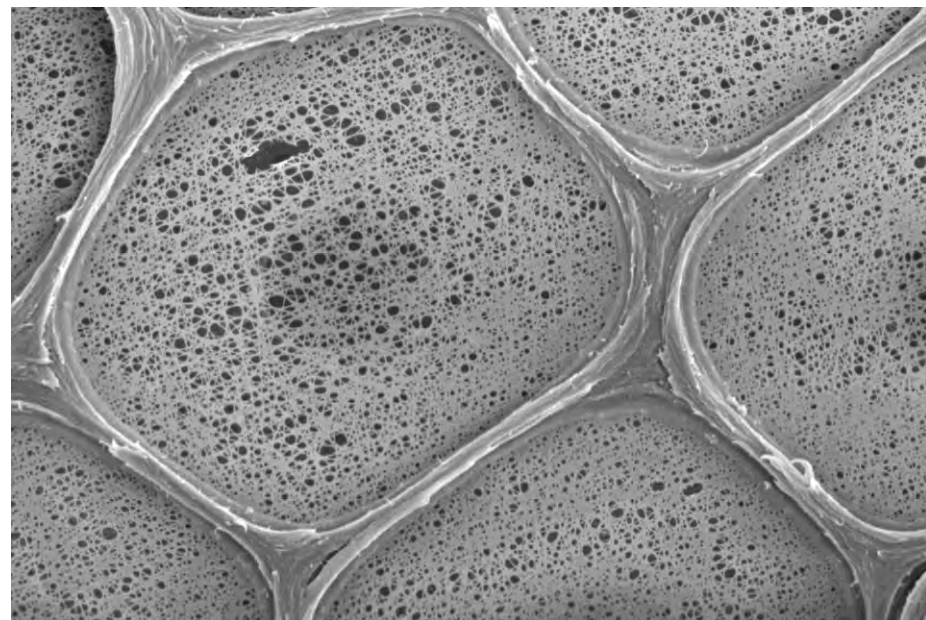
Partitioning of vascular resistance



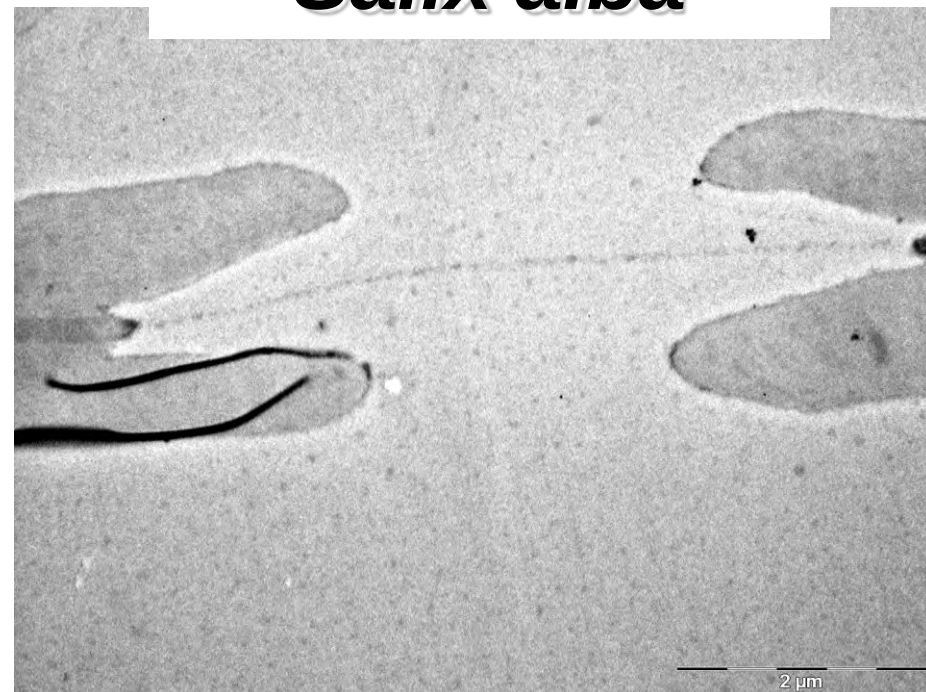
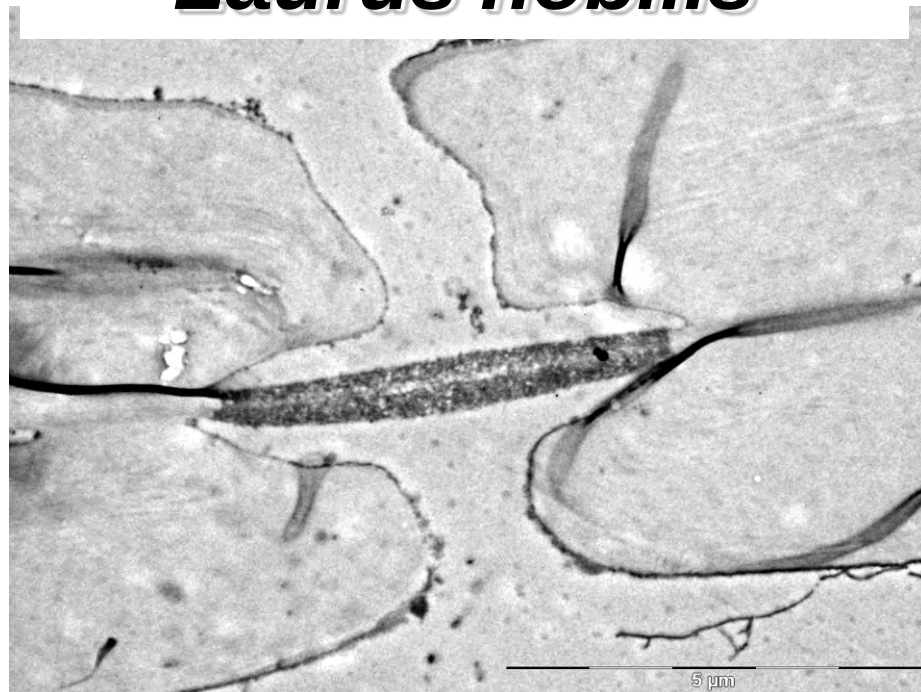
Choat, Cobb & Jansen (2008)



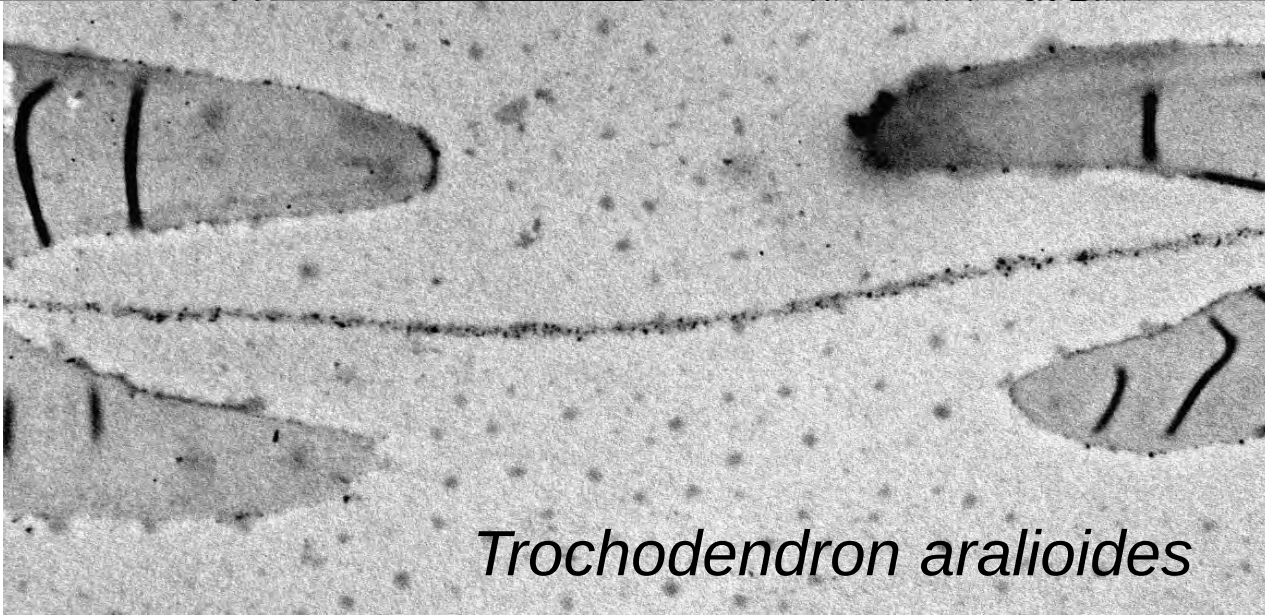
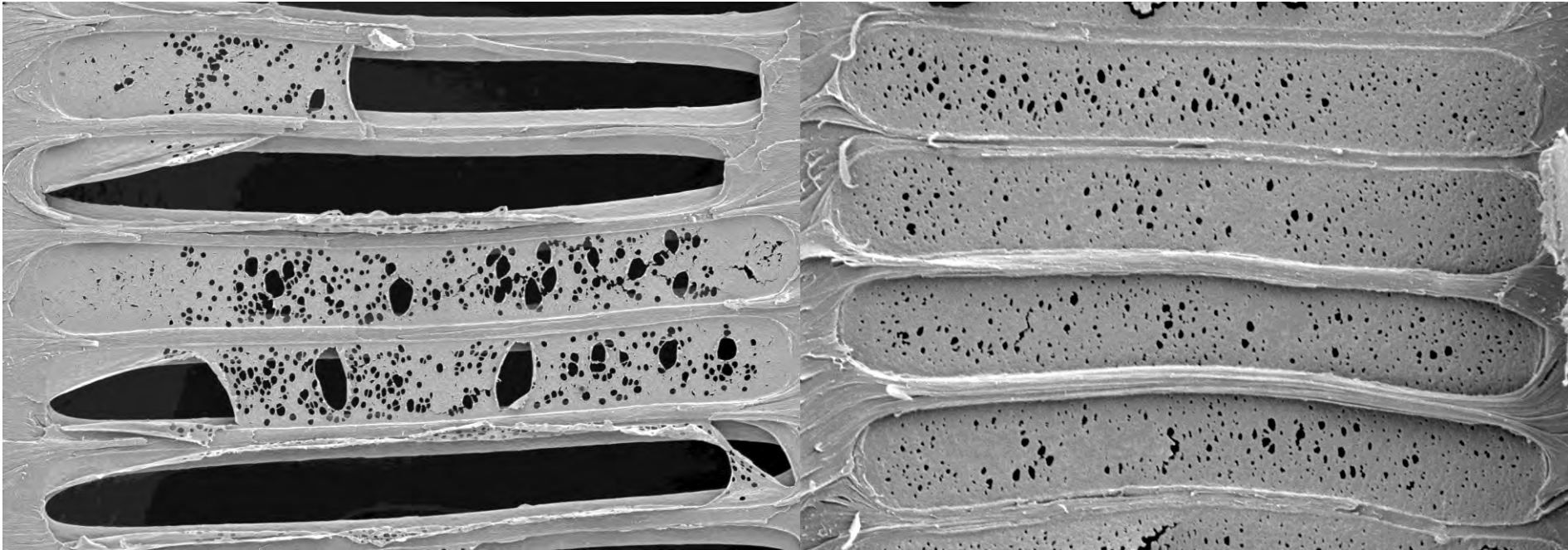
Laurus nobilis



Salix alba

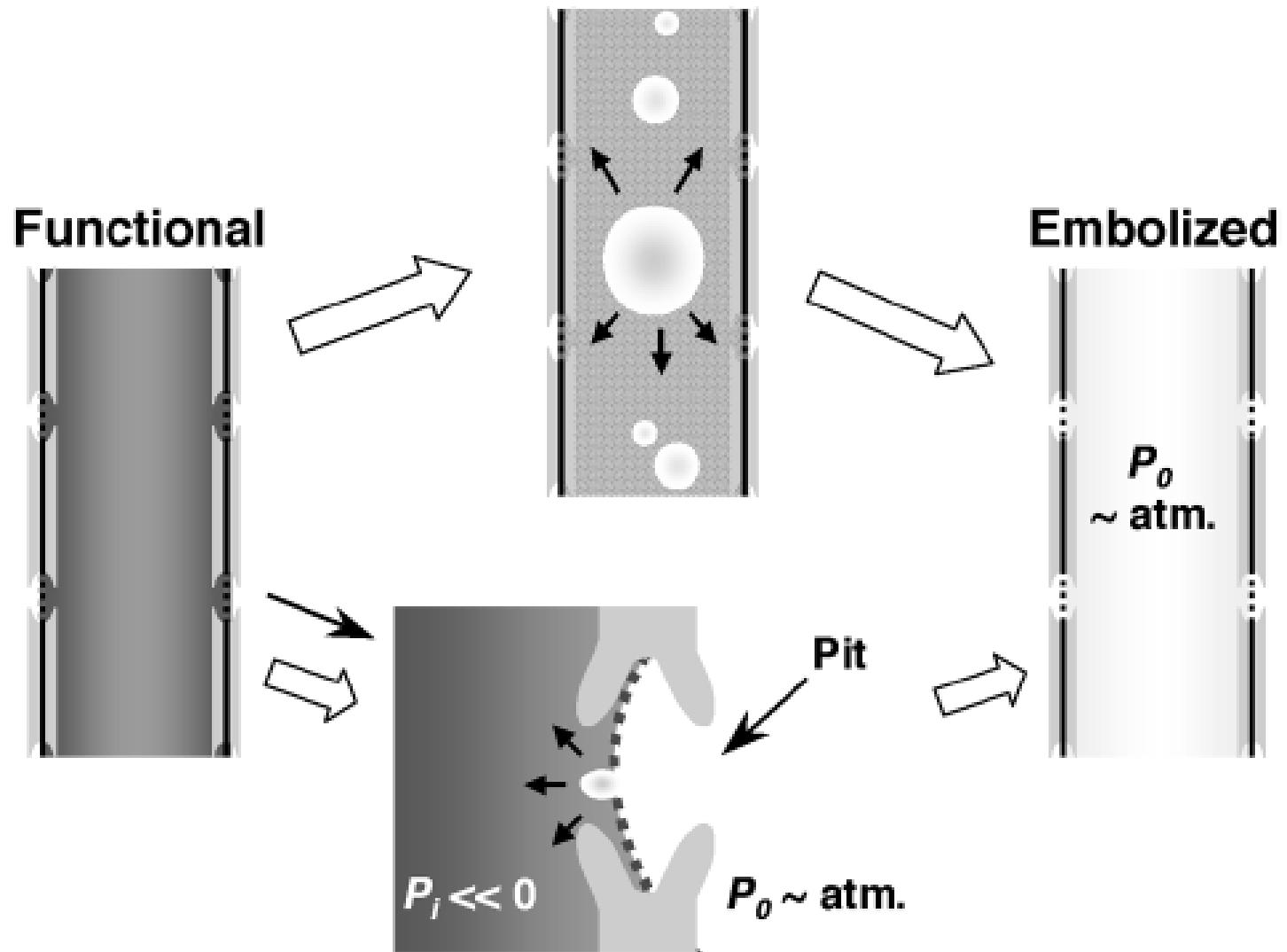


Tetracentron sinense



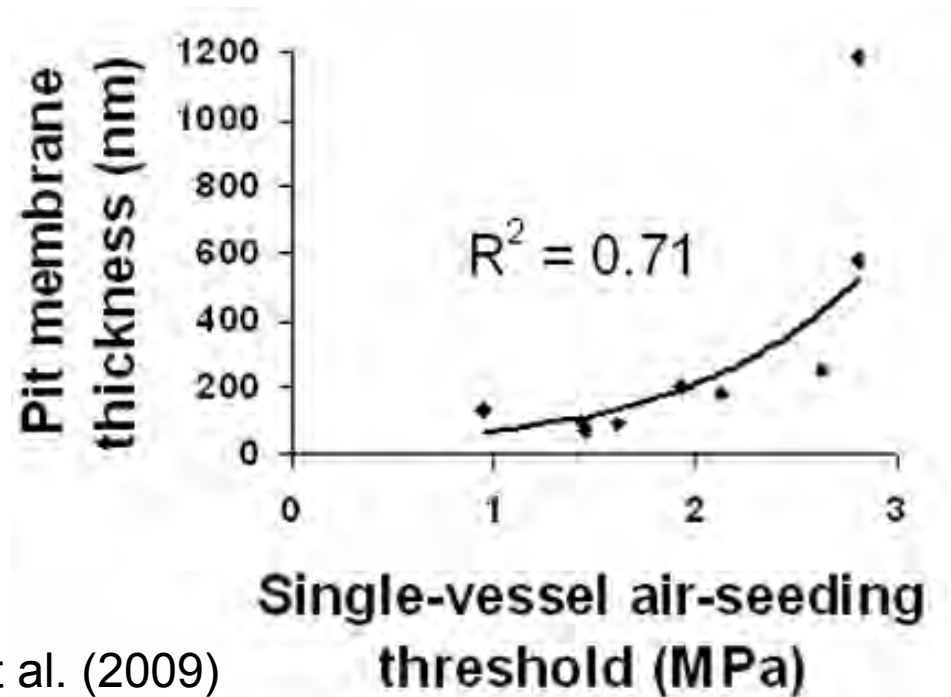
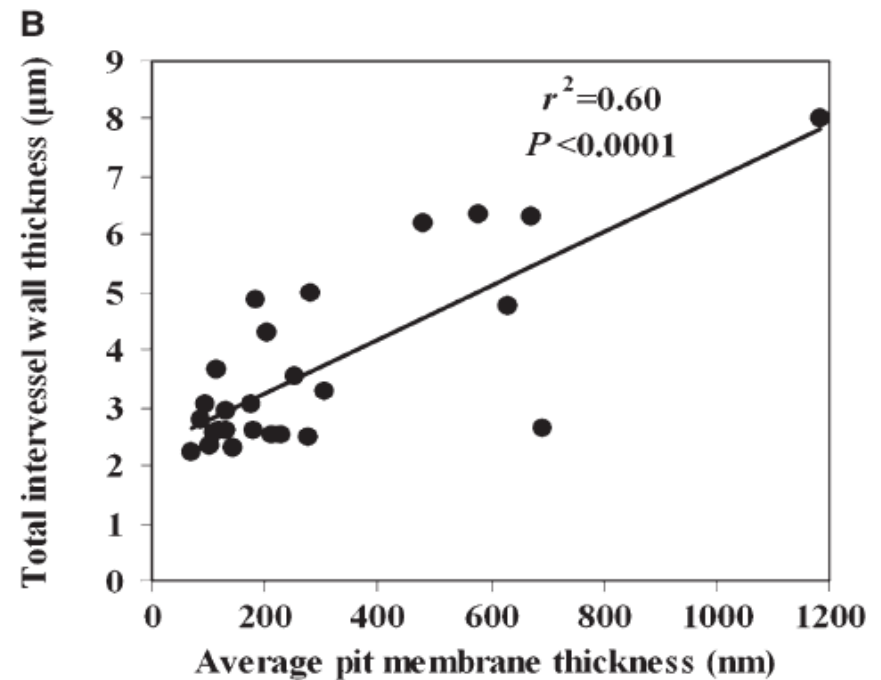
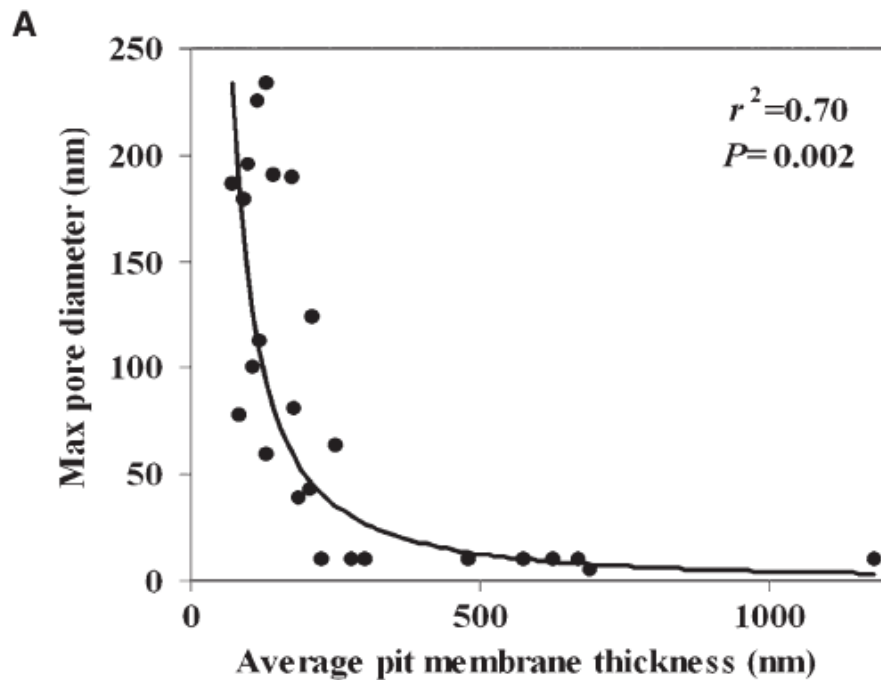
Trochodendron aralioides

Freezing-induced cavitation



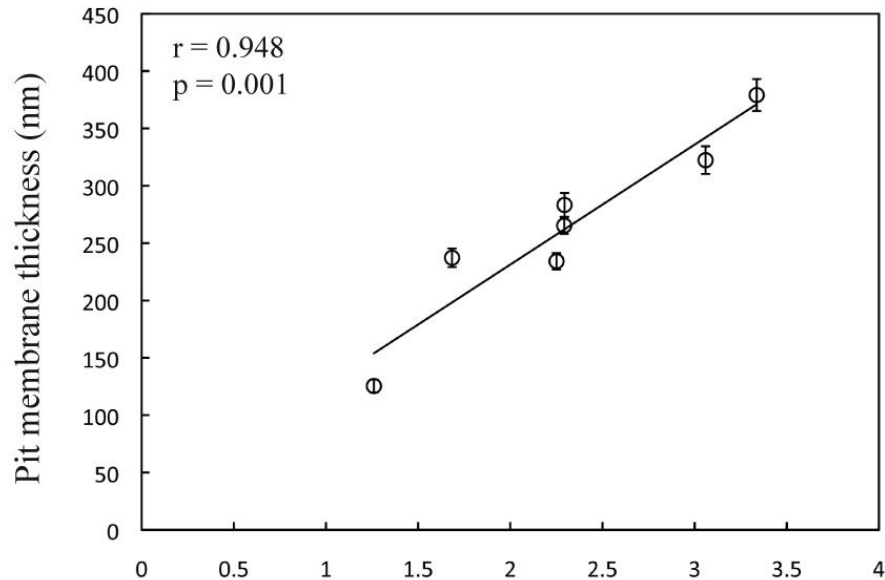
Drought-induced cavitation

Hacke & Sperry (2001)

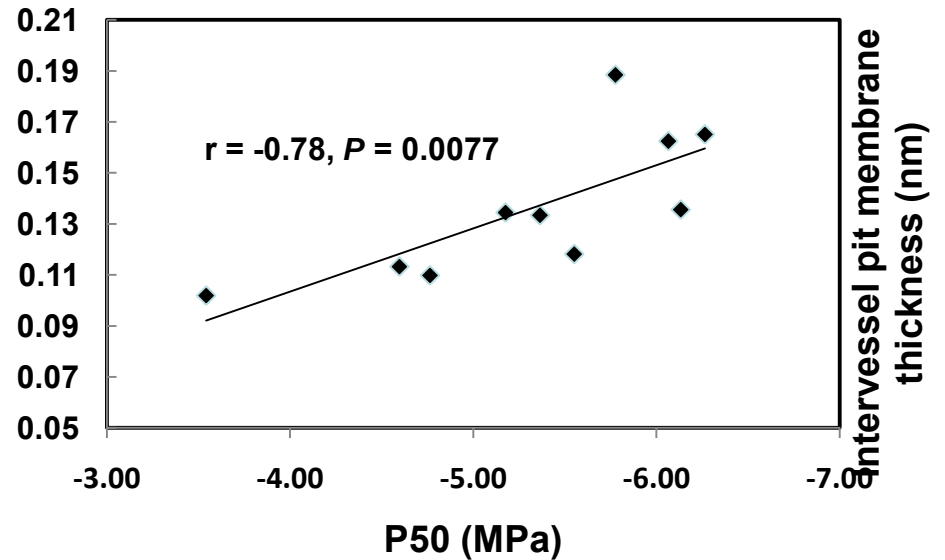


Jansen et al. (2009)

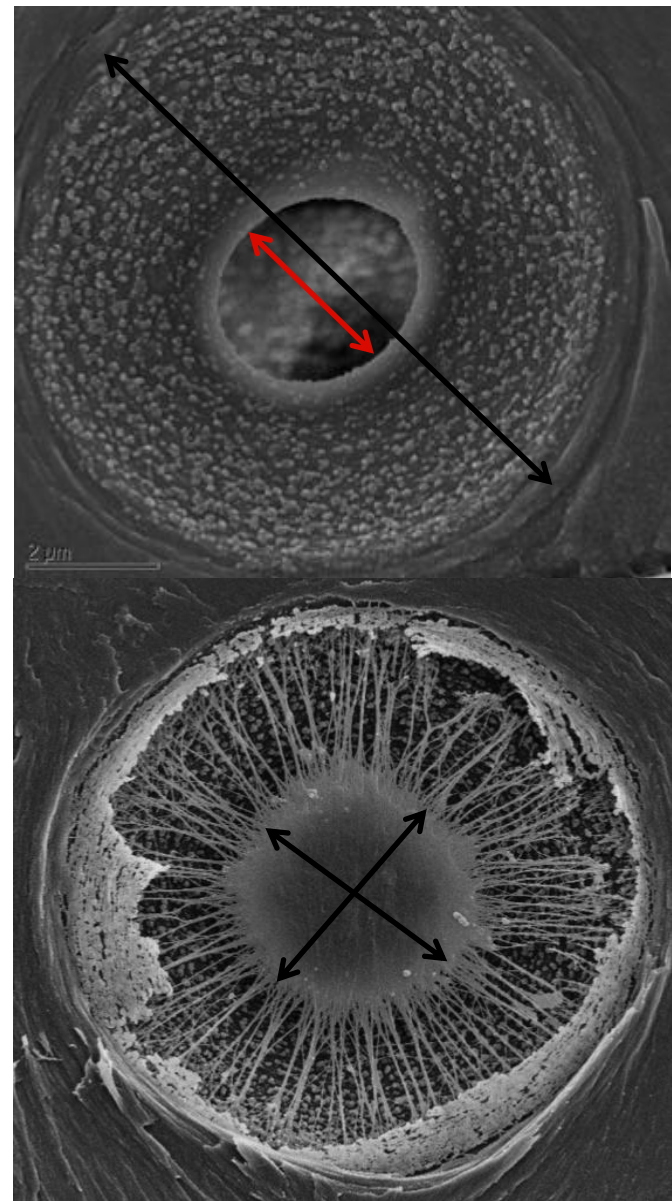
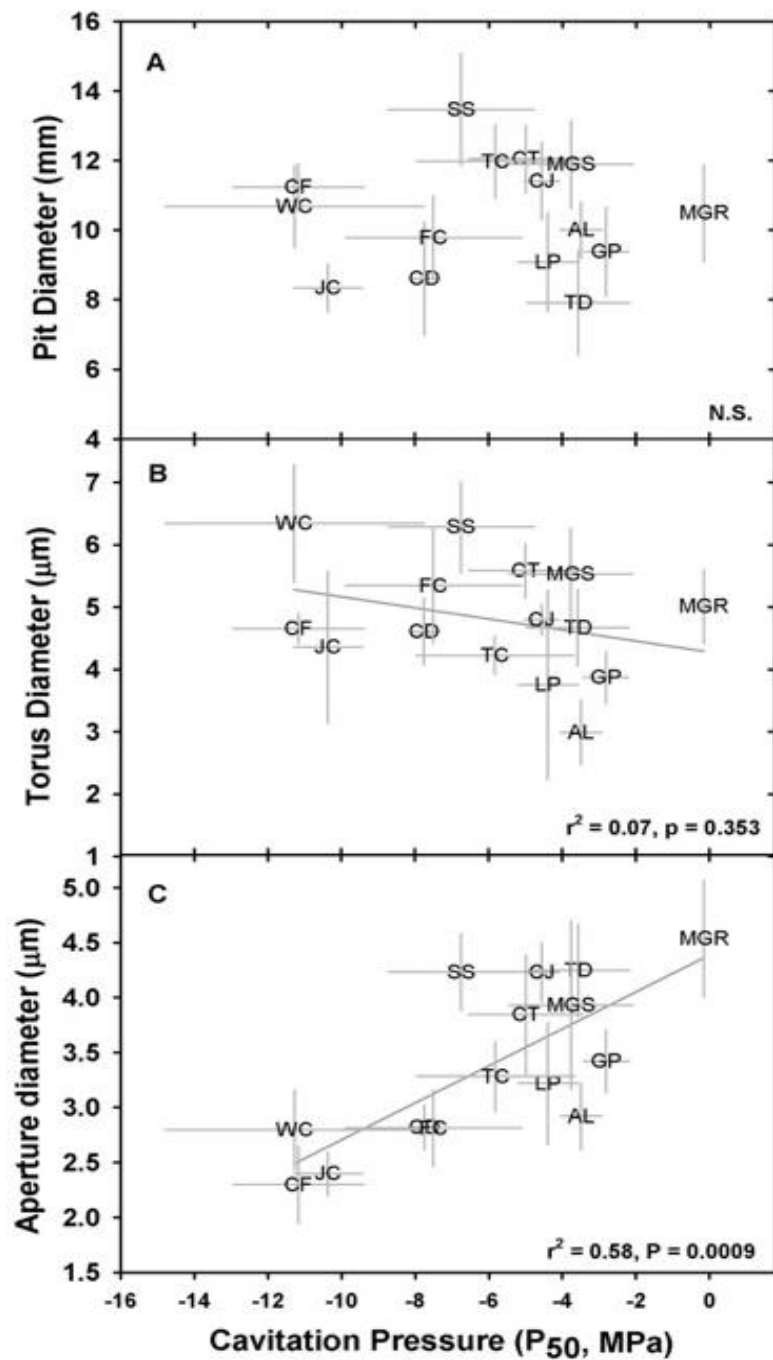
Intervessel pit membrane thickness vs. P50 in *Acer* and *Prunus*



Lens et al. (In press)

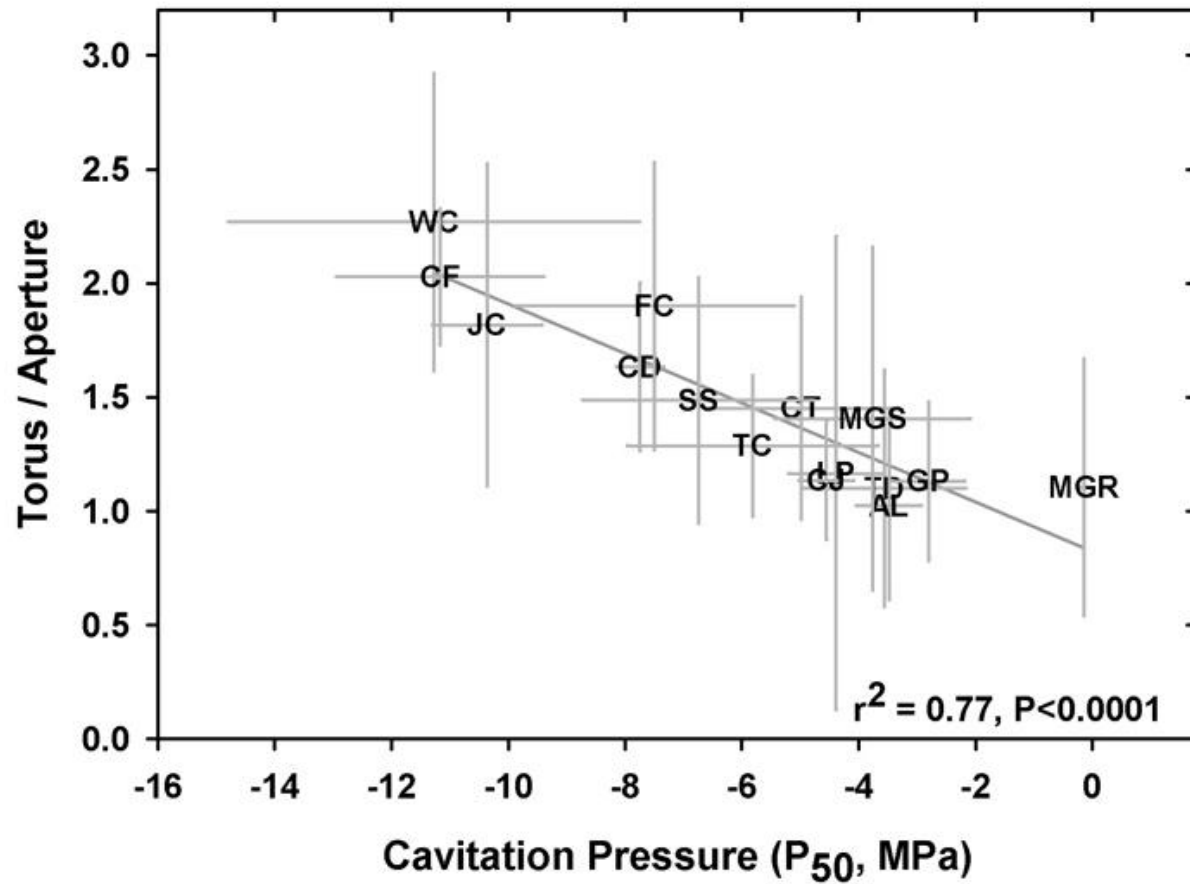


Rabaey et al. (unpublished)

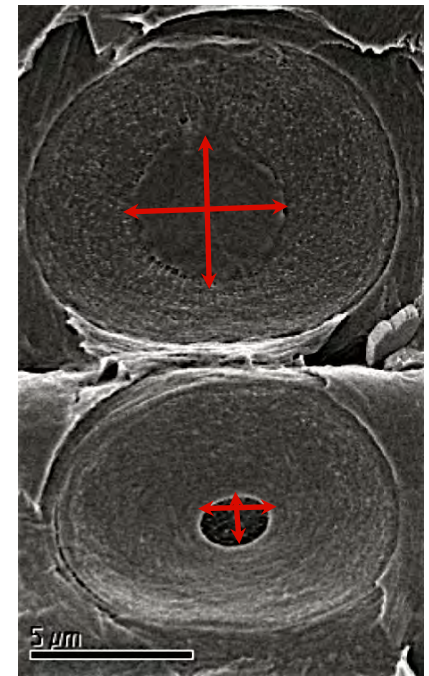


Calocedrus decurrens

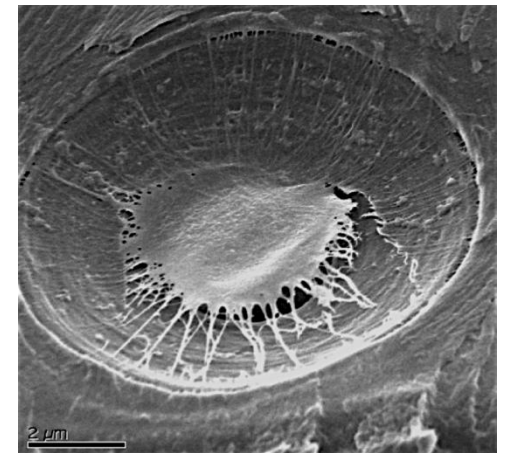
Pittermann et al. (2010)



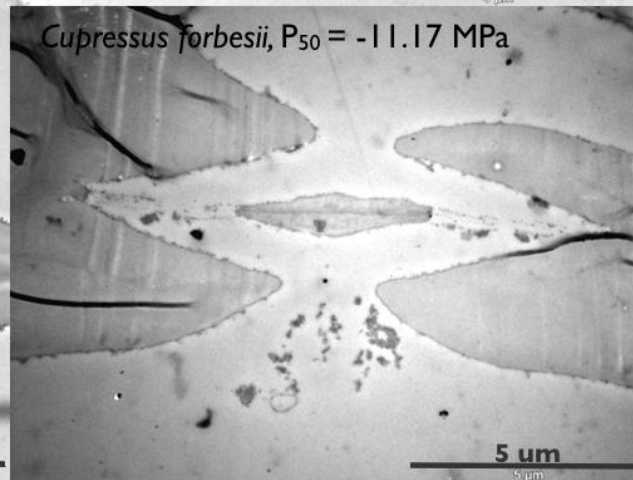
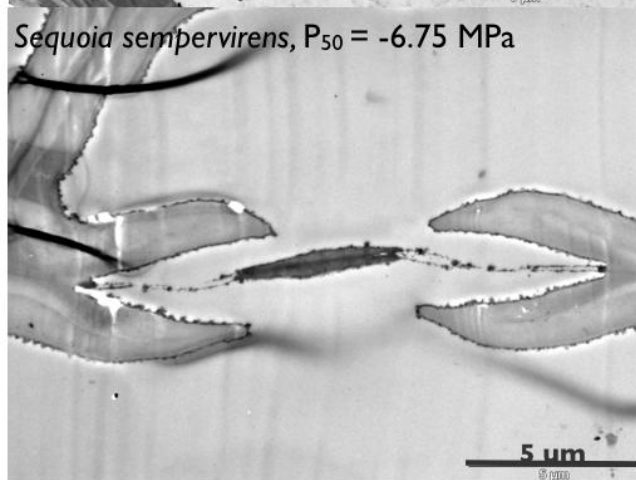
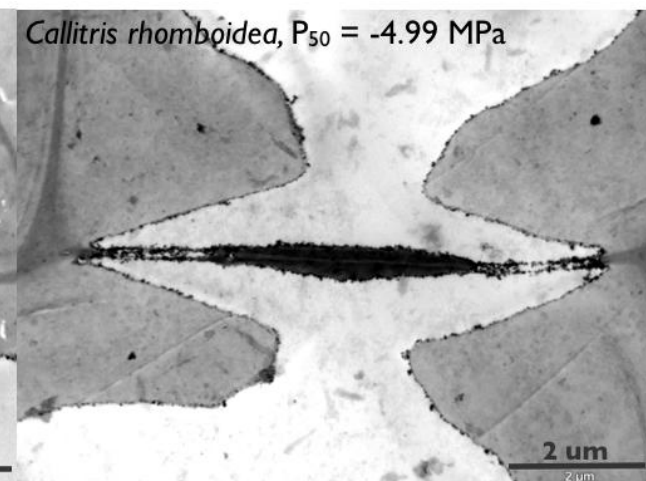
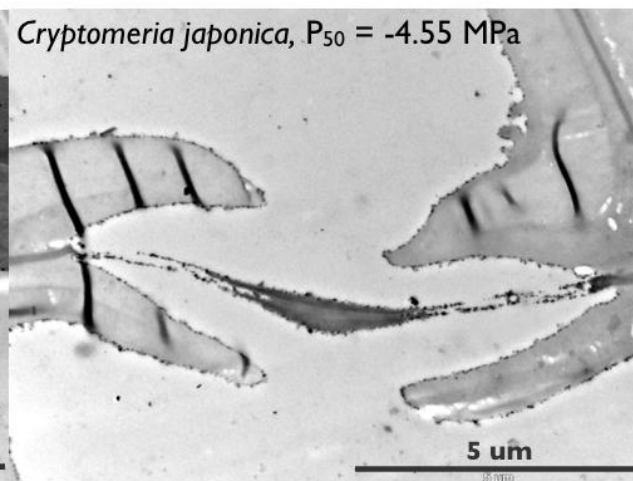
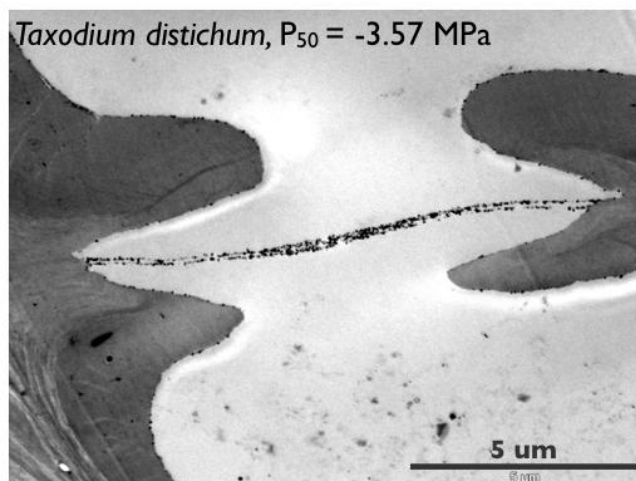
Pittermann et al. (2010)



Cupressus forbesii



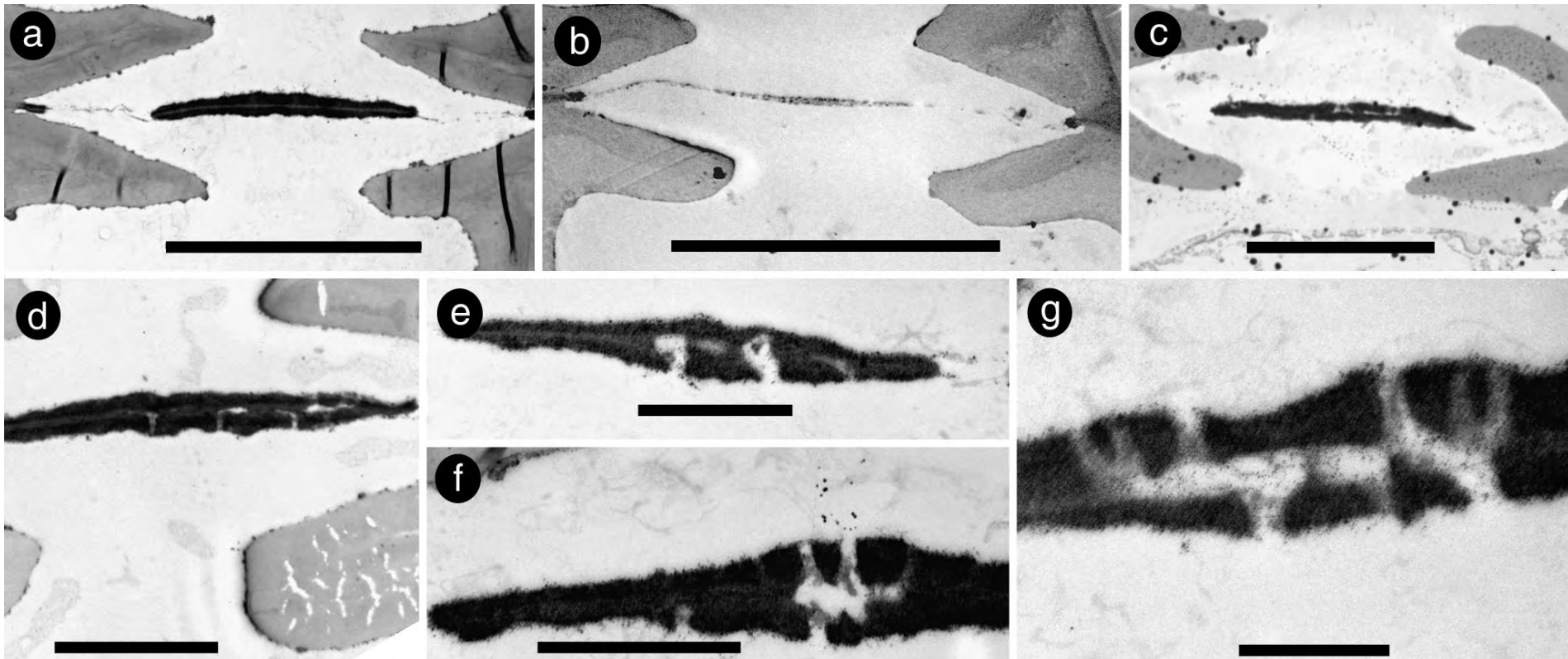
Glyptostrobus pensilis



- Torus thickness: increases with more negative cavitation pressures
- Margo thickness: invariable across P_{50}

Pittermann et al. (2010)

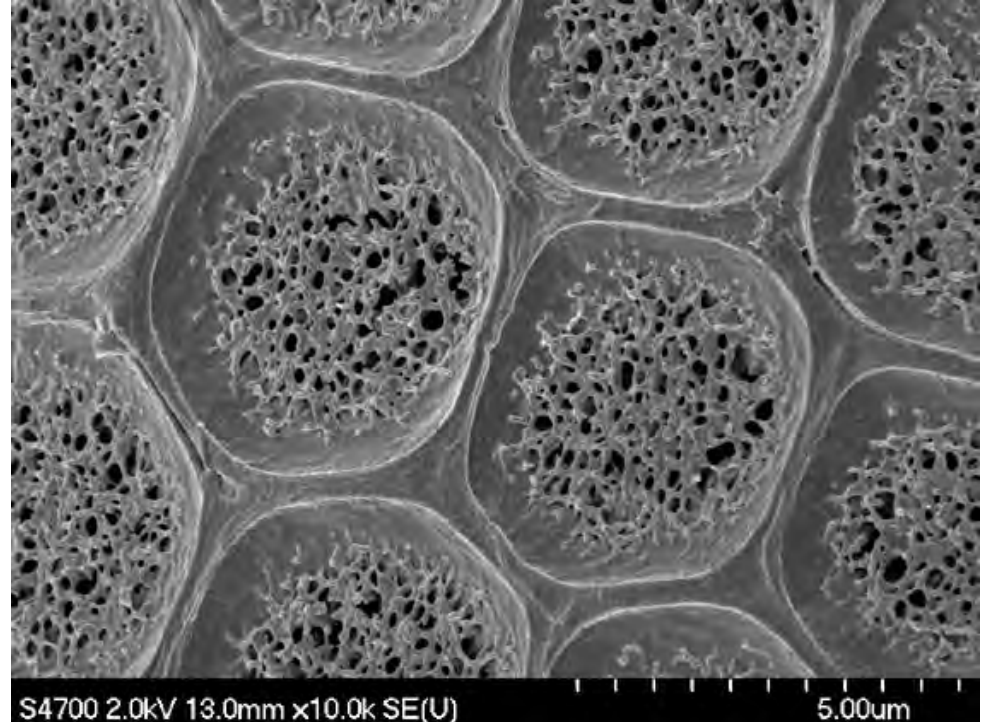
How impermeable is the torus?



Vestured pits

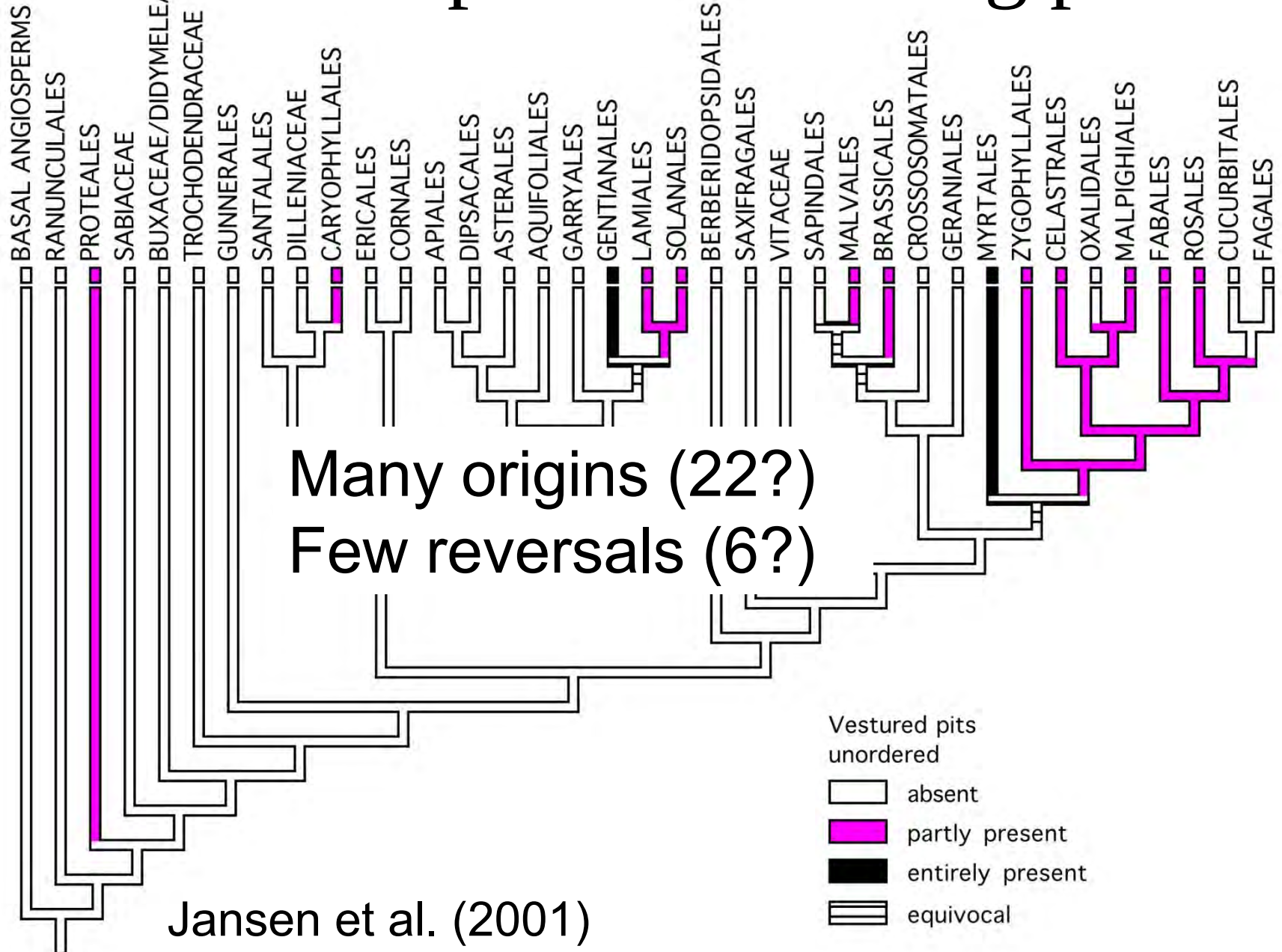


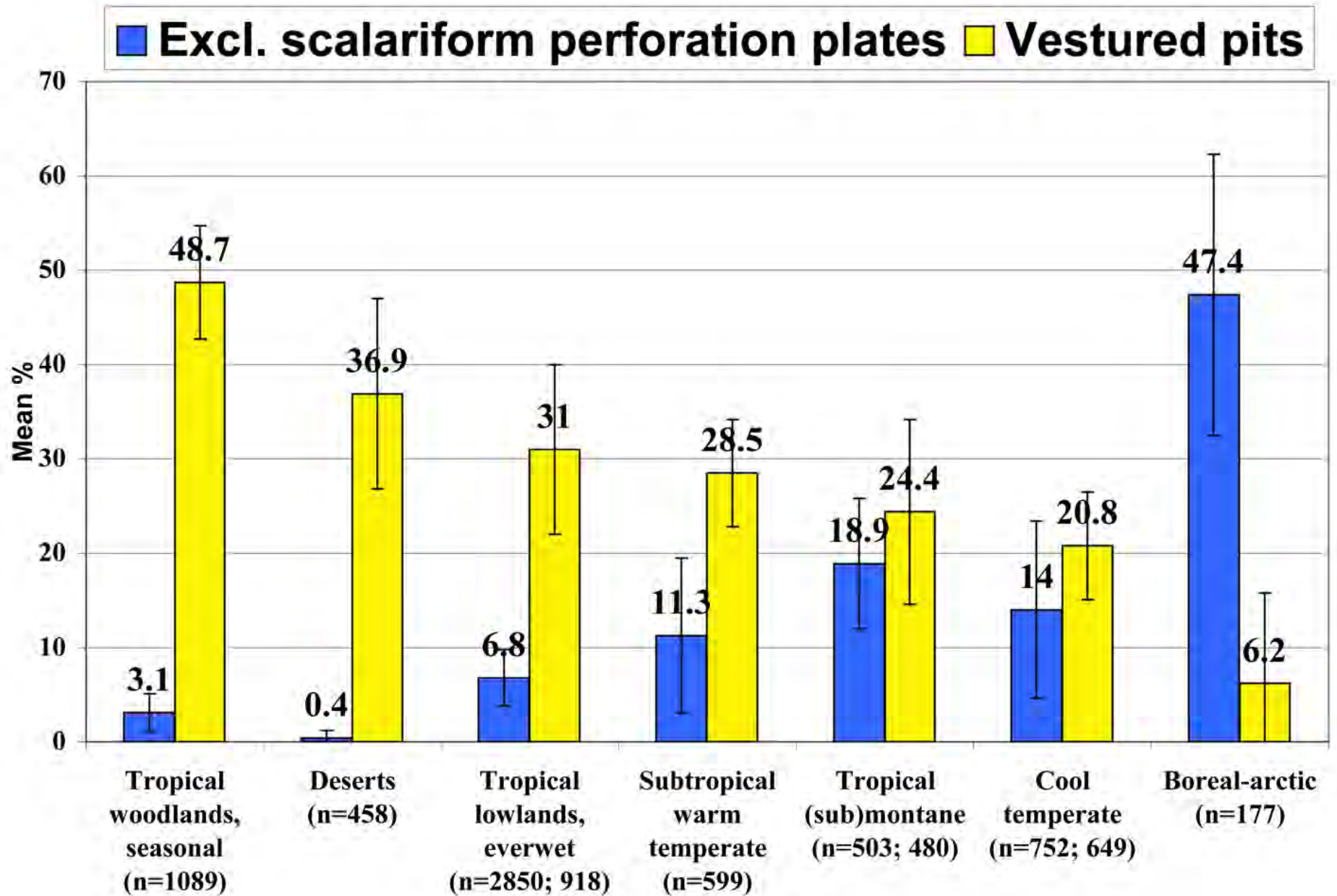
***Gynochtodes* sp.**
- Rubiaceae



Cleistocalyx ellipticus
- Myrtaceae

Vestured pits in flowering plants

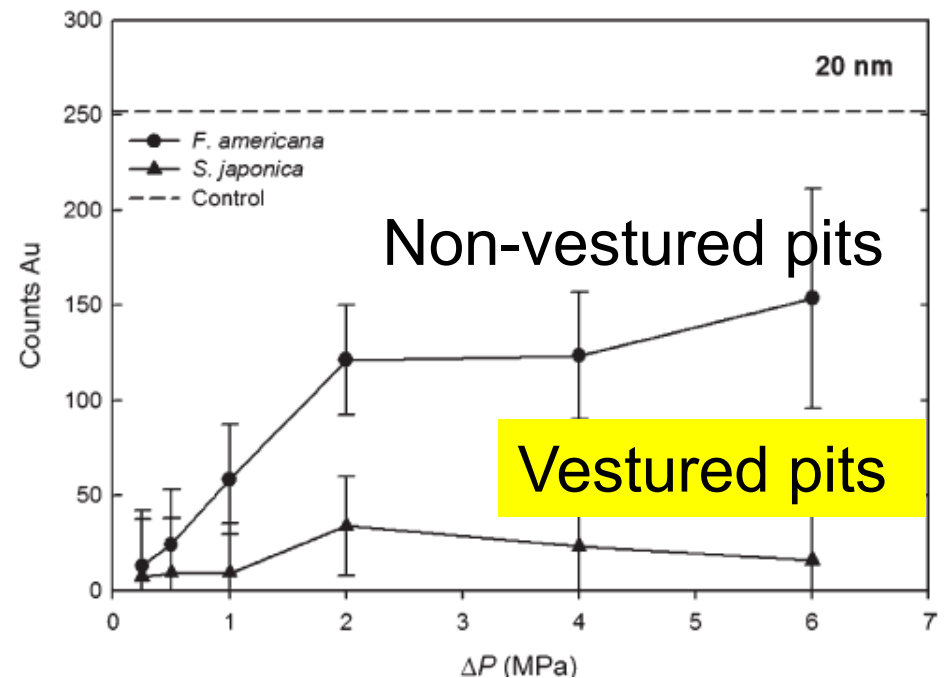
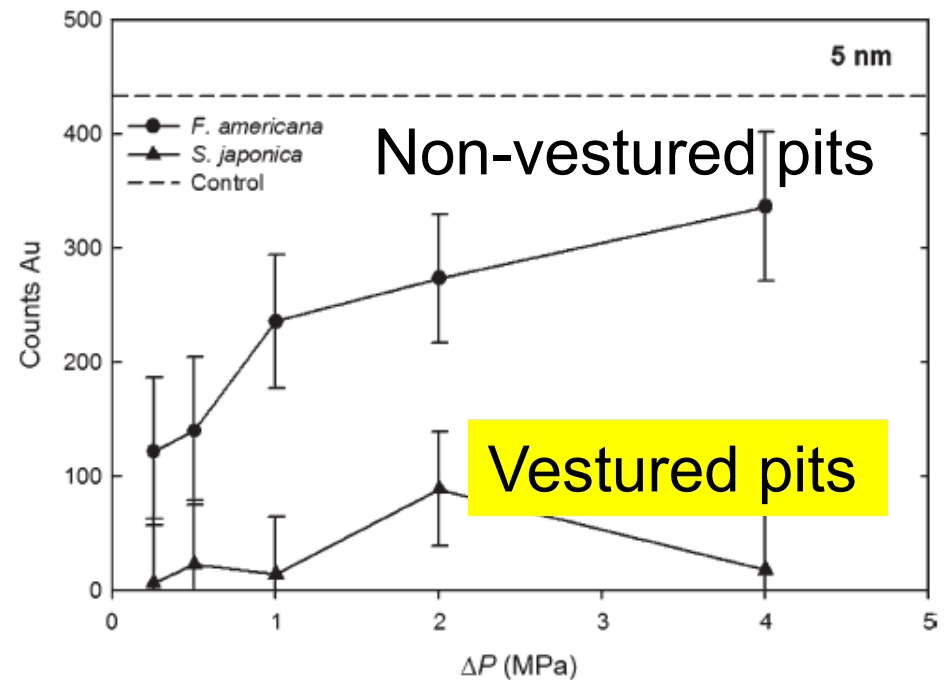
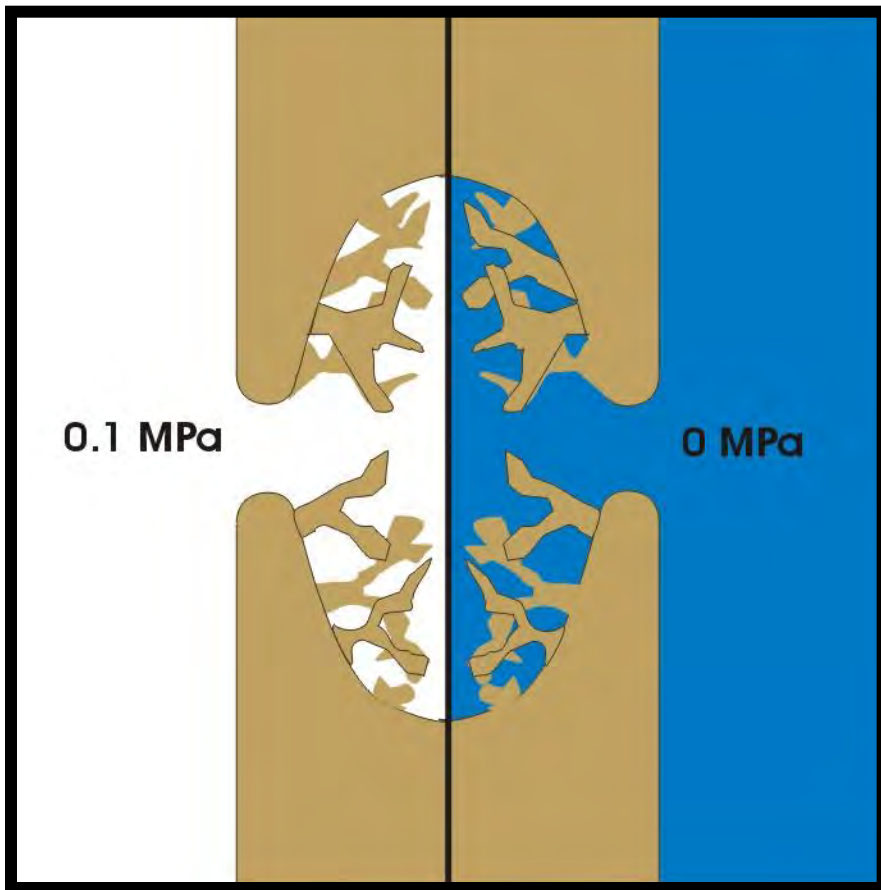




Jansen et al. (2004)

Vestures reduce the probability of air-seeding

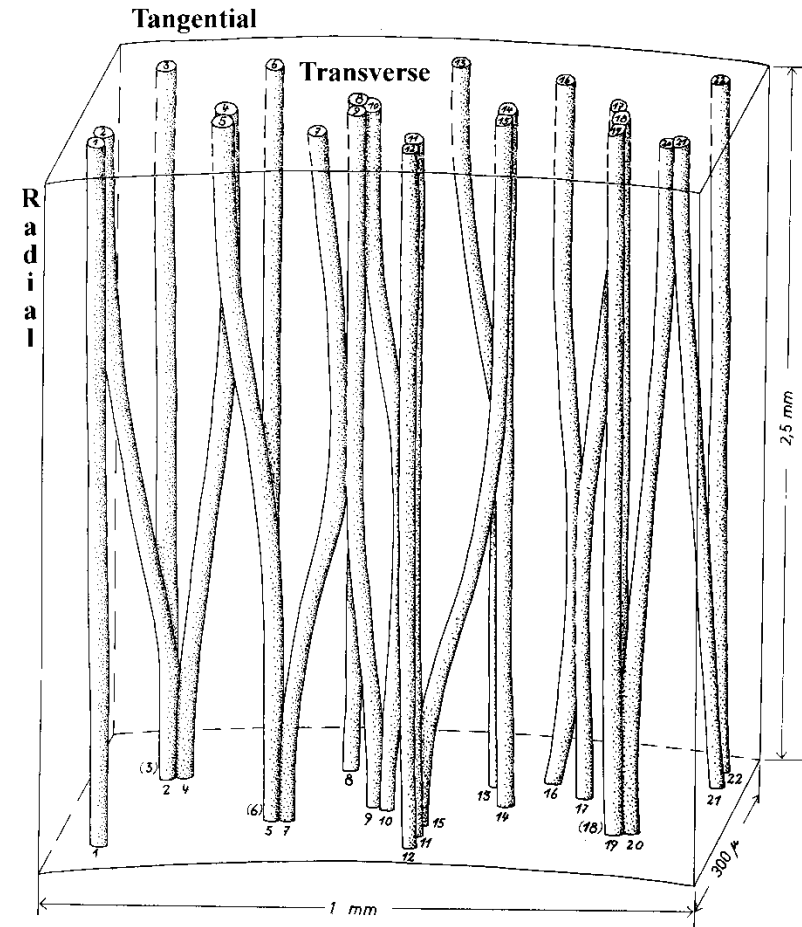
Choat et al. (2004)



‘Rare pit’ hypothesis: pit quantity vs pit quality?

Vulnerability to cavitation depends on the **single largest pit membrane pore**

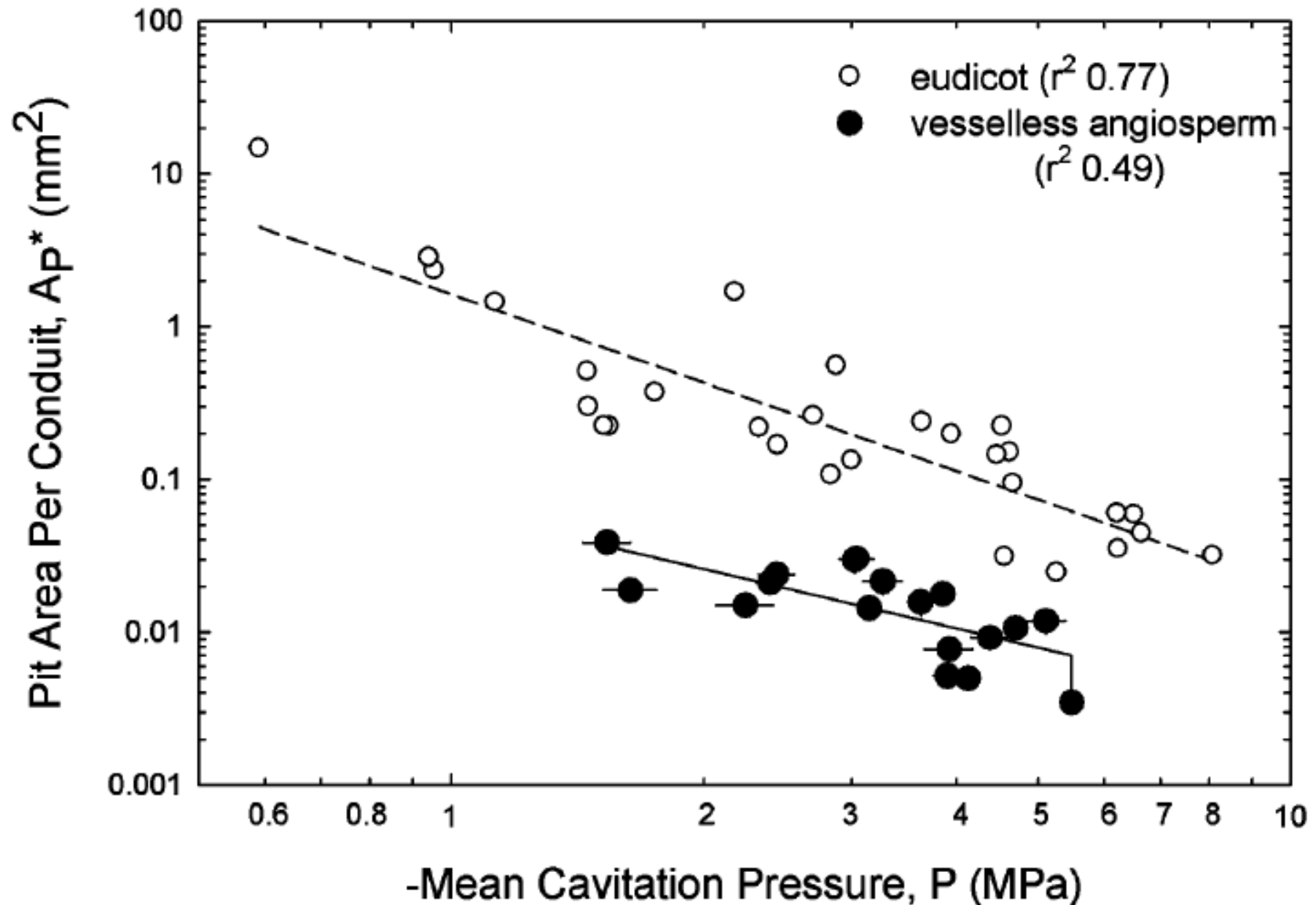
The largest pit membrane pore is influenced by **the total area of pits in a conduit**: the more pit area, by chance the larger the greatest pore (Wheeler et al. 2005).



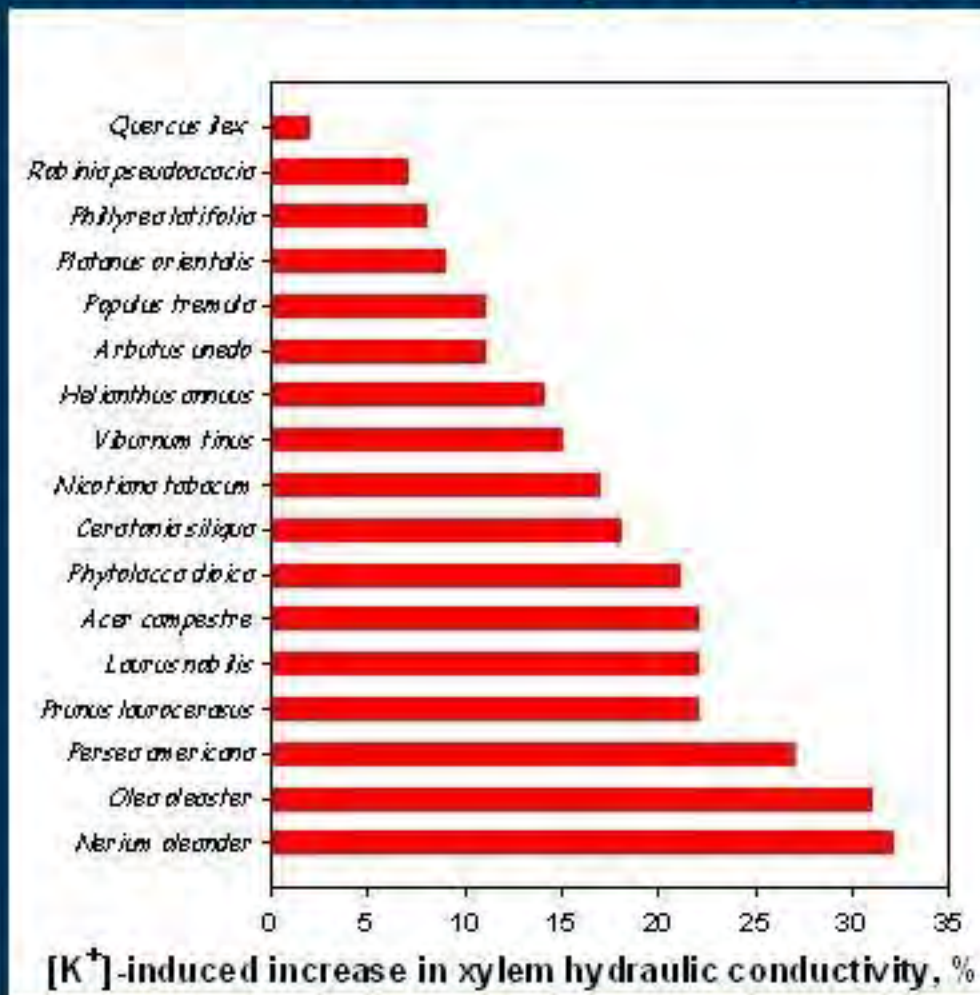
Braun 1959 – *Populus* vessel network

Cavitation protection increases in conduits with less interconduit pit area

Hacke et al. (2006, 2007)



The ionic effect is species-specific



(Published and unpublished data by the authors)

Jansen et al. (2011)

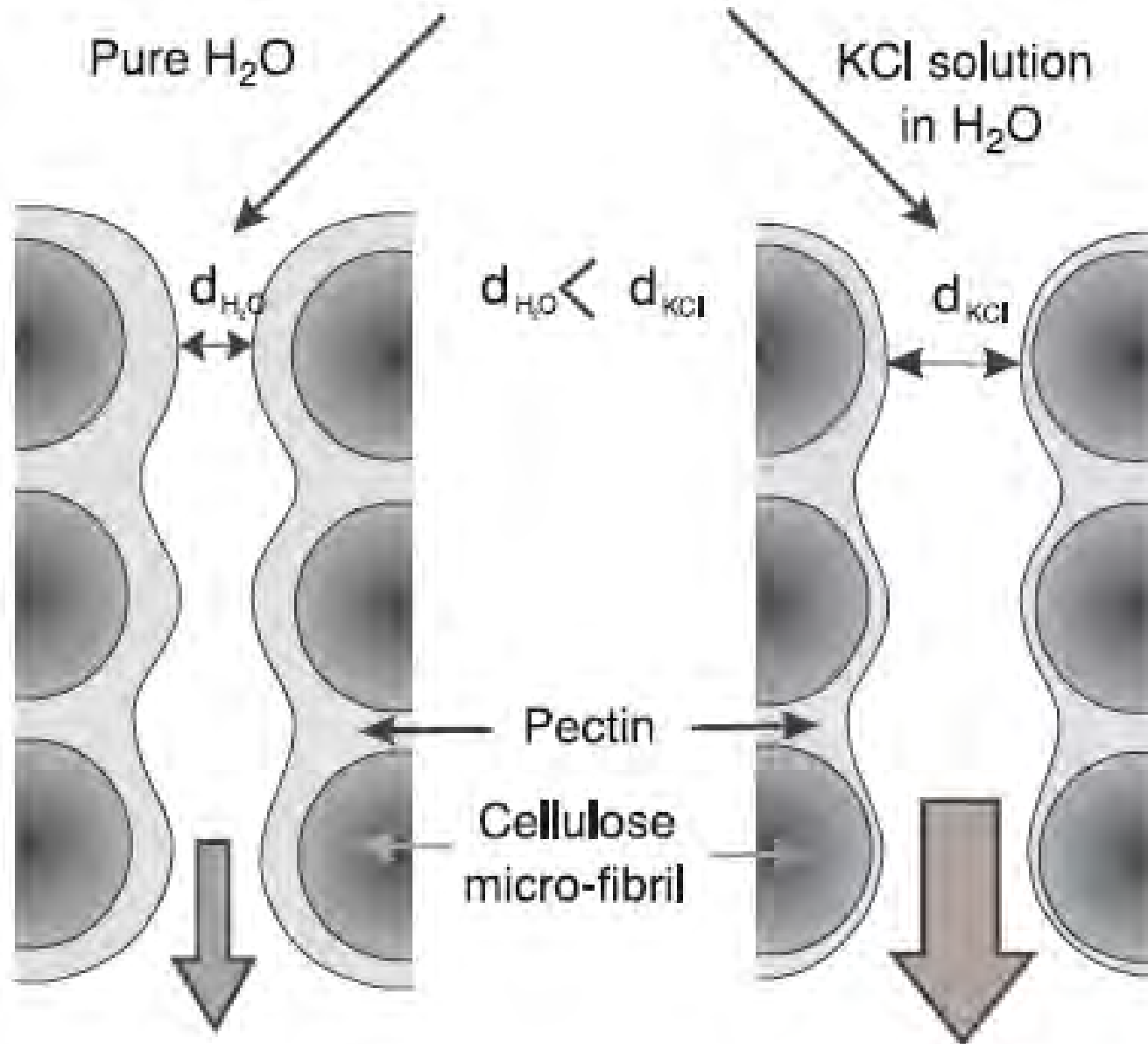
XLVII Congresso SIFV – Scuola Superiore Sant'Anna, Pisa – Luglio 2008

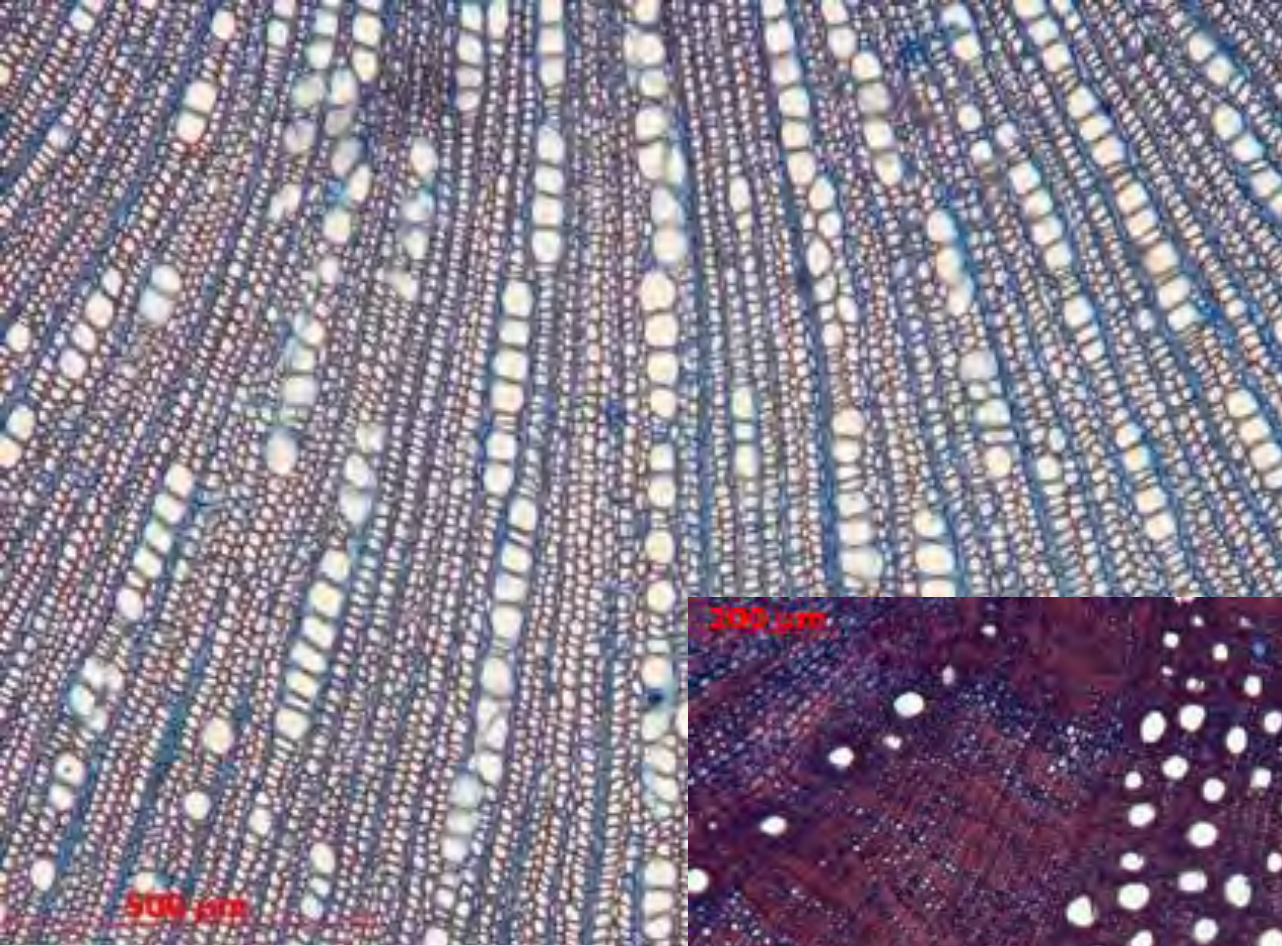


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DI TRIESTE

'Hydrogel' hypothesis

Zwieniecki et al. (2001)



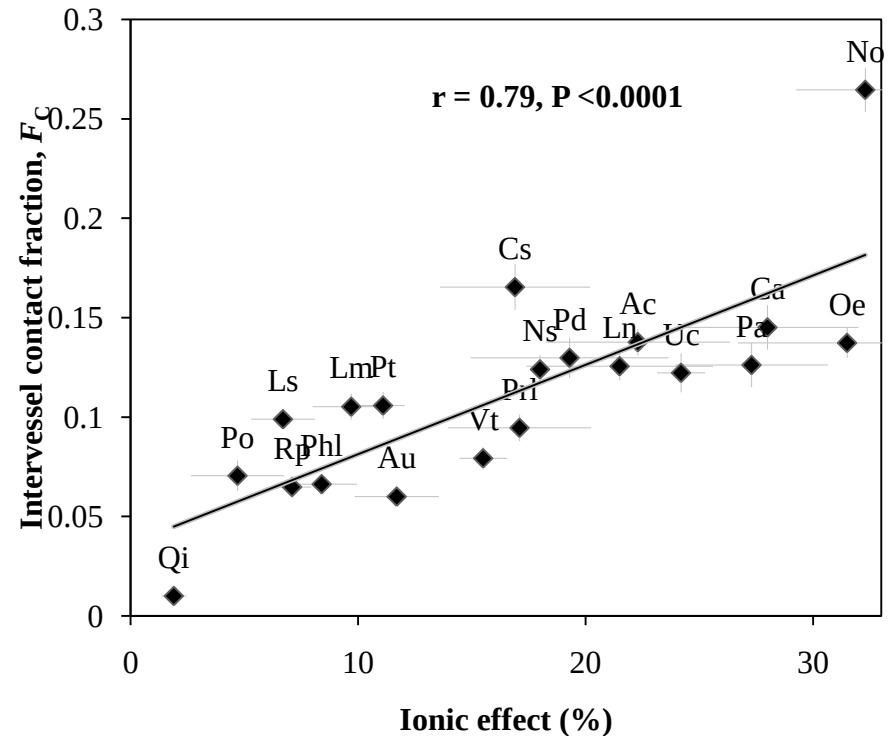
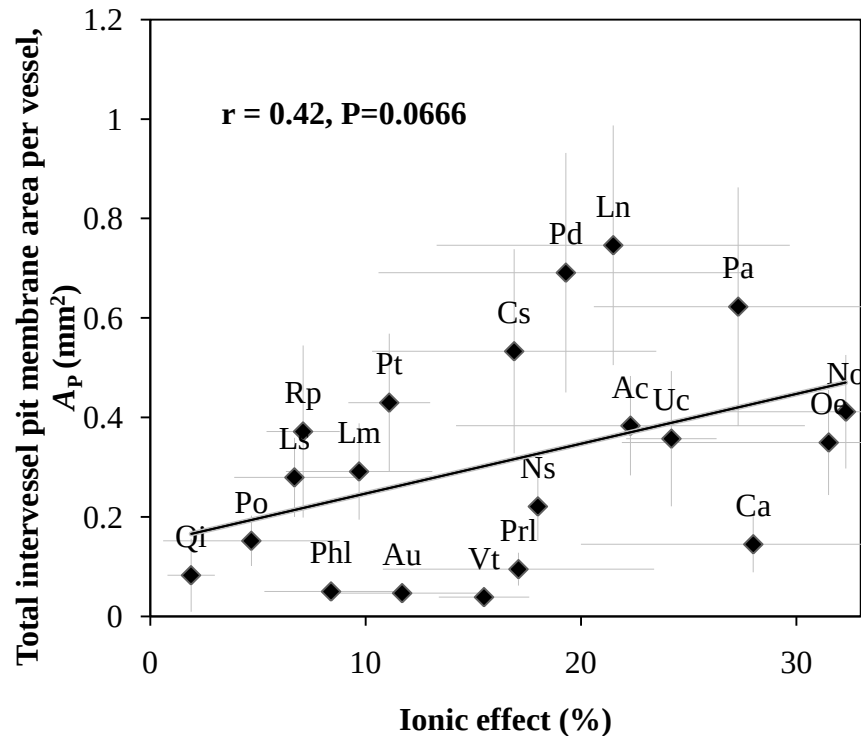


***Quercus ilex*: 1.9%
ionic effect**

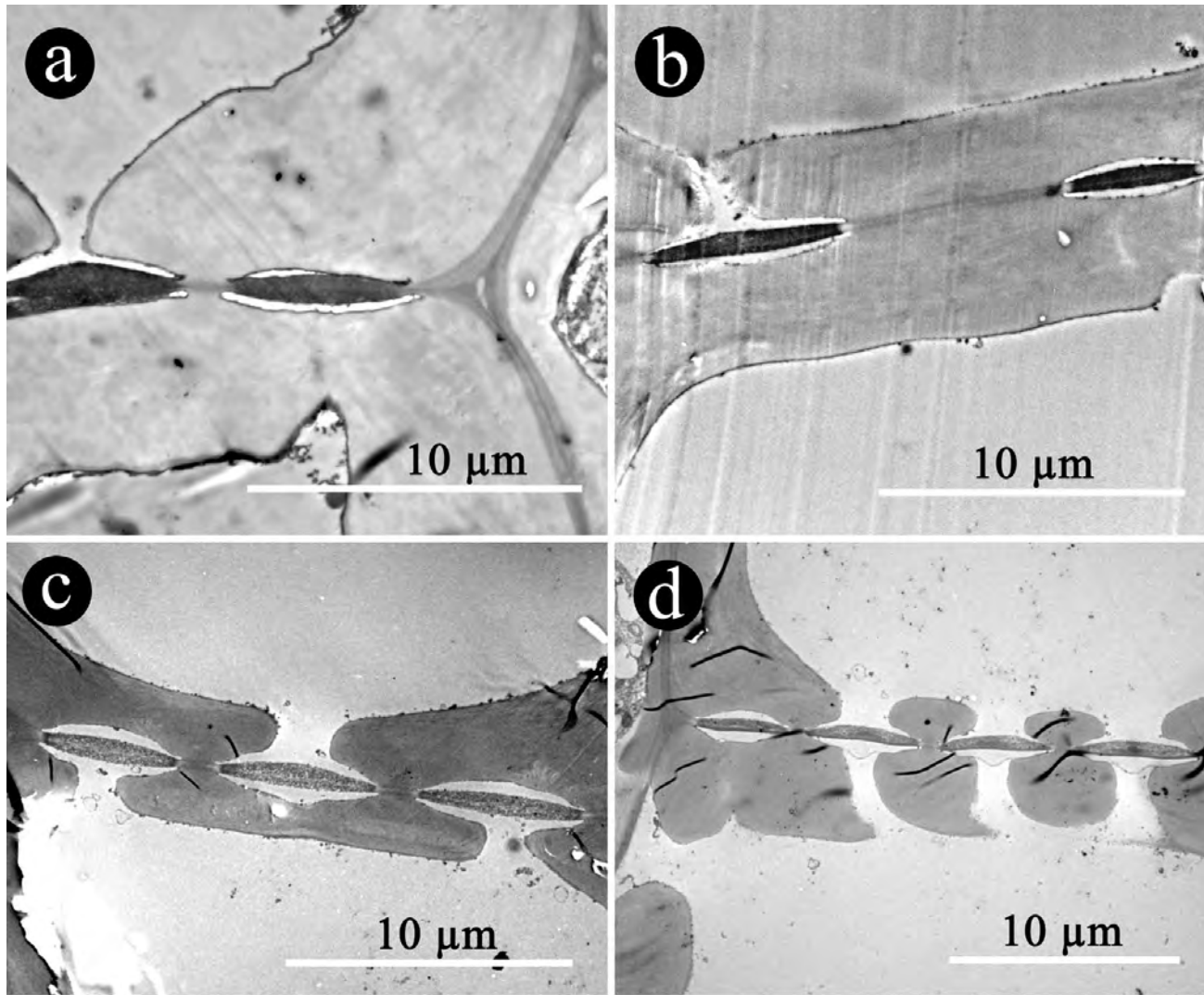
***Nerium oleander*:
32.3% ionic effect**



Do quantitative vessel and pit characters account for interspecific variation of the ionic-effect?



TEM-staining with ruthenium red for acidic pectins

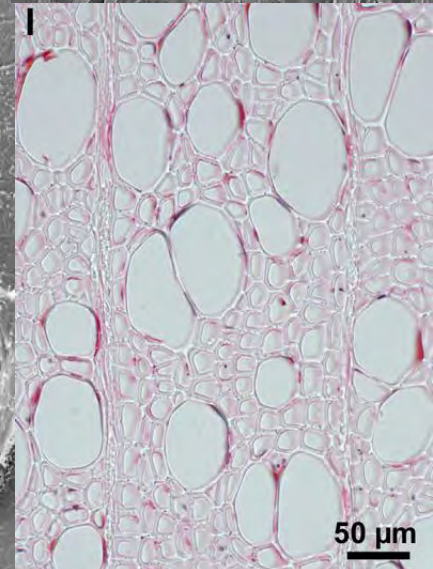
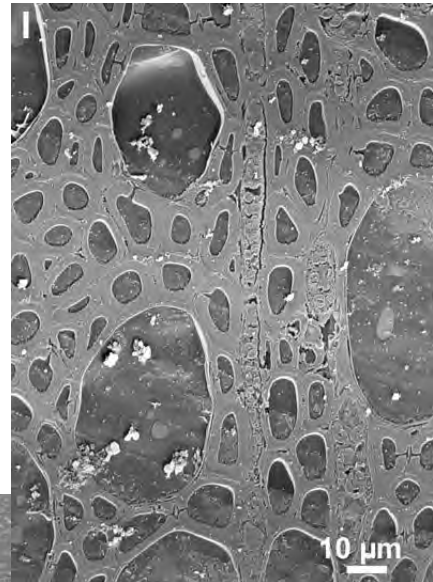
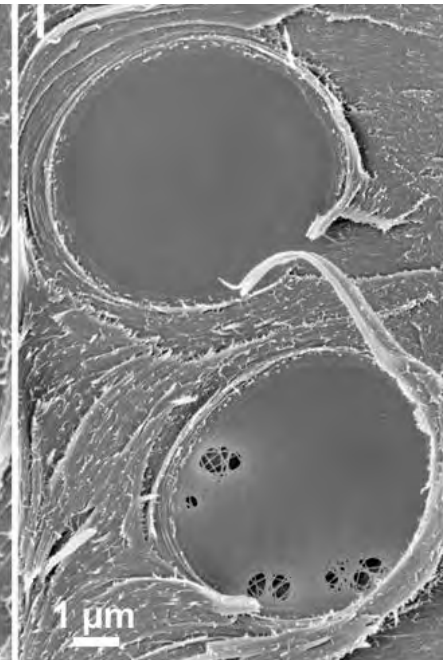
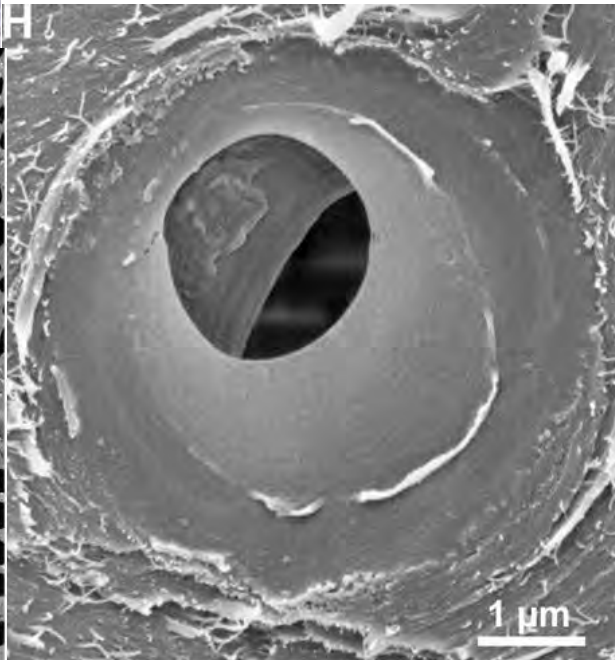
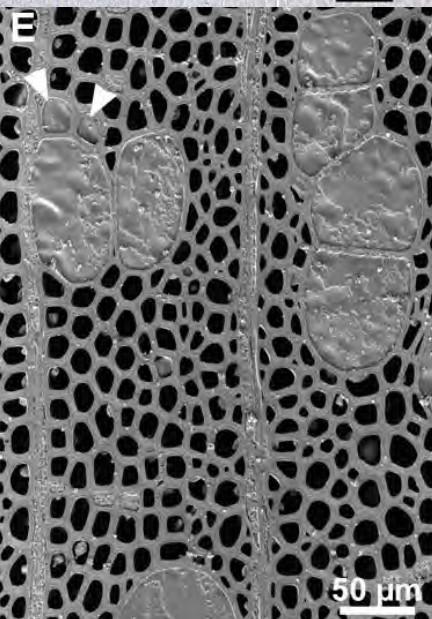


Lauraceae (ionic effect) **a.** *Umbellularia californica* (24.2%); **b.** *Laurus nobilis* (21.5%); **c.** *Litsea serica* (6.7%); **d.** *Lindera megaphylla* (9.7%)

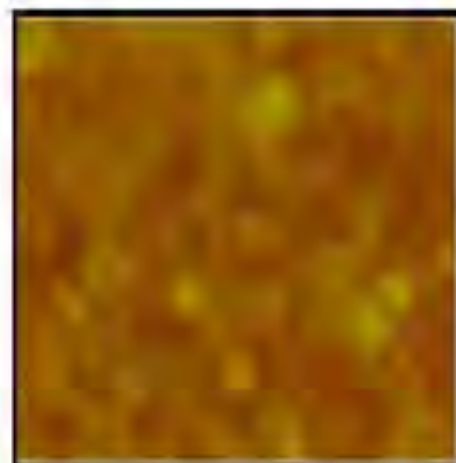
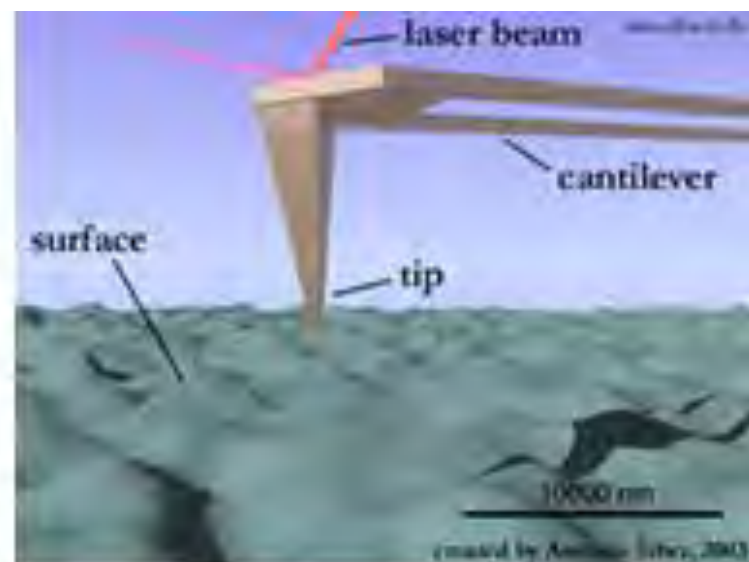
Gortan et al. (2011)

Imperforate tracheary elements

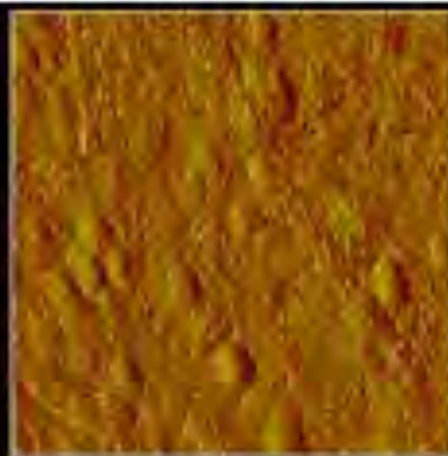
Sano et al. (2011)



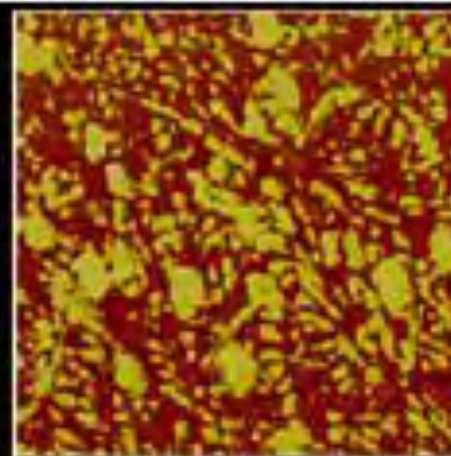
Atomic force microscopy



Height



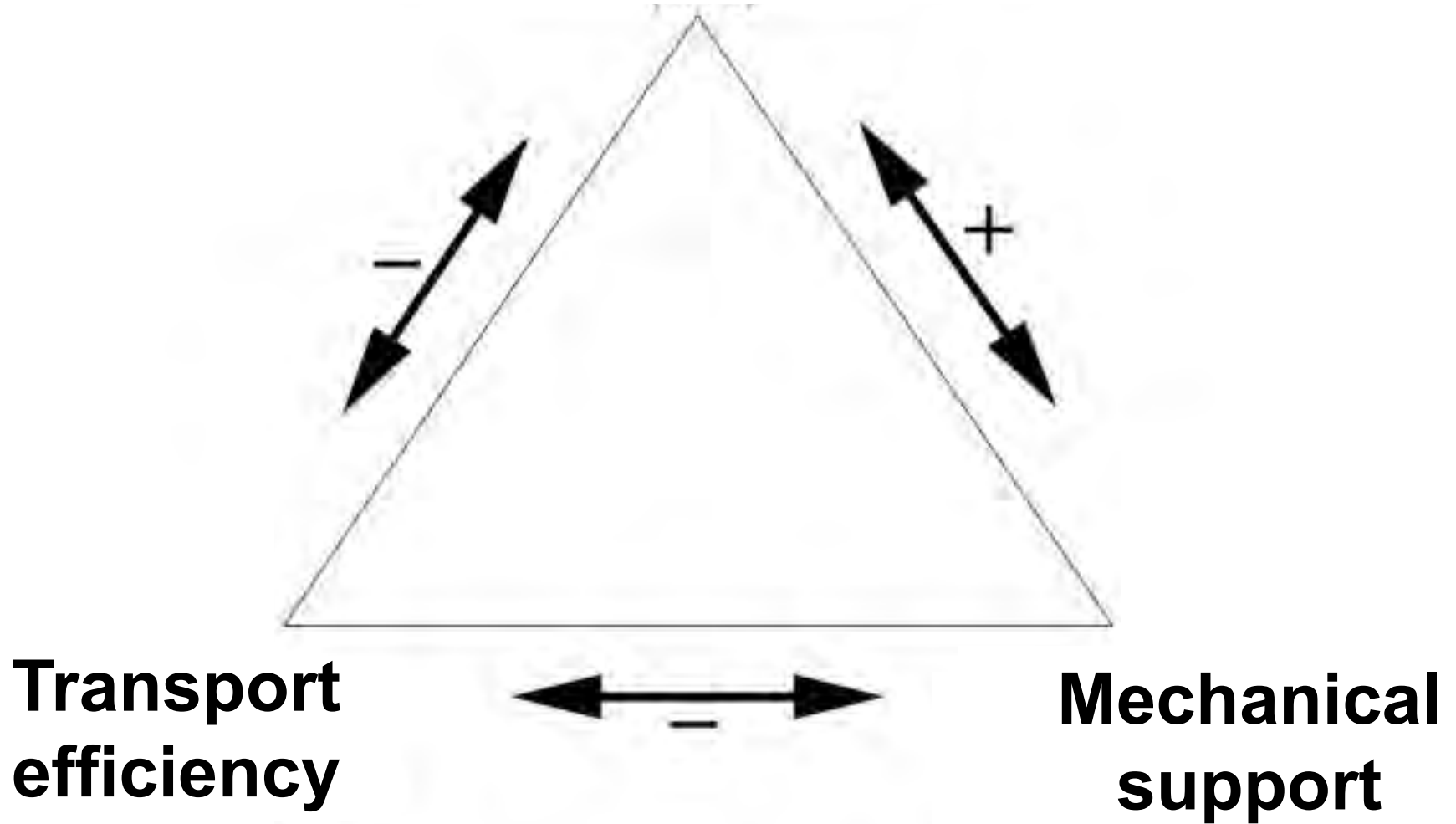
Amplitude



Phase

Laurus nobilis: air-dried pit membrane after
alcohol + acetone treatment

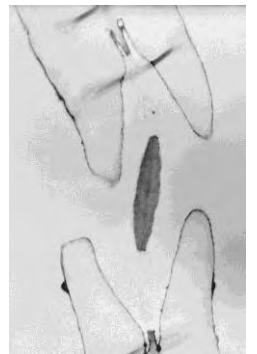
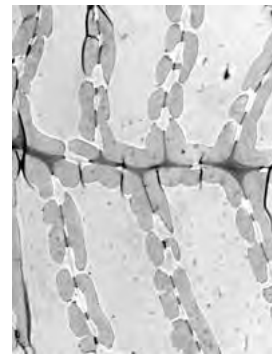
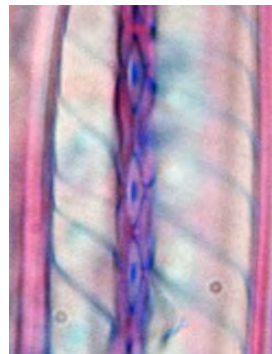
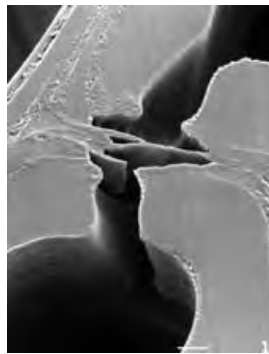
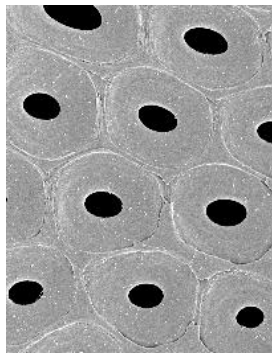
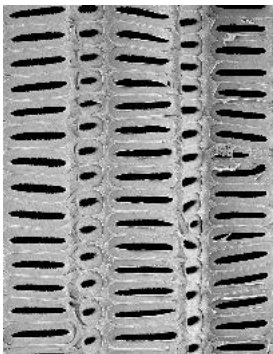
Resistance to embolism



Baas et al. (2004); Chave et al. (2009)

Acknowledgements

Erika Amano, Guillermo Angeles, Pieter Baas, Brendan Choat, Hervé Cochard, Simon Connell, Sylvain Delzon, Roland Dute, Peter Gasson, Emmanuelle Gortan, Uwe Hacke, Missy Holbrook, Matthias Klepsch, Jean-Baptiste Lamy, Frederic Lens, Jordi Martinez-Vilalta, Stefan Mayr, Hugh Morris, Andrea Nardini, Alexei Oskolski, Thomas Pesacreta, Jarmilla Pittermann, Annelies Pletsers, David Rabaey, Yuzou Sano, Nele Schmitz, Alexander Scholz, Erik Smets, Anke Stein, Maciej Zwieniecki



Ultrasonic acoustic emission signals from walnut stem during freeze-thaw cycles

Jun KASUGA, Thierry AMÉGLIO

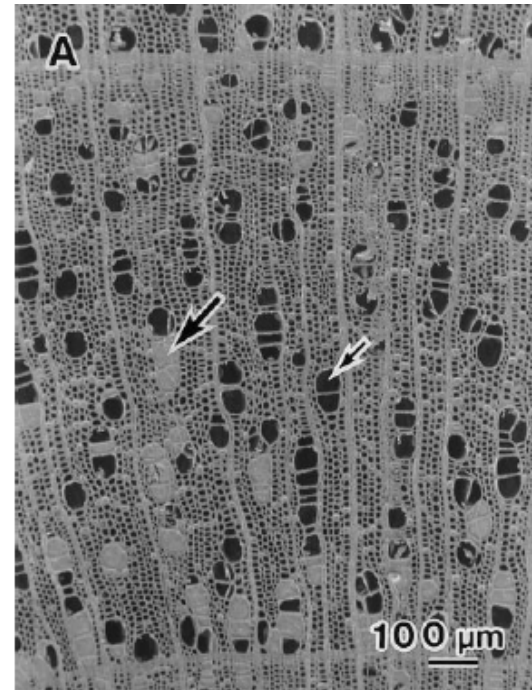
Winter embolism

- In winter, succession freeze-thaw events induce embolism formation in water conduits in xylem of plants

Betula platyphylla var. *japonica*



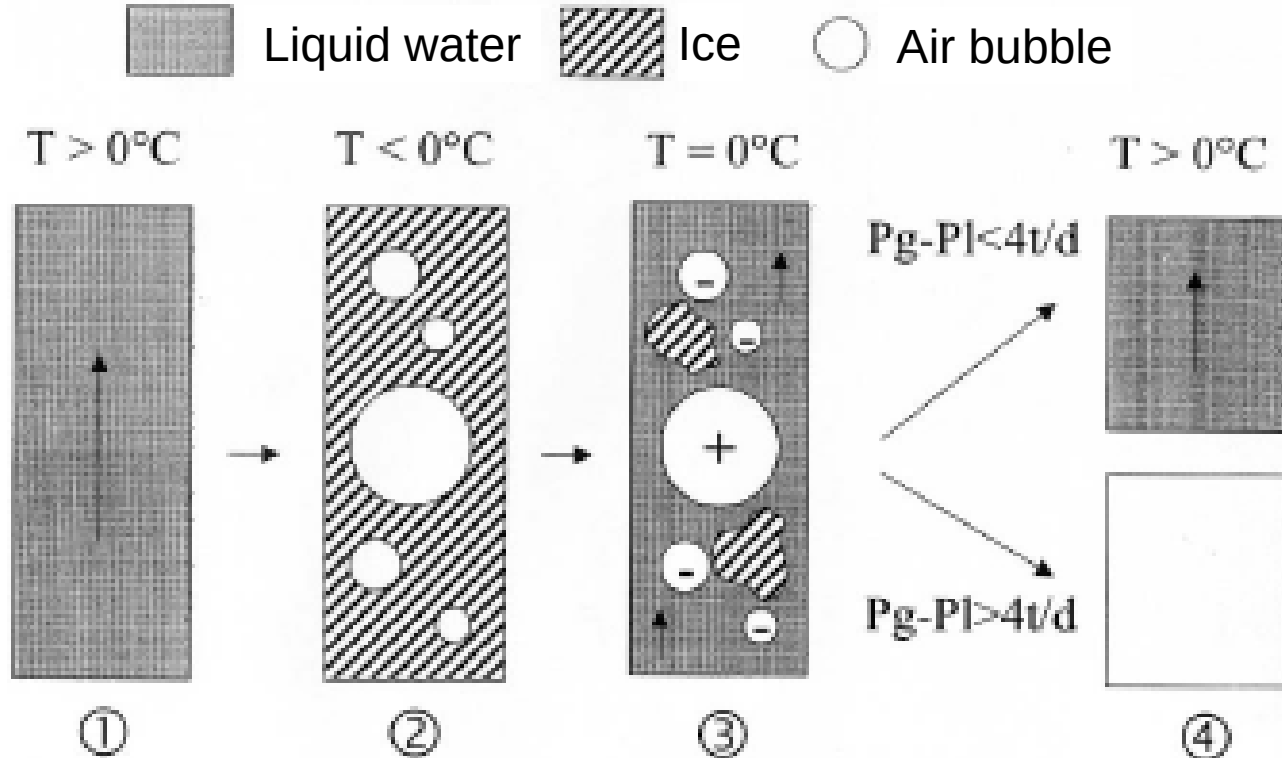
Growing season (April)



Just after winter (March)

Utsumi et al., 1998

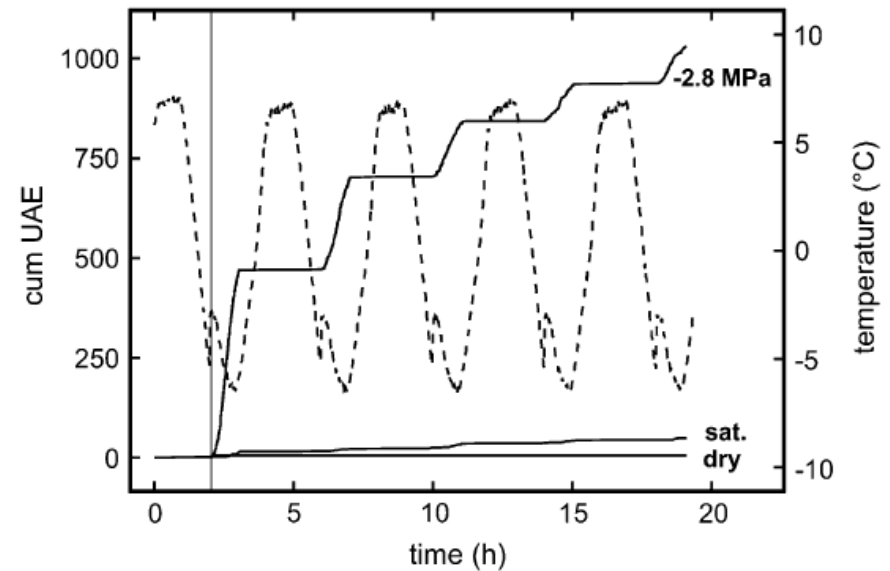
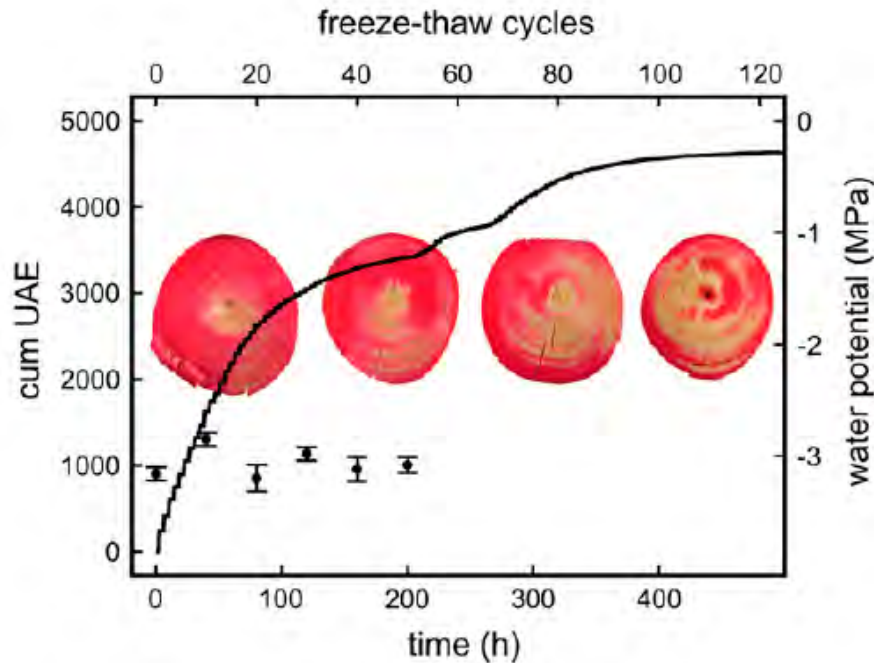
Mechanism of winter embolism generation



Cruiziat et al., 2002

- (1)-(2) During freezing, air bubbles are created in sap
- (3)-(4) During thawing, there are two possibilities
(depend on bubble size and water potential of sap):
- air bubbles dissolve in sap => no embolism
 - air bubbles expand => embolism

Results contradicting the previous theory



Mayr et al. 2007

- According to classical theory, wider tracheids in early wood are considered to be more vulnerable. However, in *Picea abies*, clustered areas of tracheids including early wood and late wood were embolized by repeated freeze-thaw cycles.
- It is thought that when cavitation happens in conduits, a rapid relaxation of a liquid tension occurs and produces an ultrasonic acoustic emission (UAE) signal. In the case of conifers, UAE were registered only in freezing process during freeze-thaw cycles.

Objectives

- We applied an ultrasonic acoustic emission (UAE) method to walnut to elucidate the relationship between embolism formation in vessels and UAE signals during freeze-thaw cycles in angiosperm species
- Our goal is on the elucidation of mechanism of winter embolism in angiosperms

Materials

- 2-6 year-old twigs were harvested from walnut trees (*Juglans regia* cv. Franquette) growing at INRA, Site de Crouël, Clermont-Ferrand in autumn (September to Early-November)
- The water potential of each sample was conditioned at -1.6 MPa (somewhat higher value than Ψ_{50} of walnut twigs)
- 50 cm-long samples were cut from the twigs after removal of side twigs
- The samples were wrapped with thin plastic film and used for freeze-thaw experiments

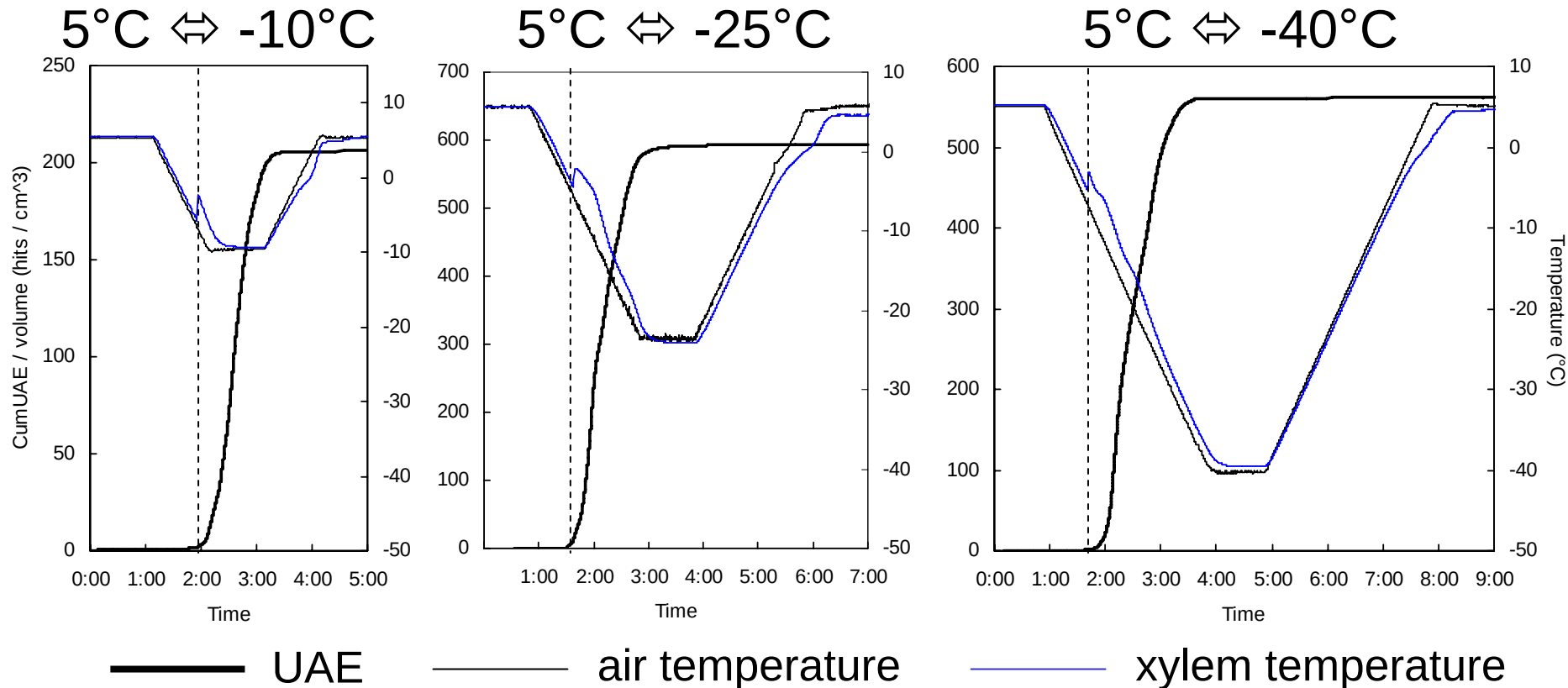
Methods

- Freeze-thaw (FT) experiment
 - One freeze-thaw cycle to different minimum temperatures (-10 , -25 , -40°C)
 - Repeated freeze-thaw cycles from 5 to -10°C
- Ultrasonic acoustic emission (UAE)
 - Peak detection parameters
 - Preamplification gain: 40 dB
 - Acquisition threshold: 45 dB
 - Time threshold: 400 μs
- Loss of water conductivity



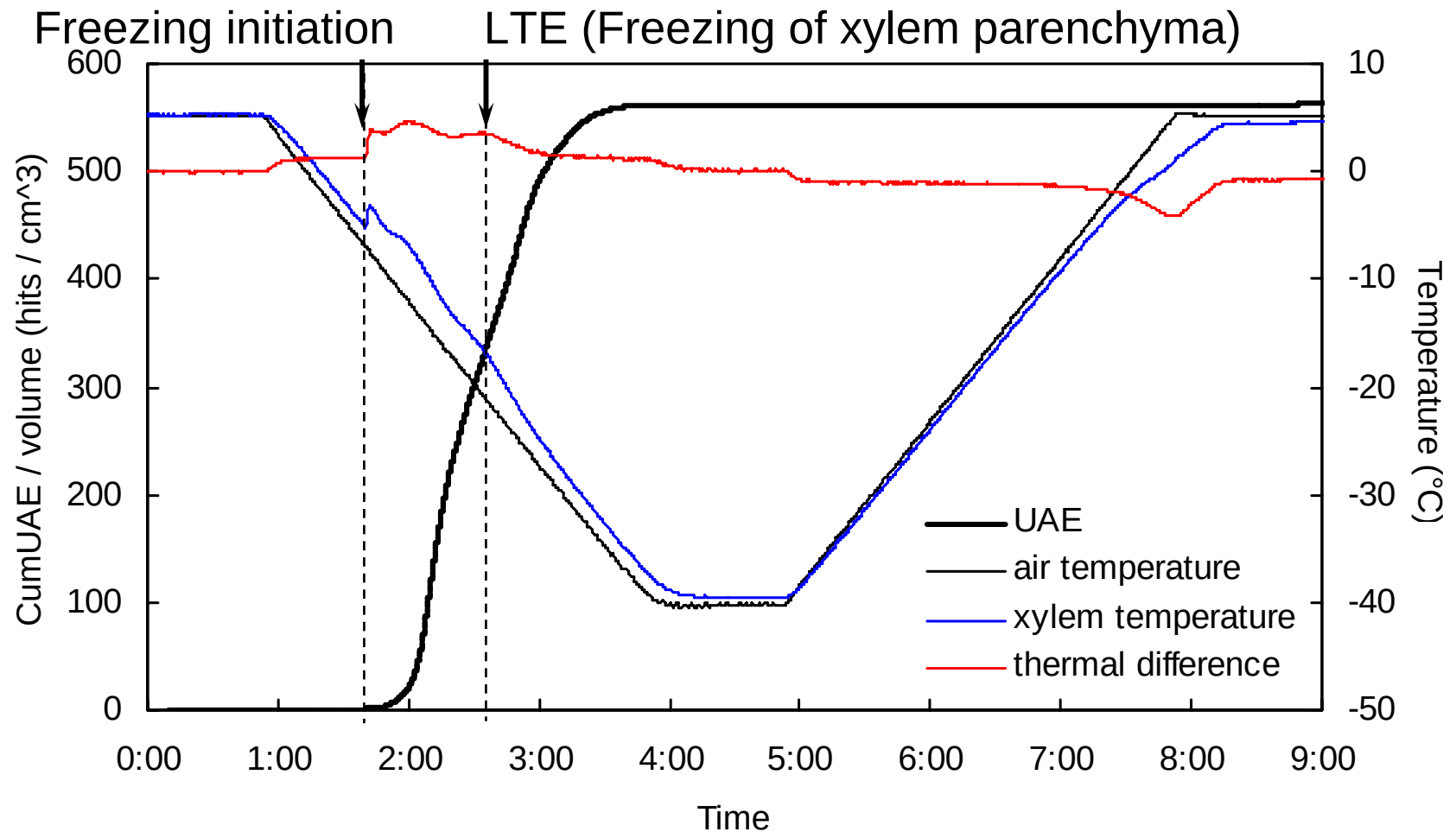
Ultrasonic acoustic emission

One FT cycle to different temperatures



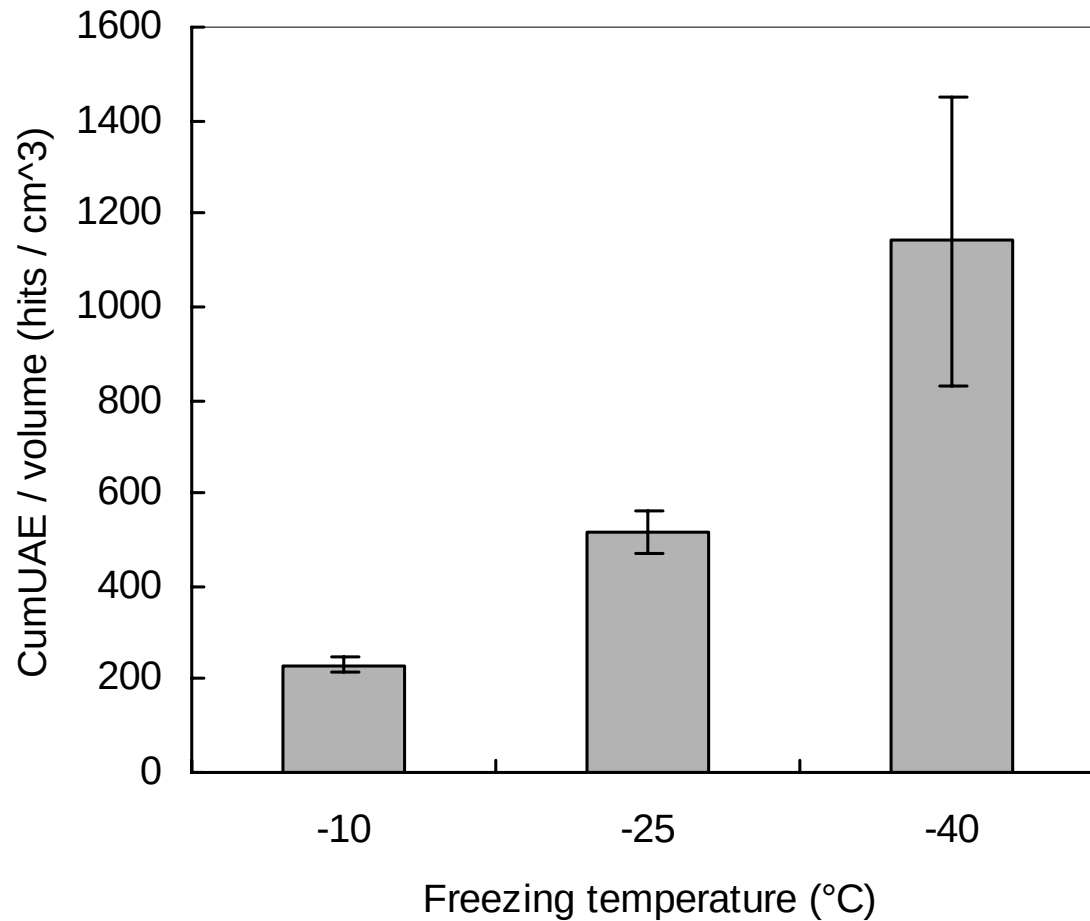
- UAE were registered only in the freezing process

One FT cycle to -40°C



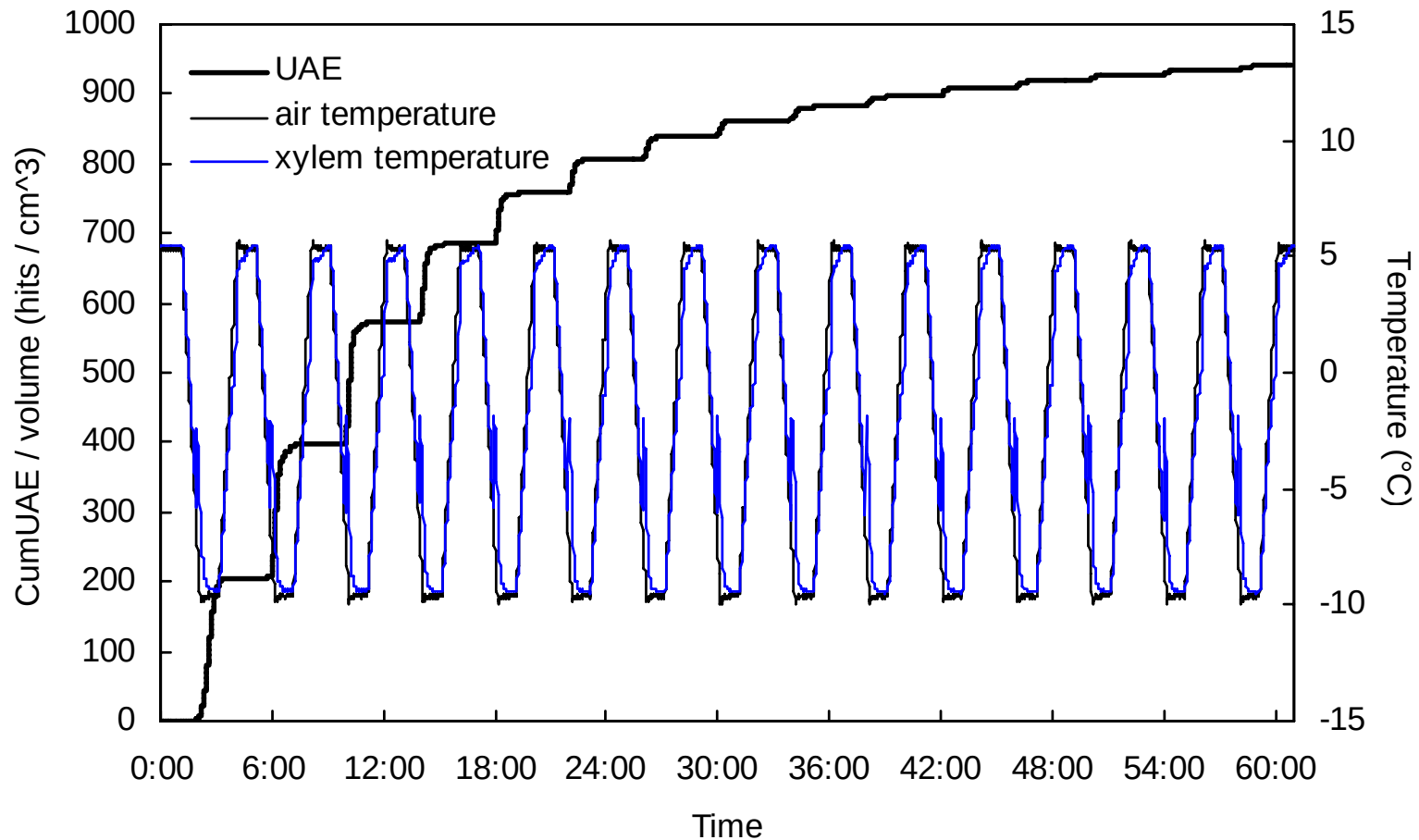
- After freezing of xylem parenchyma cells, acceleration of generation of UAE was observed

Cumulative number of UAE during one FT cycle



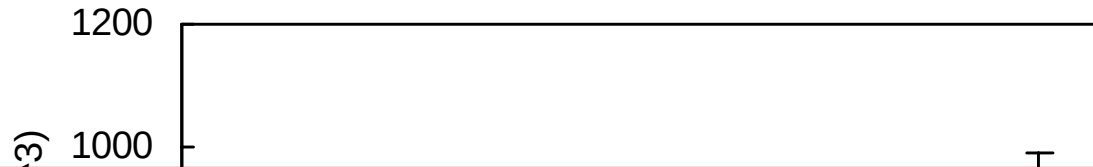
- Freezing to lower temperatures increased the cumulative number of UAE signals

Repeated FT cycles ($5^{\circ}\text{C} \leftrightarrow -10^{\circ}\text{C}$)

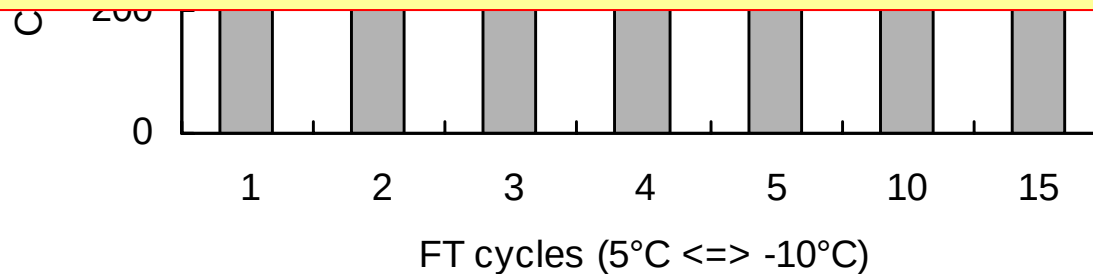


- Although there seemed to be a threshold, cumulative number of UAE kept increasing even after 15 times of FT cycles

Cumulative number of UAE during repeated FT cycles (5°C ⇔ -10°C)



Are there any relationships between UAE generation and embolism formation?

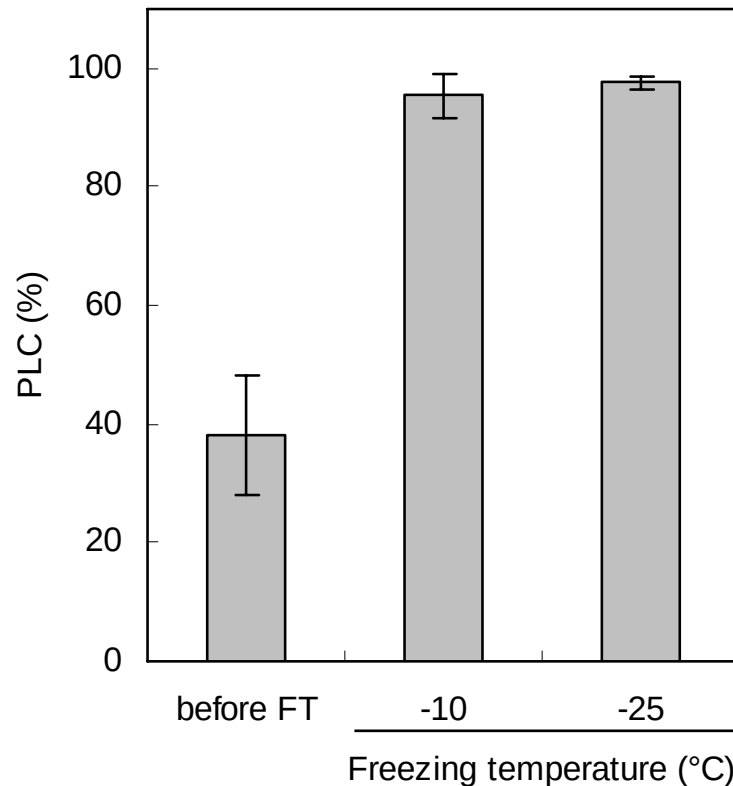


- Cumulative UAE signals were increased during repeated FT cycles

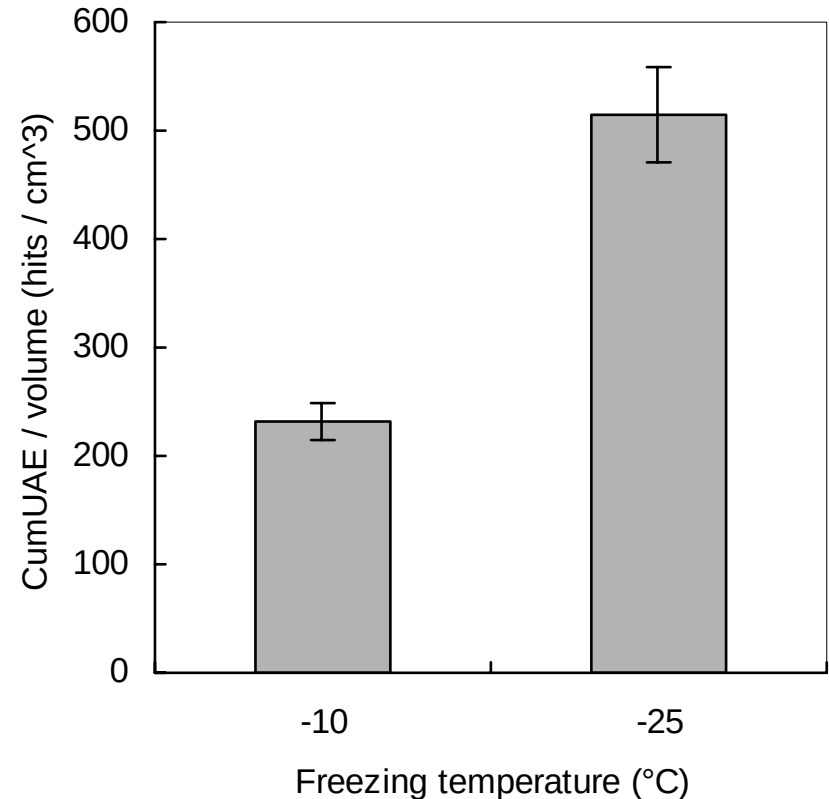
Loss of water conductivity

One FT cycle to different temperatures

Loss of conductivity



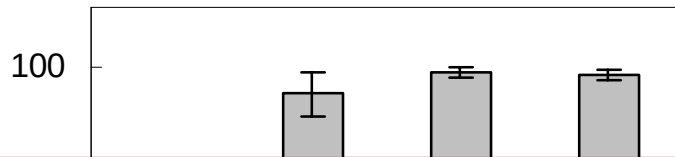
UAE



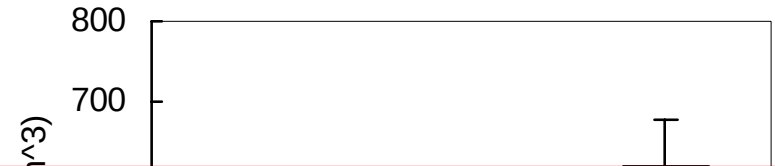
- Almost all vessels were embolized only by freezing to -10°C although a lot of UAE signals were registered between -10 and -25°C

Repeated FT cycles (5°C ⇔ -10°C)

Loss of conductivity



UAE



UAE signals were not only from vessel elements
- wood fibers?
- parenchyma cells?

- Almost all vessels were embolized only by one FT cycle although cumulative UAE signals were increased during repeated FT cycles

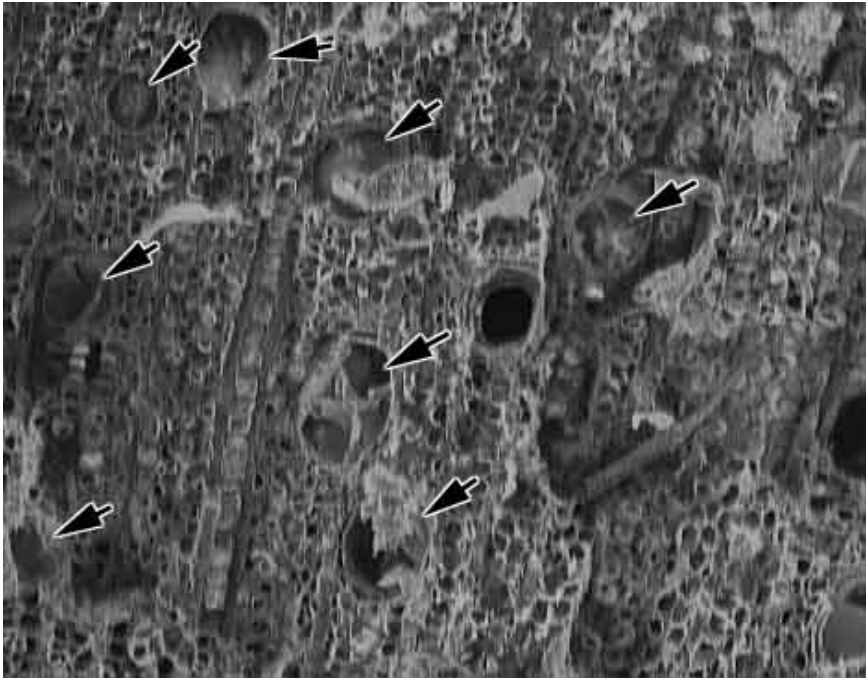
Summary of Results

- FT cycles induced UAE signals from twigs and embolism of conduits in *Juglans regia* similar to conifers
- UAE signals were registered only during the freezing process
- Freezing to lower temperatures and repetition of FT cycles increased the cumulative number of UAE signals
- After freezing of parenchyma cells, number of UAE signals increased
- Almost all vessels were embolized only by one cycle of FT between 5°C to –10°C although freezing to lower temperatures and further repetition of FT cycles increased the cumulative number of UAE signals

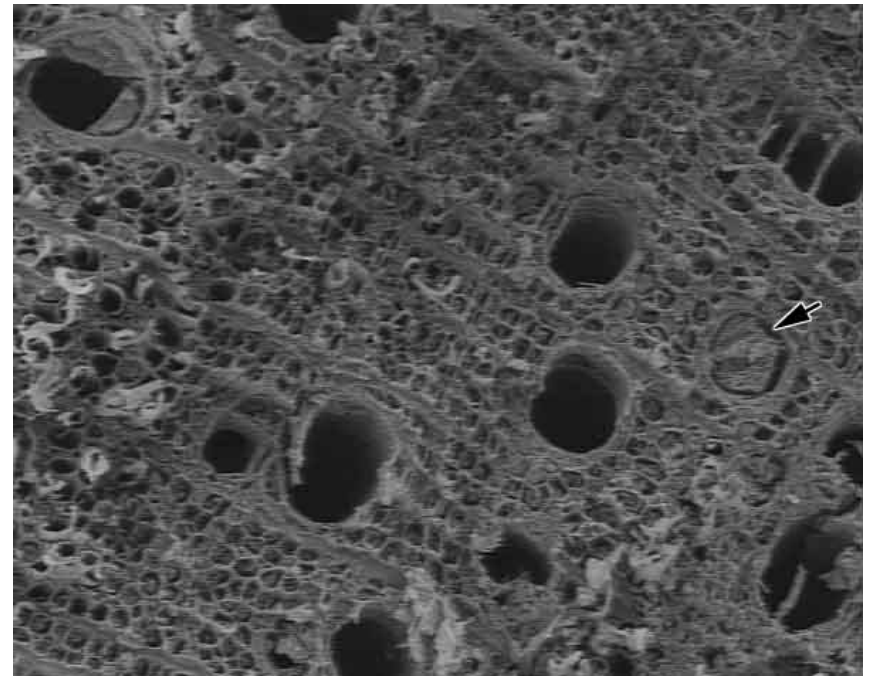
Conclusion

- In this study, we could not find any correspondence between increase of cumulative number of UAE signals and those of embolized vessels
 - How and where were the UAE signals generated?
 - Fibers, parenchyma cells, etc.?
- However, because no UAE signals were registered in the thawing process, it's possible that results in this study reflect the presence of other mechanism of embolism formation than the classical theory in angiosperms
- We are attempting to observe the changes in distribution of water in twigs during freeze-thaw cycles by cryo-scanning electron microscope

Cryo-scanning electron microscopy



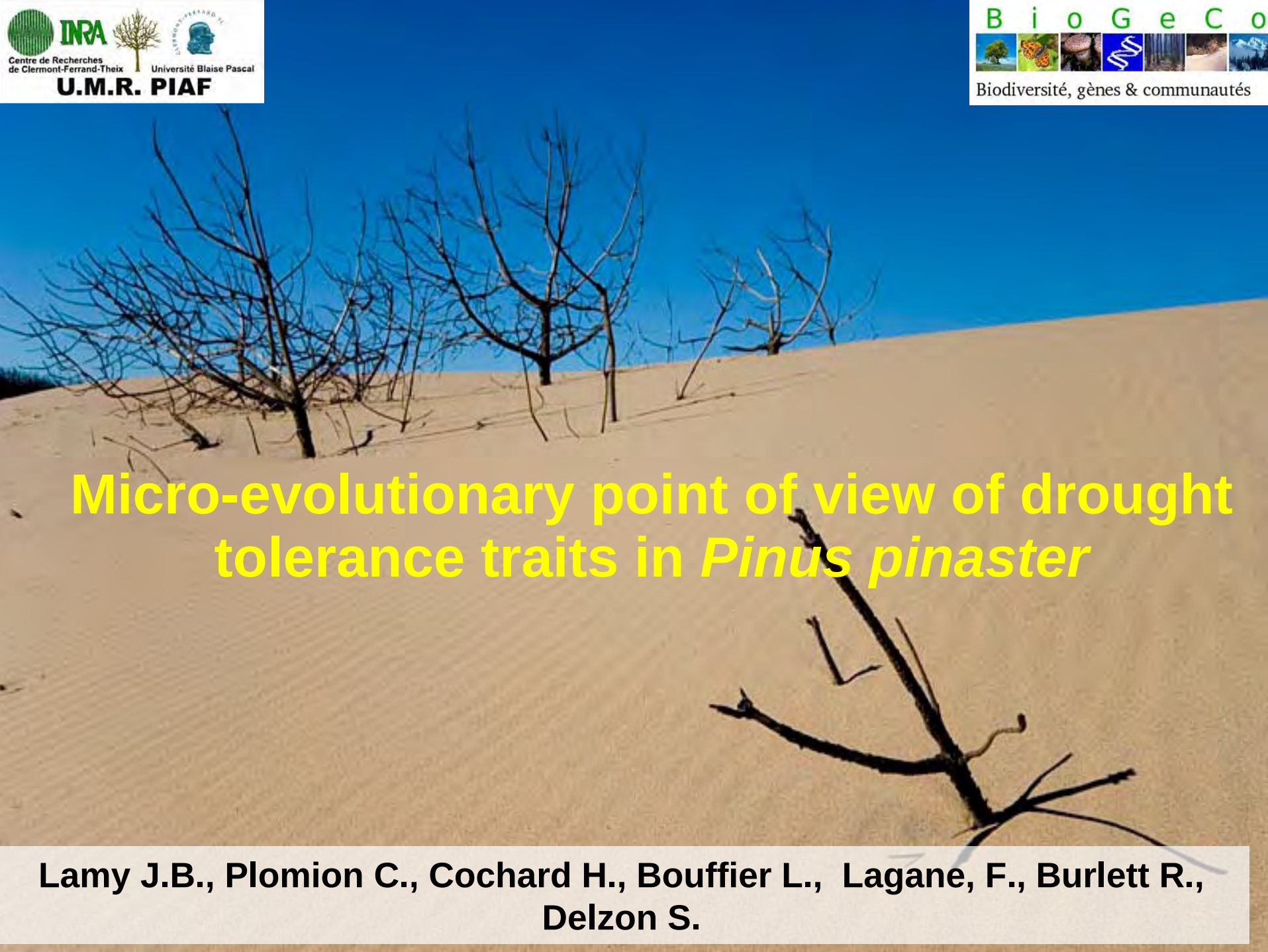
Before FT



After FT (5 $\leftrightarrow$ -25°C)

- 
- **Special thanks:**
 - **Christian BODET**
 - **Guillaume CHARRIER**
 - **This work is partly supported by Japan Society for the Promotion of Science**

Merci pour votre attention!



Micro-evolutionary point of view of drought tolerance traits in *Pinus pinaster*

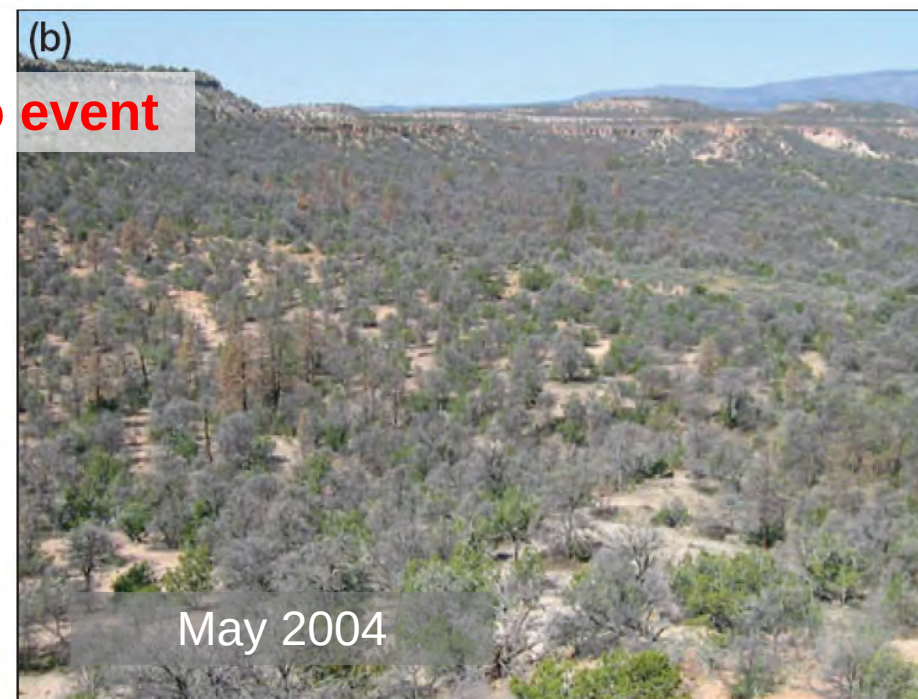
Lamy J.B., Plomion C., Cochard H., Bouffier L., Lagane, F., Burlett R.,
Delzon S.

Introduction

Extreme Climatic events



El Niño event



Breshears et al 2009

Juniperus monosperma

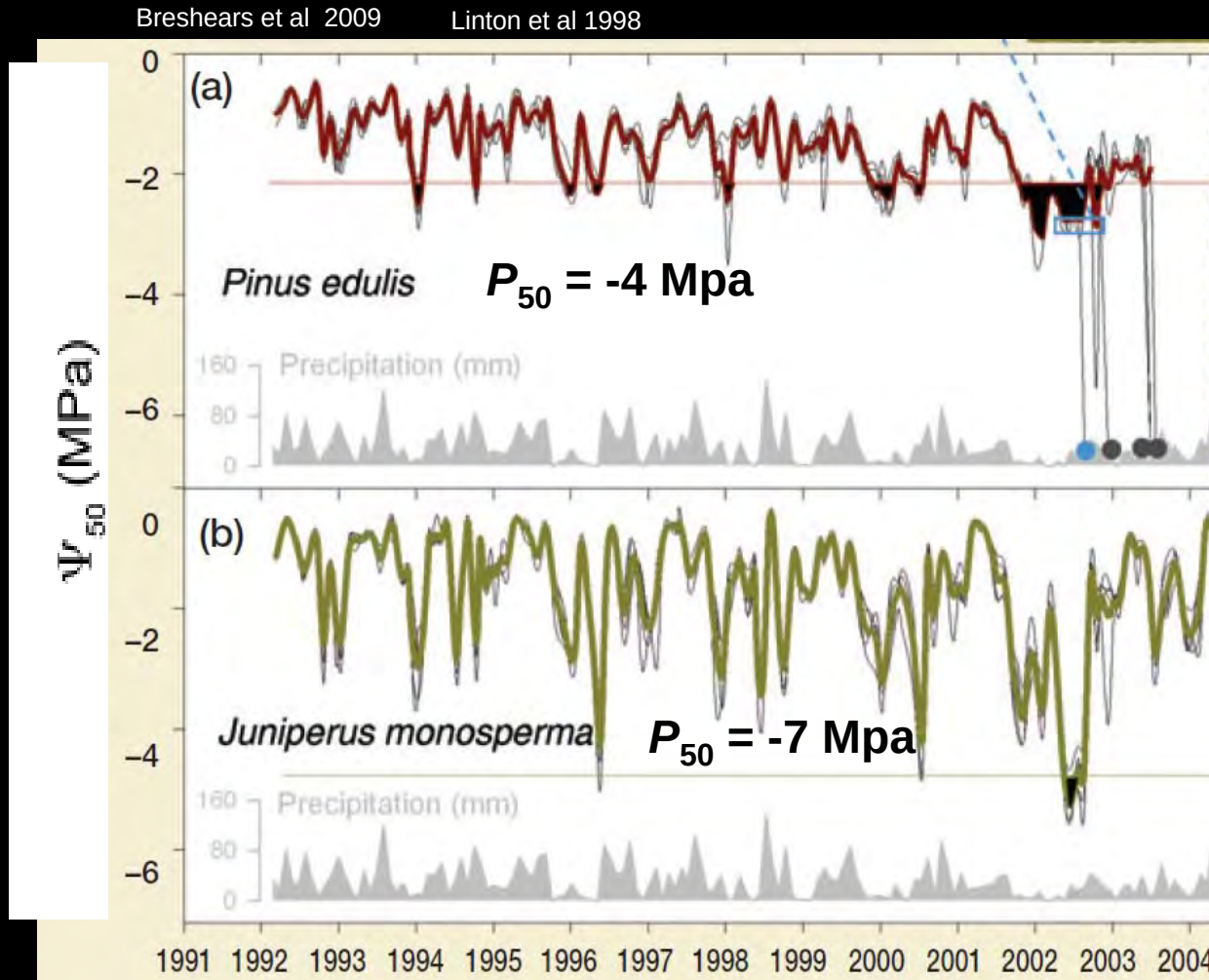
Pinus edulis



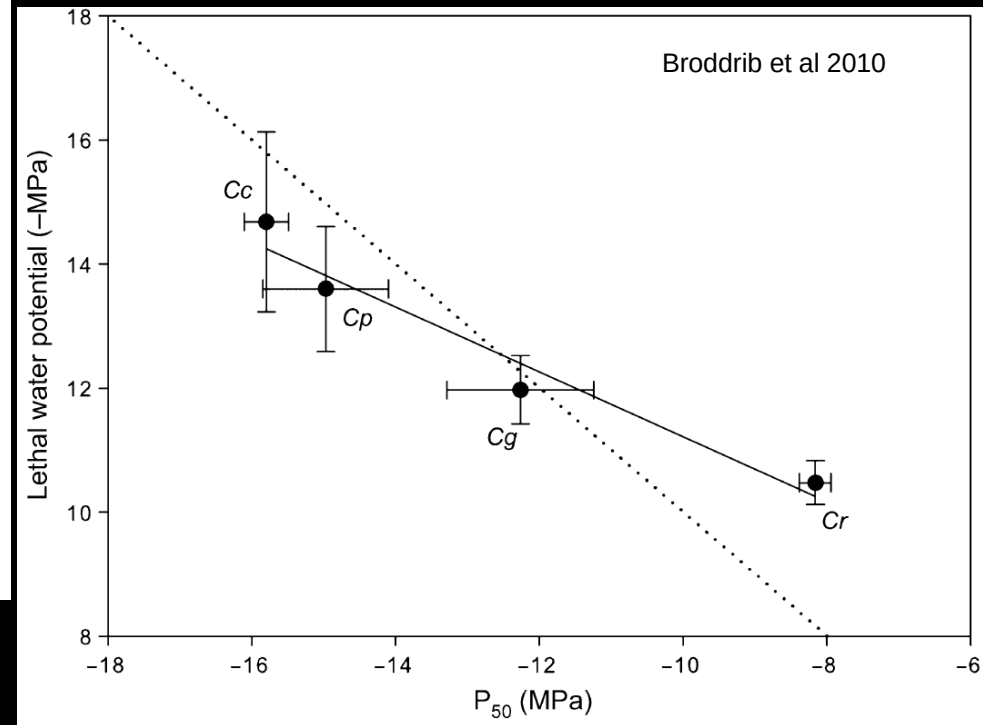
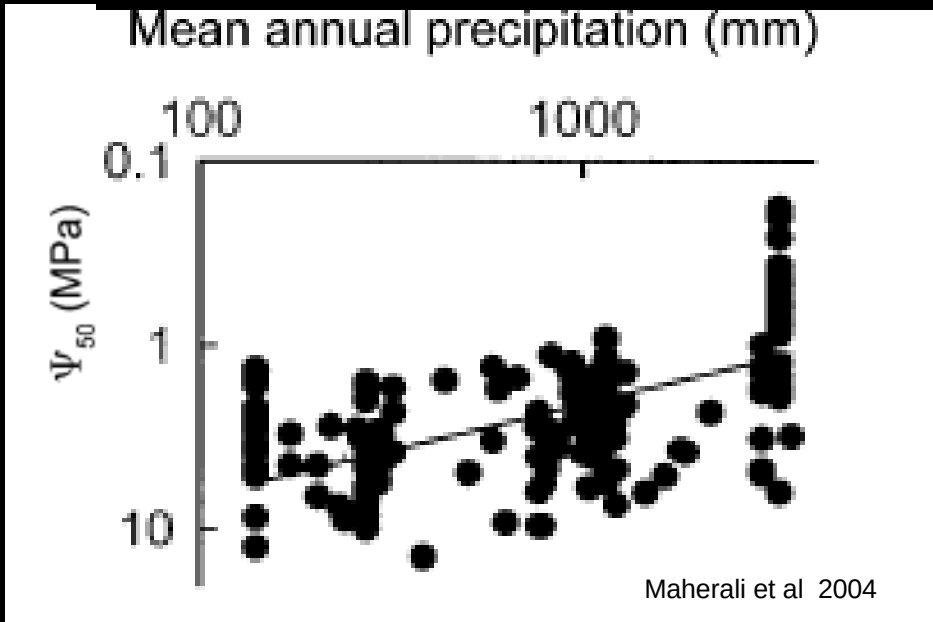
Introduction

Extreme Climatic events

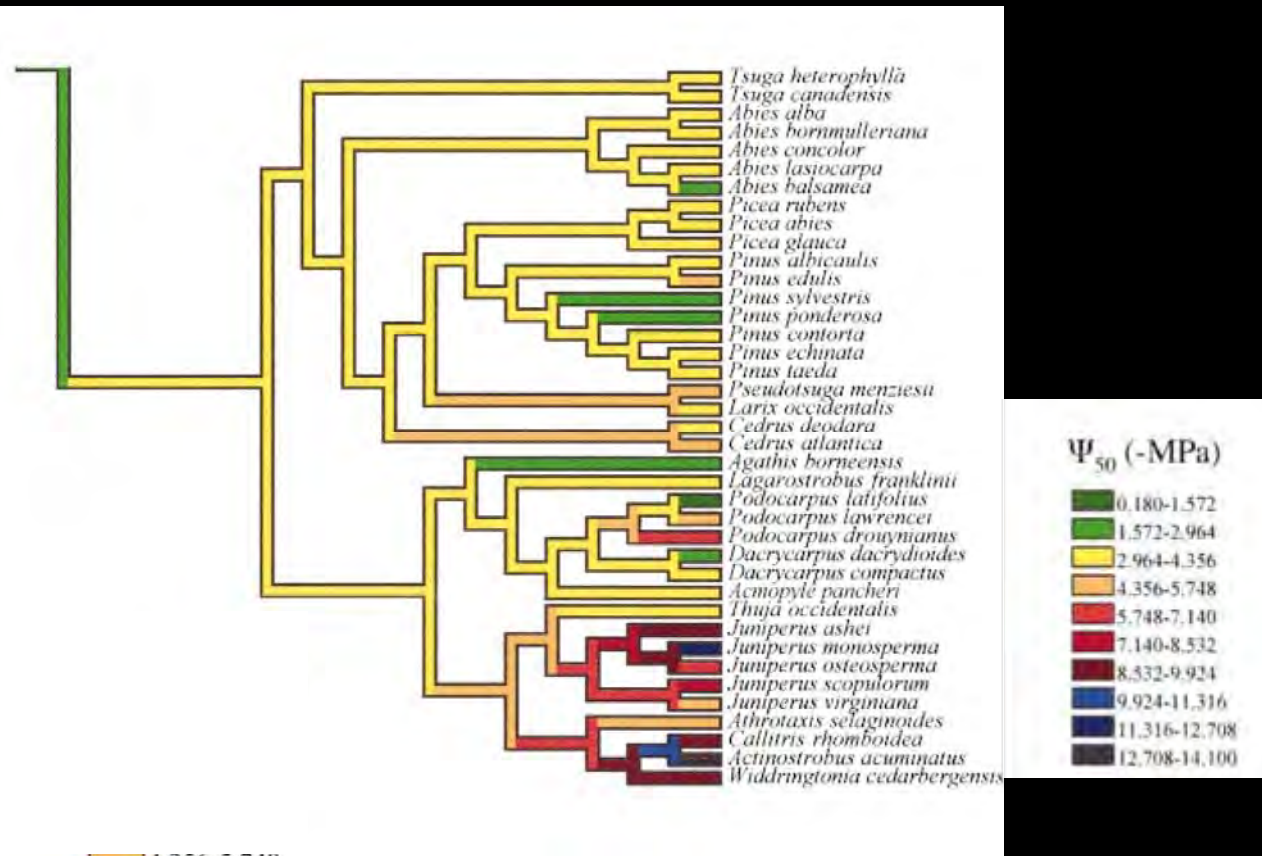
Which traits could trace the drought tolerance ?



Embolism is an direct causal factor of trees ' death



From a physiological point of view, cavitation resistance is a key drought tolerance



What evolutive forces have driven the evolution of these traits ?

...correlation between traits

...Check the postulate “cavitation resistance is a target of natural selection”

There is Genetic differences between populations ?

What evolutive forces drive the evolution of these traits ?

What are the correlation between traits

Material et Methods

characters

Pressure inducing 50 % loss of conductance

MPa⁻¹

P_{50}

Wood microdensity

dimensionless

D

Carbon 13 isotopic composition

‰

$\delta^{13}\text{C}$

Height increment between 2004 2005

mm

Δ_h

Total dry biomass 2005

g

B_{TOT}

Total needle area 2005

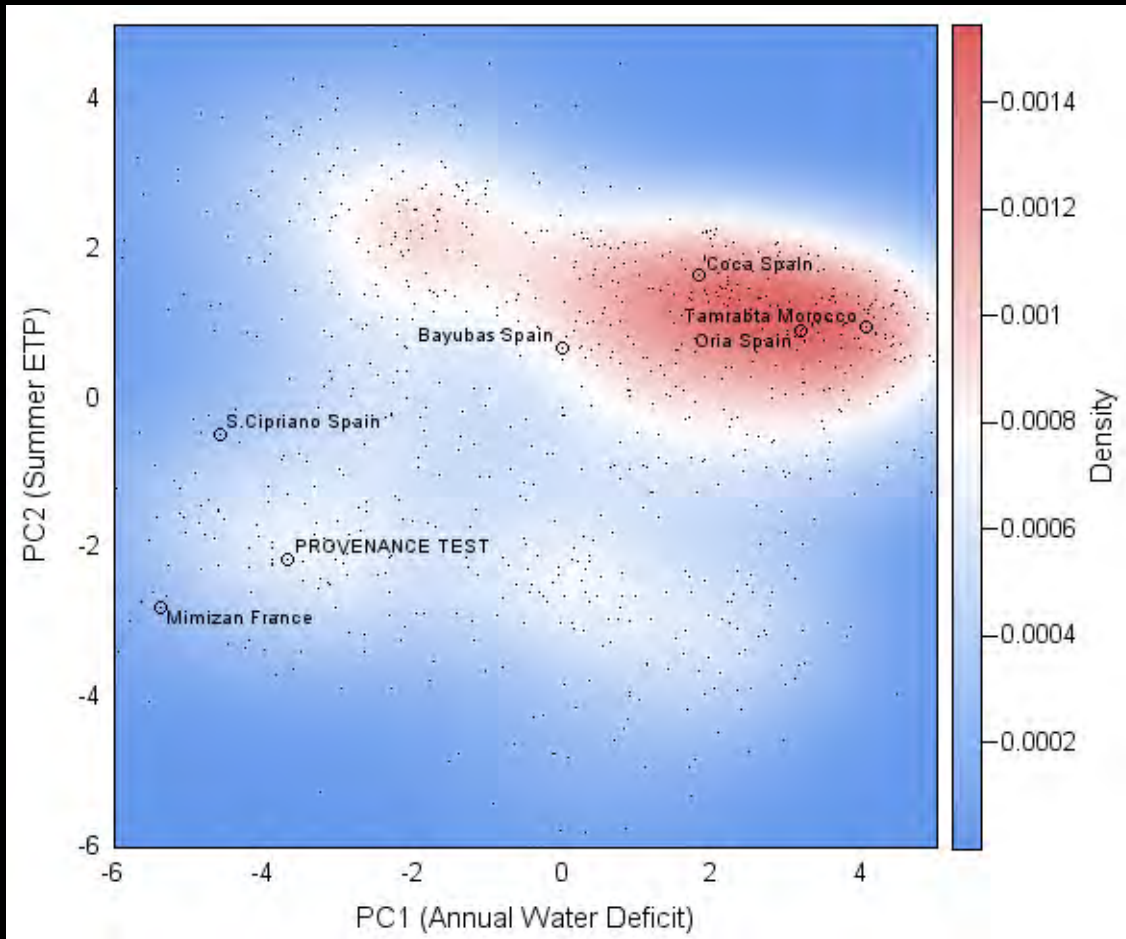
m²

A_{LEAF}

Material et Methods

Climate origin of populations

- 6 populations
 - 8 mother tree
 - 5 half-sibs
- = 240 genotype



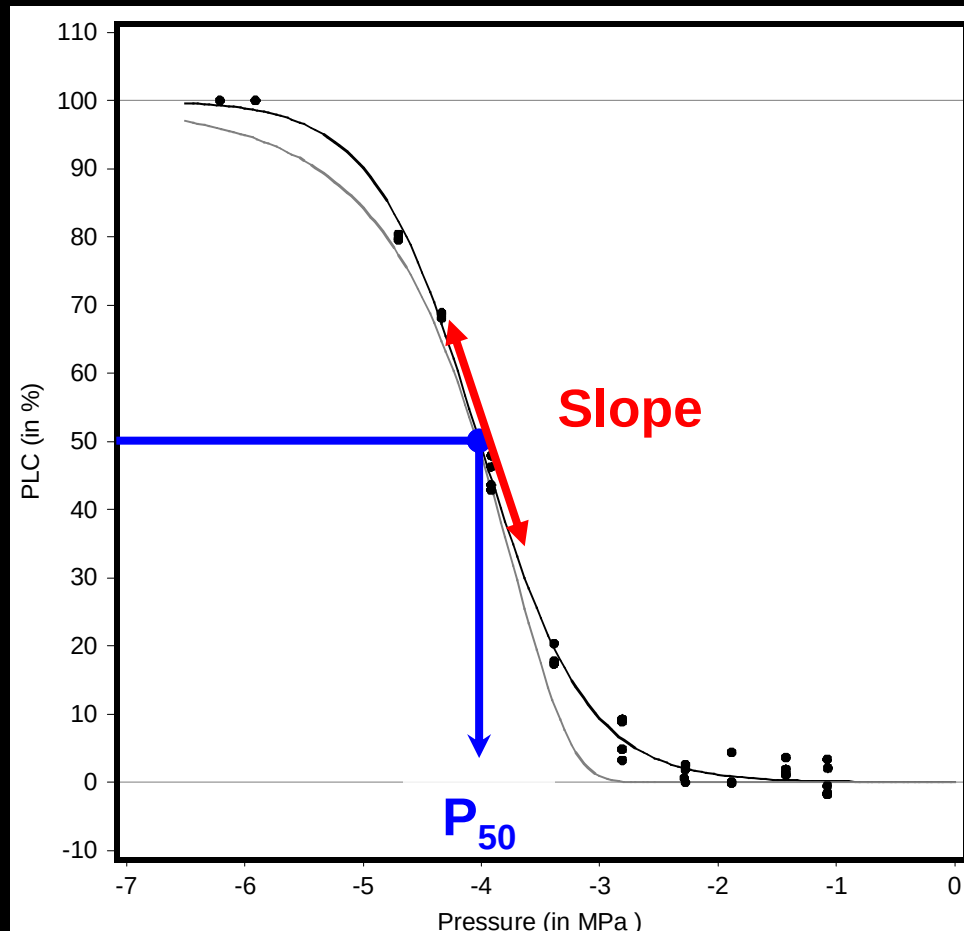
Lamy & al 2010 submitted

Spatial location of populations from Bucci et al 2007

Climatic data from New et al 2002



Hydraulic character



Vulnerability curves

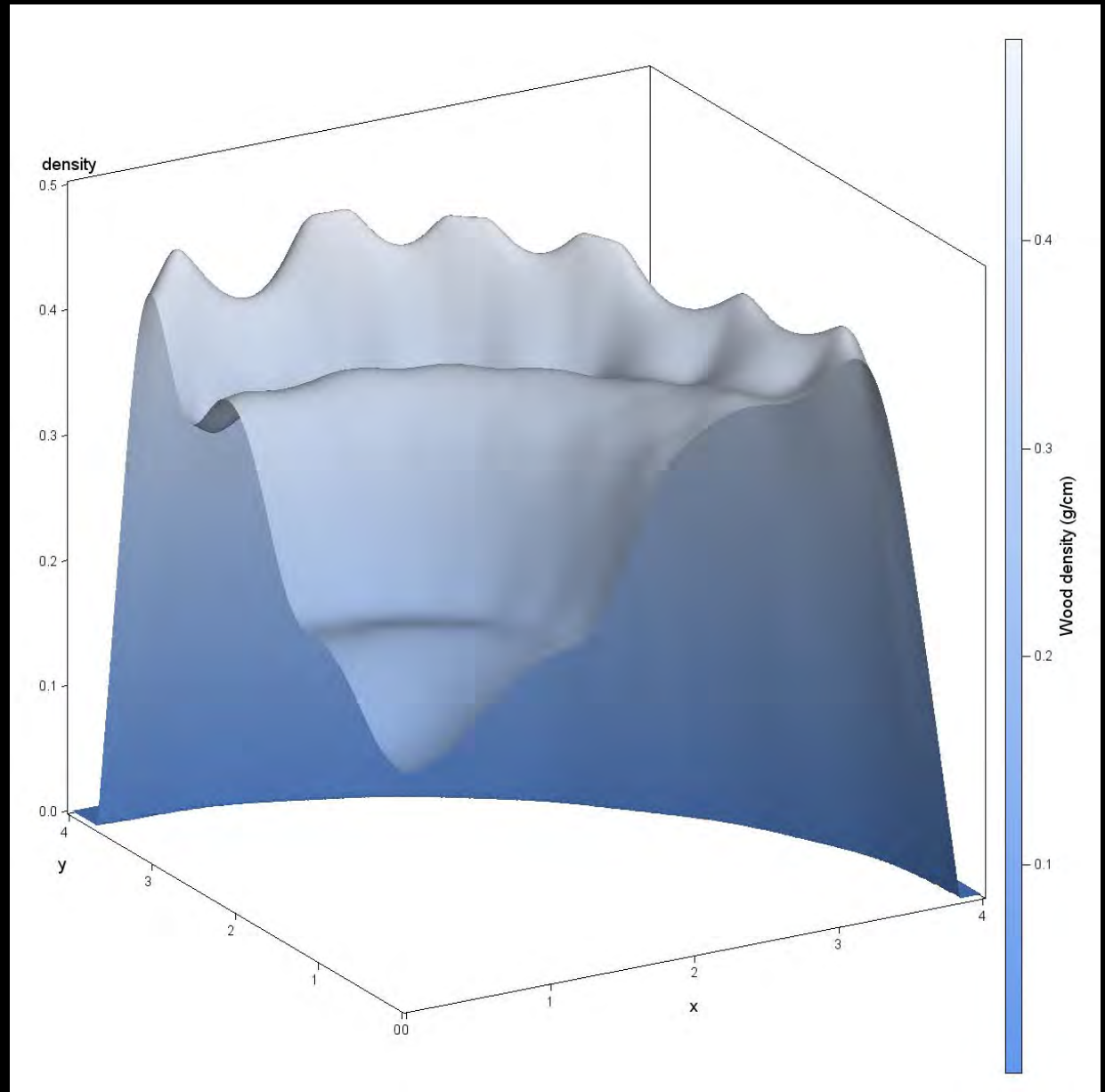
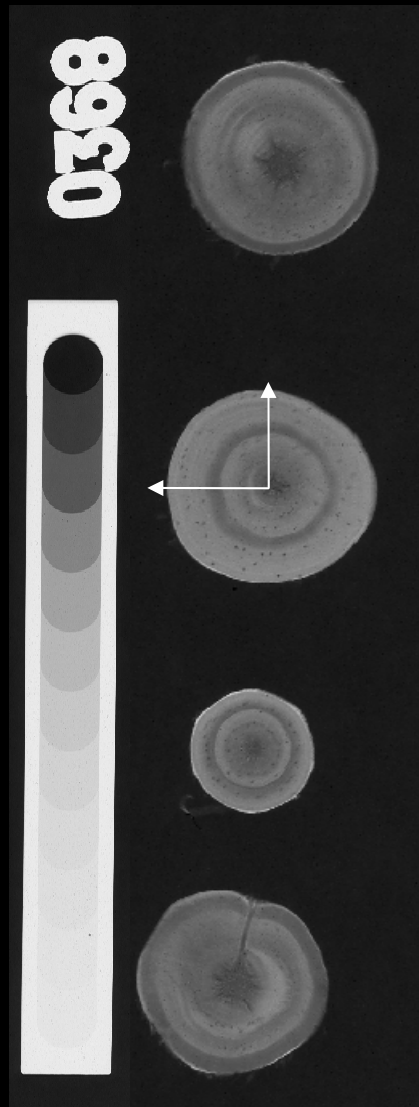
TECHNICAL FOCUS

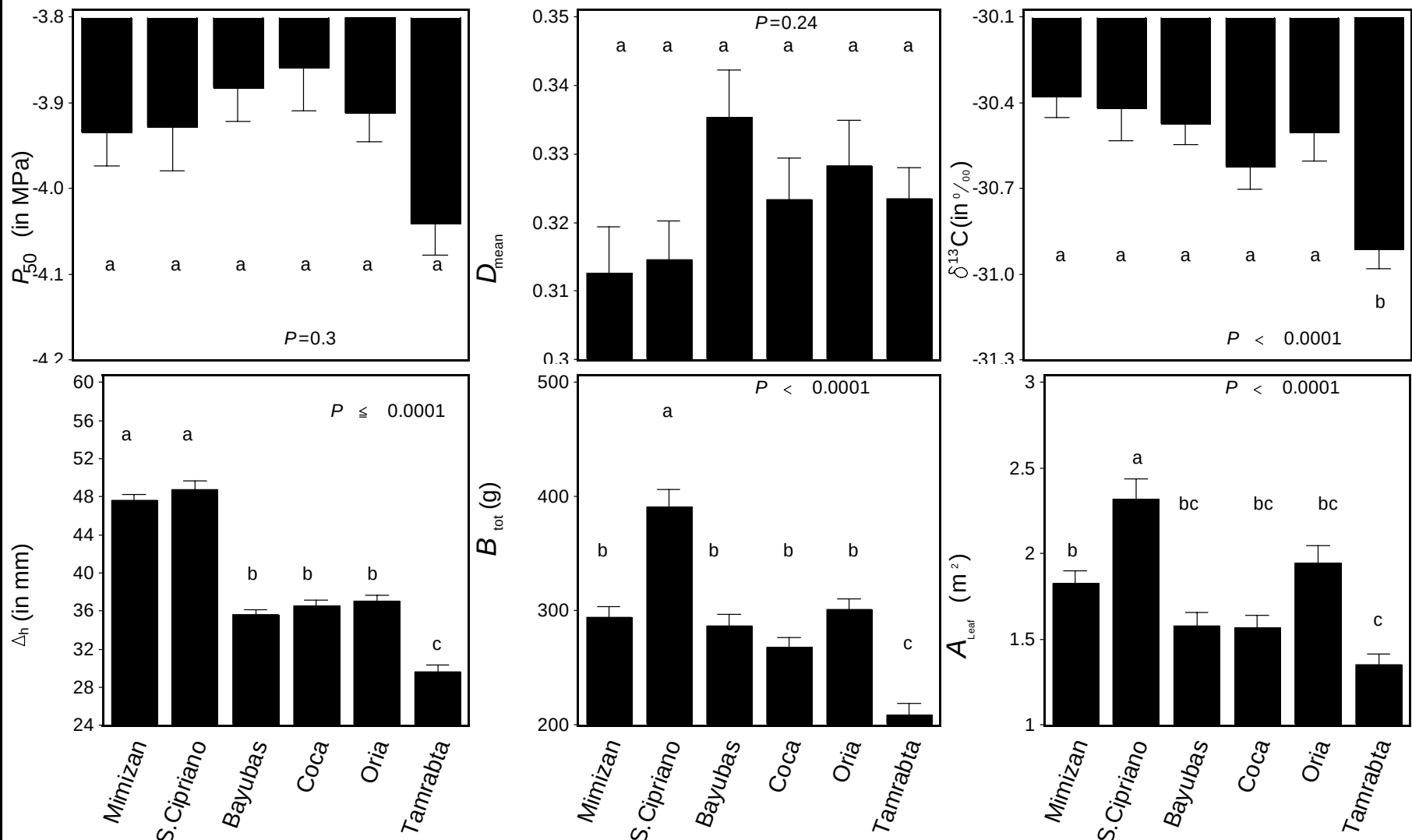
Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves

Hervé Cochard^{a,*}, Gaëlle Damour^b, Christian Bodet^a, Ibrahim Tharwat^c, Magalie Poirier^a and Thierry Améglio^a



« Feu Cavitron 2 »

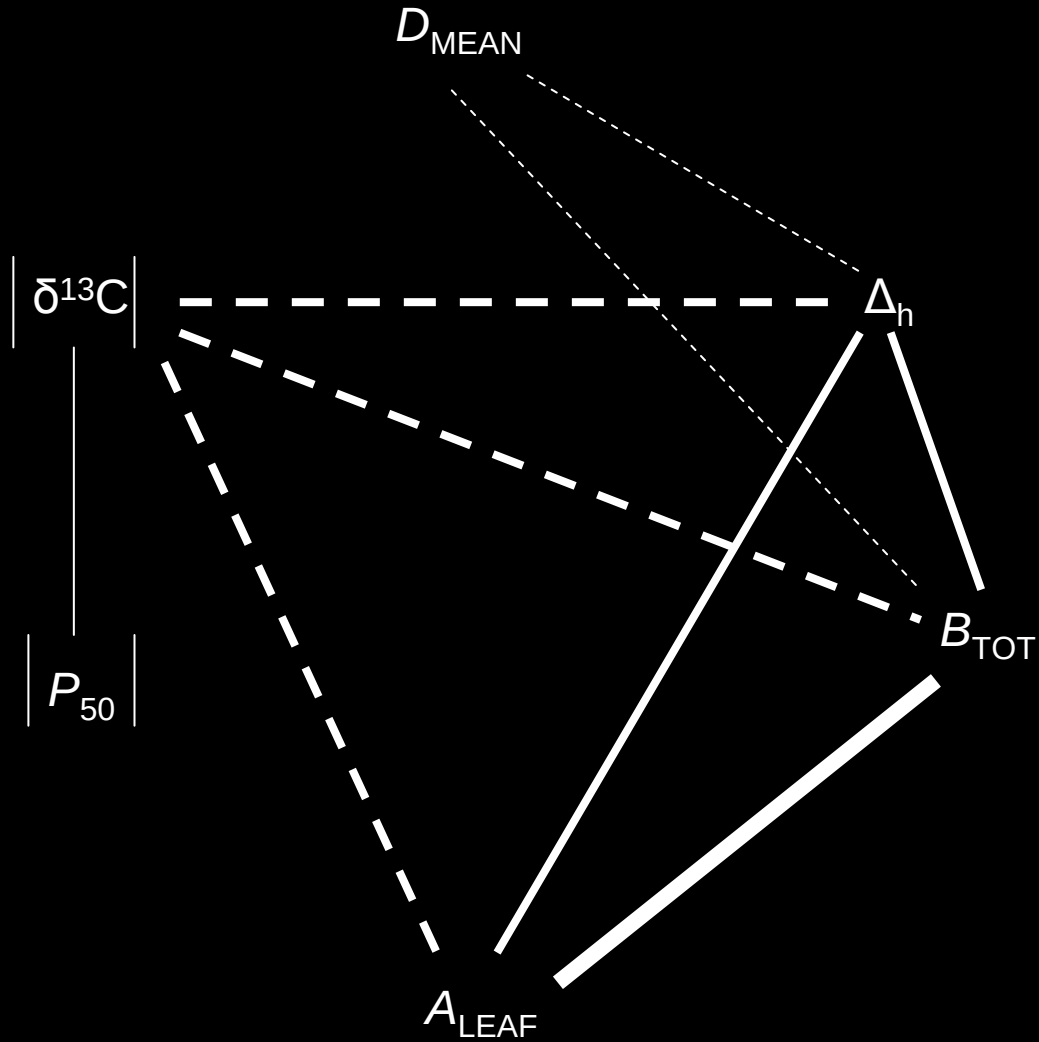




Lamy & al 2010 submitted

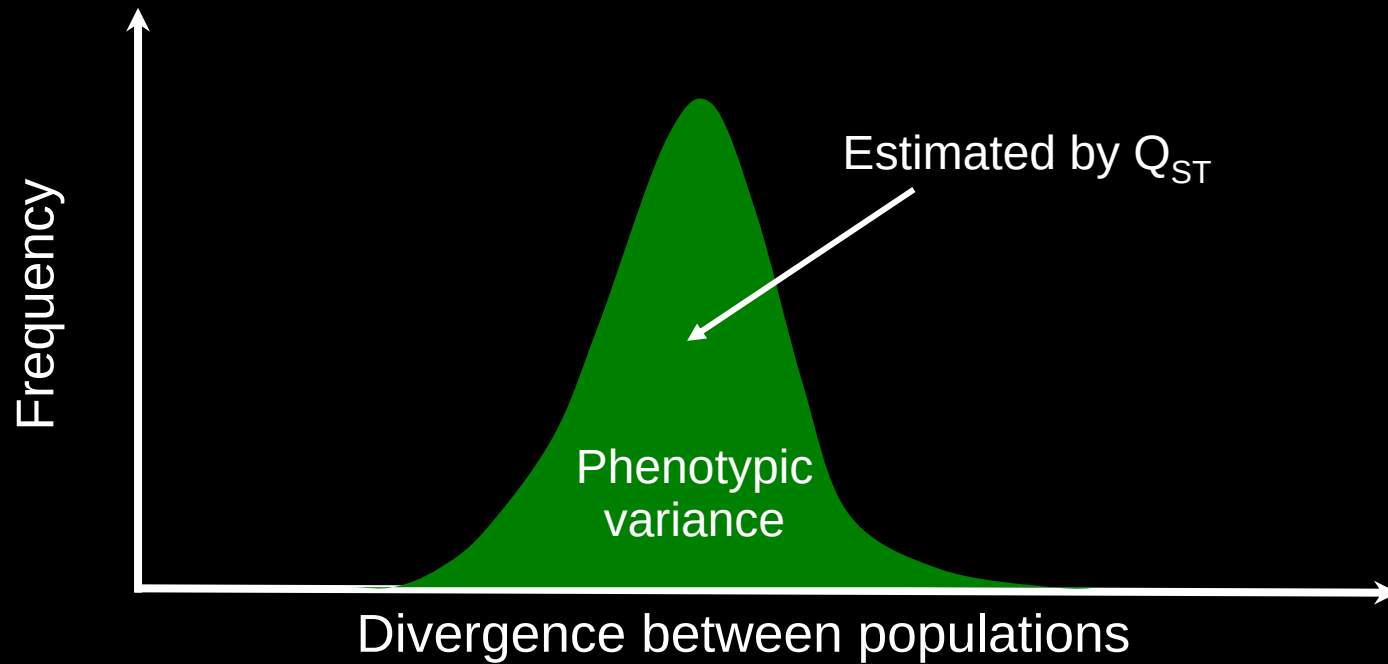
No phenotypic difference between population for wood-related traits

Phenotypic difference between population for the other traits.



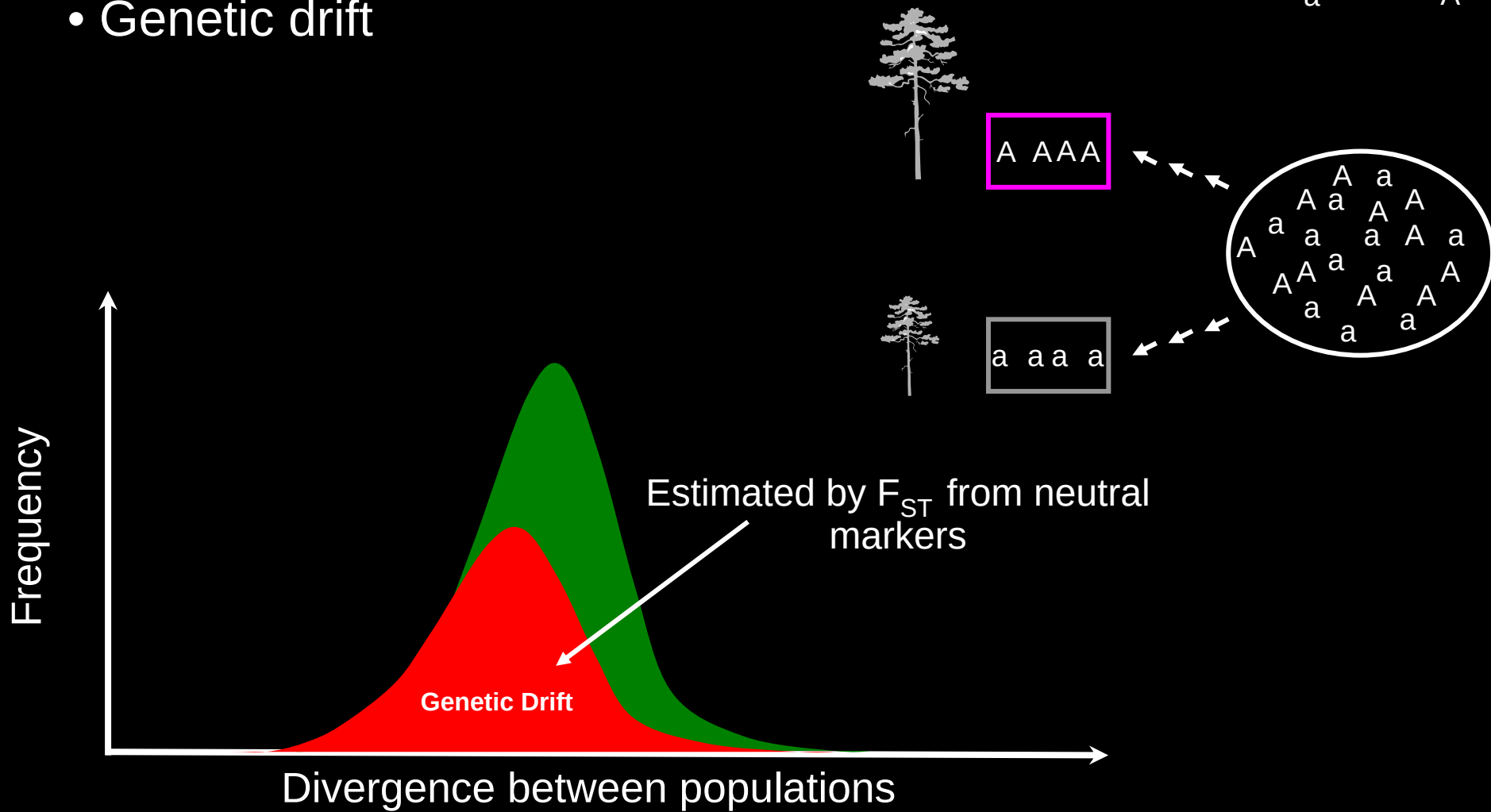
Concepts

- Phenotypic variation :



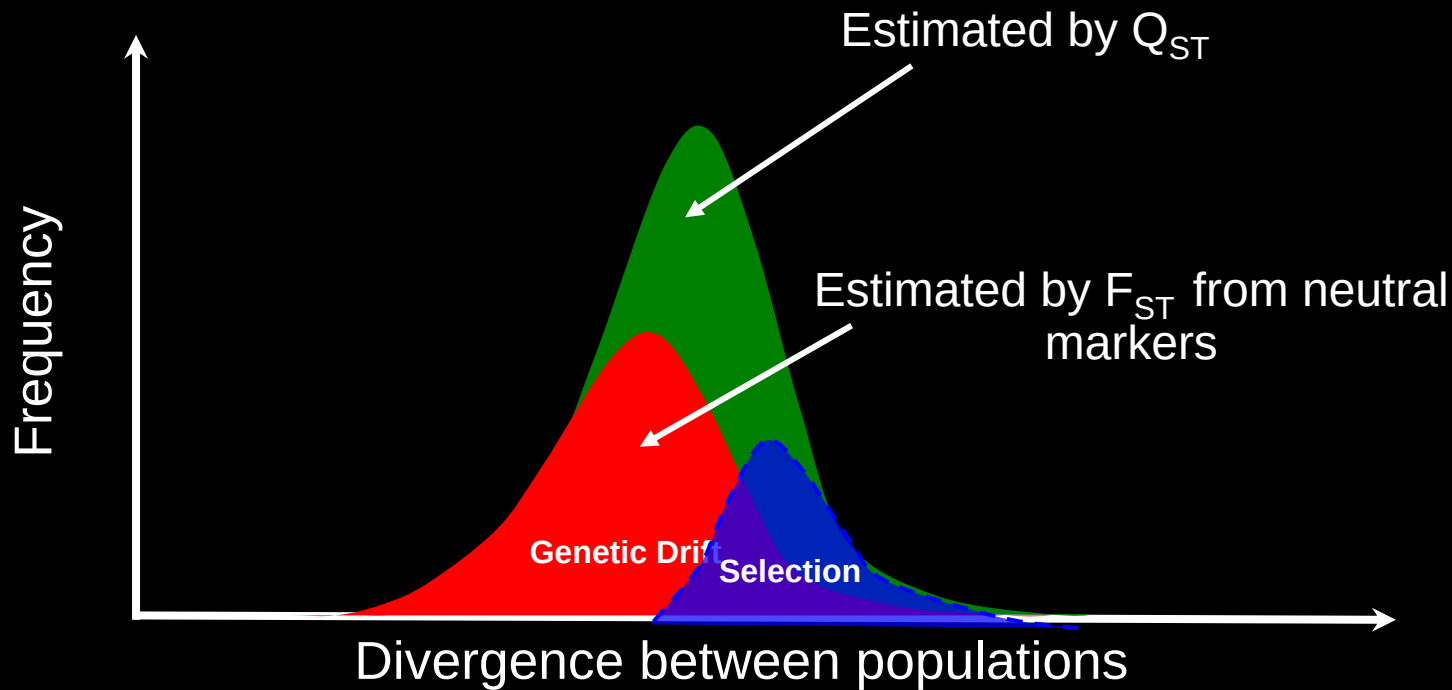
Concepts

- Genetic drift



Concepts

- Genetic drift or Selection



Concepts

Genetic drift or Selection ?

•In theory :

$$Q_{ST} = F_{ST}$$

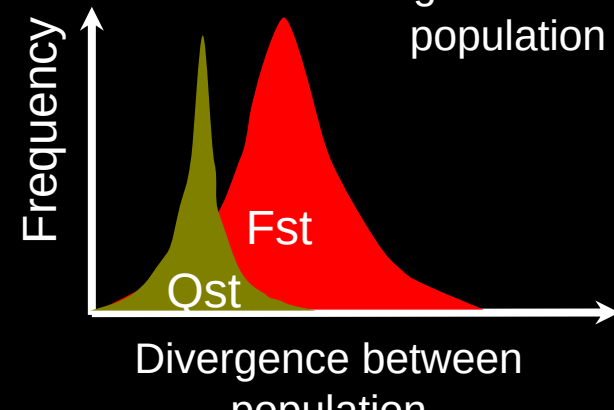
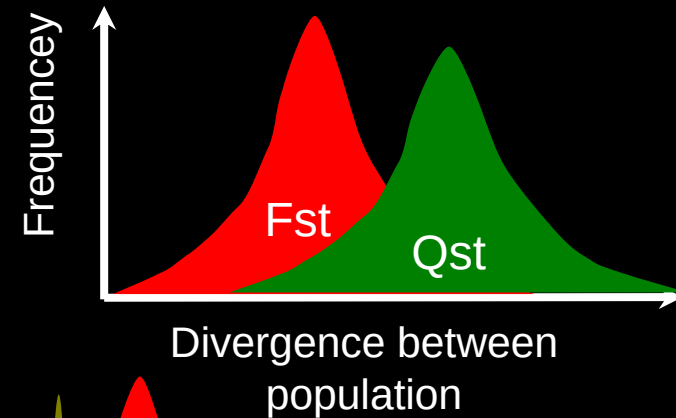
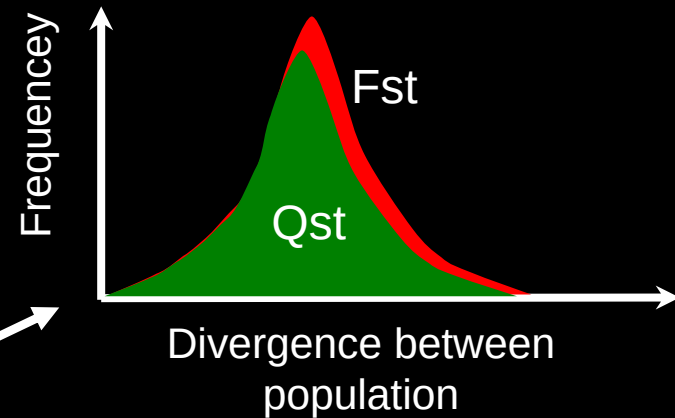
$$Q_{ST} > F_{ST}$$

$$Q_{ST} < F_{ST}$$

Drift

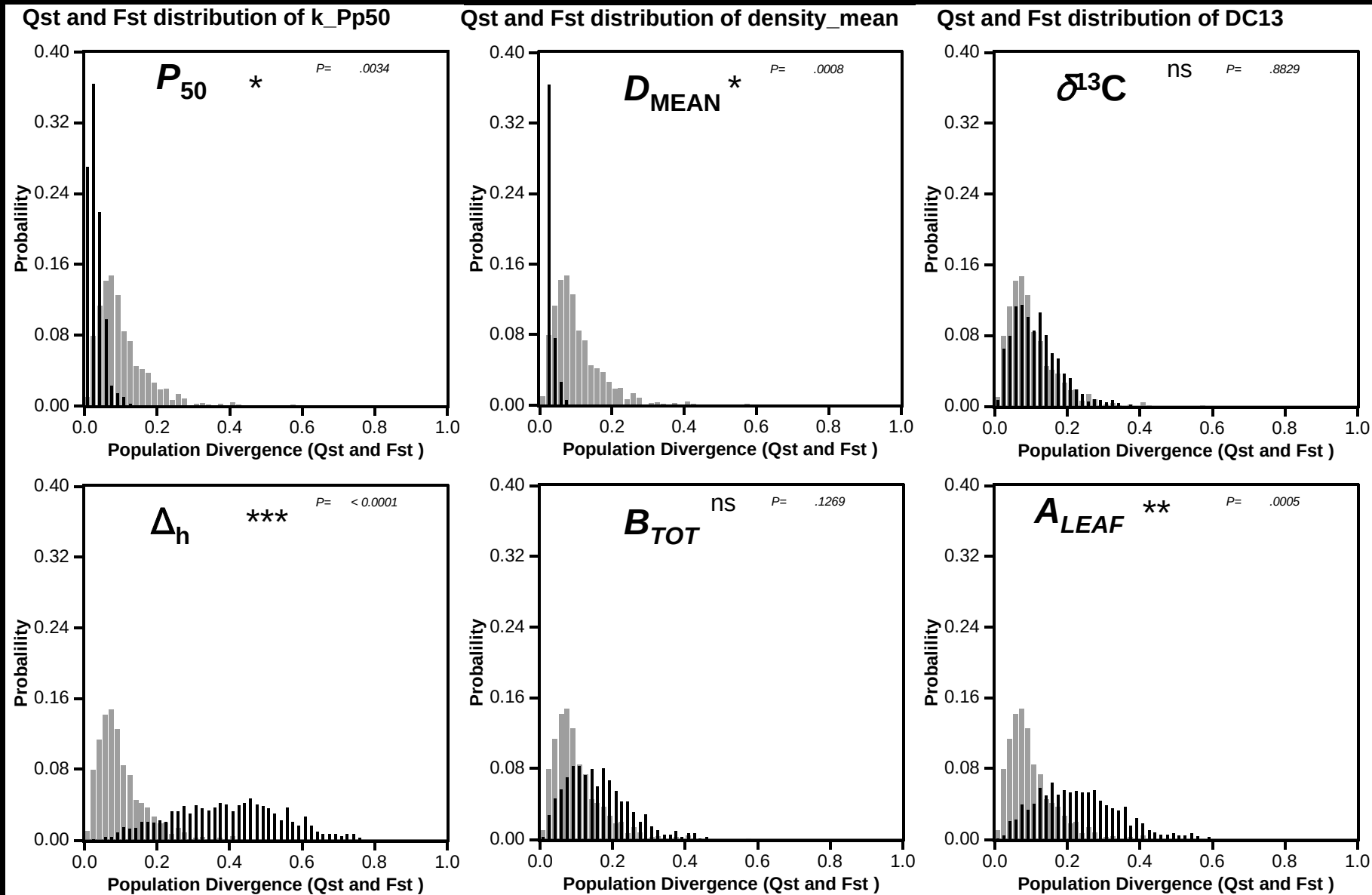
Diversifying selection

Uniform selection



Results et Discussion

V_G = Genetic variance

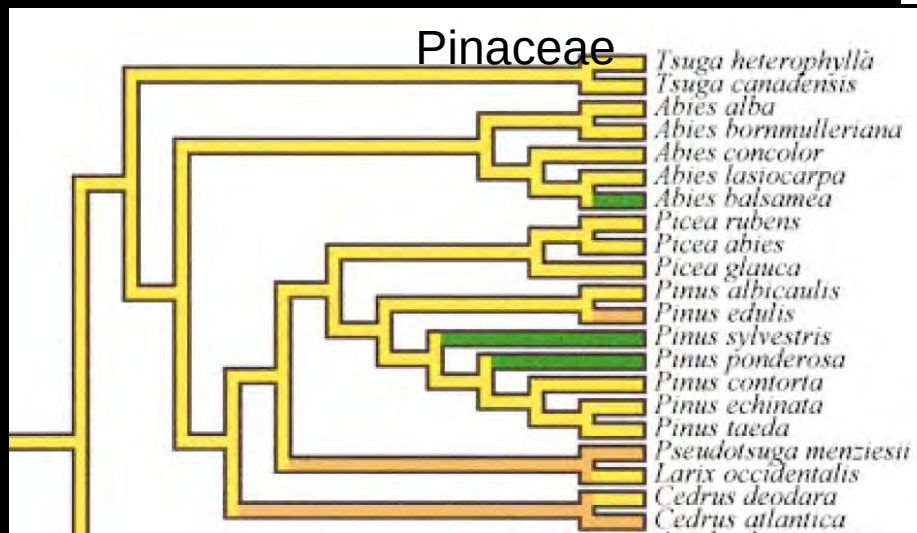
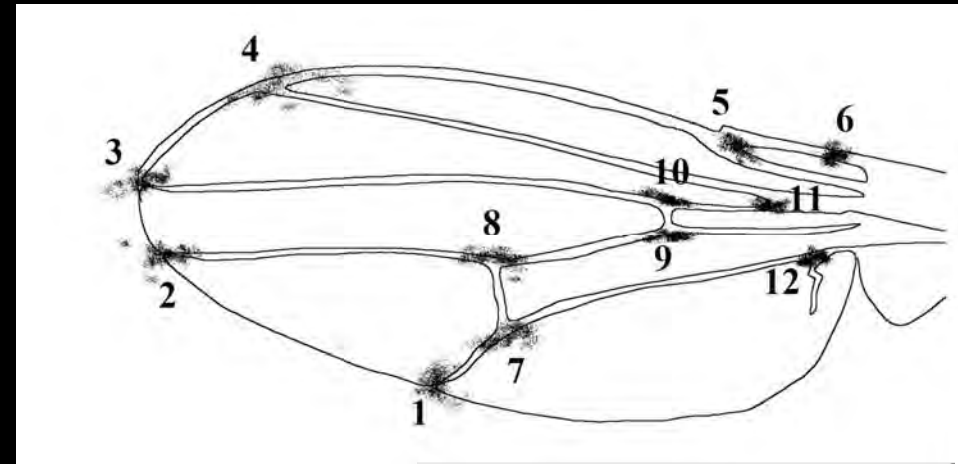


Results et Discussion

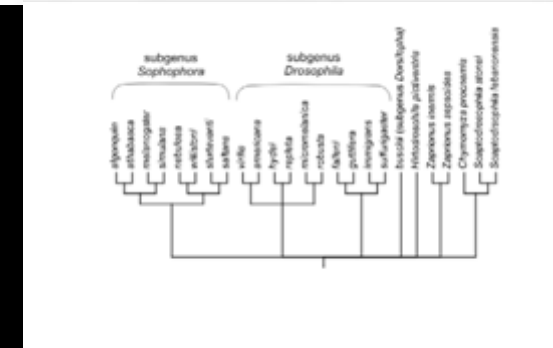
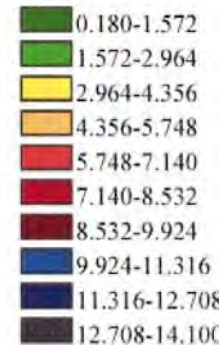
Highly climatically contrasted populations
No between-populations divergence

The first indirect proof in plant science of *uniform* selection across population

=>Canalized trait
(very old stabilizing selection)



Ψ_{50} (-MPa)

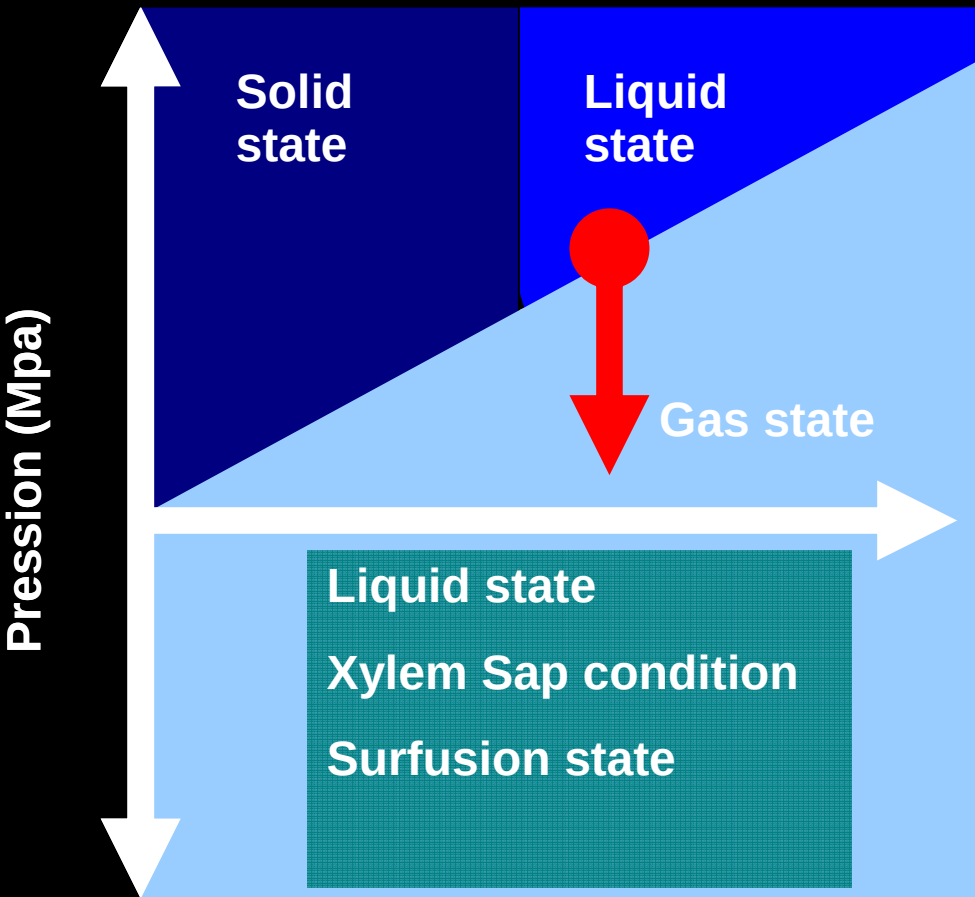




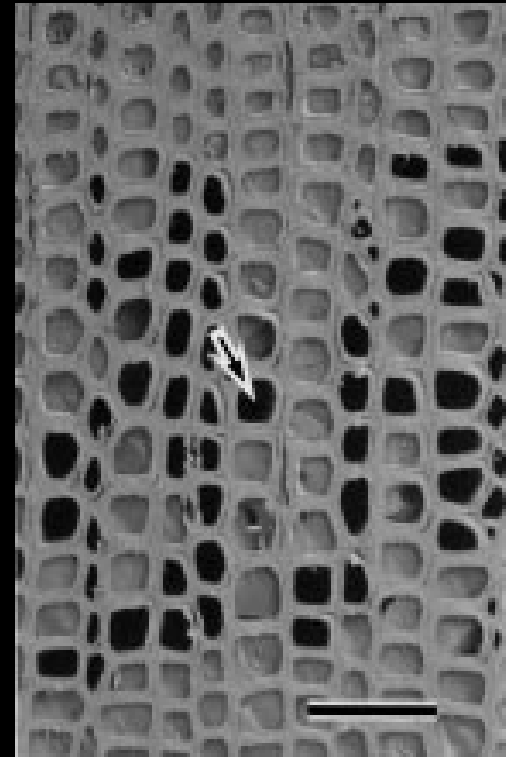
LALAQÜE HADRIEN

Introduction

Physiological process of cavitation

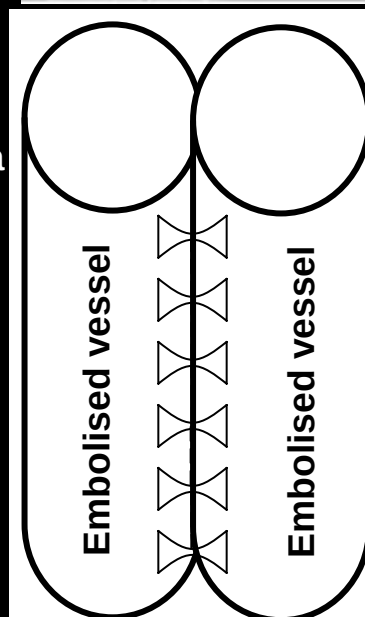
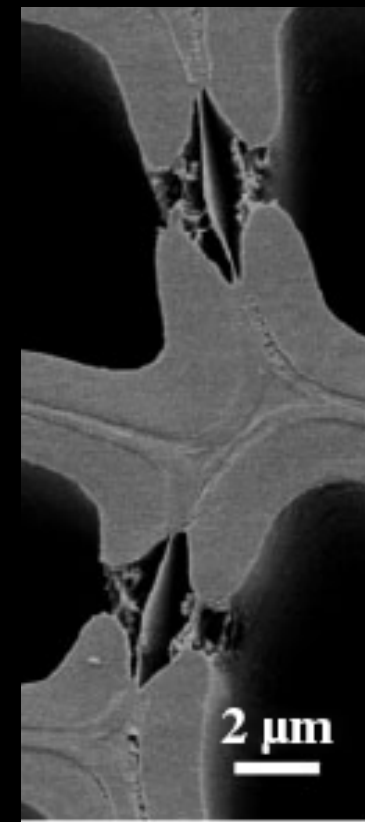
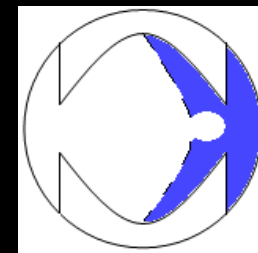
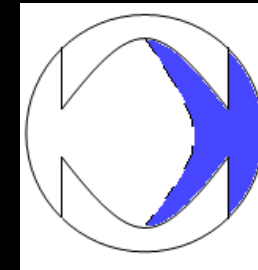
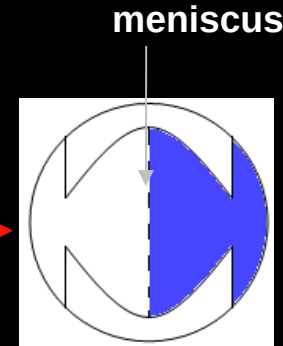
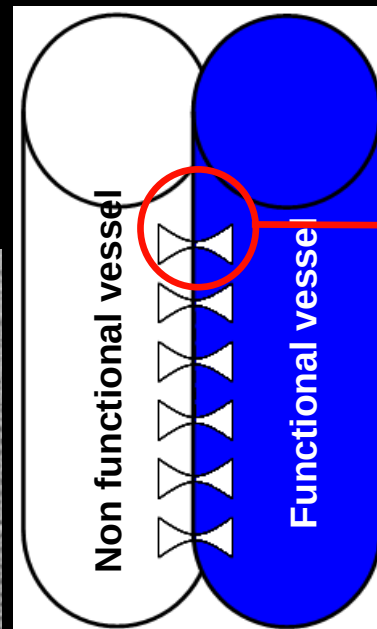
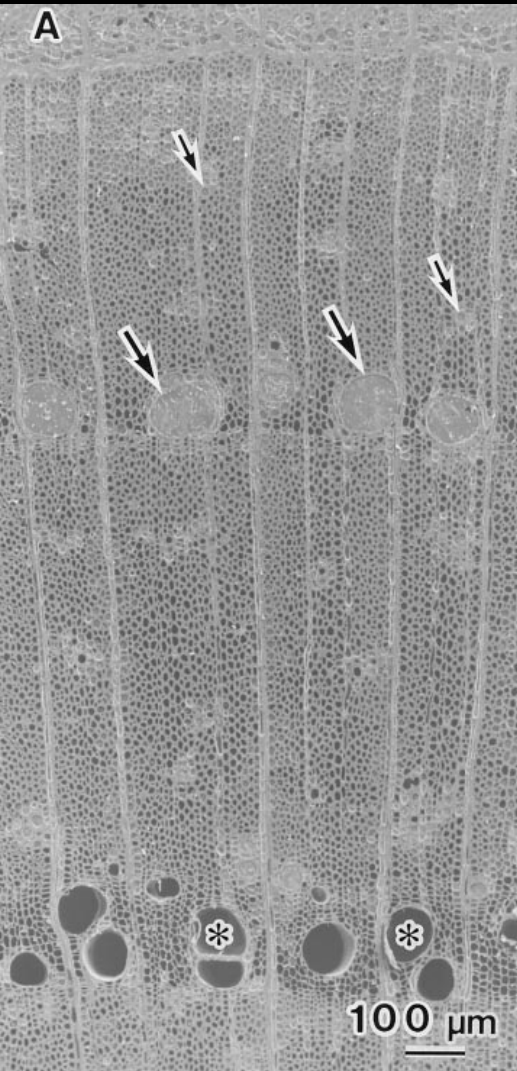


Temperature (K)



Introduction

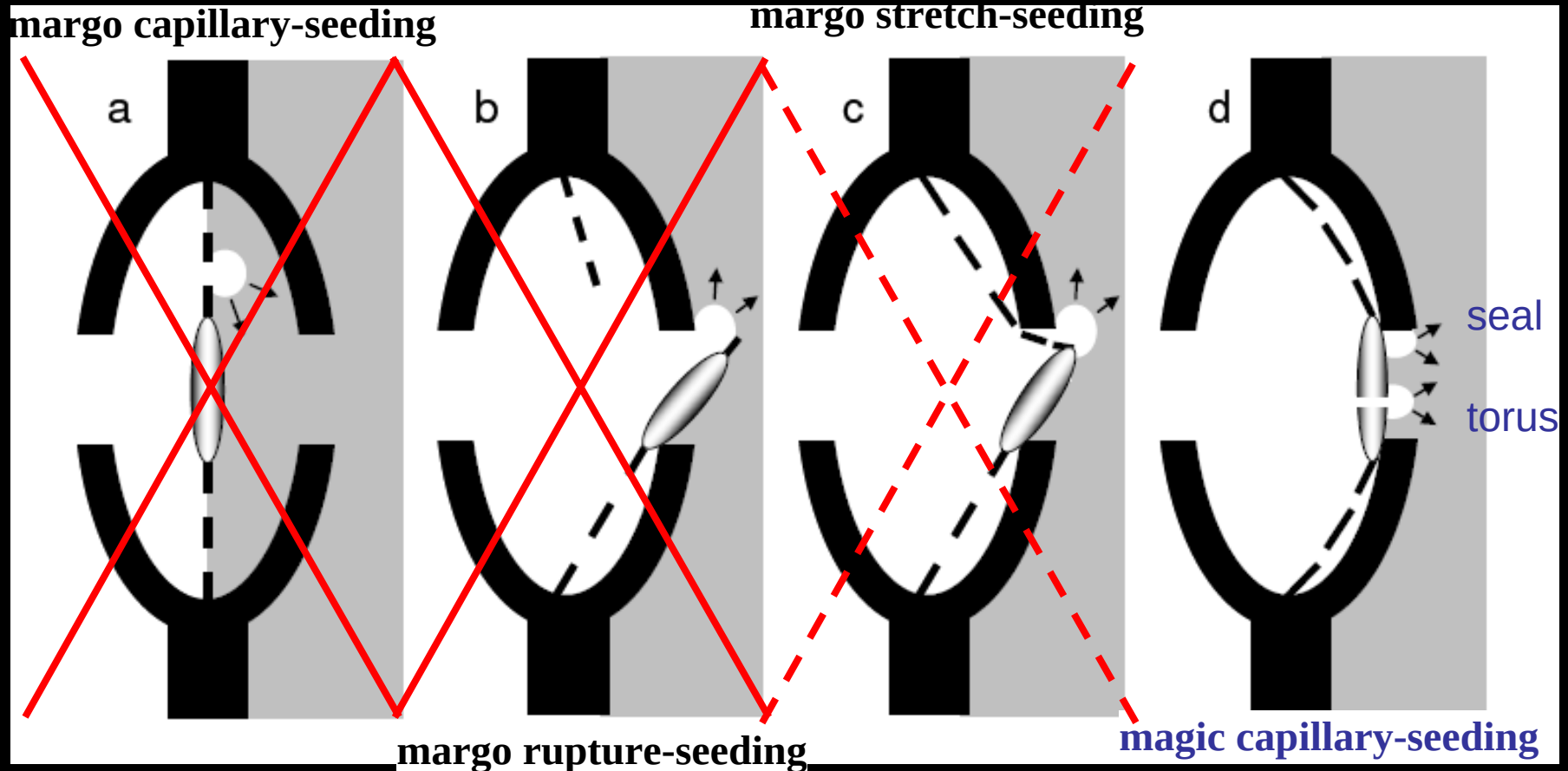
Physiological process of cavitation



Tyree and Zimmermann 1996
« Xylem Structure and the Ascent of Sap »

Utsumi et al 1996

Hypothesis for embolism propagation in Gymnosperm



DP deflection < DP
margo capillary
seeding

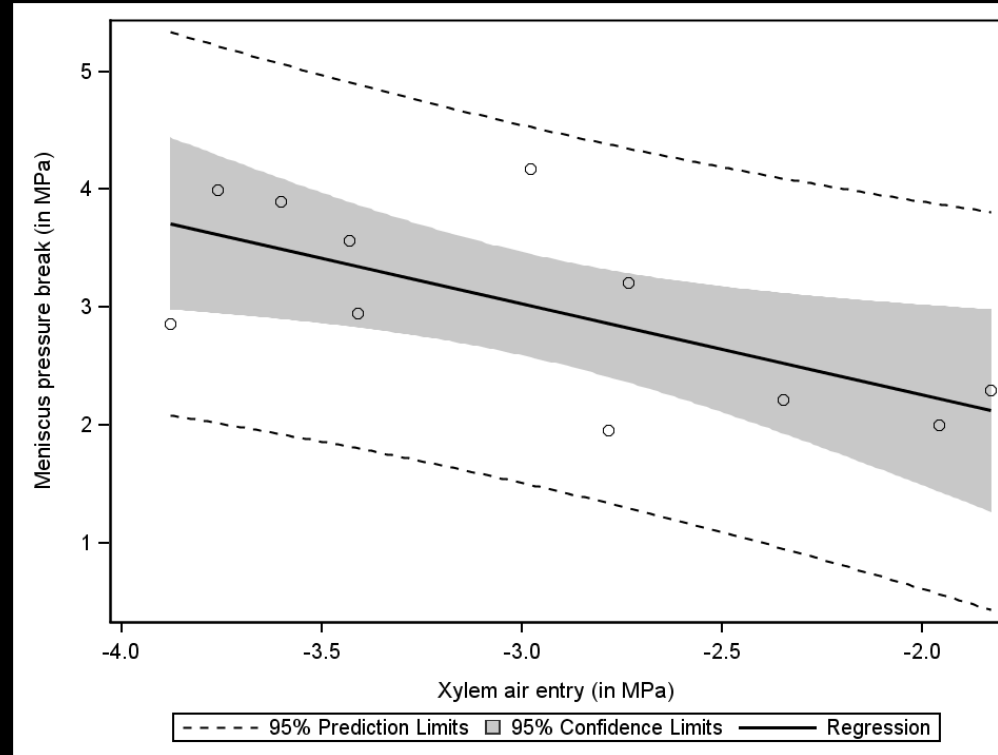
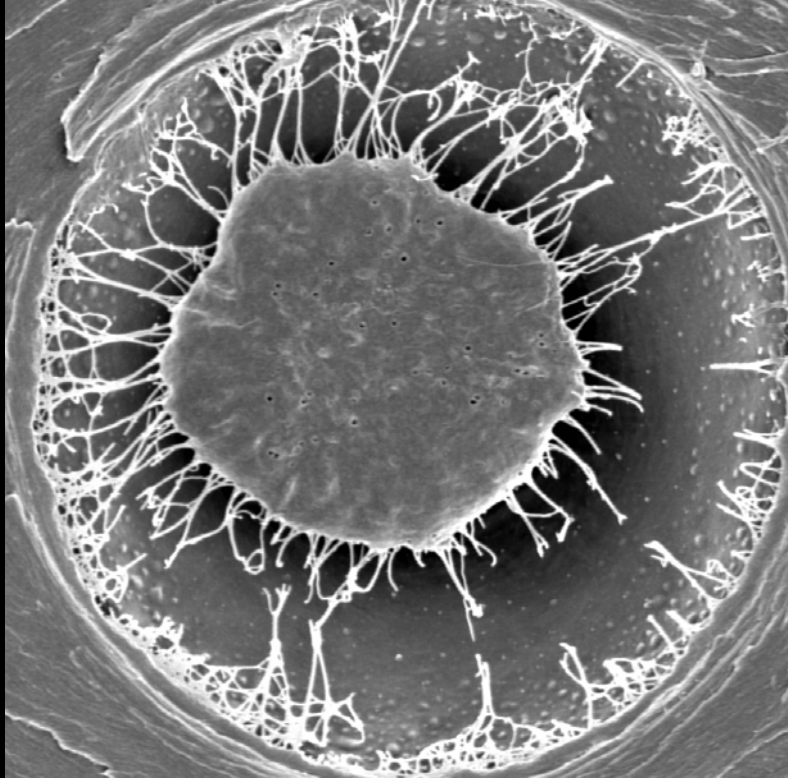
no increase in
conductance above
the cavitation
threshold

Higher margo
flexibility = more
cavitation resistant

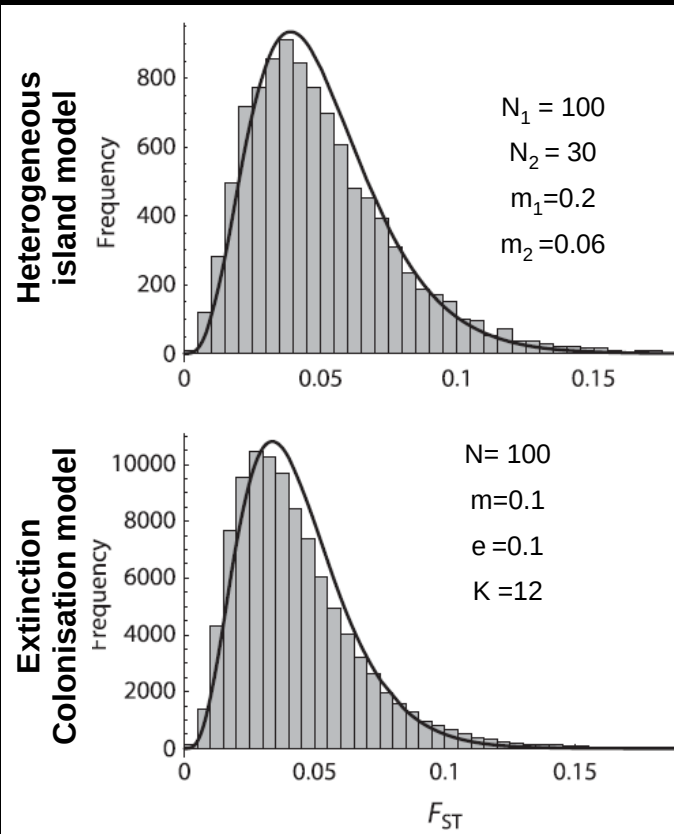
Cochard & al 2009, Delzon et al 2010

Introduction

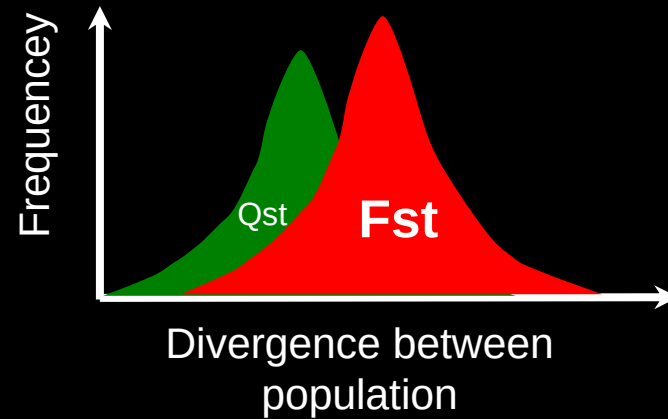
Physiological process of cavitation



Distribution of F_{ST} ?



Whitlock 2008

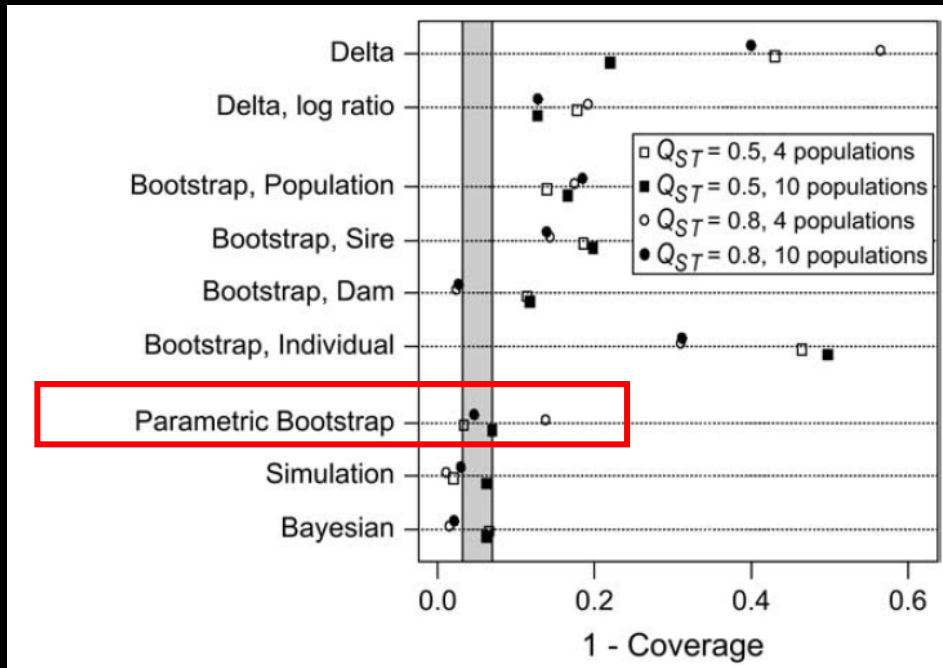


Approximation of F_{ST} distribution with Lewontin-Krakauer distribution

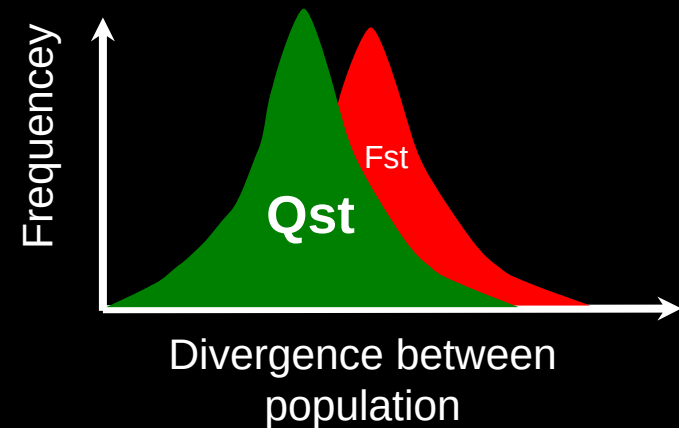
$$\hat{F}_{ST} = F_{ST}^* \times \frac{random \chi^2_{loci-1}}{df}$$

Distribution of Q_{ST} ?

Bad performance of classical methods



O'Hara et al 2005



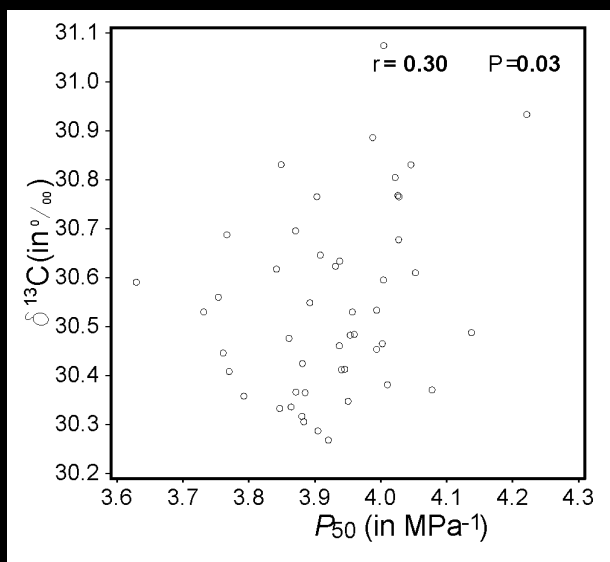
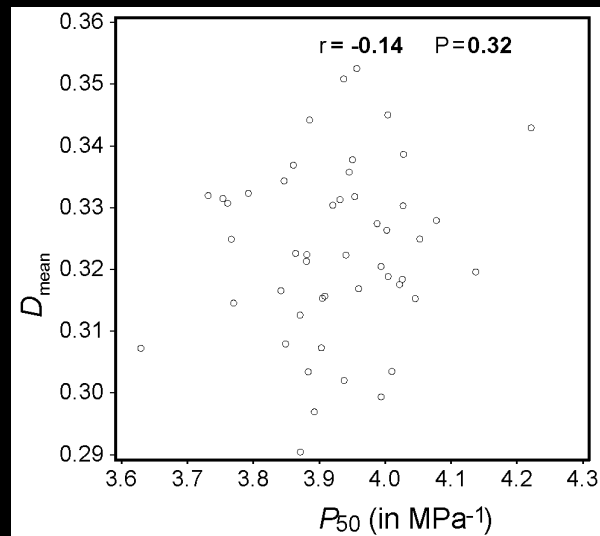
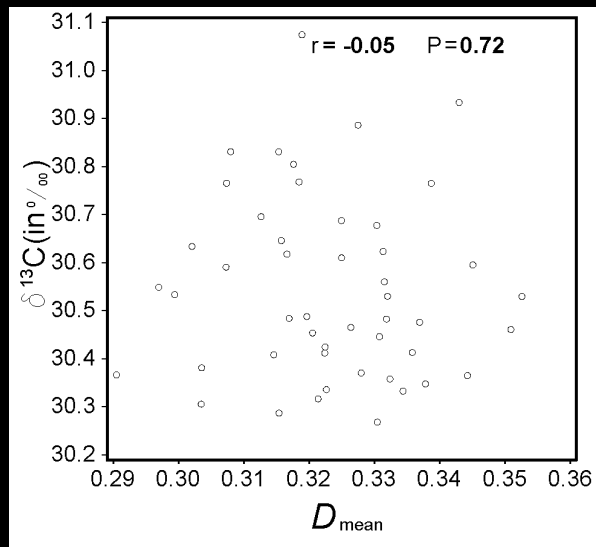
AN APPROXIMATE DISTRIBUTION OF ESTIMATES
OF VARIANCE COMPONENTS

F. E. SATTERTHWAITE

General Electric Company, Ft. Wayne, Indiana

$$\hat{V}_i = V \frac{\chi^2_{df_{effective}}}{df_{effective}} \quad \text{with}$$

$$df_{effective} = \frac{df_{observed} - 1}{1 + \frac{2V^2}{nV_{I-1}^2} + \left(\frac{V^2}{V_{I-1}^2} \right)^2 \left(\frac{1}{n^2 + \frac{I-1}{n^3I - n^2}} \right)}$$



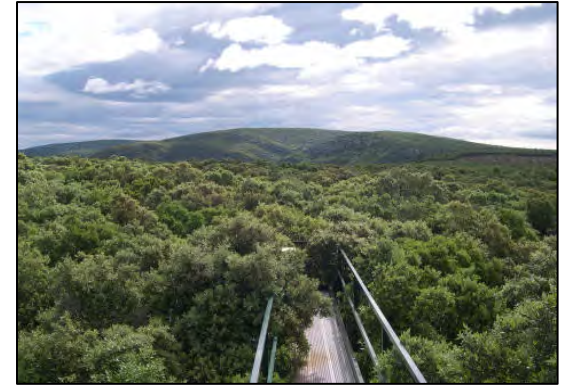
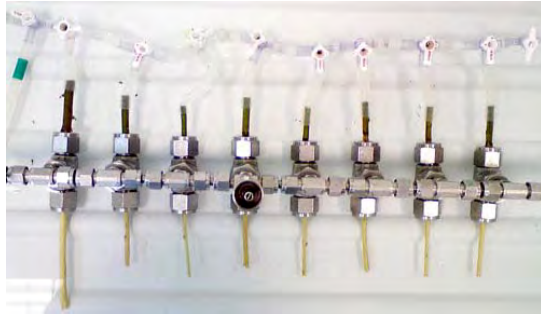
Results and Discussion

V_G = Genetic variance

Characters	h^2_{ns}	SE h^2_{ns}	CV_A	CV_{BP}	CV_R	CV_P
P50	0.438	0.117	4.4	1	6.7	6.6
$\delta^{13}C$	0.21	0.067	1.3*	0.6*	3.4*	2.2*
Δh	0.363	0.080	16.2	18.8	2	26.9

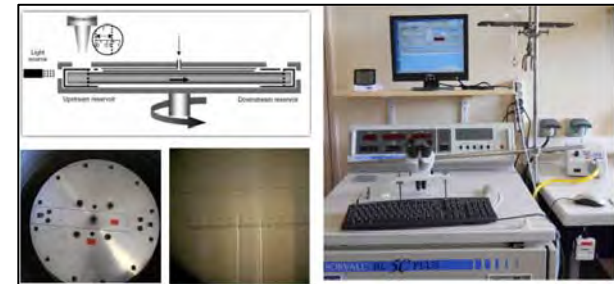
Lamy & al 2010 in prep

It is possible to select more cavitation resistance genotype, but the gain could be limited by the amount of additive variance



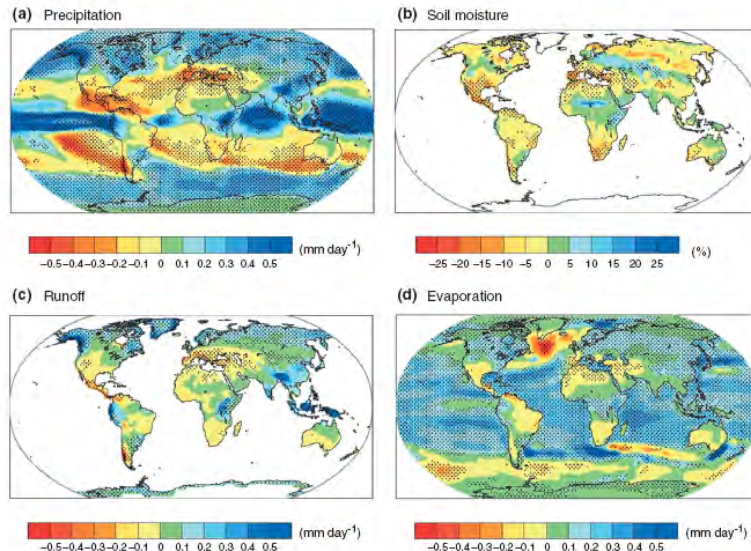
How reliable is vulnerability to cavitation measurement on *Quercus ilex*

Nicolas Martin, Damien Longepierre, Roland Huc, Hervé Cochard, Serge Rambal



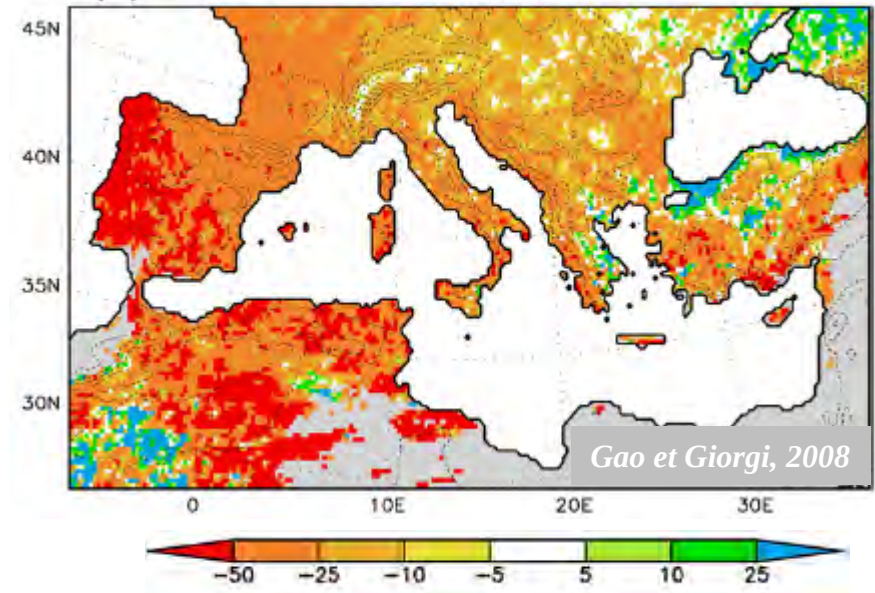
Why? Climate change etc...

- 25 to 30% des précipitations 2100



Aiguo 2010

- 25 to 50% Mediterranean bassin
(d) Precipitation change, B2-Ref, JJA, %



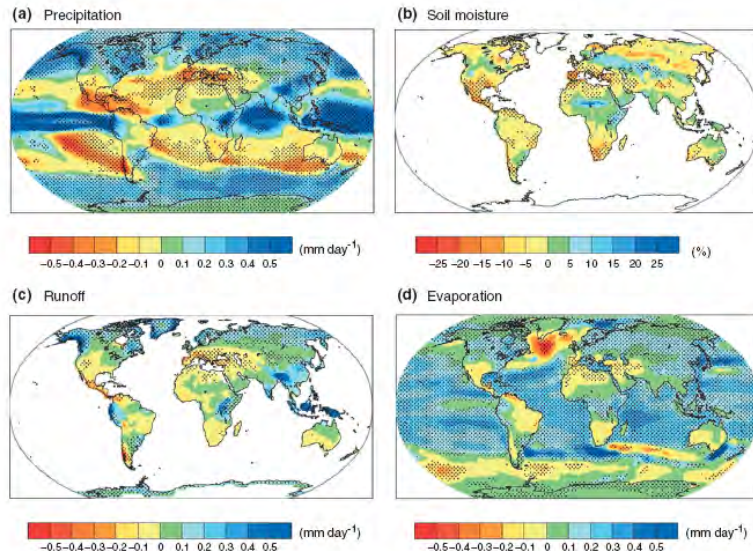
- How will Mediterranean forests respond to climate change? ➔ Trees die-off, Carbon Budget..

- How pertinent traits will vary with adverse conditions?

- Widespread Mediterranean species : *Quercus ilex*

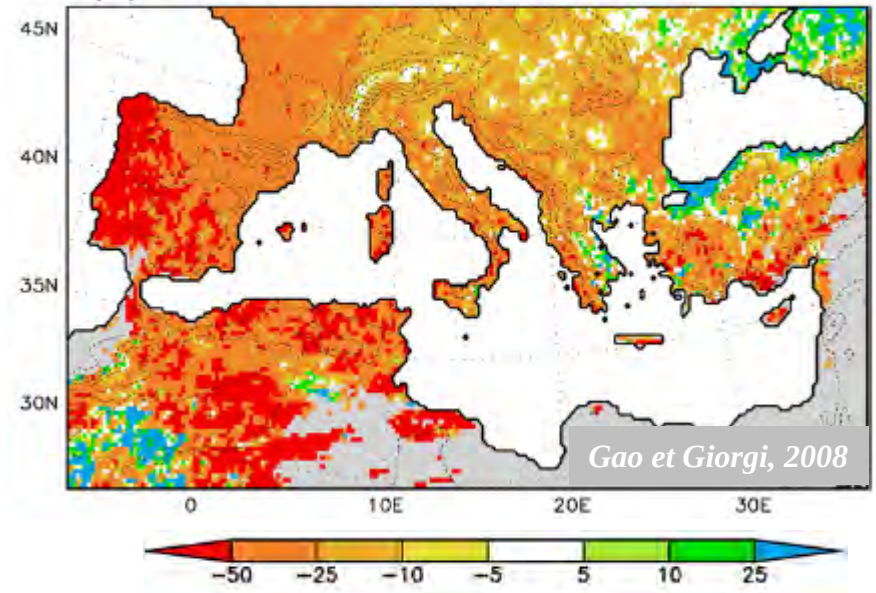
Why? Climate change etc...

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Aiguo 2010

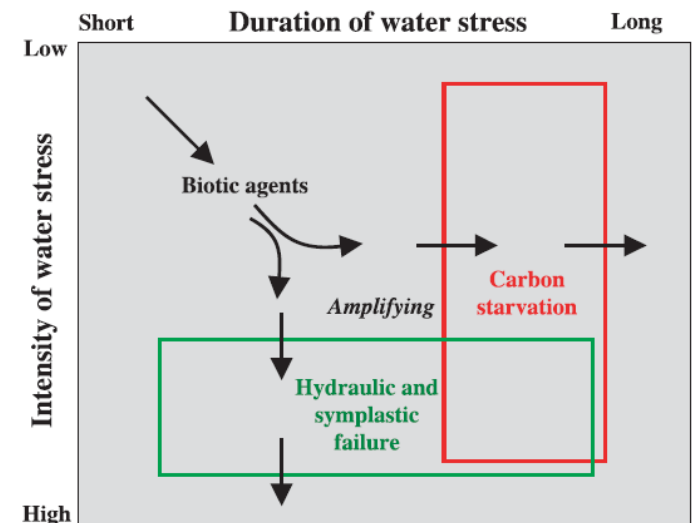
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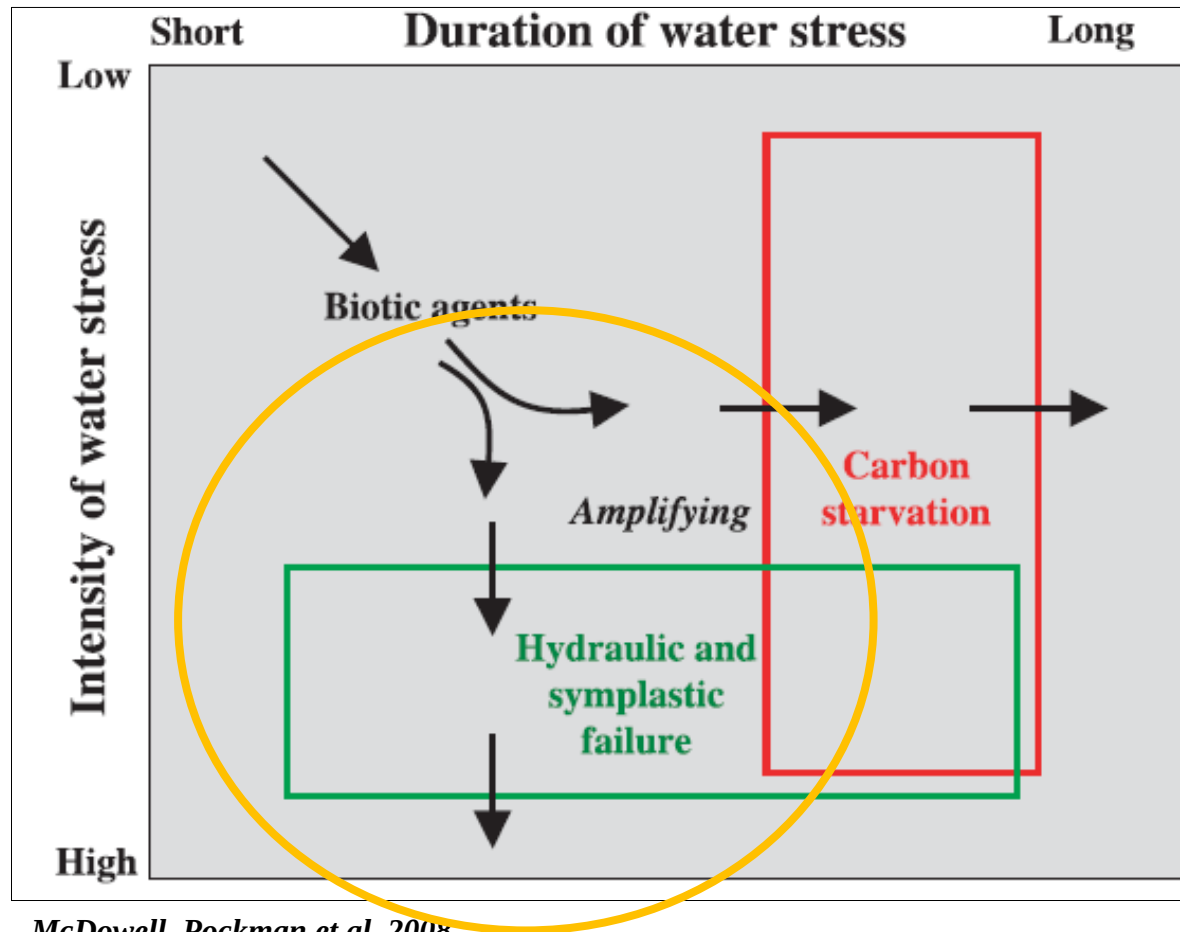
- How pertinent traits will vary with adverse conditions?

- Widespread Mediterranean species : *Quercus ilex*



McDowell, Pockman et al, 2008

Vulnerability to drought and pertinent traits



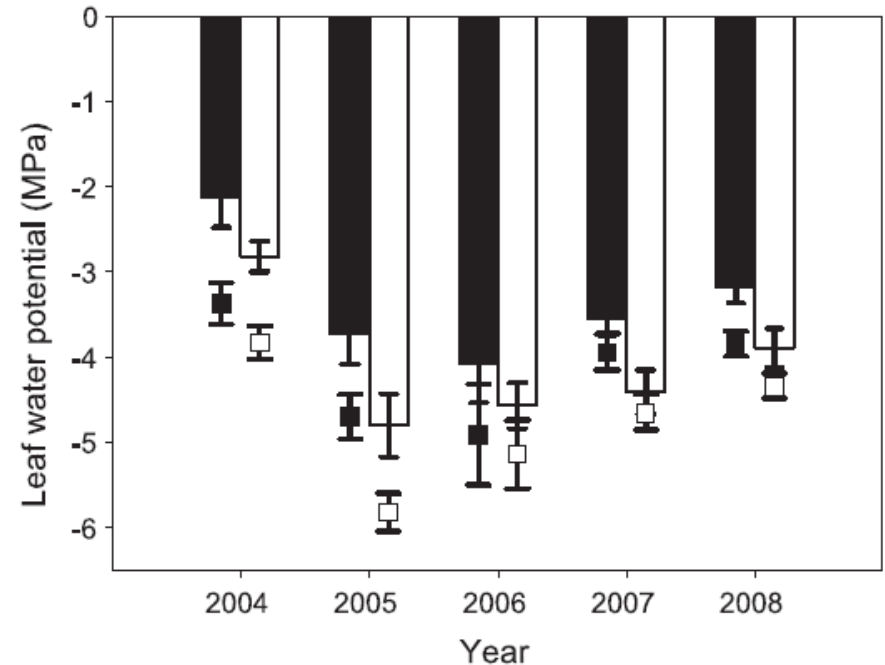
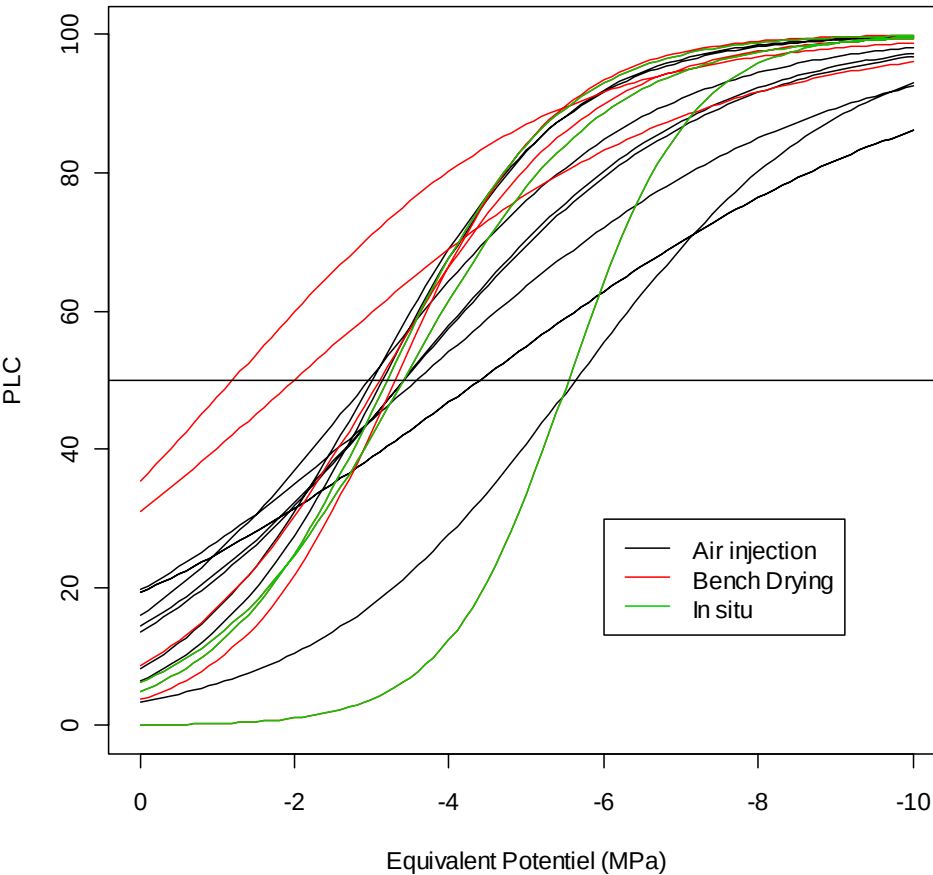
Traits related to vulnerability to cavitation are of growing interests

How reliable are their measures ? on *Quercus ilex*...

A brief littérature survey and some basics rules

Discrepancies between between field measurement and laboratory analysis

Biblio Qilex



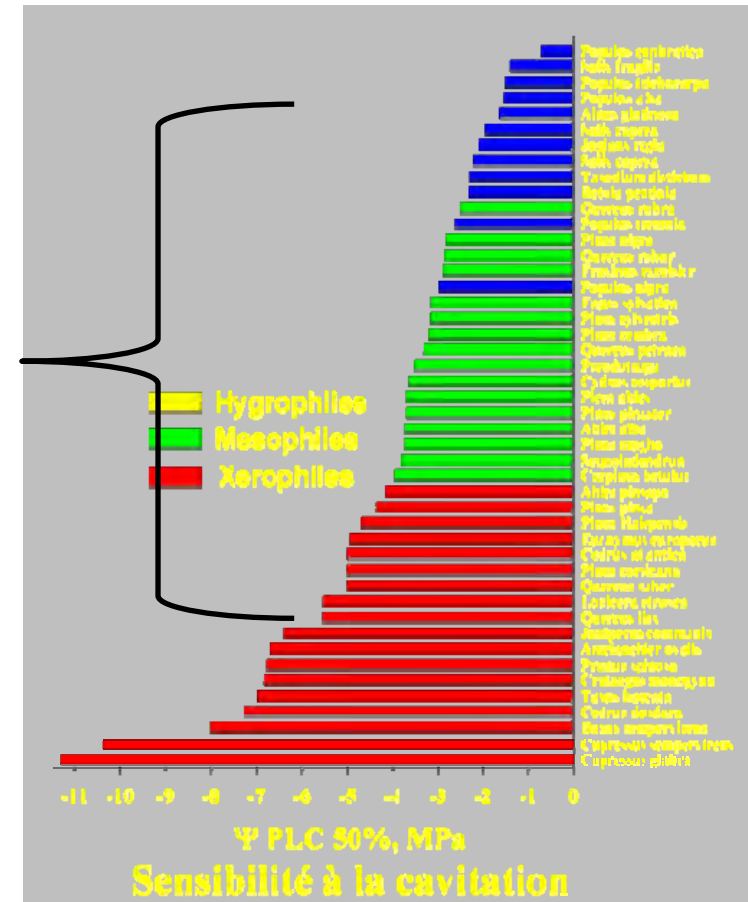
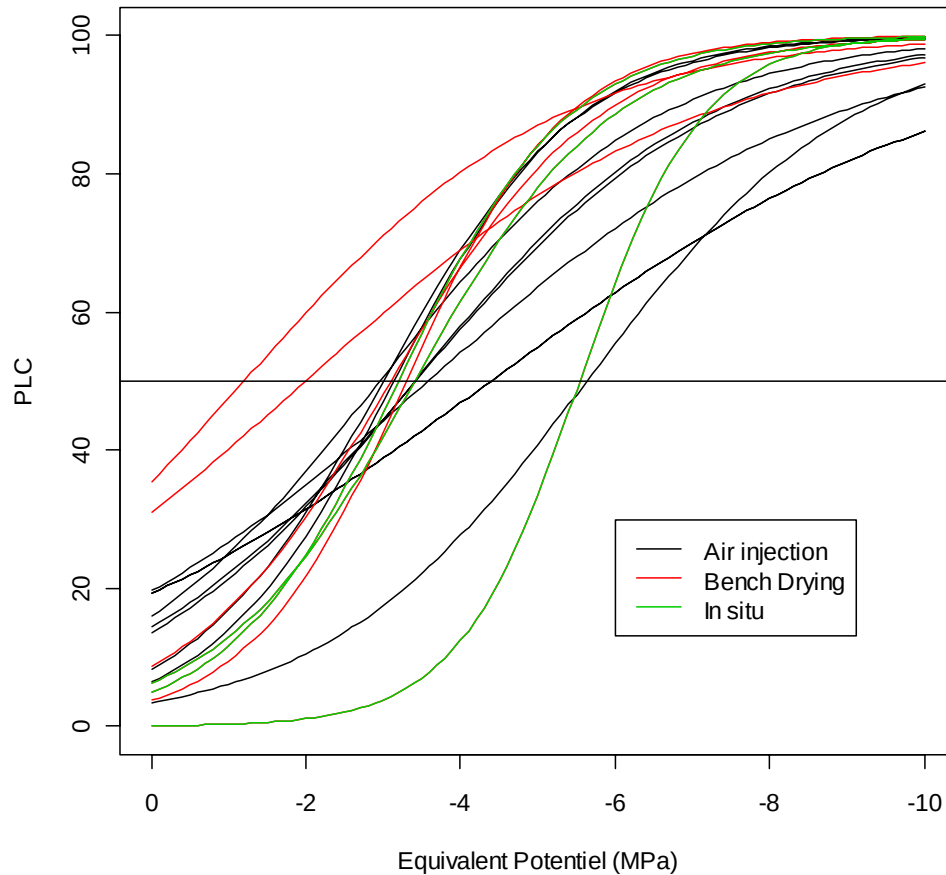
Psi min = - 4.50 (Mpa)

Tyree & Cochard 1996 : [...]a correlation between vulnerability to cavitation and other traits of drought resistance holds except for where *Q.ilex* [...]

A brief littérature survey and some basics rules

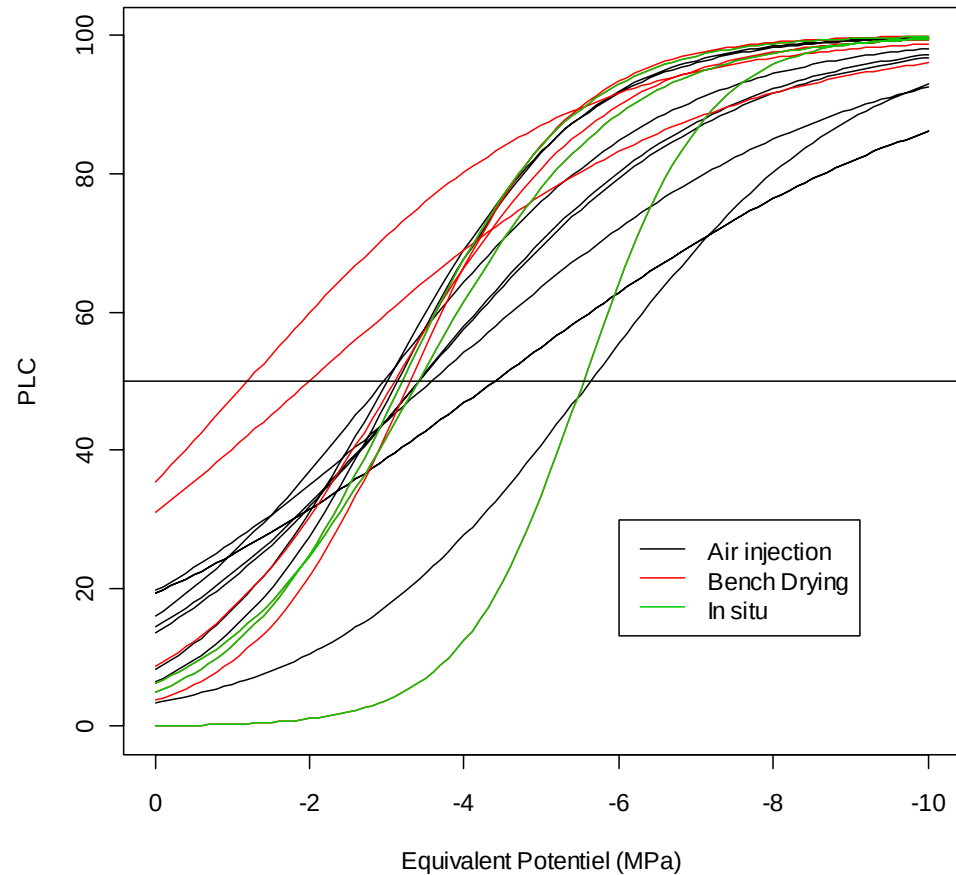
Quercus ilex : Démon darwinien?

Biblio Qilex

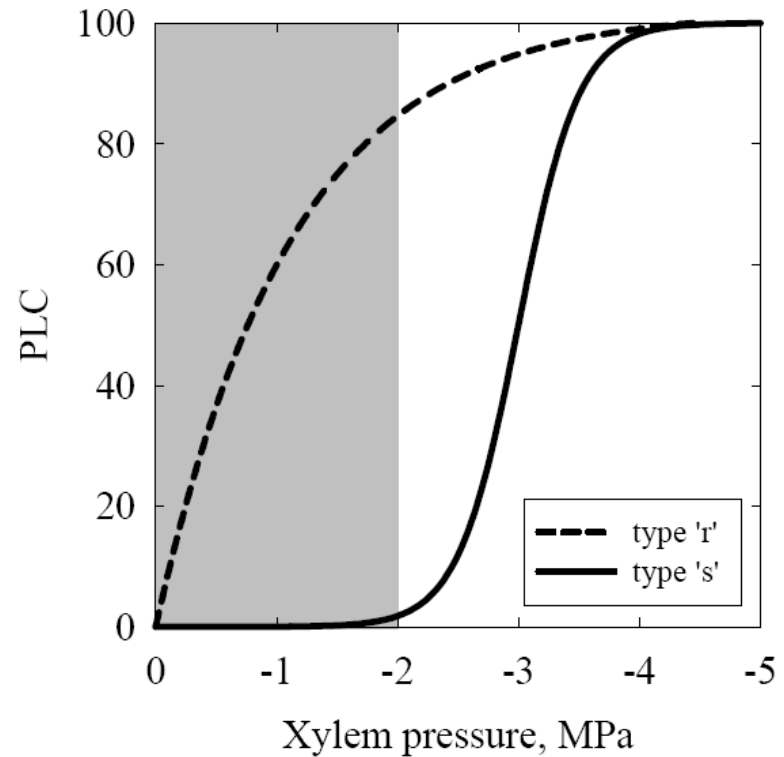


Sample length problems?

Biblio Qilex



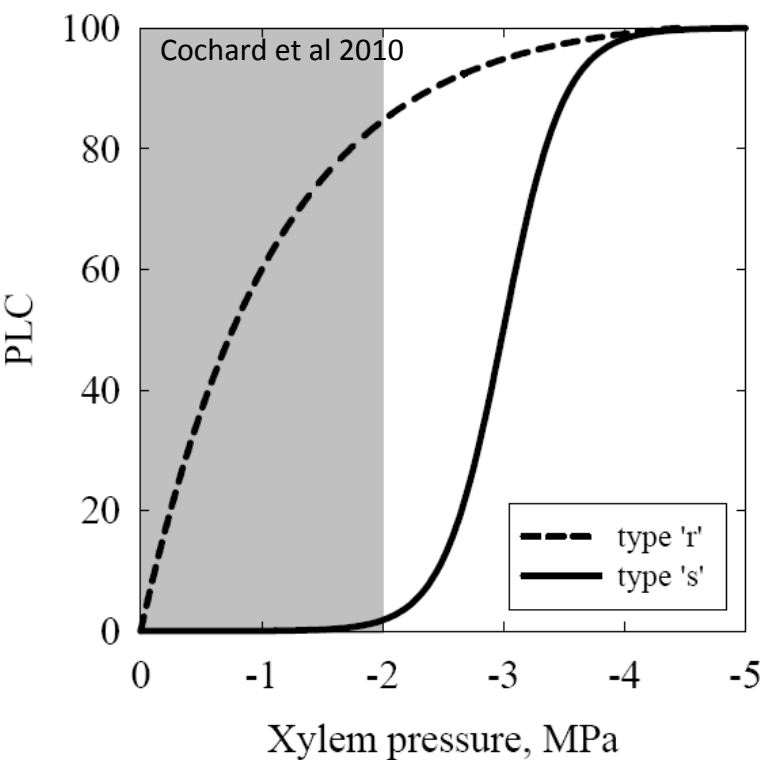
?



Cochard et al 2010

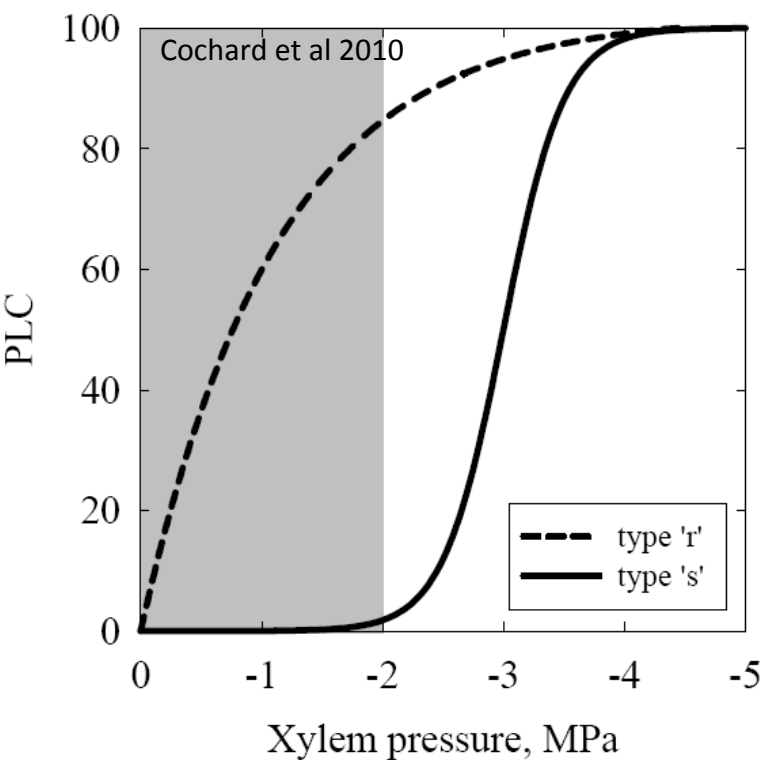
Maximal vessel length > 1.5 m

Sample length problems?



P50 Cochard <i>et al.</i> 2005	Ring porous	<i>Fraxinus excelsior</i>	-2.86 ± 0.67	-3.84 ± 2.27
		<i>Quercus ilex</i>	-5.52 ± 0.96	-4.13 ± 7.45
		<i>Quercus robur</i>	-2.83 ± 0.08	-2.08 ± 0.67
		<i>Vitis vinifera</i>	-	-0.75 ± 0.19
		<i>Celtis australis</i>	-	-1.65 ± 0.54

Sample length problems?

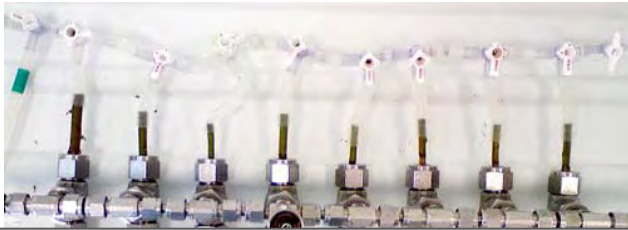


Obvious with the CAVITRON

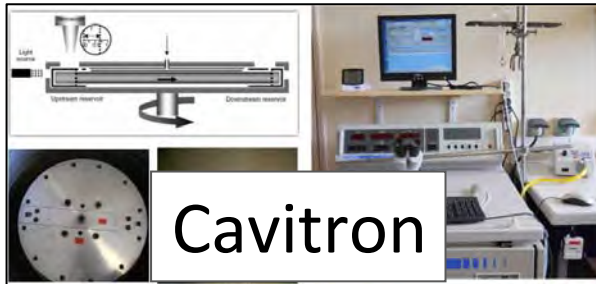
What about other methods?

Ring porous P50 Cochard <i>et al.</i> 2005	<i>Fraxinus excelsior</i>	-2.86 ± 0.67	-3.84 ± 2.27
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	<i>Celtis australis</i>	–	-1.65 ± 0.54

Sample length problems? *Methods comparison*



Air injection method



Cavitron



Reference air
drying methods

How consistent with :

1/ Minimum water potential value -4.50 MPa

2/ The R/S shaped curves

Sample length problems? *Methods comparison*



Stems

Bench Drying reference methods :

>2 meters long stems

New apical shoots (current year)



Resprouts : few water stressed

Bench Drying reference methods :

>2 meters long stems

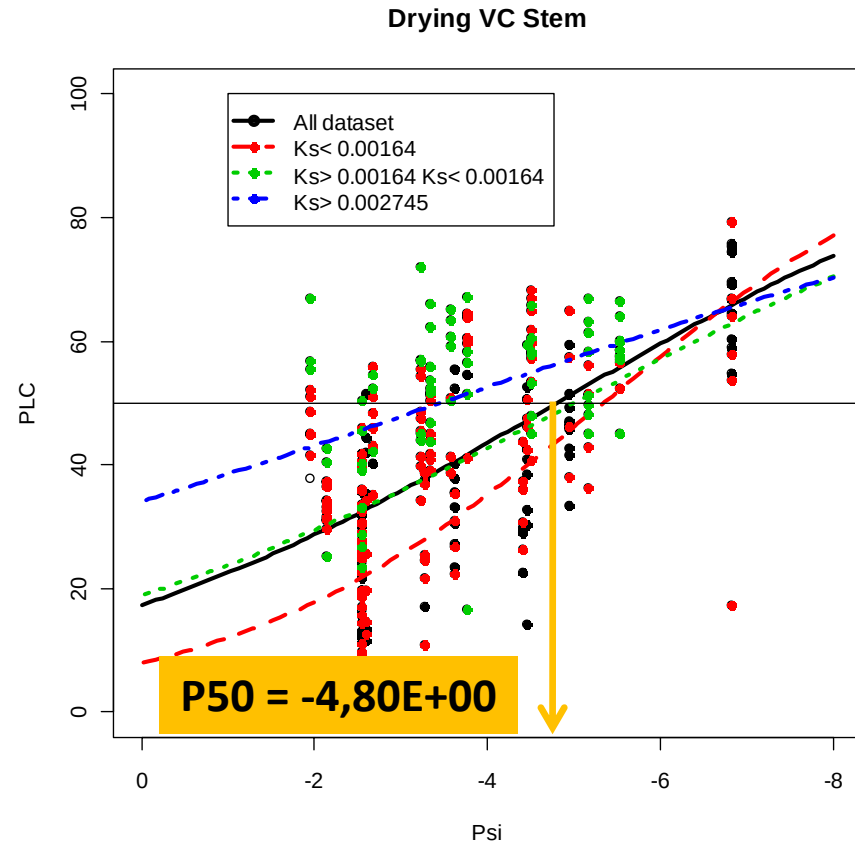
CAVITRON

28 cm

Double ended pressurisation method

15 to 25 cm

Methods comparison : Dehydration reference method **Stems**



Not very « S », very noisy:

- ? Interaction between water stress and phenology & growth
- High native embolism
- However, P50 consistent

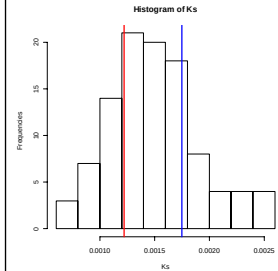
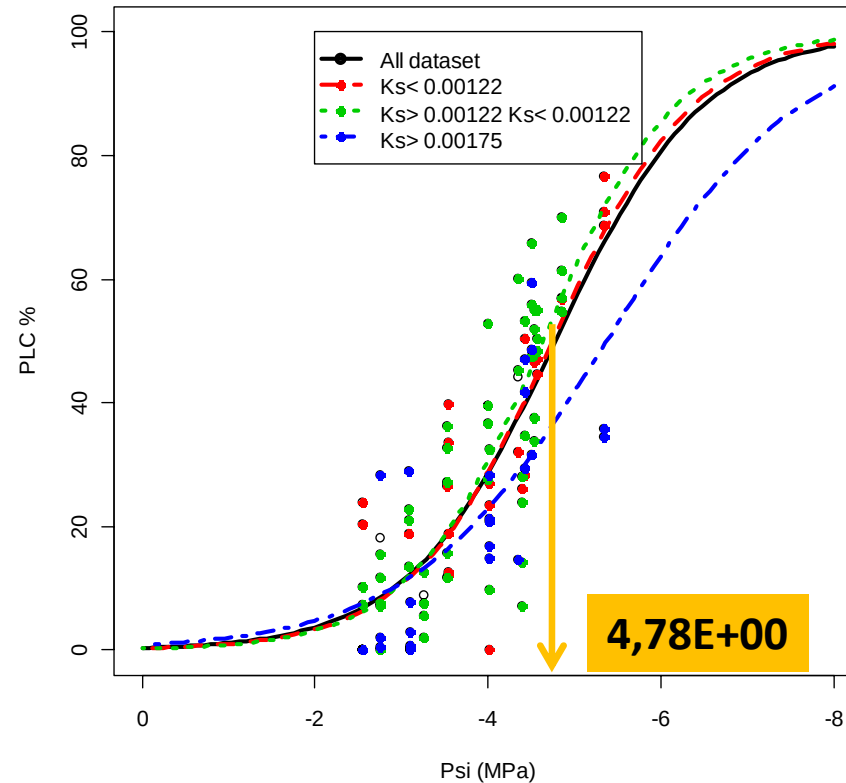
Methods comparison : Dehydration reference method **resprouts**



Few native embolism

$\Psi_{\min}$ during the season = -1.7

VC Resprout Bench Drying

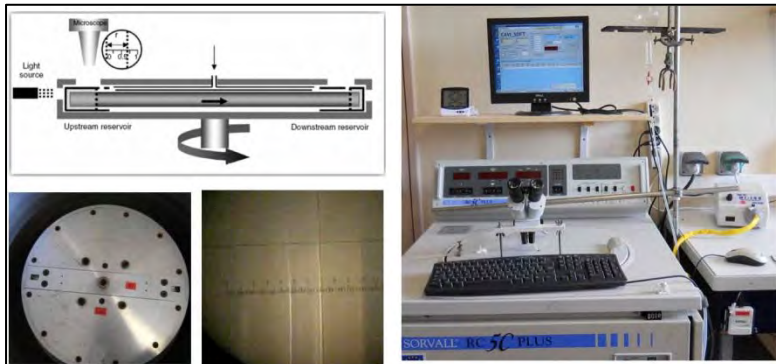


CLEANER !!

P50 in accordance with dehydration method on stems

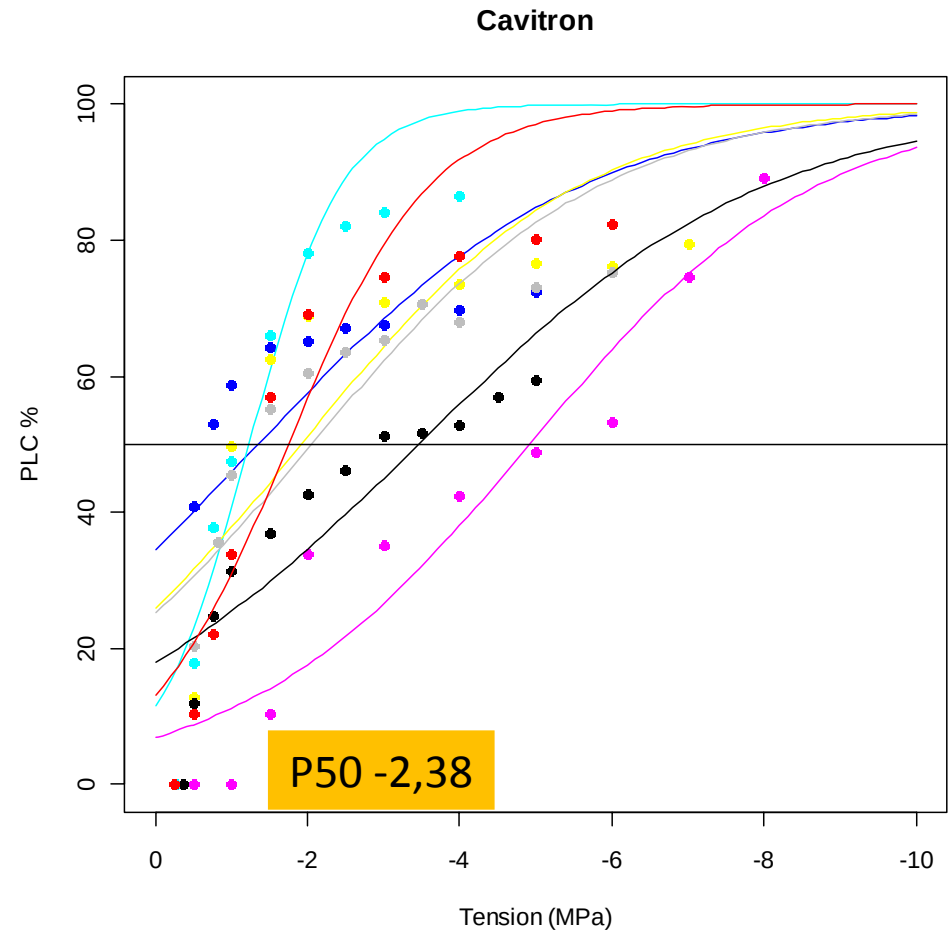
Methods comparison : Cavatron on resprouts

<1 year old resprouts



Methods comparison : Cavitron on resprouts

<1 year old resprouts

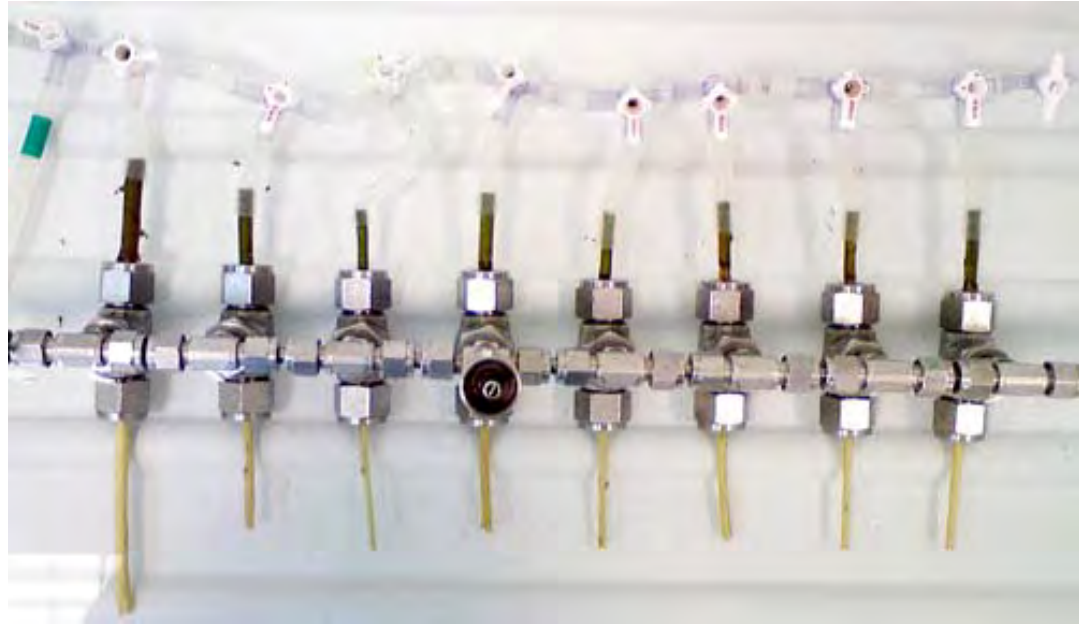


Clearly R shaped curves
Weak P50
OK...

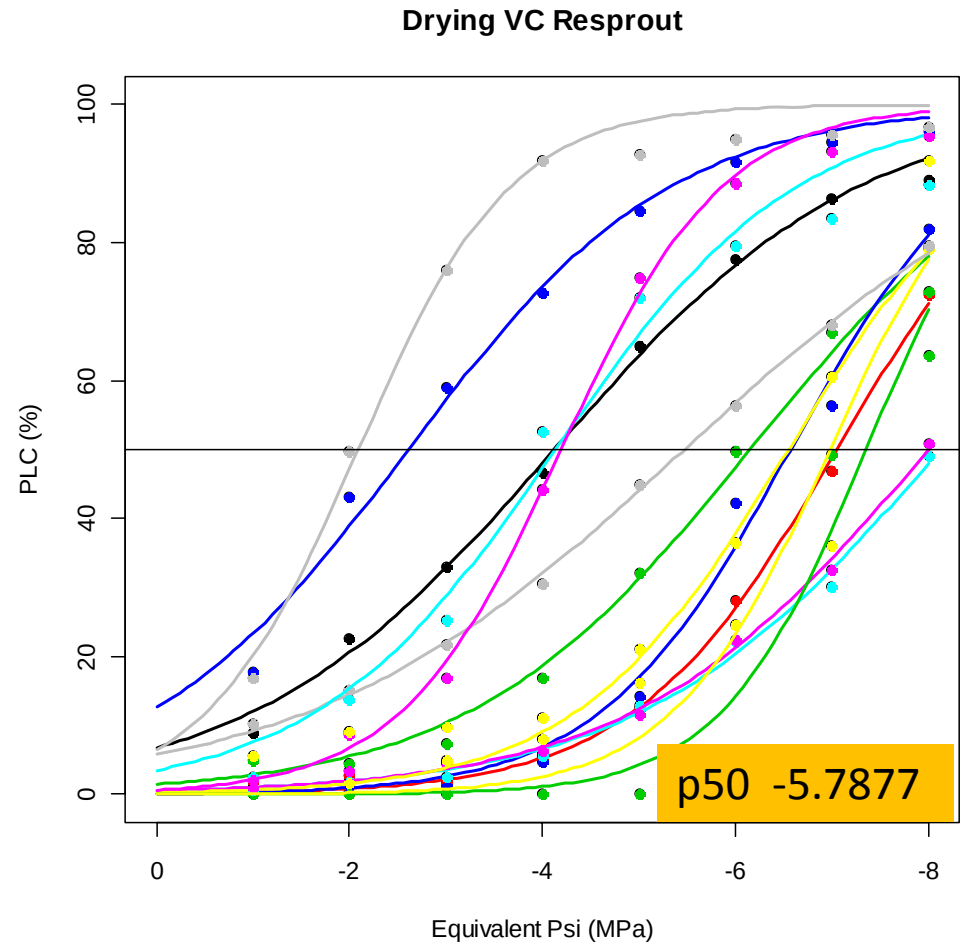
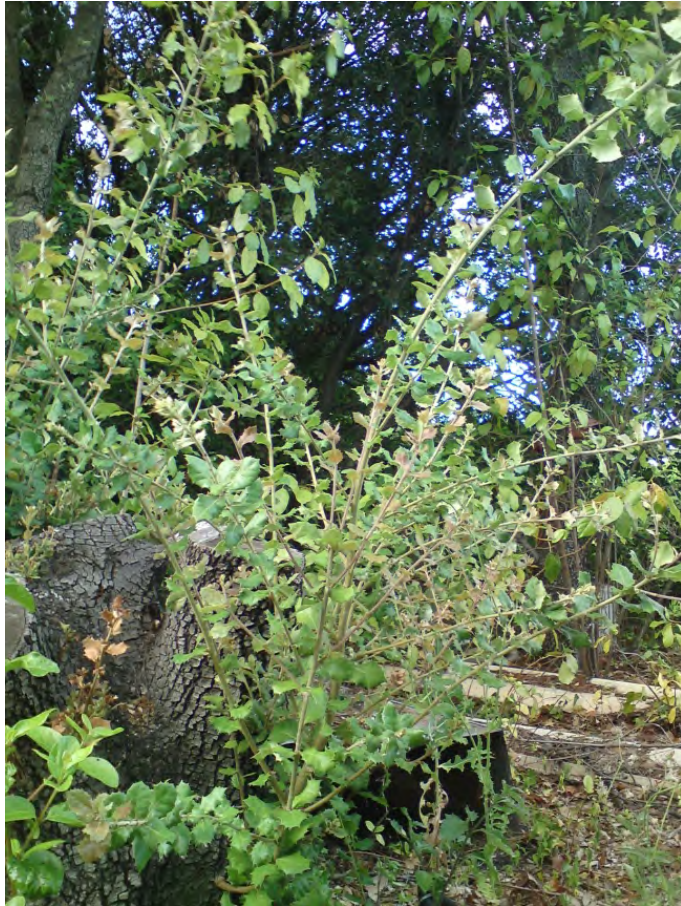
Methods comparison : Air injection on resprouts



Length : 15 & 22 cm
Different diameters



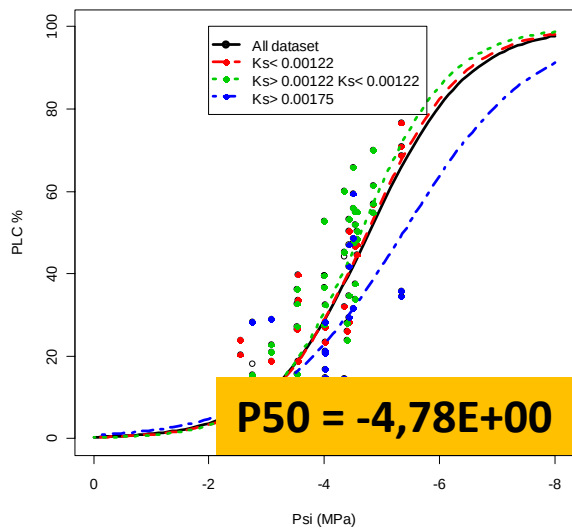
Methods comparison : Air injection on resprouts



Very high variability
Unconsistent with the reference method

Sum up

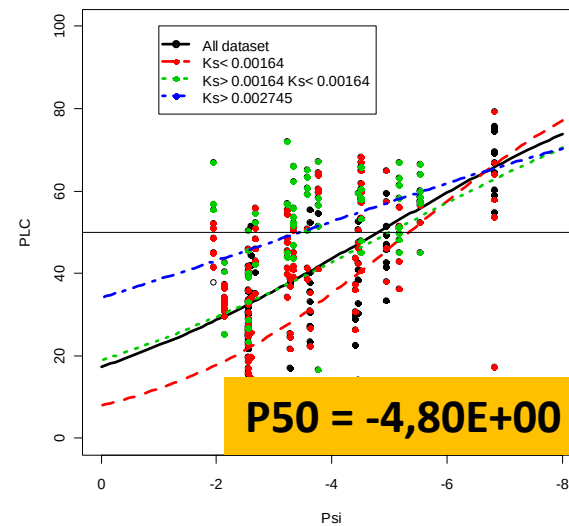
VC Resprout Bench Drying



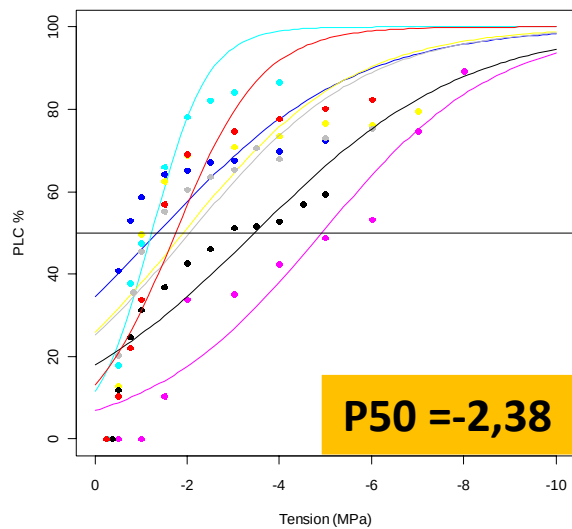
OK

±OK

Drying VC Stem



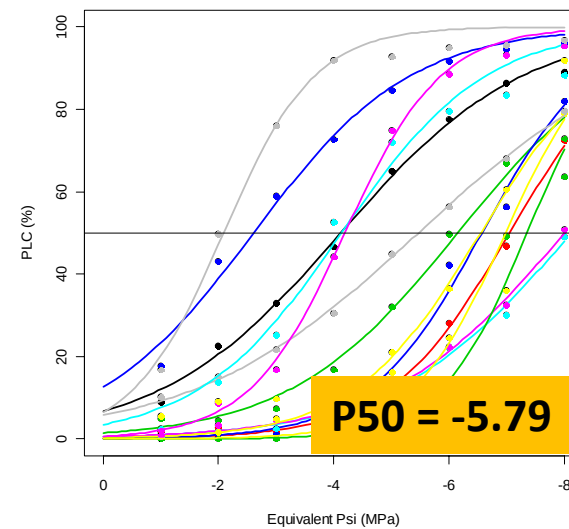
Cavitron



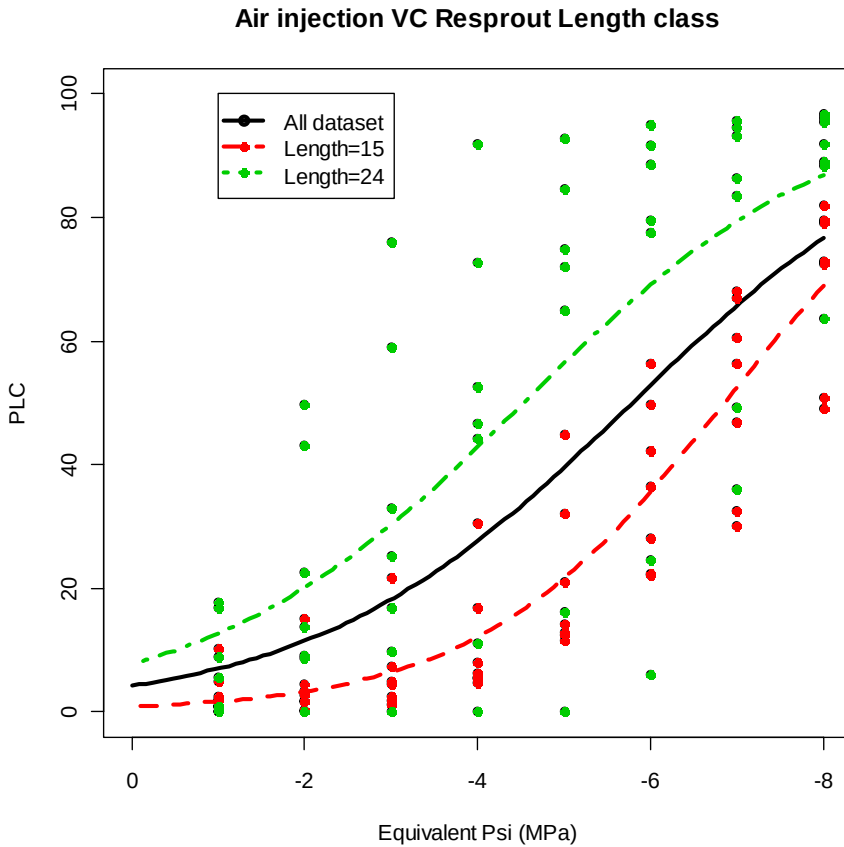
NON

...

Drying VC Resprout

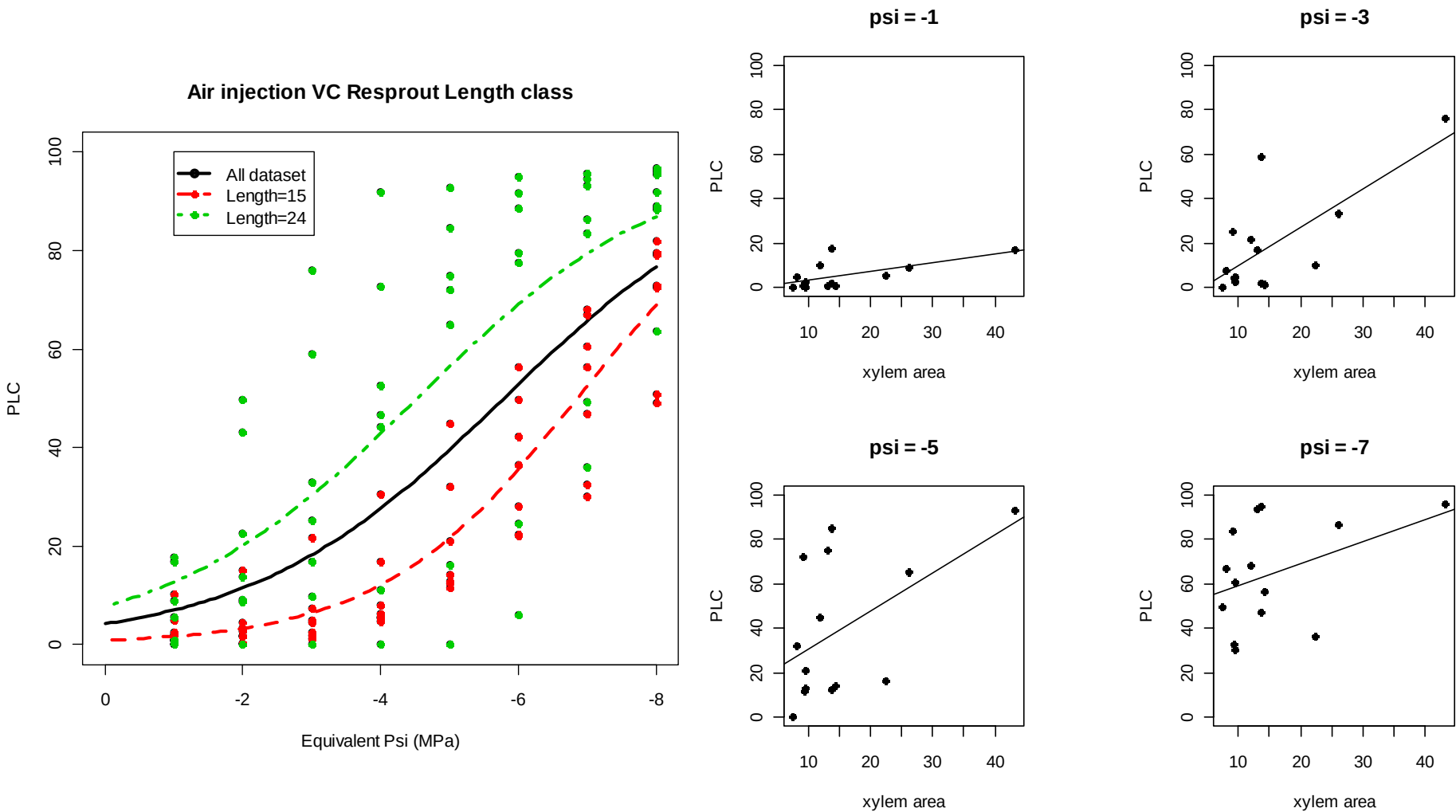


What happened with air injection?

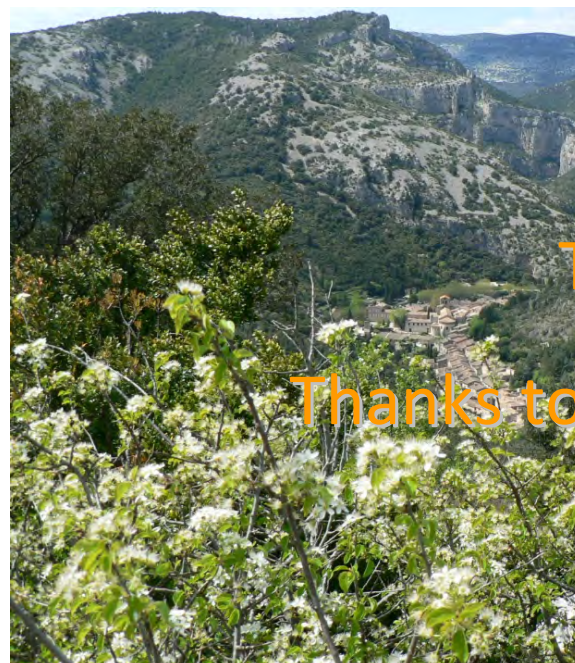
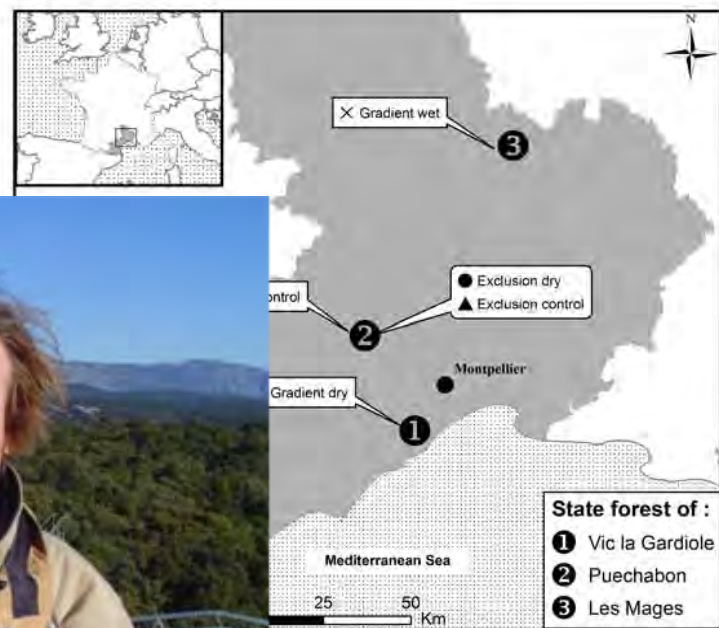


Higher length = More « R shaped» & vulnerable ?

What happened with air injection?



Effect of size distributions



Thanks for your attention
Thanks to my surpervisor *Laurent Misson...*

XYLEM GROUP MEETING

Nancy, April 2011

Establishment of hydraulic control of expansion during leaf ontogeny in *Arabidopsis thaliana*

Florent PANTIN¹

François BARBIER¹

André LACOINTE³

Geneviève CONÉJÉRO^{2,4}

Colette TOURNAIRE-ROUX²

Christophe MAUREL²

Bertrand MULLER¹

Thierry SIMONNEAU¹

Technical support

Crispulo BALSERA¹

Myriam DAUZAT¹

Gaëlle ROLLAND¹

1



2



3



4





Hydraulic control of leaf growth

- > Leaf expansion: a major component of plant performance

- > A key role for hydraulics in the control of growth
 - ✓ Plant hydraulic properties are coordinated with relative growth rates, gas exchanges, and species evolution [SACK & HOLBROOK, 2006; McKOWN et al., 2010]

 - ✓ Growth is among the first processes affected by water stress [HSIAO, 1973]

 - ✓ Cell growth is driven by water relations [LOCKHART, 1965; ORTEGA, 1985]

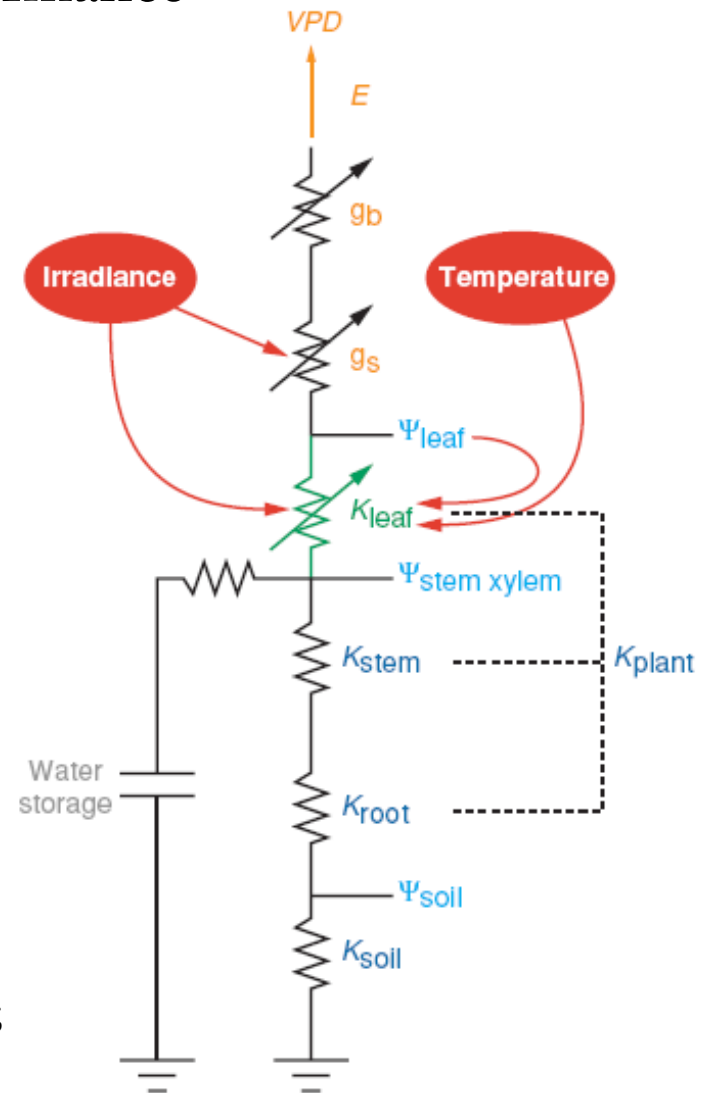
 - ✓ This holds true at the organ level [e.g. BOYER, 1968; BEN HAJ SALAH & TARDIEU, 1997; BOYER & SILK, 2004; BOUCHABKE et al., 2006; ORTEGA, 2010]

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Hydraulic control of leaf growth

> Growth depressions during the daytime have been attributed to hydraulics in the literature

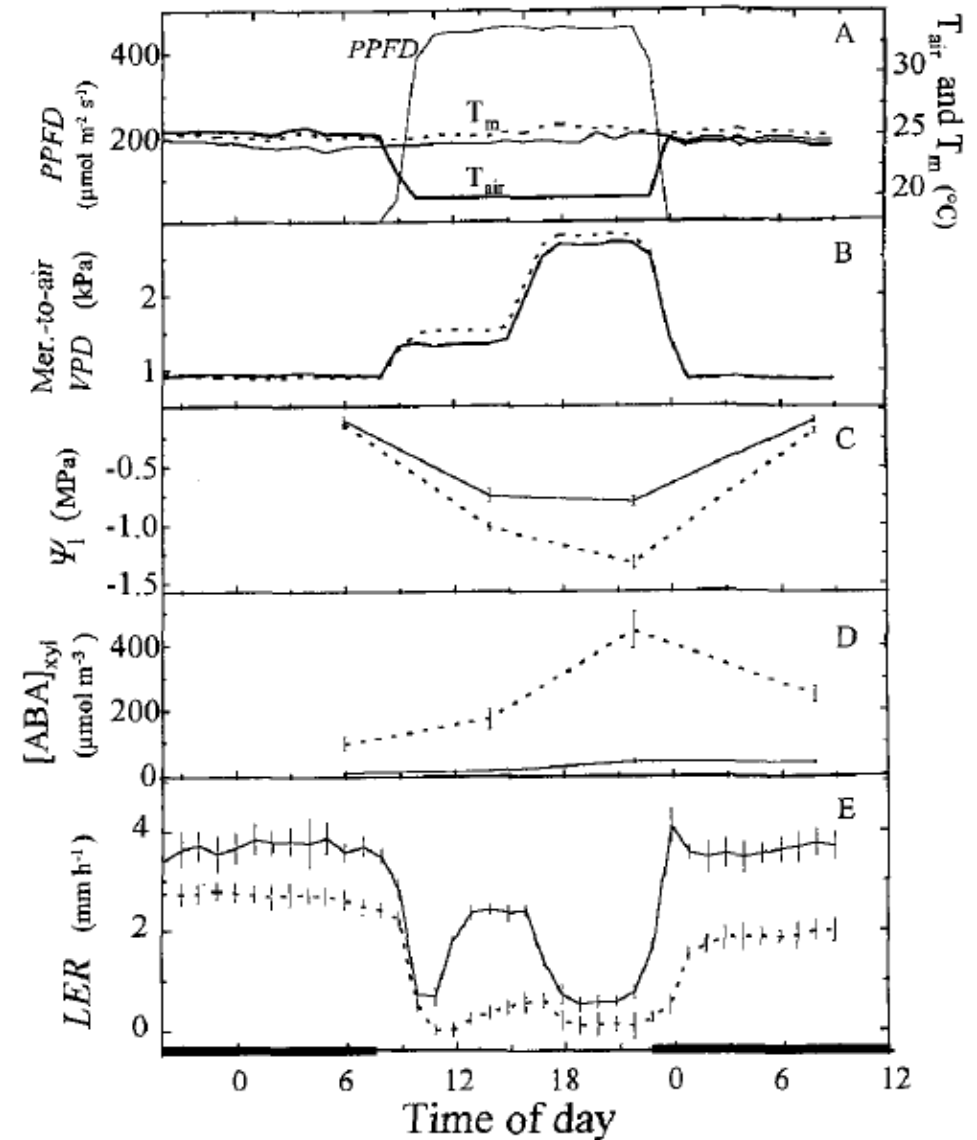
Plant Physiol. (1997) 114: 893–900

Control of Leaf Expansion Rate of Droughted Maize Plants under Fluctuating Evaporative Demand¹

A Superposition of Hydraulic and Chemical Messages?

Halim Ben Haj Salah and François Tardieu*

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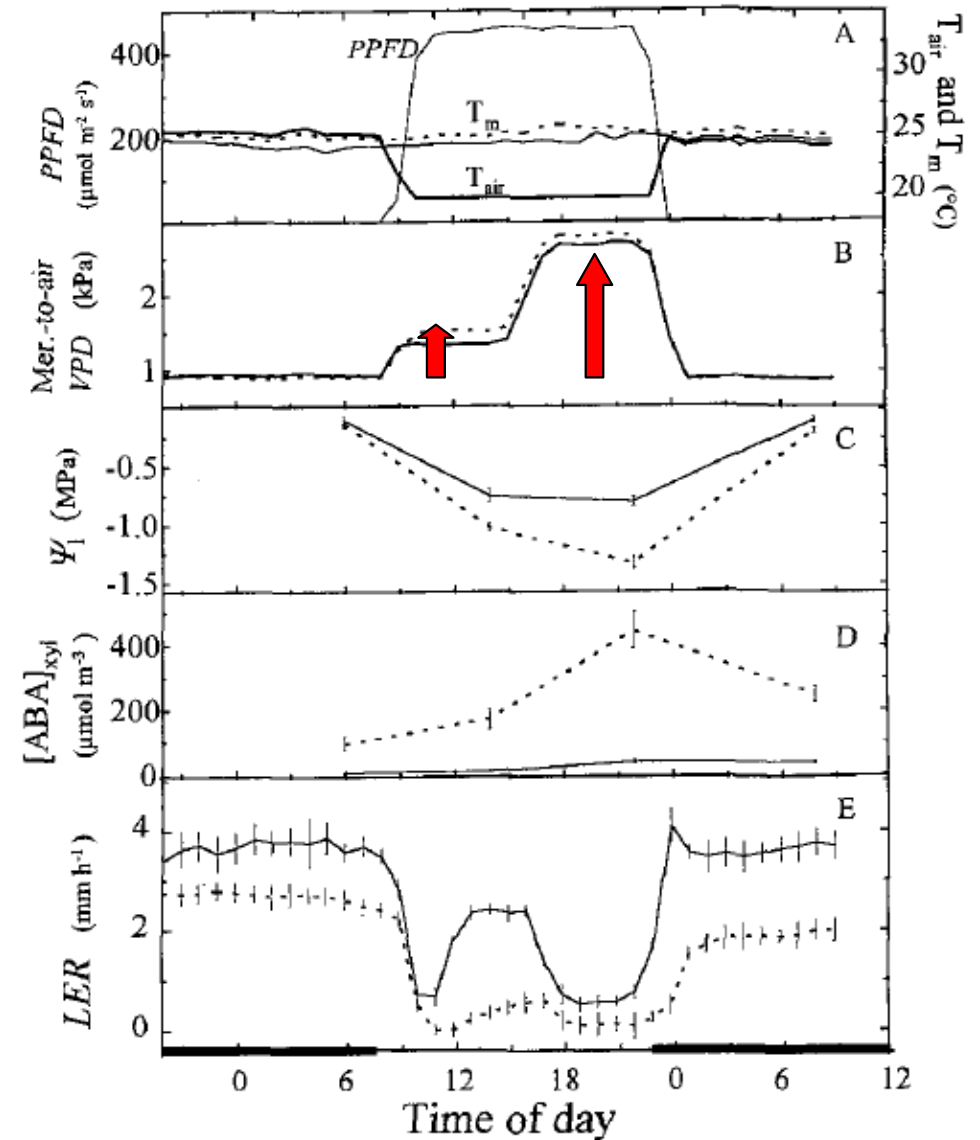
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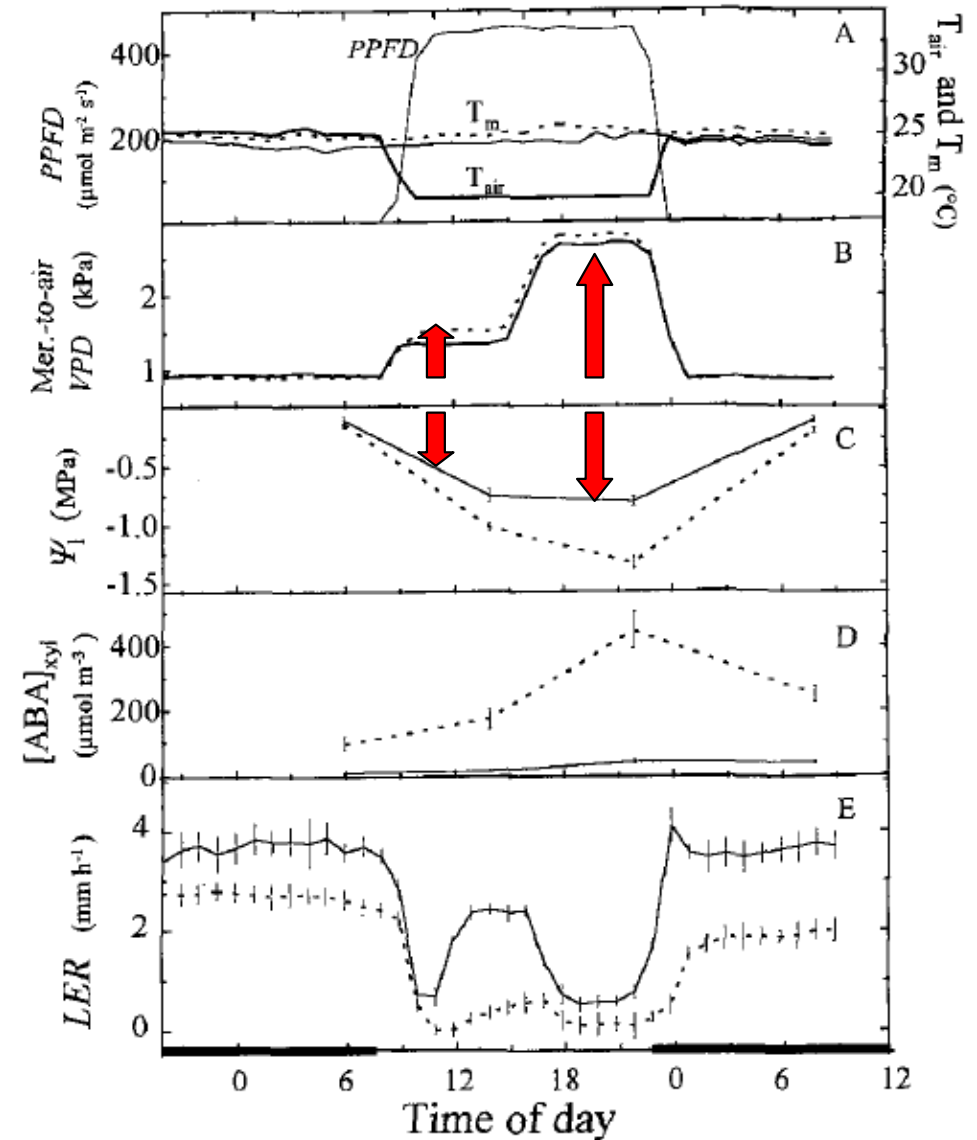
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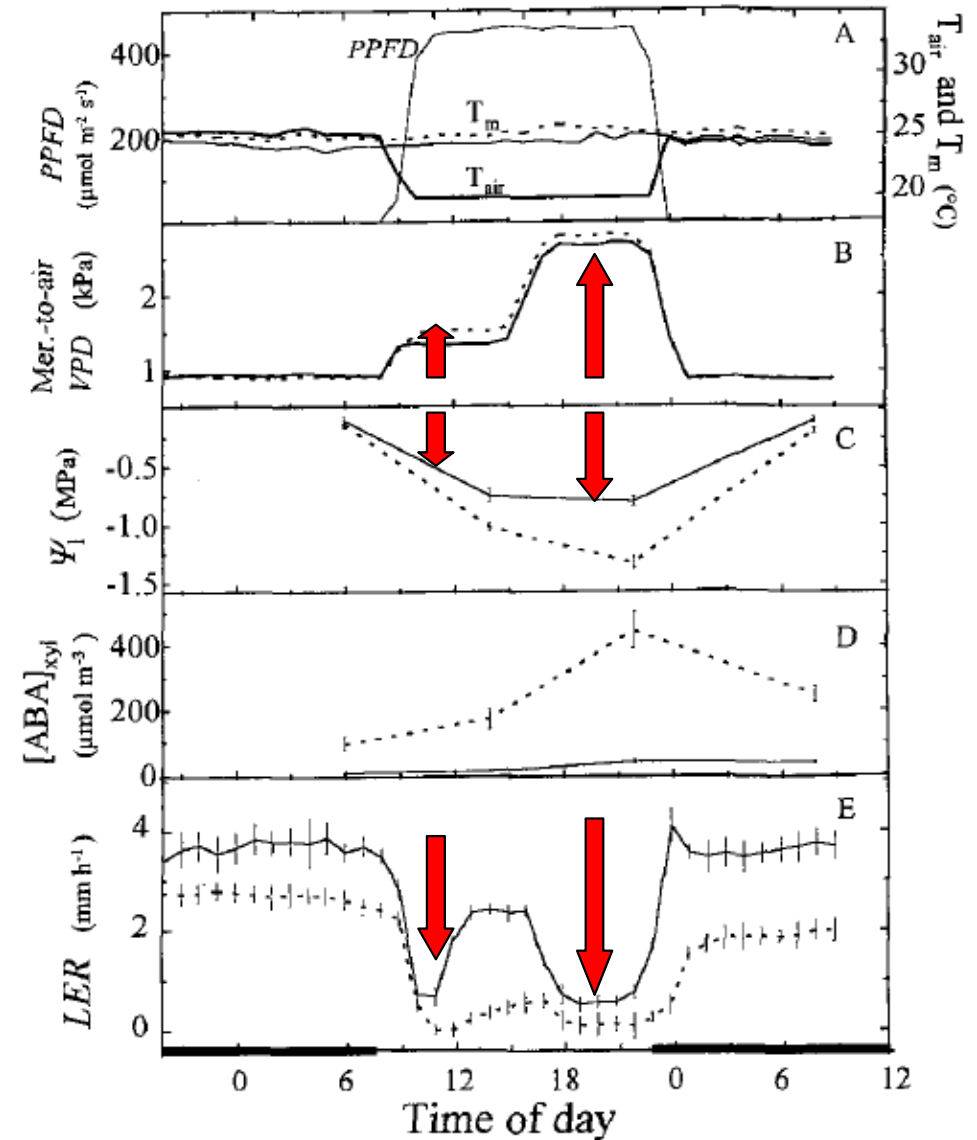
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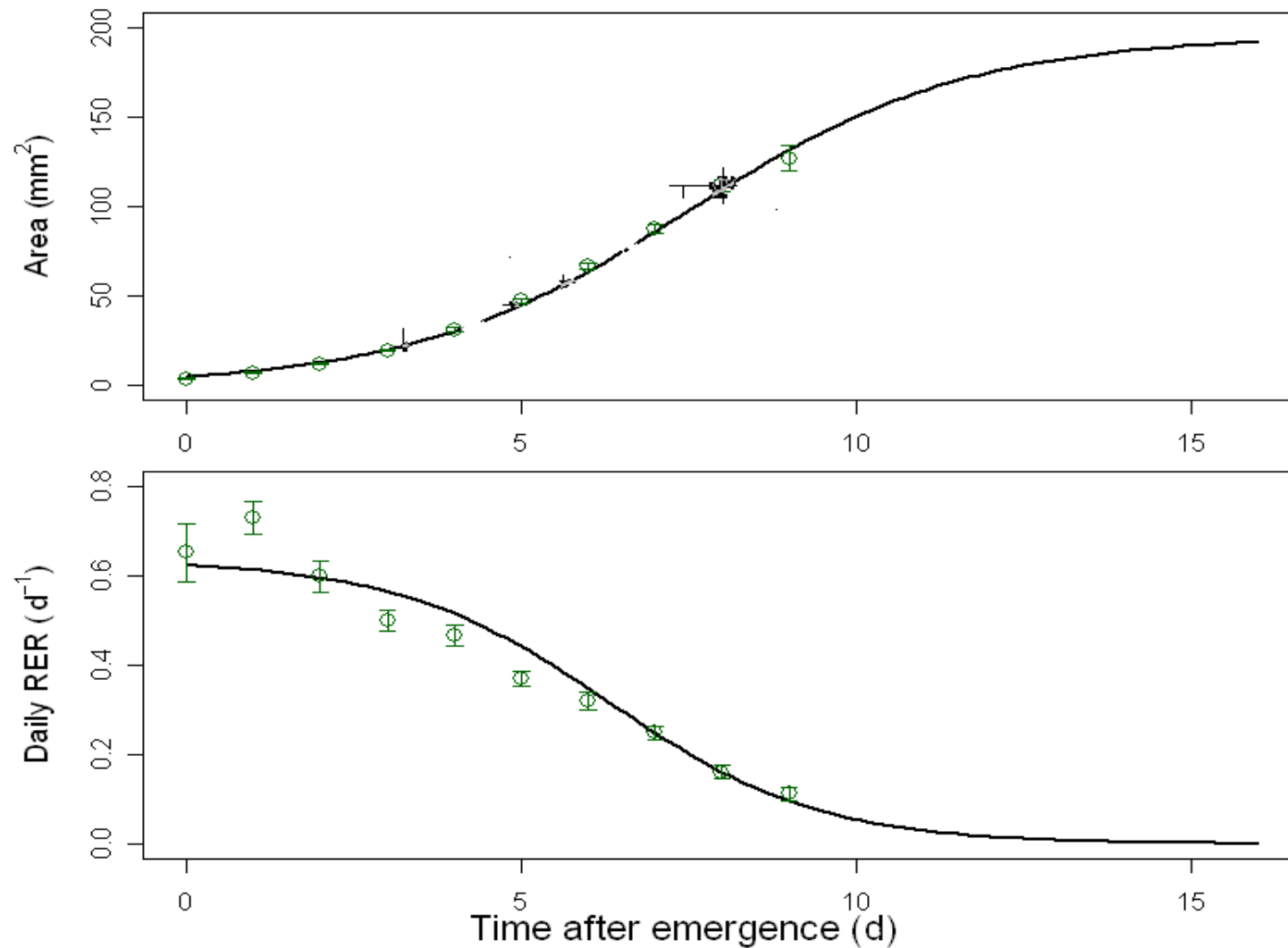
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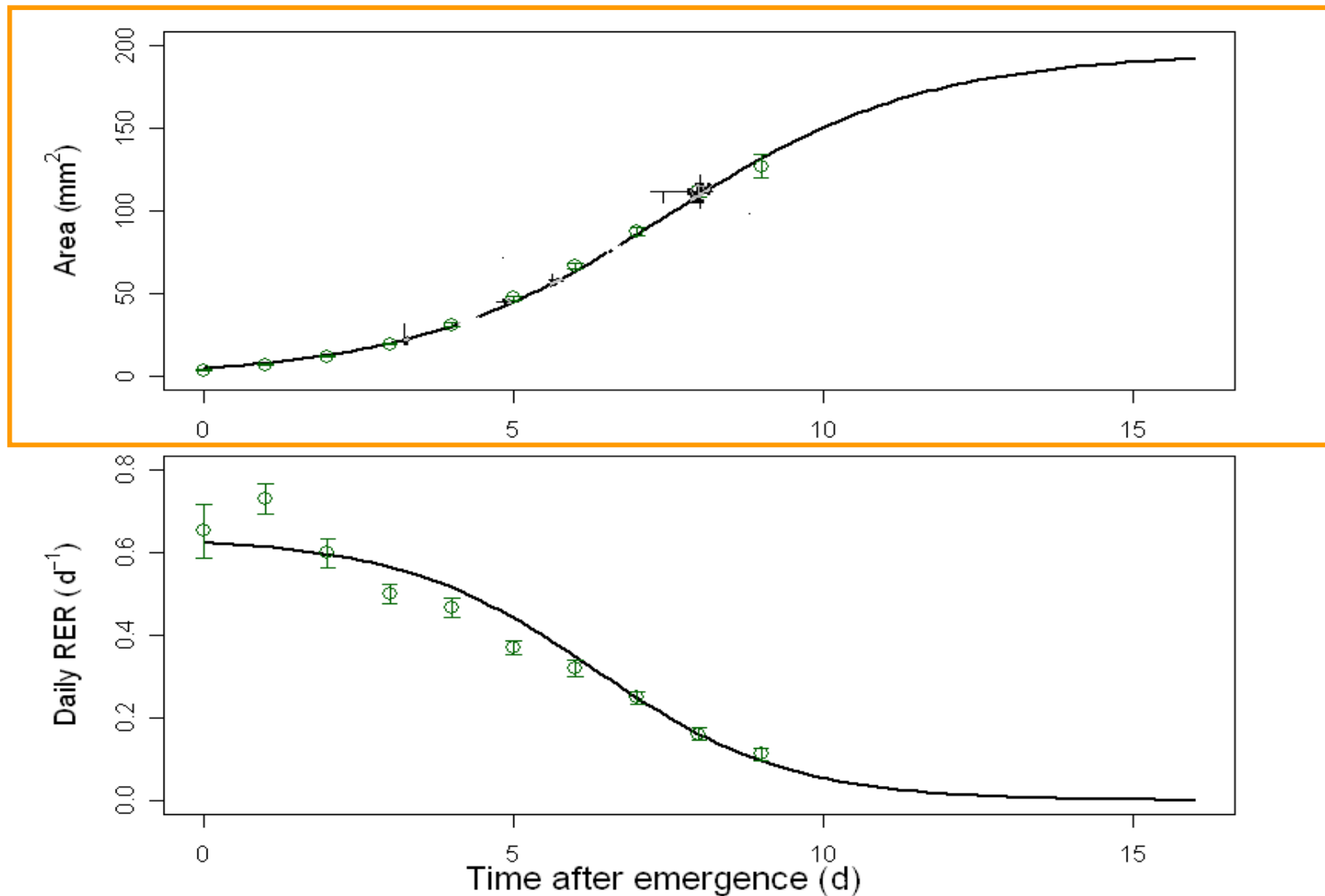
Hydraulic control of leaf growth

> Using **Relative Expansion Rate** to study leaf growth in *Arabidopsis thaliana*



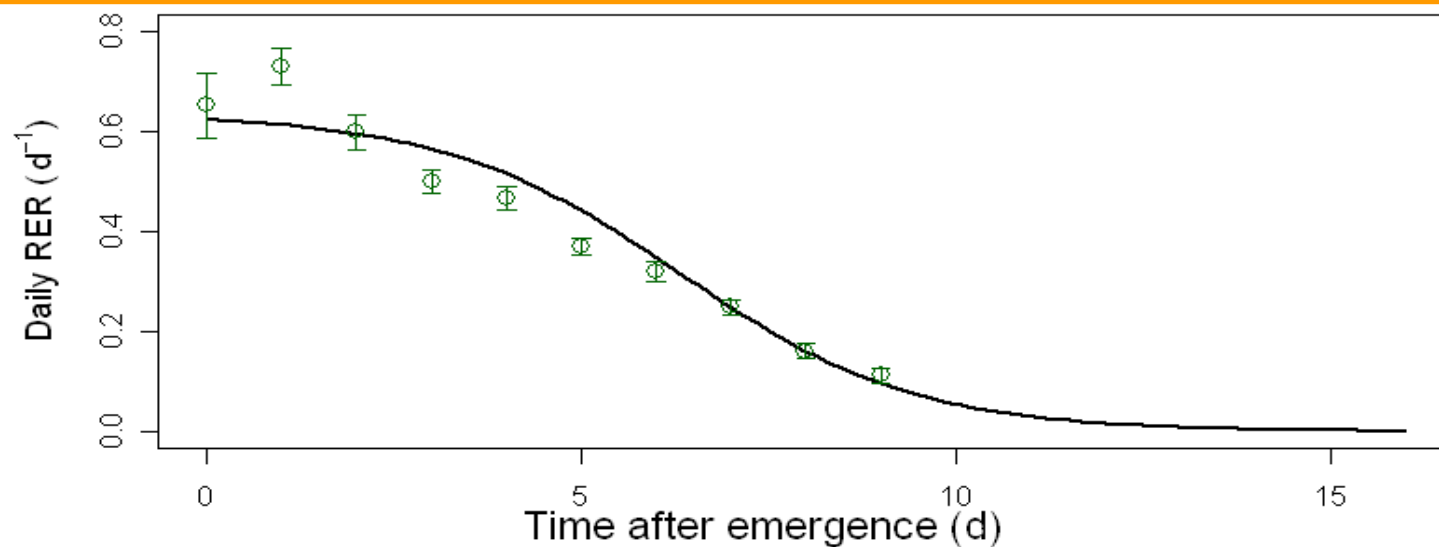
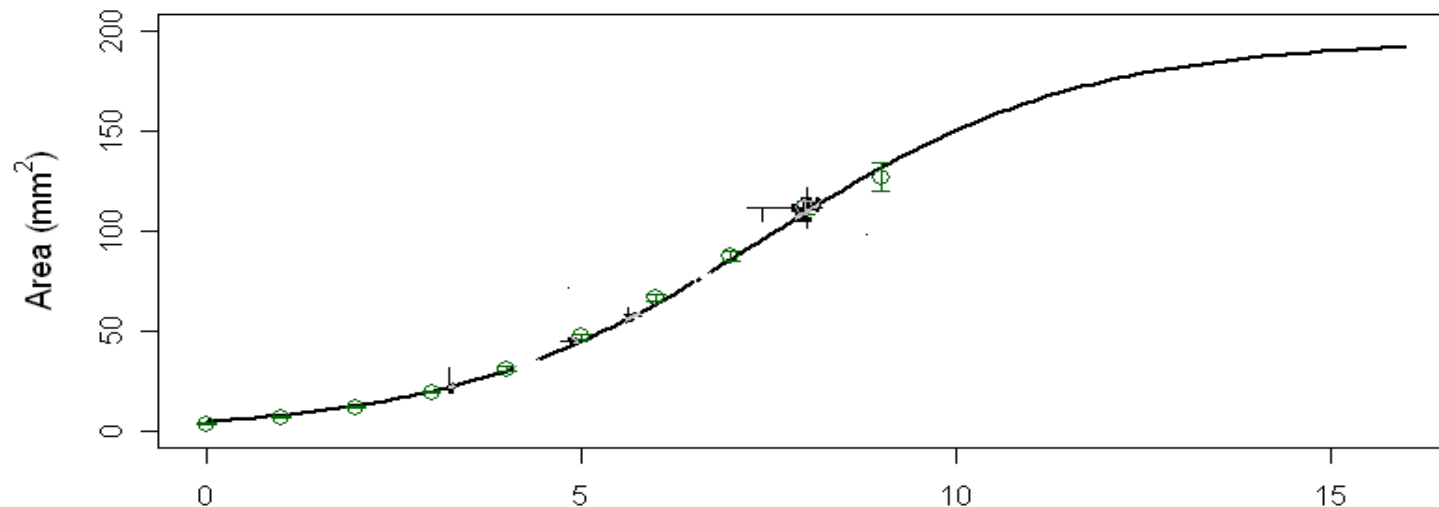
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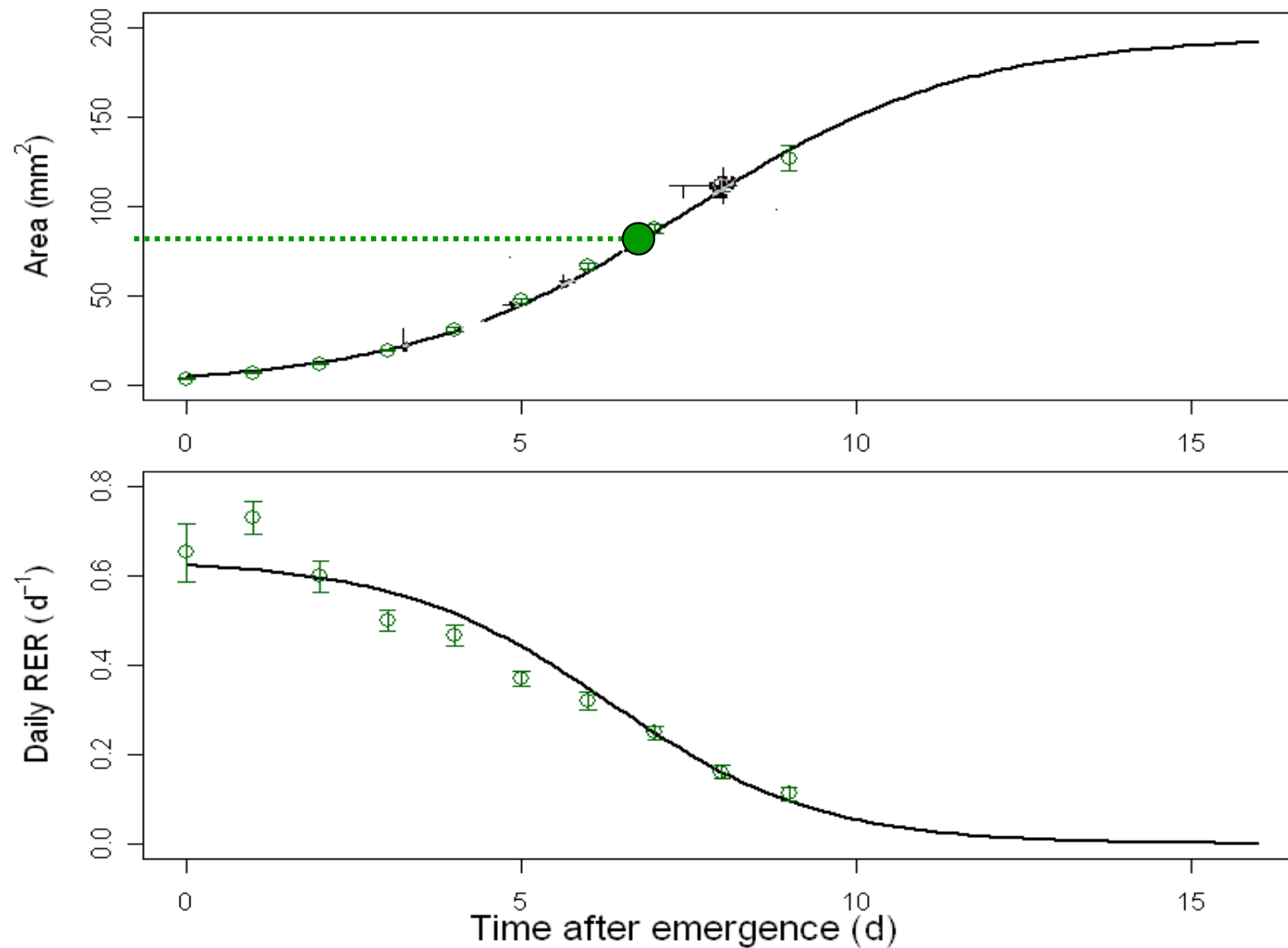
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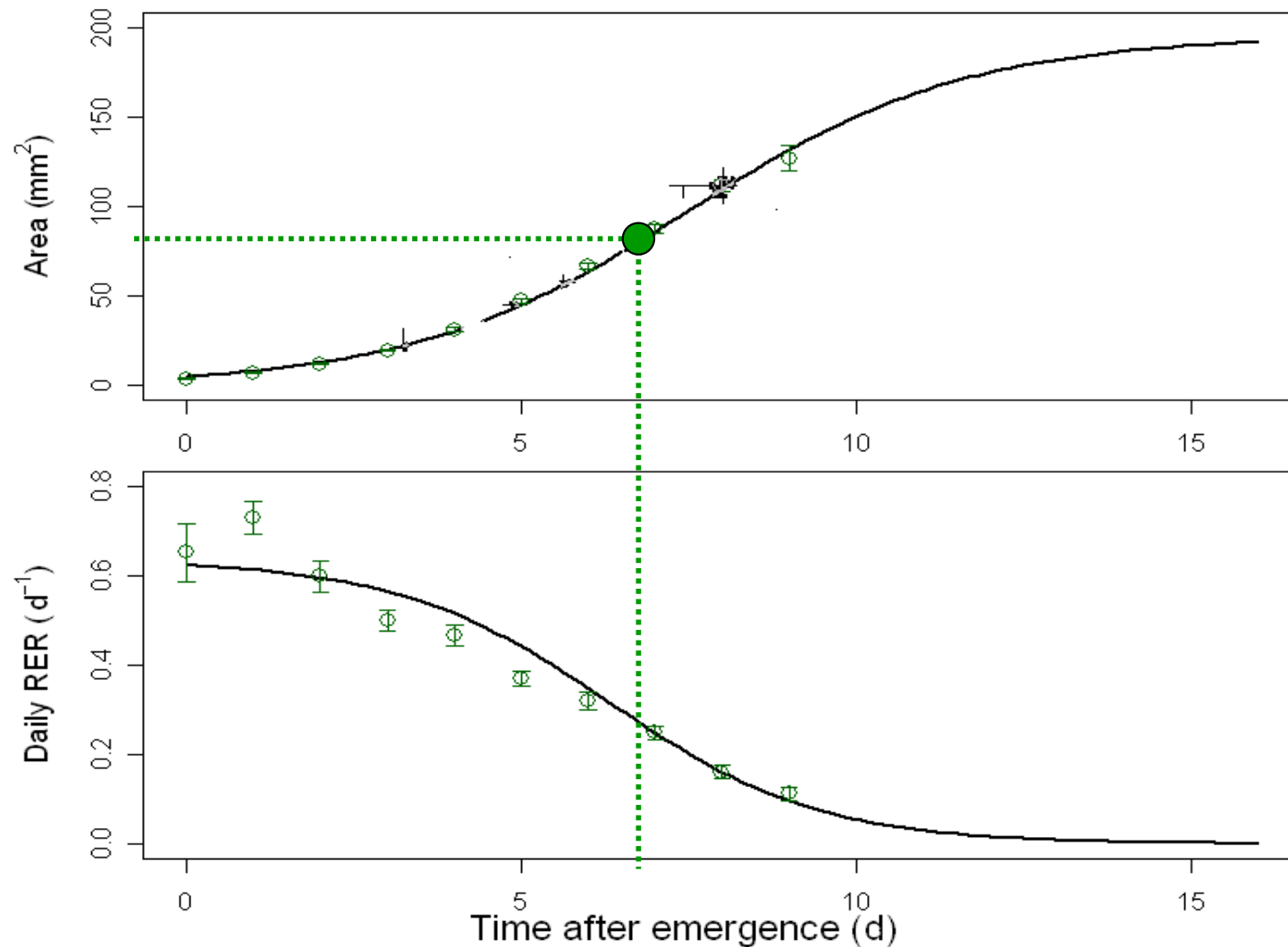
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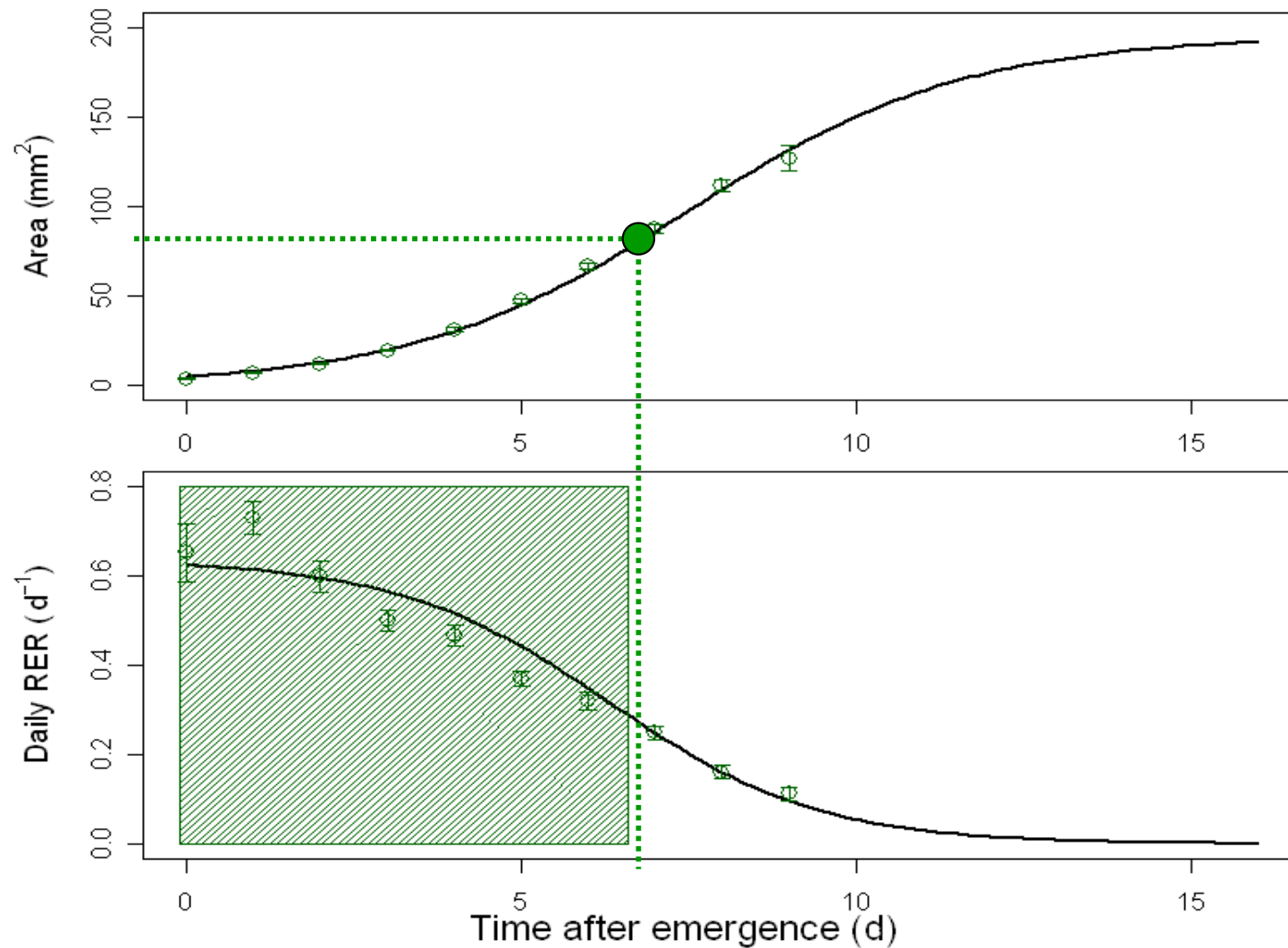
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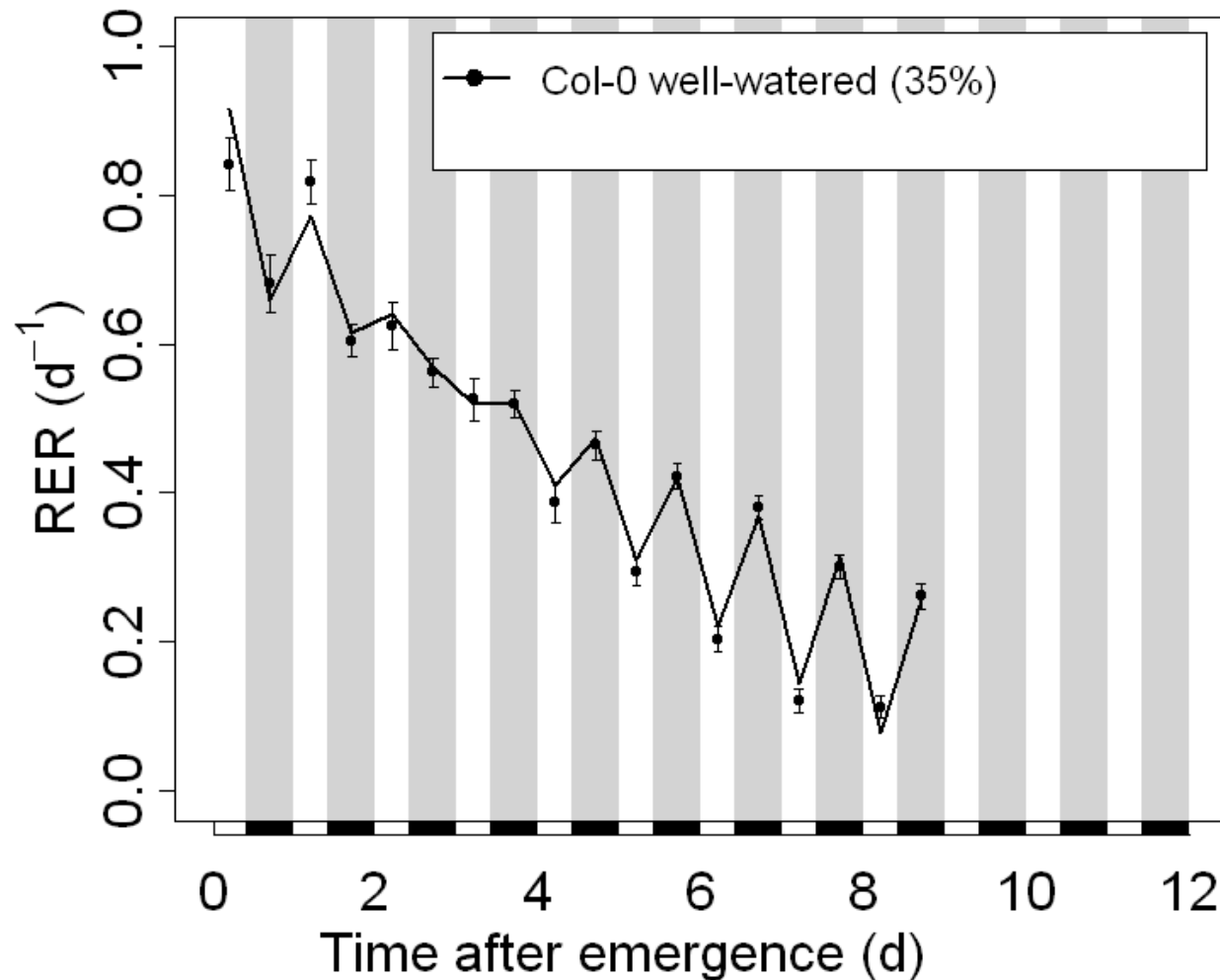
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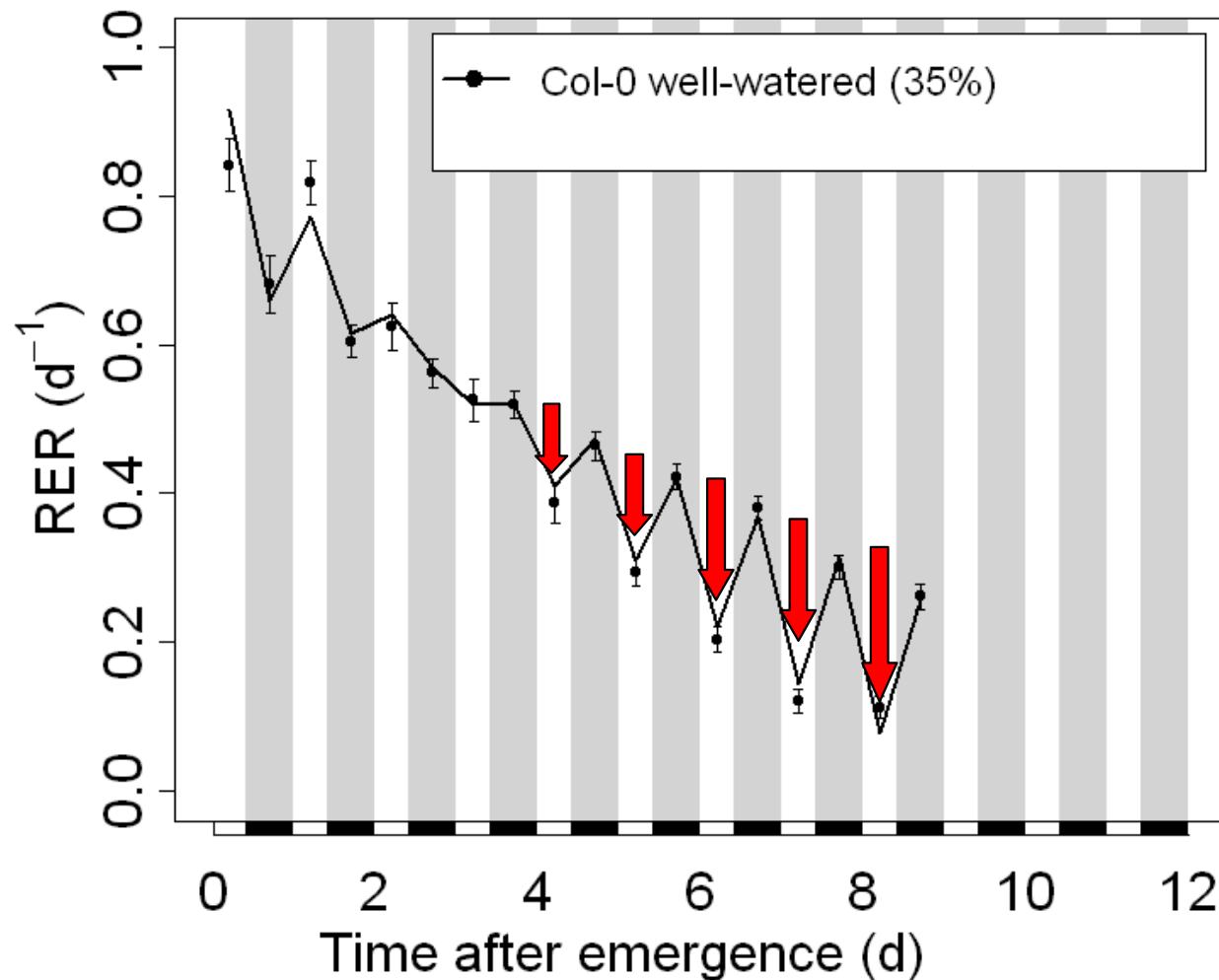
> A day/night analysis of leaf growth reveals **growth depressions in the daytime** which amplify during leaf development



[PANTIN et al., 2011]

Hydraulic control of leaf growth

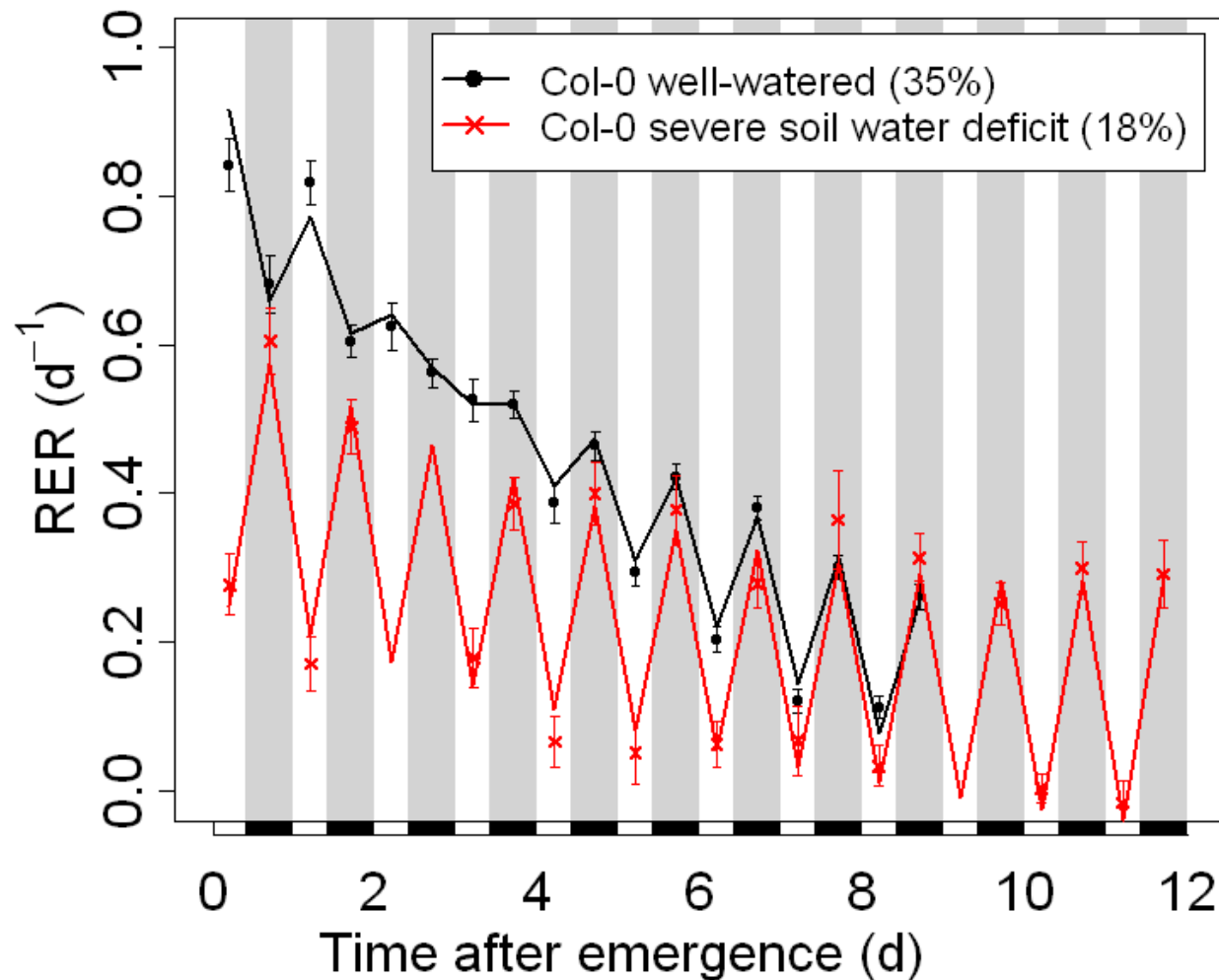
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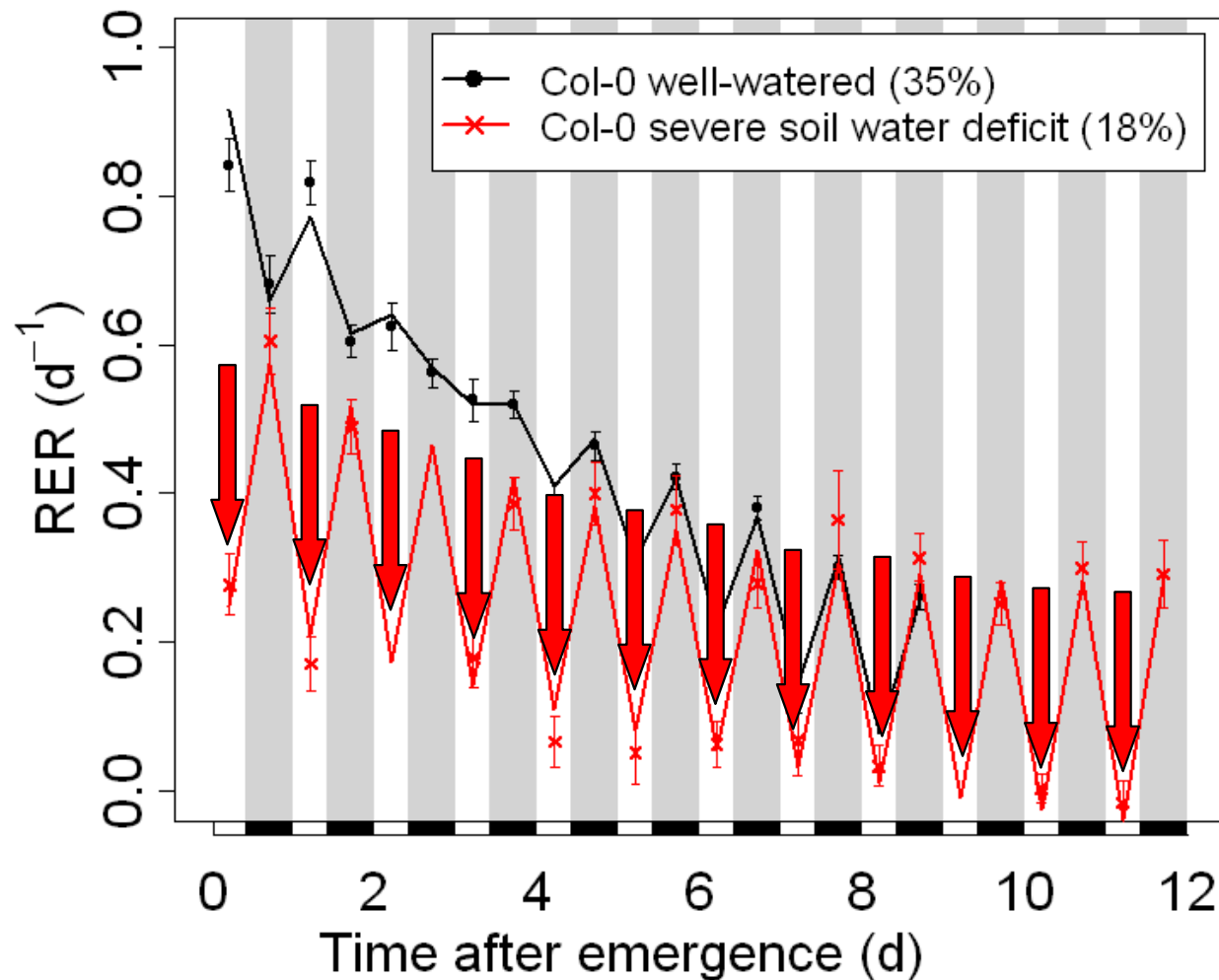
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Hydraulic control of leaf growth

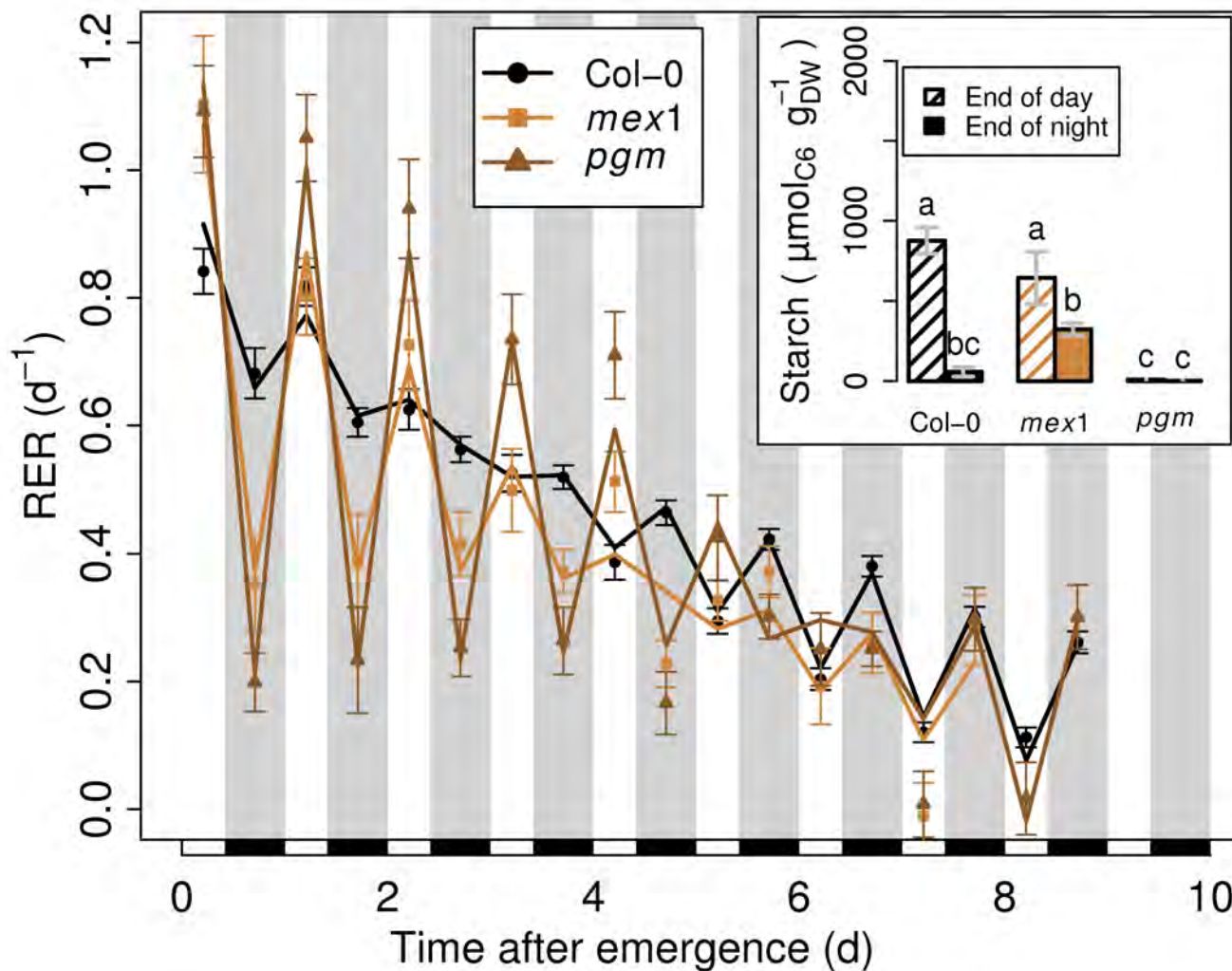
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[PANTIN et al., 2011]

Metabolic control of leaf growth

> Amplification of **early** depressions in the **nighttime** in starch mutants suggests their **metabolic origin**



[PANTIN et al., 2011]

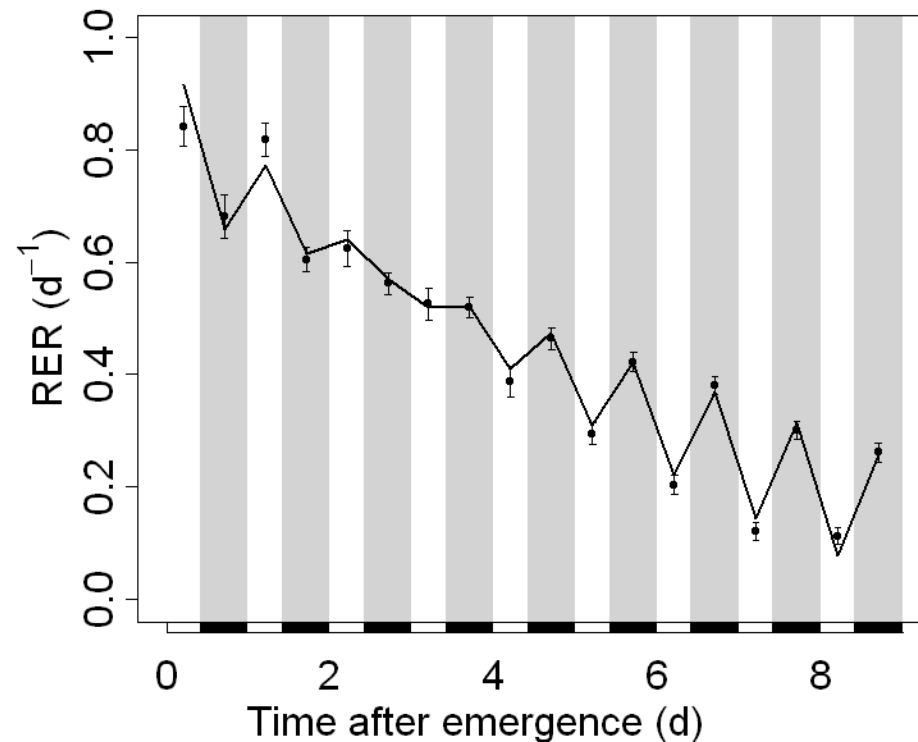
Hydraulic control of leaf growth

> Using a set of mutants grown under several environments, it was concluded that during the course of its ontogeny, **the predominant control of leaf expansion switches from metabolics to hydraulics**

Metabolic control



Hydraulic control

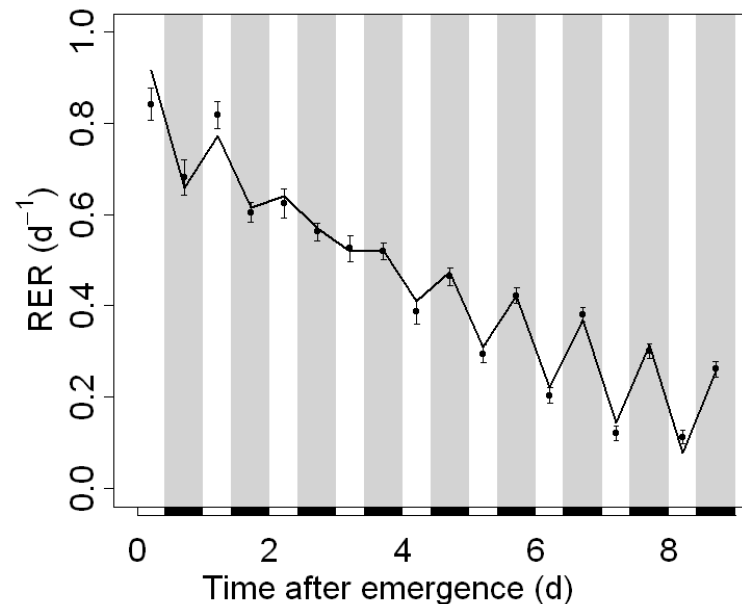


[PANTIN et al., 2011]

Leaf growth: establishment of hydraulic control

> AIMS:

- check the **hydraulic nature** of diurnal depressions
- understand **what underlies the establishment** of water predominance in the control of leaf growth



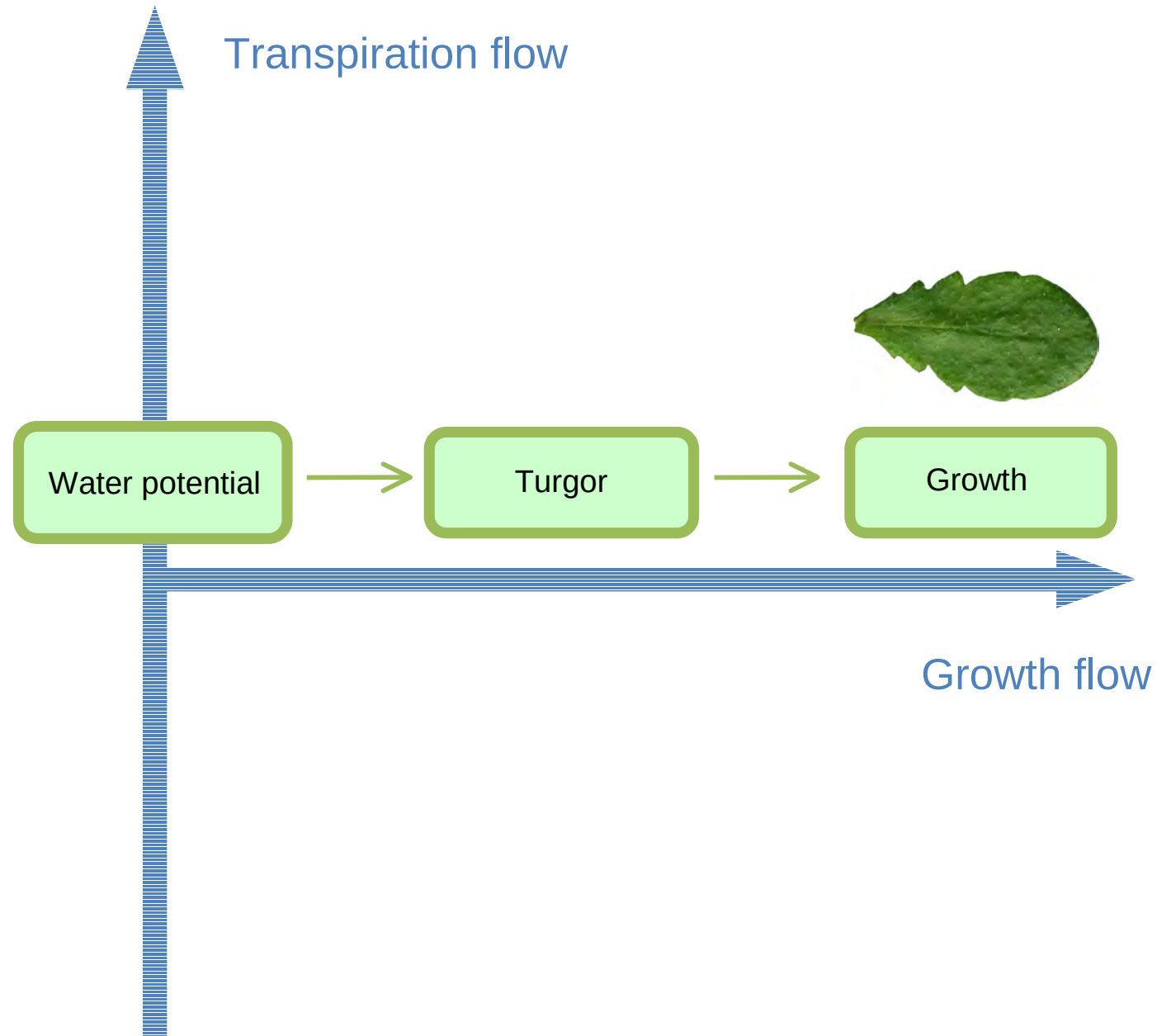


Leaf growth: establishment of hydraulic control

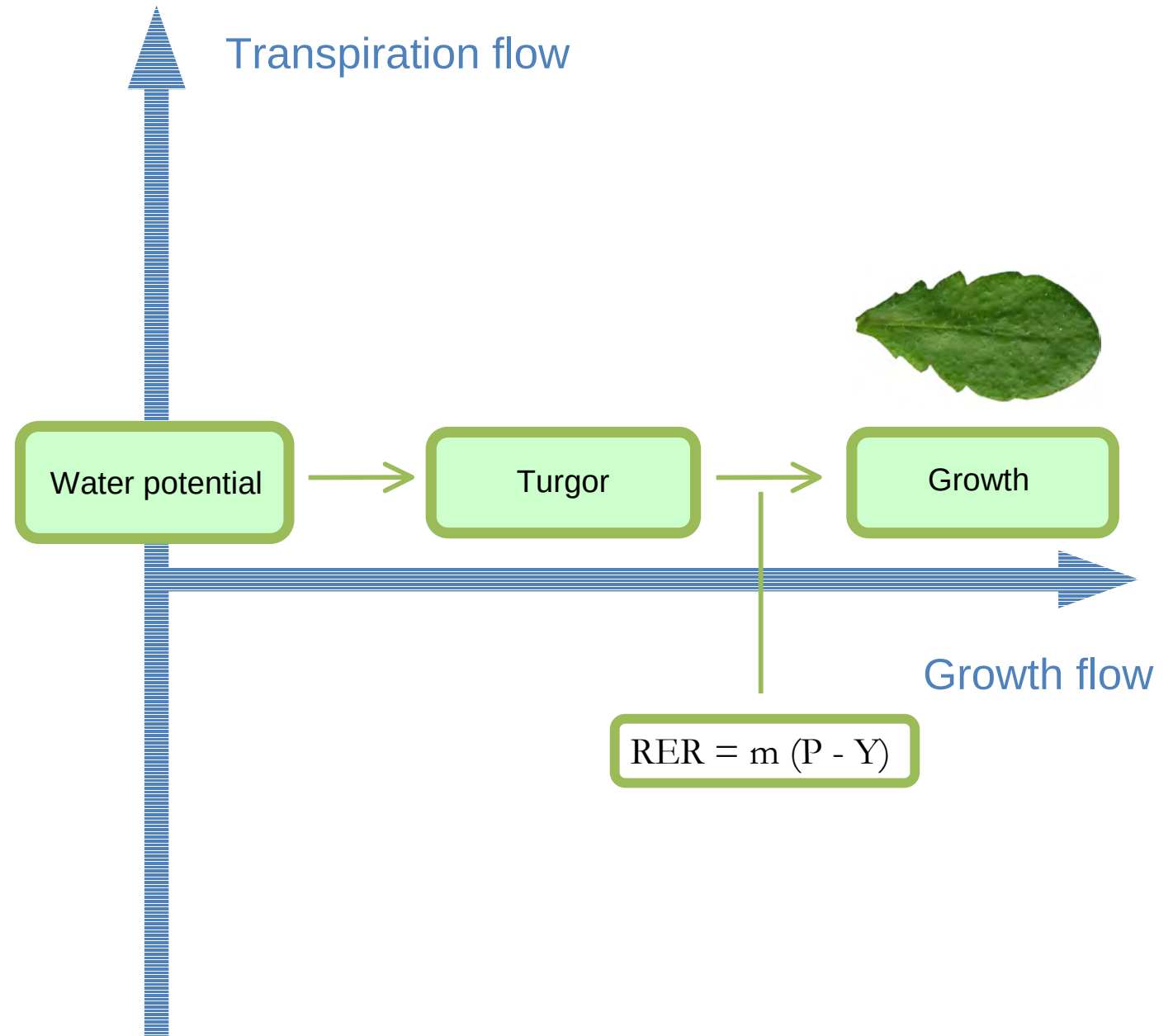
– I –

Emergence of diurnal hydraulic limitations:
evidence from day/night water potentials
and transpiration

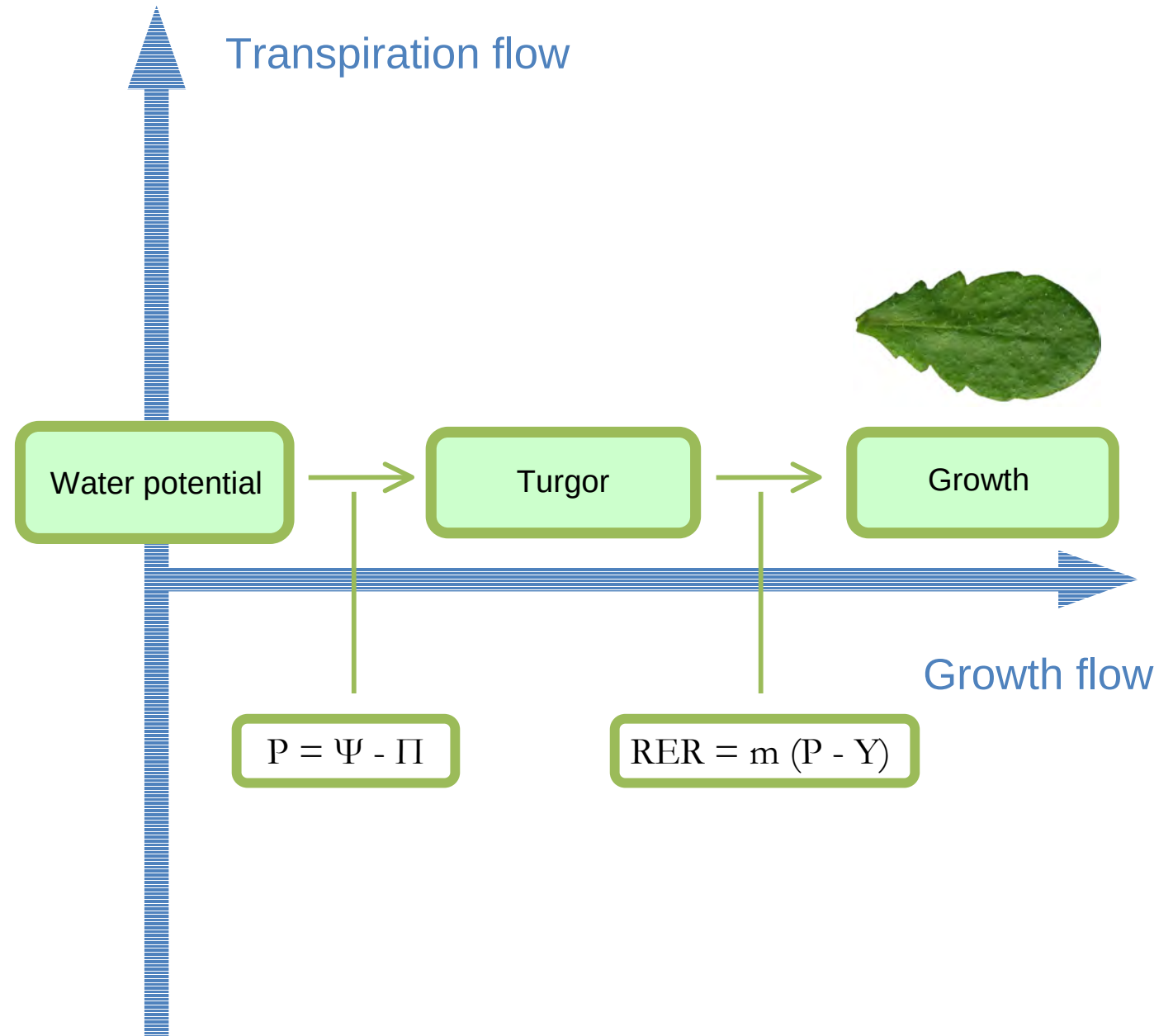
Leaf growth: establishment of hydraulic control



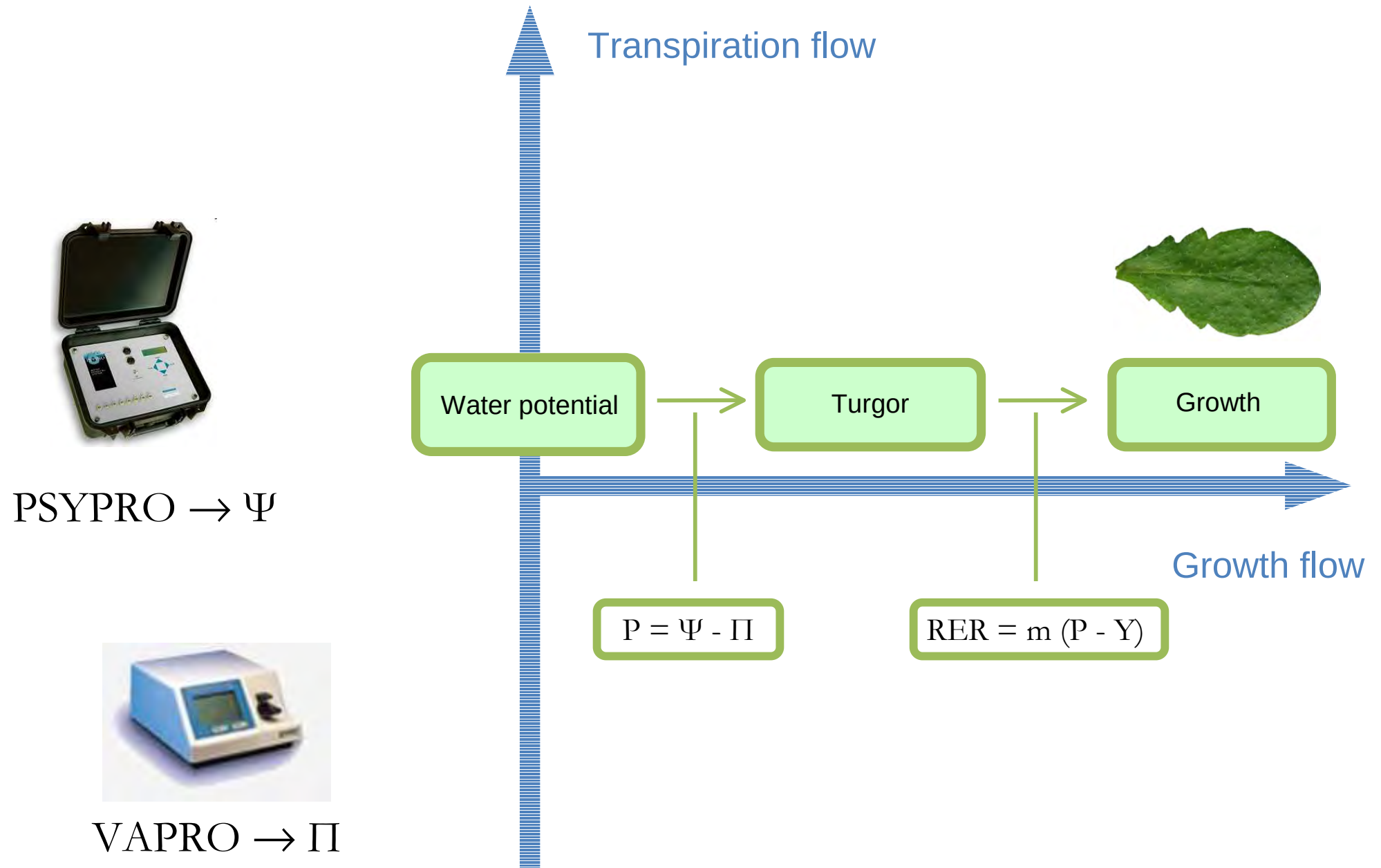
Leaf growth: establishment of hydraulic control



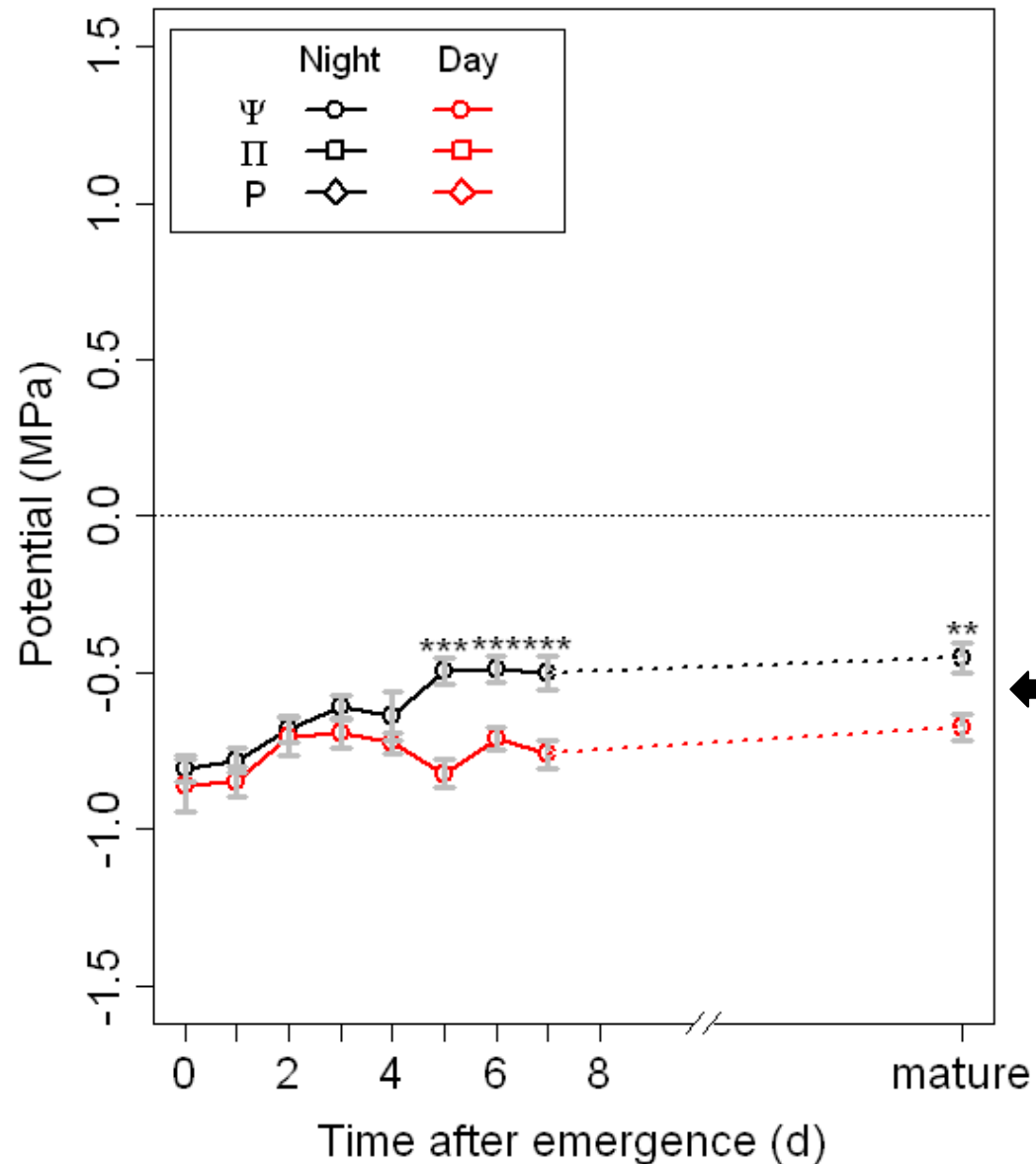
Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control



Leaf water status



> Water potential Ψ increases with leaf development

> **Differences between day and night** progressively emerge

← Ψ

Leaf water status

Journal of Experimental Botany, Vol. 53, No. 368, pp. 489–503, March 2002

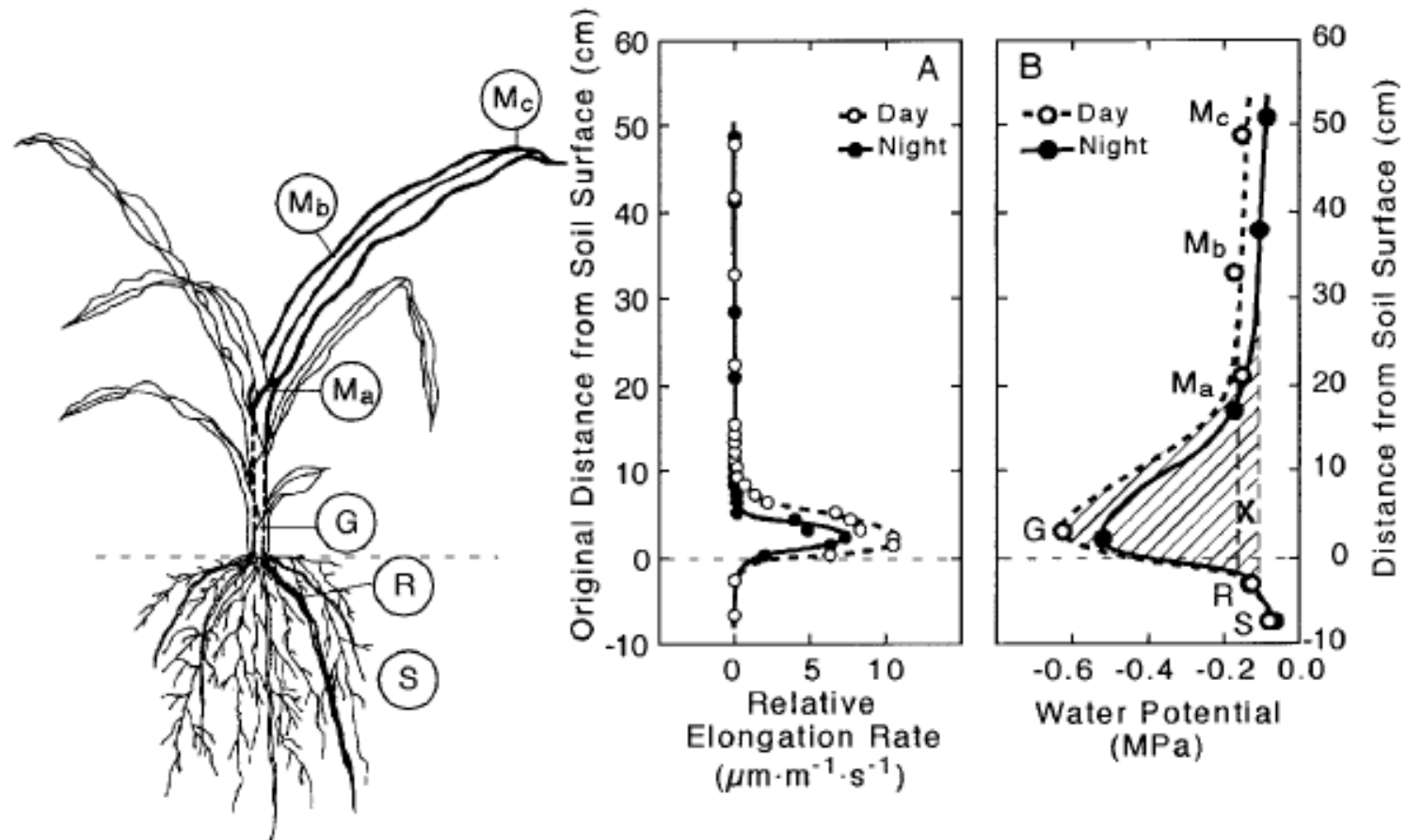


Journal of
Experimental
Botany

Growth-induced water potentials and the growth of maize leaves

An-Ching Tang and John S. Boyer¹

College of Marine Studies and College of Agriculture and Natural Resources, University of Delaware, Lewes, DE 19958, USA



Leaf water status

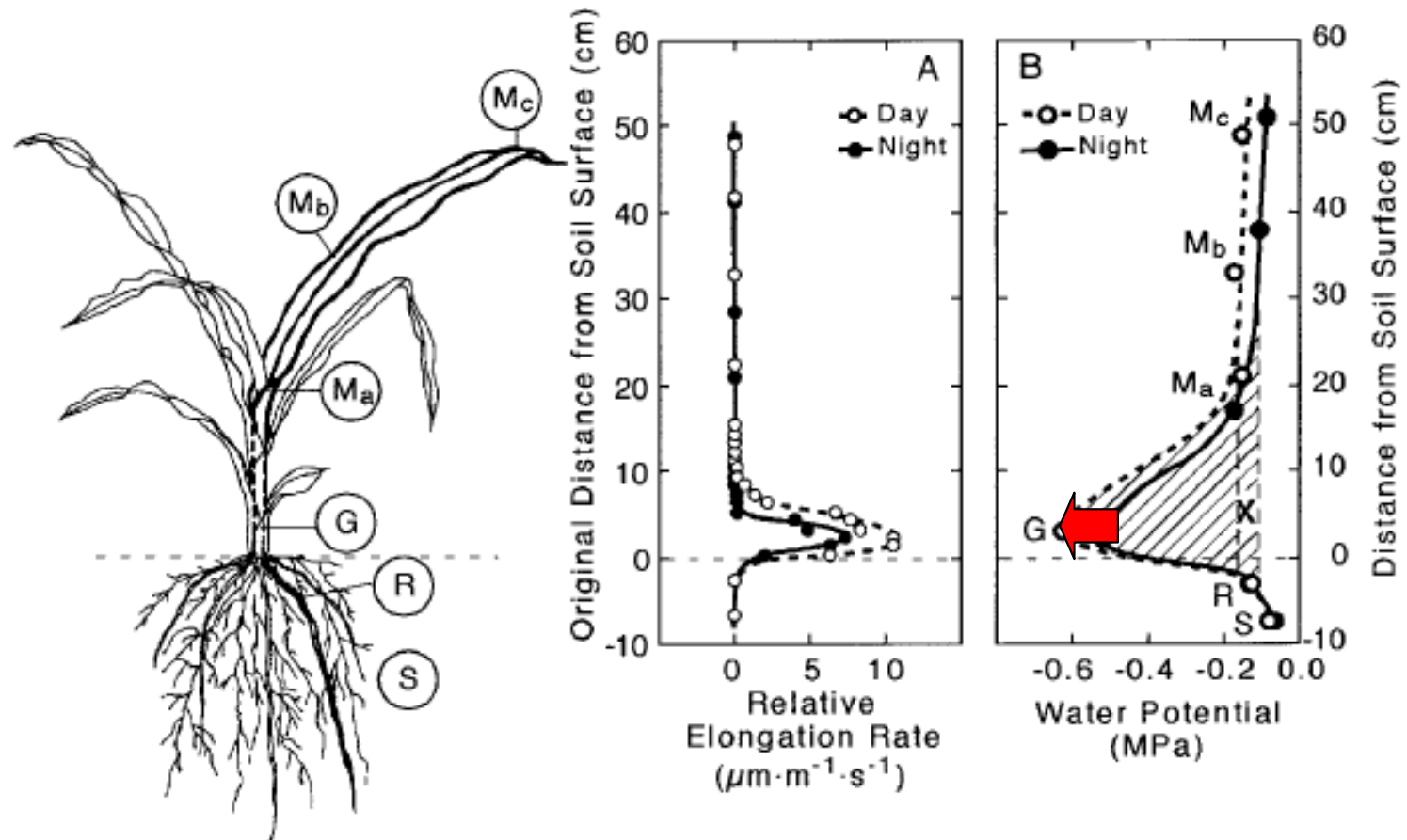
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Growth-induced water potentials and the growth of maize leaves

An-Ching Tang and John S. Boyer¹

College of Marine Studies and College of Agriculture and Natural Resources, University of Delaware, Lewes, DE 19958, USA



Leaf water status

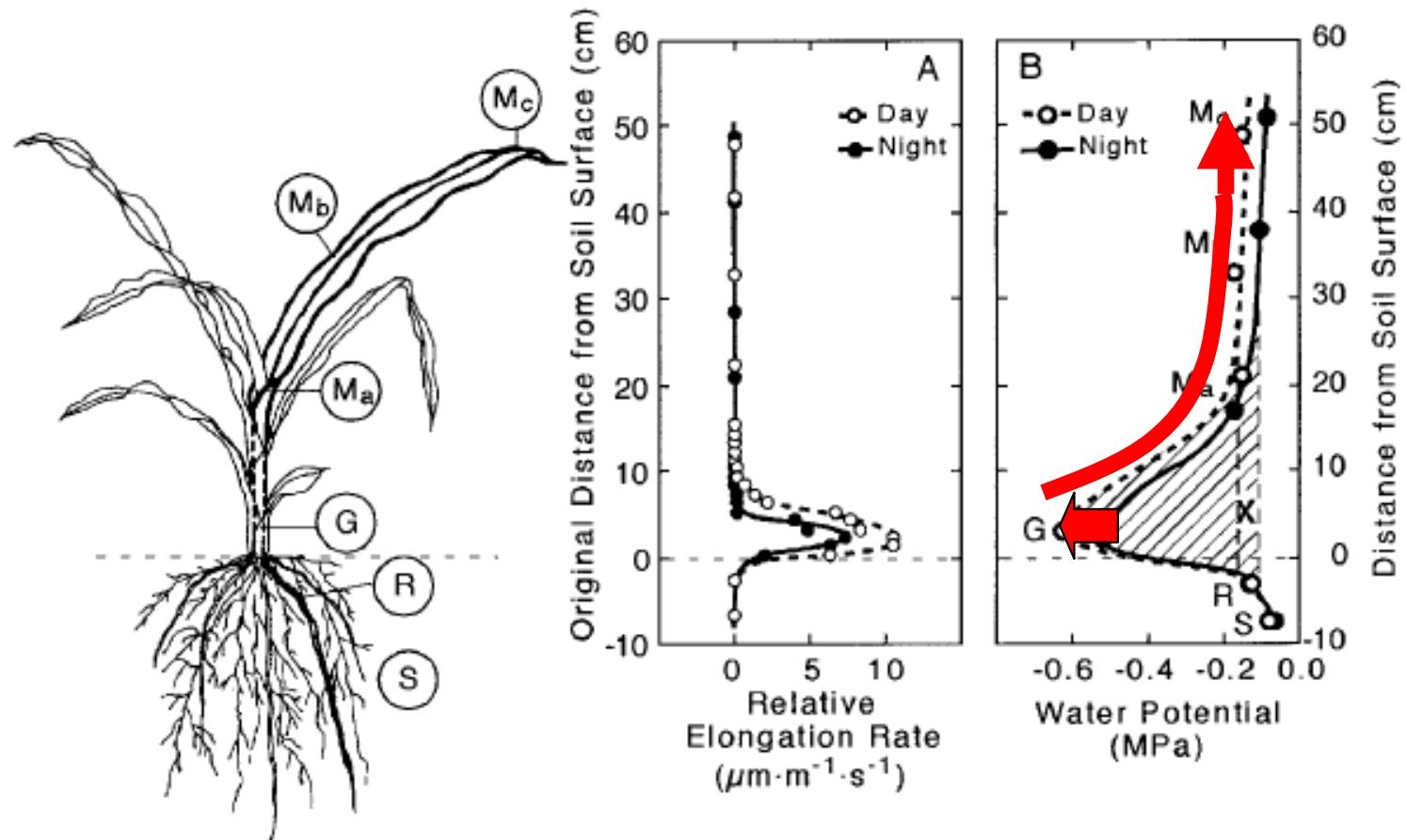
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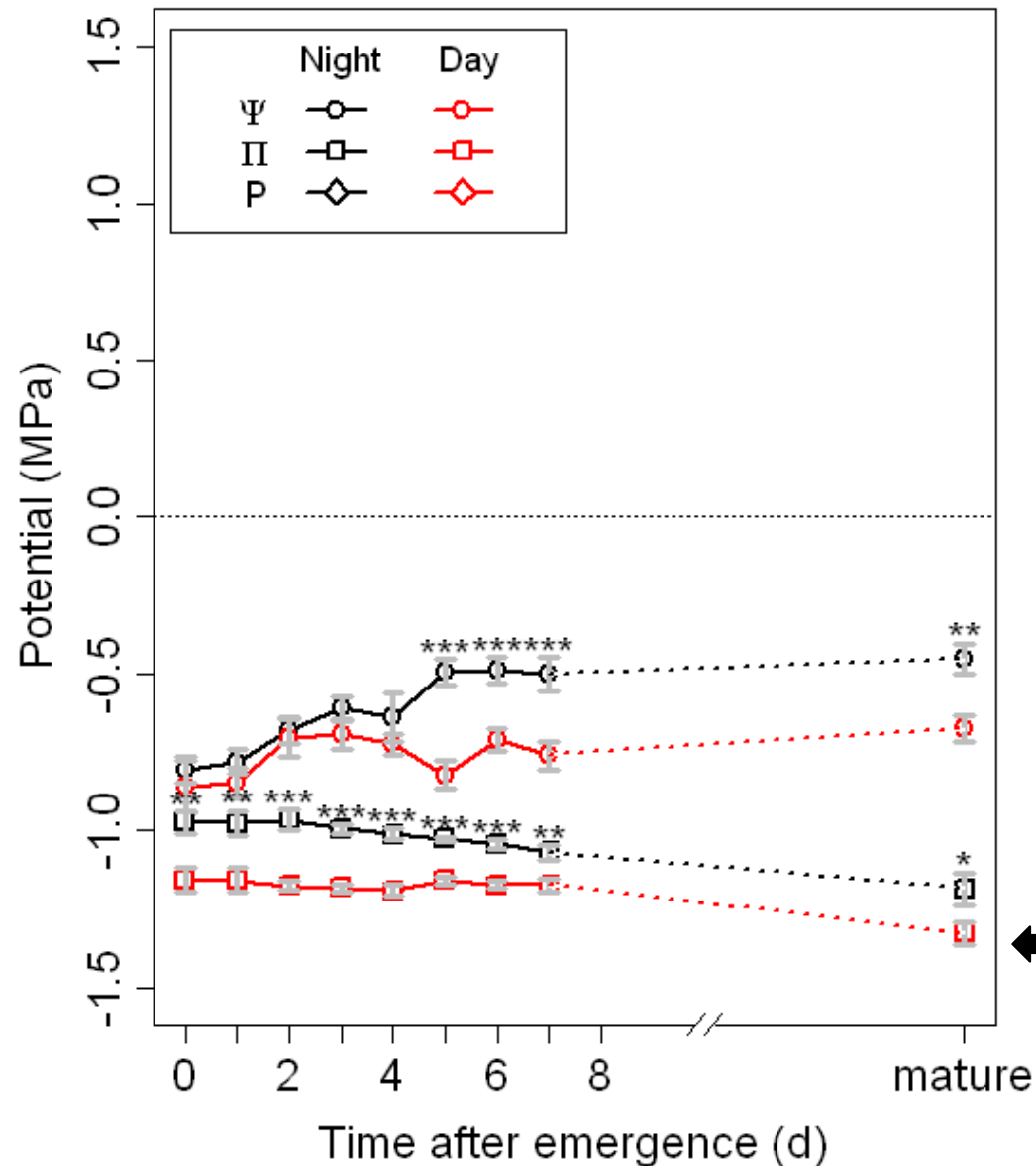
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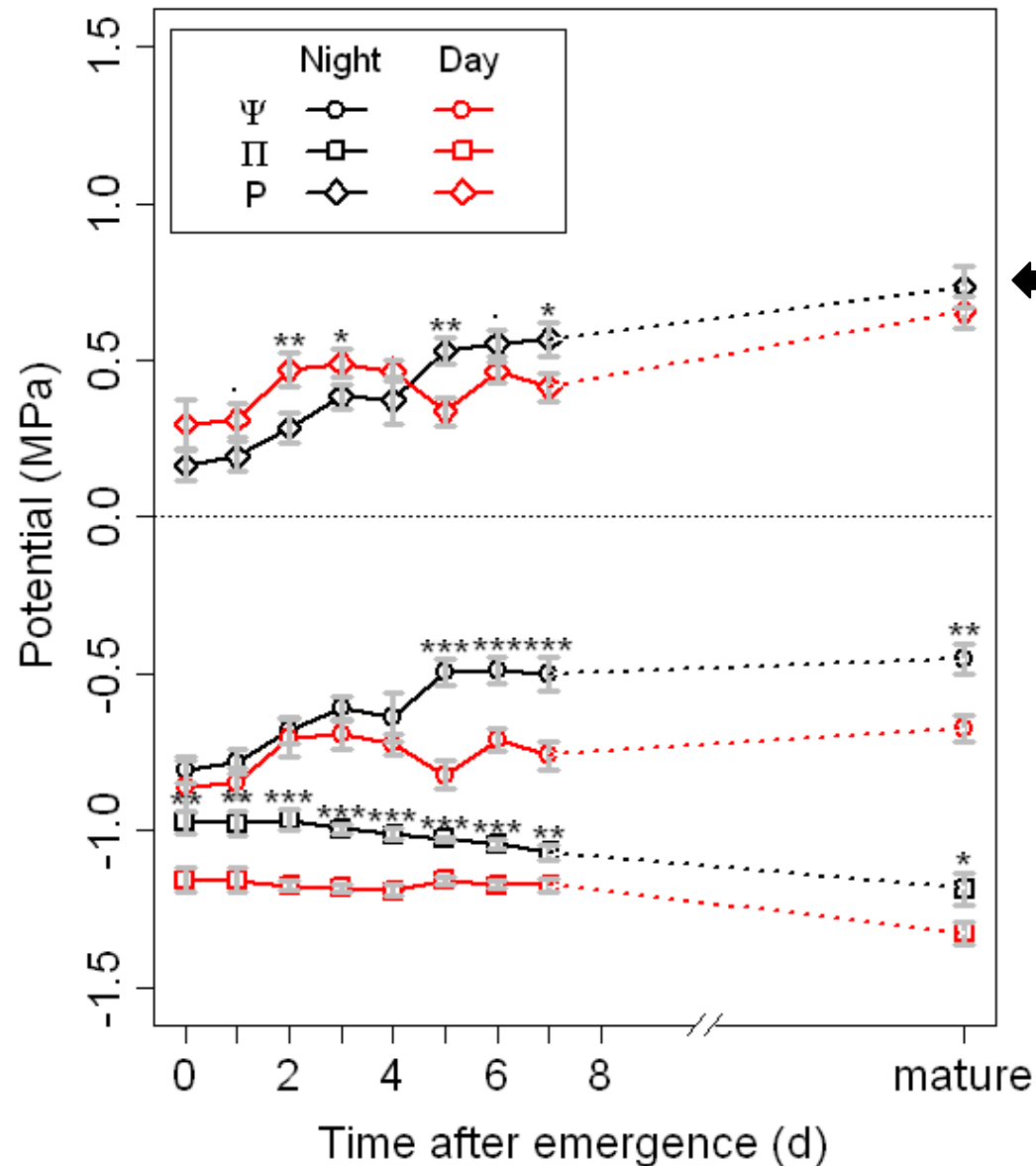


> Osmotic potential Π is nearly stable

> **Diurnal Π** is lower than **nocturnal Π** because solutes accumulate (i) actively with sugar and organic acids biosynthesis and (ii) passively with water loss due to transpiration

← Π

Leaf water status



← **P**

> **Day/night differences in turgor P reverse** during the course of leaf development

> Diurnal depressions of P are **consistent with the diurnal depressions of growth**

Leaf water status

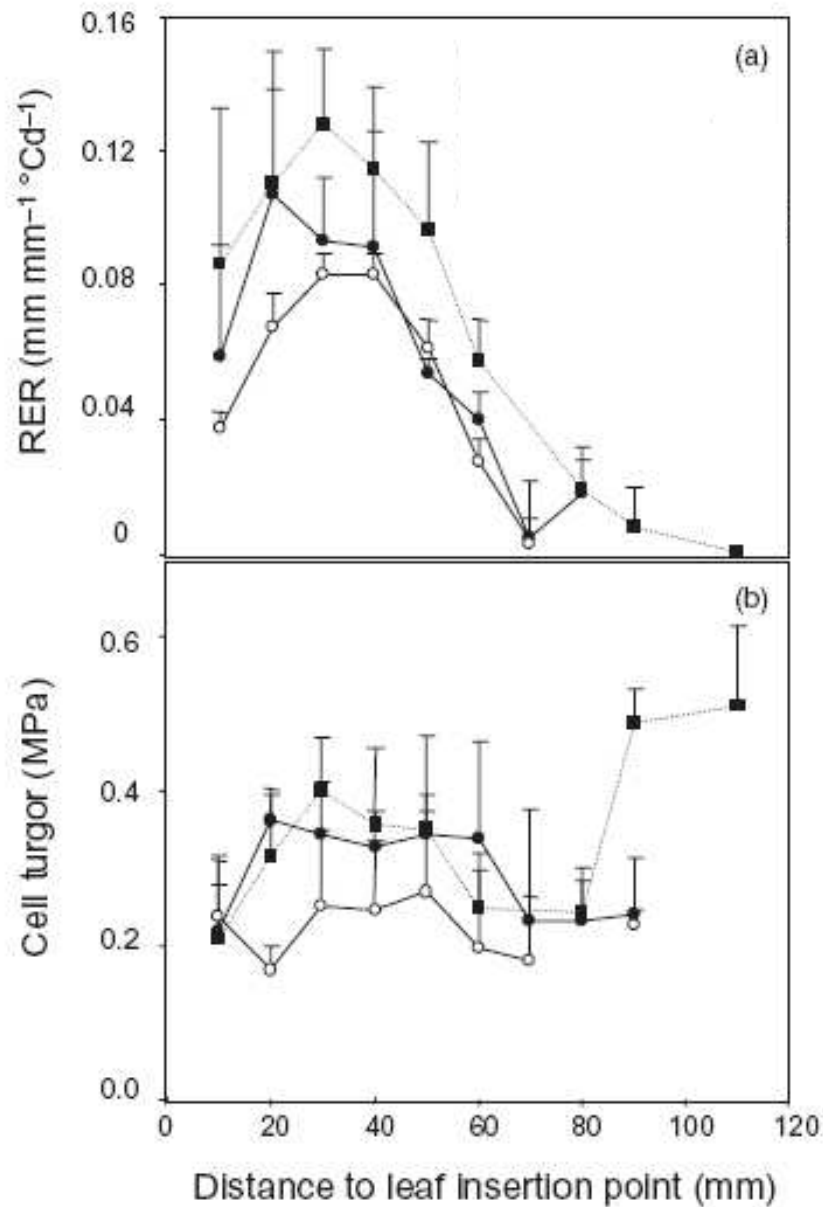
Plant, Cell and Environment (2006) 29, 1138–1148

doi: 10.1111/j.1365-3040.2005.01494.x

Leaf growth and turgor in growing cells of maize (*Zea mays* L.) respond to evaporative demand under moderate irrigation but not in water-saturated soil

OUMAYA BOUCHABKÉ, FRANÇOIS TARDIEU & THIERRY SIMONNEAU

Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, UMR Institut National de la Recherche Agronomique, École Nationale Supérieure d'Agronomie de Montpellier, F- 34060 Montpellier cedex 1, France



- Night
- Day – low VPD
- Day – high VPD

Leaf water status

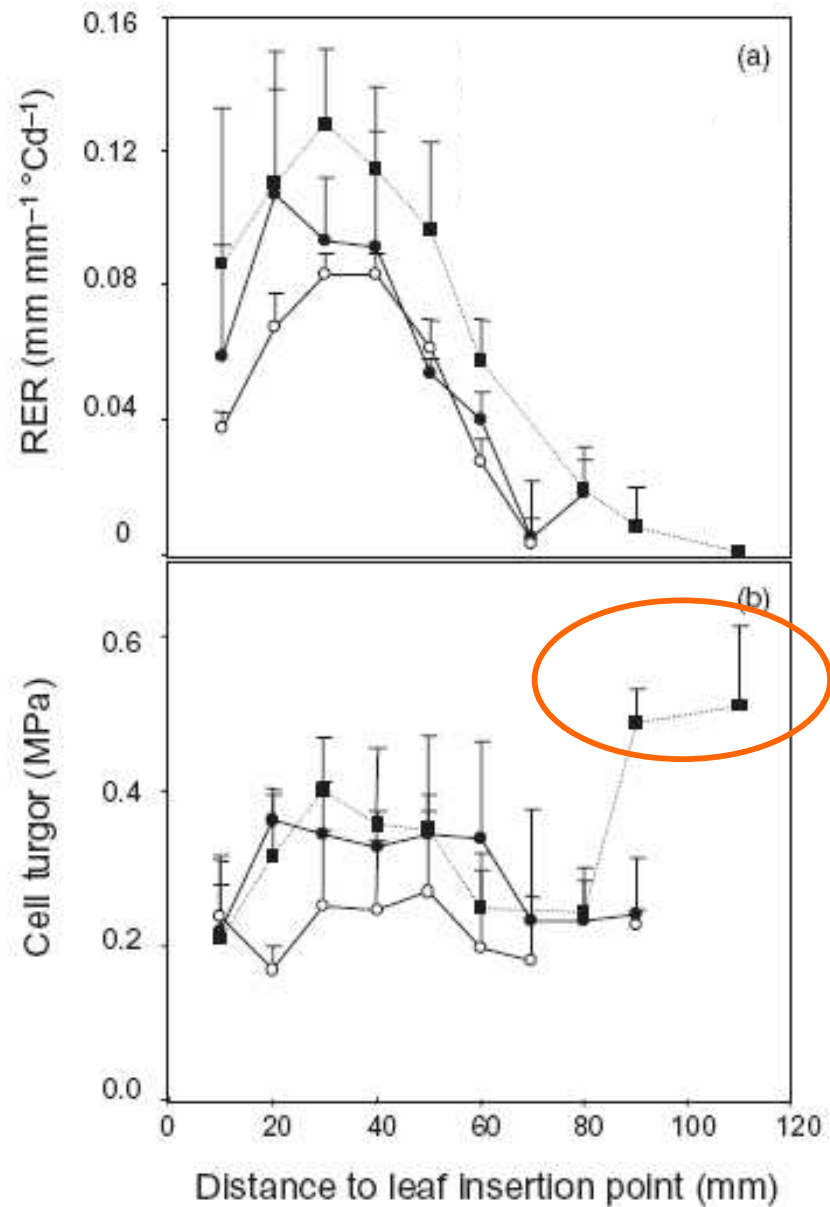
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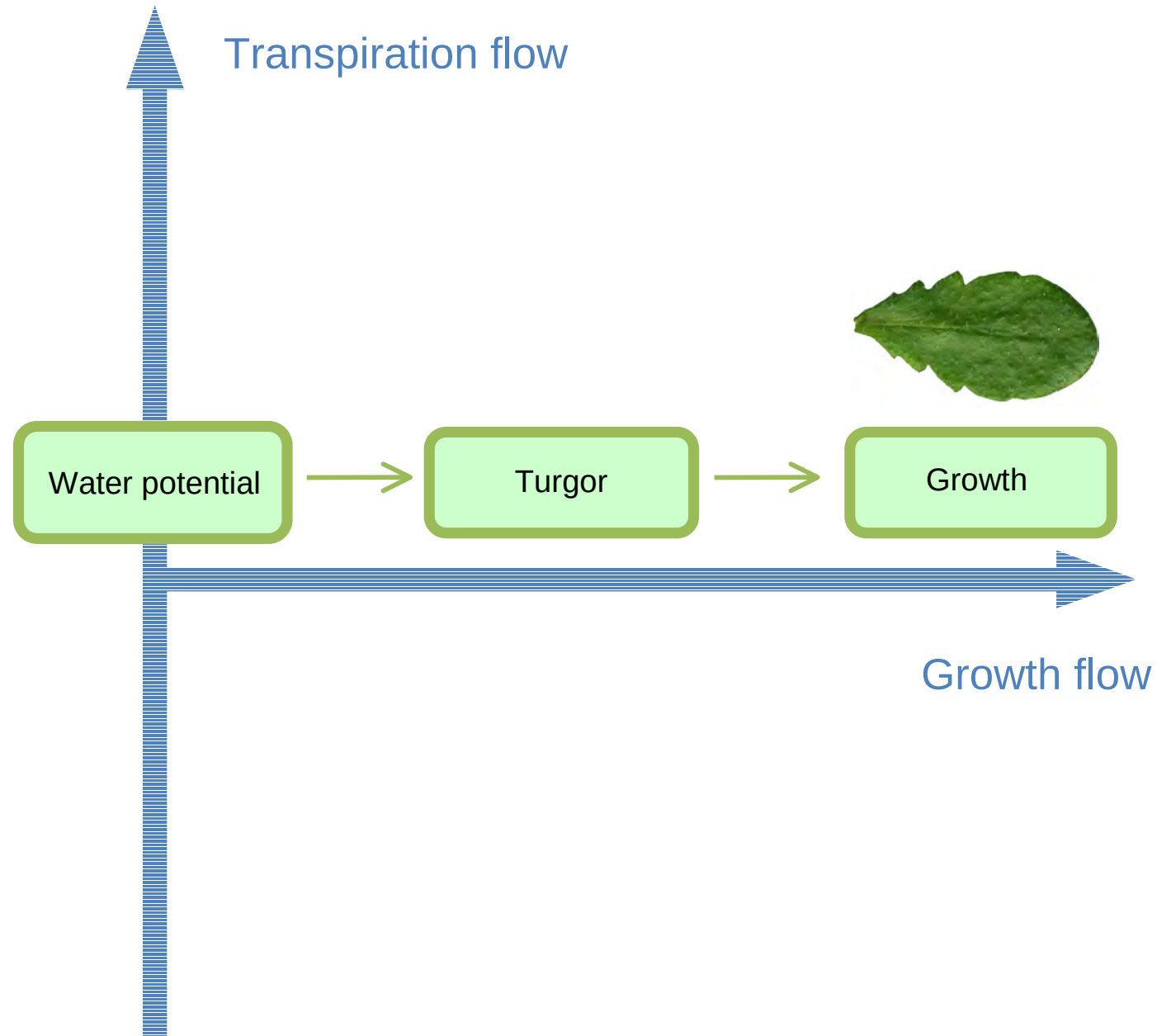
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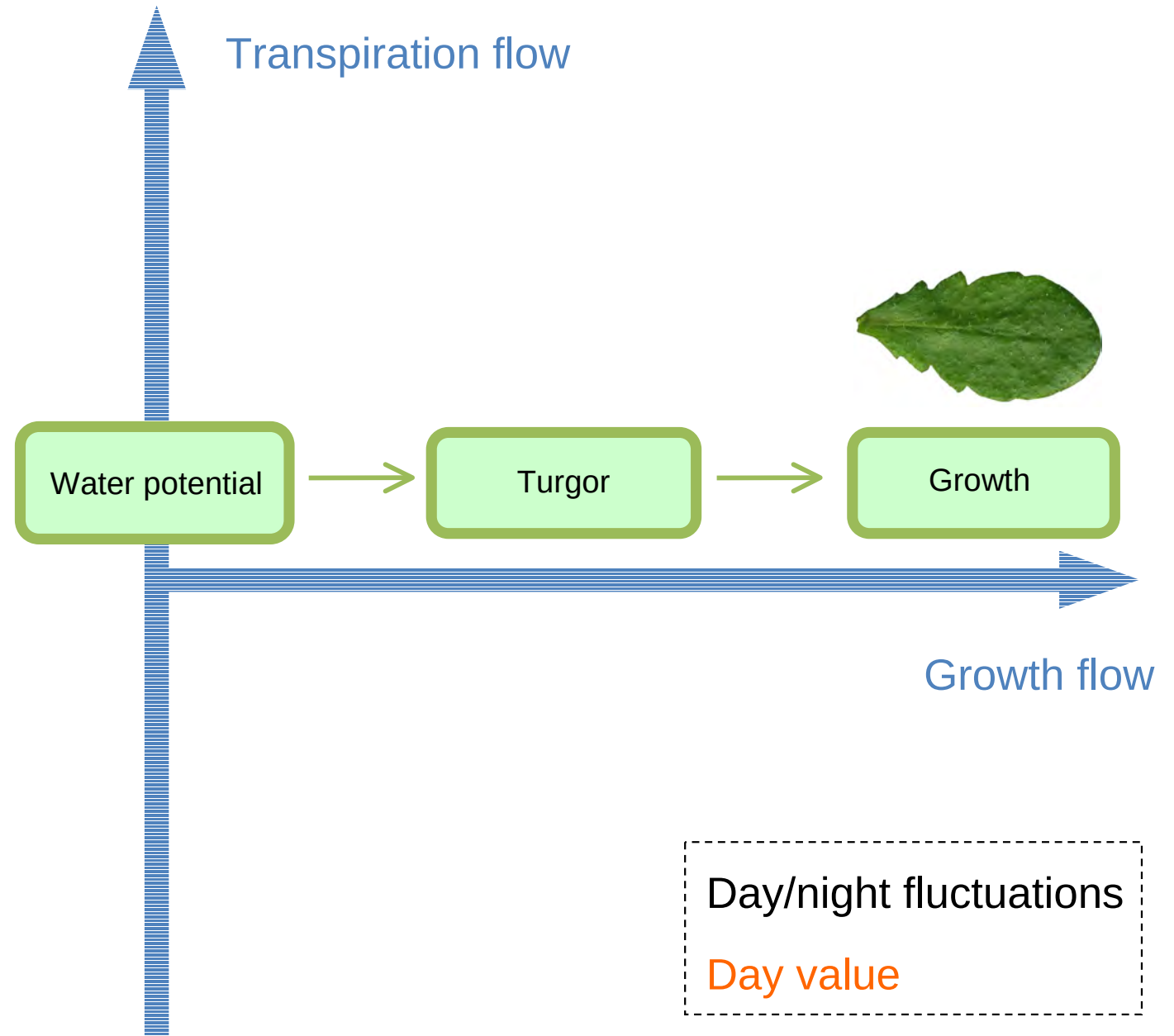


Decrease in wall extensibility?

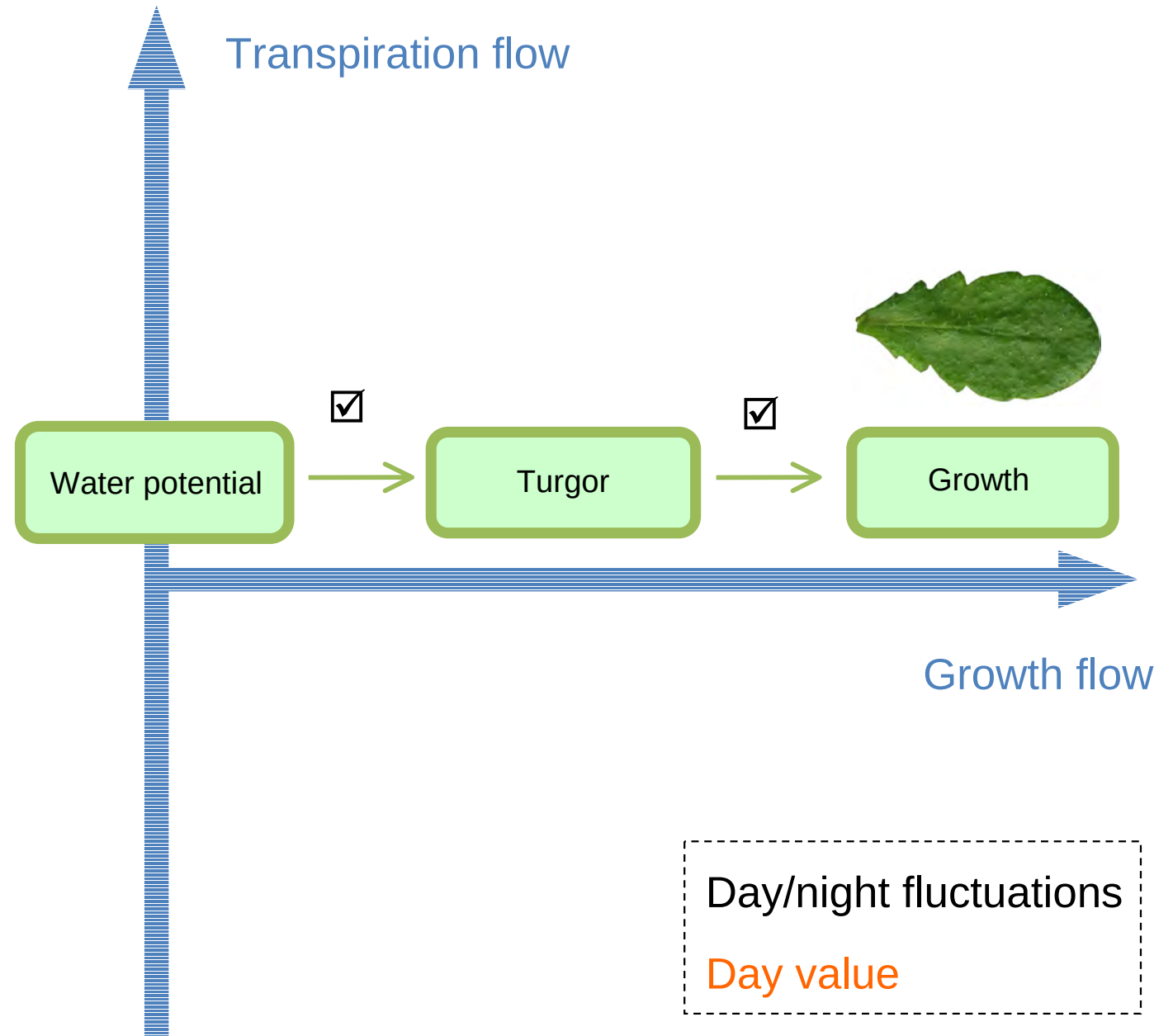
Leaf growth: establishment of hydraulic control



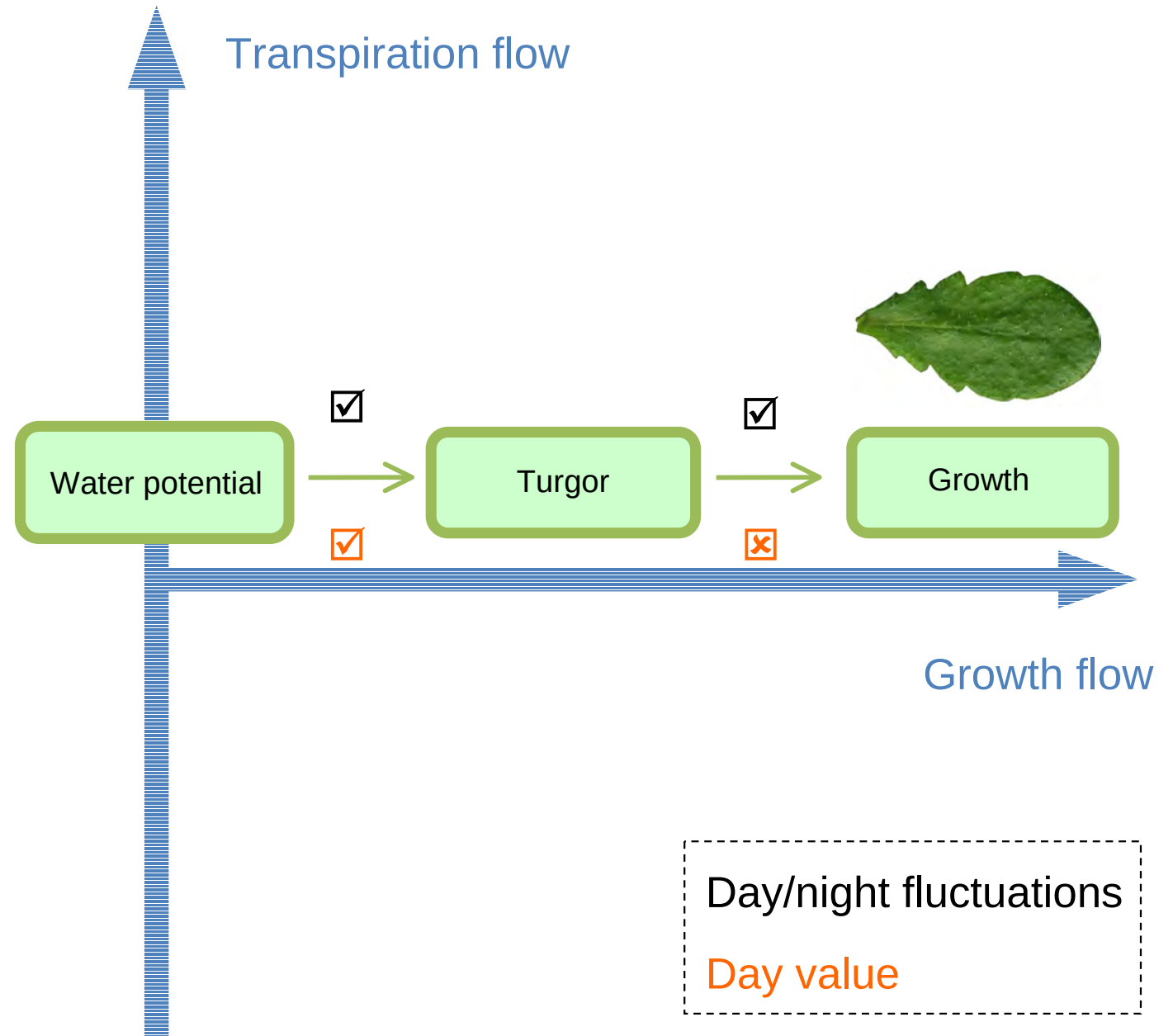
Leaf growth: establishment of hydraulic control



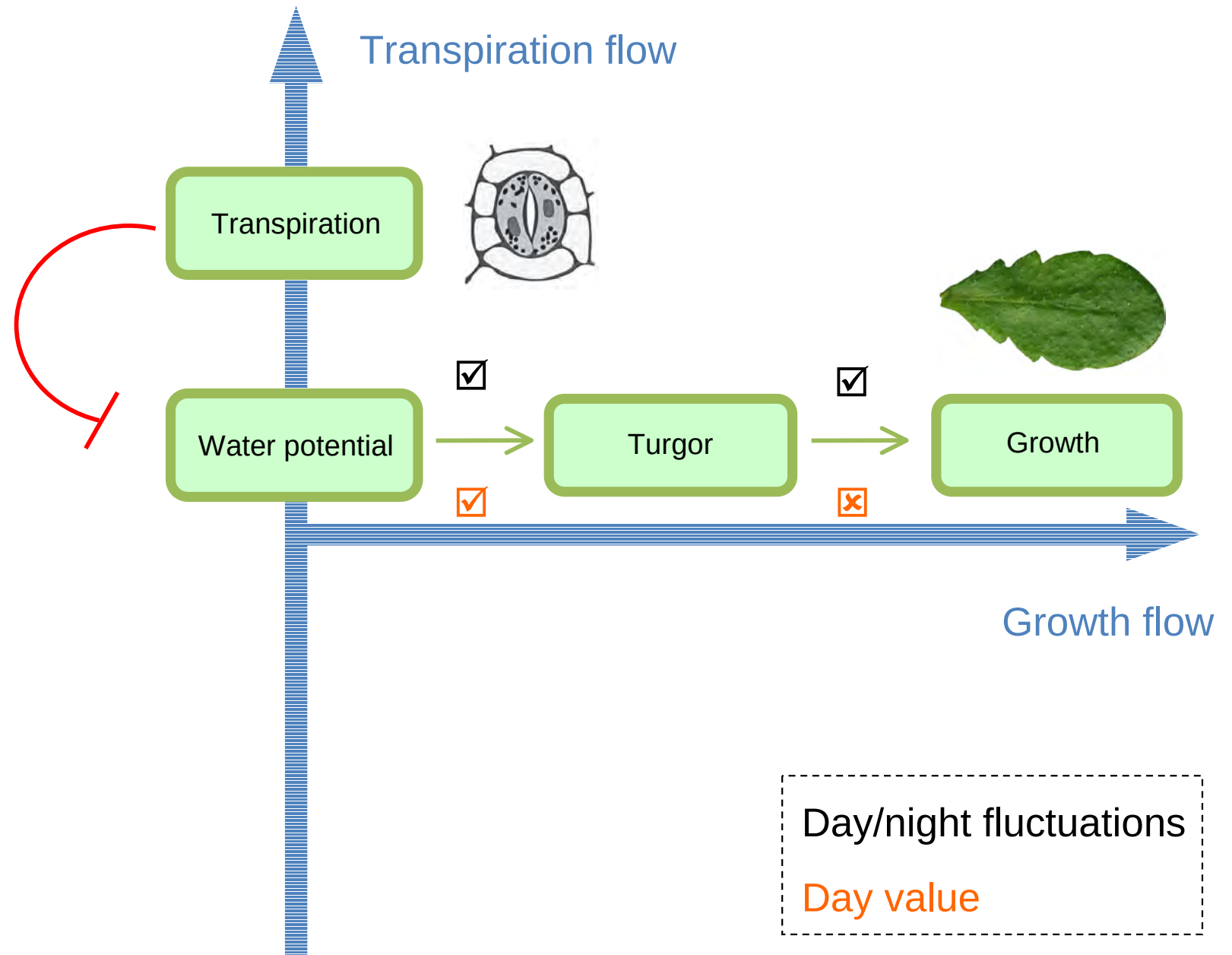
Leaf growth: establishment of hydraulic control



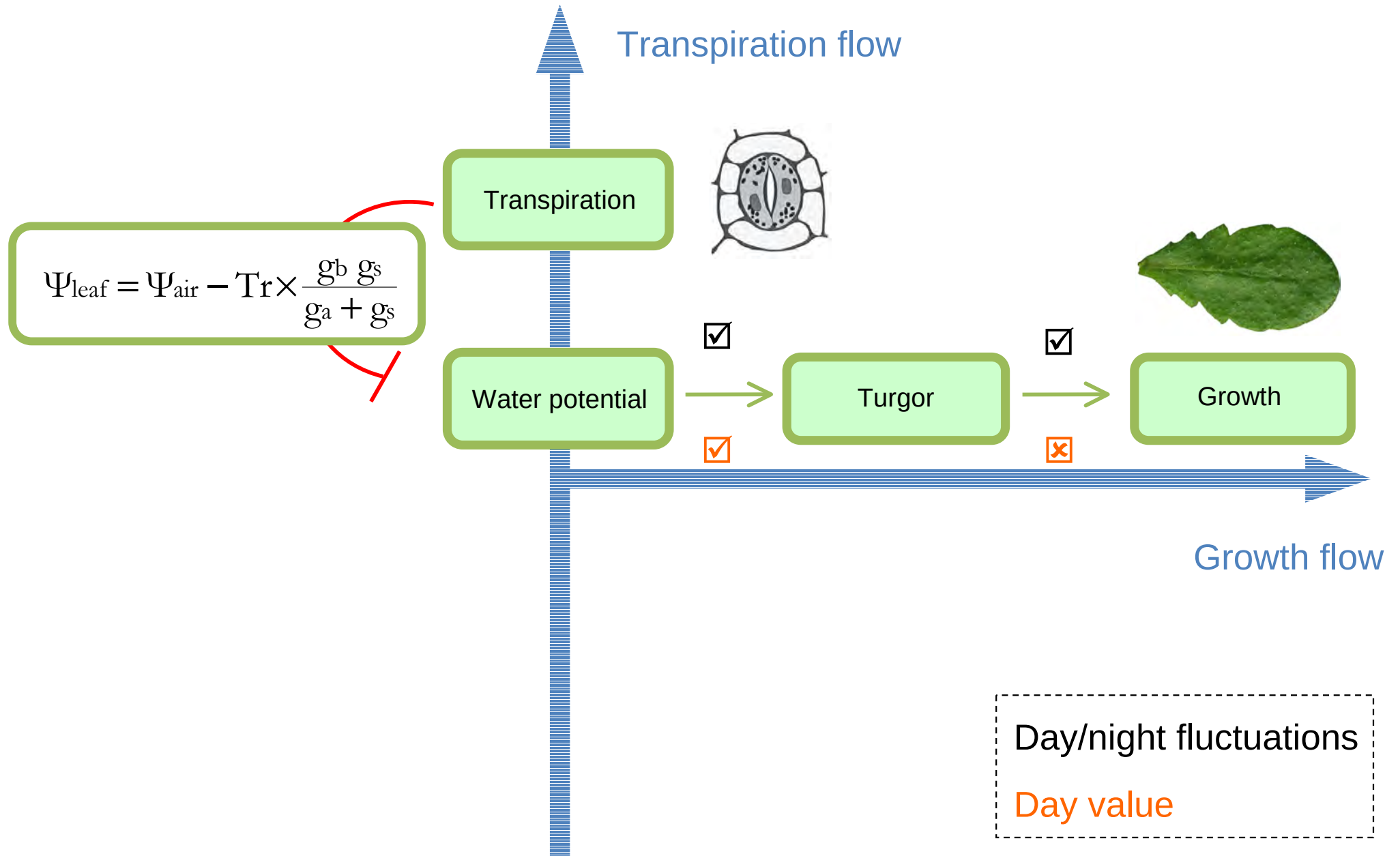
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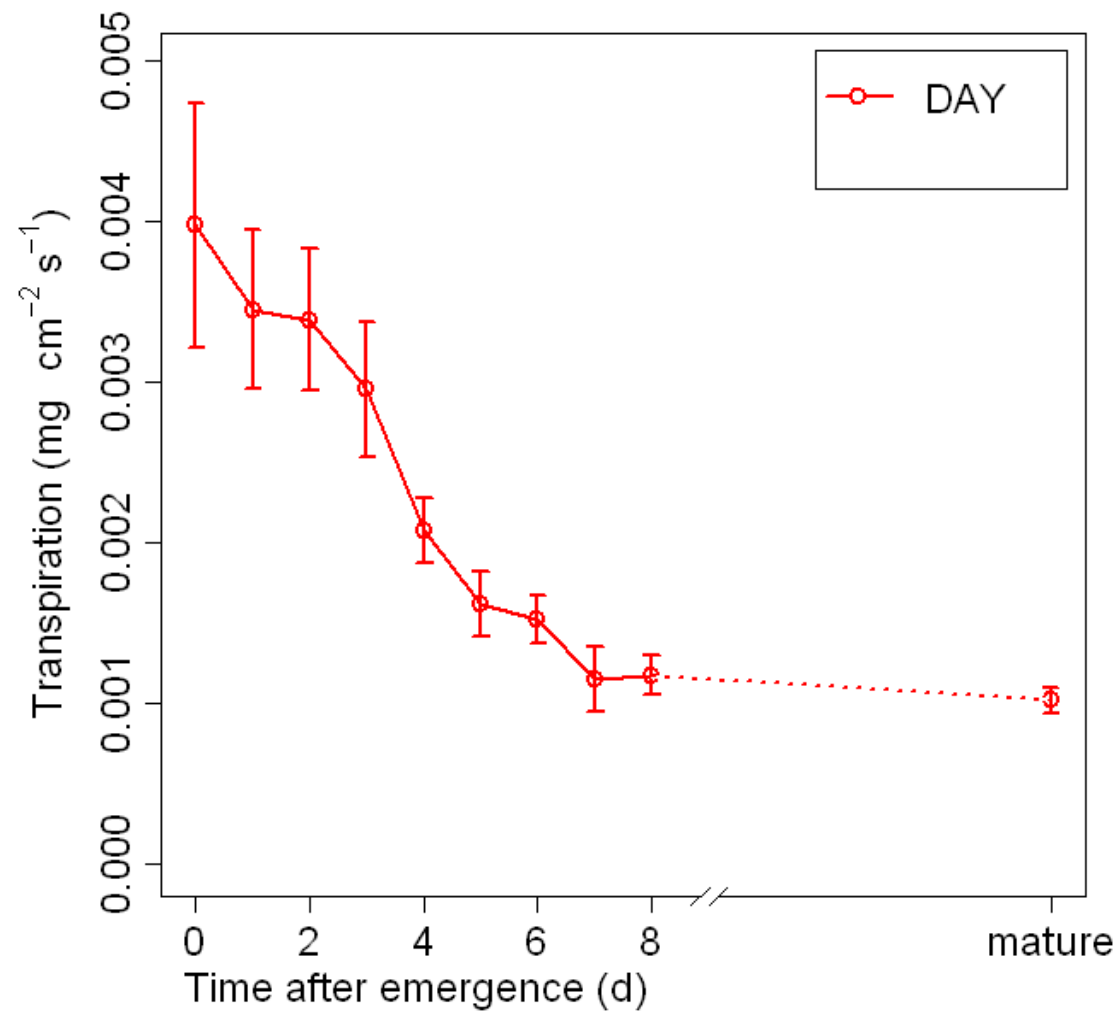
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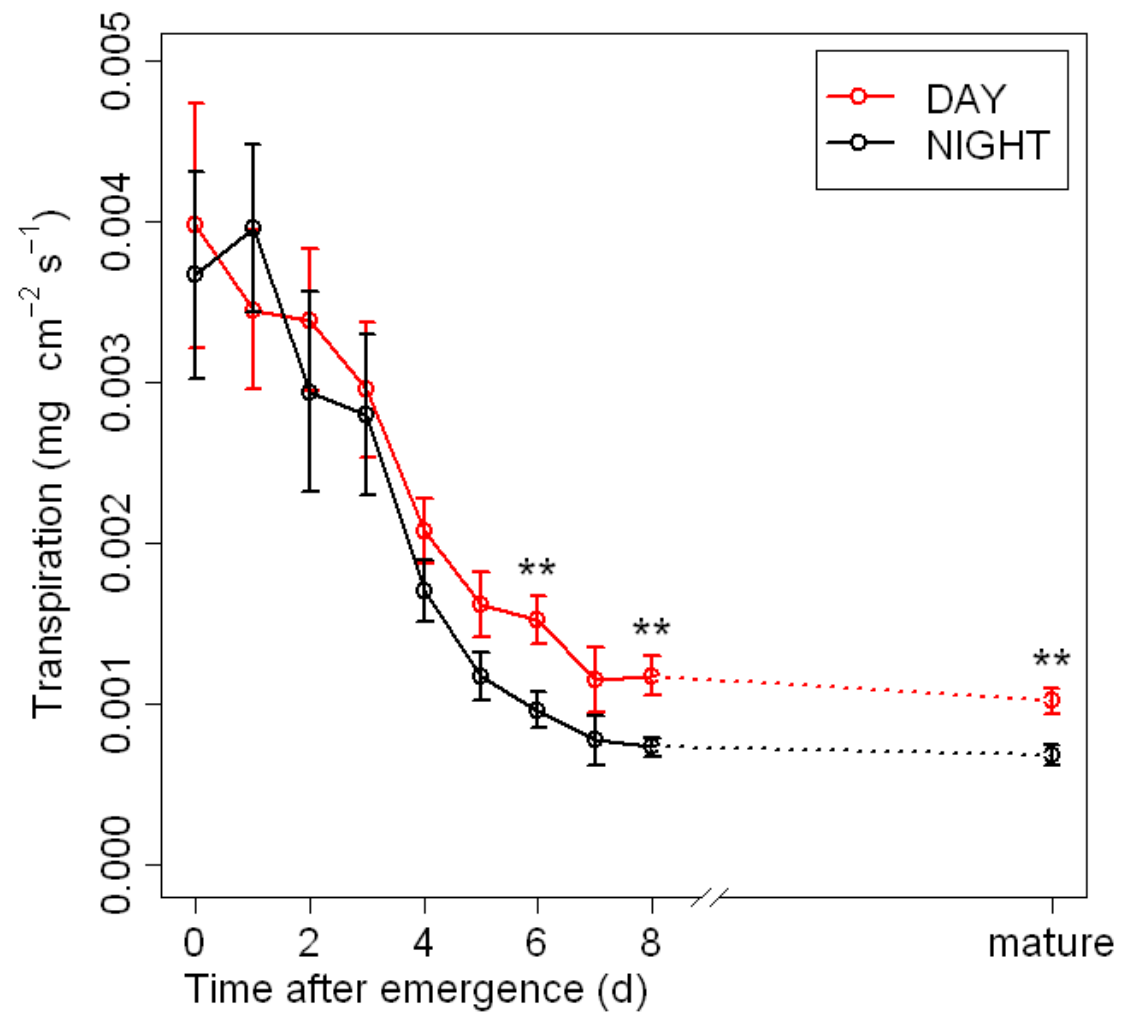
Leaf growth: establishment of hydraulic control



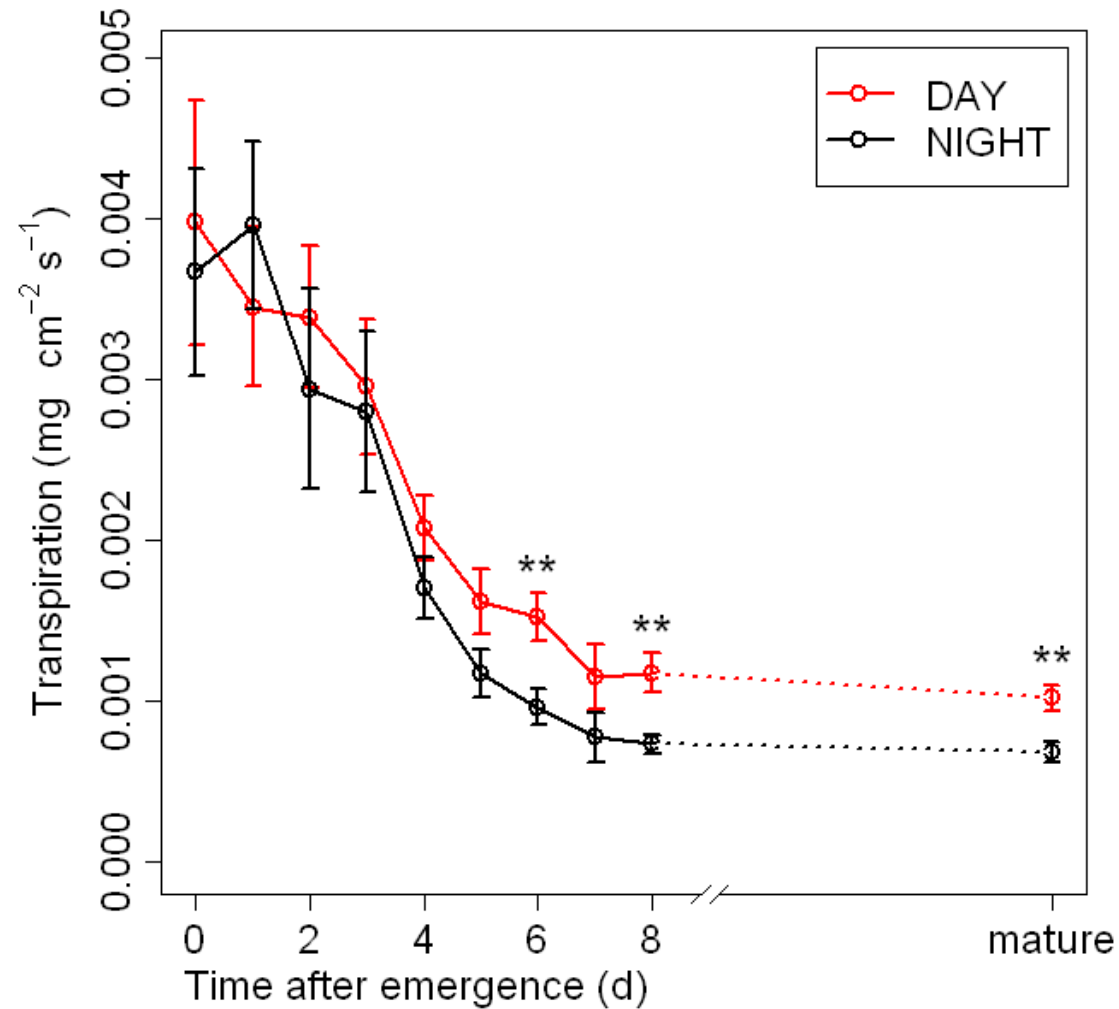
Transpiration



Transpiration

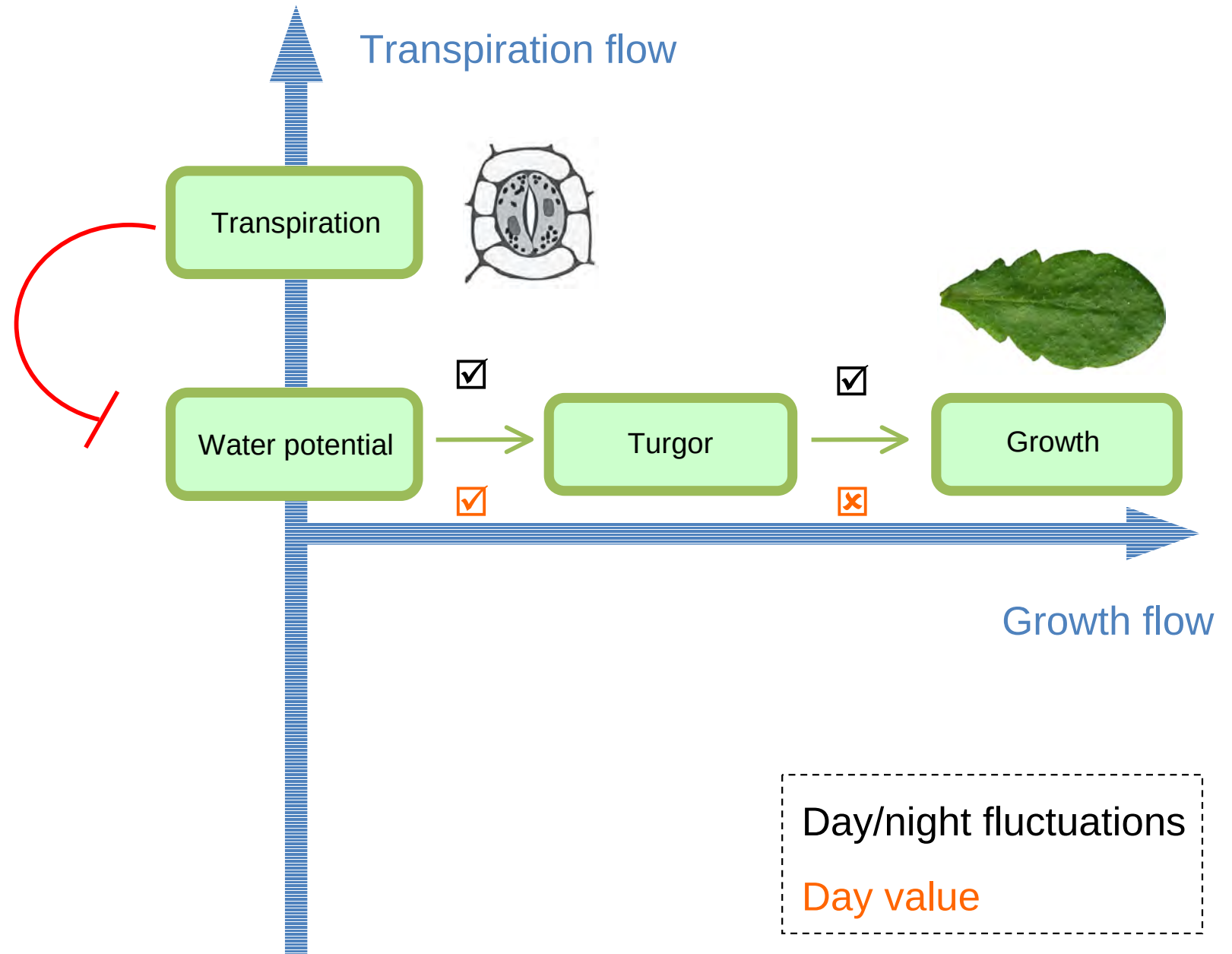


Transpiration

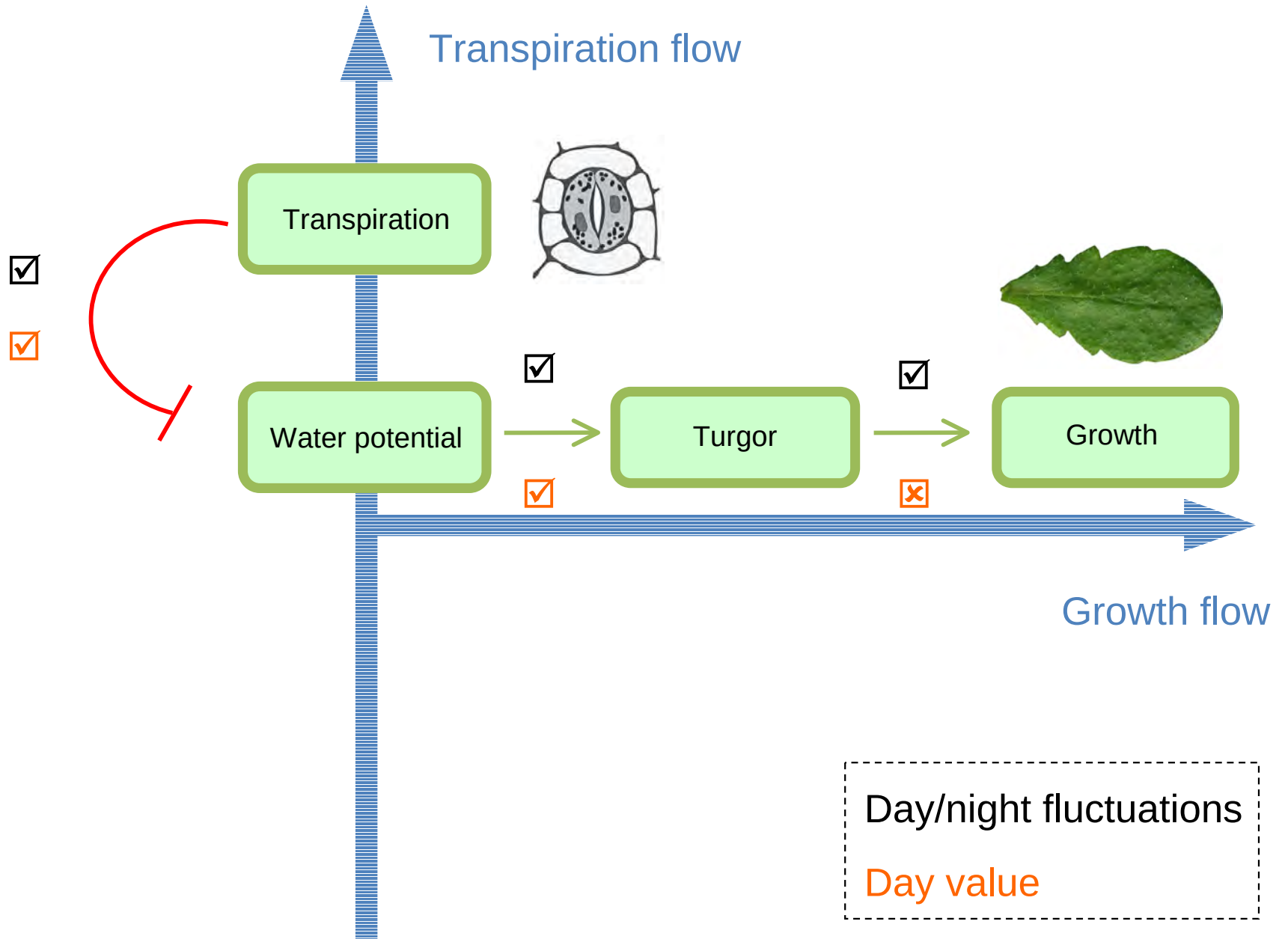


⇒ Changes in day/night differences of transpiration are consistent with potentials and expansion developmental patterns

Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control





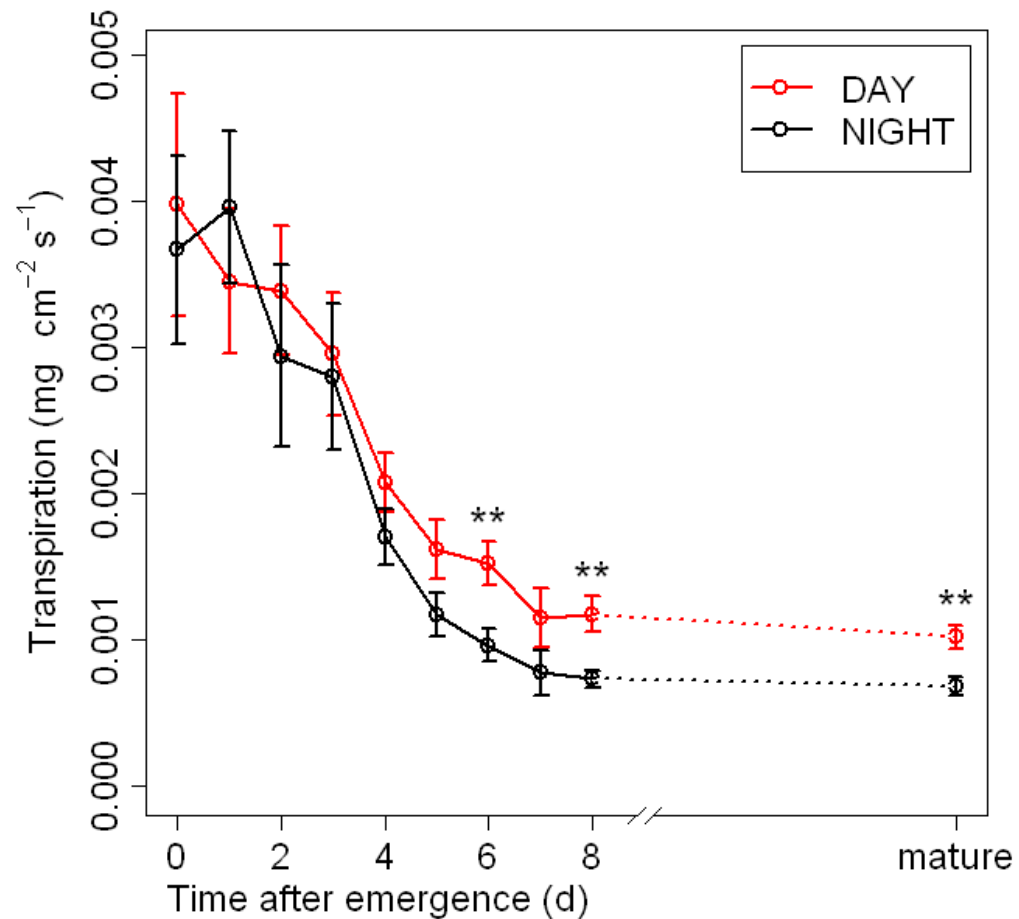
Leaf growth: establishment of hydraulic control

– II –

Why no difference between day and night
transpiration in the young leaf?
Putative role of stomata and cuticle

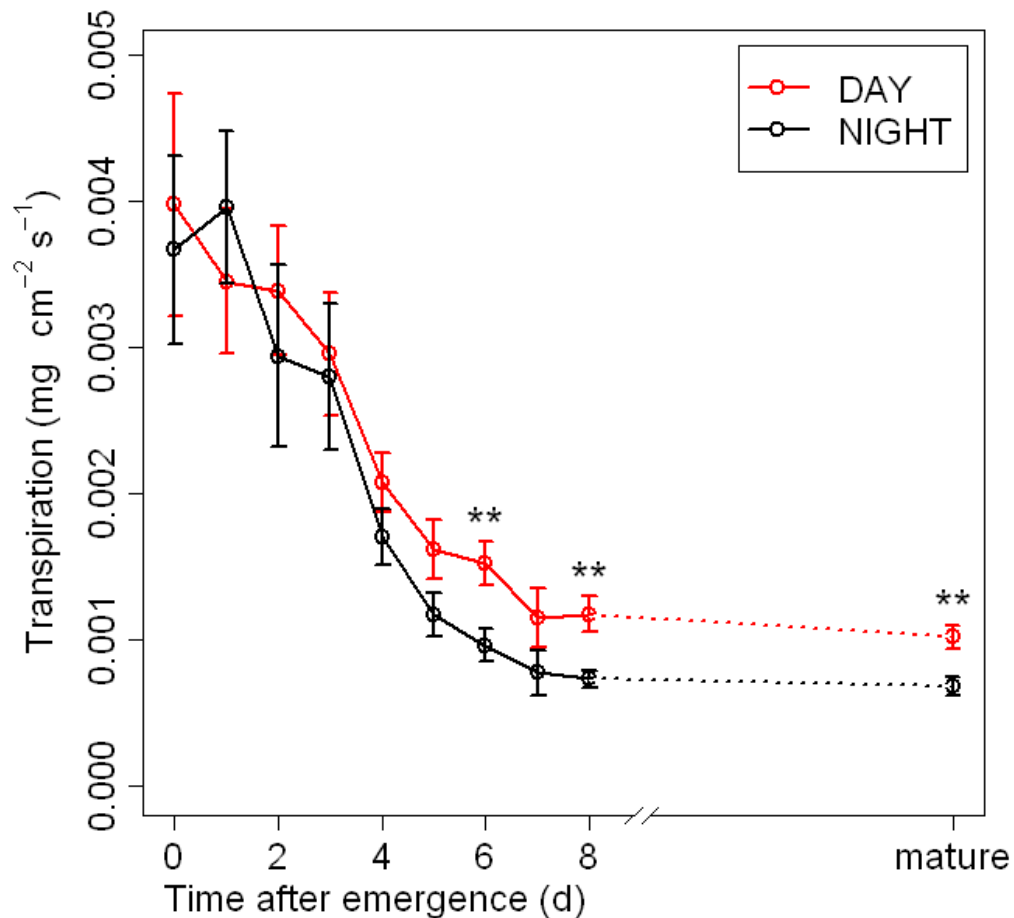
Changes in day/night difference of transpiration

In the early stages, why is transpiration higher than in later stages and not lower at night?



Changes in day/night difference of transpiration

In the early stages, why is transpiration higher than in later stages and not lower at night?

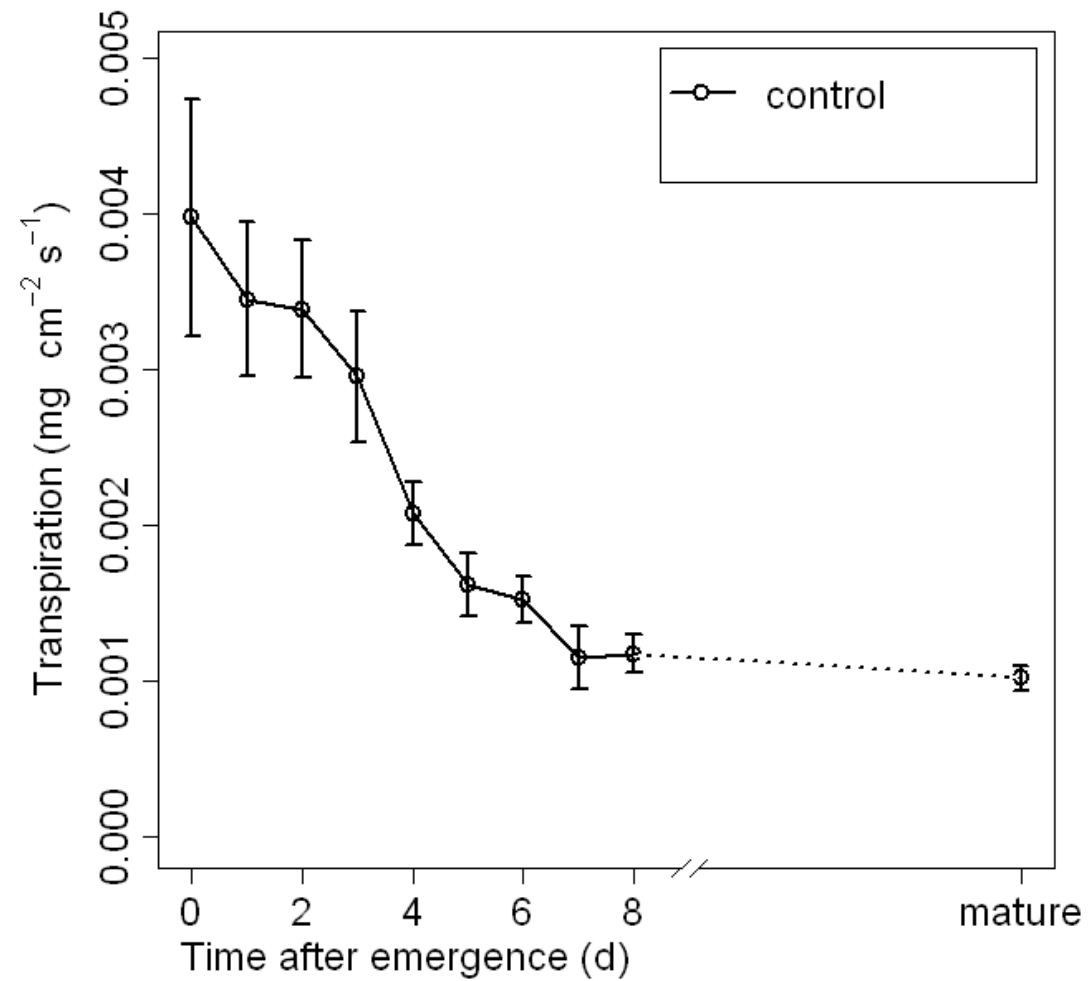


- Stomata contribution to transpiration is negligible in the early stages?

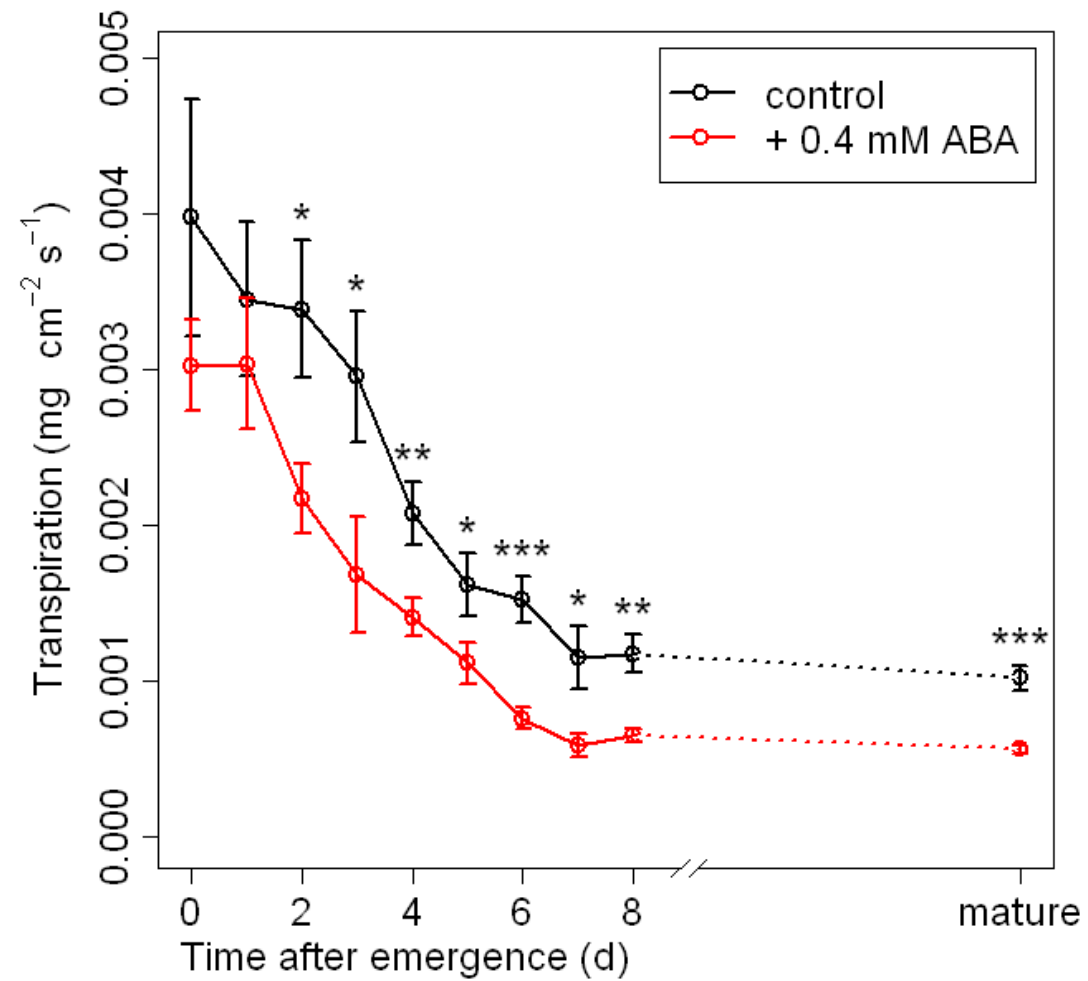
└ Young cuticle is more permeable (thickness? composition?)

- Stomata are always largely open in the early stages?

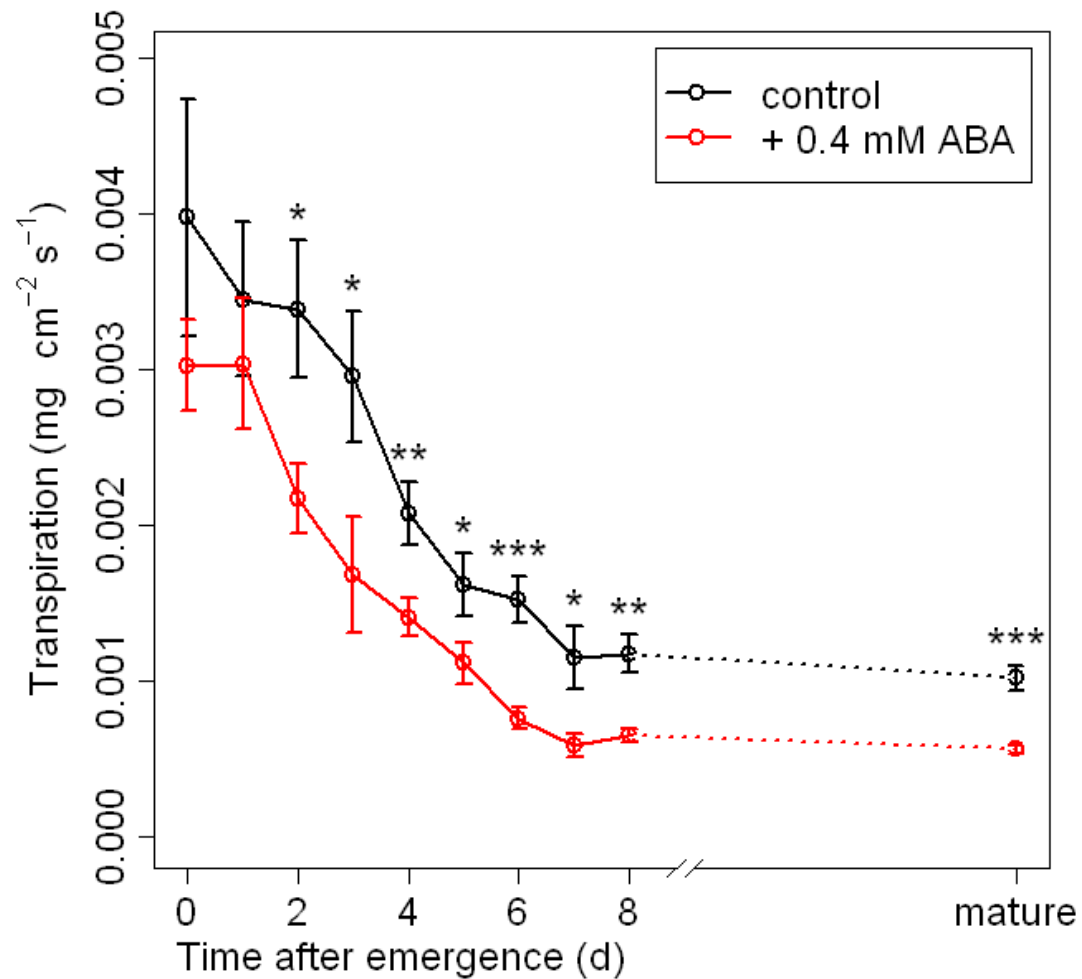
Response to abscisic acid



Response to abscisic acid



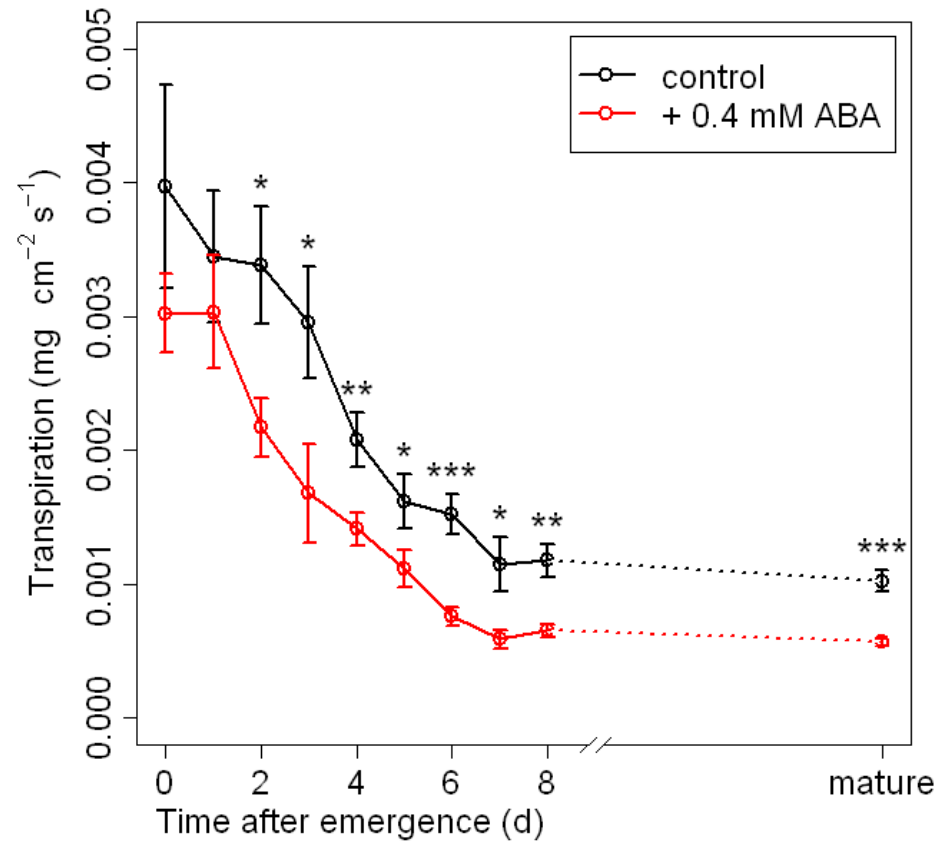
Response to abscisic acid



→ The effect of ABA in the early stages, even weak, is not consistent with a transpiration from the cuticle solely

Transpiration of an open stomata mutant

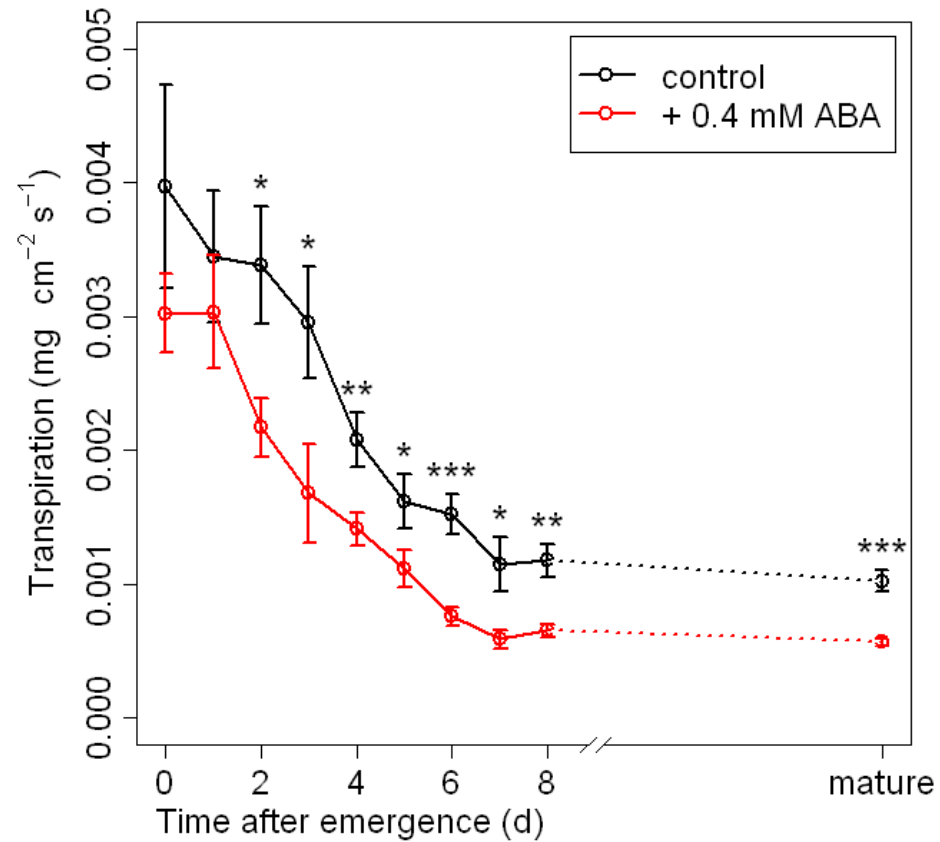
Wild-type Col-0



Transpiration of an open stomata mutant

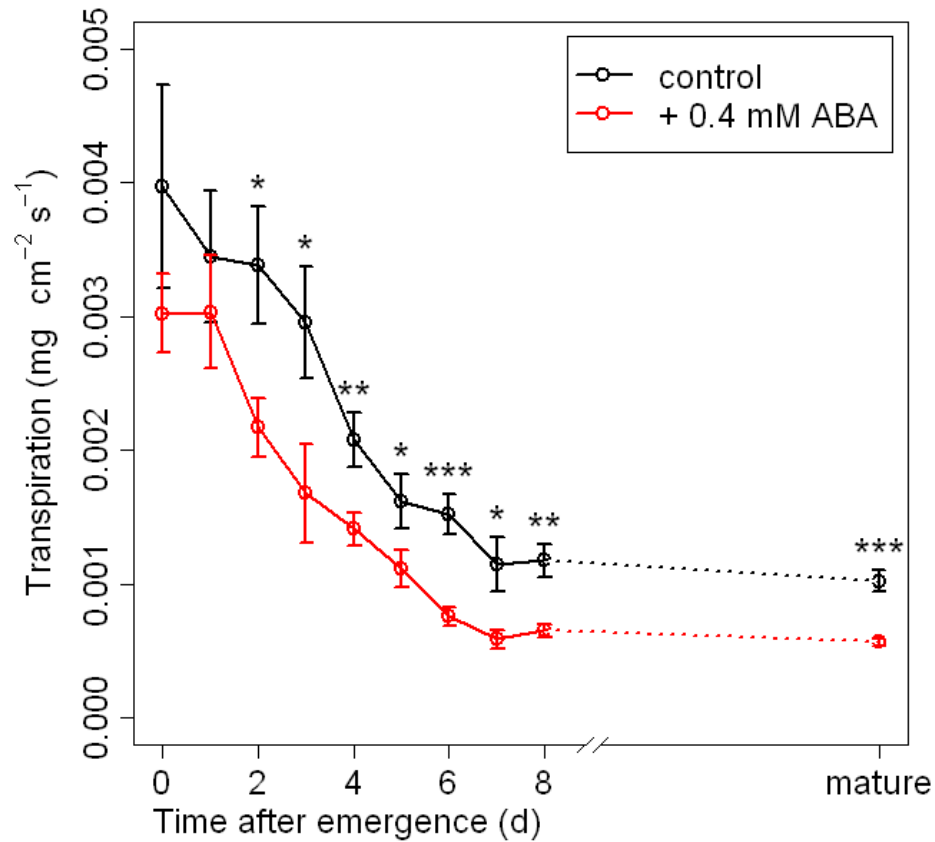
Wild-type Col-0

Open stomata *ost2* (ABA insensitive)

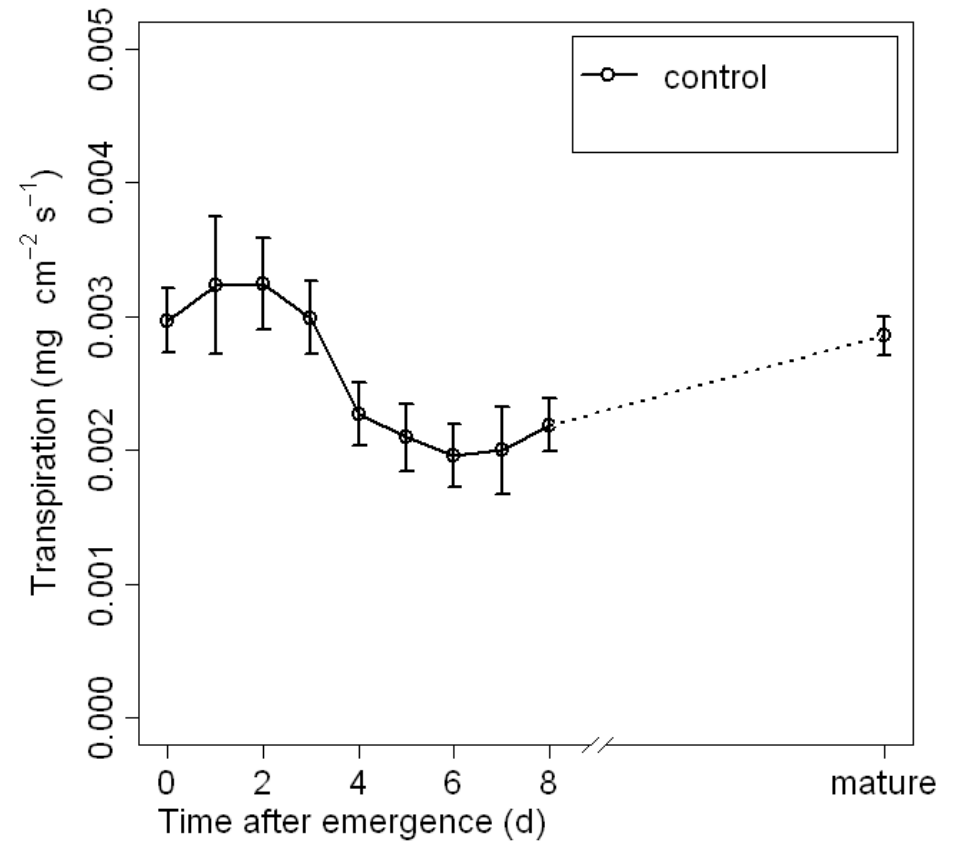


Transpiration of an open stomata mutant

Wild-type Col-0

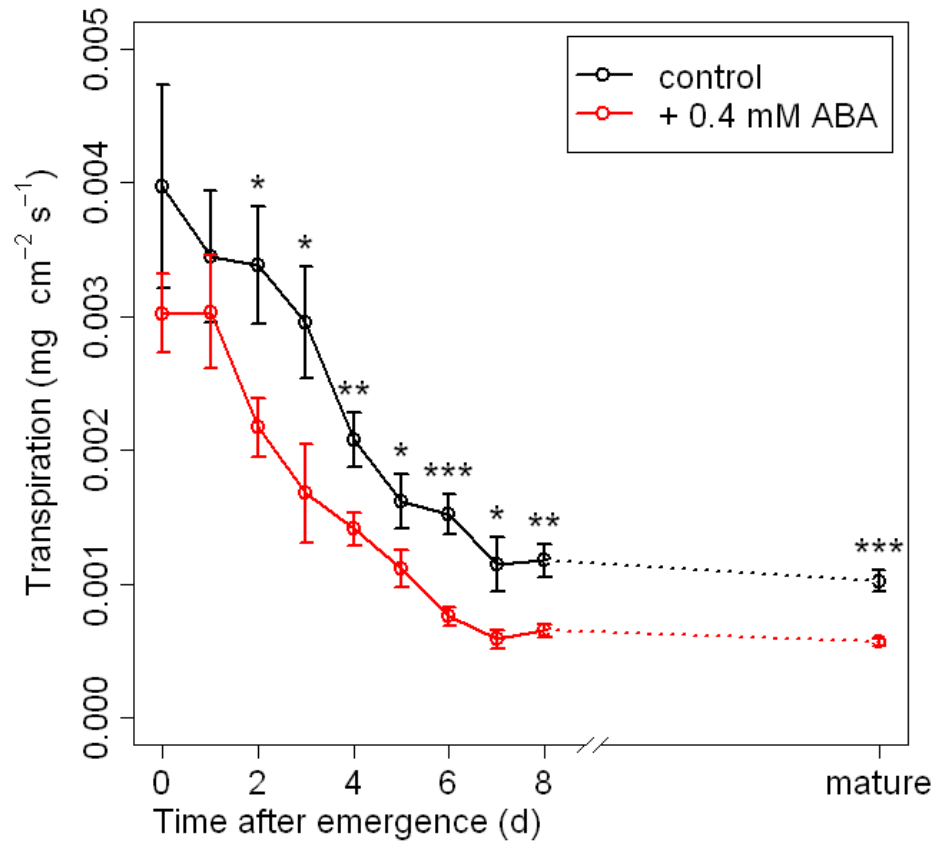


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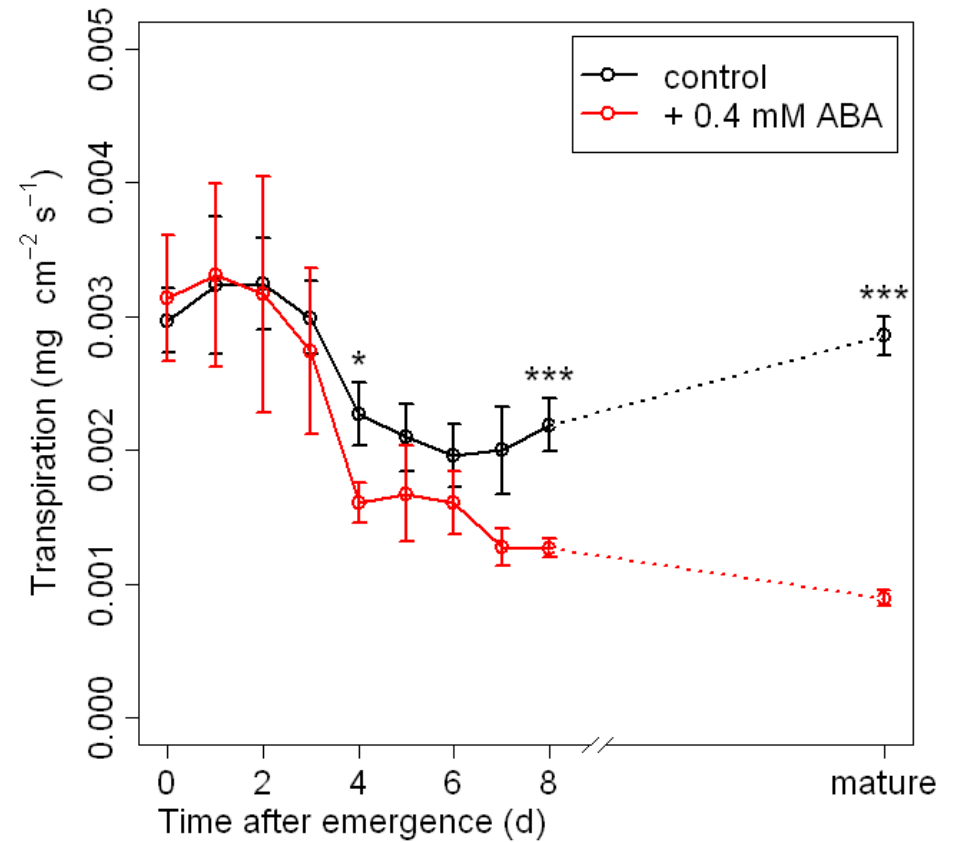


Transpiration of an open stomata mutant

Wild-type Col-0

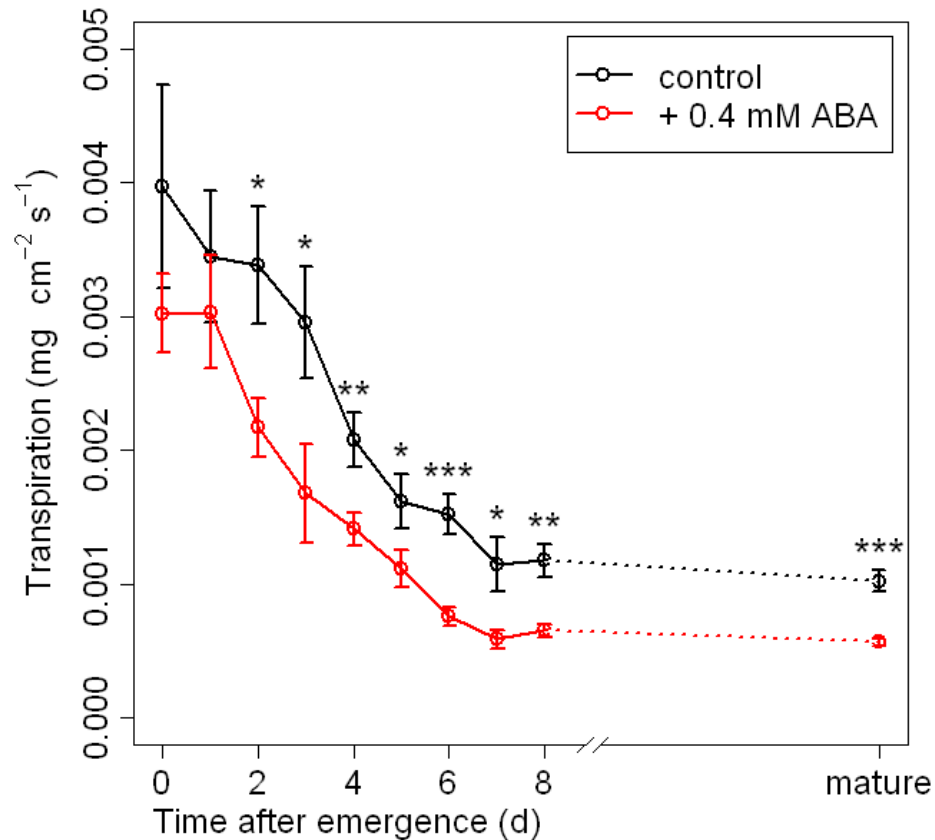


Open stomata *ost2* (ABA insensitive)

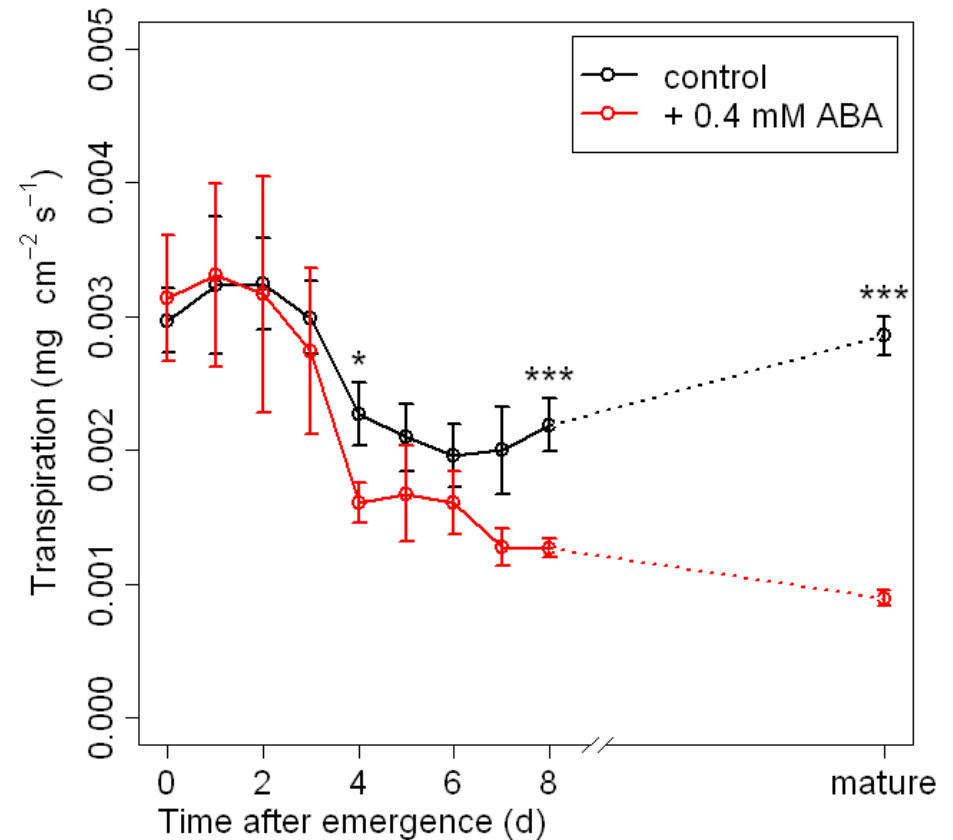


Transpiration of an open stomata mutant

Wild-type Col-0



Open stomata *ost2* (ABA insensitive)



A developmental trend in stomatal aperture in line with stomatal sensitivity or functioning?



Changes in day/night difference of transpiration

**In the early stages, why is transpiration higher than in later stages
and not lower at night?**

- Young stomata are insensitive?
- Young stomata are functional AND subjected to an endogenous stimulus?



Changes in day/night difference of transpiration

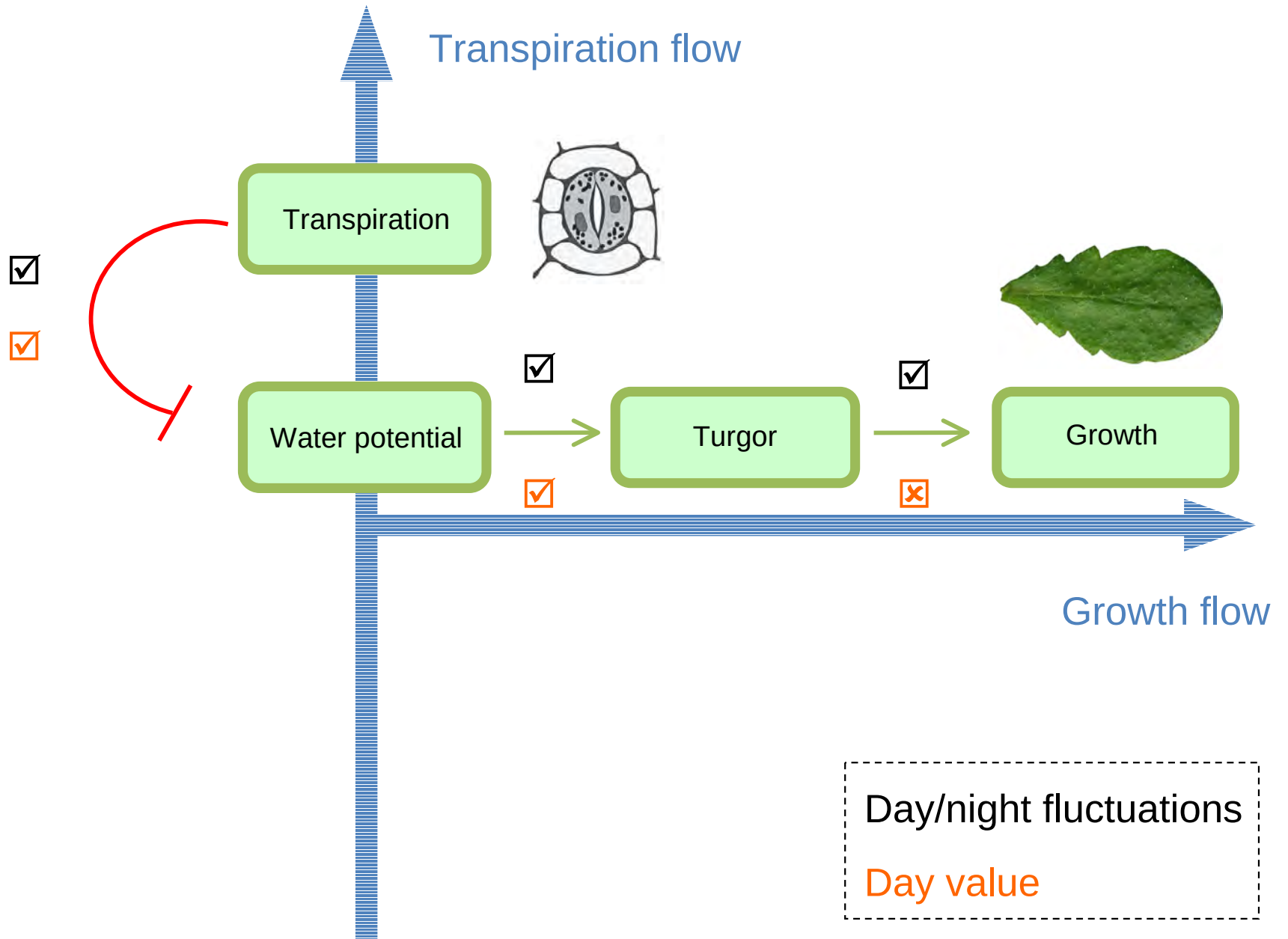
In the early stages, why is transpiration higher than in later stages and not lower at night?

- Young stomata are insensitive?
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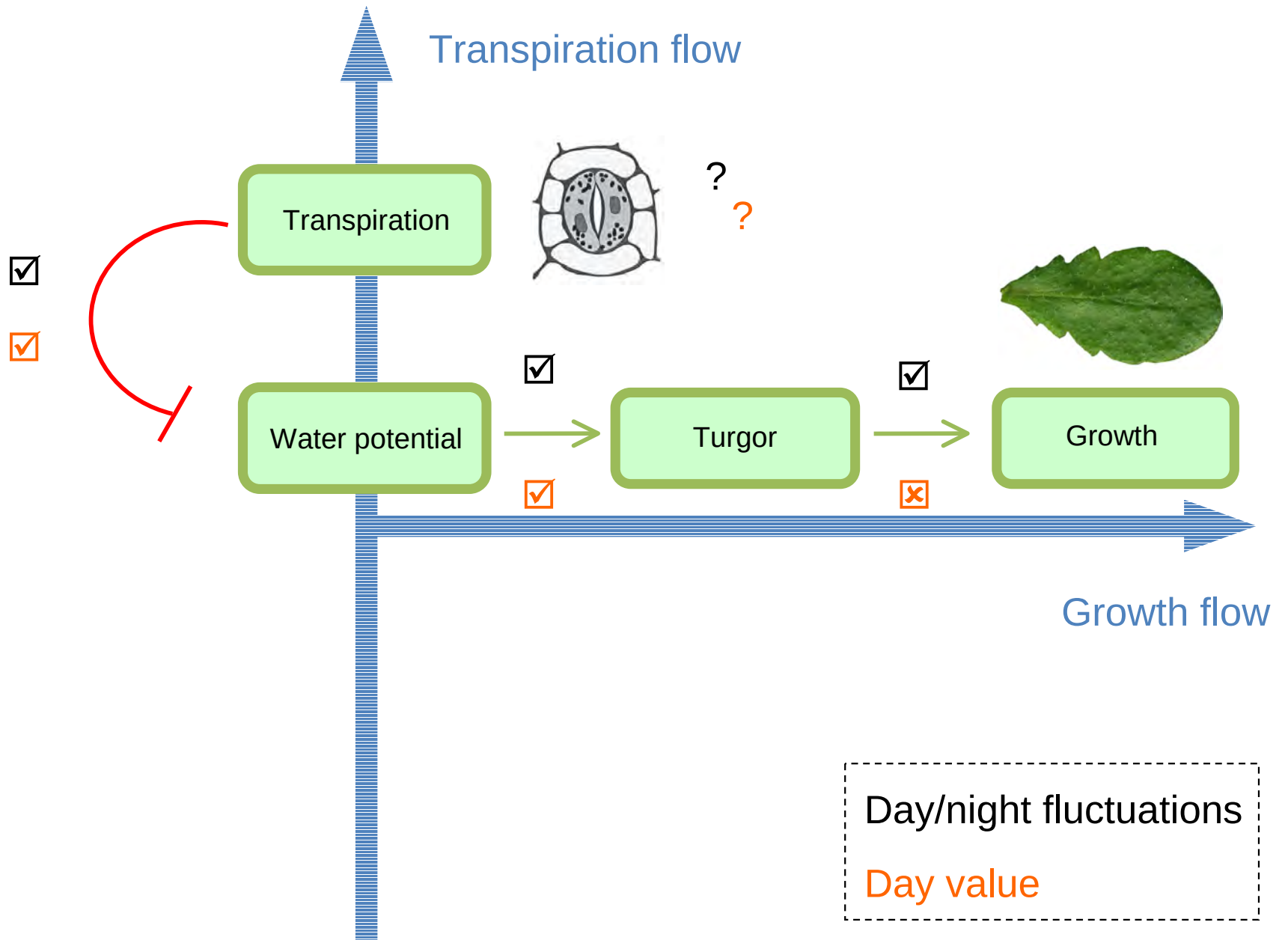
To be performed (Jeanne Renaud, M2):

- **Morphological observations** under cryoscanning electron microscopy look at native stomatal aperture
- Experiments on **epidermal strips** (coll. A. Vavasseur, CEA Cadarache) to evaluate a putative developmental acquisition of stomatal sensitivity to several effectors (fusaricocin, light, ABA, CO₂)
- **Day/night transpiration** on several mutants or environments to test the nature of the stimulus that would open the young stoma at night

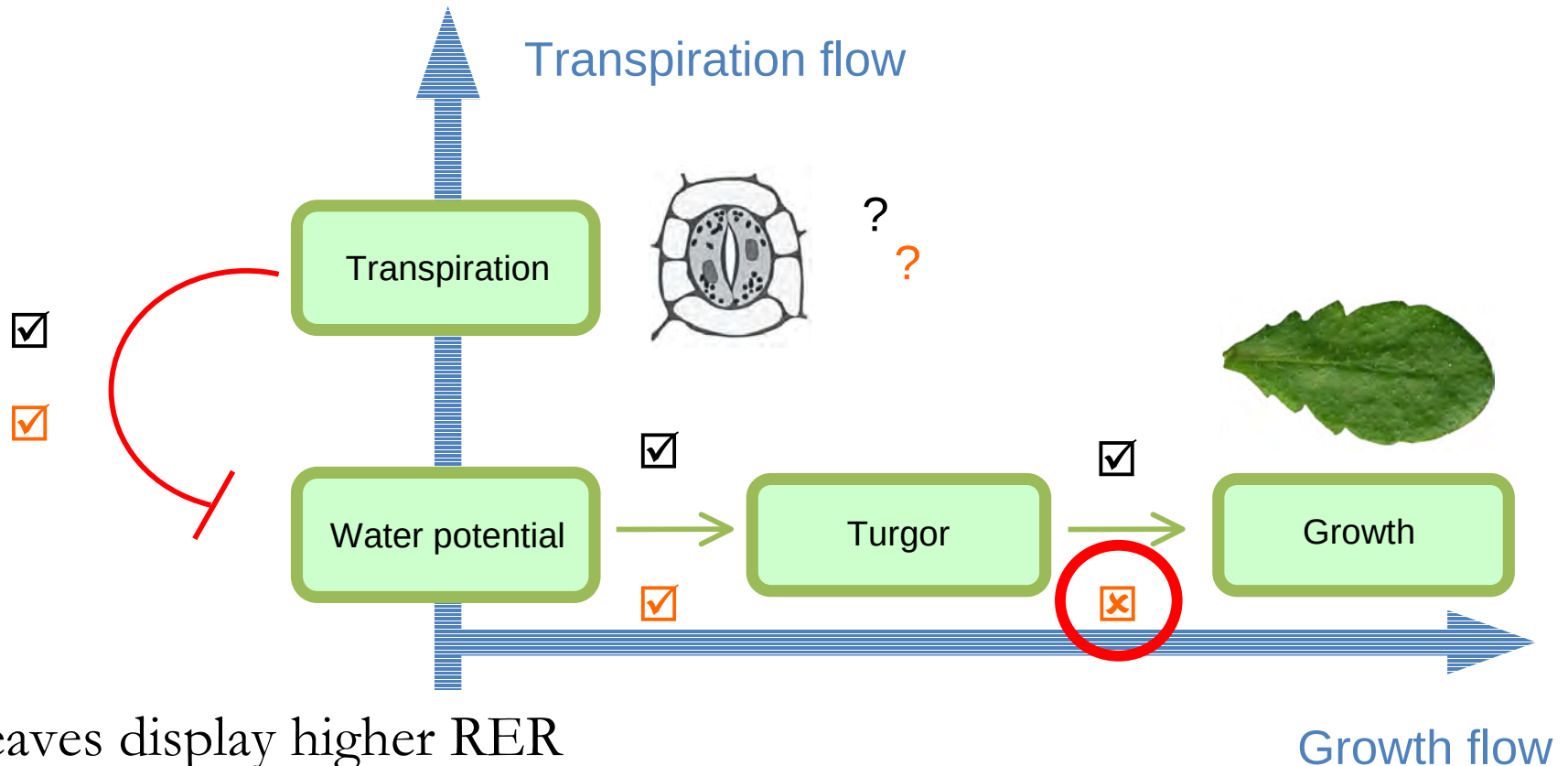
Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control



Young leaves display higher RER despite lower turgor and higher transpiration

- High wall extensibility allows expansion at lower water potentials?
- Transpiration is balanced by a high supply capacity?

Day/night fluctuations

Day value



Leaf growth: establishment of hydraulic control

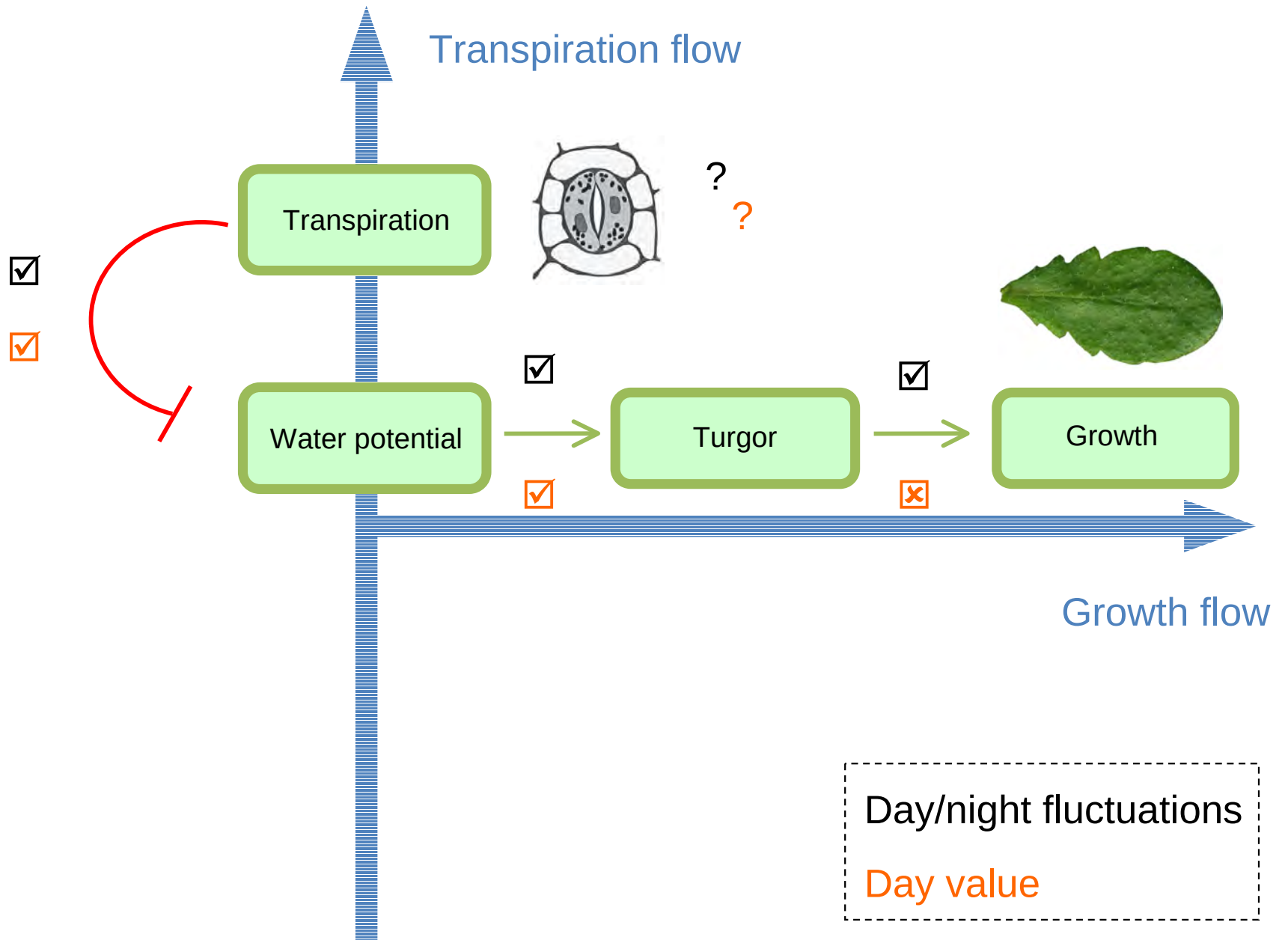
– III –

How does young leaf sustain high growth
rate despite high transpiration?

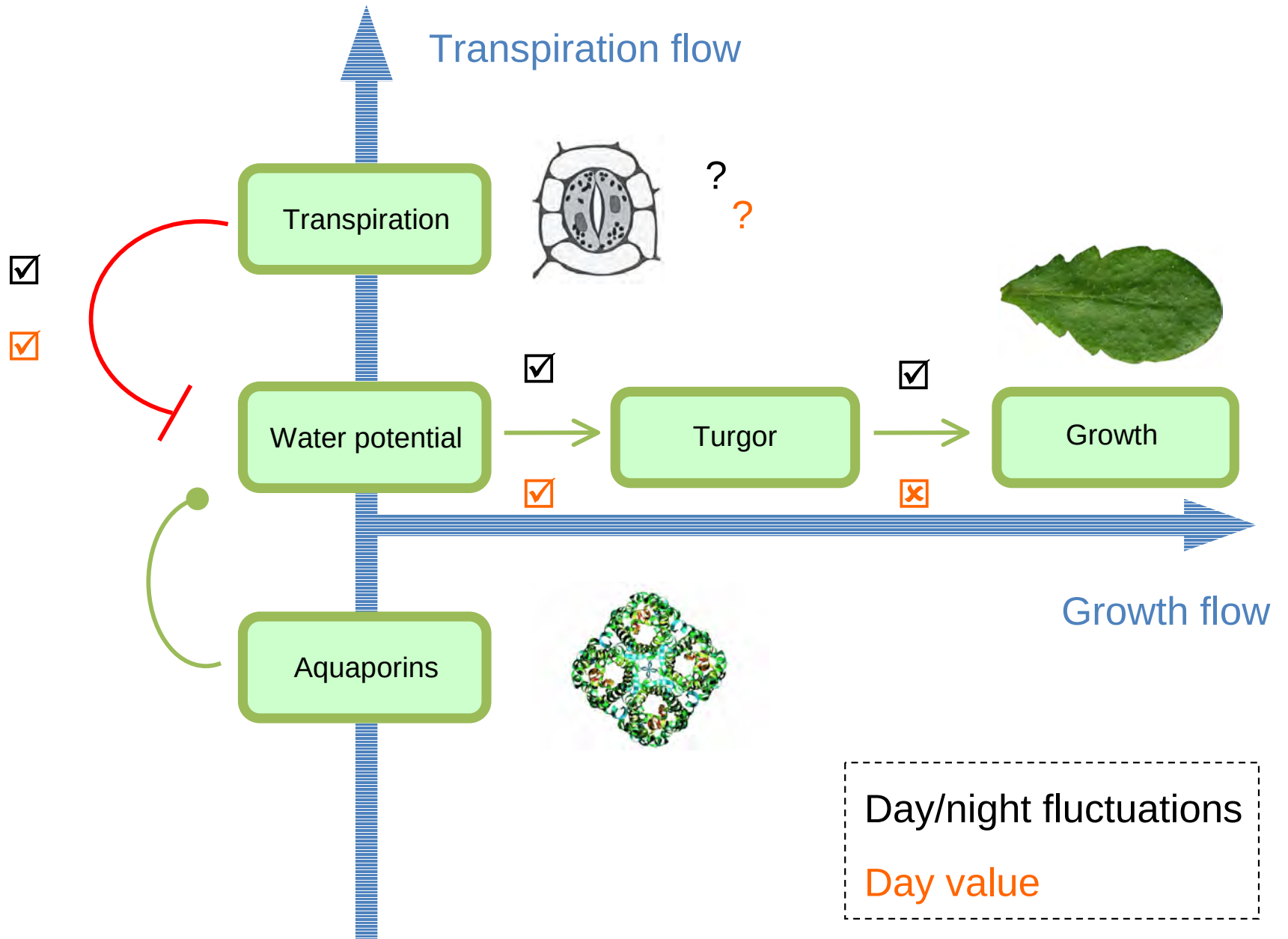
Contribution of xylem and aquaporins
to leaf hydraulic conductance

(preliminary results)

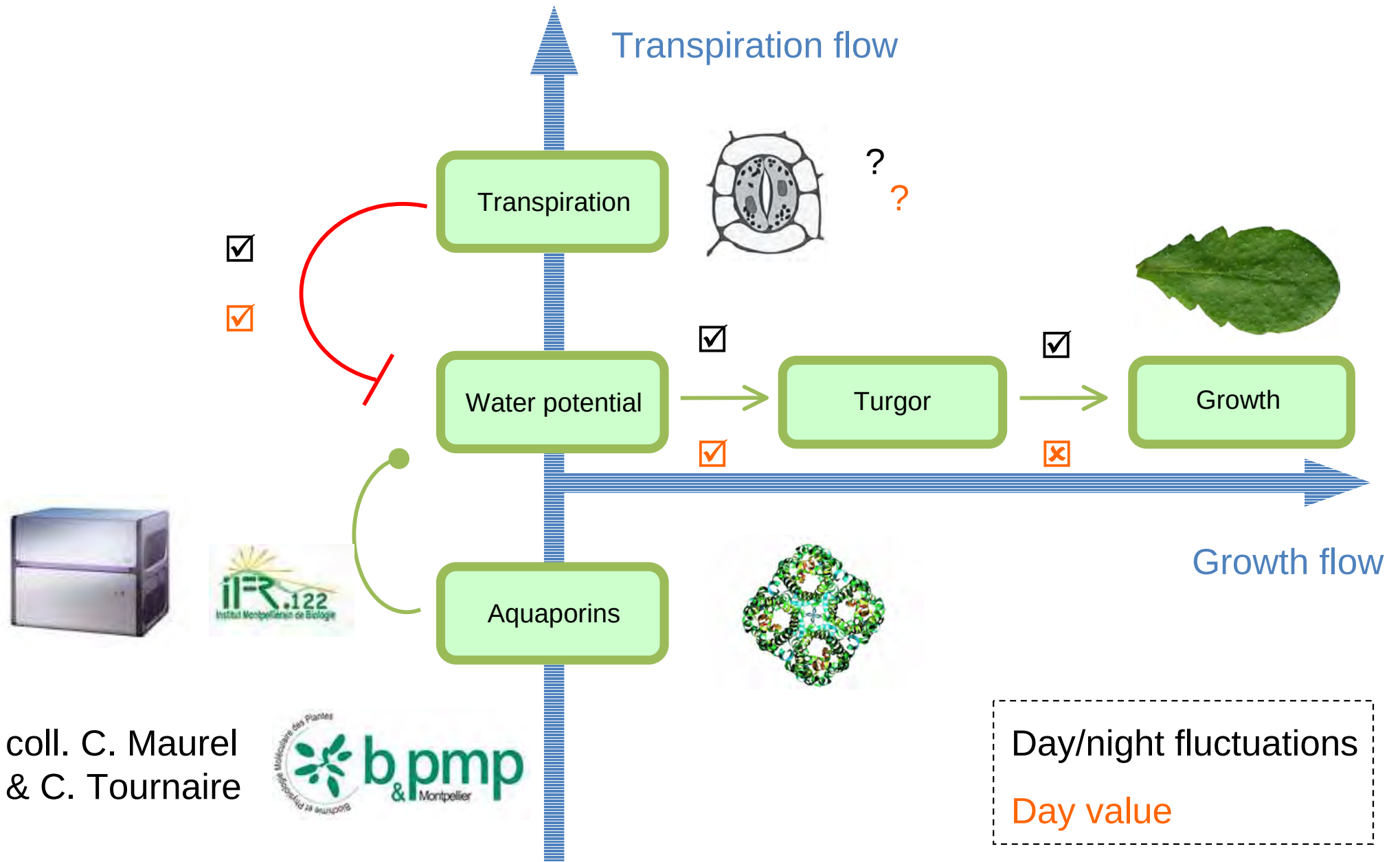
Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control

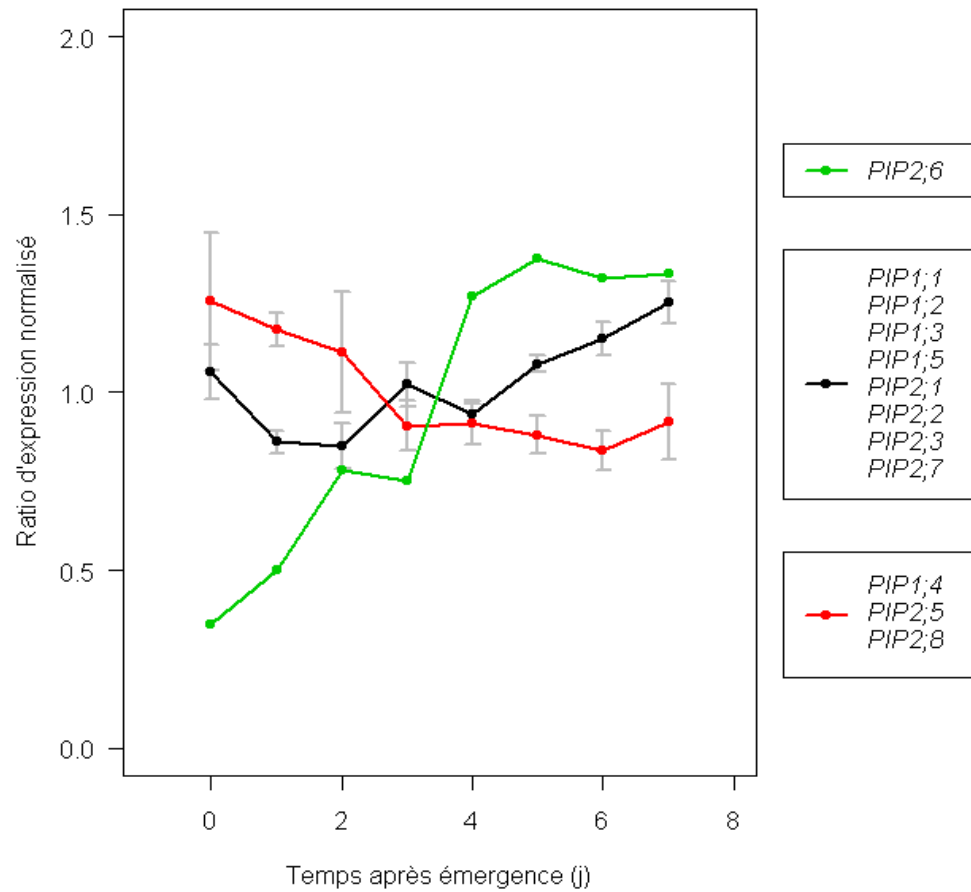


Leaf growth: establishment of hydraulic control



PIPs expression

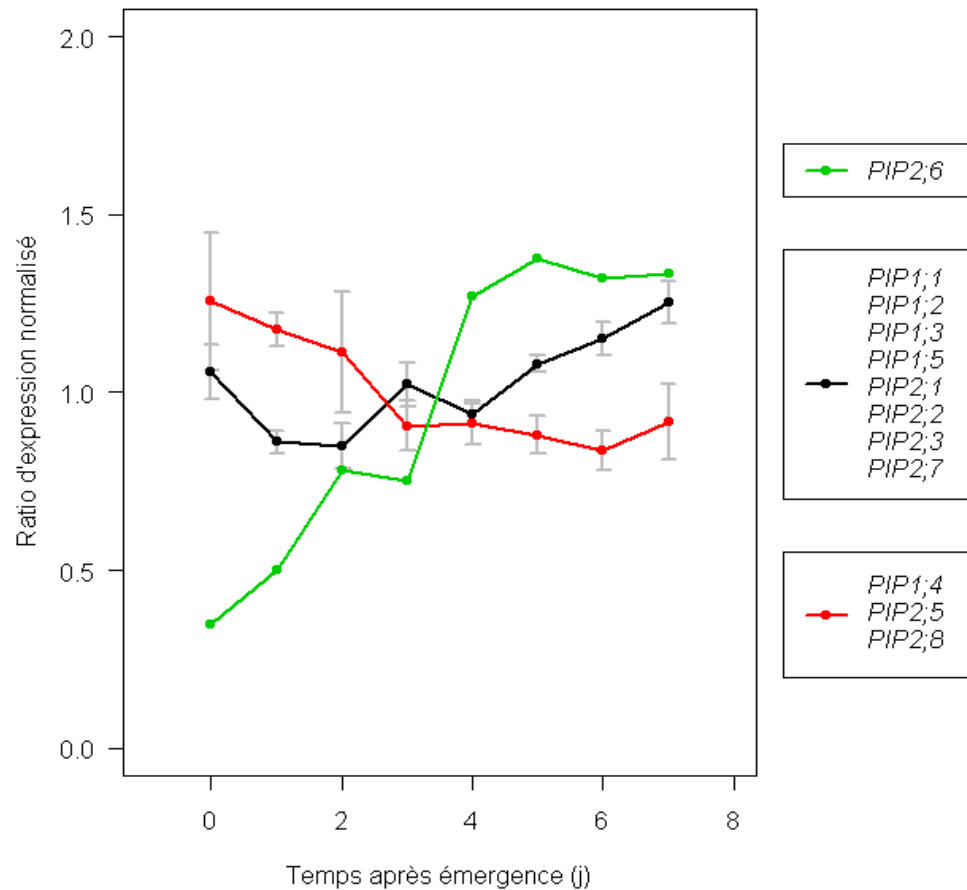
> *PIPs* are differentially expressed during leaf development



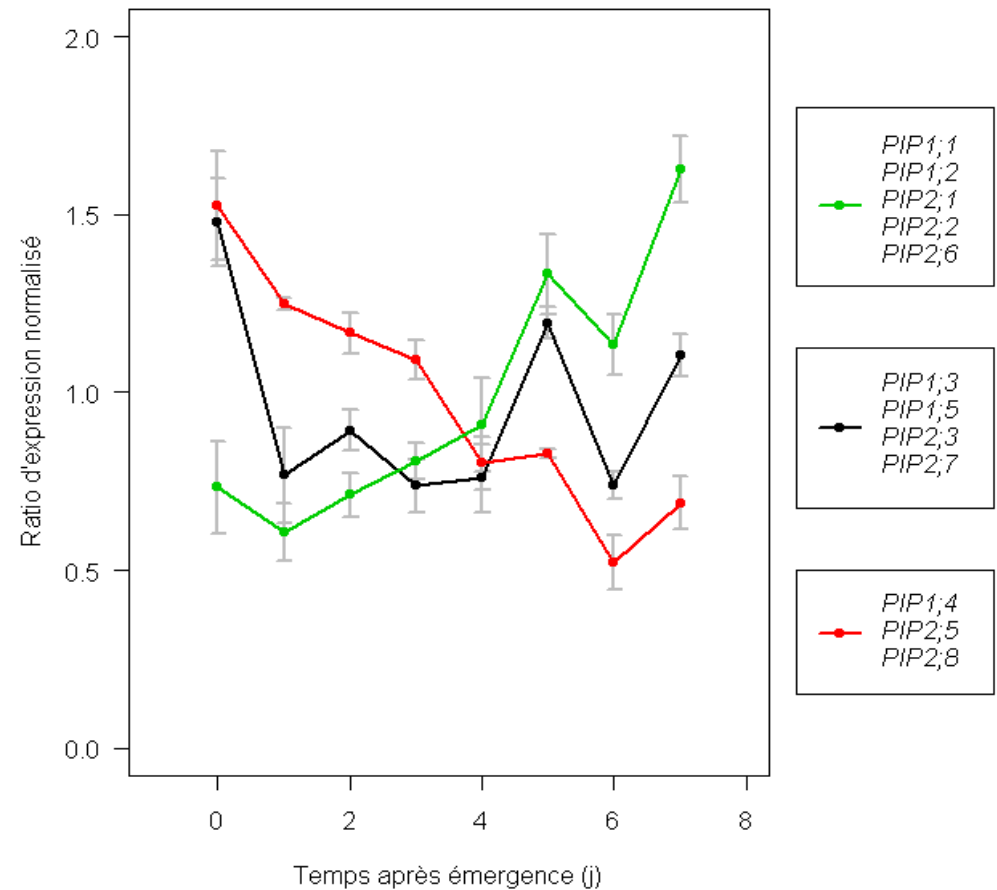
DAY

PIPs expression

- > *PIPs* are differentially expressed during leaf development
- > At night, "ascending" cluster is appended with 4 more *PIPs*



DAY



NIGHT

Set of *PIP*s mutants



Col-0(1)



Col-0(13)



PG4 (empty vector)



PIP2;1-OE(1)



PIP2;1-OE(2)



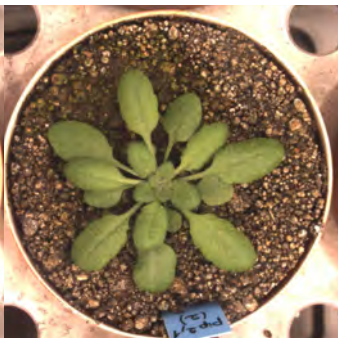
pip1;2(1)



pip1;2(2)



pip2;1(1)



pip2;1(2)



pip2;6(1)



pip2;6(2)



pip1;2pip2;1



pip1;2pip2;6



pip2;1pip2;6

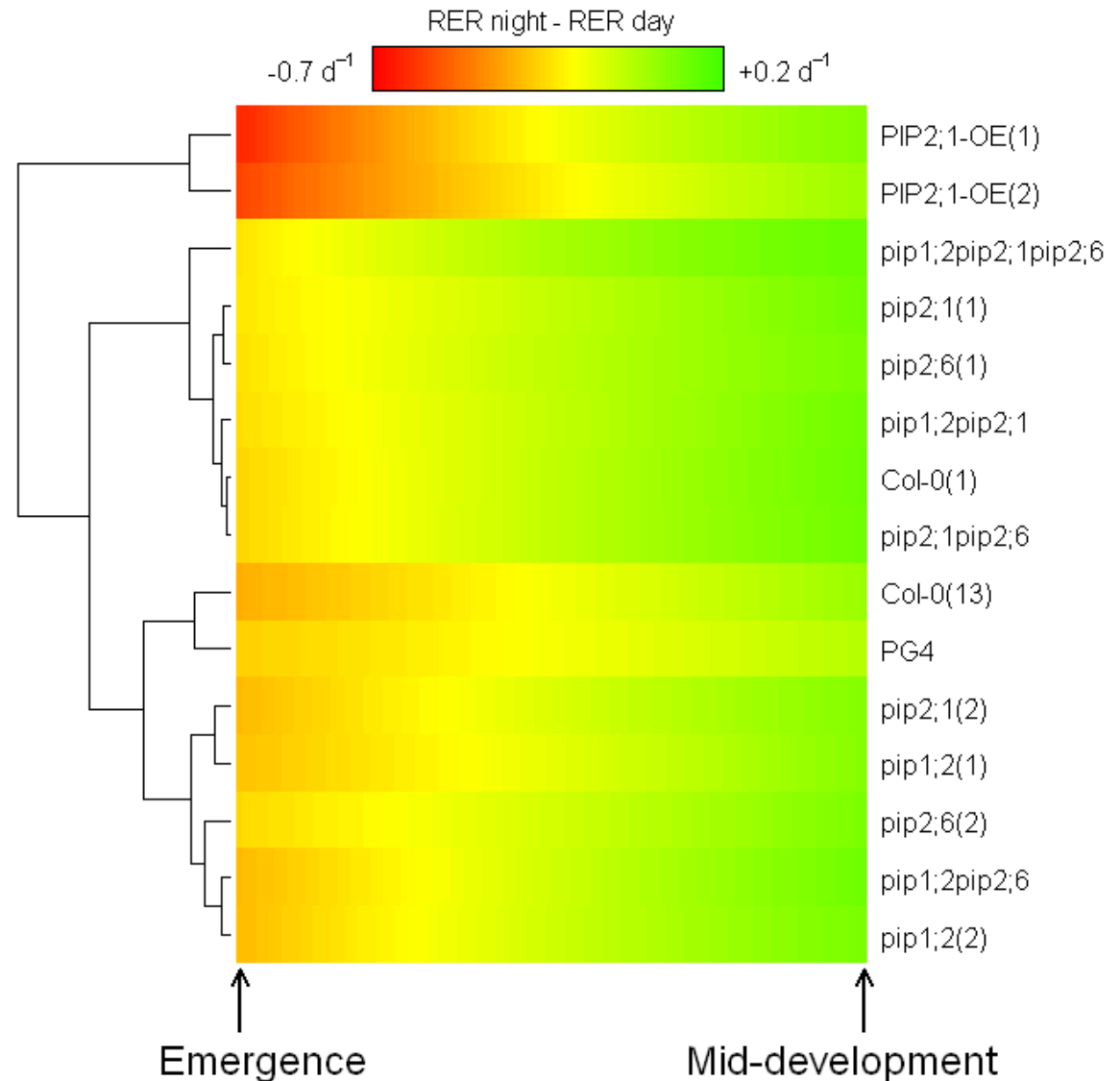


pip1;2pip2;1;pip2;6

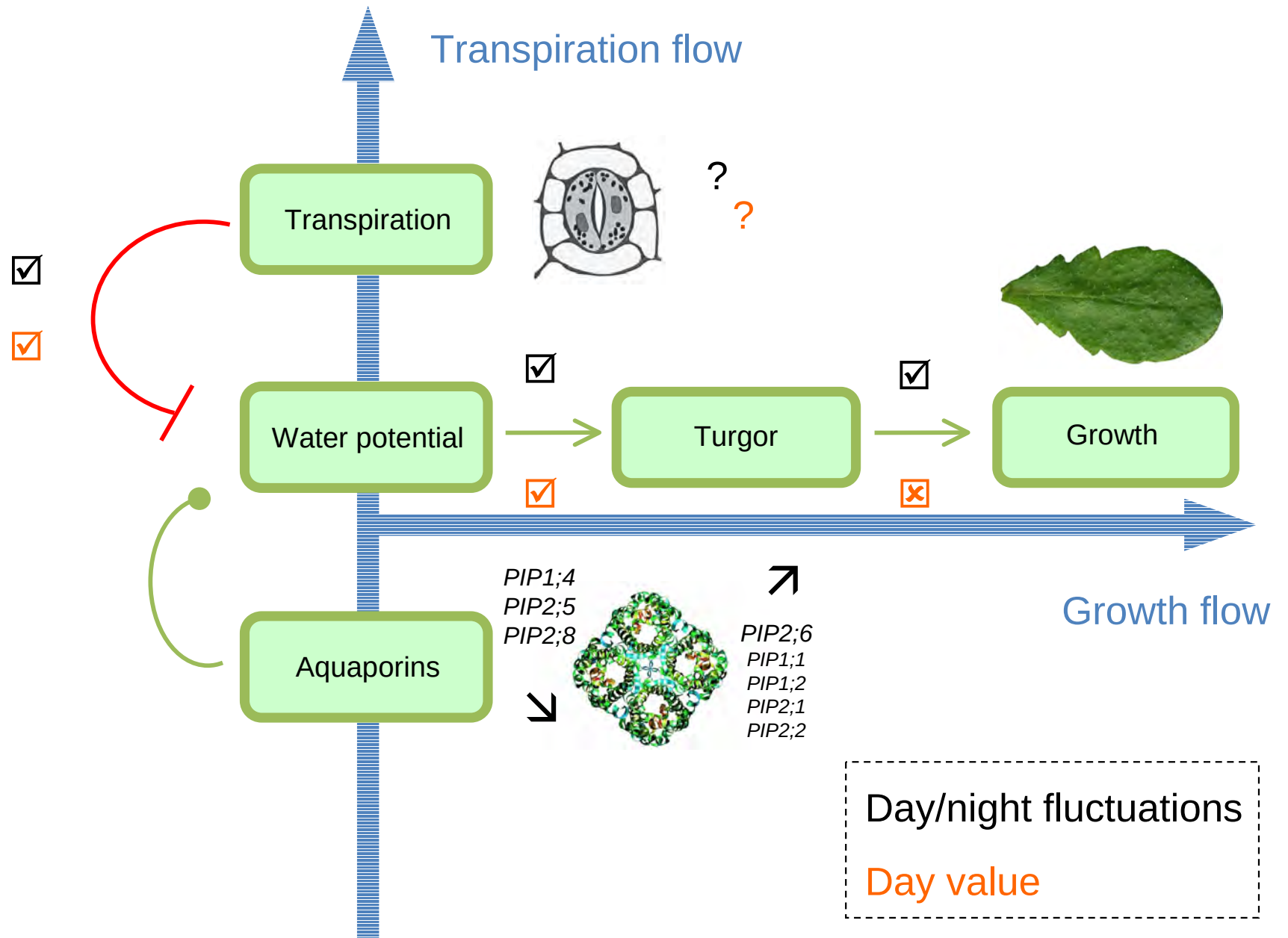
Leaf elongation of *PIP*s mutants

> No obvious response in the KOs, including double and triple mutants

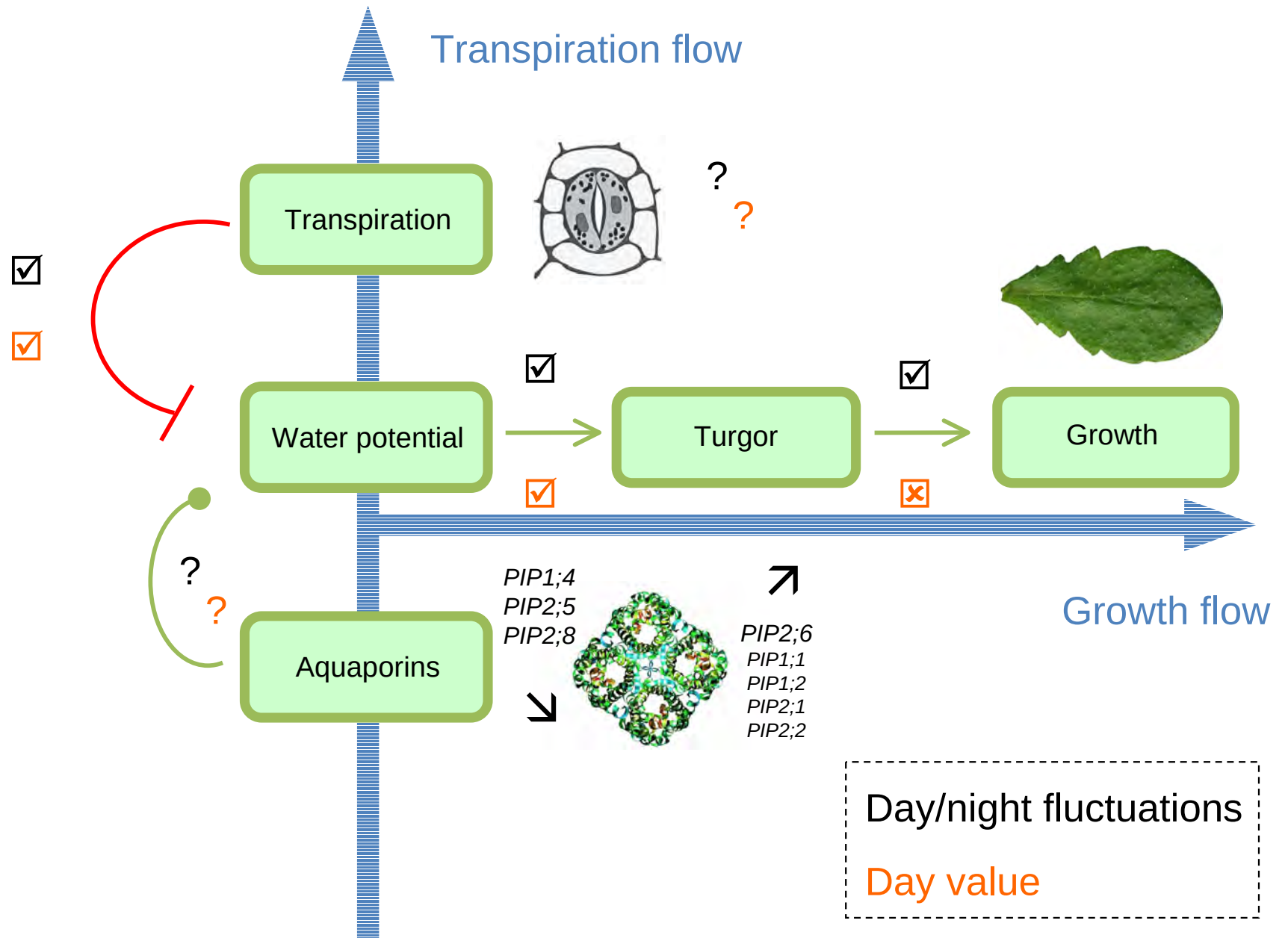
> ***PIP2;1-OEs*** have a distinct phenotype: early (metabolic) variations are amplified while later (hydraulic) oscillations are attenuated



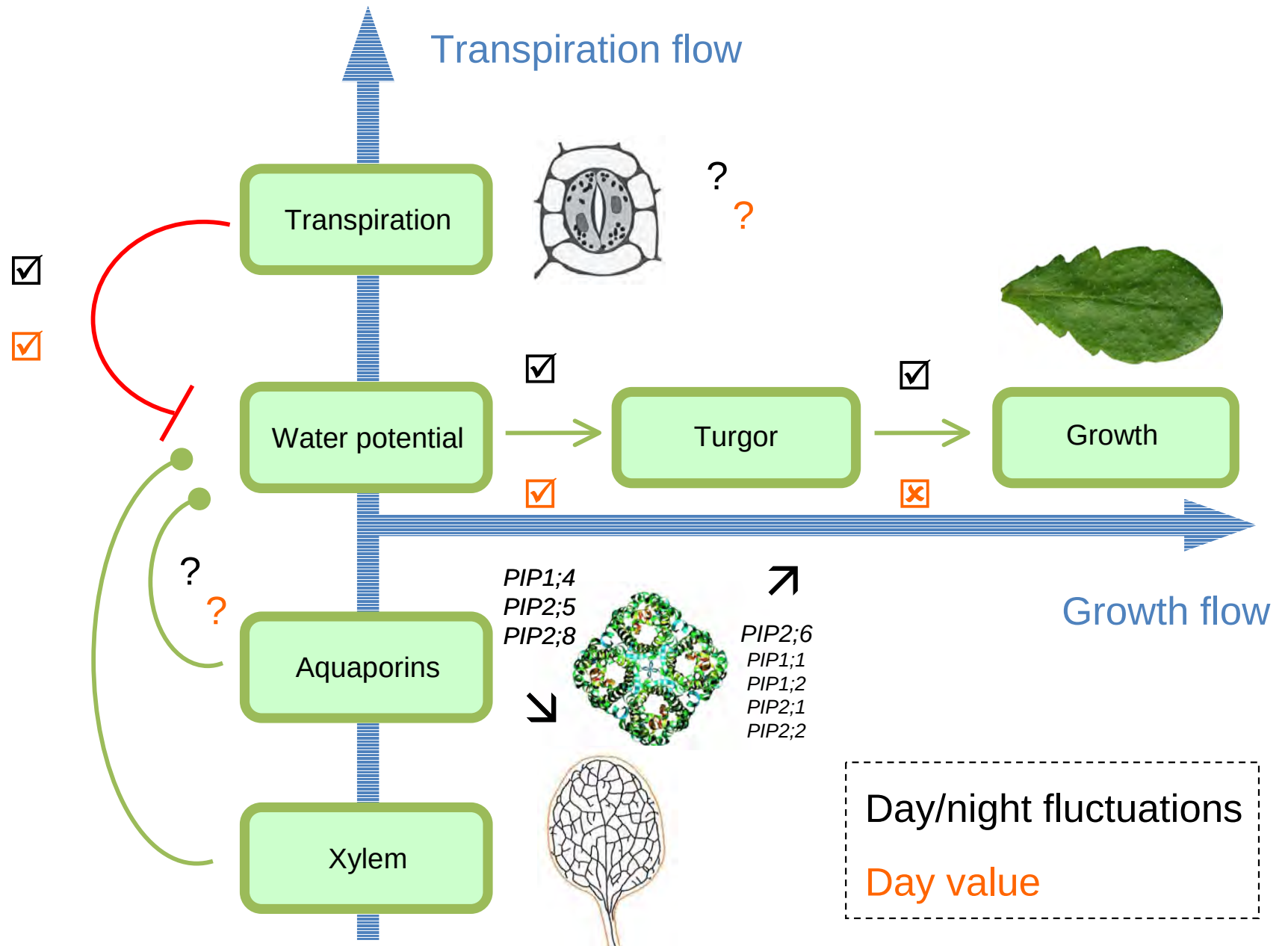
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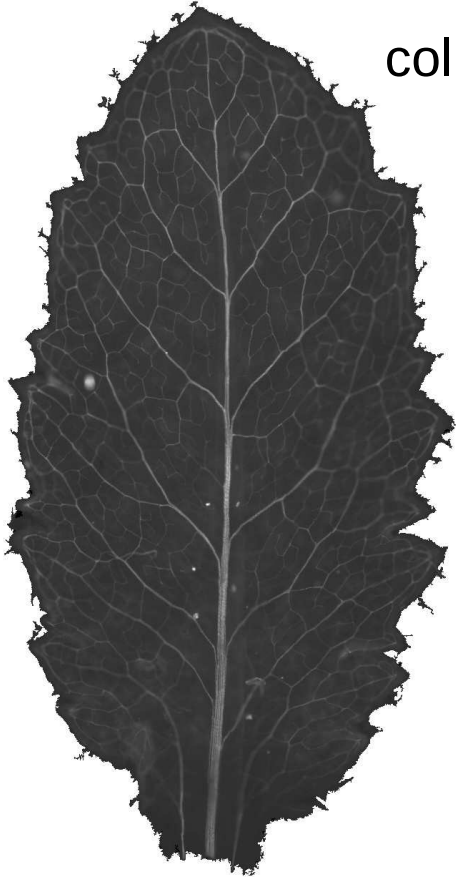
Xylem network



PHIV

Plate-forme
d'Histocytologie
et d'Imagerie cellulaire Végétale

coll. G. Conéjéro



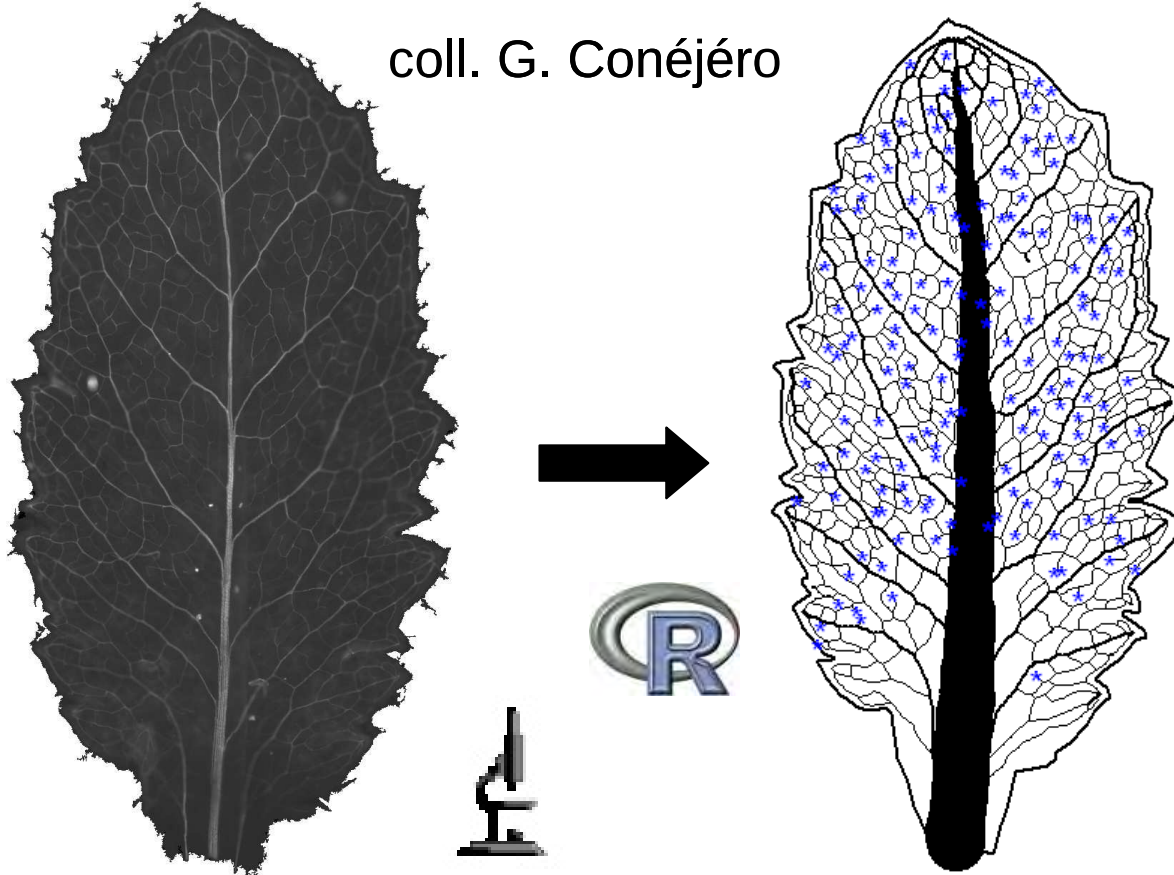
Epifluorescence
microscopy

Xylem network



Plate-forme
d'Histocytologie
et d'Imagerie cellulaire Végétale

coll. G. Conéjéro



Epifluorescence
microscopy

Network digitizing

Xylem network

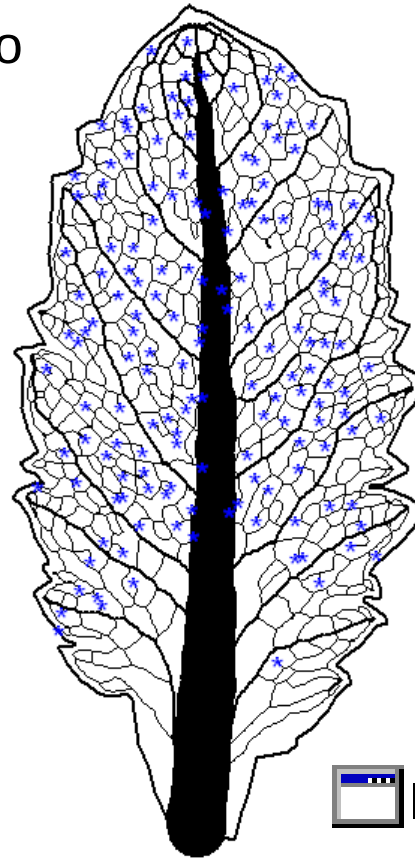
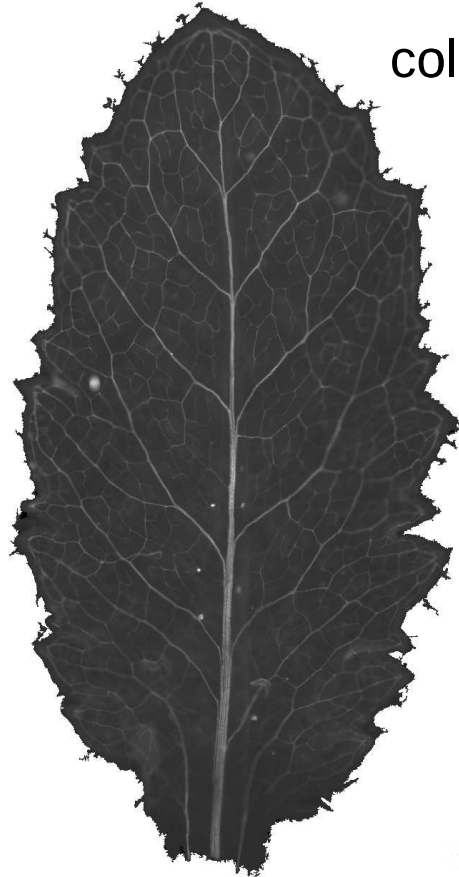


Plate-forme
d'Histocytologie
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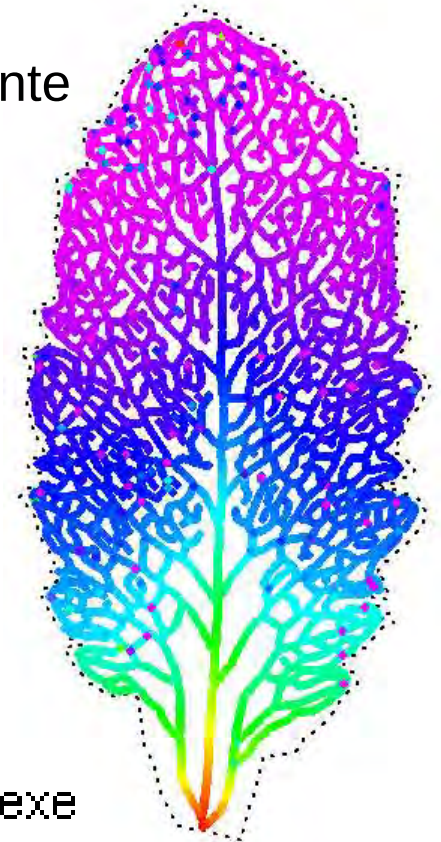


coll. G. Conéjéro

coll. A. Lacointe



Hydric!-Reseau.exe



-0.1 MPa  -8.8 MPa

Epifluorescence
microscopy

Network digitizing

Functional modeling

Xylem network

 Hydric!-Reseau.exe

- Developed in C++ by A. Lacointe to model sieve fluxes within complex architectures (including loops)
- Computes the exact solution of a system of equations following **Ohm's law** and **Kirchoff's node law** given a potential difference or a flux



Xylem network



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Model hypotheses

- Each xylem node is branched in series with a mesophyllic resistance followed by a stoma
- Stomatal and mesophyllic resistances are set very high
- Anatomy: each individual vessel has the same lumen diameter (to be checked in PHIV)

Xylem network

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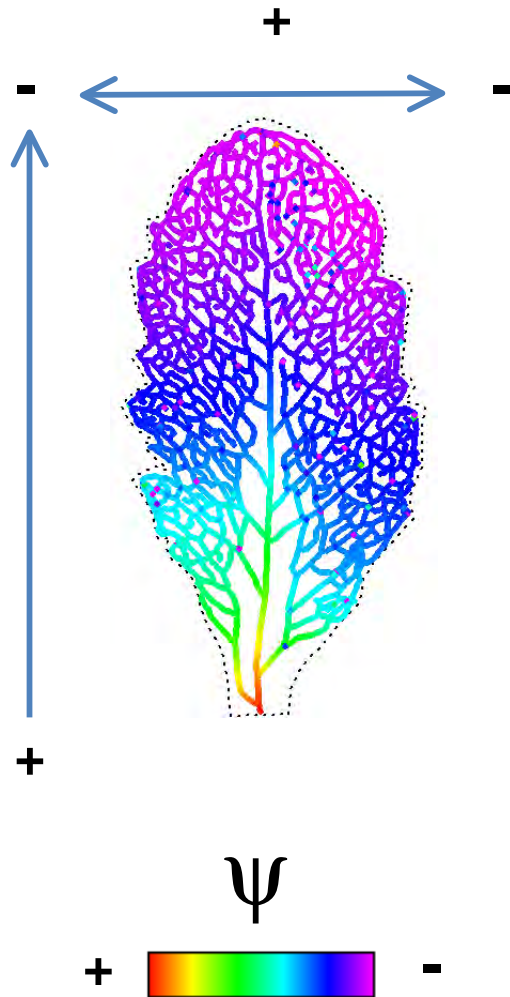
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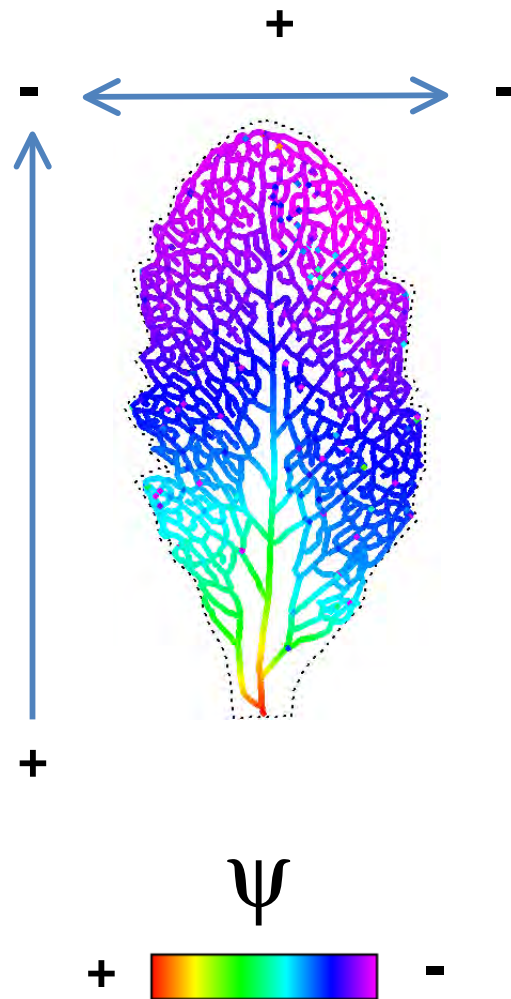
Xylem network

> A double potential gradient establishes spatially in the xylem



Xylem network

> A double potential gradient establishes spatially in the xylem

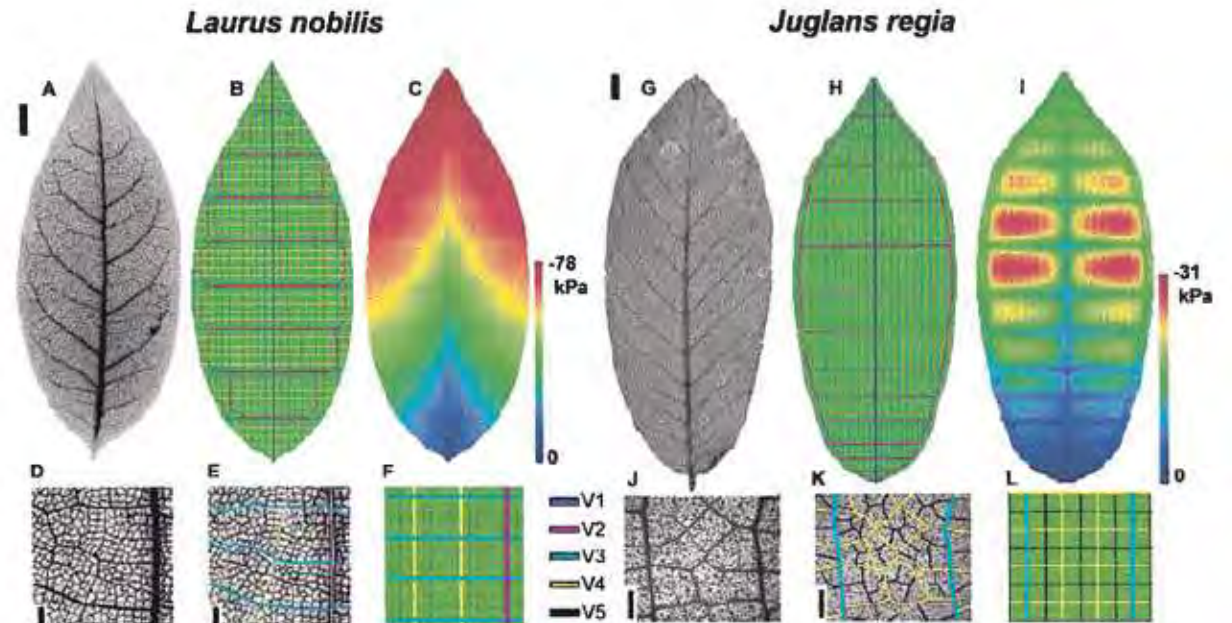


Plant, Cell and Environment (2004) 27, 1257–1267

Hydraulic architecture of leaf blades: where is the main resistance?

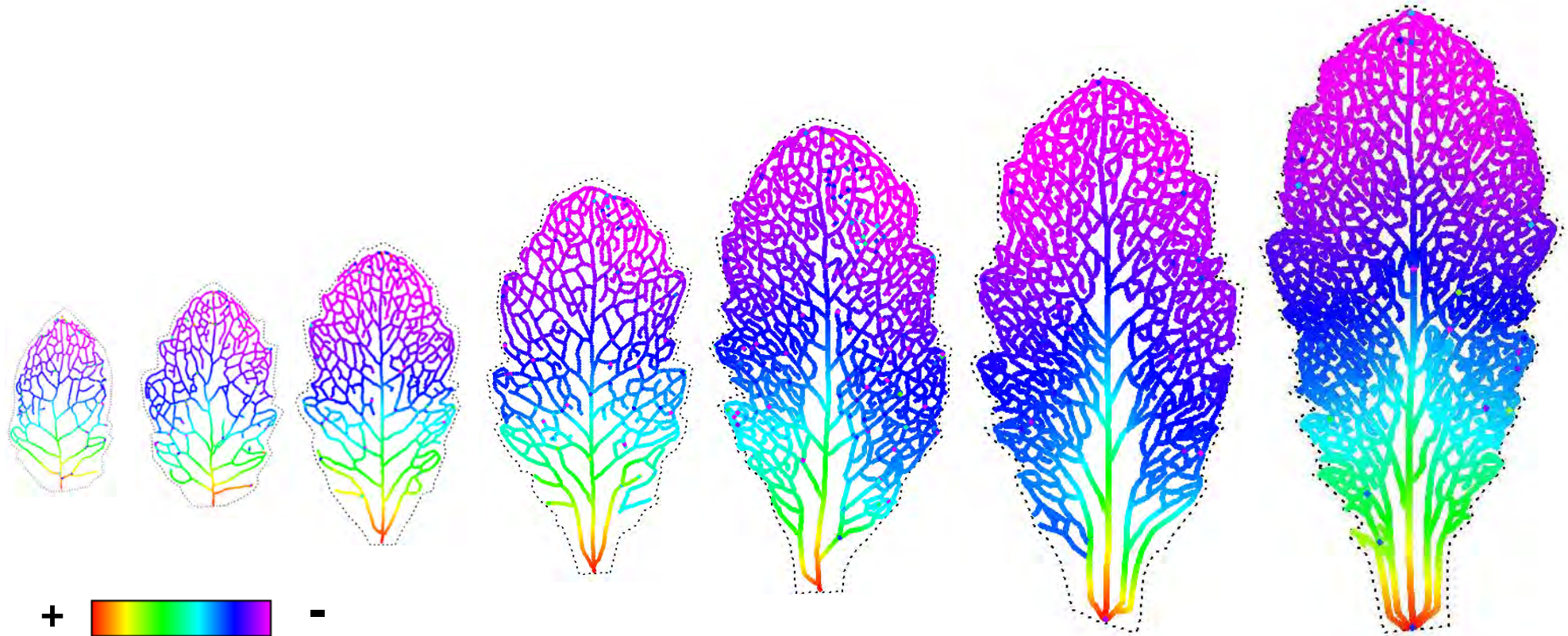
H. COCHARD¹, A. NARDINI² & L. COLL¹

¹UMR PIAF, Site INRA de Crouel, 63039 Clermont-Ferrand Cedex 2, France and ²Dipartimento di Biologia, Università di Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy



Xylem network

> This potential gradient is conserved during leaf development



> Absolute values are unrealistic because it would require development-dependent parameterization (stomatal and mesophyllic resistance, growth-induced water potential), but it does not influence the xylem conductance values



Xylem network

> Apparent xylem conductance is computed as

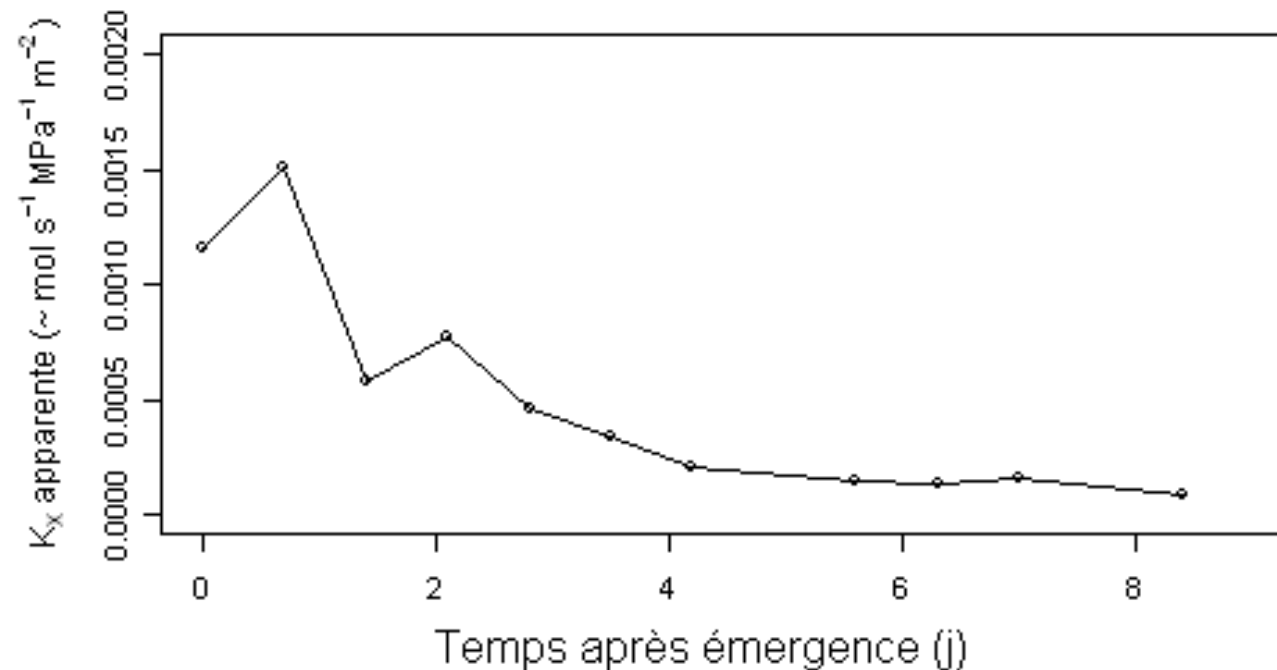
$$K_{\text{xylem}} = \frac{\text{Transpiration}}{\Psi_{\text{petiole}} - \frac{\sum_{i=1}^n \Psi_{\text{node } i}}{n}} \times \frac{1}{\text{Leaf area}}$$

Xylem network

> Apparent xylem conductance is computed as

$$K_{\text{xylem}} = \frac{\text{Transpiration}}{\Psi_{\text{petiole}} - \frac{\sum_{i=1}^n \Psi_{\text{node } i}}{n}} \times \frac{1}{\text{Leaf area}}$$

> Apparent xylem conductance decreases during leaf development



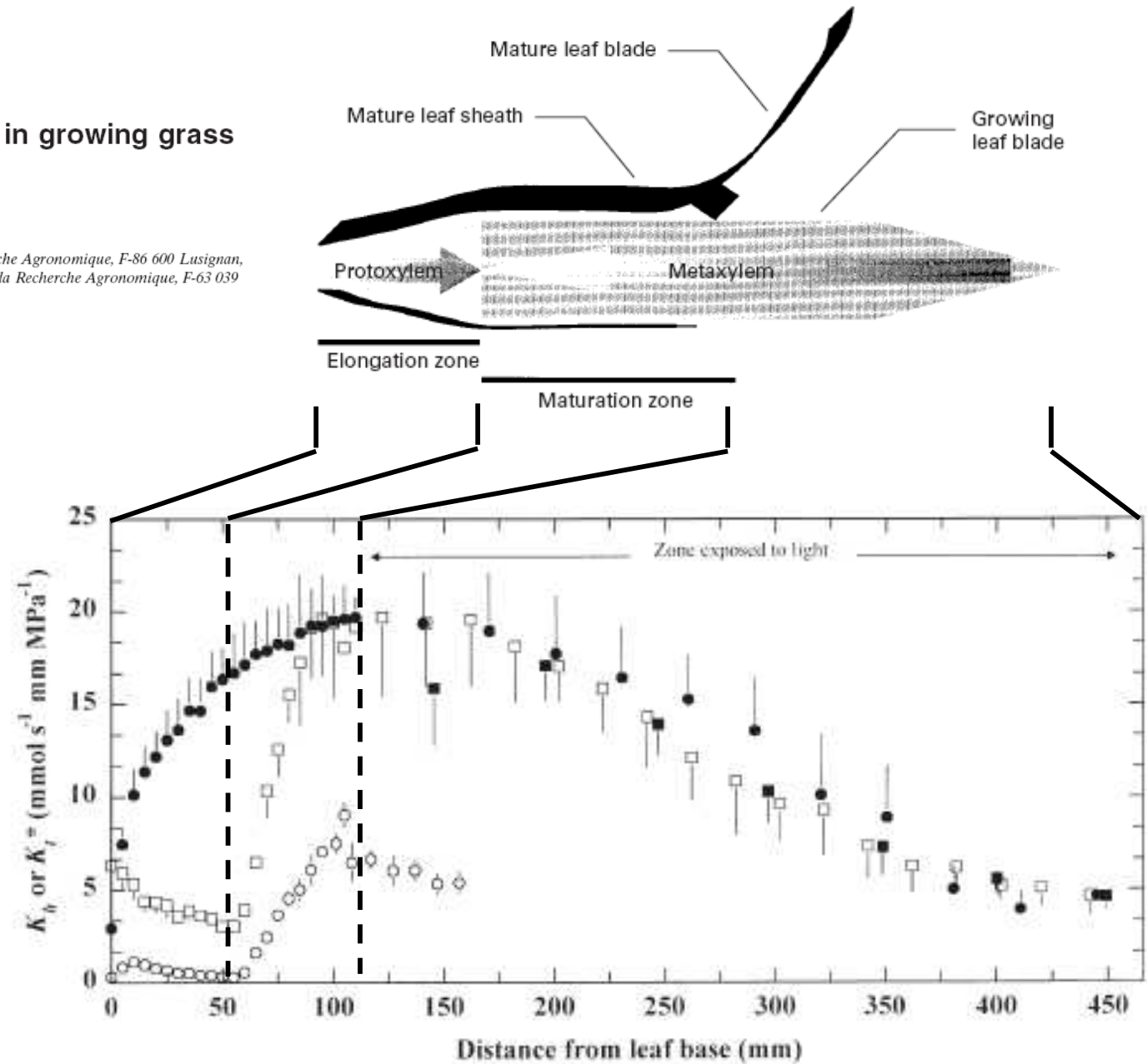
Xylem network

Plant, Cell and Environment (2001) 24, 65–76

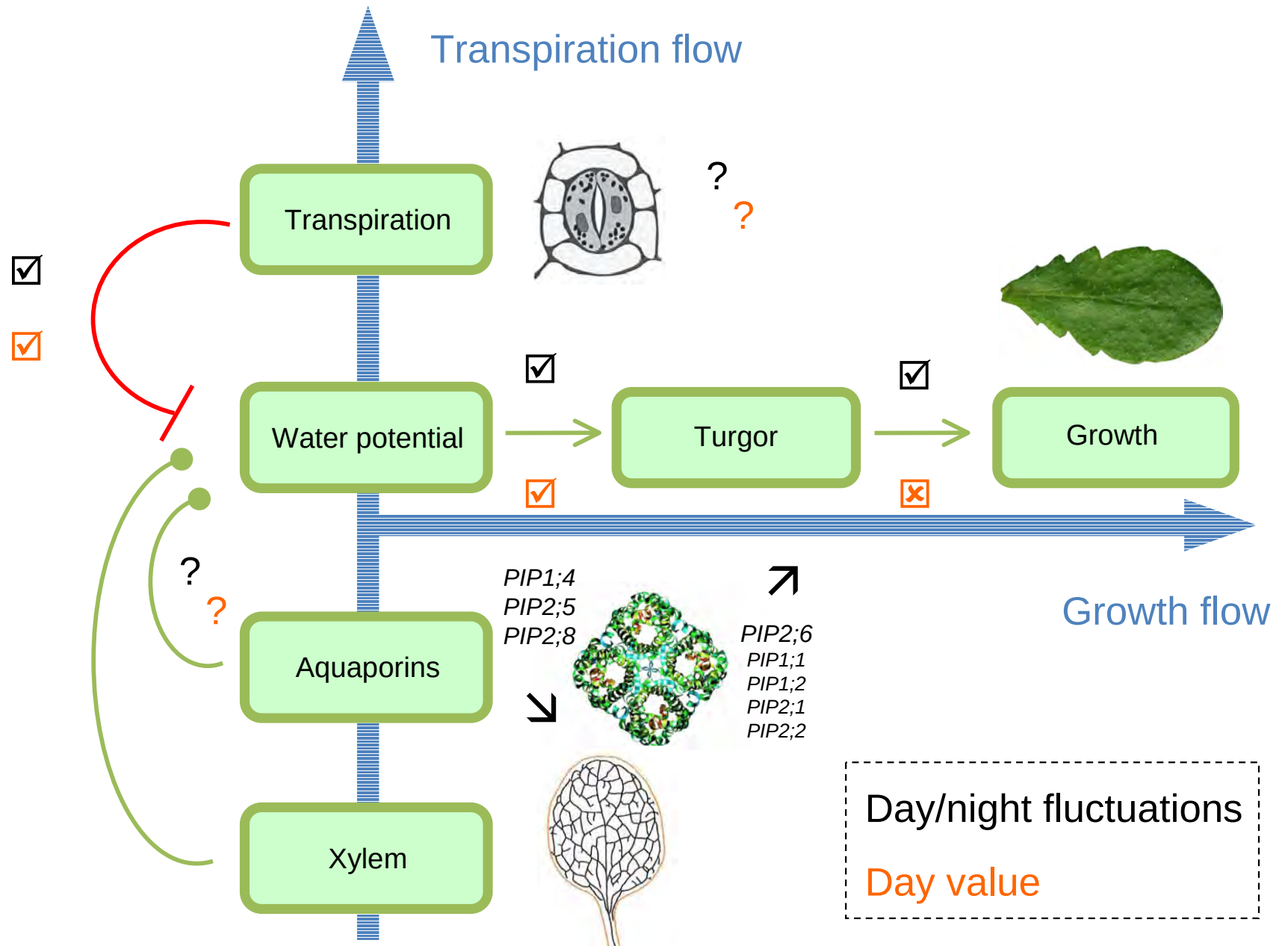
Hydraulic architecture and water flow in growing grass tillers (*Festuca arundinacea* Schreb.)

P. MARTRE,¹ H. COCHARD² & J.-L. DURAND¹

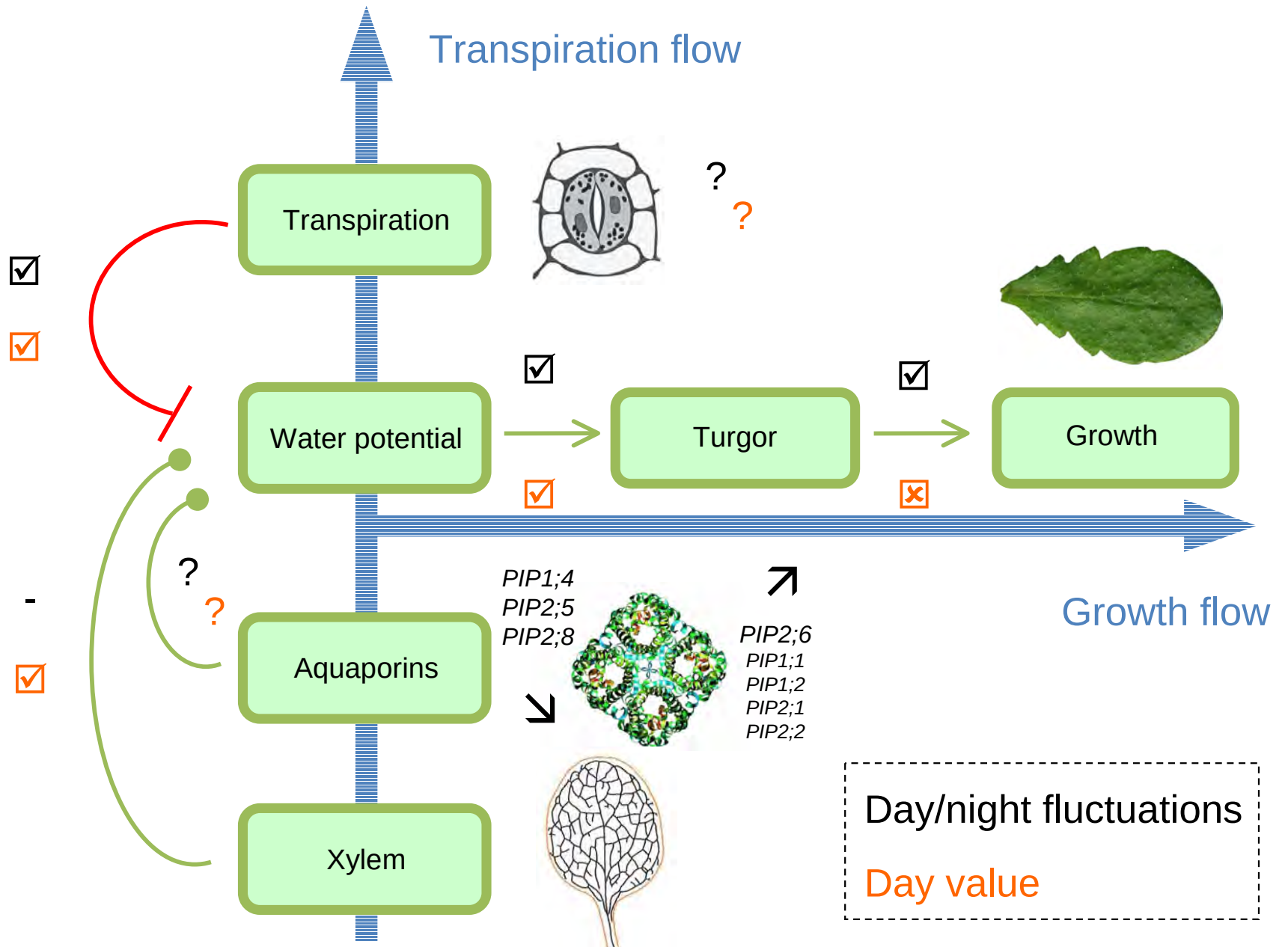
¹Unité d'Ecophysiologie des Plantes Fourragères, Institut National de la Recherche Agronomique, F-86 600 Lusignan, France, ²Unité de Physiologie Intégrée des Arbres Fruitiers, Institut National de la Recherche Agronomique, F-63 039 Clermont-Ferrand, France



Leaf growth: establishment of hydraulic control



Leaf growth: establishment of hydraulic control





Conclusion

- > **Hydraulics drives diurnal depressions of growth** as evidenced by turgor, water potentials, and transpiration fluctuations between day and night
- > **Establishment of diurnal hydraulic control** during leaf development in *Arabidopsis* is likely to result of both
 - (i) an **absence of transpiration fluctuation** in the early stages
 - (ii) a progressive **limitation of water supply by xylem** (and maybe aquaporins)
- > The reason why **transpiration in the early stages** is higher than in the later stages and not lower at night remains elusive

Thanks for attention

