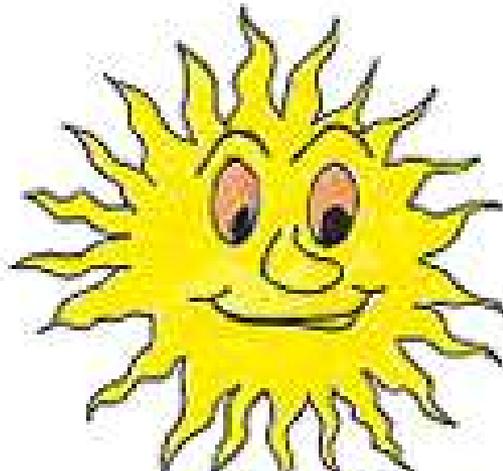


Introduction to vascular plant anatomy for understanding water relations and hydraulic function

Functional agrobiodiversity and agrosystem resilience (Task 2.2., Topic 1)

Virginia Hernandez-Santana

Funchal, 27-31/01/2025



I REALLY DON'T KNOW HOW I DO IT.

I JUST SIT HERE,
SUCK UP A LITTLE
WATER, ABSORB
A LITTLE CO₂,
AND SOAK UP A
LITTLE SUNSHINE



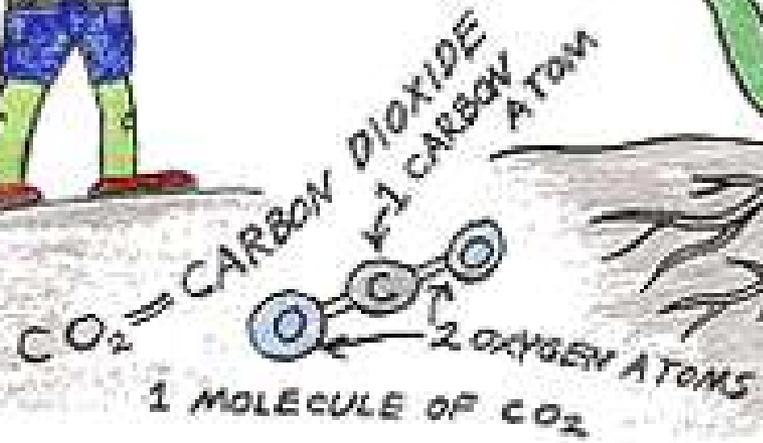
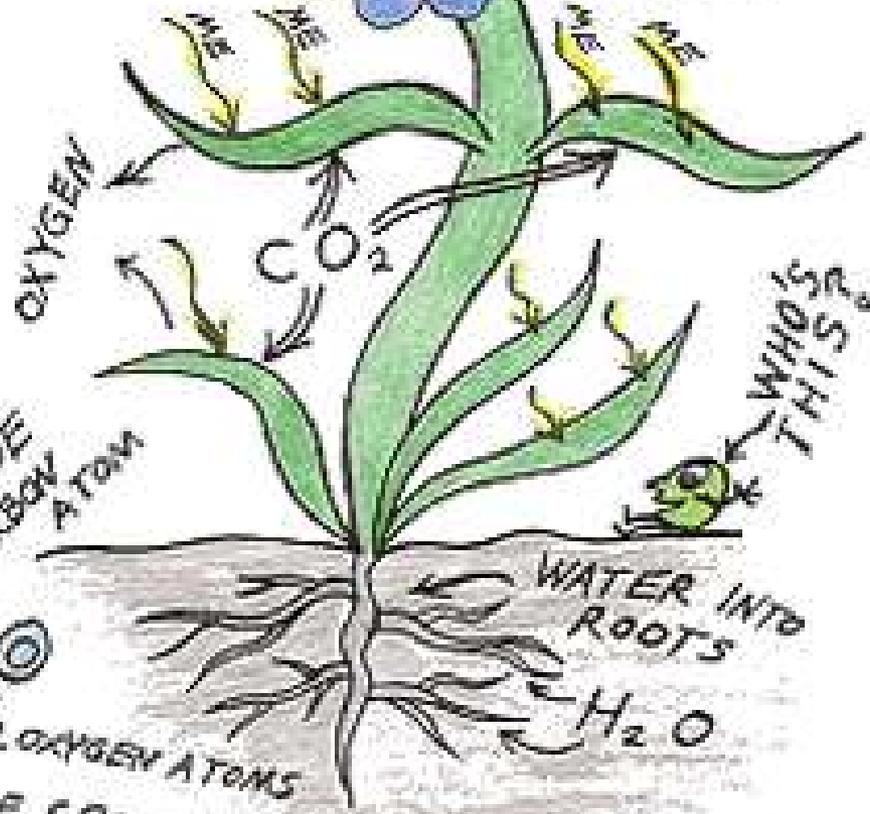
NEXT THING
I KNOW,
I'M FULL
OF HOME MADE
SUGAR.

I PAINTED
MYSELF GREEN.
I STOOD IN
THE SUN
ALL DAY.



I FEEL
YUCKY.

BUT I JUST
GOT THIRSTY.
AND I'M STILL
HUNGRY.



1.- Key anatomical structures for water absorption, transport and regulation

- Root Anatomy: structures and their role in water absorption
- Stem anatomy: vascular tissues and hydraulic function
- Leaf anatomy: water regulation through transpiration

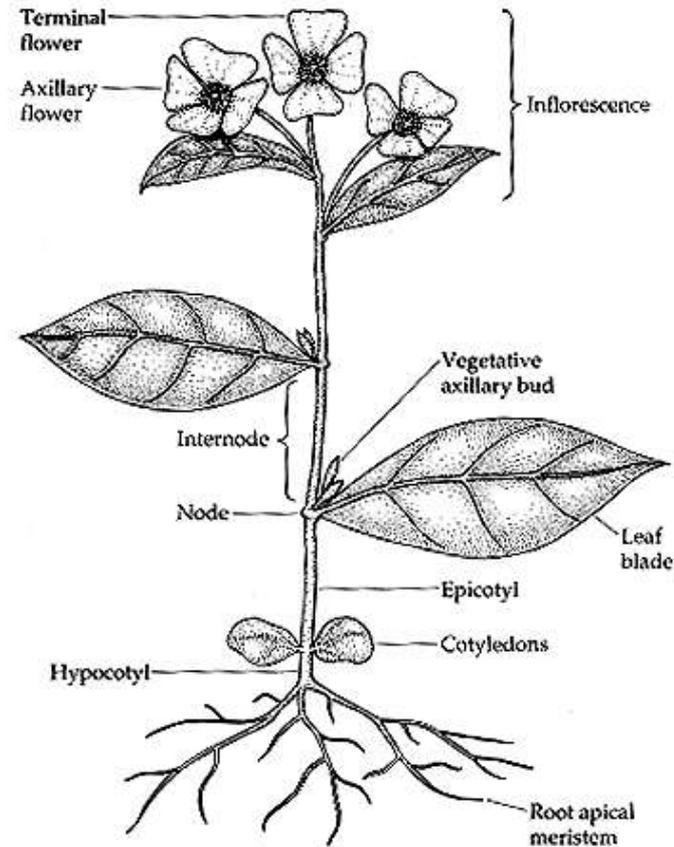
2.- Anatomy research in plant physiology and agriculture

- Strategies of water and carbon use regulation across fruit tree species
- Anatomical characterisation to localize carbohydrates

1.- Key anatomical structures for water absorption, transport and regulation

The three basic structures of plants for water relations

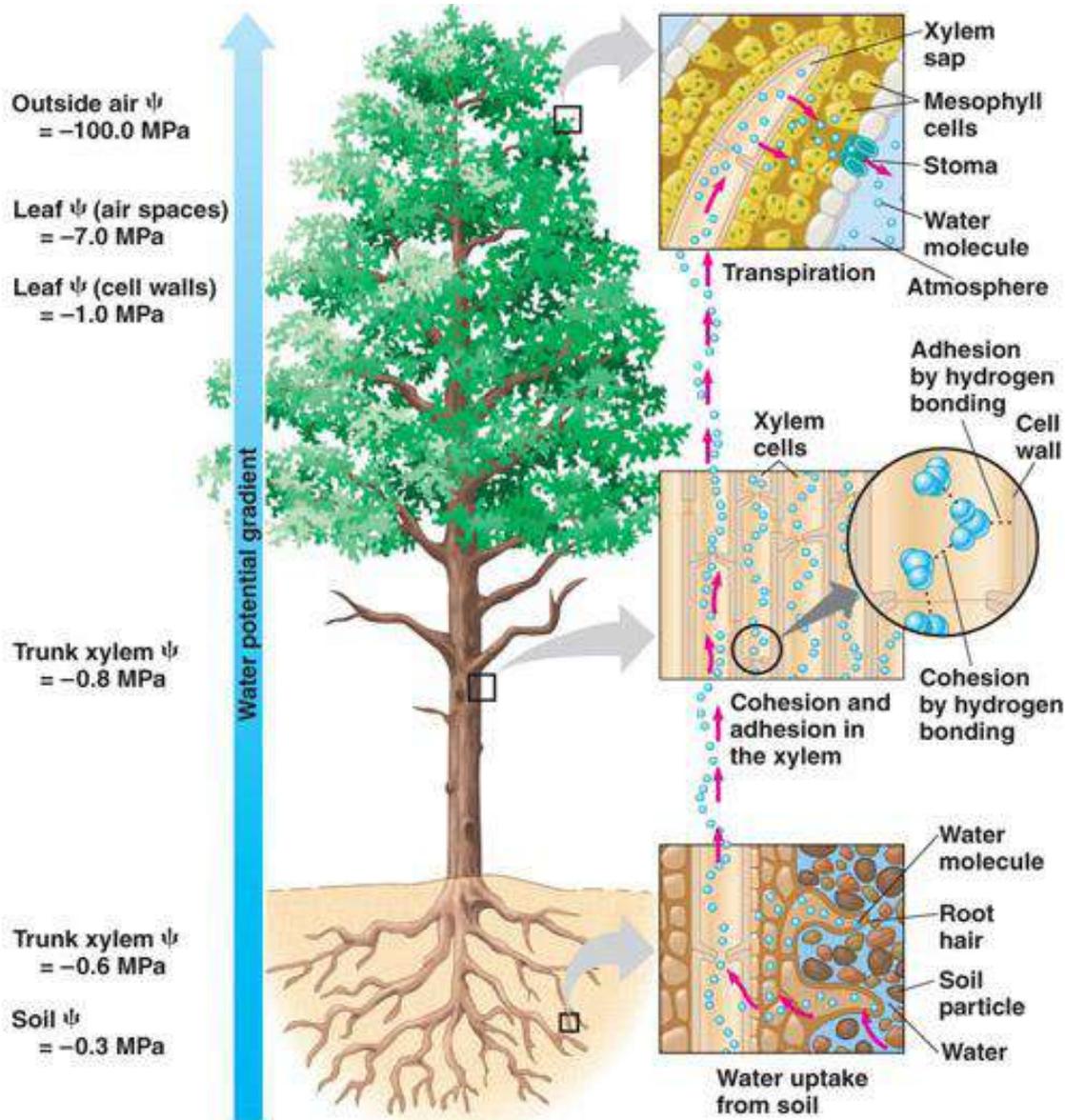
- Leaves
- Stems
- Roots
- (Flowers)



REGULATION

TRANSPORT

ABSORPTION



Roots

Functions:

- Anchor the plant
- Absorb water & minerals
- Store carbohydrates
- Propagation



Different Types of Roots

Tap Root

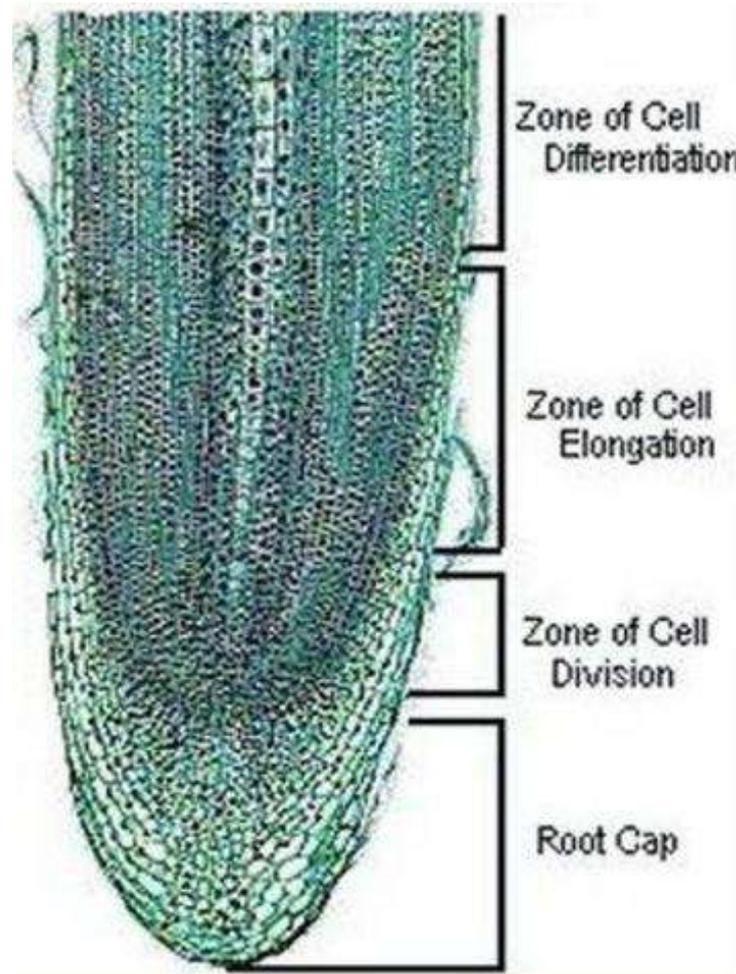
- One main root, no nodes
- Ideal for anchorage
- Penetration is greater for water
- Storage area for food

Fibrous Roots

- Many finely branched secondary roots
- Shallow roots cover a large area

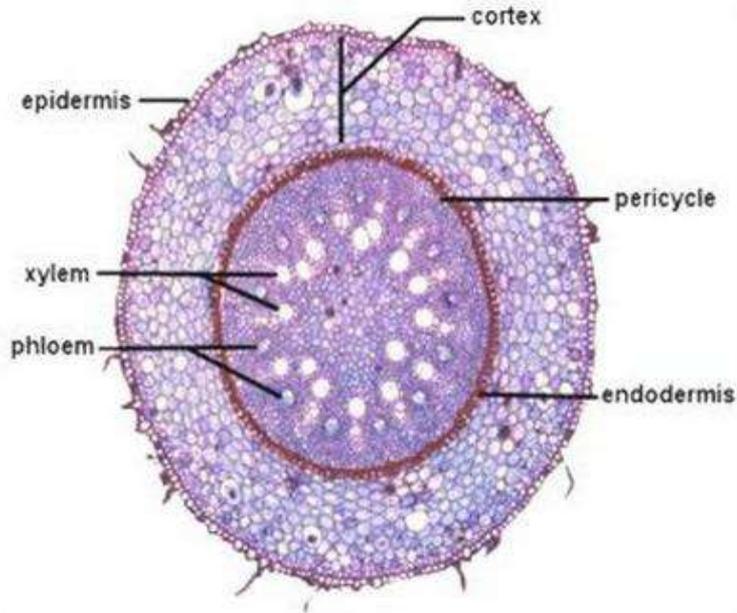


Structures and their role in water absorption



Root tip longitudinal cross section

Structures and their role in water absorption



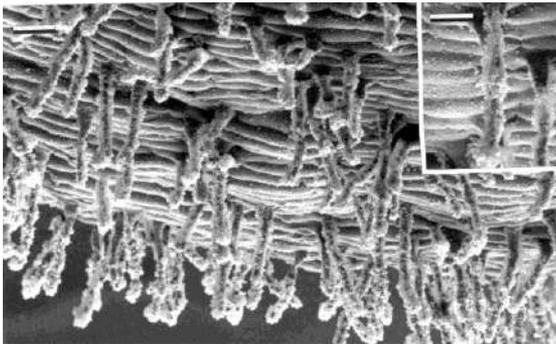
Epidermis: presence of root hairs increases surface area for water uptake.

Cortex: parenchyma cells allow water movement through apoplast and symplast pathways.

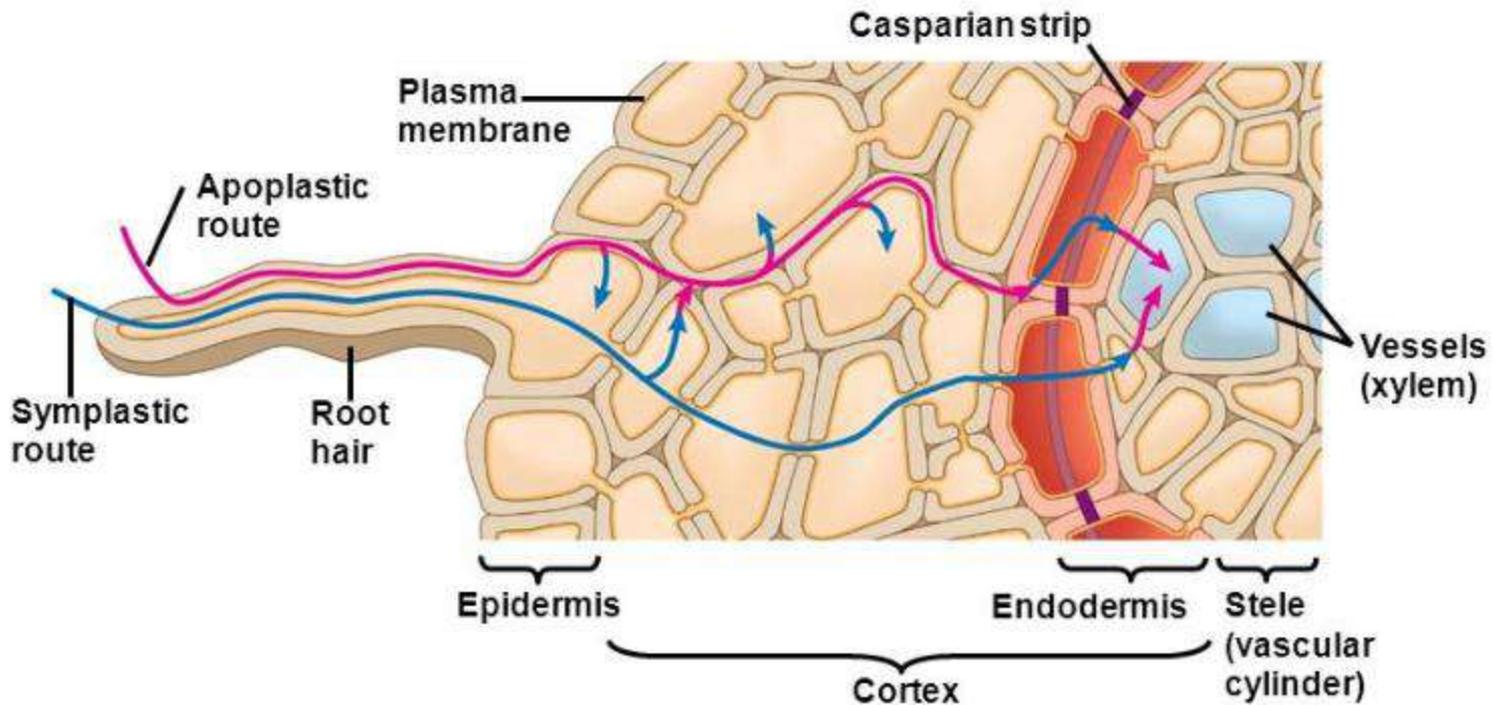
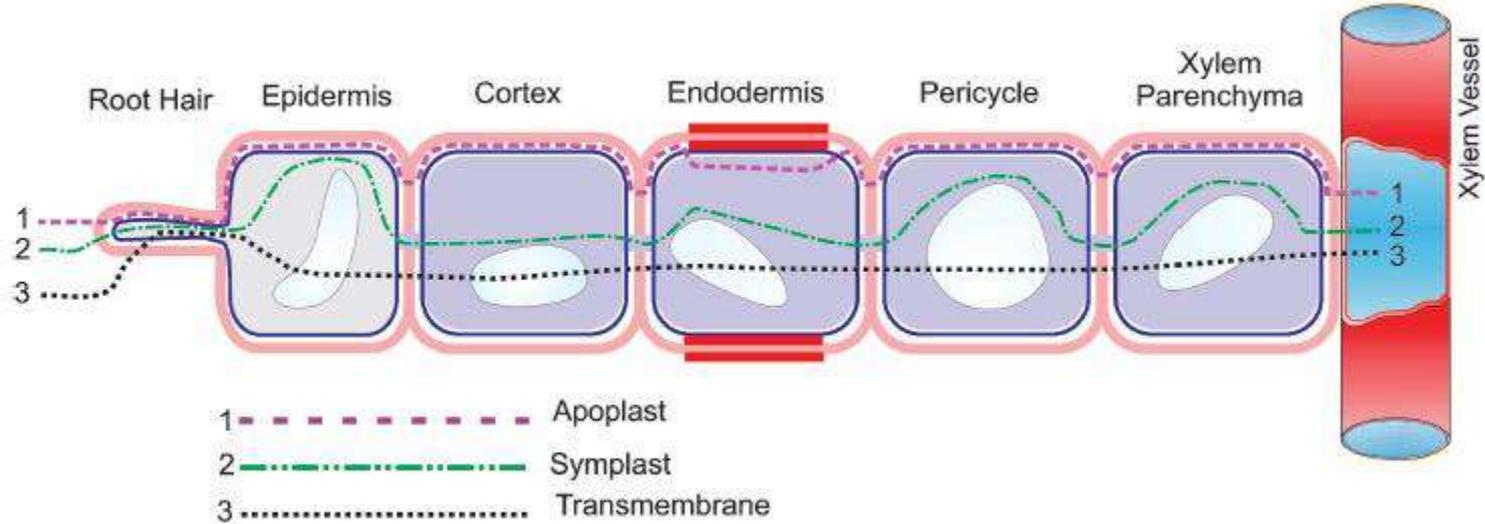
Endodermis: contains the Casparian strip (suberin layer) that forces water into the symplast, regulating entry into the stele.

Pericycle: involved in lateral root formation; helps maintain water conduction continuity.

Xylem in roots: Conducts absorbed water upward through tracheary elements.



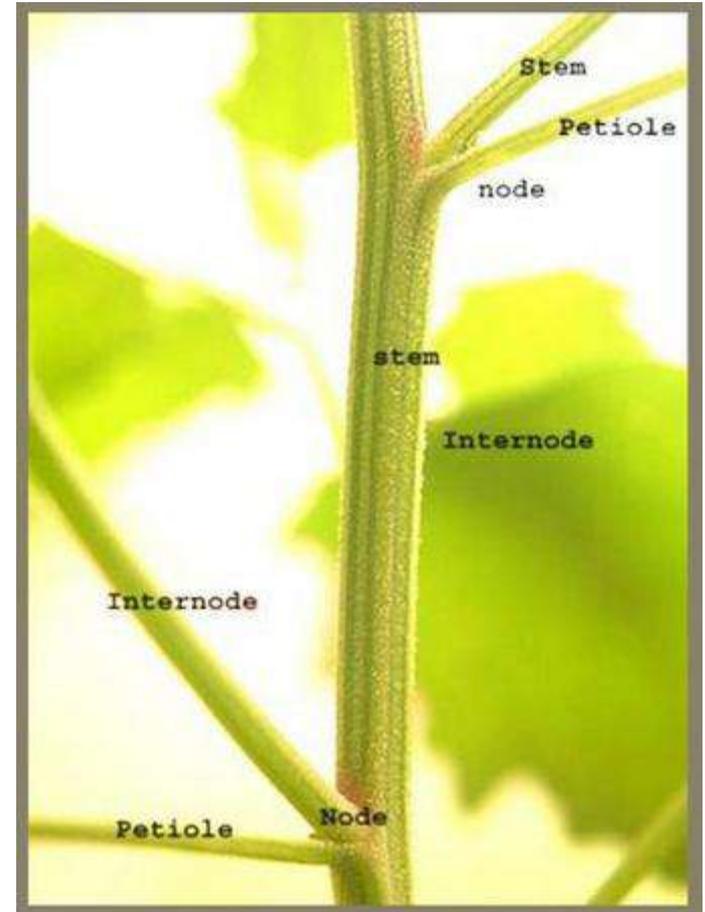
Water absorption pathways



Stems

Functions

- Support leaves & reproductive structures
- Contain the vascular tissues: movement of materials – Water, sugars, hormones and ions
- Carbohydrates storage



Vascular Tissues and Hydraulic Function

Xylem

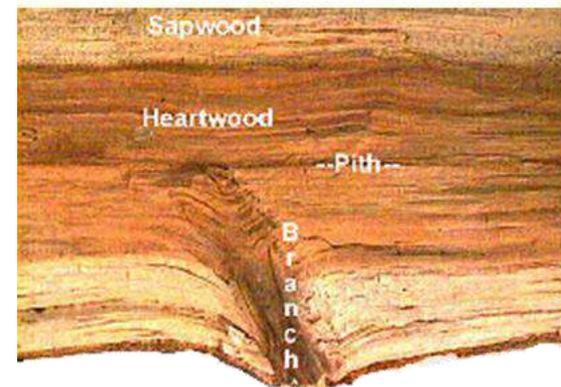
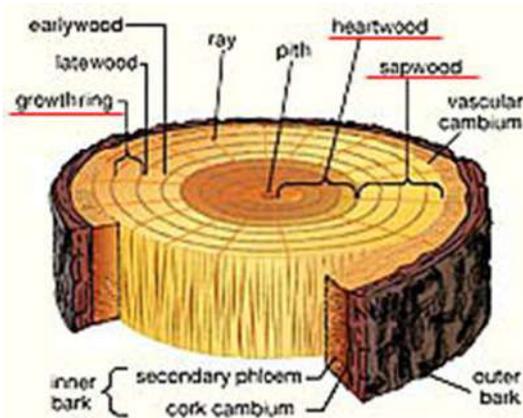
- The tissue that transports water and minerals up from roots to stems/leaves

Phloem

- Tissue that transports organic molecules (sugars, hormones...) down from leaves to roots

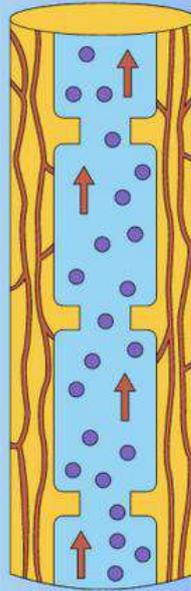
Cambium

- Thin, green, actively growing tissue located between bark & wood and produces all new stems cells



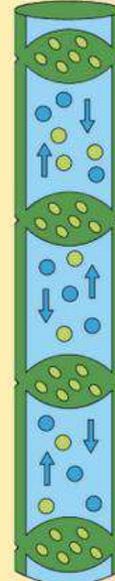
Plant vascular system

- Transports water and minerals
- One-way (upward) flow
- Transport is bulk flow due to negative pressure
- Hollow dead cells
- No end walls
- Form center of the vascular bundle
- Rich in lignin, which supports the plant



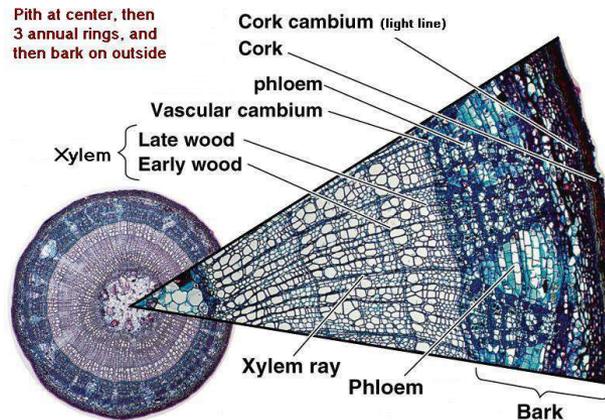
Xylem

- Transports food in the form of sugars
- Two-way flow
- Transport is by osmosis/turgor pressure
- Live cells with no nucleus
- Perforated end walls
- Outside edge of vascular bundle
- Also transports amino acids, mRNA, and hormones

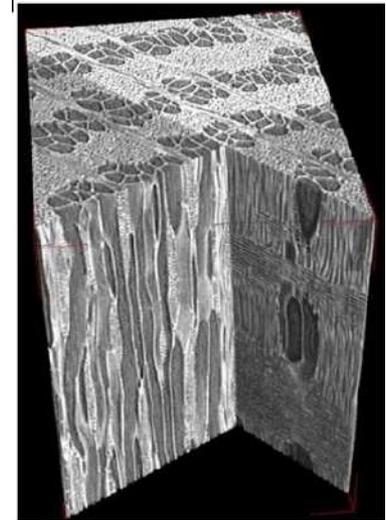
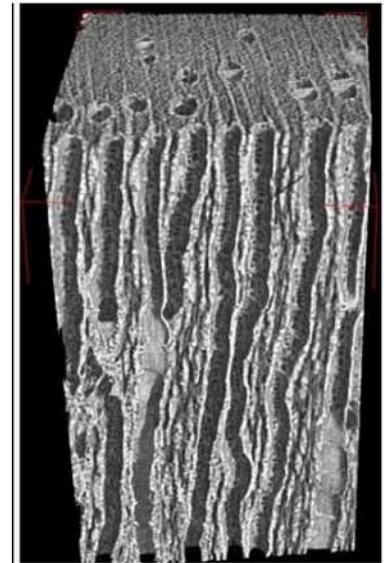
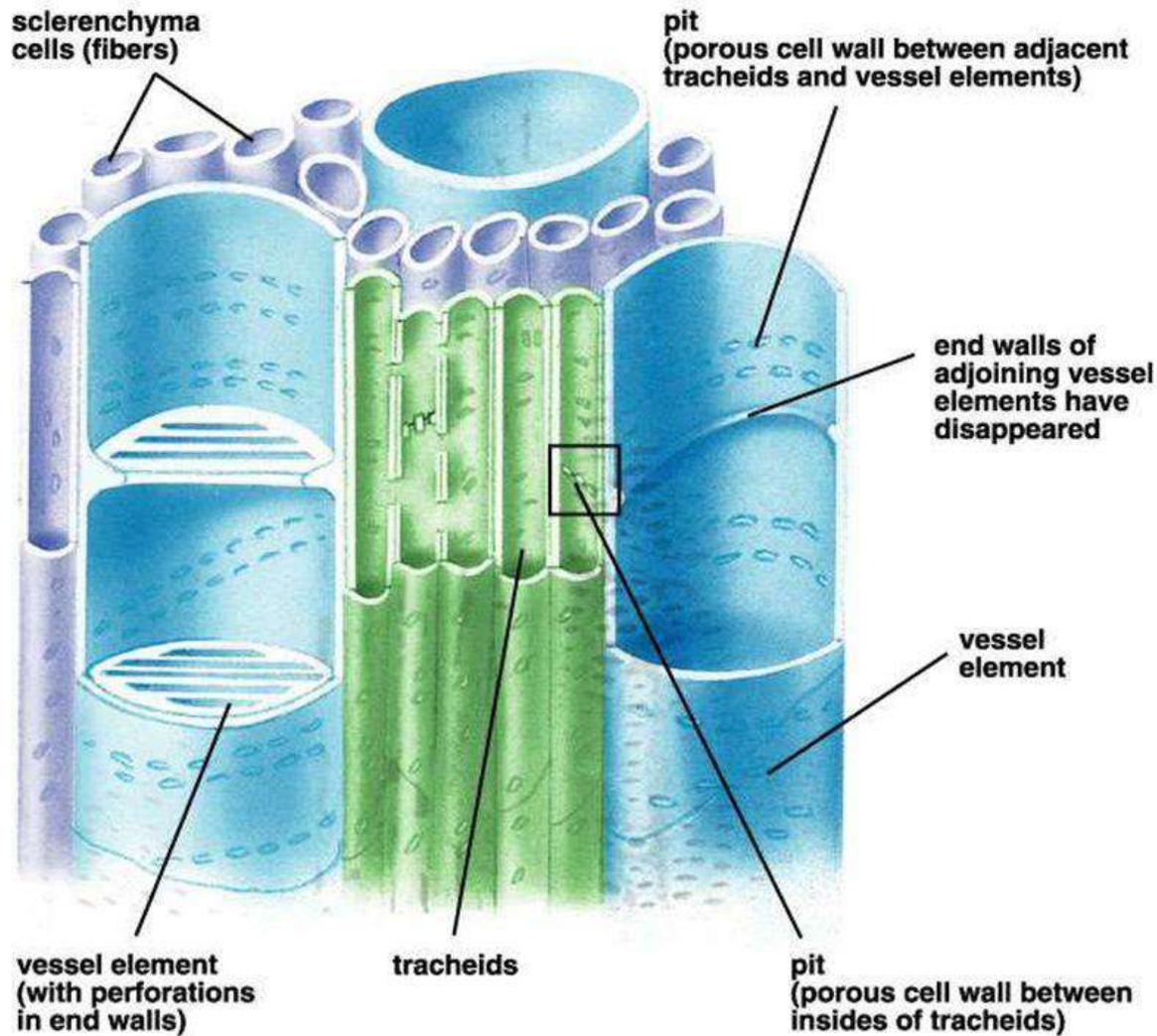


Phloem

sciencenotes.org

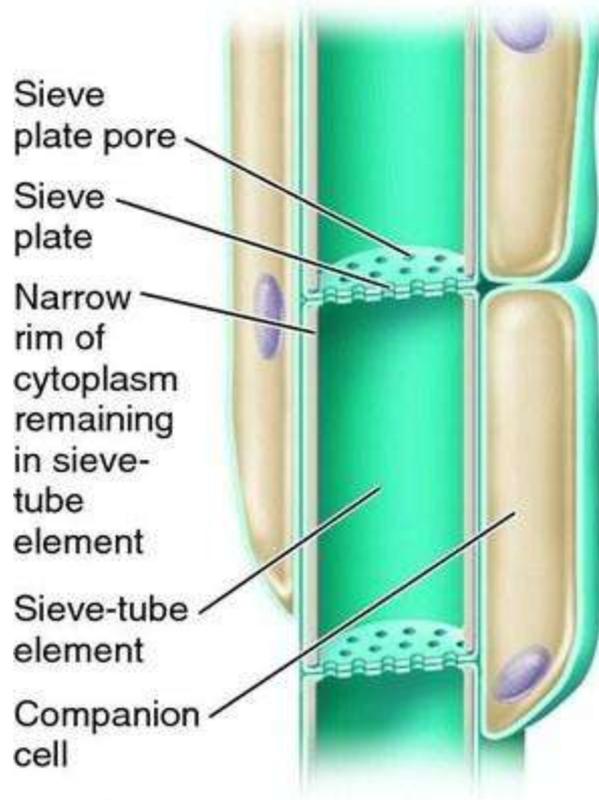


Plant vascular system: xylem

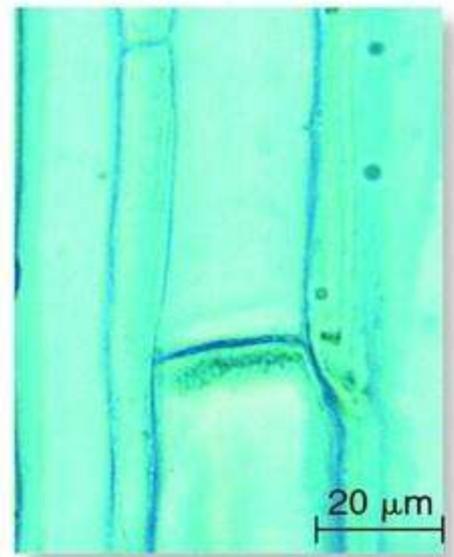


McElrone et al. (2013)

Plant vascular system: phloem



(a) Sieve-tube elements and companion cells

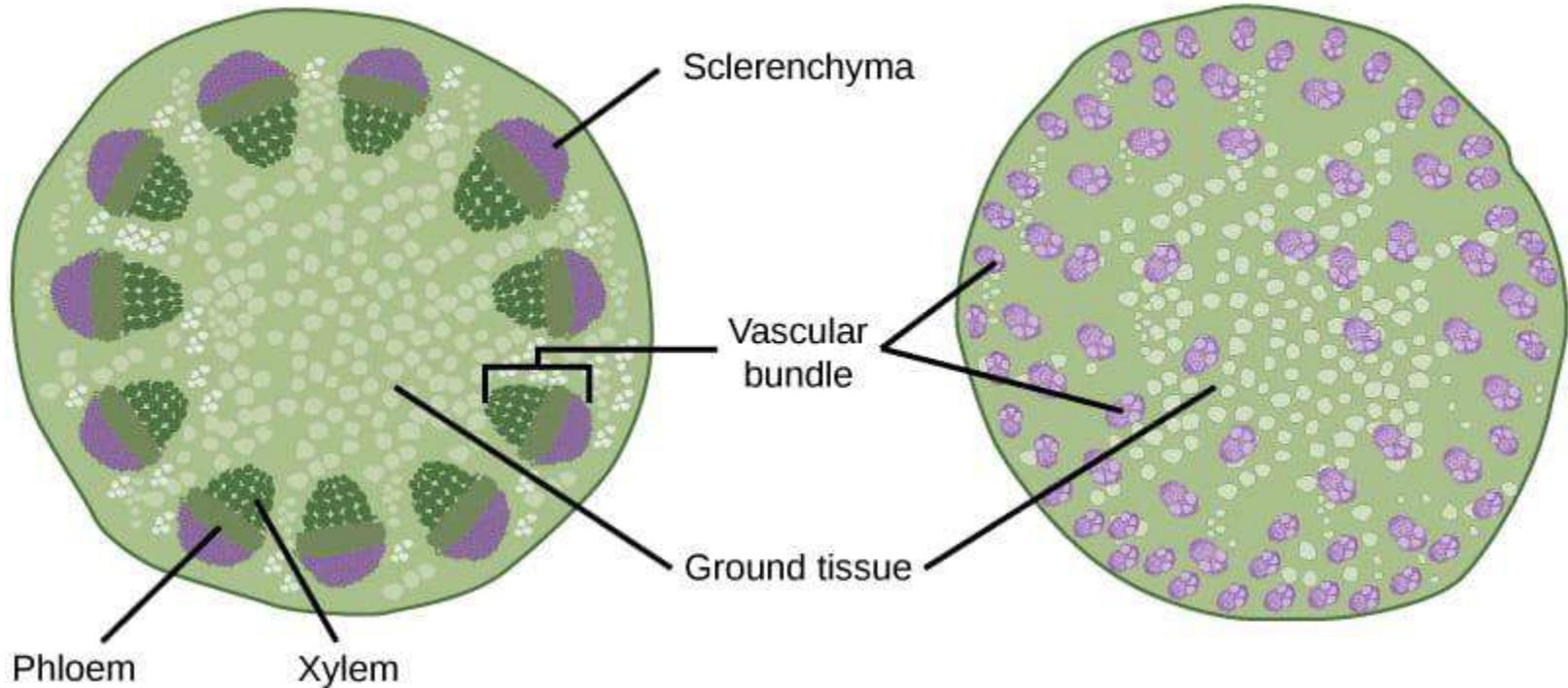


(b) Light micrograph of phloem stained with blue dye, showing sieve-tube elements

Arrangement of Vascular bundles

Dicot stem

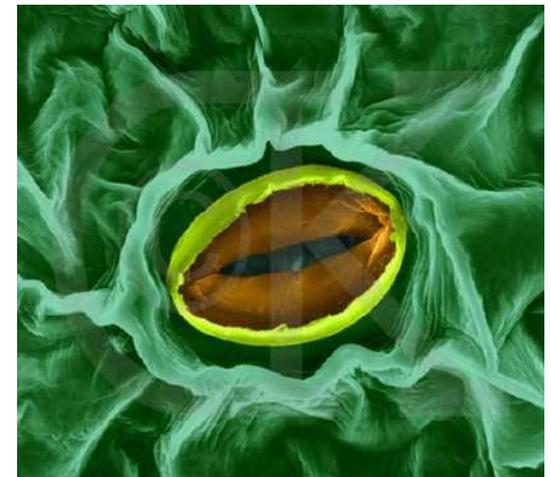
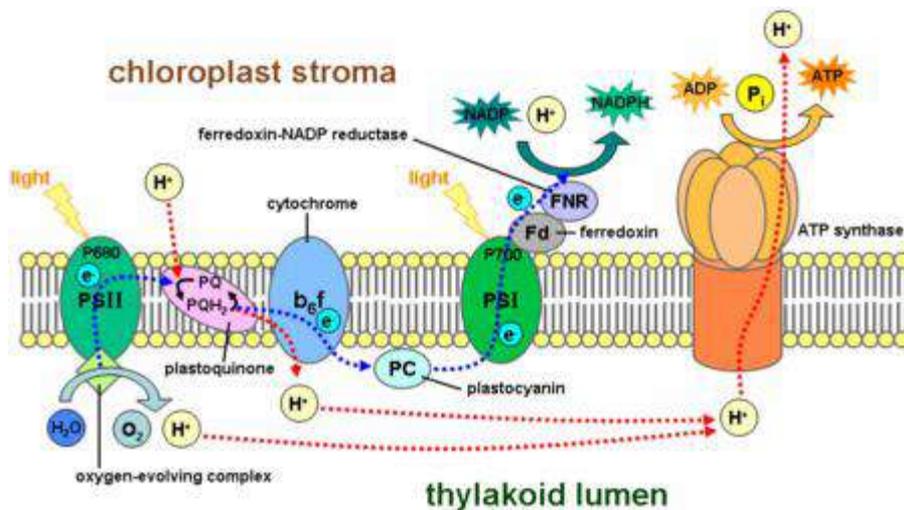
Monocot stem



Leaves

Functions

- Site of gas exchange ($H_2O + CO_2$)
- Photosynthesis
- Store carbohydrates



External Parts of the Leaf

Petiole

- Leaf stalk or part that connects the leaf to the stem

Blade

- The large, flat part of a leaf

Midrib

- The large center vein

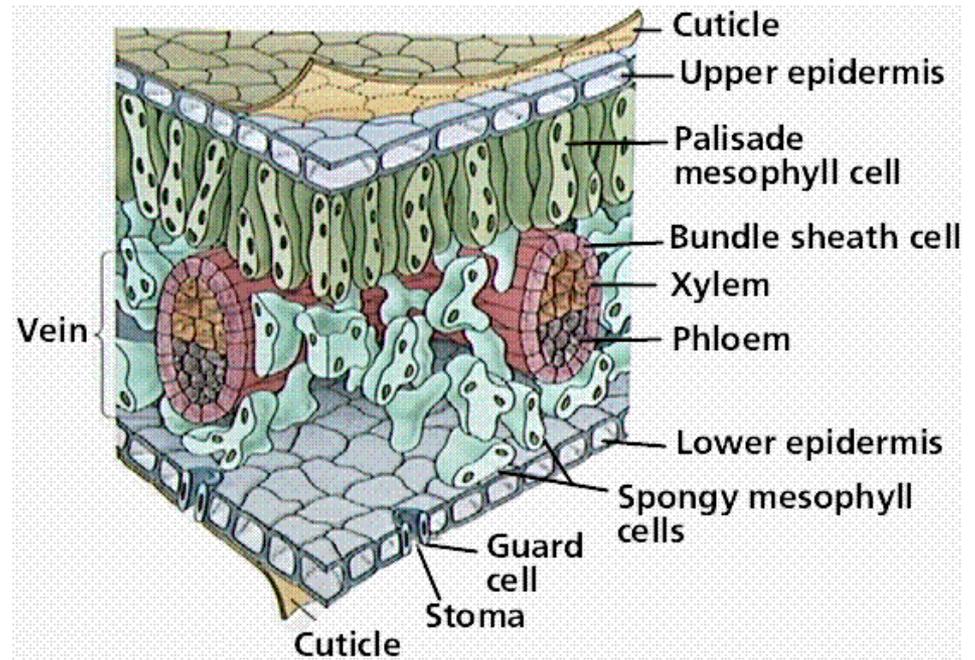
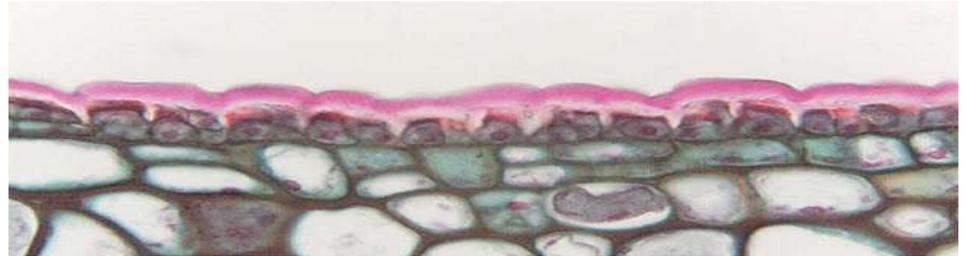


Tissues of the Leaf

Epidermis

Cuticle

- Waxy substance that covers the leaves & stems
- Waterproof layer that keeps water in plants



Tissues of the Leaf

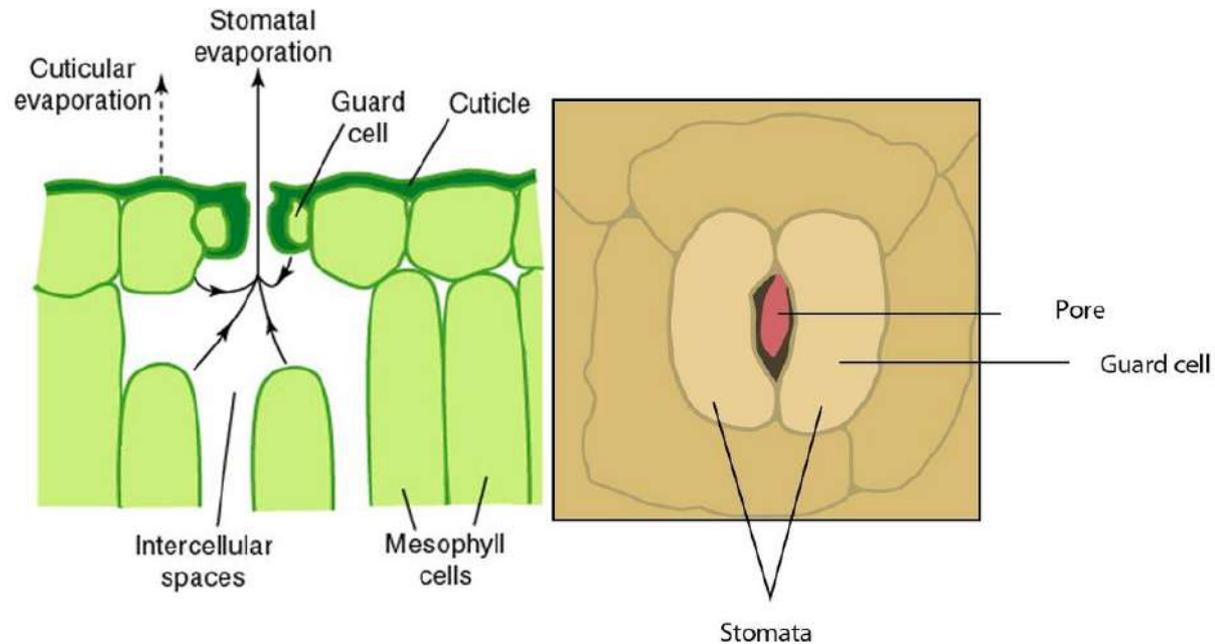
Epidermis

Guard Cells

- Primary site of photosynthesis

Stomata

- Site of gas exchange



Tissues of the Leaf

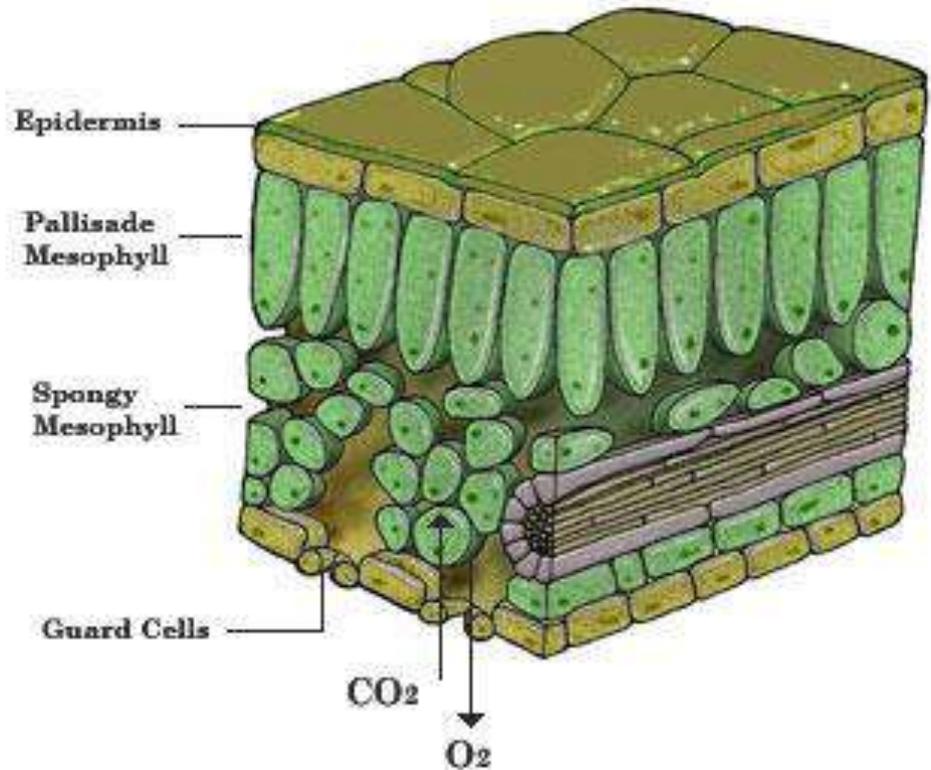
Mesophyll

Palisade mesophyll

- Primary site of photosynthesis

Spongy mesophyll

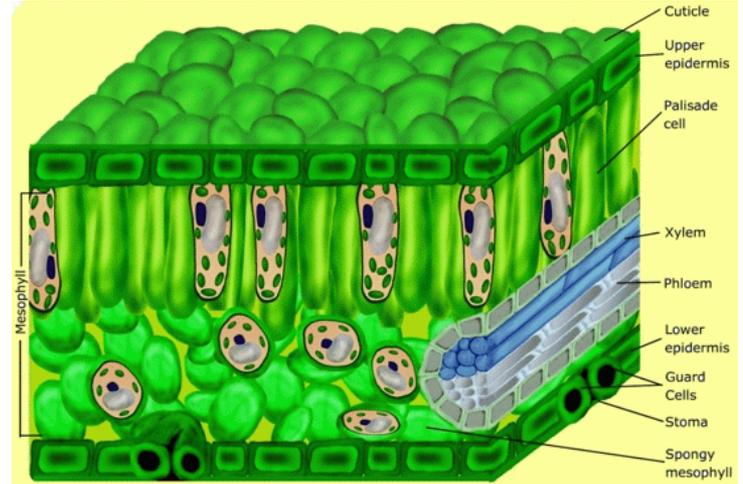
- Contains air and chloroplasts
- Site of photosynthesis and gas exchange



Tissues of the Leaf

Vascular Bundles

- In spongy mesophyll
- Xylem + phloem



2.- Anatomy research in plant physiology and agriculture

Strategies of water and carbon use regulation across fruit tree species based on anatomical measurements



Tree Physiology 00, 1–11
doi:10.1093/treephys/tpv146



Research paper

Role of leaf hydraulic conductance in the regulation of stomatal conductance in almond and olive in response to water stress

Virginia Hernandez-Santana^{1,2}, Celia M. Rodriguez-Dominguez¹, J. Enrique Fernández¹ and Antonio Diaz-Espejo¹



Journal of Experimental Botany

<https://doi.org/10.1093/jxb/erad157> Advance Access Publication 28 April 2023



RESEARCH PAPER

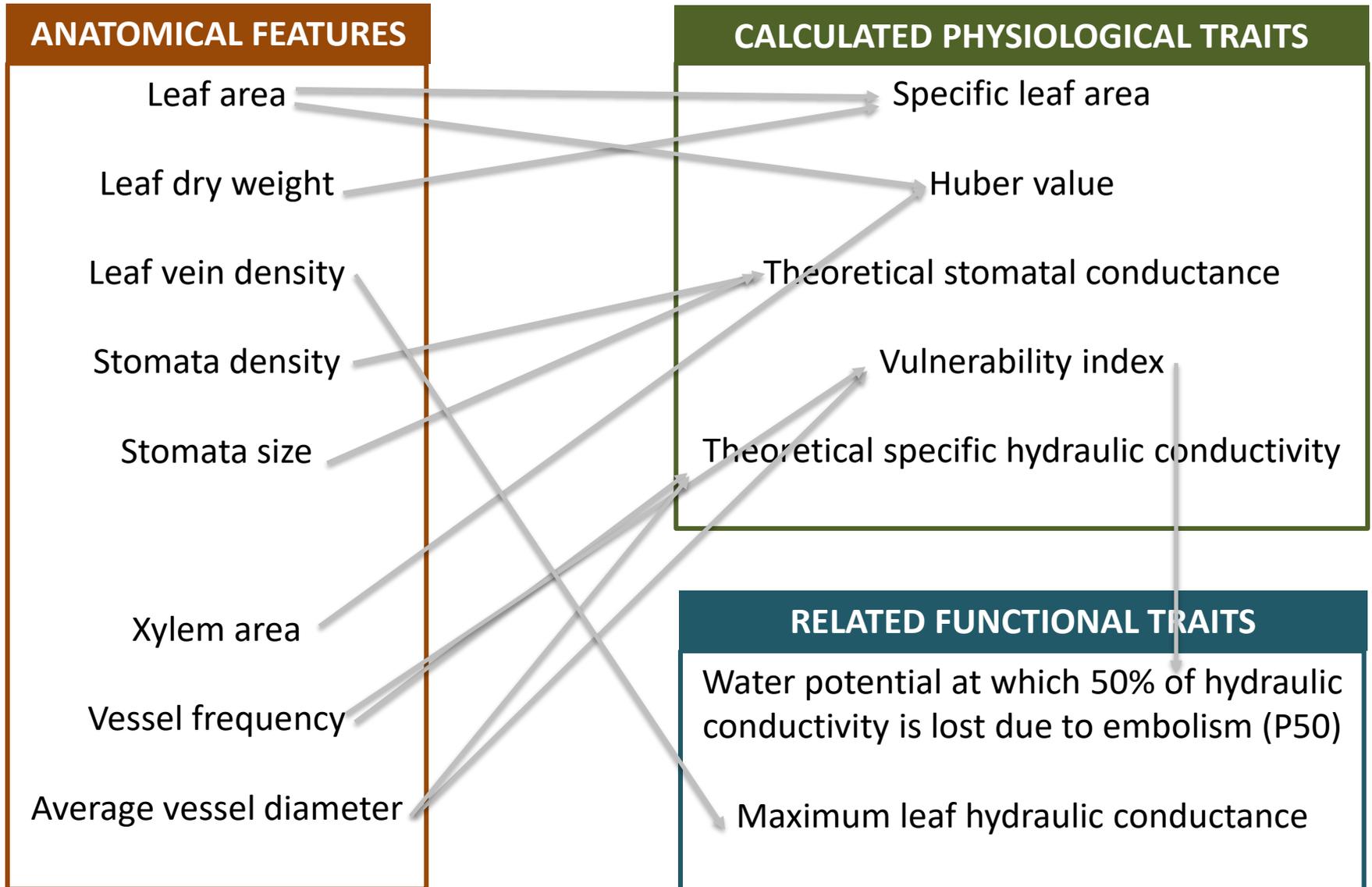
Role of hydraulic traits in stomatal regulation of transpiration under different vapour pressure deficits across five Mediterranean tree crops

Virginia Hernandez-Santana^{1,2,*}, Celia M. Rodriguez-Dominguez^{1,2} , Jaime Sebastian-Azcona¹, Luis Felipe Perez-Romero³  and Antonio Diaz-Espejo^{1,2}

Correlations of key traits shape common strategies of water and carbon use regulation across fruit tree species

Virginia Hernandez-Santana^{1*}; Jaime Sebastian-Azcona¹; Celia M. Rodriguez-Dominguez¹; Alfonso Perez-Martin¹; Antonio Montero¹; Daniel Benzal¹; Federica Rossi²; Luis F. Perez-Romero³, Antonio Diaz-Espejo¹.

Anatomical-related measurements and physiological traits

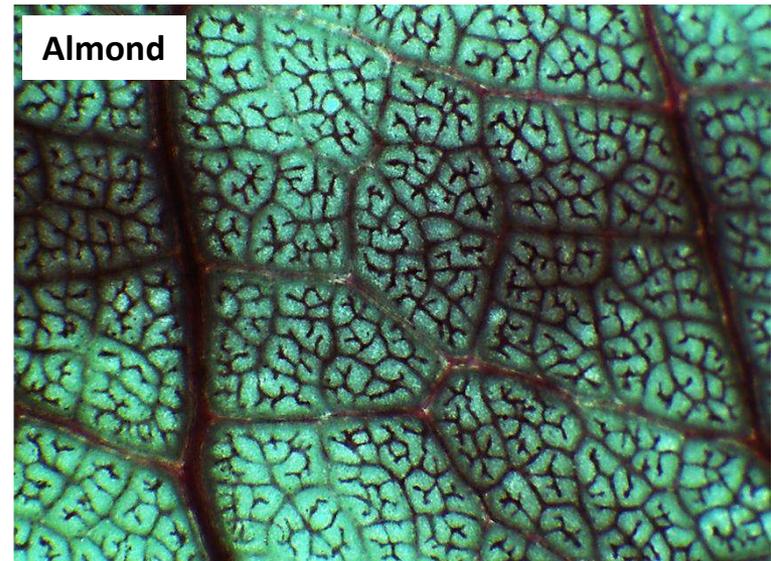
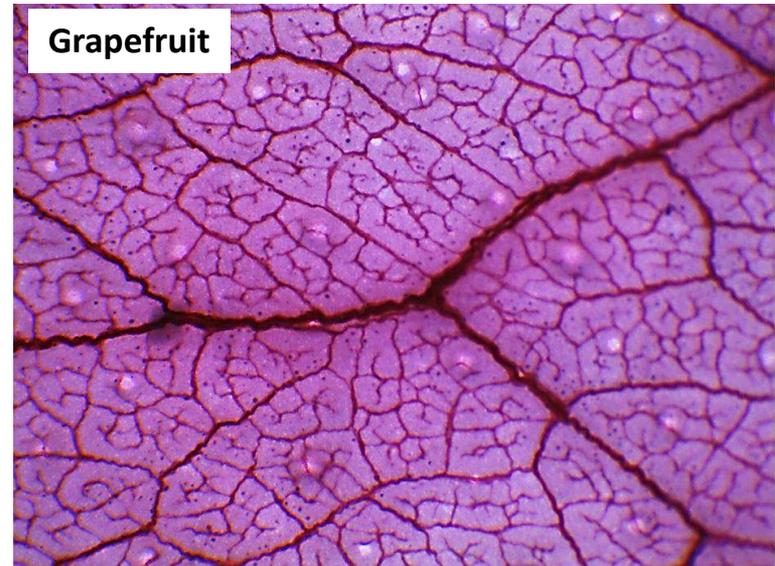
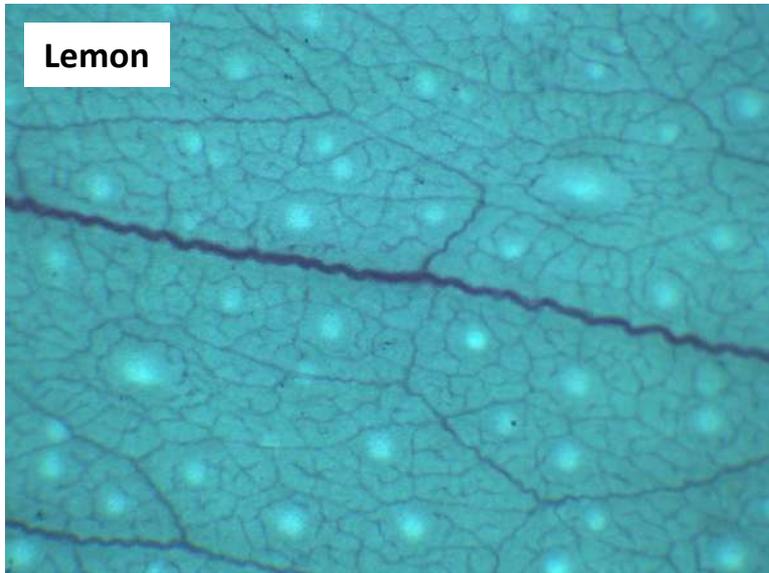


Experimental orchard

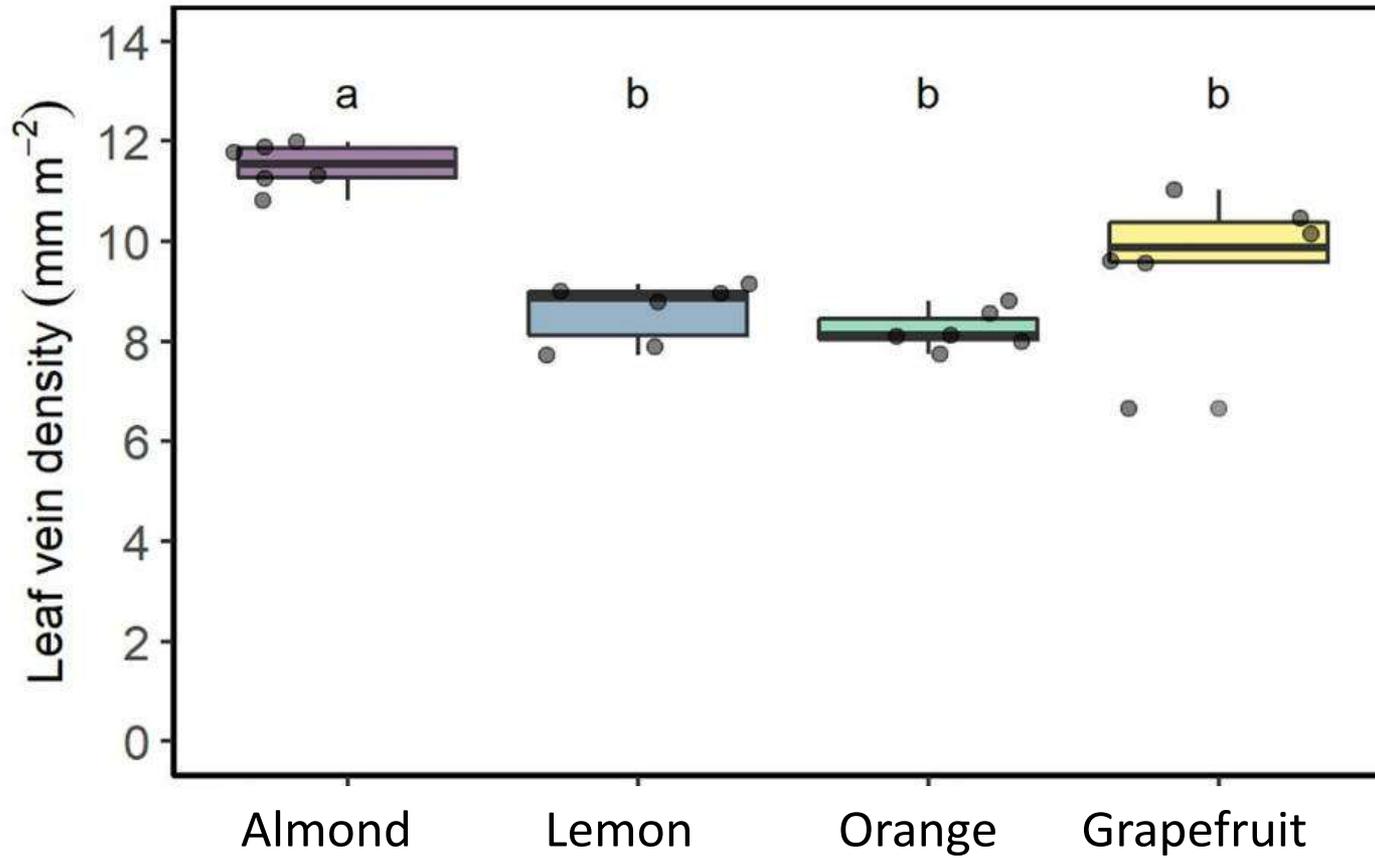


Finca La Hampa
13 fruit tree species

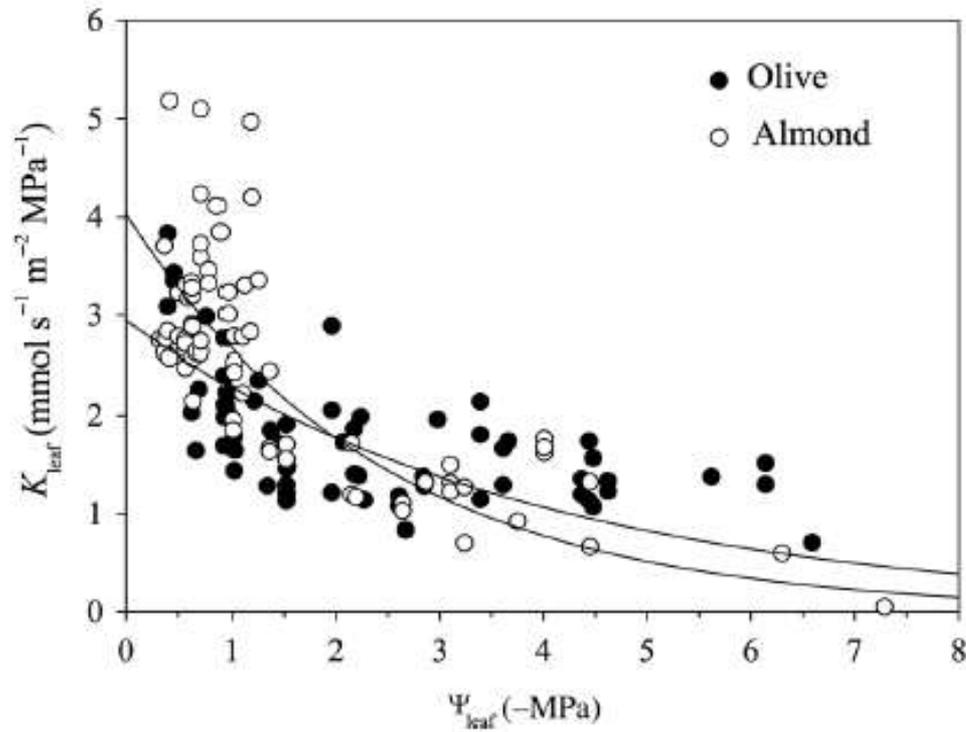
Leaf vein density



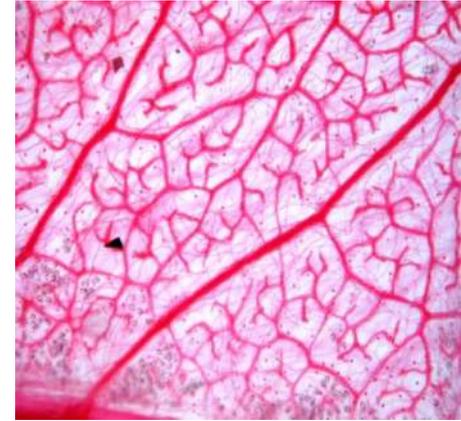
Leaf vein density



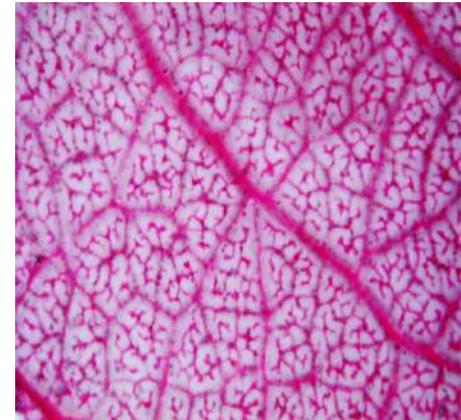
Leaf vein density



olive $6.74 \pm 0.19 \text{ mm mm}^{-2}$

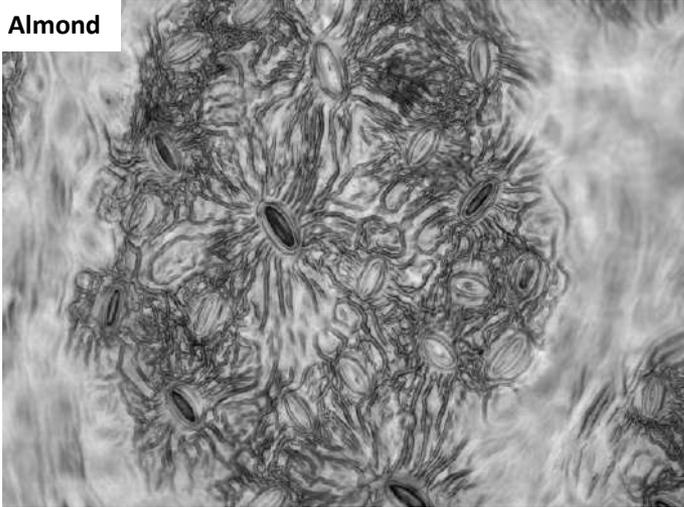


almond $11.33 \pm 0.28 \text{ mm mm}^{-2}$

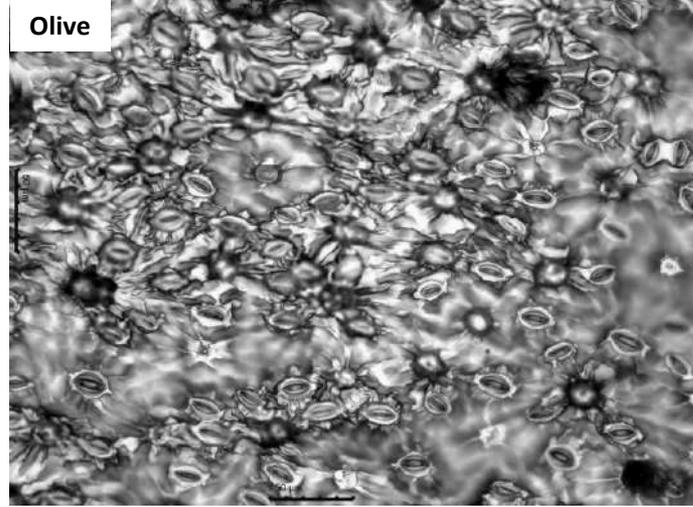


Anatomical measurements to calculate theoretical stomatal conductance

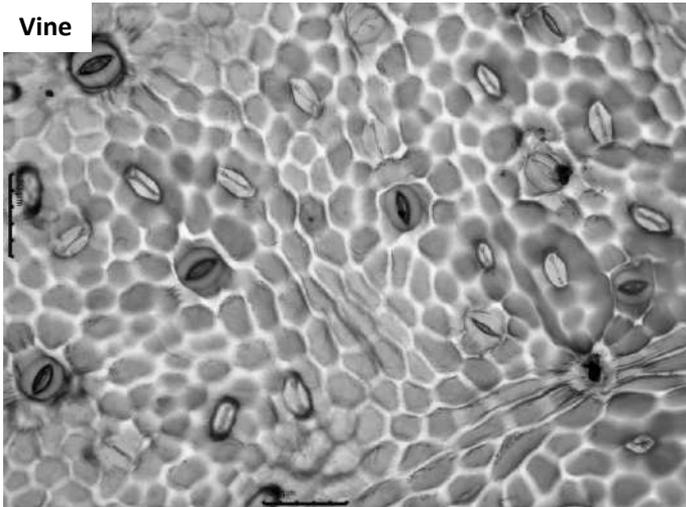
Almond



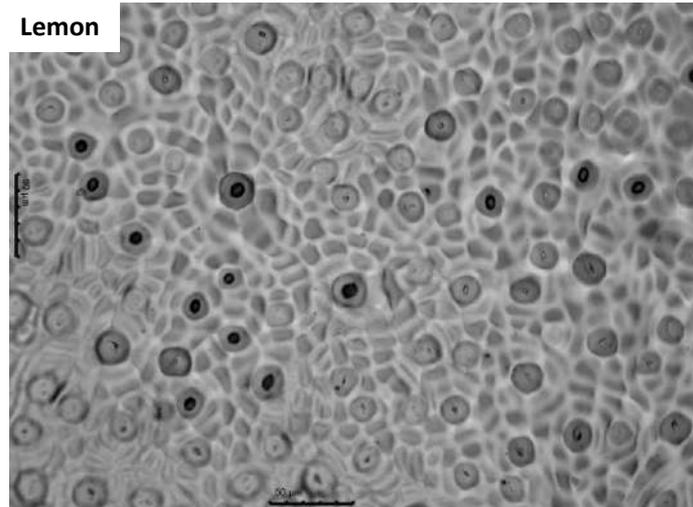
Olive



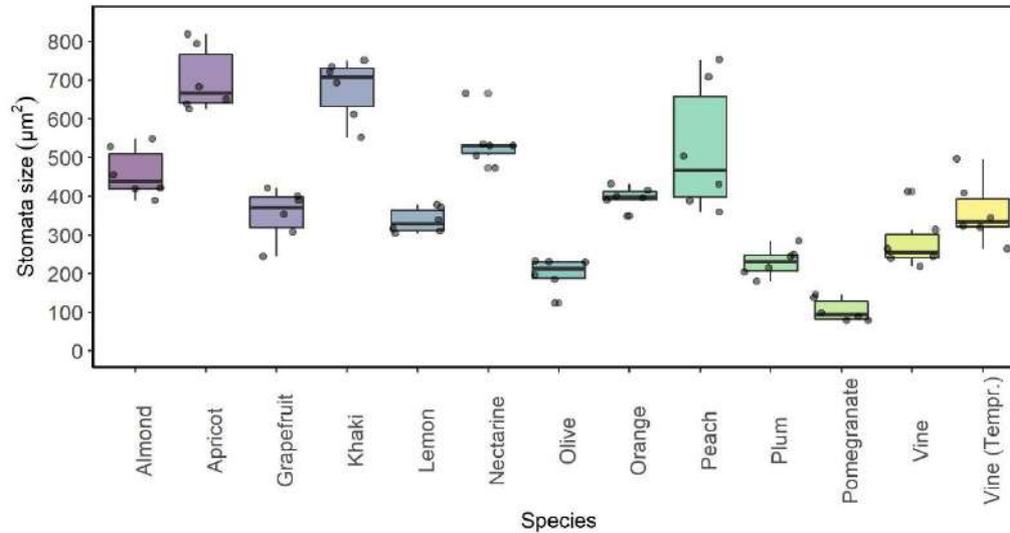
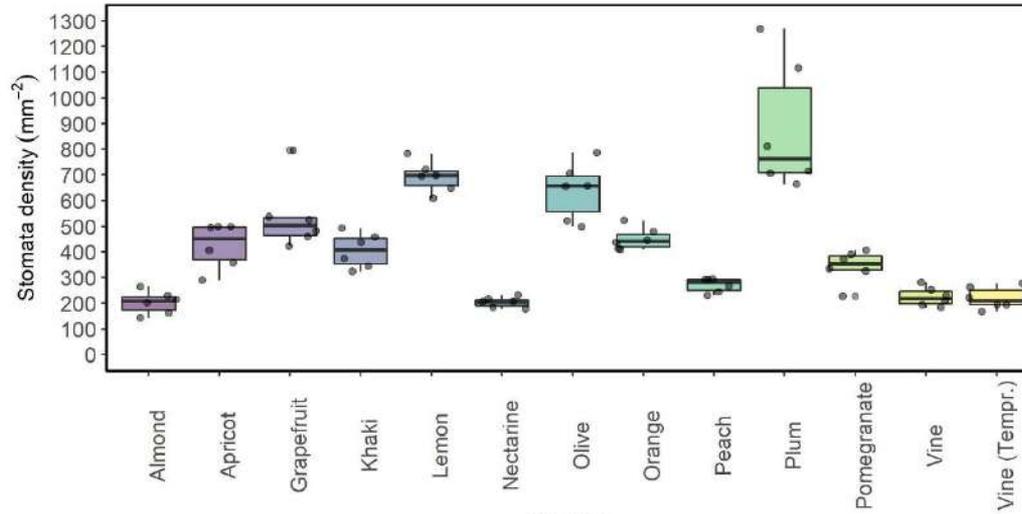
Vine



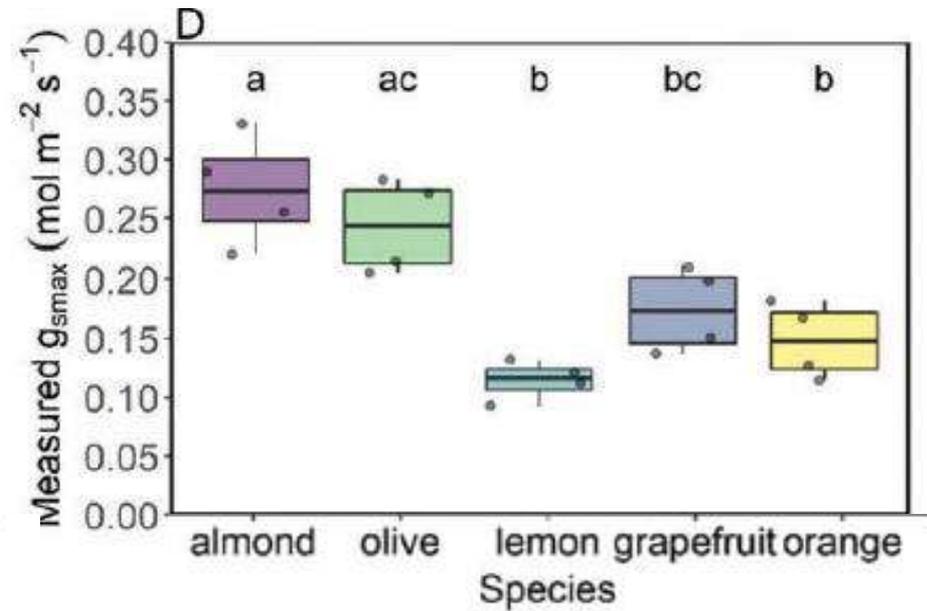
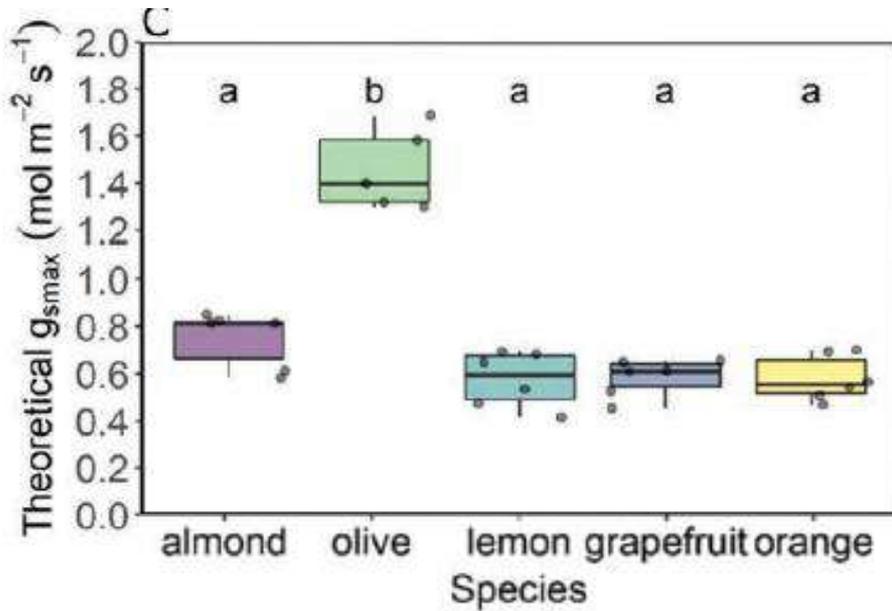
Lemon



Anatomical measurements to calculate theoretical stomatal conductance

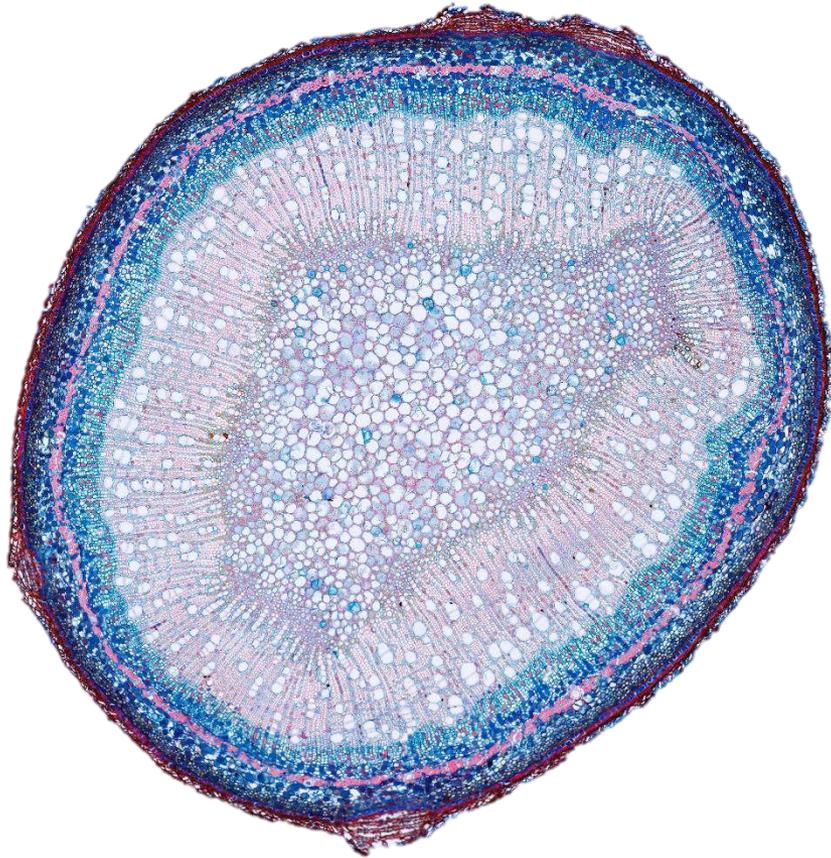


Anatomical measurements to calculate theoretical stomatal conductance

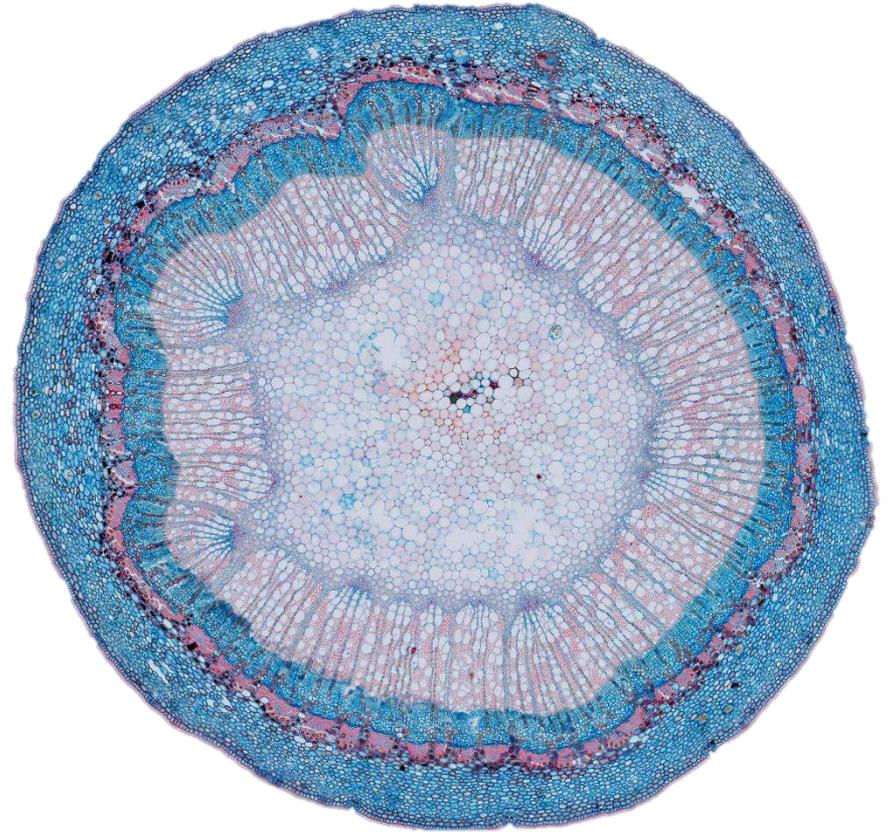


Calculated using Franks et al. (2009)

Anatomical measurements to calculate hydraulic traits



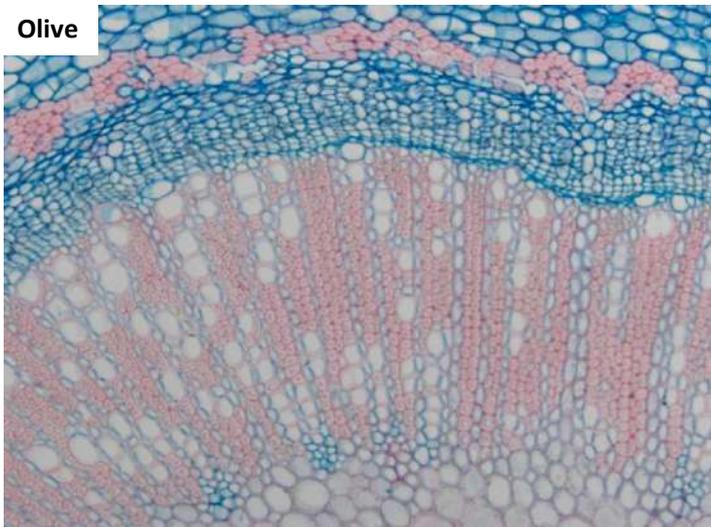
Khaki



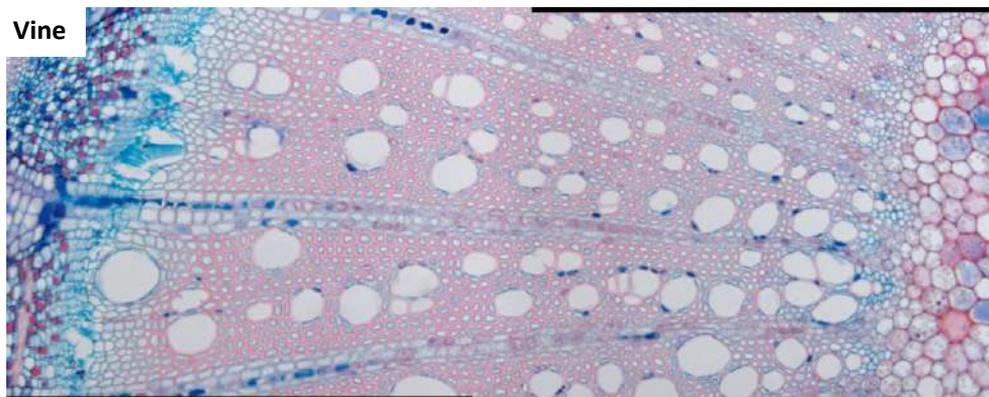
Peach

Anatomical measurements to calculate hydraulic traits

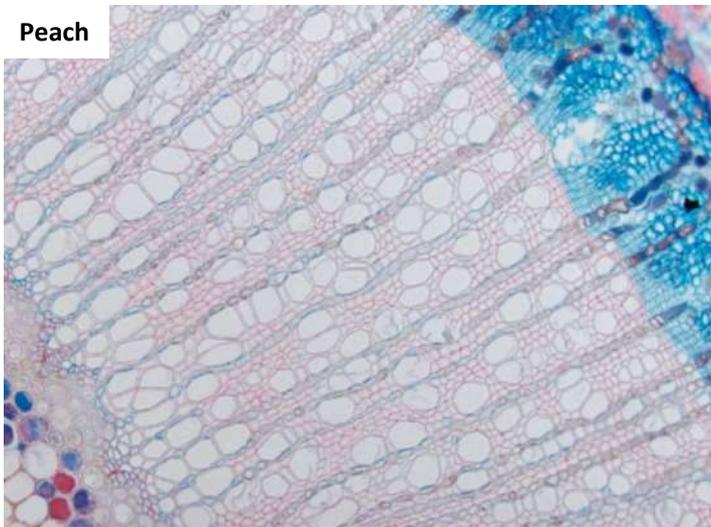
Olive



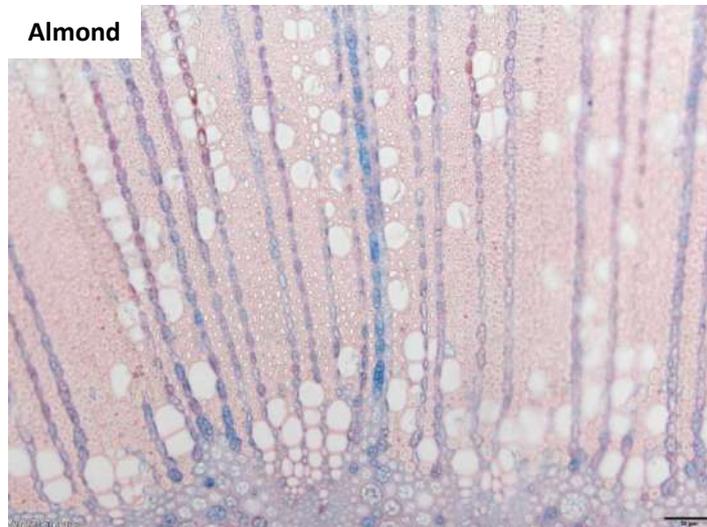
Vine



Peach

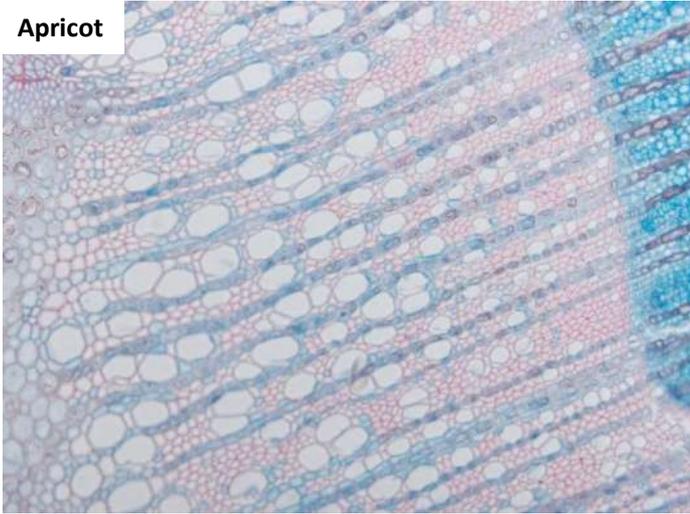


Almond

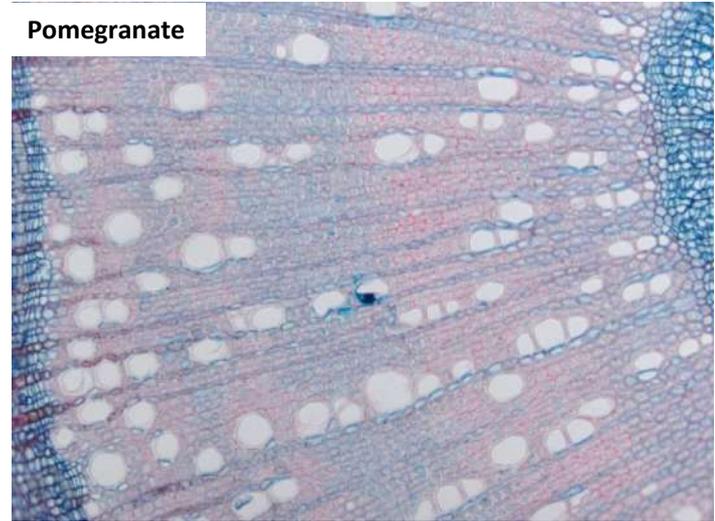


Anatomical measurements to calculate hydraulic traits

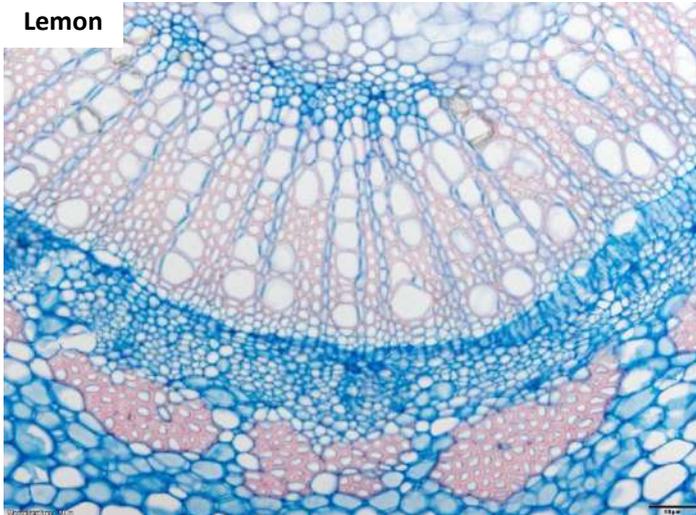
Apricot



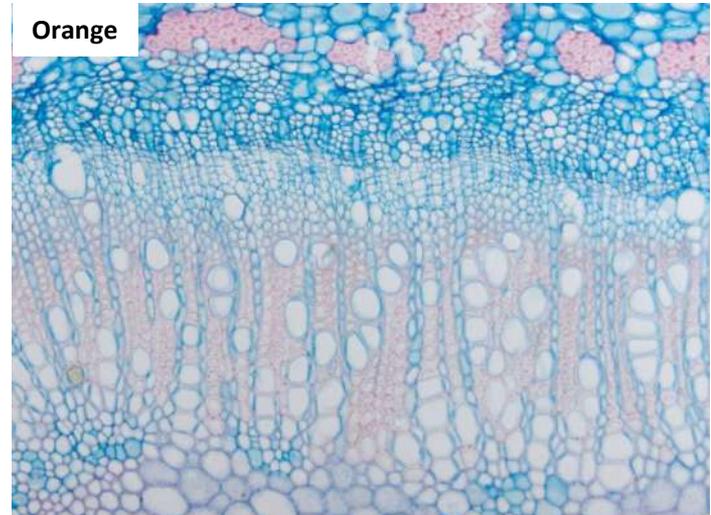
Pomegranate



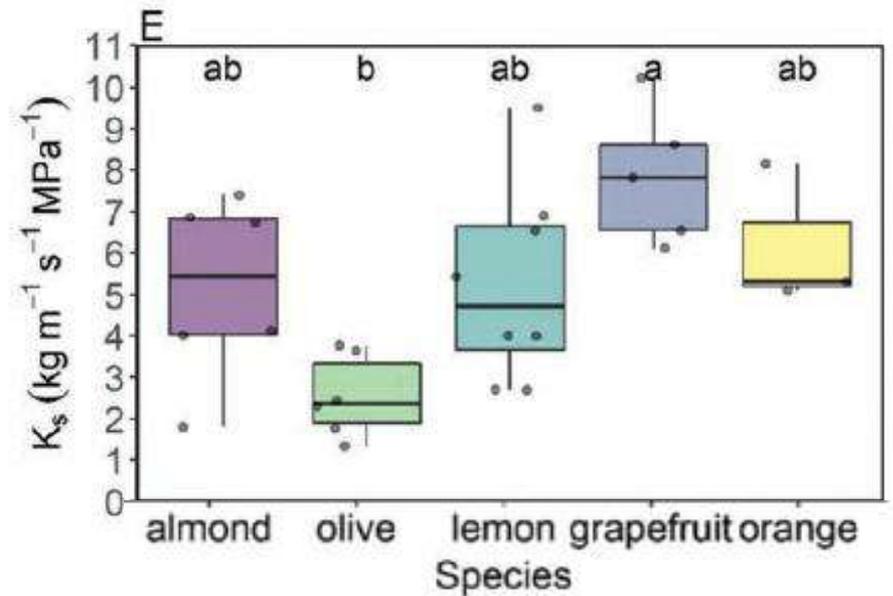
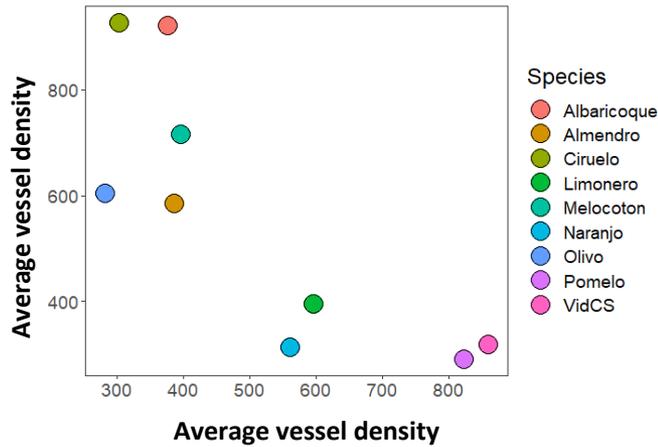
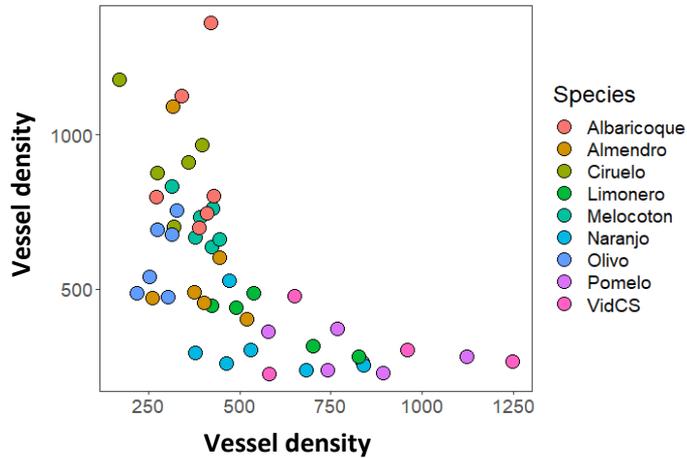
Lemon



Orange

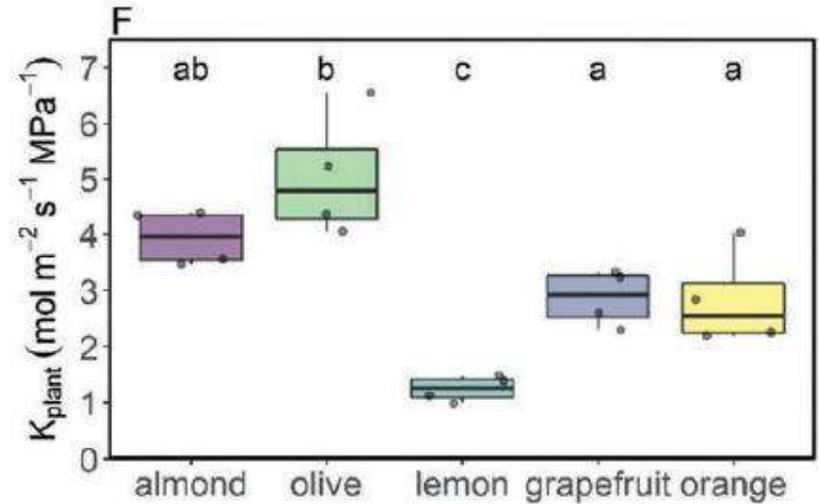
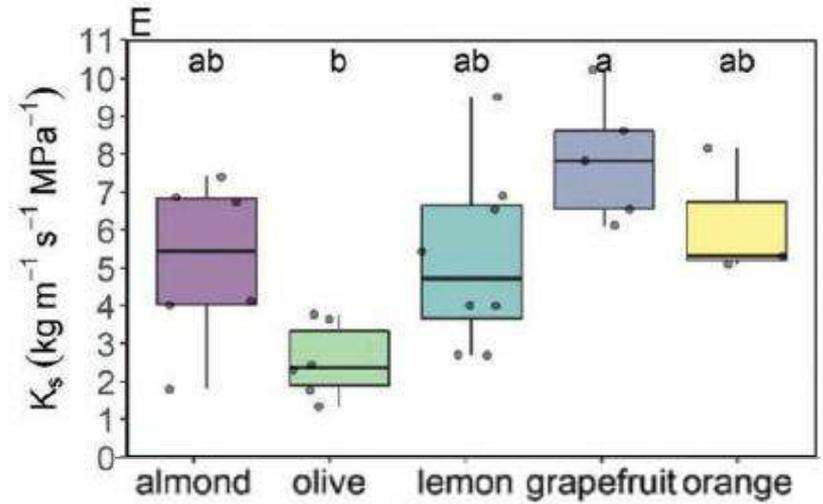
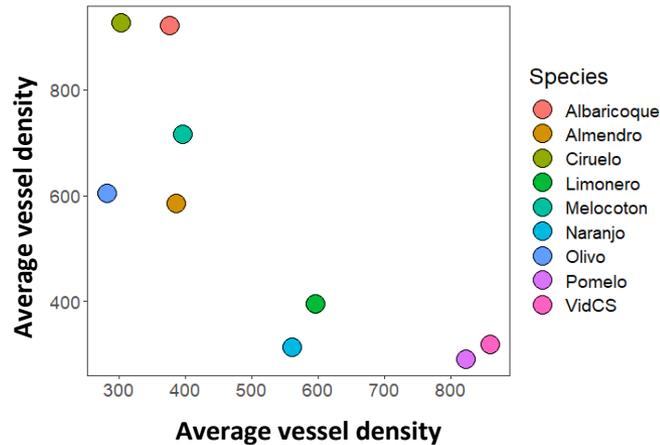
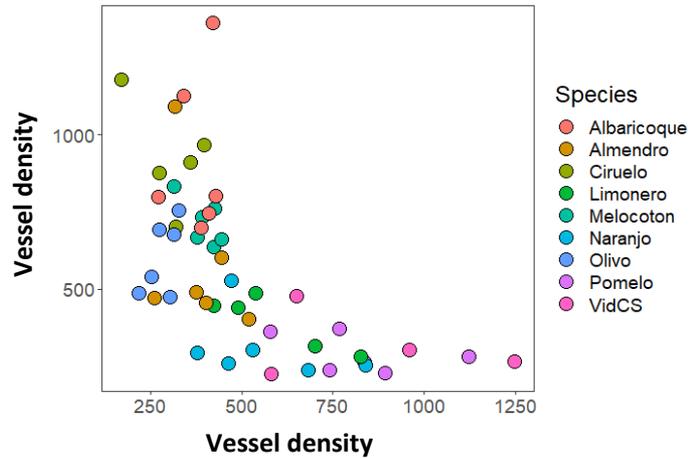


Anatomical measurements to calculate hydraulic traits

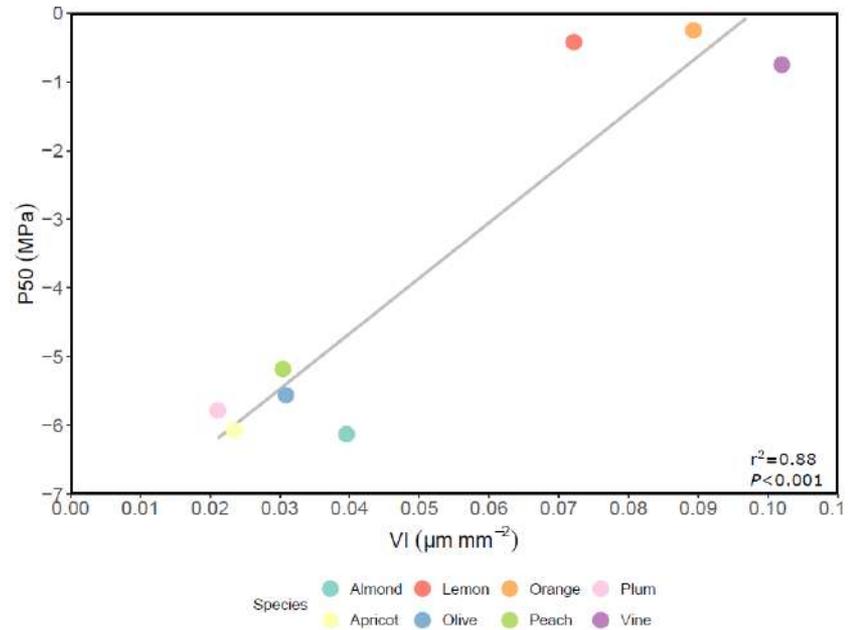
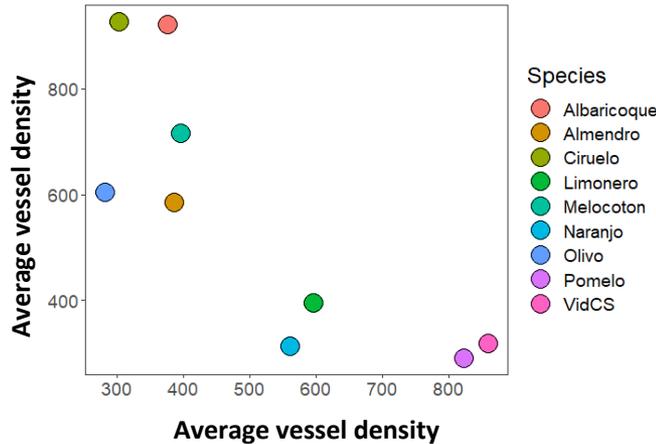
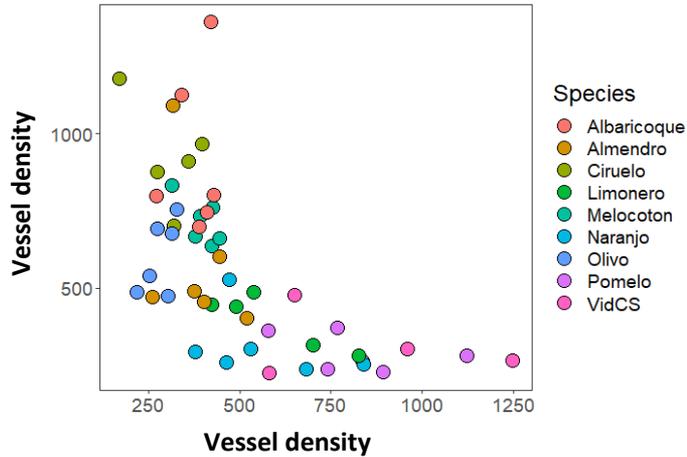


Hagen–Poiseuille equation (Tyree and Ewers, 1991)

Anatomical measurements to calculate hydraulic traits



Anatomical measurements to calculate hydraulic traits



VI: estimation of the ability of a plant to resist cavitation (Carlquist, 1977)

2.- Anatomy research in physiology and agriculture

Anatomical characterisation to localize carbohydrates

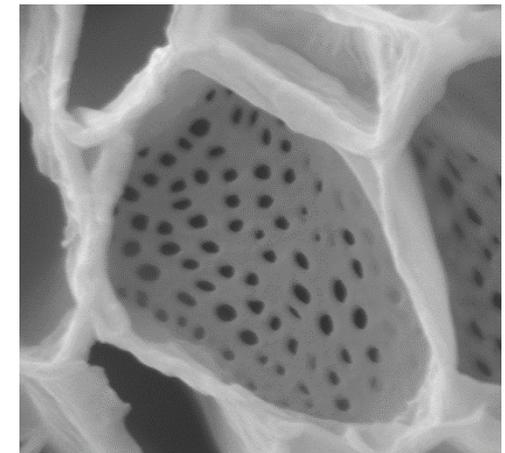
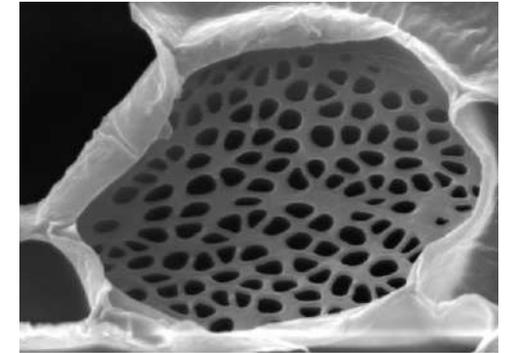
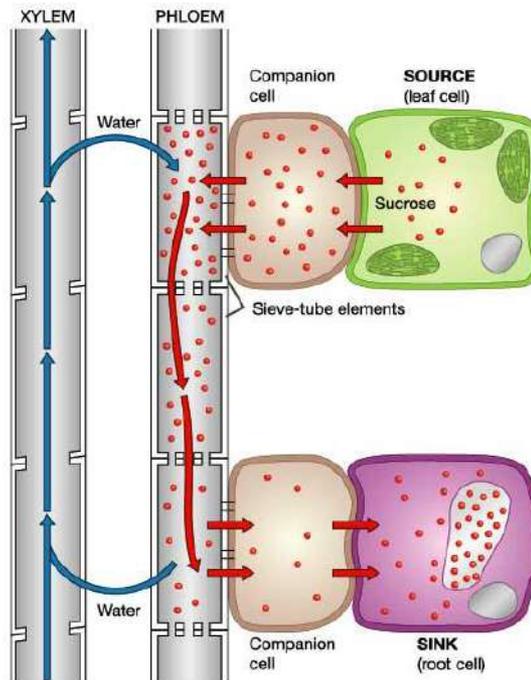
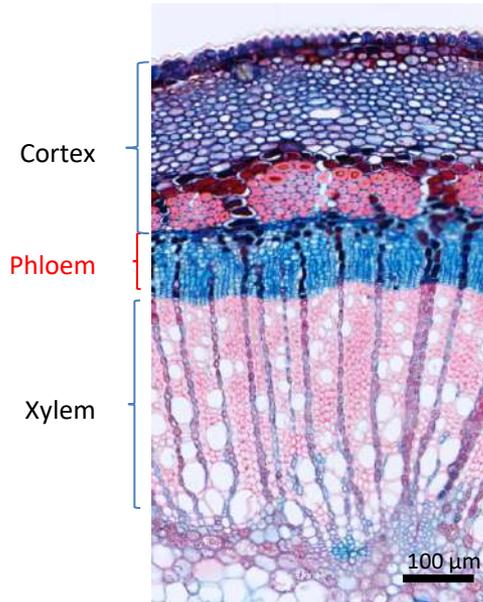


<https://watchplantproject.eu/>

A biohybrid system technology for **in-situ self-powered monitoring** that allows plants to wear AI components and technological interfaces, which results in creating of “smart biohybrid organisms” for environmental monitoring

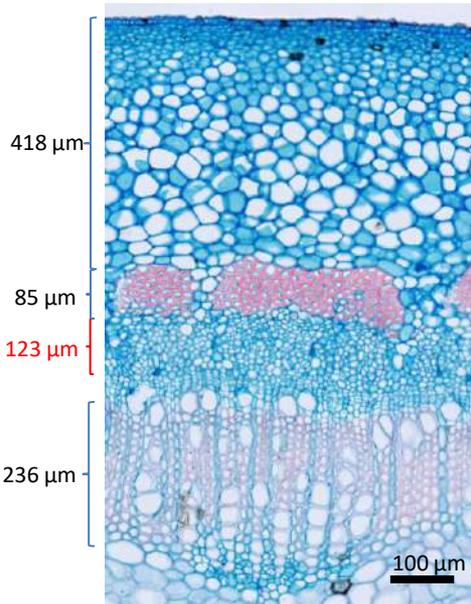
A new source of renewable energy from plants: plants carbohydrates

Where are the sugars? Phloem and pith



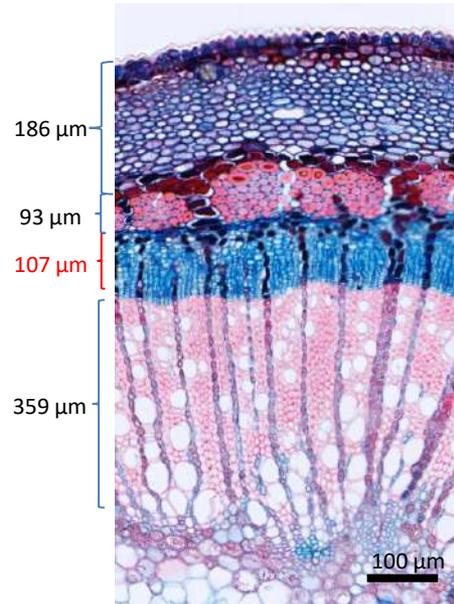
Phloem location in fruit tree stems

Lemon tree



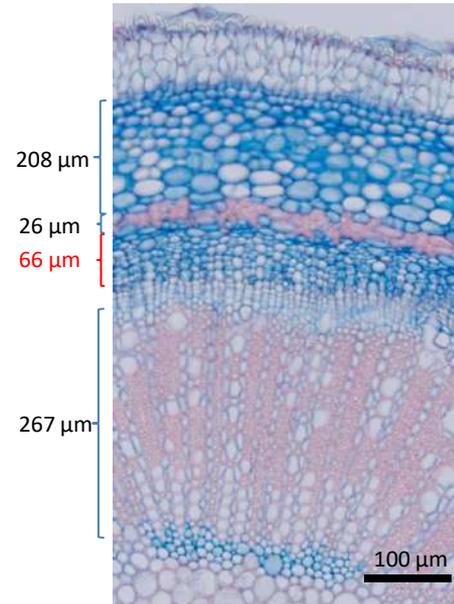
Phloem between 500 and 623 μm from cortex (similar to tomato)

Almond tree



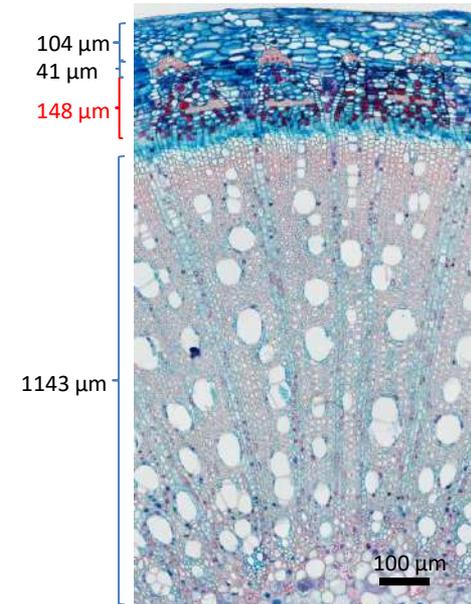
Phloem between 280 and 400 μm from cortex

Olive tree



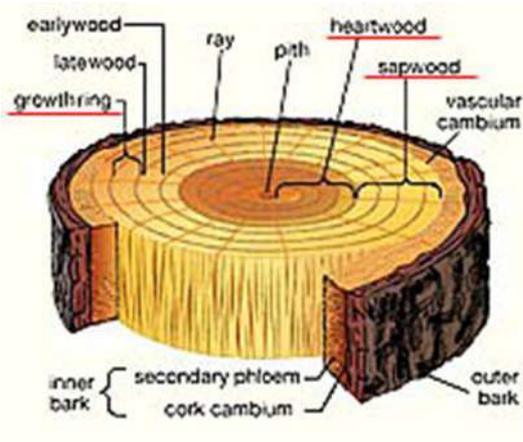
Phloem between 280 and 350 μm from cortex

Grapevine

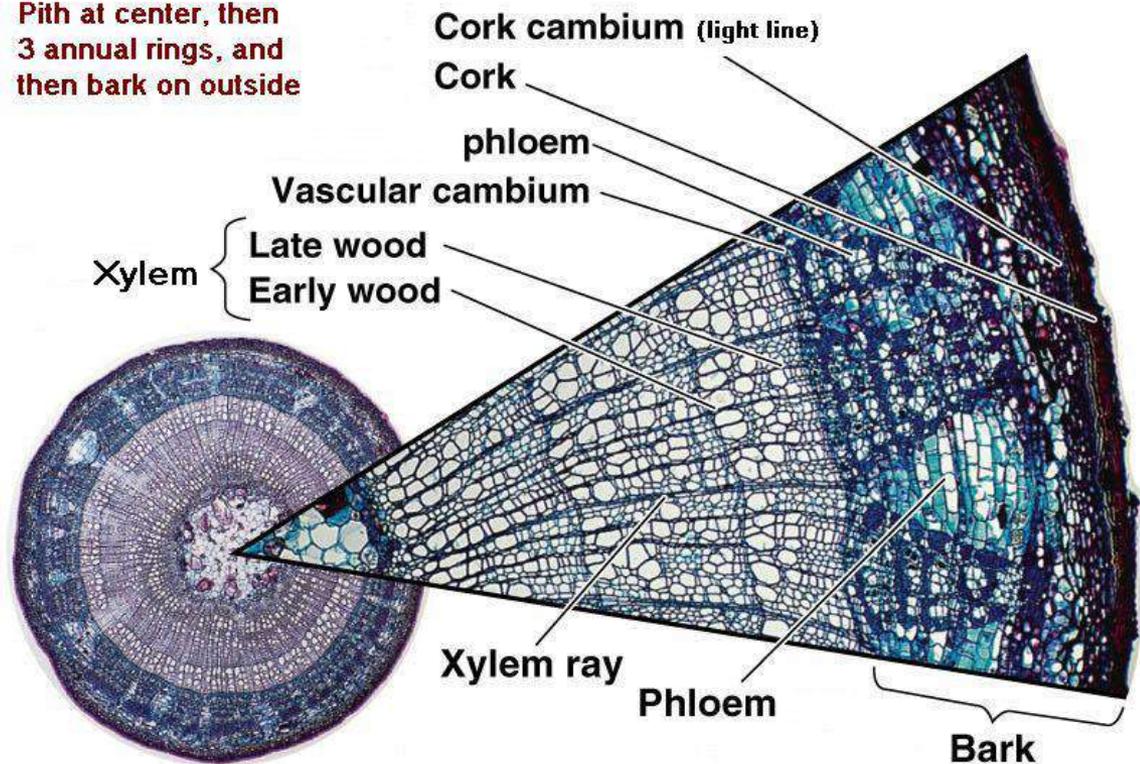


Phloem between 150 and 300 μm from cortex

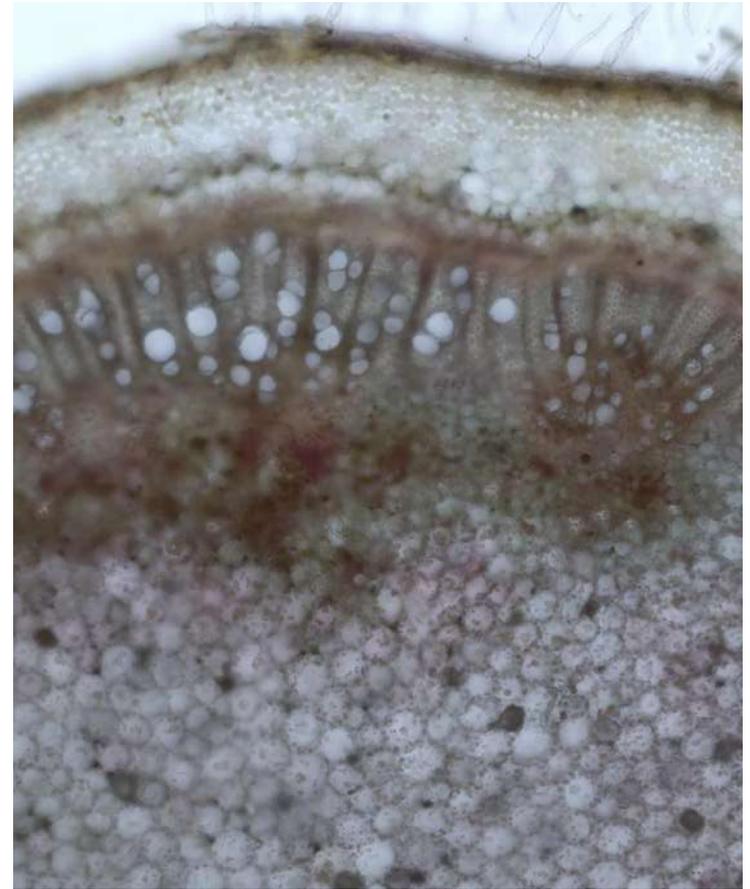
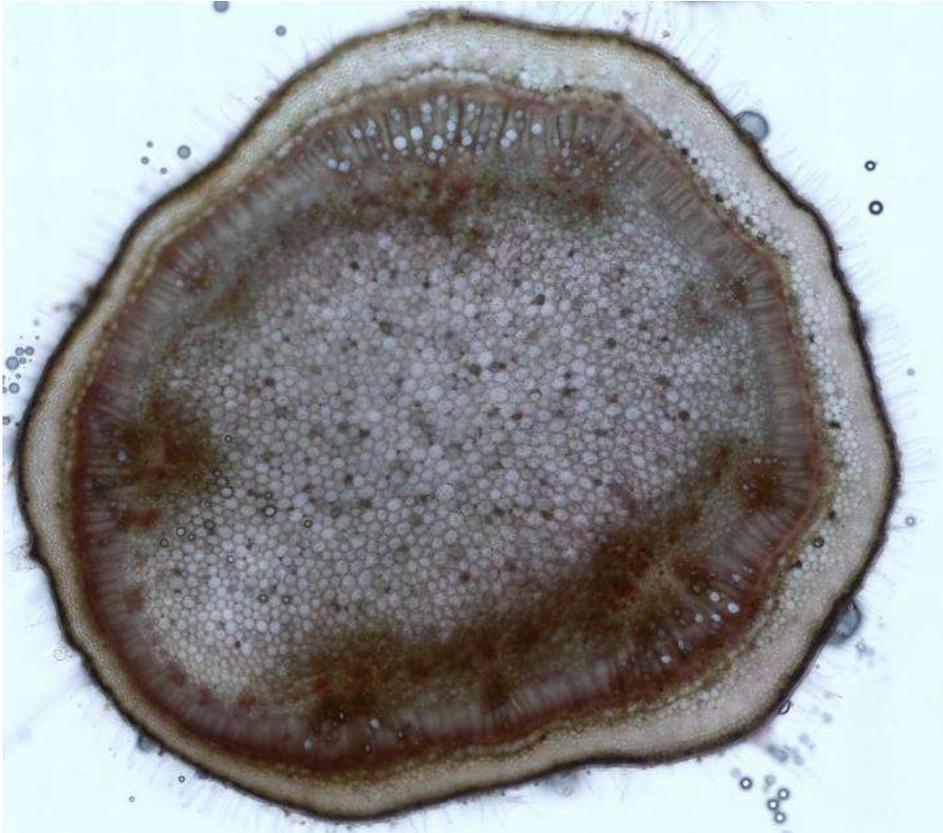
Where are the sugars?



**Pith at center, then
3 annual rings, and
then bark on outside**

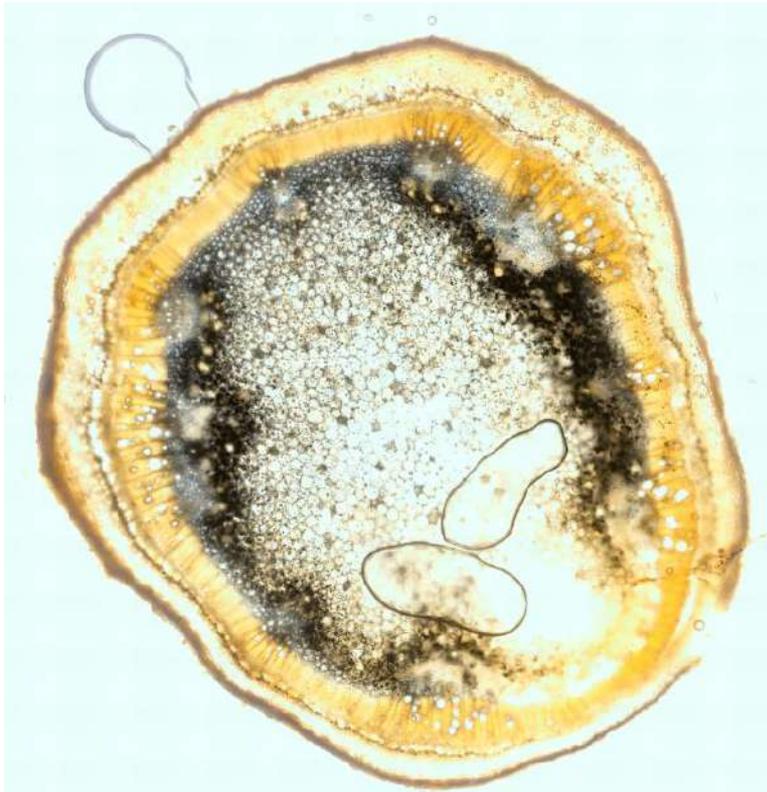


Where are the sugars?



Where are the sugars?

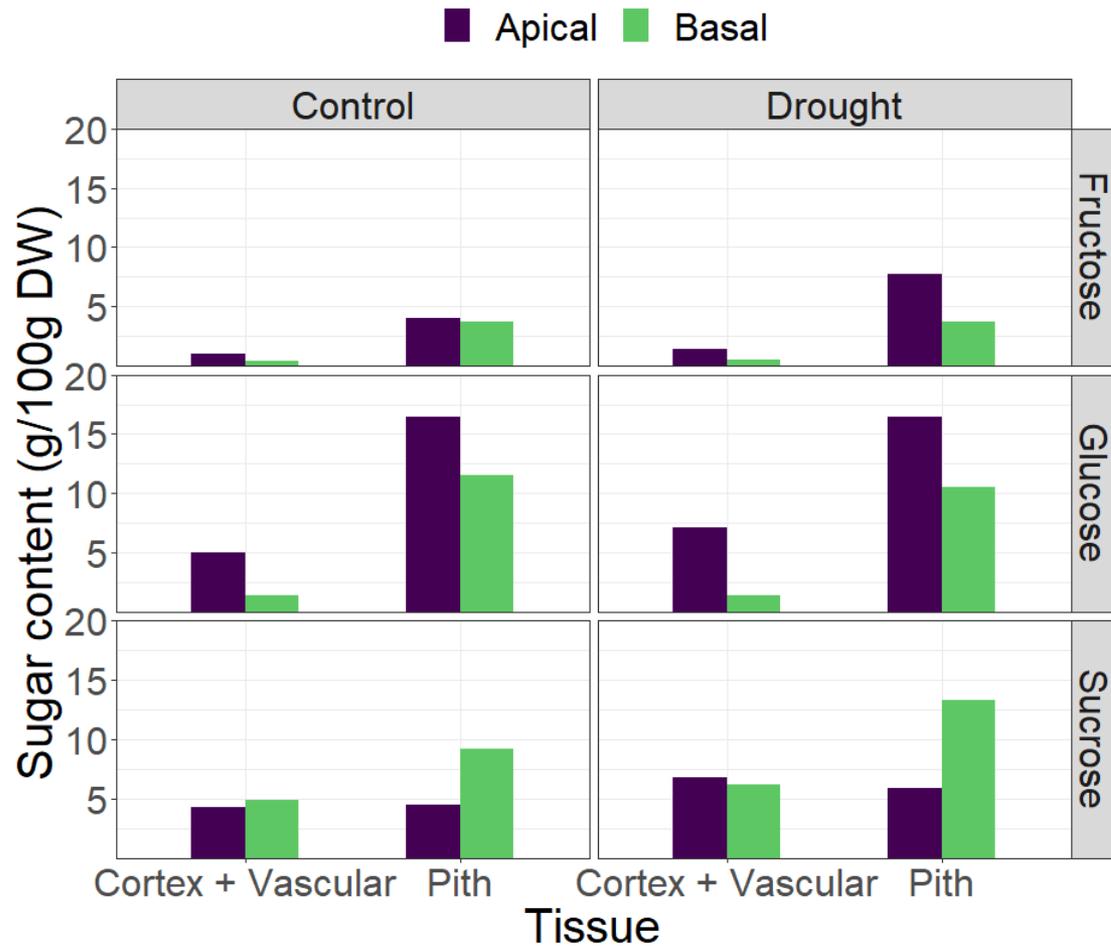
APICAL SECTION



BASAL SECTION



Where are the sugars?



Thanks!



Functional agrobiodiversity and agrosystem resilience: understanding plant traits to respond to water scarcity

Task 2.2., Topic 1

Virginia Hernandez-Santana

✉ virginiahsa@irnas.csic.es

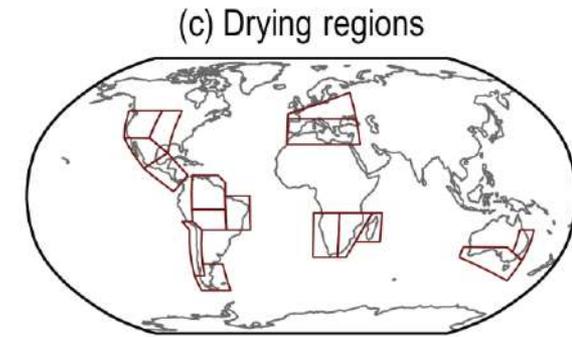
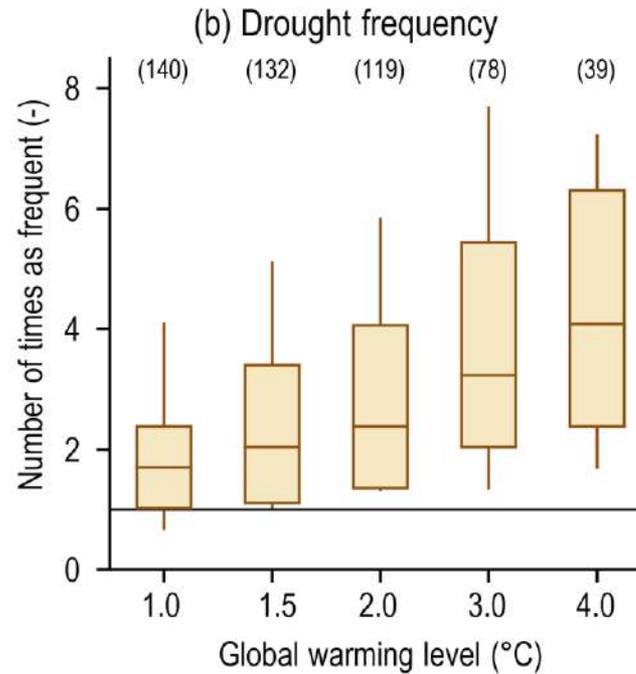
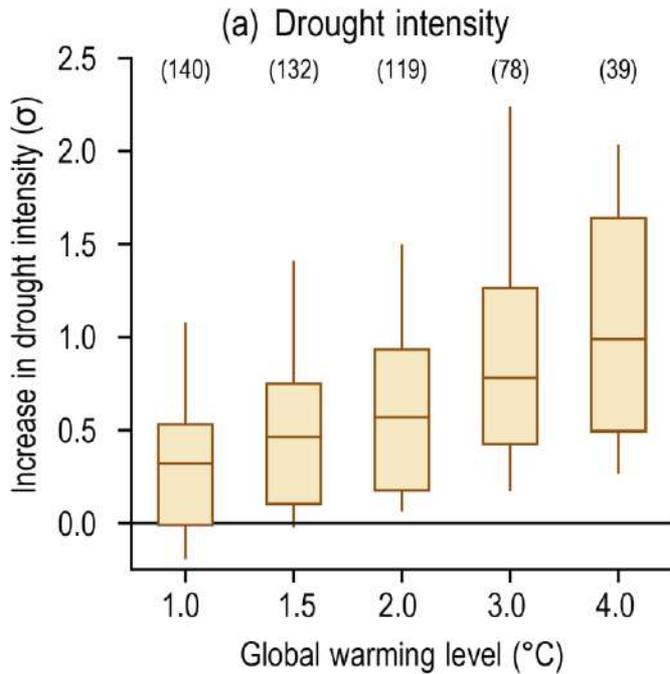
Funchal, 27-31/01/2025

- **Plant traits**
 - **Overview of plant traits and their importance**
 - **Leaf and whole-plant economics spectrum**
 - **Hydraulic traits and their impact on water use**
 - **Trait correlations and their drivers**
- **Implications for agricultural practices and sustainability**
- **(Examples of key traits methods)**

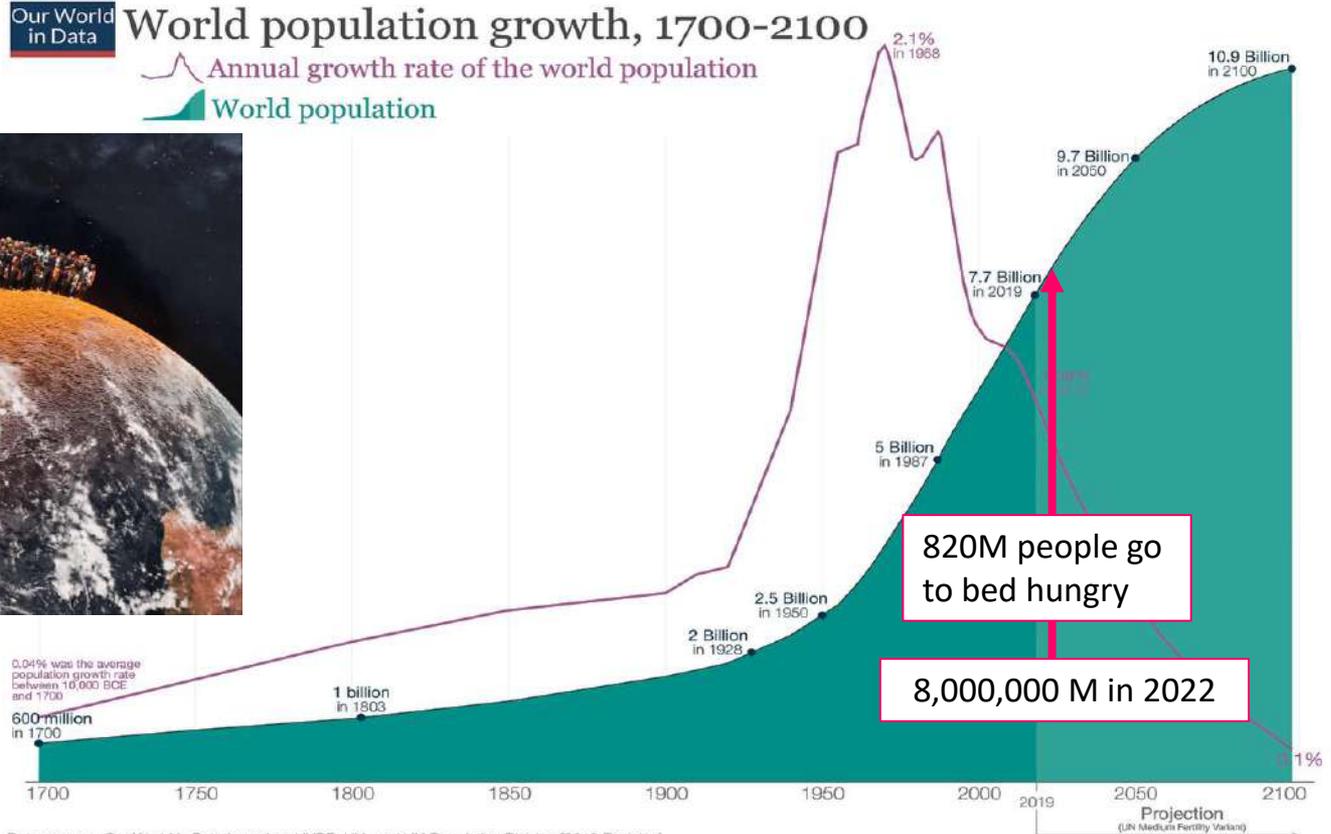
Plant traits

Overview of plant traits and their importance

Changes in 10-year soil moisture drought in drying regions



Overview of plant traits and their importance



Data sources: Our World in Data based on HYDE, UN, and UN Population Division [2019 Revision]
This is a visualization from OurWorldInData.org, where you find data and research on how the world is changing.

Licensed under CC-BY by the author Max Roser.



Overview of plant traits and their importance



SUSTAINABLE DEVELOPMENT GOALS

17 GOALS TO TRANSFORM OUR WORLD



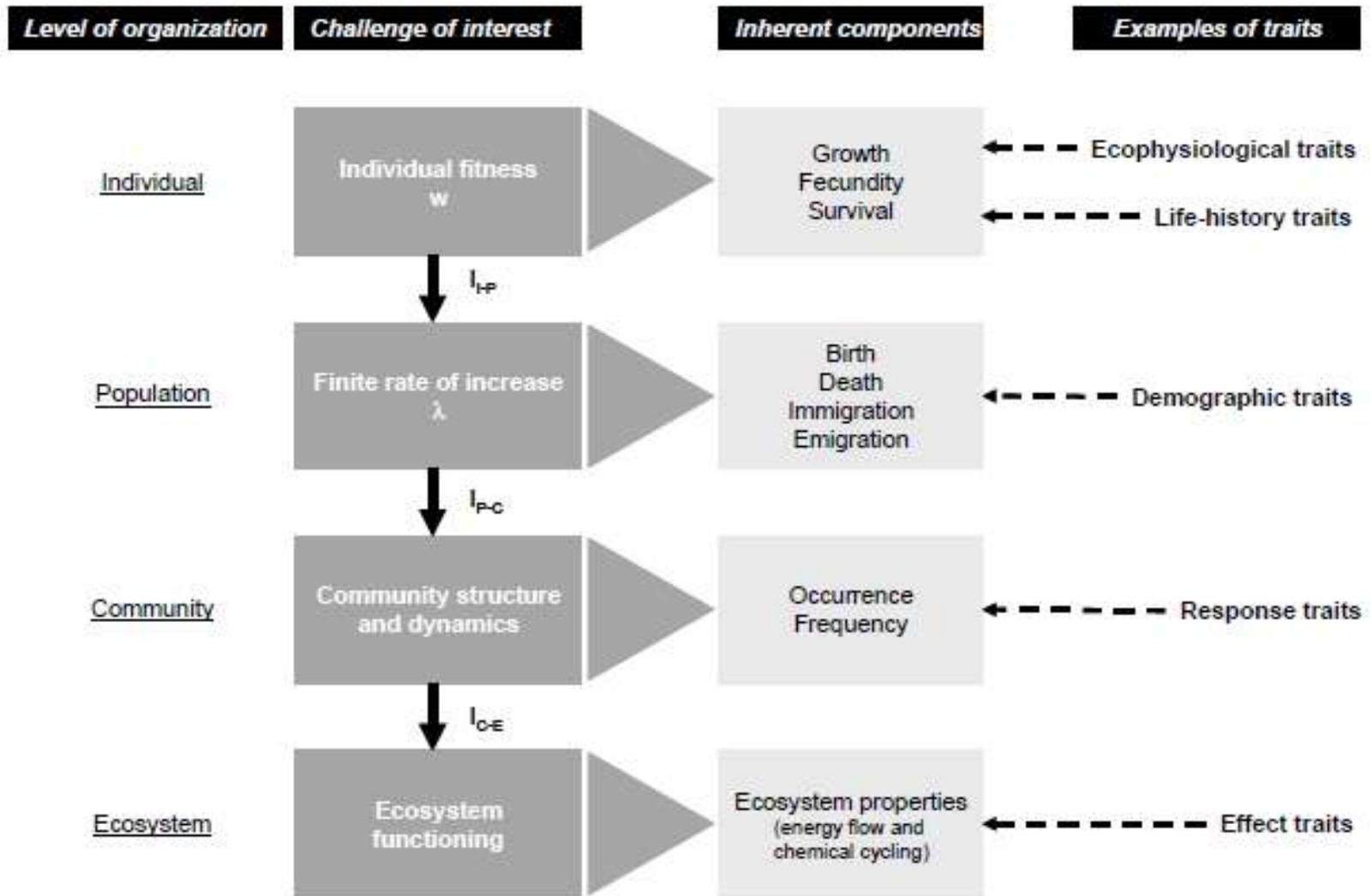
Understand plant response to drought

Overview of plant traits and their importance

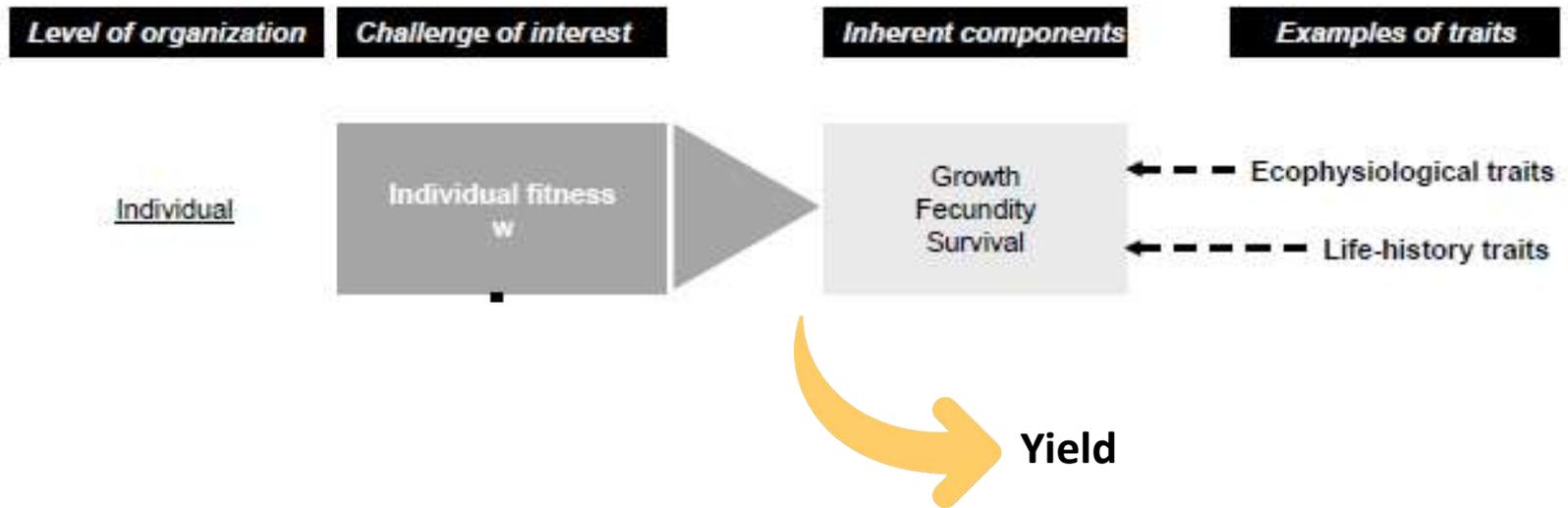
Trait-based approach

Terms	Definitions
Individual level:	
Overall definitions:	
● Trait	● Any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization.
● Attribute	● Value or modality taken by a trait at a point of an environmental gradient.
● Functional trait	● Any trait which impacts fitness indirectly via its effects on growth, reproduction and survival.
● Performance trait	● Direct measure of fitness. In plants, only three types of performance traits are recognized: vegetative biomass, reproductive output (e.g. seed biomass, seed number), plant survival.
Interactions with environment:	
● Response trait	● Any trait the attribute of which varies in response to changes in environmental conditions.
● Ecological performance	● Response of the whole-organism performance, assessed by one or more performance traits (maximum, mean or variance), to an environmental gradient.
● Effect trait	● Any trait which reflects the effects of a plant on environmental conditions; community or ecosystem properties.
Population, community and ecosystem levels:	
● Demographic parameter	● Population feature which directly conditions the finite rate of increase (λ) of the population: age- or stage-specific rates of survival, reproduction, growth, development.
● Community or ecosystem property	● Any feature or process measured at the community or ecosystem level
● Community functional parameter	● Any feature resulting from the community-aggregation of functional traits

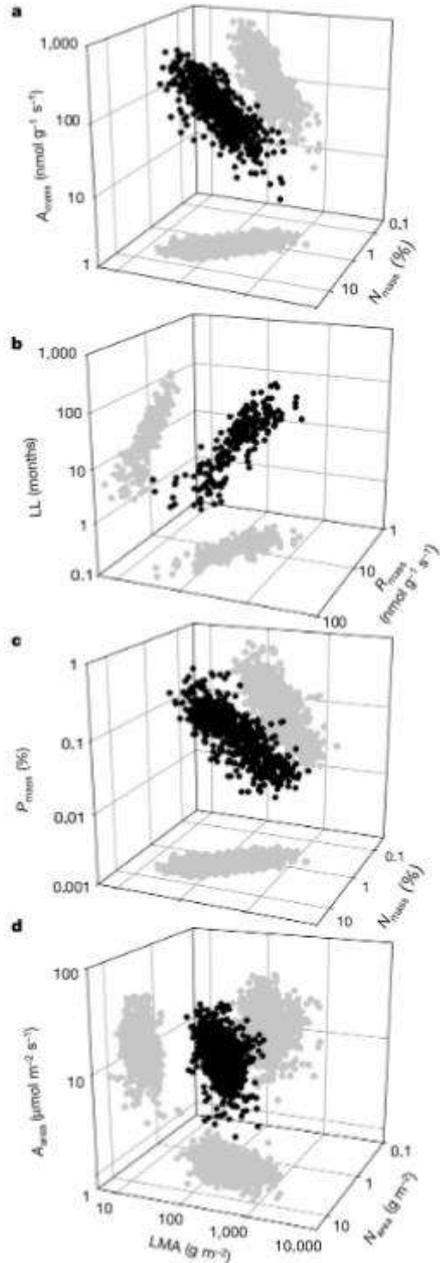
Overview of plant traits and their importance



Overview of plant traits and their importance



Leaf and whole-plant economics spectrum



Leaf Economics Spectrum (LES): LES links leaf traits that relate to costs of construction, nutrient content, and carbon fixation rates, providing insight into plant resource use strategies.

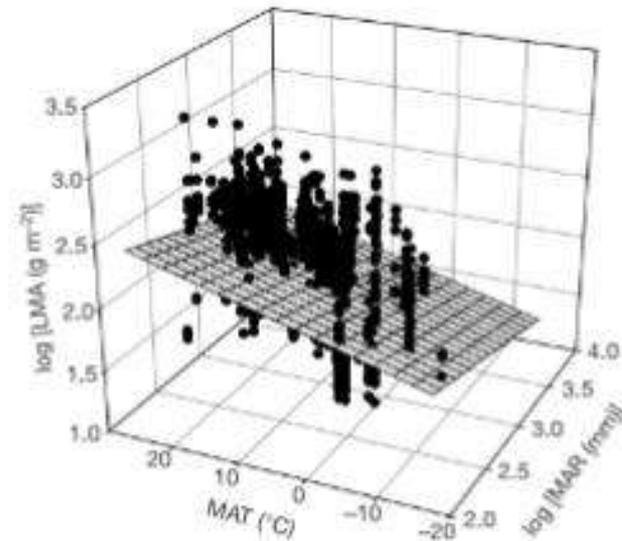


Figure 3 LMA as a function of MAT and MAR at the study sites (data for 2,370 species from 163 sites; rainfall and LMA are log₁₀-scaled). The coefficients for MAT and log rainfall were highly significant in a multiple regression (both $P < 0.0001$; further details given in Supplementary Information).

Leaf and whole-plant economics spectrum



Whole-Plant Economics Spectrum: This expands on LES by incorporating traits related to water, carbon, and nutrient use throughout the plant.

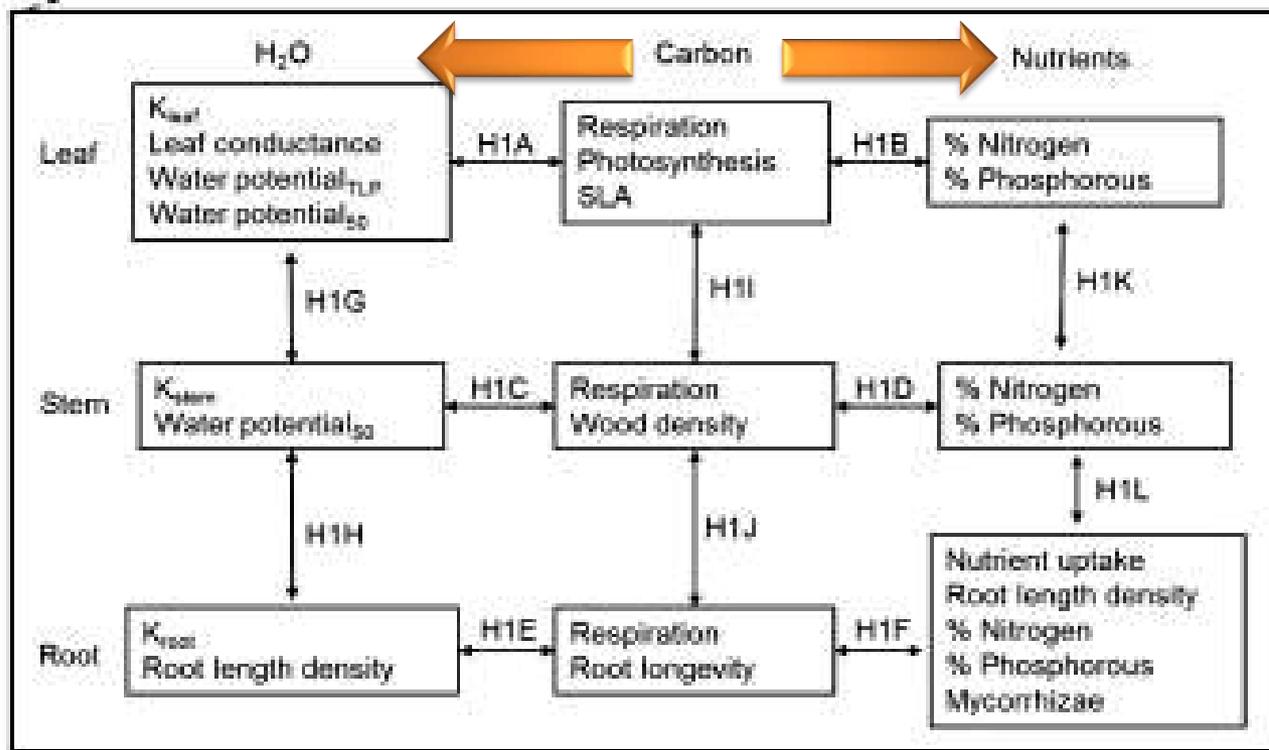


Fig. 1. Illustration of traits, resources and linkages across scales. Relationships between organ traits across scales shown otherwise. Hypotheses (H1–H9) are shown; when in parenthesis, it suggests an in-attributes at organism, community and ecosystem scales can be considered traits and represent the inter-community-weighted mean and variance in the value of any given trait at the scale of community, ecos

Leaf and whole-plant economics spectrum



Whole-Plant Economics Spectrum: This expands on LES by incorporating traits related to water, carbon, and nutrient use throughout the plant.

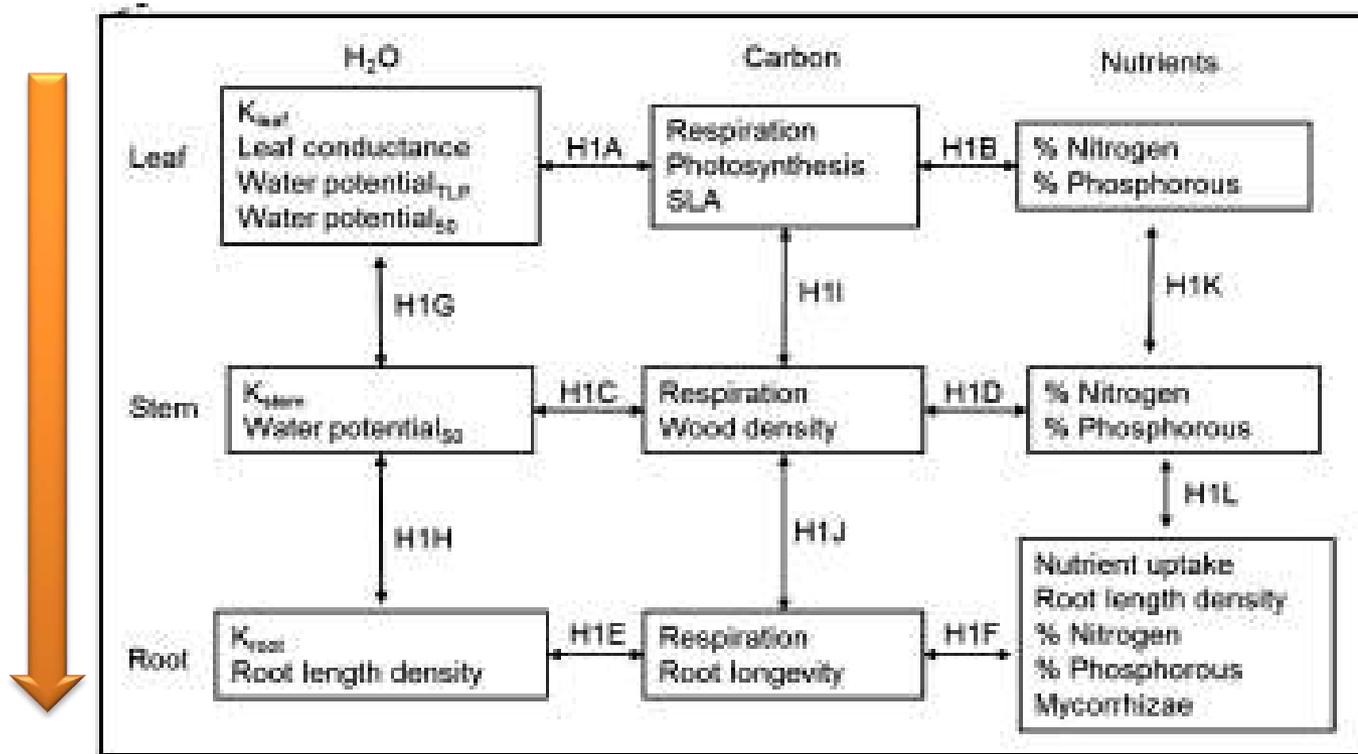


Fig. 1. Illustration of traits, resources and linkages across scales. Relationships between organ traits across scales shown otherwise. Hypotheses (H1–H9) are shown; when in parenthesis, it suggests an in-attributes at organism, community and ecosystem scales can be considered traits and represent the inter-community-weighted mean and variance in the value of any given trait at the scale of community, ecos

Hydraulic traits and their impact on water use



Hydraulic Traits are crucial for understanding plant water use and response to drought.

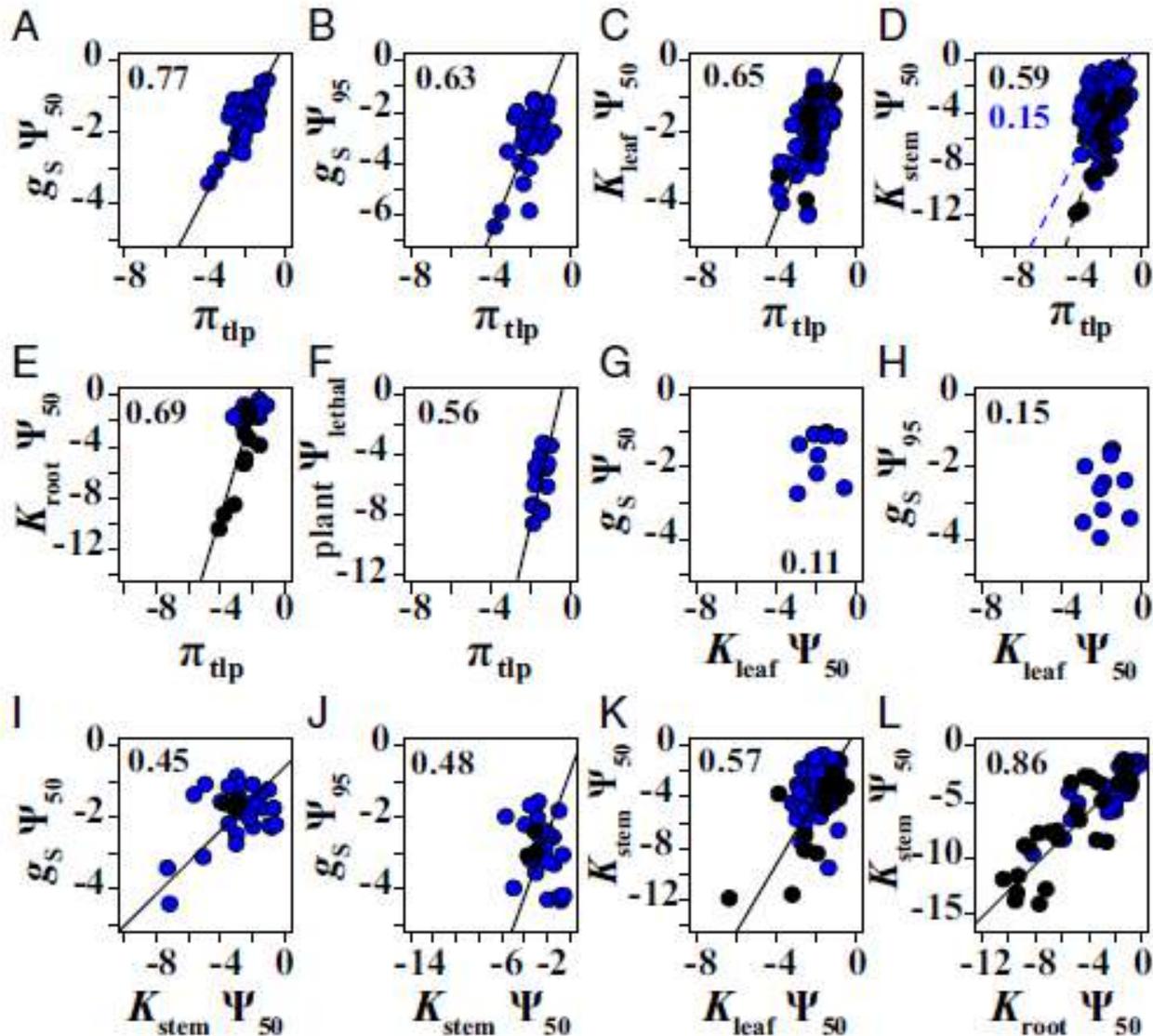
Table 1. The symbol, definition, and functional significance of the drought tolerance traits and the environmental water supply and general plant water status variables

Symbol	Definition	<i>n</i>	Significance
Ψ_W	Water potential		Potential energy of water; a thermodynamically explicit and scalable index of water status
$\Psi_{leaf}, \Psi_{stem}, \Psi_{root}$	Ψ_W of the leaf, stem, and root		Index of hydration and the demand for water of each organ
π_{tip}	Bulk leaf turgor loss point, the Ψ_{leaf} where turgor potential = 0	285	Point at which, on average, leaf cells lose turgor and the leaf wilts (7)
$g_s \Psi_{50}$	Ψ_{leaf} at 50% loss of stomatal conductance	49	Ψ_W at 50% loss is a standard and, thus, comparable measure of drought tolerance across physiological processes (6)
$g_s \Psi_{95}$	Ψ_{leaf} at 95% loss of stomatal conductance	49	Approximates the maximum leaf water stress a plant can tolerate while maintaining gas exchange and C uptake
$K_{leaf} \Psi_{50}$	Ψ_{leaf} at 50% loss of leaf conductivity	117	Hydraulic traits measure drought impacts on the water supply for transpiration, which limits gas exchange and C uptake (17). Leaf water supply is hypothesized to be the most direct hydraulic constraint on transpiration (8)
$K_{stem} \Psi_{12}$	Ψ_{stem} at 12% loss of stem conductivity	208	Early declines in stem water supply are expected to impact gas exchange and C uptake more directly than later declines (10)
$K_{stem} \Psi_{50}$	Ψ_{stem} at 50% loss of stem conductivity	286	Hypothesized to correspond closely to the maximum water stress plants tolerate in natural conditions (4)
$K_{stem} \Psi_{88}$	Ψ_{stem} at 88% loss of stem conductivity	204	Hypothesized to be the point of irreversible xylem damage (18)
$K_{root} \Psi_{50}$	Ψ_{root} at 50% loss of root conductivity	44	Roots are hypothesized to be the “weakest link” (least tolerant organ), limiting tolerance of the entire hydraulic system (45)
Plant Ψ_{lethal}	Ψ_{leaf} at plant death; here, the Ψ_{leaf} at which all leaves show tissue damage	15	Integrates physiological and metabolic drought responses and recovery and directly links drought to performance (11)
$\Psi_{min, MD}, \Psi_{min, PD}$	Seasonal minimum water potential (Ψ_{min}), the most negative Ψ_{leaf} measured in the growing season at predawn (PD) or midday (MD)	174	Midday measurements quantify the strongest water stress the leaves experience in a typical year, whereas predawn measurements characterize the most negative soil water potential (13)

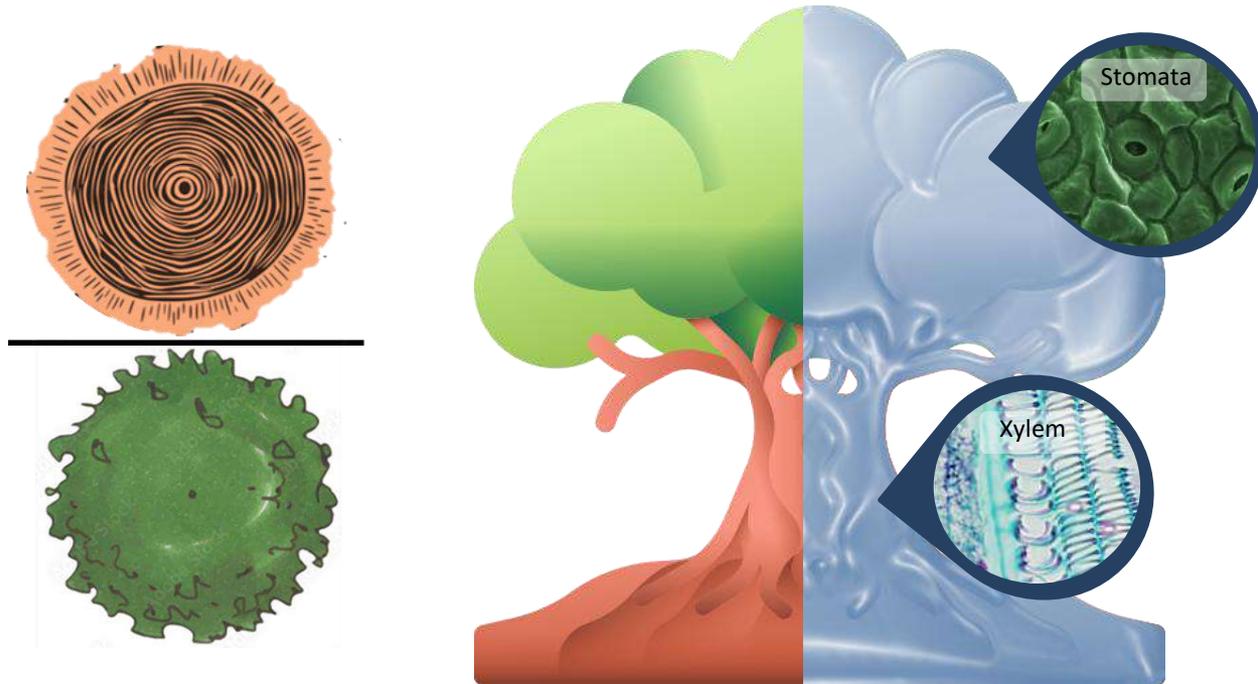
n is the number of species compiled for each trait. All units are MPa.

Bartlett et al. (2016)

Hydraulic traits and their impact on water use

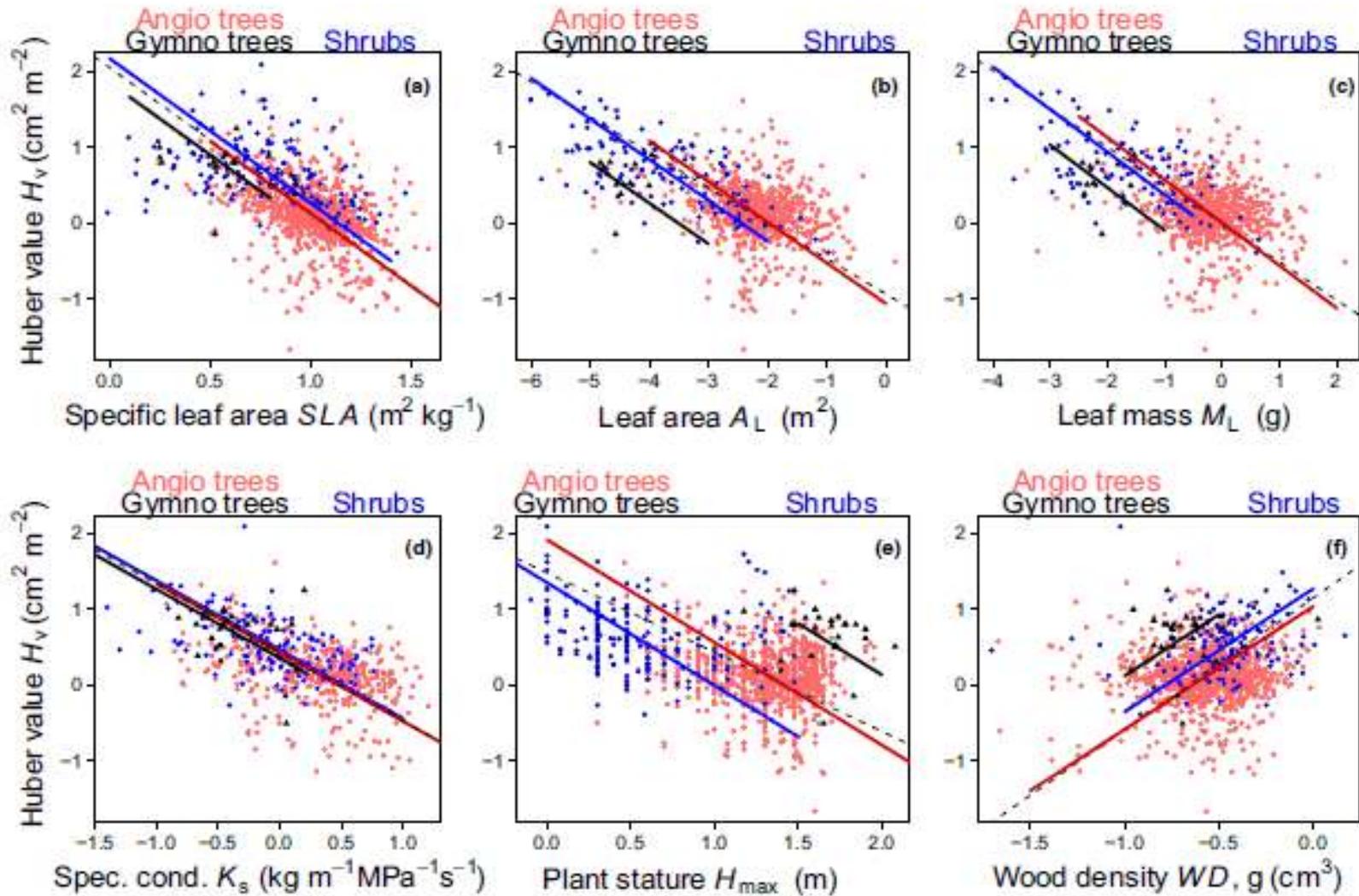


Hydraulic traits: Huber value

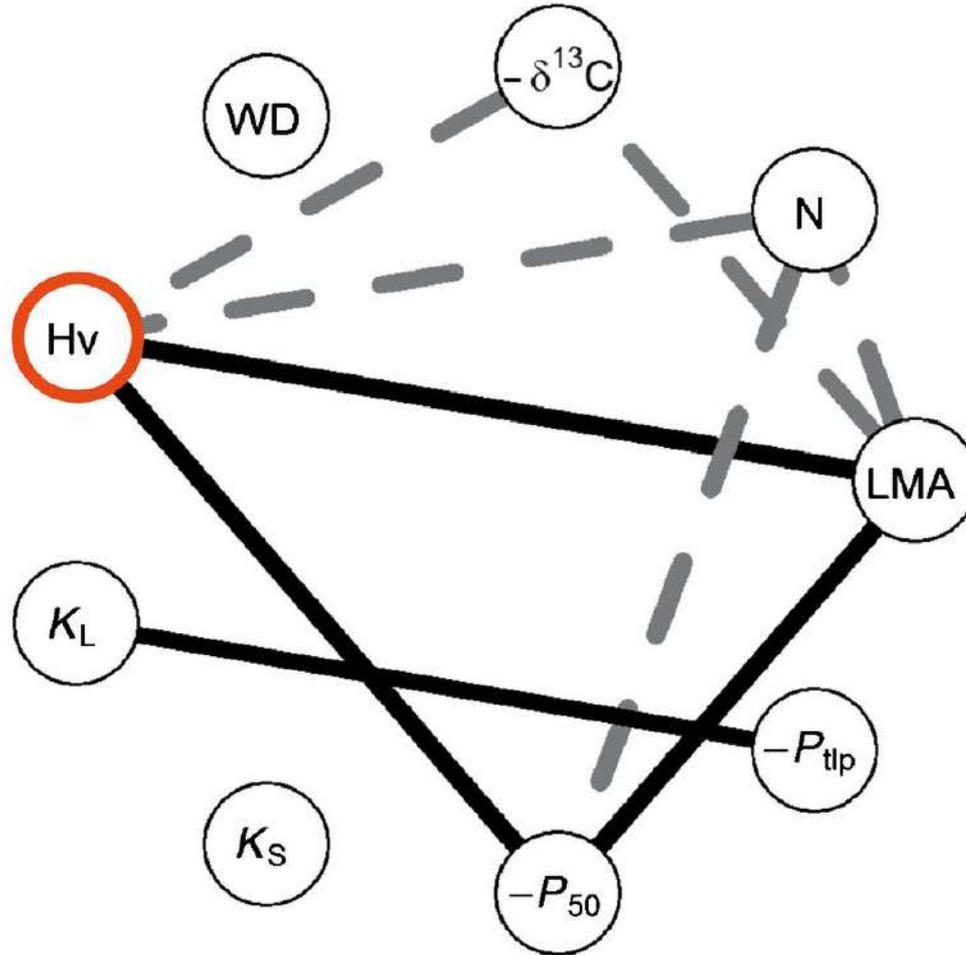


Hv seems to play a central role linking C through the different allocation of sapwood and leaf area which would influence the functional capacity to supply water through the sapwood and lose it through the leaves

Hydraulic traits: Huber value



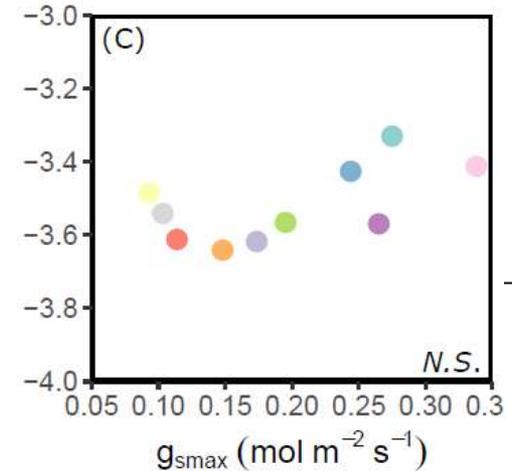
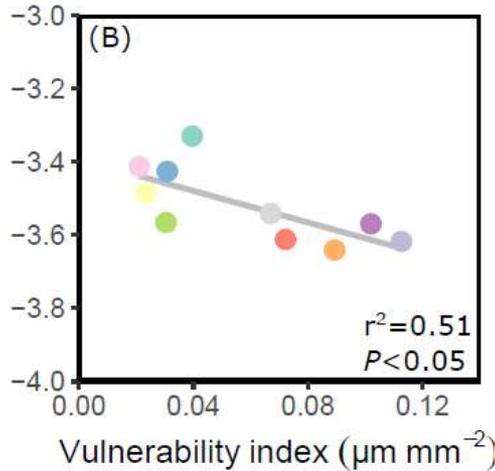
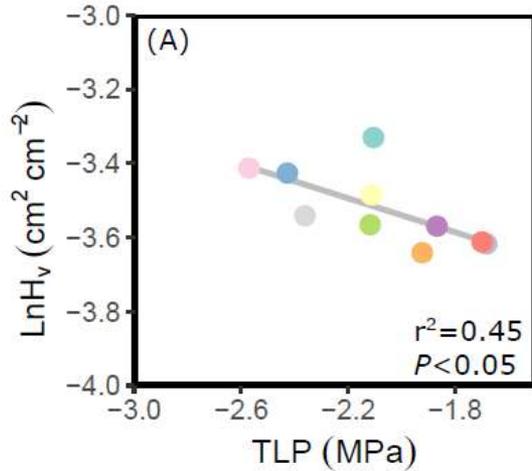
Hydraulic traits: Huber value



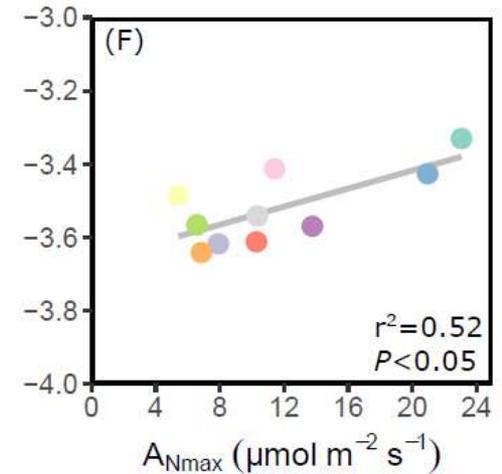
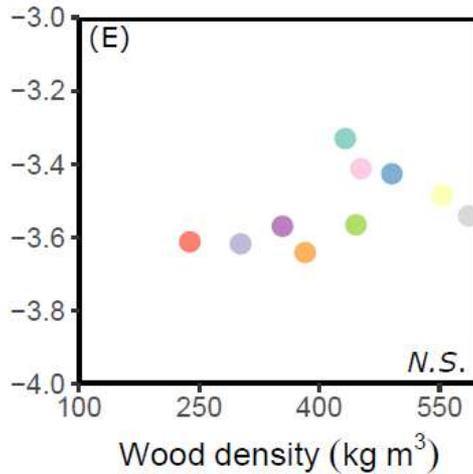
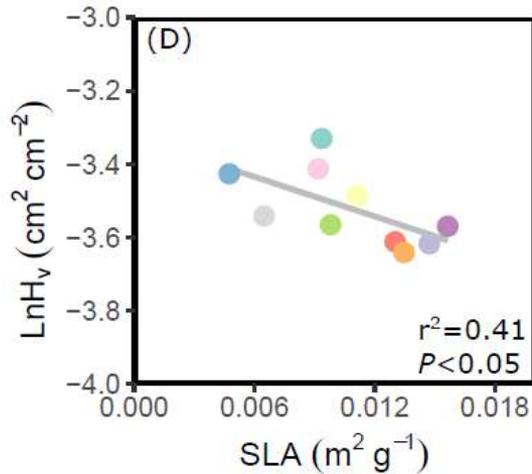
Rosas et al. (2019)

Hydraulic traits: Huber value

Water related traits



Carbon related traits



- species
- Almond
 - Apricot
 - Grapefruit
 - Lemon
 - Orange
 - Olive
 - Peach
 - Plum
 - Pomegranate
 - Vine

Trait correlations and their drivers

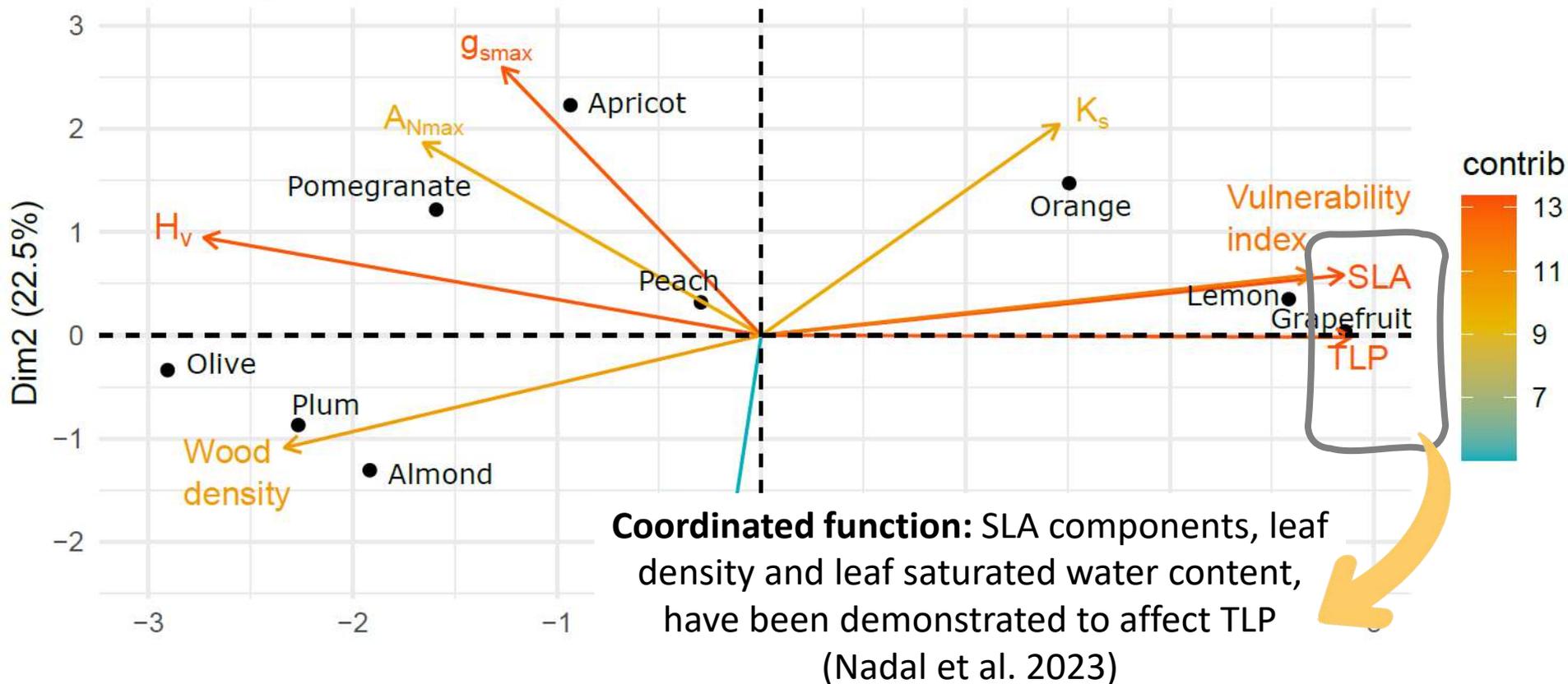
Drought tolerance traits can be correlated across species because of:

- **functional coordination**, such as mechanistic linkages
- **concerted convergence**, non-coordinated evolution of traits driven by drought stress, i.e., coselection by the environment, wherein traits are directionally but independently selected by water supply to optimize overall plant function
- **shared ancestry**

Trait correlations and their drivers

- Functional coordination
- Concerted convergence
- Shared ancestry

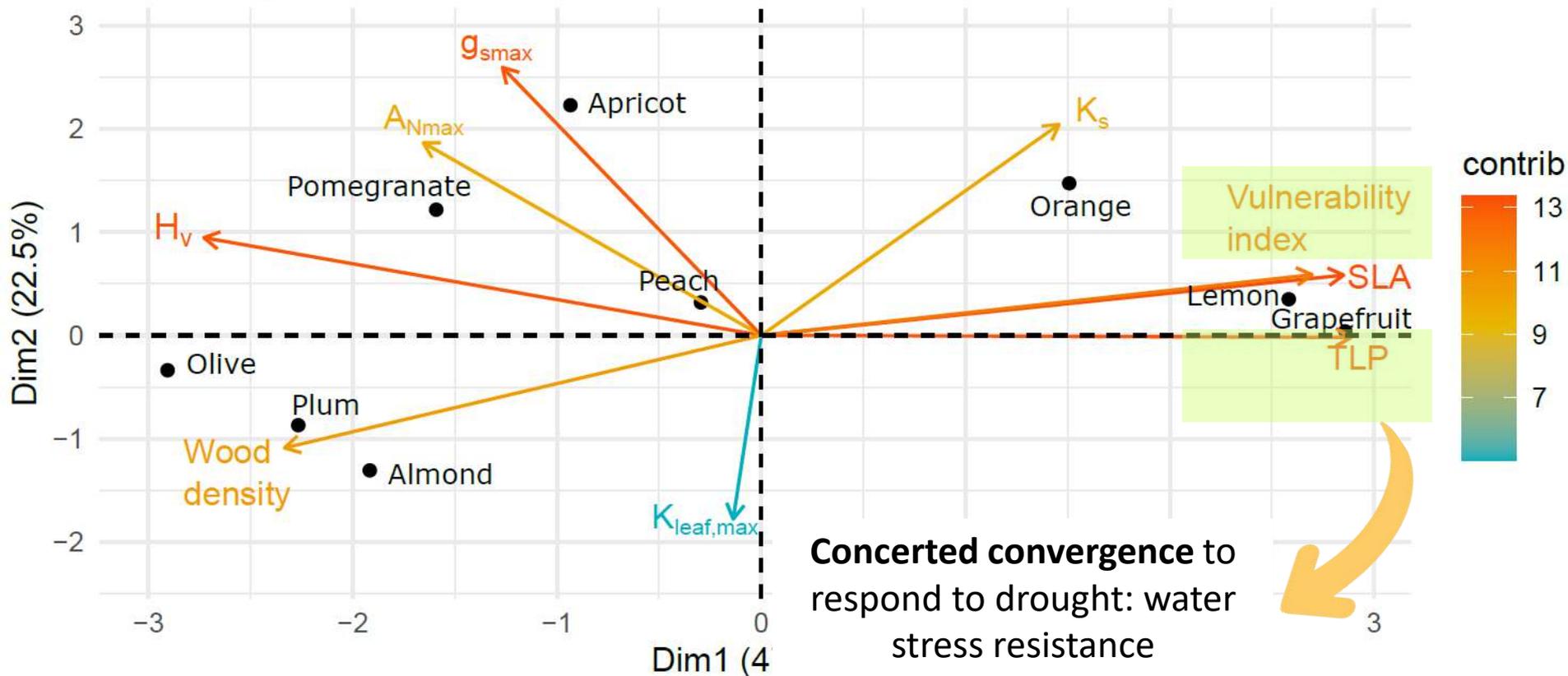
PCA – Biplot



Trait correlations and their drivers

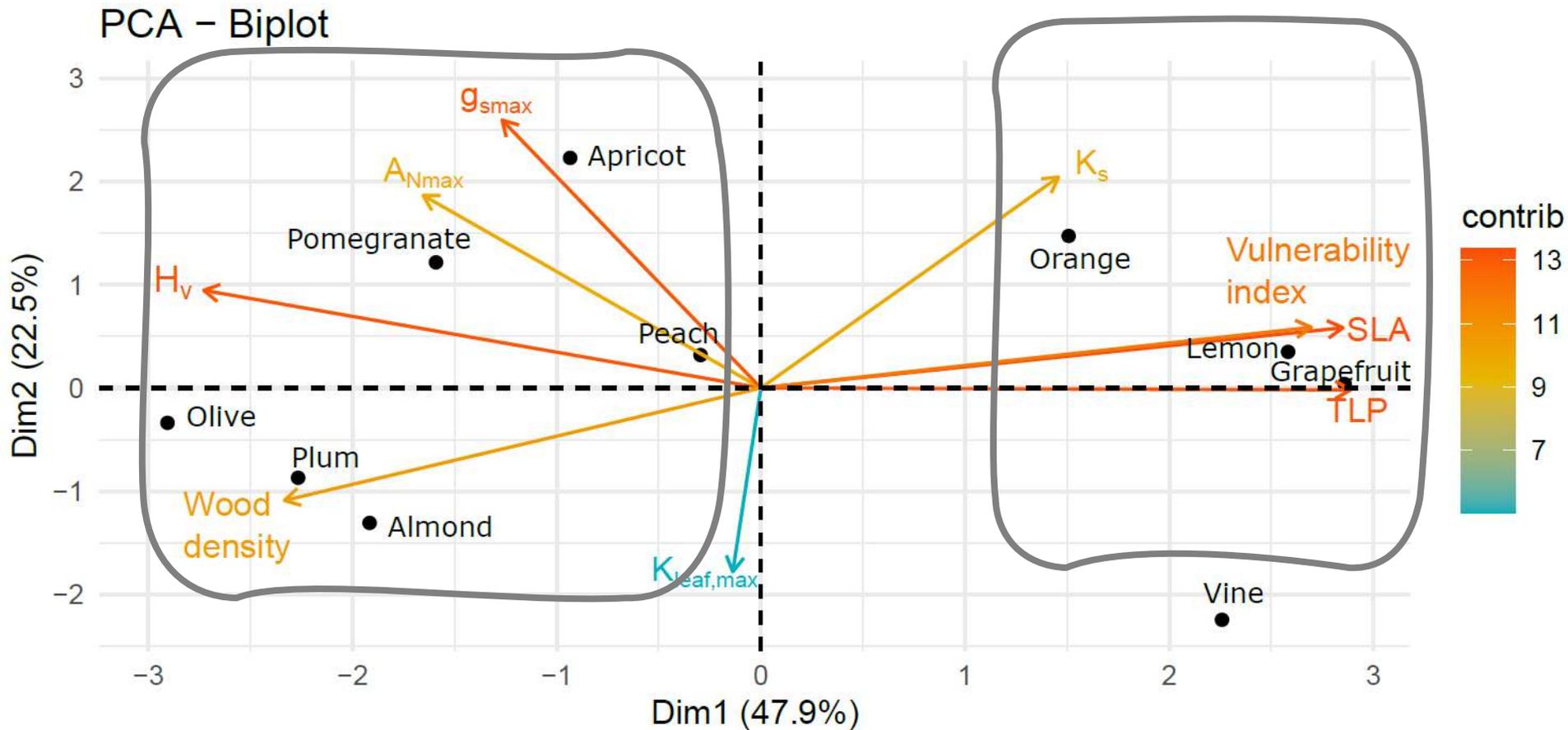
- Functional coordination
- **Concerted convergence**
- Shared ancestry

PCA – Biplot



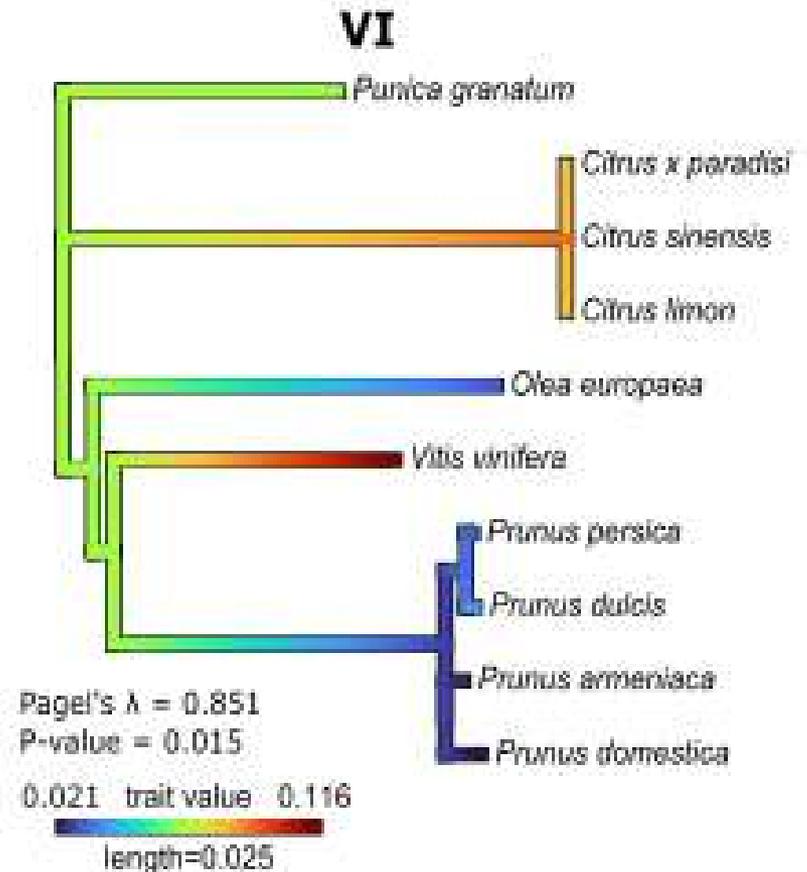
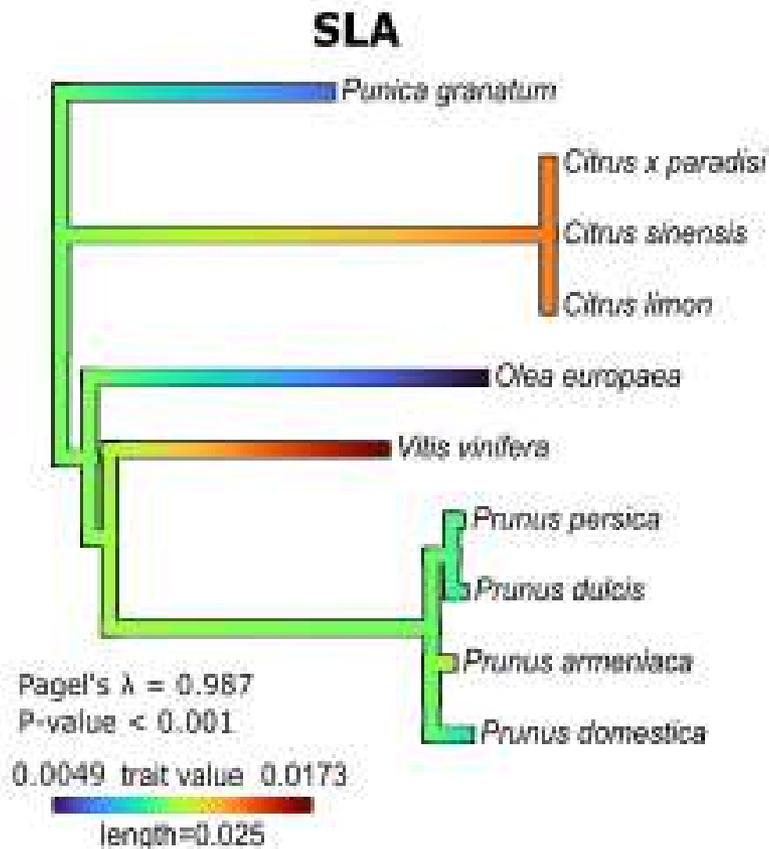
Trait correlations and their drivers

- Functional coordination
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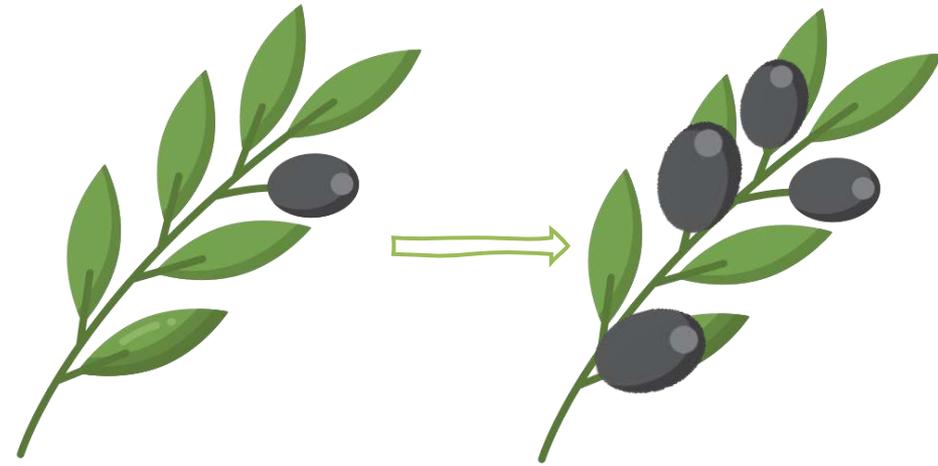
Trait correlations and their drivers

- Functional coordination
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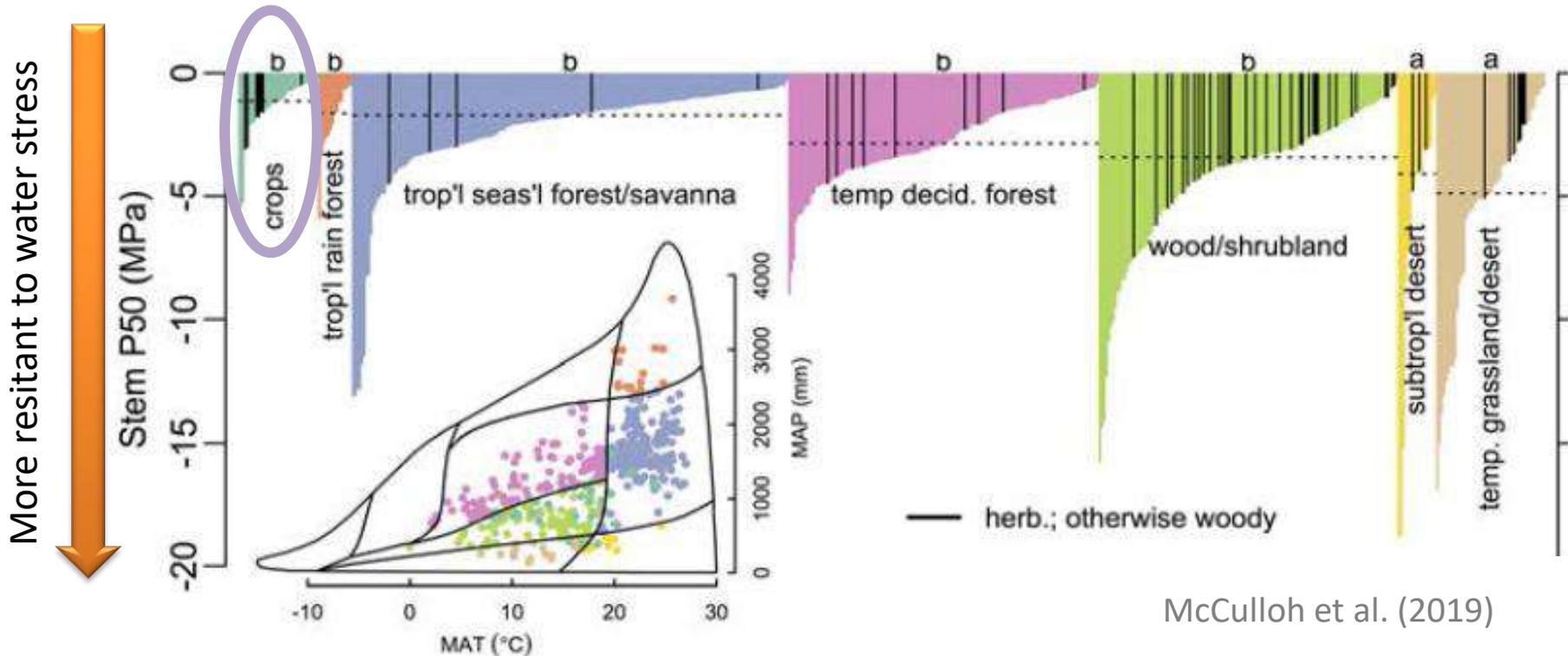


*Implications for agricultural
practices and sustainability??*

Why studying traits in crop species?



- Crop species largely selected for reproductive traits
- Not for vegetative physiology traits that determine plant response to water deficit
- Less studied: current and future climate conditions?



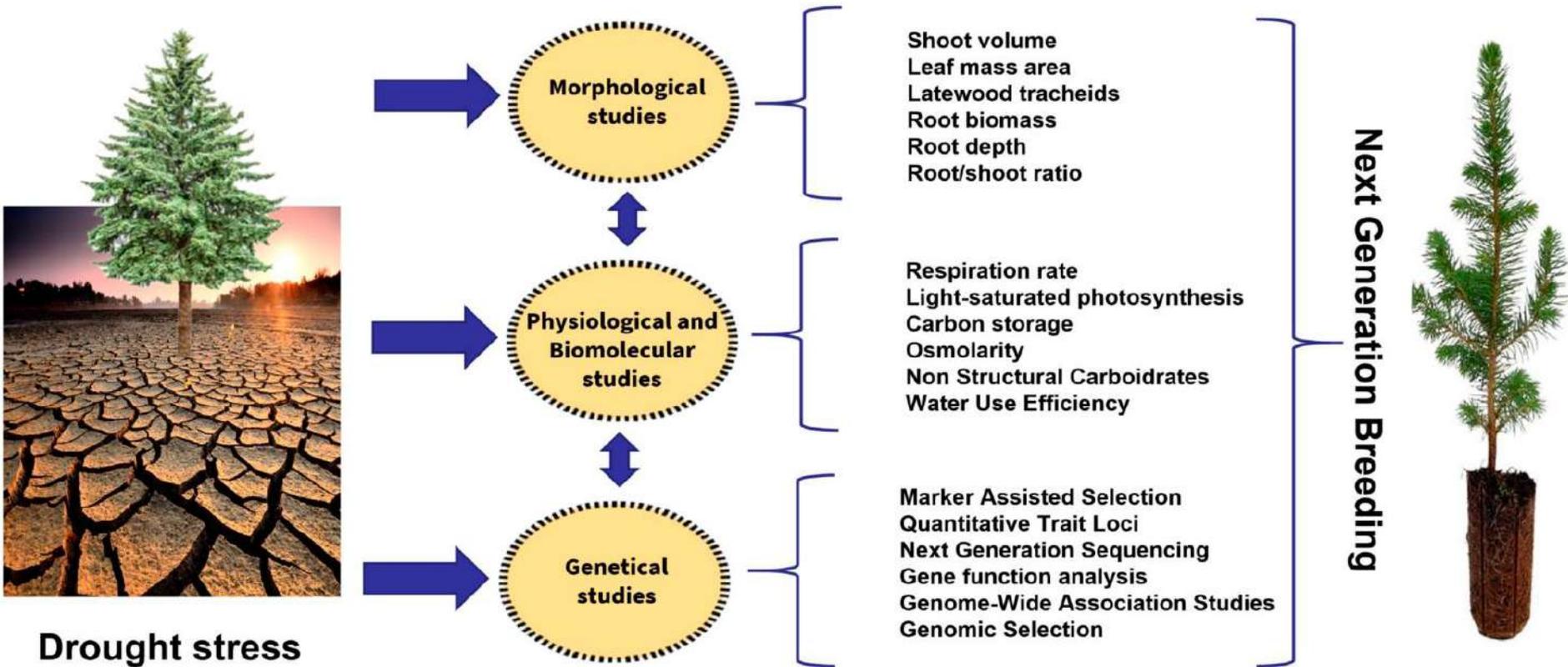
Why studying fruit trees?

Fruit trees are specially vulnerable to water deficit and high temperatures:

- Long crop life
- Long vegetative season
- Product depends on water availability



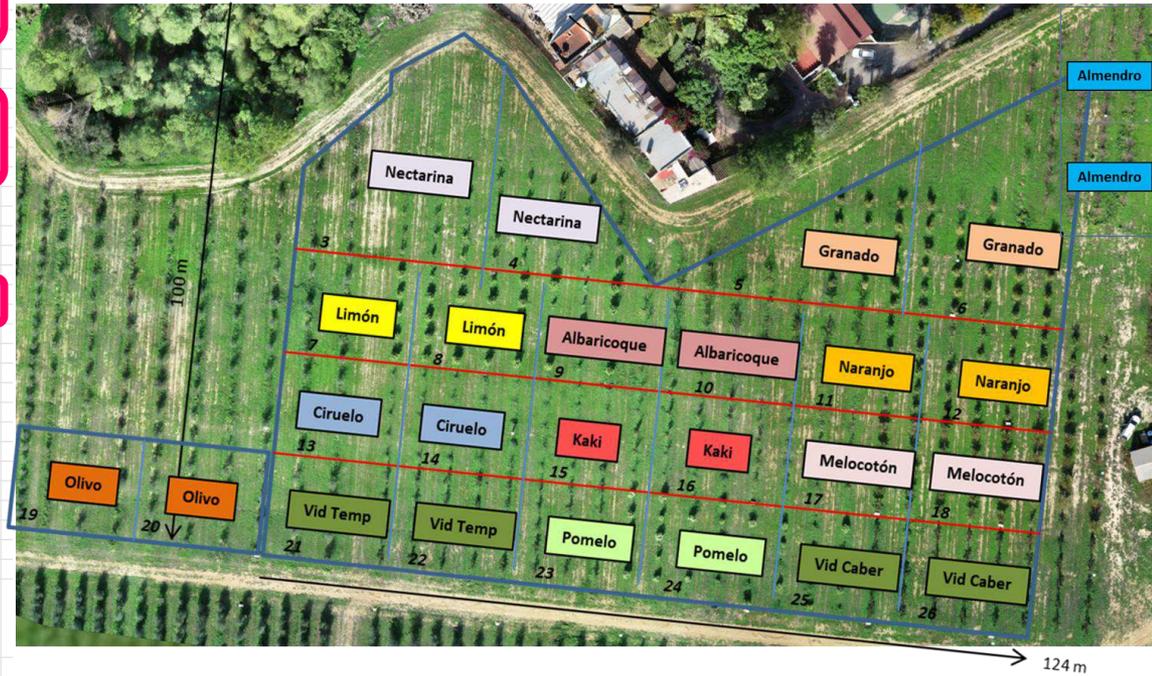
Implications for agricultural practices and sustainability



Baldi & La Porta (2022)

Implications for agricultural practices and sustainability

	A	B	C	D	E	F
1	Plant traits	Leaf area density		Stomatal traits	Stomata length	
2		Leaf area index			Stomata width	
3		Huber value			Stomatal density upper	
4		Shoot growth rate			Stomatal density lower	
5		Leaf growth rate			Stomatal ratio (up/low)	
6					gs max	
7	Wood traits	Wood density*		Photosynthesis traits	gm, max	
9		RWC wood*			Vcmax,25	
10		Vessel density*			Jmax,25	
11		Vessel diameter*			Rd,25	
12		Vessel length*			FTRmax 1800	
13		Twigs P50		Sm (gm)		
14		Twigs P12		Sc (gm)		
15		Twigs Kmax (leaf area)		Cell wall thickness (gm)		
16		Trunk Js max (sap flux)				
17	Diffuse/ring-porous		Water relations	RWC, min, midday		
18	Hydraulic safety margin			TLP		
19	Stomatal safety margin			Pi, 100		
20			Pi, 0			
21	Leaf traits	LMA		Root traits	Root length	
22		Leaf nitrogen			Root area	
23		Leaf width			Specific root length	
24		Leaf length		Root mass fraction		
25		Leaf thickness				
26		Leaf absorbance upper				
27		Leaf transmittance upper				
28		Leaf reflectance upper				
29		Leaf absorbance lower				
30		Leaf transmittance lower				
31		Leaf reflectance lower				
32		Leaf vein density				
33		Homo/Heterobaric (absence/presence of bundle sheath extensions)				
34	E _{max}					
35	Kleaf,max					
36	P50 Kleaf					
37	Psi, min, midday					
38						
39						



Framework for indexes used for irrigation management

Results summary

↑Hv

↓SLA

↑Anmax

↓VI

For a given sapwood,
small canopies with small
leaves but high acquisitive
leaves

Less vulnerable to water
stress

↓Hv

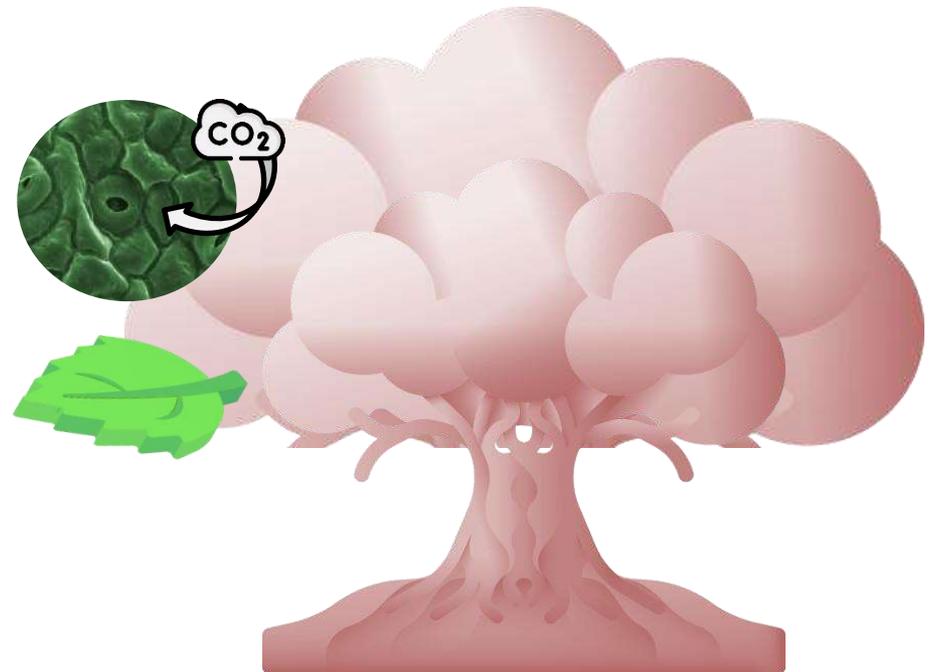
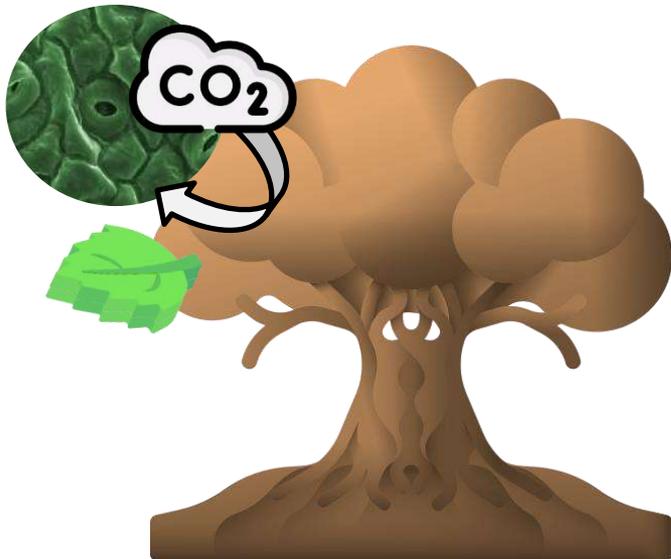
↑SLA

↓Anmax

↑VI

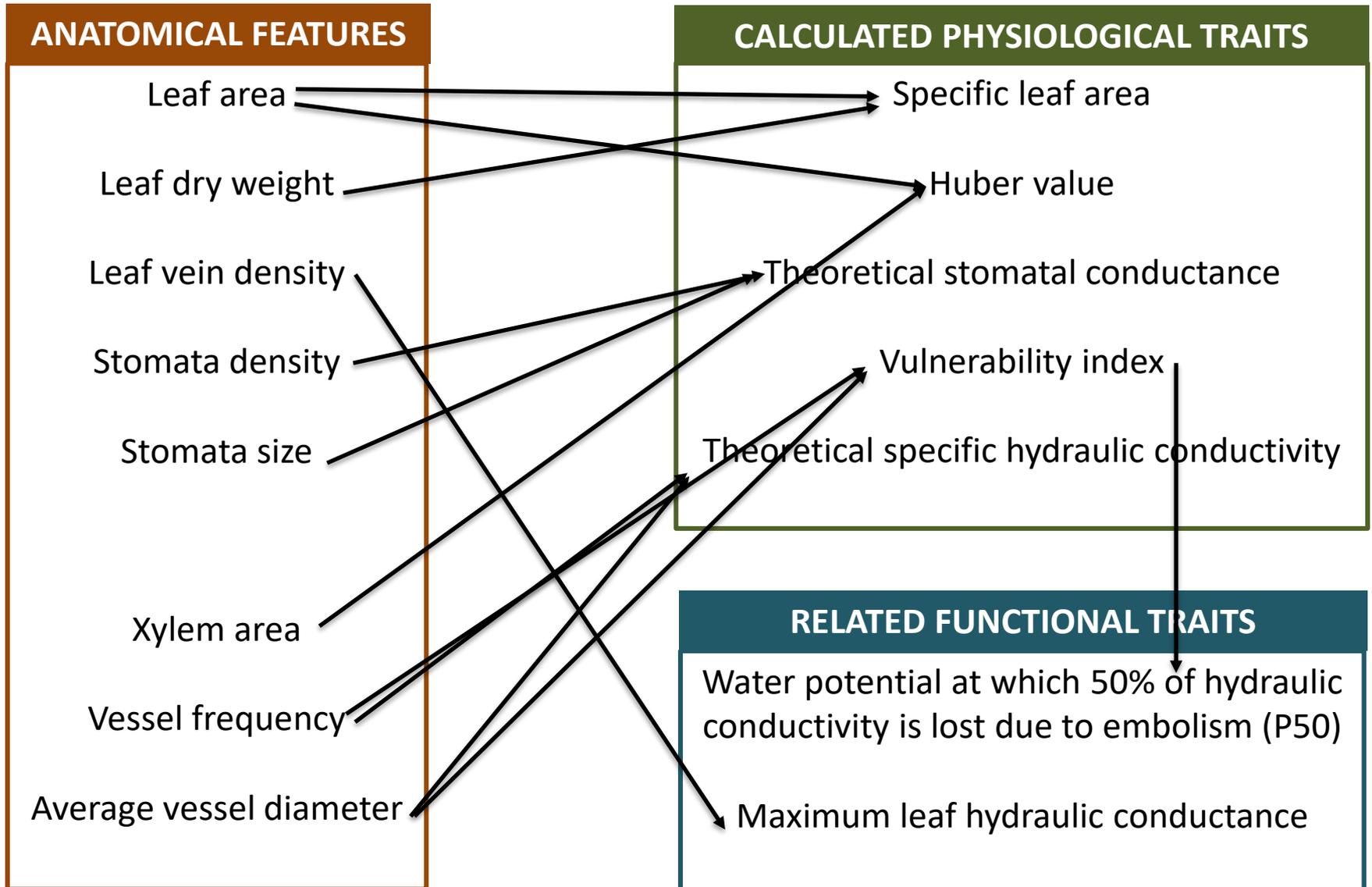
For a given sapwood,
large canopies with big
leaves but low acquisitive
leaves

More vulnerable to
water stress

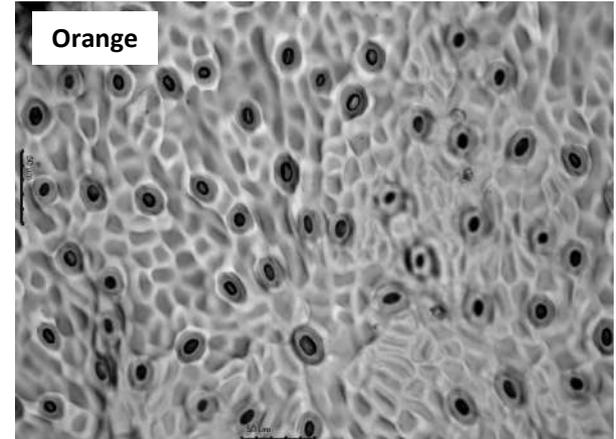
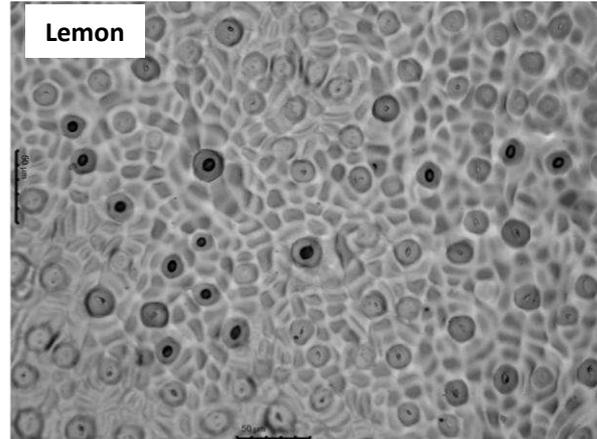
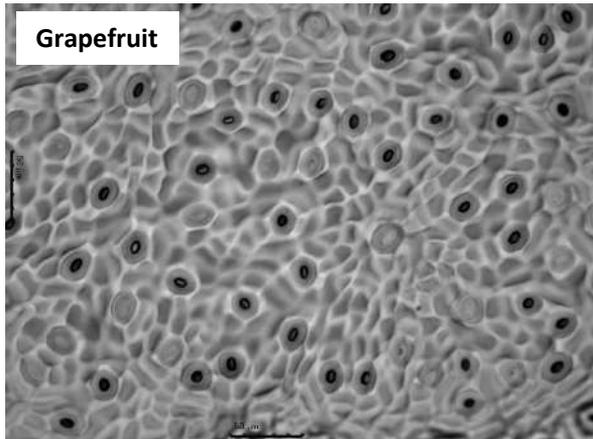
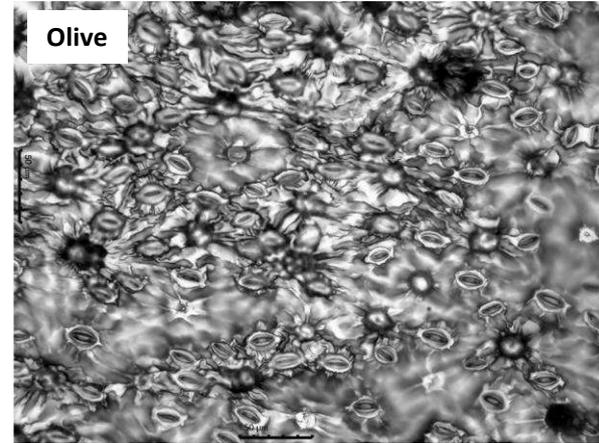
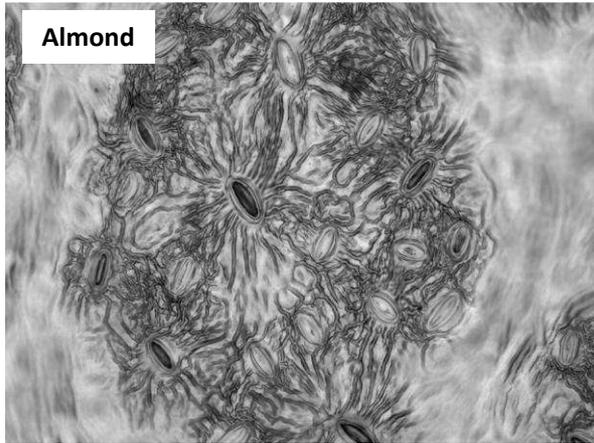


*Thanks for your
attention!*

Traits methods

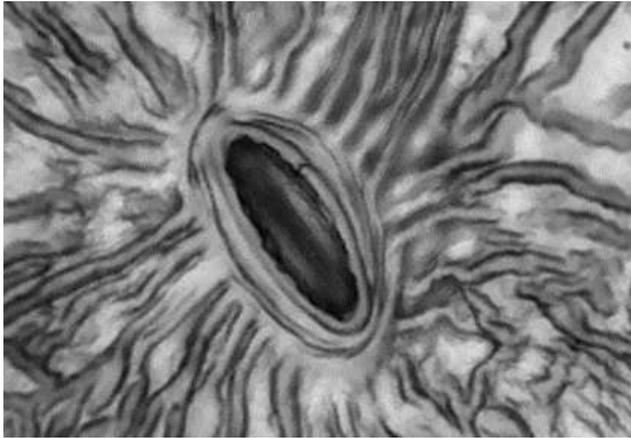


Traits methods: theoretical stomatal conductance

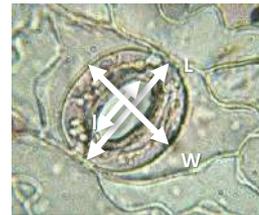
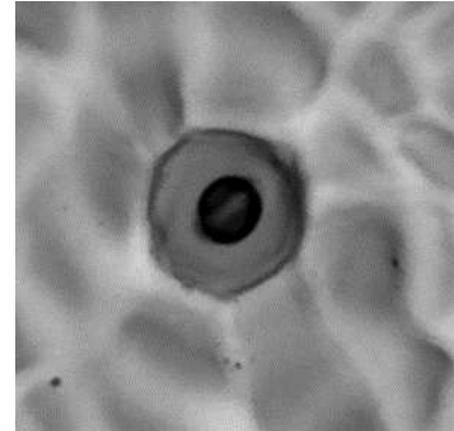


Traits methods: theoretical stomatal conductance

Almond



Lemon

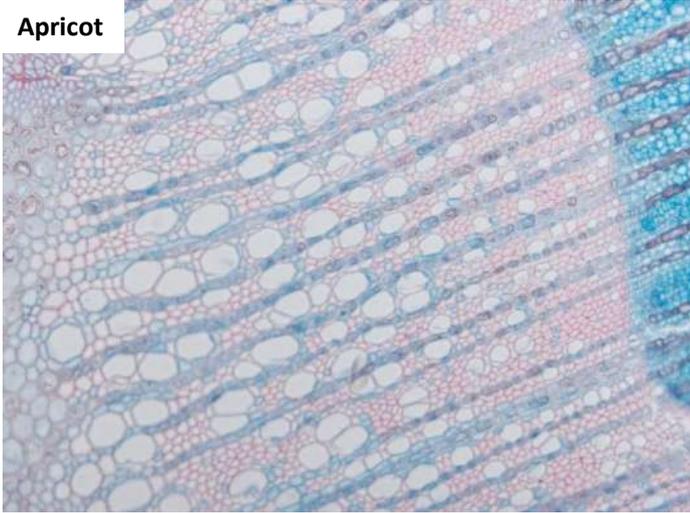


Franks et al. (2009)

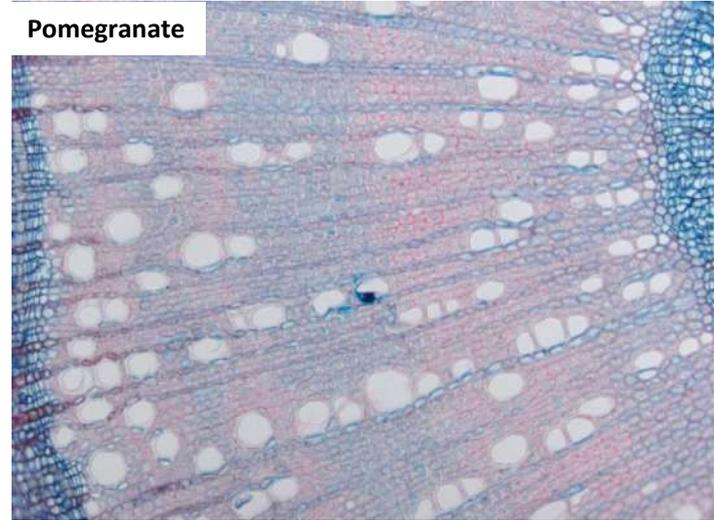
Tratamiento	Stomata length (L) (mm)	Stomata width (W) (mm)	Density of stomata (D) (número mm ⁻²)	Stomata pore length (l) (mm)	a_{max}	S (stomata size)	a (fraction stomatal size)	$g_{w,max}$	$g_{s,max}$
Almendro	28.7	15.7	202	20.1	317.31	450.59	0.704207177	0.760	0.303
Limonero	19.5	17.1	695	8.07	51.15	333.45	0.153393243	0.574	0.135

Traits methods: specific hydraulic conductivity and vulnerability index

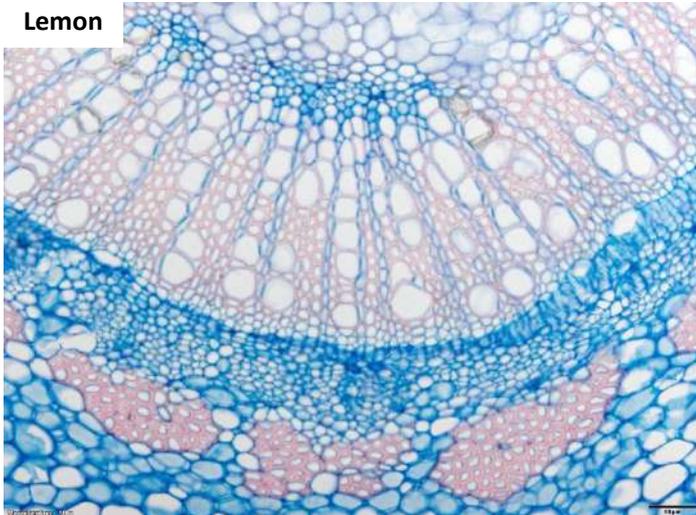
Apricot



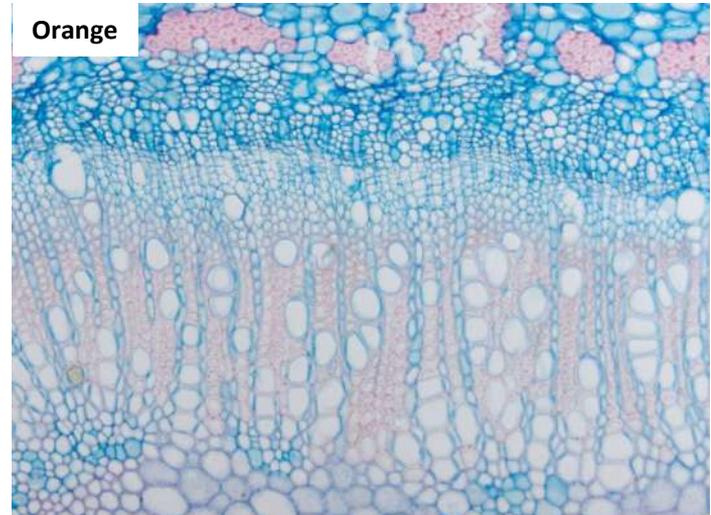
Pomegranate



Lemon



Orange



Traits methods: specific hydraulic conductivity and vulnerability index

Fixed in FAA (5% formaldehyde, 2.5% acetic acid, 50% ethanol).

Sections 15 µm thick cut using a cryostat (Leica CM 1950)

Stained with a mix of safranin and astra blue (Gartner and Schweingruber, 2013) and mounted in DPX.

Images were taken with a light microscope (OLYMPUS BX61)

Vessels area in branch transect measured using Fiji (Schindelin et al. 2012)

Calculation of K_s (kg m⁻¹ s⁻¹ MPa⁻¹) was determined using the Hagen–Poiseuille equation (Tyree and Ewers, 1991):

$$K_s = \left(\frac{\pi \rho}{128 \eta A} \right) \sum_{i=1}^n d_i^4$$

ρ and η are the density and viscosity of water at 20 °C (998.2 kg m⁻³ and 1.002 10⁻⁹ MPa*s, respectively), A is the area of the transect, and d is the equivalent diameter of every vessel within the transect.

Traits methods: specific hydraulic conductivity and vulnerability index

Fixed in FAA (5% formaldehyde, 2.5% acetic acid, 50% ethanol).

Sections 15 µm thick cut using a cryostat (Leica CM 1950)

Stained with a mix of safranin and astra blue (Gartner and Schweingruber, 2013) and mounted in DPX.

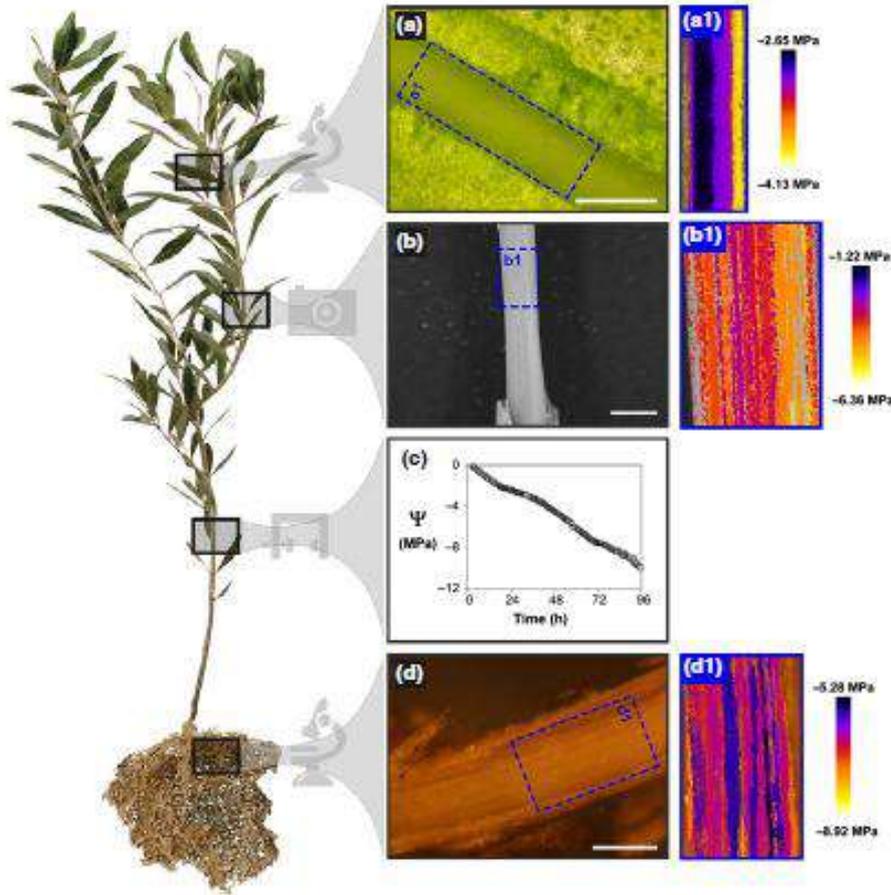
Images were taken with a light microscope (OLYMPUS BX61)

Vessels area in branch transect measured using Fiji (Schindelin et al. 2012)

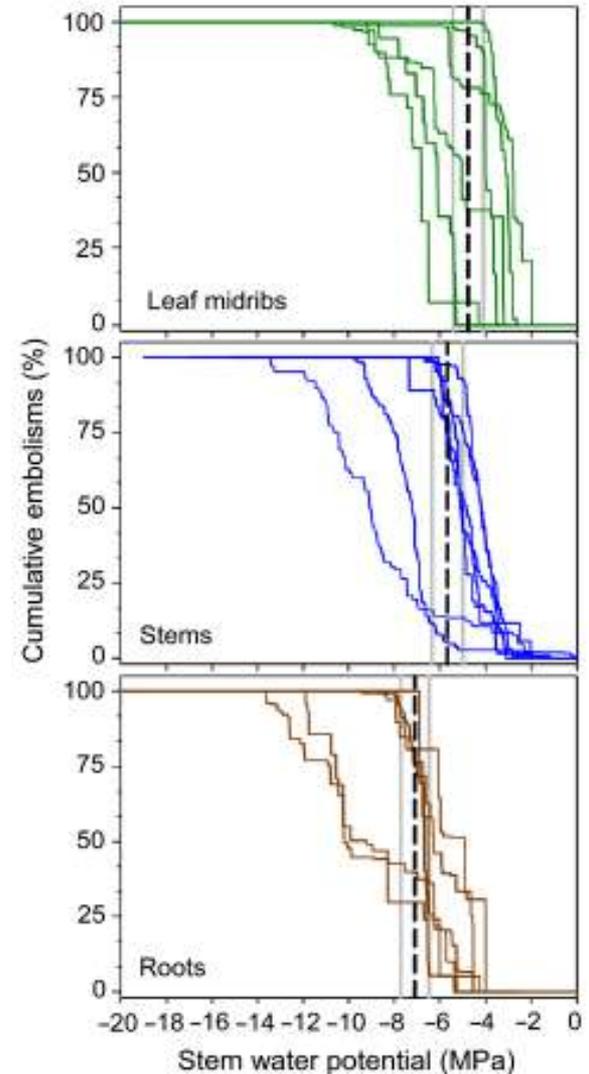
The **vulnerability index** (mm⁻²; Carlquist, 1977) is an estimation of the ability of a plant to resist cavitation and it is calculated as:

$$VI = \frac{\text{average vessel diameter}}{\text{vessel frequency}}$$

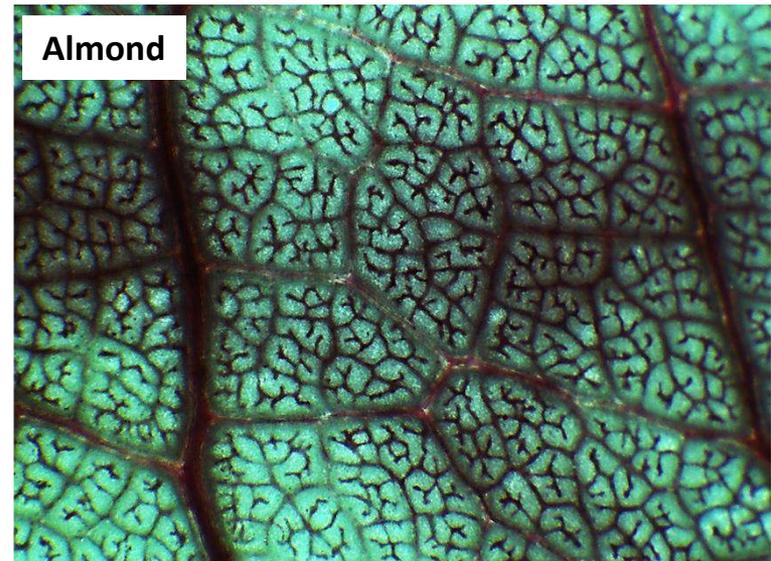
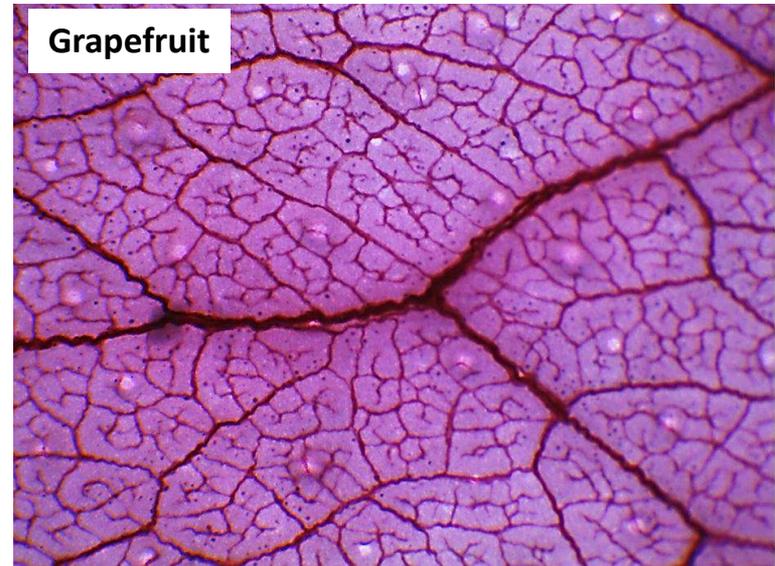
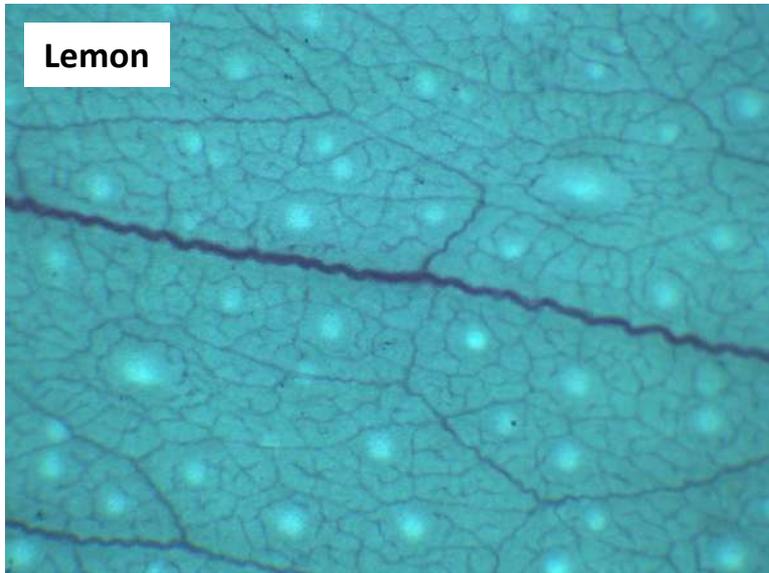
Traits methods: specific hydraulic conductivity and vulnerability index



Rodriguez-Dominguez et al. (2018)



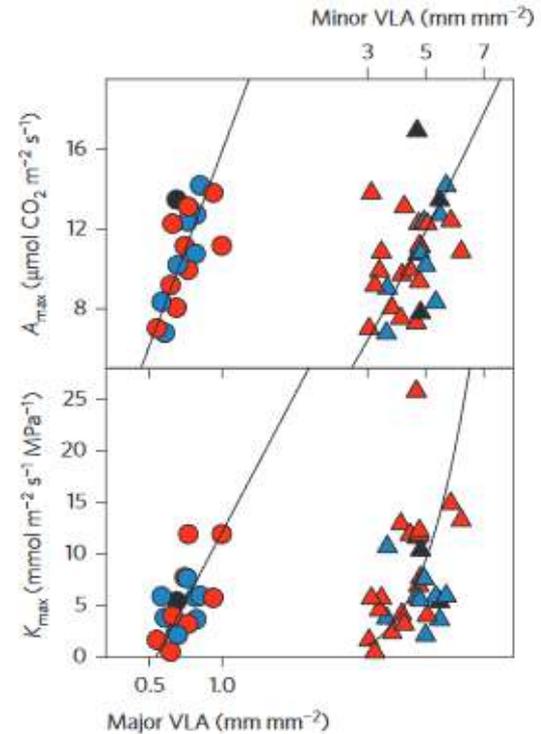
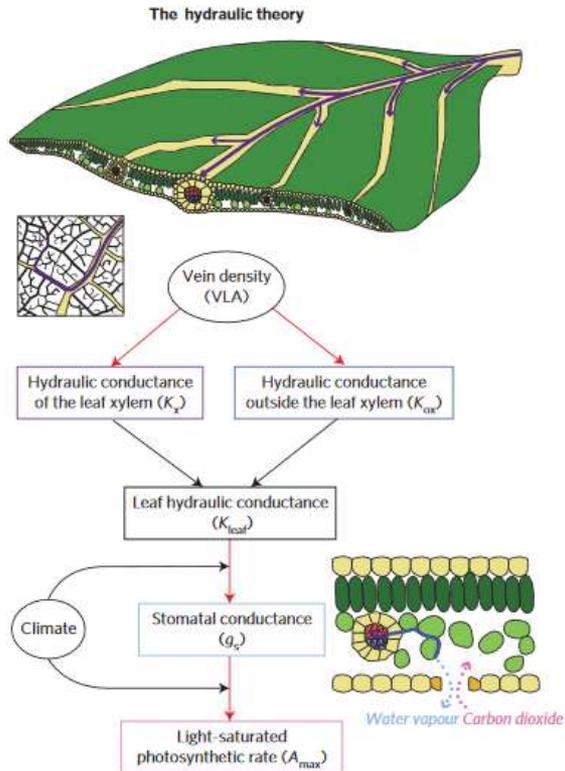
Traits methods: leaf hydraulic conductance



Traits methods: leaf hydraulic conductance

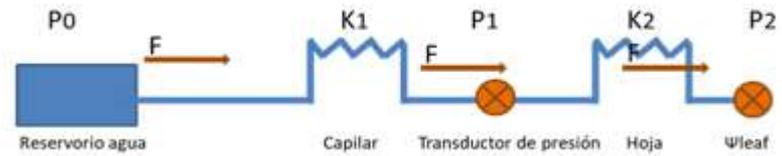
Hydraulic related traits:

- Leaf vein density



Scoffoni et al. (2016)

Traits methods: leaf hydraulic conductance



$$K_{leaf} = \frac{\text{Flujo (mmol/s)}}{((\text{Potencial hidrico foliar (MPa)} - \Delta P \text{ (MPa)}) * \text{Área (m}^2))}$$

Scoffoni et al. (2012)
 Blackman et al. (2010)
 Hernandez-Santana et al. (2016)

Sensing connected to agriculture: sap flow sensors

Task 2.3., Topic 3

WIDERA PROJECT

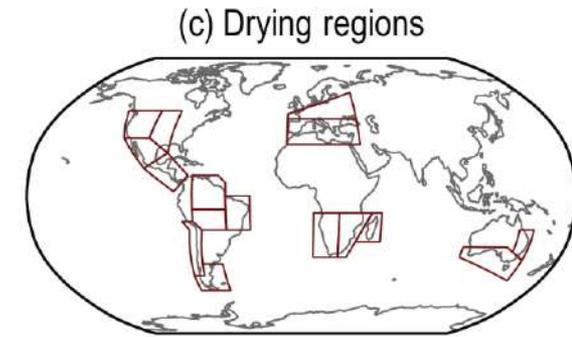
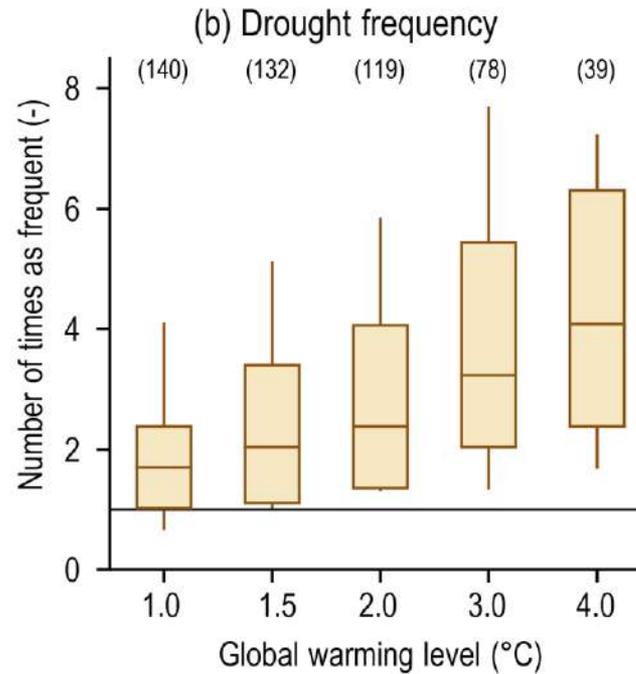
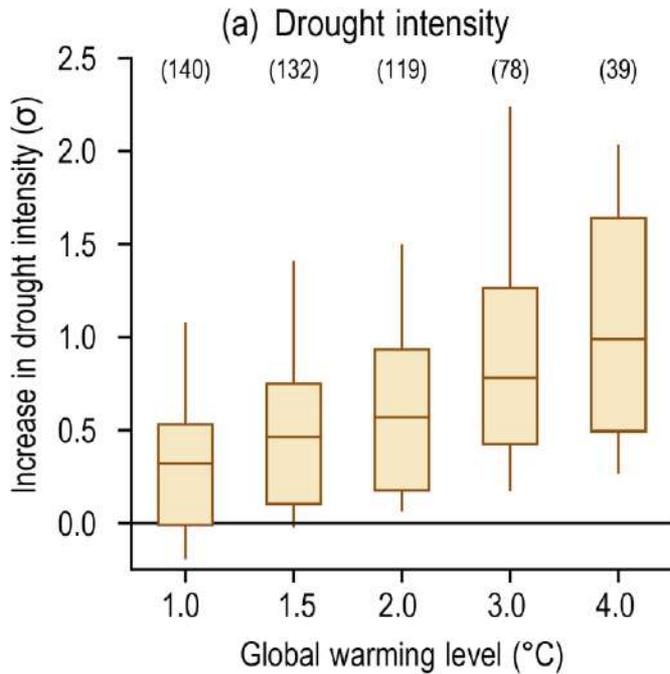
Virginia Hernandez-Santana

✉ virginiahsa@irnas.csic.es

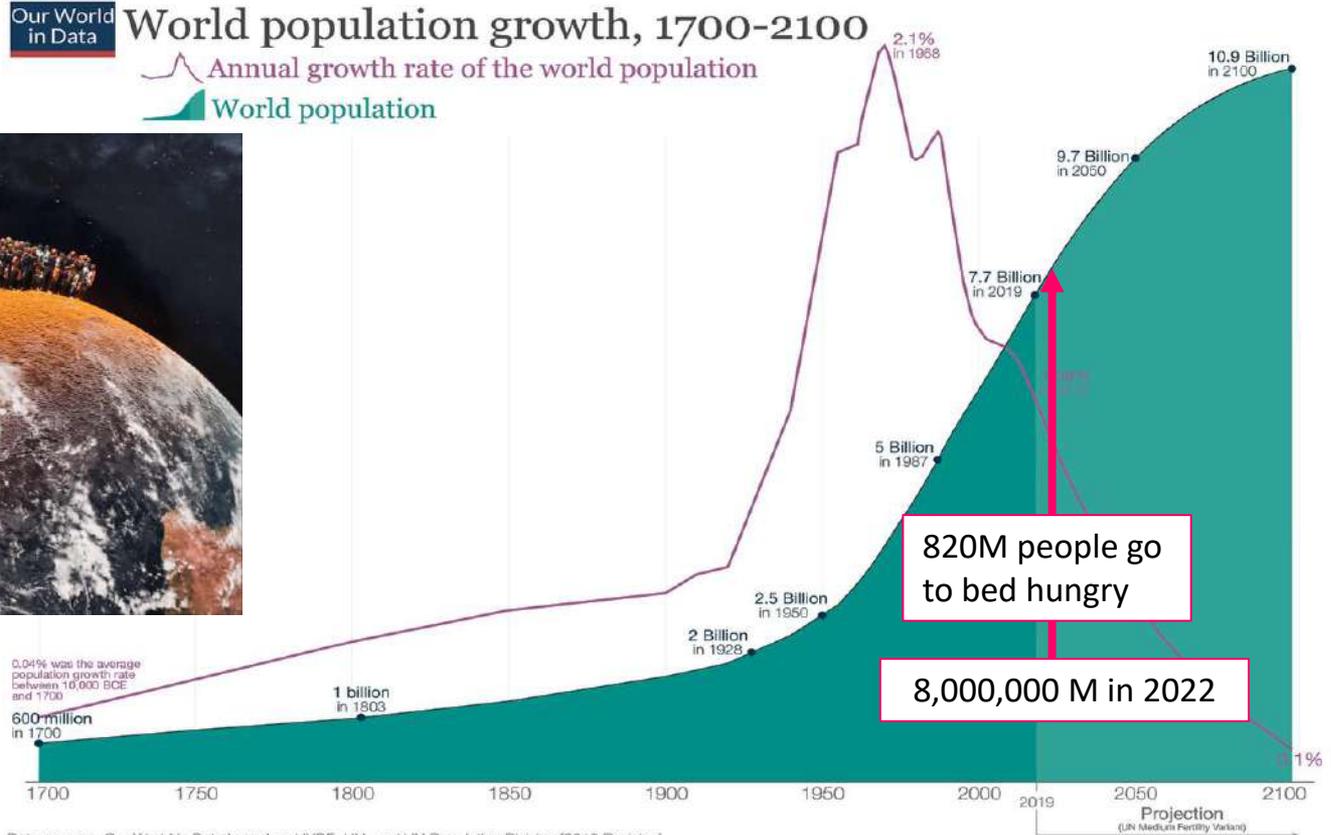
Funchal, 27-31/01/2025

Climate change scenario

Changes in 10-year soil moisture drought in drying regions



Climate change scenario



Data sources: Our World in Data based on HYDE, UN, and UN Population Division [2019 Revision]
This is a visualization from OurWorldInData.org, where you find data and research on how the world is changing.

Licensed under CC-BY by the author Max Roser.

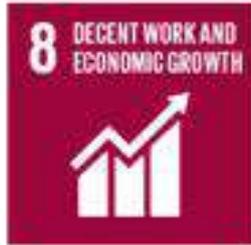


Climate change scenario



SUSTAINABLE DEVELOPMENT GOALS

17 GOALS TO TRANSFORM OUR WORLD



Irrigation to improve productivity

Irrigation



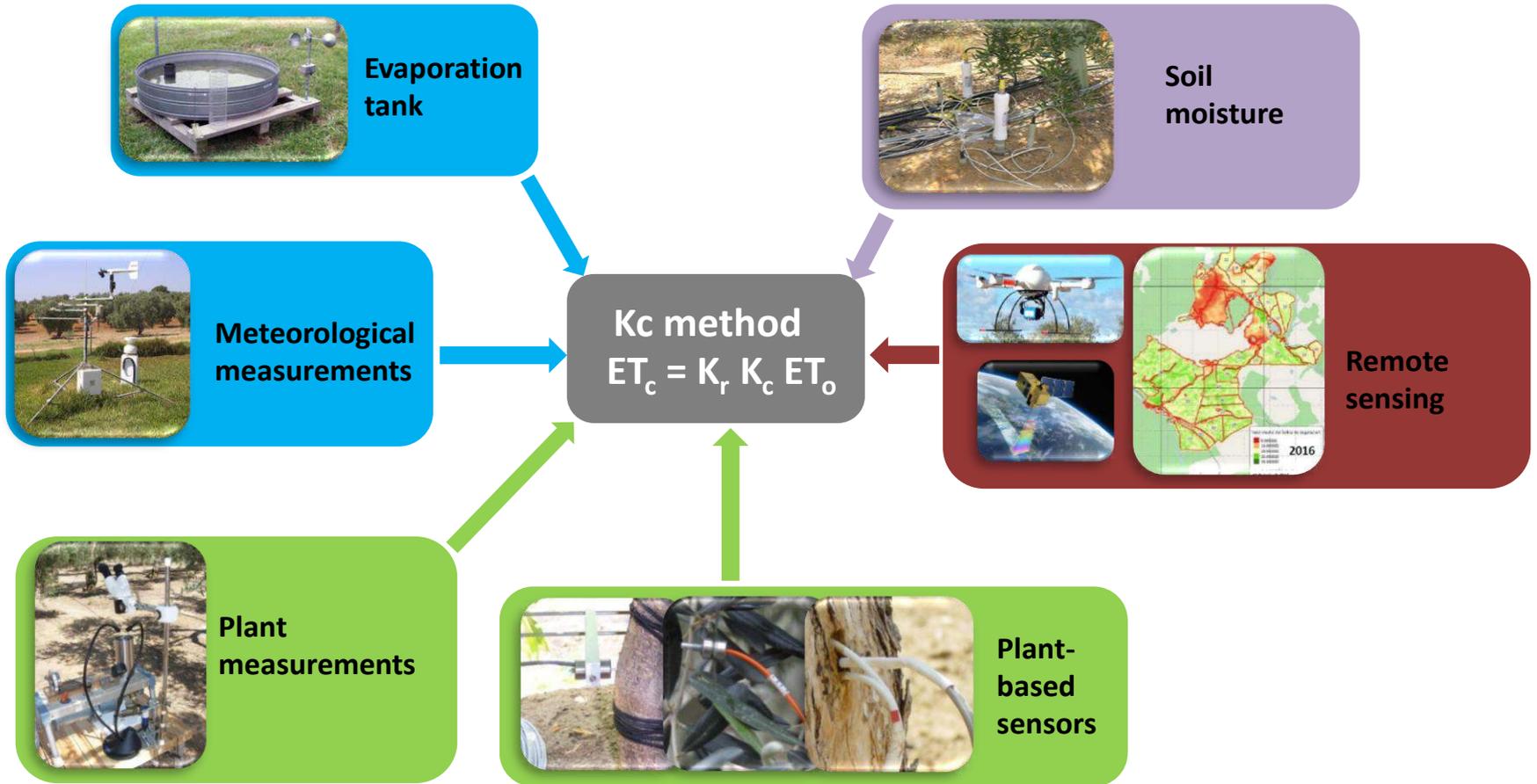
Water availability is the major limitation for crop productivity

Climate change

Water stress will limit the productivity of more than half of cultivated land in the next 50 years

Mediterranean agrarian systems: disequilibrium between water needs and availability

Irrigation





Plant-based sensors

Measuring plant water use & Scaling from leaves to ecosystems/orchards

Leaf scale



- Stomatal conductance (g_s)
Infrared gas analysis or steady-state diffusion porometry
- Photosynthetic capacity (A_{max})
Infrared gas analysis
- Instantaneous water use efficiency (A/E)
Infrared gas analysis
- Intrinsic water use efficiency ($\delta^{13}C$)
Stable C isotope composition with mass spectrometry
- Predawn and midday leaf water potential (Ψ_{pd} , Ψ_{md})
Pressure chamber

Plant scale



- Peak and total daily sap flow per unit sapwood area
Heat balance collars or heat dissipation sap flow probes
- Peak and total daily sap flow per unit leaf area
Heat balance collars or heat dissipation sap flow probes
- Total daily sap flow per plant
Heat balance collars or heat dissipation sap flow probes

ORCHARD SCALE

Ecosystem scale



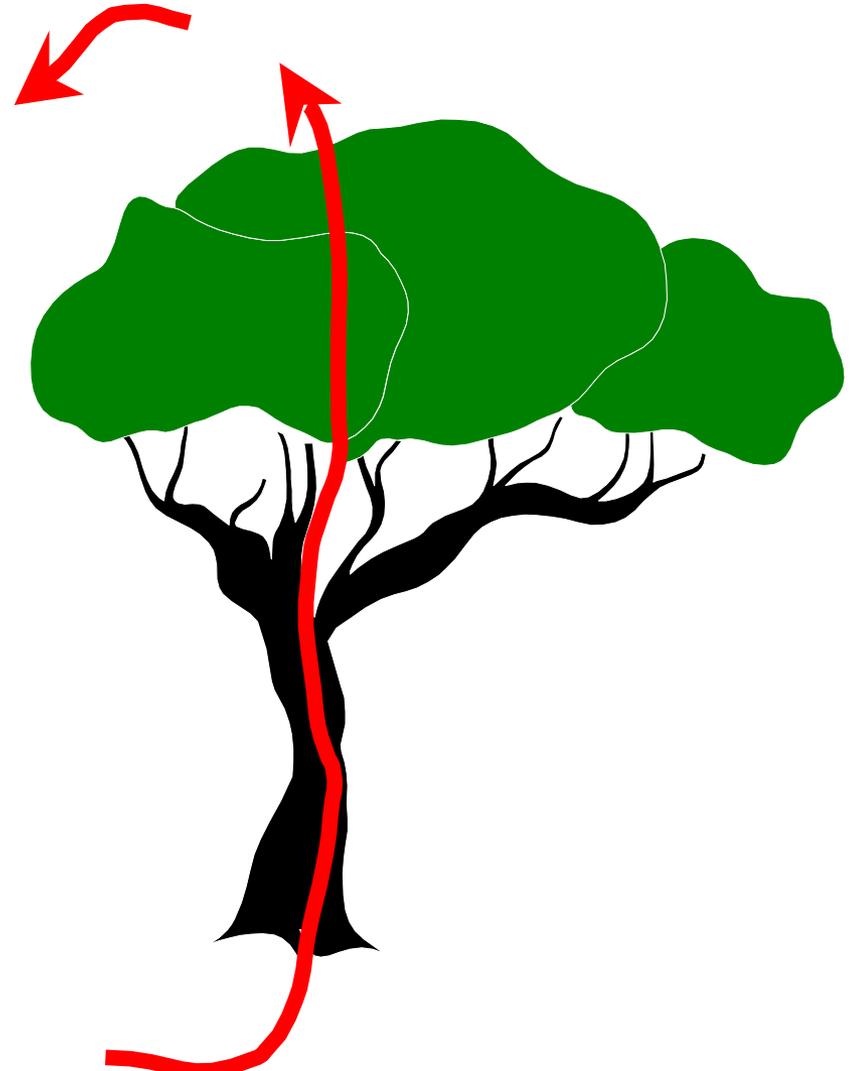
- Total daily sap flow per unit ground area
Heat balance collars or heat dissipation sap flow probes
- Evapotranspiration per unit ground area:
transpiration + interception + soil evaporation (ET)
Eddy covariance or energy balance or infrared gas analysis

How to determine the amount of water used by a whole plant?

- Transpiration:
Soil-plant-atmosphere continuum

We could measure:

- Moisture loss from the soil
- Liquid flow through the stem
- Vapor loss to the atmosphere



How to determine the amount of water used by a whole plant?

- Direct techniques:

- Weighing lysimeters
- Ventilated chambers
- Potometers

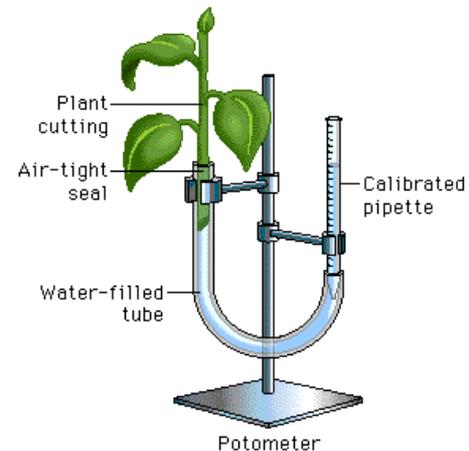


What about larger plants?

And less intrusive methods?

- Indirect techniques:

- Chemical tracers, Radio and stable isotopes
- Thermal tracers (energy balance, heat dissipation, heat-pulse)



Using heat as a tracer of sap movement

- Stream water flow analogy

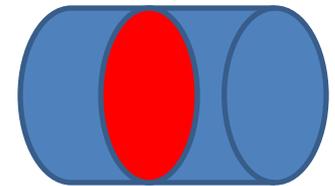


2. Use dye or chemical tracer (salt) for dilution pattern as a measure of mass flow [$M T^{-1}$] (equivalent to $L^3 T^{-1}$)

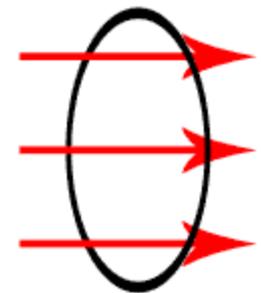


() ...strictly speaking...physics

- FLOW = matter, heat or momentum that is in motion
- FLUX = amount of something passing through a given surface per unit of time [$L^3 T^{-1}$ or $M T^{-1}$]
(integral of flux density over a finite surface)



- FLUX DENSITY = flux per unit of surface [$L^3 L^{-2} T^{-1}$]
numerically equivalent to $L T^{-1}$ (units of velocity)
Velocity, and flux density, are vectors
(scalar + direction)

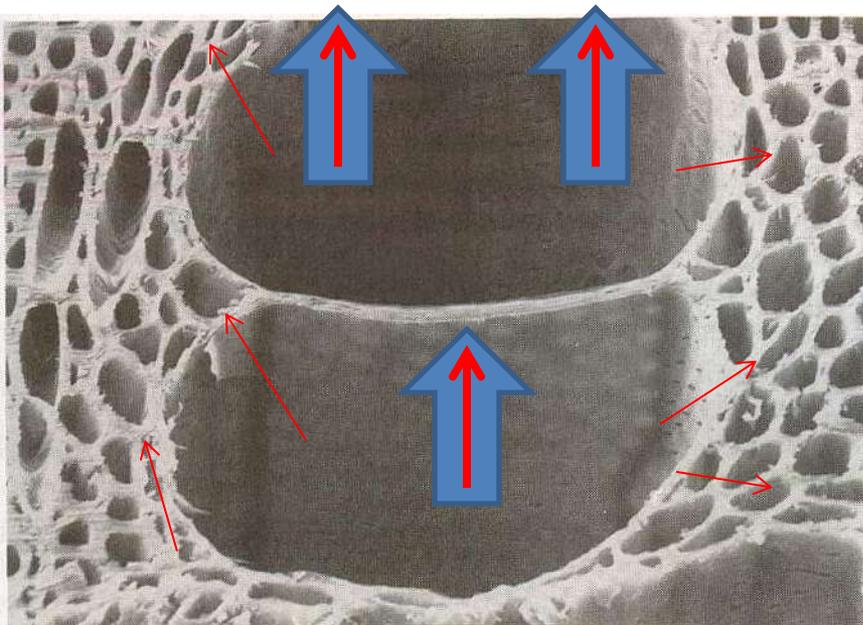


Lemur R, Fernandez JE, Steppe K. 2009. Symbols, SI units and physical quantities within the scope of sap flow studies. Acta Horticulturae 846: 21-32

<http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Sap+flow#sthash.YTSbwwwbp.dpuf>

Using heat as a tracer of sap movement

- Simple in practice but complex theoretically:
sap flows through a porous medium



Xylem vessels of *Eucalyptus marginata*

Sap transport:

convection as mass flow

Heat transport:

convection as mass flow
(w flowing sap) &
conduction through sap +
stationary tissue

Three main types of thermometric methods

1. Pulsed heat methods (HPV) Huber (1932) and Marshall (1958)

- **Compensation Heat Pulse (CHPM)** Huber & Schmidt (1937)
Cohen's HP method or T-Max Cohen et al. (1981)
Green's HPV system Green (1998)
Calibrated Average Gradient Testi and Villalobos (2009)
- **Heat Ratio Method (HRM)** Burgess et al. (2001)

2. Constant heat methods

- **Granier's or Heat Dissipation (HD)** Granier (1985)
- **Heat Field Deformation (HFD)** Nadezhdina et al. (1998)

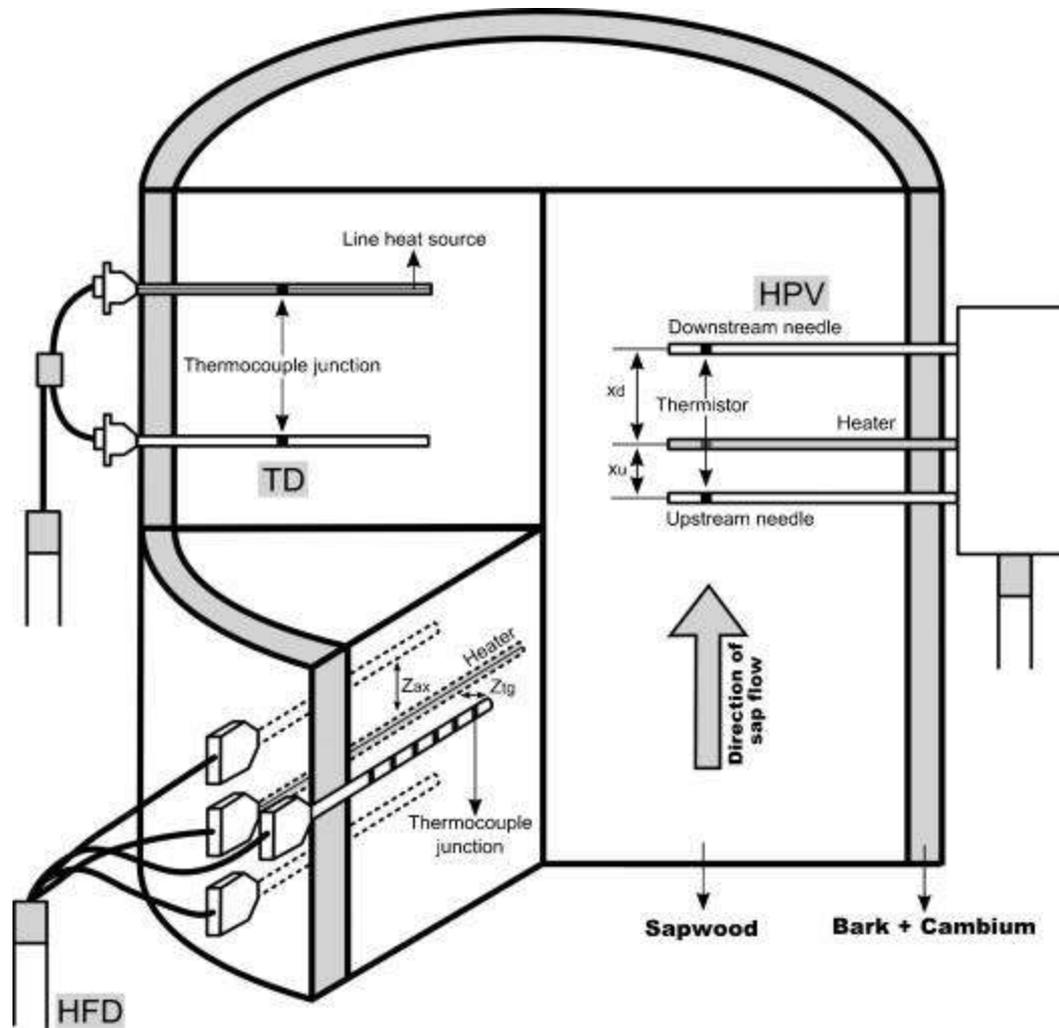
3. Heat balance methods Vieweg and Ziegler (1960) and Daum (1967)

- **Stem Heat Balance (SHB)** Sakuratani (1981)
- **Trunk Sector Heat Balance (THB)** Čermák et al. (1973, 2004) and Kučera et al. (1977)

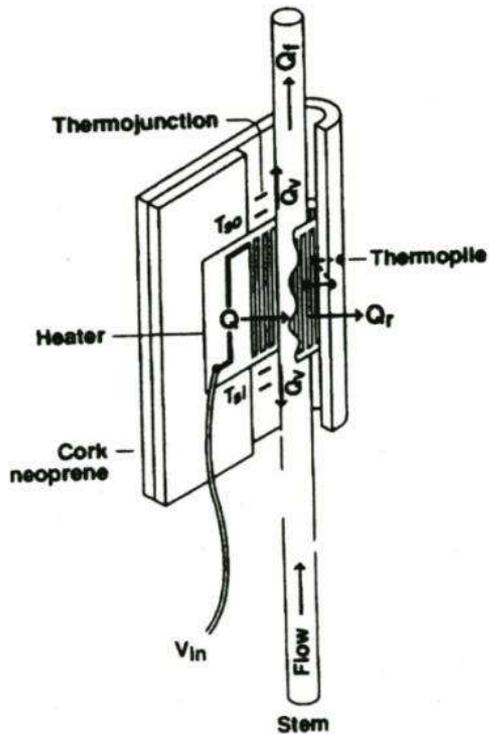
Sap velocity (sap flux density)

Sap mass flow

Sap velocity methods



Method	Probes	Primary measurement	Solves for	Main limitations
SHB	Flexible collar with heater, thermopile, T_u and T_d	Voltages (temps) u , d and radial	F_s (mass sap flow) [$M T^{-1}$]	Constant heat. Only good for small stems (<20cm diameter). Change collars to allow growth and minimize heat damage.
THB				



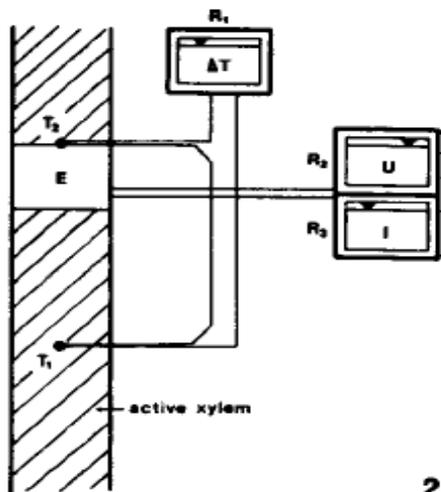
$$Q = Q_v + Q_r + Q_f + S$$

$$Q_v = \frac{K_{st} A}{dz} (\Delta T_2 - \Delta T_1)$$

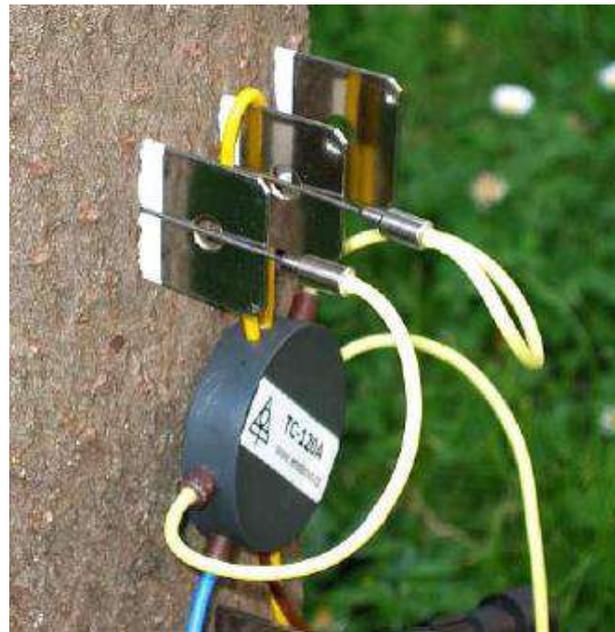
$$Q_r = K_{sh} E$$

$$F = \frac{Q_f}{(cp (\Delta T_1 - \Delta T_2)/2)}$$

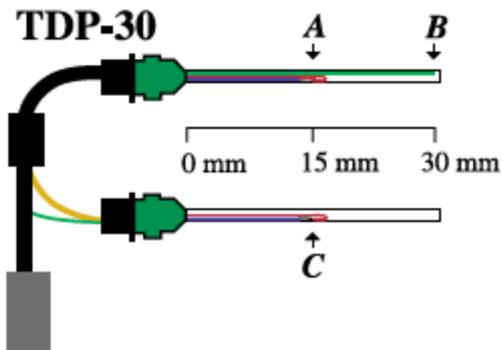
Method	Probes	Primary measurement	Solves For	Main limitations
SHB	Flexible collar with heater, thermopile, T_u and T_d	Voltages (temps) u , d and radial	F_s (mass sap flow) [$M T^{-1}$]	Constant heat. Only good for small stems (<20cm diameter). Change collars to allow growth and minimize heat damage.
THB	3-5 stainless steel heated plates parallel into sapwood	Voltage (temps) diff btwn heated and non-heated segments	F_s (mass sap flow) [$M T^{-1}$]	Constant heat. Minimum stem diameter ~ 12 cm, but "Baby system" now available.



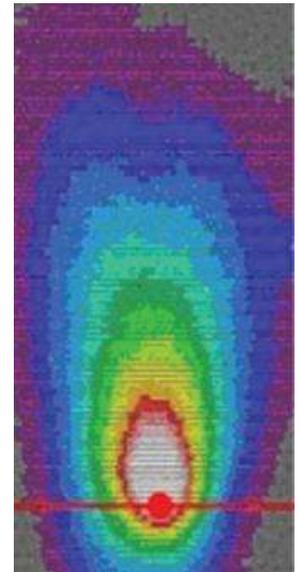
2



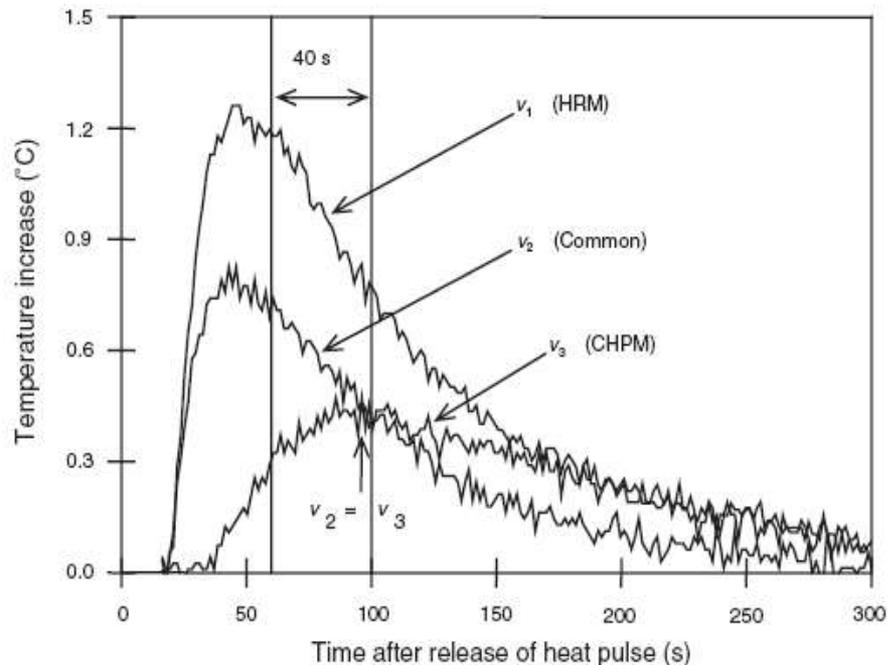
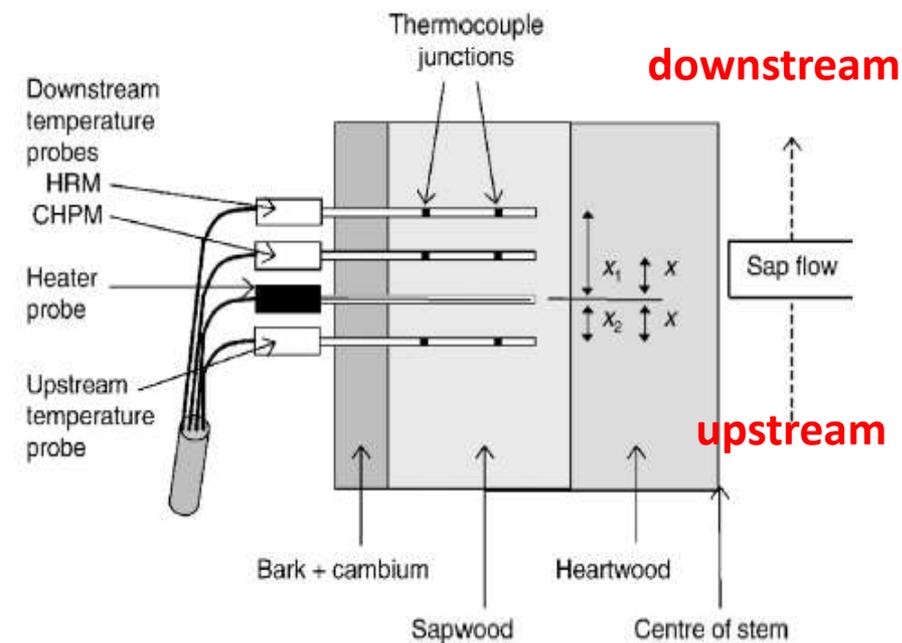
Method	Probes	Primary measurement	Solves for	Main limitations
HD or Granier's	Internal linear Td w Heater, Tu	temp diff between T probes	J_s [$L^3L^{-2} h^{-1}$] for probe length	Continuous heat. Empirical coefficients applied for all species; calibration mandatory. Sensitive to external temp gradients.
HFD	Internal linear 2 Taxial (sym), 1 Ttangential (asym), Heater	ratios of temp differences (dTsym and dTas)	J_s [$L^3L^{-2} h^{-1}$] at a point	Continuous heat. J_s not integrated across sapwood.



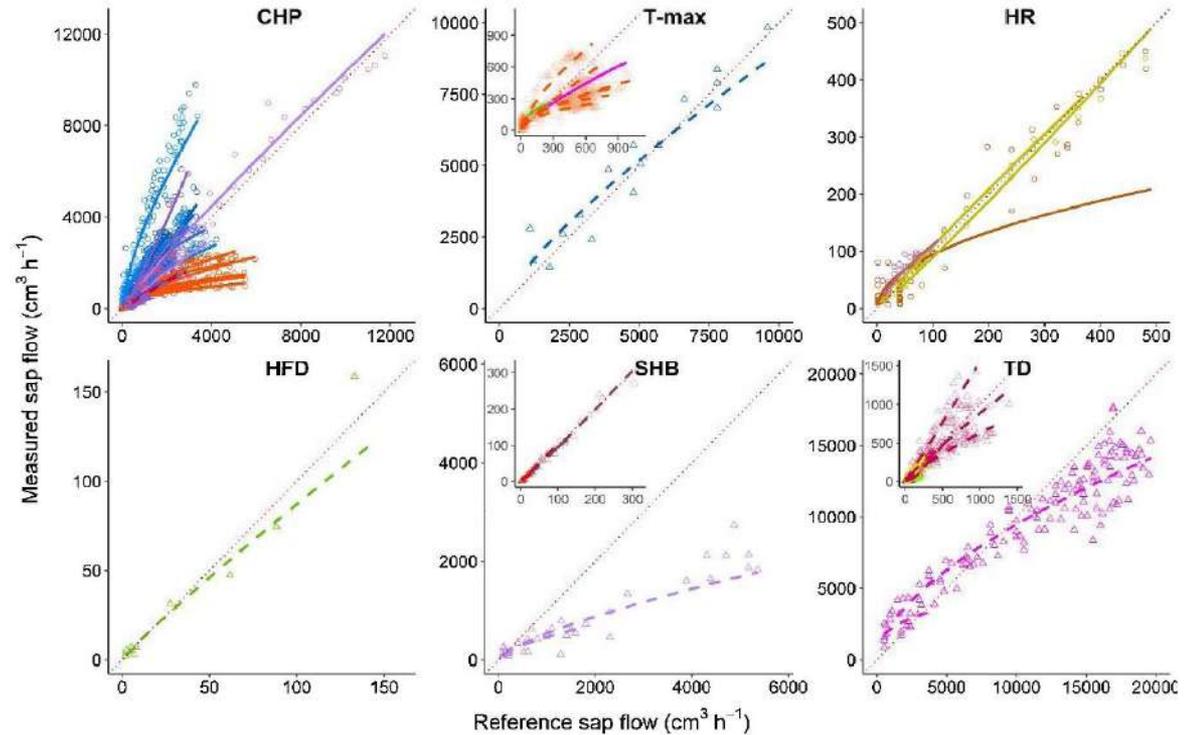
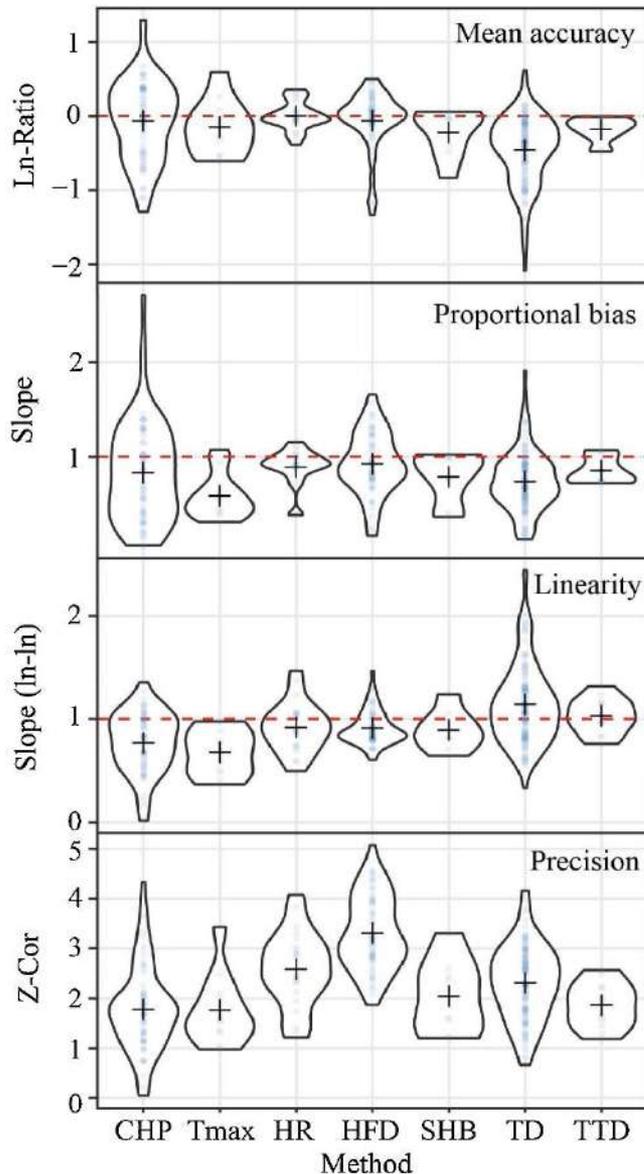
A- Thermocouple #1
 B- Heater
 C-Reference Thermocouple



Method	Probes	Primary measurement	Solves for	Main limitations
CHPM	Internal linear Tu, Td, Heater Asymmetrical	time when $T_u - T_d = 0$ after a HP	v_h [L h ⁻¹] at a point	Not good for low flows (<5 cm h ⁻¹), but algorithm developed: CAG. v_h not integrated across sapwood.
HRM	Internal linear Tu, Td, Heater Symmetrical	ratio of temp differences after a HP	v_h [L h ⁻¹] at a point	Not good for high flows (>50 cm h ⁻¹), but algorithm recently developed. v_h not integrated across sapwood.

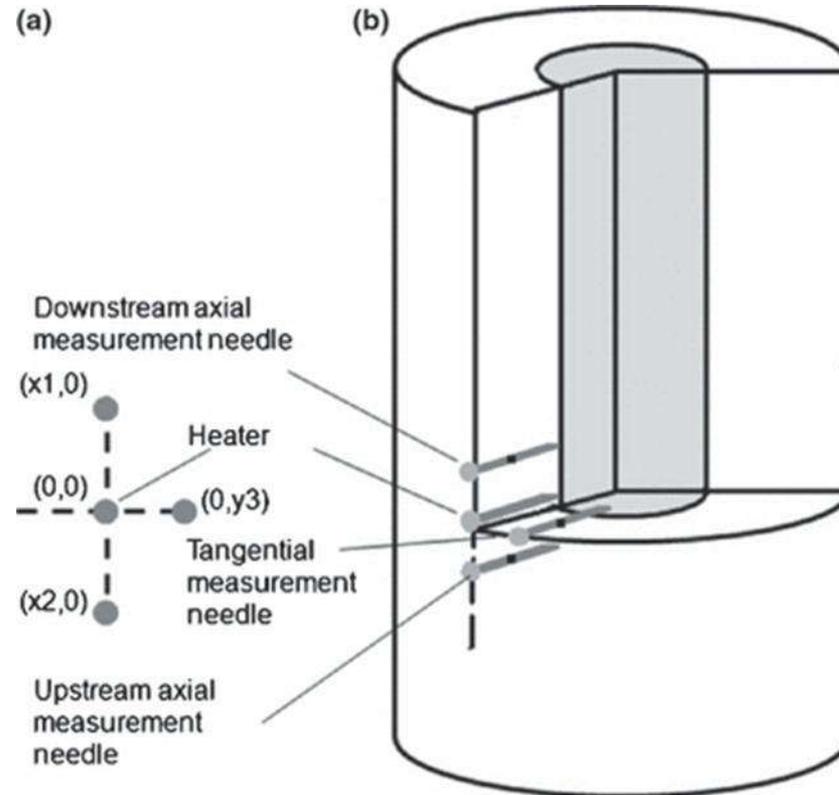


BIAS AND UNCERTAINTY



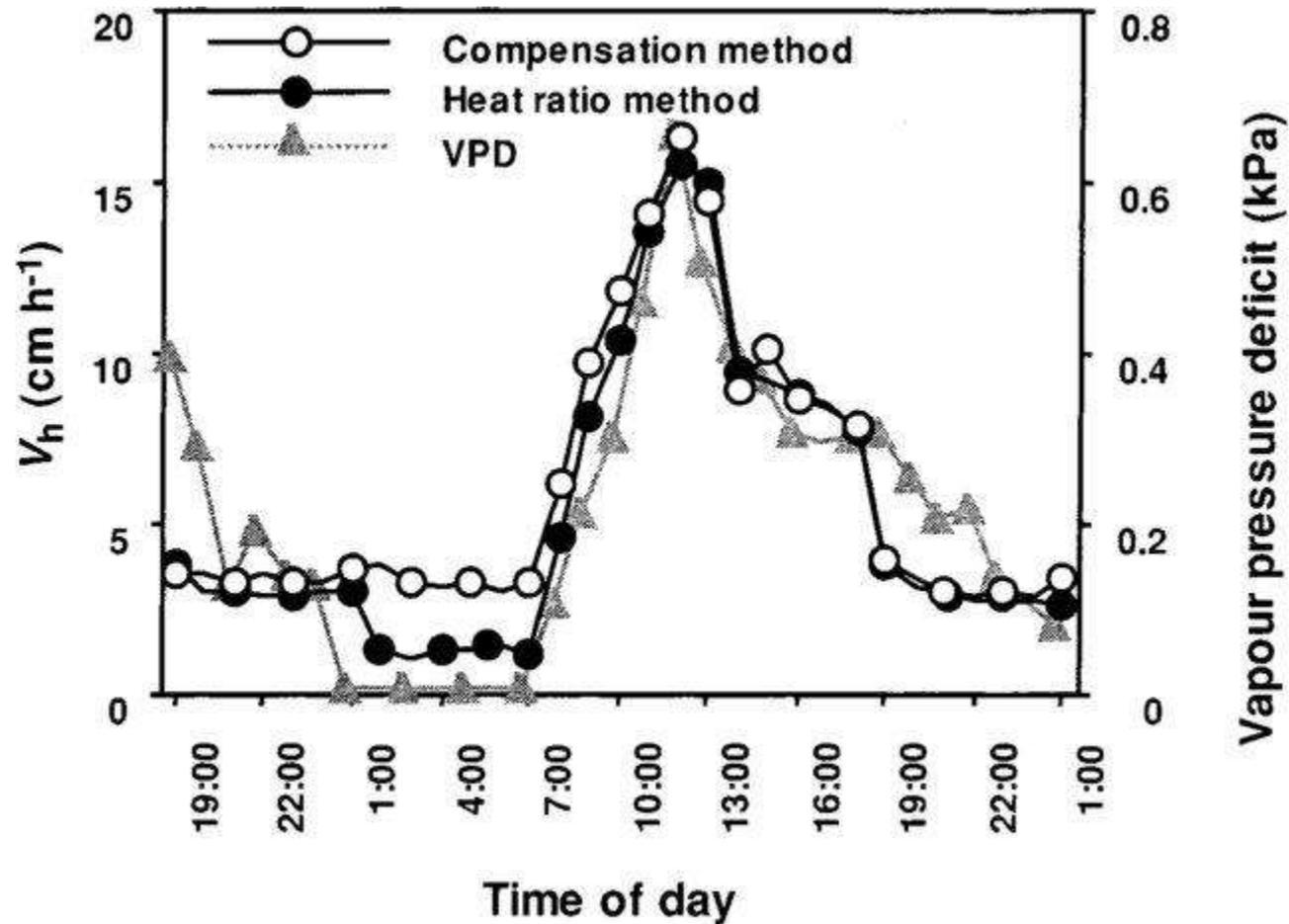
- | | | | | |
|-----------------------|-------------------------------|----------------------------|---------------------------|--------------------------|
| Alarcon et al. 2005 | Cohen et al. 1998 | Gutierrez-soto et al. 2012 | Lu and Chacko 1998 | Smith et al. 1995 |
| Ballester et al. 2011 | Dragoni et al. 2005 | Gutierrez et al. 1994 | Madurapperuma et al. 2009 | Sperling et al. 2012 |
| Barret et al. 1995 | Dye et al. 1996 | Hatton et al. 1995 | Montague et al. 2006 | Sun et al. 2012 |
| Bleby et al. 2004 | Fernandez et al. 1999 | Heilman et al. 1990 | Nadezhdina et al. 1998 | Swanson & Whitfield 1981 |
| Braun and Schmid 1999 | Fernandez et al. 2006 | Intrigliolo et al. 2009 | Nortes et al. 2009 | Urban et al. 2012 |
| Burgess et al. 2001 | Gonzalez-Altozano et al. 1998 | Johan Uddling et al. 2009 | Prendergast et al. 2007 | Vellame et al. 2010 |
| Caspari et al. 1993 | Green et al. 1988 | Lu 2002 | Shackel et al. 1992 | |

BIAS AND UNCERTAINTY: SOLUTIONS



Sapflow+: a four-needle heat-pulse sap flow sensor enabling nonempirical sap flux density and water content measurements

BIAS AND UNCERTAINTY: SOLUTIONS



Burgess et al. 2001

BIAS AND UNCERTAINTY: SOLUTIONS

Principles of the DRM

To alleviate the negative bias of the HRM at high V , we propose a new method, the DRM, that uses a three-step approach to estimate V . First, V_{12} is estimated as in the HRM. Second, an additional estimate of sap velocity (V_{23} , Eq. (6)) is computed using the temperature rises at Probes #2 and #3, where Probe #3 is located downstream to Probe #2 at the same distance as Probe #2 is from Probe #1 (so $x_3 = 3x_2$):

$$V_{23} = \frac{2k}{x_3 - x_2} \ln\left(\frac{\delta_3}{\delta_2}\right) + \frac{x_2 + x_3}{2t}. \quad (6)$$

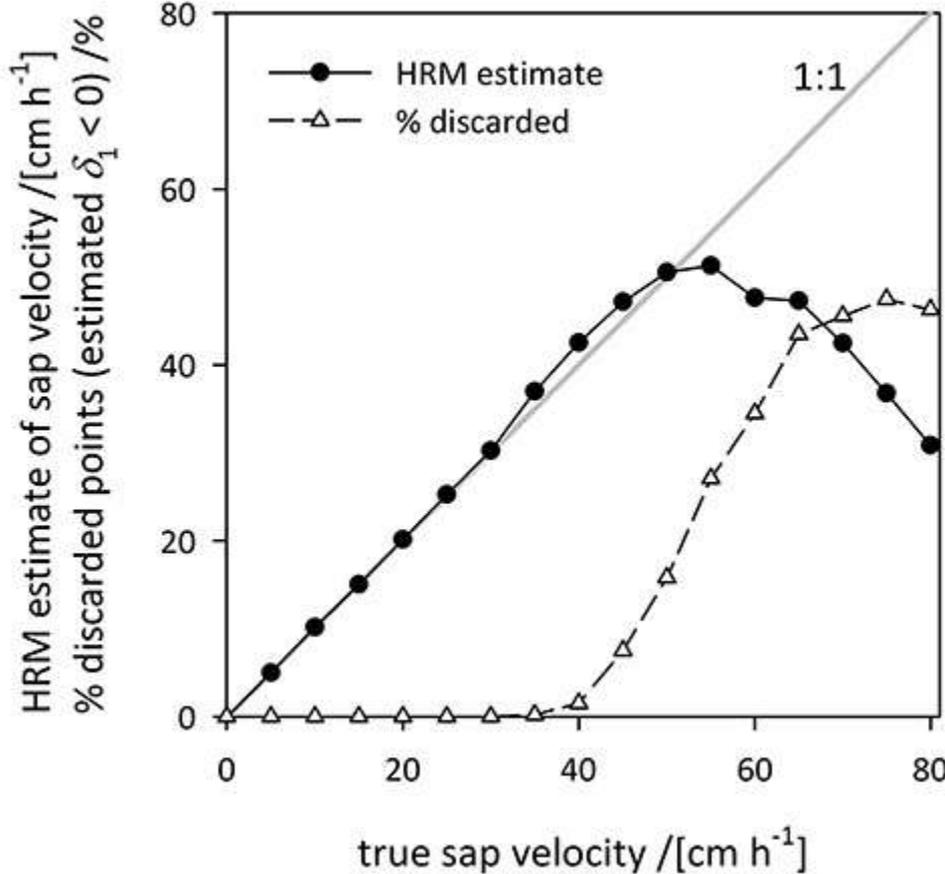
The third step is to choose between the two estimates of velocity (V_{12} and V_{23}) to give a single, final estimate of V (V_{DRM}). On the grounds that measurements with less uncertainty are intrinsically preferable to those with greater uncertainty, we chose V_{DRM} as the value with the smaller intrinsic measurement uncertainty (σ_{12} or σ_{23}):

$$V_{\text{DRM}} = \begin{cases} V_{12} & \text{if } \sigma_{12} \leq \sigma_{23} \\ V_{23} & \text{else} \end{cases}, \quad (7)$$

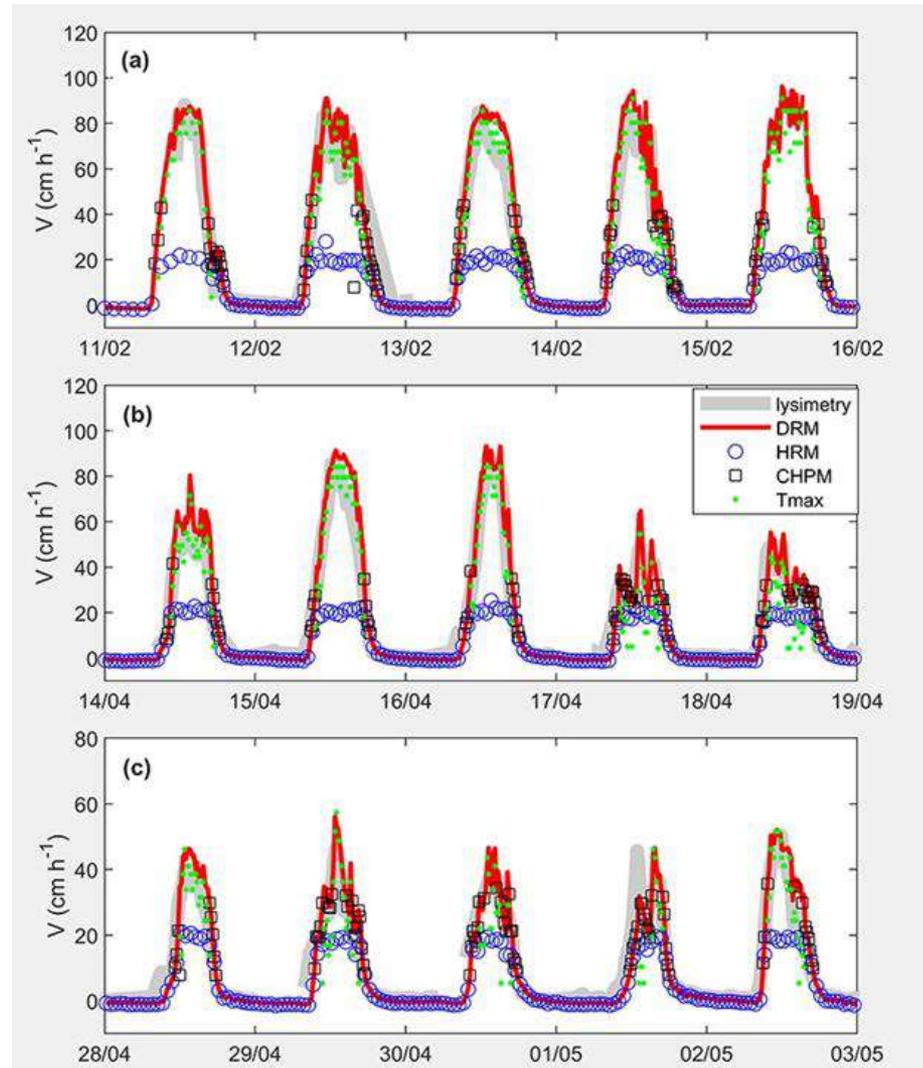
where σ_{12} and σ_{23} depend on the standard deviation of random noise for temperature sensors (σ_T) and on the estimated temperature rises at each probe (δ_1 , δ_2 and δ_3), as follows:

$$\sigma_{12} = \frac{2k\sigma_T}{x_2 - x_1} \sqrt{\frac{1}{\delta_2^2} + \frac{1}{\delta_1^2}}, \text{ and} \quad (8)$$

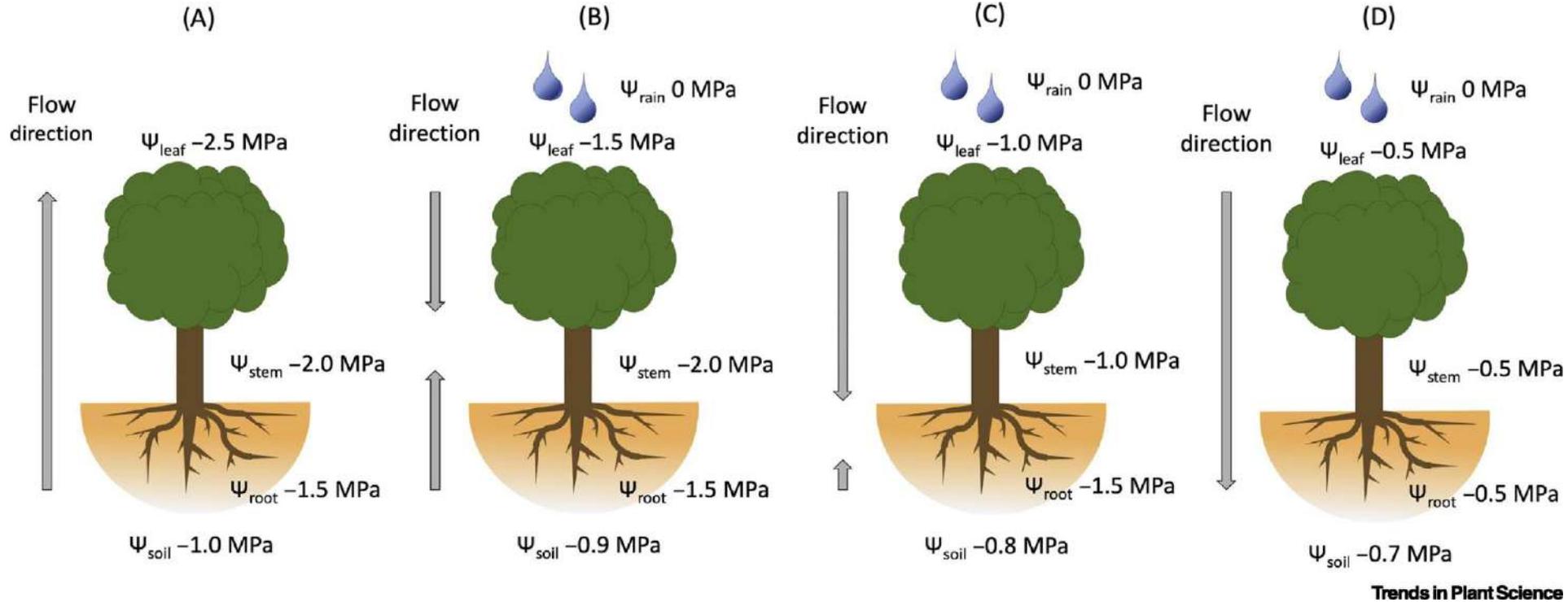
$$\sigma_{23} = \frac{2k\sigma_T}{x_3 - x_2} \sqrt{\frac{1}{\delta_3^2} + \frac{1}{\delta_2^2}}. \quad (9)$$



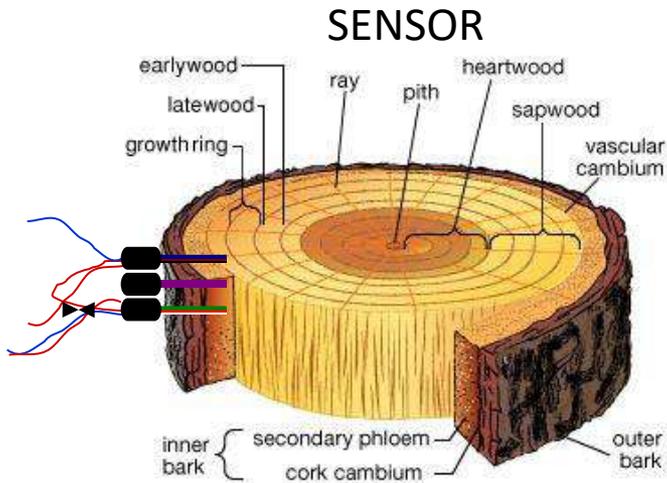
BIAS AND UNCERTAINTY: SOLUTIONS



BIAS AND UNCERTAINTY: SOLUTIONS



UPSCALING



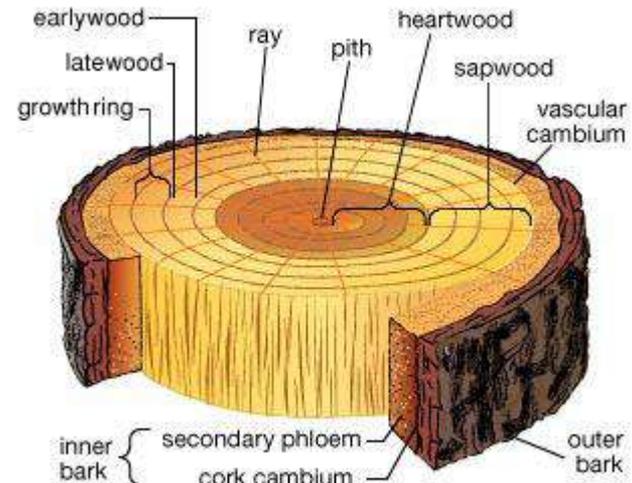
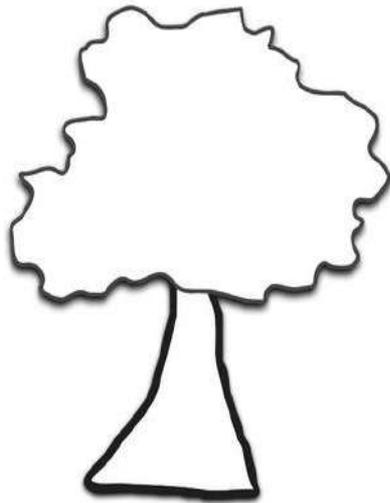
© 2006 Merriam-Webster, Inc.



STAND/ORCHARD

FROM SENSOR TO TREE

- SOURCES OF VARIABILITY AT TREE LEVEL?
 - Height
 - Radial variability
 - Circumferential variability



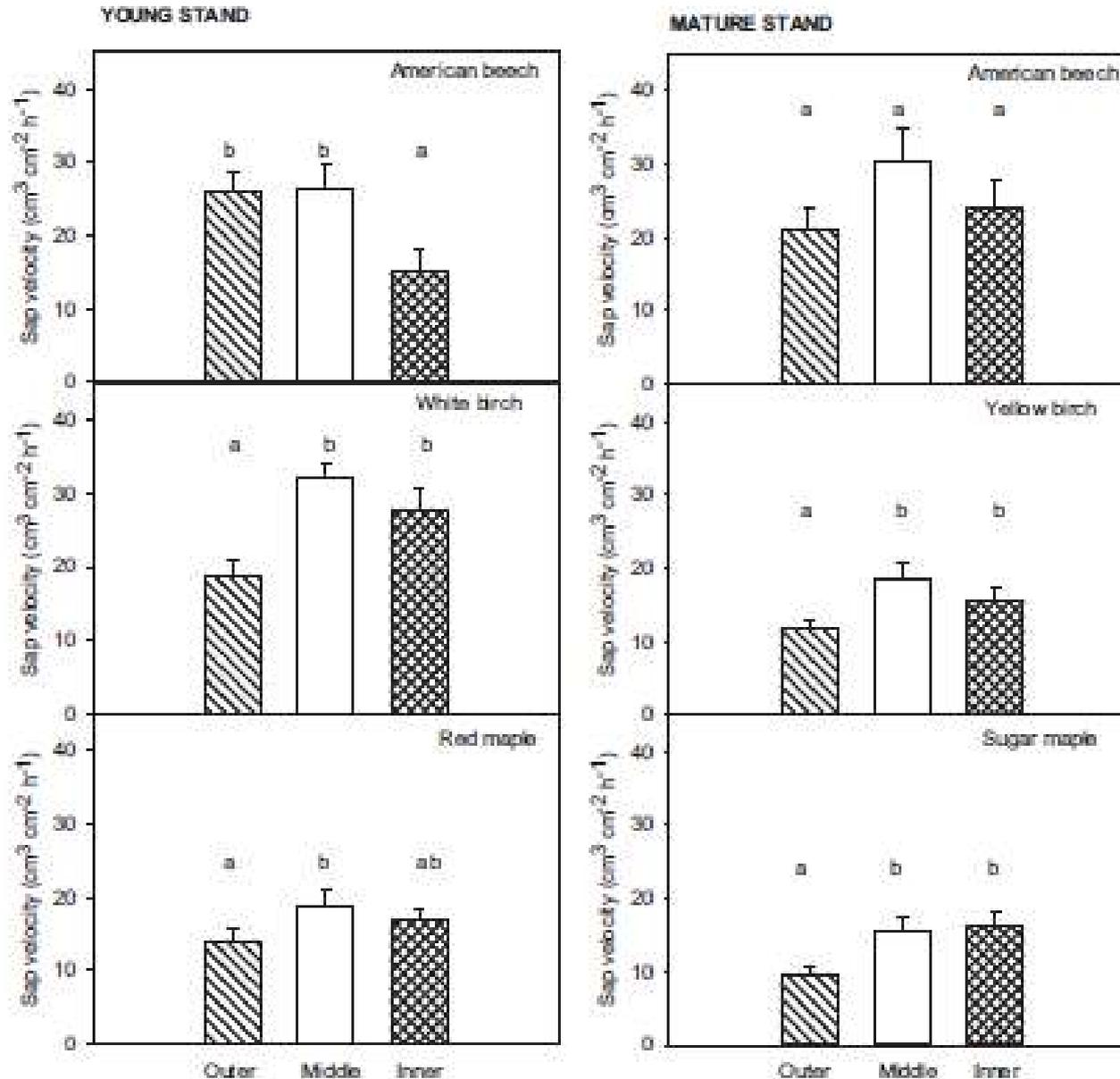
FROM SENSOR TO TREE

- SOURCES OF VARIABILITY AT TREE LEVEL?
 - Height
 - Radial variability
 - Circumferential variability

Scaling from single-point sap velocity measurements to stand transpiration in a multispecies deciduous forest: uncertainty sources, stand structure effect, and future scenarios

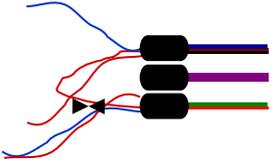
Virginia Hernandez-Santana, Adan Hernandez-Hernandez, Matthew A. Vadeboncoeur, and Heidi Asbjornsen

FROM SENSOR TO TRFF



FROM SENSOR TO TREE

- HOW TO GO FROM SENSOR TO TREE?
UNITS?



$$Q_s = J_s * A_s \text{ (Sap flux = sap flux density * sapwood area)}$$

UNITS?



$$[M T^{-1} \text{ (or } L^3 T^{-1}) = L^3 L^{-2} T^{-1} * L^{-2}]$$

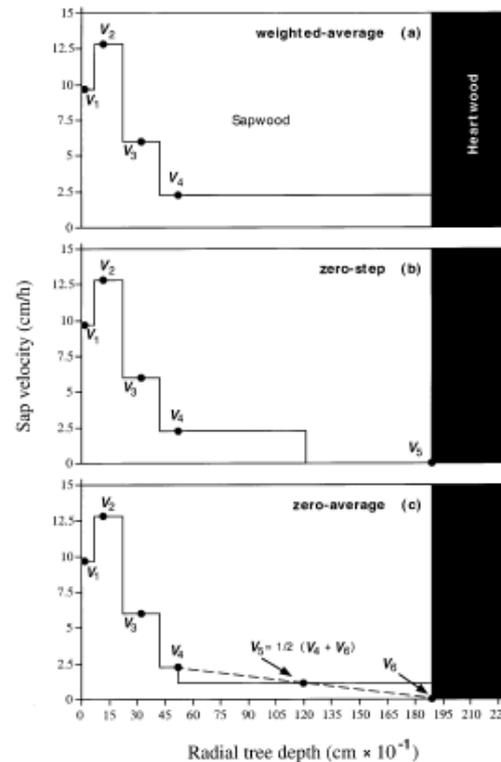
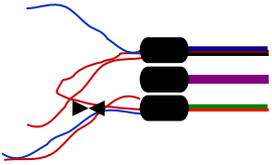
$$\text{ex. } cm^3 s^{-1}, m^3 h^{-1}, kg \text{ day}^{-1}$$

FROM SENSOR TO TREE

- HOW TO GO FROM SENSOR TO TREE?
UNITS?

$$Q_s = J_s * A_s \text{ (Sap flux = sap flux density * sapwood area)}$$

- BUT WHAT IF WE MEASURE AT DIFFERENT DEPTHS?



- Pausch et al. 2000

FROM SENSOR TO TREE

$$- Q_s = J_s * A_s$$

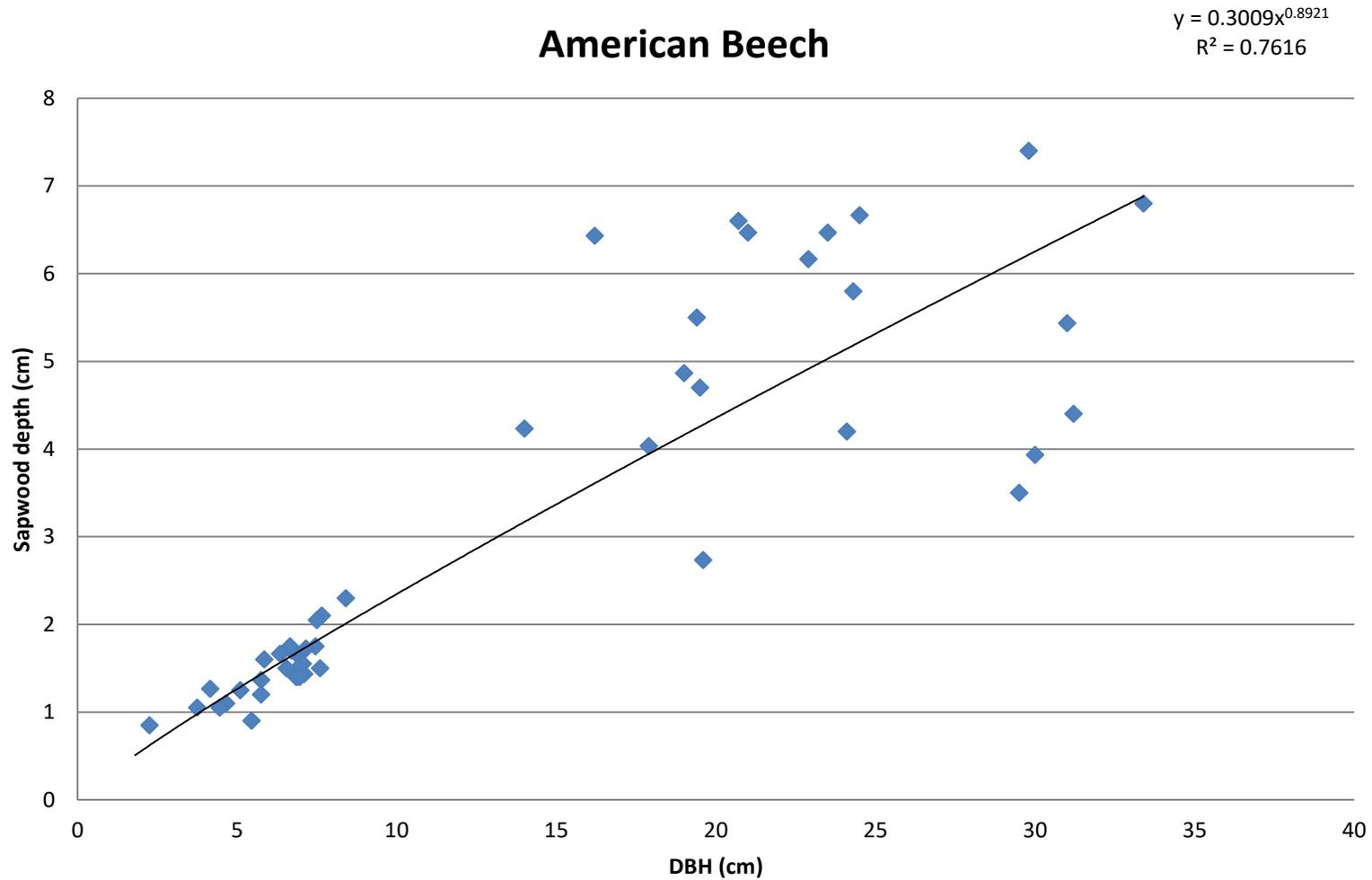
Pressler
increment borer:
different color or
translucency



Dye



FROM SENSOR TO TREE



FROM TREE TO STAND

- HOMOGENEOUS STAND (MONOSPECIFIC, SAME SIZE)

- AGRICULTURAL STAND

$$T = Q_s * \text{StandDensity}$$

$$T = Q_s * \text{StandDensity} / \text{LAI} \text{ -- leaf area basis}$$

$$[L T^{-1} \text{ (or } L^3 L^{-2} T^{-1}) = L^3 T^{-1} * L^{-2}]$$



- HETEROGENEOUS STAND (MULTISPECIFIC, DIFFERENT SIZE)

$$T = (Q_s * \text{StandDensity}) \text{ species } i, \text{ size } j$$



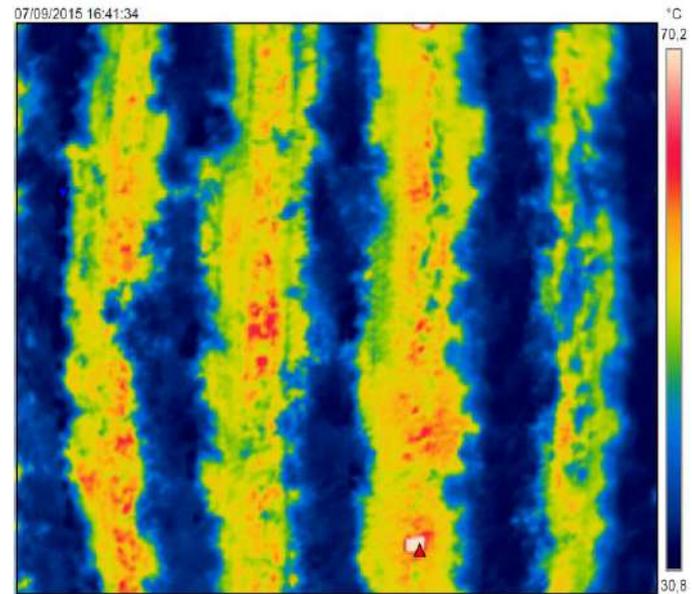
FROM TREE TO STAND

Stand-scale transpiration (T , mm h^{-1}) can be calculated for stands using stand inventories in smaller plots of each stand.

Unsamped species/individuals to estimate stand-scale T : sapwood area and J_s of studied species within the same stand having the most similar DBH range.

FROM TREE TO STAND

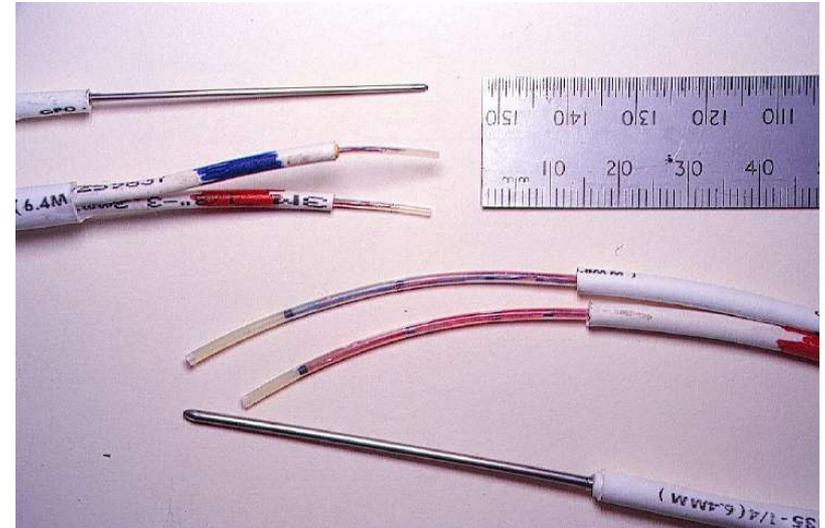
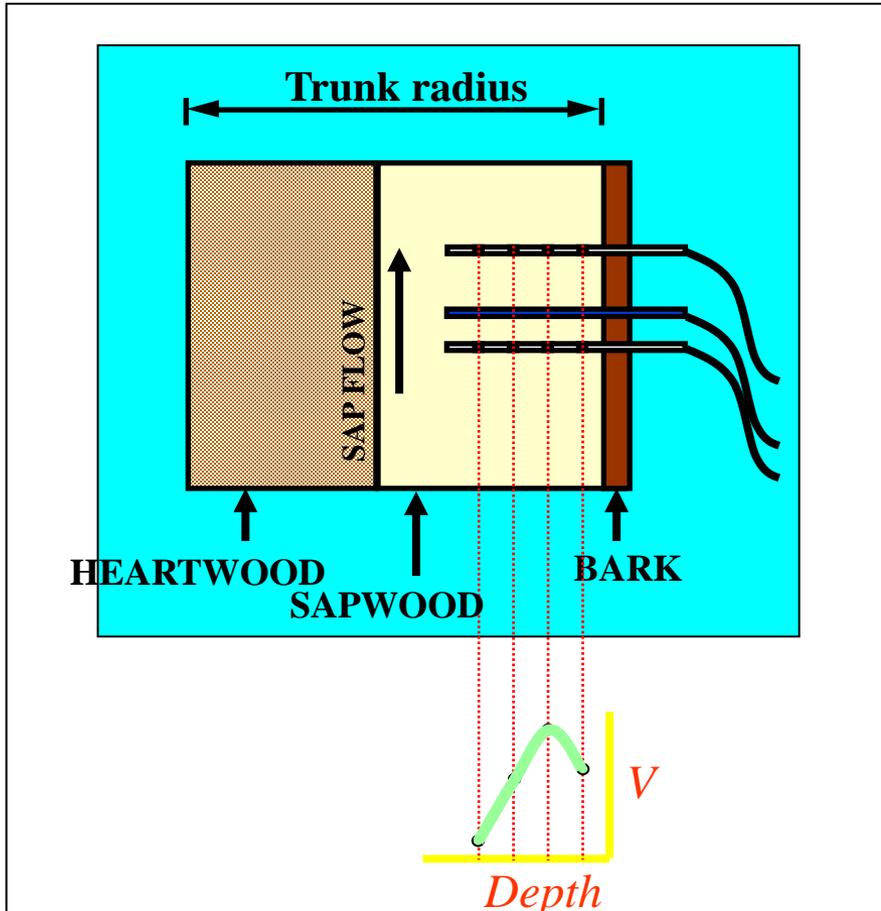
Scaling water fluxes requires an appropriate sampling design to effectively capture stand structure and spatial distribution associated with trees of differing size, dimension, canopy position, leaf area and species (Köstner et al. 1998).



Practical exercises

- Exercise1: HEAT PULSE VELOCITY.

The compensation heat-pulse method (Green and Clothier, 1988)



Fundamentals of the CHP method when using a HPV system

CHP: compensation heat pulse

HPV: heat pulse velocity

The CHP method **measures the time** required for a temperature difference between sensors to return to zero following a heat pulse input.

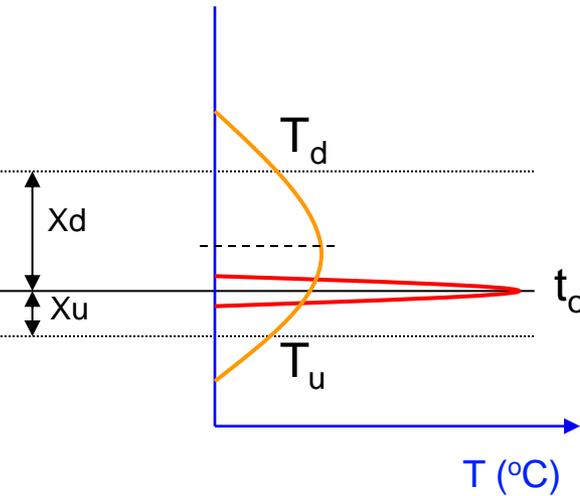
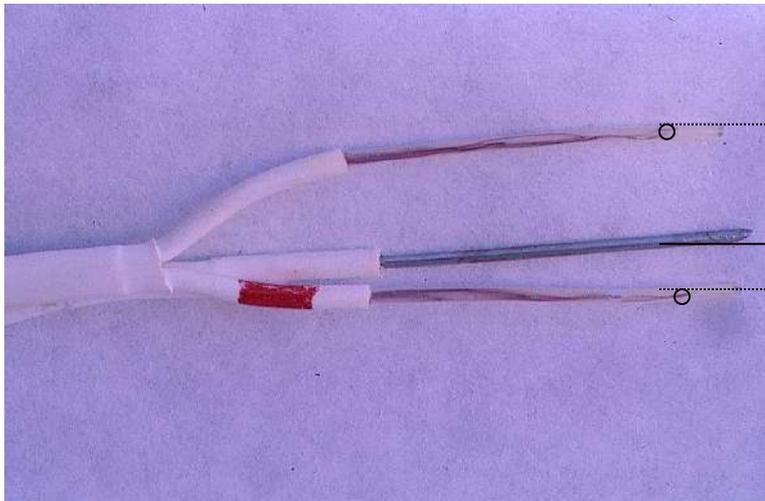
We used the CHP method to estimate sap flux in the stem

We are going to see the **HPV system of Green et al. (2003)**

Theory and Practical Application of Heat Pulse to Measure Sap Flow

Steve Green,* Brent Clothier, and Bryan Jardine

Published in Agron. J. 95:1371–1379 (2003).

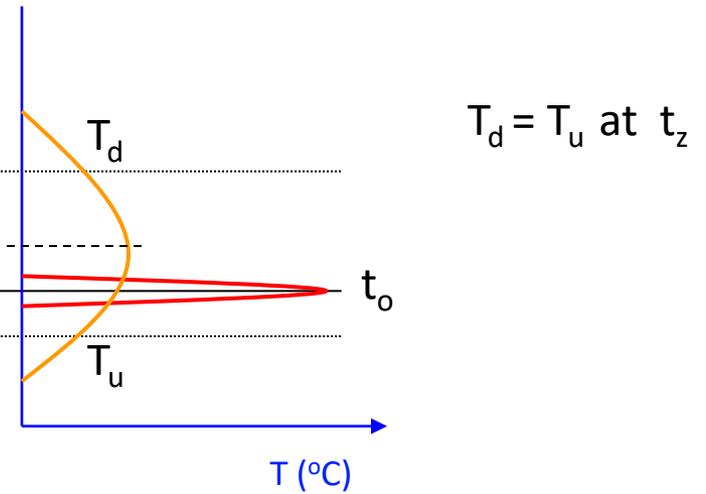
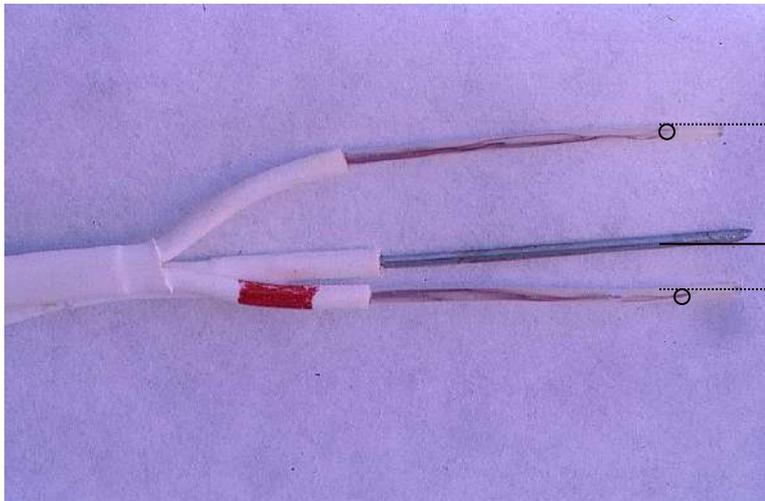


$$T_d = T_u \text{ at } t_z$$



the cross-over time

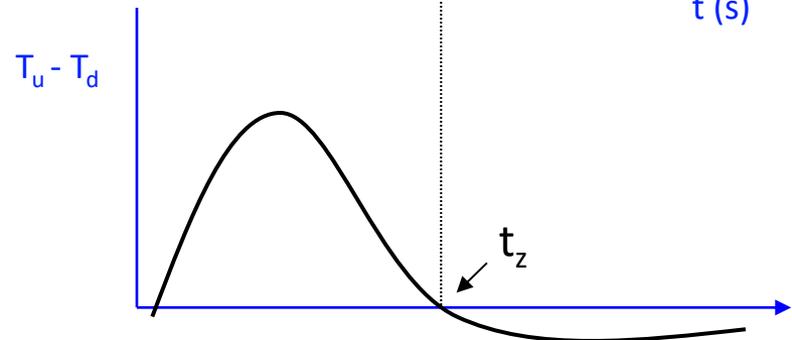
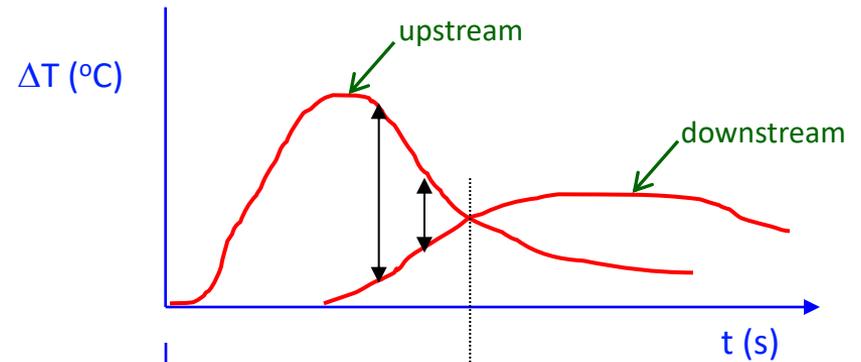
The CHP method **measures the time** required for a temperature difference between sensors to return to zero following a heat pulse input.



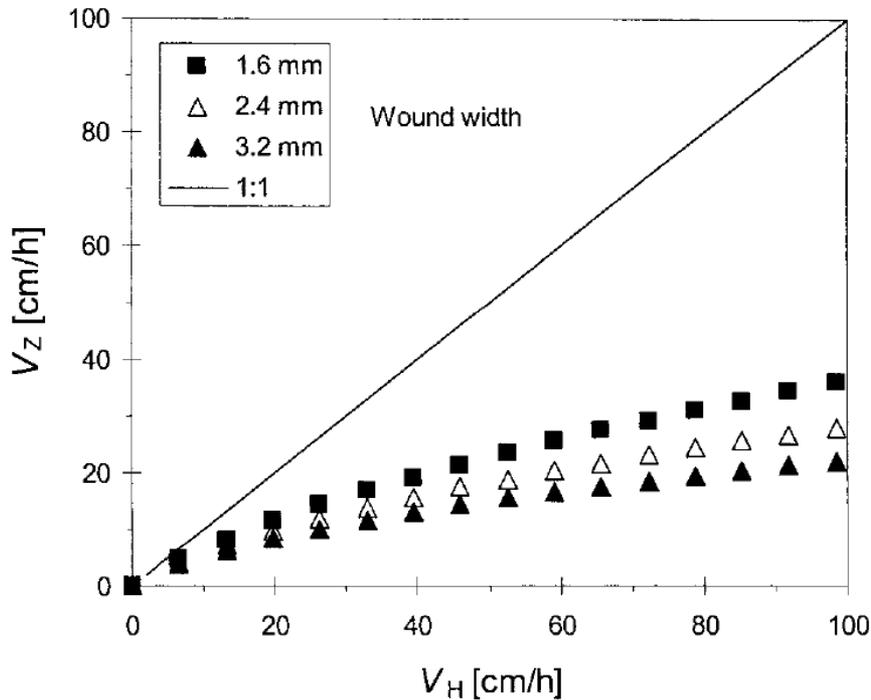
$$V_h = (X_d + X_u) / 2t_z$$

$$V_c = a_0 + a_1 V_h + a_2 V_h^2$$

$$J = (0.441F_M + F_L)V_c$$



The HPV must be corrected to account for the probe-induced effects of wounding and the thermal heterogeneity introduced by the probes



$$V = \frac{X_d + X_u}{2t_z}$$

$$V_c = a_0 + a_1 V_h + a_2 V_h^2$$

Fig. 3. Influence of wound width (mm) on the relationship between measured heat pulse velocity (V_z) and the actual heat pulse velocity (V_H). This relationship was determined using a numerical model of heat and mass flow through a 2-d slab of wet wood. The temperature probes are Teflon, and the heater is stainless steel.

The HPV must be corrected to account for the probe-induced effects of wounding and the thermal heterogeneity introduced by the probes

$$V = \frac{X_d + X_u}{2t_z}$$

Green, 1988

Where a_0 , a_1 , a_2 are the correction factors that depend on the probe size, spacing and material composition

$$V_c = a_0 + a_1 V_h + a_2 V_h^2$$

Green, 2003

Table 1. Correction factors for the compensation method using 1.6-mm-diam. probes placed at a distance of $x_U = -5$ mm upstream and $x_D = 10$ mm downstream from the heater.

Wound width	Coefficients in Eq. [7]		
	a_0	a_1	a_2
mm			
1.6	-5.48E-01	1.33E+00	4.00E-02
1.8	-4.26E-01	1.31E+00	4.94E-02
2.0	-9.63E-02	1.24E+00	6.34E-02
2.2	1.31E-01	1.19E+00	7.36E-02
2.4	3.94E-01	1.12E+00	8.78E-02
2.6	8.36E-01	9.98E-01	1.07E-01
2.8	1.51E+00	7.91E-01	1.32E-01
3.0	1.79E+00	6.86E-01	1.52E-01
3.2	2.31E+00	5.02E-01	1.78E-01
3.4	2.86E+00	2.71E-01	2.11E-01

From the corrected HPV we can infer the sap flux density:

$$J = (0.505F_M + F_L)V_c$$



Factor related to the thermal properties of the wood matrix
(others use 0.441)

Where F_M and F_L are the volume fractions of wood and water, respectively.

The volumetric sap flux (Q), can be determined by integrating the measured sap flux density profile over the area of conducting Sapwood.

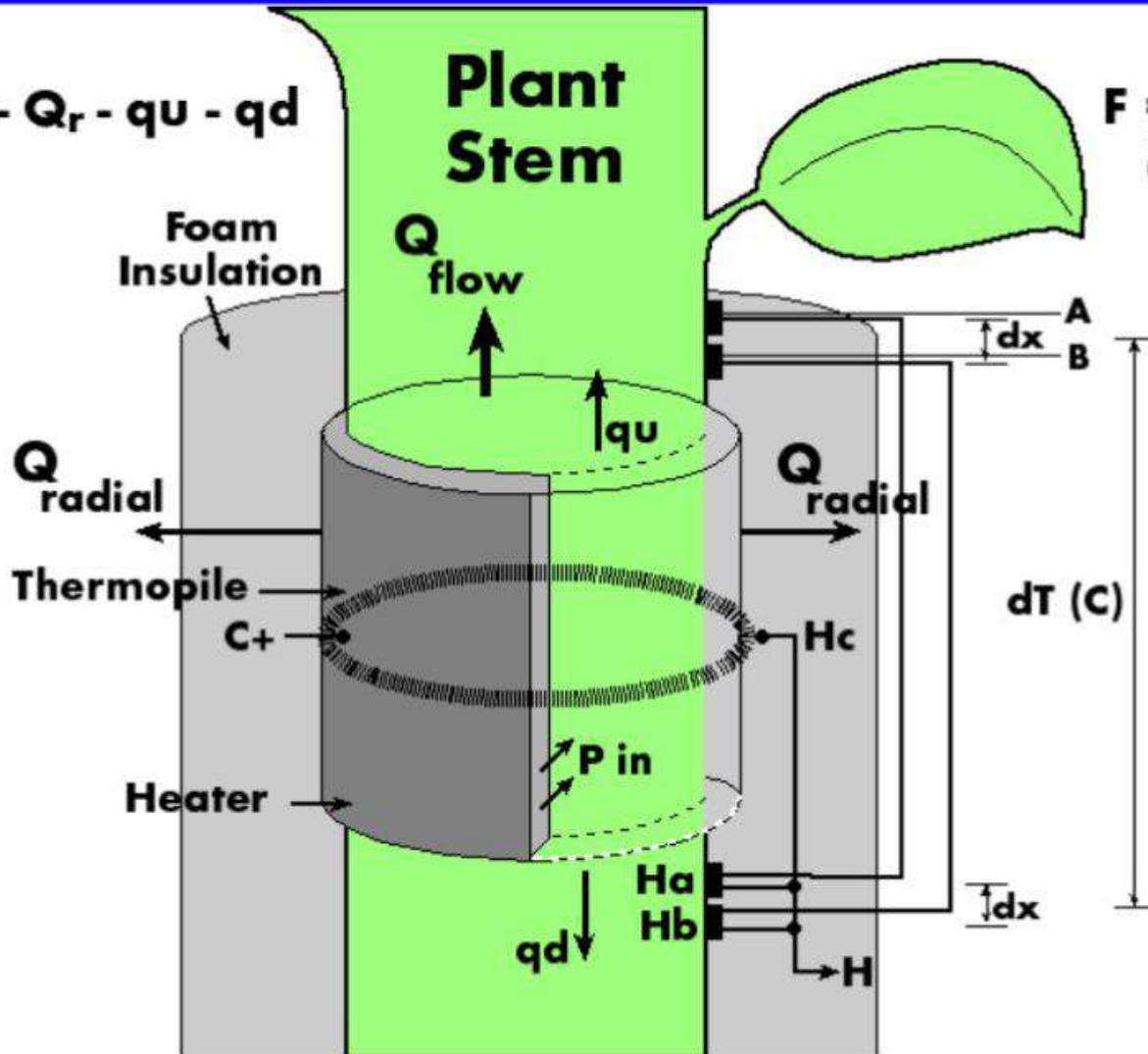
Practical exercises

- Exercise2: **STEM HEAT BALANCE.**

How Dynagage Works

$$Q_F = P_{in} - Q_r - q_u - q_d$$

$$F = \frac{Q_F}{C_p \cdot dT}$$



Energy Balance Sapflow Equation

$$F = (P_{in} - Q_v - Q_r) / C_p * dT$$

- **Where:**

- **F = Flow rate per unit of time**
- **P_{in} = Power supplied in watts**
- **Q_v = Vertical or Axial conduction**
- **Q_r = Radial heat Conduction**
- **C_p = Specific heat of water (4.186 J/g*C)**
- **dT = Temperature increase in sap**

Sheath Conductance

- $Q_r = K_{sh} \text{ (W/mV)} * C_h \text{ (mV)}$

- **KSH is determined by a Zero Set.**

- **As the radius of the cylinder affects the thermal conduction rate, the thermal conductance constant for a particular gage installation or K_{sh} must be calculated to produce accurate readings.**

- **Min KSH is the minimum level of Sheath Conductance when Radial Heat loss signal (C_h) is at it's maximum when the plant is not transpiring between 2:00am – 5:00am . Since $K_{sh} = [P_{in} - Q_v] \text{ (W)} / C_h \text{ (W/mV)}$, if $Q_f=0$.**

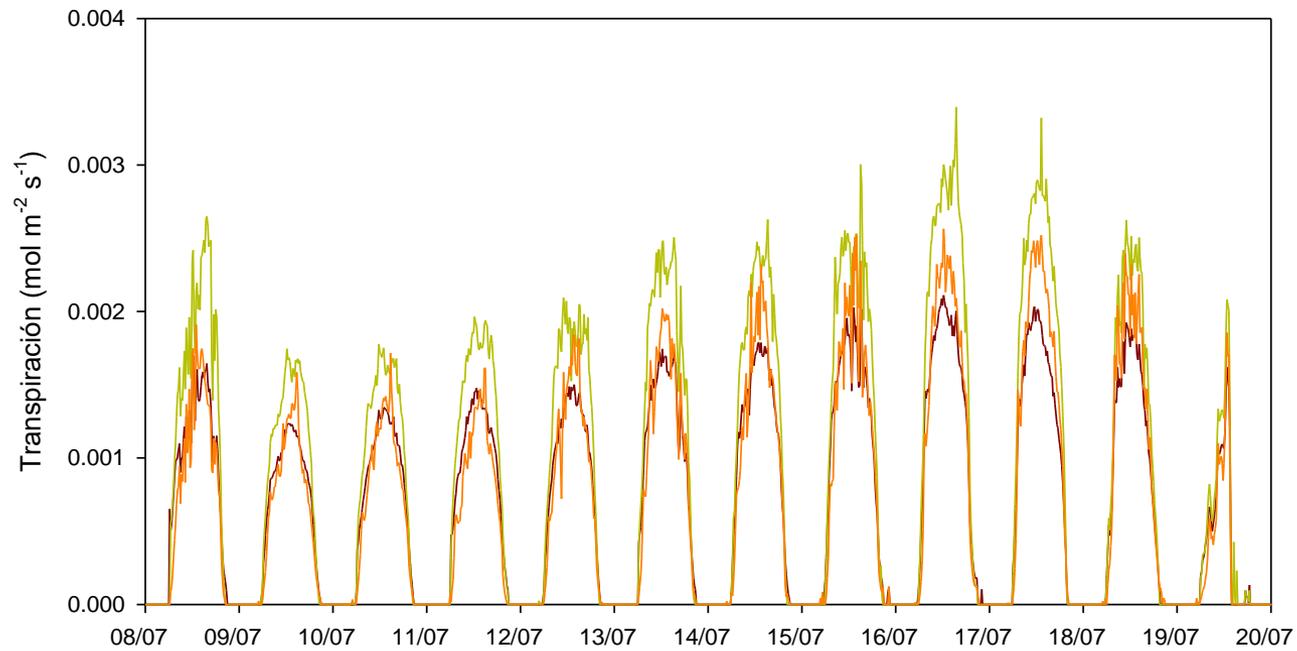
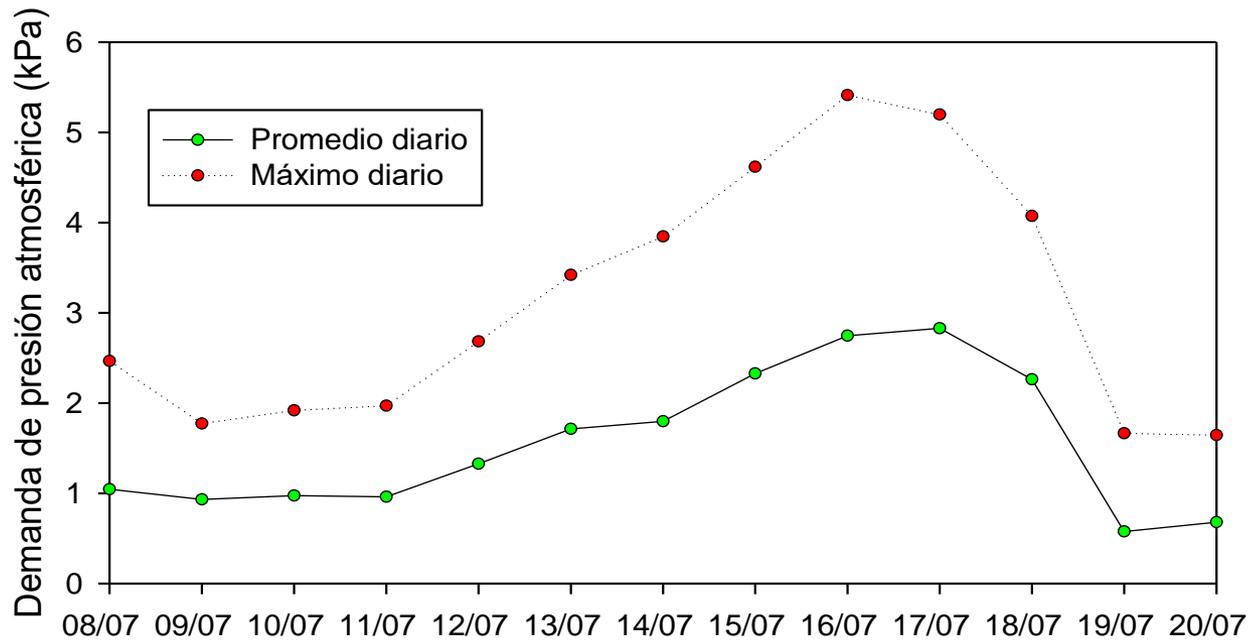
- **This minimum KSH is then used as a zero set to find the equivalent zero flow rate, pre-dawn, and the correct Q_r at any later time.**

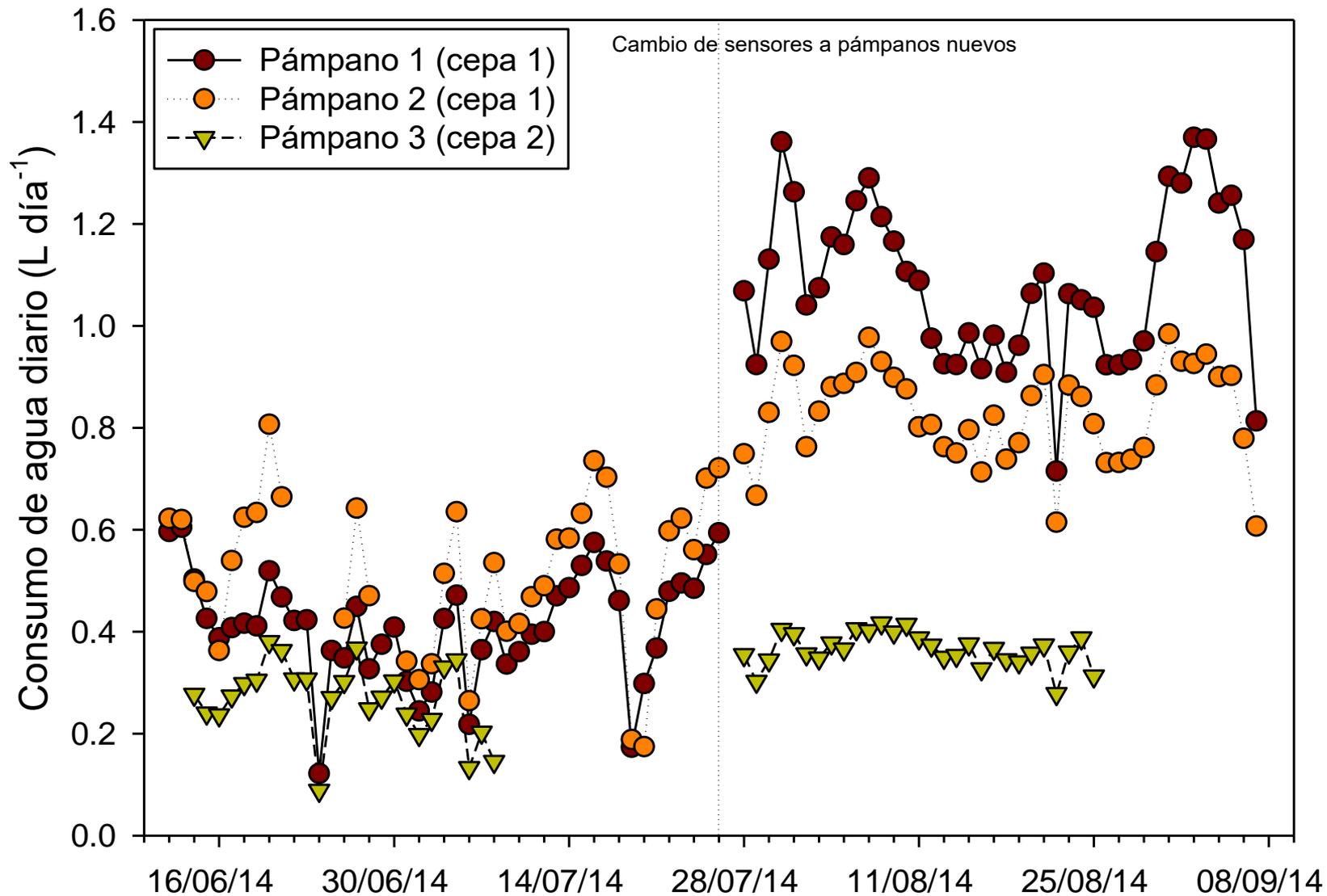


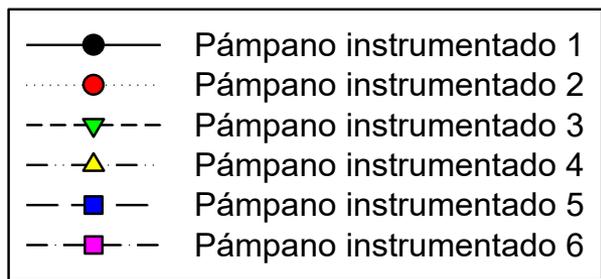
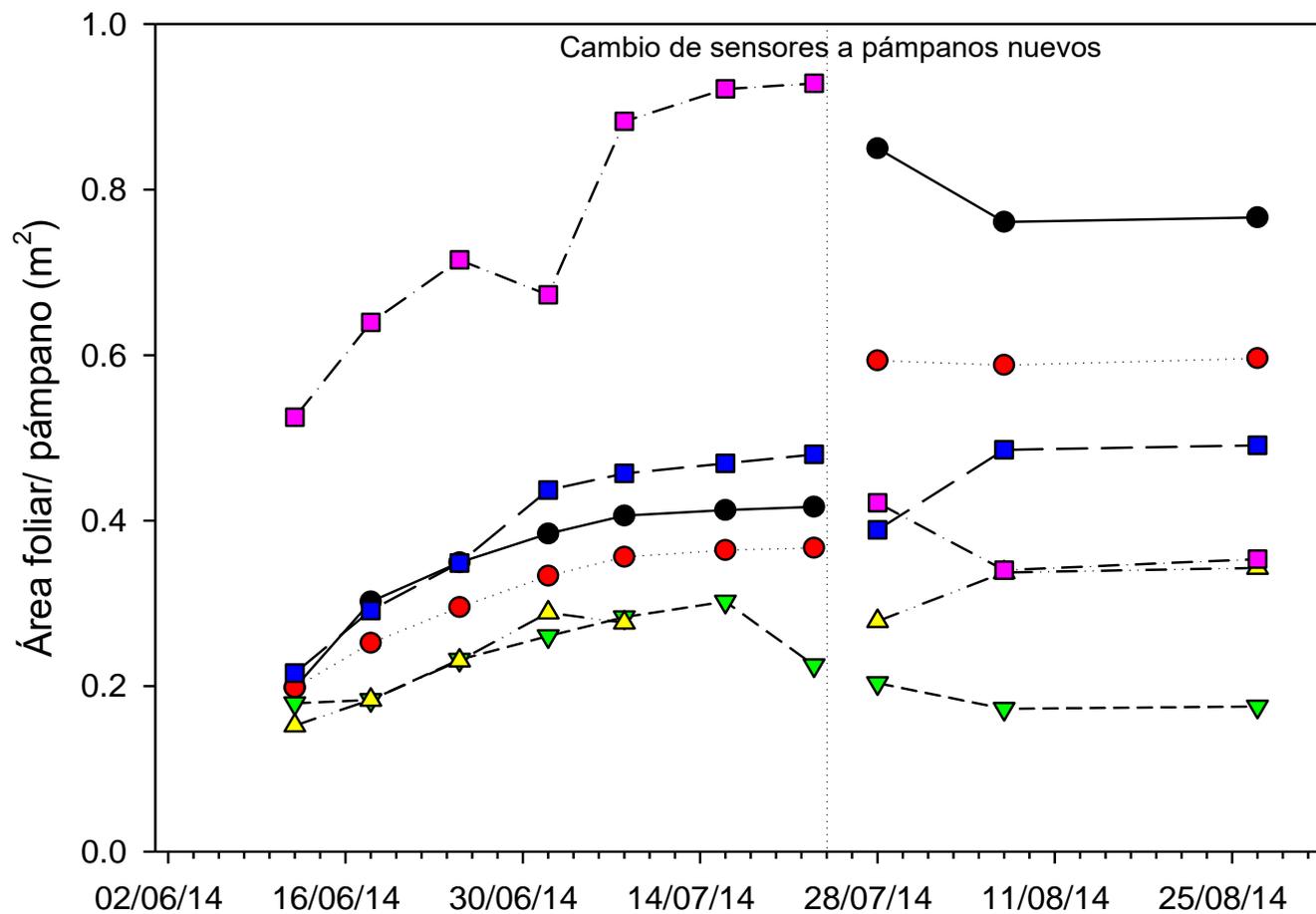
To evaluate the temporal evolution of plant water consumption and to derive canopy conductance and photosynthetic assimilation.

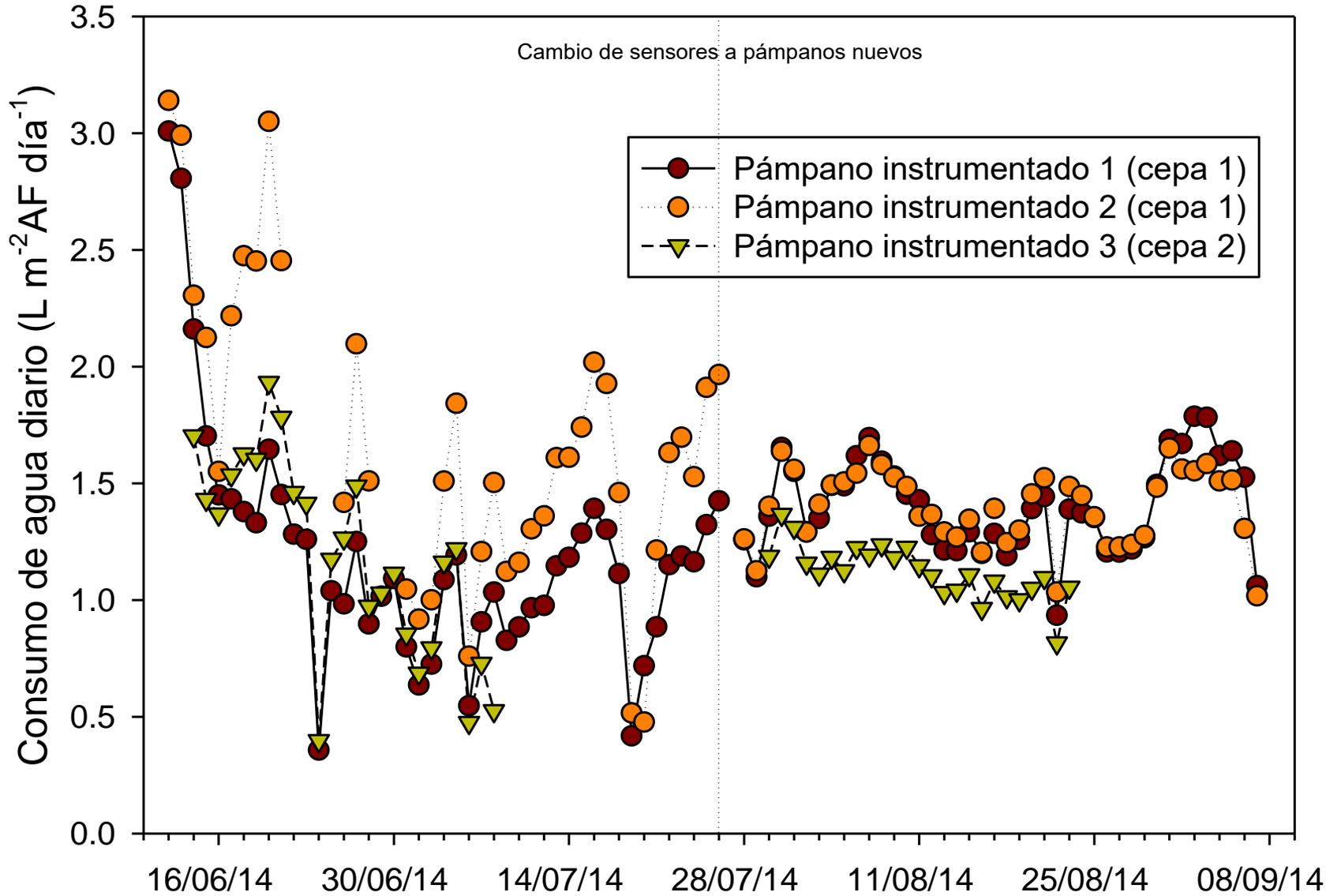
To establish relationships between these variables and water in the soil and meteorological variables to develop a predictive model of the plant's photosynthetic activity and therefore of production





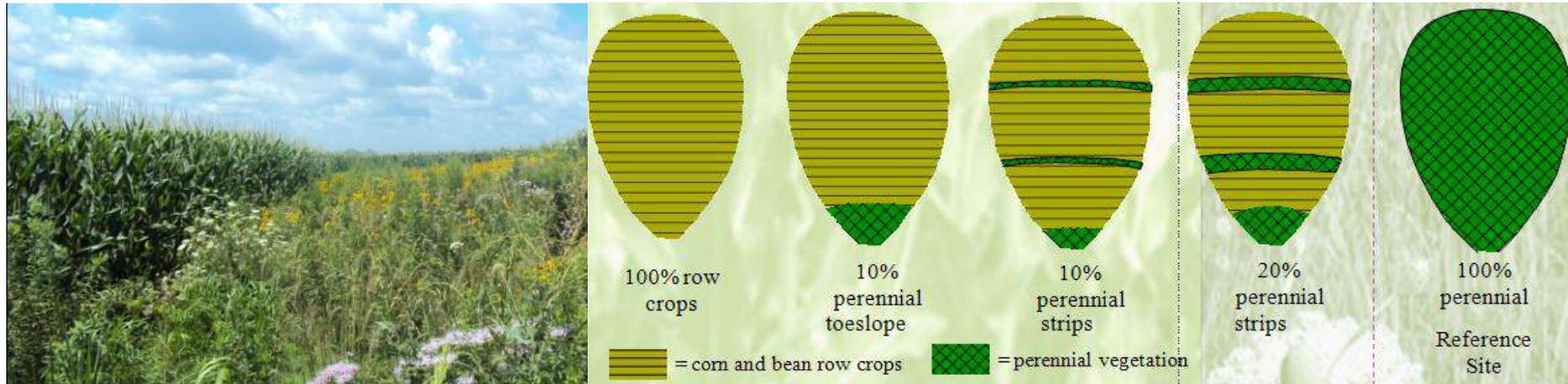






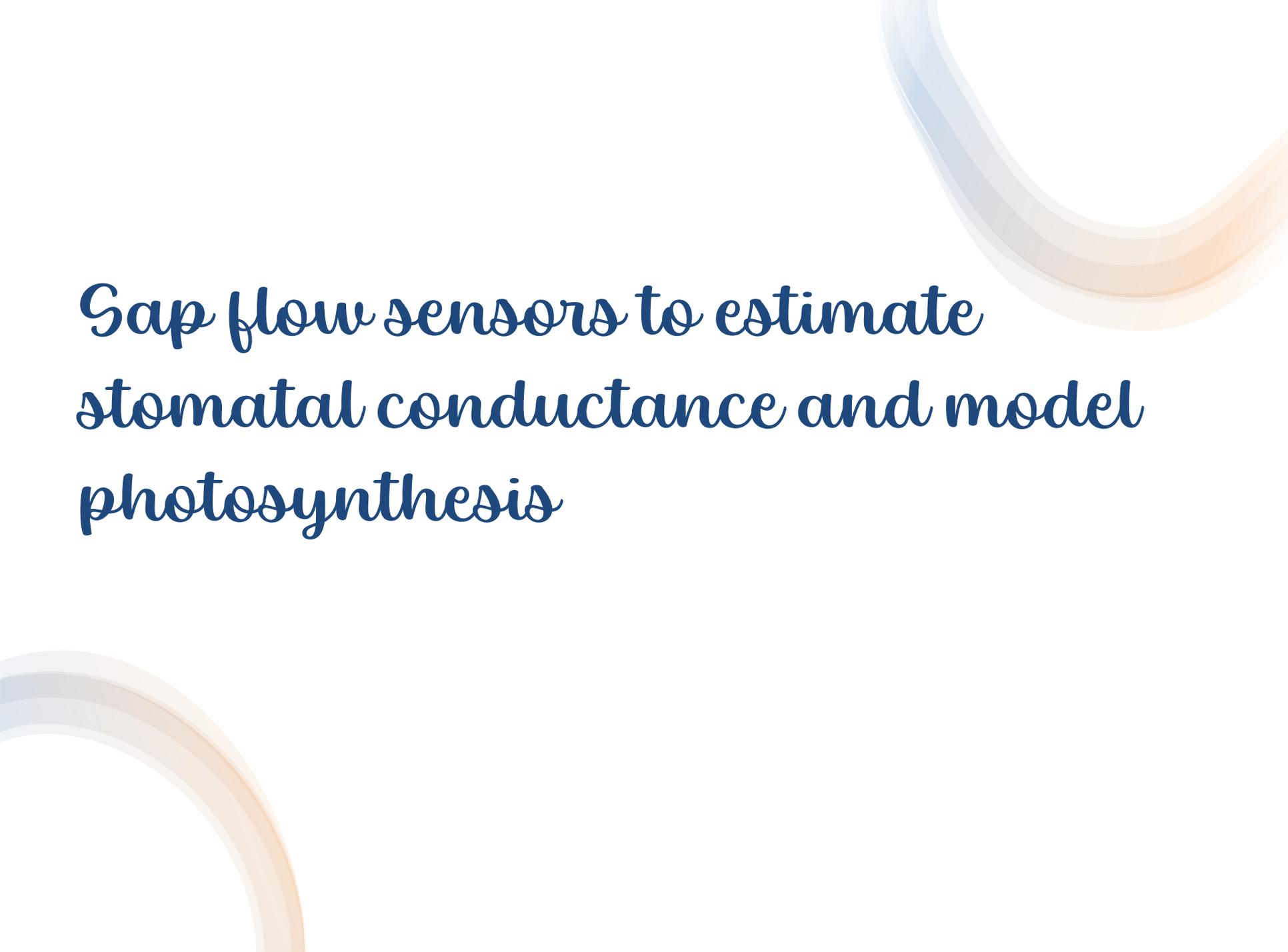
Comparison of ecohydrological function of prairie species (C4 grass, C3 forb) and corn (C4 crop) during a wet year

Vilma Mateos, H. Asbjornsen, T. Sauer, J. Tarara, D. Meek
22nd North American Prairie Conference, August 1-5, 2010



- Perennial cover within agriculturally-dominated landscapes to reduce runoff and erosion, etc.
- Enhance transpiration to remove excess water?
- Stem Heat Balance sap flow technique to compare species-specific transpiration patterns.



The slide features decorative curved lines in the corners. In the top right, there is a blue line that curves downwards and then back up, transitioning into an orange line that curves upwards. In the bottom left, there is a similar orange line curving upwards and then back down.

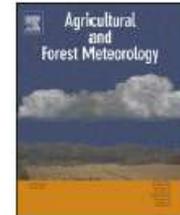
*Sap flow sensors to estimate
stomatal conductance and model
photosynthesis*



Contents lists available at [ScienceDirect](#)

Agricultural and Forest Meteorology

journal homepage: www.elsevier.com/locate/agrformet



The dynamics of radial sap flux density reflects changes in stomatal conductance in response to soil and air water deficit

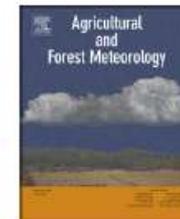
V. Hernandez-Santana^a, J.E. Fernández^a, C.M. Rodriguez-Dominguez^{a,b}, R. Romero^a,
A. Diaz-Espejo^{a,*}



Contents lists available at [ScienceDirect](#)

Agricultural and Forest Meteorology

journal homepage: www.elsevier.com/locate/agrformet



Relationships between fruit growth and oil accumulation with simulated seasonal dynamics of leaf gas exchange in the olive tree

V. Hernandez-Santana^a, R.D.M. Fernandes^a, A. Perez-Arcoiza^a, J.E. Fernández^a, J.M. Garcia^b,
A. Diaz-Espejo^{a,*}



Problem addressed

16 October 2016
World Food Day



Increasing population
demanding more food



Conservation of our
scarce water resources

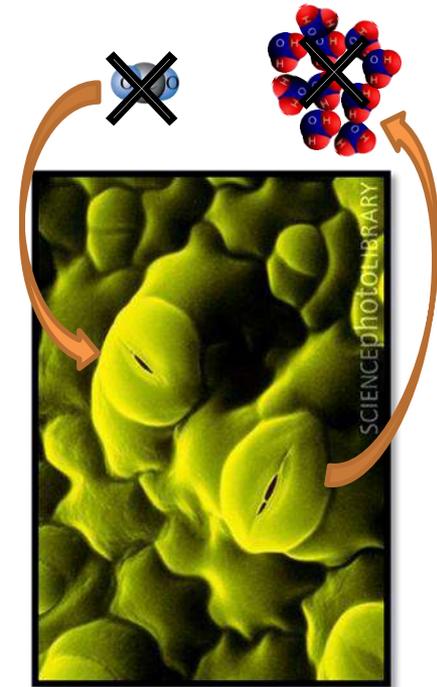
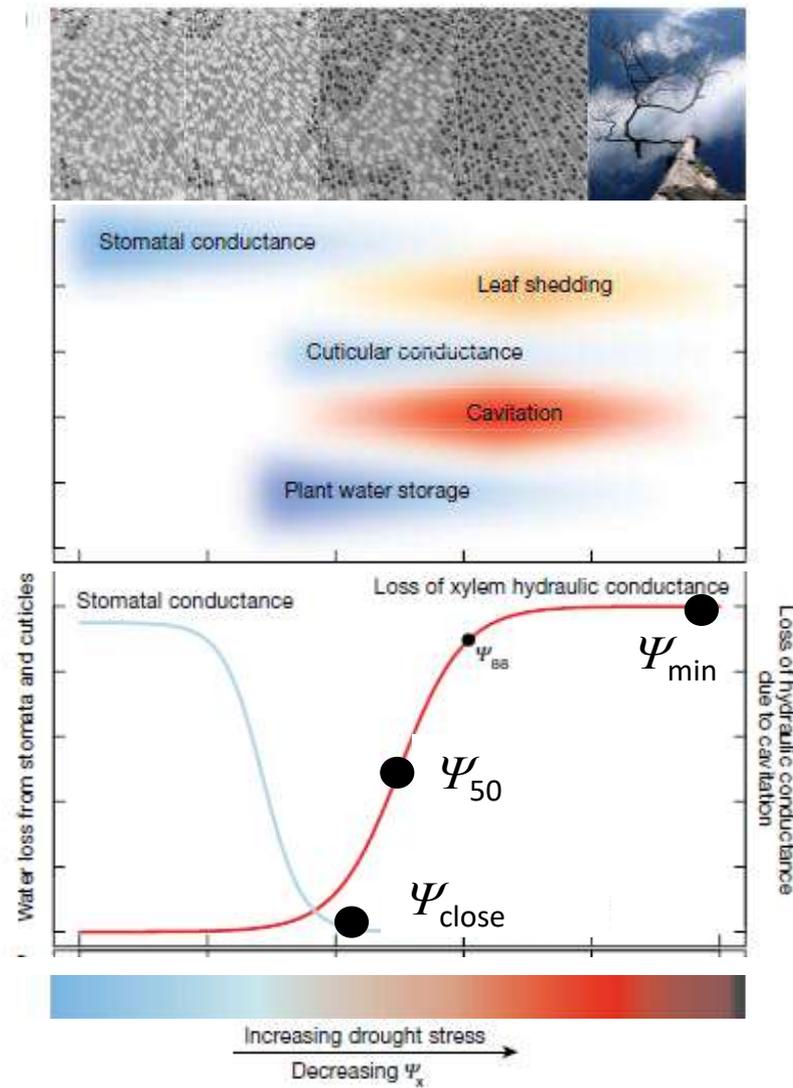
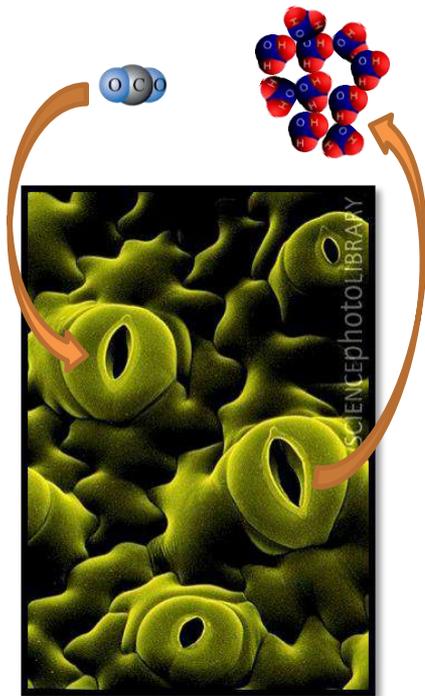
More resilient
agricultural
systems less
dependant on
rainfall



Deficit irrigation
strategies

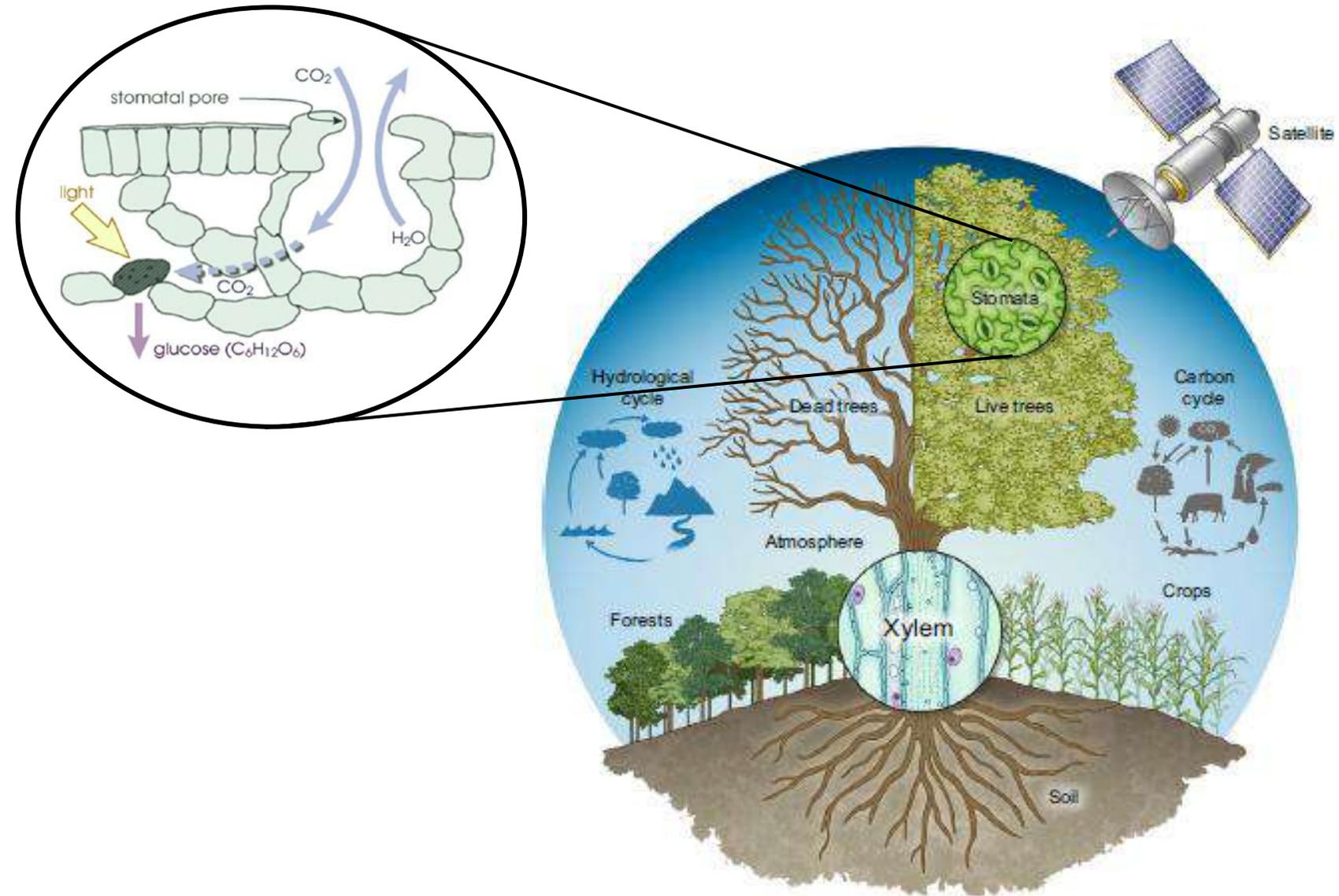


Water stress indicator



Choat et al. (2018)

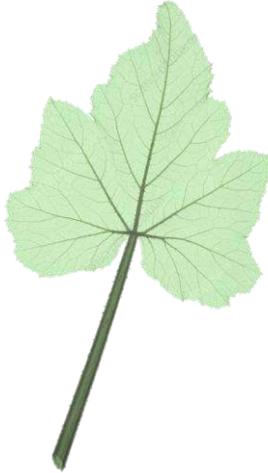
At the crossroad of water and carbon fluxes



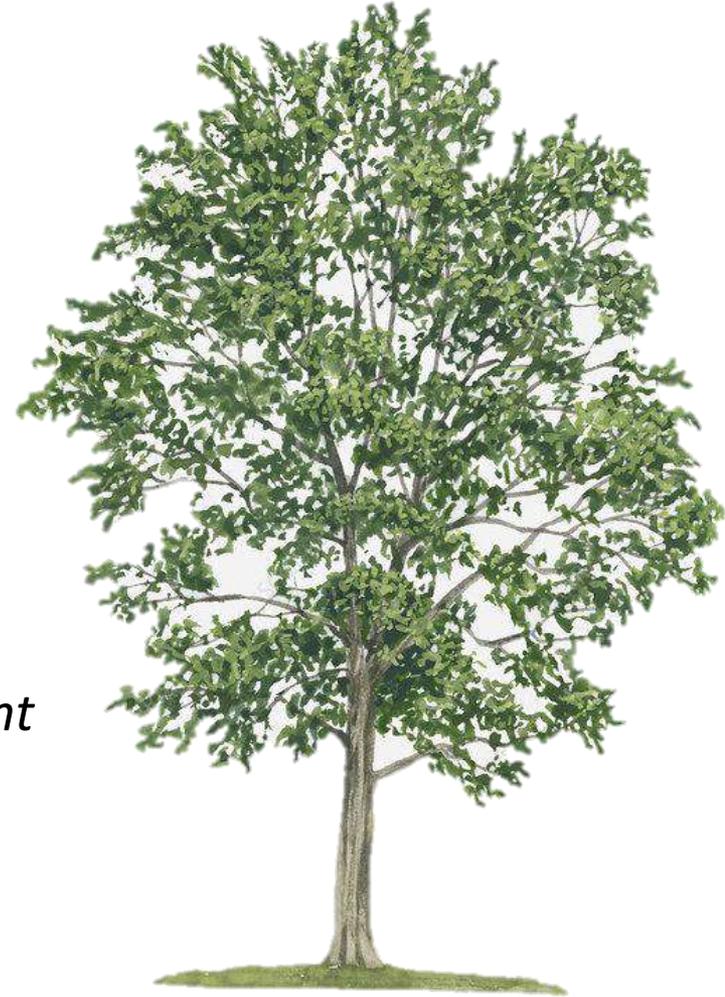
McDowell et al. (2019)

The importance for crops

g_s



LA



$$g_s D_a LA = E_{plant}$$

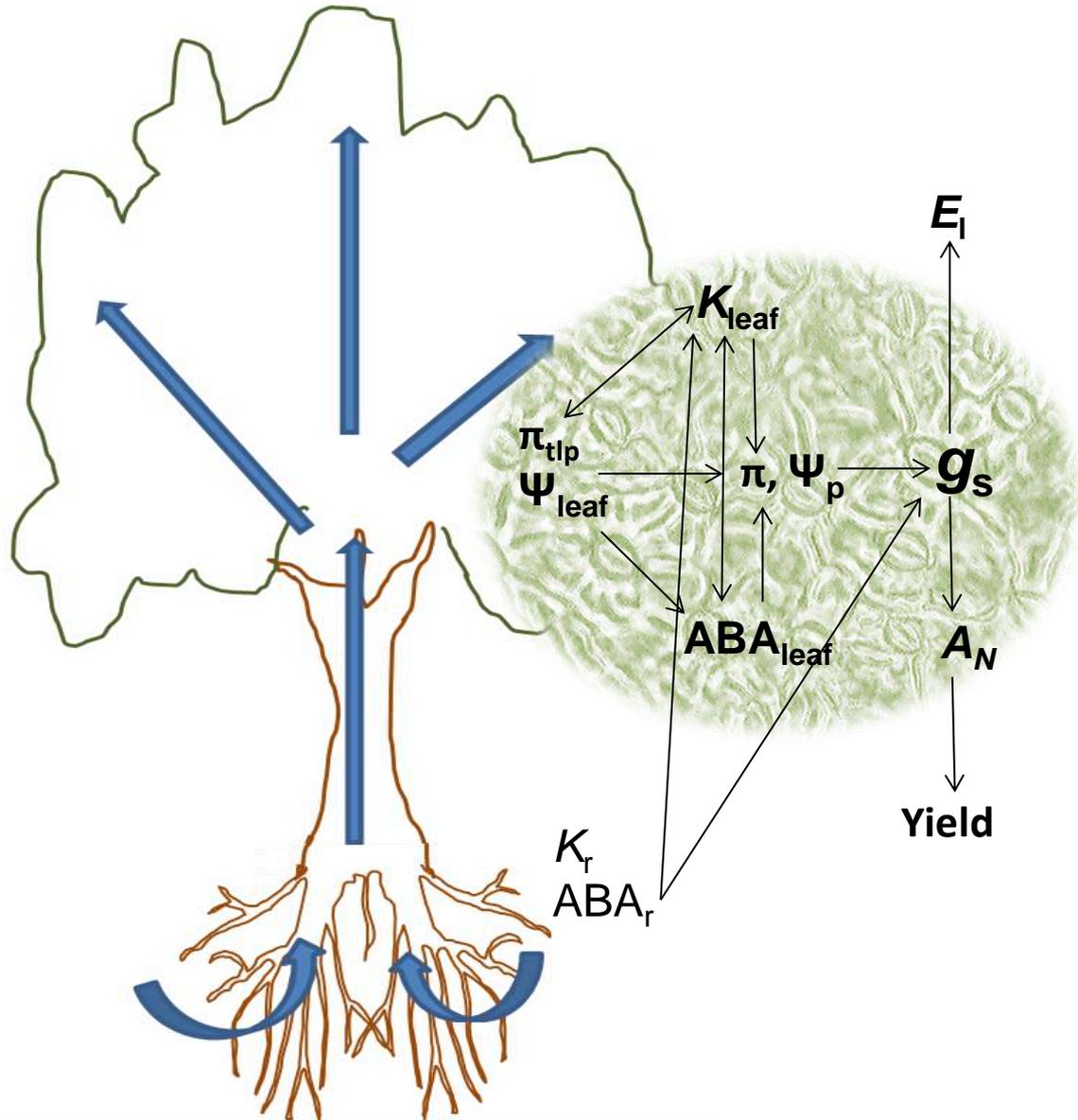
g_s : stomatal conductance

D_a : vapor pressure deficit

LA : leaf area

E_{plant} : plant transpiration

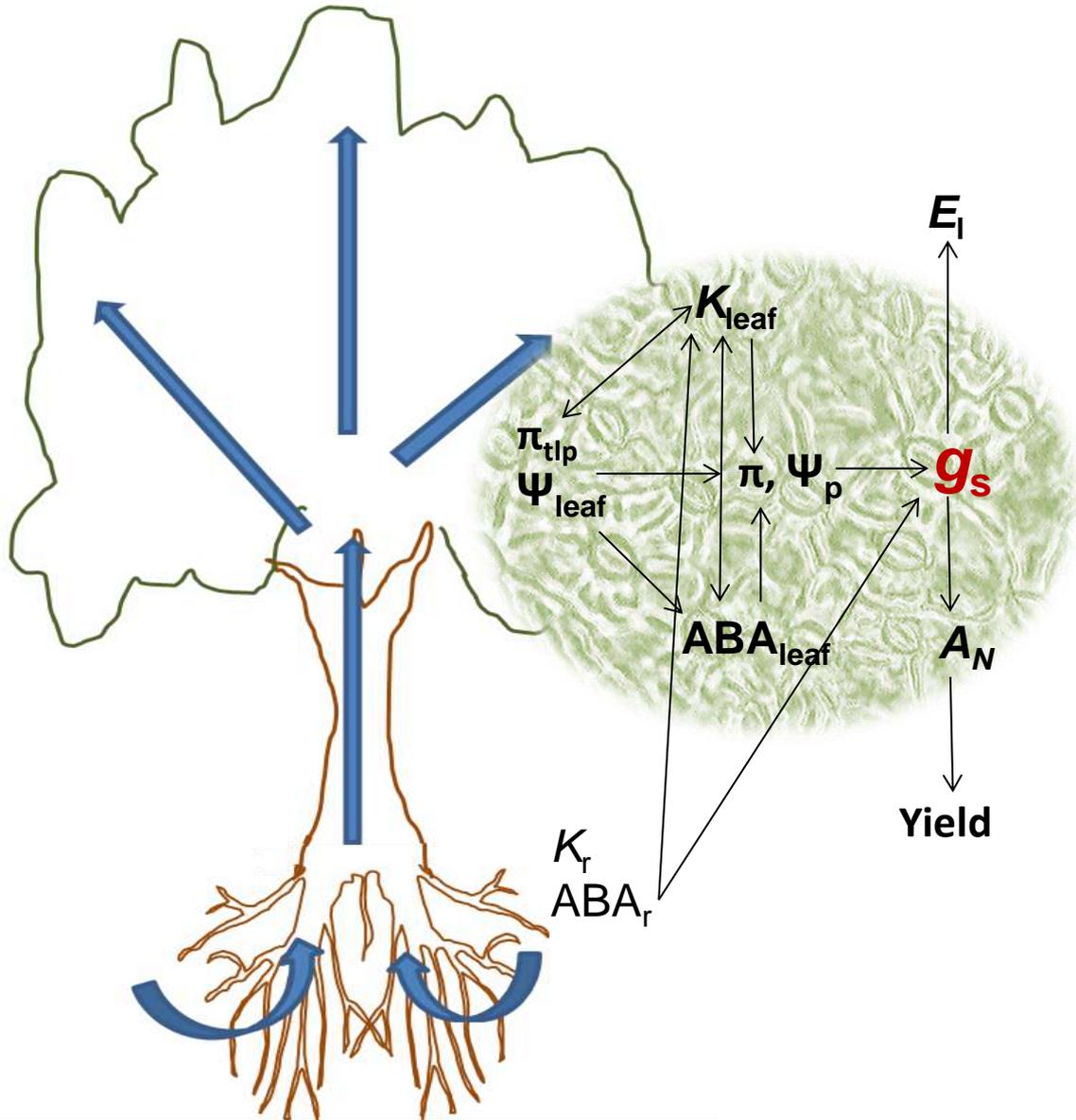
Problem addressed



Some sensor characteristics:

- Sensitive and indicative of plant water status
- Related to plant water use and, importantly, to yield through its influence on photosynthesis
- Easy to interpret, physiological meaning
- Able to be measured continuously and automatically

Problem addressed



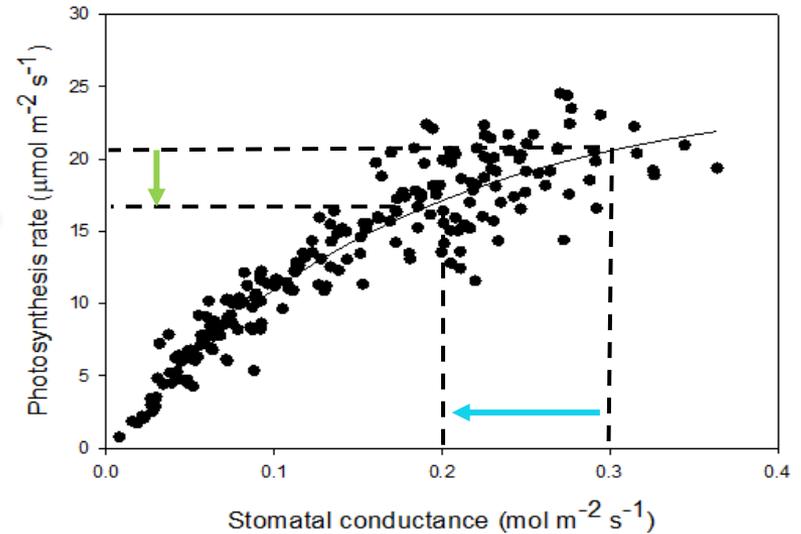
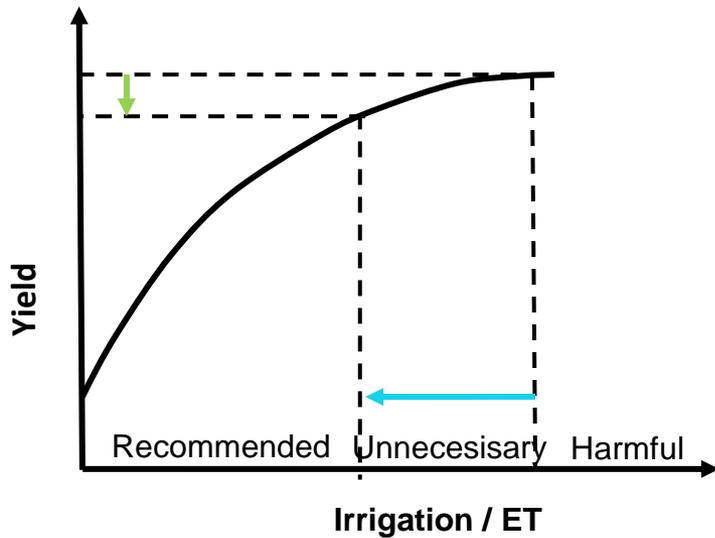
Some sensor characteristics:

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- ✓ Easy to interpret, physiological meaning
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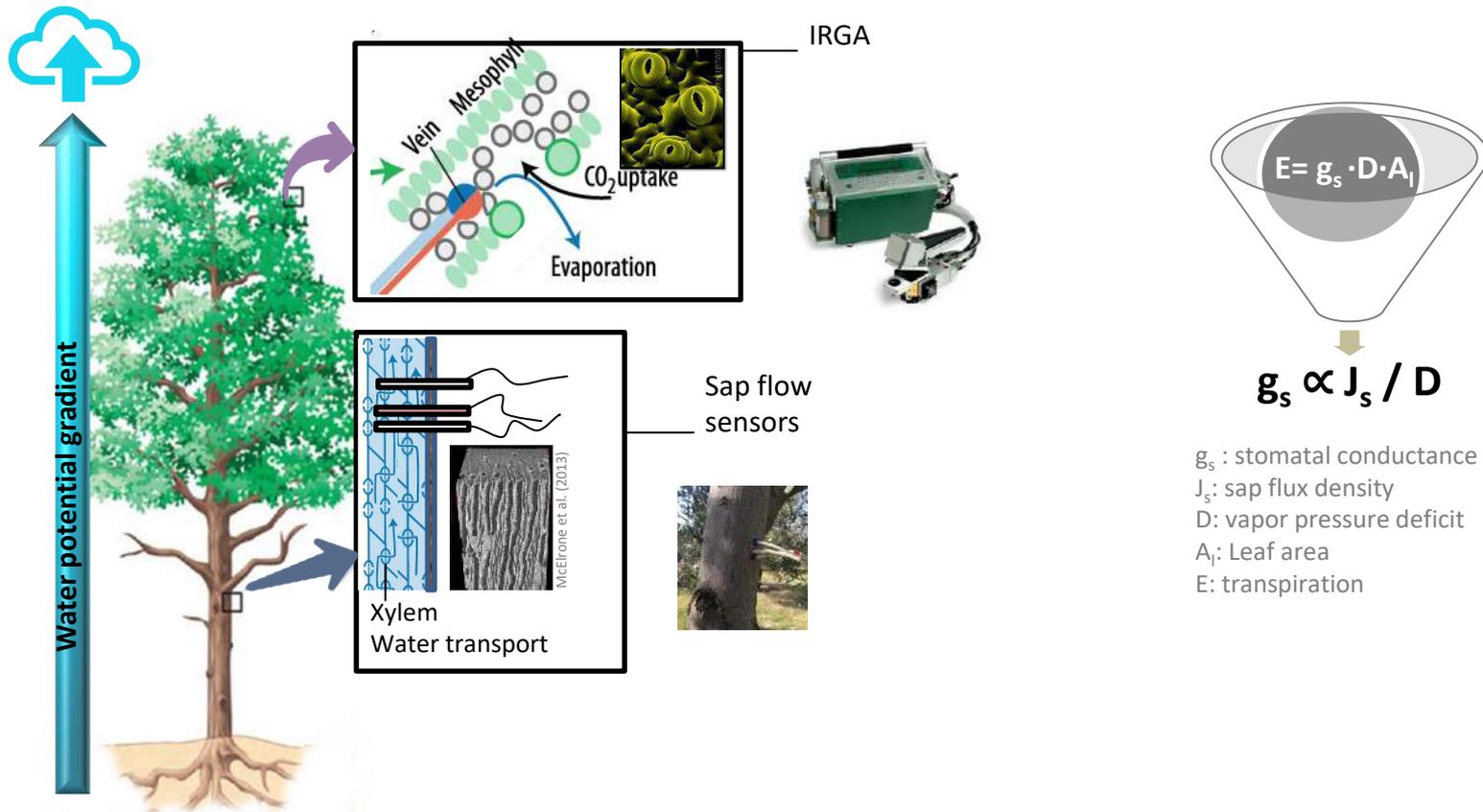
HYPOTHESIS: the relationship and the meaning between irrigation and yield is similar to the relationship between stomatal conductance and photosynthesis.

THUS, we can use stomatal conductance and photosynthesis to manage irrigation in a sustainable manner in crops



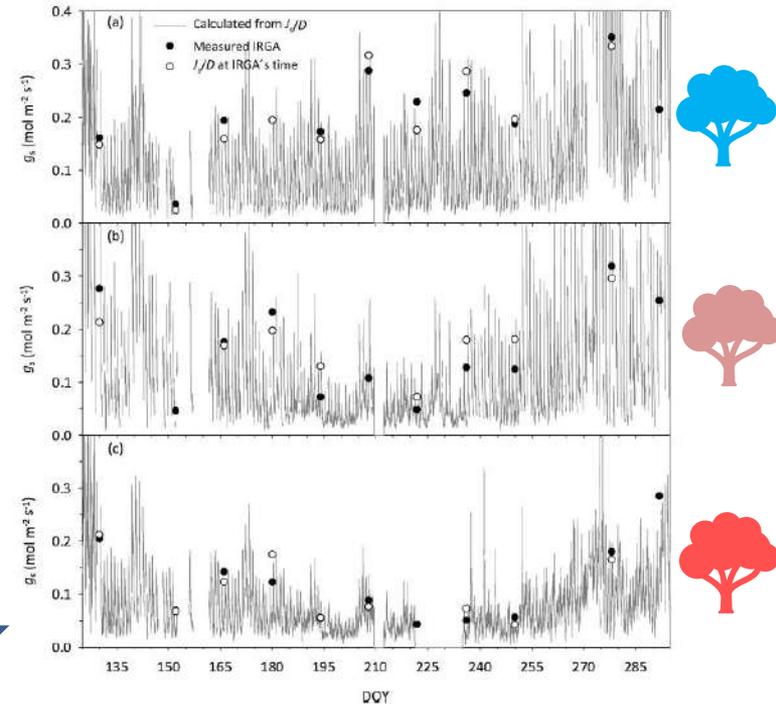
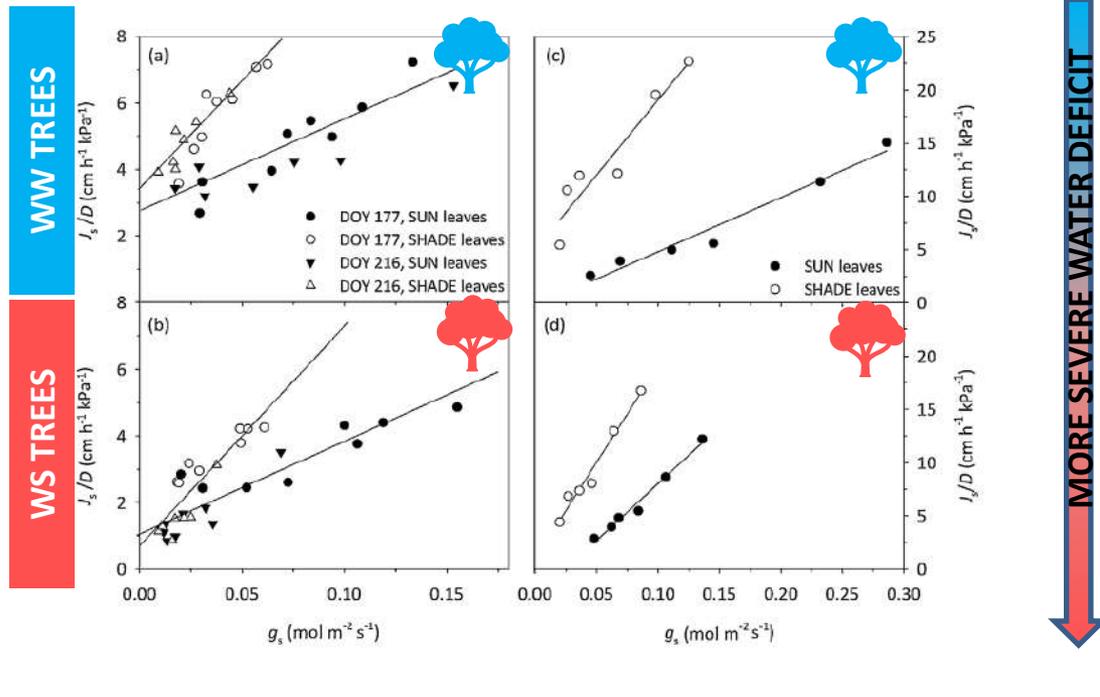
OBJECTIVE

Simular **conductancia estomática en continuo** a través de sensores en planta y variables meteorológicas fáciles de medir



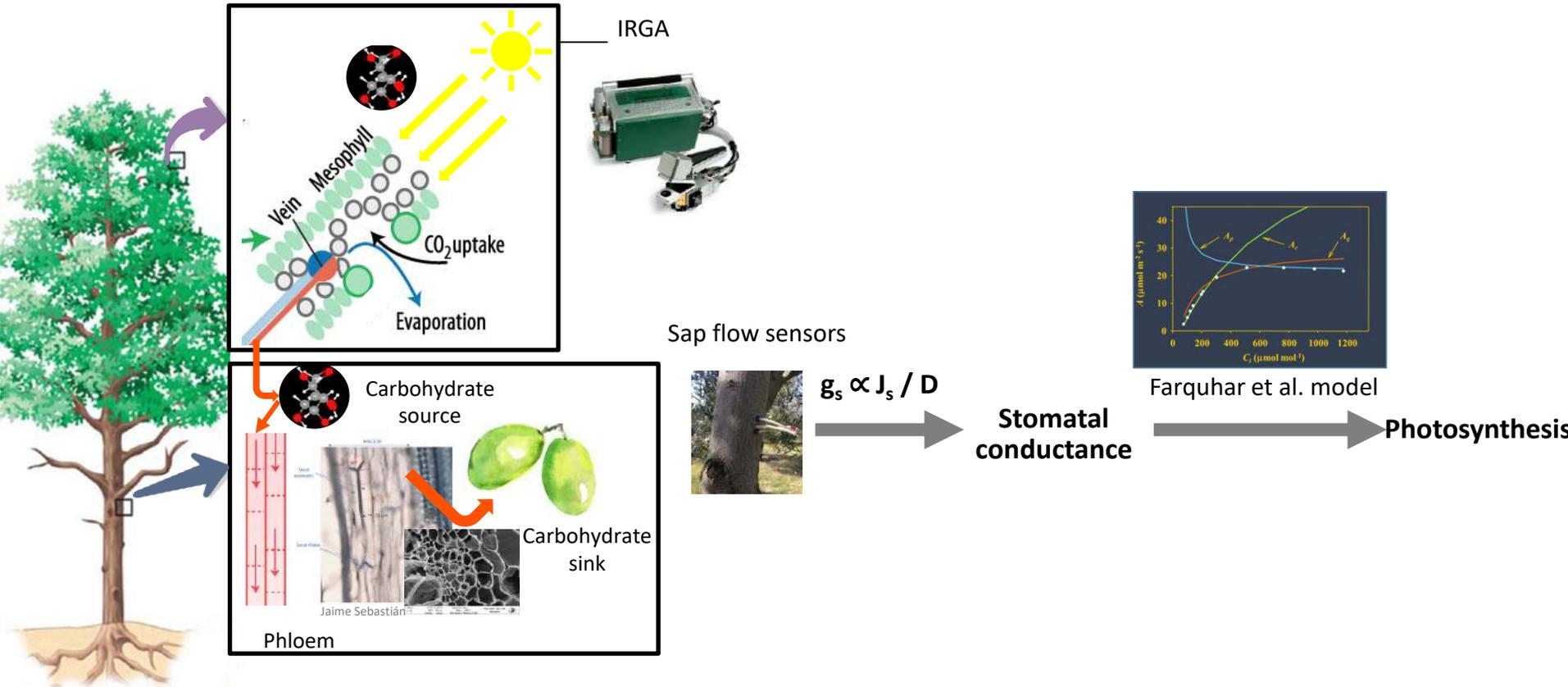
RESULTS

To simulate stomatal conductance in a continuous manner through plant sensors and meteorological variables easy to measure



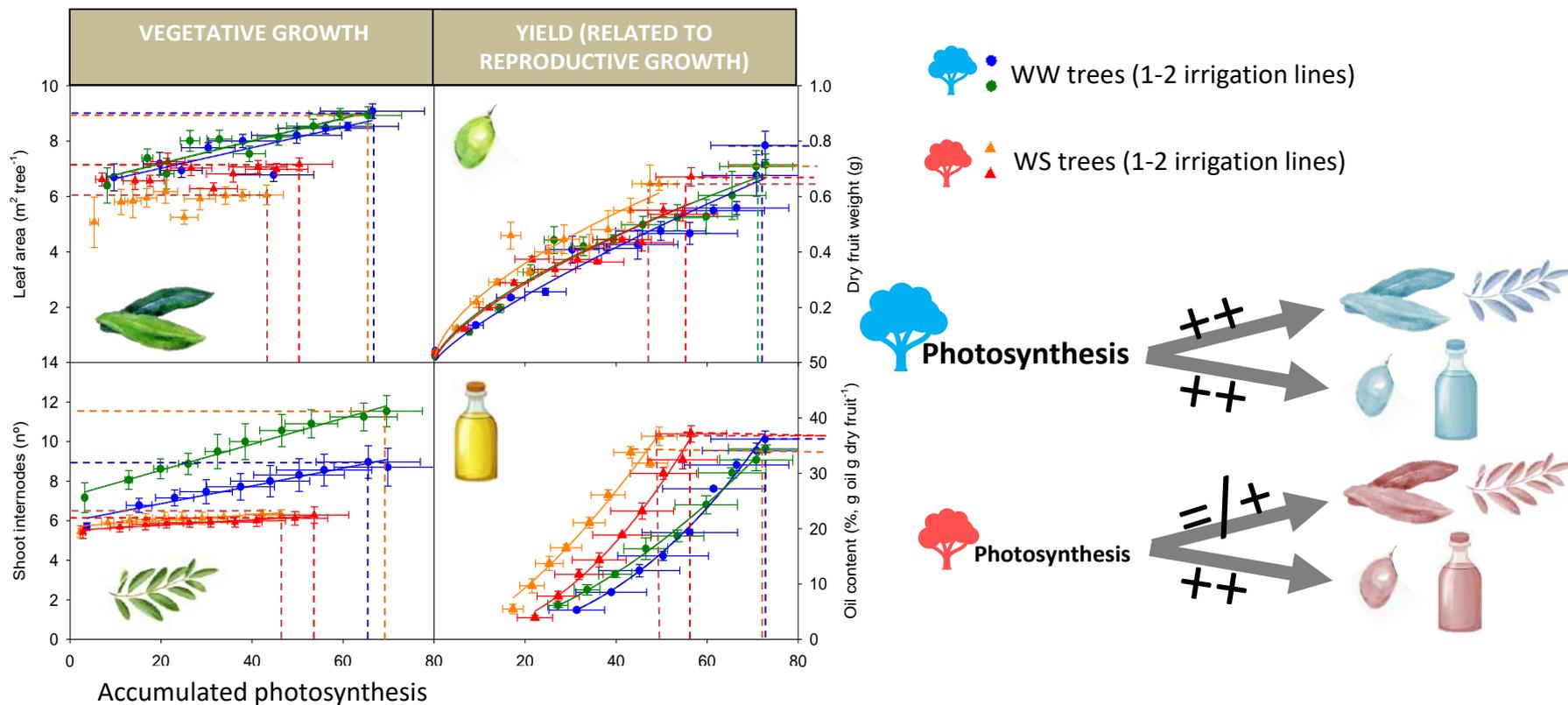
OBJECTIVE

To **model photosynthesis** in a continuous manner to quantify its effect over vegetative and reproductive growth



RESULTS

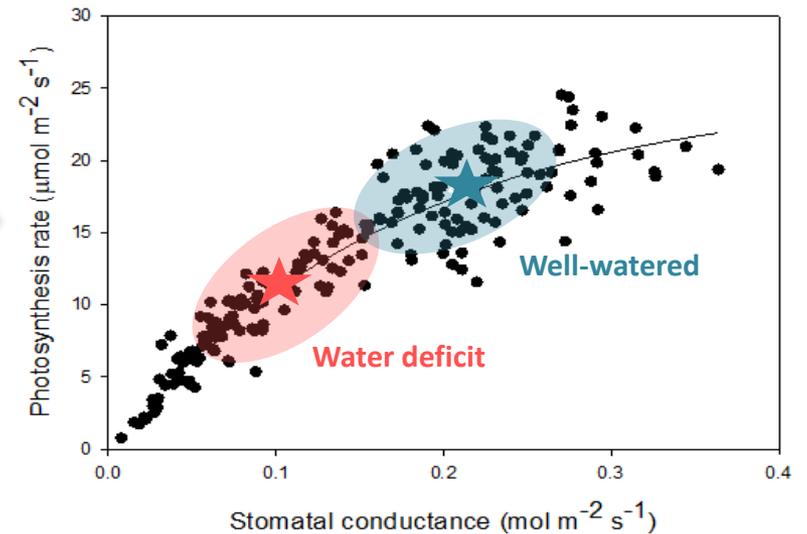
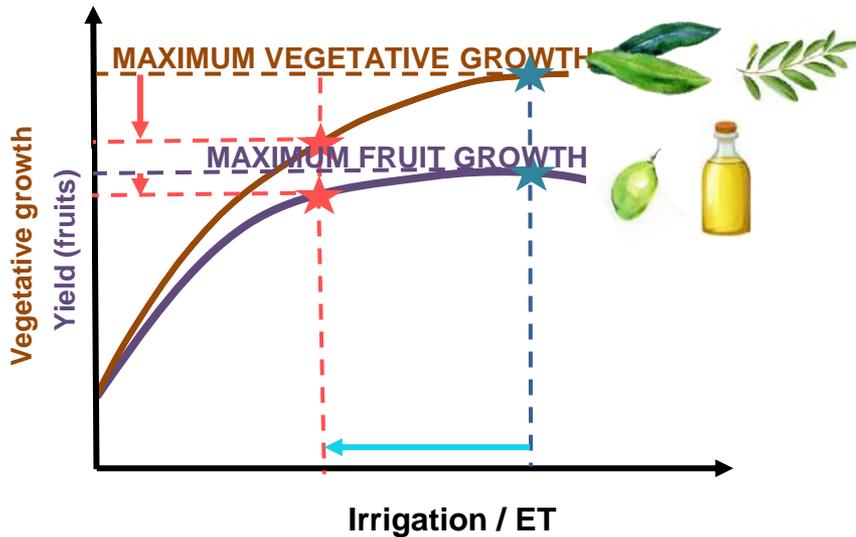
To **model photosynthesis** in a continuous manner to quantify its effect over vegetative and reproductive growth





HYPOTHESIS: the relationship and the meaning between irrigation and yield is similar to the relationship between stomatal conductance and photosynthesis.

THUS, we can use stomatal conductance and photosynthesis to manage irrigation in a sustainable manner in crops



*Thanks for your
attention!*

Experimental design and implementation layout

Task 2.2., Topic 2

WIDERA PROJECT

Virginia Hernandez-Santana

 virginiahsa@irnas.csic.es

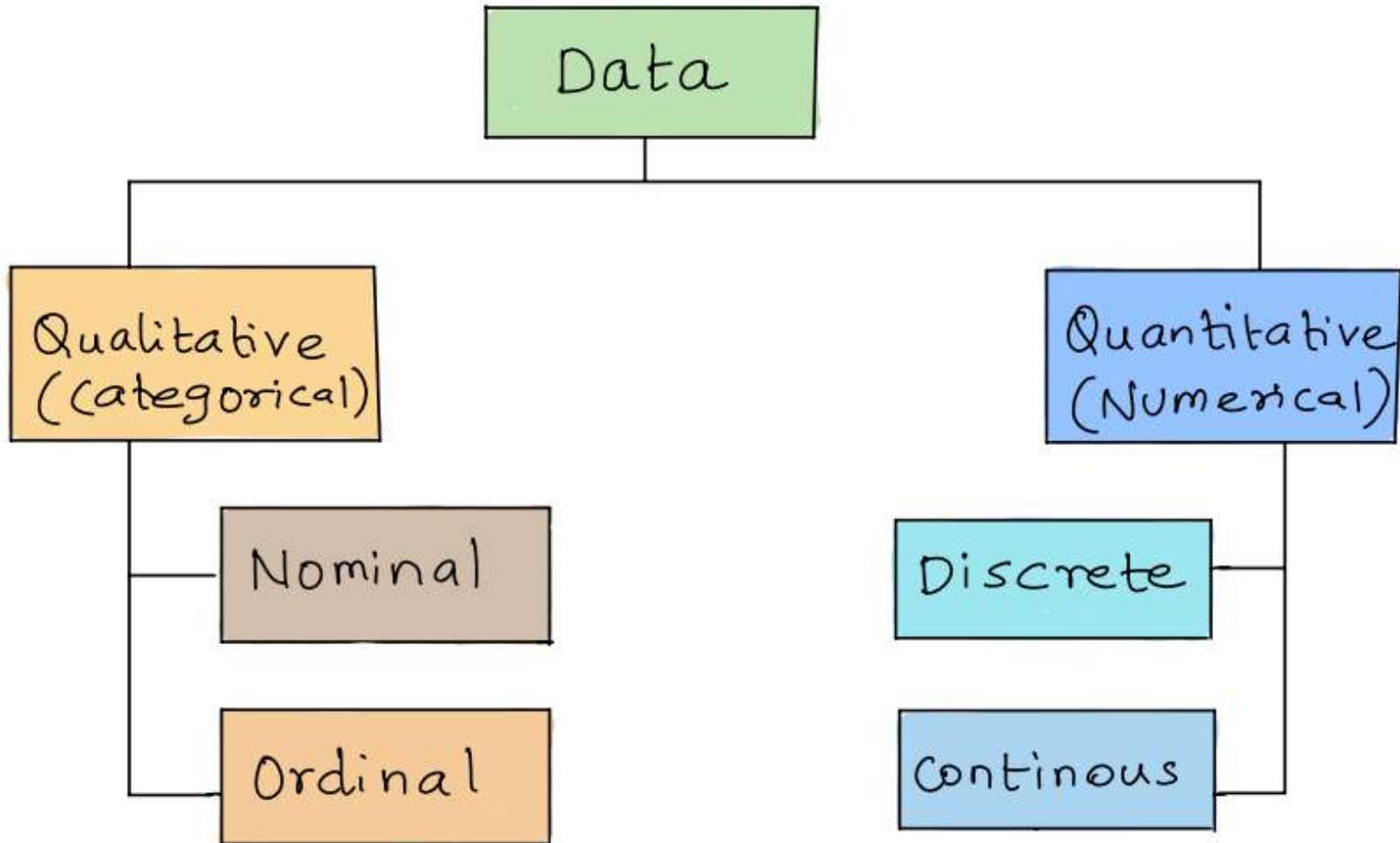
Funchal, 27-31/01/2025

- **Basic concepts**
- **Experimental design in agriculture**
- **Some practice**

BASIC CONCEPTS

- **DATA ORGANIZATION**
- **DESCRIPTIVE STATISTICS**
- **INFERENCEAL STATISTICS**

BASIC CONCEPTS: VARIABLE TYPES



BASIC CONCEPTS: DATA ORGANIZATION

The image shows a screenshot of Microsoft Excel. The title bar indicates the file is named "Ejemplo datos grupo" and is saved on the PC. The ribbon is set to "Inicio" (Home). The formula bar shows the text: "1. En este archivo se guardan los datos brutos limpios (listos para analizar) de toda una campaña". The spreadsheet grid is visible, with columns A, B, C, and D, and rows 1 through 7. A blue-bordered box highlights the following instructions:

1. En este archivo se guardan los datos brutos limpios (listos para analizar) de toda una campaña
2. Primero rellena las areas naranjas en la pestaña de metadatos.
 - 2.1 Indica el nombre del proyecto y de la finca en los campos correspondientes
 - 2.2 Escribe tu nombre y las variables que vas a rellenar
 - 2.3 Indica el nombre y la descripción de los tratamientos utilizados. **IMPORTANTE. Los nombres deben ser simples y los mismos en todas las pestañas**
 - 2.3 En el campo "Variables", anota la variable que vas a añadir indicando un nombre simple, la descripción de la variable, las unidades de medida y cualquier comentario que creas relevante sobre la medida
3. Introduce los datos de cada variable en una nueva pestaña
 - 3.1 Crea una pestaña, nombrala con el nombre simple de la variable y pega TODAS las columnas de la pestaña "Variable"
 - 3.2 Rellena todas las columnas siguiendo las referencias descritas en la pestaña de Metadatos, en caso de no utilizar todas las columnas, deja vacias las que no se apliquen a la variable introducida
 - 3.3 Nombra la ultima columna *Variable* con el nombre simple de la variable a introducir y pega los valores de esa variable
4. Es **MUY IMPORTANTE** utilizar la misma nomenclatura en todas las pestañas, por lo que si ya se han añadido datos anteriormente, asegurate de que se utilizan los mismos nombres para los tratamientos, especie, localizacion, etc
5. Guarda el archivo utilizando el siguiente nombre genérico: AÑO_PROYECTO_CAMPO/INVERNADERO.xlsx; E.g. 2022_RIEGAFRUIT_CAMPO.xlsx

The bottom of the screen shows the worksheet tabs: "Instrucciones", "Metadatos", "Variable", "LAI", "Licor", "TDV", and a plus sign for additional tabs.

BASIC CONCEPTS: DATA ORGANIZATION

Autoguardado Ejemplo datos grupo • Guardado en Este PC

Inicio Insertar Disposición de página Fórmulas Datos Revisar Vista Ayuda Acrobat

Portapapeles Fuente Alineación Número Estilos Celdas

B31 ID de la subreplica; E.g. 1

Proyecto		Variables			
Nombre	Variable	Unidad	Comentario		
ECOLIMA	LAI	Leaf Area Index	m3/m3		
Finca	An	Fotosíntesis neta	µmolm CO2 m-2s-1		
Sanabria	gs	Conductancia estomatica	µmolm H2O m-2s-1		
Responsible datos (Variables)	TDV	Trunk diameter variation	µm		
Menganito	An, gs				
Fulanita	LAI				
Sebastián	TDV				
Tratamientos (Definición)					
Control	100% ETC irrigation				
FS40RDI	40% ETC RDI controlled with sap flow				
PT40RDI	40% ETC RDI controlled with turgor probes				
Definiciones					
Proyecto	Nombre del proyecto; E.g. Riegafruit				
Localización	Nombre de la localización del estudio; E.g. La Hampa				
Especie	Nombre comun de la especie y variedad si es relevante; E.g. Olivo				
Variedad	Nombre de la variedad; E.g. Arbequina				
Tratamiento	Nombre corto del tratamiento, como se define en el apartado superior; E.g. FS40RDI				
Tratamiento 2	En caso de haber combinacion de tratamientos, nombre del segundo tratamiento aplicado				
Parcela	ID de la parcela o bloque; E.g. 1				
Individuo	ID del individuo; E.g. 1				
Fecha	Fecha de muestreo; E.g. 12/05/2023				
DOY	Dia del año o dia juliano; E.g. 145				
Hora	Hora de muestreo; E.g. 14:30				
Replica	ID de la replica; E.g. 1				
Subreplica	ID de la subreplica; E.g. 1				
Variable	Valor de la variable. Rellenar como indicado en la pestaña de instrucciones				

Instrucciones Metadatos Variable LAI Licor TDV

BASIC CONCEPTS: DATA ORGANIZATION

Autoguardado Ejemplo datos grupo • Guardado en Este PC

Inicio Insertar Disposición de página Fórmulas Datos Revisar Vista Ayuda Acrobat

Portapapeles Fuente Alineación Número Estilos Celdas

B31 ID de la subreplica; E.g. 1

Proyecto		Variables			
Nombre	Variable	Unidad	Comentario		
ECOLIMA					
Finca					
Sanabria					
Responsable datos	(Variables)				
Menganito	An, gs				
Fulanita	LAI				
Sebastián	TDV				
Tratamientos	(Definición)				
Control	100% Etc irrigation				
FS40RDI	40% Etc RDI controlled with sap flow				
PT40RDI	40% Etc RDI controlled with turgor probes				
Definiciones					
Proyecto	Nombre del proyecto; E.g. Riegafruit				
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Variedad	Nombre de la variedad; E.g. Arbequina				
Tratamiento	Nombre corto del tratamiento, como se define en el apartado superior; E.g. FS40RDI				
Tratamiento 2	En caso de haber combinación de tratamientos, nombre del segundo tratamiento aplicado				
Parcela	ID de la parcela o bloque; E.g. 1				
Individuo	ID del individuo; E.g. 1				
Fecha	Fecha de muestreo; E.g. 12/05/2023				
DOY	Día del año o día juliano; E.g. 145				
Hora	Hora de muestreo; E.g. 14:30				
Replica	ID de la replica; E.g. 1				
Subreplica	ID de la subreplica; E.g. 1				
Variable	Valor de la variable. Rellenar como indicado en la pestaña de instrucciones				

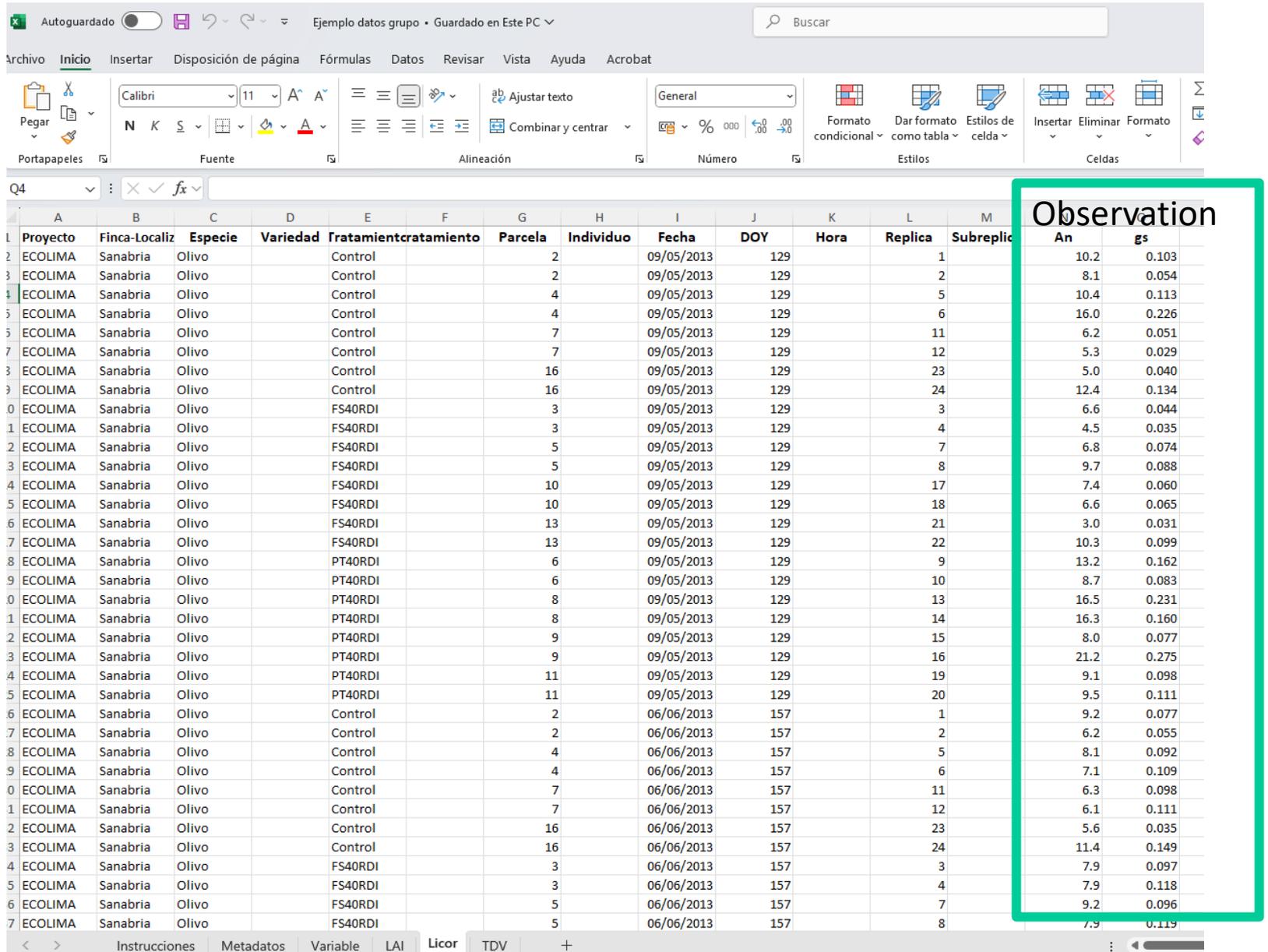
Instrucciones Metadatos Variable LAI Licor TDV

BASIC CONCEPTS: DATA ORGANIZATION

The image shows a screenshot of Microsoft Excel with a data table. The header row is highlighted in green and labeled 'header' in a green box on the left. The table contains 17 columns and 47 rows of data. The columns are labeled: Proyecto, Finca-Localiz, Especie, Variedad, Tratamientratamiento, Parcela, Individuo, Fecha, DOY, Hora, Replica, Subreplica, An, and gs. The data rows show various entries for 'ECOLIMA' and 'Sanabria' across different 'Especie' and 'Variedad' categories, with associated 'Tratamientratamiento', 'Parcela', 'Individuo', 'Fecha', 'DOY', 'Hora', 'Replica', 'Subreplica', 'An', and 'gs' values.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
header	Proyecto	Finca-Localiz	Especie	Variedad	Tratamientratamiento	Parcela	Individuo	Fecha	DOY	Hora	Replica	Subreplica	An	gs	
2	ECOLIMA	Sanabria	Olivo	Control			2	09/05/2013	129			1	10.2	0.103	
3	ECOLIMA	Sanabria	Olivo	Control			2	09/05/2013	129			2	8.1	0.054	
4	ECOLIMA	Sanabria	Olivo	Control			4	09/05/2013	129			5	10.4	0.113	
5	ECOLIMA	Sanabria	Olivo	Control			4	09/05/2013	129			6	16.0	0.226	
5	ECOLIMA	Sanabria	Olivo	Control			7	09/05/2013	129			11	6.2	0.051	
7	ECOLIMA	Sanabria	Olivo	Control			7	09/05/2013	129			12	5.3	0.029	
3	ECOLIMA	Sanabria	Olivo	Control			16	09/05/2013	129			23	5.0	0.040	
3	ECOLIMA	Sanabria	Olivo	Control			16	09/05/2013	129			24	12.4	0.134	
0	ECOLIMA	Sanabria	Olivo	FS40RDI			3	09/05/2013	129			3	6.6	0.044	
1	ECOLIMA	Sanabria	Olivo	FS40RDI			3	09/05/2013	129			4	4.5	0.035	
2	ECOLIMA	Sanabria	Olivo	FS40RDI			5	09/05/2013	129			7	6.8	0.074	
3	ECOLIMA	Sanabria	Olivo	FS40RDI			5	09/05/2013	129			8	9.7	0.088	
4	ECOLIMA	Sanabria	Olivo	FS40RDI			10	09/05/2013	129			17	7.4	0.060	
5	ECOLIMA	Sanabria	Olivo	FS40RDI			10	09/05/2013	129			18	6.6	0.065	
6	ECOLIMA	Sanabria	Olivo	FS40RDI			13	09/05/2013	129			21	3.0	0.031	
7	ECOLIMA	Sanabria	Olivo	FS40RDI			13	09/05/2013	129			22	10.3	0.099	
8	ECOLIMA	Sanabria	Olivo	PT40RDI			6	09/05/2013	129			9	13.2	0.162	
9	ECOLIMA	Sanabria	Olivo	PT40RDI			6	09/05/2013	129			10	8.7	0.083	
0	ECOLIMA	Sanabria	Olivo	PT40RDI			8	09/05/2013	129			13	16.5	0.231	
1	ECOLIMA	Sanabria	Olivo	PT40RDI			8	09/05/2013	129			14	16.3	0.160	
2	ECOLIMA	Sanabria	Olivo	PT40RDI			9	09/05/2013	129			15	8.0	0.077	
3	ECOLIMA	Sanabria	Olivo	PT40RDI			9	09/05/2013	129			16	21.2	0.275	
4	ECOLIMA	Sanabria	Olivo	PT40RDI			11	09/05/2013	129			19	9.1	0.098	
5	ECOLIMA	Sanabria	Olivo	PT40RDI			11	09/05/2013	129			20	9.5	0.111	
6	ECOLIMA	Sanabria	Olivo	Control			2	06/06/2013	157			1	9.2	0.077	
7	ECOLIMA	Sanabria	Olivo	Control			2	06/06/2013	157			2	6.2	0.055	
8	ECOLIMA	Sanabria	Olivo	Control			4	06/06/2013	157			5	8.1	0.092	
9	ECOLIMA	Sanabria	Olivo	Control			4	06/06/2013	157			6	7.1	0.109	
0	ECOLIMA	Sanabria	Olivo	Control			7	06/06/2013	157			11	6.3	0.098	
1	ECOLIMA	Sanabria	Olivo	Control			7	06/06/2013	157			12	6.1	0.111	
2	ECOLIMA	Sanabria	Olivo	Control			16	06/06/2013	157			23	5.6	0.035	
3	ECOLIMA	Sanabria	Olivo	Control			16	06/06/2013	157			24	11.4	0.149	
4	ECOLIMA	Sanabria	Olivo	FS40RDI			3	06/06/2013	157			3	7.9	0.097	
5	ECOLIMA	Sanabria	Olivo	FS40RDI			3	06/06/2013	157			4	7.9	0.118	
6	ECOLIMA	Sanabria	Olivo	FS40RDI			5	06/06/2013	157			7	9.2	0.096	
7	ECOLIMA	Sanabria	Olivo	FS40RDI			5	06/06/2013	157			8	7.9	0.119	

BASIC CONCEPTS: DATA ORGANIZATION



The image shows a Microsoft Excel spreadsheet with the following data structure:

	A	B	C	D	E	F	G	H	I	J	K	L	M	Observation	
														An	gs
1	Proyecto	Finca-Localiz	Especie	Variedad	Tratamiento	tratamiento	Parcela	Individuo	Fecha	DOY	Hora	Replica	Subreplic		
2	ECOLIMA	Sanabria	Olivo		Control		2		09/05/2013	129		1		10.2	0.103
3	ECOLIMA	Sanabria	Olivo		Control		2		09/05/2013	129		2		8.1	0.054
4	ECOLIMA	Sanabria	Olivo		Control		4		09/05/2013	129		5		10.4	0.113
5	ECOLIMA	Sanabria	Olivo		Control		4		09/05/2013	129		6		16.0	0.226
5	ECOLIMA	Sanabria	Olivo		Control		7		09/05/2013	129		11		6.2	0.051
7	ECOLIMA	Sanabria	Olivo		Control		7		09/05/2013	129		12		5.3	0.029
3	ECOLIMA	Sanabria	Olivo		Control		16		09/05/2013	129		23		5.0	0.040
3	ECOLIMA	Sanabria	Olivo		Control		16		09/05/2013	129		24		12.4	0.134
0	ECOLIMA	Sanabria	Olivo		FS40RDI		3		09/05/2013	129		3		6.6	0.044
1	ECOLIMA	Sanabria	Olivo		FS40RDI		3		09/05/2013	129		4		4.5	0.035
2	ECOLIMA	Sanabria	Olivo		FS40RDI		5		09/05/2013	129		7		6.8	0.074
3	ECOLIMA	Sanabria	Olivo		FS40RDI		5		09/05/2013	129		8		9.7	0.088
4	ECOLIMA	Sanabria	Olivo		FS40RDI		10		09/05/2013	129		17		7.4	0.060
5	ECOLIMA	Sanabria	Olivo		FS40RDI		10		09/05/2013	129		18		6.6	0.065
6	ECOLIMA	Sanabria	Olivo		FS40RDI		13		09/05/2013	129		21		3.0	0.031
7	ECOLIMA	Sanabria	Olivo		FS40RDI		13		09/05/2013	129		22		10.3	0.099
8	ECOLIMA	Sanabria	Olivo		PT40RDI		6		09/05/2013	129		9		13.2	0.162
9	ECOLIMA	Sanabria	Olivo		PT40RDI		6		09/05/2013	129		10		8.7	0.083
0	ECOLIMA	Sanabria	Olivo		PT40RDI		8		09/05/2013	129		13		16.5	0.231
1	ECOLIMA	Sanabria	Olivo		PT40RDI		8		09/05/2013	129		14		16.3	0.160
2	ECOLIMA	Sanabria	Olivo		PT40RDI		9		09/05/2013	129		15		8.0	0.077
3	ECOLIMA	Sanabria	Olivo		PT40RDI		9		09/05/2013	129		16		21.2	0.275
4	ECOLIMA	Sanabria	Olivo		PT40RDI		11		09/05/2013	129		19		9.1	0.098
5	ECOLIMA	Sanabria	Olivo		PT40RDI		11		09/05/2013	129		20		9.5	0.111
6	ECOLIMA	Sanabria	Olivo		Control		2		06/06/2013	157		1		9.2	0.077
7	ECOLIMA	Sanabria	Olivo		Control		2		06/06/2013	157		2		6.2	0.055
8	ECOLIMA	Sanabria	Olivo		Control		4		06/06/2013	157		5		8.1	0.092
9	ECOLIMA	Sanabria	Olivo		Control		4		06/06/2013	157		6		7.1	0.109
0	ECOLIMA	Sanabria	Olivo		Control		7		06/06/2013	157		11		6.3	0.098
1	ECOLIMA	Sanabria	Olivo		Control		7		06/06/2013	157		12		6.1	0.111
2	ECOLIMA	Sanabria	Olivo		Control		16		06/06/2013	157		23		5.6	0.035
3	ECOLIMA	Sanabria	Olivo		Control		16		06/06/2013	157		24		11.4	0.149
4	ECOLIMA	Sanabria	Olivo		FS40RDI		3		06/06/2013	157		3		7.9	0.097
5	ECOLIMA	Sanabria	Olivo		FS40RDI		3		06/06/2013	157		4		7.9	0.118
6	ECOLIMA	Sanabria	Olivo		FS40RDI		5		06/06/2013	157		7		9.2	0.096
7	ECOLIMA	Sanabria	Olivo		FS40RDI		5		06/06/2013	157		8		7.9	0.119

BASIC CONCEPTS: DATA ORGANIZATION

Autoguardado Ejemplo datos grupo • Guardado en Este PC

Archivo Inicio Insertar Disposición de página Fórmulas Datos Revisar Vista Ayuda Acrobat

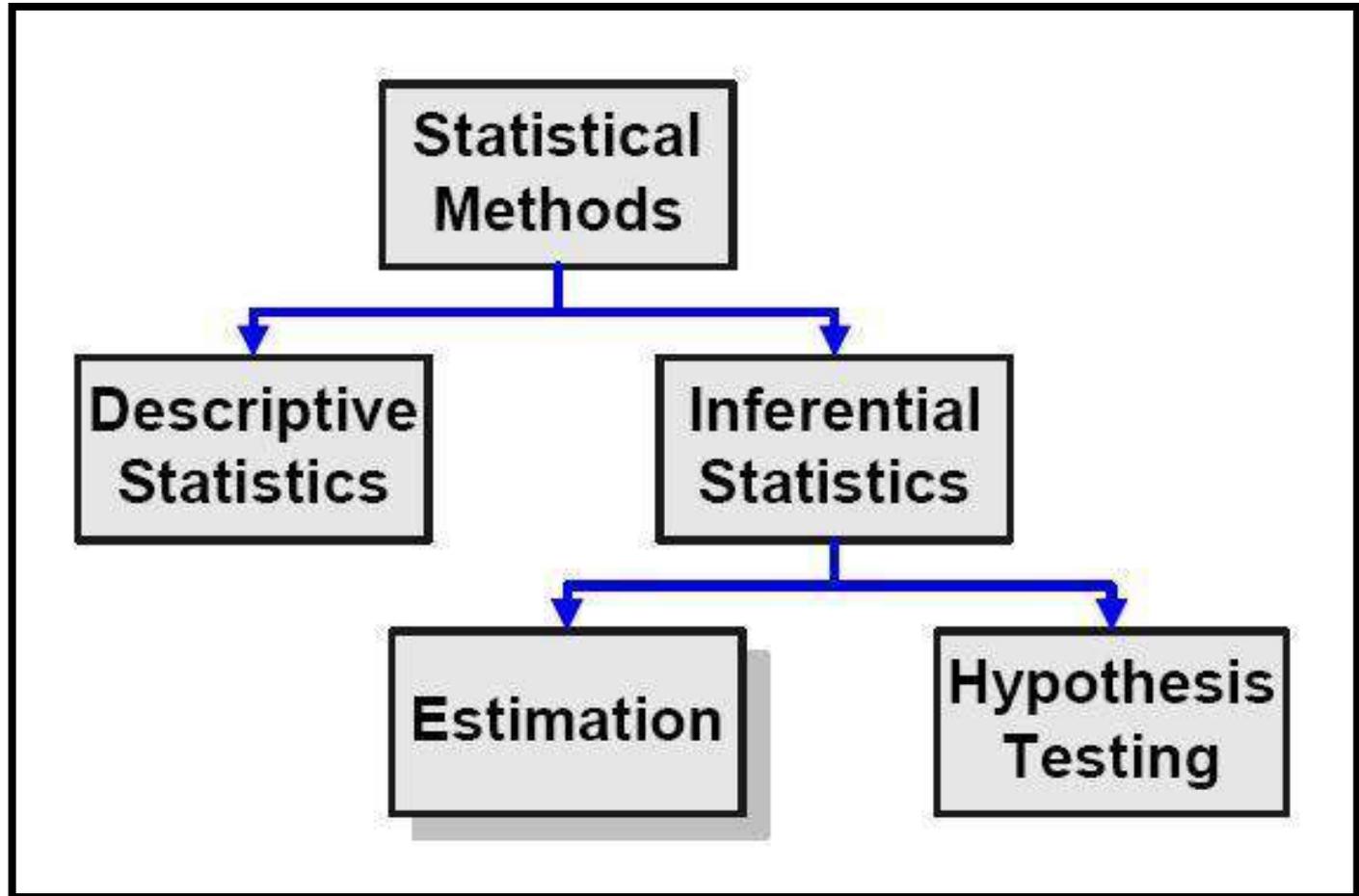
Calibri 11 Fuente Alineación Número Estilos Celdas

Portapapeles Pegar Ajustar texto Combinar y centrar Formato condicional Dar formato como tabla Estilos de celda Insertar Eliminar Formato

Q4	Experimental design structure							I	J	K	L	M	N	O	
	Proyecto	Finca-Localiz	Especie	Variedad	Tratamientratamiento	Parcela	Individuo	Fecha	DOY	Hora	Replica	Subreplica	An	gs	
1	ECOLIMA	Sanabria	Olivo		Control		2	09/05/2013	129			1		10.2	0.103
2	ECOLIMA	Sanabria	Olivo		Control		2	09/05/2013	129			2		8.1	0.054
3	ECOLIMA	Sanabria	Olivo		Control		4	09/05/2013	129			5		10.4	0.113
4	ECOLIMA	Sanabria	Olivo		Control		4	09/05/2013	129			6		16.0	0.226
5	ECOLIMA	Sanabria	Olivo		Control		7	09/05/2013	129			11		6.2	0.051
6	ECOLIMA	Sanabria	Olivo		Control		7	09/05/2013	129			12		5.3	0.029
7	ECOLIMA	Sanabria	Olivo		Control		16	09/05/2013	129			23		5.0	0.040
8	ECOLIMA	Sanabria	Olivo		Control		16	09/05/2013	129			24		12.4	0.134
9	ECOLIMA	Sanabria	Olivo		FS40RDI		3	09/05/2013	129			3		6.6	0.044
10	ECOLIMA	Sanabria	Olivo		FS40RDI		3	09/05/2013	129			4		4.5	0.035
11	ECOLIMA	Sanabria	Olivo		FS40RDI		5	09/05/2013	129			7		6.8	0.074
12	ECOLIMA	Sanabria	Olivo		FS40RDI		5	09/05/2013	129			8		9.7	0.088
13	ECOLIMA	Sanabria	Olivo		FS40RDI		10	09/05/2013	129			17		7.4	0.060
14	ECOLIMA	Sanabria	Olivo		FS40RDI		10	09/05/2013	129			18		6.6	0.065
15	ECOLIMA	Sanabria	Olivo		FS40RDI		13	09/05/2013	129			21		3.0	0.031
16	ECOLIMA	Sanabria	Olivo		FS40RDI		13	09/05/2013	129			22		10.3	0.099
17	ECOLIMA	Sanabria	Olivo		PT40RDI		6	09/05/2013	129			9		13.2	0.162
18	ECOLIMA	Sanabria	Olivo		PT40RDI		6	09/05/2013	129			10		8.7	0.083
19	ECOLIMA	Sanabria	Olivo		PT40RDI		8	09/05/2013	129			13		16.5	0.231
20	ECOLIMA	Sanabria	Olivo		PT40RDI		8	09/05/2013	129			14		16.3	0.160
21	ECOLIMA	Sanabria	Olivo		PT40RDI		9	09/05/2013	129			15		8.0	0.077
22	ECOLIMA	Sanabria	Olivo		PT40RDI		9	09/05/2013	129			16		21.2	0.275
23	ECOLIMA	Sanabria	Olivo		PT40RDI		11	09/05/2013	129			19		9.1	0.098
24	ECOLIMA	Sanabria	Olivo		PT40RDI		11	09/05/2013	129			20		9.5	0.111
25	ECOLIMA	Sanabria	Olivo		Control		2	06/06/2013	157			1		9.2	0.077
26	ECOLIMA	Sanabria	Olivo		Control		2	06/06/2013	157			2		6.2	0.055
27	ECOLIMA	Sanabria	Olivo		Control		4	06/06/2013	157			5		8.1	0.092
28	ECOLIMA	Sanabria	Olivo		Control		4	06/06/2013	157			6		7.1	0.109
29	ECOLIMA	Sanabria	Olivo		Control		7	06/06/2013	157			11		6.3	0.098
30	ECOLIMA	Sanabria	Olivo		Control		7	06/06/2013	157			12		6.1	0.111
31	ECOLIMA	Sanabria	Olivo		Control		16	06/06/2013	157			23		5.6	0.035
32	ECOLIMA	Sanabria	Olivo		Control		16	06/06/2013	157			24		11.4	0.149
33	ECOLIMA	Sanabria	Olivo		FS40RDI		3	06/06/2013	157			3		7.9	0.097
34	ECOLIMA	Sanabria	Olivo		FS40RDI		3	06/06/2013	157			4		7.9	0.118
35	ECOLIMA	Sanabria	Olivo		FS40RDI		5	06/06/2013	157			7		9.2	0.096
36	ECOLIMA	Sanabria	Olivo		FS40RDI		5	06/06/2013	157			8		7.9	0.119

Instrucciones Metadatos Variable LAI Licor TDV +

DESCRIPTIVE STATISTICS



DESCRIPTIVE STATISTICS

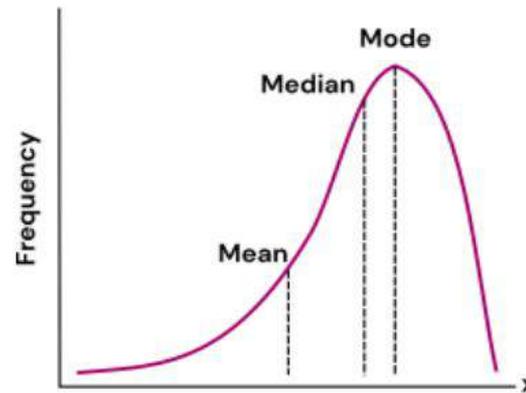
Descriptive Statistics

Measures of Central Tendency

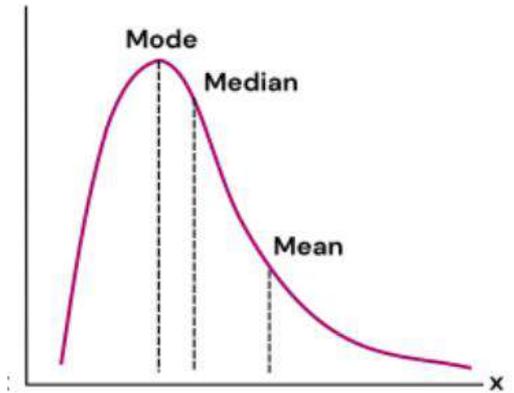
Mean

Median

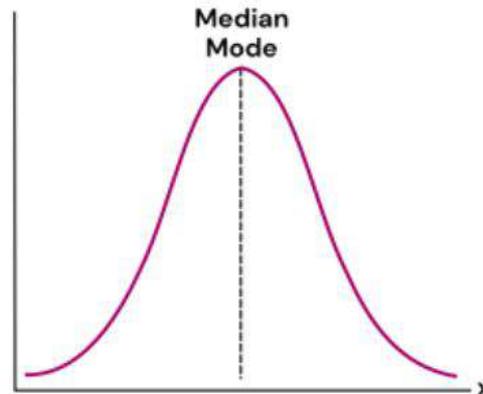
Mode



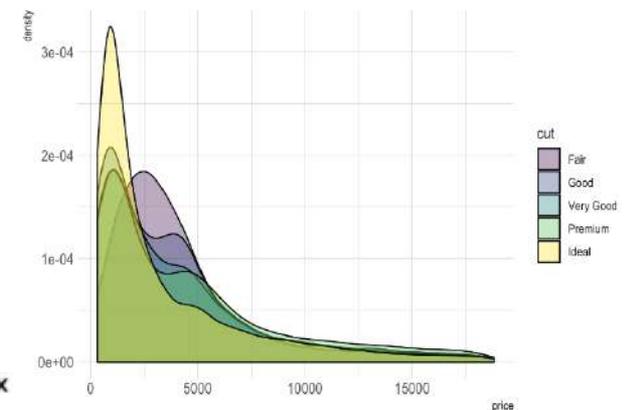
Negatively skewed



Positively skewed



Normal (no skew)



DESCRIPTIVE STATISTICS

Descriptive Statistics

Measures
of Dispersion

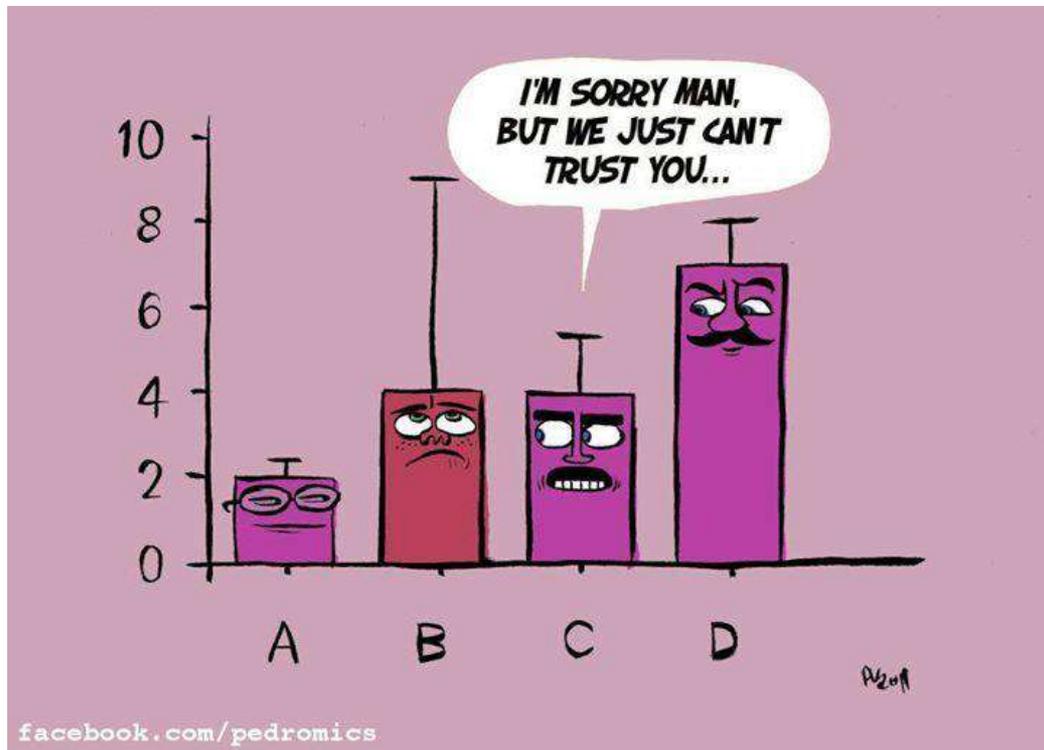
Range

Standard Deviation

Quartile Deviation

Variance

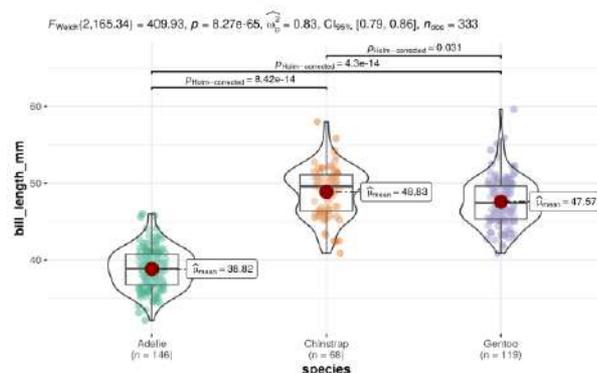
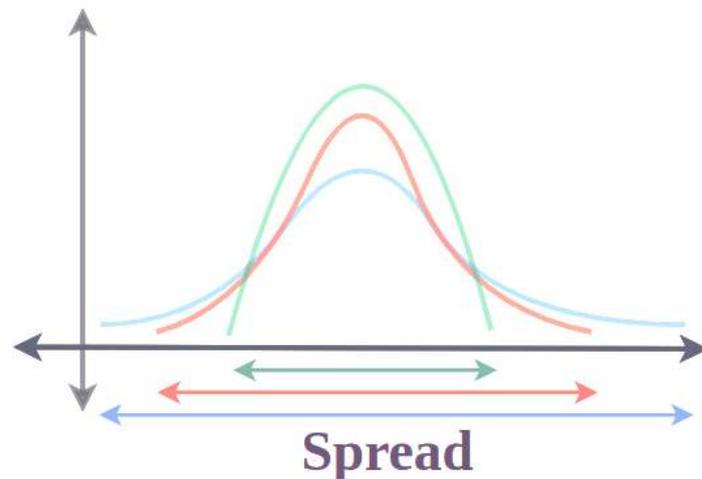
Absolute Deviation



DESCRIPTIVE STATISTICS

Descriptive Statistics

Measure of Dispersion



$\log_{10}(BF_{10}) = -195.59, \hat{R}^2_{\text{corrected}} = 0.70, CI_{95\%} [0.67, 0.73], \hat{C}_{\text{Covary}} = 0.71$
Pairwise test: Games-Howell test, Comparisons shown: only significant

Measures
of Dispersion

Range

Standard Deviation

Quartile Deviation

Variance

Absolute Deviation

INFERENCEAL STATISTICS



Descriptive Statistics

Descriptive statistics are used to describe the characteristics or features of a dataset.

Examples:

- Mean
- Median
- Mode
- Skewness
- Standard Deviation
- Kurtosis



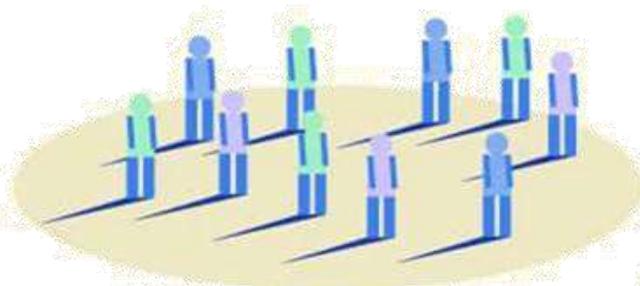
Inferential Statistics

Inferential statistics focus on generalizing about a larger population based on a representative sample of that population.

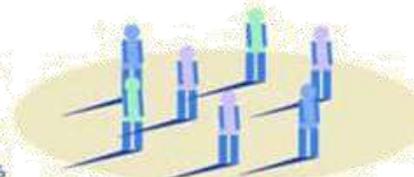
Examples:

- T-test (1 or 2 samples)
- Pearson correlation (2)
- Spearman correlation (2)
- Linear Regression (2)

Population



Sample



Inferential Statistics

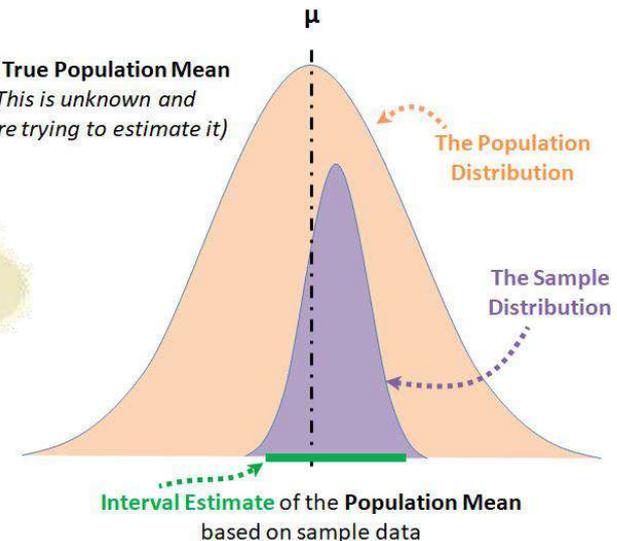
Descriptive Statistics

The True Population Mean
(This is unknown and we're trying to estimate it)

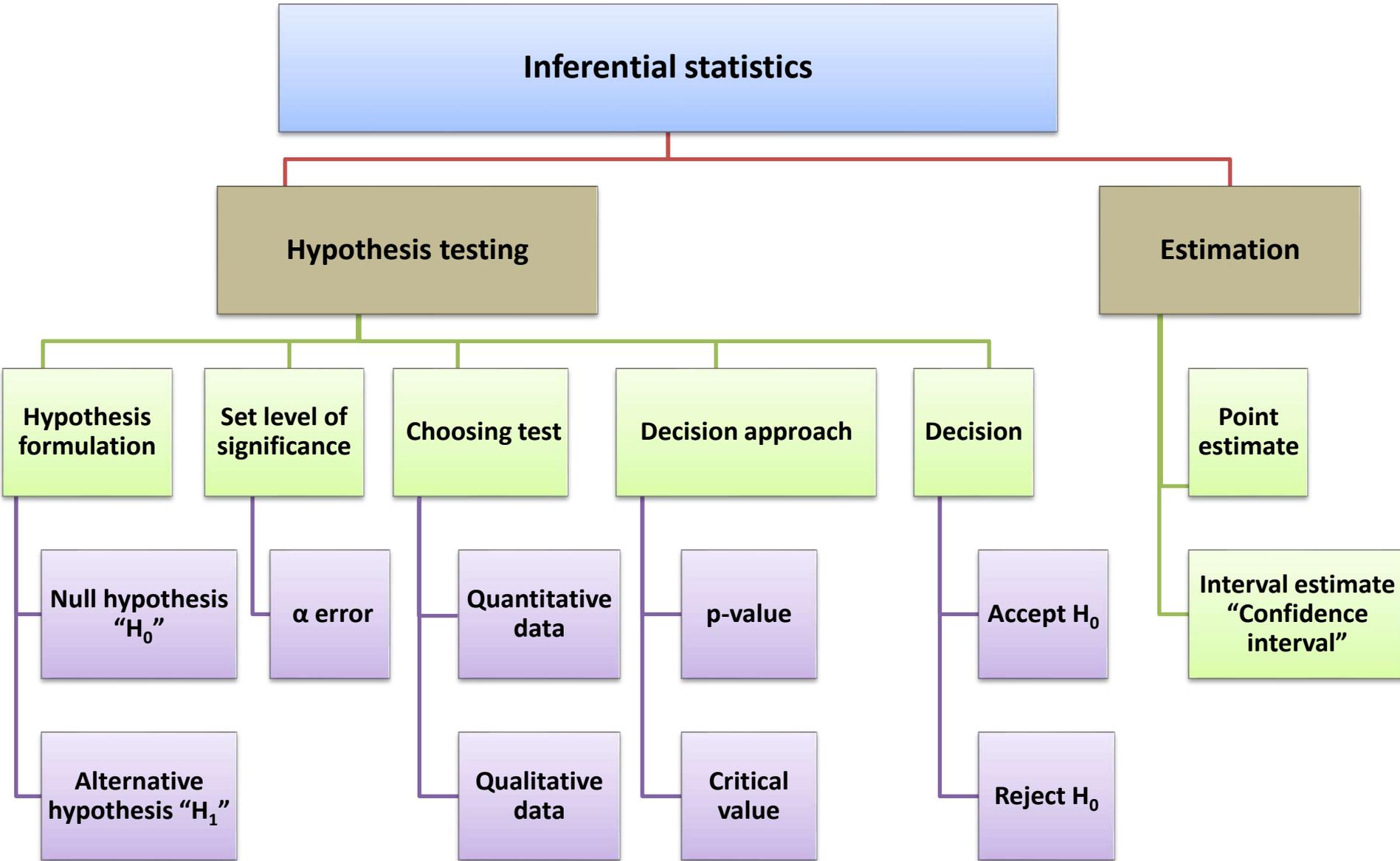
The Population Distribution

The Sample Distribution

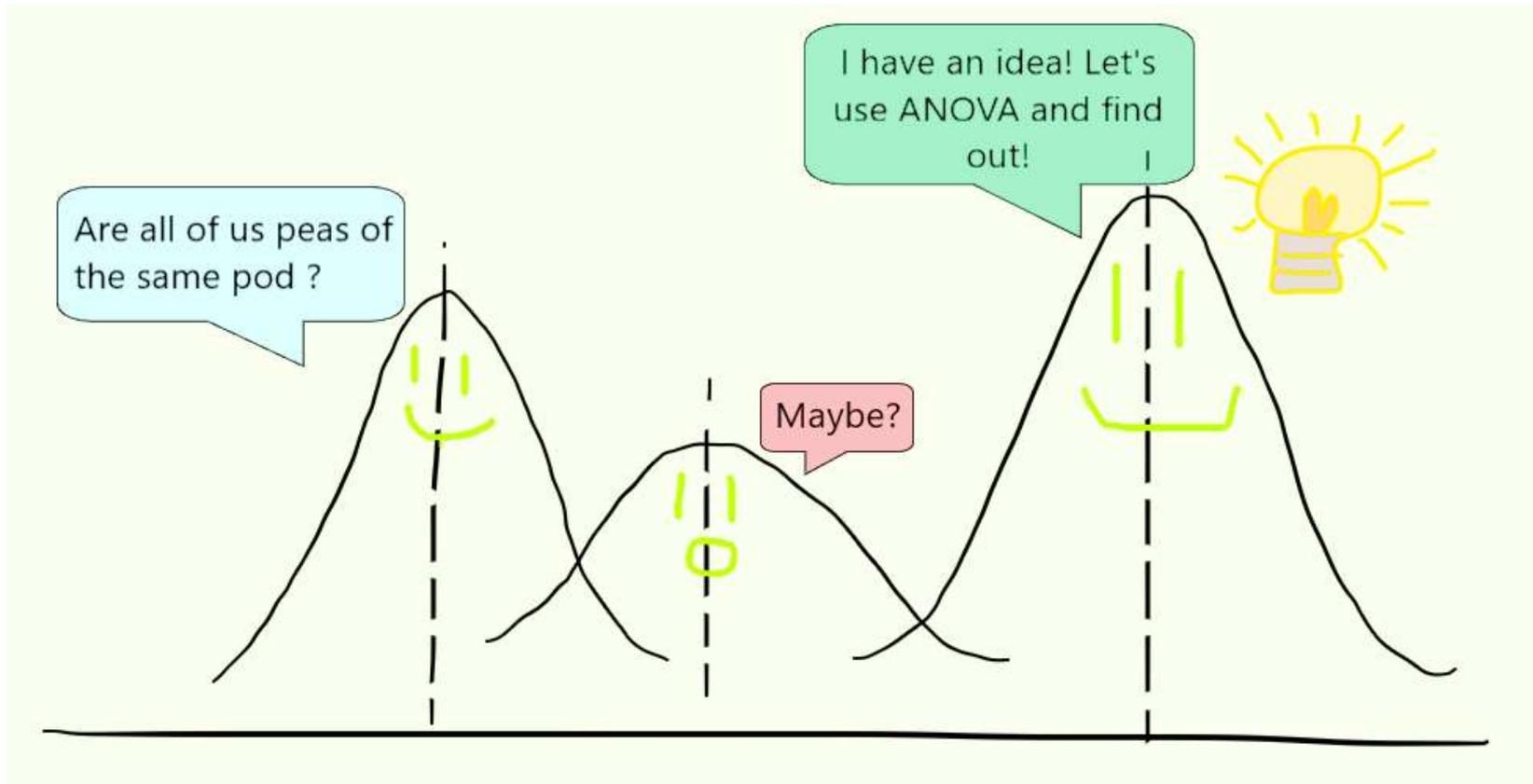
Interval Estimate of the Population Mean
based on sample data



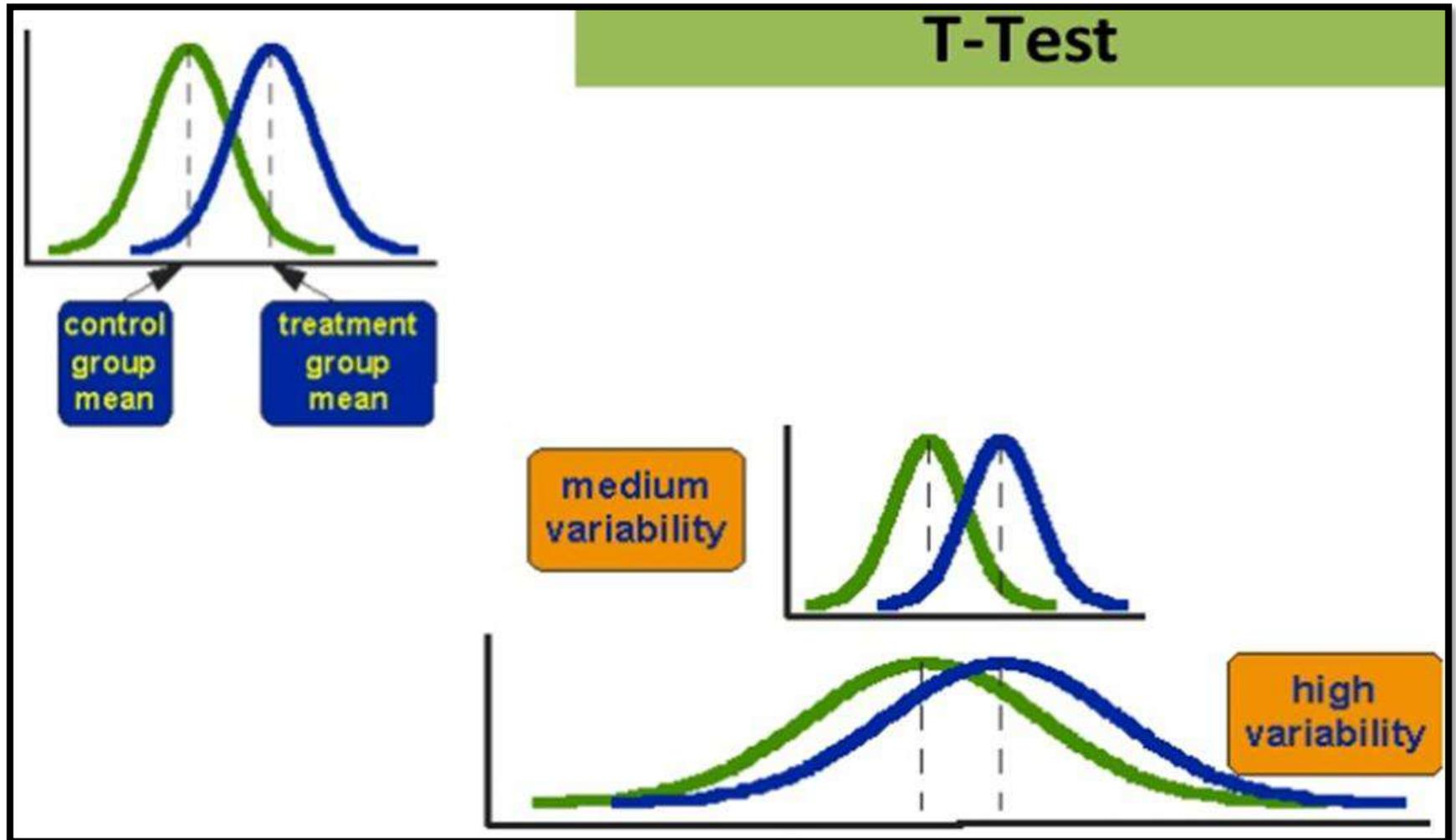
INFERENCEAL STATISTICS



INFERENCE STATISTICS: examples



INFERENCEAL STATISTICS: examples

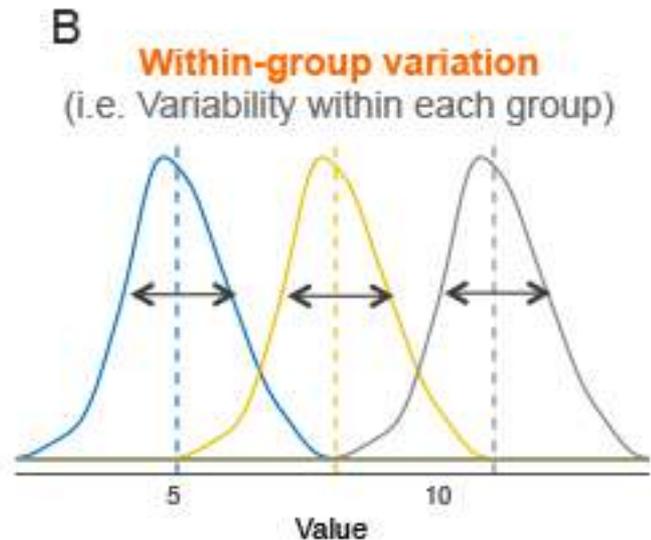
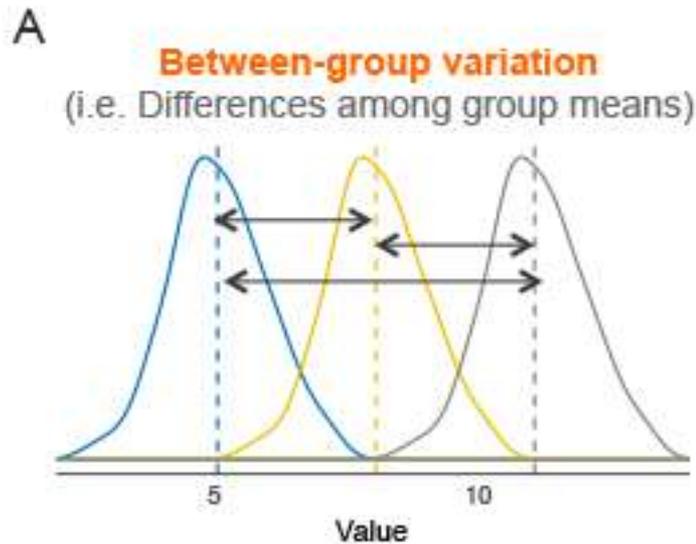


INFERENCEAL STATISTICS: examples

- Used for Comparing several means.
- To compare >2 means, this can be done by use of several t-tests that can consume more time & lead to spurious significant results. So, we must use analysis of variance or **ANOVA**.

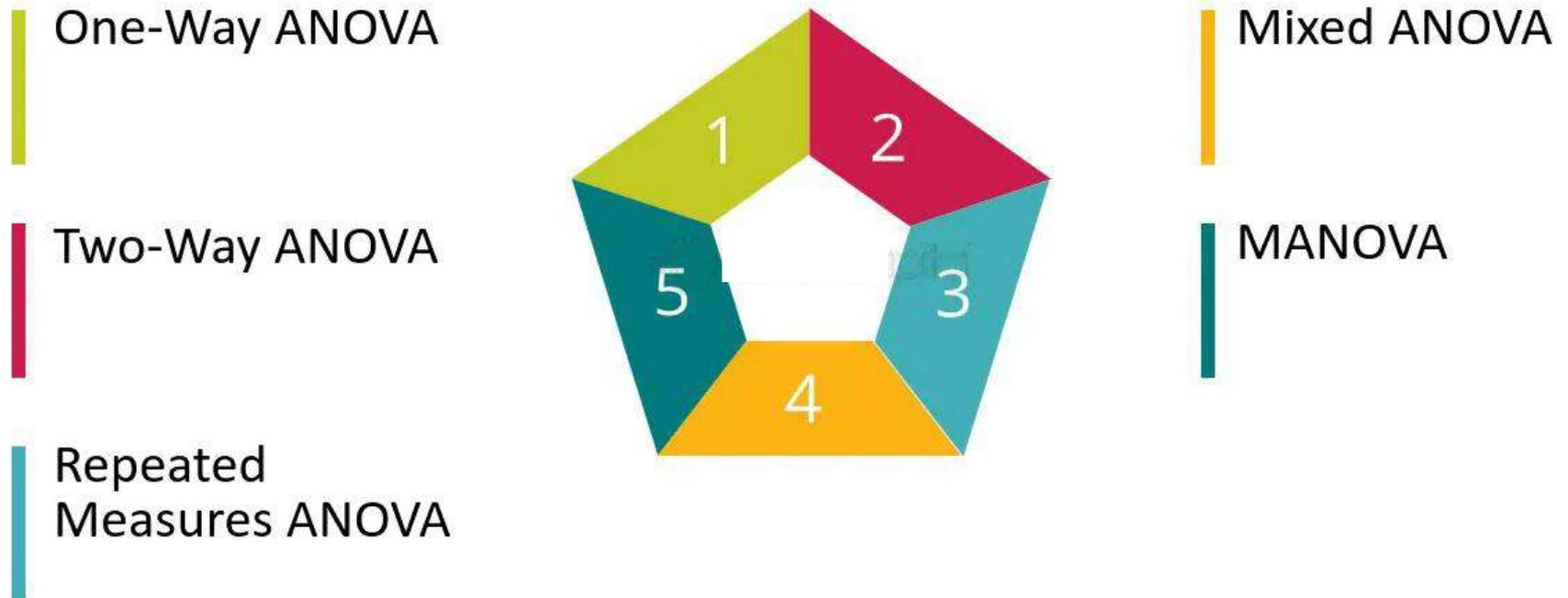
The main idea in the ANOVA is that we have to take into account **the variability within the groups and between the groups** and value of F is equal to the ratio between the means sum square of between the groups and within the groups.

$$F = \text{between-groups MS} / \text{within-groups MS}.$$



INFERENCEAL STATISTICS: examples

Types of ANOVA

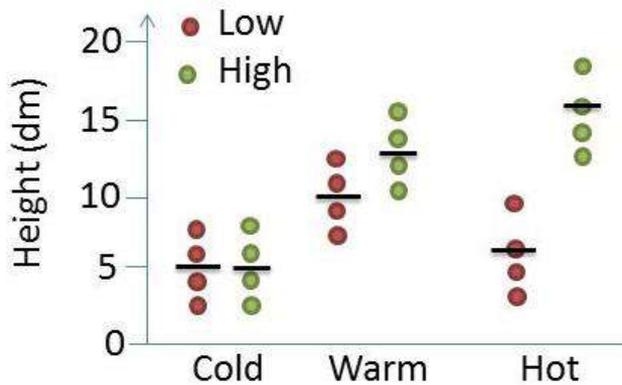
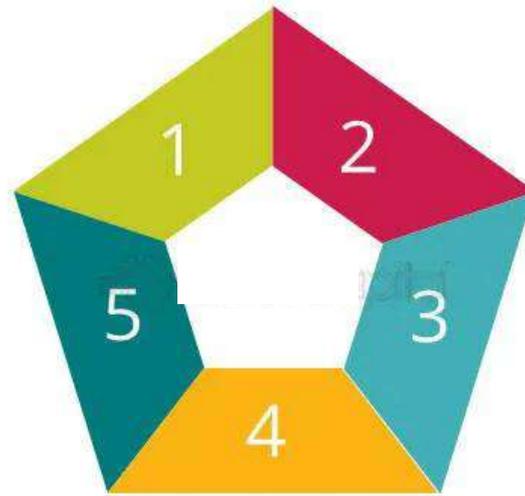


INFERENCEAL STATISTICS: examples

Types of ANOVA

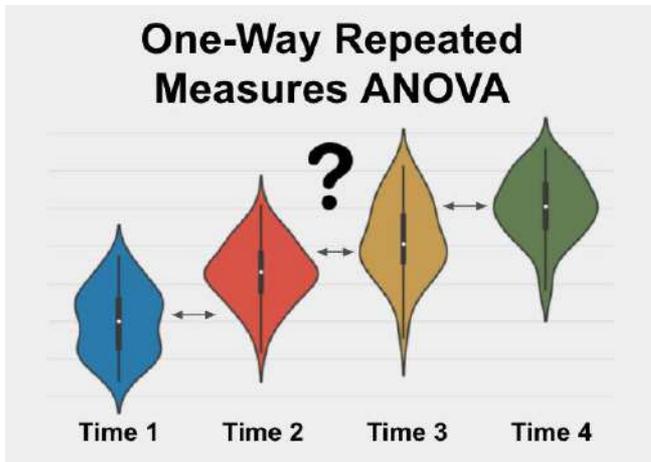
One-Way ANOVA

Two-Way ANOVA

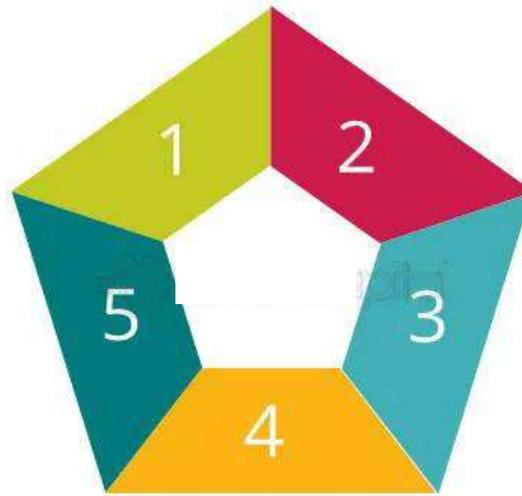


INFERENTIAL STATISTICS: examples

Types of ANOVA

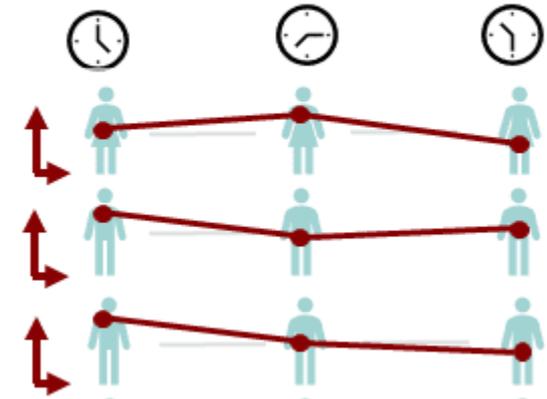


Repeated Measures ANOVA

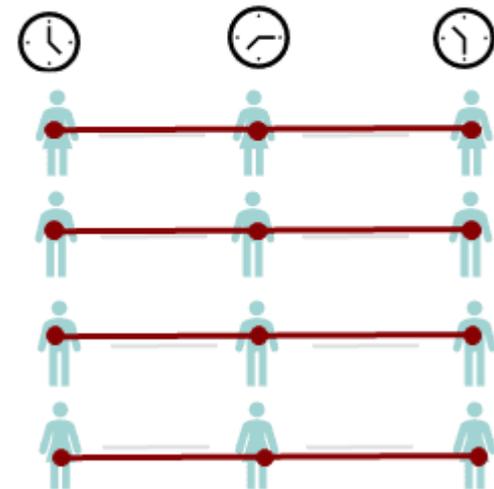


Simplified...

...therapy has an influence.

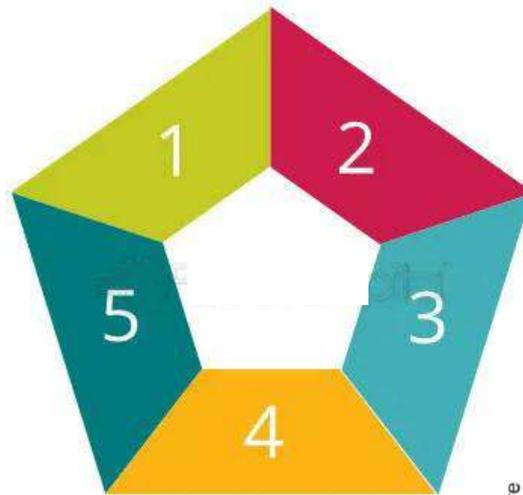


...therapy has no influence.

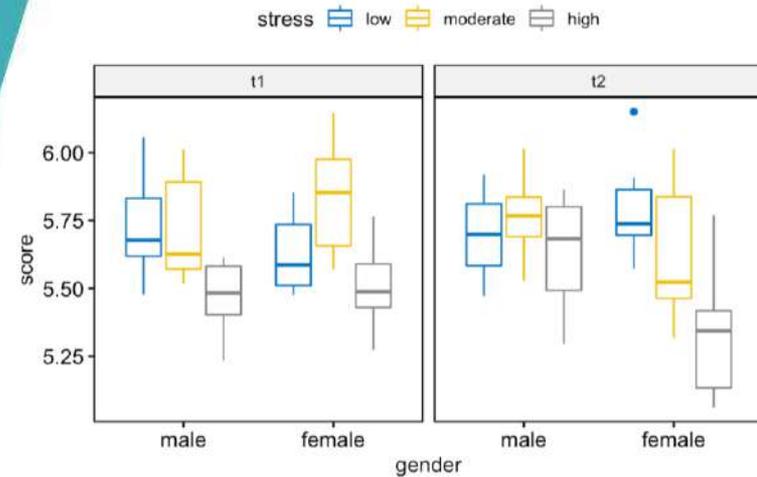


INFERENCEAL STATISTICS: examples

Types of ANOVA

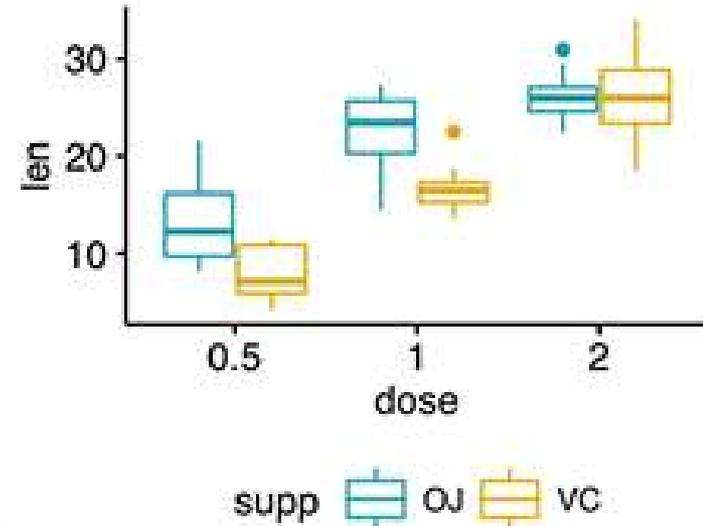
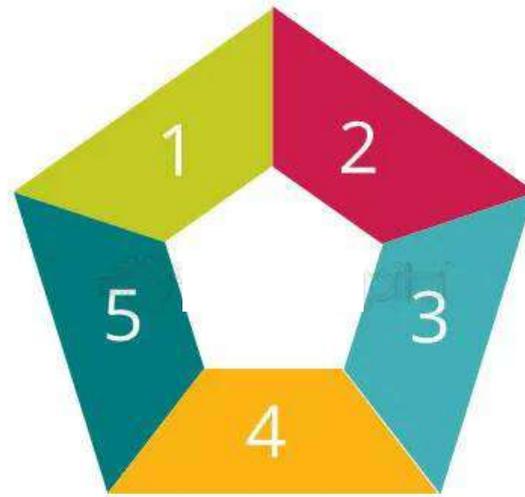


Mixed ANOVA



INFERENCEAL STATISTICS: examples

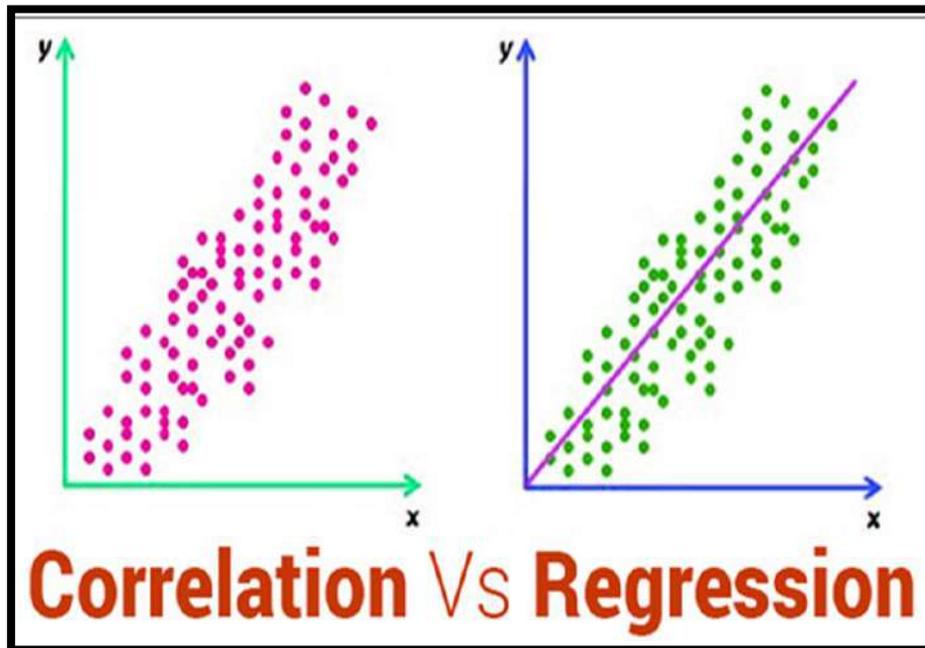
Types of ANOVA



MANOVA

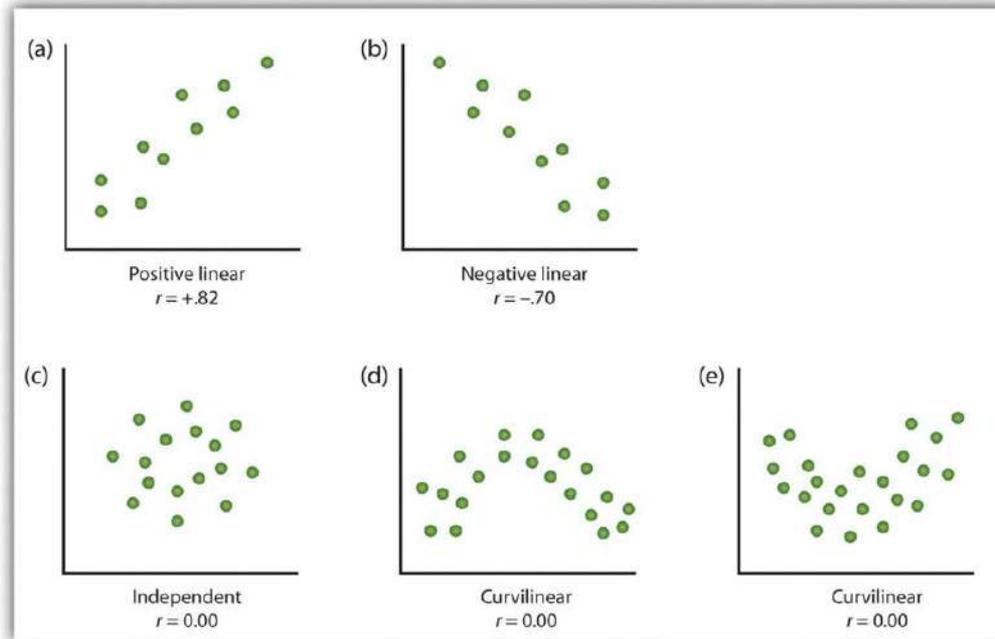
INFERENCEAL STATISTICS: examples

CORRELATION		REGRESSION	
1	Relationship 	One variable affects the other 	
2	Movement together 	cause and effect 	

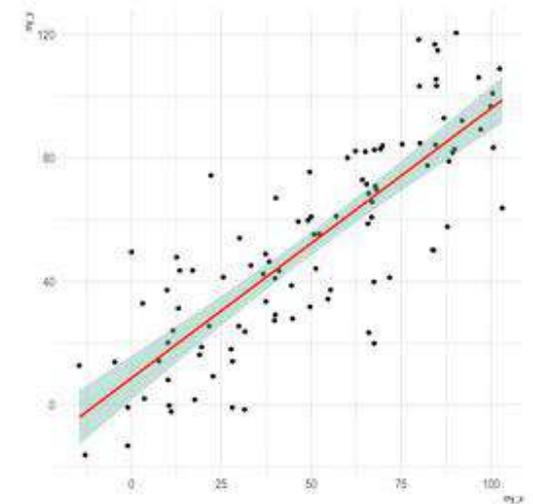
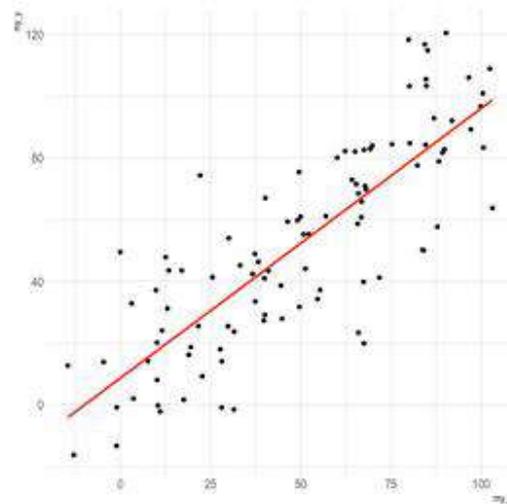
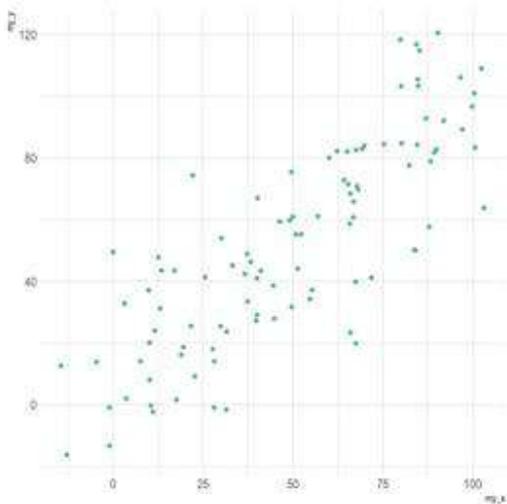


INFERENTIAL STATISTICS: examples

CORRELATION

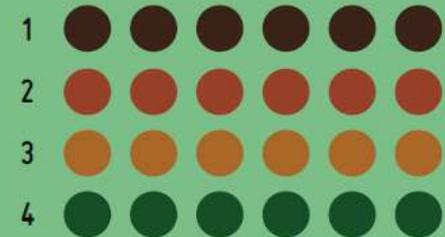
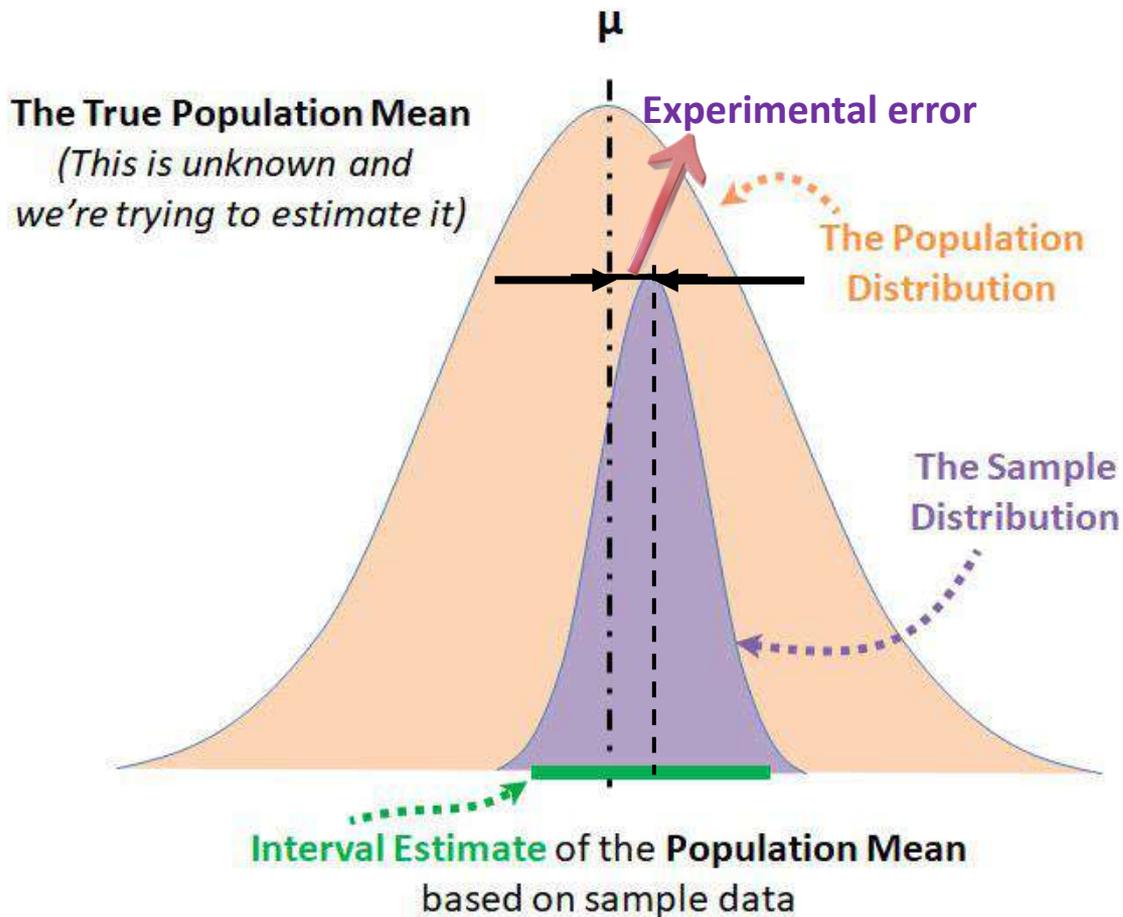


REGRESSION

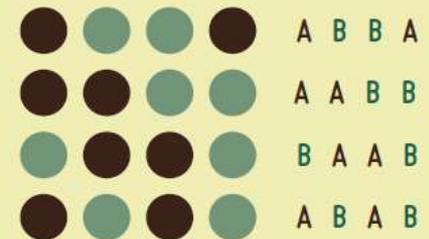


EXPERIMENTAL DESIGN IN AGRICULTURE

Experimental design



Replication
helps to make more precise estimates of the experimental error



Randomisation
prevents any other uncontrolled sources of variation from biasing the results

Experimental design

Replication + randomisation



EXPERIMENTAL DESIGN

The most appropriate design for a trial depends on:



1 Number of varieties being tested



2 size of the machinery used to apply treatments

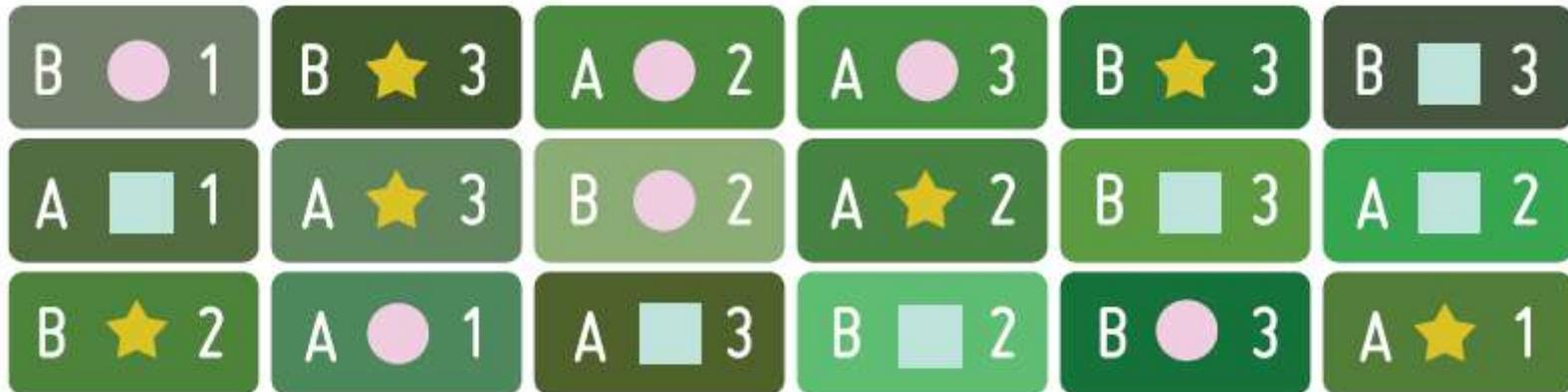


3 size of the detectable differences between treatments



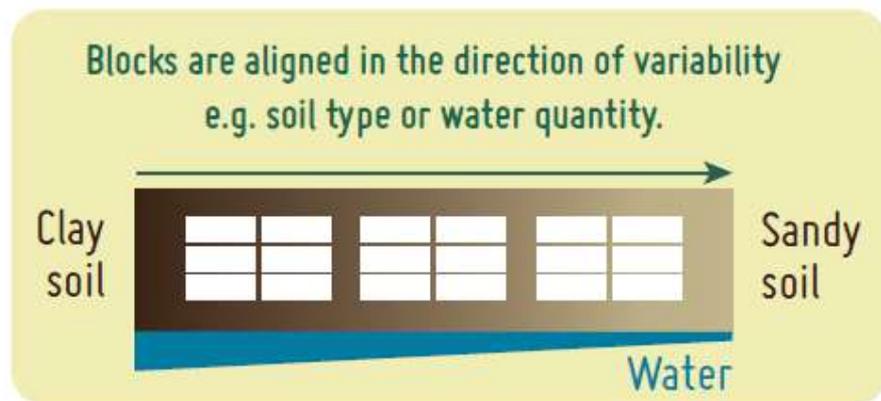
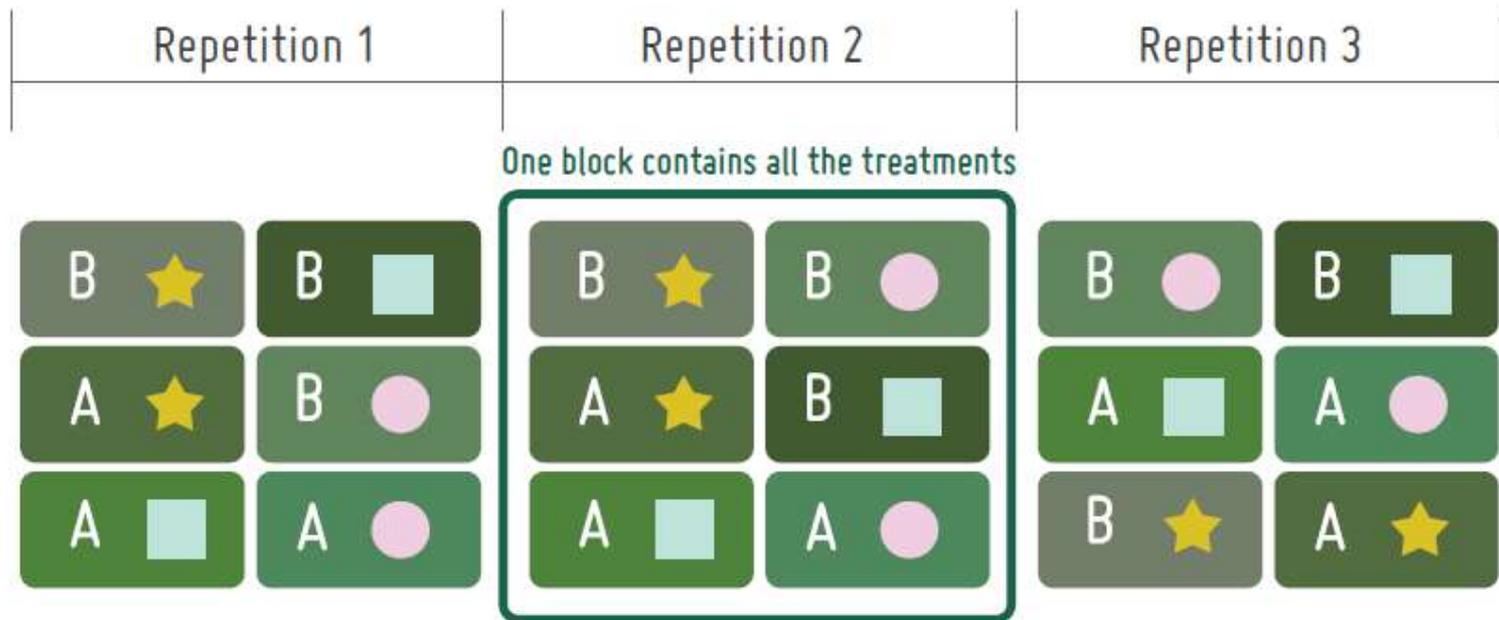
4 differences between treatments, and the resources required (time, space and money) to operate the trial.

Completely randomised design

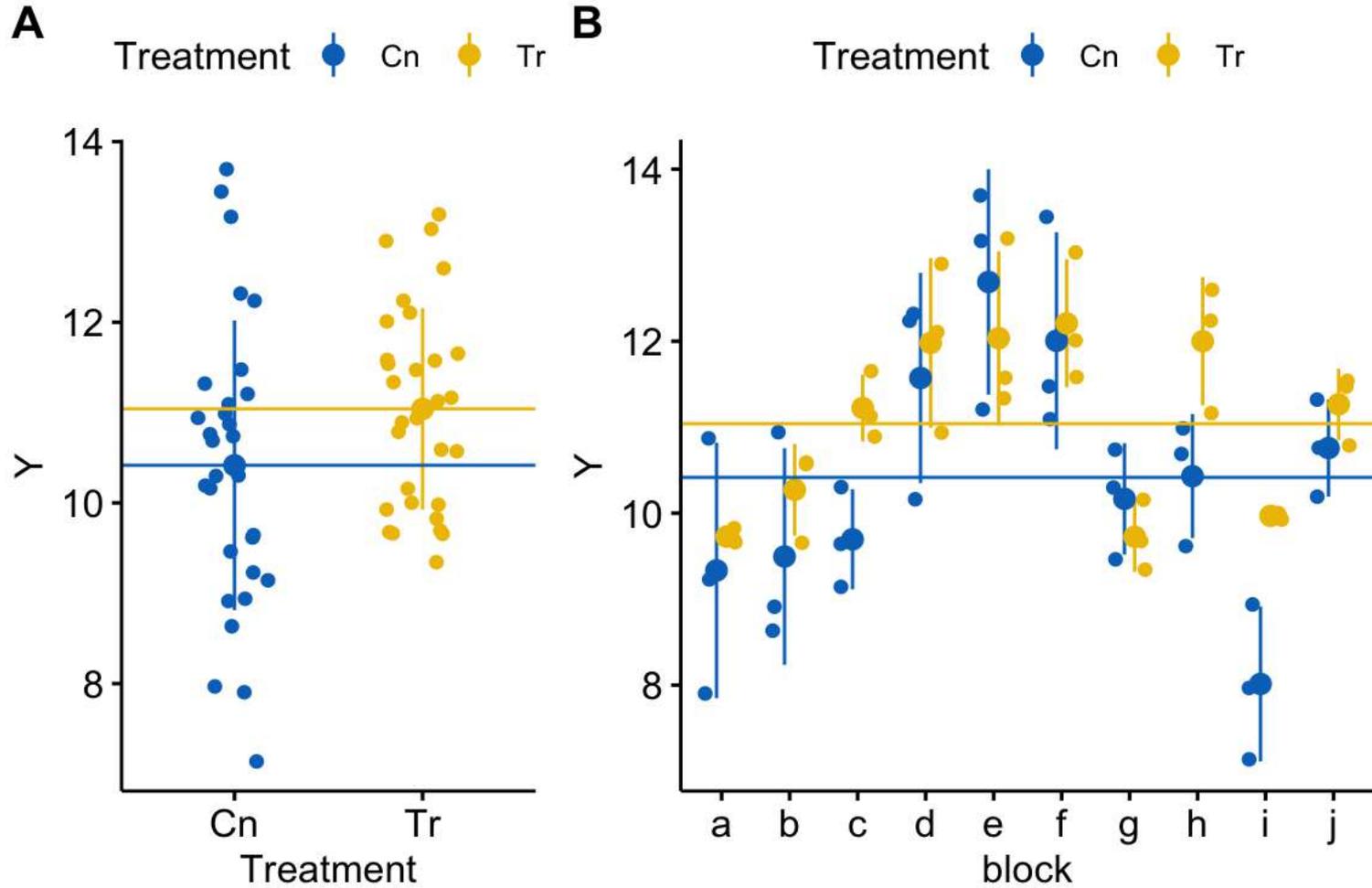


A B Seed varieties
★ ■ ● Treatments
1, 2, 3 Repetitions

Randomised complete block design

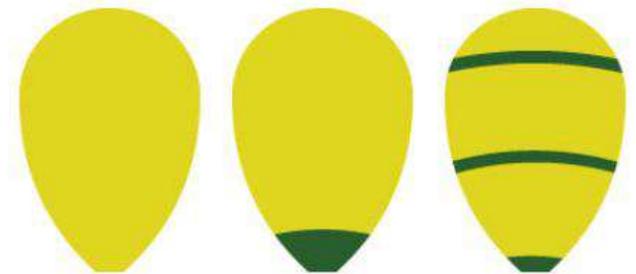
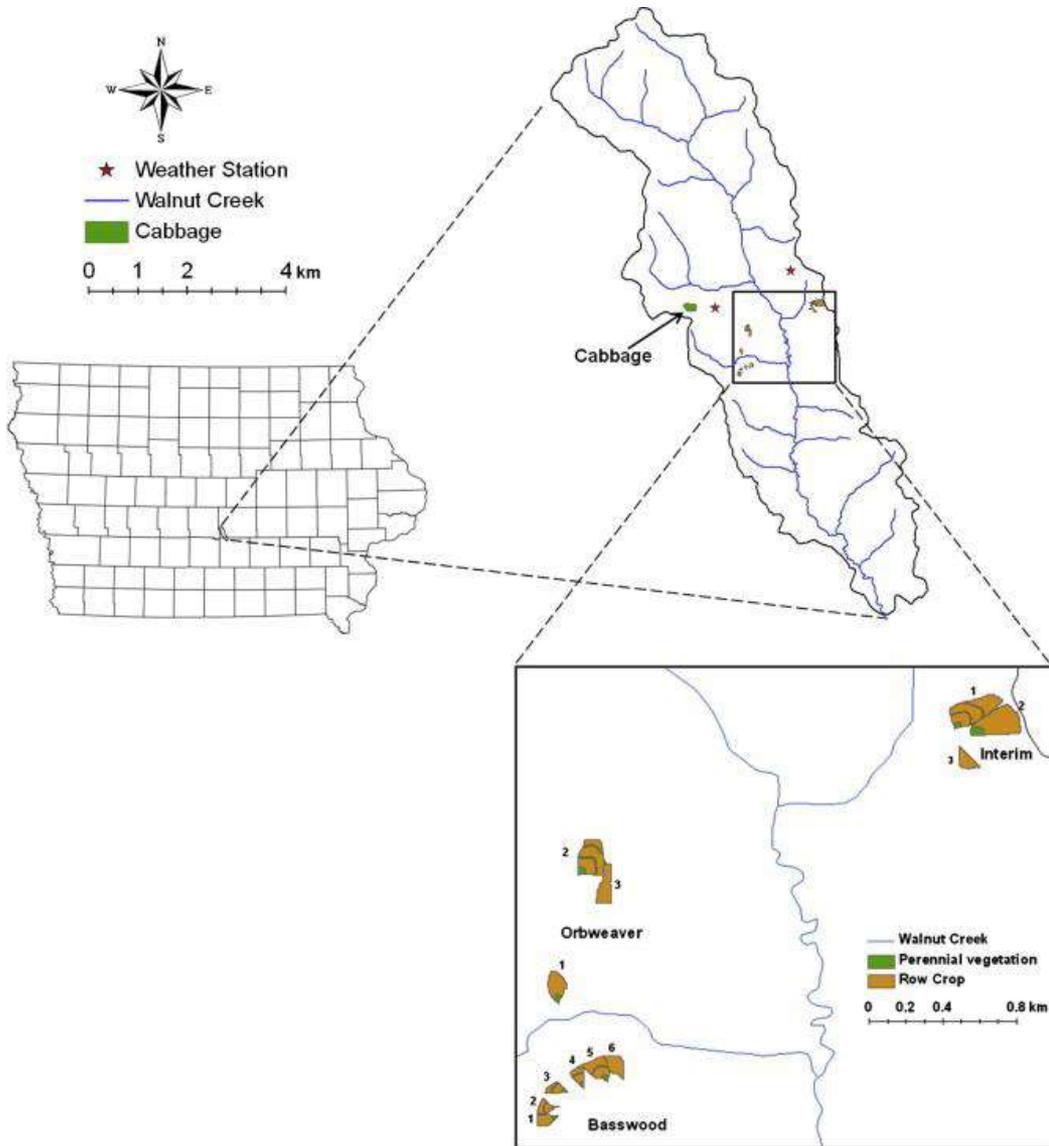


Randomised complete block design

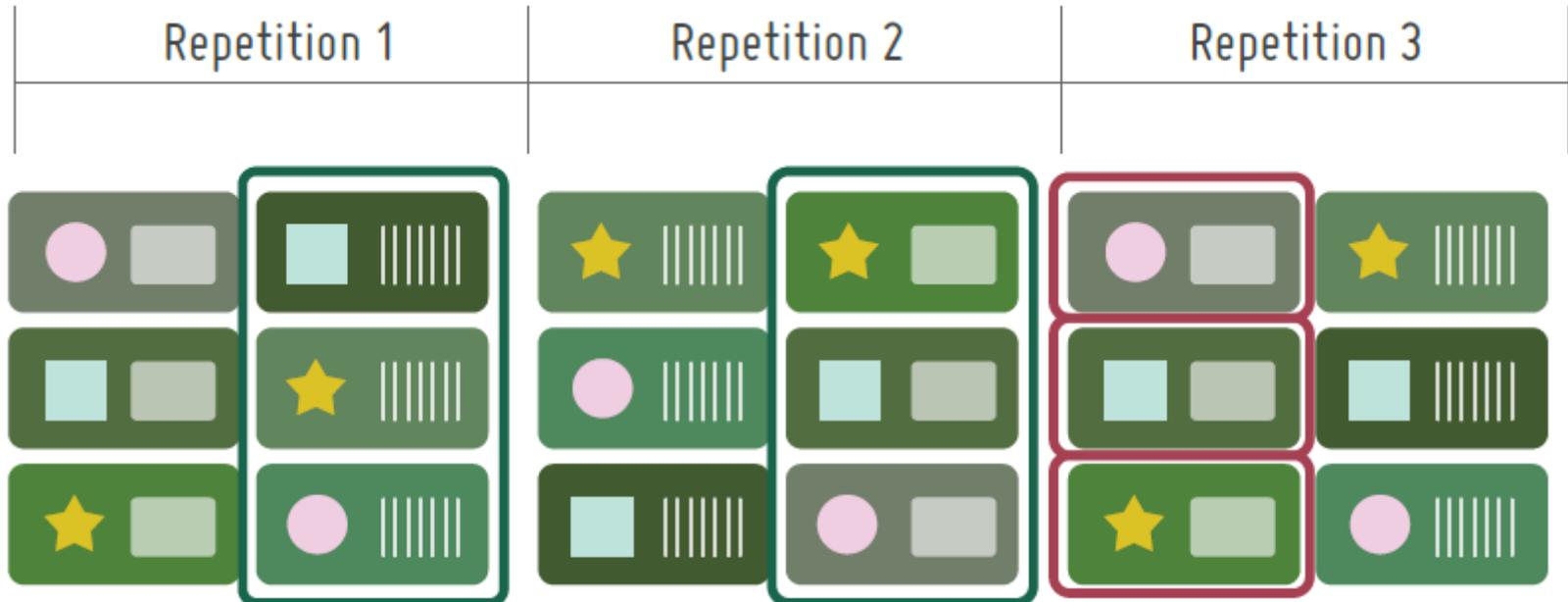


Fixed factors: treatment
Random factors: block

Randomised complete block design

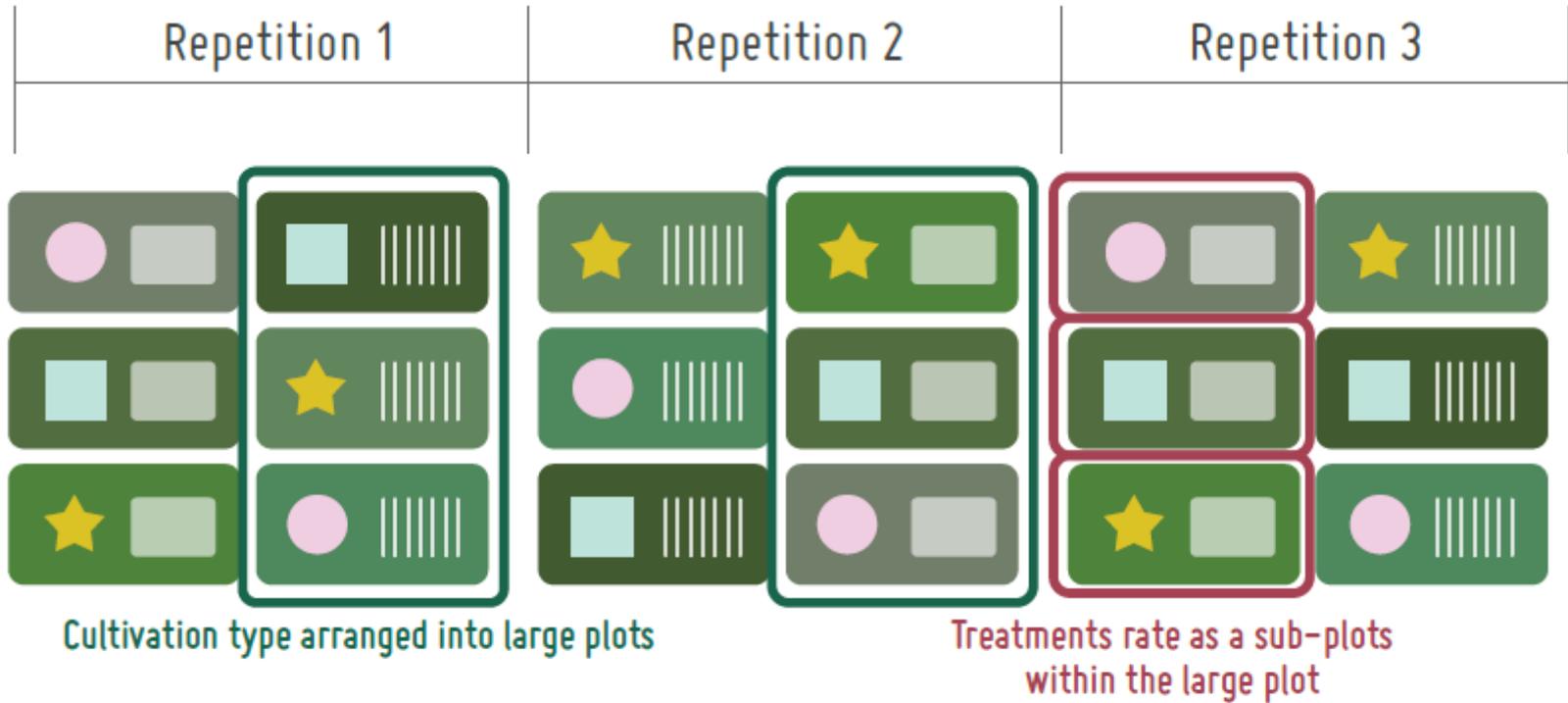


Split plot design

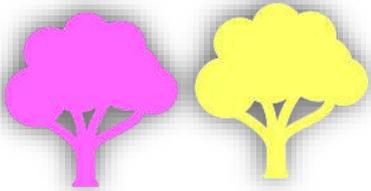


★ ■ ● Treatments
■ No till ||||| Plough

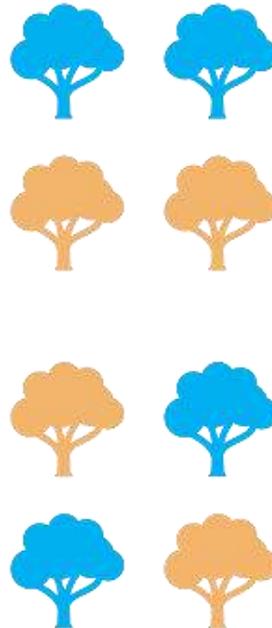
Split plot design



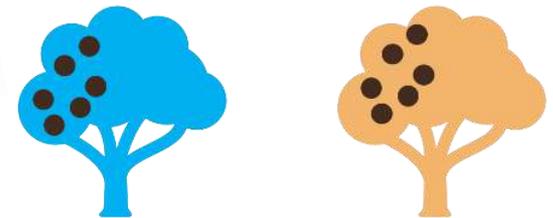
Split plot design



- Factor #1: species
- Level1: almond
 - Level2: lemon

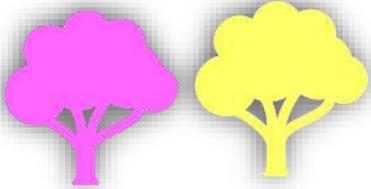


- Factor #2: irrigation treatments
- Level1: Well-watered
 - Level2: Water stress

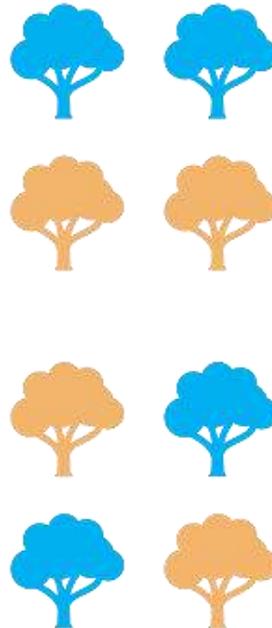


- Subfactor: fruit removal treatments
- Sublevel1: Fruits
 - Sublevel2: No fruits

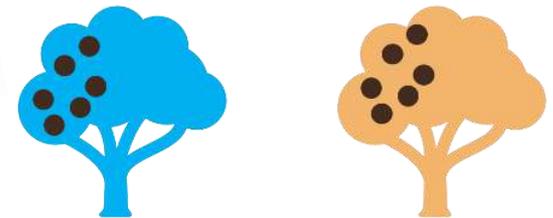
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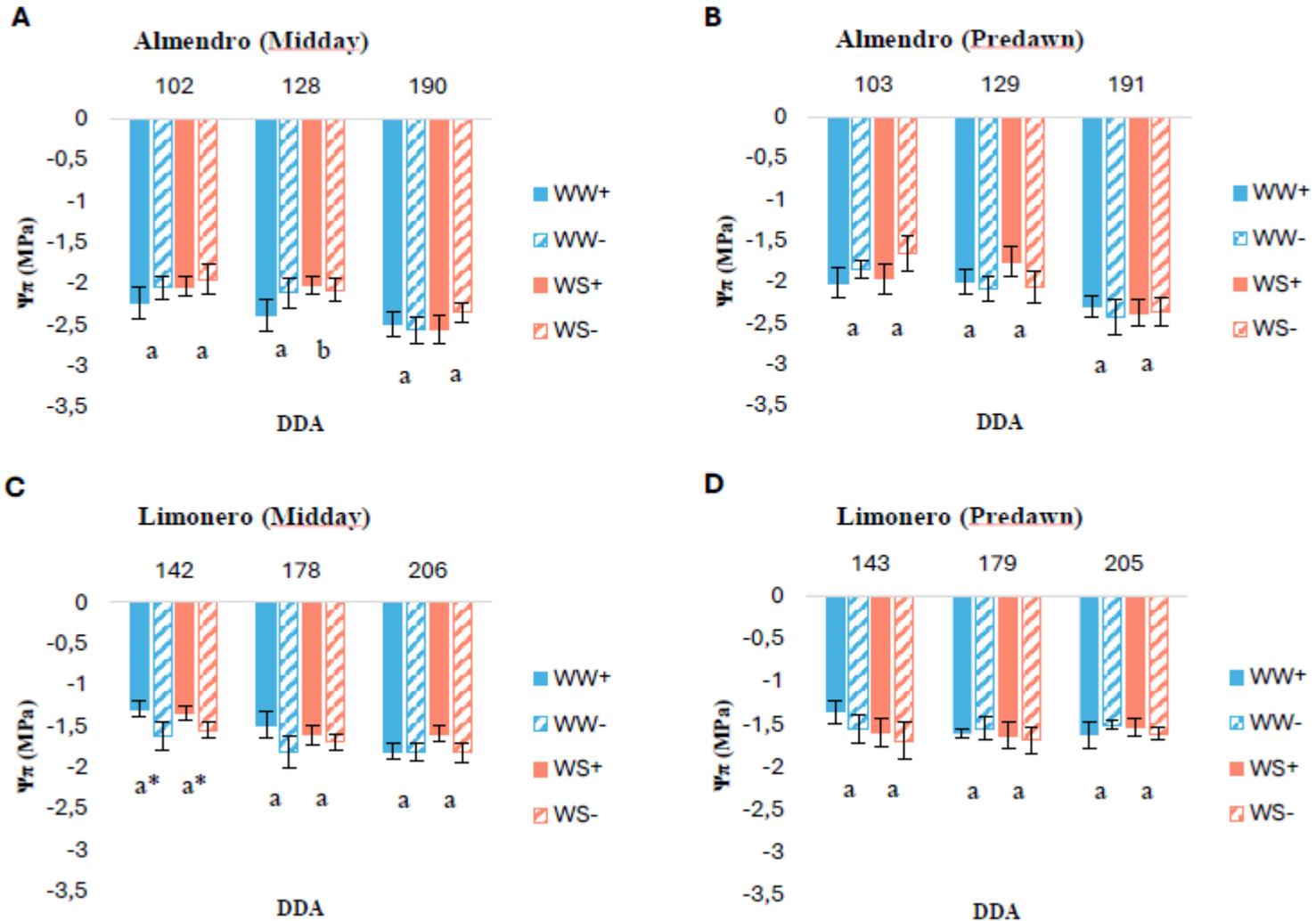


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Split plot design





On-Farm Experimentation to transform global agriculture

Myrtille Lacoste ^{1,2} , Simon Cook ^{1,3}, Matthew McNee⁴, Danielle Gale ¹, Julie Ingram ⁵,
Véronique Bellon-Maurel^{6,7}, Tom MacMillan ⁸, Roger Sylvester-Bradley⁹, Daniel Kindred ⁹,
Rob Bramley ¹⁰, Nicolas Tremblay ¹¹, Louis Longchamps ¹², Laura Thompson ¹³, Julie Ruiz ¹⁴,
Fernando Oscar García ^{15,16}, Bruce Maxwell¹⁷, Terry Griffin ¹⁸, Thomas Oberthür ^{19,20},
Christian Huyghe²¹, Weifeng Zhang²², John McNamara²³ and Andrew Hall ²⁴

Restructuring farmer-researcher relationships and addressing complexity and uncertainty through joint exploration are at the heart of On-Farm Experimentation (OFE). OFE describes new approaches to agricultural research and innovation that are embedded in real-world farm management, and reflects new demands for decentralized and inclusive research that bridges sources of knowledge and fosters open innovation. Here we propose that OFE research could help to transform agriculture globally. We highlight the role of digitalization, which motivates and enables OFE by dramatically increasing scales and complexity when investigating agricultural challenges.

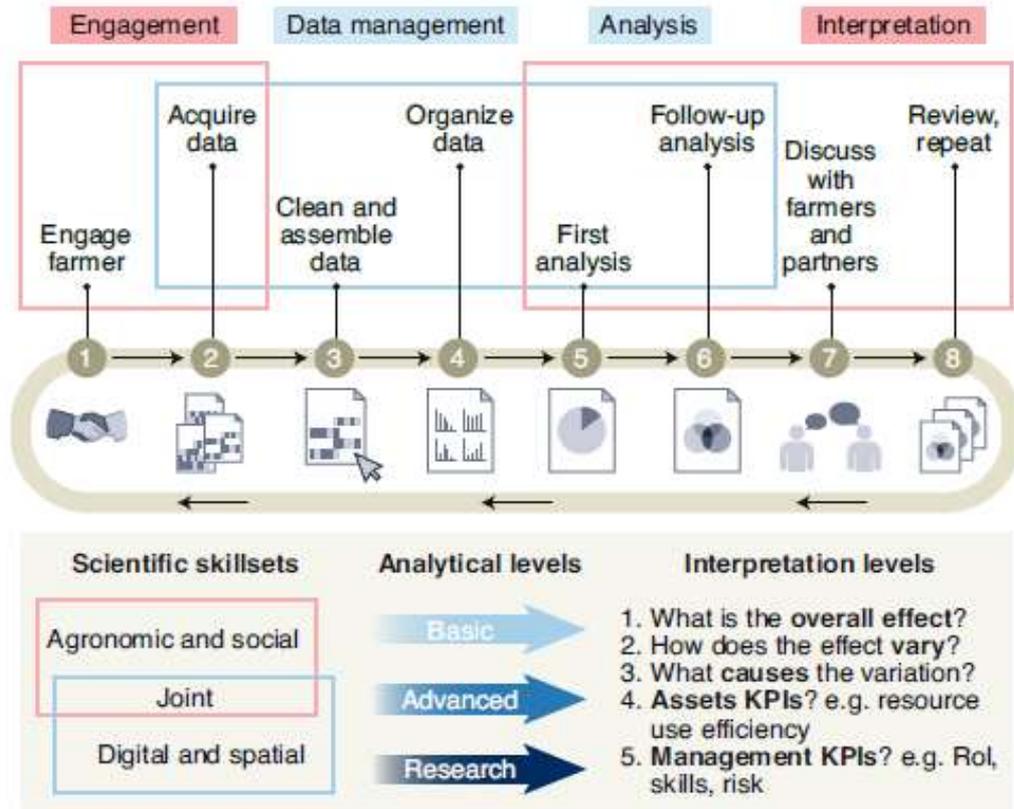


Fig. 1 | The OFE process. OFE follows an iterative process during which practical information is generated that farmers can easily understand, assess and readily convert to farm practices. Practically, OFE involves

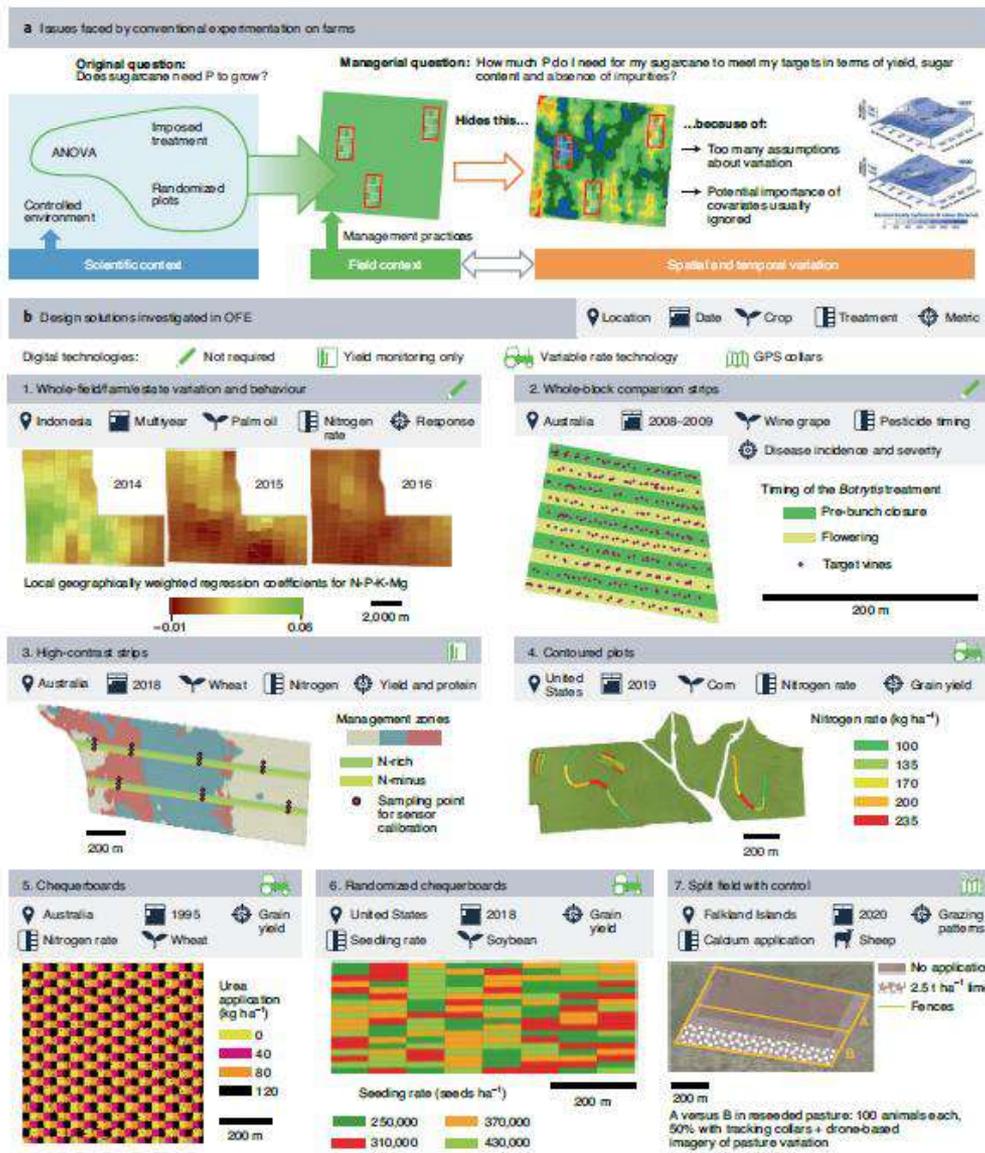


Fig. 2 | OFE designs to capture field-scale variations. **a.** Experimenting at the field scale may involve straightforward assessments of variation, especially in smallholder and subsistence farming, but also because farmers may attach low priority to statistical results and replications. One objective of OFE is to capture and utilize spatial and temporal variability. This is a problem that conventional trial methods cannot solve. ANOVA, analysis of variance. **b.** OFE initiatives across the world are developing a range of field-scale designs to address the issue. Challenges include addressing machinery requirements, data collection, spatial analytics and managerial relevance. Strategies range from the observations of yearly changes (1) to purposeful sampling (3, 4) or the utilization of the entire field (2, 5-7), especially in precision agriculture (3-6). Digital technologies add benefits (for example large datasets, ease of implementation, automation) as well as challenges (such as data processing).

*Thanks for your
attention!*

Practical exercise: Designing an Agricultural Research Experiment

Objective: Develop a comprehensive experimental plan to address a research question in agriculture.

1. Formulate a Research Question:

Define a clear and focused question that your experiment will address.

- 1. E.g. What variety is more resistant to cavitation? How much tree water use is reduced when irrigation is reduced to 50% Etc?*

2. Design the Experiment:

- 1. Choose an appropriate experimental design (e.g., randomized complete block, split-plot, or factorial design).*
- 2. Specify the independent variable(s) (e.g., irrigation regimes, fertilizer levels,) and dependent variable(s) (e.g., crop yield, plant height).*
- 3. Determine the number of treatments and their levels, replications, and plot sizes.*

3. Measurements:

Specify the variables to be measured, how and when.

4. Methods and Sensors:

Describe the methods for data collection and the tools or sensors you will use (use preferably some explained during the week).

5. Statistical Analyses:

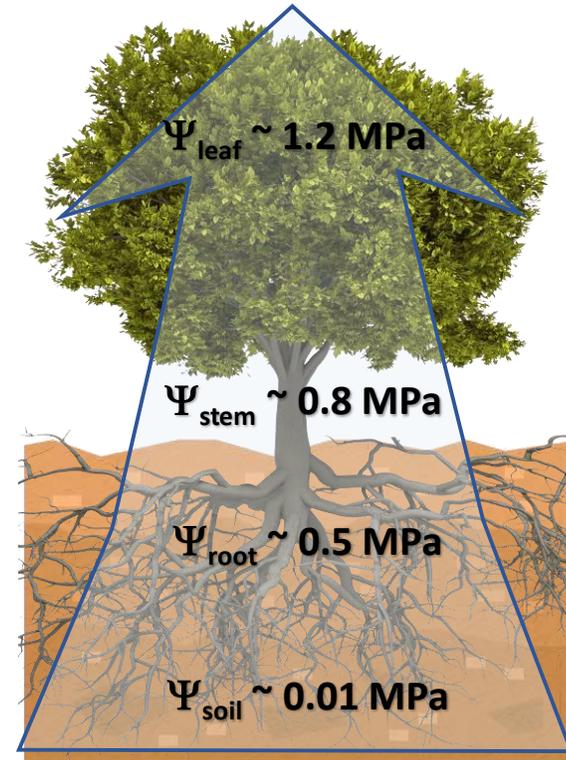
Propose the statistical methods needed to analyze the data (techniques explained such as ANOVA for treatment effects, regression analysis for relationships, etc). Justify your choice of methods.

Introduction to Vascular Plant Physiology for Understanding Water Relations and Hydraulic Function

Celia M. Rodríguez Domínguez

2nd Training School

27th January to 31st January 2025



CSIC

isUP-Agr 

UNLOCKING THE POTENTIAL FOR AGRICULTURAL
RESEARCH ON AN EU OUTMOST REGION:
BOOSTING ISOPLEXIS CENTRE



ISOPlexis
Centro de Agricultura Sustentável e Tecnologia Alimentar

UNIVERSIDADE da MADEIRA

OBJECTIVES

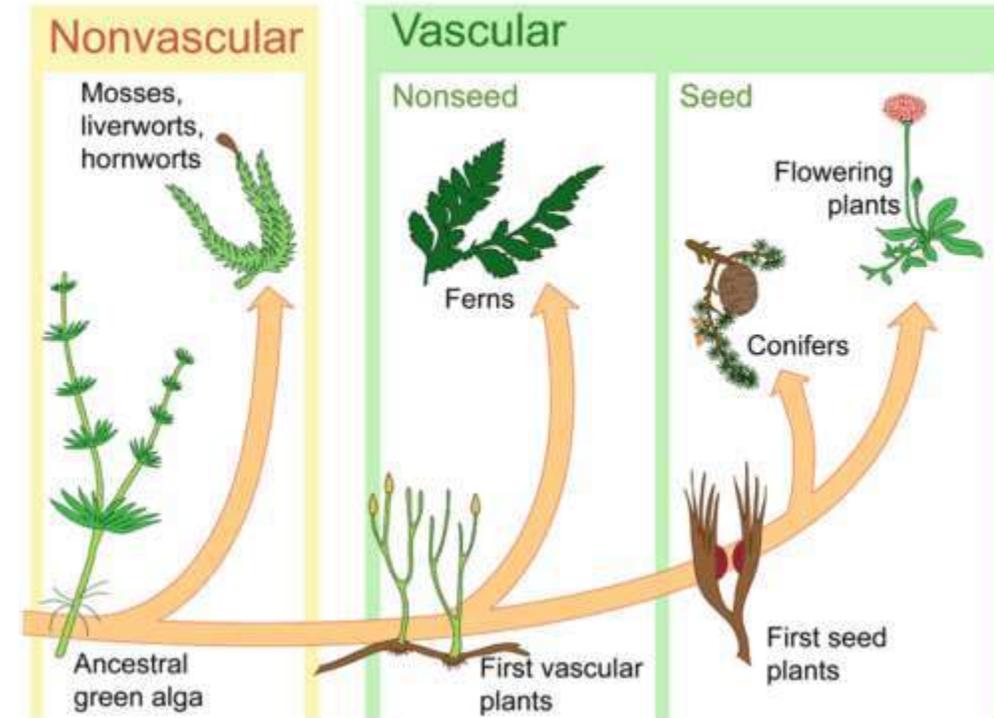
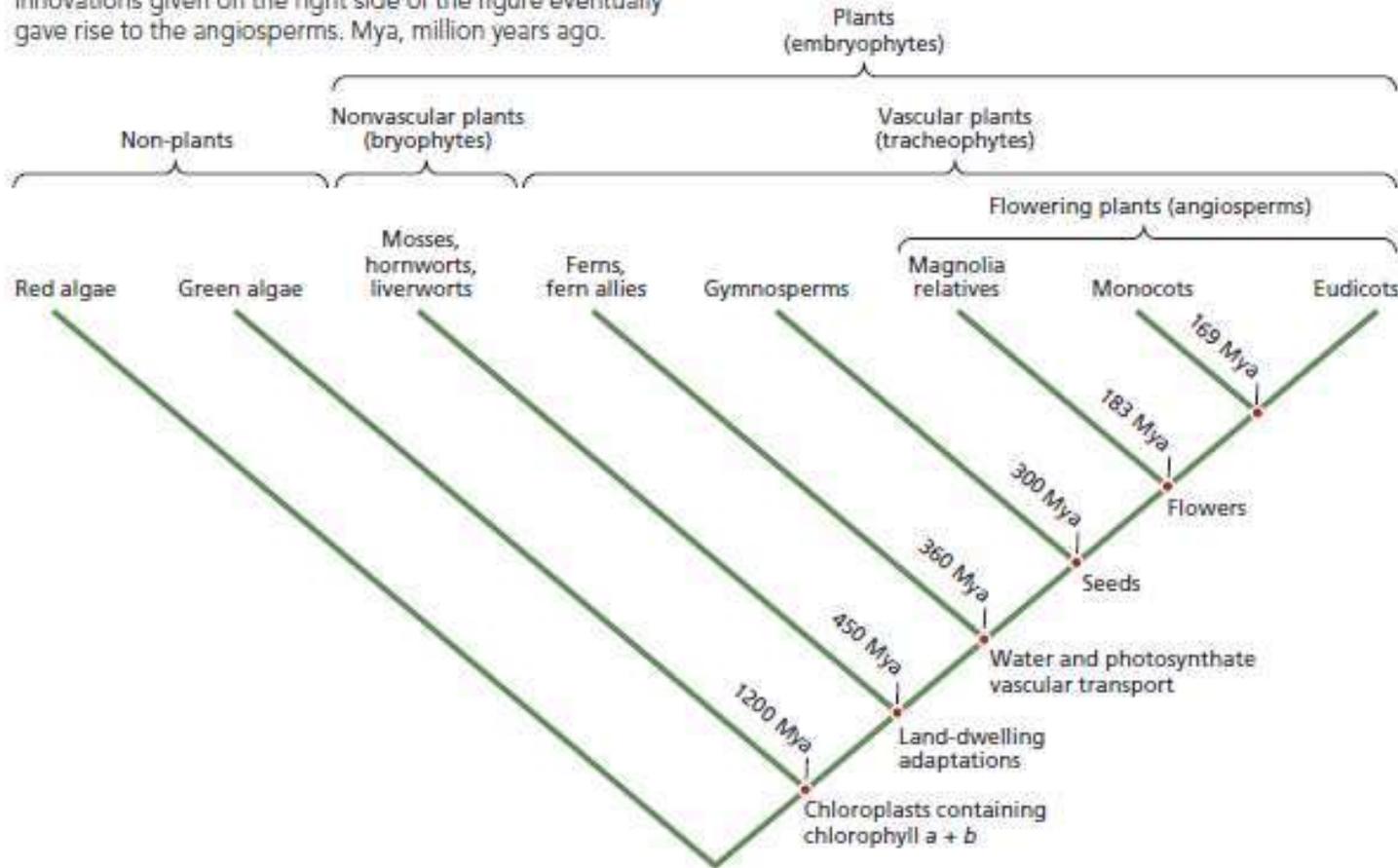
- To understand the basic structure of vascular plants and their role in water transport.
- To introduce the fundamental concepts related to water relations in plants, such as water potential, transpiration, and hydraulic conductivity.
- To understand the physiological mechanisms of plant response to environmental factors.
- To recognize the significance of these physiological processes in the context of agriculture.



BEFORE WE START... WHAT IS A VASCULAR PLANT?

To answer this question, we need to go back 450 Mya, when plants (bryophytes) first colonized land...

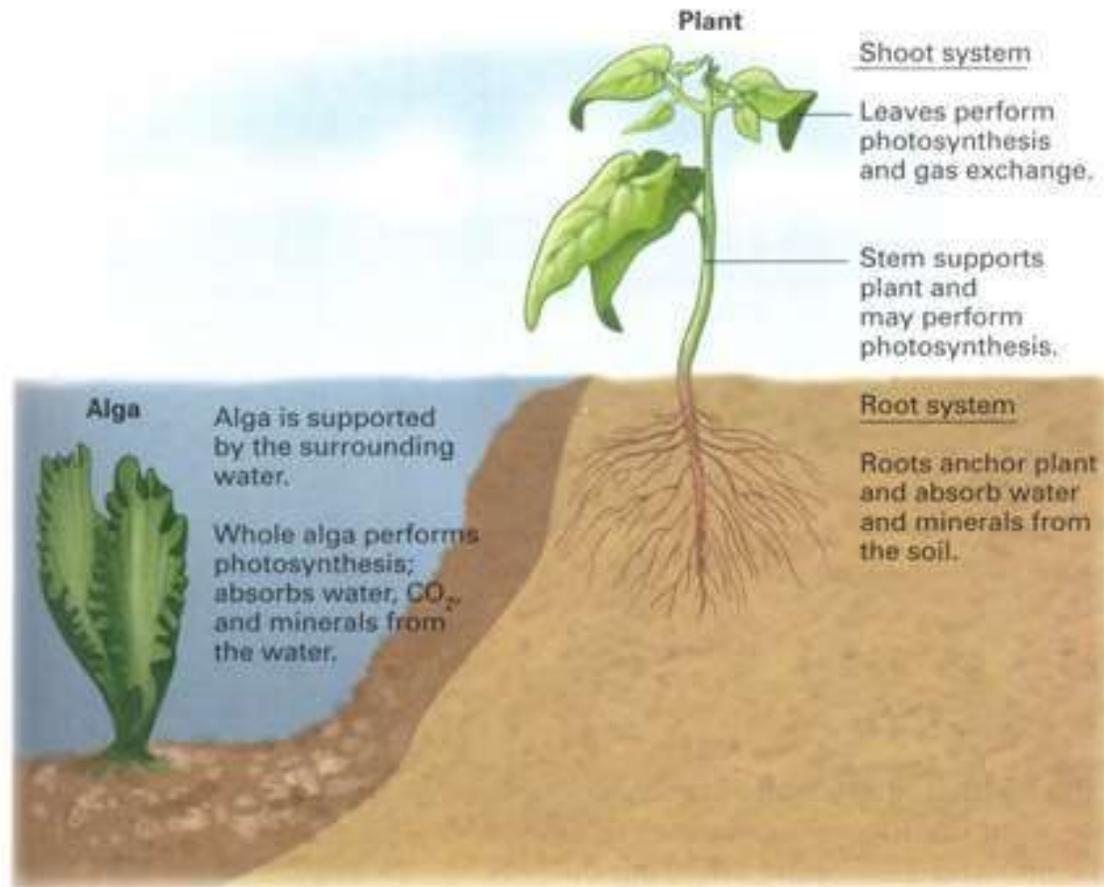
Figure 1.1 Cladogram showing the evolutionary relationships among the various members of the plants and their close relatives, the algae. The sequence of evolutionary innovations given on the right side of the figure eventually gave rise to the angiosperms. Mya, million years ago.



BEFORE WE START... WHAT IS A VASCULAR PLANT?

...During this conquer of Earth, plants had to face many challenges to adapt to the new dry conditions...

From a simple, green alga ancestor that lived in the water, plants eventually evolved several major adaptations for life on land.



◀ **Figure 19-2** While algae live entirely in water, a plant lives in two environments: air and soil. A plant's organ systems are adapted to these two environments.

BEFORE WE START... WHAT IS A VASCULAR PLANT?

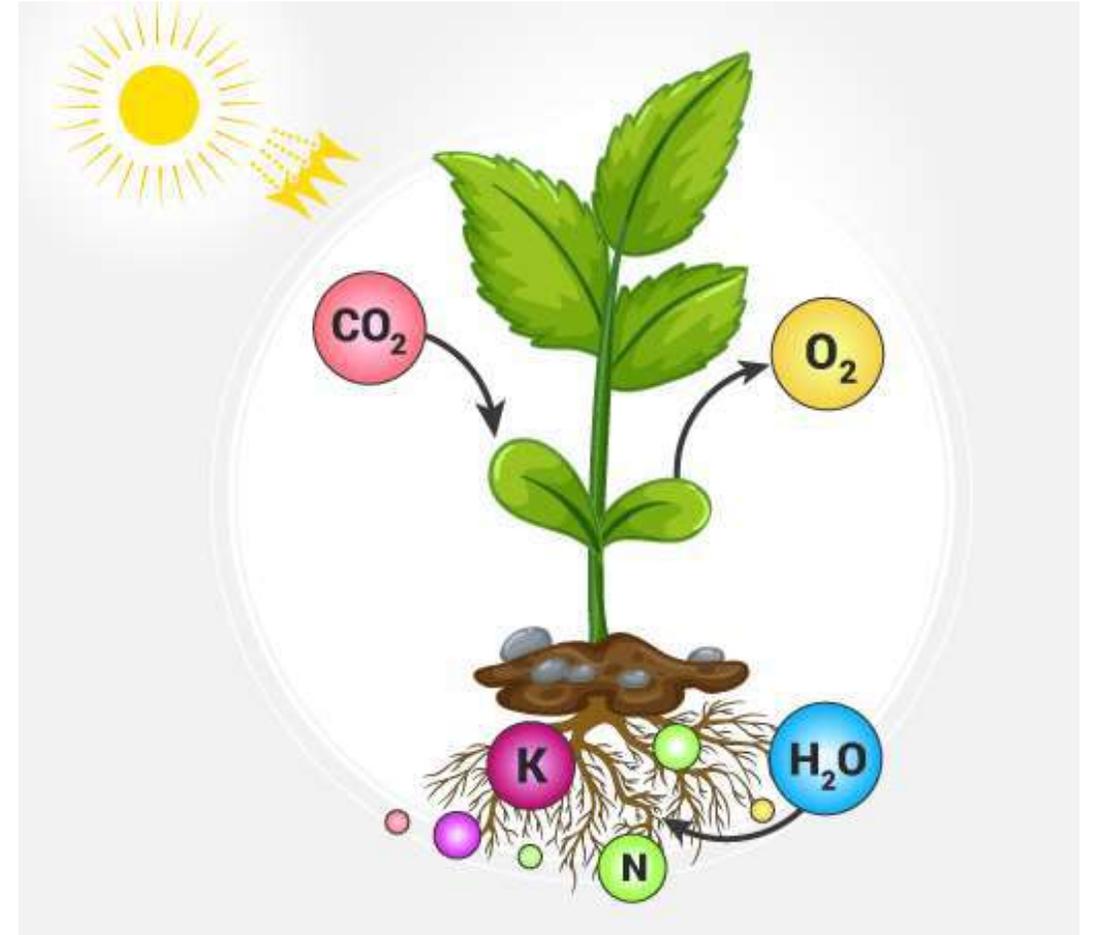
Six major unifying principles common to all plants (non-vascular and vascular)

❖ *Plants, and green algae, are the ultimate solar collectors, the Earth's primary producers.*

Light energy --> Chemical energy, CO₂ + H₂O --> Carbohydrates

❖ Other than certain reproductive cells, plants are sessile.

They have evolved the ability to grow toward essential resources: light, water, mineral nutrients



BEFORE WE START... WHAT IS A VASCULAR PLANT?

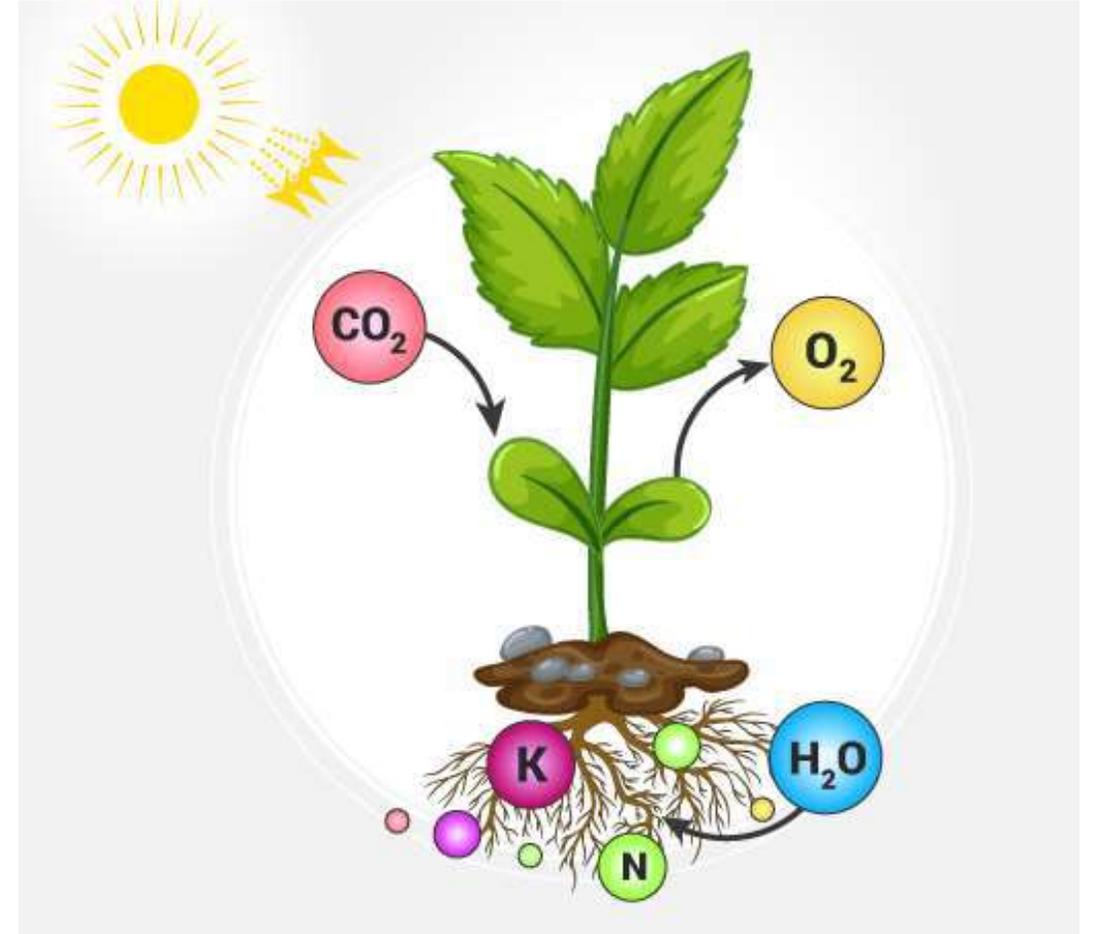
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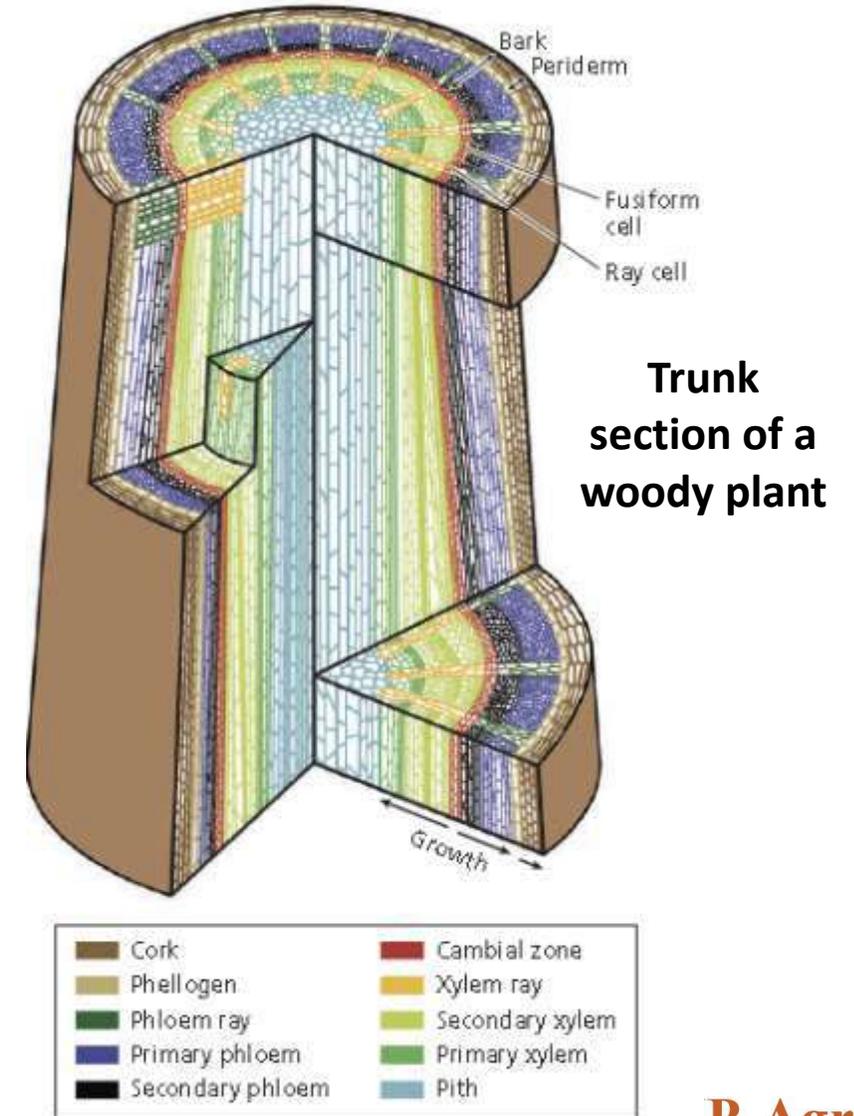
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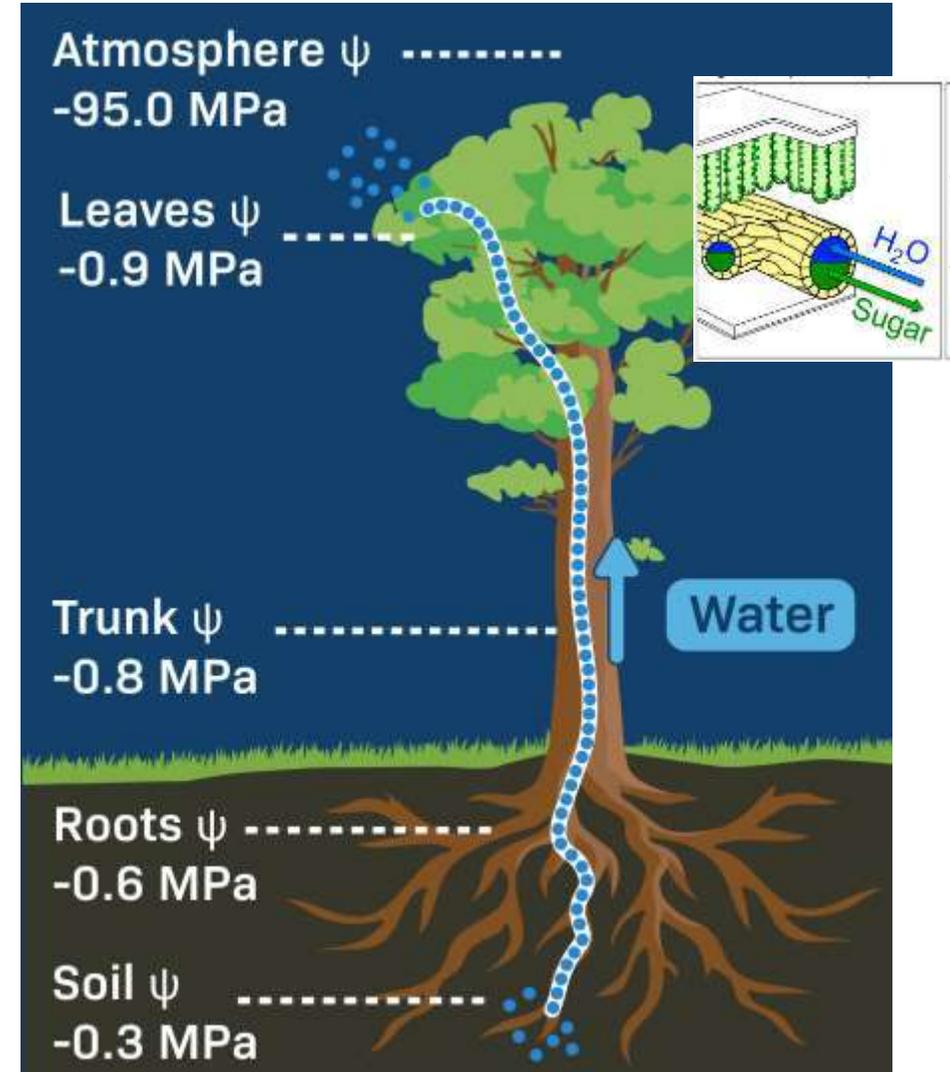
- ❖ *Plants are structurally reinforced to support their mass as they grow toward sunlight against the pull of gravity.*
- ❖ Plants have mechanisms for moving water and minerals from the soil to the sites of photosynthesis and growth, as well as mechanisms for moving the products of photosynthesis to non-photosynthetic organs and tissues.
- ❖ Plants lose water continuously by evaporation and have evolved mechanisms for avoiding desiccation.
- ❖ Plants develop from embryos that derive nutrients from the mother plant, and these additional food stores facilitate the production of large self-supporting structures on land.



BEFORE WE START... WHAT IS A VASCULAR PLANT?

Six major unifying principles common to all plants (non-vascular and vascular)

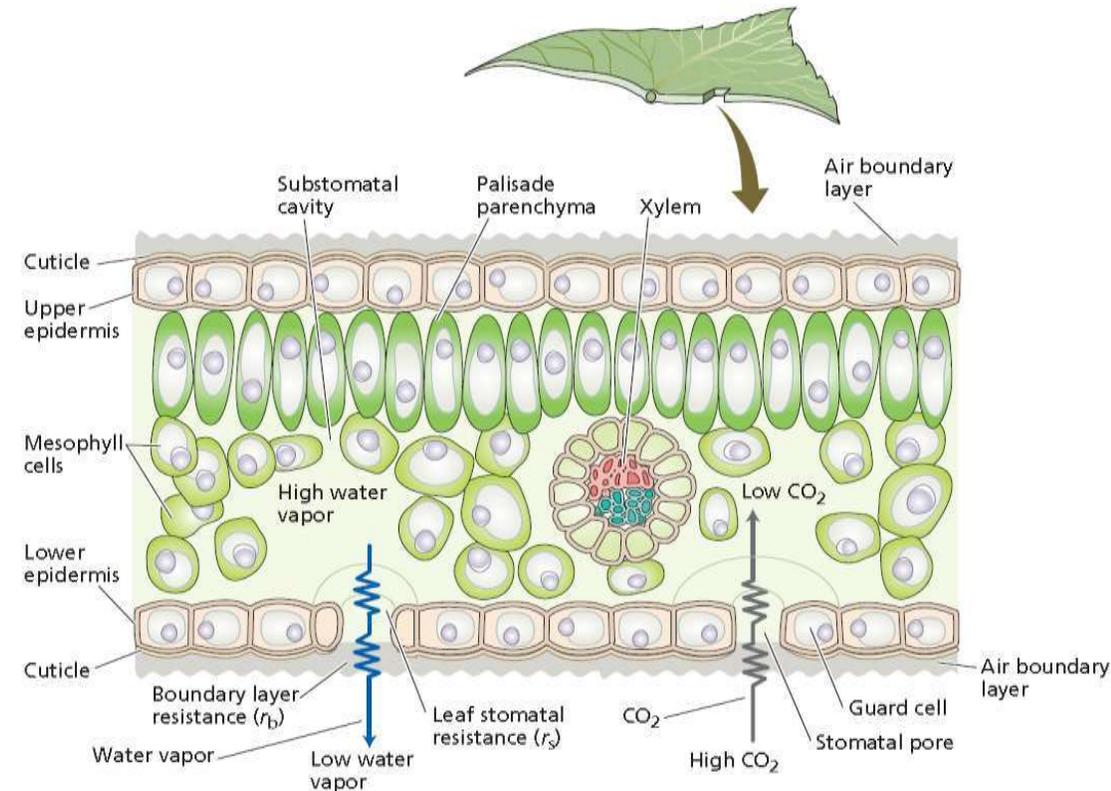
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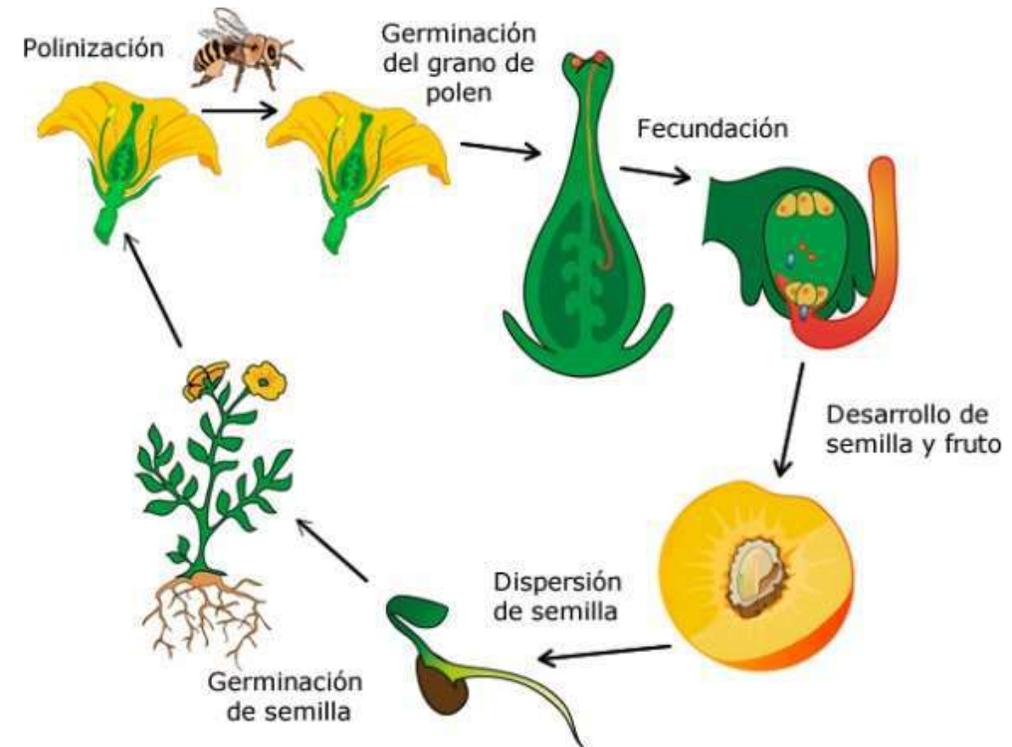
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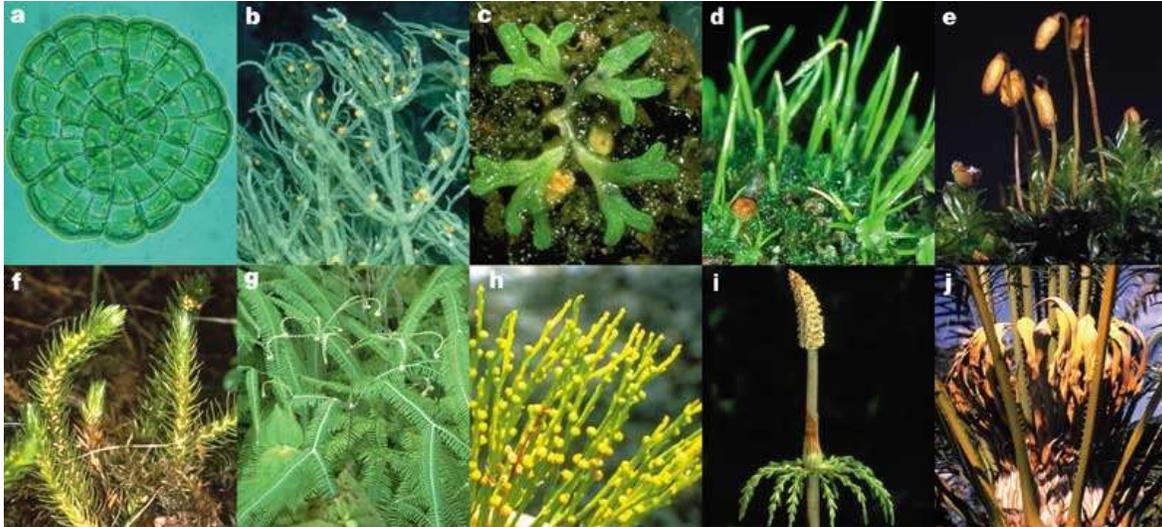
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BEFORE WE START... WHAT IS A VASCULAR PLANT?

...Returning to the question... We can categorize plants into two main groups:

Non-vascular plants



Mosses, liverworts, hornworts

Vascular plants

Ferns



Gymnosperms

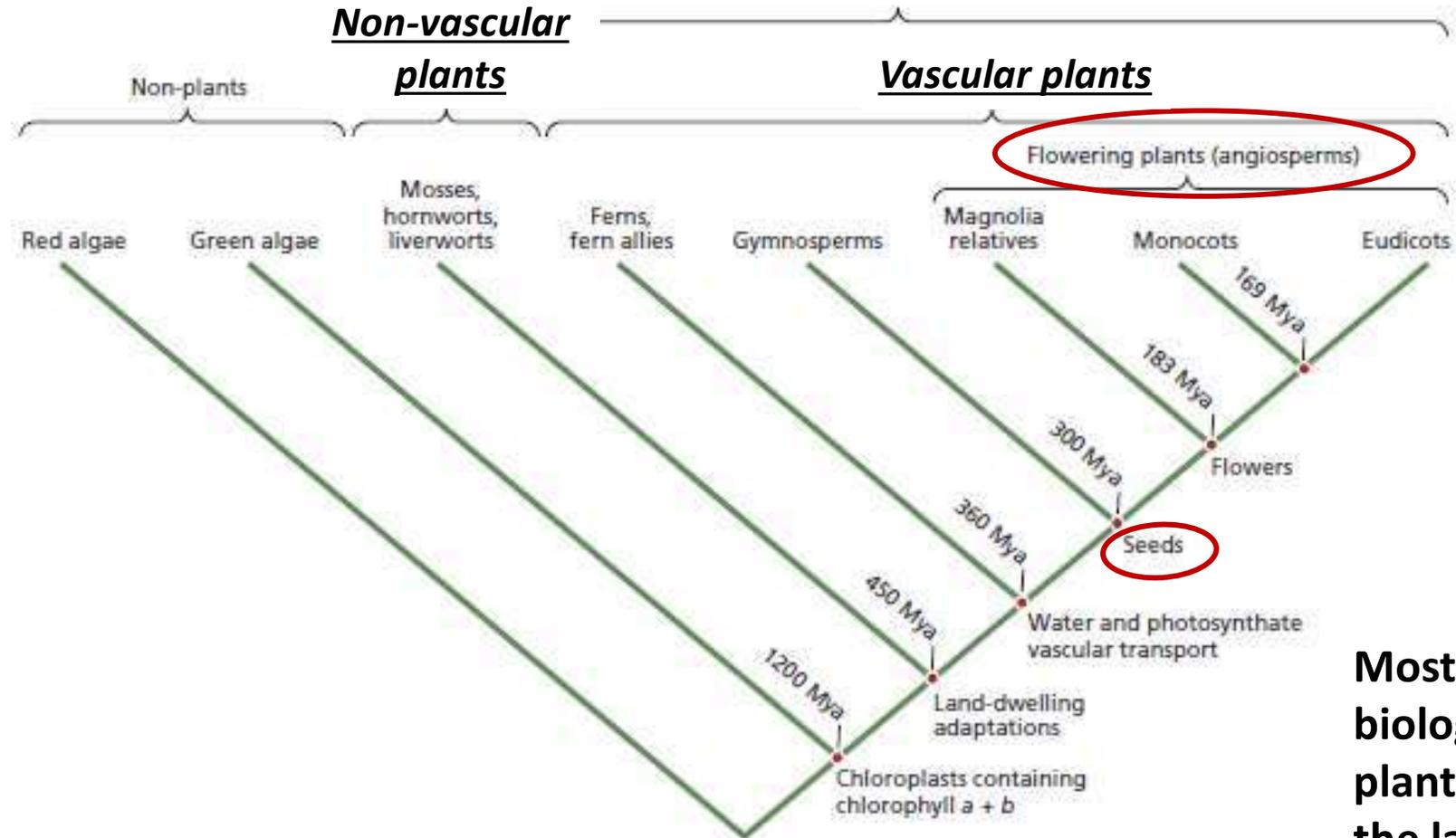


Angiosperms



BEFORE WE START... WHAT IS A VASCULAR PLANT?

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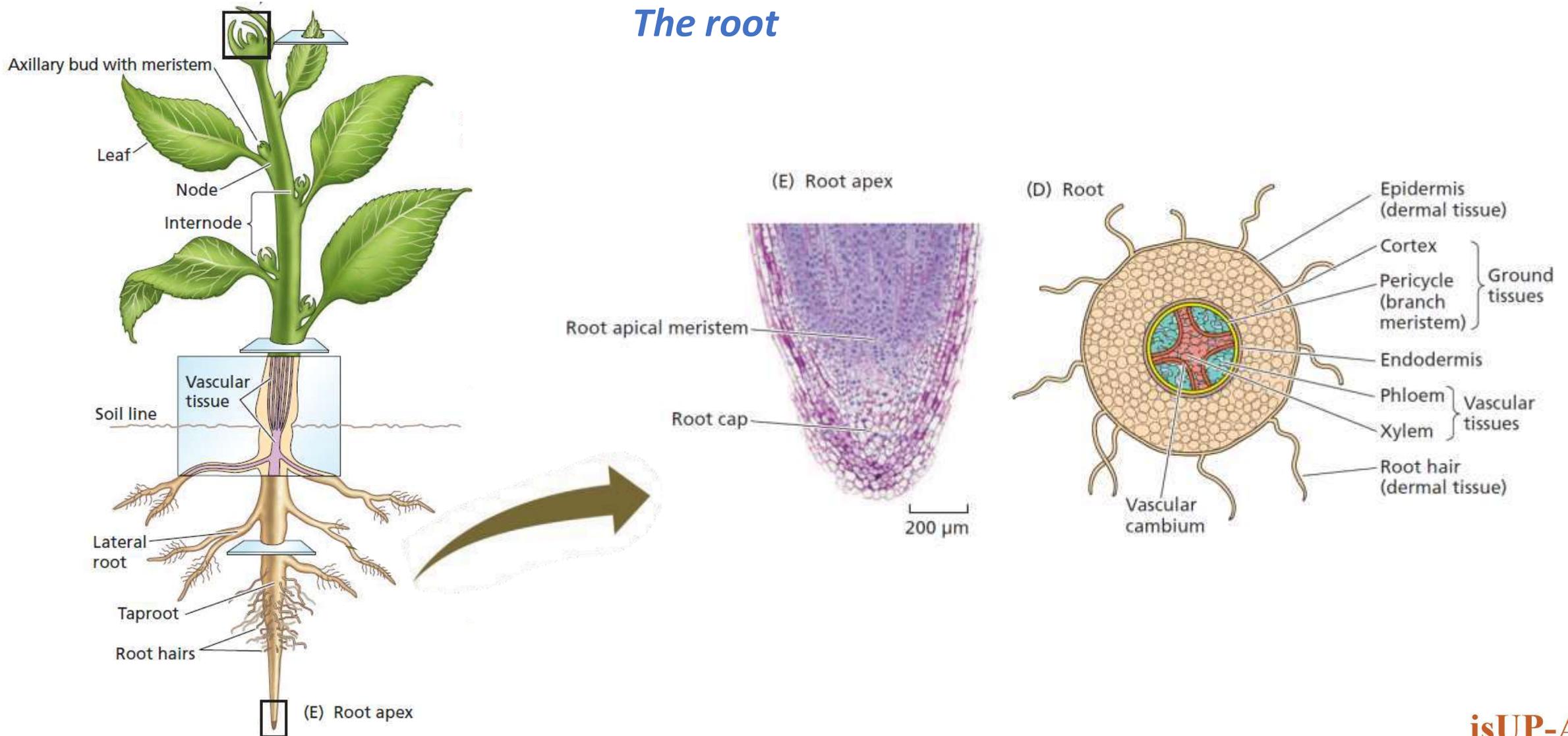


Most research in plant biology has focused on the plants that have evolved in the last 300 million years, the seed plants

BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

High diversity, but same basic vegetative body plan

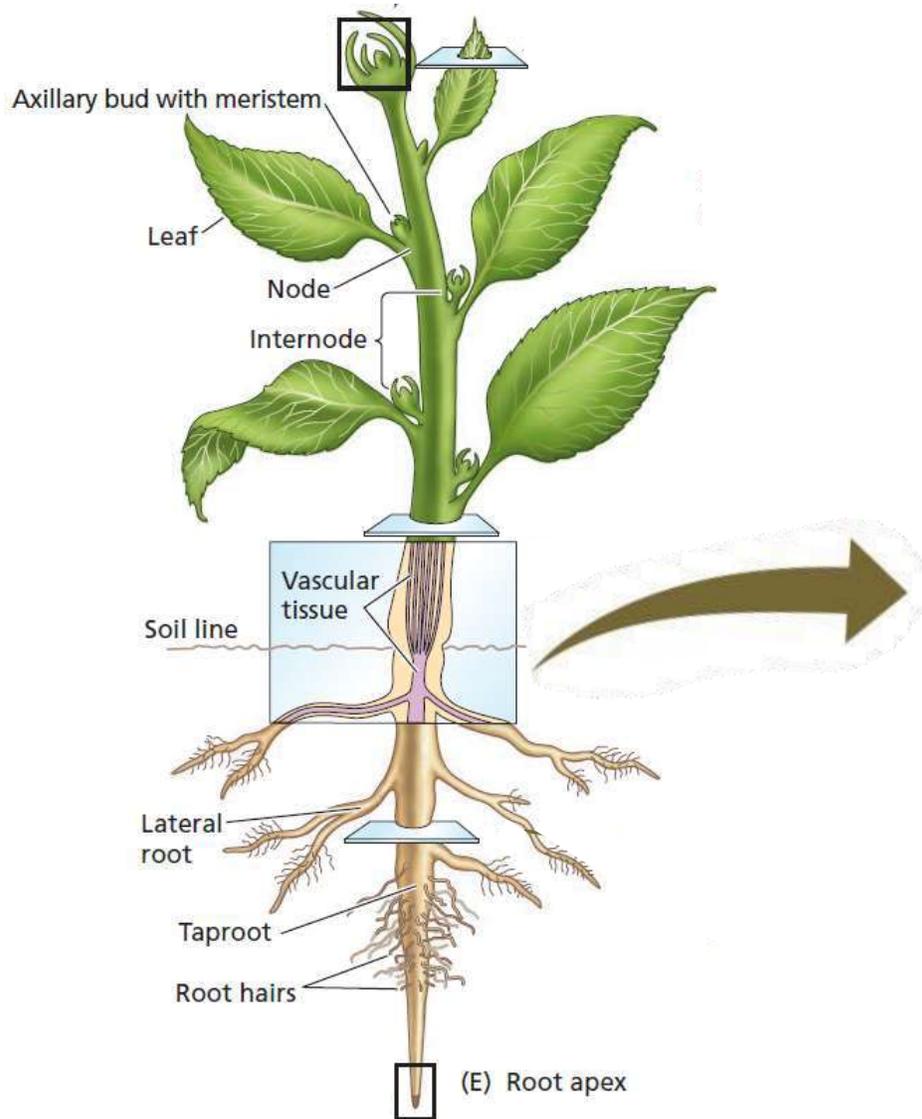
The root



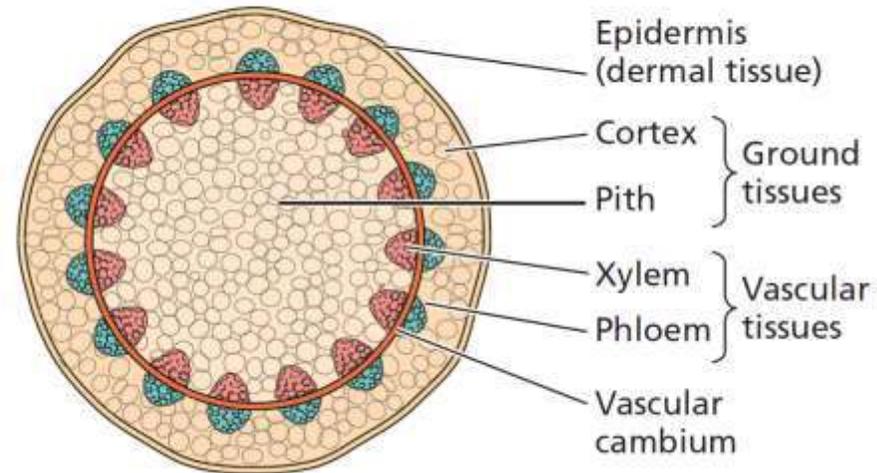
BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

High diversity, but same basic vegetative body plan

The stem

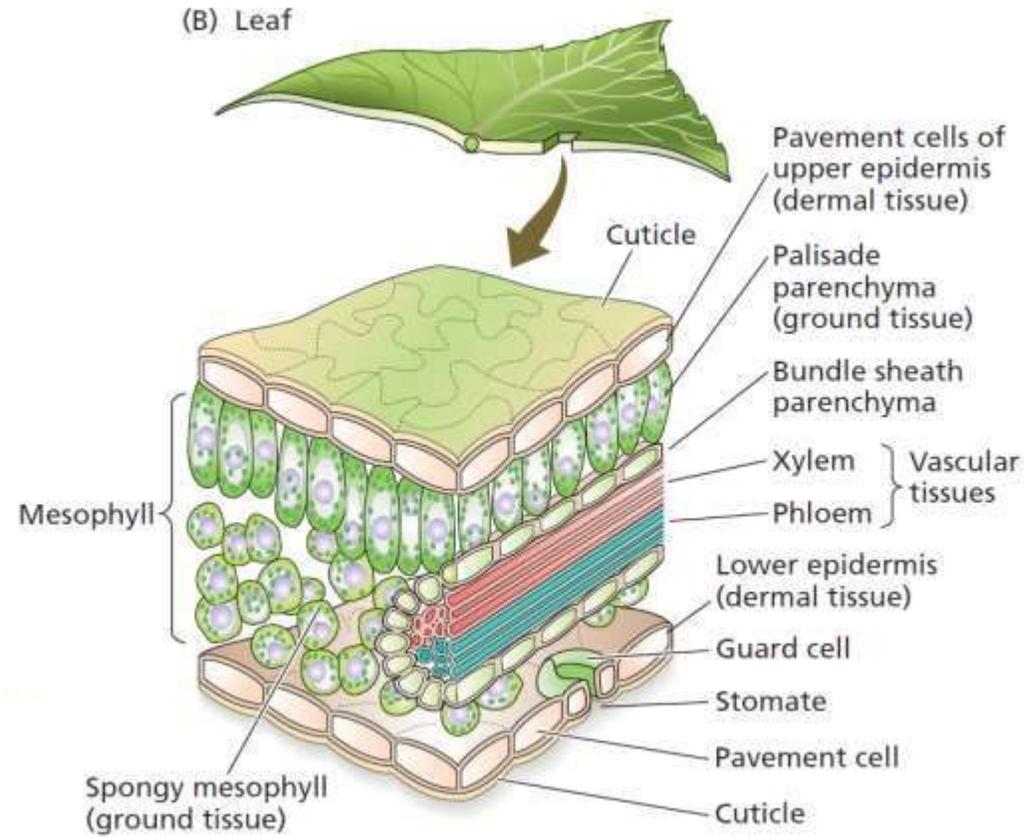
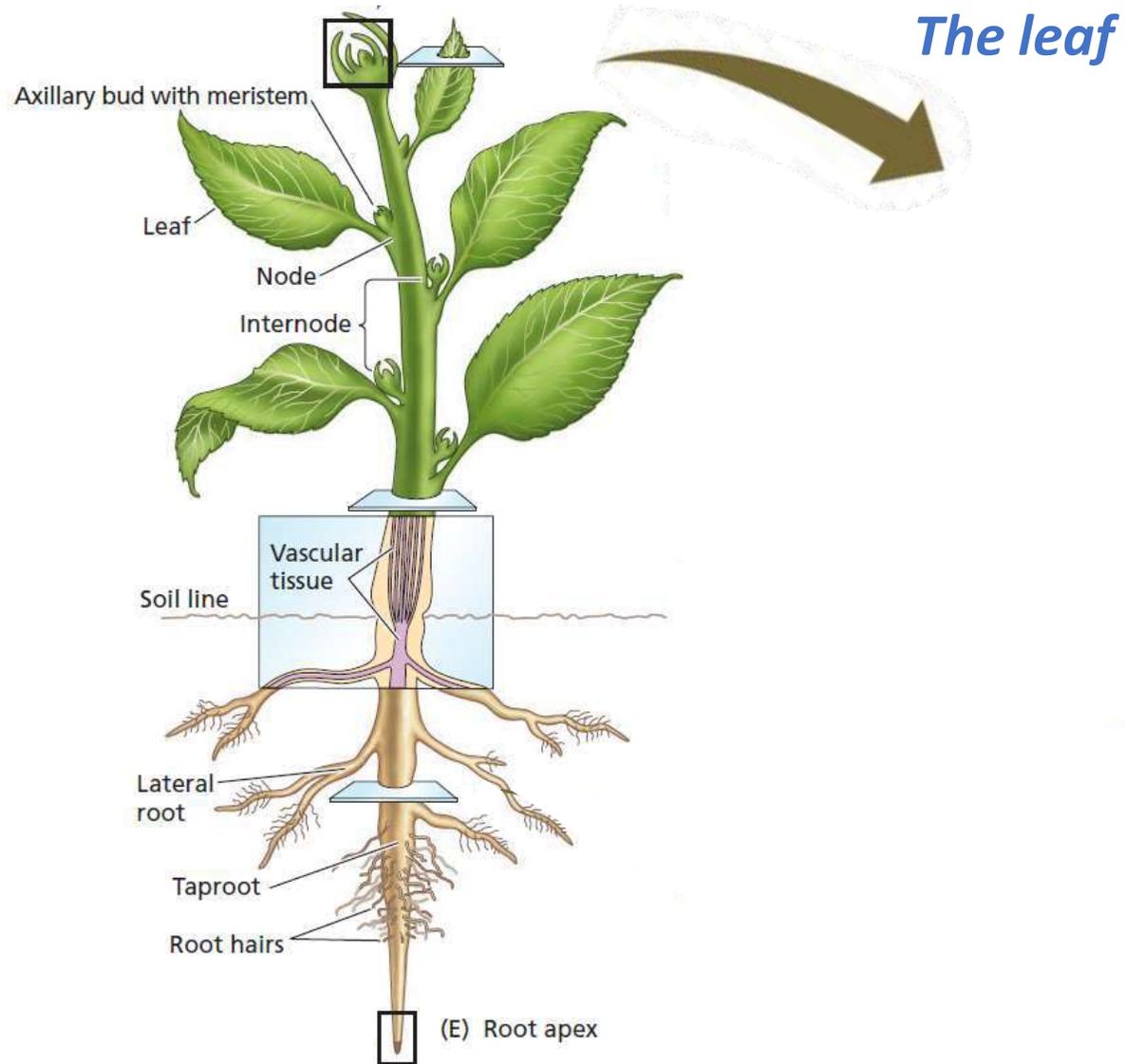


(C) Stem



BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

High diversity, but same basic vegetative body plan

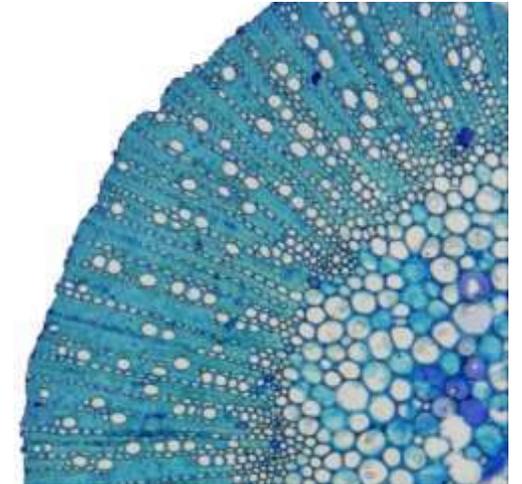
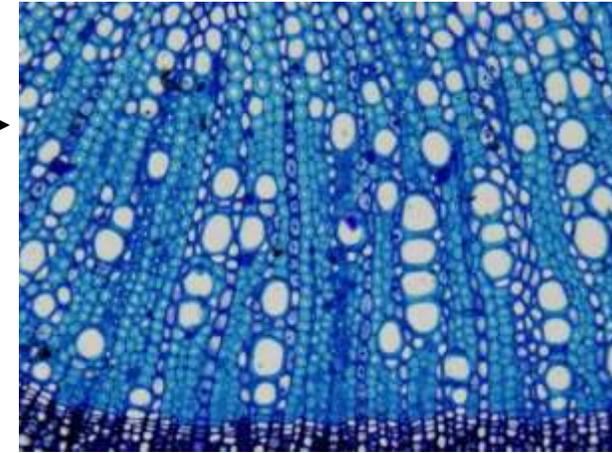
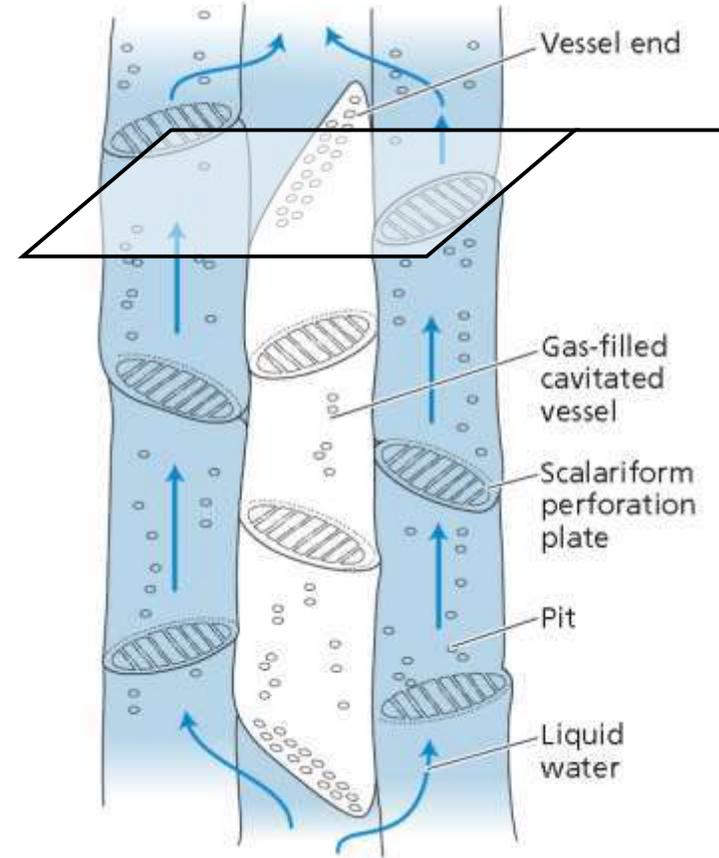


BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Three key structures have the greatest impact on plants' hydraulic function and water relations

☐ XYLEM

Dead cells (vessels) - loss of the cytoplasm - in which remain the thick, lignified cell walls, which form hollow tubes through which water can flow with relatively little resistance, enabling to transport large quantities of water with great efficiency.



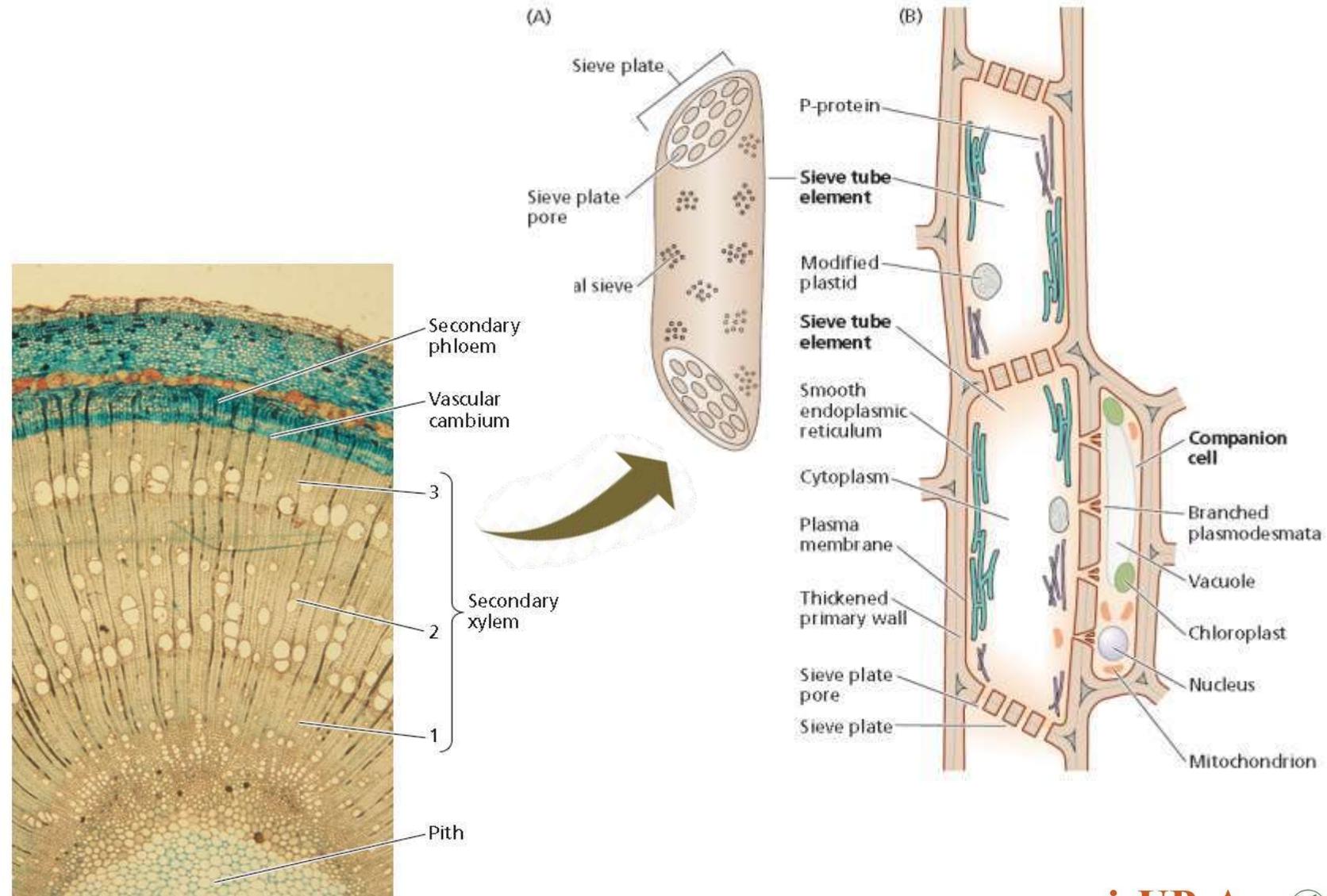
BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Three key structures have the greatest impact on plants' hydraulic function and water relations

☐ PHLOEM

Tissue, living cells, that translocates the products of photosynthesis—particularly sugars—from mature leaves to areas of growth and storage, including the roots.

The phloem also transmits signals between sources and sinks in the form of regulatory molecules, and redistributes water and various compounds throughout the plant body.

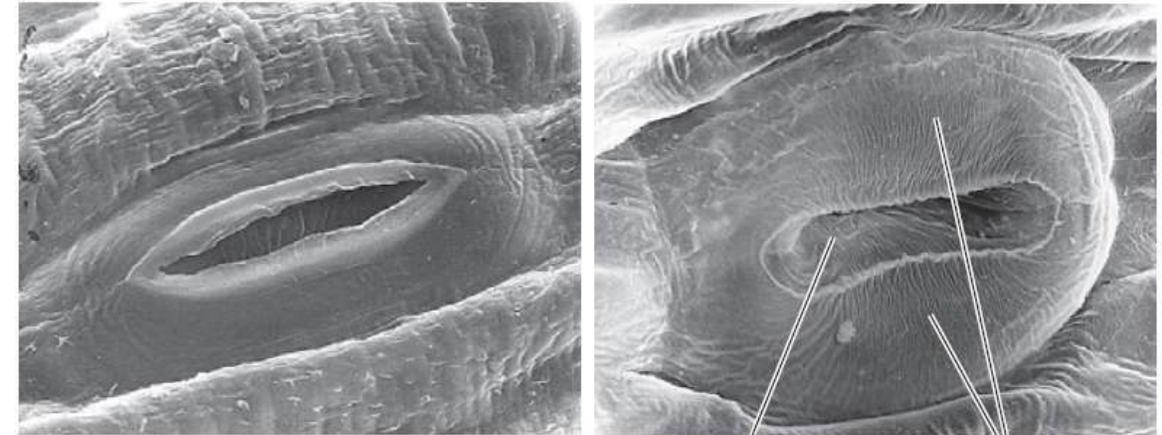


BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Three key structures have the greatest impact on plants' hydraulic function and water relations

□ STOMATA

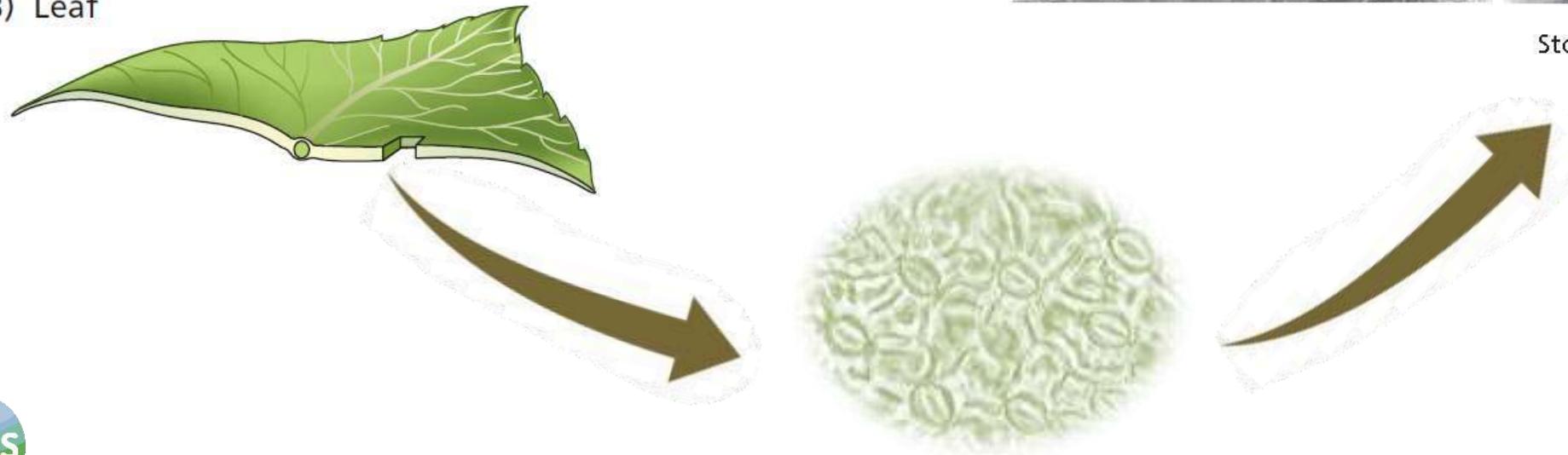
Formed by two modified epidermal cells, called the guard cells, control water loss and carbon dioxide uptake needed for carbon assimilation during photosynthesis.



Stomatal pore

Guard cells

(B) Leaf



BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

All of these structures are fundamental for maintaining the water balance of plants

What does the plant do to maintain this equilibrium?

Demand

Atmosphere

Leaf area

Stomata

Crop load



Supply

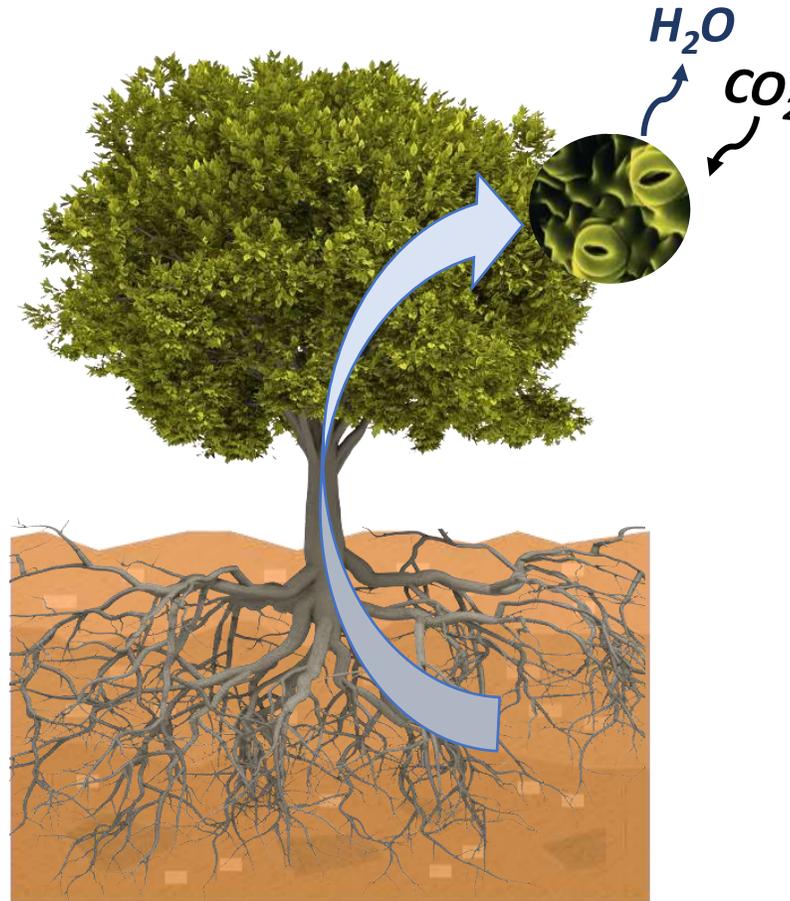
Soil

Soil water

Soil-root hydraulics

Plant hydraulics

Root area



Stomatal conductance
x
Atmospheric demand (VPD)

||

Water potential gradient
x
Hydraulic conductivity

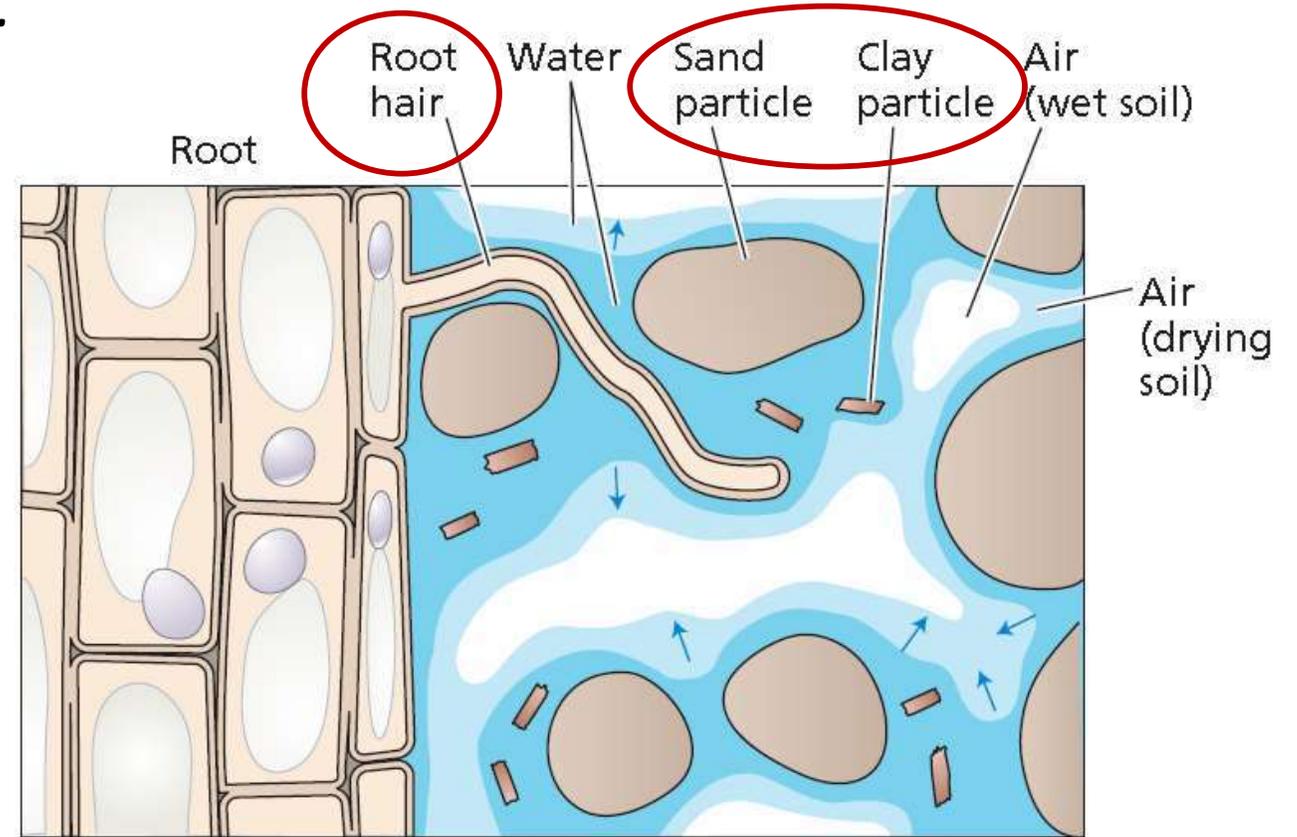
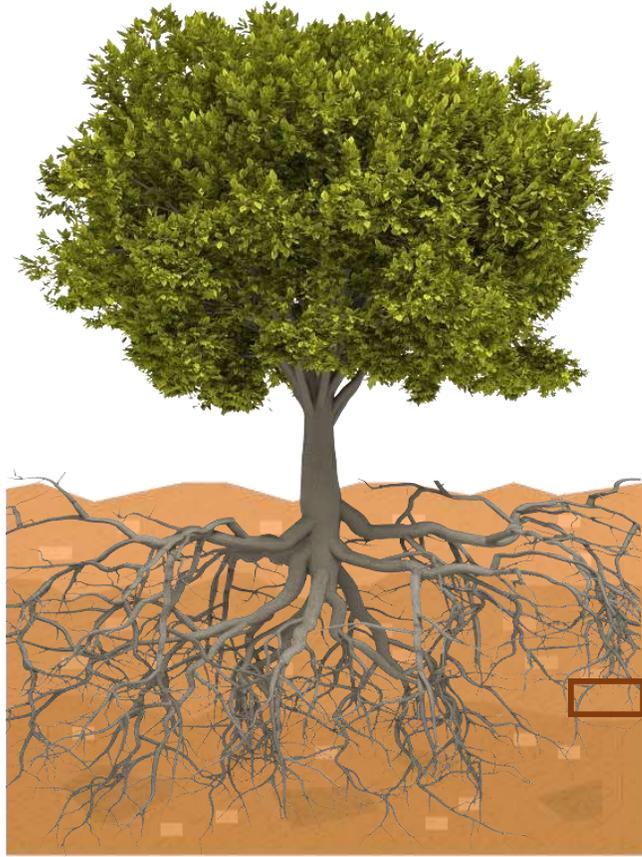


BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Starting at the soil-root processes...

How water moves in the soil...

And roots absorb the water needed by the plant...

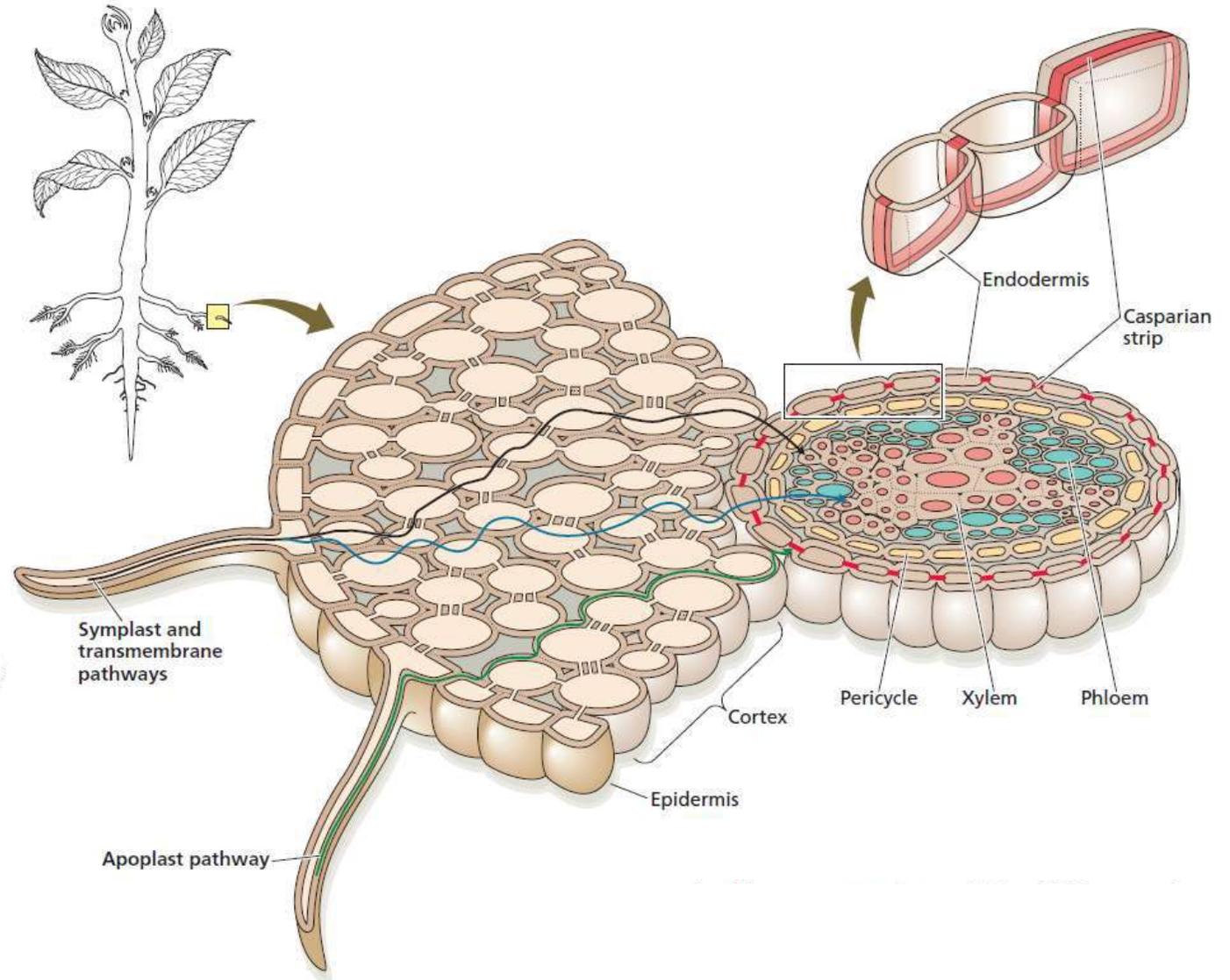
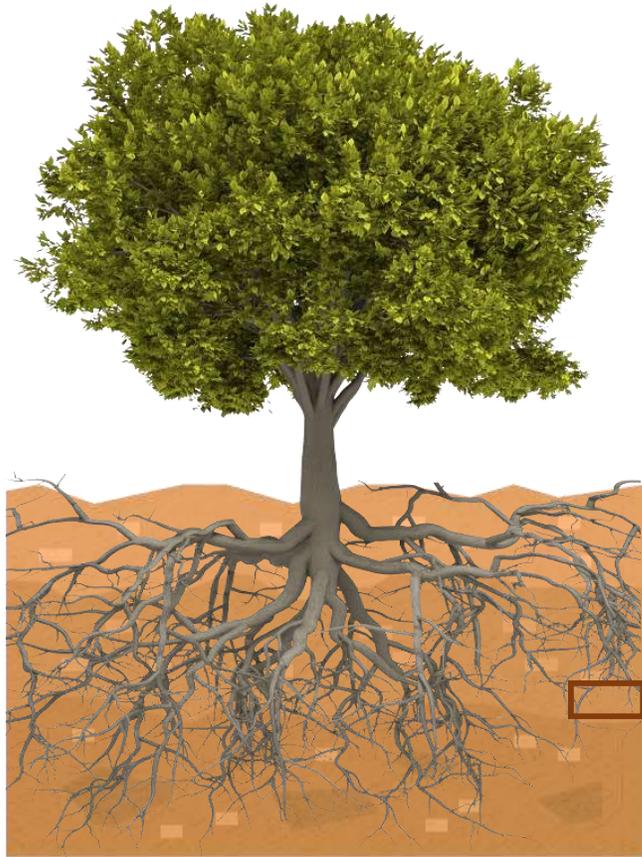


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BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Water absorption by roots



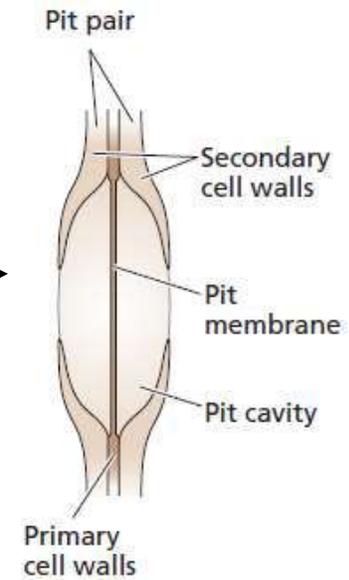
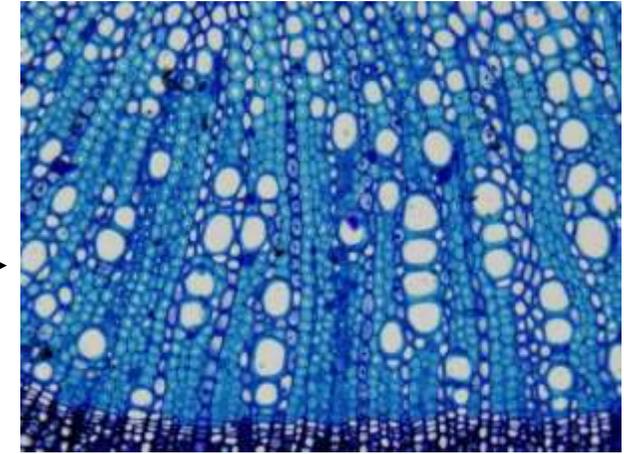
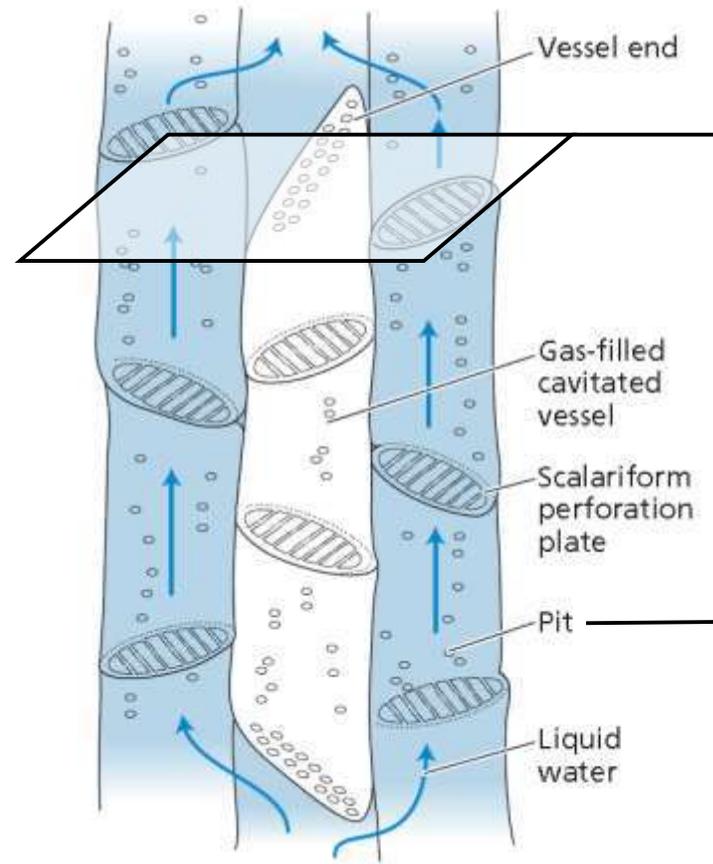
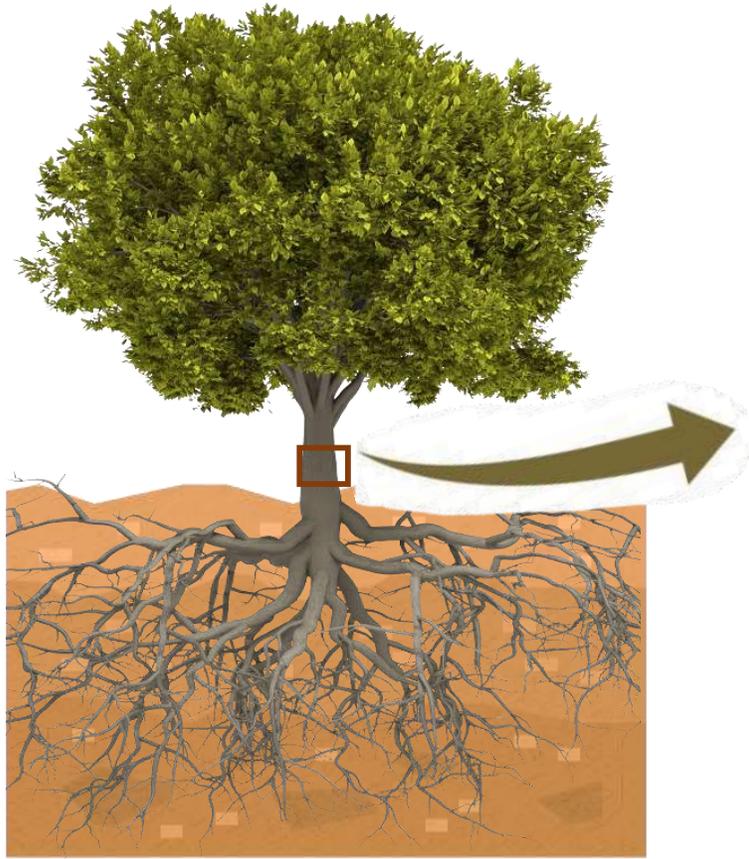
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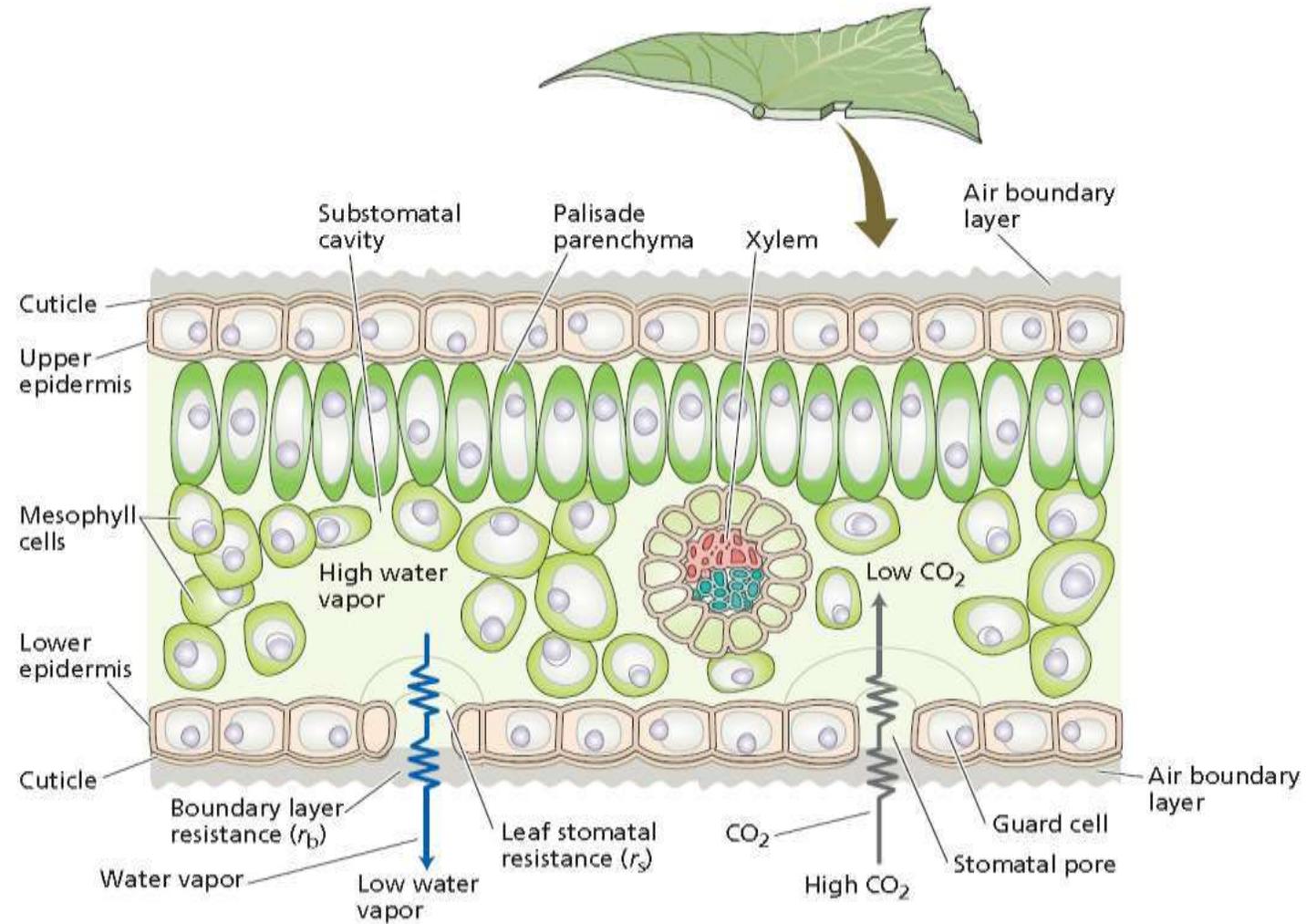
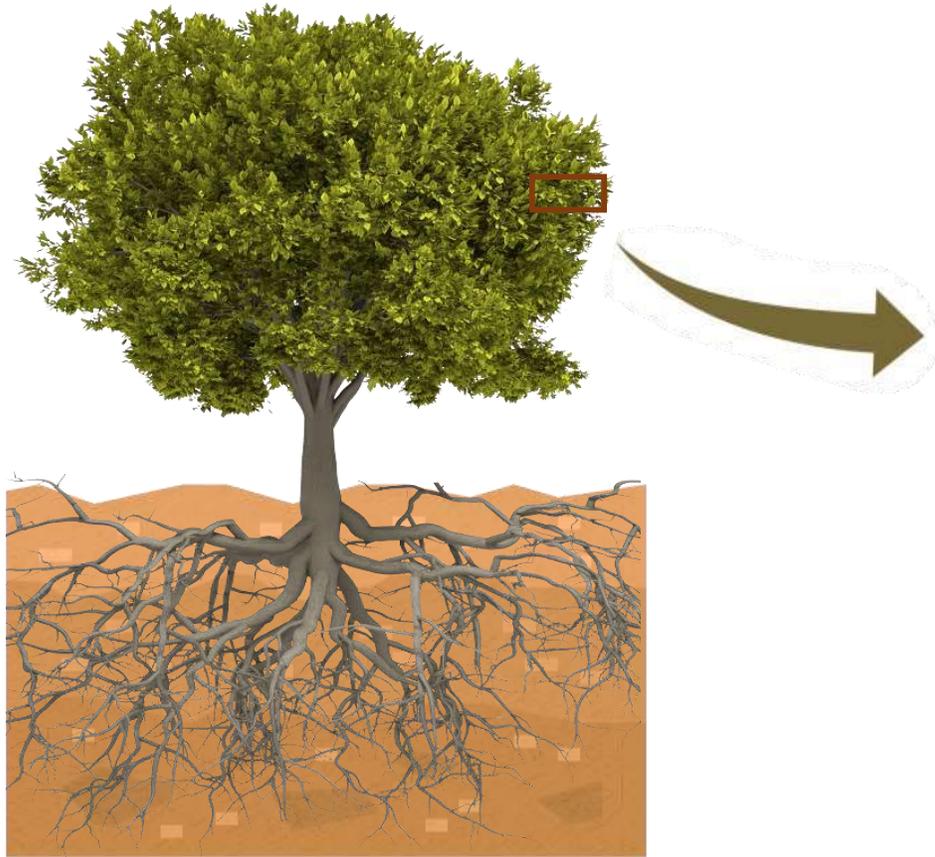
BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Water transport through the xylem



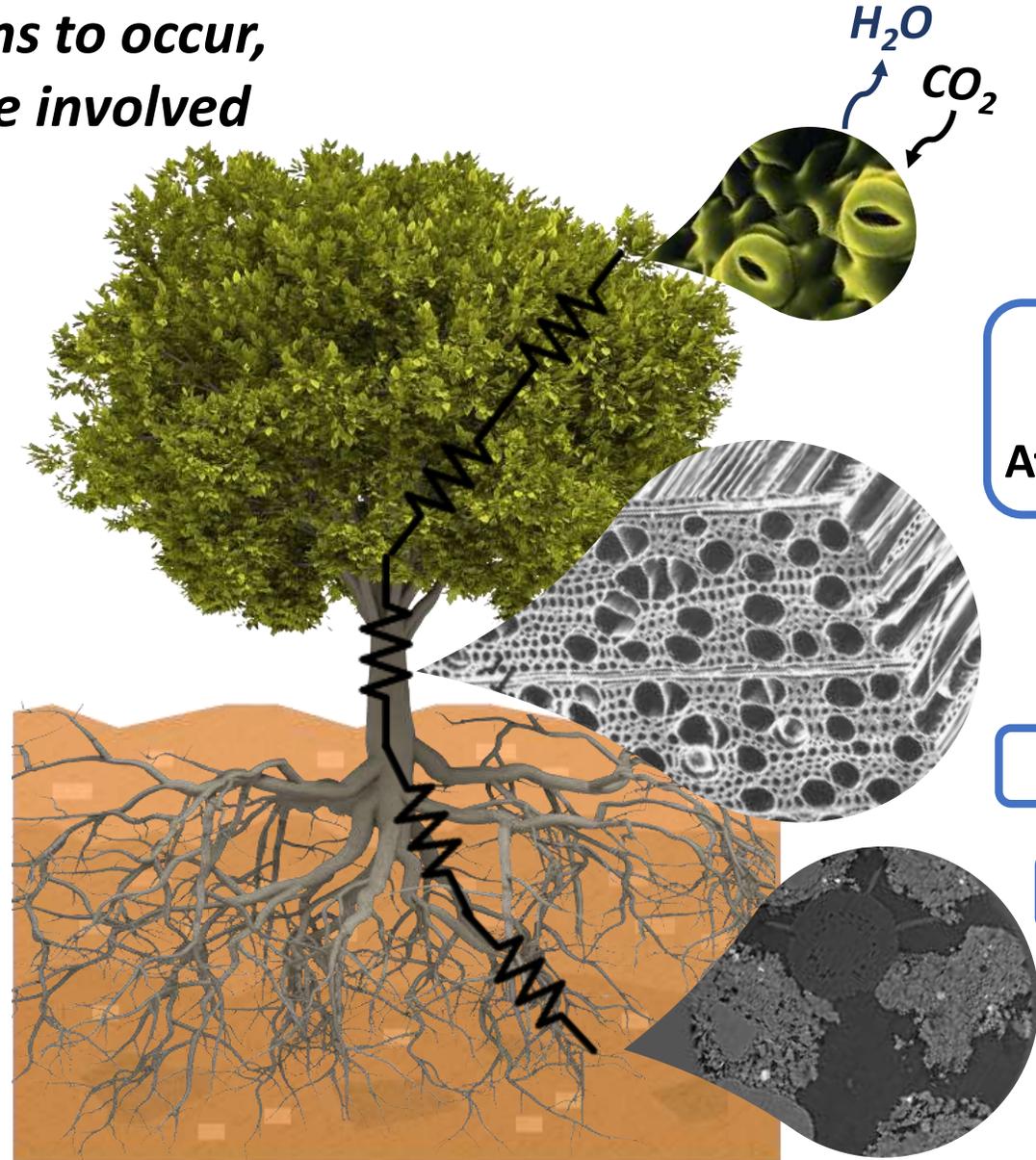
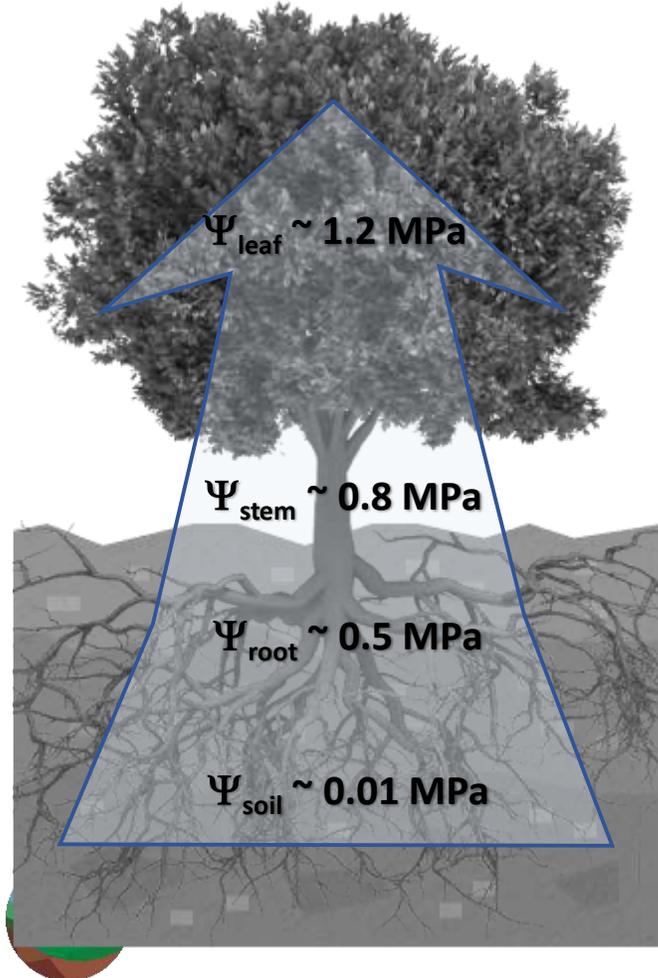
BASIC STRUCTURE OF VASCULAR PLANTS AND THEIR ROLE IN WATER TRANSPORT

Water movement from the leaf to the atmosphere



BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

For all of the previous functions to occur, three main plant processes are involved



TRANSPIRATION

Stomatal conductance
x
Atmospheric demand (VPD)

||

WATER POTENTIAL

Water potential gradient

x

Hydraulic conductivity

HYDRAULIC CONDUCTIVITY

BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water potential, a fundamental thermodynamic concept to understand water movement



Represents the ability of a system to gain or give up water

It comes from the thermodynamic concept of **FREE ENERGY**
[capacity of a system to perform work]

Ψ **high** tendency to **give up** water

Ψ **low** tendency to **gain** water

Movement of water

high Ψ
[-0.5 MPa]



low Ψ
[-1.5 MPa]



BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water potential at cell level & Components



It is measured in pressure units (bar, MPa...) and it is **NEGATIVE**

[pure water: $\Psi = 0$ MPa]

Reflects the gravitational pull of the water column

Ψ_g

$$\Psi_g = \rho_w g h$$

0.01 MPa m⁻¹

$$\Psi = \Psi_s + \Psi_p + \cancel{\Psi_g}$$

solutes *pressure* *gravity*



$h = 10$ m



**0.1 MPa
increase in Ψ**

BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water potential at cell level & Components

Ψ_s

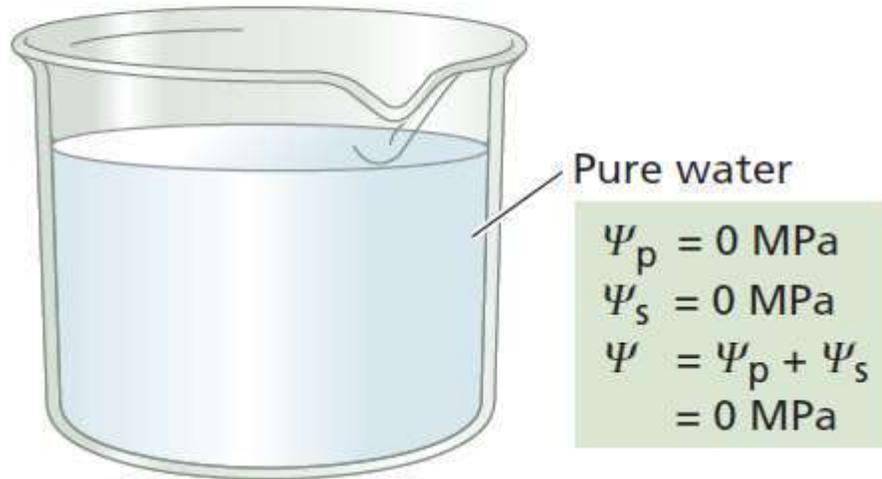
Represents the effect of dissolved solutes on Ψ

It is **NEGATIVE** and the addition of solutes always decreases Ψ

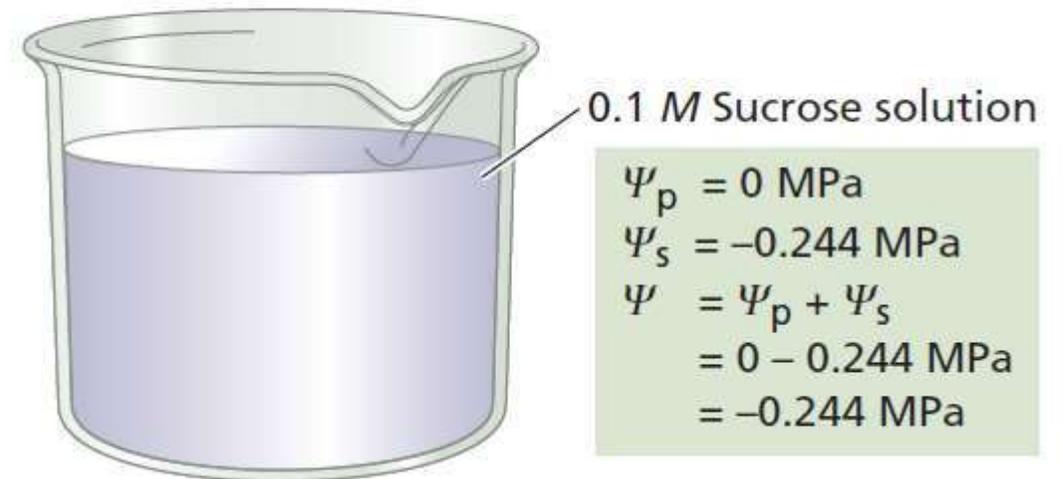
$$\Psi = \Psi_s + \Psi_p$$

solutes *pressure*

(A) Pure water



(B) Solution containing 0.1 M sucrose

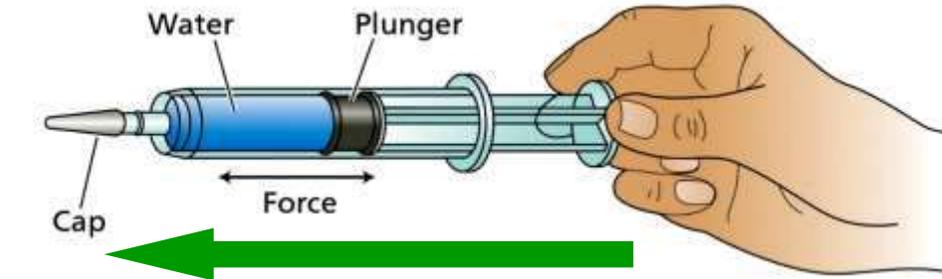


BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water potential at cell level & Components

Ψ_p

Represents the effect of hydrostatic pressure on Ψ
 Depending on the system, it can be **POSITIVE** or **NEGATIVE**
 $+\Psi_p \rightarrow$ increases Ψ
 $-\Psi_p \rightarrow$ decreases Ψ



HYDROSTATIC PRESSURE
 $+\Psi_p$

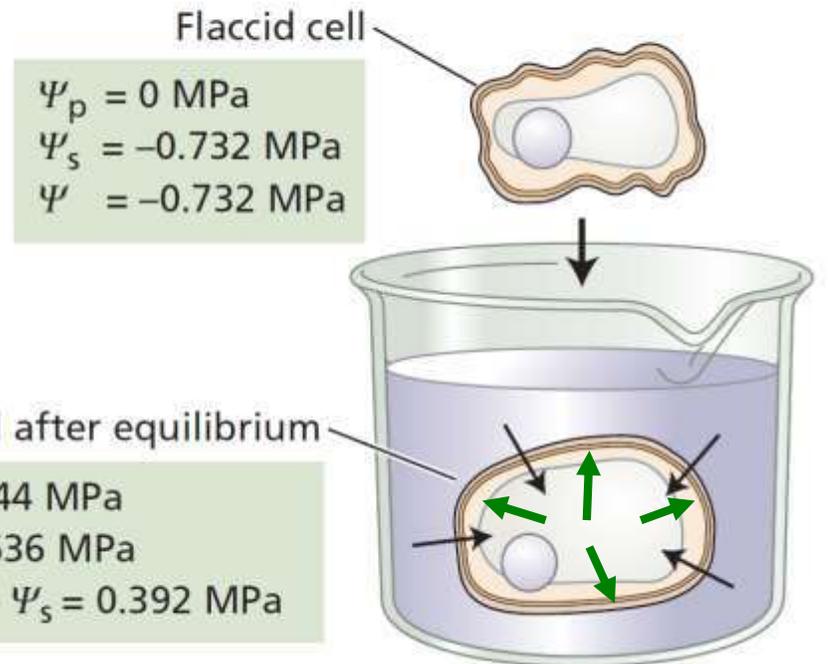
TURGOR PRESSURE

TENSION
 $-\Psi_p$

$$\Psi = \Psi_s + \Psi_p$$

solutes *pressure*

(C) Flaccid cell dropped into sucrose solution



BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water potential at cell level & Components

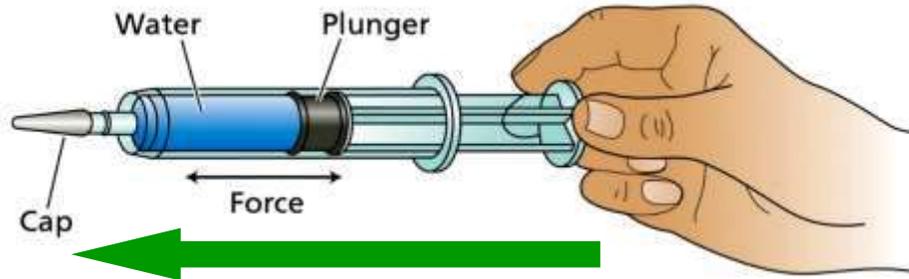
Ψ_p

Represents the effect of hydrostatic pressure on Ψ

Depending on the system, it can be **POSITIVE** or **NEGATIVE**

+ Ψ_p -> increases Ψ

- Ψ_p -> decreases Ψ



HYDROSTATIC PRESSURE

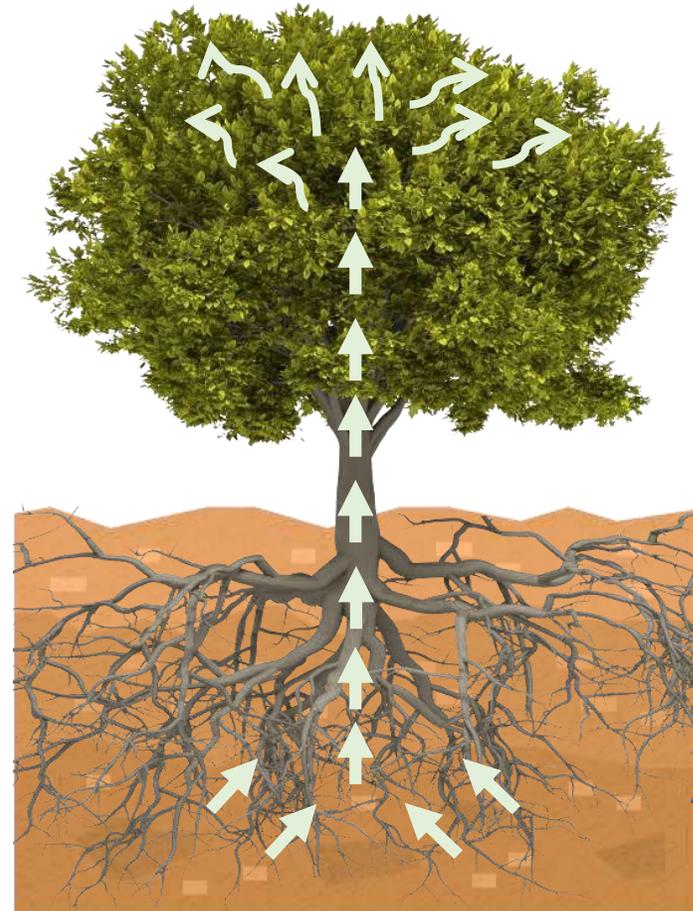
+ Ψ_p

TENSION

- Ψ_p

$$\Psi = \Psi_s + \Psi_p$$

solutes *pressure*



$\Psi_{\text{leaf}} \sim 1.2 \text{ MPa}$

$\Psi_{\text{stem}} \sim 0.8 \text{ MPa}$

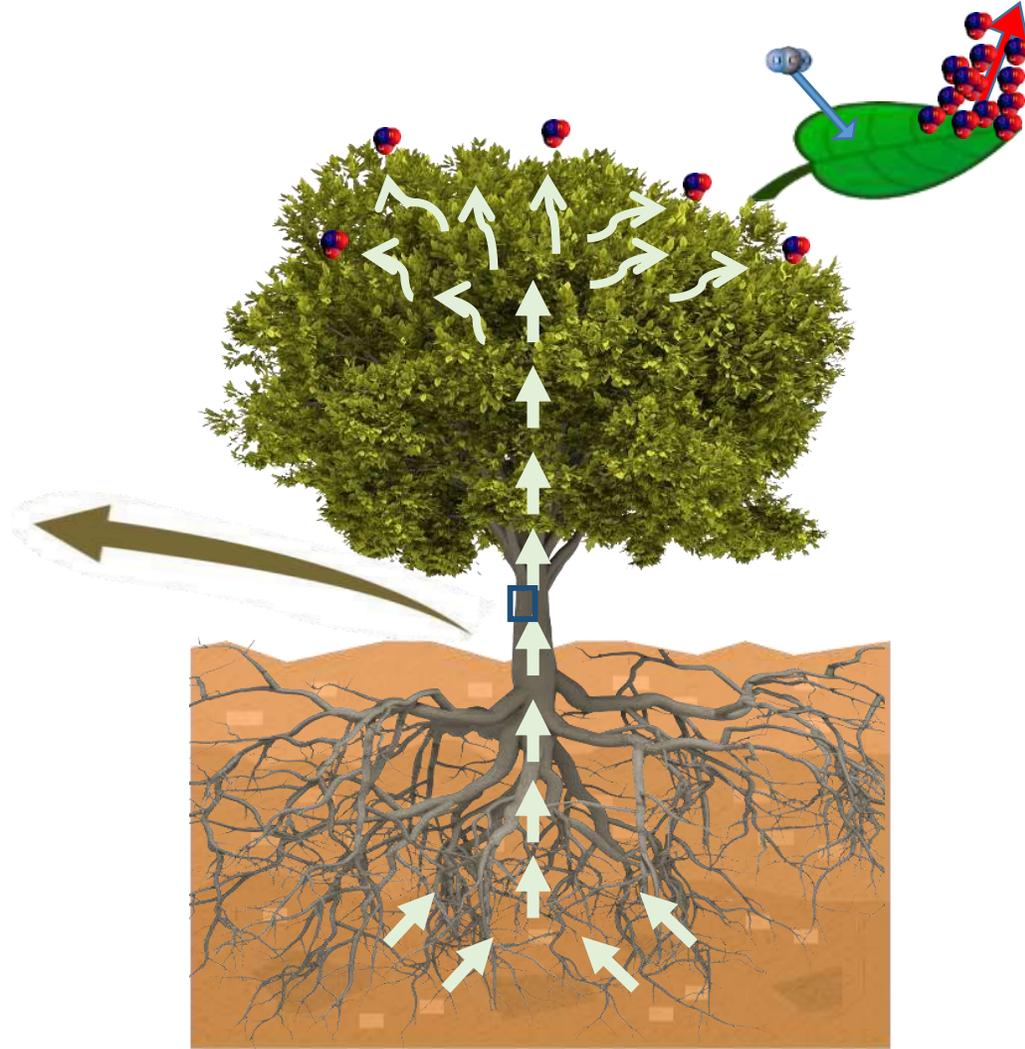
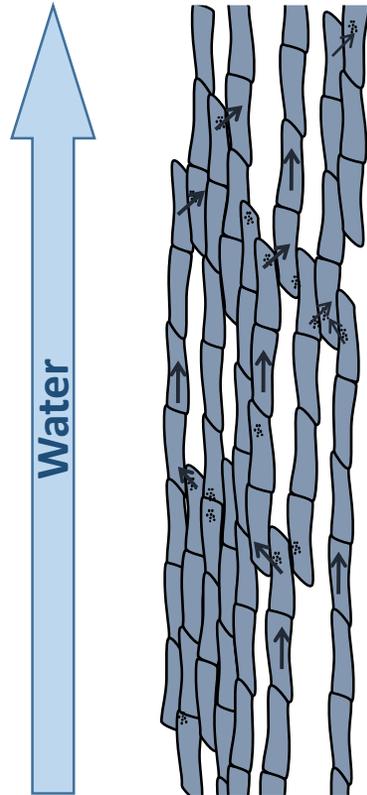
$\Psi_{\text{root}} \sim 0.5 \text{ MPa}$

$\Psi_{\text{soil}} \sim 0.01 \text{ MPa}$

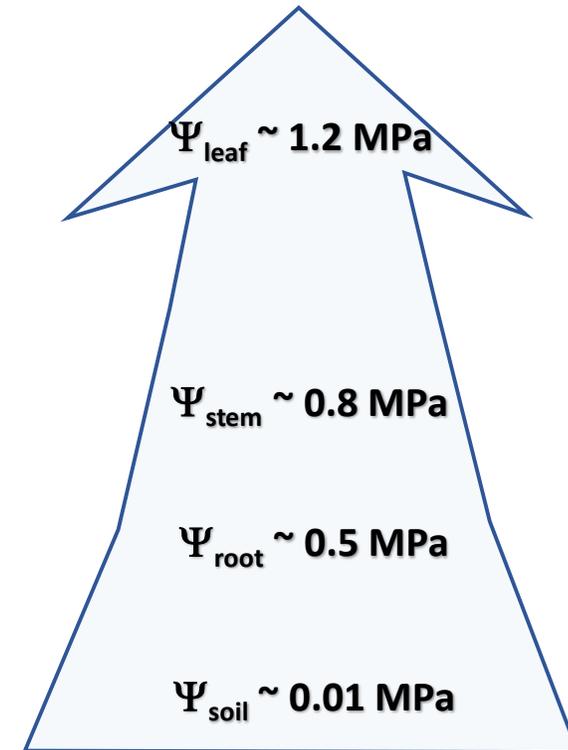
BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Cohesion-tension theory of sap ascent

XYLEM VESSELS



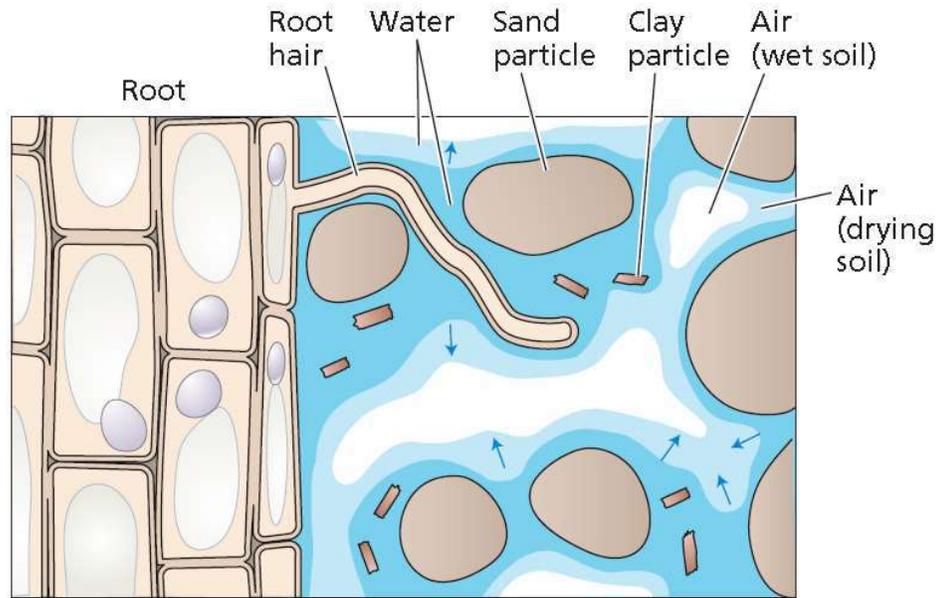
Transpiration



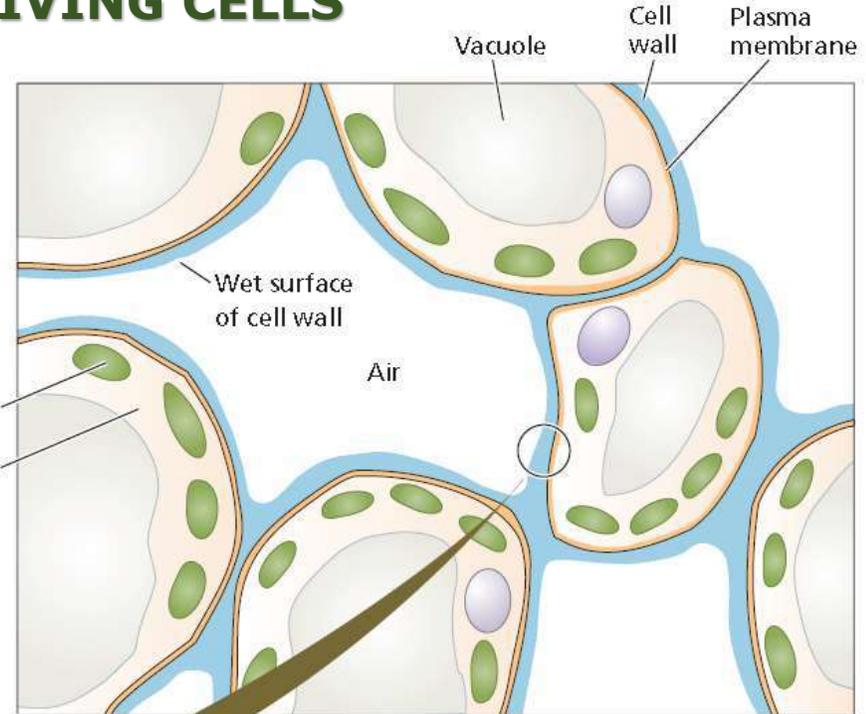
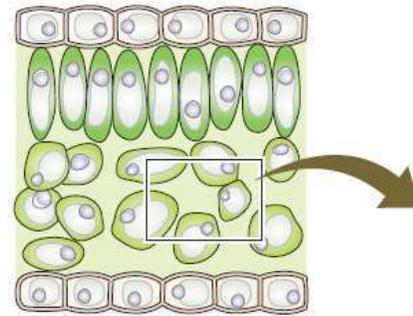
BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Cohesion-tension theory of sap ascent

SOIL



LIVING CELLS

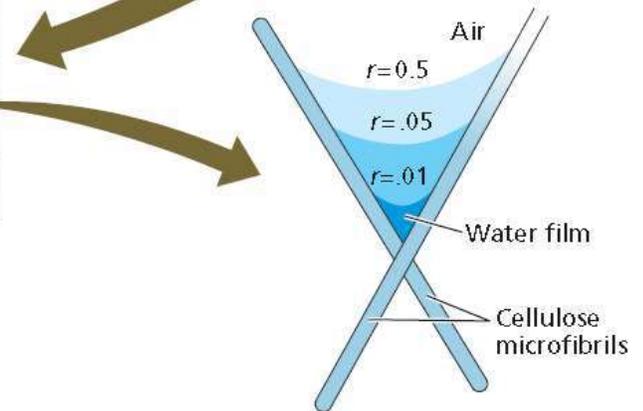
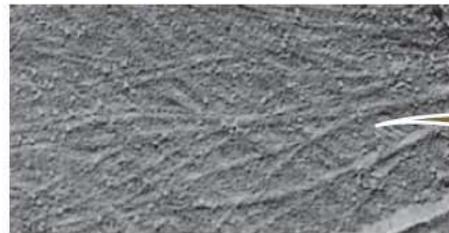


Radius of curvature (μm)	Hydrostatic pressure (MPa)
0.5	-0.3
0.05	-3
0.01	-15

Surface tension of water

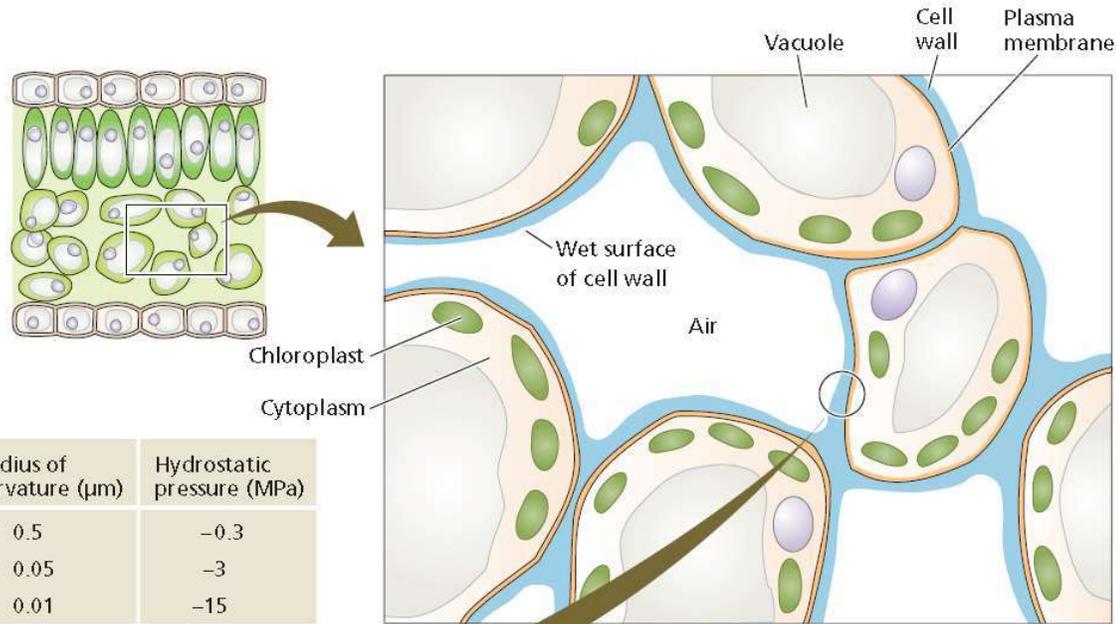
$$\Psi_p = \frac{-2T}{r}$$

Radius of curvature of air-water interface

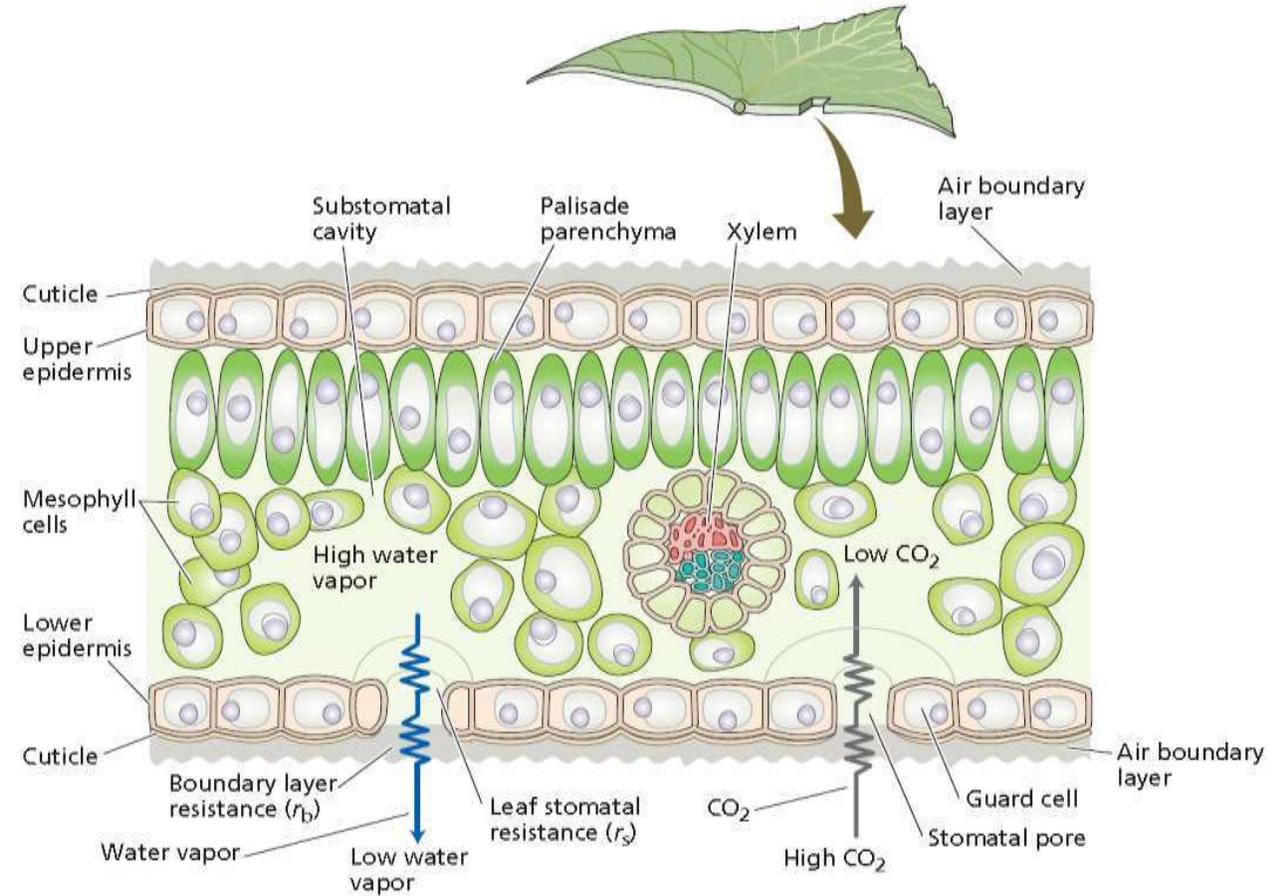
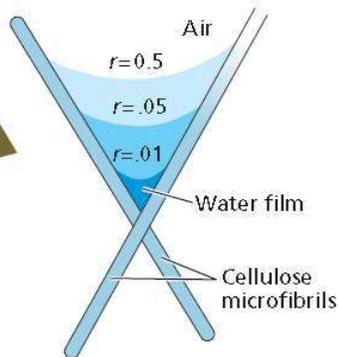
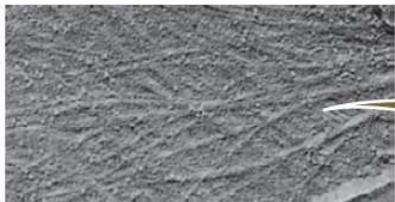


BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

*Another process essential for water balance of plants is **TRANSPIRATION***



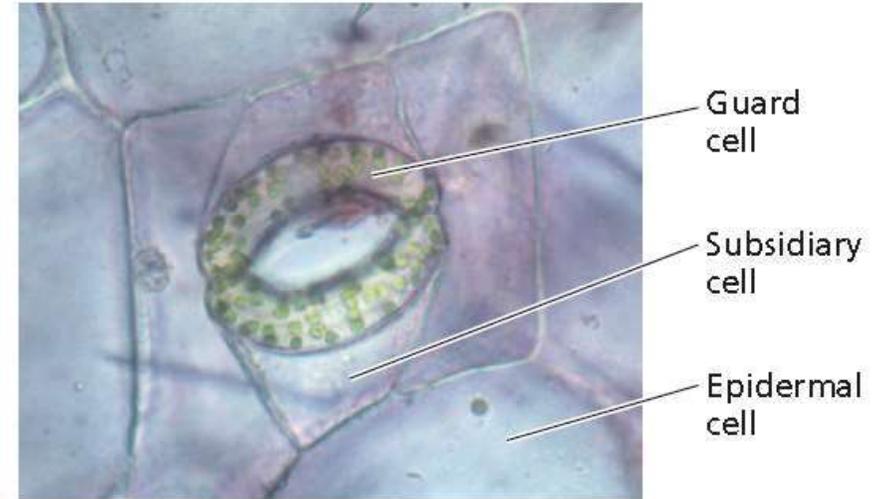
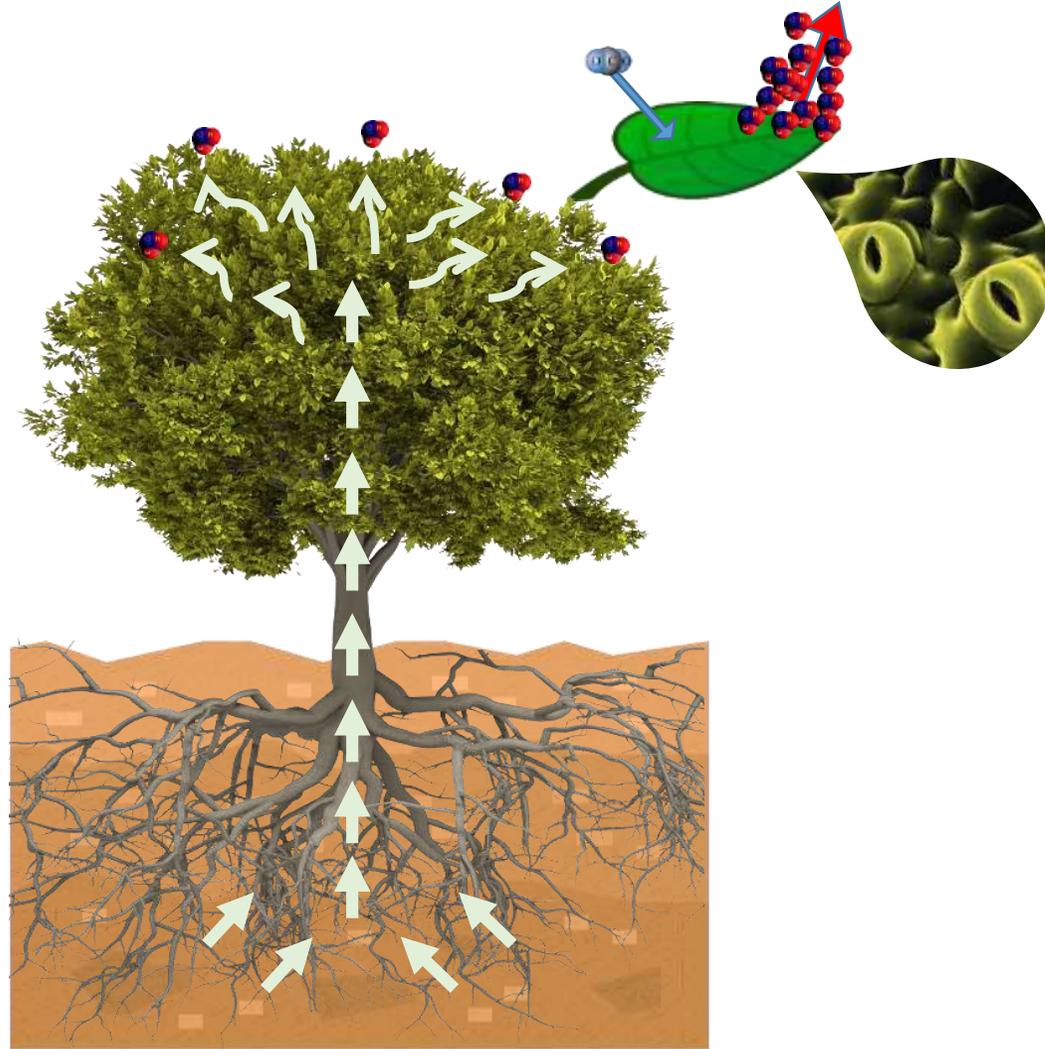
Radius of curvature (μm)	Hydrostatic pressure (MPa)
0.5	-0.3
0.05	-3
0.01	-15



> 95% of water evaporates from leaves through **STOMATA**
 < 5% of water for photosynthesis and growth

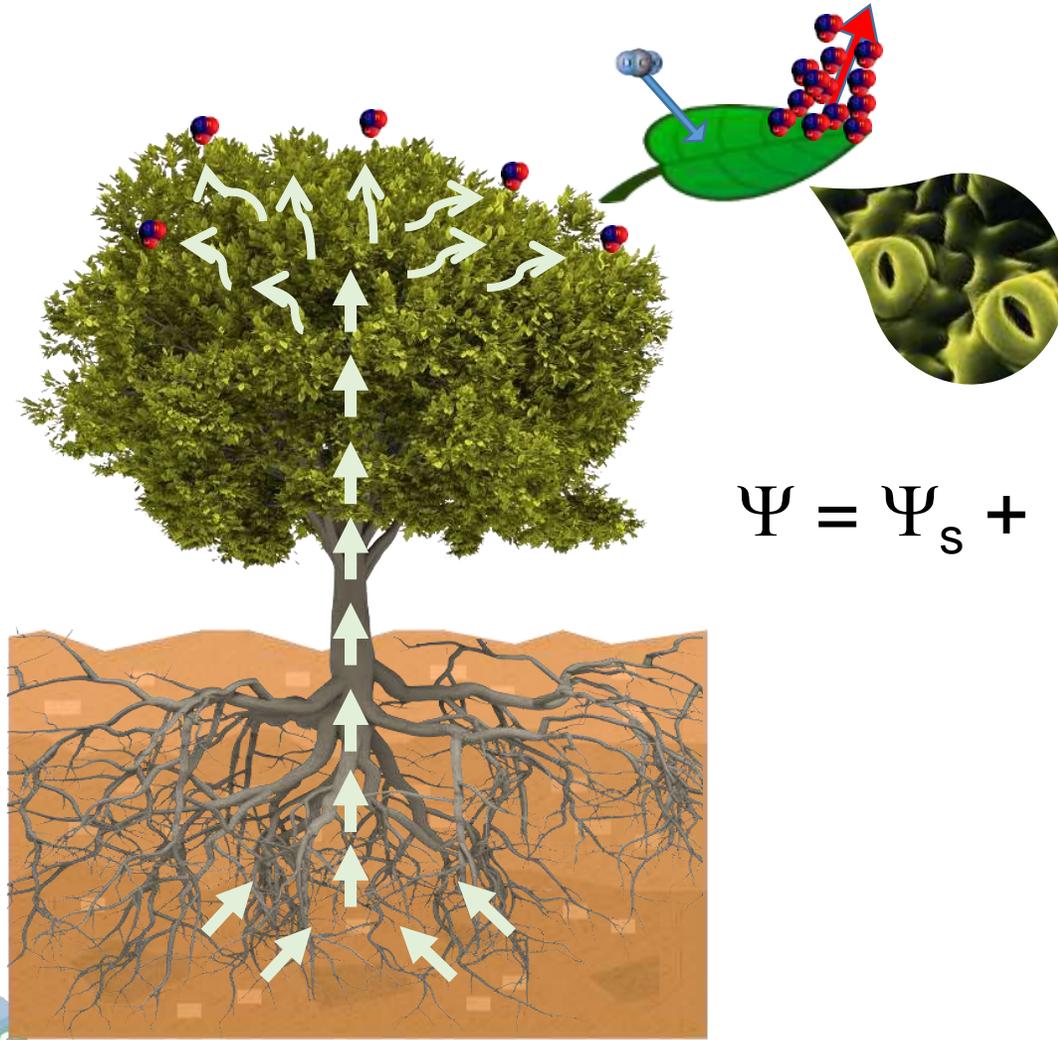
BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Stomatal control of transpiration

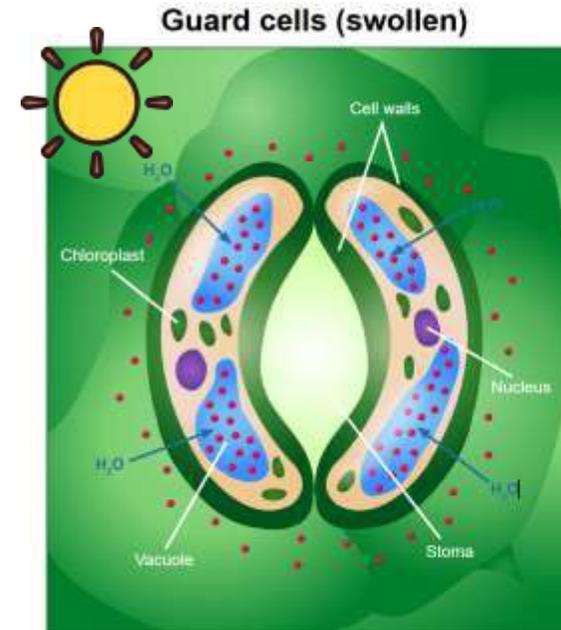


BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

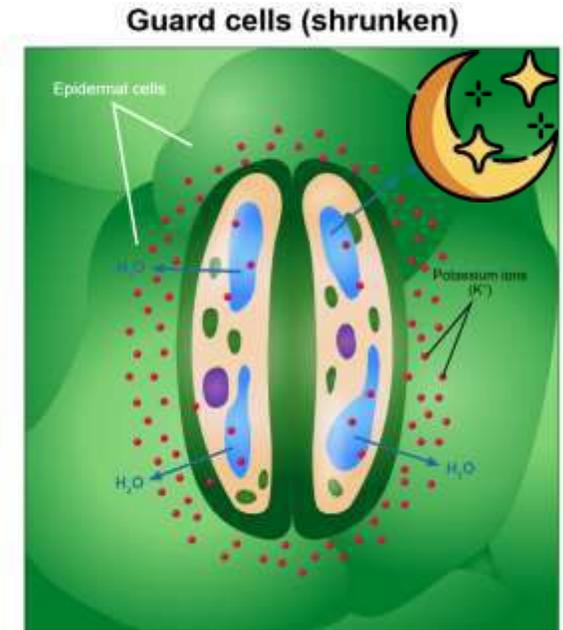
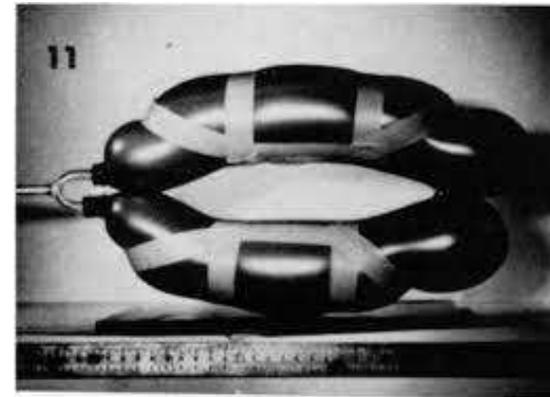
Stomatal control of transpiration



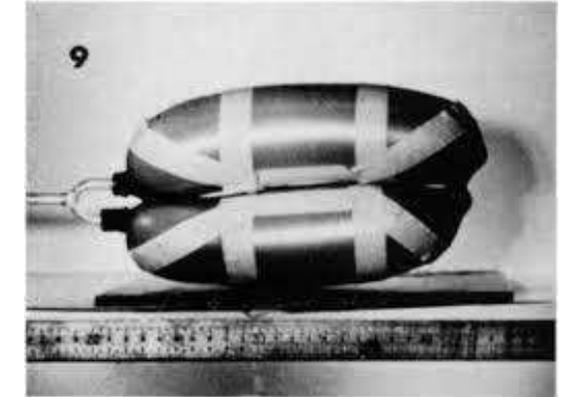
$$\Psi = \Psi_s + \Psi_p$$



↑ Ψ_p

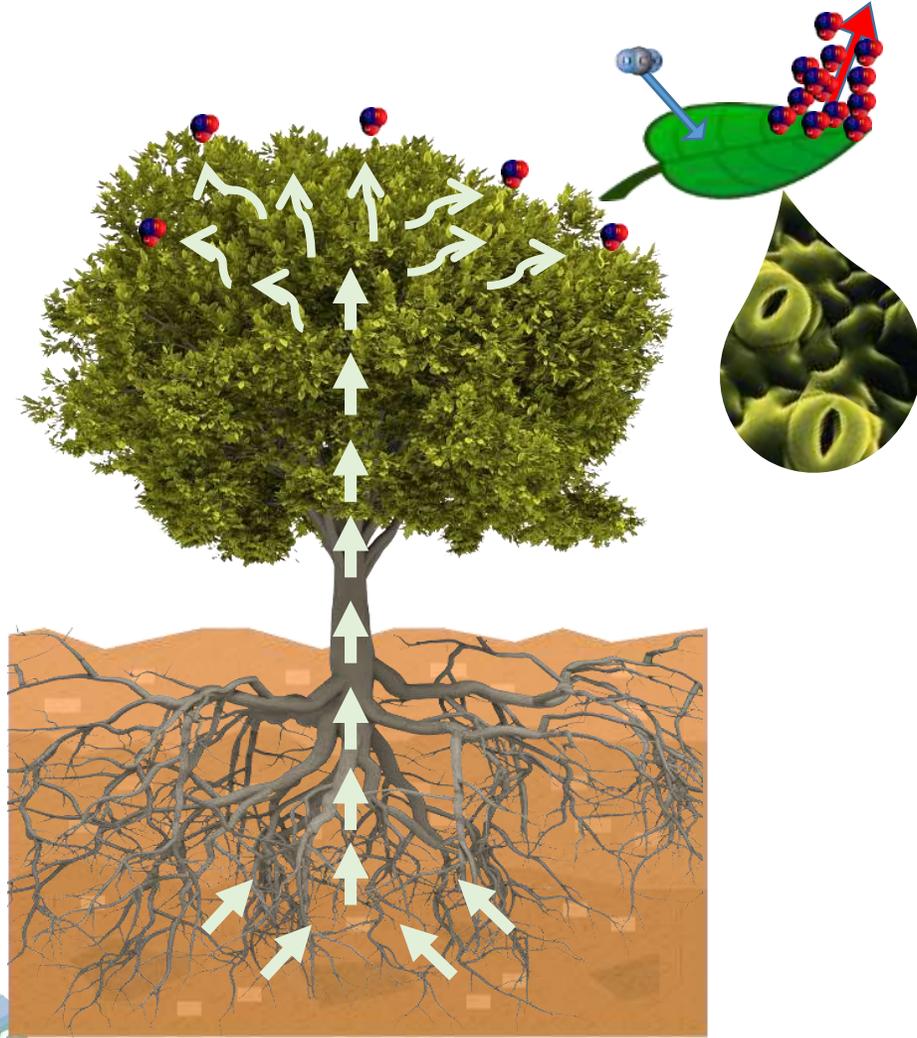


↓ Ψ_p



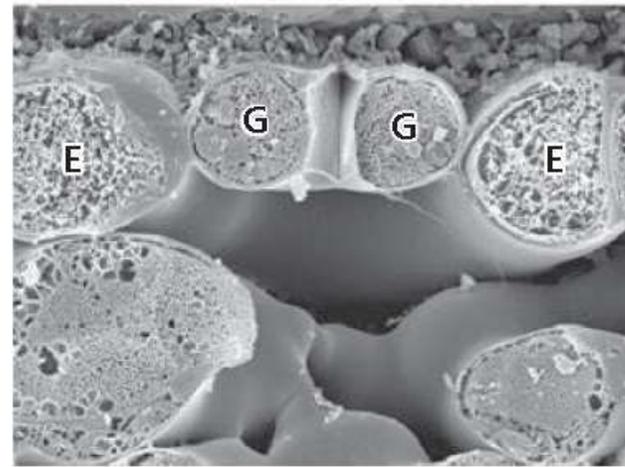
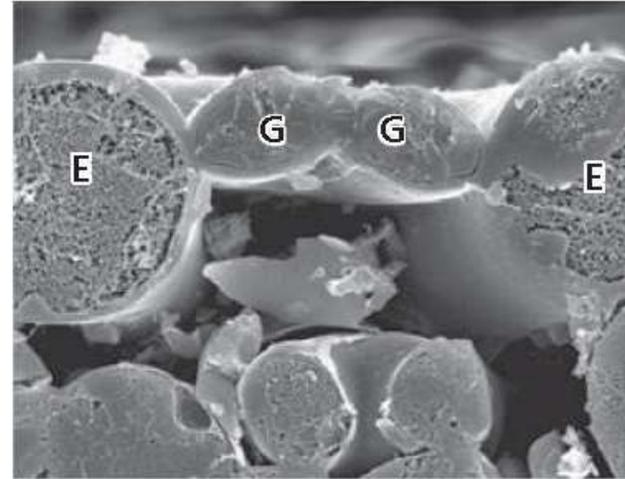
BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Stomatal control of transpiration



FERN

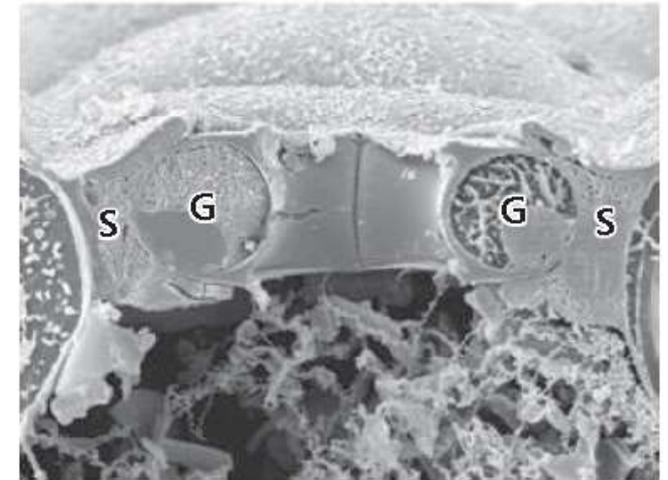
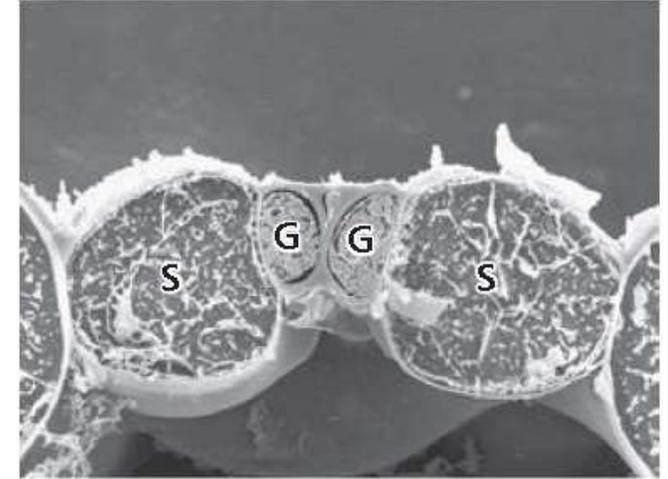
(A) *Nephrolepis exaltata*



20 μm

ANGIOSPERM

(C) *Tradescantia virginiana*

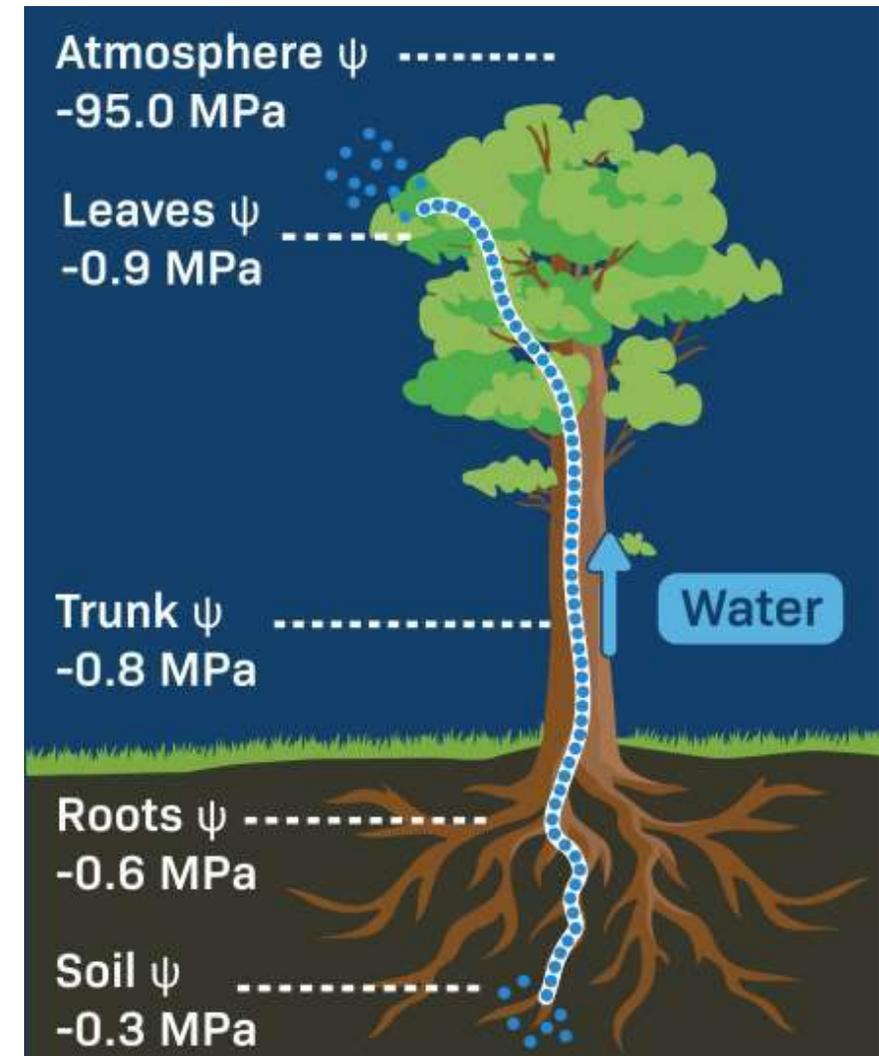
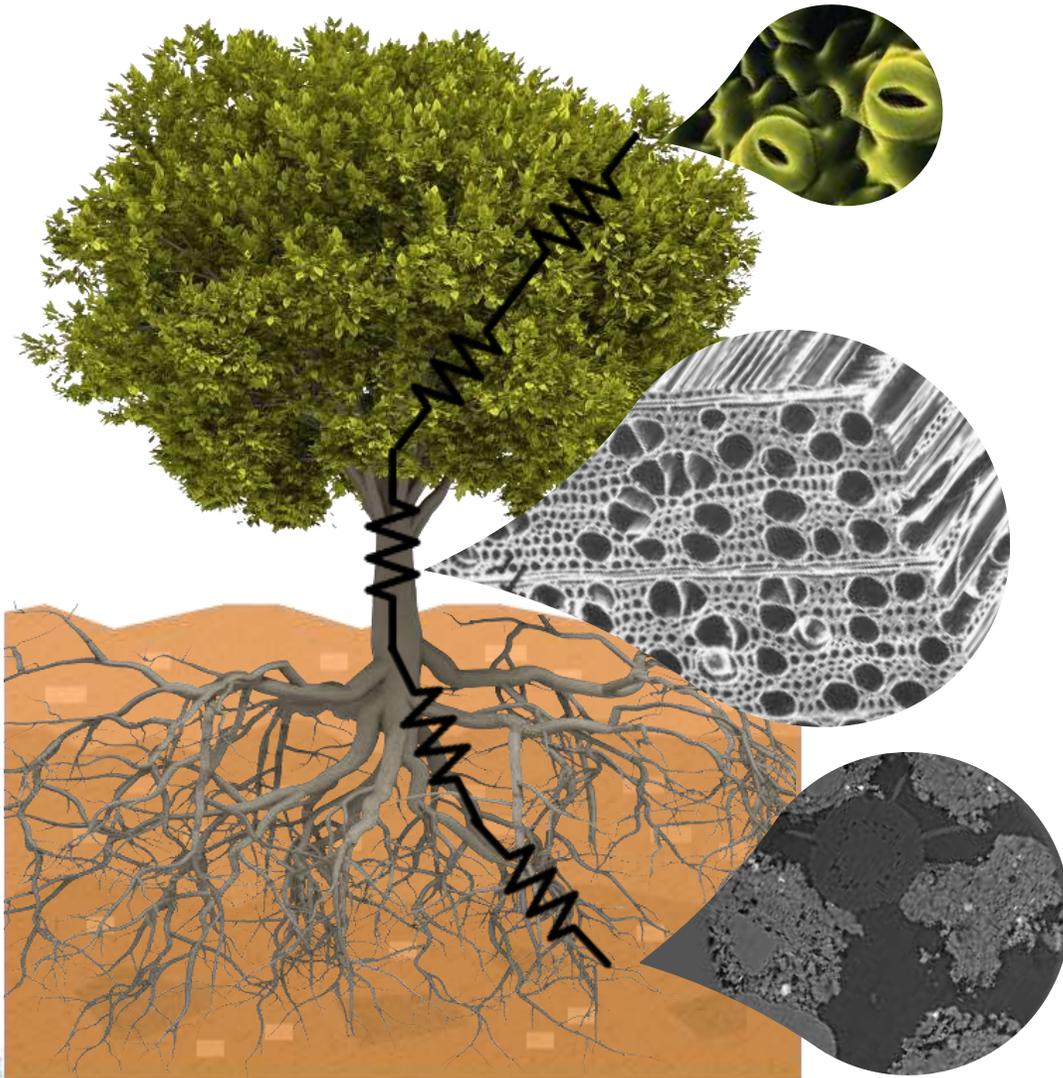


20 μm

BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

HYDRAULIC CONDUCTIVITY is the third essential concept needed to fully understand water balance of plants and their interactions with the environment

$$\text{Transpiration} / \Delta\Psi = \text{Hydraulic conductivity}$$



BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

HYDRAULIC CONDUCTIVITY

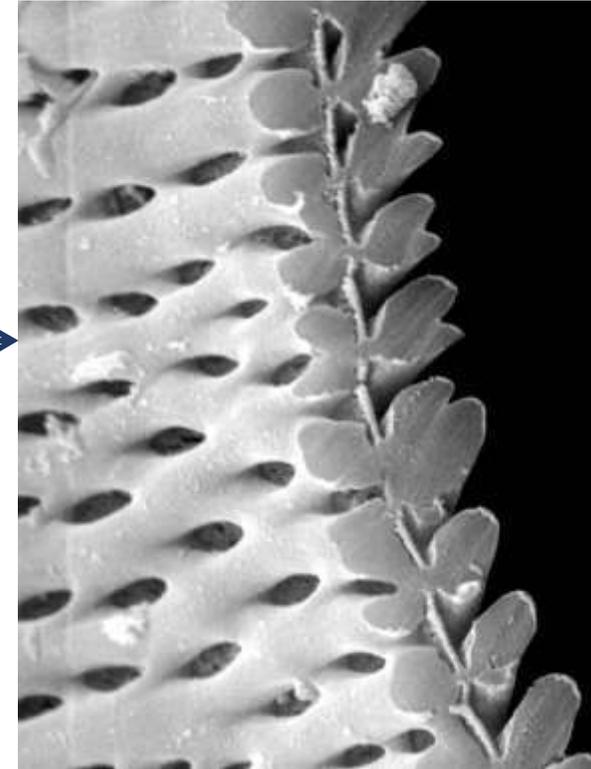
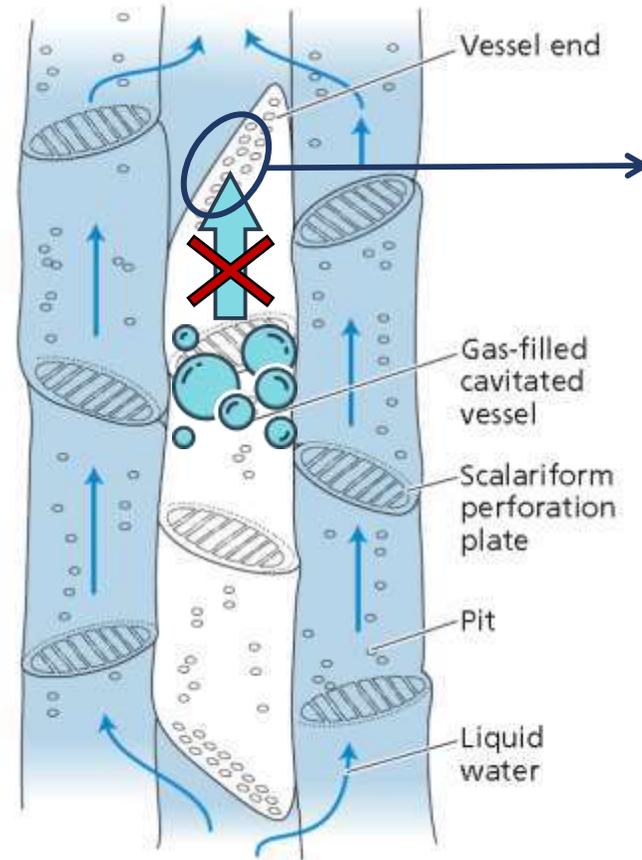
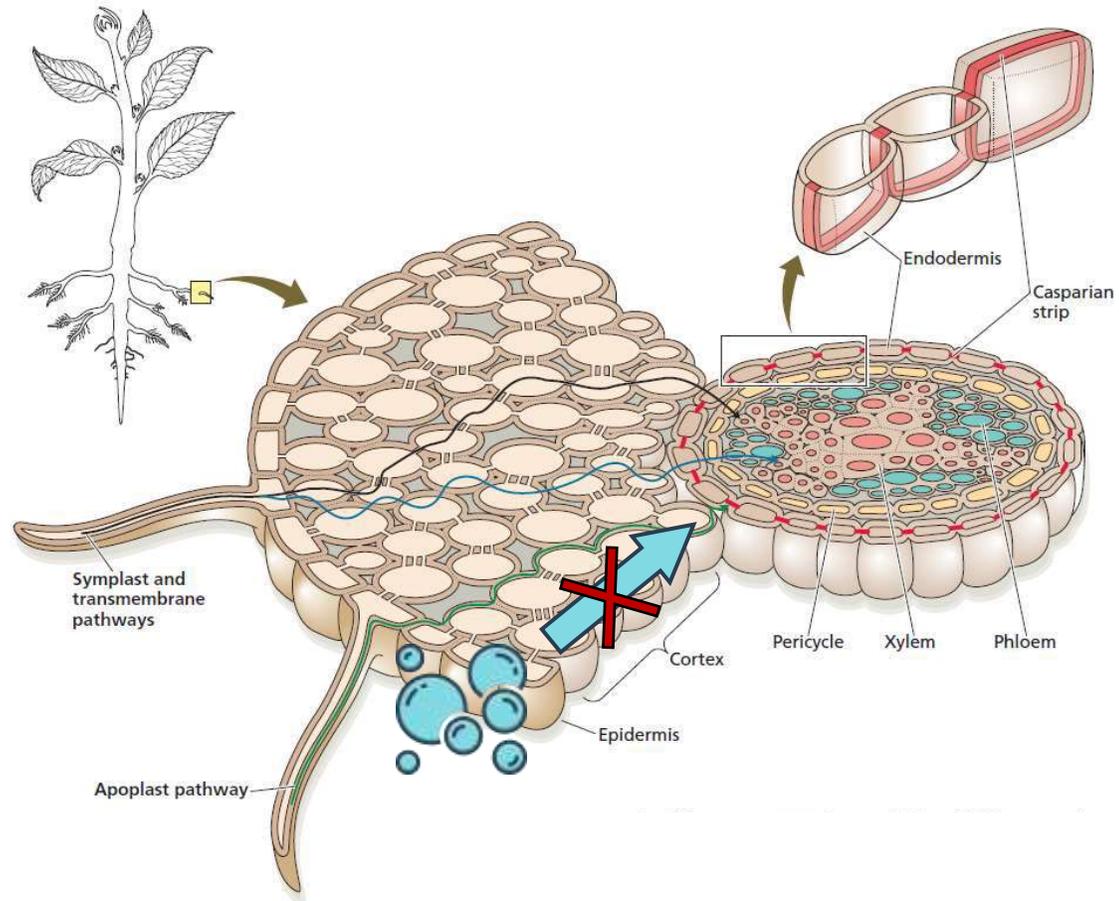
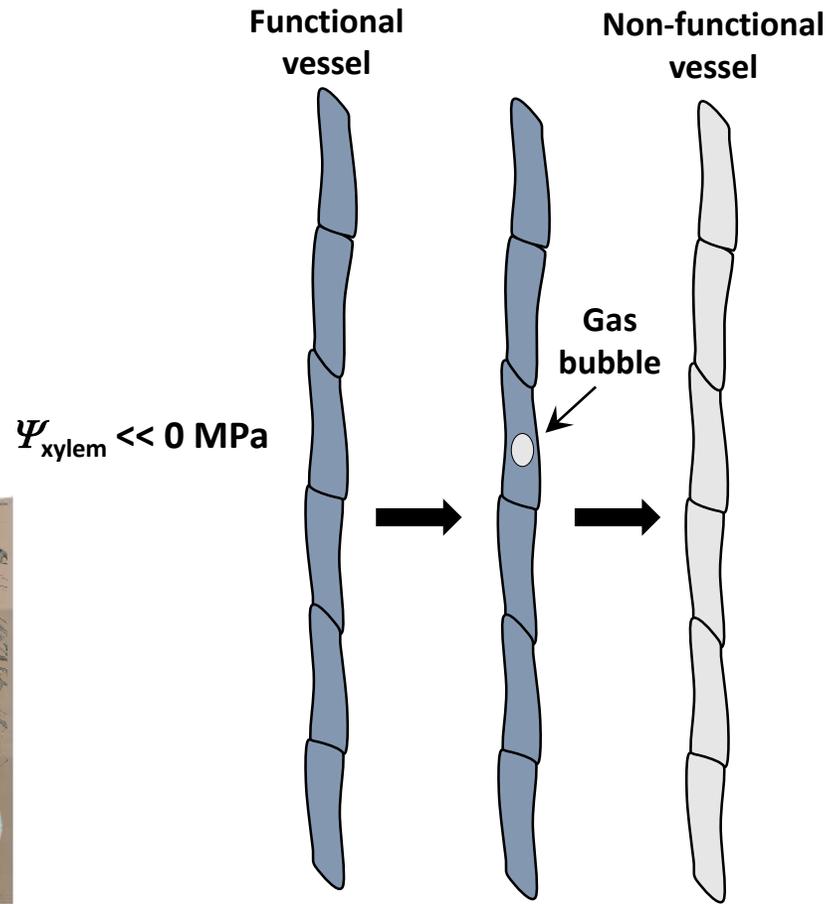
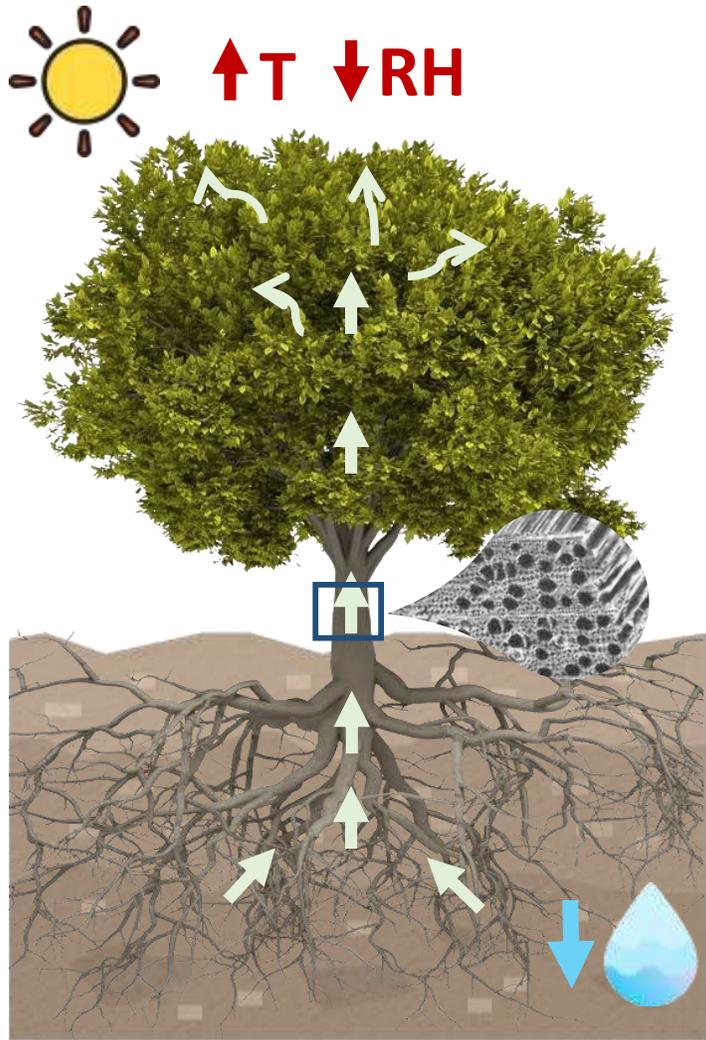


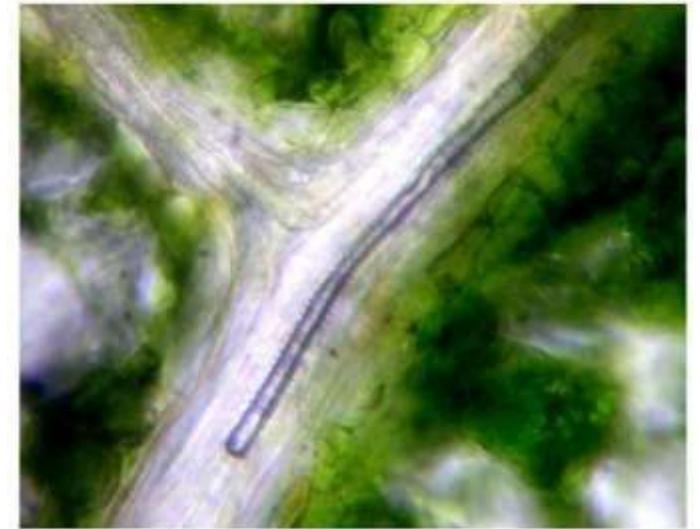
Photo: Jarmila Pittermann

BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS



Cavitation

Abrupt change from liquid water under tension to water vapor



©INRA/Hervé Cochard

Embolism



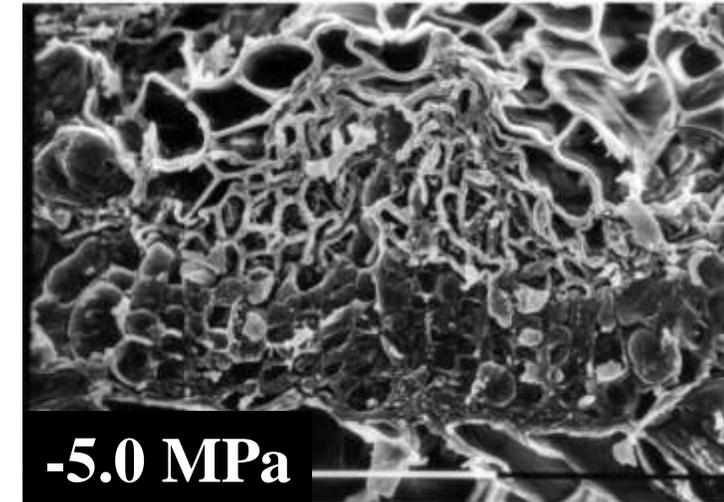
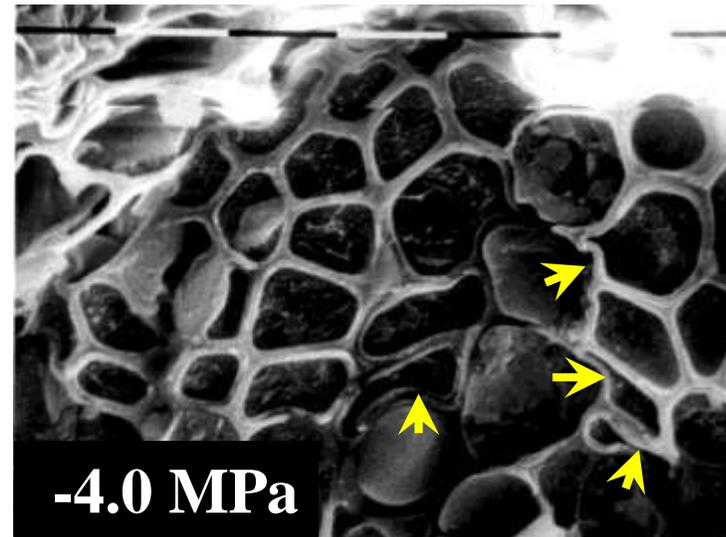
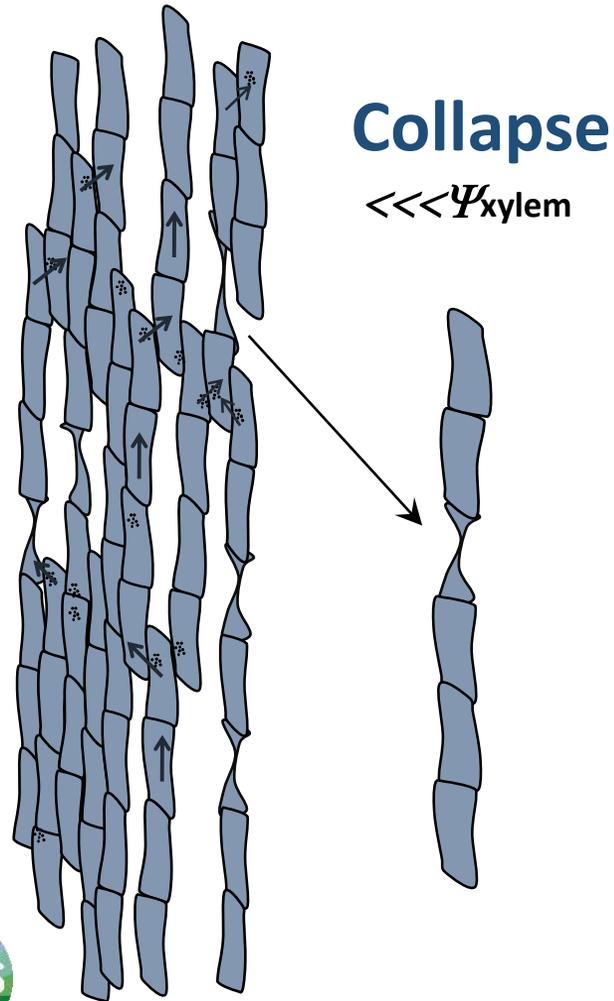
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BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water stress conditions \rightarrow $\downarrow \Psi_{\text{xylem}}$ \rightarrow \uparrow Cavitated conduits \rightarrow $\downarrow K$

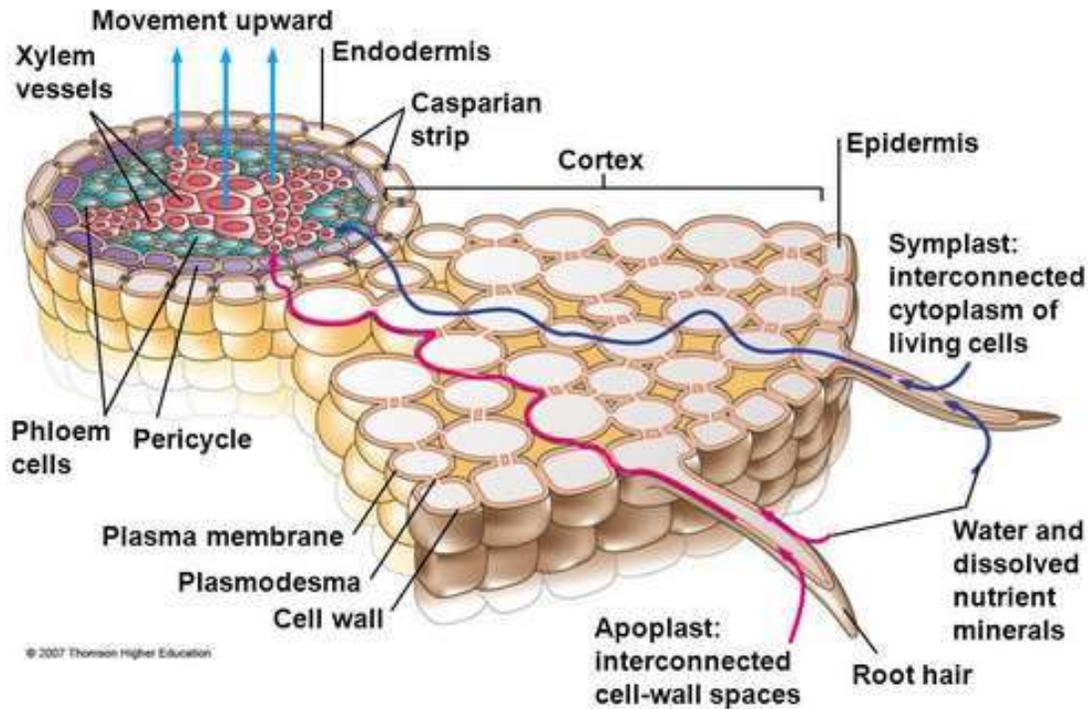


Pinus needles
(*Pinus cembra*)

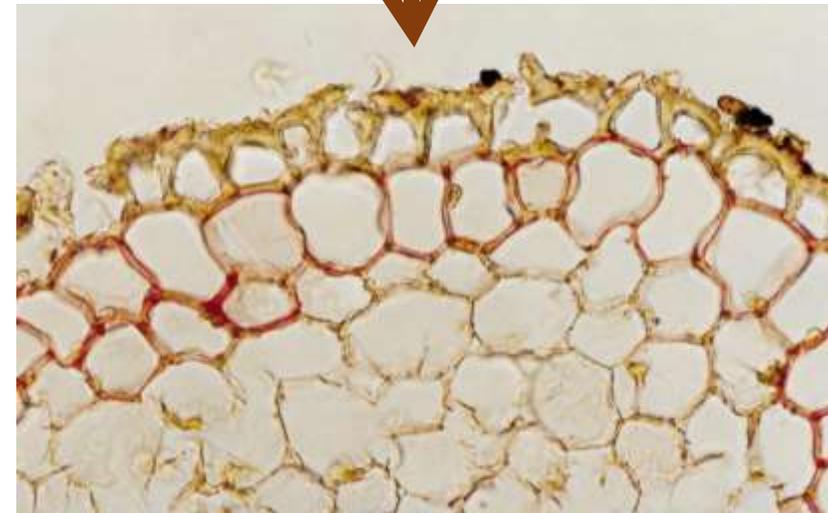
Images from Herve Cochard's website

BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water stress conditions $\rightarrow \downarrow \Psi_{\text{xylem}} \rightarrow \uparrow$ Cavitated conduits $\rightarrow \downarrow K$

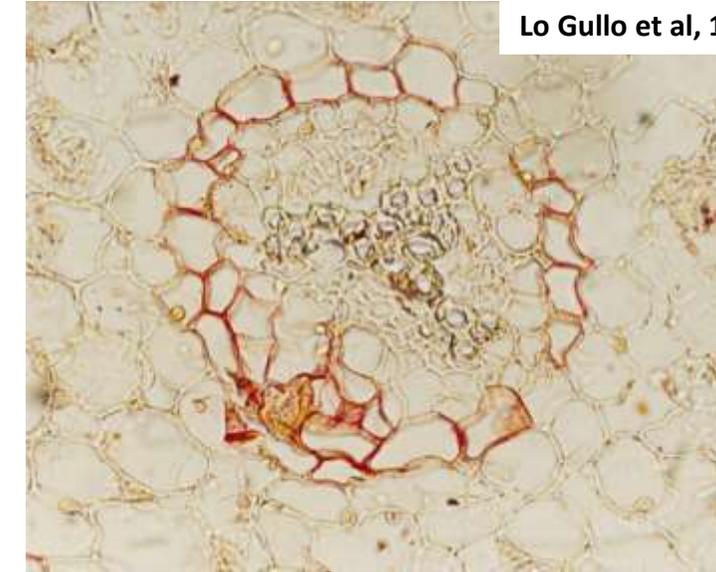
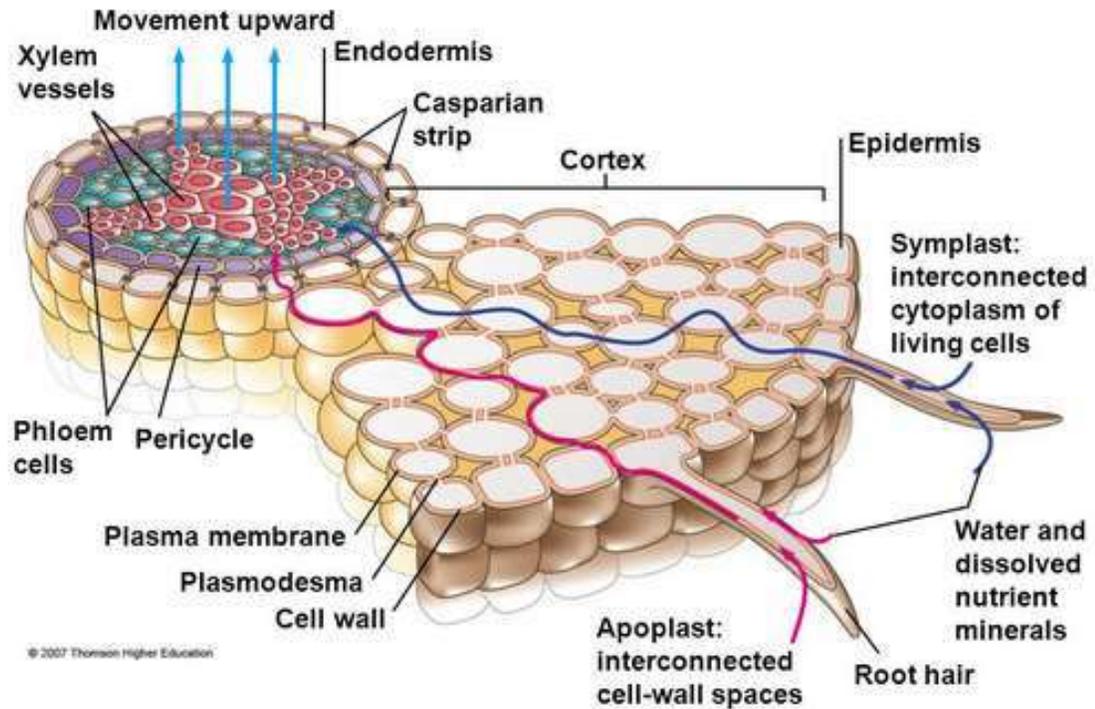


severe drought

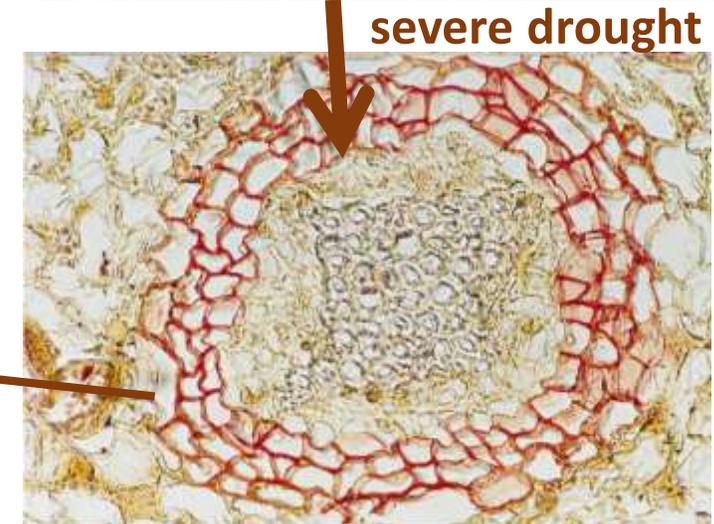


BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

Water stress conditions \rightarrow $\downarrow \Psi_{\text{xylem}}$ \rightarrow \uparrow Cavitated conduits \rightarrow $\downarrow K$



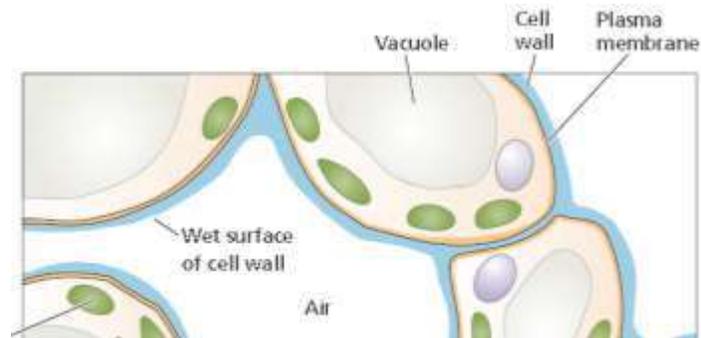
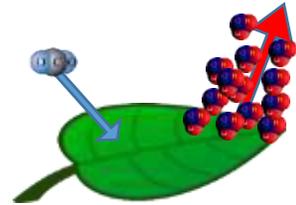
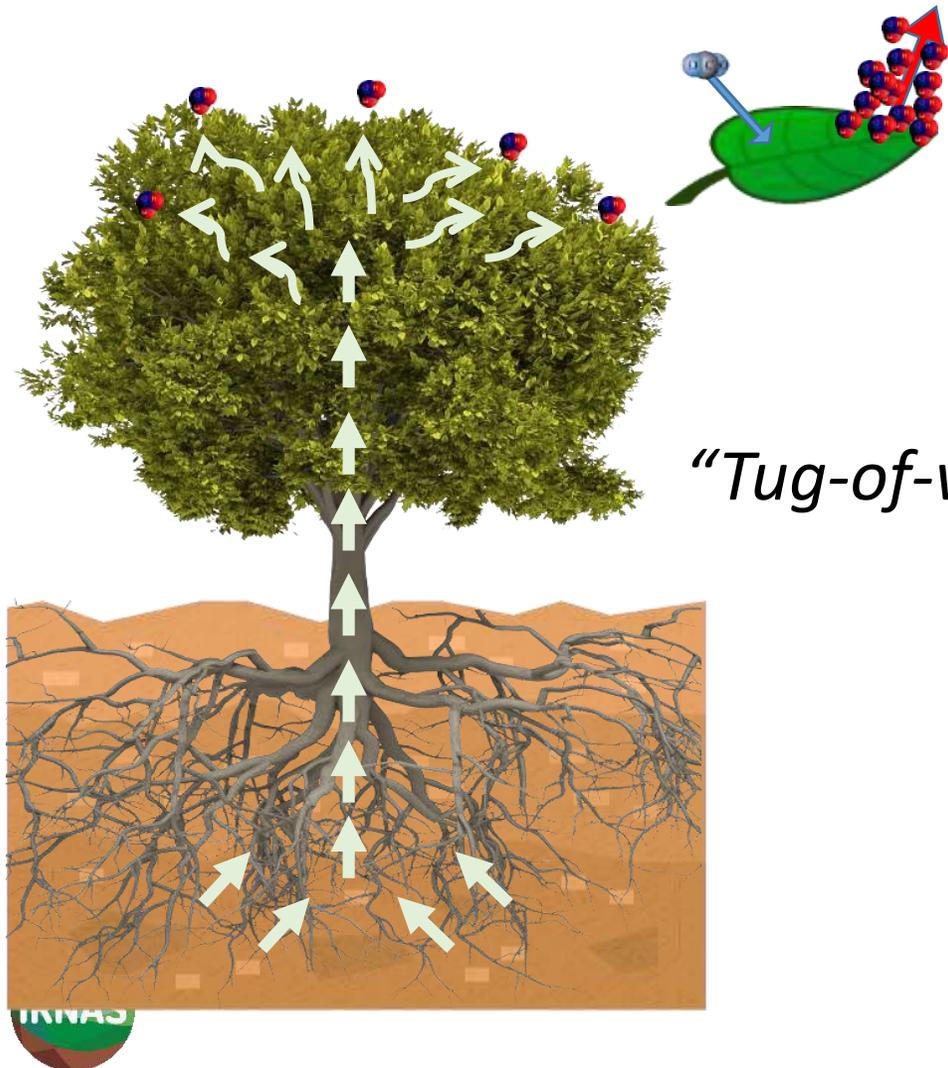
Lo Gullo et al, 1998, *New Phyt*



BASIC CONCEPTS RELATED TO WATER RELATIONS IN PLANTS

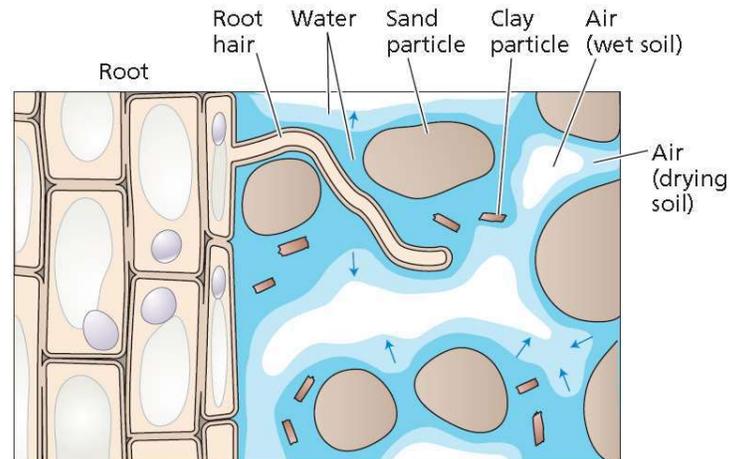
Take-home message

Key element in the transport of water from the soil to the leaves is the generation of negative pressures within the xylem



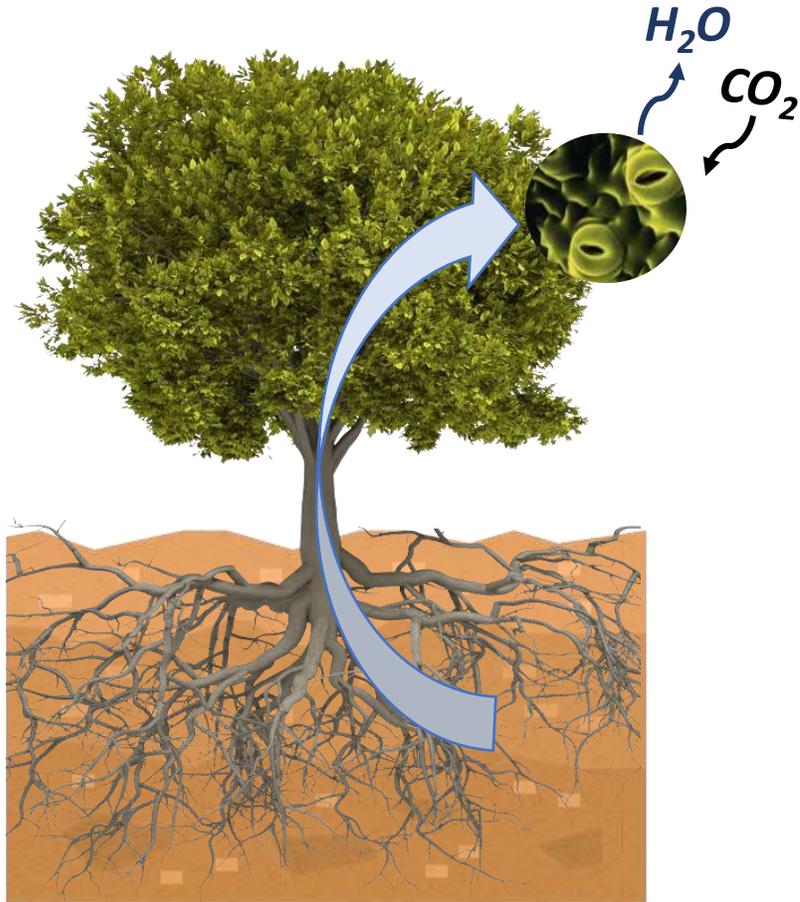
Leaf water at cell walls held

“Tug-of-war” (“Tira y afloja”)



Soil water held

PLANT RESPONSE TO ENVIRONMENTAL FACTORS



Stomatal conductance
x
Atmospheric demand

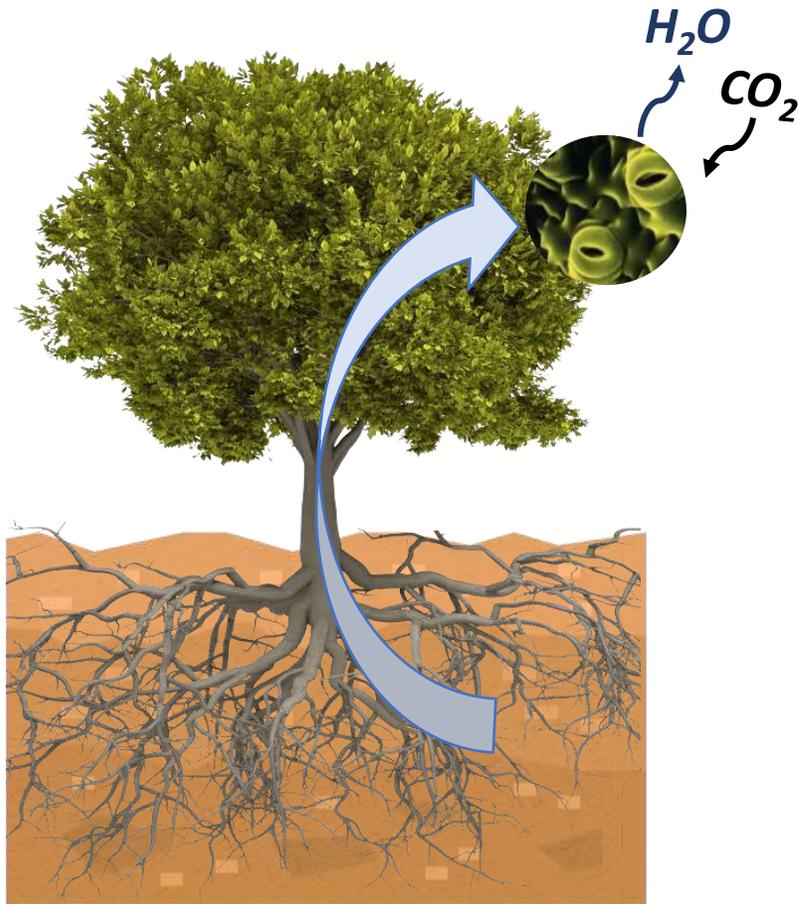
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Water potential gradient
x
Hydraulic conductivity



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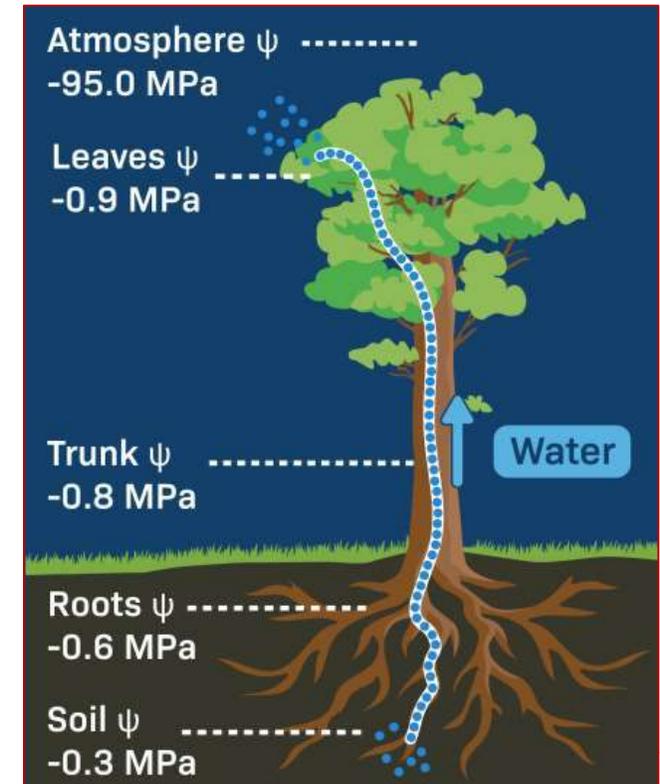
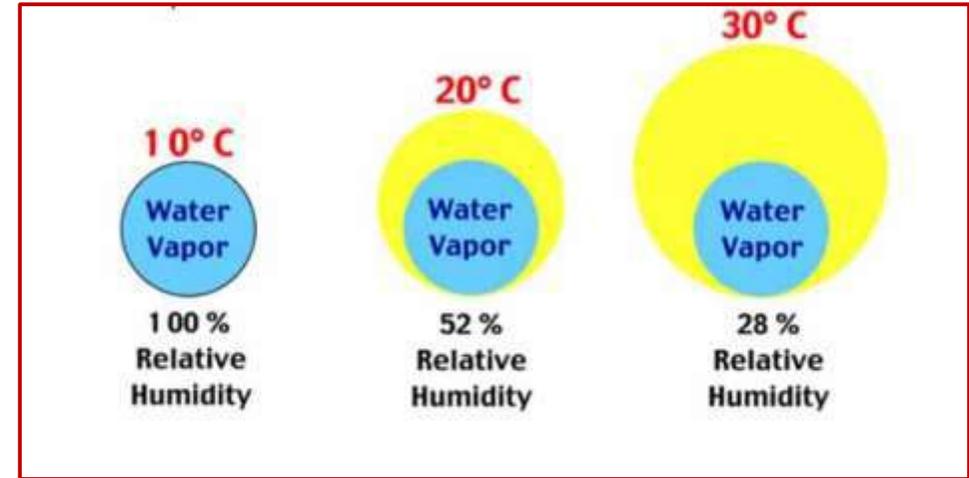
PLANT RESPONSE TO ENVIRONMENTAL FACTORS



Stomatal conductance
 x
 Atmospheric demand

||

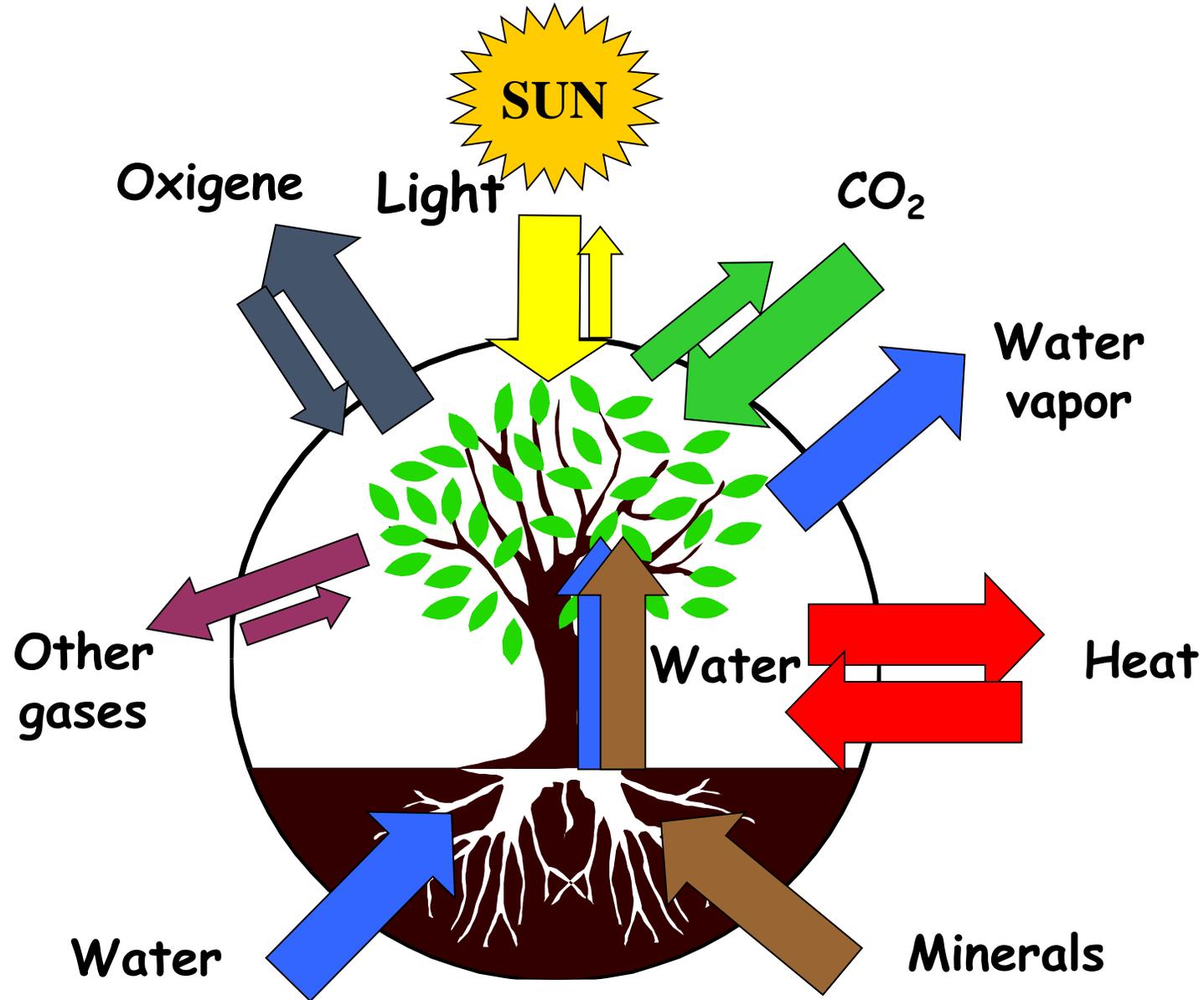
Water potential gradient
 ($\Psi_{\text{soil}} - \Psi_{\text{leaf}}$)
 x
 Hydraulic conductivity



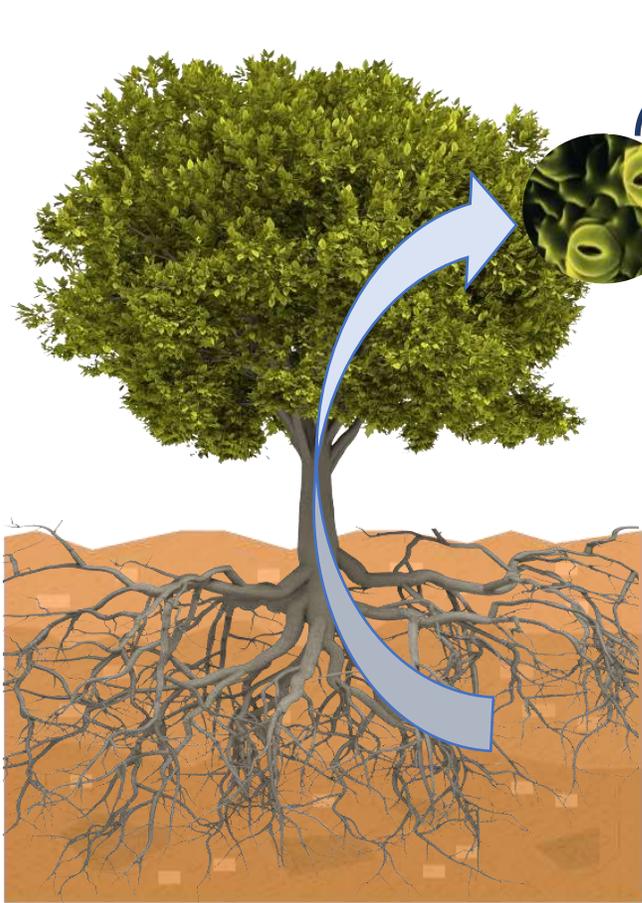
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PLANT RESPONSE TO ENVIRONMENTAL FACTORS



PLANT RESPONSE TO ENVIRONMENTAL FACTORS



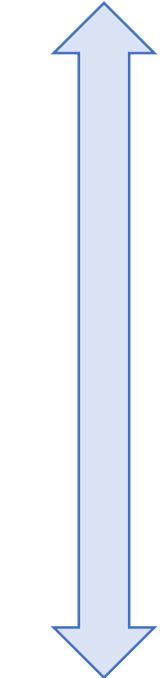
H_2O
 CO_2

Stomatal conductance
x
Atmospheric demand

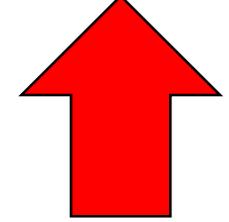
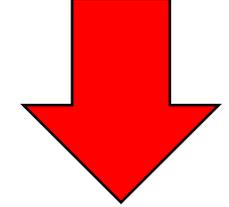
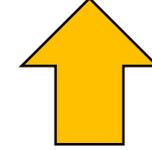
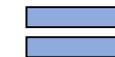
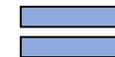
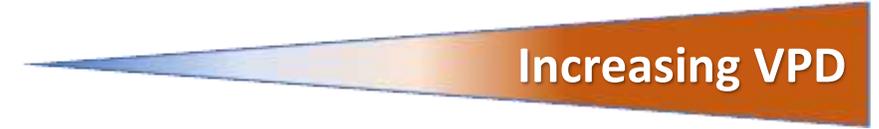
||

Water potential gradient
($\Psi_{soil} - \Psi_{leaf}$)
x
Hydraulic conductivity

Demand

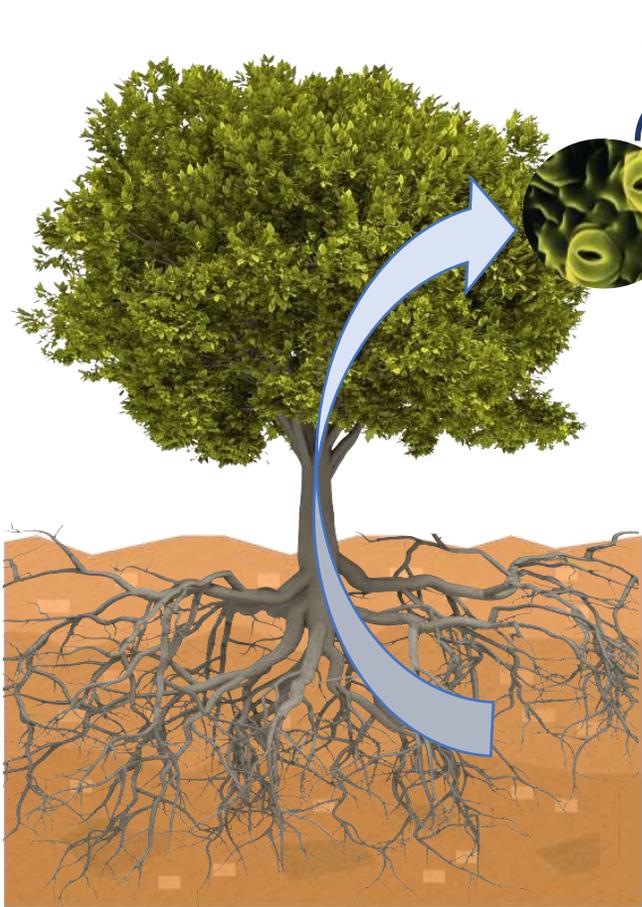
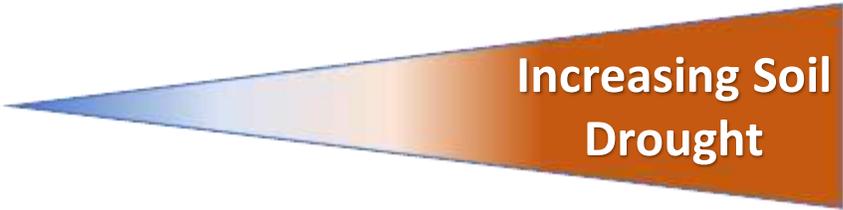


Supply



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PLANT RESPONSE TO ENVIRONMENTAL FACTORS

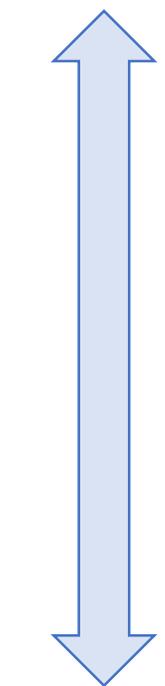


H_2O
 CO_2
 Stomatal conductance
 x
 Atmospheric demand

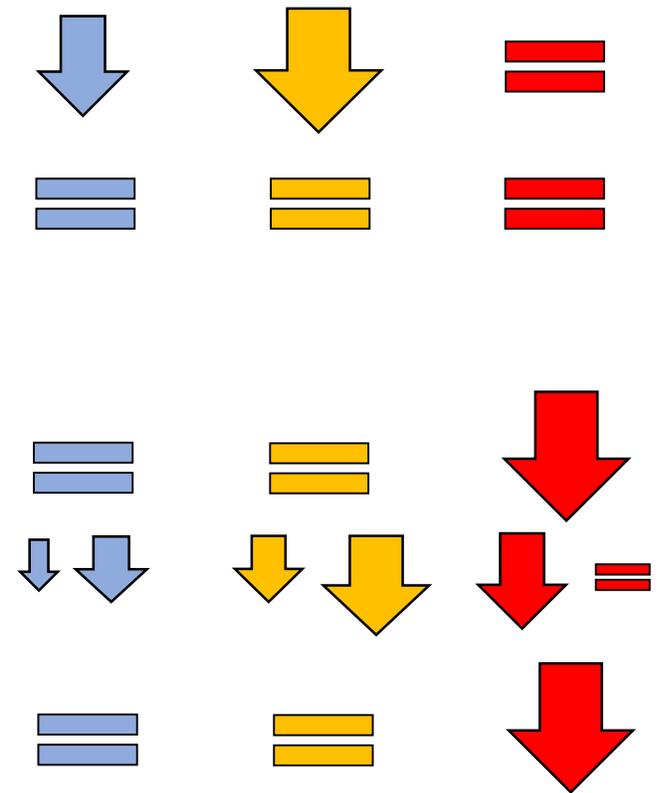
||

Water potential gradient
 ($\Psi_{soil} - \Psi_{leaf}$)
 x
 Hydraulic conductivity

Demand



Supply

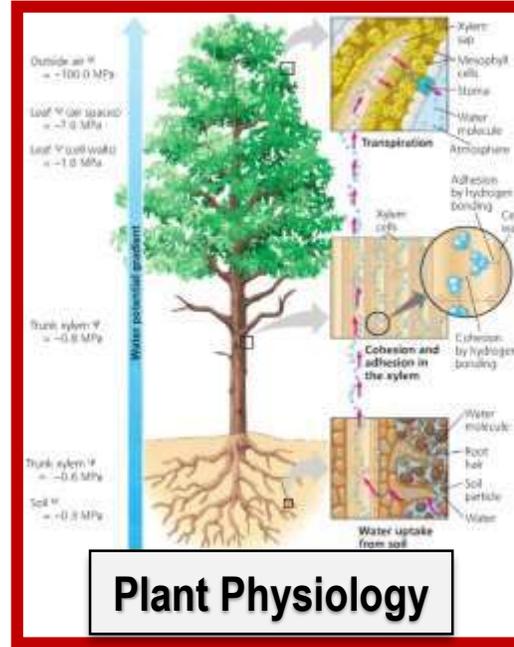


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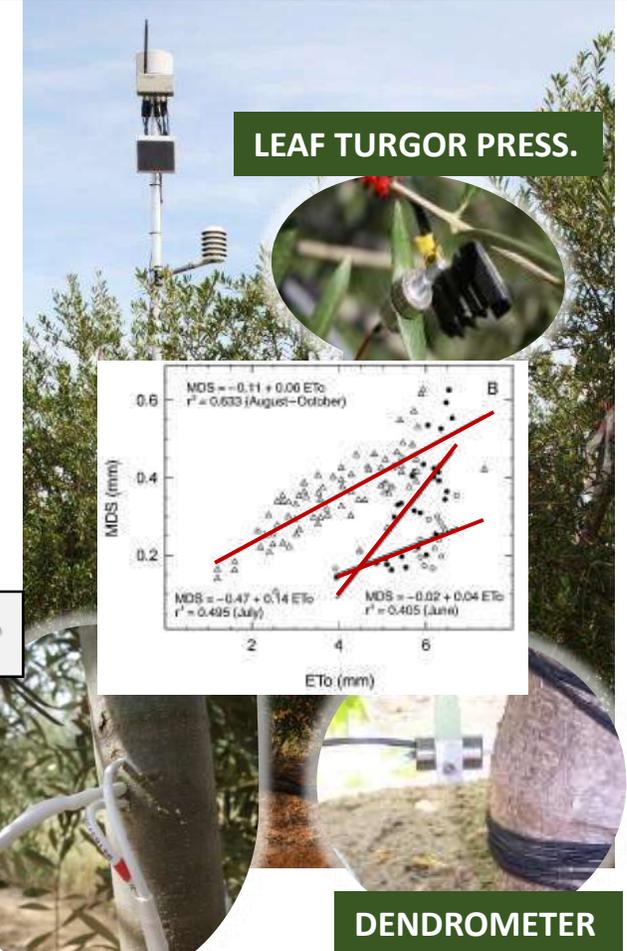
WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

- Essential to optimize water use in Agriculture.
- To place plant sensor outputs within a physiological context.
- To simplify physiological models.



Plant ecophysiology under water stress

To optimize irrigation strategies by monitoring water stress in real time



$$A_c = V_{cmax} \frac{C_i - \Gamma^*}{C_i + K_c [1 + (o_i / K_o)]}$$

$$g_s = \chi \frac{\alpha(\psi_s + \pi_e) - \pi_e}{1 + \chi(\alpha R + f_g r_{cg}) D_s}$$

$$E_p = K(\psi) (\psi_{sr} - \psi_{pc})$$

Process-based model

Plant-based sensor

To predict global change impacts on plants by integrating disparate physiological responses



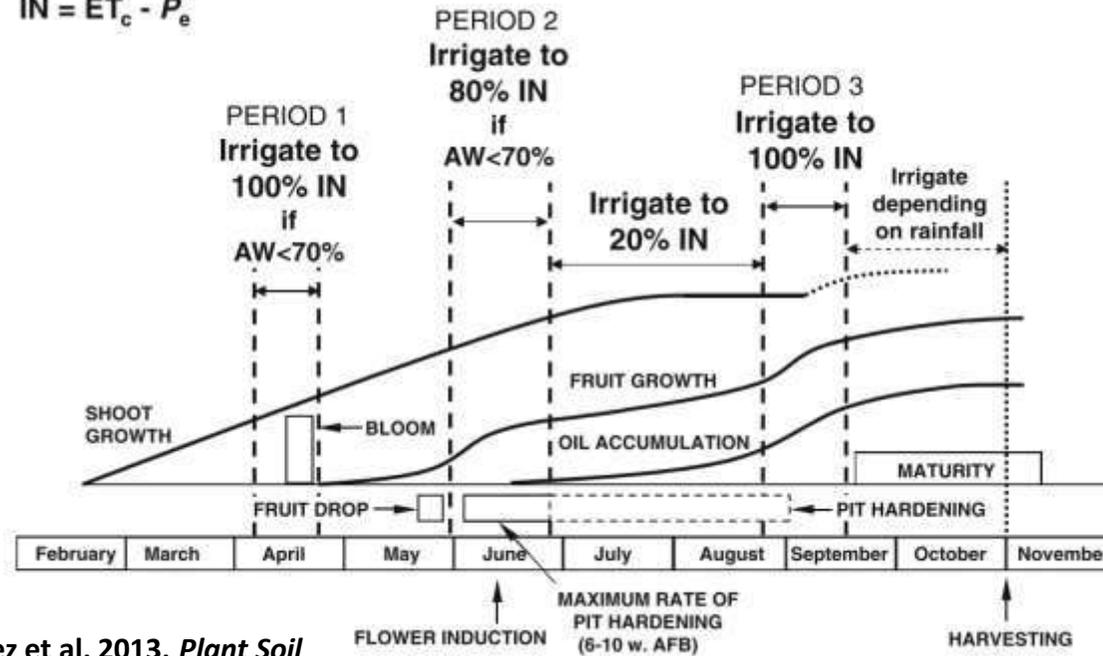
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WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

Regulated Deficit Irrigation

$$IN = ET_c - P_e$$



Fernández et al, 2013, *Plant Soil*

To optimize irrigation strategies by monitoring water stress in real time



Plant Physiology

Process-based model

Plant-based sensor

To predict global change impacts on plants by integrating disparate physiological responses



SAP FLOW



DENDROMETER

$$g_s = \chi \frac{\alpha(\psi_s + \pi_e) - \pi_e}{1 + \chi(\alpha R + f_g r_{cg}) D_s}$$

$$A_c = V_{cmax} \frac{C_i - \Gamma^*}{C_i + K_c [1 + (\alpha_i / K_o)]}$$

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 $E_p = K(\psi)(\psi_{sr} - \psi_{pc})$

WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

Combination of plant sensor and mechanistic model to monitor plant water stress in a hedgerow olive orchard

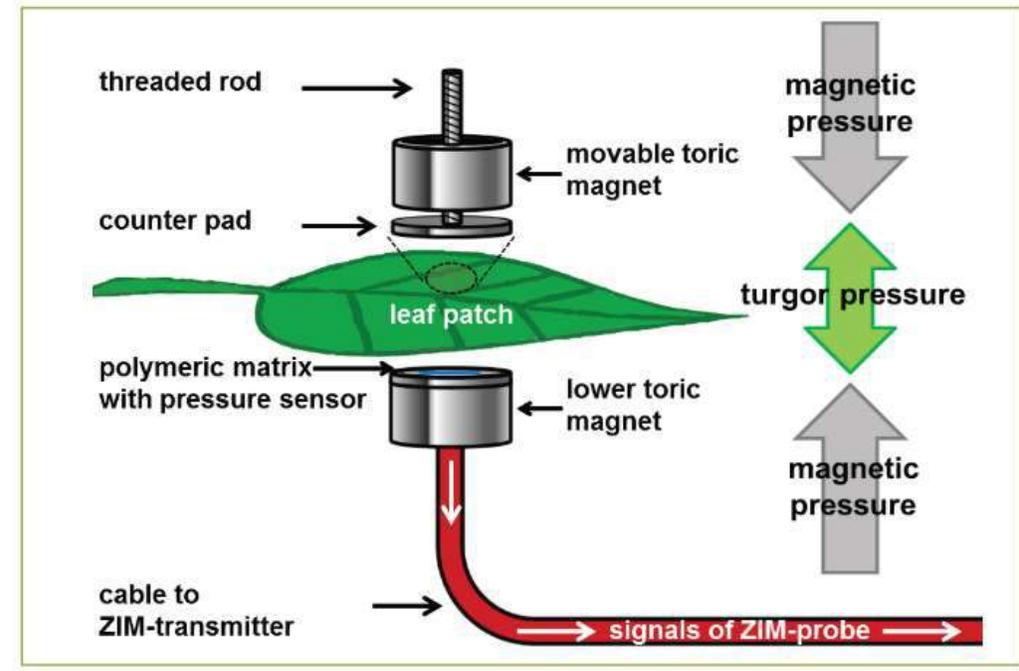
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$$E_p = K(\psi) (\psi_{sr} - \psi_{pc})$$



Zimmermann et al. 2013 *Theor Exp Plant Phys*

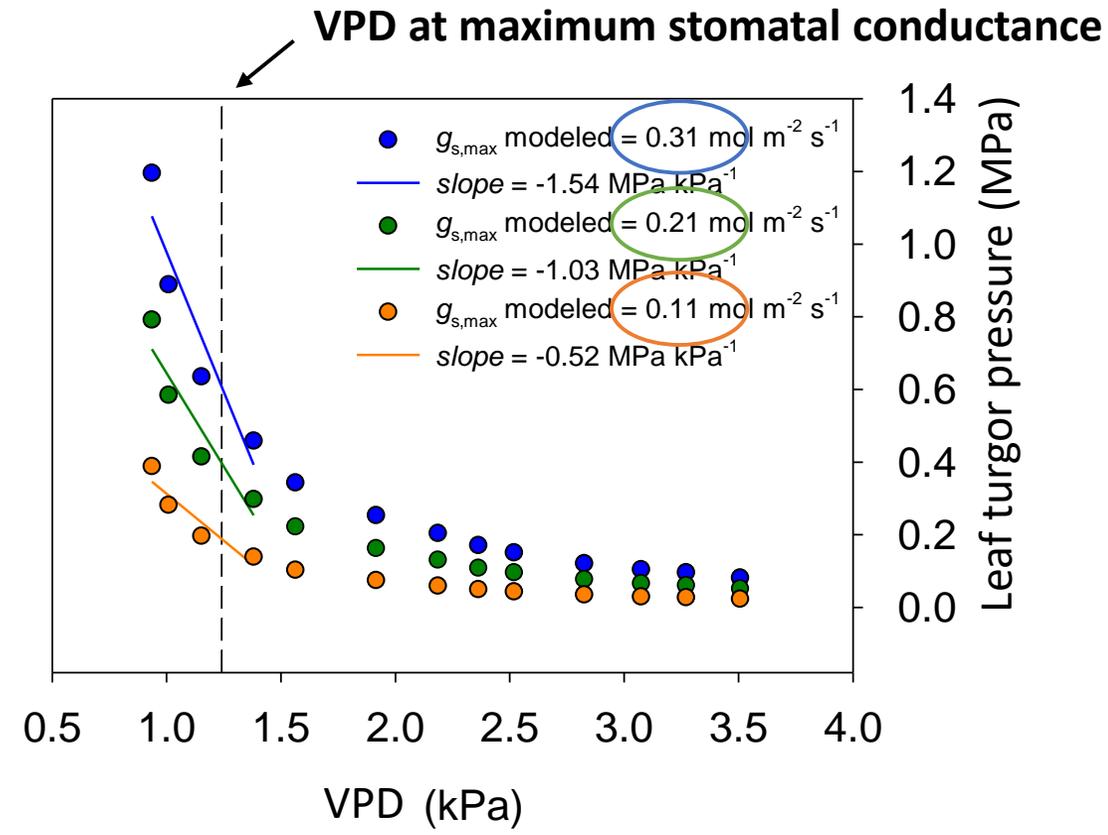


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WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

Combination of plant sensor and mechanistic model to monitor plant water stress in a hedgerow olive orchard

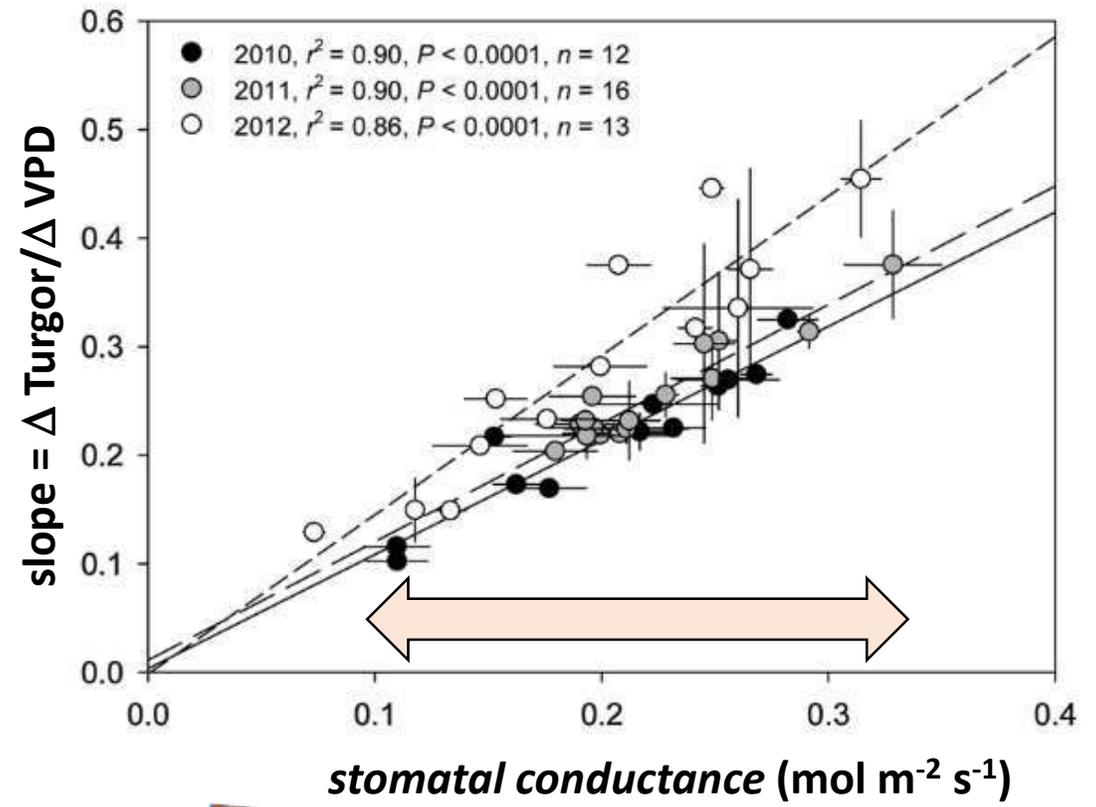
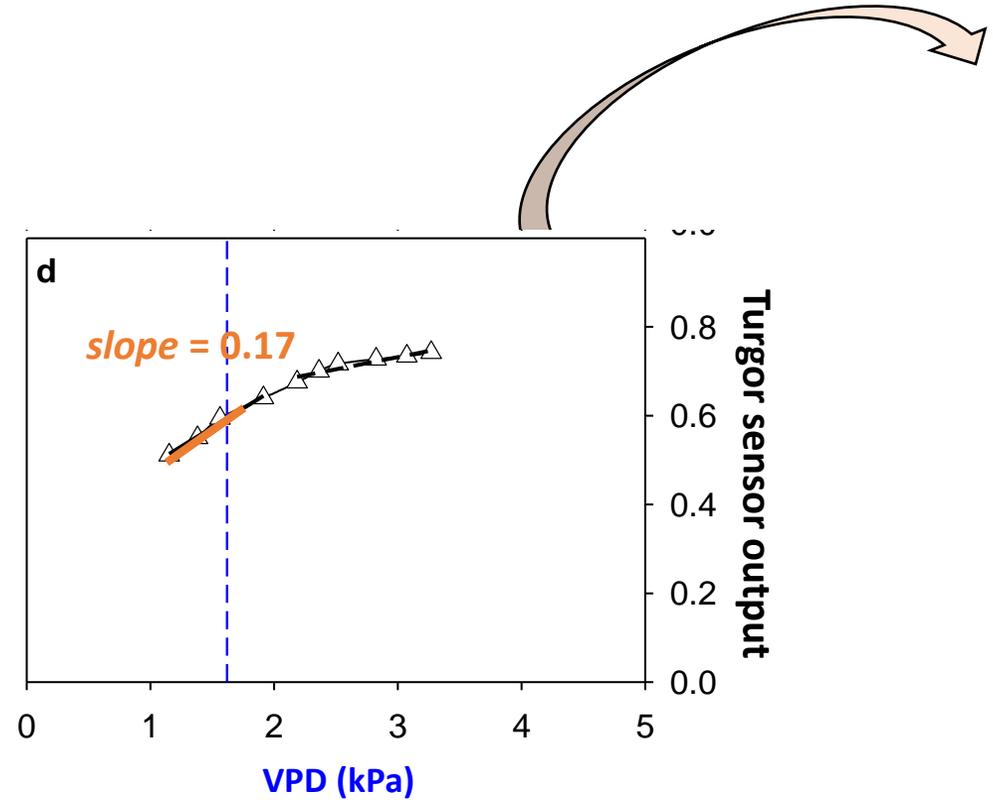


Rodriguez-Dominguez et al. 2016 *Agr Forest Meteo*

WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

Combination of plant sensor and mechanistic model to monitor plant water stress in a hedgerow olive orchard

Continuous monitoring of stomatal conductance from leaf turgor and vapor pressure deficit measurements



Increasing water stress



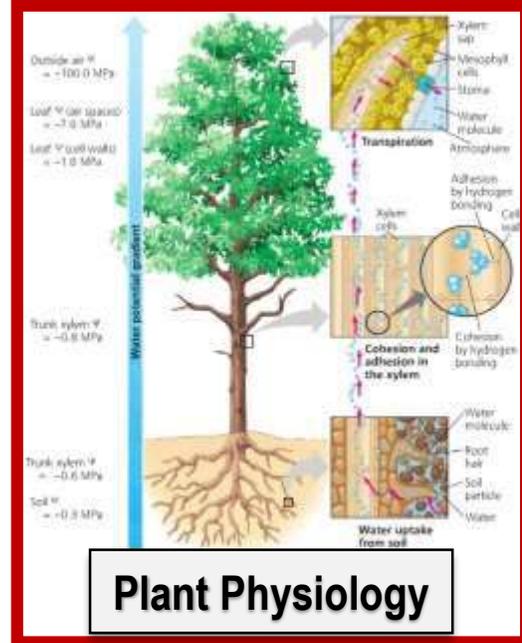
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Rodriguez-Dominguez et al. 2016 Agr Forest Meteo



WHY IS THIS KNOWLEDGE IMPORTANT? RELEVANCE TO AGRICULTURE

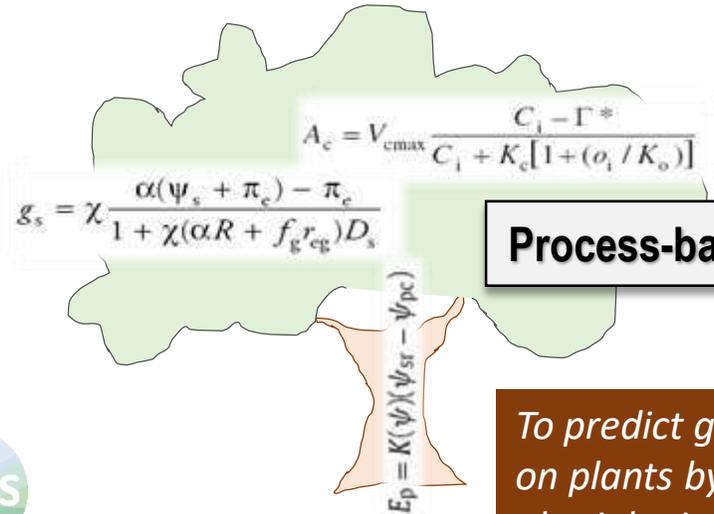
PHYSIOLOGICALLY-BASED APPROACH



Plant ecophysiology under water stress

To optimize irrigation strategies by monitoring water stress in real time

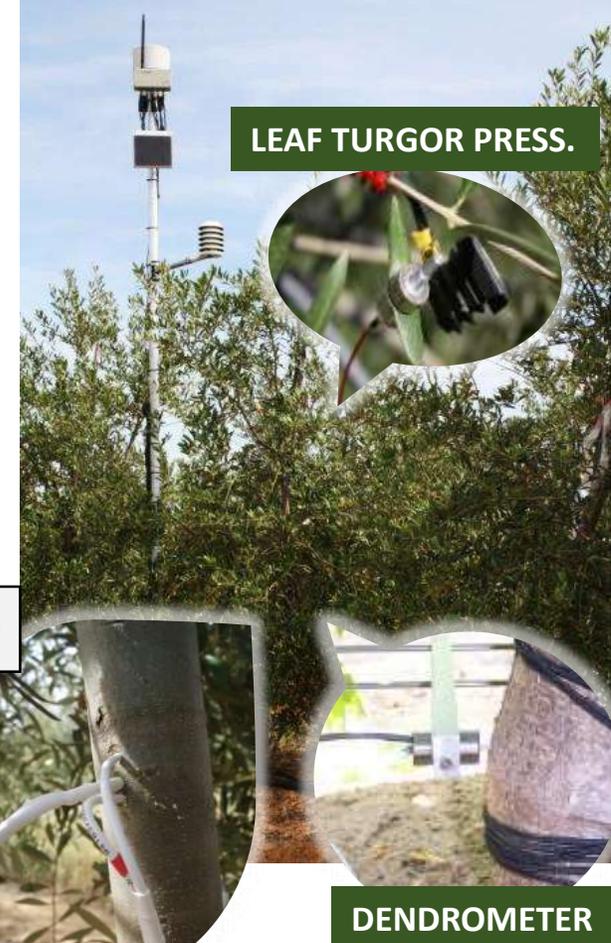
Plant Physiology



Process-based model

Plant-based sensor

To predict global change impacts on plants by integrating disparate physiological responses



LEAF TURGOR PRESS.

SAP FLOW

DENDROMETER

THANK YOU!!



crodriguez@irnas.csic.es

2nd Training School - 27th January to 31st January 2025



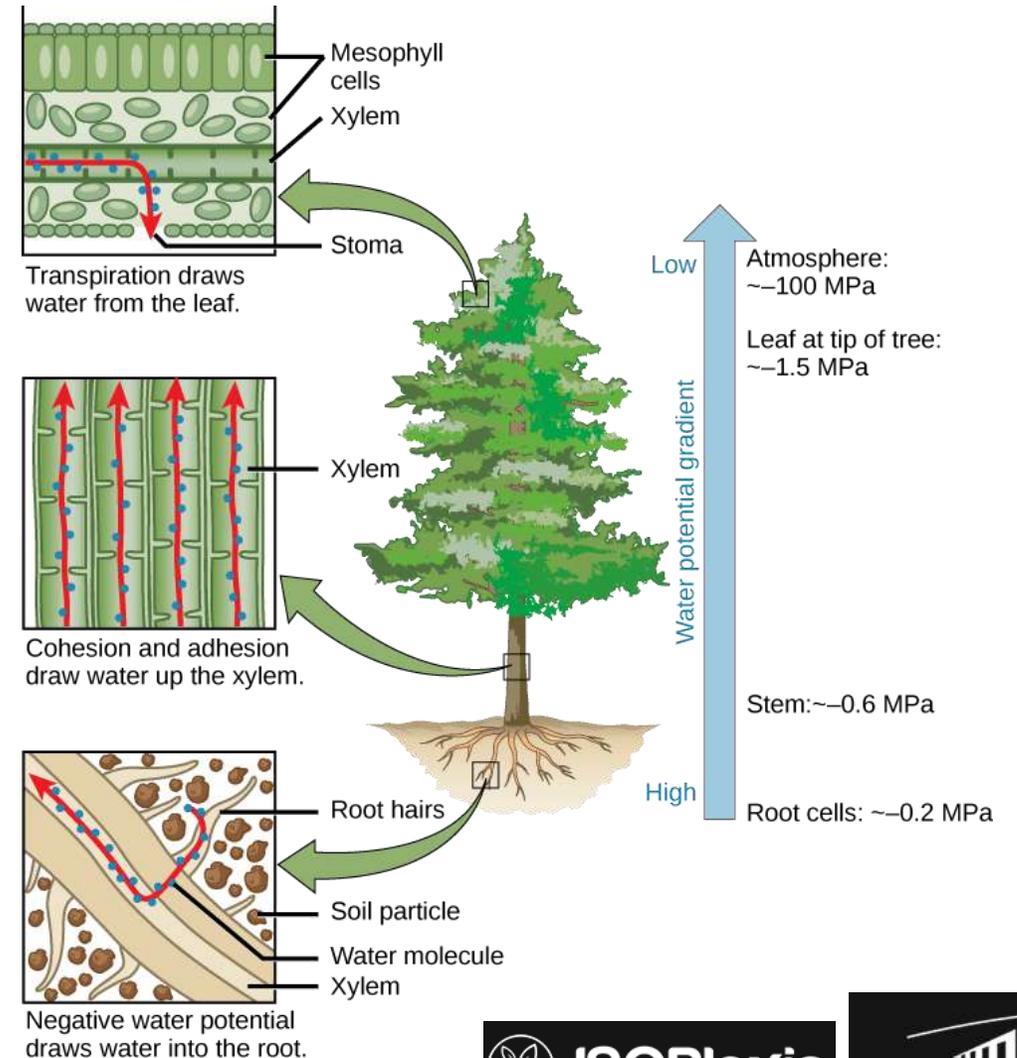
Water Potential

A Fundamental Plant Water Status Indicator

Celia M. Rodríguez Domínguez

2nd Training School

27th January to 31st January 2025



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isUP-Agr 

UNLOCKING THE POTENTIAL FOR AGRICULTURAL
RESEARCH ON AN EU OUTMOST REGION:
BOOSTING ISOPLEXIS CENTRE



ISOPlexis
Centro de Agricultura Sustentável e Tecnologia Alimentar

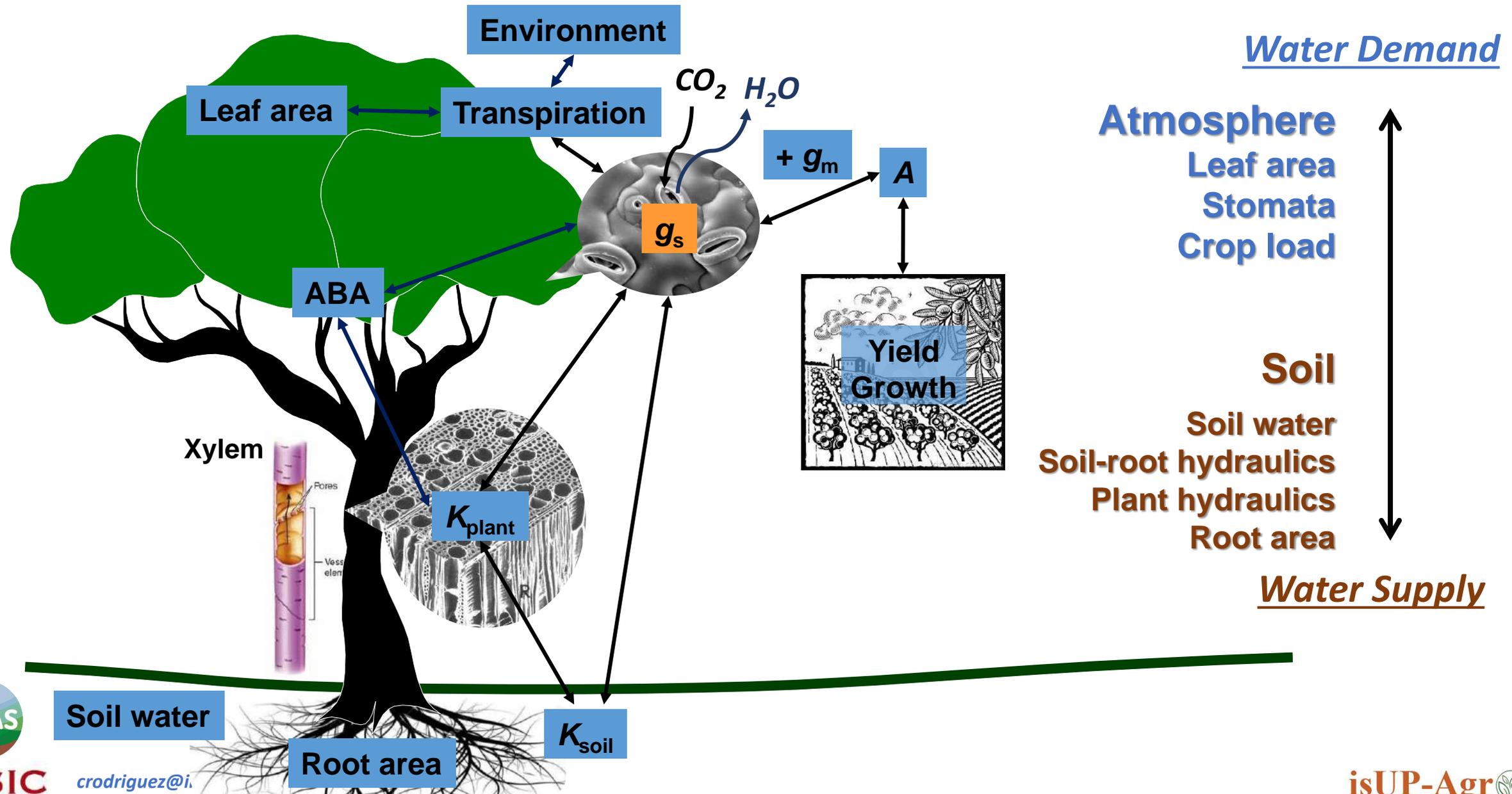


OBJECTIVES

- To understand the fundamental principles of water potential and its components.
- To familiarize with key methodologies for measuring water potential in plants.
- To learn practical strategies for optimizing water potential measurements in research and agricultural management.



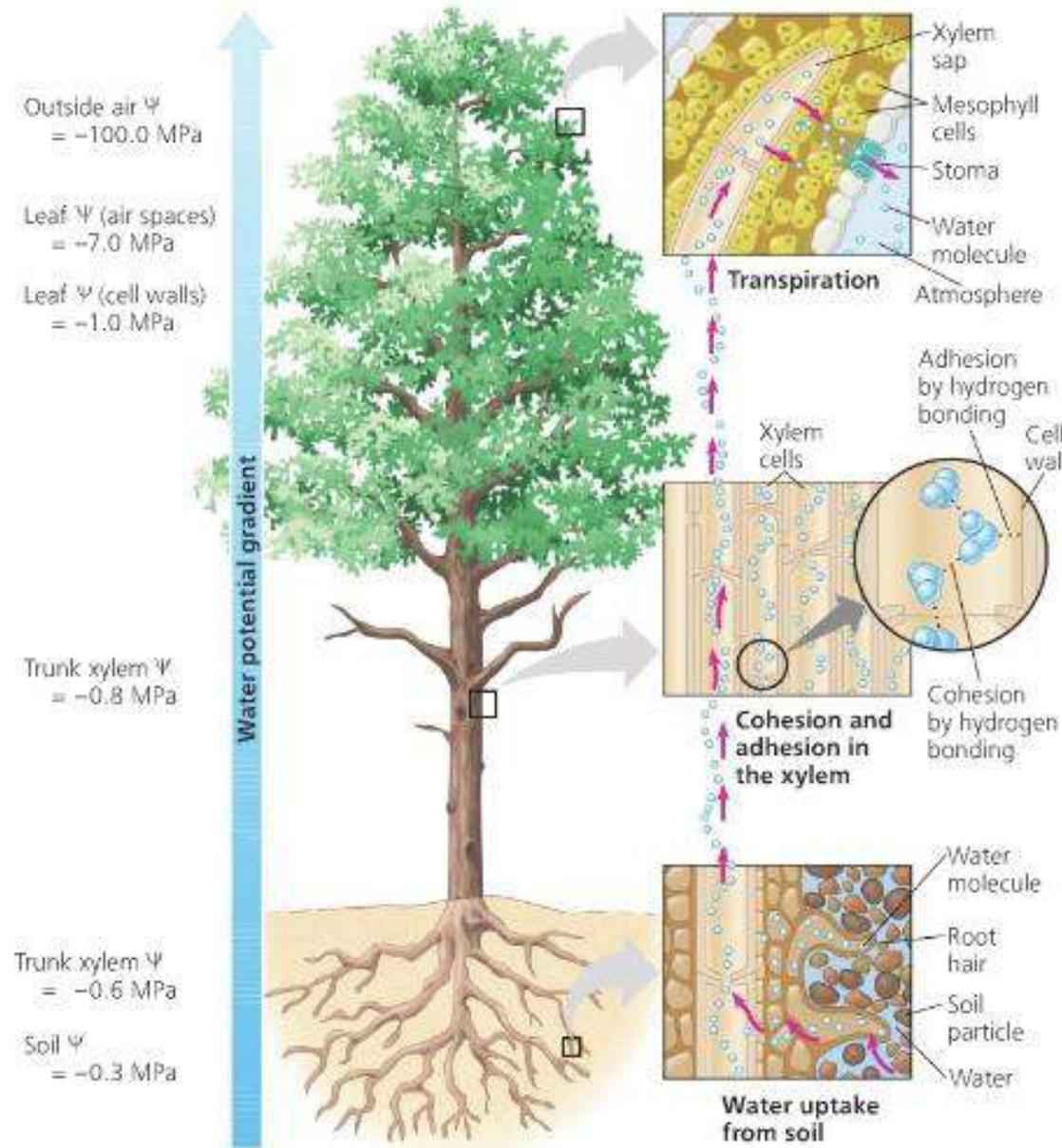
IMPORTANCE OF UNDERSTANDING WATER POTENTIAL FOR PLANT PHYSIOLOGY



IMPORTANCE OF UNDERSTANDING WATER POTENTIAL FOR PLANT PHYSIOLOGY



- ❑ Fundamental to understand **WATER MOVEMENT**. It governs transport across plasma membranes.
- ❑ It allows unambiguous description of **PLANT WATER STATUS**.



BASIC PRINCIPLES

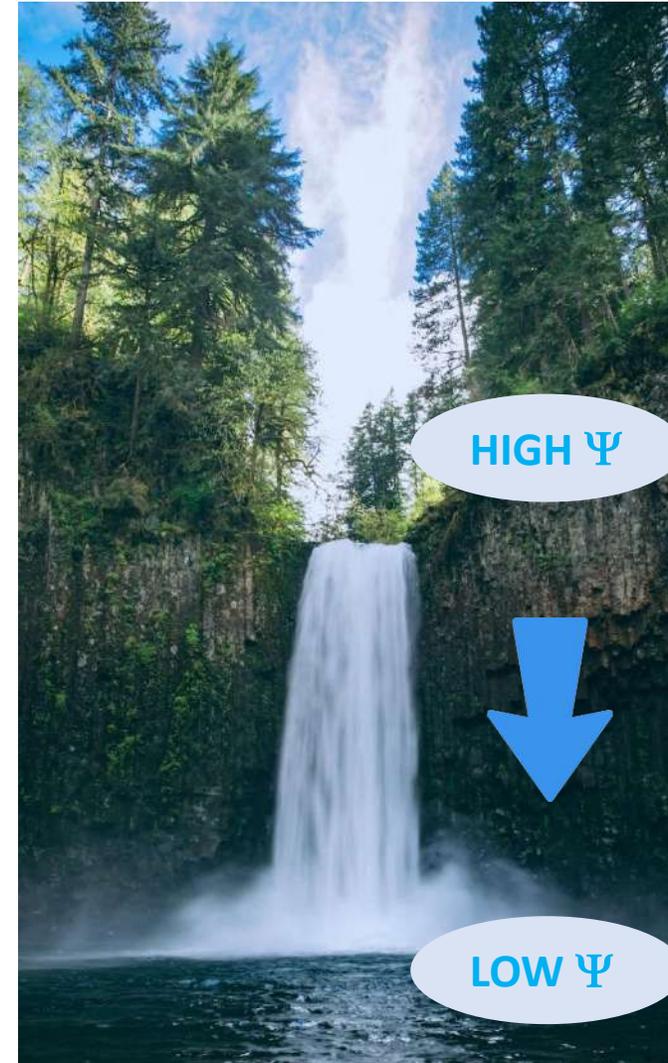
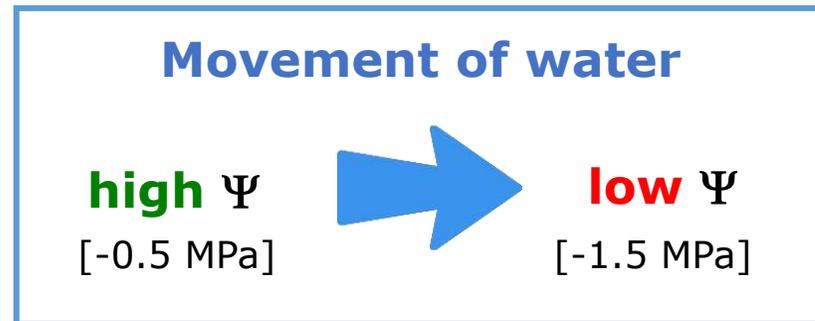


It comes from the thermodynamic concept of **FREE ENERGY**
[capacity (potential) of a system to perform work]

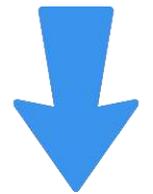
Represents the ability of a system to gain or give up water

Ψ **high** tendency to **give up** water

Ψ **low** tendency to **gain** water



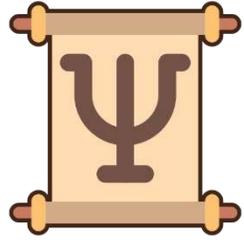
Higher potential for performing work



Lower potential for performing work



BASIC PRINCIPLES & COMPONENTS



It is measured in pressure units (bar, MPa...) and it is **NEGATIVE**

[*pure water*: $\Psi = 0$ MPa]

$$1 \text{ Pa} = 10^{-5} \text{ atm}$$

$$1 \text{ MPa} = 10^6 \text{ Pa} = 10 \text{ atm}$$

$$1 \text{ atm} = 0.1 \text{ MPa} \sim 1 \text{ bar} = 760 \text{ mm Hg}$$

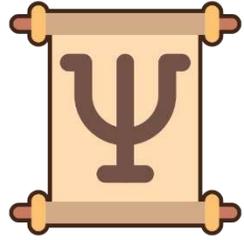
Factors that **increase** FREE ENERGY of water, **increase** Ψ (capacity to **give up** water)
[*e.g. high pressure*]

Factors that **decrease** FREE ENERGY of water, **decrease** Ψ (capacity to **gain** water)
[*e.g. presence of solutes*]

Major factors influencing FREE ENERGY of water, and hence, Ψ :

$$\Psi = \underbrace{\Psi_s}_{[solutes]} + \underbrace{\Psi_p}_{pressure} + \underbrace{\Psi_g}_{gravity}$$

BASIC PRINCIPLES & COMPONENTS: THE GRAVITATIONAL POTENTIAL



It is measured in pressure units (bar, MPa...) and it is **NEGATIVE**

[pure water: $\Psi = 0$ MPa]

$$\Psi = \Psi_s + \Psi_p + \cancel{\Psi_g}$$

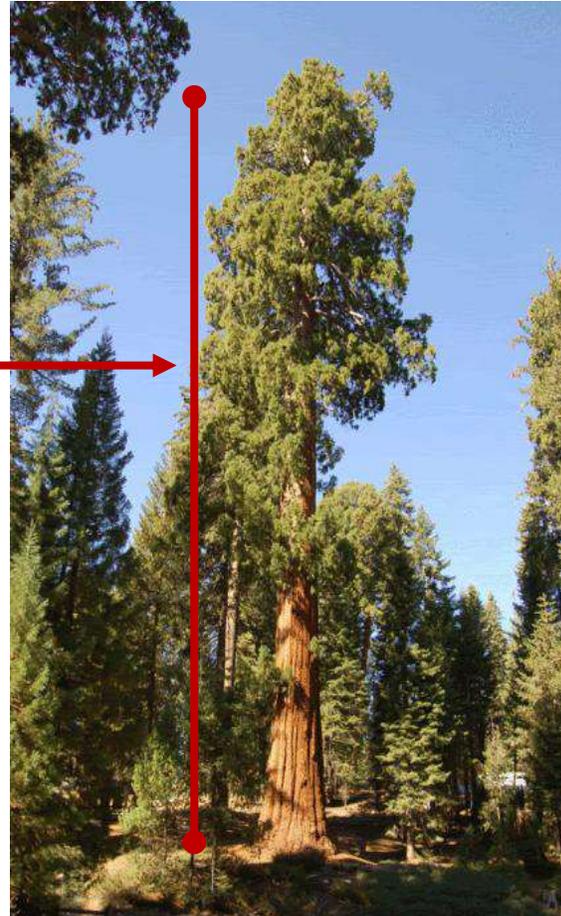
[solutes] *pressure* *gravity*

Ψ_g

Reflects the gravitational pull of the water column

$$\Psi_g = \rho_w g h$$

0.01 MPa m⁻¹



$h = 10$ m



**0.1 MPa
increase in Ψ**



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BASIC PRINCIPLES & COMPONENTS: THE OSMOTIC POTENTIAL

 Ψ_s

Represents the effect of dissolved solutes on Ψ

It is **NEGATIVE** and the addition of solutes always **decreases** Ψ

$$\Psi = \Psi_s + \Psi_p$$

[solute] pressure

Van't Hoff EQUATION

$$\Psi_s = -RTc_s$$

R : 0,00831 MPa L mol⁻¹ K⁻¹

T: Temperature K

c_s: Osmolarity (mol L⁻¹)

SOLUTE	MOLARITY	OSMOLARITY (molarity x number of ions)	Ψ_s (20°C = 293 K)
Sucrose	0.1	0.1	-0.244 MPa
NaCl	0.1	0.2	-0.488 MPa



BASIC PRINCIPLES & COMPONENTS: THE OSMOTIC POTENTIAL

Ψ_s

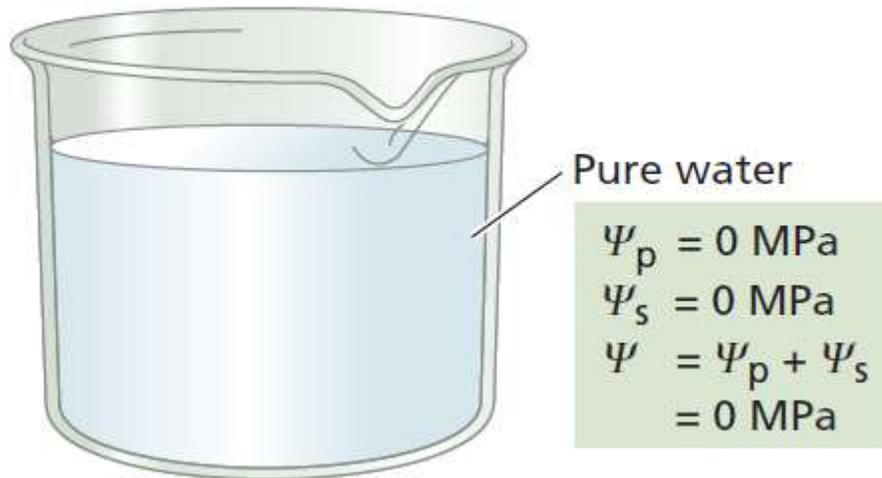
Represents the effect of dissolved solutes on Ψ

It is **NEGATIVE** and the addition of solutes always **decreases** Ψ

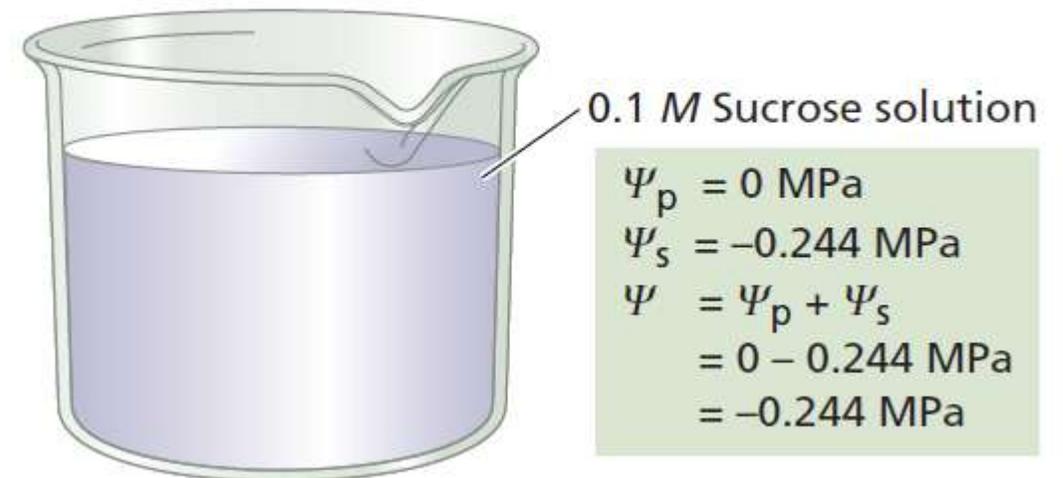
$$\Psi = \Psi_s + \Psi_p$$

[solute] pressure

(A) Pure water



(B) Solution containing 0.1 M sucrose



BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

Ψ_p

Represents the effect of hydrostatic pressure on Ψ

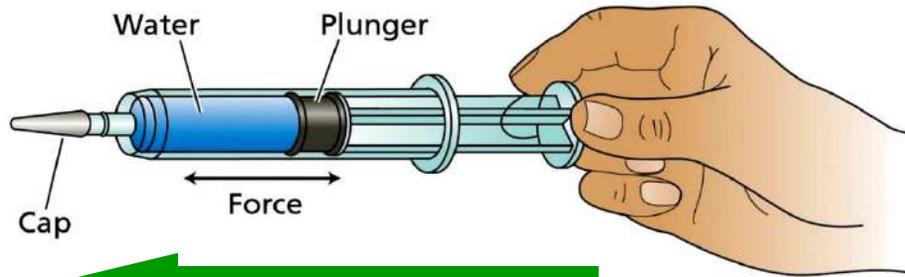
Depending on the system, it can be **POSITIVE** or **NEGATIVE**

+ Ψ_p -> increases Ψ

- Ψ_p -> decreases Ψ

$$\Psi = \Psi_s + \Psi_p$$

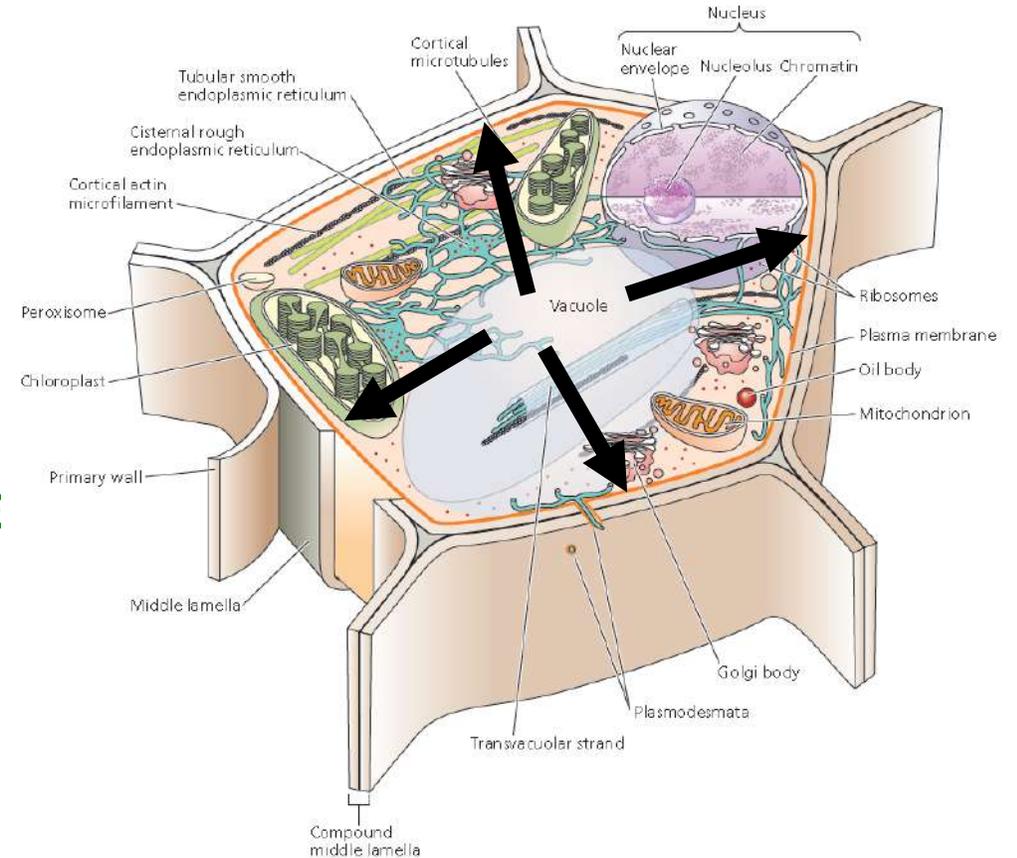
[solute] pressure



HYDROSTATIC PRESSURE
+ Ψ_p

TURGOR PRESSURE

TENSION
- Ψ_p



BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

TURGOR PRESSURE

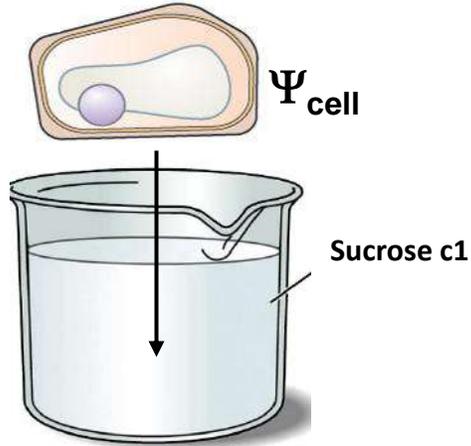
 Ψ_p

OSMOSIS: Net movement of water through a semipermeable membrane

$$\Psi = \Psi_s + \Psi_p$$

[solute] *pressure*

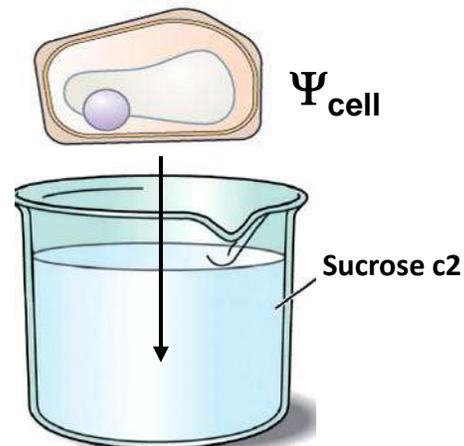
Hypotonic solution

 Ψ sucrose 1

$$\Psi = -RTc_1$$

$$\Psi_{\text{cell}} < \Psi_{\text{sucrose 1}}$$

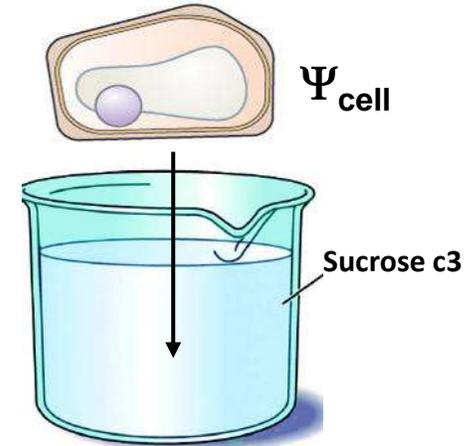
Isotonic solution

 Ψ sucrose 2

$$\Psi = -RTc_2$$

$$\Psi_{\text{cell}} = \Psi_{\text{sucrose 2}}$$

Hypertonic solution

 Ψ sucrose 3

$$\Psi = -RTc_3$$

$$\Psi_{\text{cell}} > \Psi_{\text{sucrose 3}}$$



BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

TURGOR PRESSURE

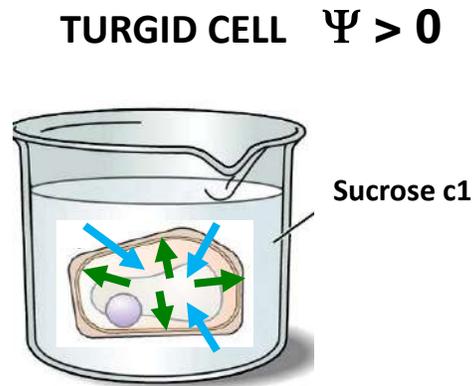
 Ψ_p

OSMOSIS: Net movement of water through a semipermeable membrane

$$\Psi = \Psi_s + \Psi_p$$

[solute] pressure

Hypotonic solution

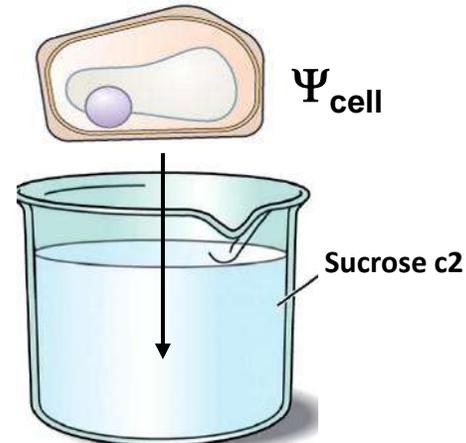
 $\Psi_{\text{sucrose 1}}$

$$\Psi = -RTc_1$$

$$\Psi_{\text{cell}} < \Psi_{\text{sucrose 1}}$$

The cell gains water

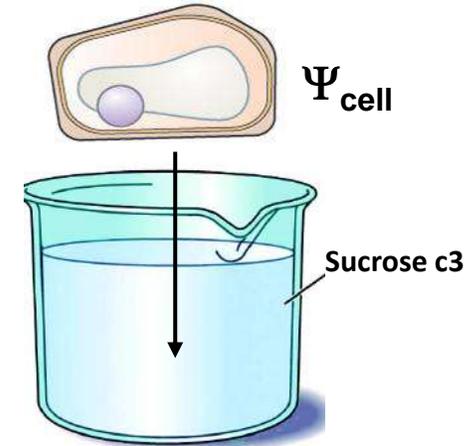
Isotonic solution

 $\Psi_{\text{sucrose 2}}$

$$\Psi = -RTc_2$$

$$\Psi_{\text{cell}} = \Psi_{\text{sucrose 2}}$$

Hypertonic solution

 $\Psi_{\text{sucrose 3}}$

$$\Psi = -RTc_3$$

$$\Psi_{\text{cell}} > \Psi_{\text{sucrose 3}}$$



BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

TURGOR PRESSURE

 Ψ_p

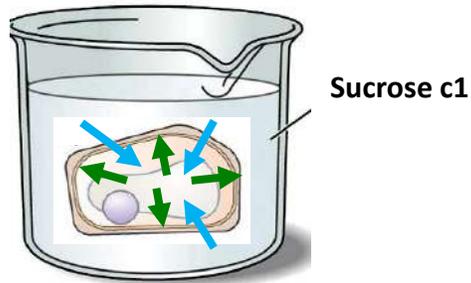
OSMOSIS: Net movement of water through a semipermeable membrane

$$\Psi = \Psi_s + \Psi_p$$

[solute] pressure

Hypotonic solution

TURGID CELL $\Psi > 0$



Ψ sucrose 1

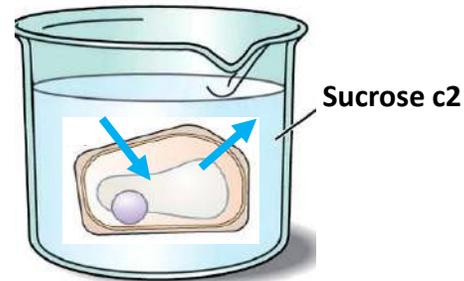
$$\Psi = -RTc_1$$

$$\Psi_{\text{cell}} < \Psi_{\text{sucrose 1}}$$

The cell gains water

Isotonic solution

FLACCID CELL



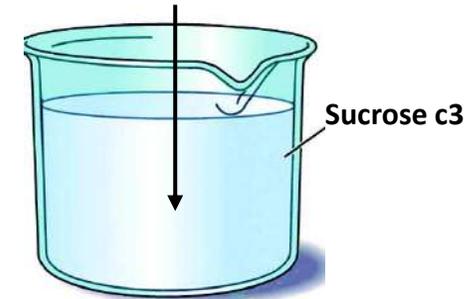
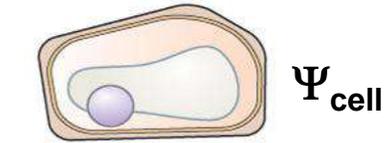
Ψ sucrose 2

$$\Psi = -RTc_2$$

$$\Psi_{\text{cell}} = \Psi_{\text{sucrose 2}}$$

The cell is in equilibrium

Hypertonic solution



Ψ sucrose 3

$$\Psi = -RTc_3$$

$$\Psi_{\text{cell}} > \Psi_{\text{sucrose 3}}$$



BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

Ψ_p

TURGOR PRESSURE

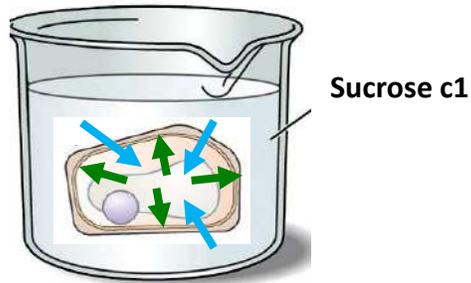
OSMOSIS: Net movement of water through a semipermeable membrane

$$\Psi = \Psi_s + \Psi_p$$

[solute] pressure

Hypotonic solution

TURGID CELL $\Psi > 0$



Ψ sucrose 1

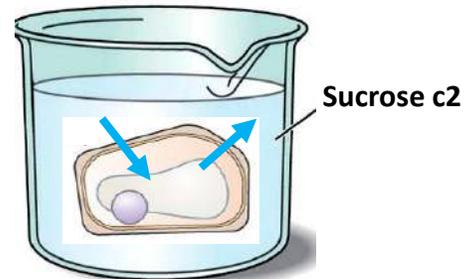
$$\Psi = -RTc1$$

$$\Psi_{\text{cell}} < \Psi_{\text{sucrose 1}}$$

The cell gains water

Isotonic solution

FLACCID CELL



Ψ sucrose 2

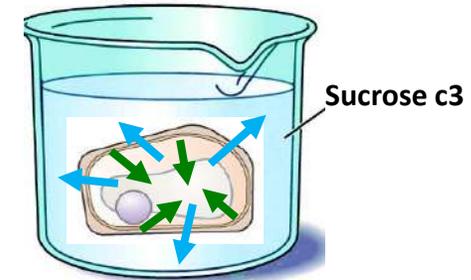
$$\Psi = -RTc2$$

$$\Psi_{\text{cell}} = \Psi_{\text{sucrose 2}}$$

The cell is in equilibrium

Hypertonic solution

PLASMOLYZED CELL $\Psi = 0$



Ψ sucrose 3

$$\Psi = -RTc3$$

$$\Psi_{\text{cell}} > \Psi_{\text{sucrose 3}}$$

The cell loses water



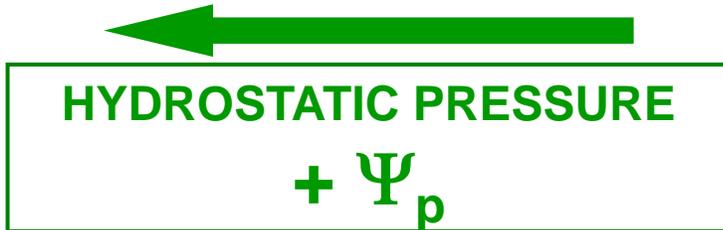
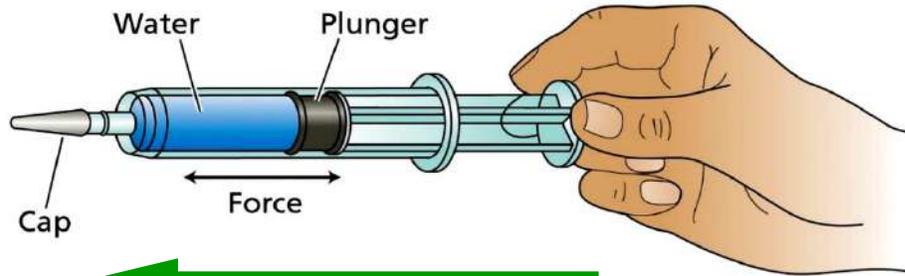
BASIC PRINCIPLES & COMPONENTS: THE PRESSURE POTENTIAL

Ψ_p

Represents the effect of hydrostatic pressure on Ψ

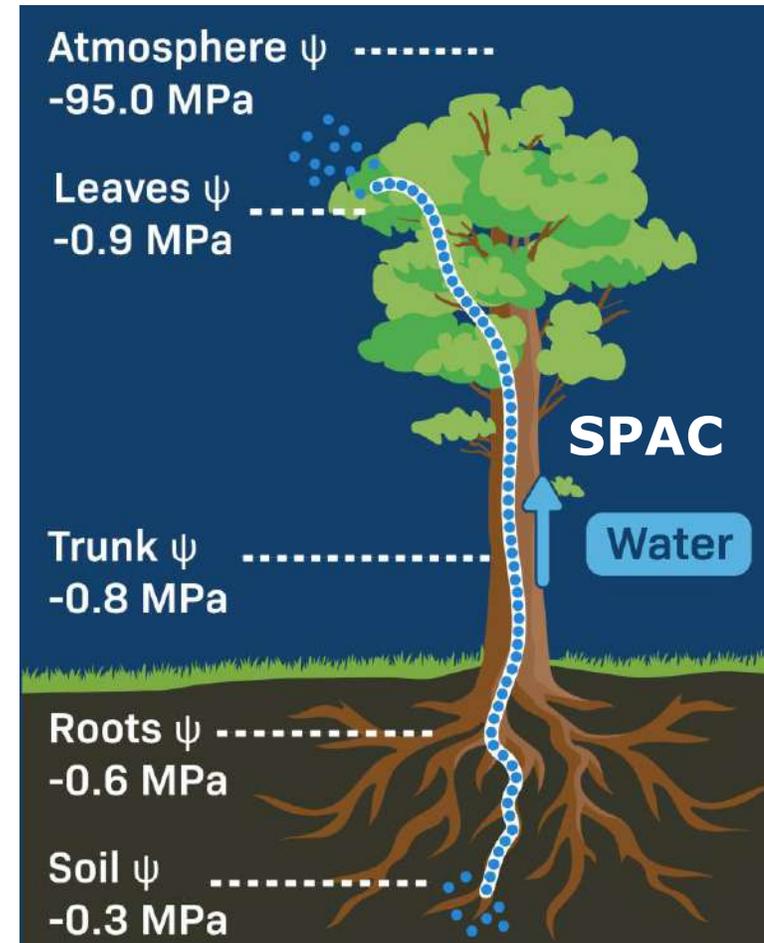
Depending on the system, it can be **POSITIVE** or **NEGATIVE**

+ Ψ_p -> increases Ψ
 - Ψ_p -> decreases Ψ

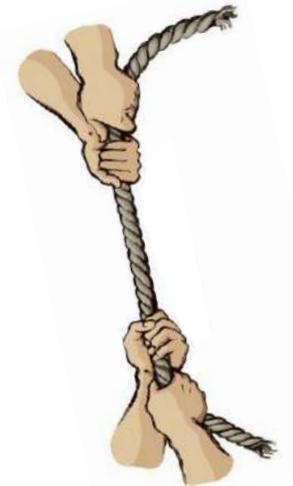


$$\Psi = \Psi_s + \Psi_p$$

[solutes] pressure



Leaf water at cell walls held



Soil water held

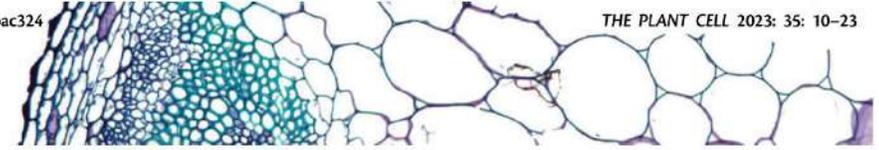


BASIC PRINCIPLES: PLANT WATER STATUS

Ψ describes unambiguously plant water status, which influence **PHYSIOLOGICAL PROCESSES**

<https://doi.org/10.1093/plcell/koac324>

THE
PLANT
CELL



THE PLANT CELL 2023: 35: 10-23

Time for a drought experiment: Do you know your plants' water status?

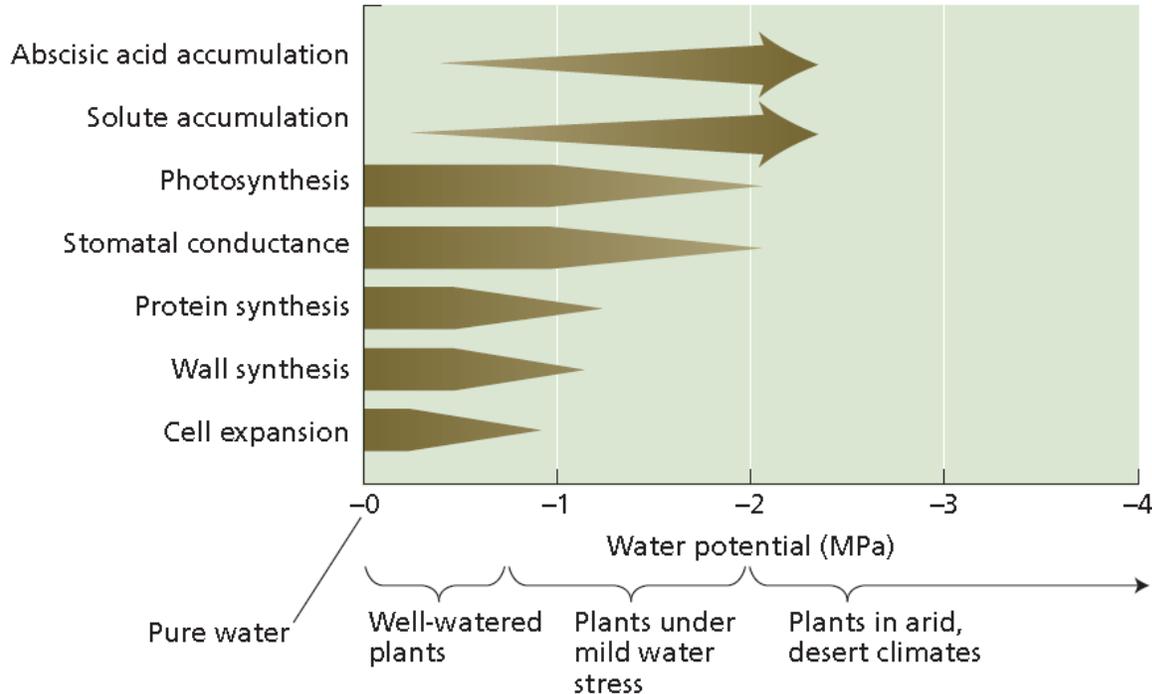
Thomas E. Juenger ^{1,*} and Paul E. Verslues ^{2,*}

Because of transpirational water loss to the atmosphere, **plants are seldom fully hydrated.**

At different points in the **SPAC**, the **components of water potential differ.**

- Soil, matric forces of water adhering to soil particles.
- Plant cell, active accumulation of solutes to drive water uptake, cells must *osmoregulate at all time* to maintain cell volume or regulate turgor.
- Apoplast and xylem, tension generated by adhesion of water molecules moving up through the plant.

Physiological changes due to dehydration:



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BASIC PRINCIPLES: CELL WATER RELATIONS

Höfler - Thoday Diagram

$R = 100 - RWC$

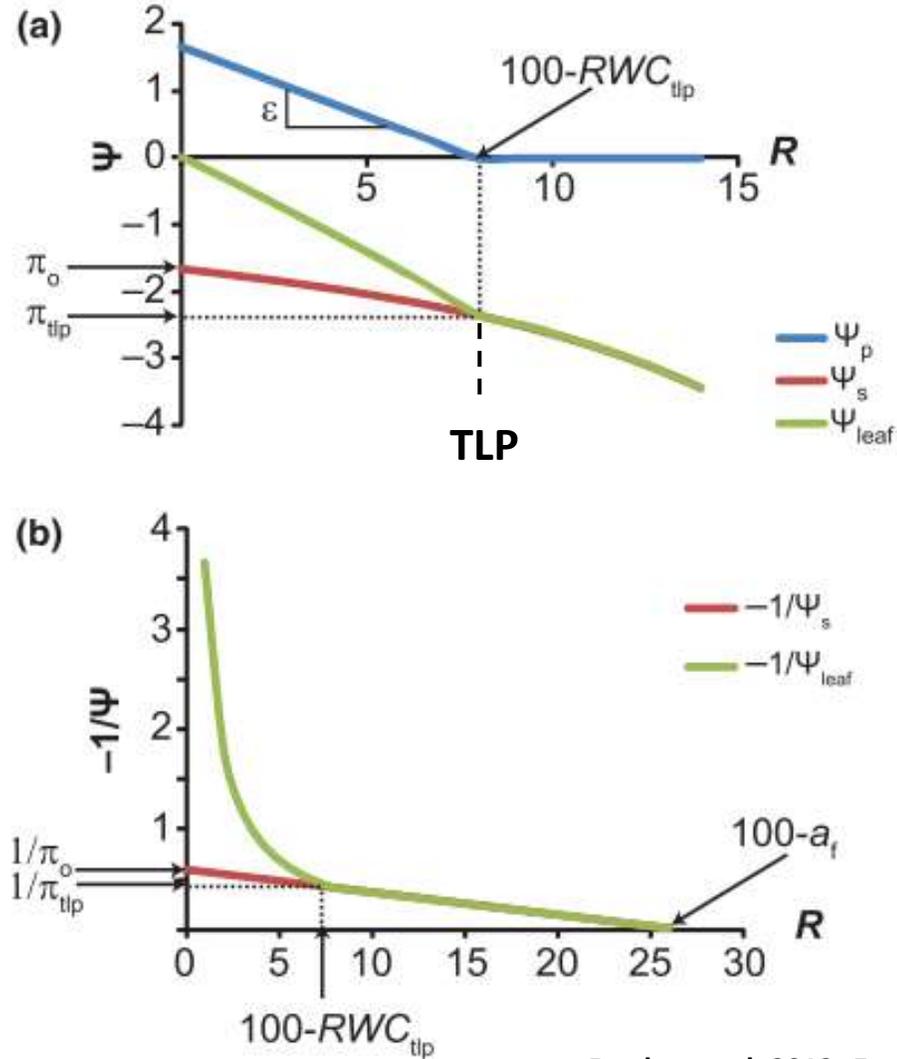
RWC = Relative Water Content

ϵ = Elastic modulus (cell wall rigidity)

↑ ϵ -> cell walls more rigid

↓ ϵ -> cell walls more flexible

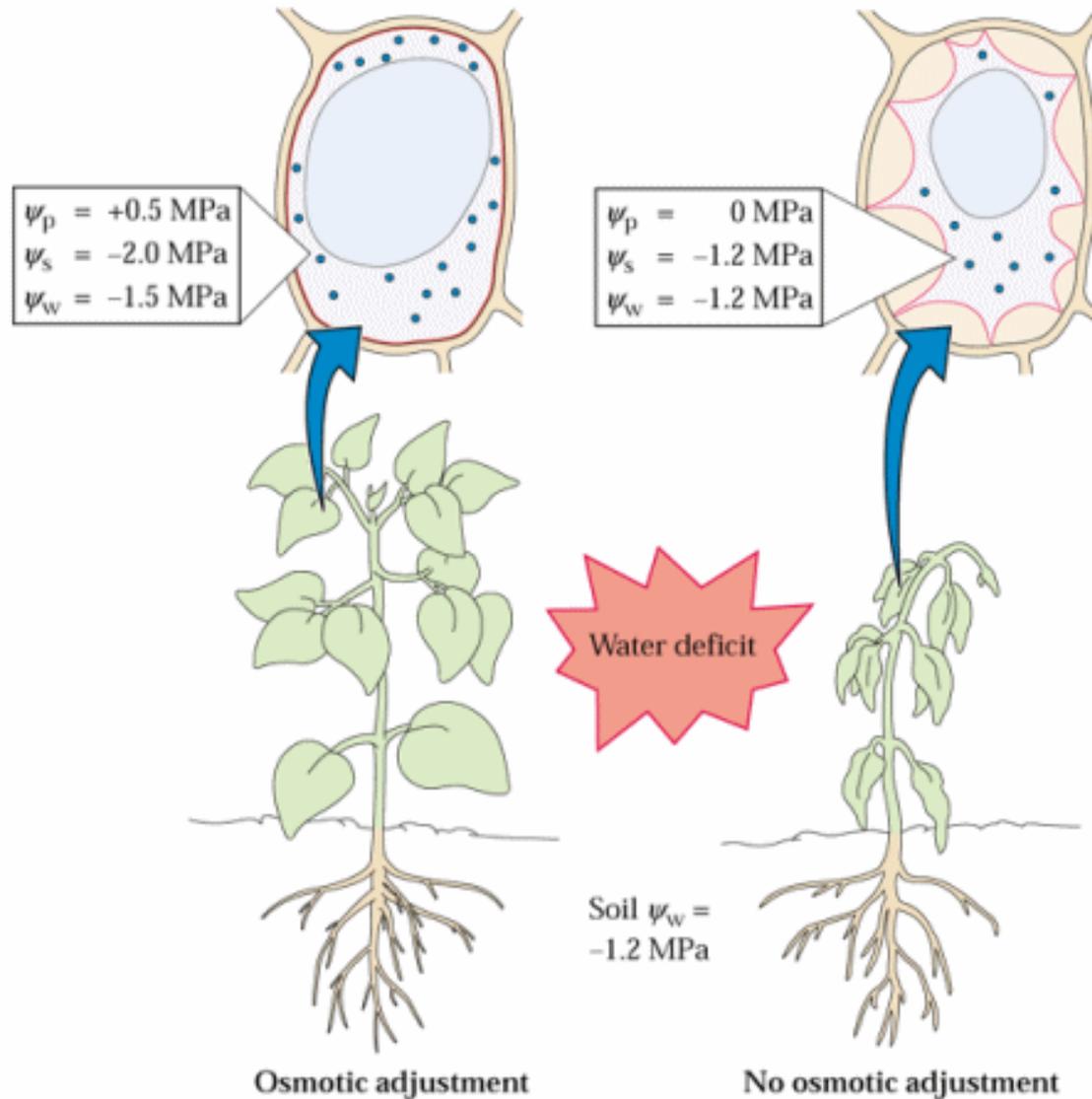
TLP = Turgor Loss Point



Bartlett et al. 2012, *Ecology Letters*



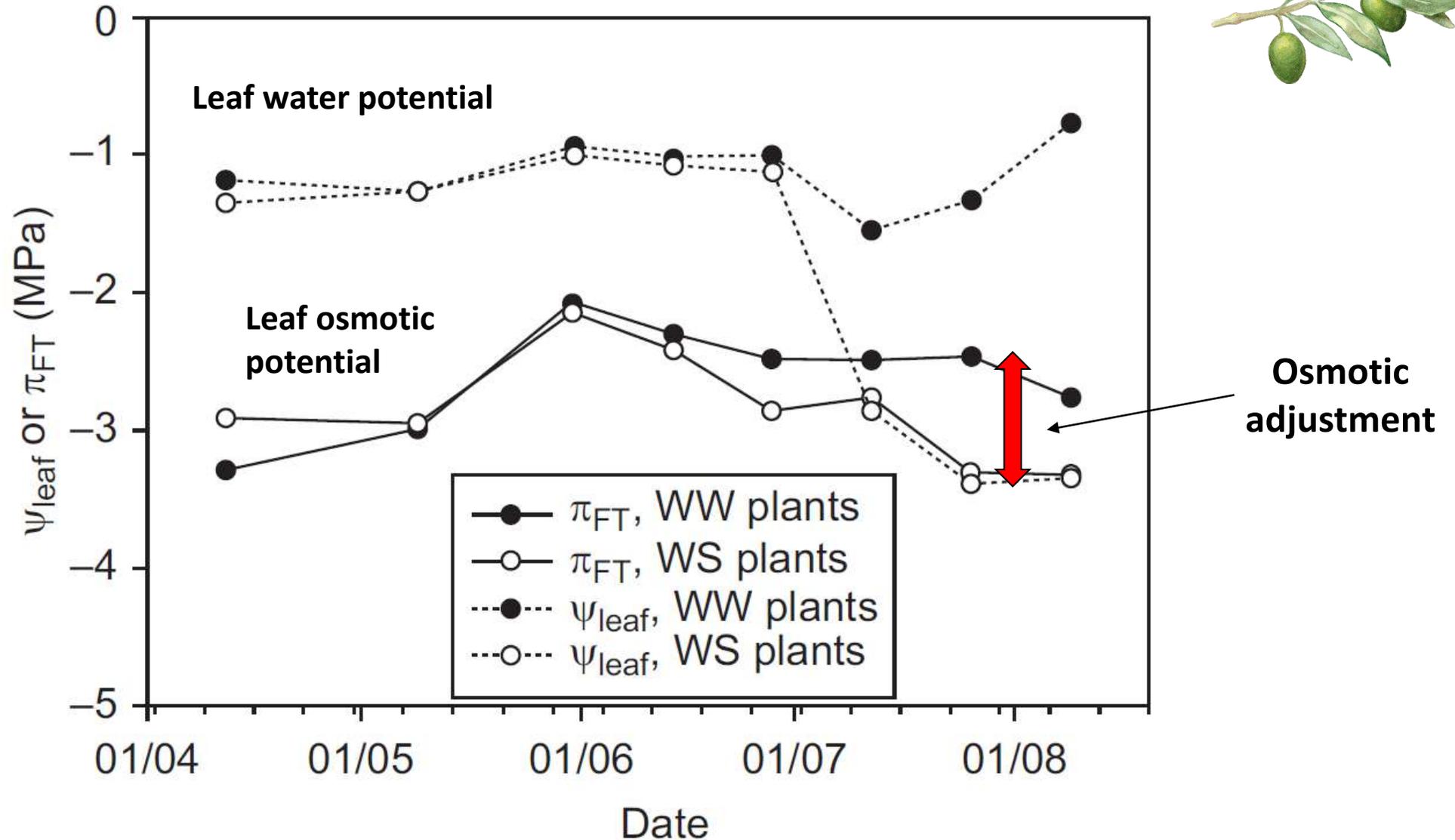
BASIC PRINCIPLES: OSMOTIC ADJUSTMENT



- ❑ Active (+ energy) accumulation of solutes.
- ❑ Essential to maintain cell turgor and volume -> cell growth, cell and tissue rigidity.
- ❑ Under soil drought conditions, a decrease in Ψ_s allows roots to extract water.

BASIC PRINCIPLES: OSMOTIC ADJUSTMENT

Example in olive trees



Osmotic adjustment

METHODOLOGIES TO MEASURE Ψ IN PLANTS

Training school: Agrosystems and Crops Monitoring

PRESSURE CHAMBER



THERMOCOUPLE PSYCHROMETERS



METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

VEGETABLE STATICKS:
Or, An ACCOUNT of some
Statical Experiments
ON THE
SAP in VEGETABLES:
Being an ESSAY towards a
Natural History of Vegetation.
Also, a SPECIMEN of
An ATTEMPT to Analyse the AIR,
By a great Variety of
CHYMIO-STATICAL EXPERIMENTS;
Which were read at several Meetings before
the ROYAL SOCIETY.

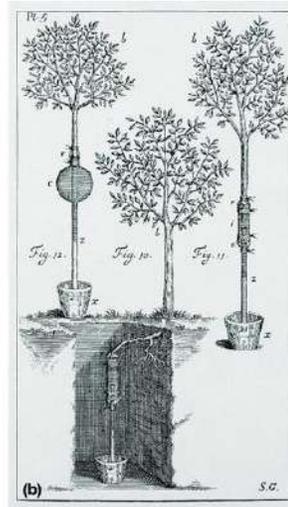
Quid est in his, in quo non naturæ ratio intelligentis appareat? Tul. de Nat. Deor.

—Etenim Experimentorum longe major est subtilitas, quam sensus ipsius— Itaque eo rem deducimus, ut sensus tantum de Experimento, Experimentum de re judicet. Fran. de Verul. Instauratio magna.

By **STEPH. HALES**, B. D. F. R. S.
Rector of Farringdon, Hampshire, and Minister of
Teddington, Middlesex.

LONDON:

Printed for W. and J. INNYS, at the West End of St. Paul's;
and T. WOODWARD, over-against St. Dunstan's Church
in Fleetstreet. M, DCC, XXVII.



s. XVIII

"PRESSURE BOMB"

By means of this method useful results were obtained, but danger attended the determinations. Despite the strength of the glass cylinders used, two explosions occurred, fortunately attended by delay in the work only, so that after a comparatively small number of observations, a more suitable method was looked for.

TRANSPIRATION AND THE ASCENT OF SAP IN PLANTS

BY

HENRY H. DIXON, Sc.D., F.R.S.

University Professor of Botany in Trinity College, Dublin; Director of
Trinity College Botanic Gardens

MACMILLAN AND CO., LIMITED
ST. MARTIN'S STREET, LONDON

1914

METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

Sap Pressure in Vascular Plants

Negative hydrostatic pressure can be measured in plants.

P. F. Scholander, H. T. Hammel, Edda D. Bradstreet, and
E. A. Hemmingen

Scholander et al, 1965, *Science*

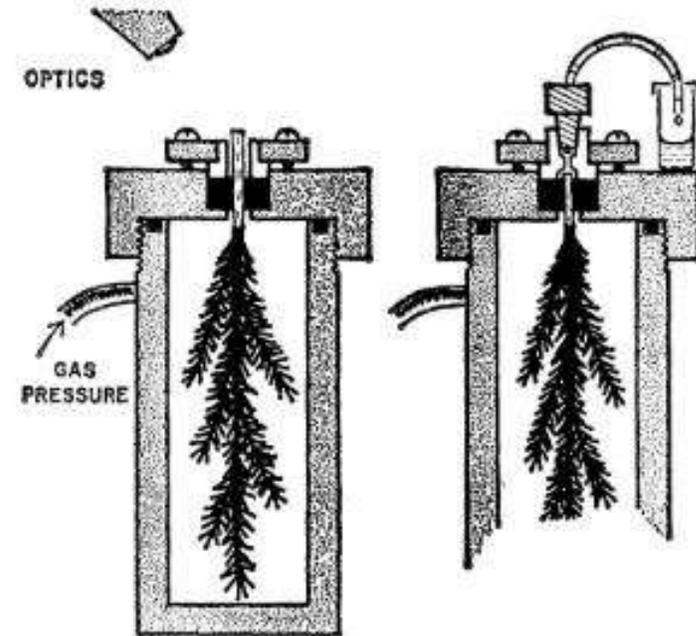


Fig. 2. Pressure bomb for measurement of sap pressure in the xylem of a twig. Left, direct observation; right, stepwise sap extrusion and pressure measurement to obtain a pressure-volume curve.

METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

Today's versions...



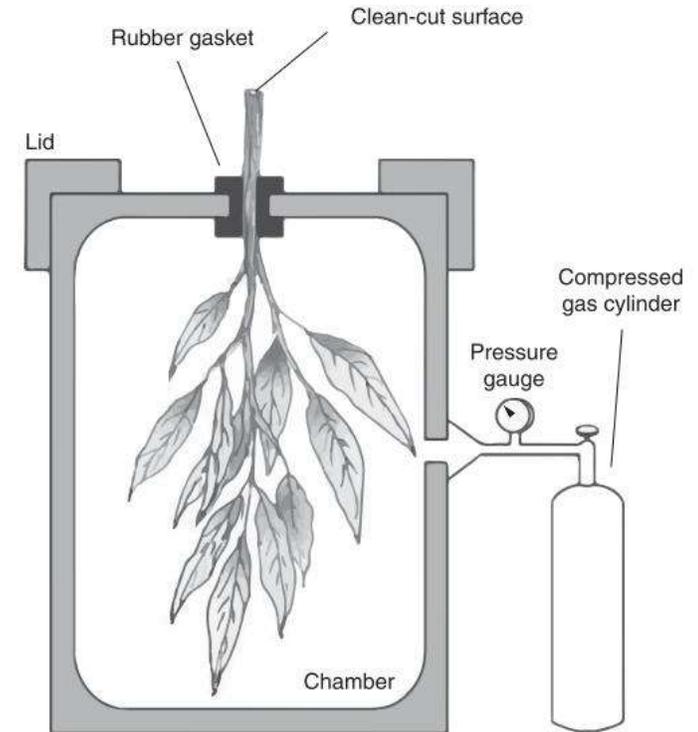
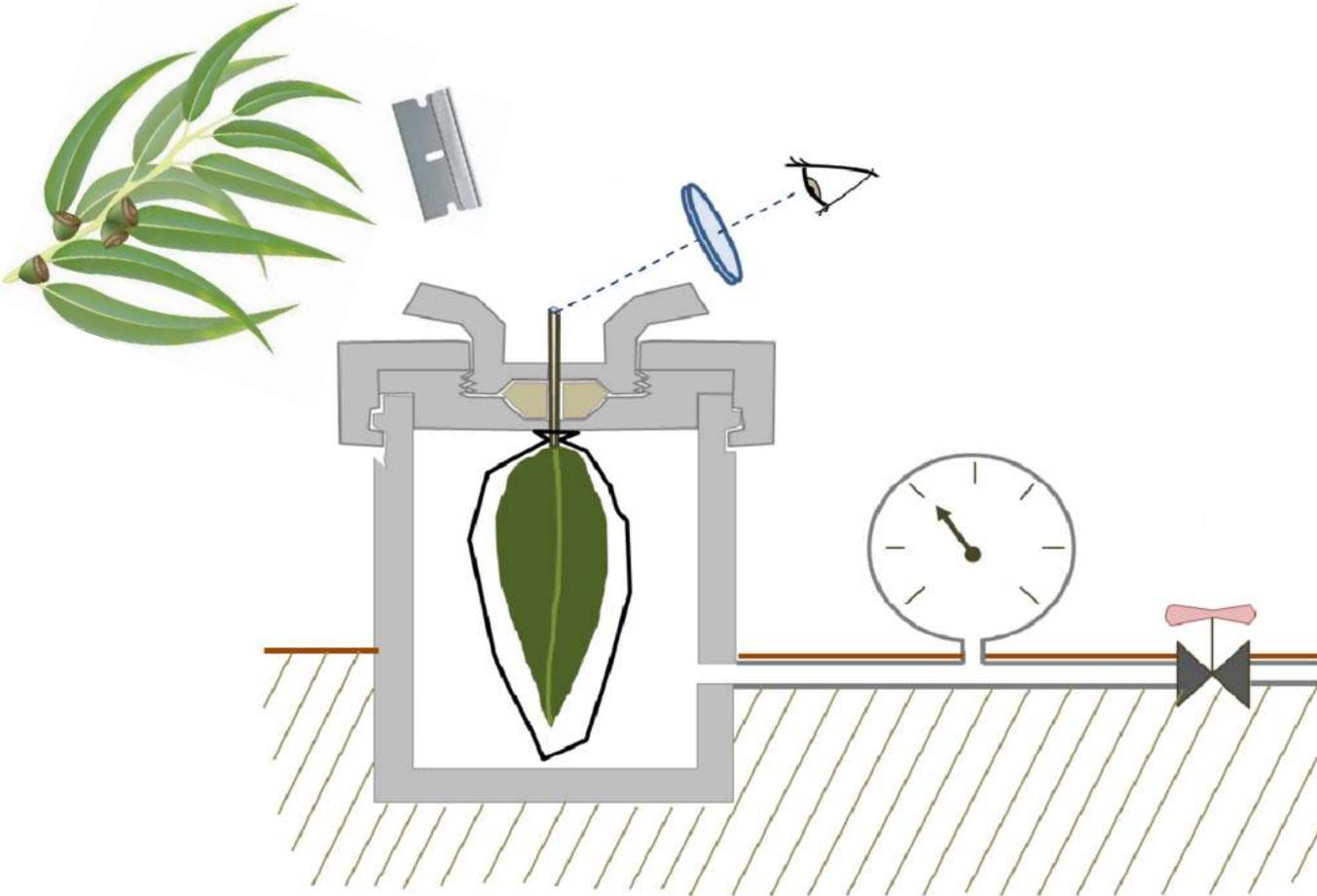
Pressure-release valves



Digital pressure transducers

METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

In the typical procedure...



Pérez-Harguindeguy et al. 2013

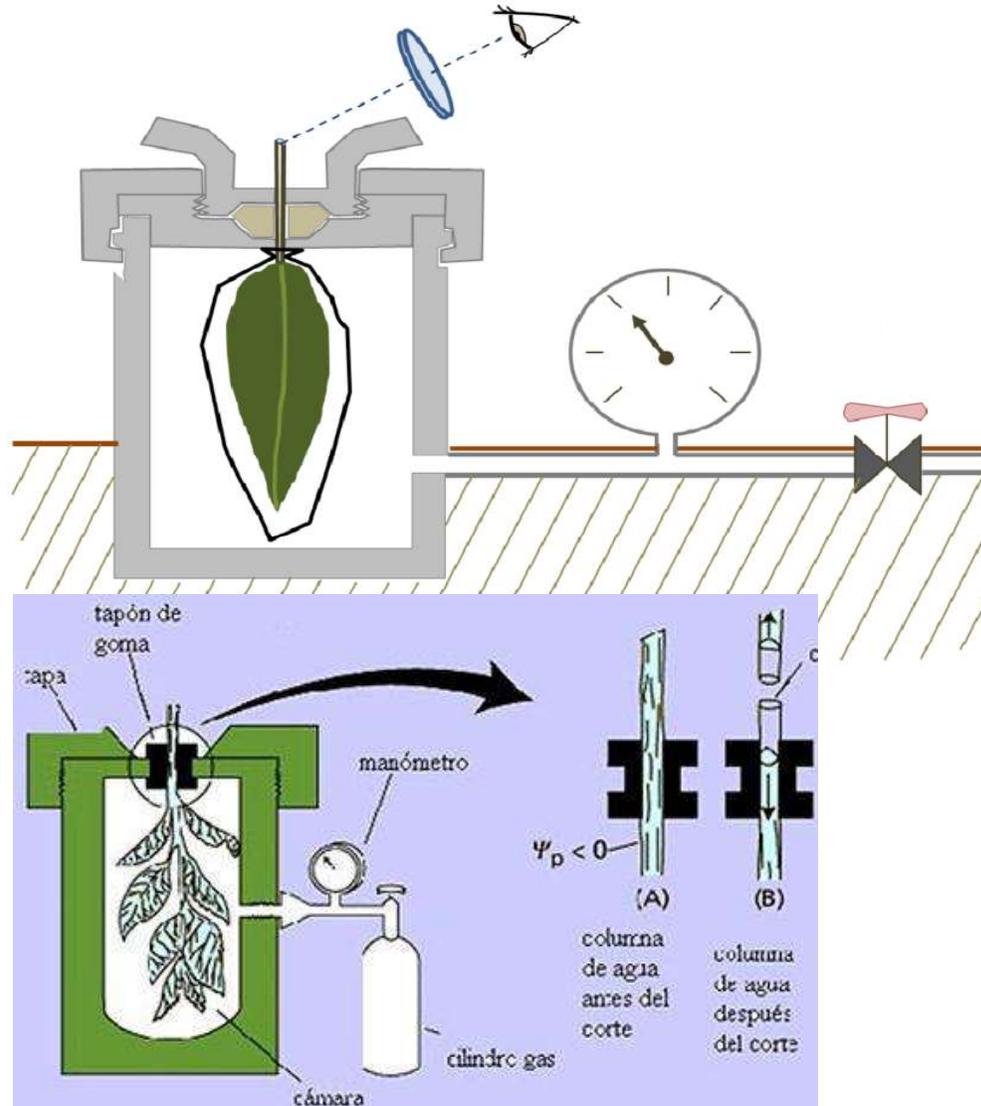


METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER



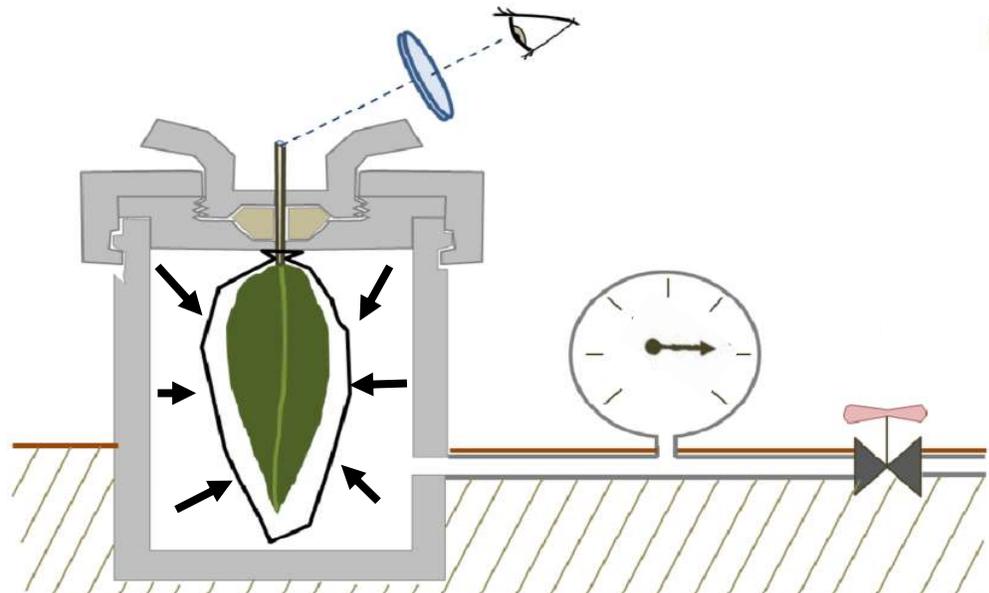
METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

The measurement assumes the Cohesion Tension theory for the ascent of sap



METHODOLOGIES TO MEASURE Ψ IN PLANTS: THE PRESSURE CHAMBER

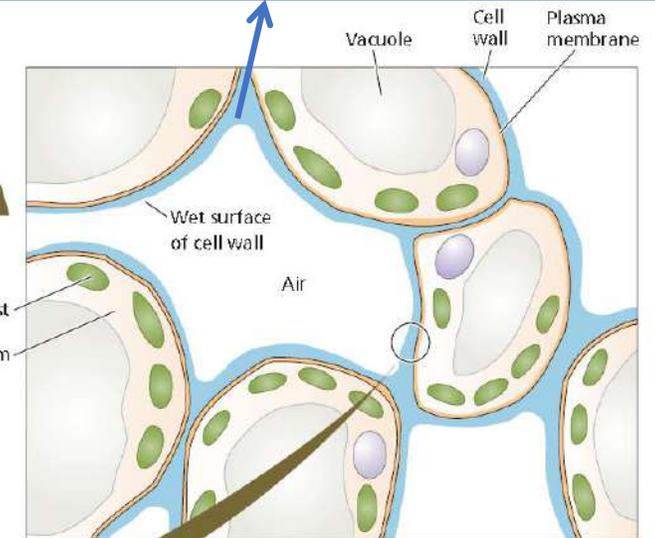
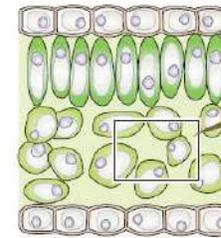
The measurement assumes the Cohesion Tension theory for the ascent of sap



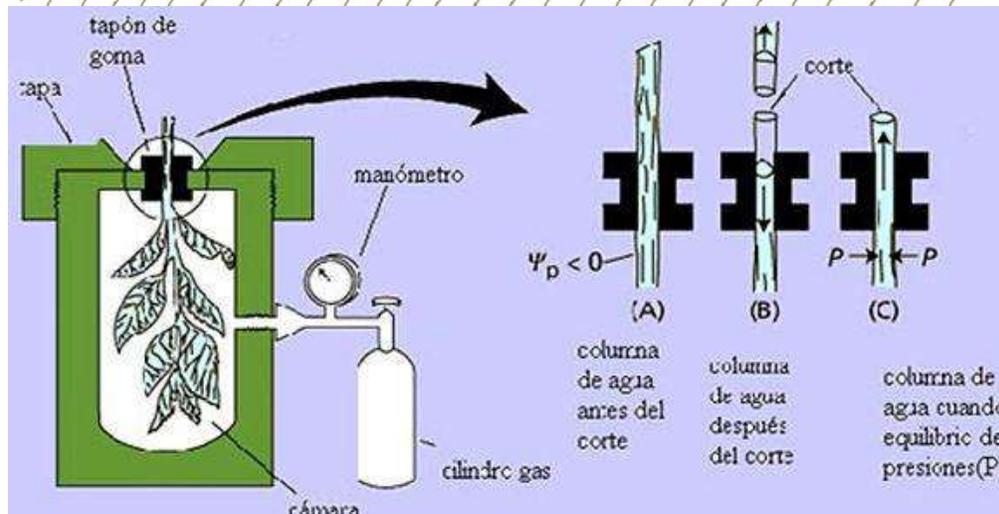
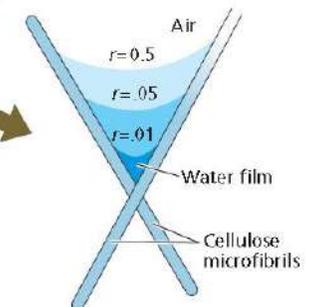
(B) Leaf



Apoplast and xylem sap $\Psi_s \sim 0$ MPa



Radius of curvature (μm)	Hydrostatic pressure (MPa)
0.5	-0.3
0.05	-3
0.01	-15

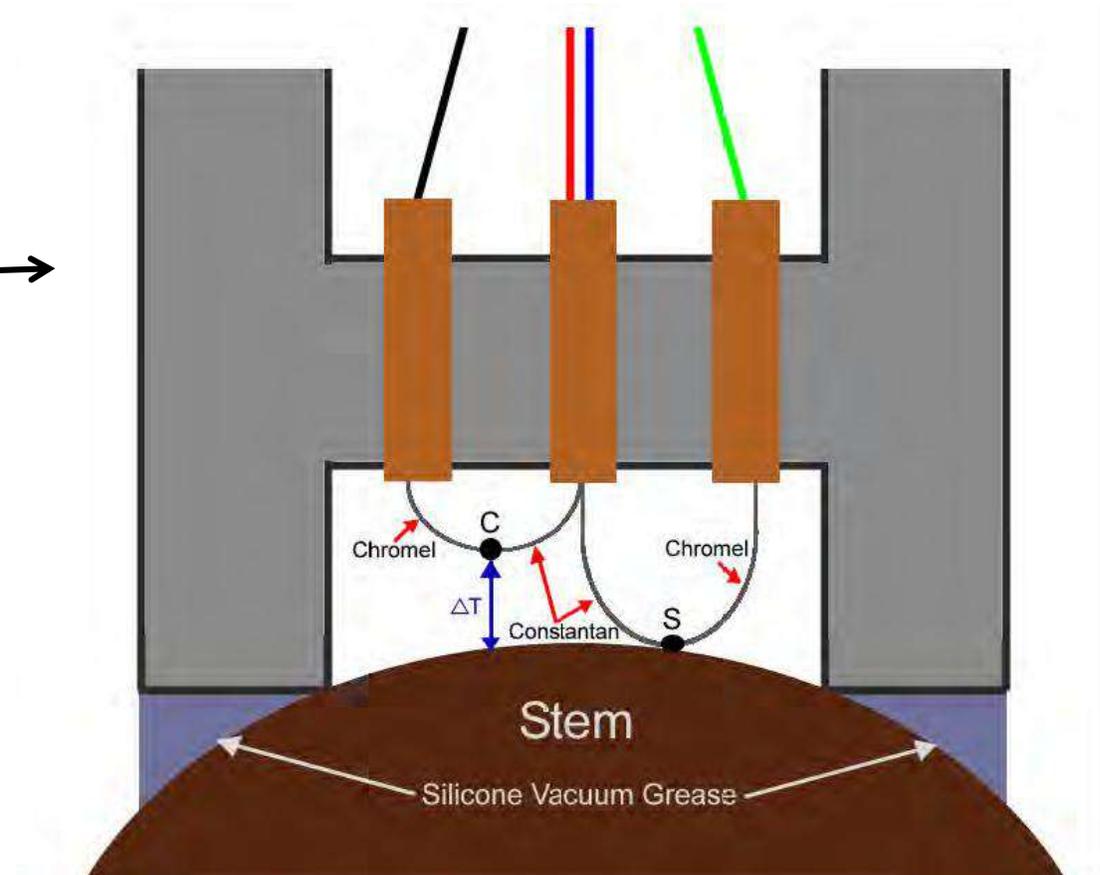


METHODOLOGIES TO MEASURE Ψ IN PLANTS: PSYCHROMETERS

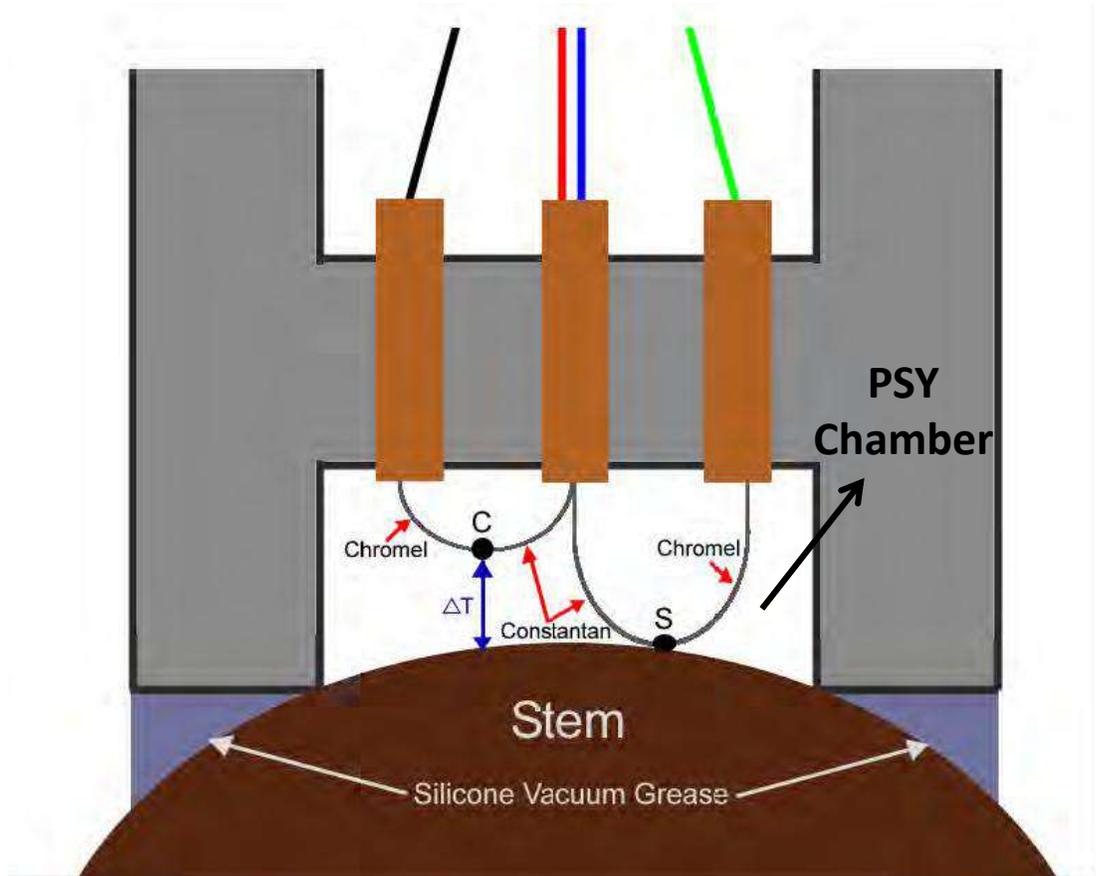
STEM THERMOCOUPLE PSYCHROMETERS



METHODOLOGIES TO MEASURE Ψ IN PLANTS: PSYCHROMETERS



METHODOLOGIES TO MEASURE Ψ IN PLANTS: PSYCHROMETERS



- ❑ It measures xylem Ψ through equilibrium via vapor phase of the air in equilibrium with the xylem water.
- ❑ Psychrometric measurement based on Peltier cooling pulse to cool Thermocouple-C sufficiently to condense water on the thermocouple. The wet-bulb depression (drop of water evaporating), after correcting by the gradient in temperature between Chamber and Sample (ΔT), is used to determine Ψ .
- ❑ The dryer the sample (lower Ψ), the dryer the air in the chamber and the faster the evaporation.

METHODOLOGIES TO MEASURE Ψ IN PLANTS

PRESSURE CHAMBER

VS

THERMOCOUPLE PSYCHROMETERS



Destructive



Semi-invasive



Reliable within a wide range of Ψ
*** with exceptions*



Reliable within a wide range of Ψ
except Ψ close to zero



Not suitable for continuous monitoring



Suitable for automatic and continuous monitoring

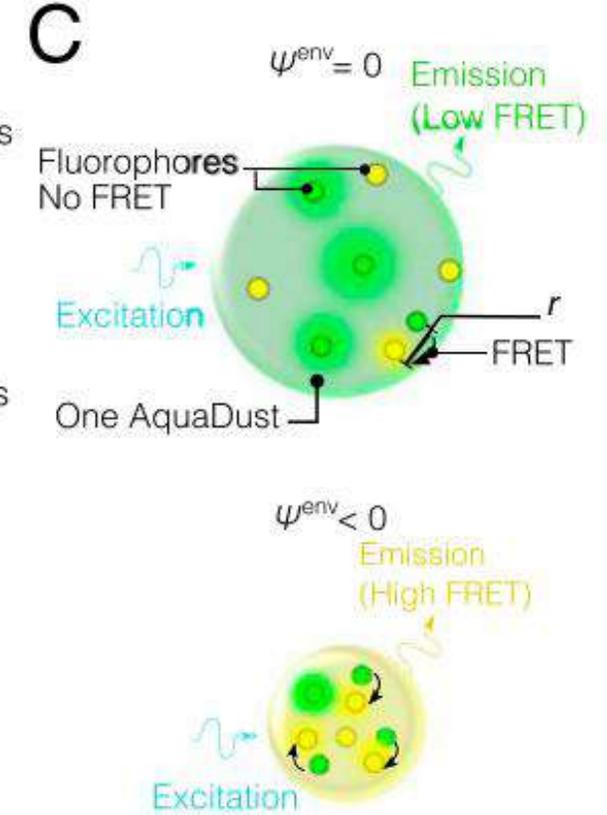
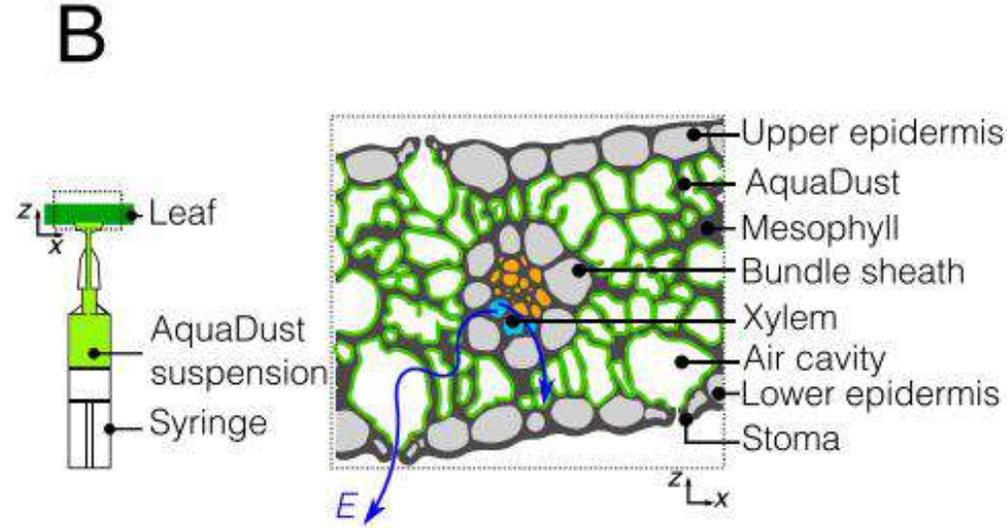
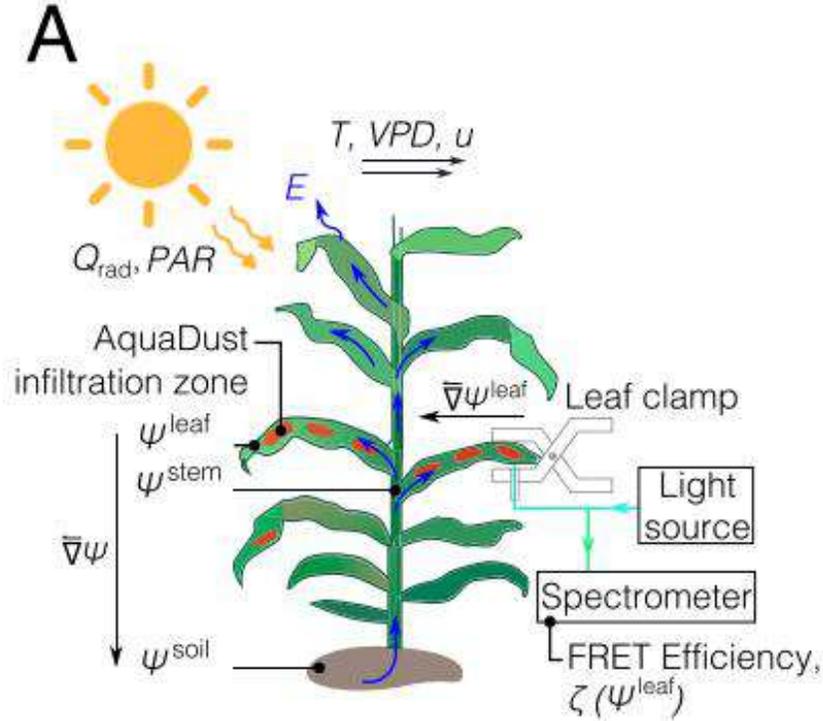


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METHODOLOGIES TO MEASURE Ψ IN PLANTS: ADVANCED AND ALTERNATIVES

"AQUADUST"



Jain *et al.*, 2021, PNAS



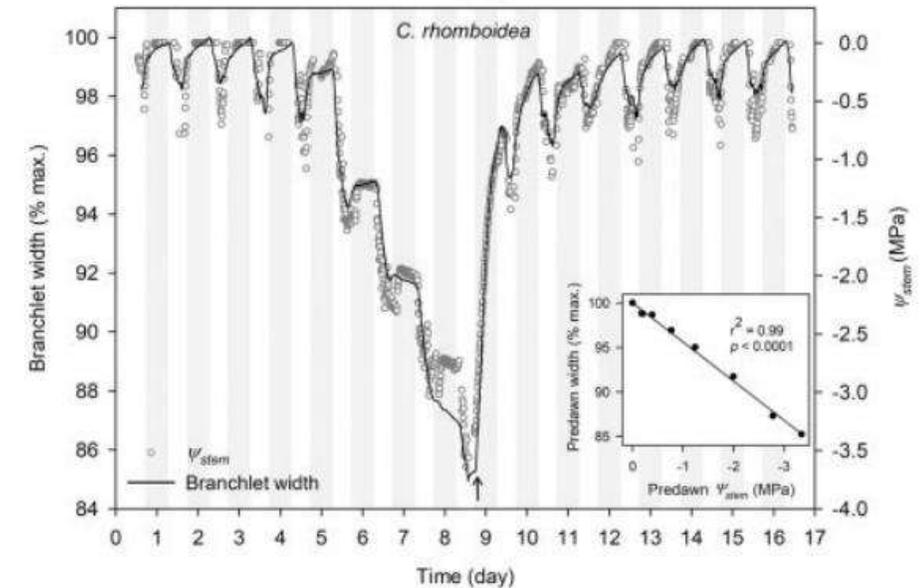
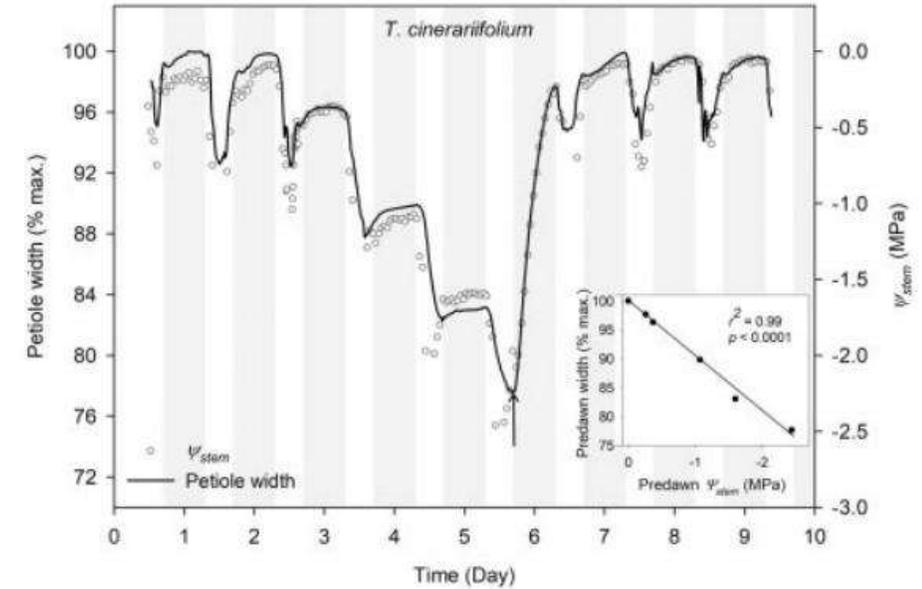
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METHODOLOGIES TO MEASURE Ψ IN PLANTS: ADVANCED AND ALTERNATIVES

"DENDROCAMS"



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER



1972:

TYREE, M.T. & H.T. HAMMEL. The relationship between turgor pressure and the water relations of plant tissue. The pressure-bomb technique. *J. Exp. Bot.* 23: 1-14, 1972.

23 O

1973:

TYREE, M.T., J. DAINITY & M. BENIS. The water relations of hemlock (*Tsuga canadensis*) II. The equilibrium water relations as measured by the pressure-bomb technique. *Can. J. Bot.* 51: 1481-1489, 1973.

TYREE, M.T. & J. DAINITY. The water relations of hemlock (*Tsuga canadensis*) II. The kinetics of exchange between the symplast and apoplast. *Can. J. Bot.* 51: 1481-1489, 1973.

TYREE M.T., M. BENIS & J. DAINITY. The water relations of hemlock (*Tsuga canadensis*) III. The dependence of water exchange in a pre-emptive state. *Can. J. Bot.* 51: 1537-1543, 1973.

1974:

TYREE, M.T., J. DAINITY & D.M. HUNTER. The water relations of hemlock (*Tsuga canadensis*) IV. The dependence of the balance pressure on water potential as measured by the pressure-bomb technique. *Can. J. Bot.* 52: 973-978, 1974.

1977:

TYREE, M.T. & Y.N.S. CHEUNG. Resistance to water flow in *Fagus grandifolia* leaves. *Can. J. Bot.* 55: 2591-2599, 1977.

1978:

TYREE, M.T., Y.N.S. CHEUNG, M.E. MacGREGOR & A.J.B. TALBOT. The characteristics of seasonal and ontogenetic changes in the tissue-water relations of *Acer*, *Populus*, *Tsuga* and *Picea*. *Can. J. Bot.* 56: 635-647, 1978.

TYREE, M.T., M.E. MacGREGOR, A. PETROV & M.I. UPENIEKS. A comparison of systematic errors between the Richards and Hammel methods of measuring tissue-water relations parameters. *Can. J. Bot.* 56: 2153-2161, 1978.

1979:

TYREE, M.T., M.E. MacGREGOR & S.I. CAMERON. Ontogenetic factors affecting the osmotic pressure and bulk modulus of elasticity of poplar leaves. *Poplar Research, Management and Utilization in Canada*, (D.C.F. Fayle, L. Zuffa & H.W. Anderson, Eds.) Ontario Ministry of Natural Resources, Forest Research Information Paper No. 102. Report 14 pp 1-11. 1979.

1980:

TYREE, M.T. & A.J. KARAMANOS. Water stress as an ecological factor. In: *Plants and Their Atmospheric Environment* (J. Grace, E.D. Ford & P.G. Jarvis, Eds) Blackwells, Oxford. pp 237-261, 1980.

CRUZZIAT P., M.T. TYREE, C. BODET & M.A. LoGULLO. The kinetics of rehydration of detached sunflower leaves following substantial water loss. *New Phytol.* 84: 293-306, 1980.

1981:

TYREE M.T., P. CRUZZIAT, M. BENIS, M.A. LoGULLO & S. SALLEO. The kinetics of rehydration of detached sunflower leaves from different initial water deficits. *Plant, Cell and Environ.* 4: 309-317, 1981.

TYREE, M.T. The relationship between the bulk modulus of elasticity of a complex tissue and the mean modulus of its cells. *Ann. Bot.* 47: 547-559, 1981.

TYREE, M.T. & H. RICHTER. Alternative methods of analysing water potential isotherms: Some cautions and clarifications. I. The impact of non-ideality and of some experimental errors. *J. Exp. Bot.* 32: 643-653, 1981.

MT Tyree, AJ Karamanos Water stress as an ecological factor Symposium-British Ecological Society 1981

1982:

TYREE, M.T. & H. RICHTER. Alternate methods of analysing water potential isotherms: Some cautions and clarifications. II. Curvilinearity in water potential isotherms. *Can. J. Bot.* 60: 911-920, 1982.

TYREE, M.T. & P.G. JARVIS. Water in tissues and cell. In: *Encyclopedia of Plant Physiology N.S. Physiological Plant Ecology Vol. 12B* pp 35-77, 1982.

OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

Table S2. Seminal reviews and studies focused on or related to leaf water potential measurements with the pressure chamber.

Reference	Brief description	Assumptions/Precautions/Recommendations	Relation to our study
Waring and Cleary, 1967, Plant moisture stress: Evaluation by pressure bomb, Science.	Early design of a portable pressure chamber and one of the first studies demonstrating the relation between plant distribution and water stress measured with the pressure chamber with attempts to standardize collection and preparation of samples.	Errors in the method related to rate of pressure increase and sample preparation are discussed. They used atm and positive values for water potential units. They mentioned that higher readings were obtained at rapid rates of pressure increase, and recommended to minimize the amount of stem outside the chamber to less than 2 cm.	Technically related to pressure chamber measurements, but the very few recommendations included are not supported with other reports.
Boyer, 1969, Measurement of the water status of plants, Annual Review of Plant Physiology.	Basic concepts related to the water status of plants and techniques available by then for measuring it, such as pressure-volume curves.	More related to the theory behind pressure chamber measurements. He mentioned that accuracy tend to vary from species to species and explained by variable compressions of the tissue and consideration of other tissues than xylem filled with sap during the measurement.	Not specifically focused on pressure chamber measurements.
Ritchie and Hinckley, 1975, The pressure chamber as an instrument for ecological research, Advances in Ecological Research.	To the best of our knowledge, this is one of the most complete review of the pressure chamber technique. It includes theoretical considerations, a historical perspective (very enjoyable to read), applications on ecological studies plus other applications in other fields, and most importantly, assumptions tested by then, independent measurements of water potentials with other techniques to validate the pressure chamber technique and unresolved questions. They also included unpublished data in an attempt at synthesis and evaluation.	Recommendation of peeling phloem and bark in conifers, but for facilitating insertion into the rubber gland. They also already pointed at the disagreement between what they called the combined gravitational and frictional potentials (P) and the water potential (ψ) at low levels of water potentials, and they explained these discrepancies due to the appearance of embolized vessels. P and ψ should be equal when matric and osmotic potentials are negligible. Concluding remarks: Pressure chamber measures the gravitational and frictional components of the total water potential in the xylem; Assumption that osmotic and matric potential of the xylem sap are negligible seemed to be valid for many species; Recutting is not recommended; Amount of tissue inside and outside the chamber should be standardize; Measurement should be done rapidly after excision; Equilibrium must be permitted during the measurement, so low rates of pressure application (0.1 - 0.3 bars s ⁻¹) are recommended. Specific procedures are	Specifically focused on pressure chamber measurements. However, some recommendations / precautions may need to be updated. For instance, they reported that recutting the stem of twigs or the petiole of leaves can induce errors in some species. Recent studies, in contrast, did not find an effect in vine leaves (Levin, 2019, Agricultural Water Management). They recommended to measure rapidly after excision, and when this is not possible, to store the sample in a humid chamber (avoiding rehydration) and in darkness (in agreement with our results, but without mentioning time of storage or if the leaves are transpiring or not).

OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER



UCLA: Celia M. Rodriguez-Dominguez, Alicia Forner, Sebastia Martorell, Lawren Sack.

SYDNEY: Brendan Choat, Rosana Lopez, Sebastian Pfautsch, Jennifer M.R. Peters.

UTAS: Madeline R. Carins-Murphy, Freya Richardson.

INNSBRUCK: Stefan Mayr.

IRNAS: Antonio Diaz-Espejo, Virginia Hernandez-Santana.

PURDUE: Scott A.M. McAdam, Timothy A. Batz.

INRAE: Paulo E. Menezes-Silva, Jose M. Torres-Ruiz.



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

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DOI: 10.1111/pce.14330

ORIGINAL ARTICLE

Plant, Cell & Environment  WILEY

Leaf water potential measurements using the pressure chamber: Synthetic testing of assumptions towards best practices for precision and accuracy

Celia M. Rodriguez-Dominguez^{1,2}  | Alicia Forner^{3,4} | Sebastia Martorell⁵ |
Brendan Choat⁶  | Rosana Lopez⁷ | Jennifer M. R. Peters⁸ |
Sebastian Pfautsch⁹  | Stefan Mayr¹⁰ | Madeline R. Carins-Murphy¹¹ |
Scott A. M. McAdam¹²  | Freya Richardson¹¹  | Antonio Diaz-Espejo^{1,2}  |
Virginia Hernandez-Santana^{1,2}  | Paulo E. Menezes-Silva¹³  |
Jose M. Torres-Ruiz¹³  | Timothy A. Batz¹² | Lawren Sack¹⁴ 

Validation of 8 assumptions commonly used in Ψ_{leaf} measurements with the pressure chamber by a multitude of laboratories around the world

Assumptions related to:

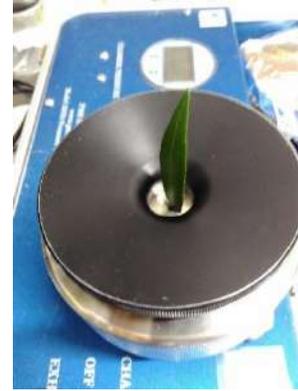
- *Sampling conditions or sample handling prior to measurement.*
- *Measurement technique.*



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

How many of you measure / have measured with the pressure chamber??

1. I'll present the ASSUMPTION.



WE NEED TO
DISCUSS
ABOUT THIS...

2. Participate!! What do you think??



3. Empirical check. What did the data tell us??



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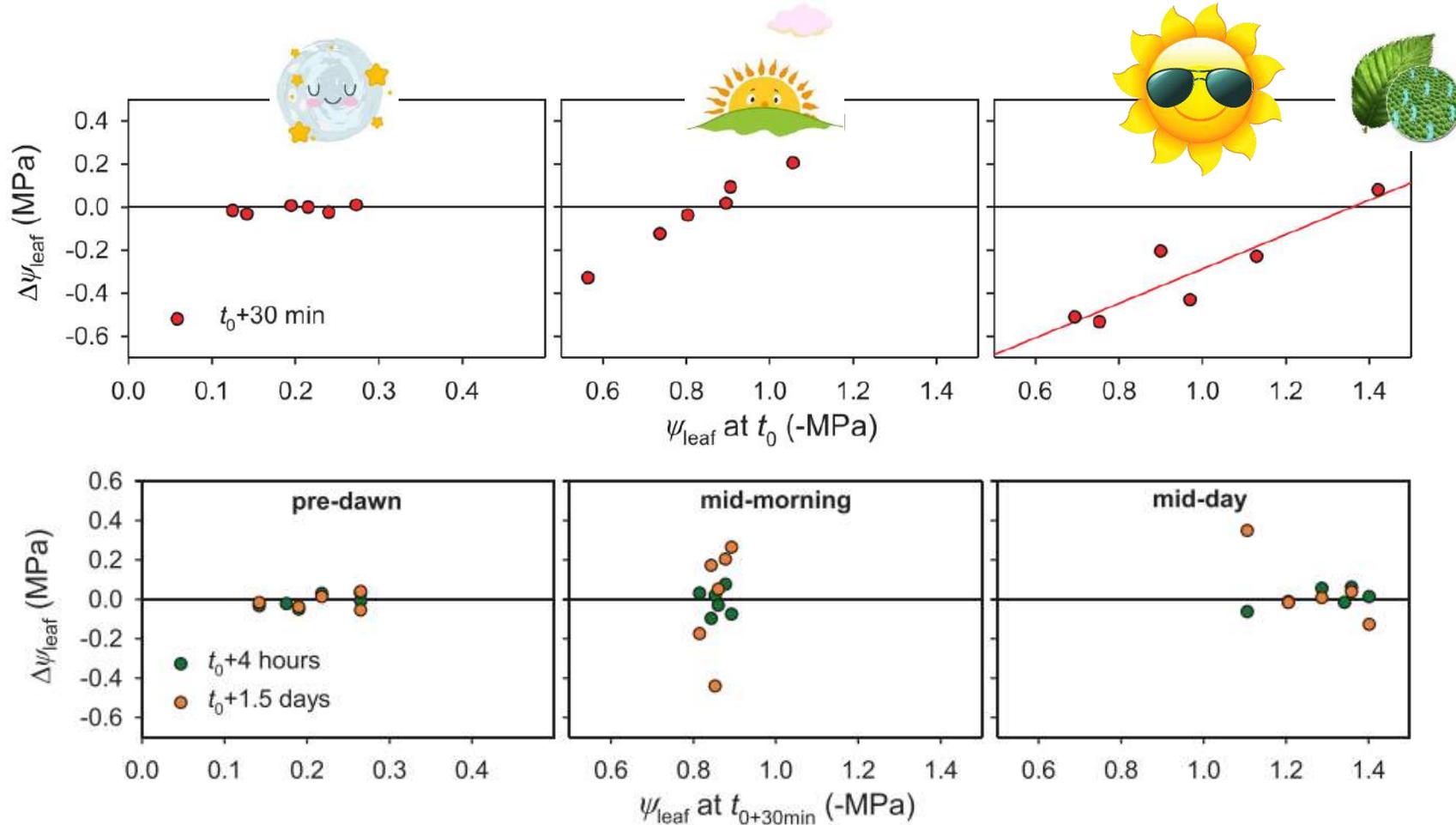
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE CONDITIONS

Do we need to equilibrate the leaf after excision? How?

Assumption #1. After leaf excision, especially when the leaf is transpiring, Ψ_{leaf} changes to an equilibrium.

Assumption #2. Once equilibrium is reached, it is maintained if leaves are stored conveniently.



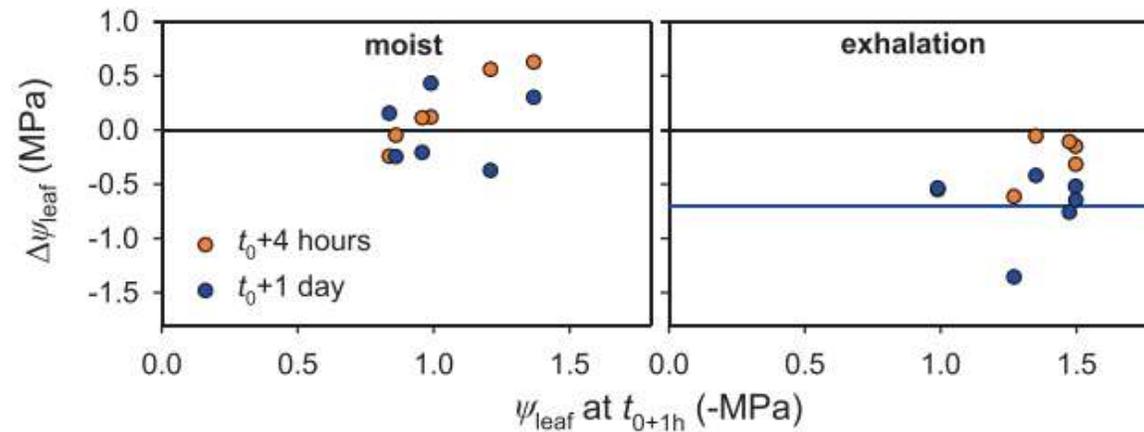
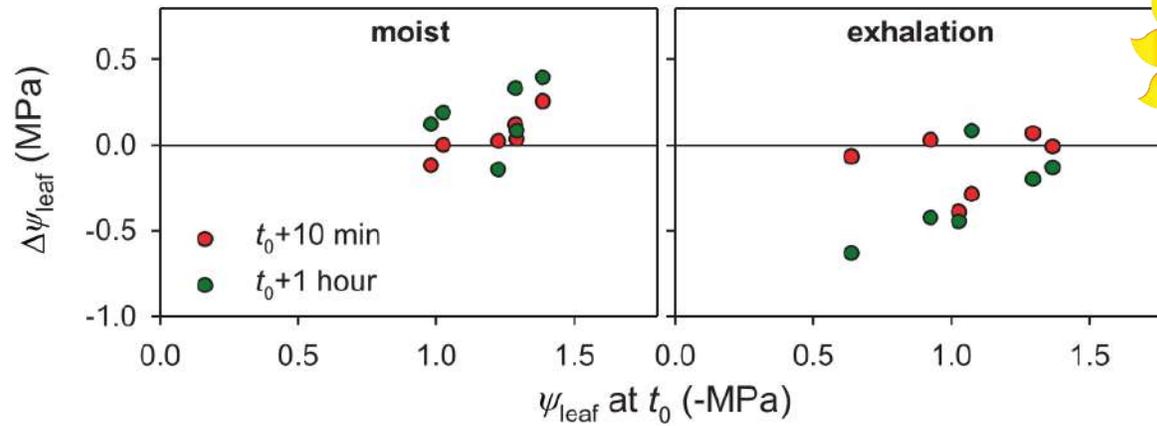
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

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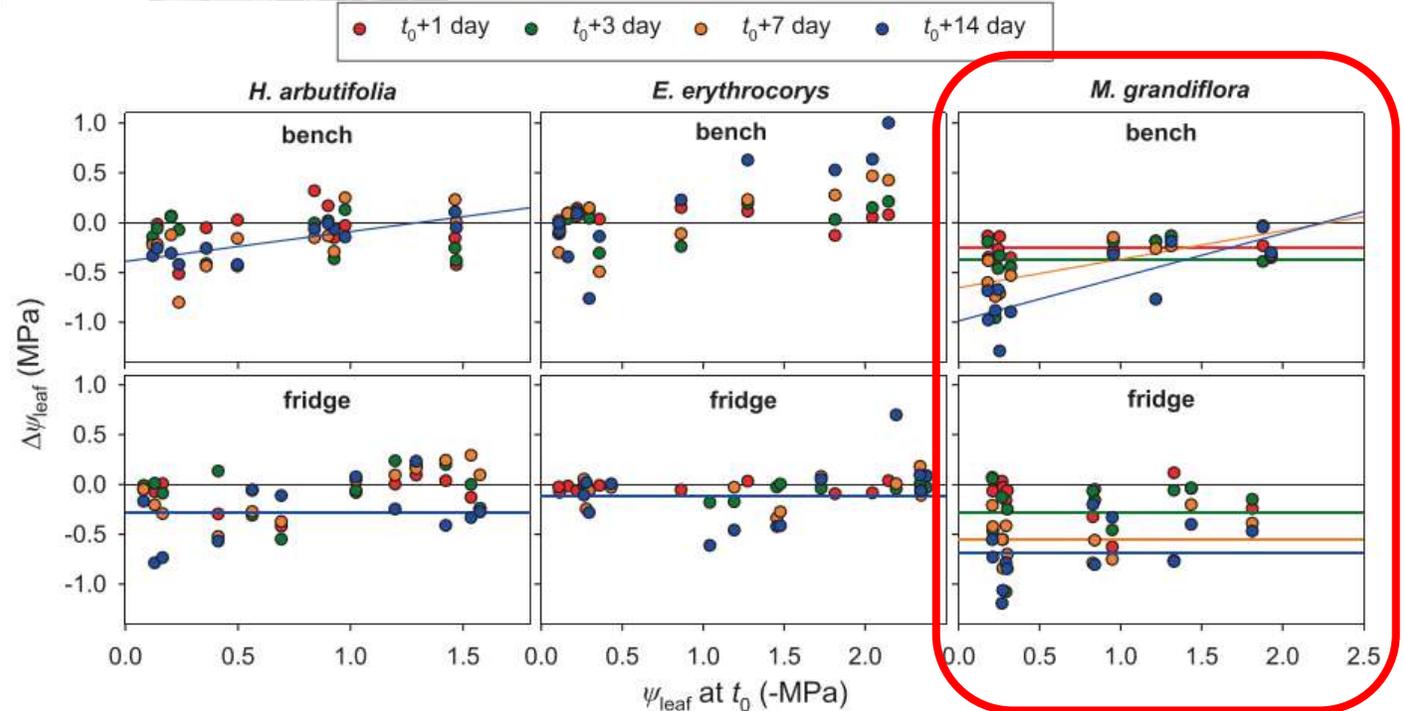
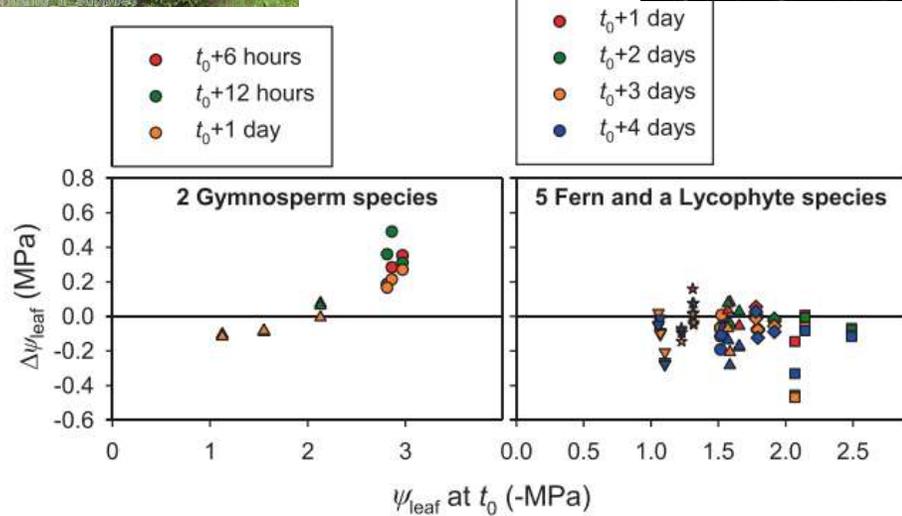
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE CONDITIONS

Do we need to equilibrate the leaf after excision? How?

Assumption #1. After leaf excision, especially when the leaf is transpiring, Ψ_{leaf} changes to an equilibrium.

Assumption #2. Once equilibrium is reached, it is maintained if leaves are stored conveniently.



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

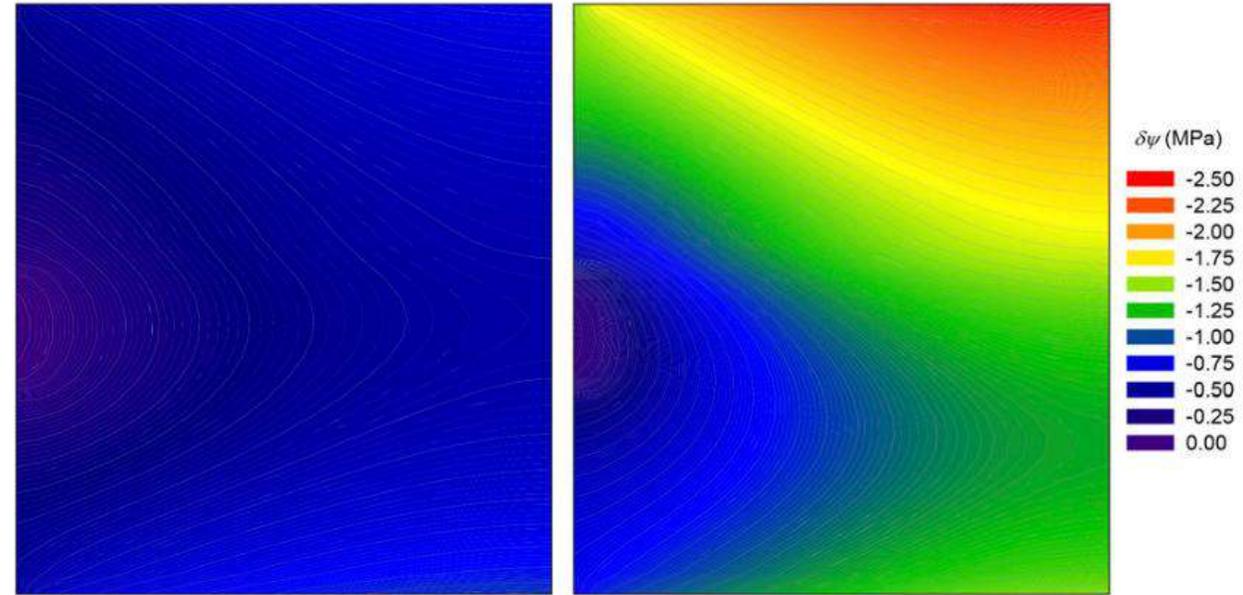
SAMPLE CONDITIONS

Do we need to equilibrate the leaf after excision? How?

Conclusions after testing assumptions #1 and #2

- ✓ *It is advisable to equilibrate leaves, especially if they were previously transpiring, and to measure once the equilibrium is reached.*
- ✓ *Equilibrium time may vary depending on the species.*
- ✓ *Storage conditions:
High humidity, Darkness, Confined space.*

Helianthus annuus



$$E_{\text{upper epid}} = 2.09 \text{ mmol m}^{-2} \text{ s}^{-1}$$
$$E_{\text{lower epid}} = 2.91 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$\delta\psi_{\text{upper epid}} = -0.77 \text{ MPa}$$
$$\delta\psi_{\text{lower epid}} = -0.95 \text{ MPa}$$

$$E_{\text{upper epid}} = 6.27 \text{ mmol m}^{-2} \text{ s}^{-1}$$
$$E_{\text{lower epid}} = 2.91 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$\delta\psi_{\text{upper epid}} = -2.48 \text{ MPa}$$
$$\delta\psi_{\text{lower epid}} = -1.44 \text{ MPa}$$

Buckley et al, 2015, *Plant Phys*



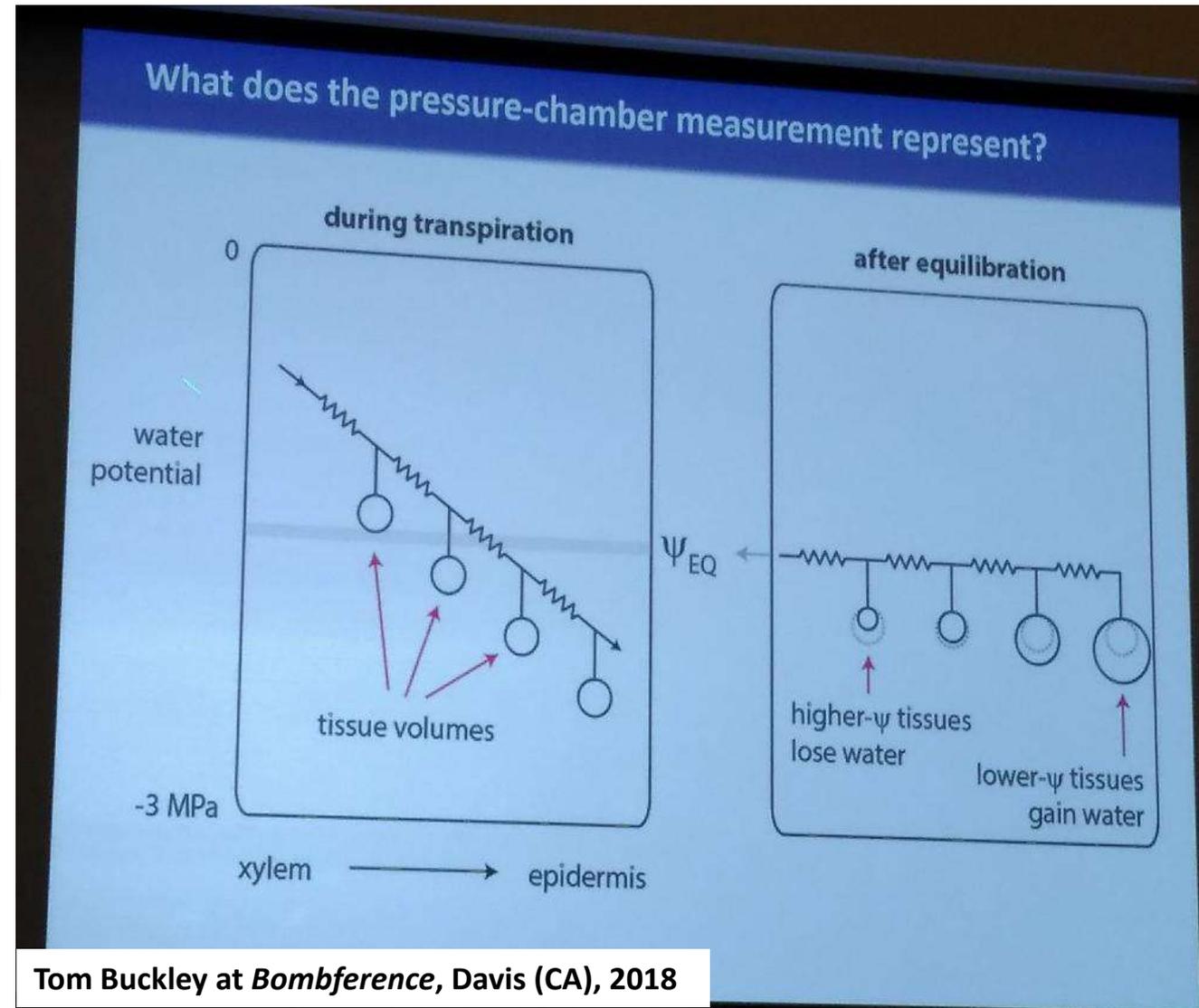
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE CONDITIONS

Do we need to equilibrate the leaf after excision? How?

Conclusions after testing assumptions #1 and #2

- ✓ *It is advisable to equilibrate leaves, especially if they were previously transpiring, and to measure once the equilibrium is reached.*
- ✓ *Equilibrium time may vary depending on the species.*
- ✓ *Storage conditions: High humidity, Darkness, Confined space.*



Tom Buckley at *Bombference*, Davis (CA), 2018



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OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

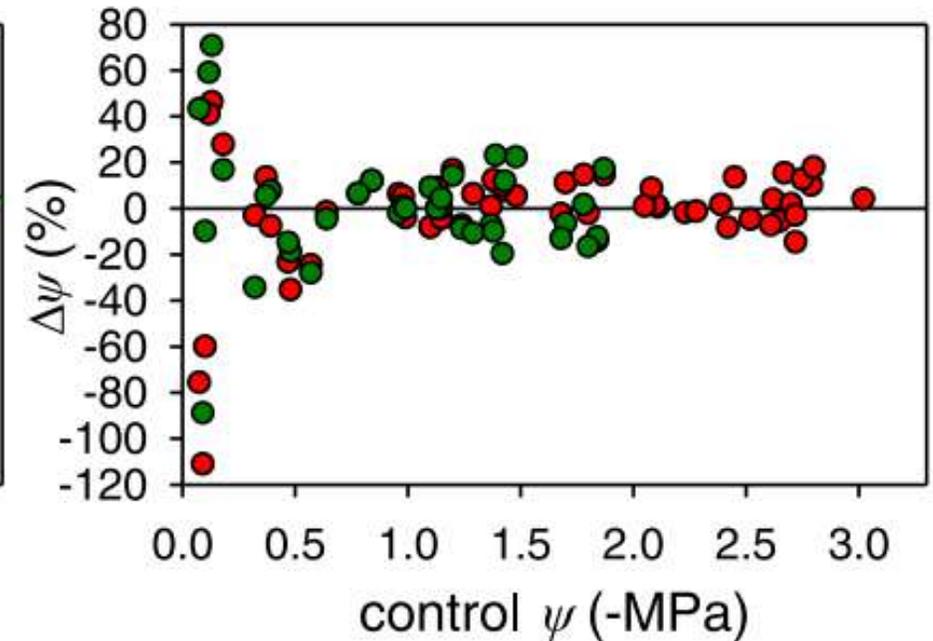
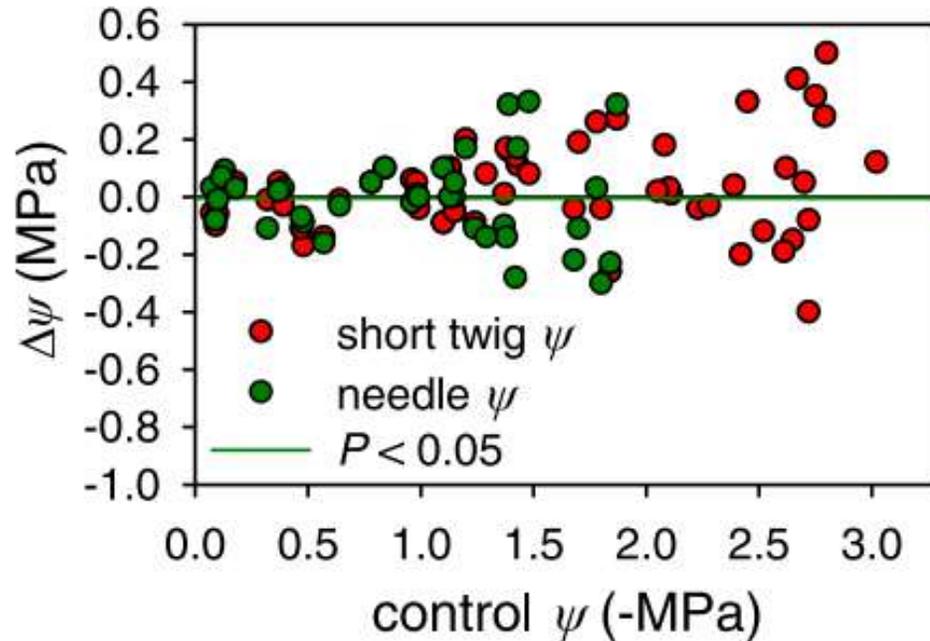
SAMPLE HANDLING

Do leaves equilibrate with their bearing stems when placed under non-transpiring conditions?

Assumption #3. Covered leaves or branches under conditions of non-transpiration equilibrate their water potentials.



Pinus cembra

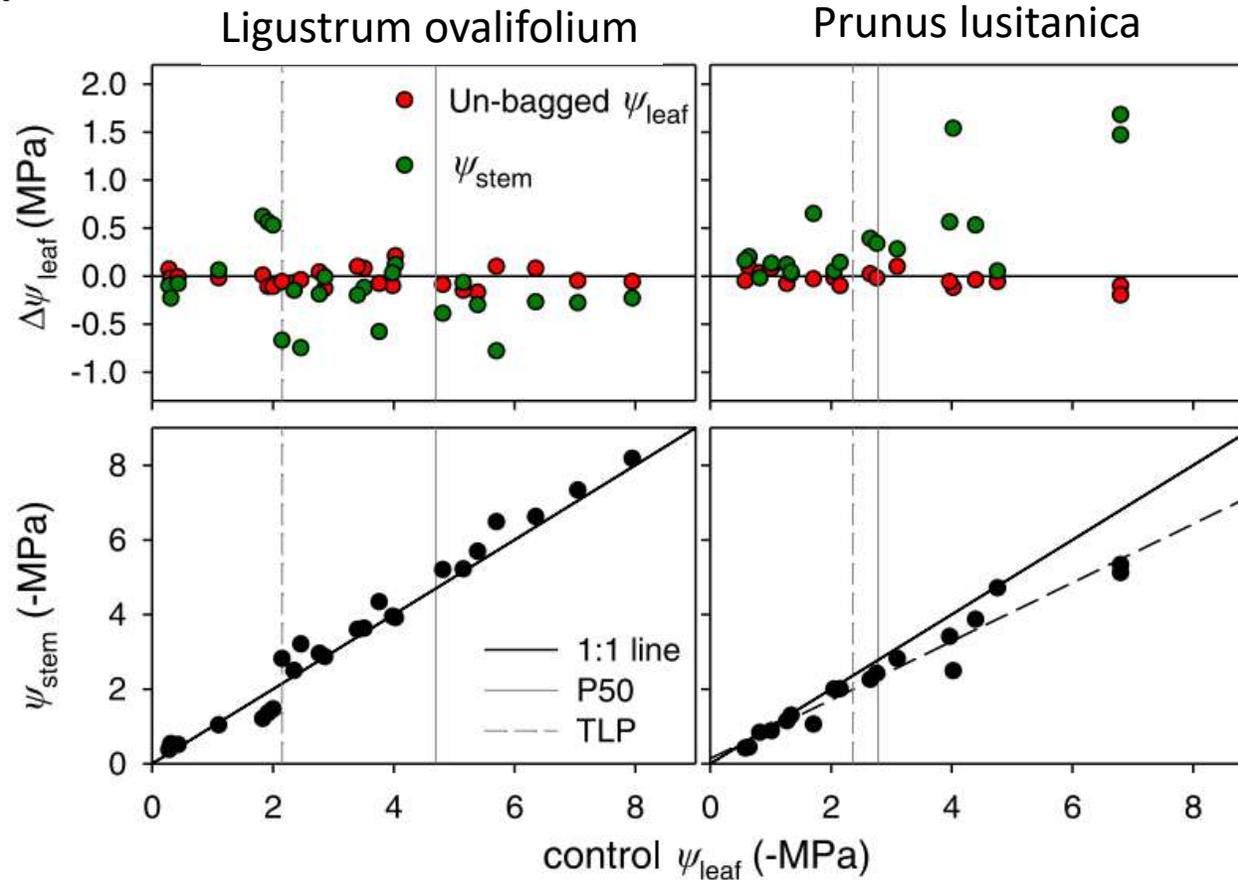


OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

**SAMPLE
HANDLING**

Do leaves equilibrate with their bearing stems when placed under non-transpiring conditions?

Assumption #3. Covered leaves or branches under conditions of non-transpiration equilibrate their water potentials.



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE HANDLING

Do leaves equilibrate with their bearing stems when placed under non-transpiring conditions?

Assumption #3. Covered leaves or branches under conditions of non-transpiration equilibrate their water potentials.

- ✓ *In general, Ψ equilibrates between connected tissues that have been under non-transpiring conditions*
- ✓ *At certain Ψ levels some particularities may occur depending on the species***

**** Case study later**



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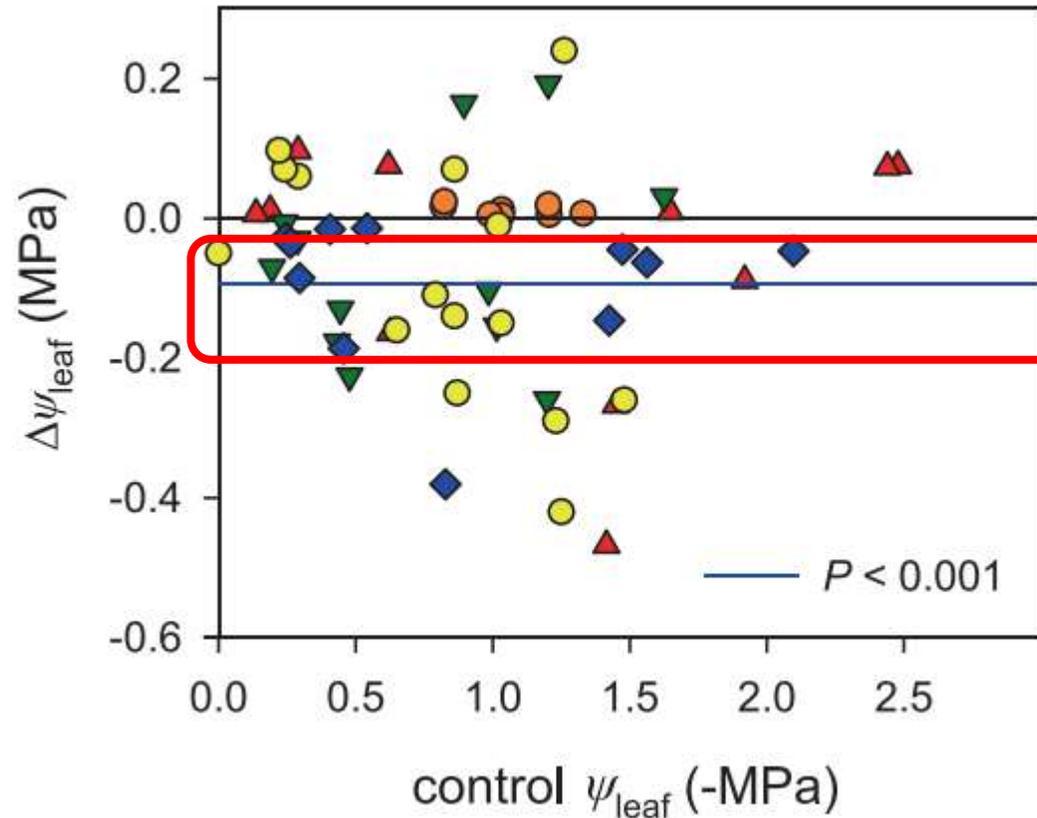
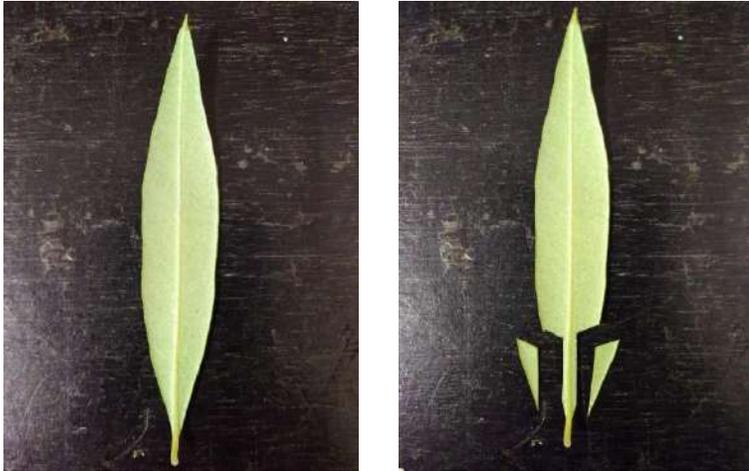
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OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE HANDLING

Can we cut part of the leaf lamina to lengthen the petiole?

Assumption #4. If the Ψ_{leaf} in the leaf is in equilibrium, sub-sample measurements give the same Ψ_{leaf} value.



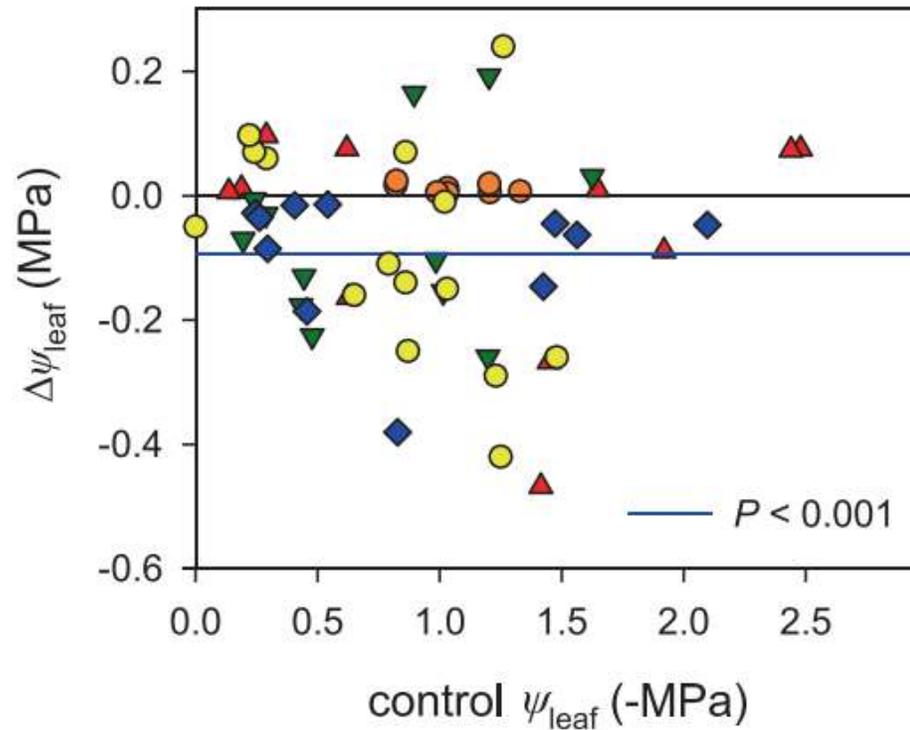
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

SAMPLE HANDLING

Can we cut part of the leaf lamina to lengthen the petiole?

Assumption #4. If the Ψ_{leaf} in the leaf is in equilibrium, sub-sample measurements give the same Ψ_{leaf} value.

- ✓ *In general, part of the leaf lamina can be trimmed without affecting the Ψ_{leaf} very much*
- ✓ *High variability, recommended test in study species*

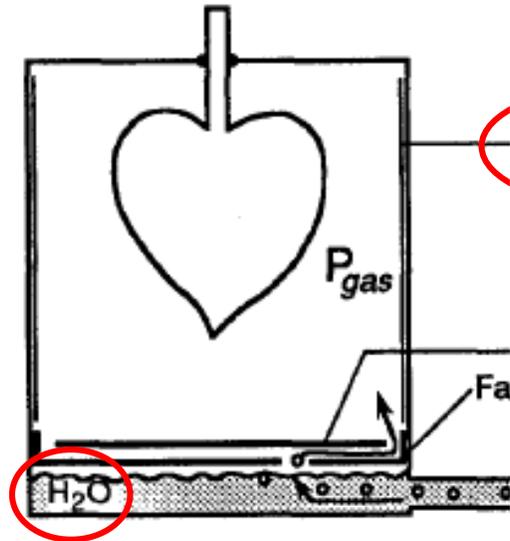


OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

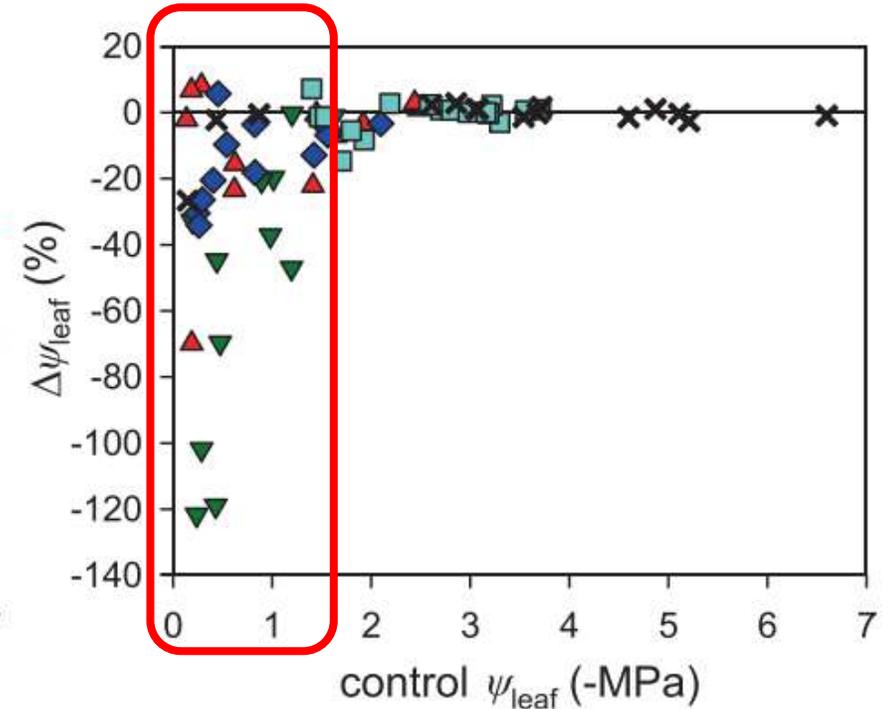
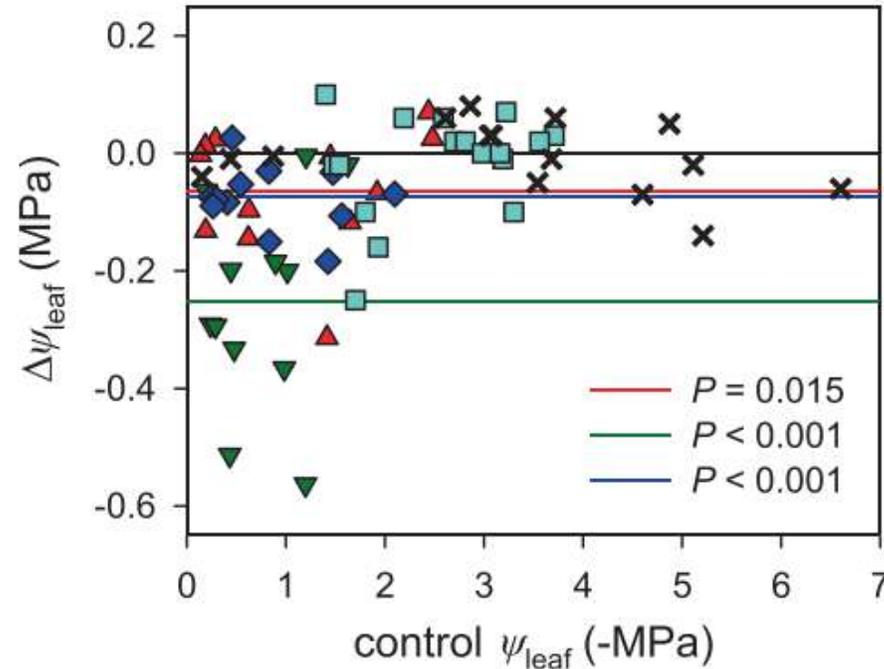
MEASUREMENT TECHNIQUE

Can we measure "bare" leaves inside the chamber?

Assumption #5. Ψ_{leaf} can change rapidly in contact with air, especially when measured with the pressure chamber.



Boyer, *Measuring the Water Status of Plants and Soils*, 1995

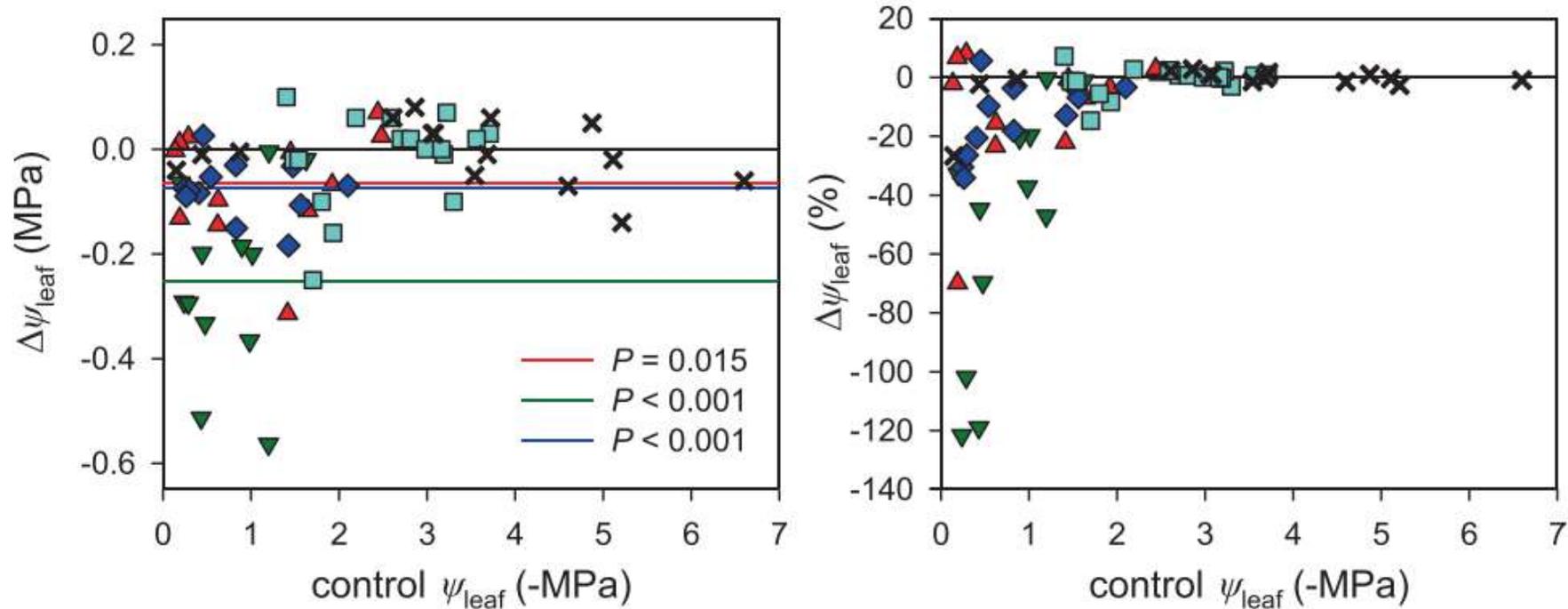


OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we measure "bare" leaves inside the chamber?

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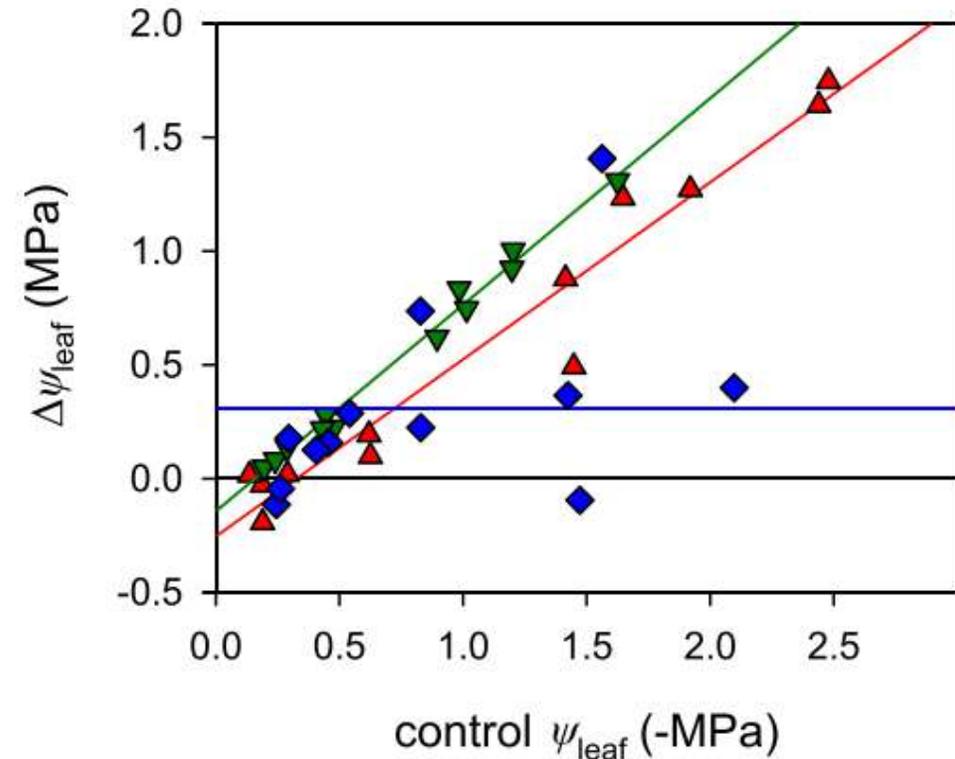
✓ *Although the effect was clearer at higher potentials, it is recommended to always use high humidity and a covered leaf inside the chamber.*

OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we measure leaves whose surfaces are wet?

Assumption #6. Ψ_{leaf} measurement assumes pressurization of air through the intercellular spaces, not crushing of the leaf.



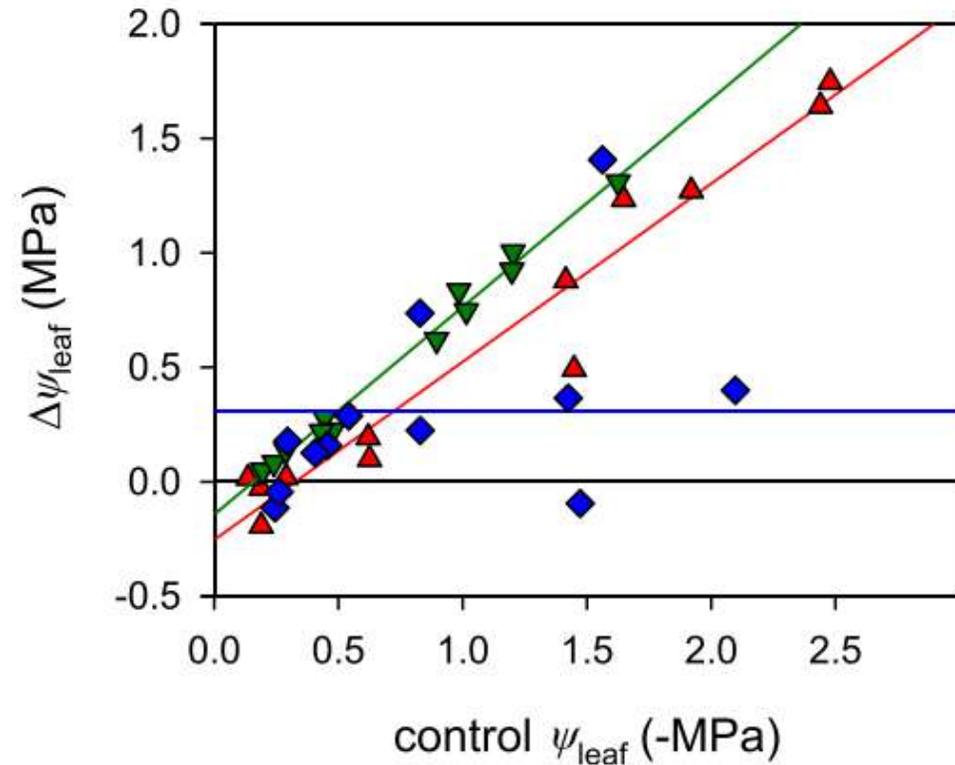
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we measure leaves whose surfaces are wet?

Assumption #6. Ψ_{leaf} measurement assumes pressurization of air through the intercellular spaces, not crushing of the leaf.

- ✓ *Measuring leaves with liquid water on their surfaces increases Ψ_{leaf}*
- ✓ *Recommendation to dry leaves before measuring*

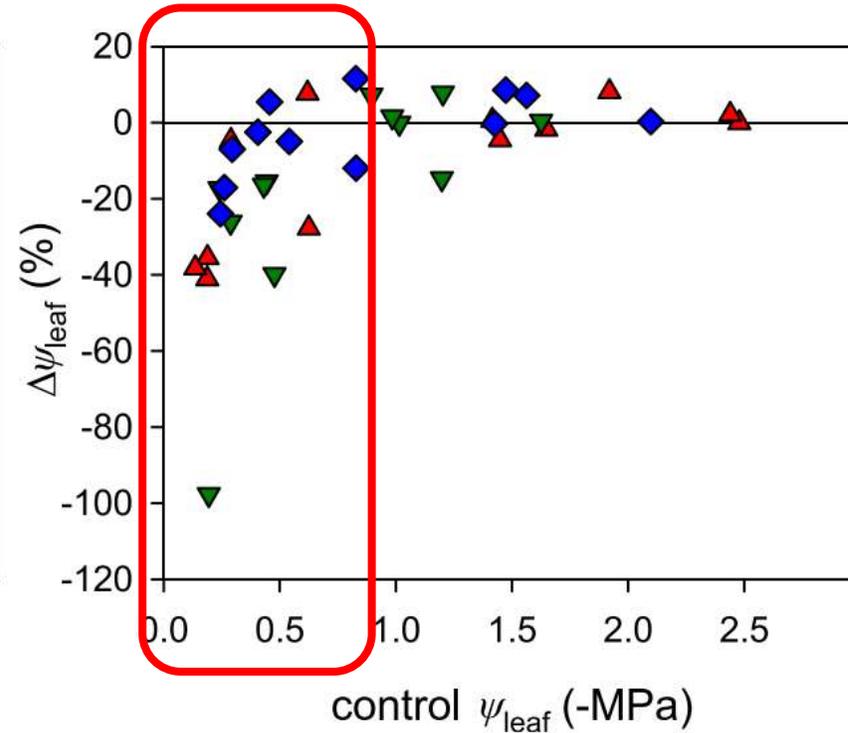
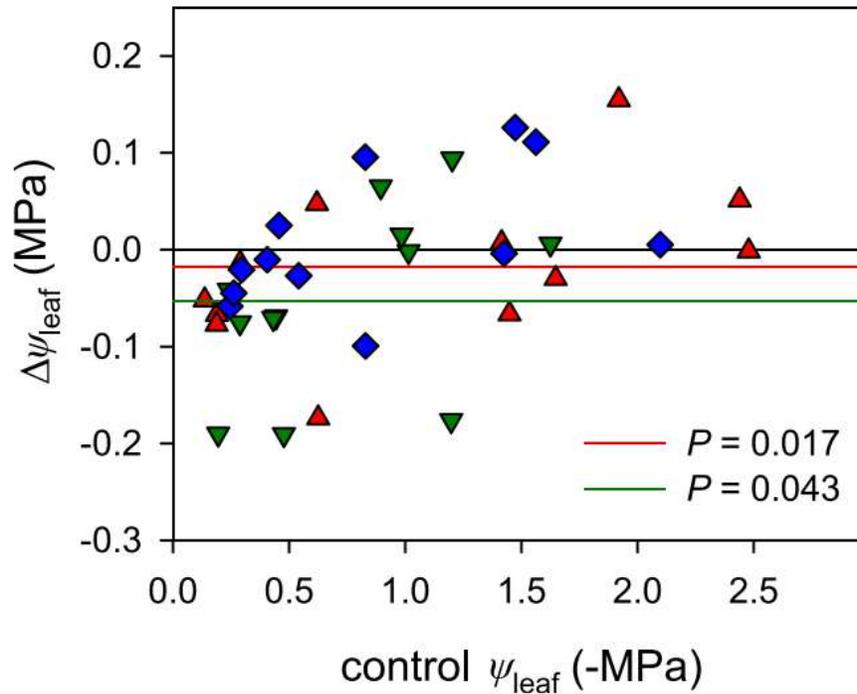


OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we measure either continuously or by applying pressure "pulses"?

Assumption #7. For accurate Ψ_{leaf} measurement, apply pressure in 'pulses' until the balance pressure is reached.



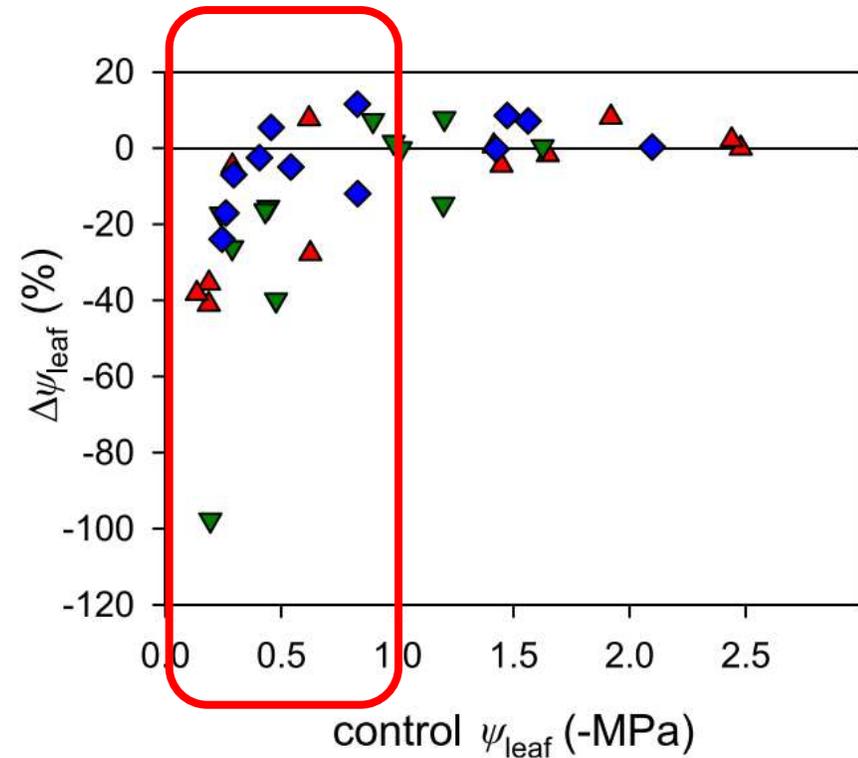
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we measure either continuously or by applying pressure "pulses"?

Assumption #7. For accurate Ψ_{leaf} measurement, apply pressure in 'pulses' until the balance pressure is reached.

- ✓ *Clearer effect at high Ψ_{leaf}*
- ✓ *Combination of recommendations depending on (i) level of water stress, (ii) degree of accuracy required, and (iii) speed of measurements required.*

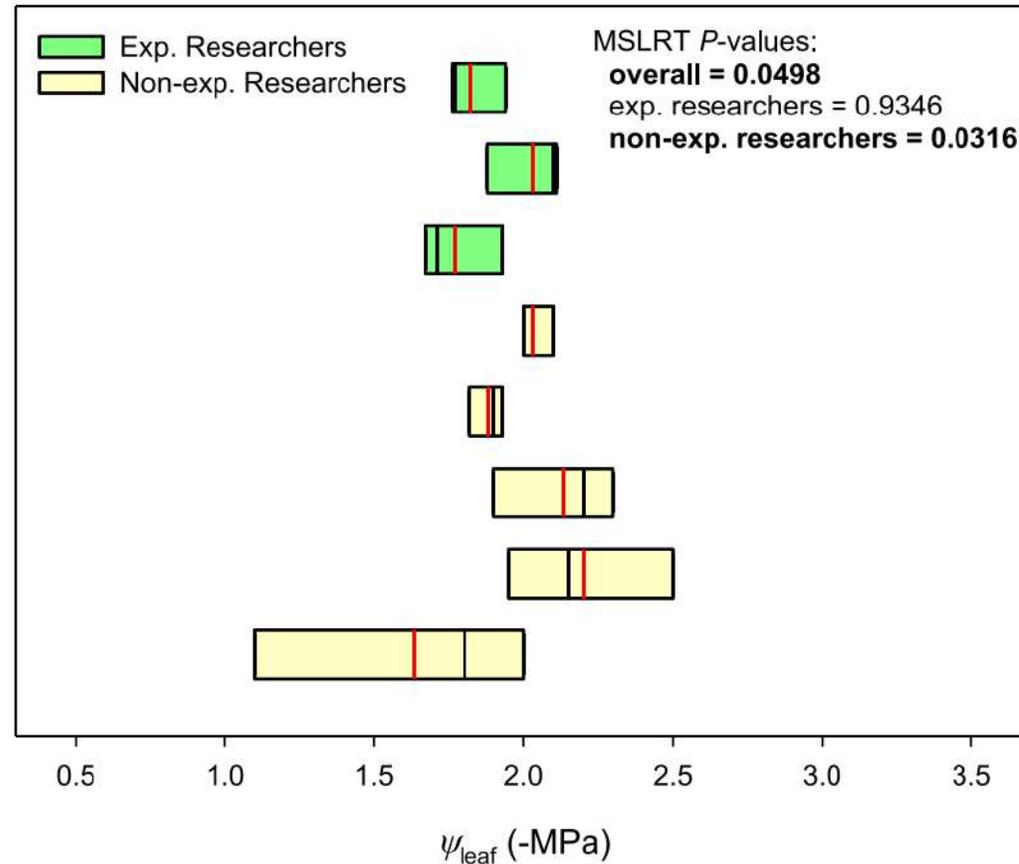


OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we rely on measurements made by our laboratory colleagues?

Assumption #8. For comparable Ψ_{leaf} measurements, a consensus among the team members on the various steps needed is required.



Carpinus betulus



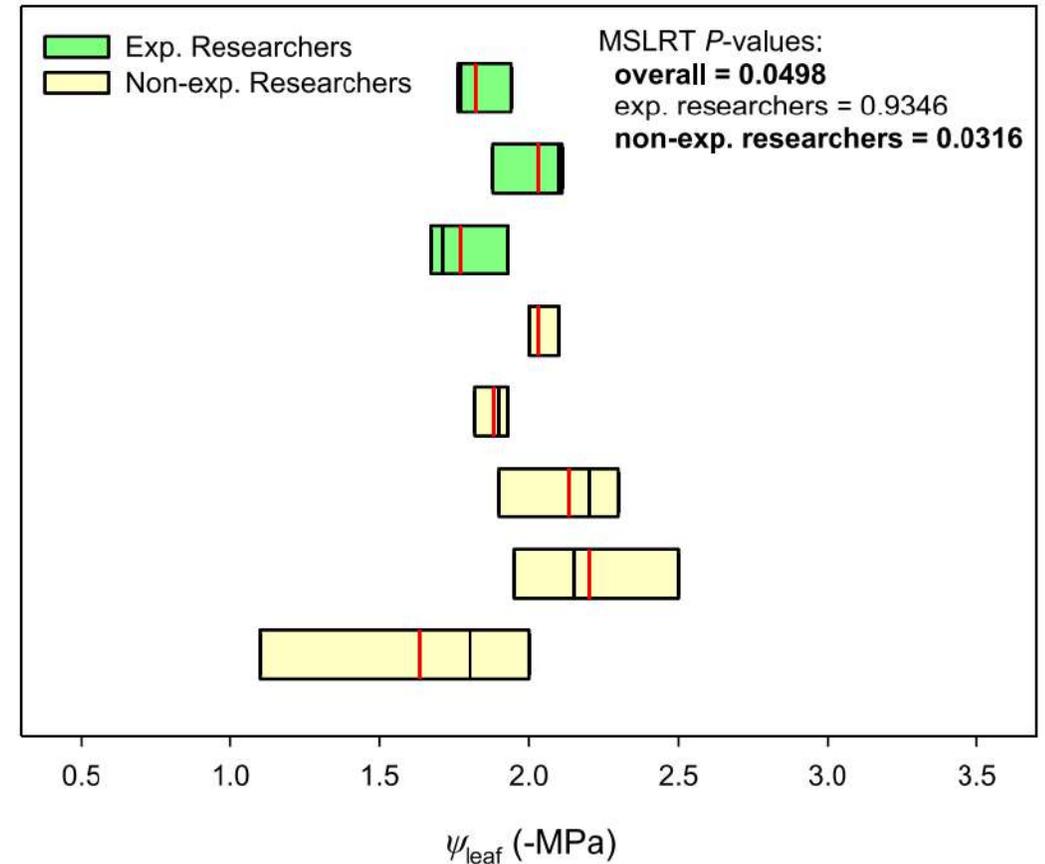
OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

MEASUREMENT TECHNIQUE

Can we rely on measurements made by our laboratory colleagues?

Assumption #8. For comparable Ψ_{leaf} measurements, a consensus among the team members on the various steps needed is required.

✓ *Recommendation of training to reach a consensus among team members, especially new members (students, visitors...) incorporating to the research group*



OPTIMIZING PRACTICAL USE: THE PRESSURE CHAMBER

Most important outcome

*Practical optimization of the technique and development of protocols and **best practice recommendations***

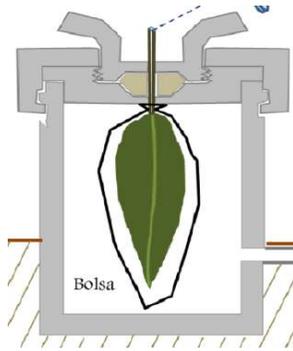
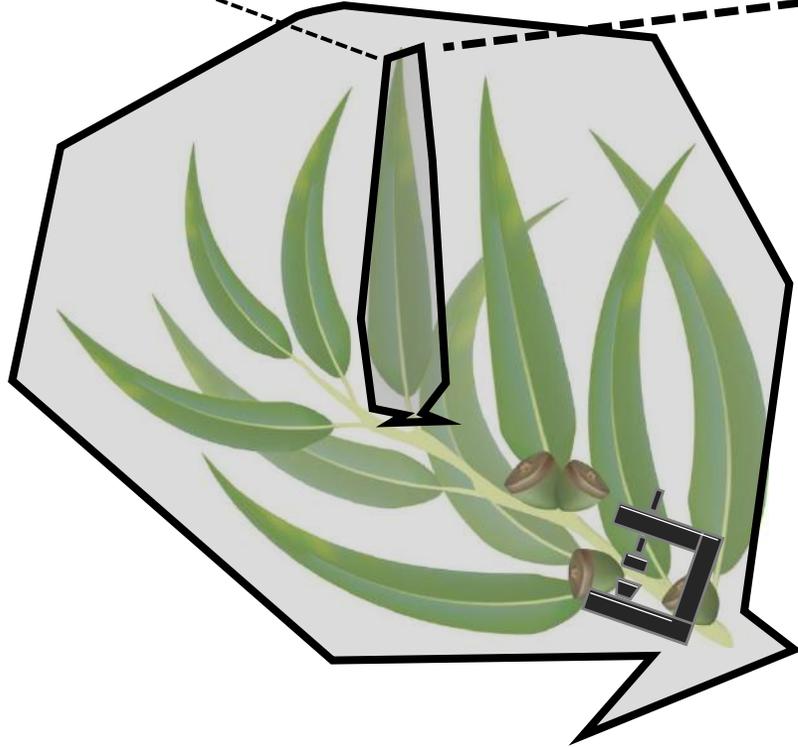
APPENDIX: Best practice recommendations for ψ_{leaf} measurement with the pressure chamber and suggestions for further tests to be performed

1. Collecting samples
2. Equilibration and storage of samples before measurements
3. Manipulation of samples before measurements
4. Measurement technique
5. Some extra recommendations on measurement technique



OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

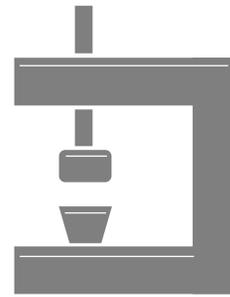
1.5 - 2 hours



PRESSURE CHAMBER

VS

STEM PSYCHROMETERS



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OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

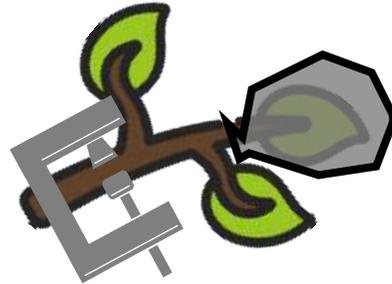
Question, Hypothesis, Objective



Could both methods be used interchangeably in these studies?



Theoretically:



$$\Psi_{\text{stem}} = \Psi_{\text{equilibrated leaf}}$$



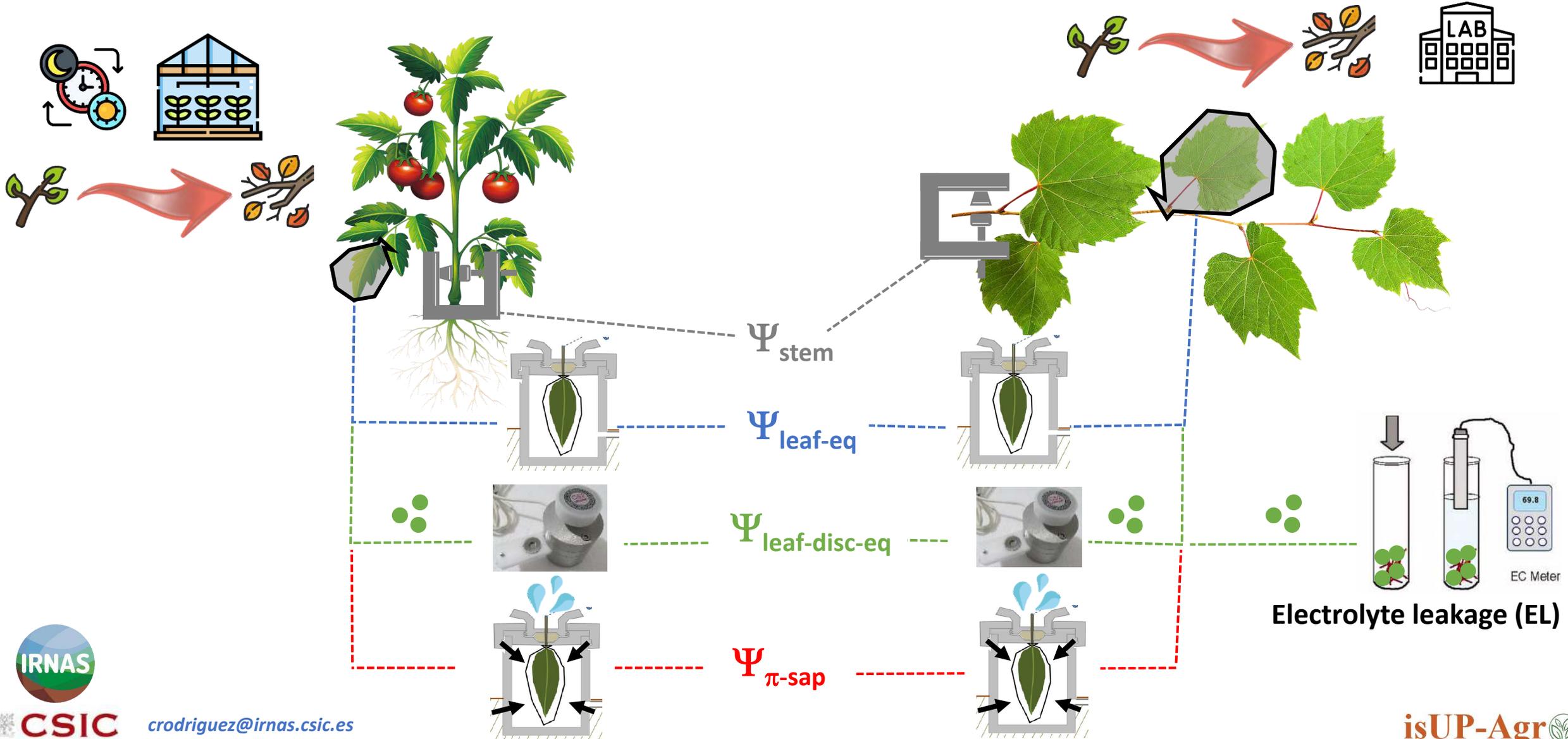
Conduct two experiments in which the plant material spans a wide range of dehydration levels to test the hypothesis



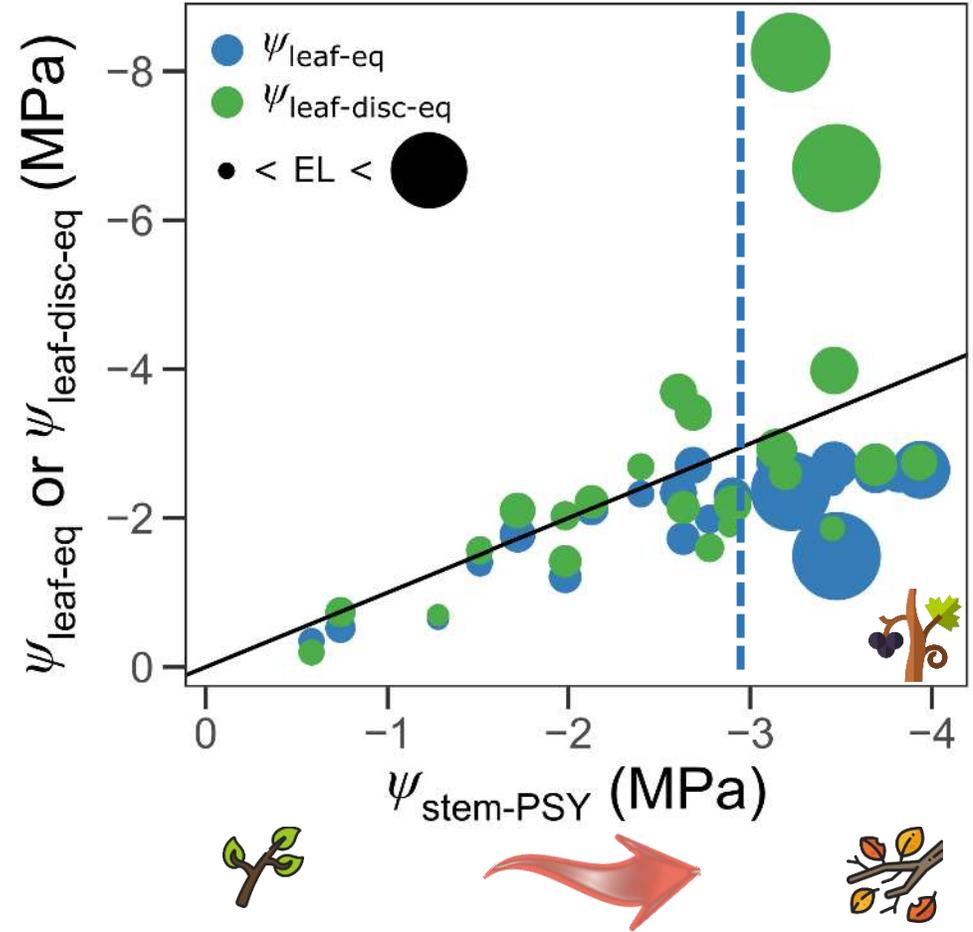
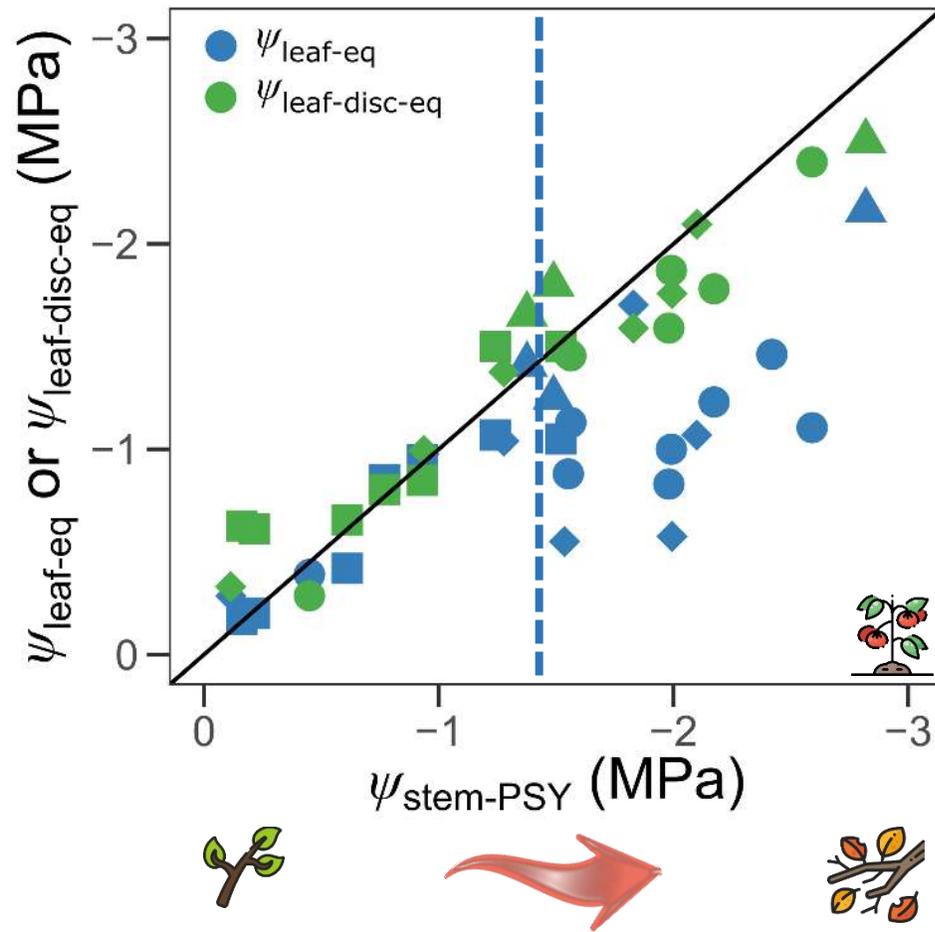
OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

Exp. #1. Study of drought-induced plant and tissue mortality

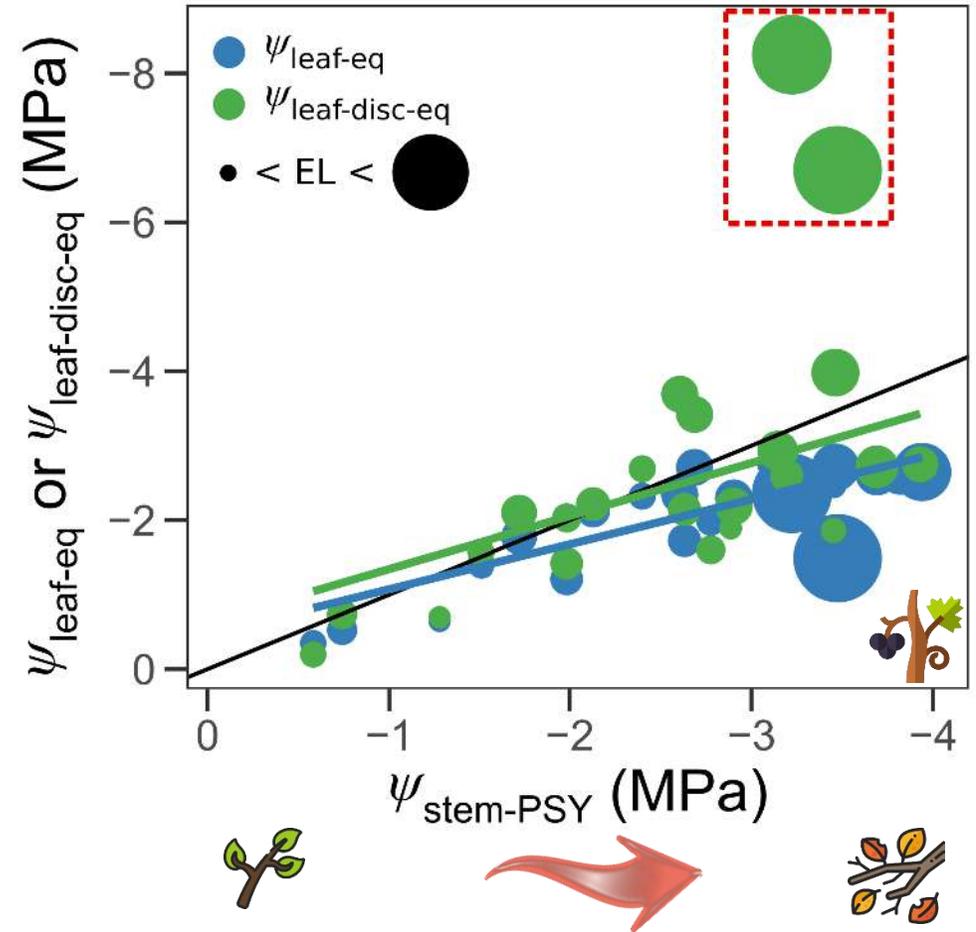
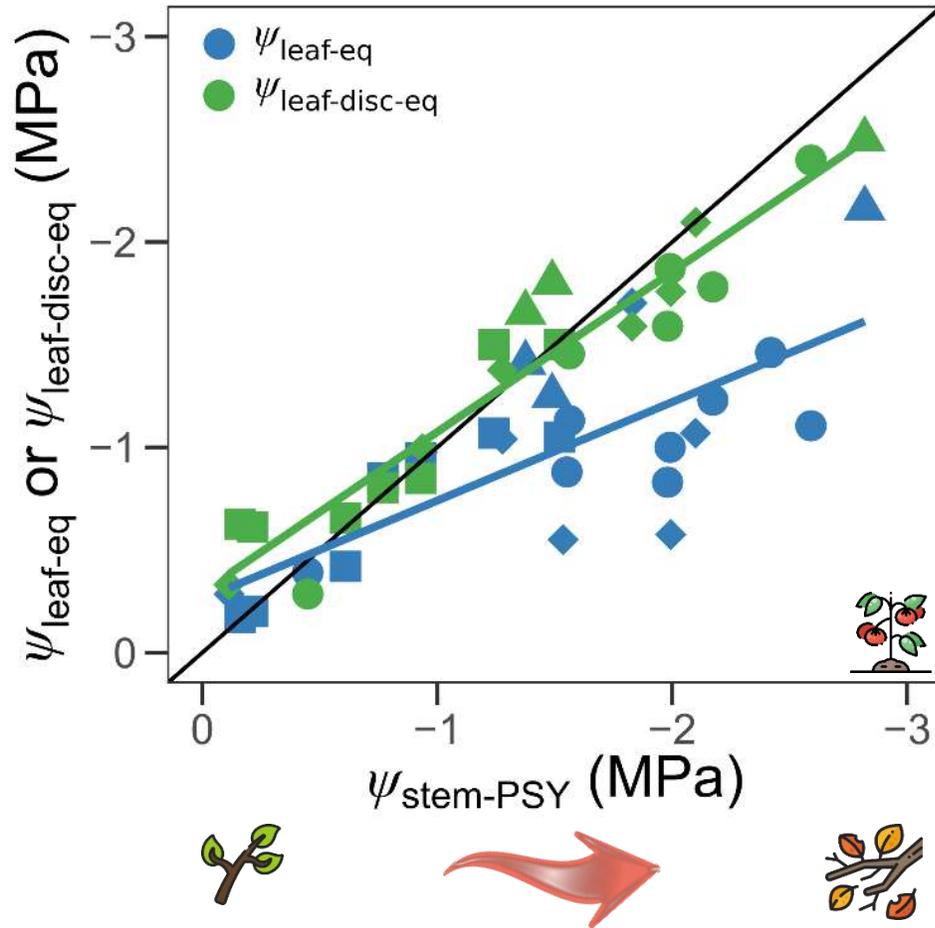
Exp. #2. Study of drought-induced embolism formation



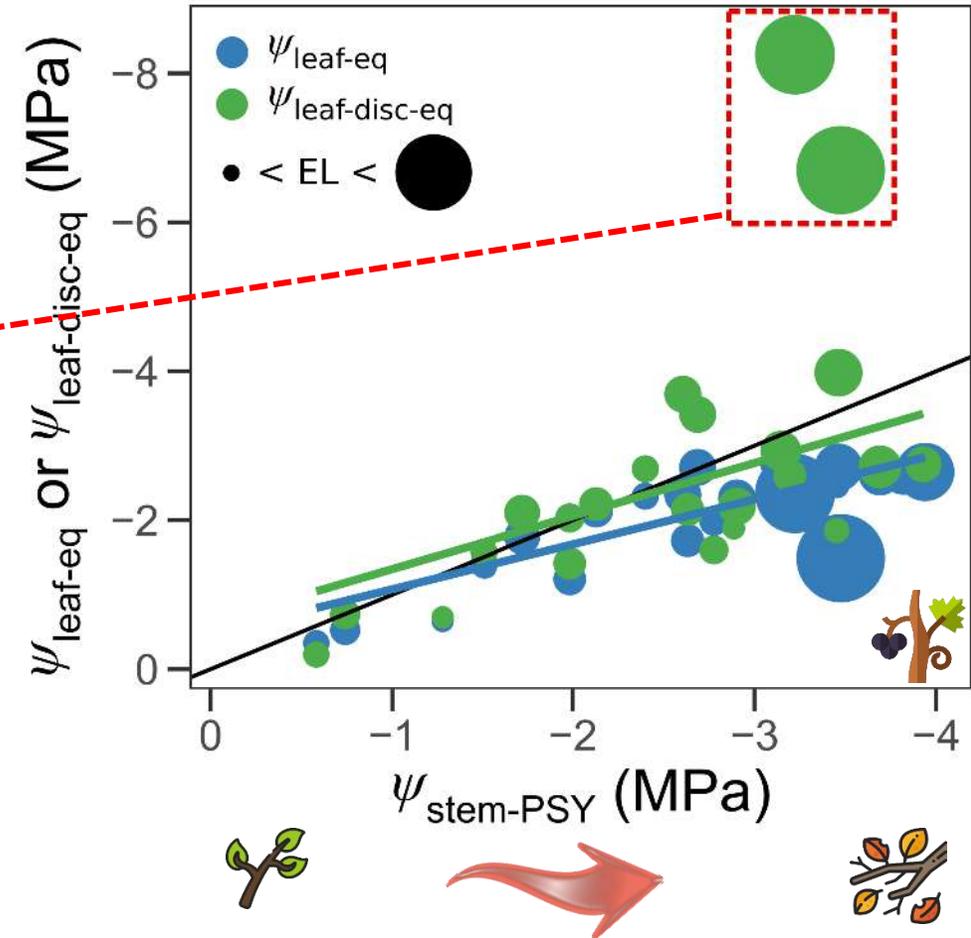
OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ



OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ



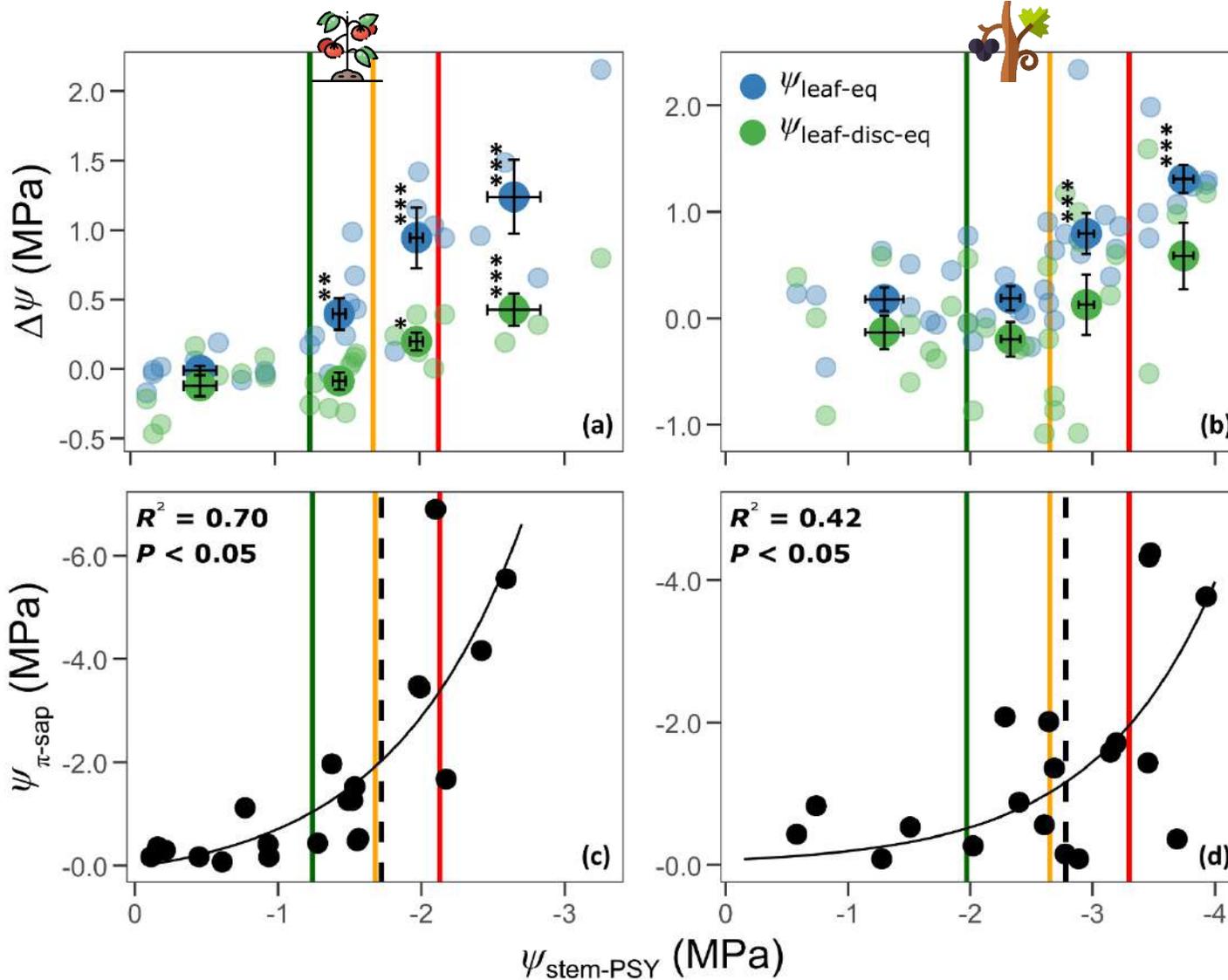
OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ



OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

Divergence thresholds between Ψ_{stem} and $\Psi_{leaf-eq}$

- $\Psi_{leaf-eq}$
- $\Psi_{leaf-disc-eq}$
- P50
- P88
- - - breakpoint $\psi_{leaf-eq}$
- - - breakpoint $\psi_{leaf-disc-eq}$
- - - breakpoint $\psi_{\pi-sap}$
- < EL < ●

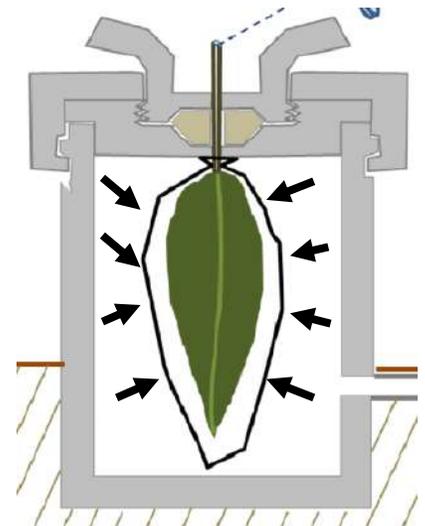


Leaf xylem cavitation



Leaf cells damage

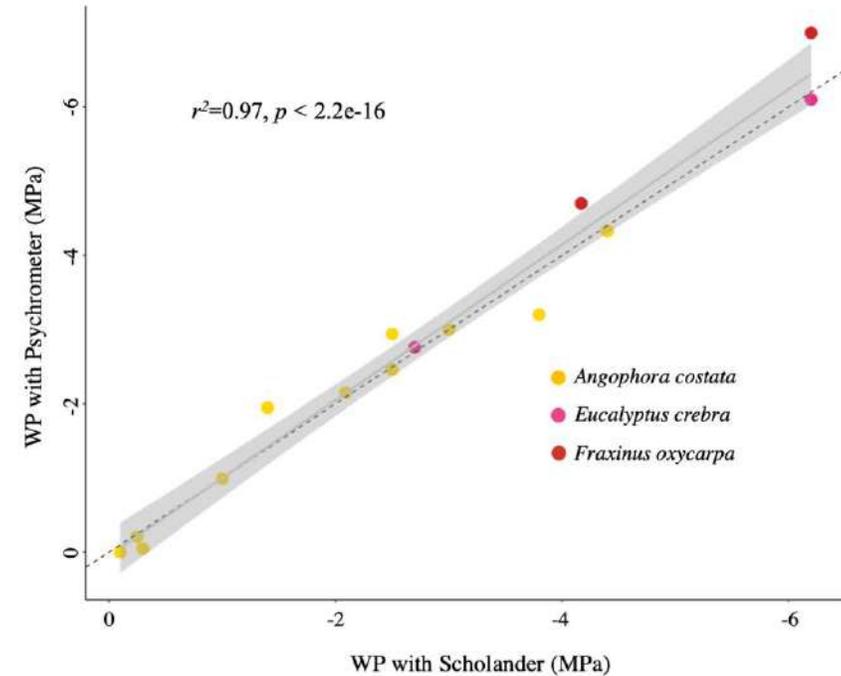
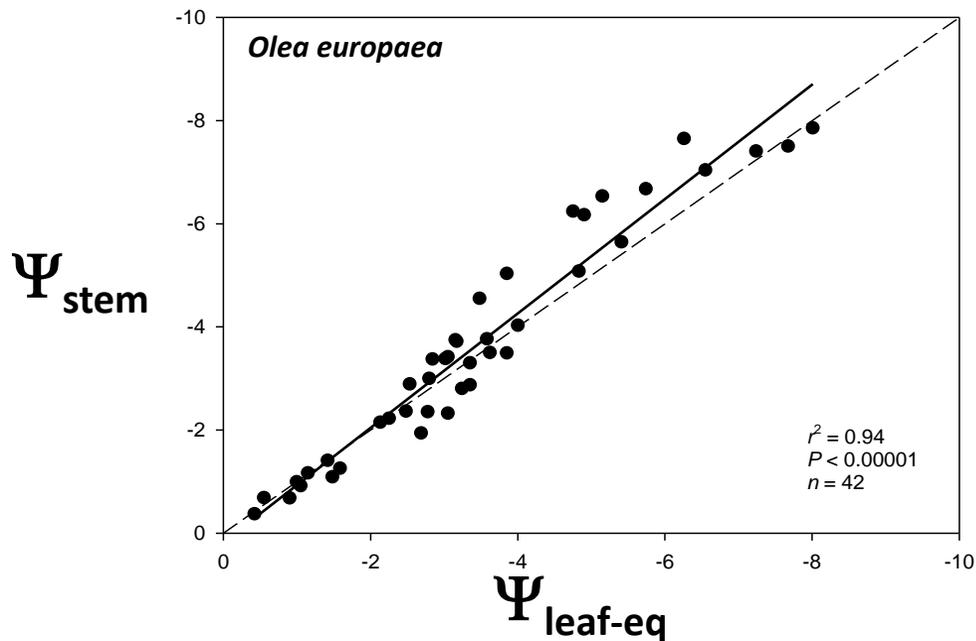
(Brodribb et al. 2021, *New Phyt*; Mantova et al. 2022, *Trends Plant Sci*; Mantova et al. 2023)



OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

Conclusions and Implications

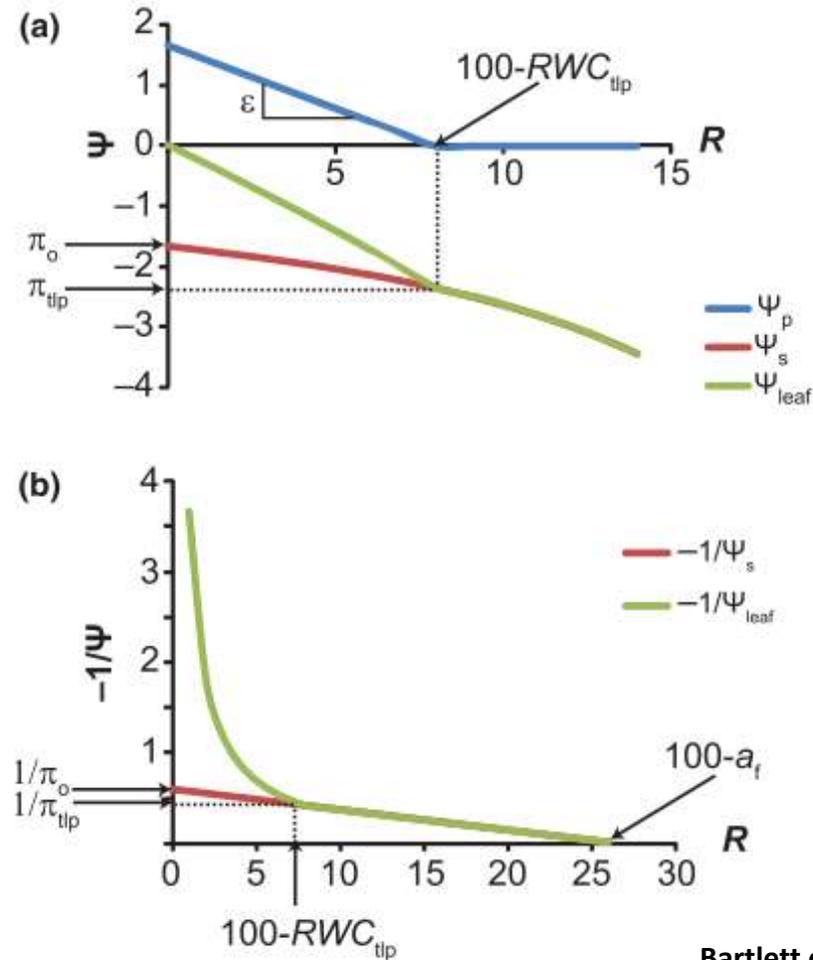
- Damaged leaves give erroneous Ψ values measured with the pressure chamber due to the extra contribution of the symplastic cell content
- Leaf damage, as has been shown in other works, occurs at stress levels that induce cavitation
- However, these results depend on the anatomical leaf structures of each species (e.g., it does not occur in other woody species)



OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

Conclusions and Implications

» Implicaciones importantes a la hora de medir curvas presión-volumen para derivar parámetros fundamentales en relaciones hídricas



Bartlett et al. 2012, *Ecology Letters*

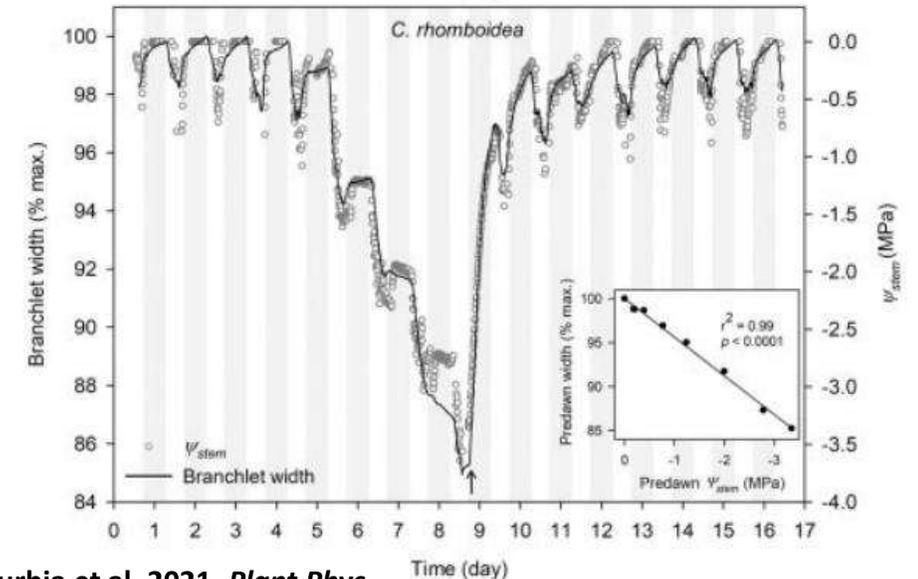
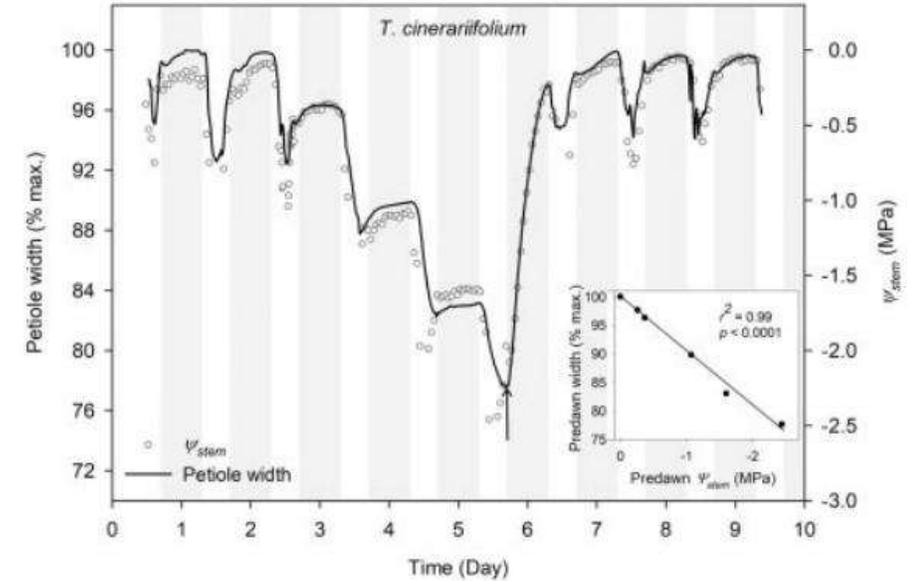
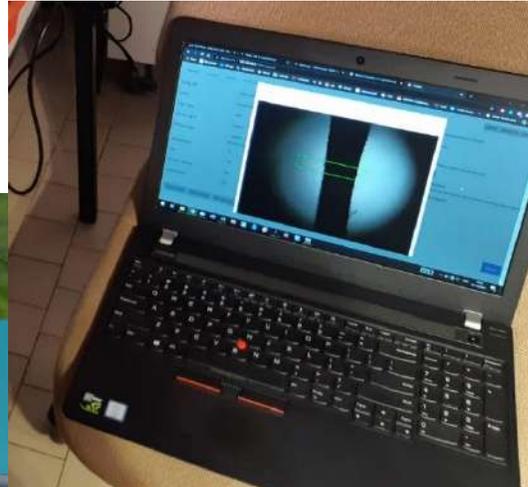


OPTIMIZING PRACTICAL USE: A CASE STUDY ON STEM Ψ

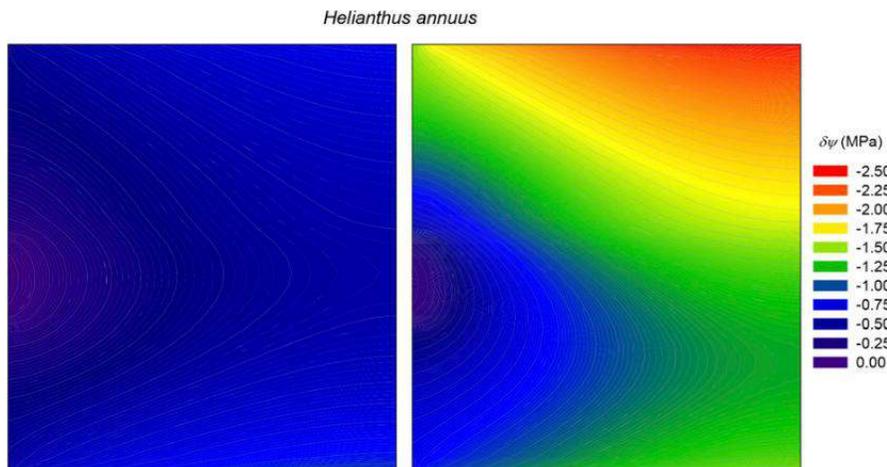
Conclusions and Implications

» Important to find alternative or combination of methods capable for measuring Ψ within wide ranges of drought levels

“DENDROCAMS”



QUESTIONS??



$$E_{\text{upper epid}} = 2.09 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$E_{\text{lower epid}} = 2.91 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$\delta\psi_{\text{upper epid}} = -0.77 \text{ MPa}$$

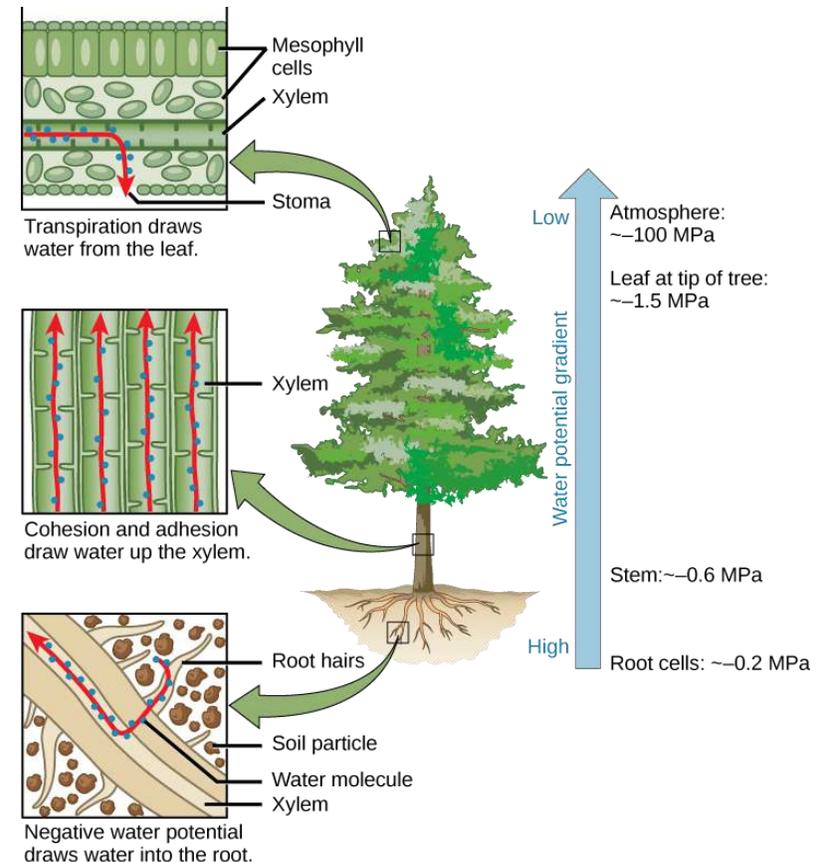
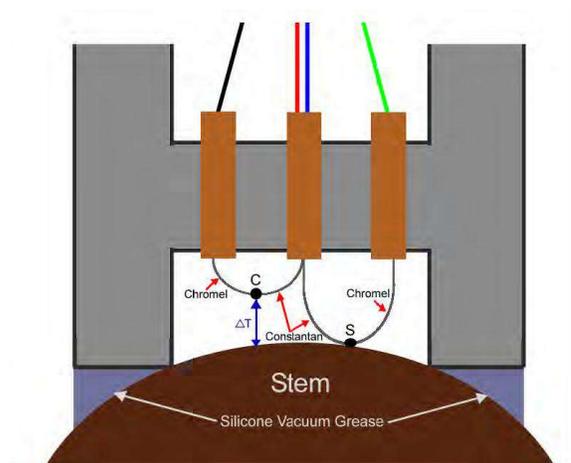
$$\delta\psi_{\text{lower epid}} = -0.95 \text{ MPa}$$

$$E_{\text{upper epid}} = 6.27 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$E_{\text{lower epid}} = 2.91 \text{ mmol m}^{-2} \text{ s}^{-1}$$

$$\delta\psi_{\text{upper epid}} = -2.48 \text{ MPa}$$

$$\delta\psi_{\text{lower epid}} = -1.44 \text{ MPa}$$

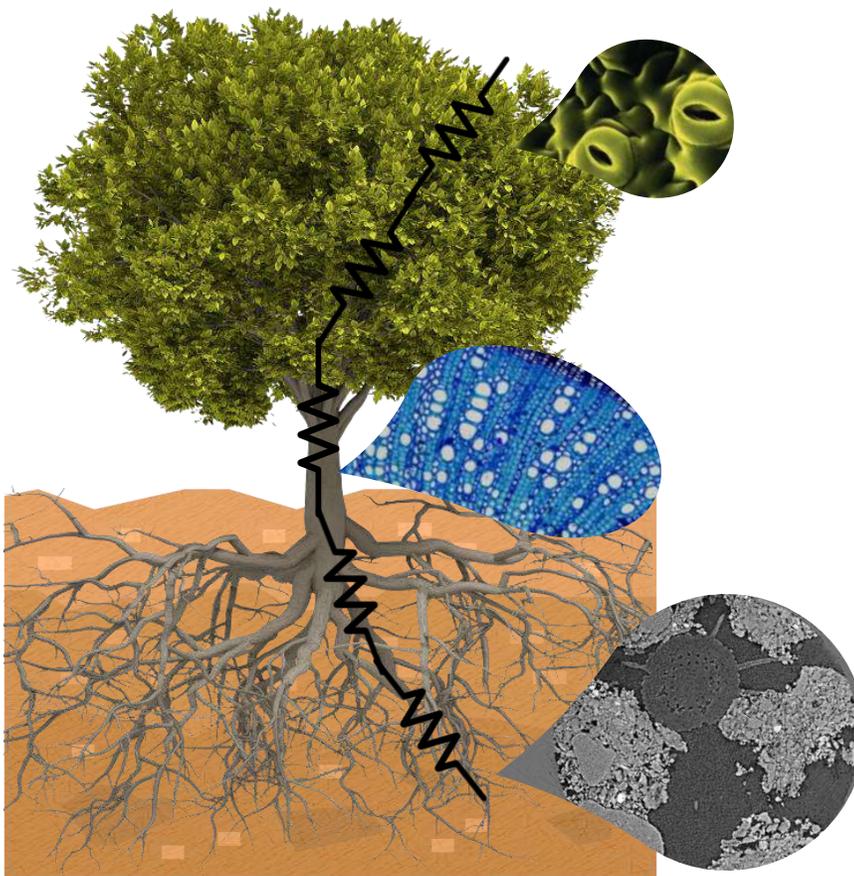


PLANT HYDRAULICS

Celia M. Rodríguez Domínguez

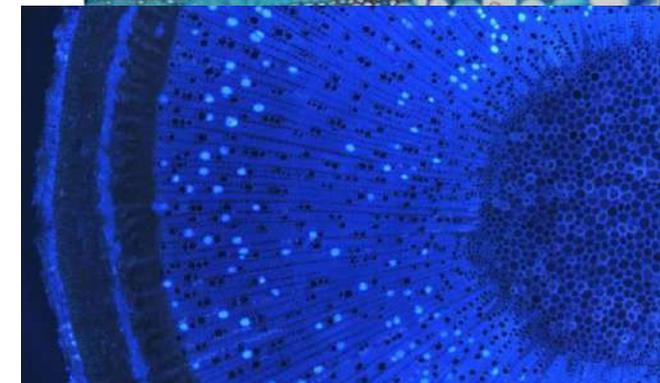
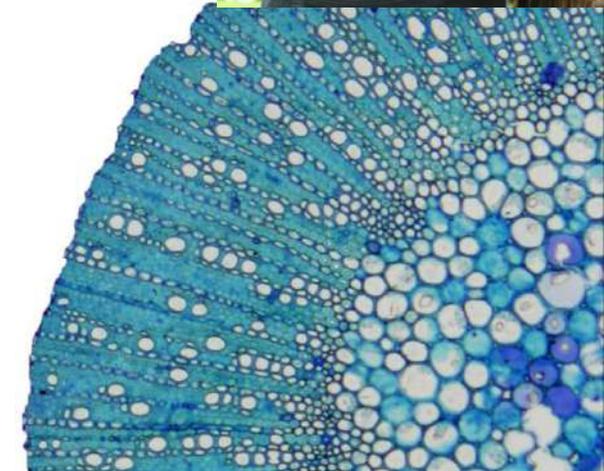
2nd Training School

27th January to 31st January 2025



Thanks!

José M.
Torres



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UNLOCKING THE POTENTIAL FOR AGRICULTURAL
RESEARCH ON AN EU OUTMOST REGION:
BOOSTING ISOPLEXIS CENTRE



ISOPlexis
Centro de Agricultura Sustentável e Tecnologia Alimentar

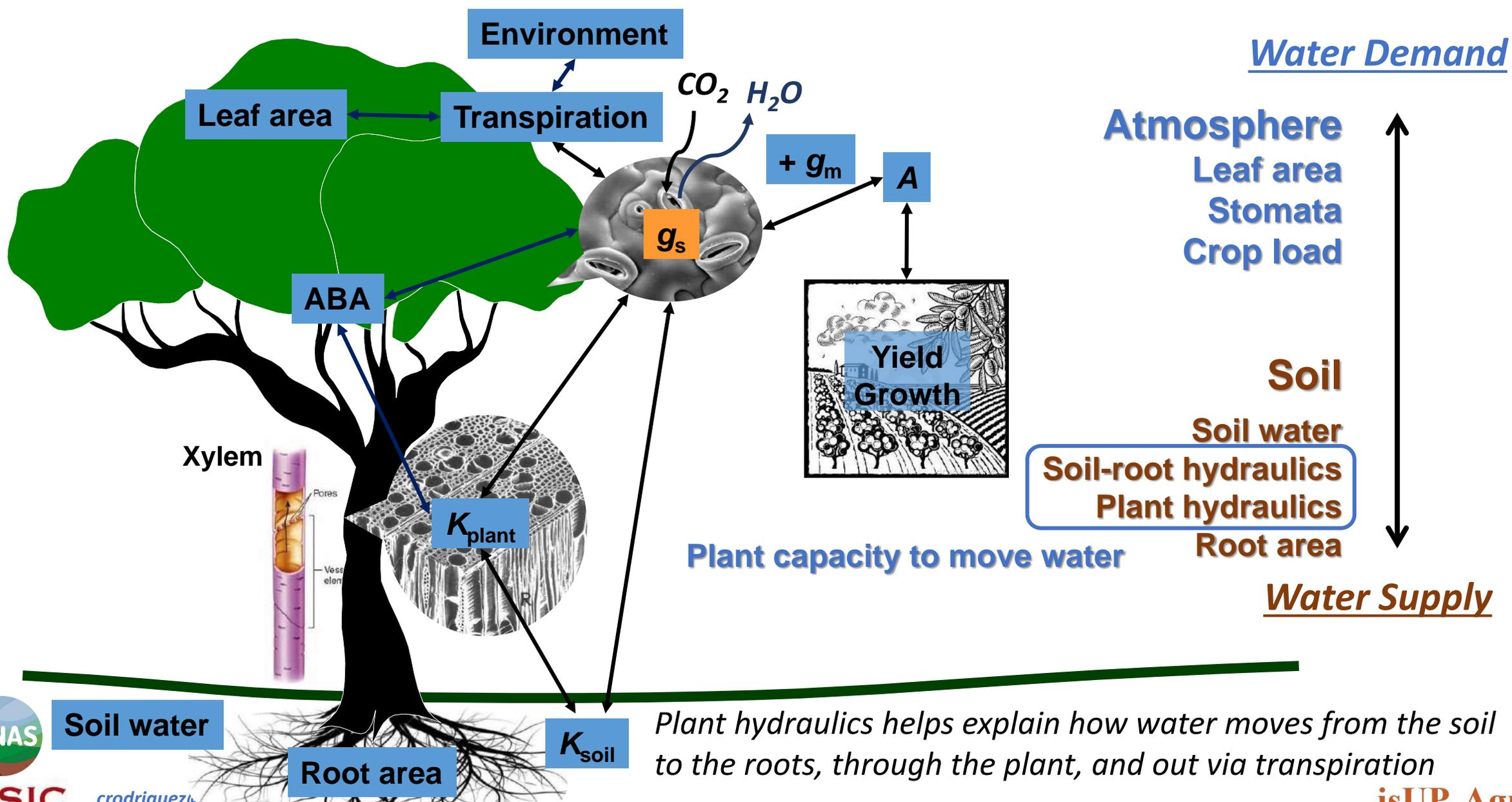


OBJECTIVES

- **To understand the fundamental principles of plant hydraulics and its relevance to plant function and performance.**
- **To learn about methodologies to measure hydraulic conductance in leaves, stems, and roots.**
- **To explore the importance of soil-root hydraulic interactions and their role in precision agriculture.**
- **To understand the application of imaging techniques for plant hydraulics in agriculture.**



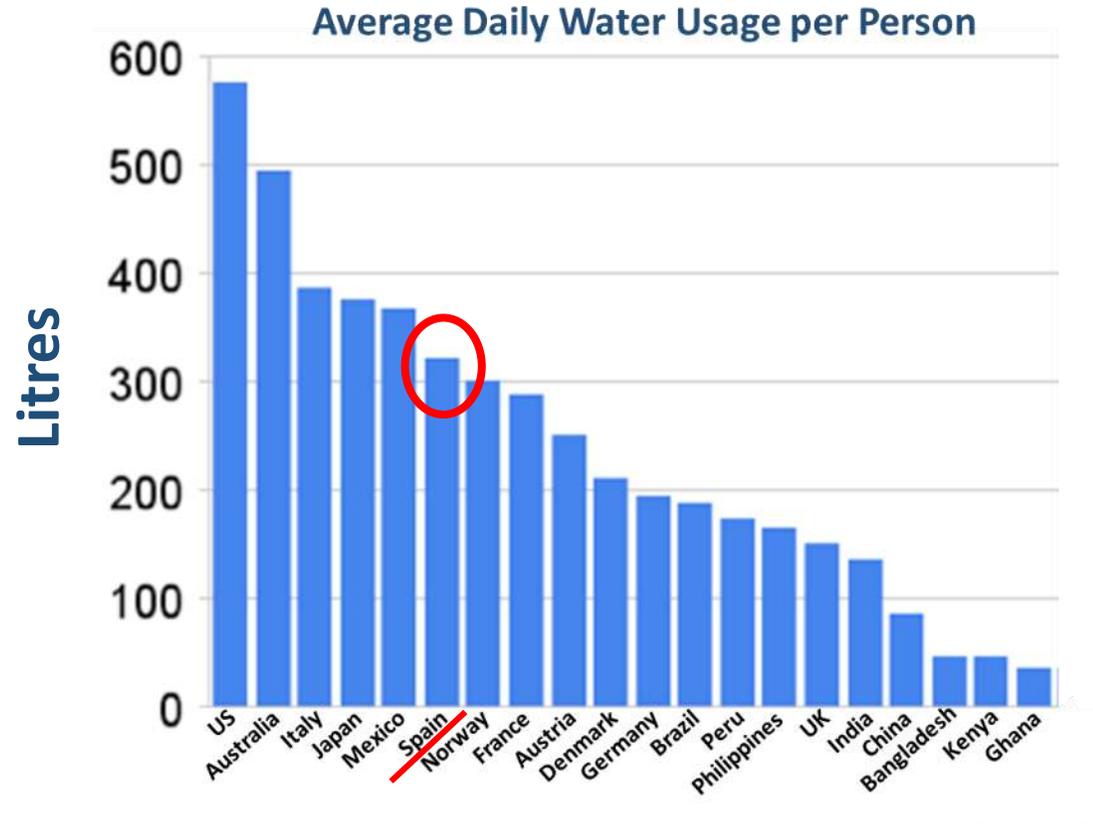
IMPORTANCE OF PLANT HYDRAULICS IN AGRICULTURAL AND ENVIRONMENTAL CONTEXTS



Plant hydraulics helps explain how water moves from the soil to the roots, through the plant, and out via transpiration

IMPORTANCE OF PLANT HYDRAULICS IN AGRICULTURAL AND ENVIRONMENTAL CONTEXTS

Amount of water used by trees

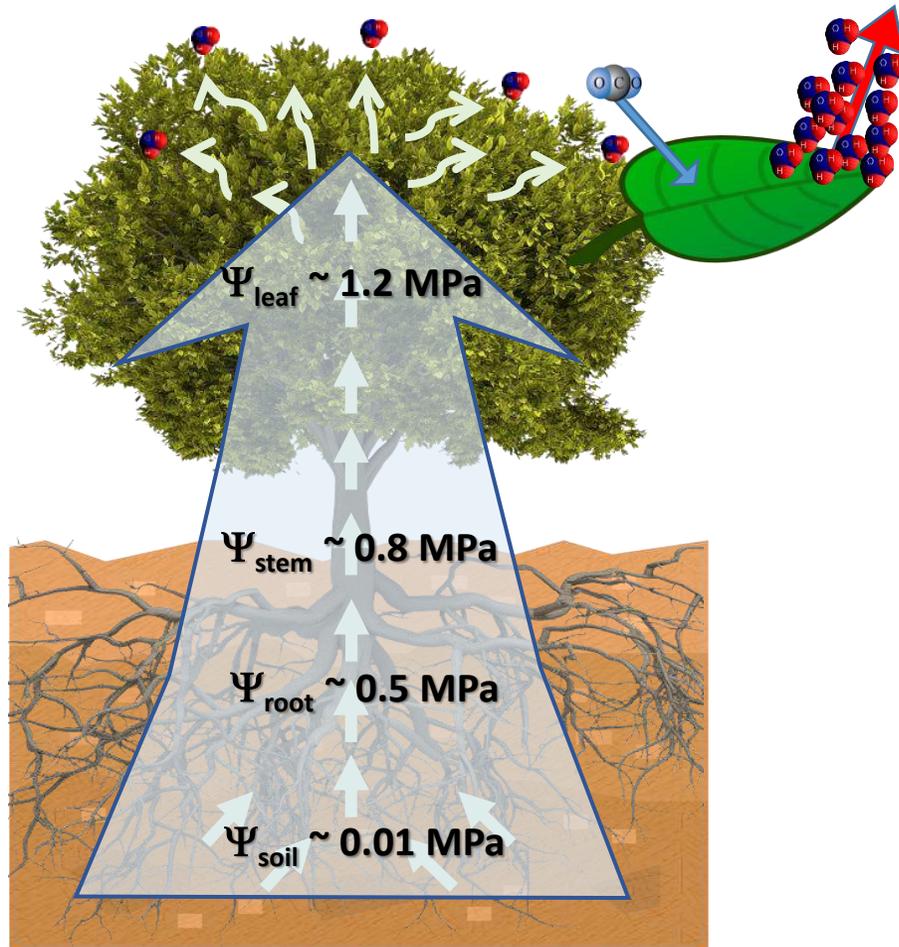


80 cm basal-diameter trees \approx Conifers 50-60 litres day⁻¹

Angiosperms 400-500 litres day⁻¹

IMPORTANCE OF PLANT HYDRAULICS IN AGRICULTURAL AND ENVIRONMENTAL CONTEXTS

*This huge movement of water is driven by **TRANSPIRATION** and creates $\Delta\Psi$ through **XYLEM***

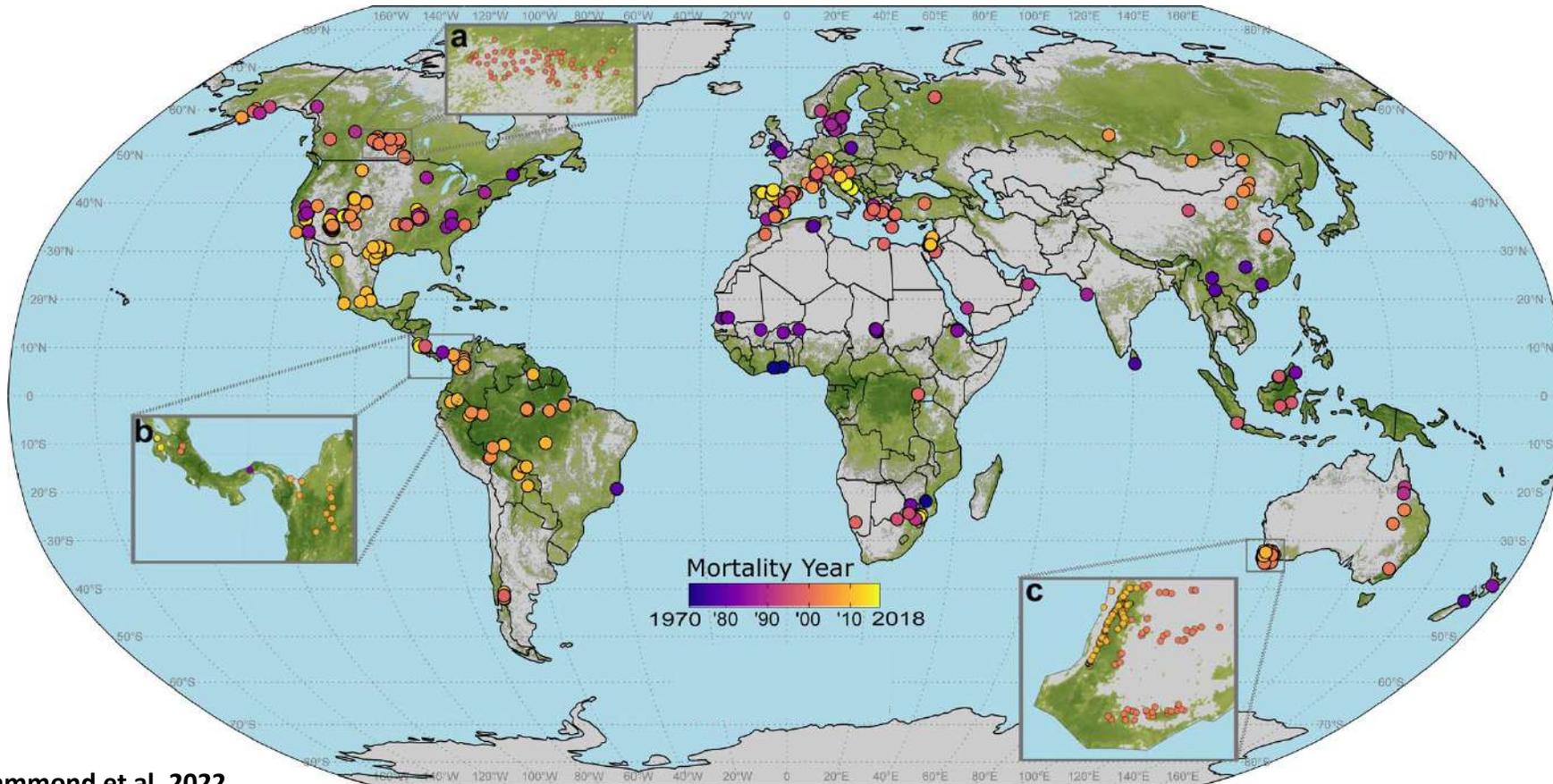


> 95% of water evaporates from leaves through **STOMATA**
< 5% of water for photosynthesis and growth

- ❑ Understanding these processes allows farmers to optimize irrigation practices, minimizing water waste and ensuring crops get the right amount of water at the right time.
- ❑ Some plants have mechanisms to maintain hydraulic function under water stress, making them better suited for arid or water-limited environments.
- ❑ Plants with optimized hydraulic functioning can photosynthesize more efficiently, leading to better growth and higher yields.

IMPORTANCE OF PLANT HYDRAULICS IN AGRICULTURAL AND ENVIRONMENTAL CONTEXTS

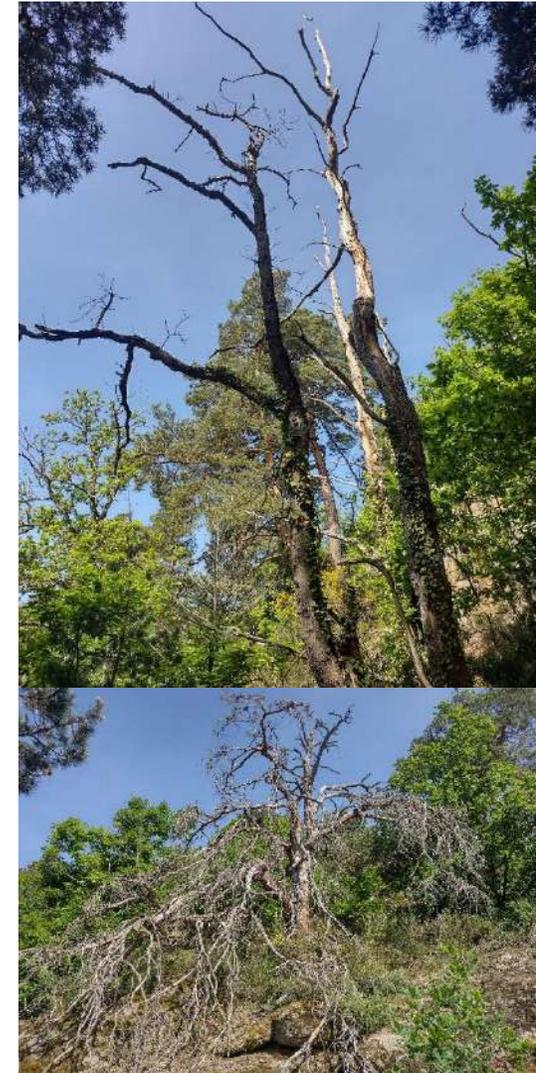
Drought-induced mortality – Hydraulic failure



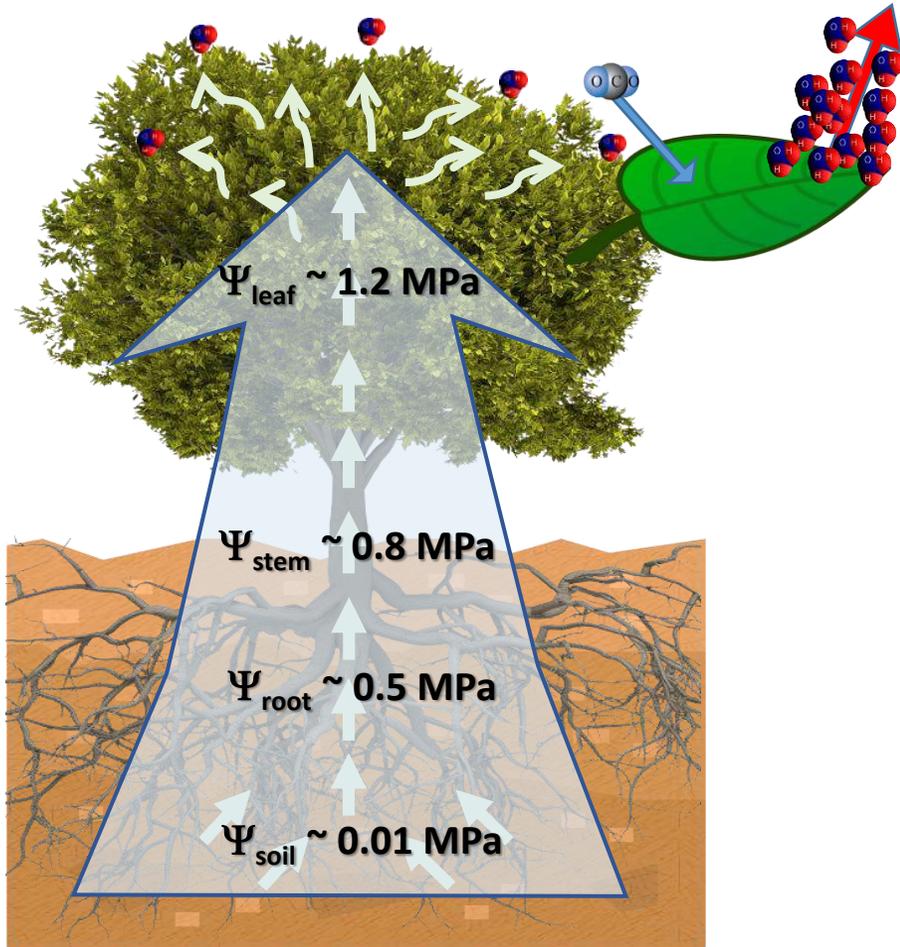
Hammond et al. 2022

66% Linked to warmer and drier conditions

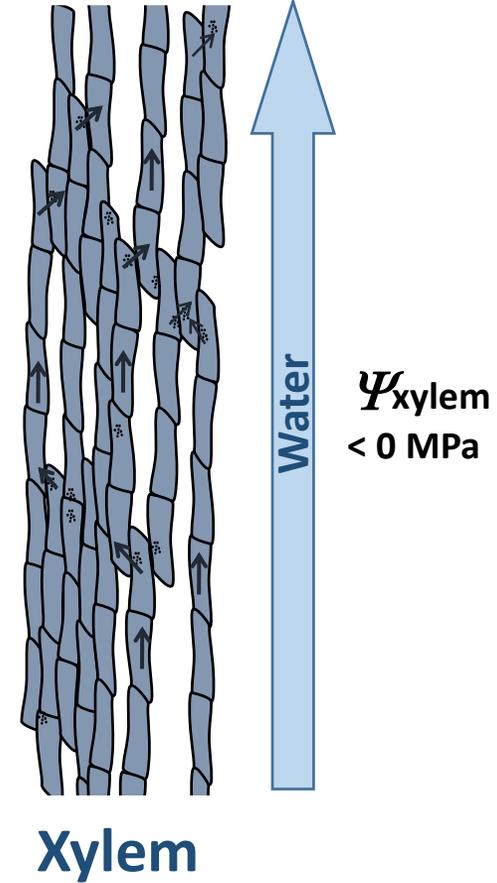
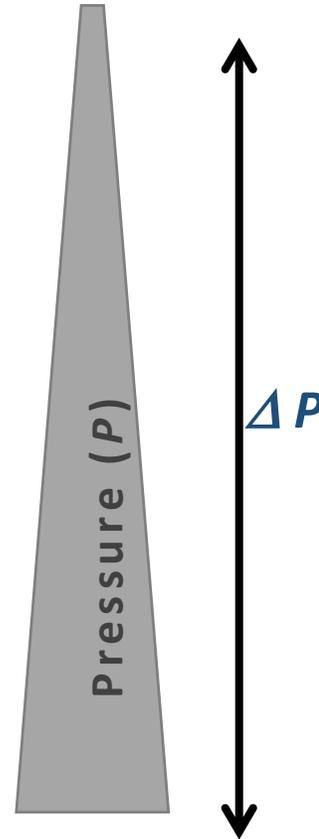
Knowing how and when plants are at risk of hydraulic failure can help farmers intervene before crops are irreparably damaged.



BASIC PRINCIPLES IN PLANT HYDRAULICS

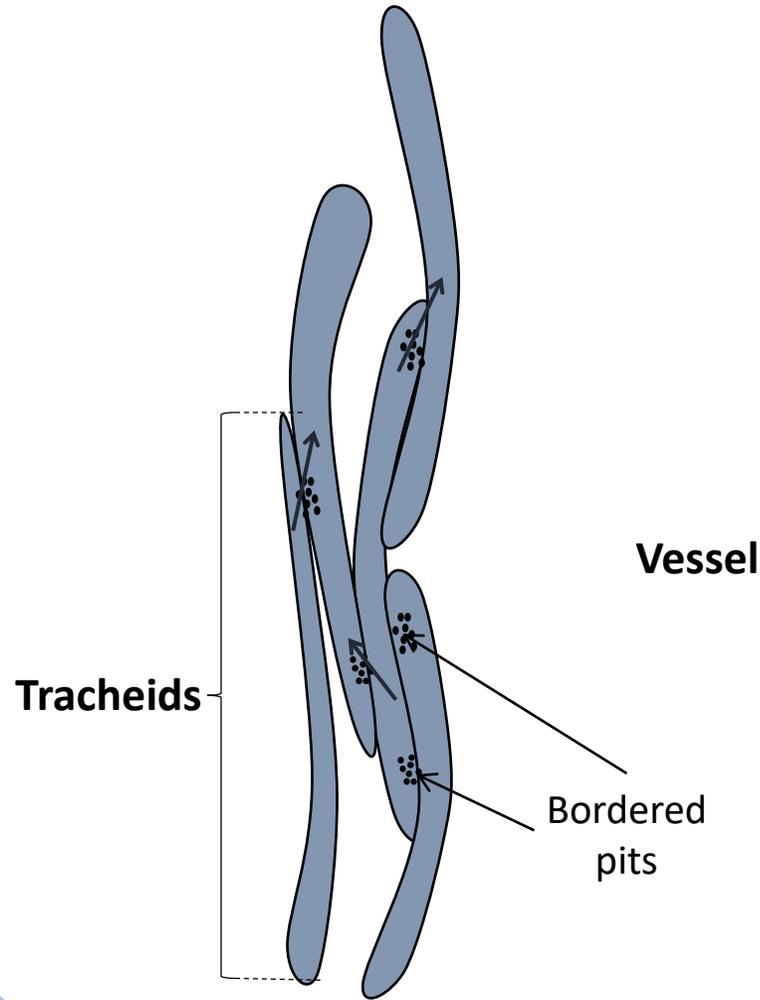


Leaf xylem pressure
 $\lll 0 \text{ MPa}$

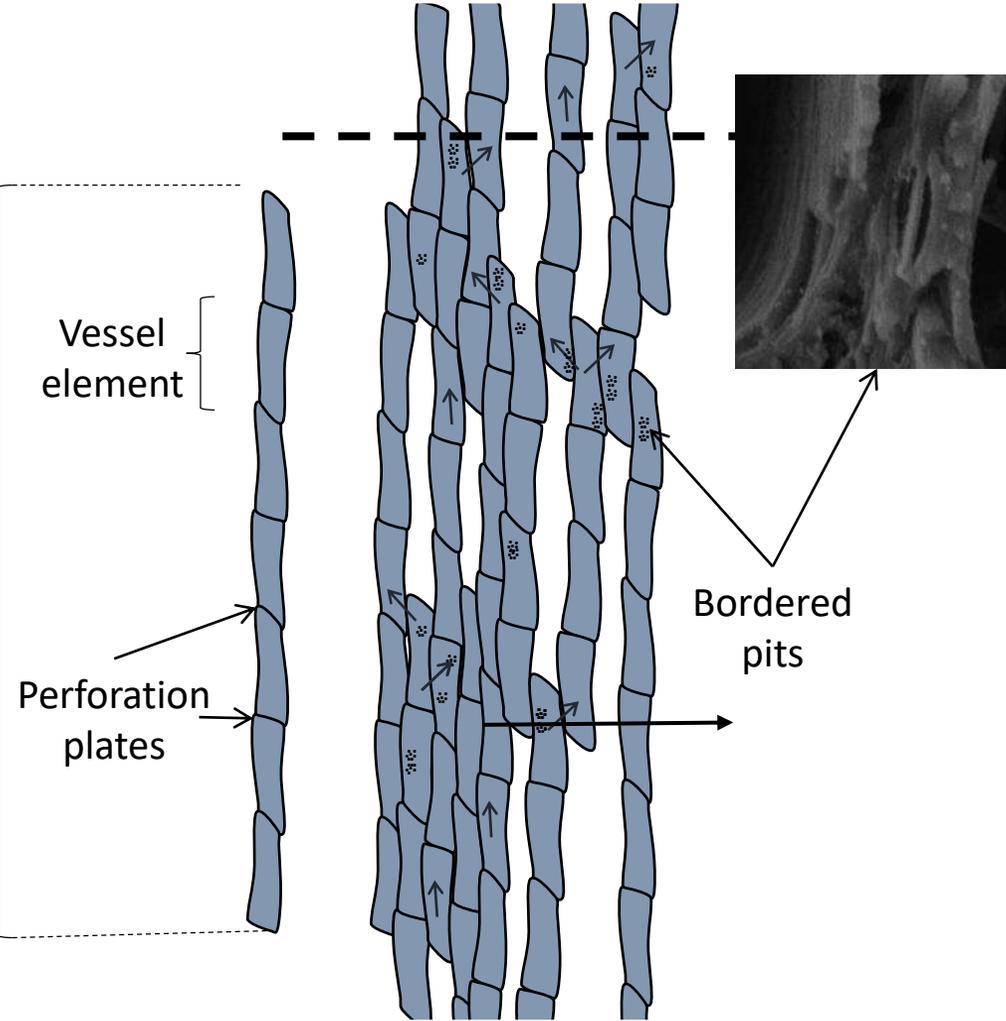


BASIC PRINCIPLES IN PLANT HYDRAULICS

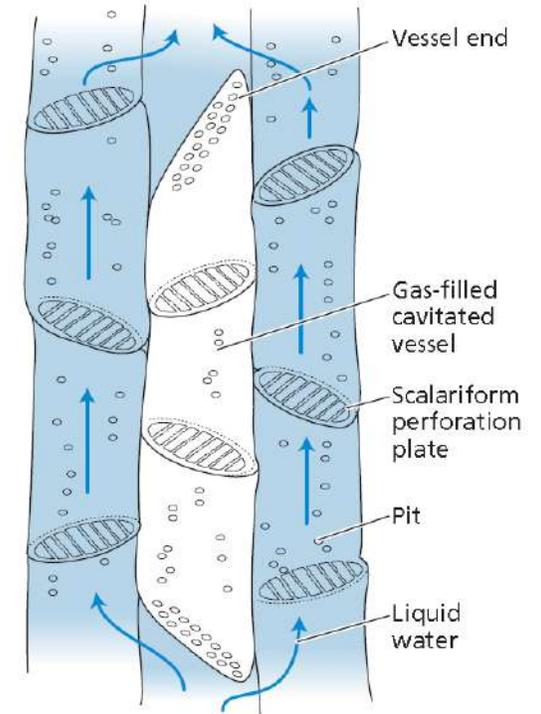
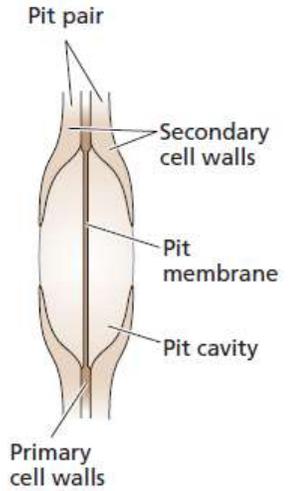
The XYLEM



Gymnosperms



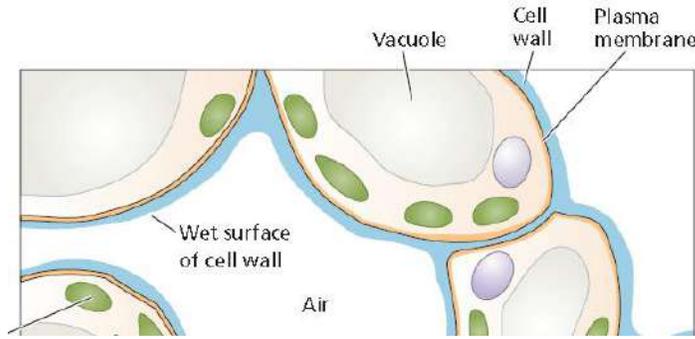
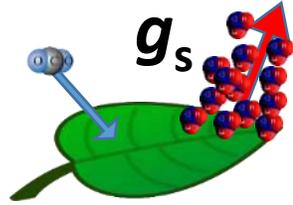
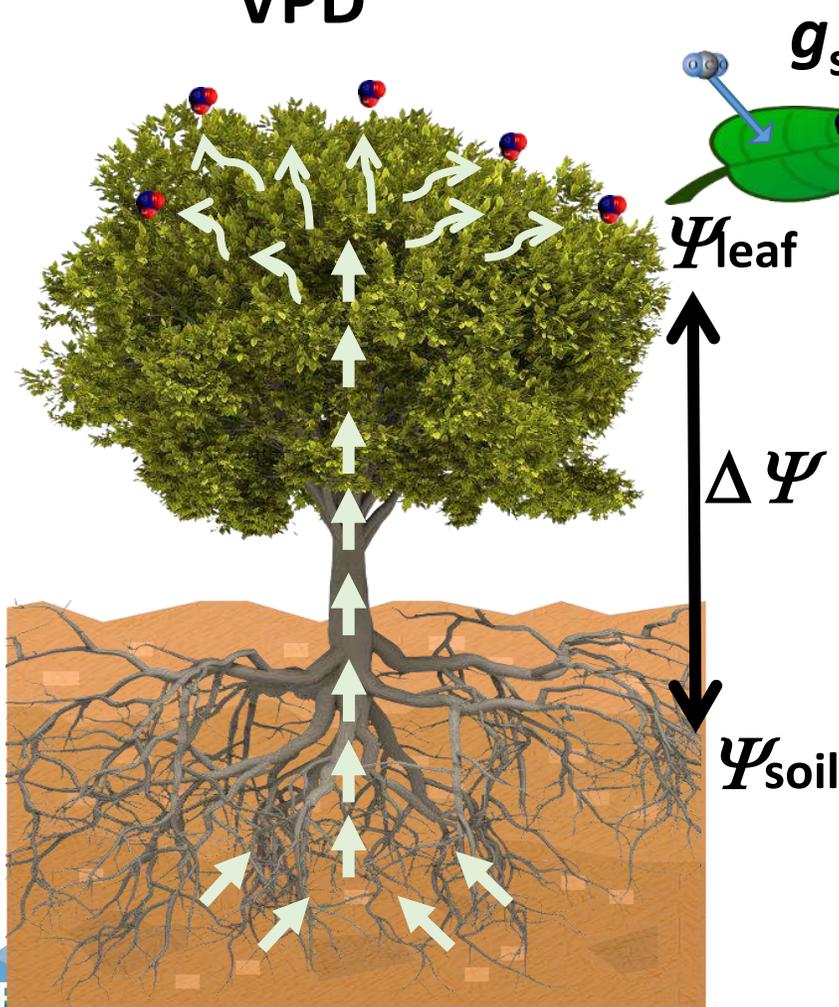
Angiosperms



BASIC PRINCIPLES IN PLANT HYDRAULICS

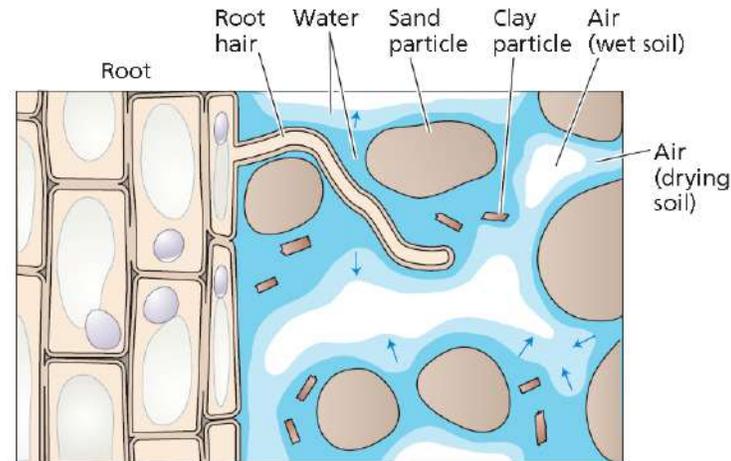
Key element in the transport of water from the soil to the leaves is the generation of negative pressures within the XYLEM

VPD



Leaf water at cell walls held

$$\text{Transpiration } (E_p) = \text{VPD } g_s = K \Delta \Psi$$

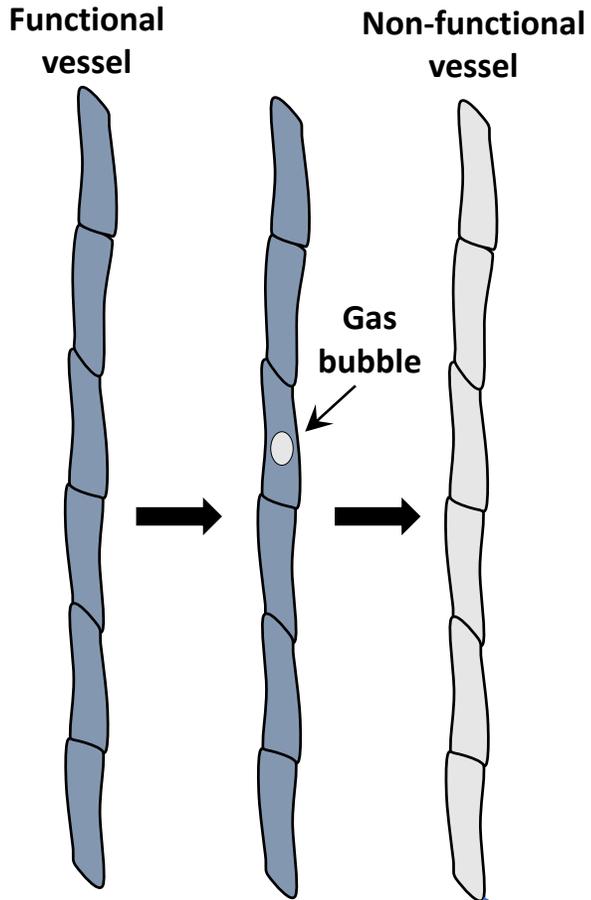
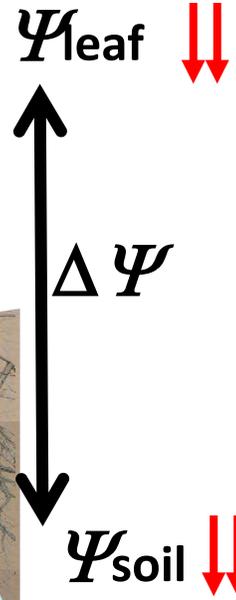
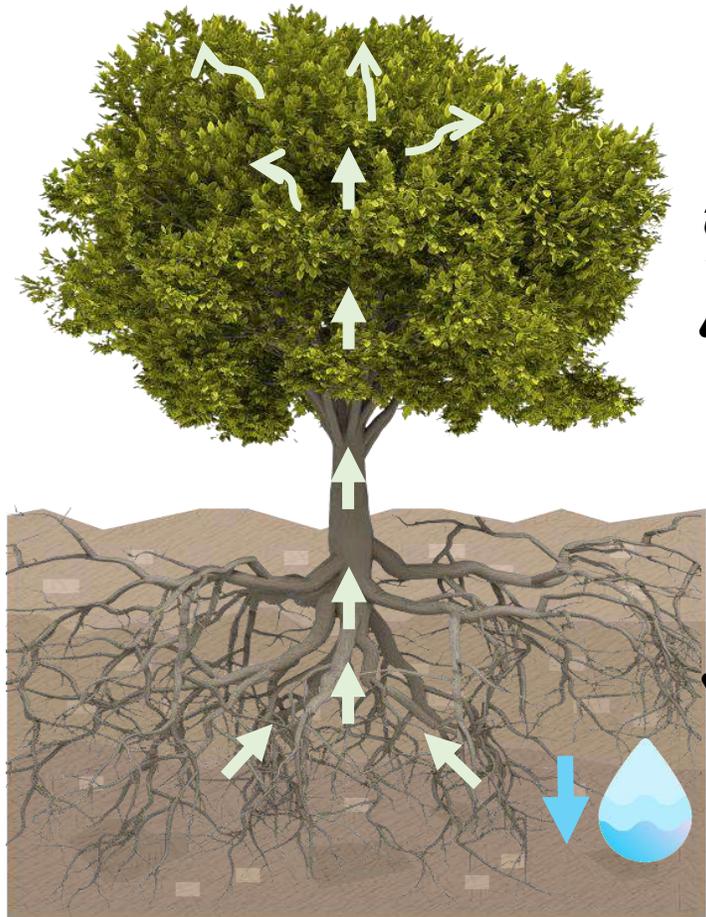


Soil water held



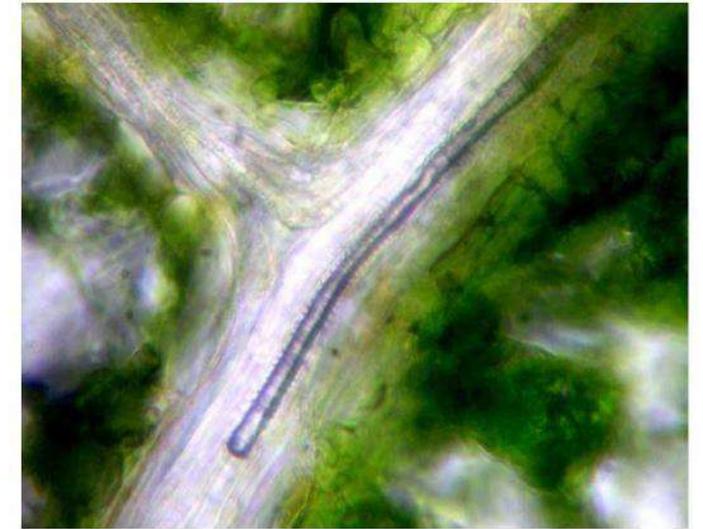
BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions

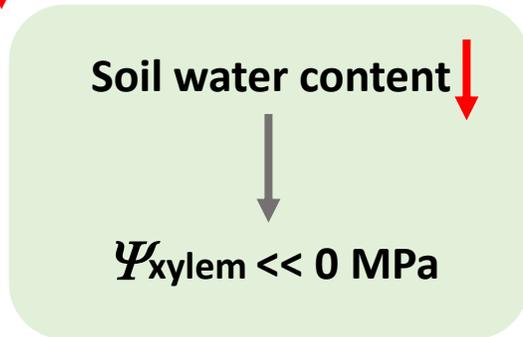


Cavitation

Abrupt change from liquid water under tension to water vapor



©INRA/Hervé Cochard

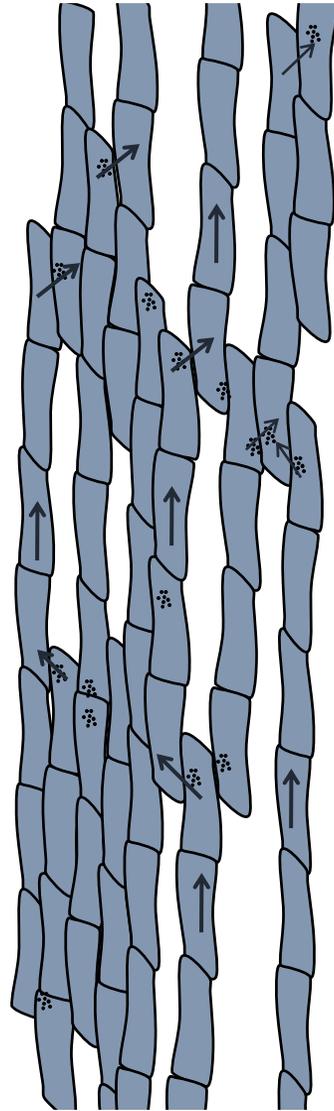


Embolism

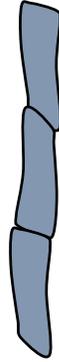


BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions and Cavitation



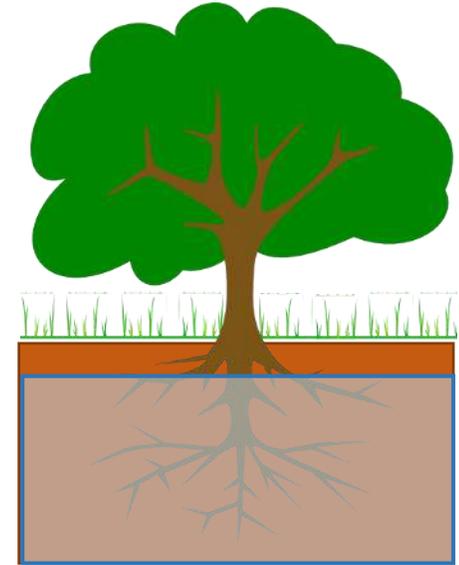
$$\Psi_{\text{xylem}} = -0.5 \text{ MPa}$$



Functional vessel

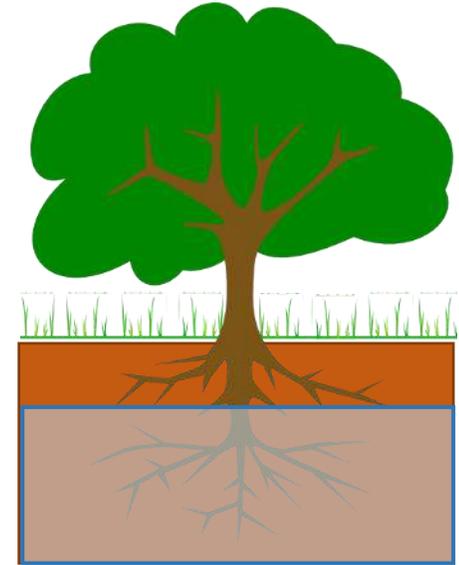
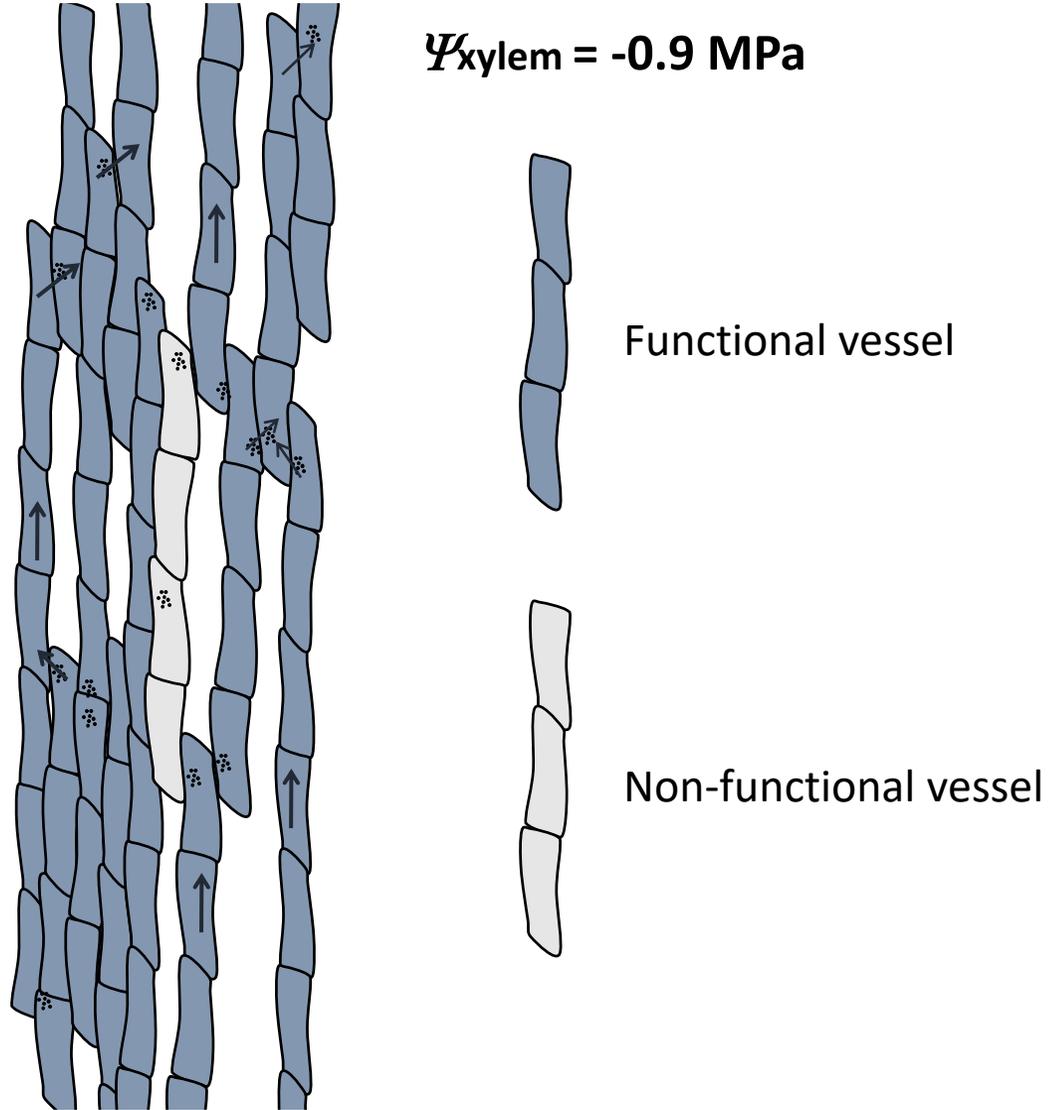


Non-functional vessel



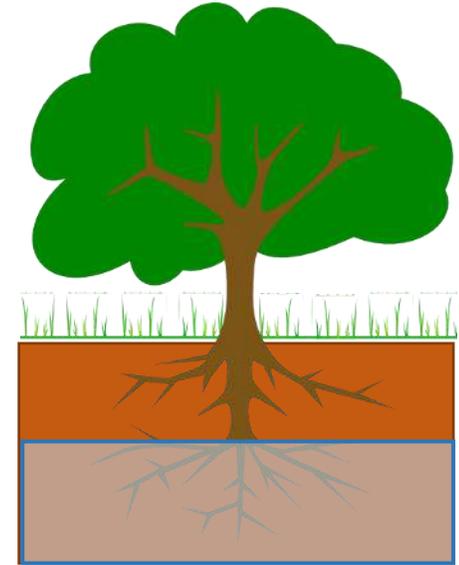
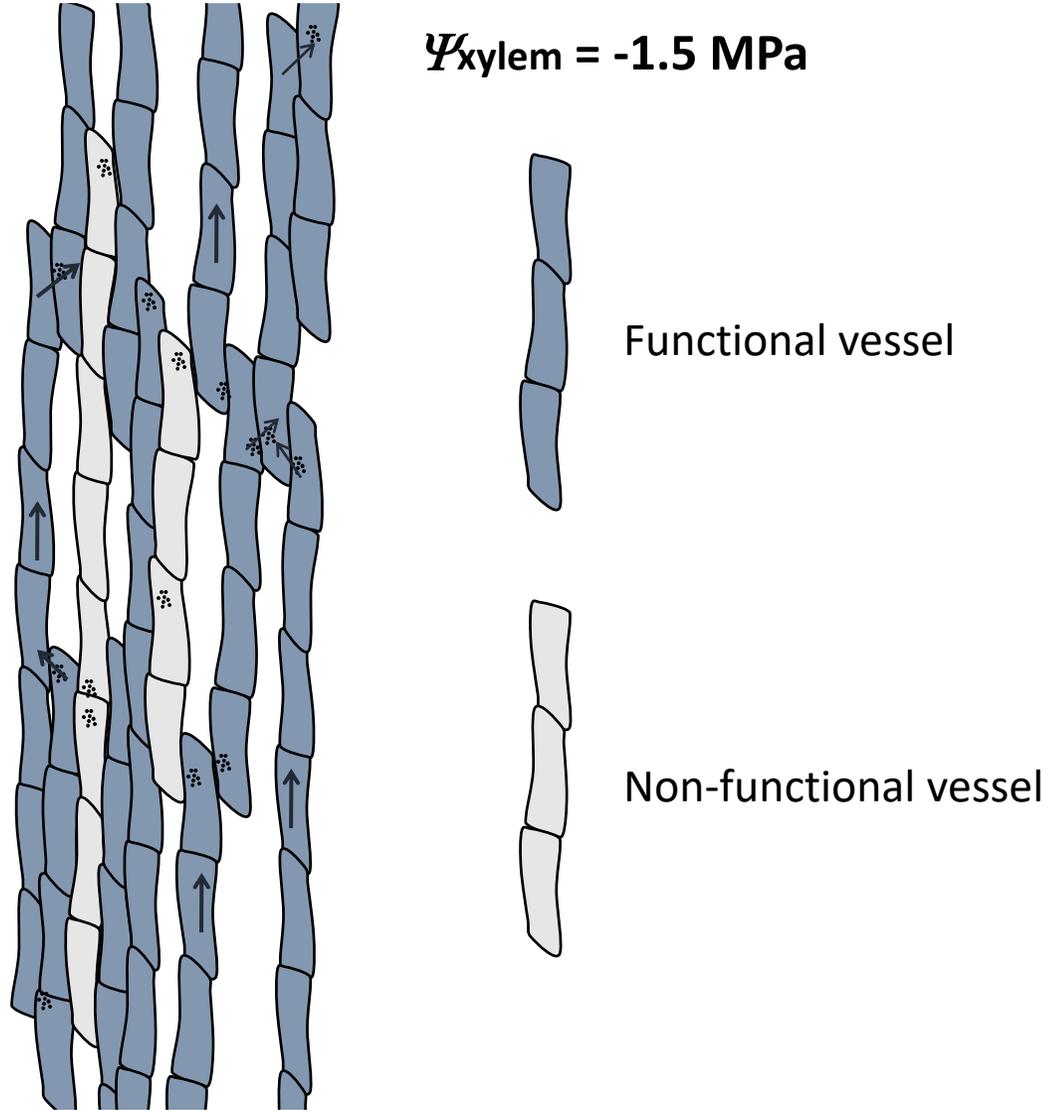
BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions and Cavitation



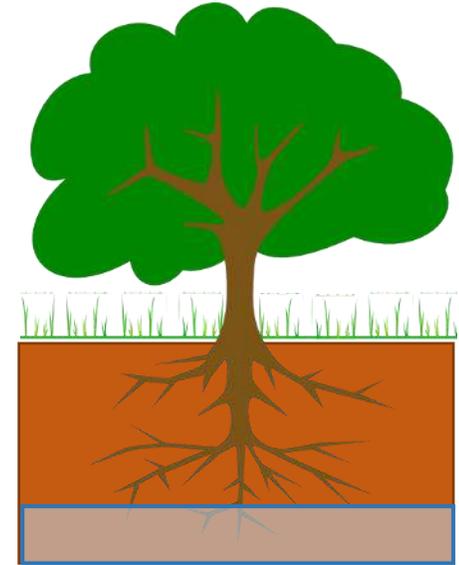
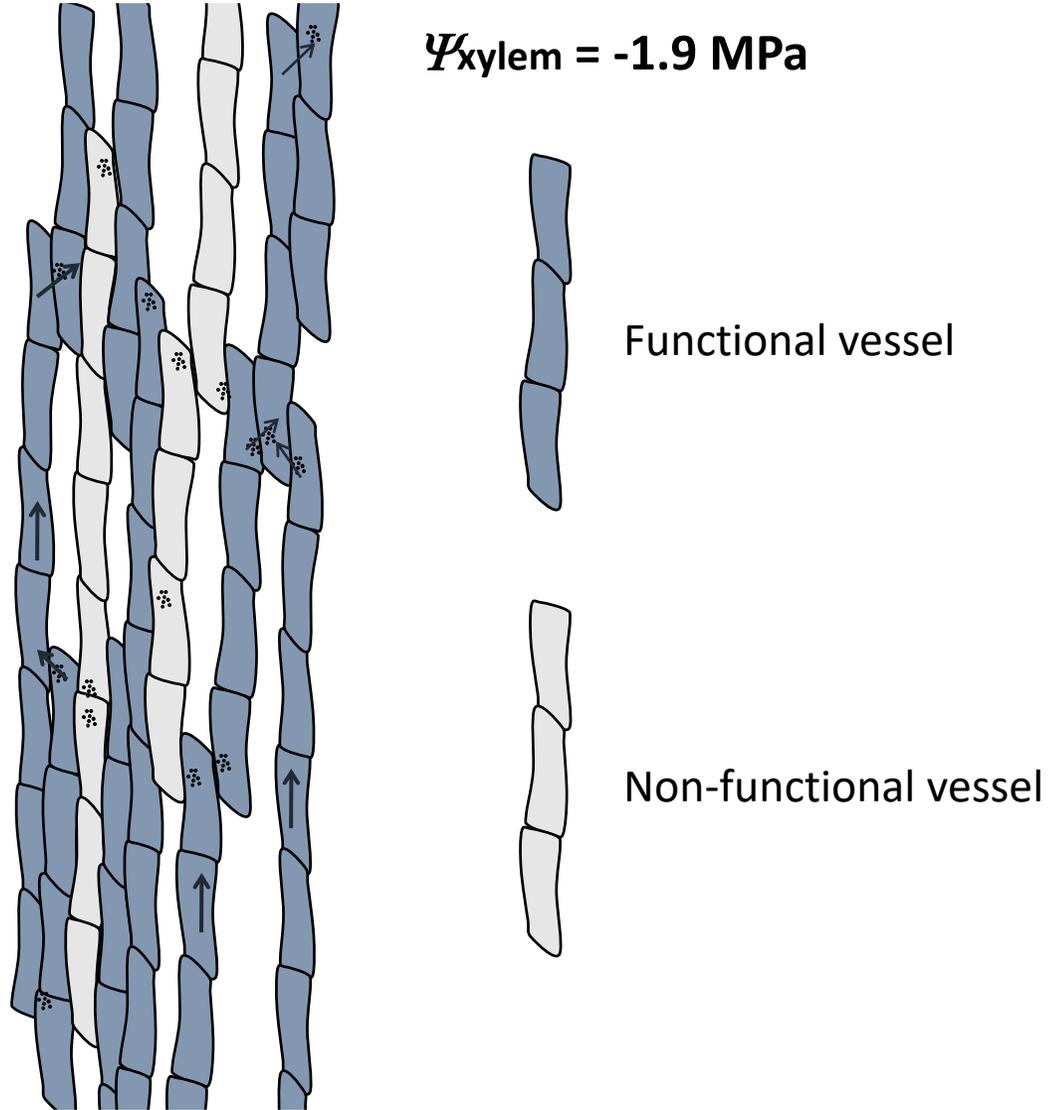
BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions and Cavitation



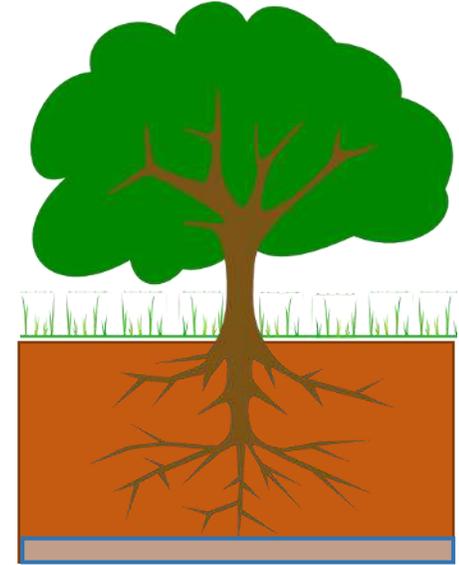
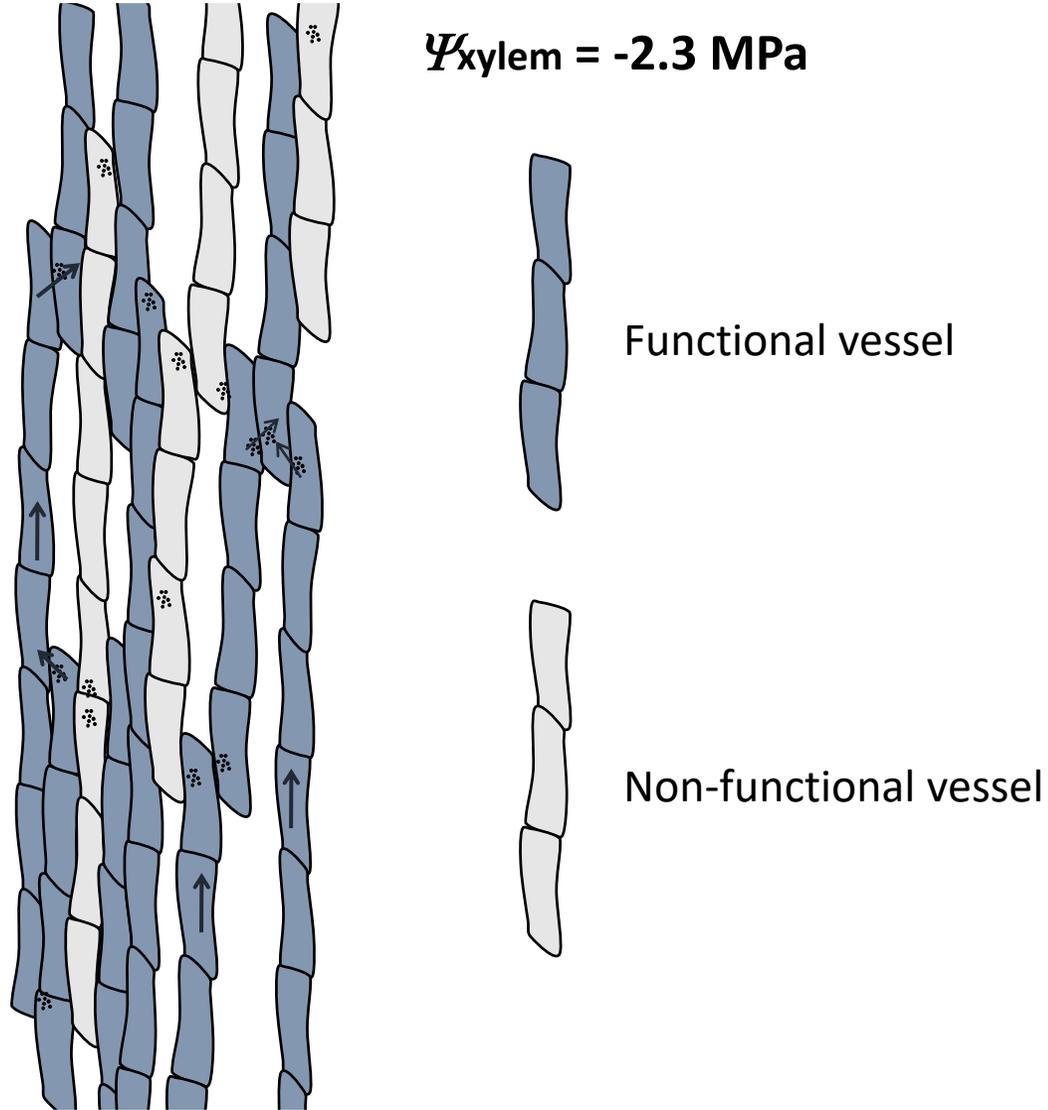
BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions and Cavitation



BASIC PRINCIPLES IN PLANT HYDRAULICS

Soil water limiting conditions and Cavitation



High xylem tensions induce losses in plant hydraulic functioning

PLC =
Percent loss of conductivity

BASIC PRINCIPLES IN PLANT HYDRAULICS

$$K = (F / \Delta P) l$$

↓ ↓ = =

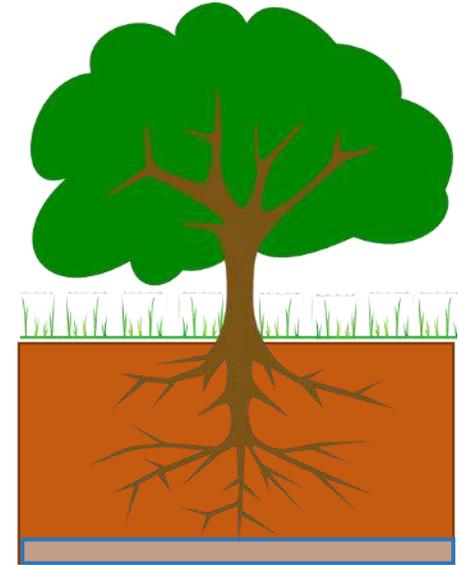
F = water flow rate

l = sample length

ΔP = pressure difference
between sample ends

$$E_p = VPD g_s = K \Delta \Psi$$

↓ ↓



High xylem tensions induce losses in plant hydraulic functioning

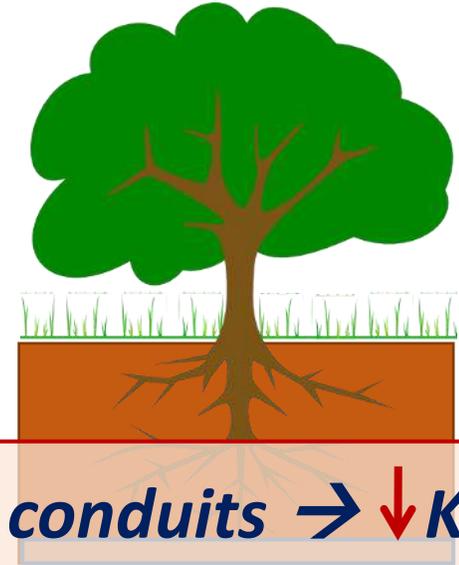
PLC =
Percent loss of conductivity

BASIC PRINCIPLES IN PLANT HYDRAULICS

$$K = (F / \Delta P) l$$

↓ ↓ = =

F = water flow rate
 l = sample length
 ΔP = pressure difference



Water stress conditions → ↓ Ψ_{xylem} → ↑ *Cavitated conduits* → ↓ K

$$E_p = \text{VPD } g_s = K \Delta \Psi$$

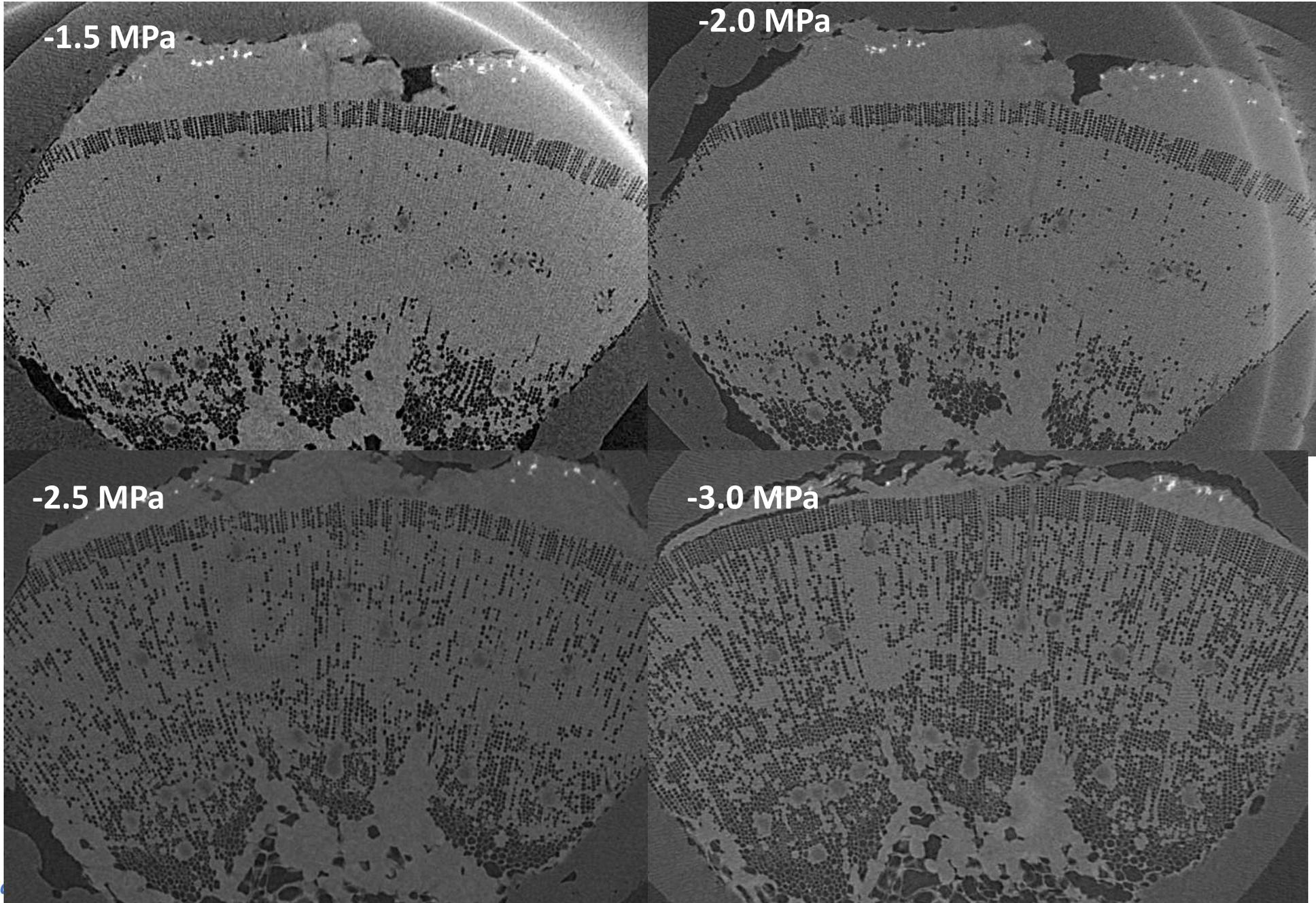
↓ ↓

High xylem tensions induce losses in plant hydraulic functioning

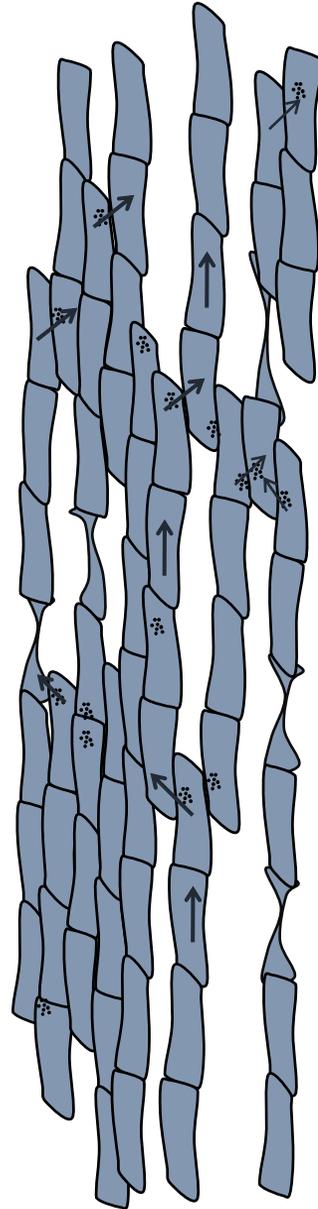
PLC =
Percent loss of conductivity



Pinus sylvestris



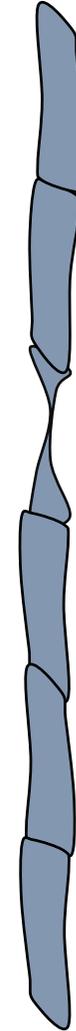
BASIC PRINCIPLES IN PLANT HYDRAULICS



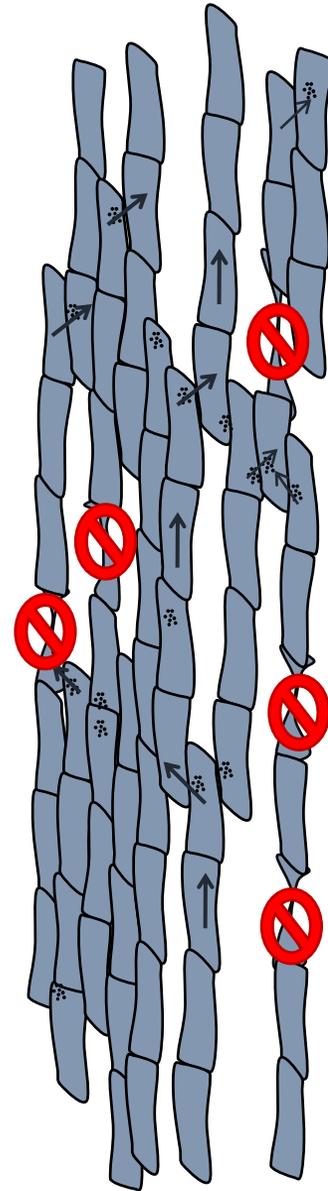
$\lll \Psi_{\text{xylem}}$

$$K = (F / \Delta P) l$$

Collapse



BASIC PRINCIPLES IN PLANT HYDRAULICS



$\lll \Psi_{\text{xylem}}$

$$K = (F / \Delta P) l$$

↓ ↓ = =

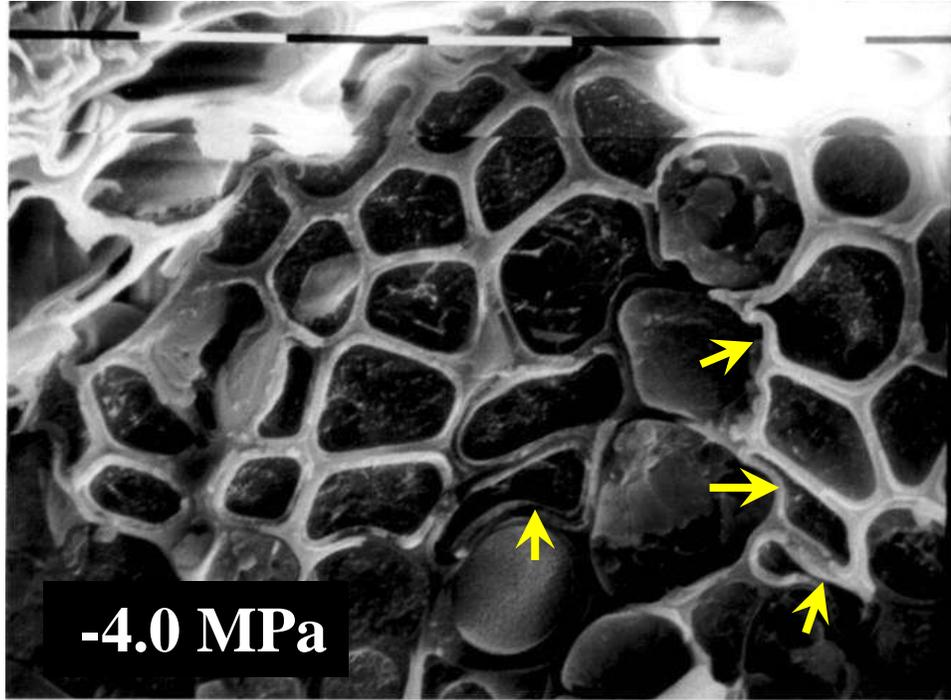
$$E_p = \text{VPD } g_s = K \Delta \Psi$$

↓ ↓

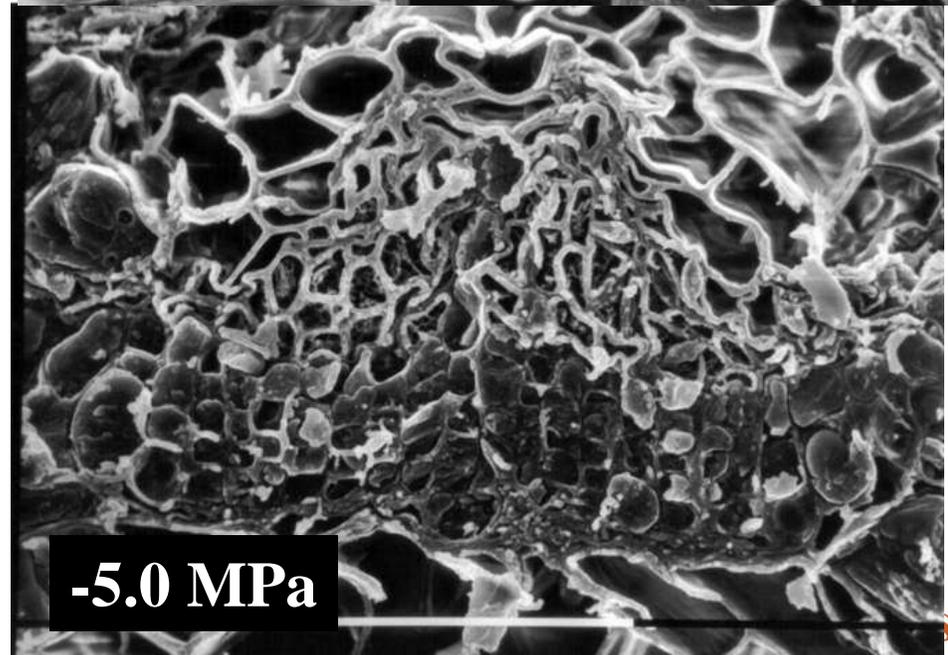
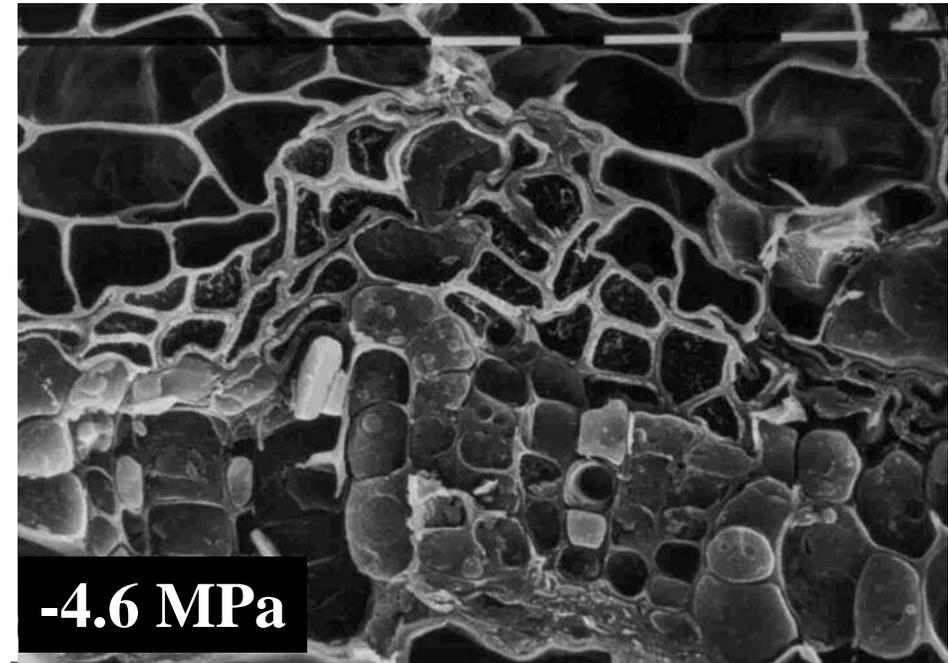
Collapse



BASIC PRINCIPLES IN PLANT HYDRAULICS



Pinus needles
(Pinus cembra)



Cryo-scanning electron microscopy
Images from Herve Cochard's website



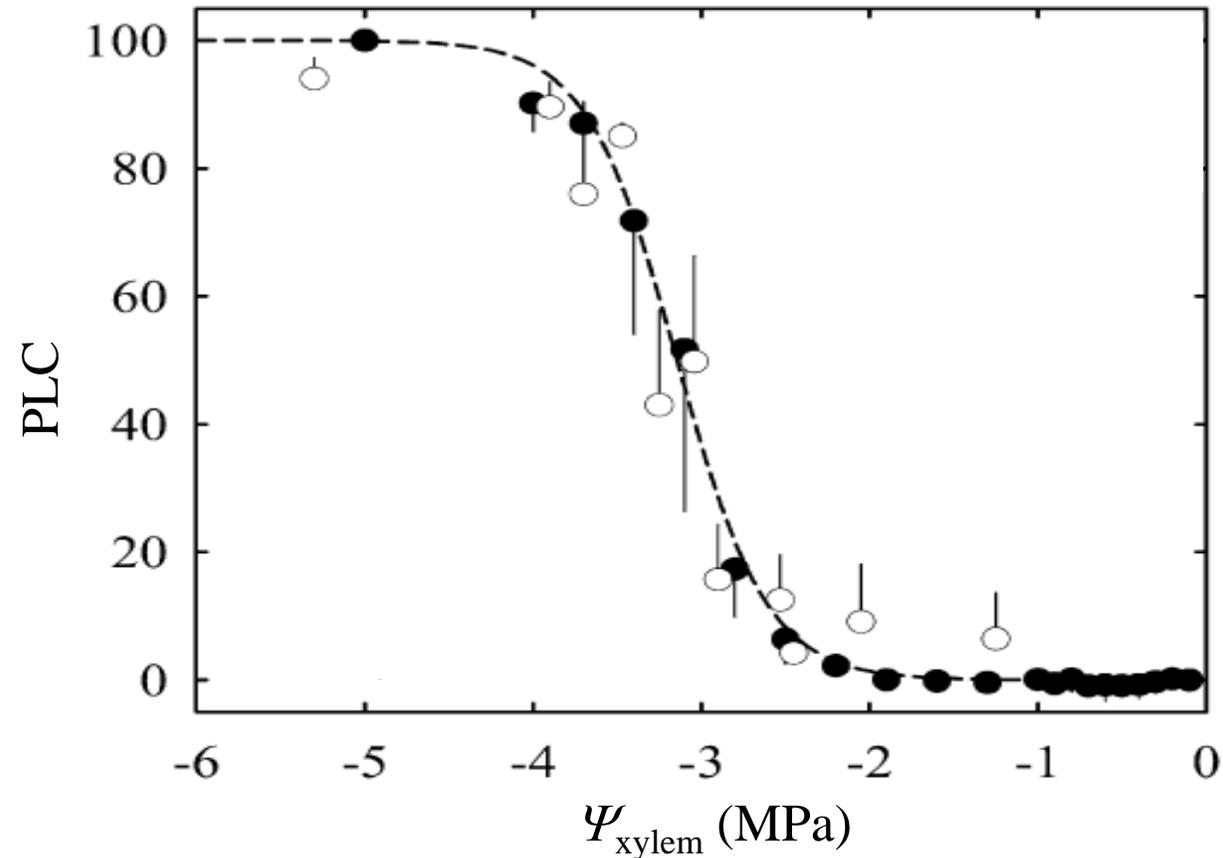
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BASIC PRINCIPLES IN PLANT HYDRAULICS

How do we quantify these losses of K?

Vulnerability curves (VCs)



PLC = Percent loss of conductivity

BASIC PRINCIPLES IN PLANT HYDRAULICS

How do we quantify these losses of K?

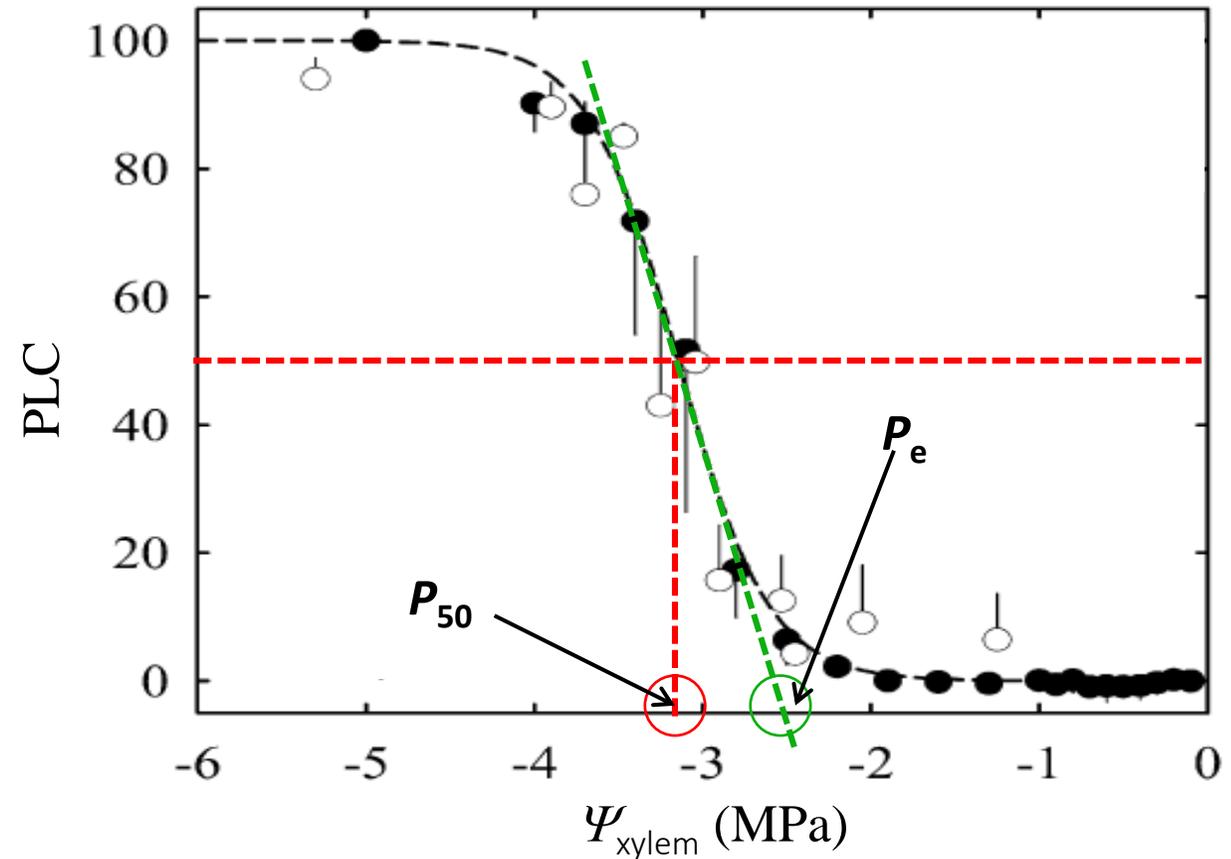
PLC = Percent loss of conductivity

P_{50} = Ψ at PLC=50%

P_e = Air entry pressure

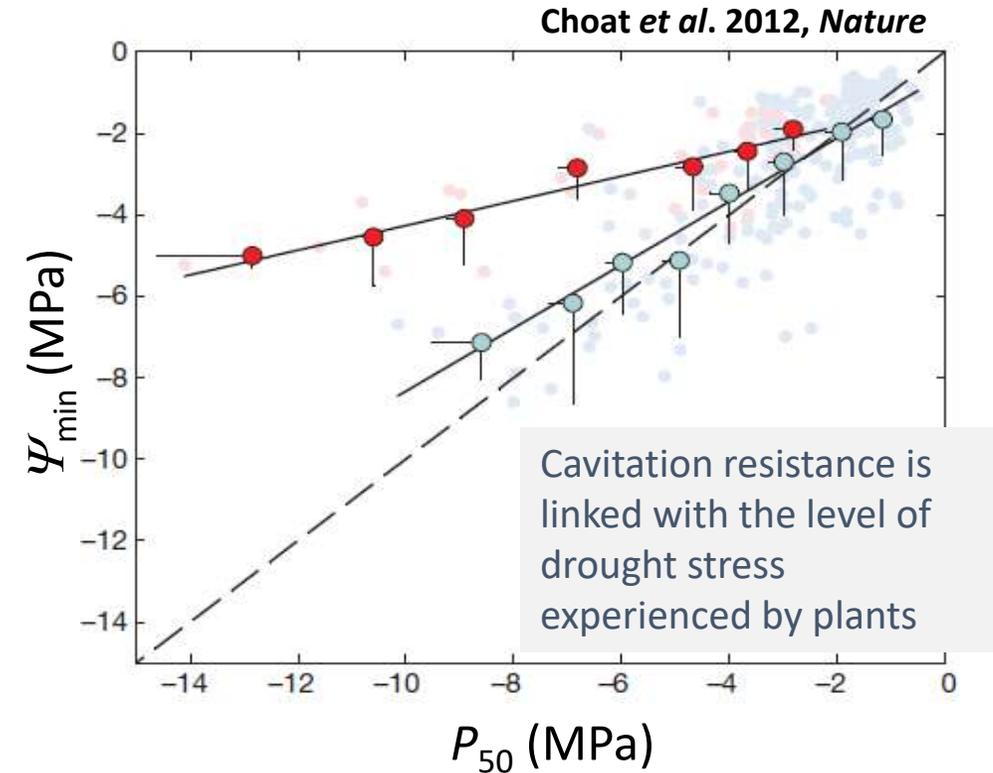
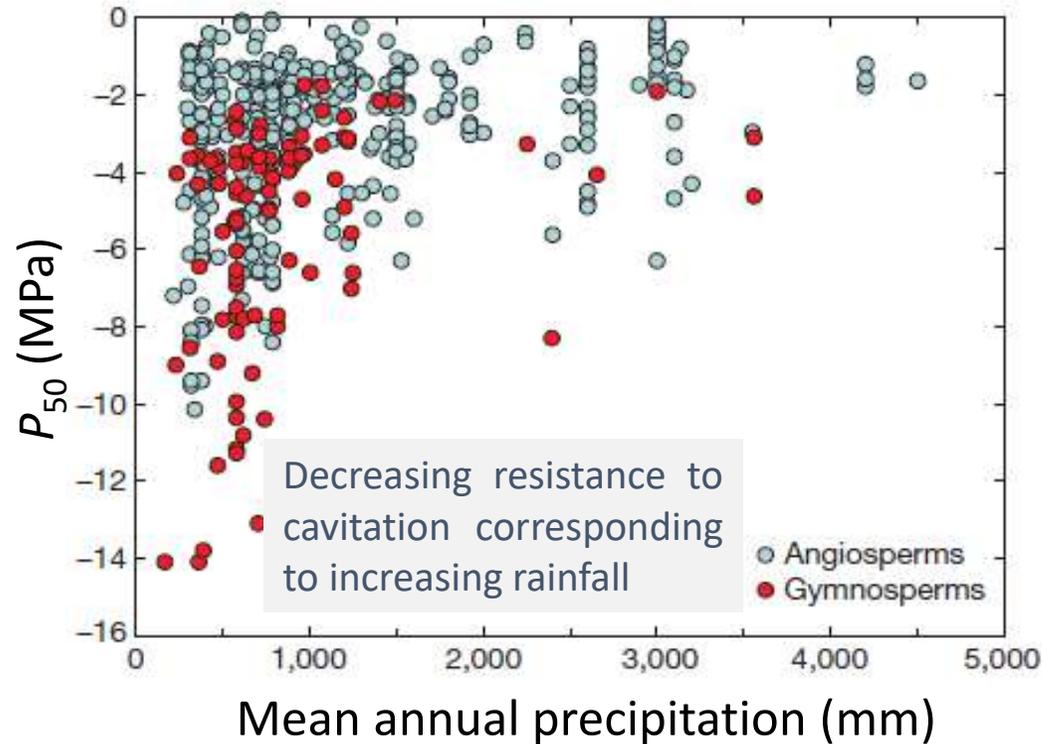
P_{88} = Full embolism point \rightarrow PLC = 88%

Vulnerability curves (VCs)



BASIC PRINCIPLES IN PLANT HYDRAULICS

Why is important to determine the vulnerability to cavitation of the species?



- Cavitation is a key mechanism of vegetation shifts and forest decline
- Useful information to predict the responses of forest ecosystems to climate change.

BASIC PRINCIPLES IN PLANT HYDRAULICS

Cavitation resistance of conifer trees

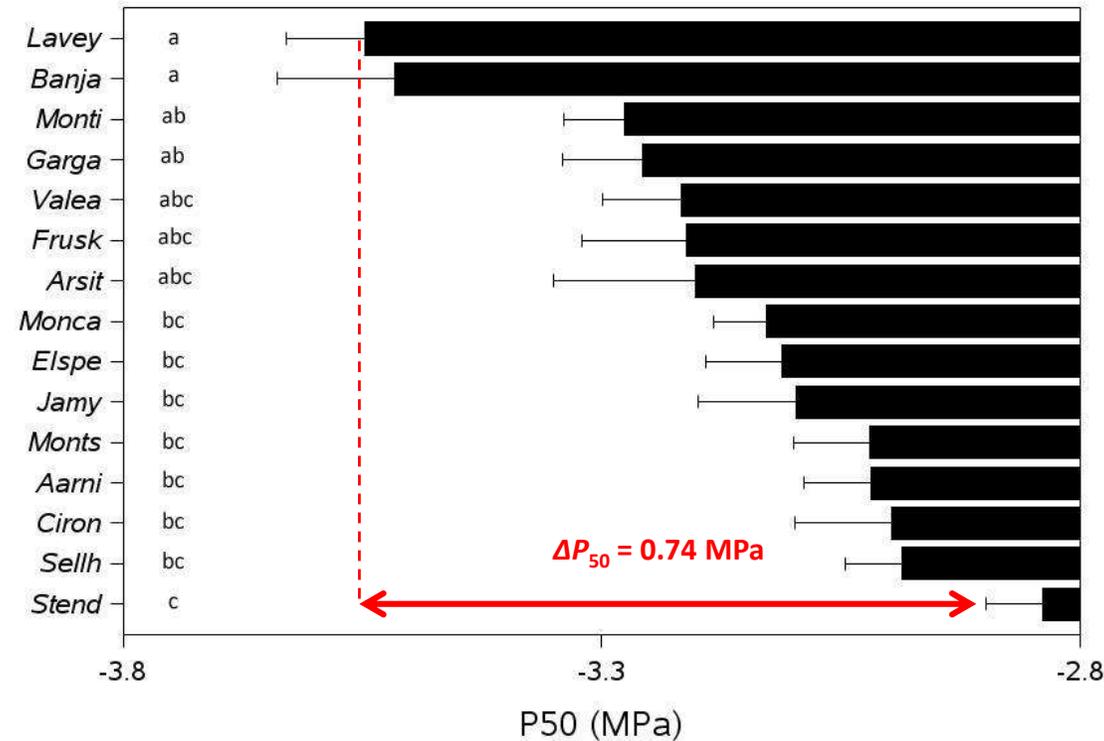


Data courtesy of S. Delzon



BASIC PRINCIPLES IN PLANT HYDRAULICS

Variability of cavitation resistance across European beech (*Fagus sylvatica* L.)



Cavitation resistance (P_{50}) is a very consistent trait within species



BASIC PRINCIPLES IN PLANT HYDRAULICS

Cavitation resistance of conifer trees

Callitris tuberculata
P50 = -18.8Mpa!!

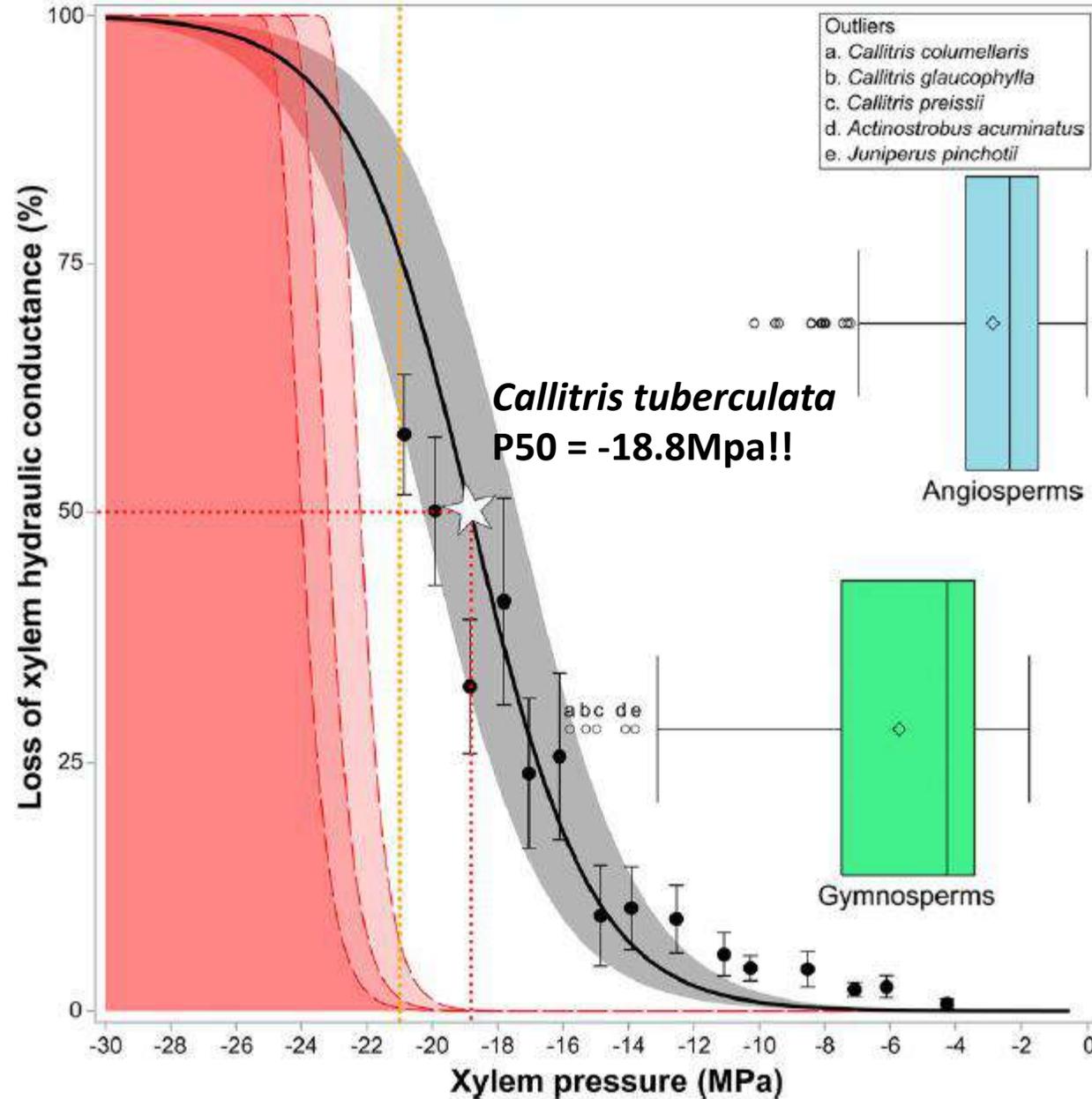


Data courtesy of S. Delzon

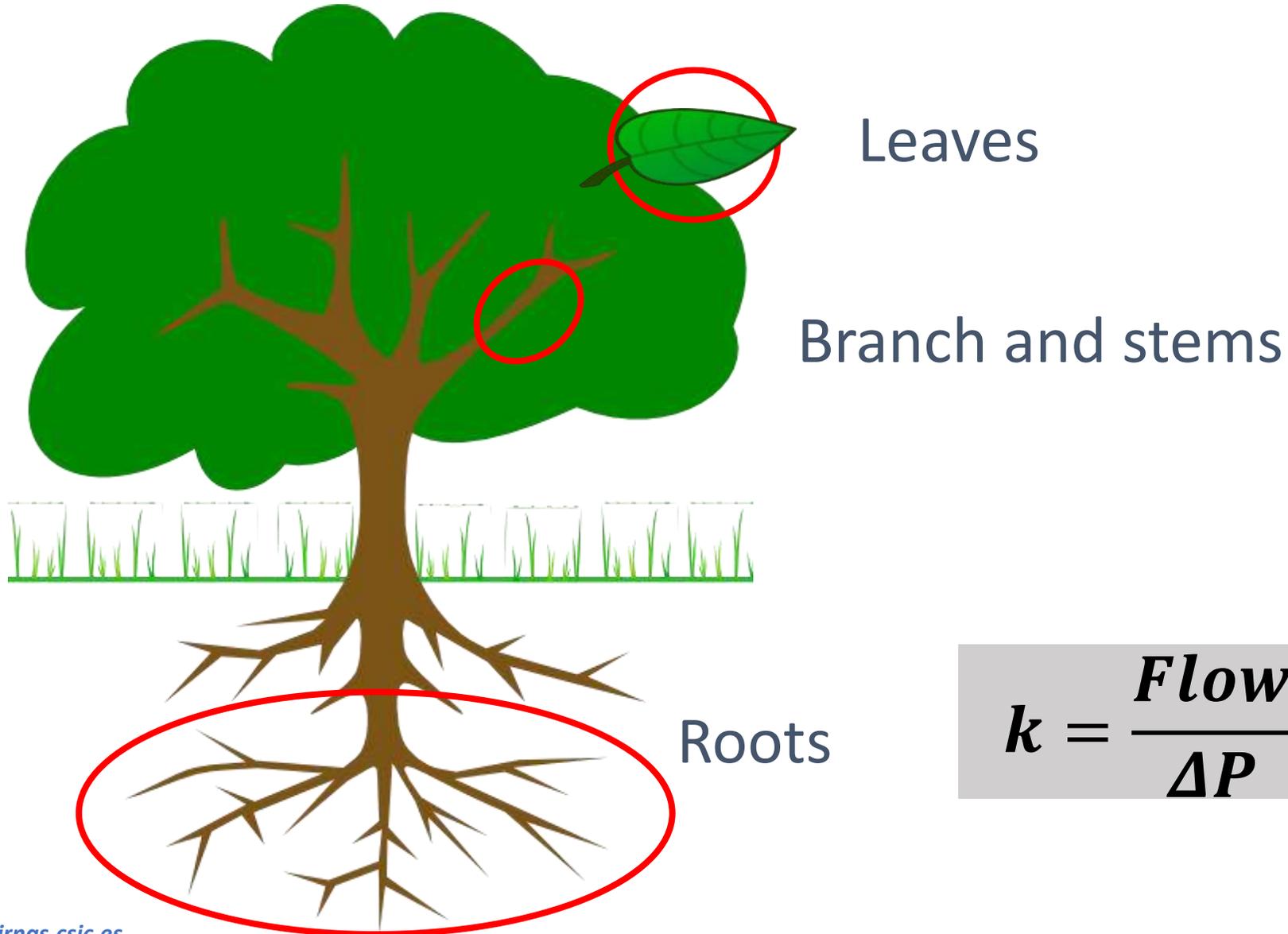


BASIC PRINCIPLES IN PLANT HYDRAULICS

Bulk cavitation occurs rapidly at tensions of around -22 MPa, setting an absolute physical limit for water transport in trees during drought

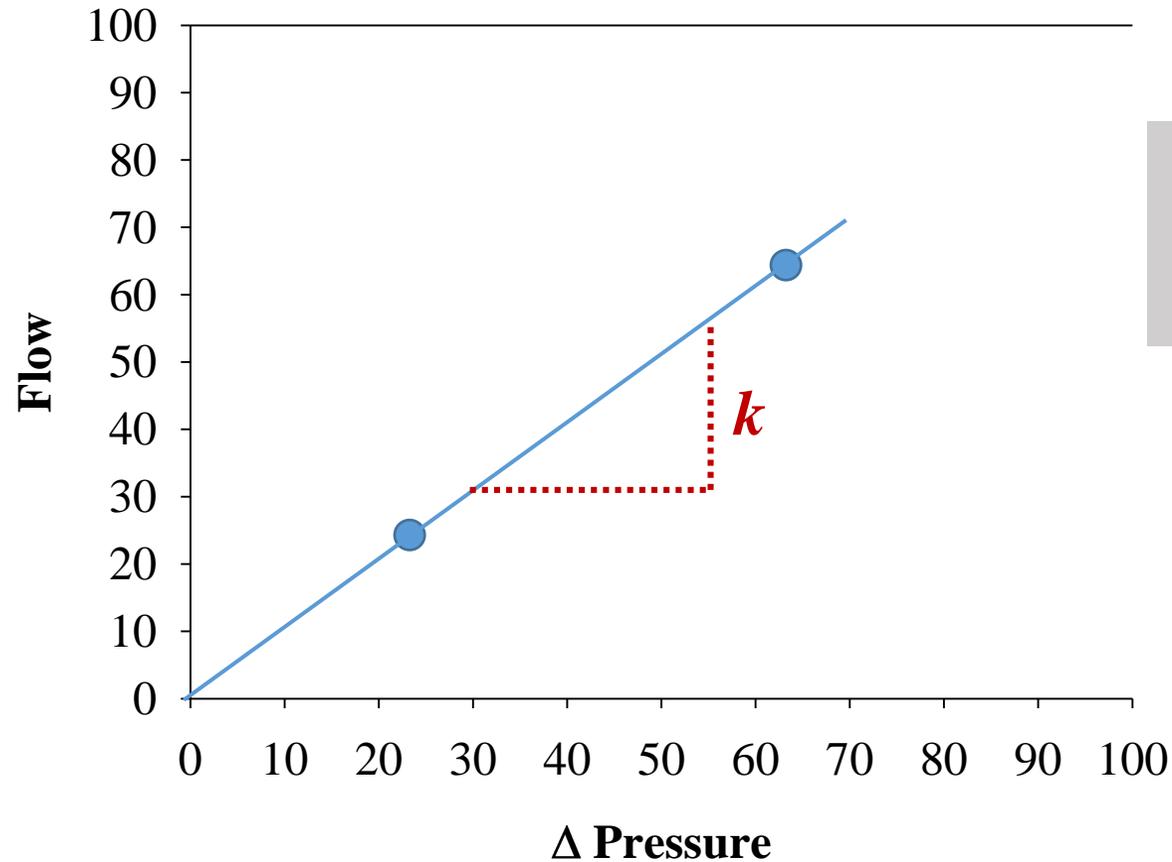


METHODS FOR MEASURING k AND GENERATING VCS



$$k = \frac{\textit{Flow}}{\Delta P}$$

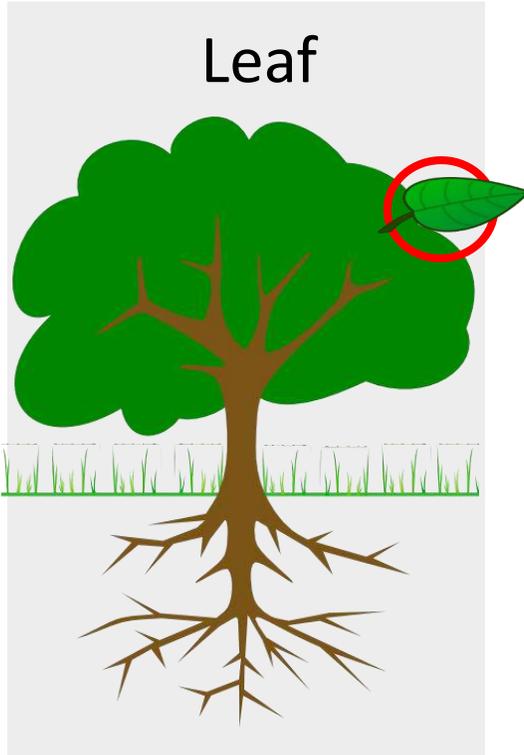
METHODS FOR MEASURING k AND GENERATING VCS



$$PLC = \frac{k_i * 100}{k_{max}}$$

$$k = \frac{Flow}{\Delta P}$$

METHODS FOR MEASURING K AND GENERATING VCS

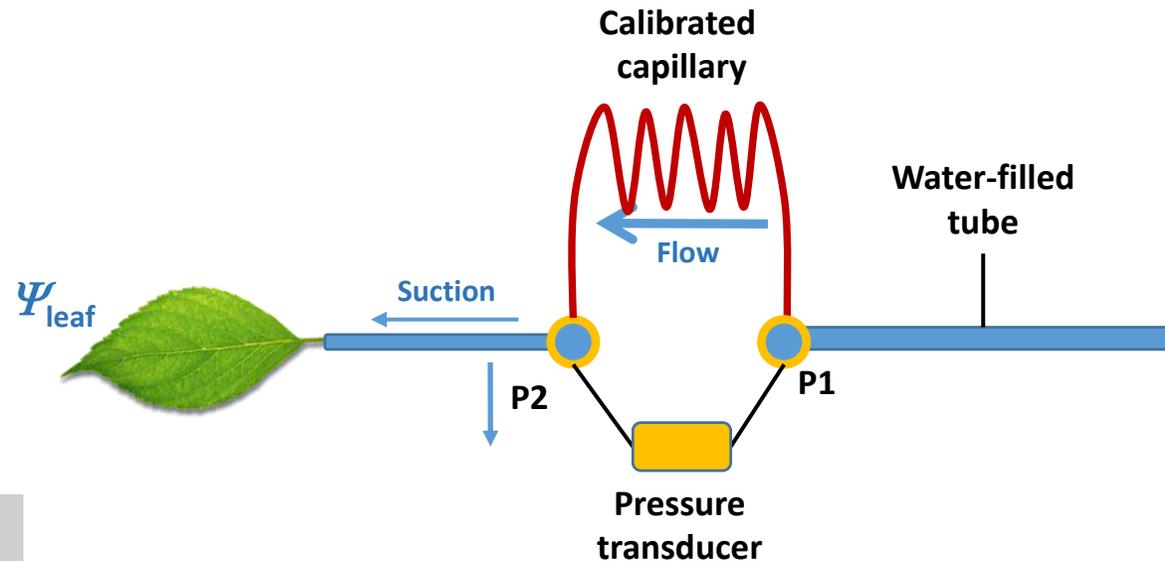


Rehydration kinetics method

Brodrigg and Cochard 2009

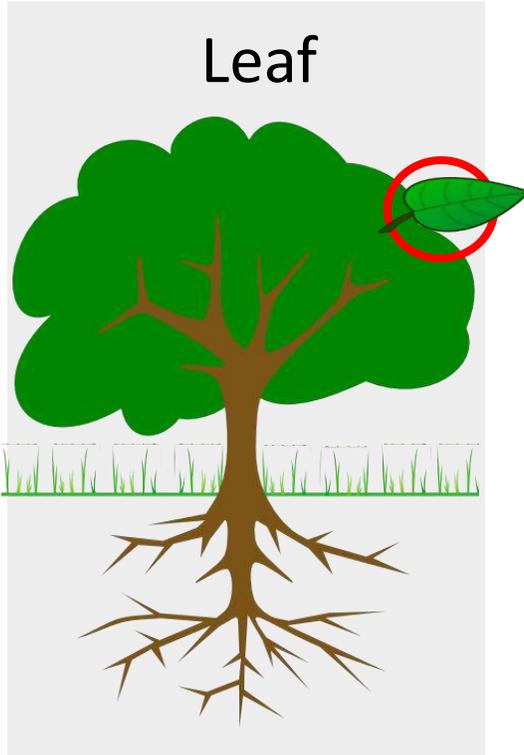
K_{leaf} is measured under non-steady-state conditions whereby leaves are allowed to rehydrate while connected to a flowmeter

$\Delta P \rightarrow \Delta mV \rightarrow \text{Flow}$



$$K_{\text{leaf}} = \frac{\text{Flow}}{\Psi_{\text{leaf}} \times \text{Leaf area}}$$

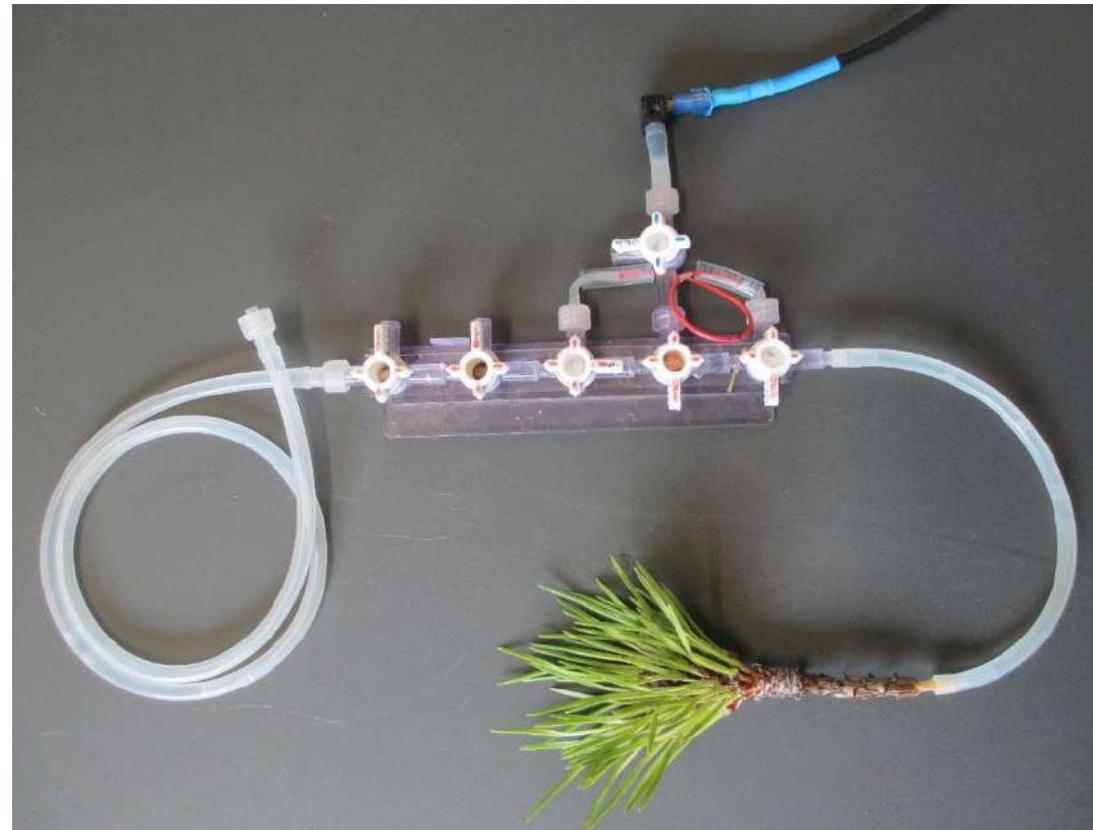
METHODS FOR MEASURING K AND GENERATING VCS



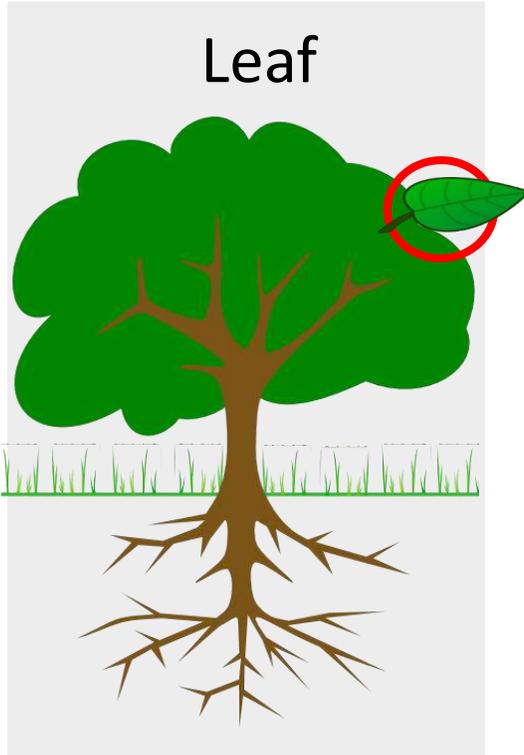
Rehydration kinetics method

Brodrribb and Cochard 2009

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METHODS FOR MEASURING K AND GENERATING VCS



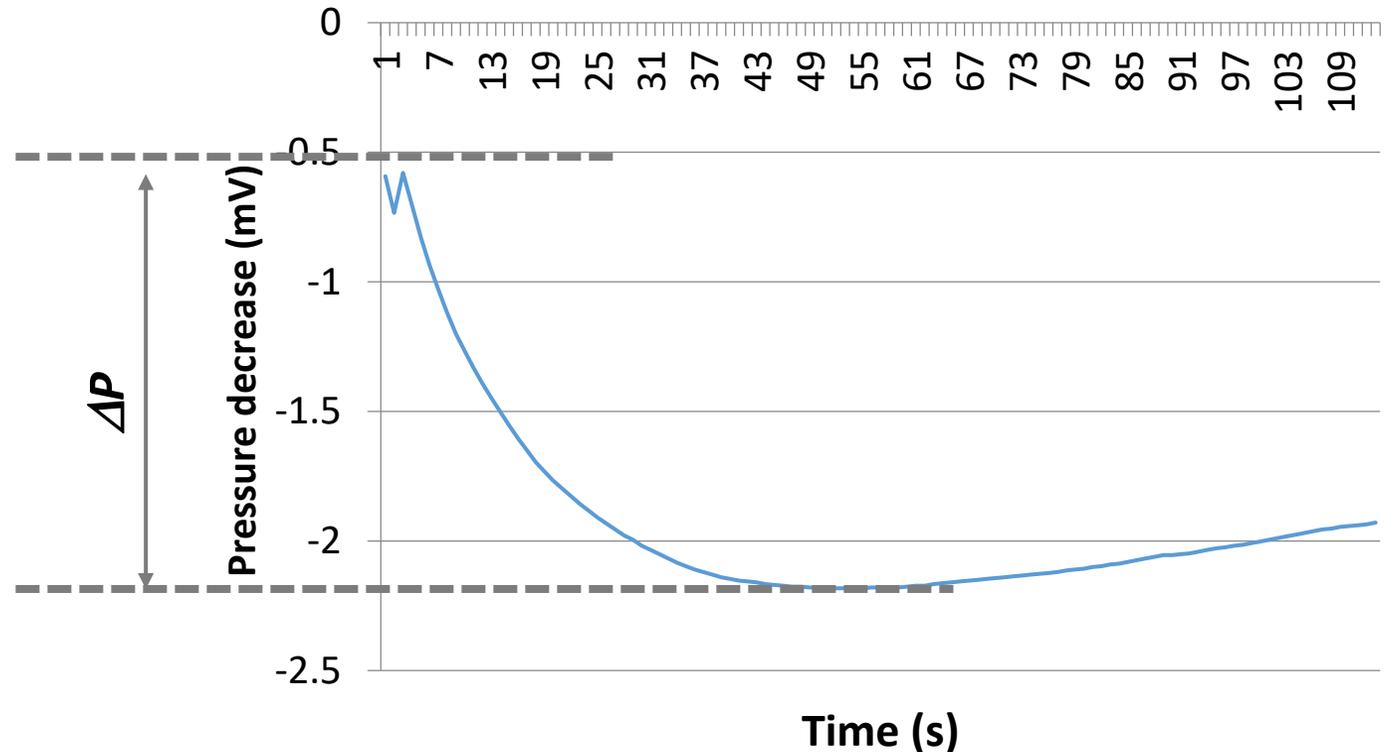
Rehydration kinetics method

Brodribb and Cochard 2009

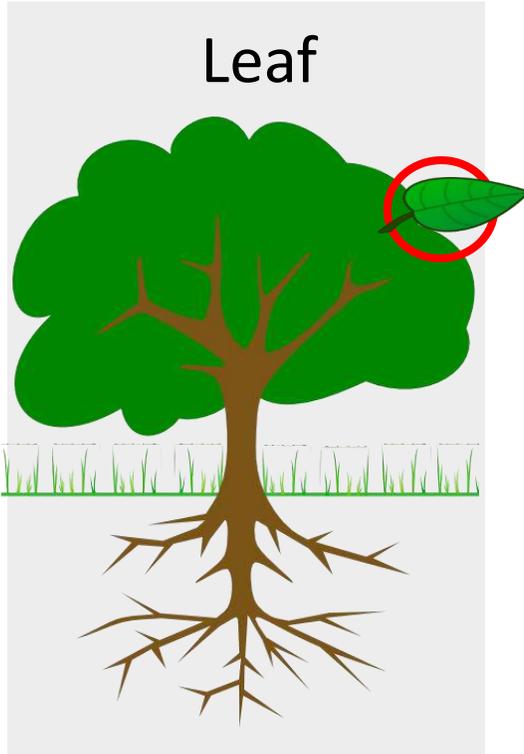
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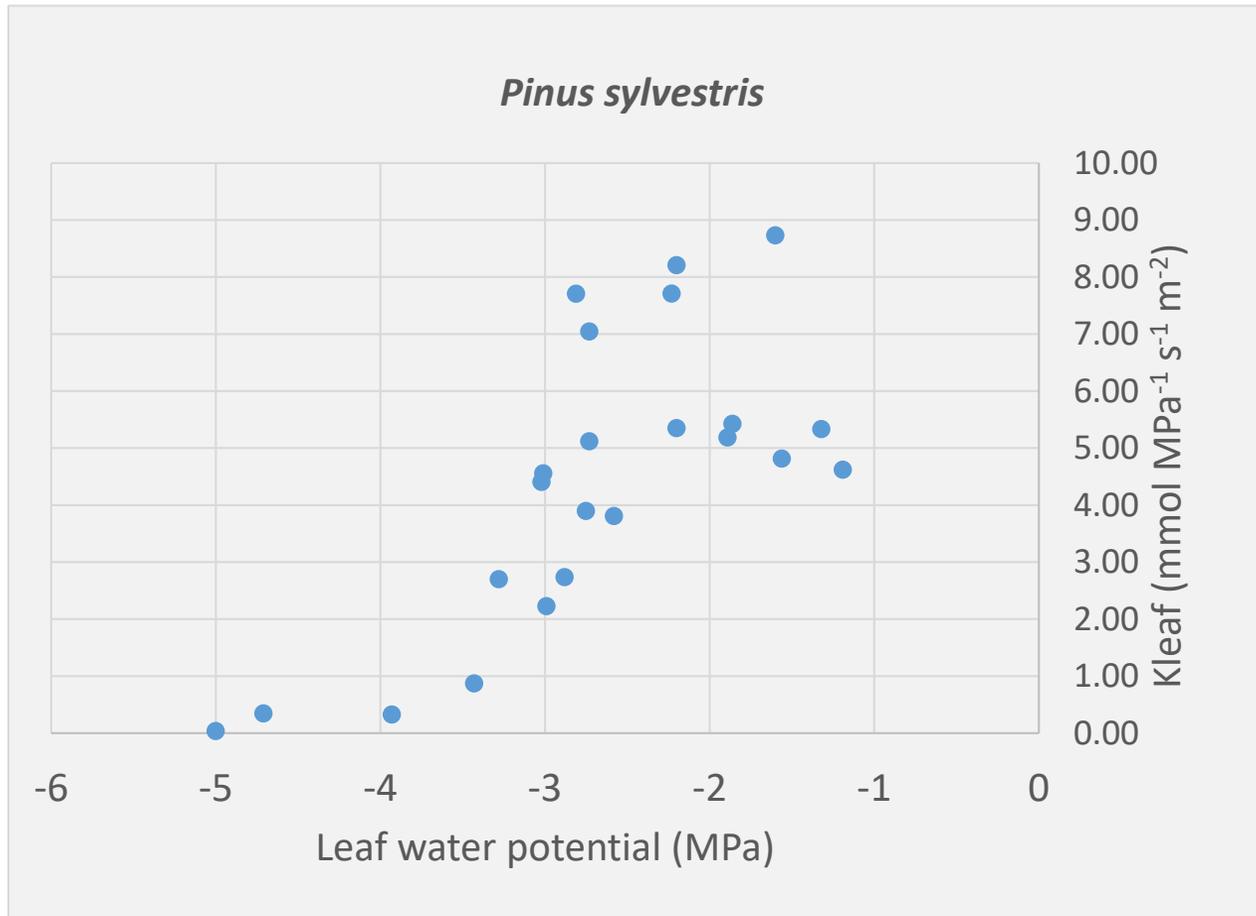


METHODS FOR MEASURING K AND GENERATING VCS

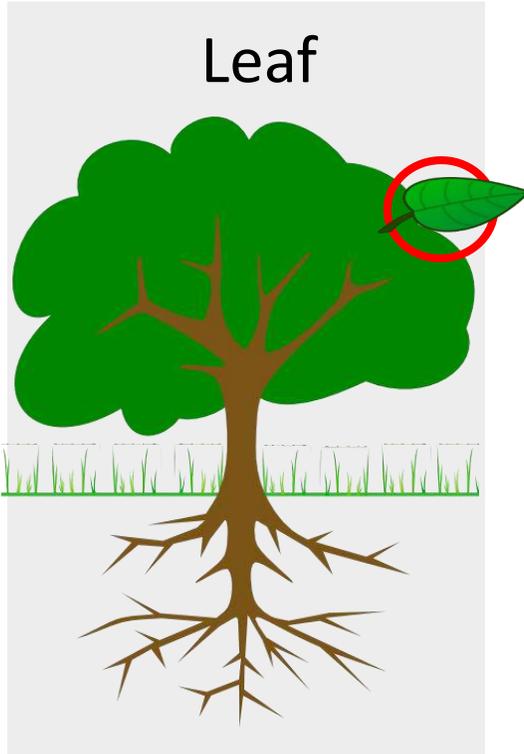


Rehydration kinetics method

Brodribb and Cochard 2009



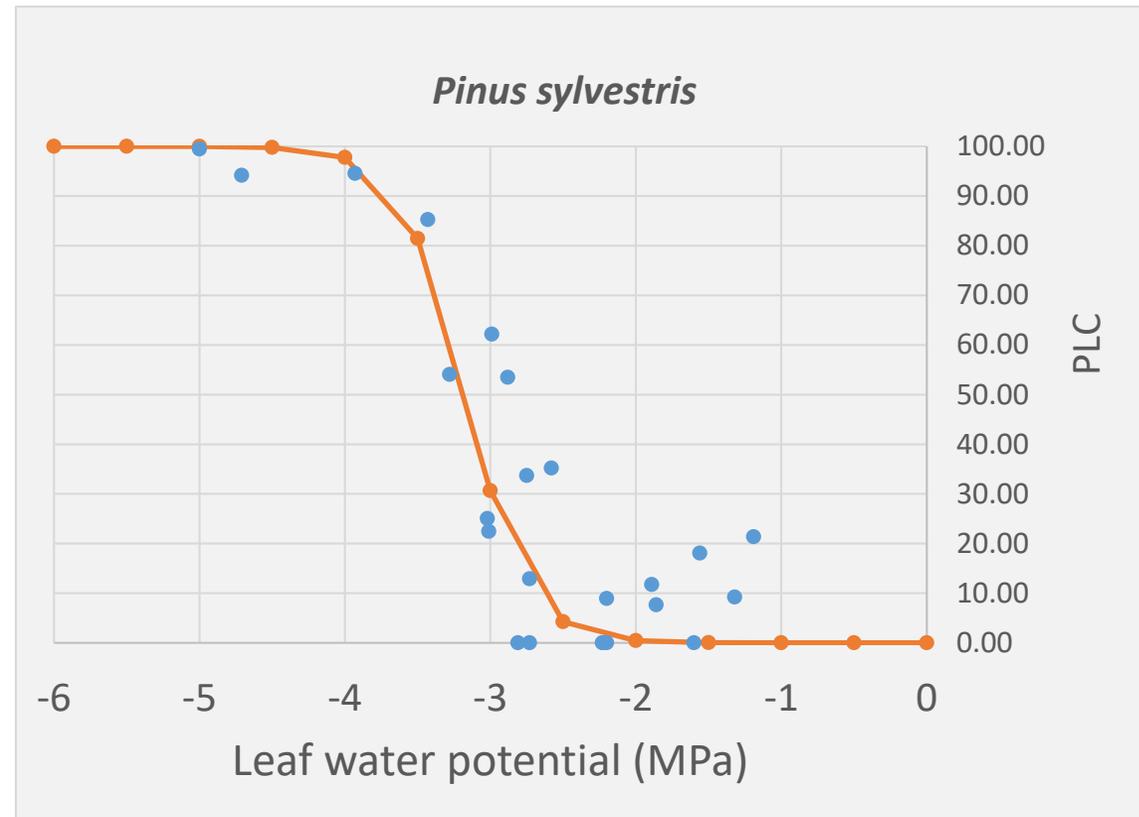
METHODS FOR MEASURING K AND GENERATING VCS



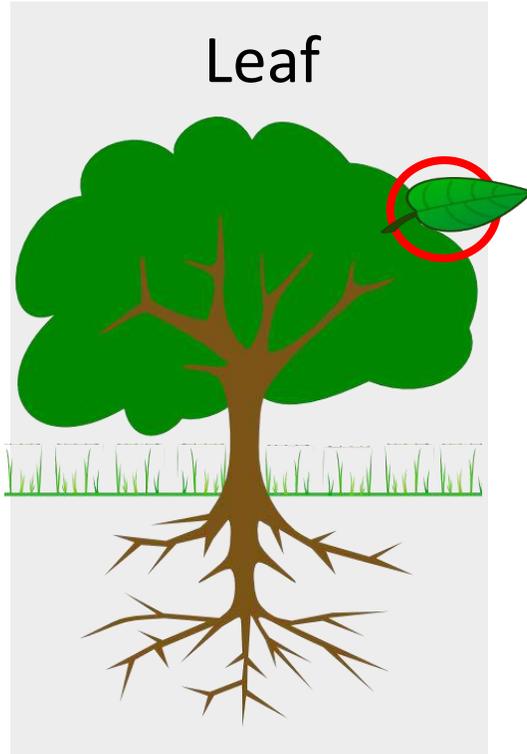
Rehydration kinetics method

Brodribb and Cochard 2009

$$PLC = 100 \times (1 - \text{native } K / \text{maximum } K)$$



METHODS FOR MEASURING K AND GENERATING VCS

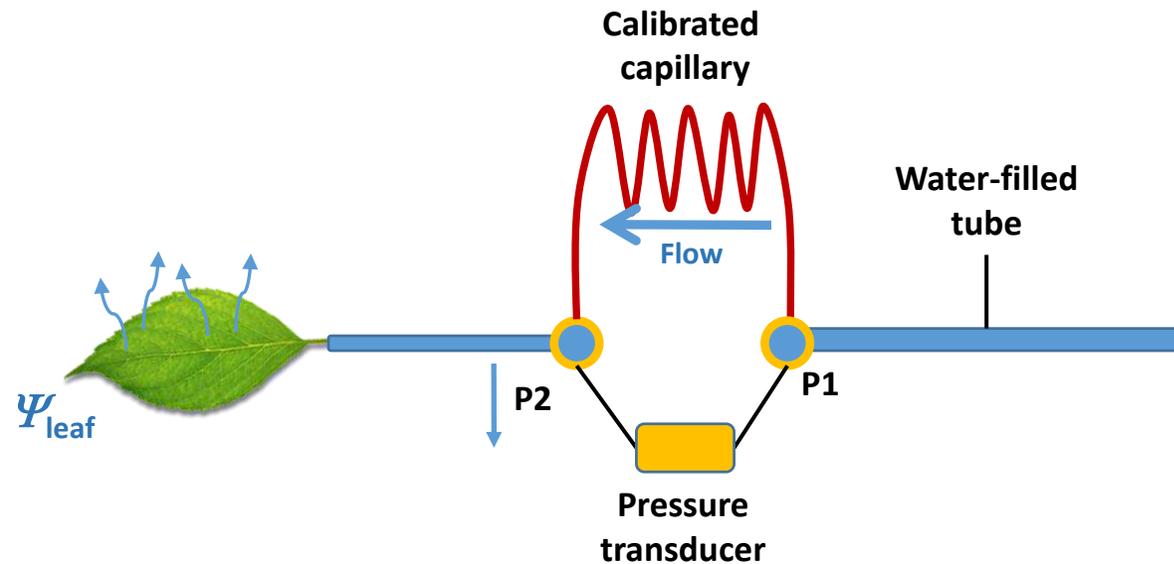


Evaporative flux method

Sack and Scoffoni 2012

K_{leaf} is measured under steady-state transpiration conditions

$\Delta P \rightarrow \Delta mV \rightarrow \text{Flow}$



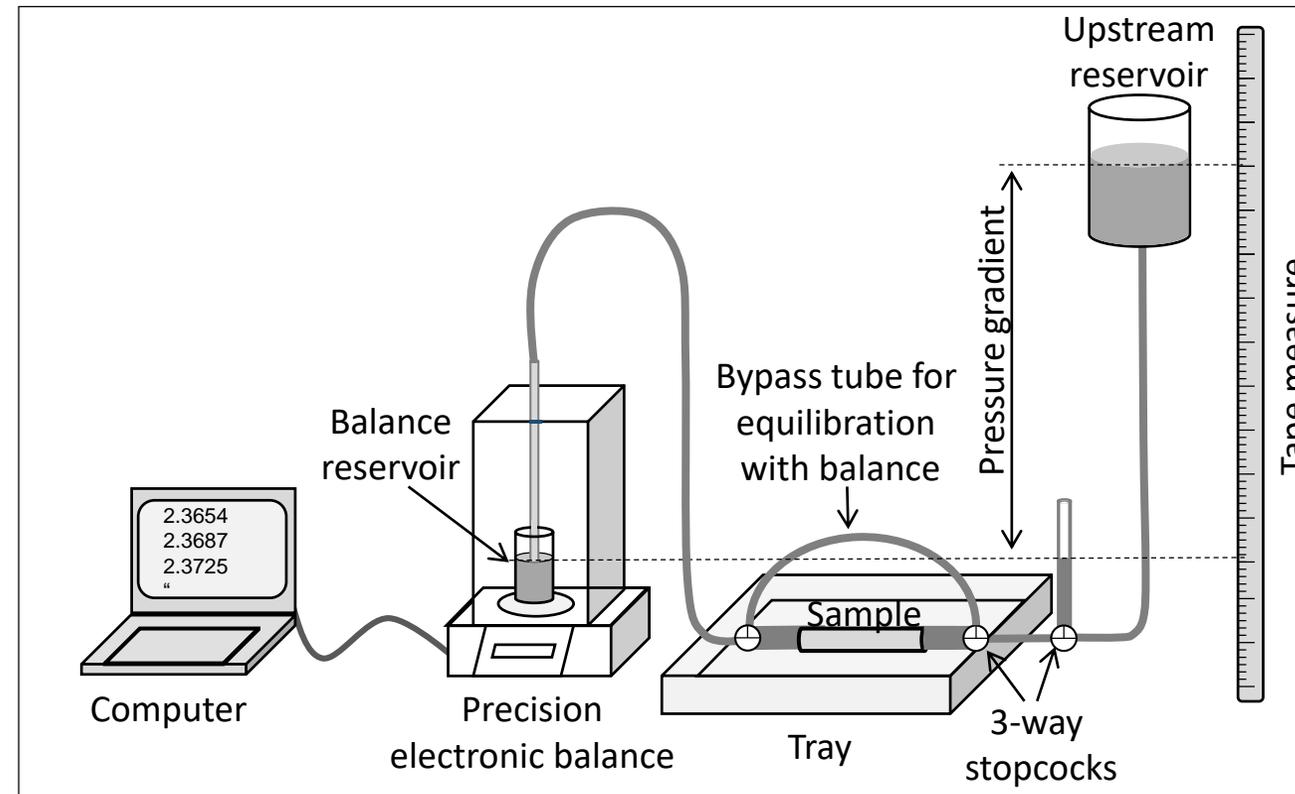
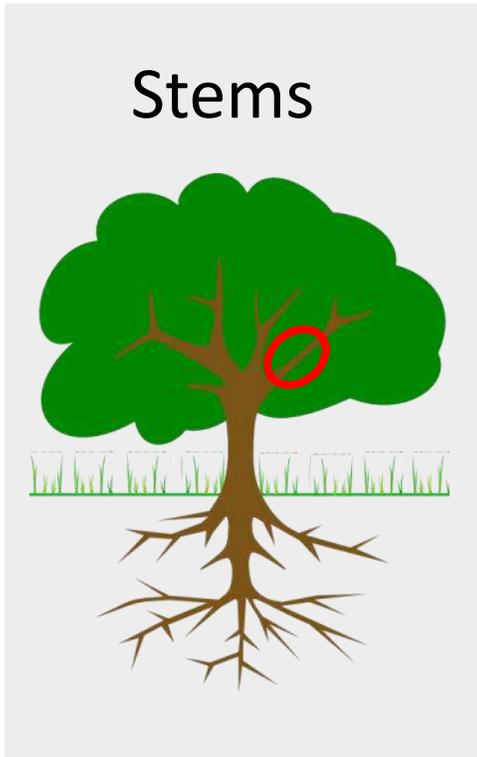
$$K_{\text{leaf}} = \frac{E}{\Delta\Psi \times \text{Leaf area}}$$



METHODS FOR MEASURING K AND GENERATING VCS

Gravimetric method

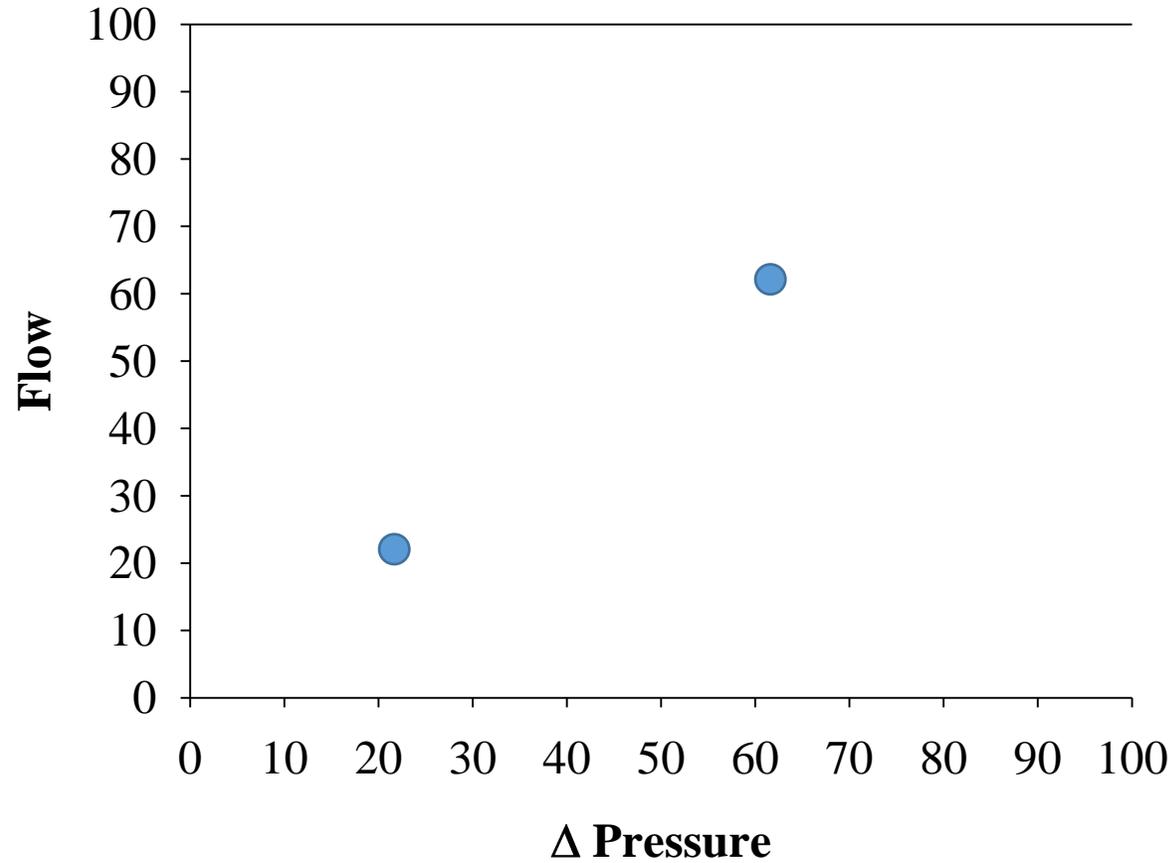
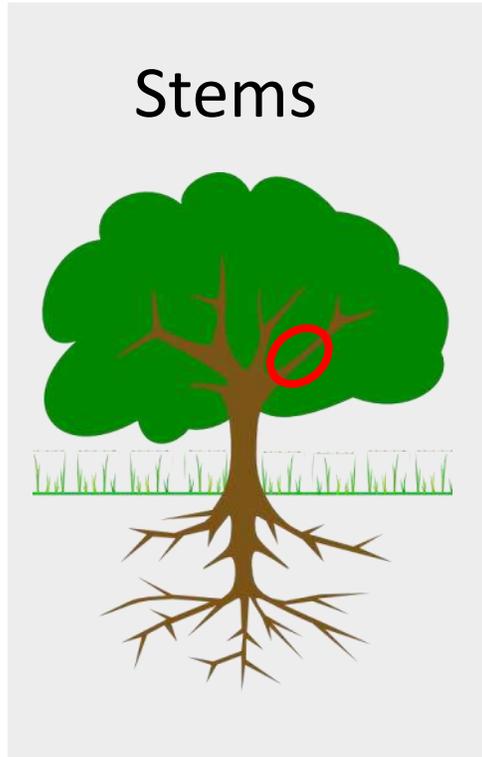
Sperry et al, 1988



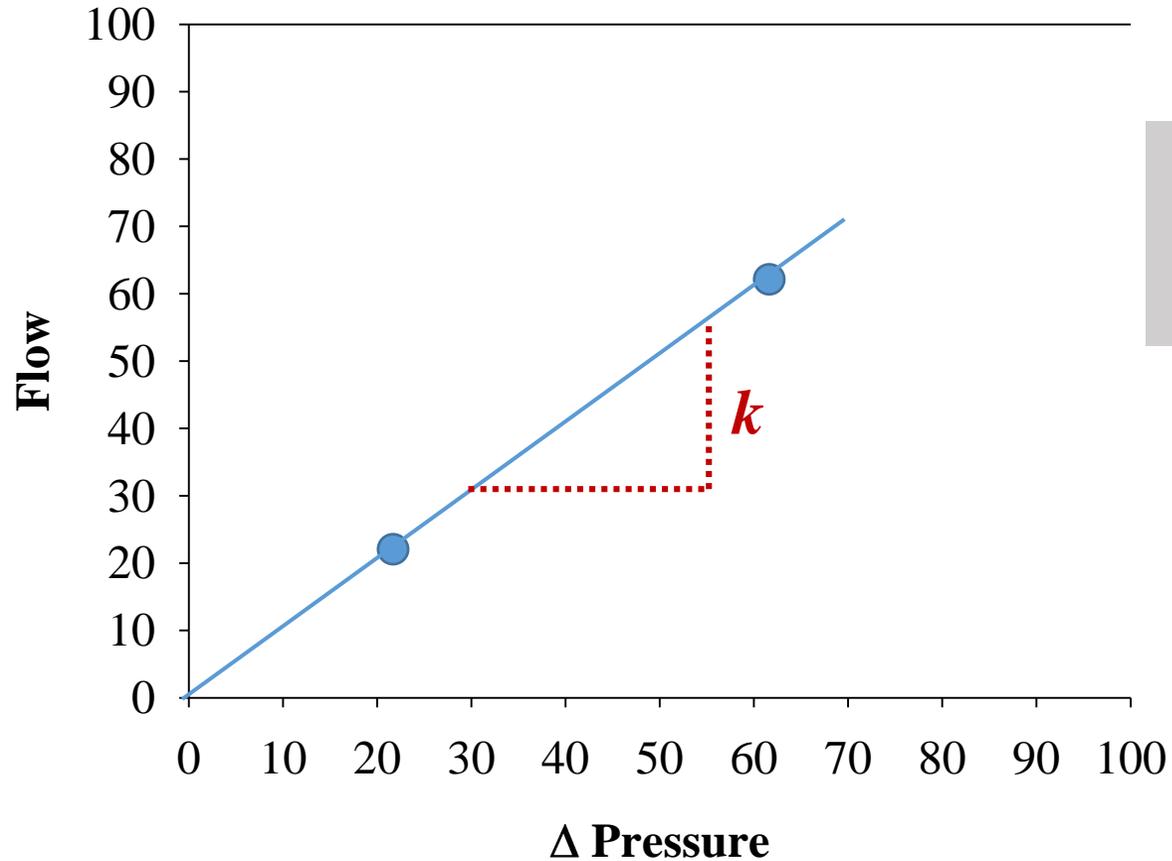
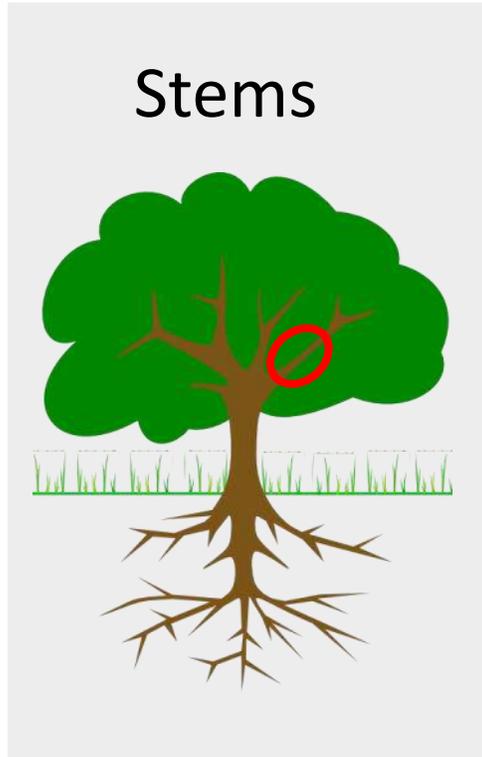
Torres-Ruiz et al. 2012

$$K = \frac{\text{Flow (g s}^{-1}\text{)}}{\Delta P \text{ (MPa)}} \times L \text{ (m)}$$

METHODS FOR MEASURING K AND GENERATING VCS



METHODS FOR MEASURING k AND GENERATING VCS

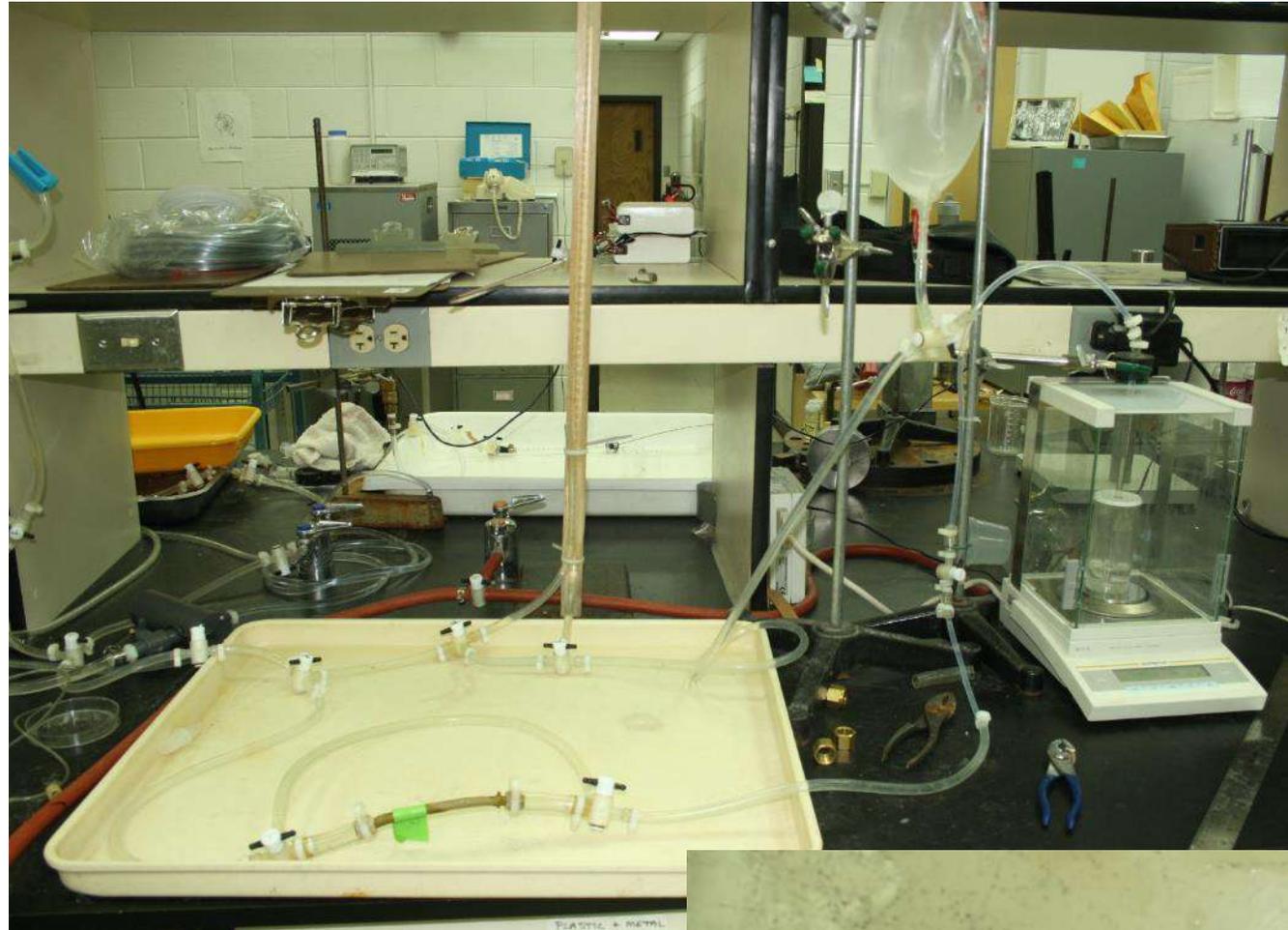
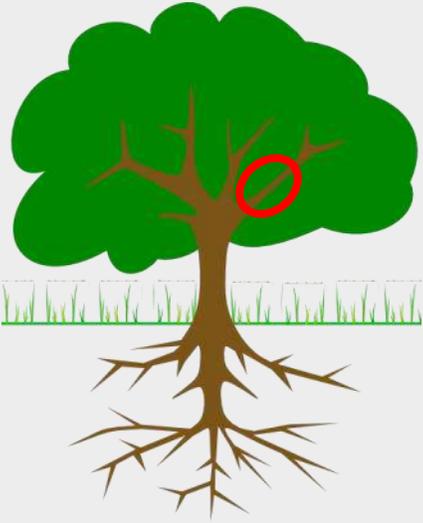


$$PLC = \frac{k_i * 100}{k_{max}}$$

$$k = \frac{Flow}{\Delta P}$$

METHODS FOR MEASURING K AND GENERATING VCS

Stems

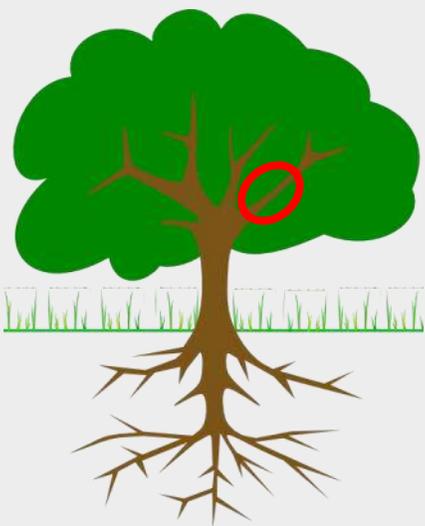


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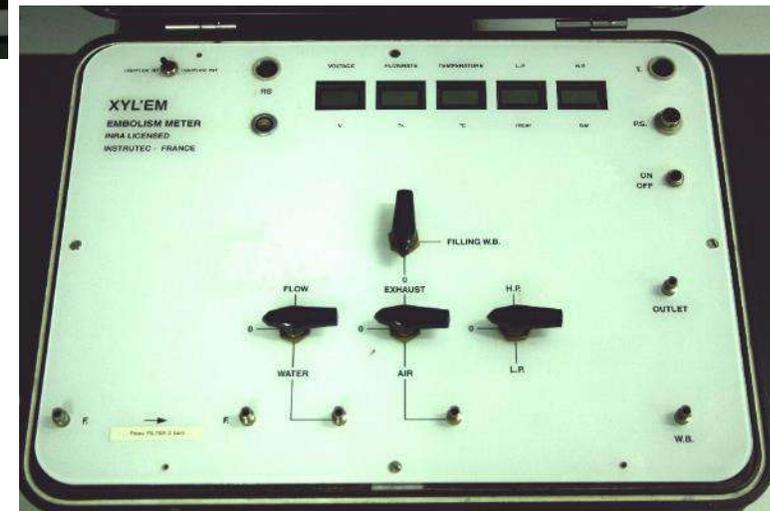
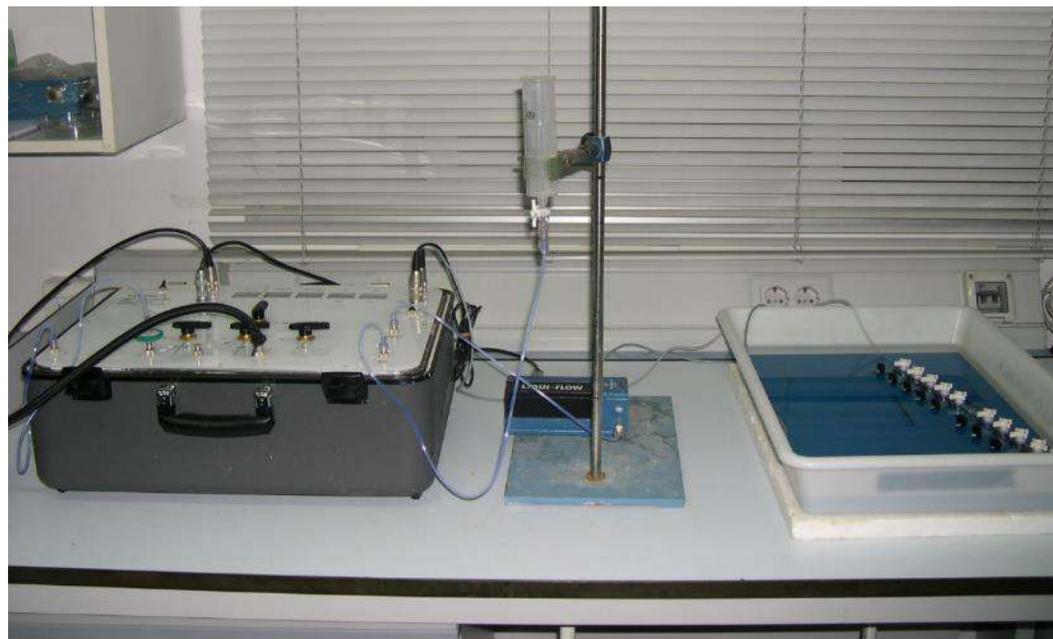
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METHODS FOR MEASURING K AND GENERATING VCS

Stems



XYL'EM apparatus



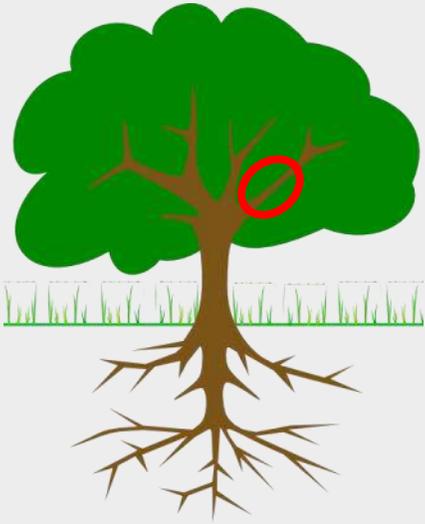
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METHODS FOR MEASURING K AND GENERATING VCS

Stems



Bench dehydration

Sperry & Tyree , 1988. Plant Physiol



Air pressurization

Cochard et al. , 1992. Plant Physiol



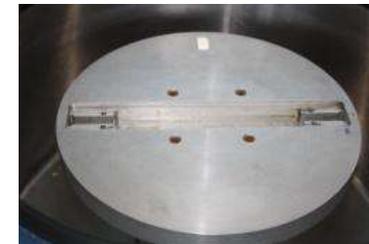
Standard-centrifugation

Pockman et al. , 1995. Nature



Flow-centrifugation (Cavitron)

Cochard et al. , 2005. Plant Cell Environ



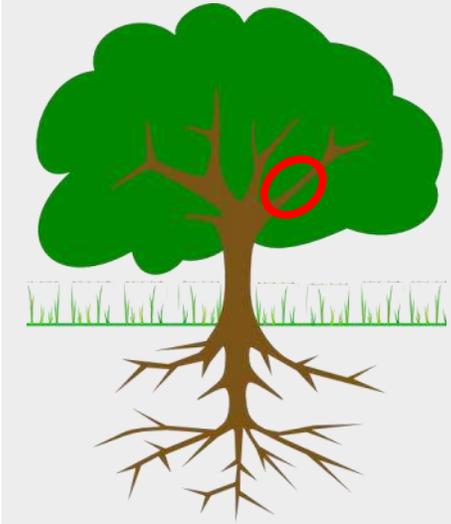
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METHODS FOR MEASURING K AND GENERATING VCS

Stems

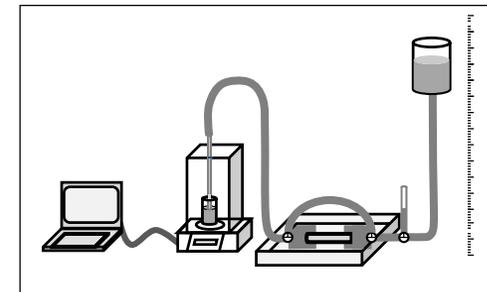
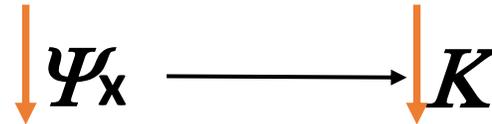


Bench dehydration

Sperry & Tyree, 1988. Plant Physiol

Intact plant or large branches are allowed to dehydrate freely in the air.

During dehydration, xylem water potential (ψ_x) and K are measured in intervals.

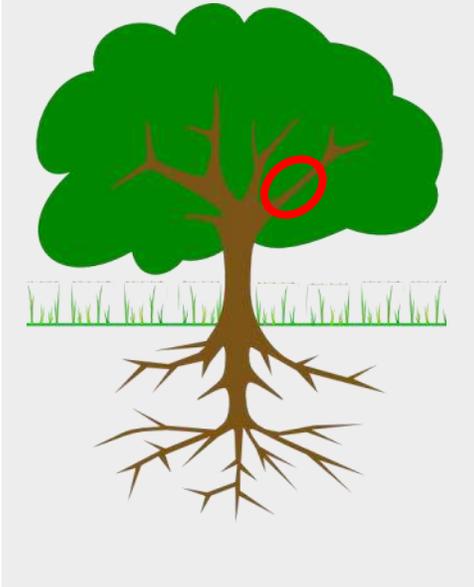


METHODS FOR MEASURING K AND GENERATING VCS

Bench dehydration

Sperry & Tyree , 1988. Plant Physiol

Stems



Percent loss of conductivity (**PLC**)

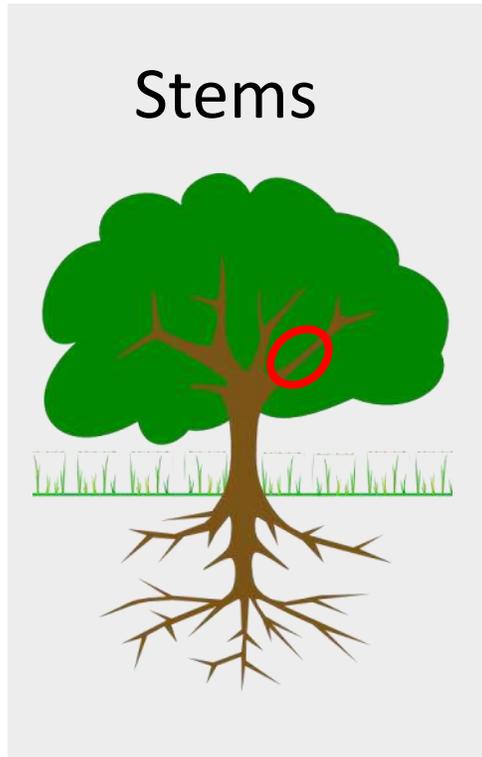
$$PLC = 100 \times (1 - \text{native } K / \text{maximum } K)$$

Native K $\xrightarrow[\text{to remove the embolism}]{\text{Flushing at high pressure}}$ Maximum K



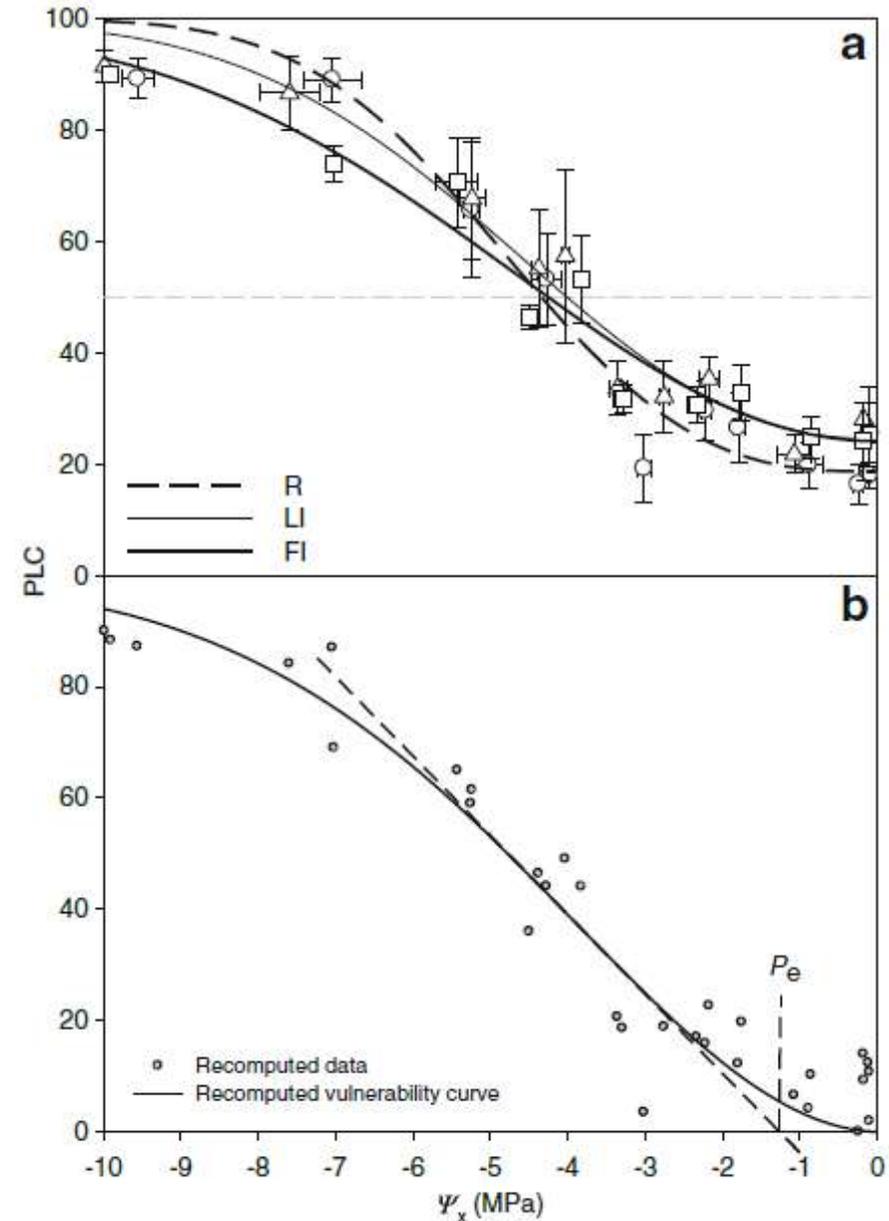
We have the **PLC** for each Ψ_x value

METHODS FOR MEASURING K AND GENERATING VCS



Bench dehydration
Sperry & Tyree, 1988. Plant Physiol

Olea europaea



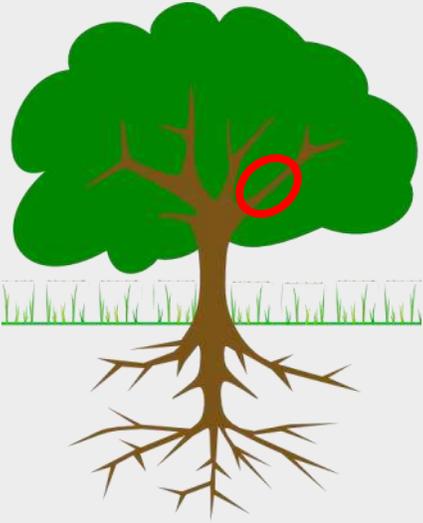
Torres-Ruiz et al. 2013



crodriguez@irnas.csic.es

METHODS FOR MEASURING K AND GENERATING VCS

Stems

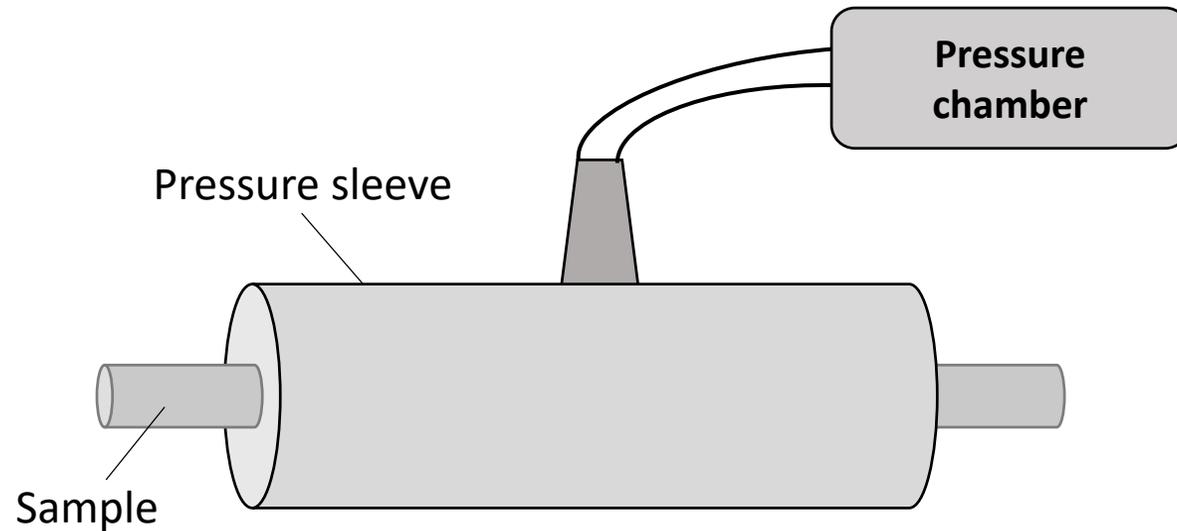


Air pressurization

Cochard et al. , 1992. Plant Physiol

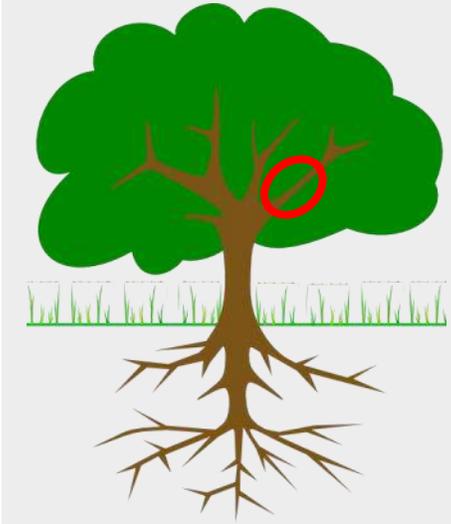
Based on the air-seeding hypothesis:

Decreasing Ψ_{xylem} by dehydration or increasing air pressure while has the same effect on embolism induction.



METHODS FOR MEASURING K AND GENERATING VCS

Stems

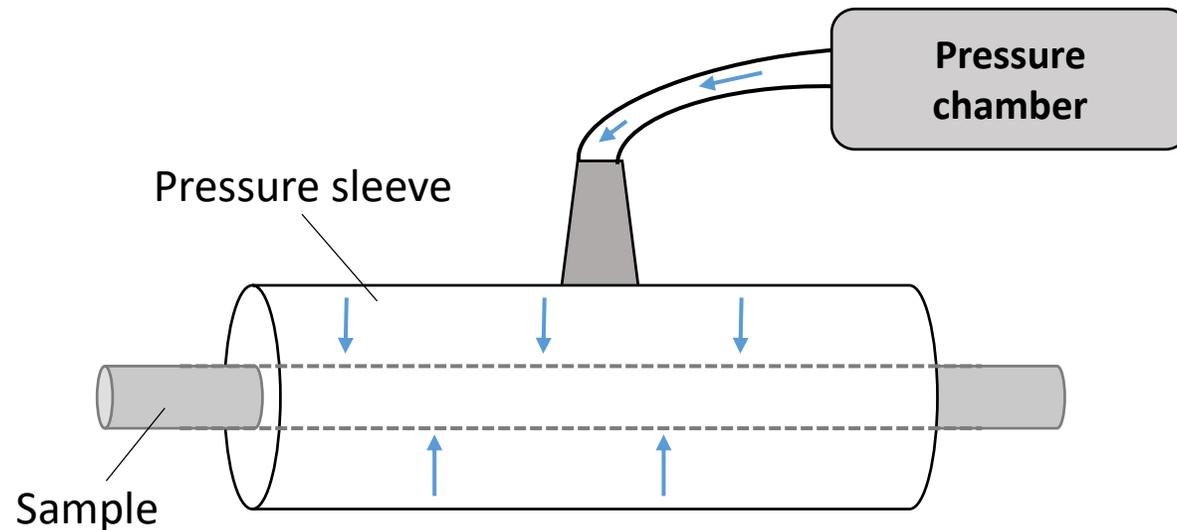


Air pressurization

Cochard et al. , 1992. Plant Physiol

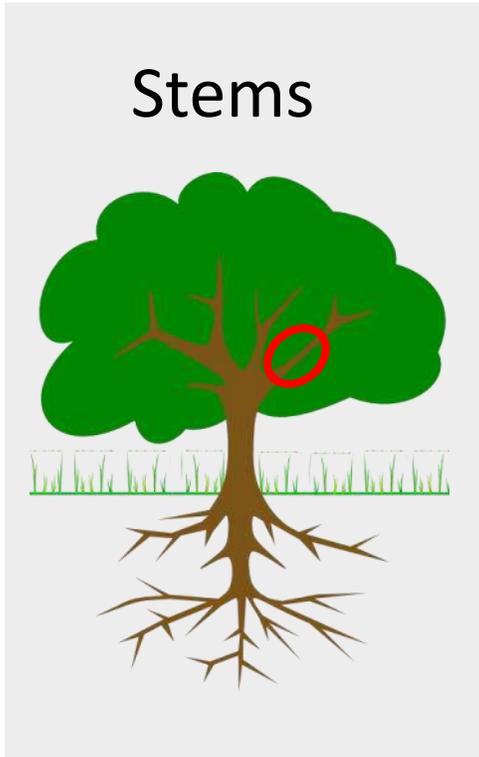
Based on the air-seeding hypothesis:

Decreasing Ψ_{xylem} by dehydration or increasing air pressure while has the same effect on embolism induction.



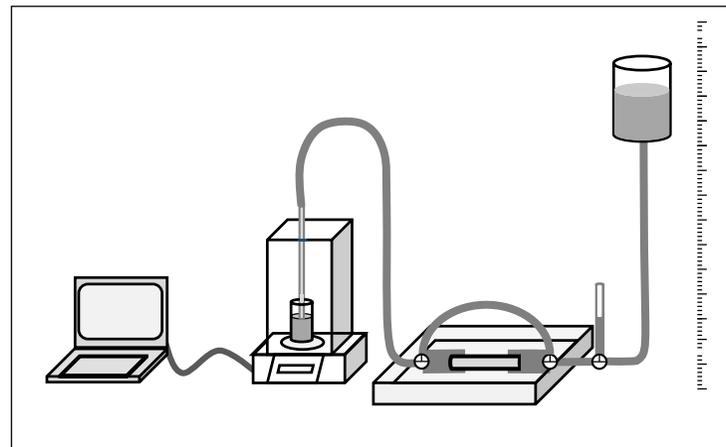
$$\text{Air pressure} = \Psi_{\text{xylem}}$$

METHODS FOR MEASURING K AND GENERATING VCS



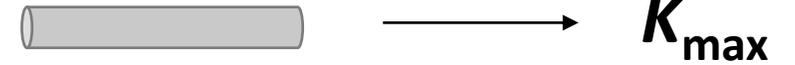
Air pressurization

Cochard et al., 1992. Plant Physiol

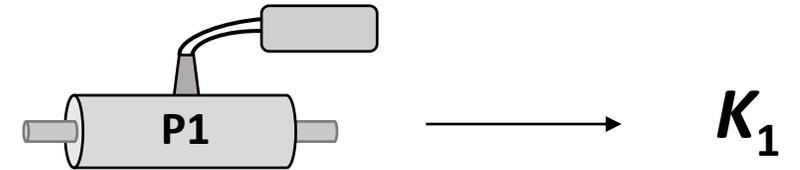


Steps:

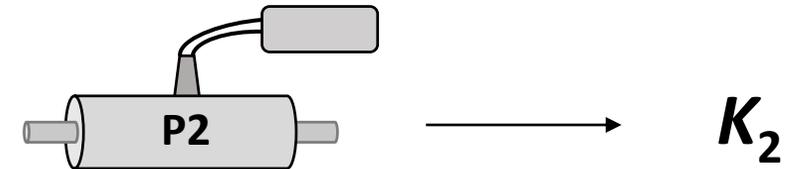
i) Remove the embolisms



ii) Air pressurization: P1



iii) Air pressurization: P2

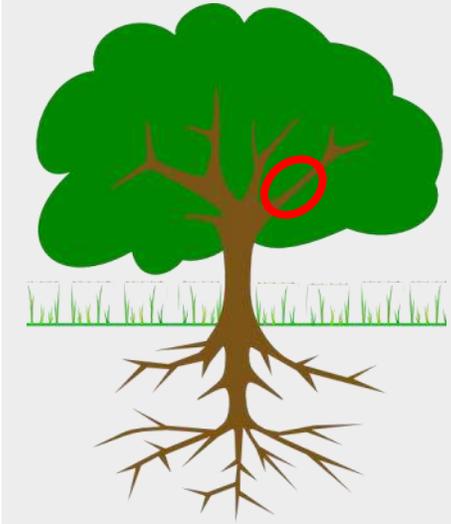


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METHODS FOR MEASURING K AND GENERATING VCS

Stems

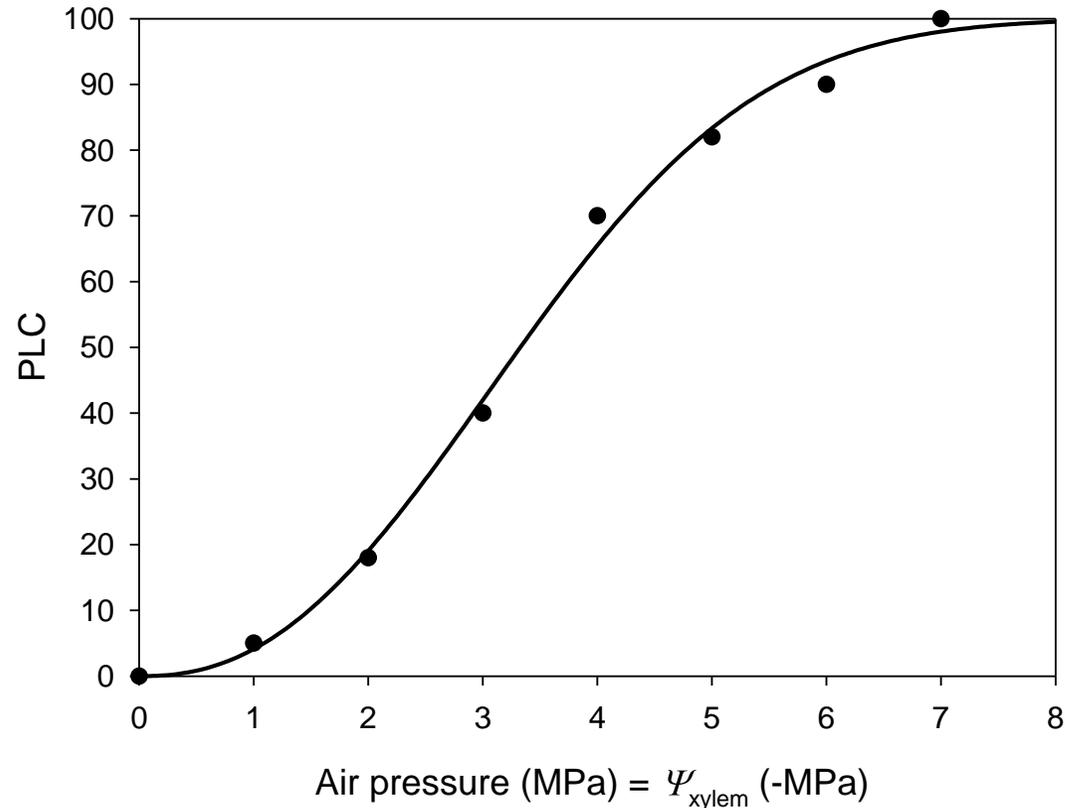


Air pressurization

Cochard et al. , 1992. Plant Physiol

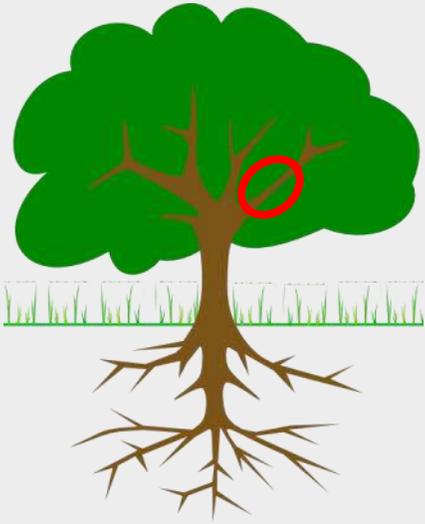
Percent loss of conductivity (PLC)

$$PLC = 100 \times (1 - K_{1,2,\dots} / \text{maximum } K)$$



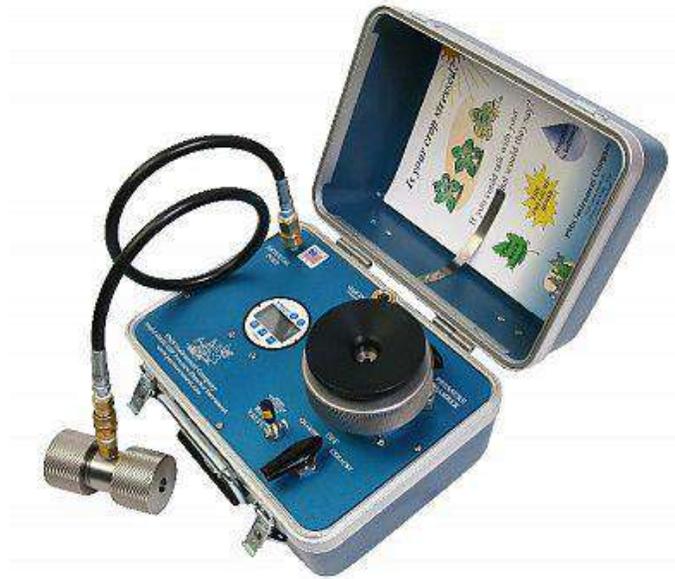
METHODS FOR MEASURING K AND GENERATING VCS

Stems



Air pressurization

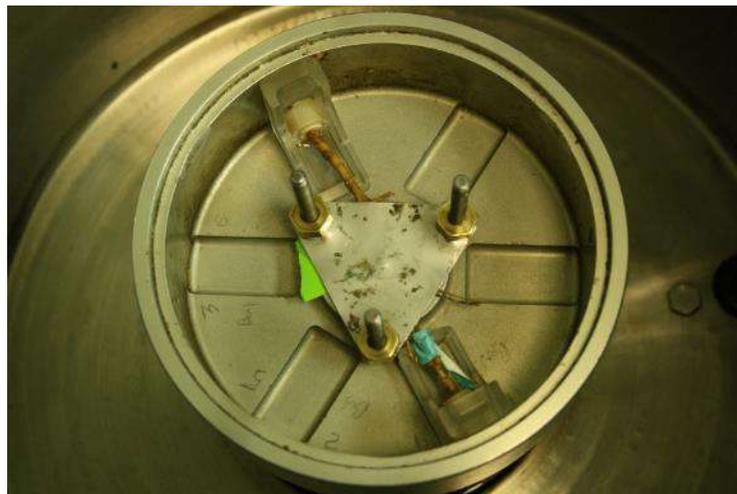
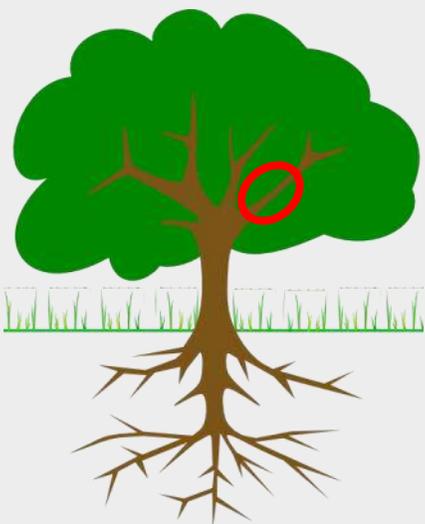
Cochard et al. , 1992. Plant Physiol



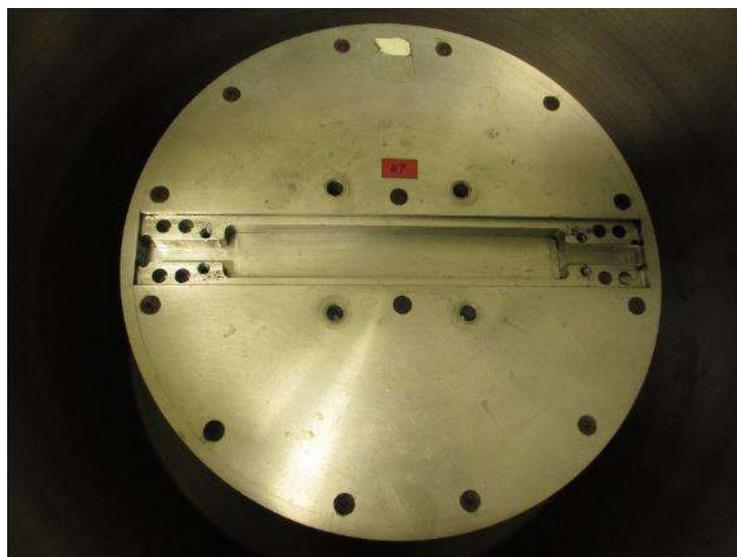
METHODS FOR MEASURING K AND GENERATING VCS

Centrifugation methods

Stems



- Standard centrifugation

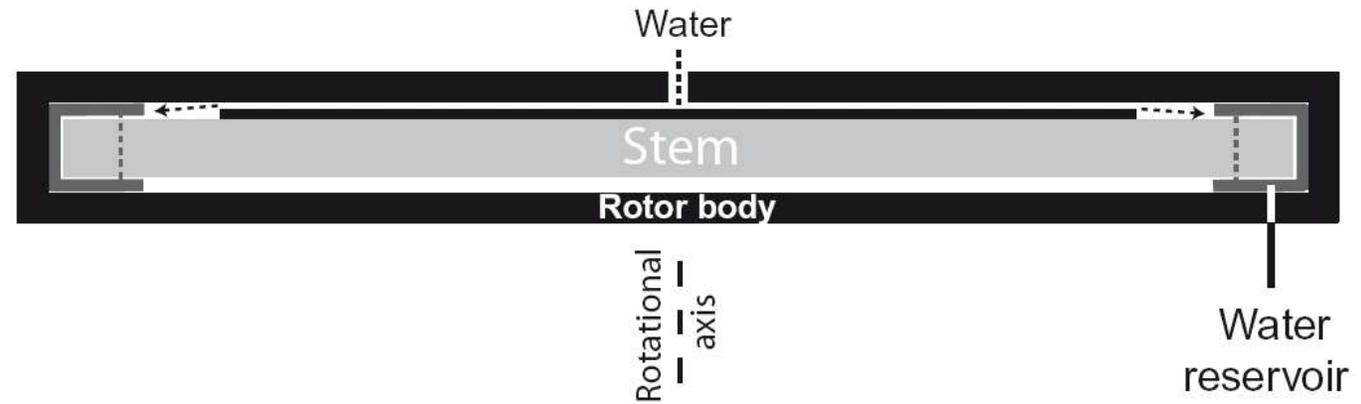
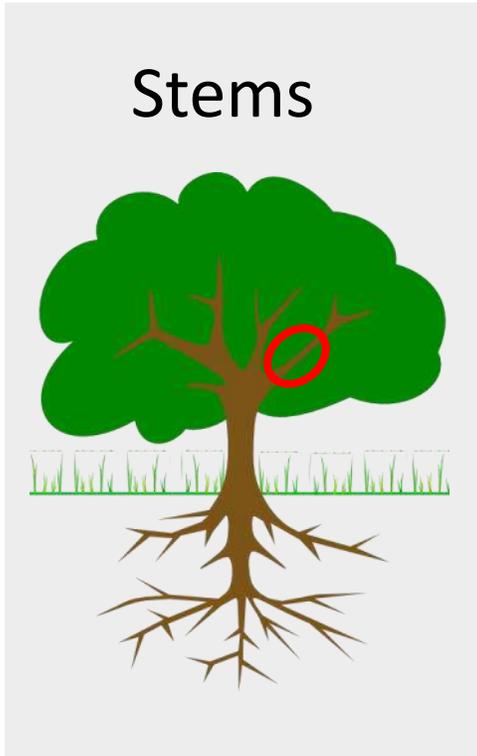


- Flow centrifugation
(Cavitron)

METHODS FOR MEASURING K AND GENERATING VCS

Standard-centrifugation

Pockman et al. , 1995. Nature

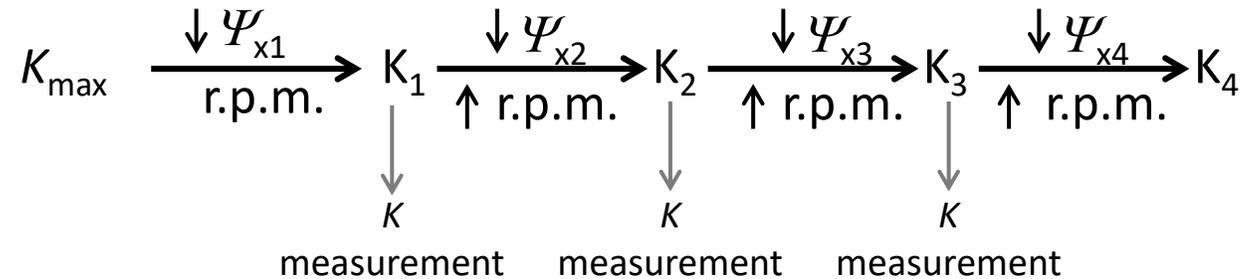
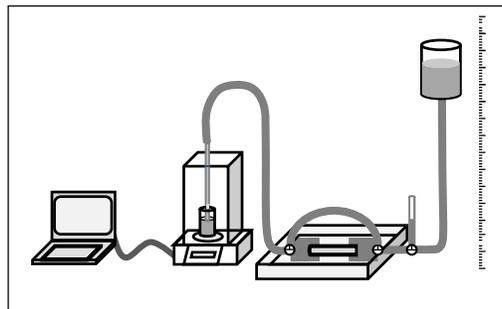
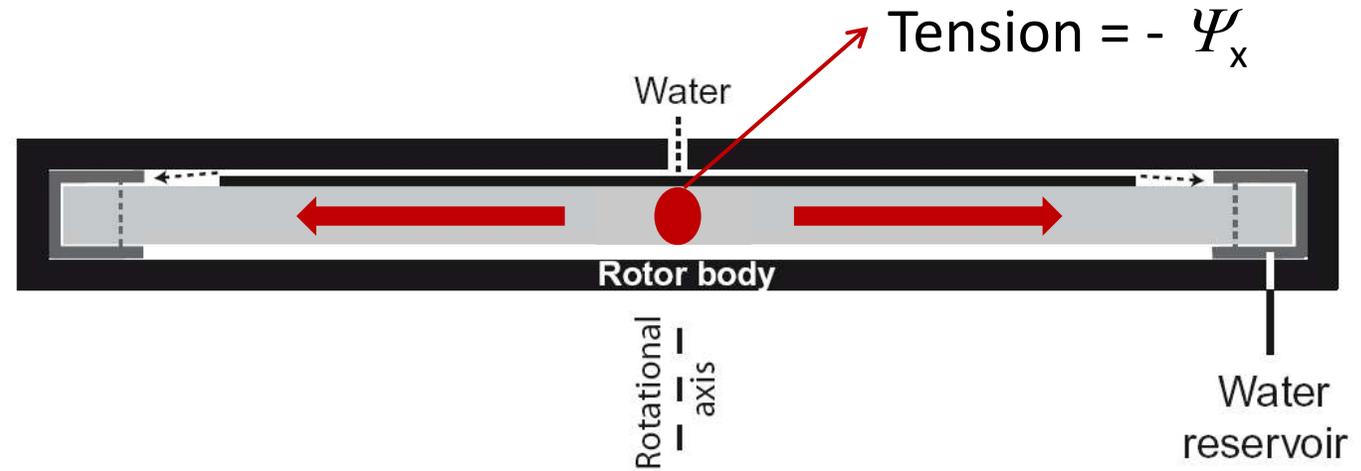
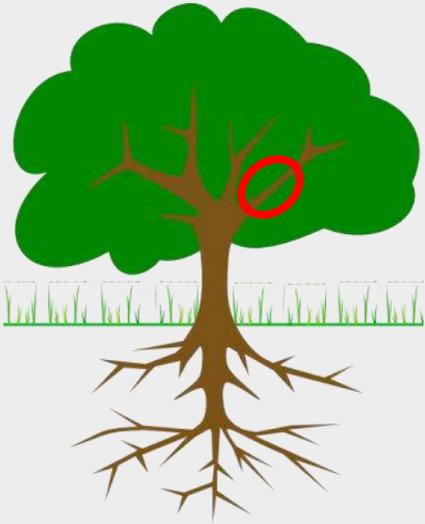


METHODS FOR MEASURING K AND GENERATING VCS

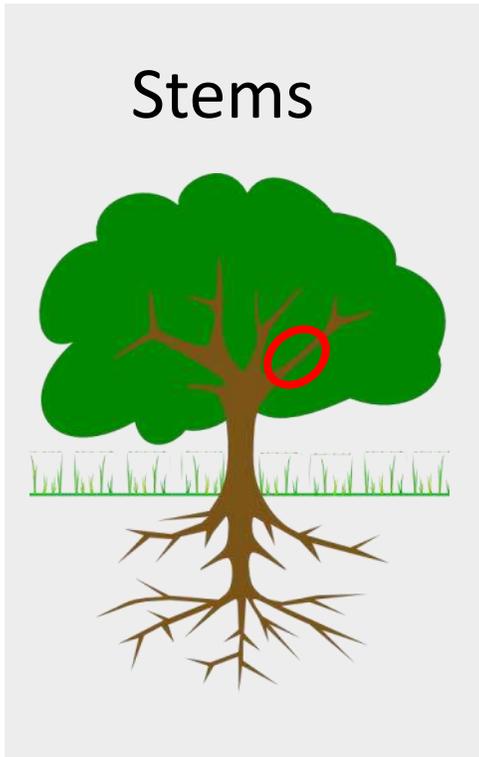
Standard-centrifugation

Pockman et al. , 1995. Nature

Stems

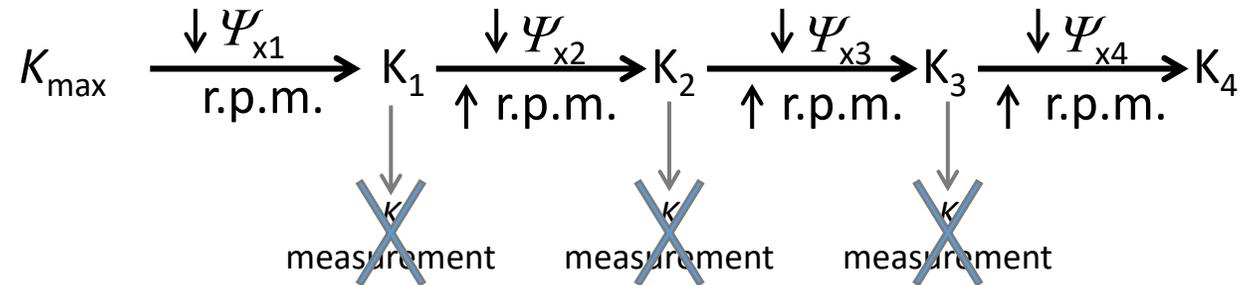
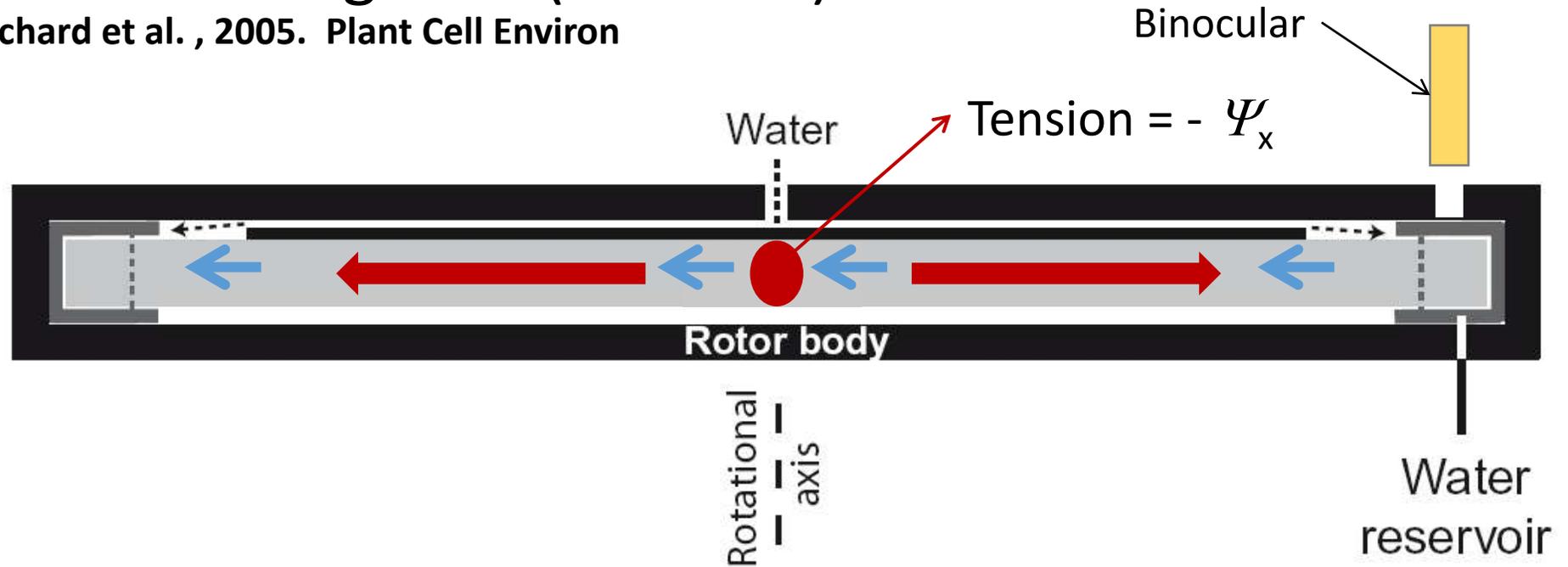


METHODS FOR MEASURING K AND GENERATING VCS



Flow-centrifugation (Cavitron)

Cochard et al., 2005. Plant Cell Environ



'in situ' K measurements



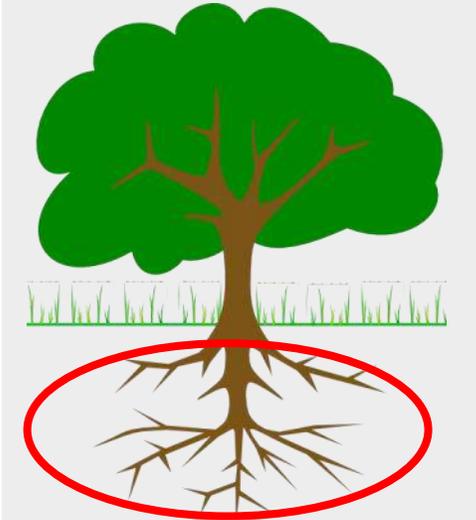
CSIC

crodriguez@irnas.csic.es



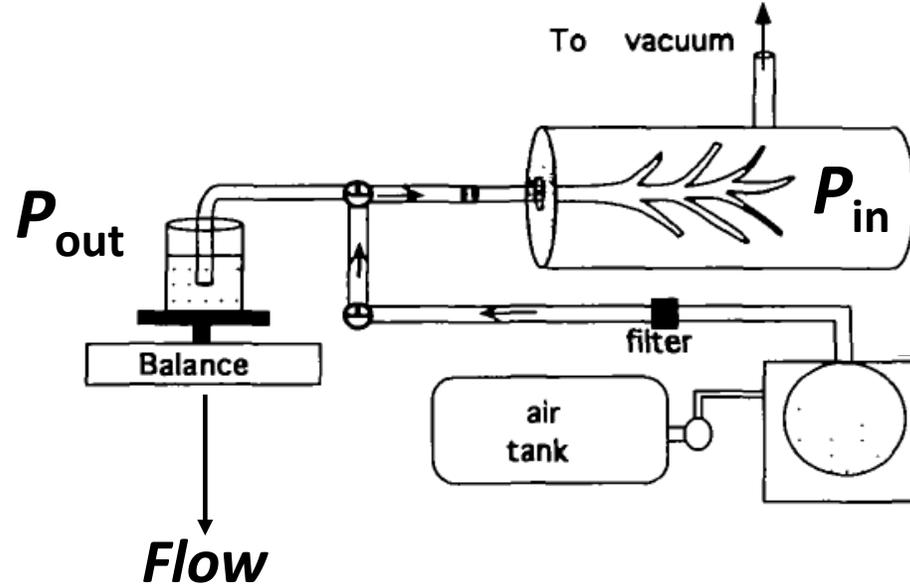
METHODS FOR MEASURING K AND GENERATING VCS

Roots



Vacuum chamber method

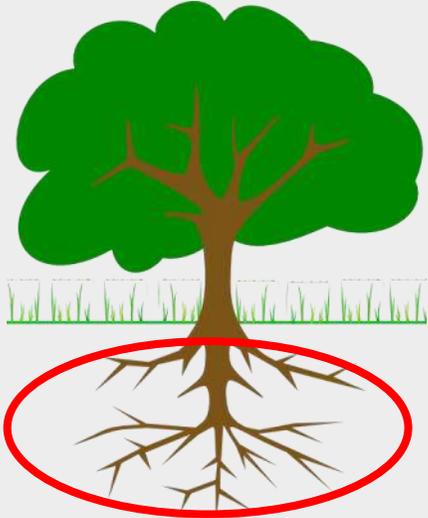
Kolb et al. 1996, *J Exp Bot*



$$K = \frac{Flow (g s^{-1})}{\Delta P (MPa)} \times L (m)$$

METHODS FOR MEASURING K AND GENERATING VCS

Roots



Vacuum chamber method

Kolb et al. 1996, *J Exp Bot*

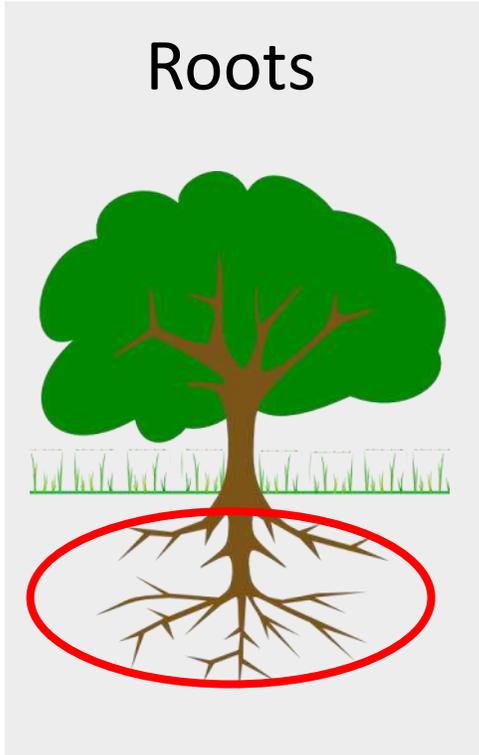


CSIC

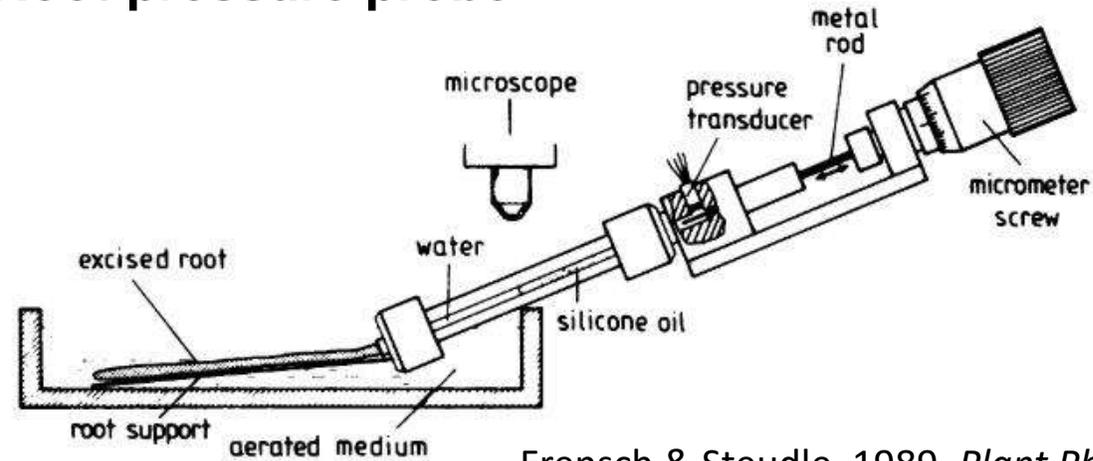
crodriguez@irnas.csic.es

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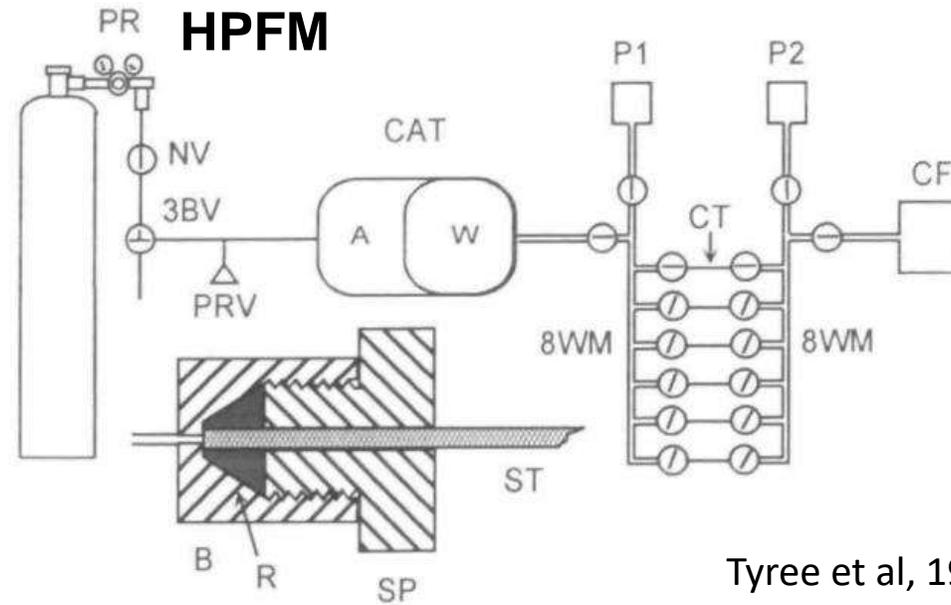
METHODS FOR MEASURING K AND GENERATING VCS



Root pressure probe



Frensch & Steudle, 1989, *Plant Phys*



Tyree et al, 1995, *J Exp Bot*



METHODS FOR MEASURING K AND GENERATING VCS

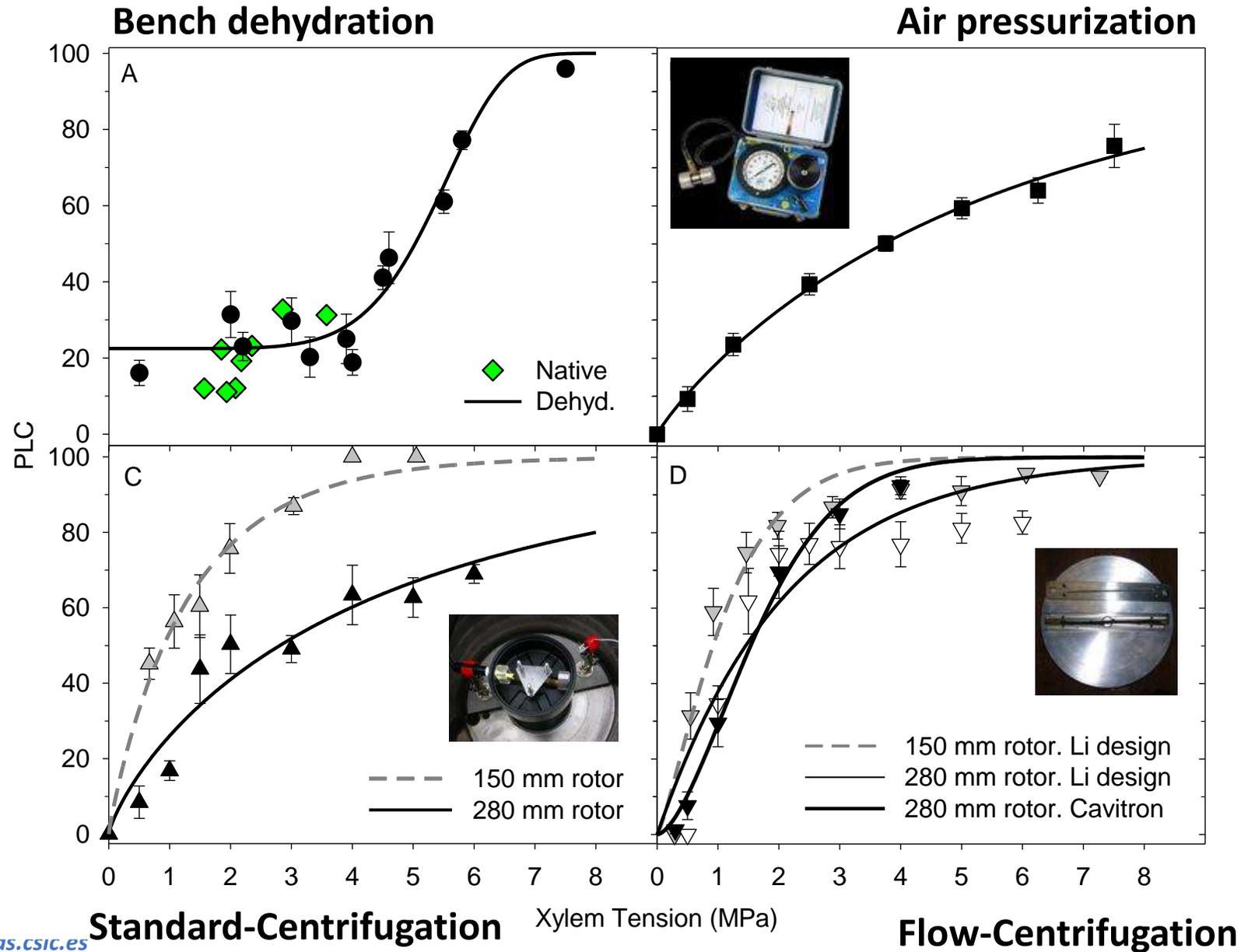
Accuracy of the methods for
generating VCs



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY

Olea europaea

Torres-Ruiz et al. 2014



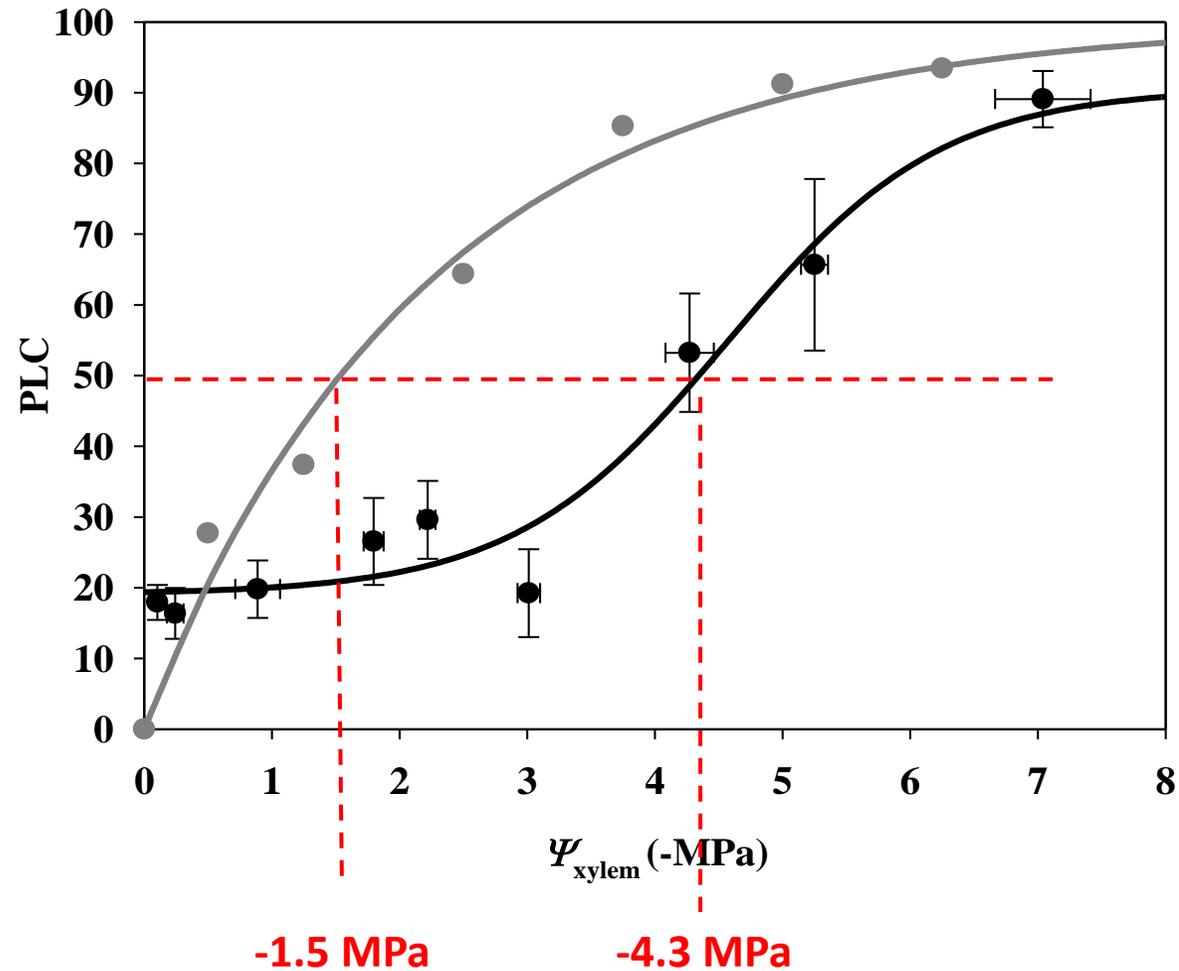
crodriguez@irnas.csic.es



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY

Olea europaea

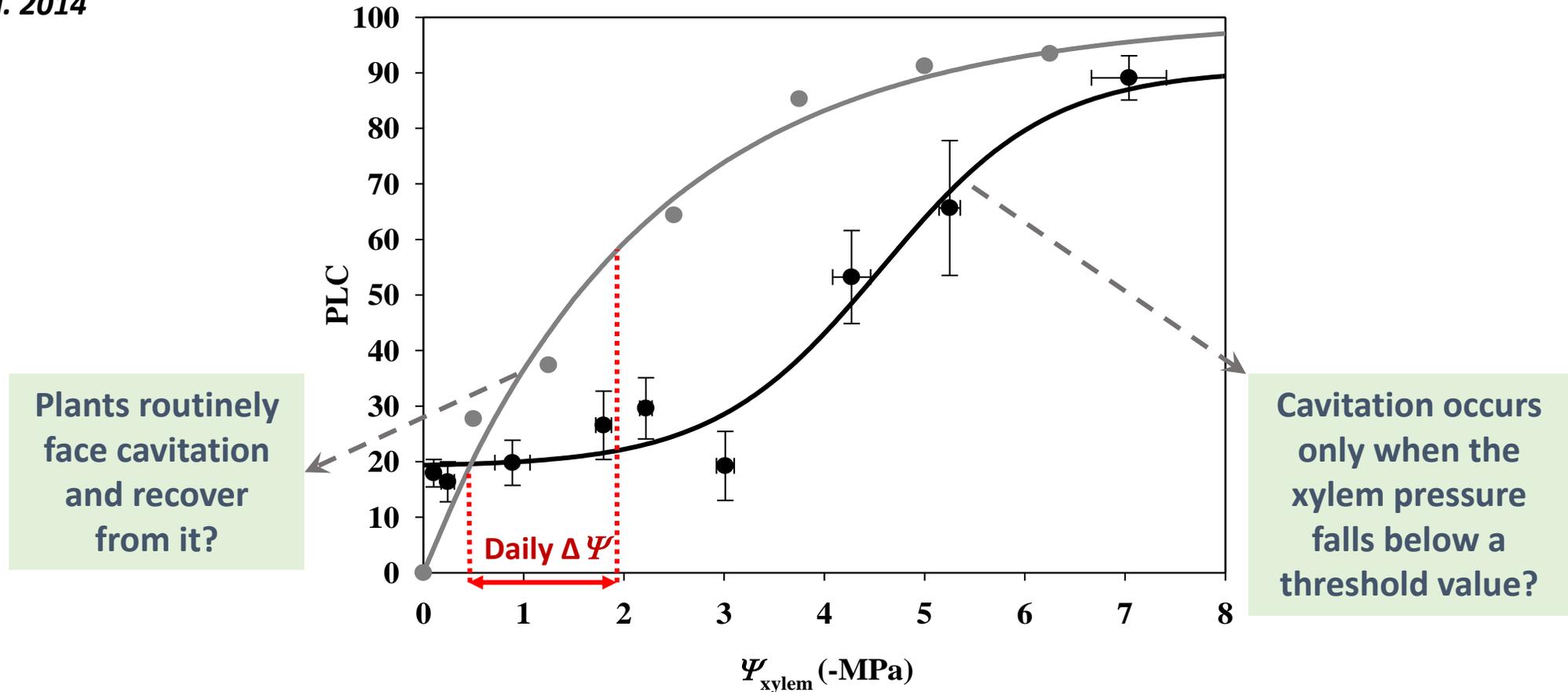
Torres-Ruiz et al. 2014



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY

Olea europaea

Torres-Ruiz et al. 2014



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY

Techniques for generating vulnerability curves (VCs)

Accuracy

In short-vesseled sps

In long-vesseled sps

Bench dehydration
(Sperry & Tyree , 1988. *Plant Physiol*)



Pressure-sleeves
(‘Air-injection’)
(Cochard et al. , 1992. *Plant Physiol*)



Static-centrifugation
(Pockman et al. , 1995. *Nature*)



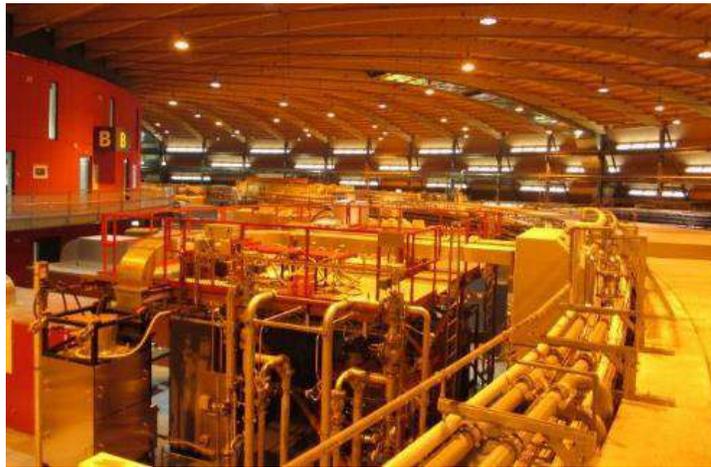
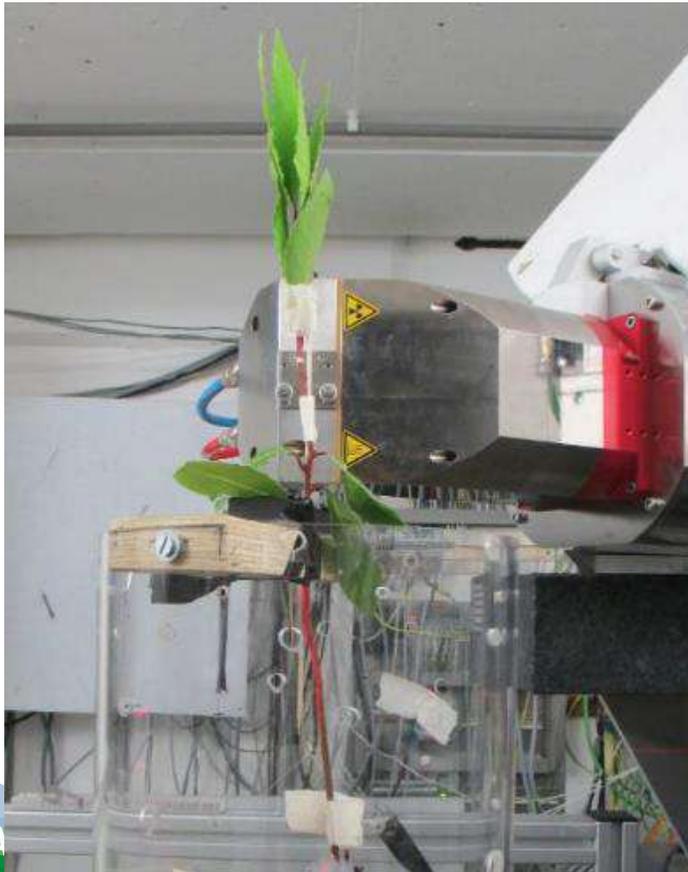
Flow-centrifugation
(Cochard et al. , 2005. *Plant Cell Environ*)



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

X-ray microtomography (micro-CT)

Powerful technique to visualize xylem embolism in intact plants with a spatial resolution around $1\ \mu\text{m}$

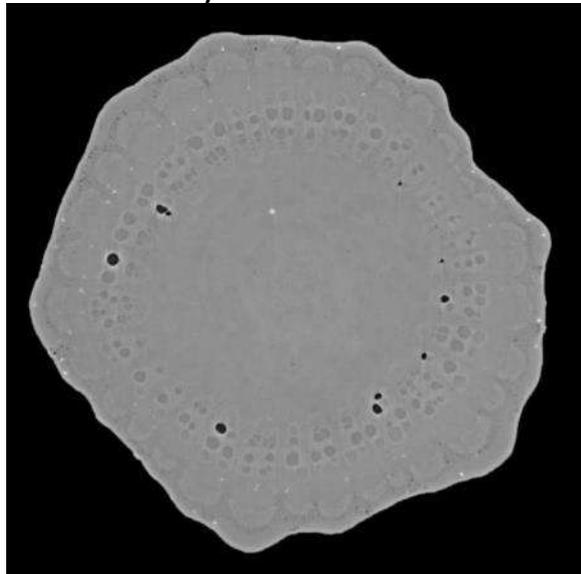


METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

X-ray microtomography (micro-CT)

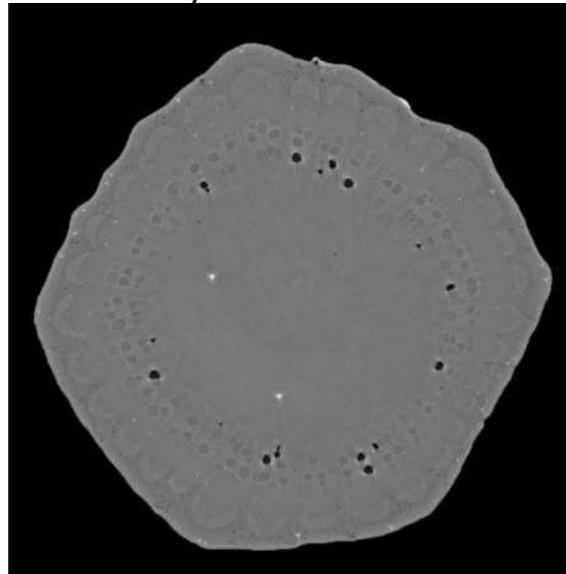
Vitis vinifera cv. Cabernet sauvignon

$\Psi_{\text{xylem}} = - 0.4 \text{ MPa}$



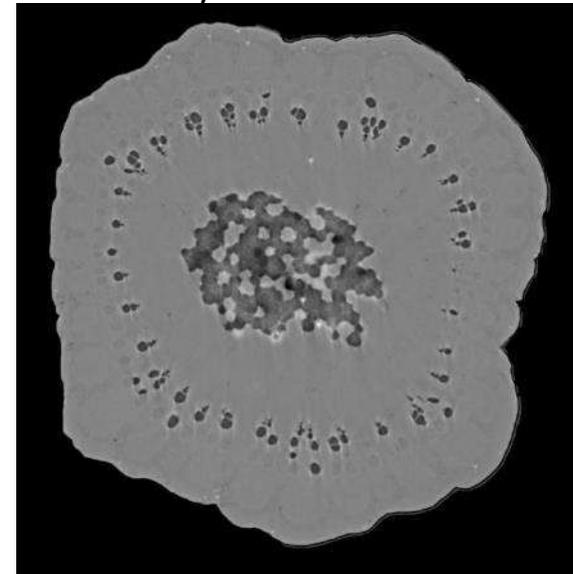
PLC=5-10%

$\Psi_{\text{xylem}} = - 1.0 \text{ MPa}$



PLC=10%

$\Psi_{\text{xylem}} = - 1.7 \text{ MPa}$



PLC=55%



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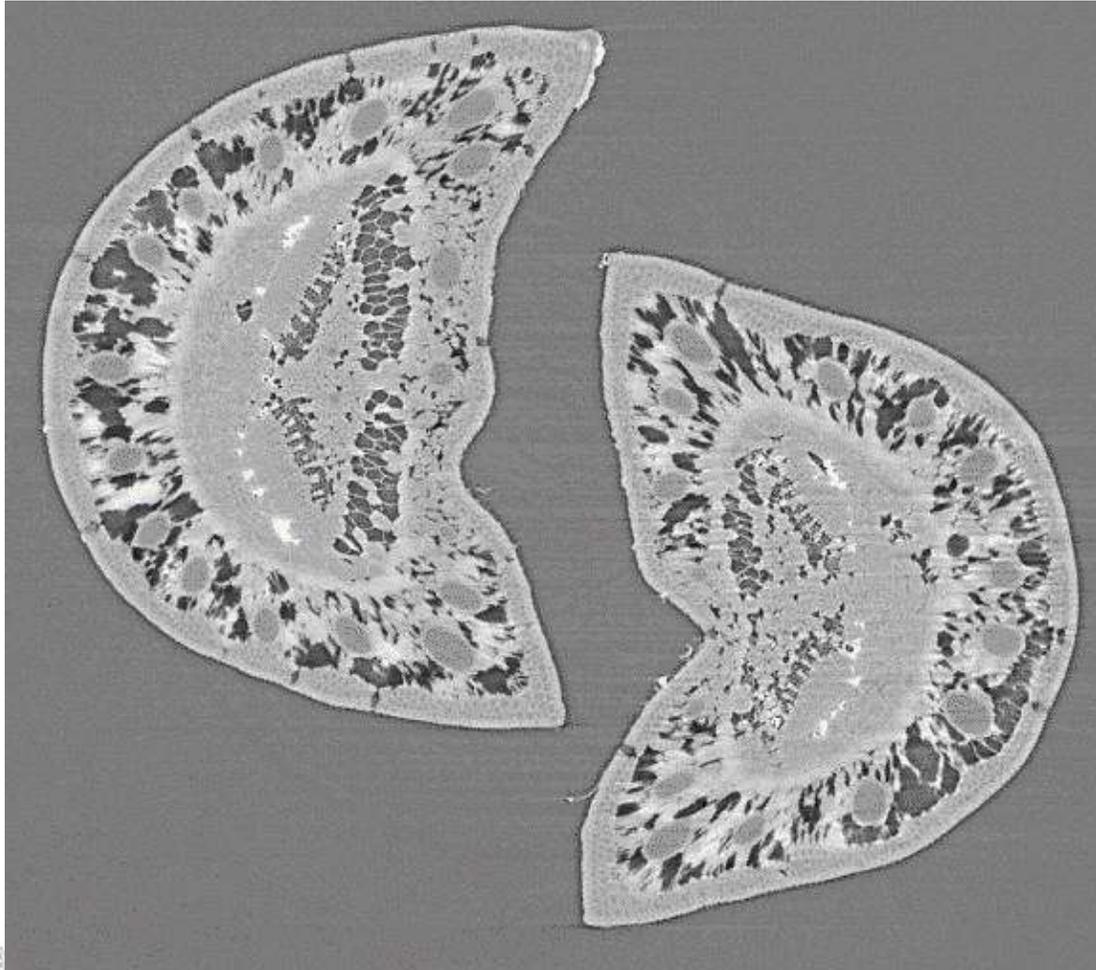
crodriguez@irnas.csic.es

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METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

X-ray microtomography (micro-CT)

Pinus sps - Needle



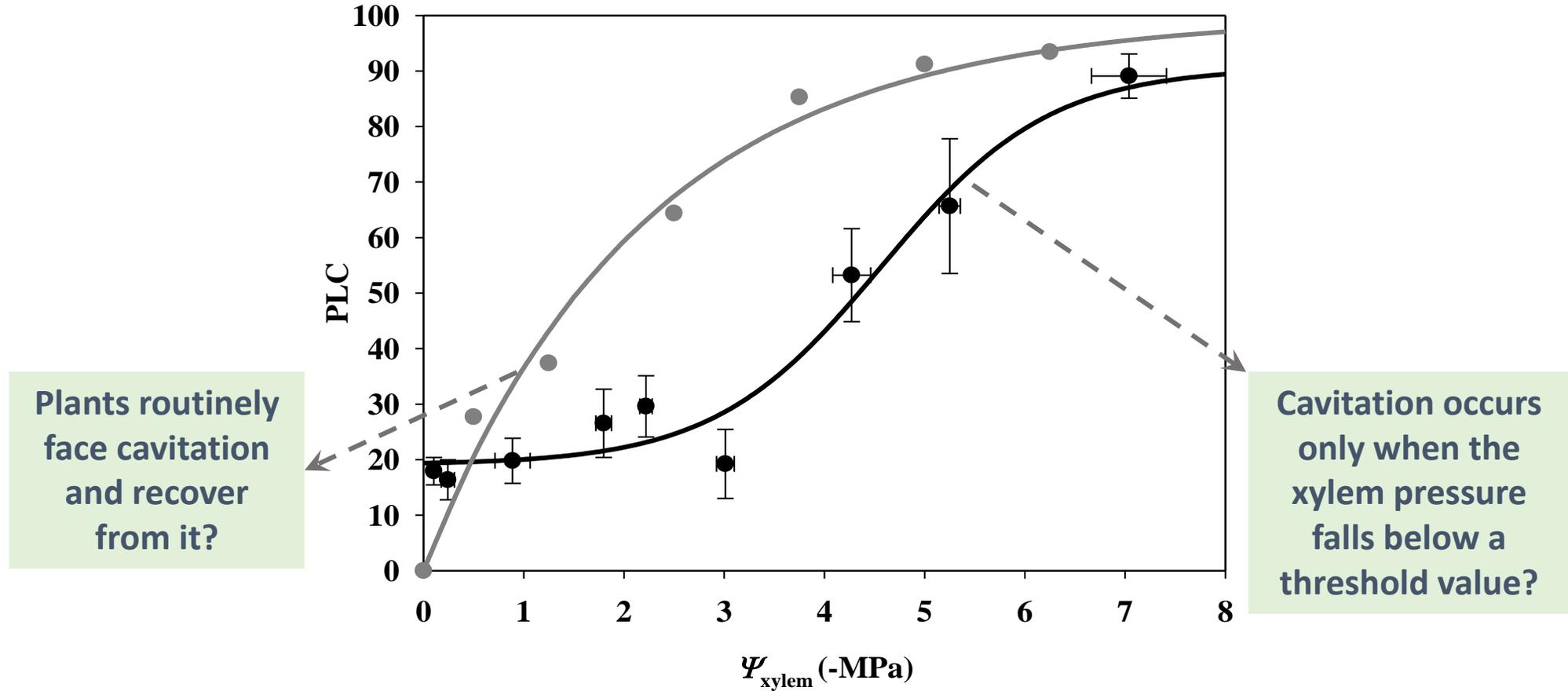
Olea europea - Leaf



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

Olea europaea

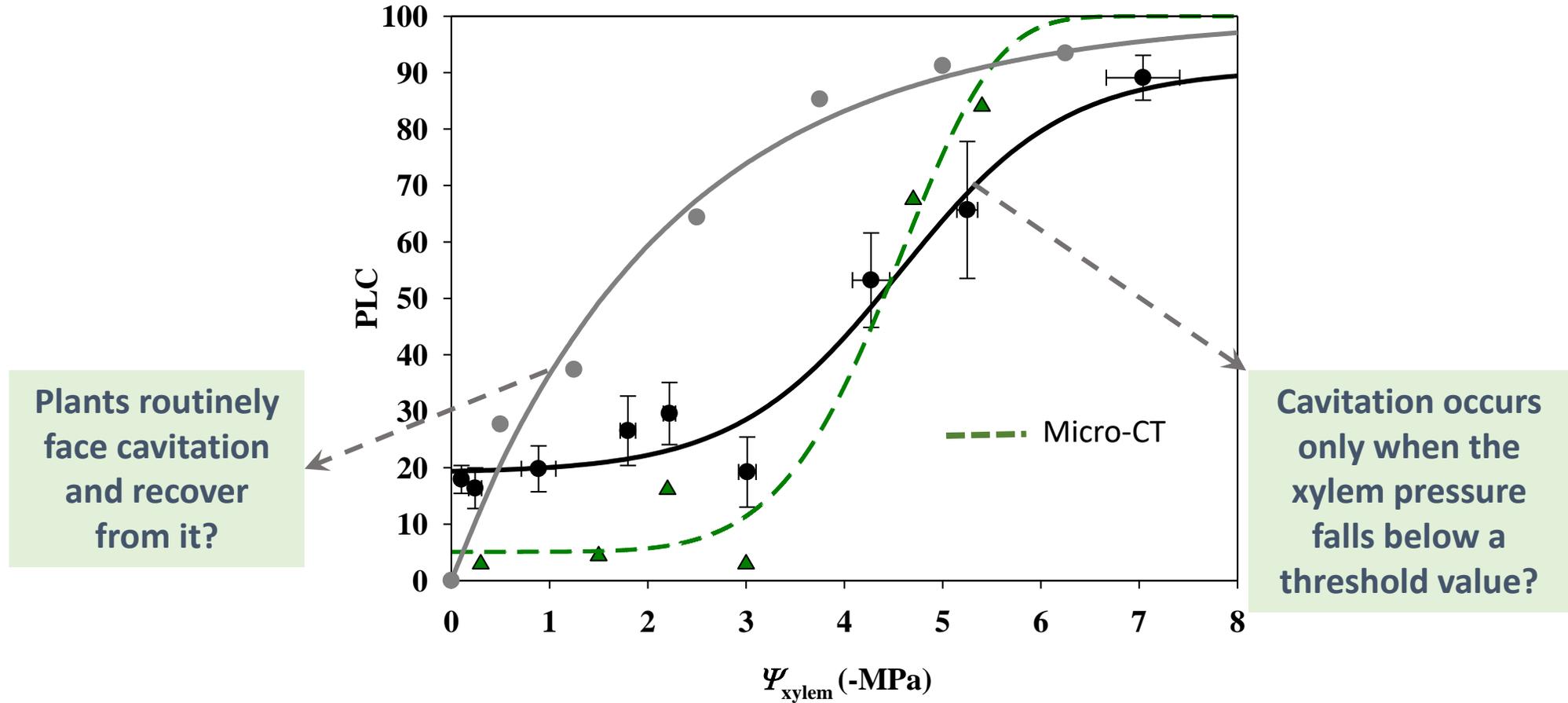
Torres-Ruiz et al. 2014



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

Olea europaea

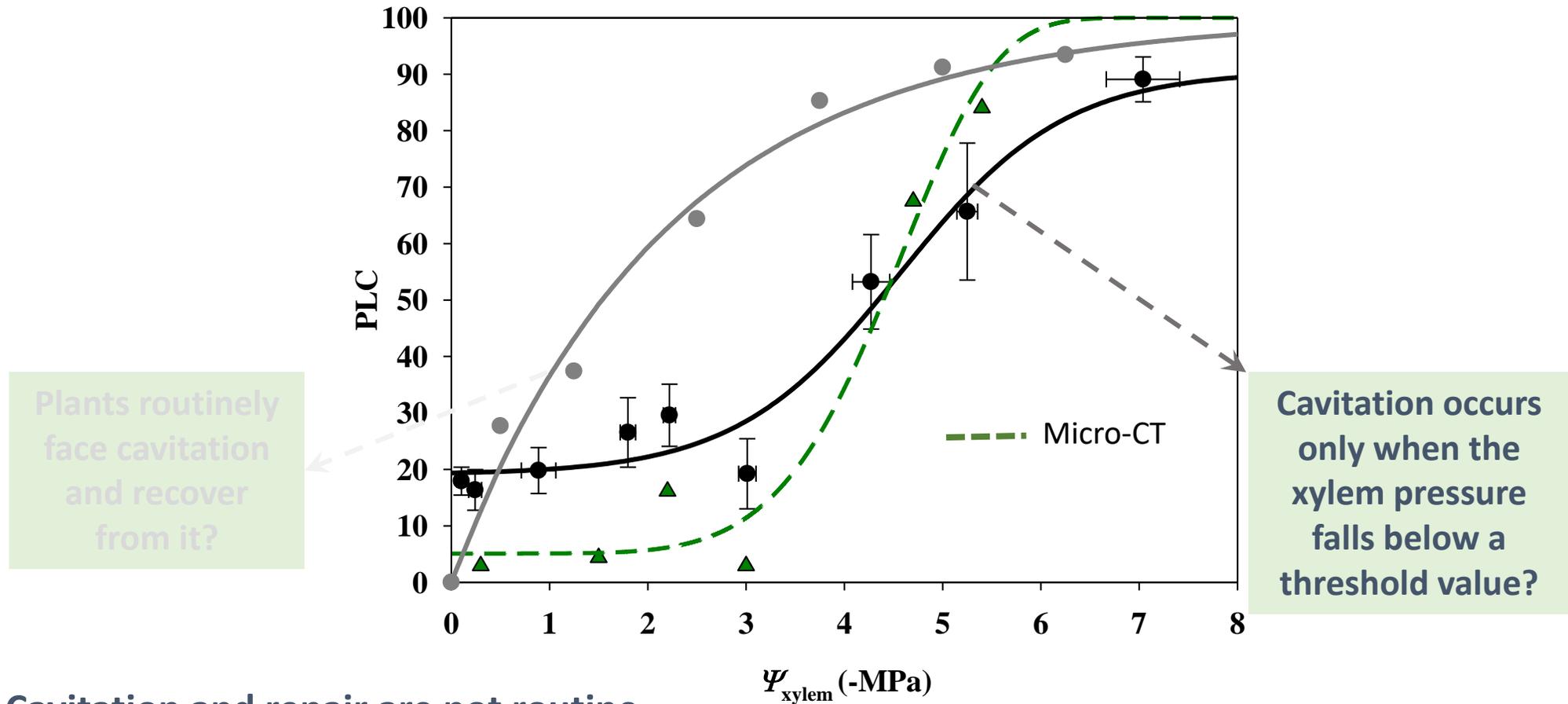
Torres-Ruiz et al. 2014



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

Olea europaea

Torres-Ruiz et al. 2014



Cavitation and repair are not routine



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

Techniques for generating vulnerability curves (VCs)

Accuracy

In short-vesseled sps

In long-vesseled sps

Bench dehydration
(Sperry & Tyree , 1988. *Plant Physiol*)



Pressure-sleeves
(‘Air-injection’)
(Cochard et al. , 1992. *Plant Physiol*)



Static-centrifugation
(Pockman et al. , 1995. *Nature*)



Flow-centrifugation
(Cochard et al. , 2005. *Plant Cell Environ*)



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

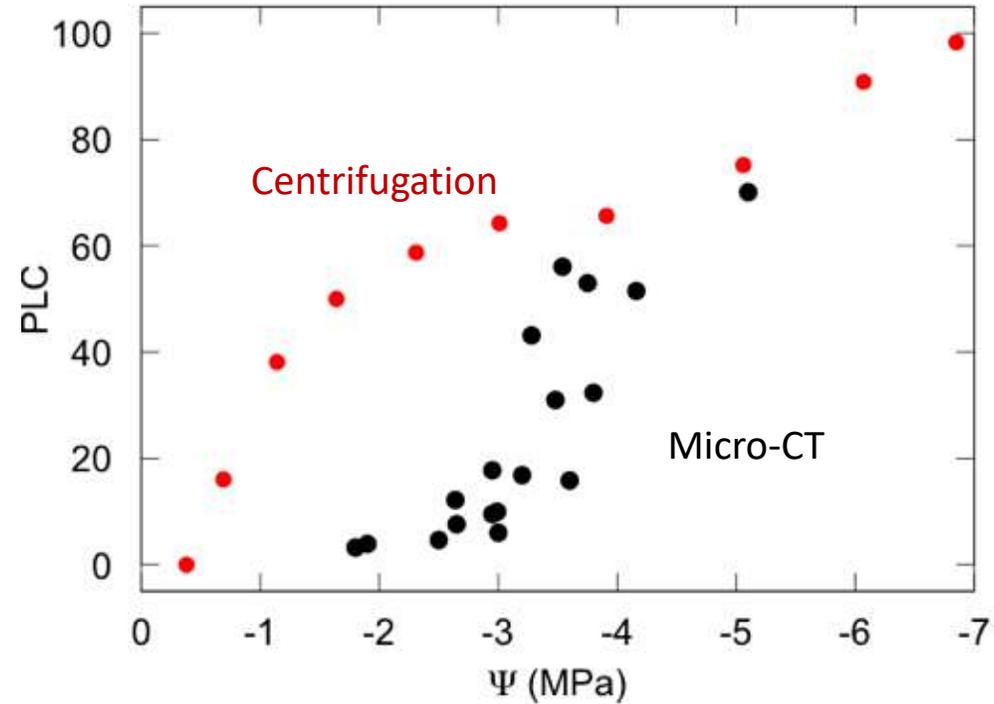
Techniques for generating vulnerability curves (VCs)

	<i>Accuracy</i>	
	<u>In short-vesseled sps</u>	<u>In long-vesseled sps</u>
Bench dehydration <small>(Sperry & Tyree , 1988. <i>Plant Physiol</i>)</small>	✓	✓
Pressure-sleeves <i>(‘Air-injection’)</i> <small>(Cochard et al. , 1992. <i>Plant Physiol</i>)</small>	✓	✗
Static-centrifugation <small>(Pockman et al. , 1995. <i>Nature</i>)</small>	✓	✗
Flow-centrifugation <small>(Cochard et al. , 2005. <i>Plant Cell Environ</i>)</small>	✓	✗

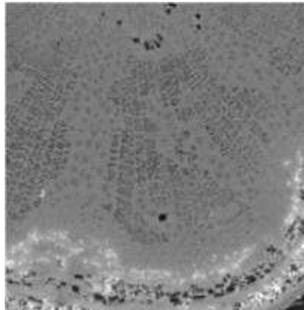


METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

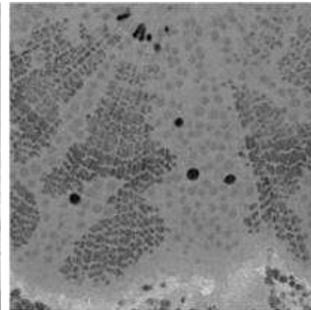
Quercus robur
Ring-porous long-vesseled species



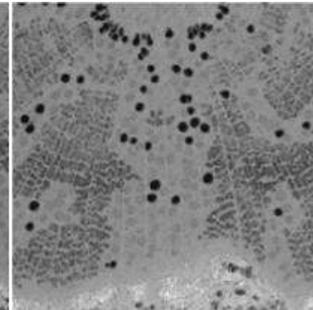
-1.8 MPa



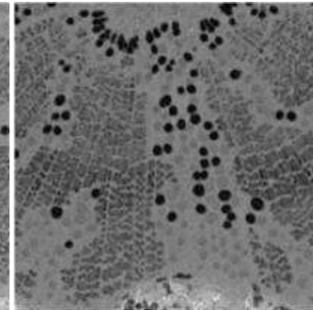
-2.9 MPa



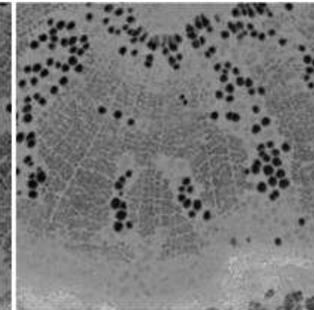
-3.5 MPa



-4.2 MPa

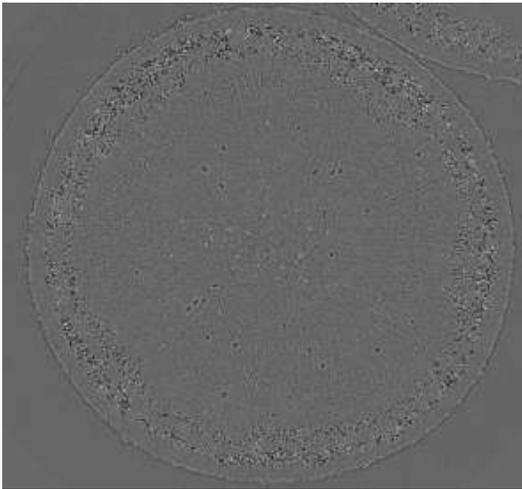


-5.1 MPa

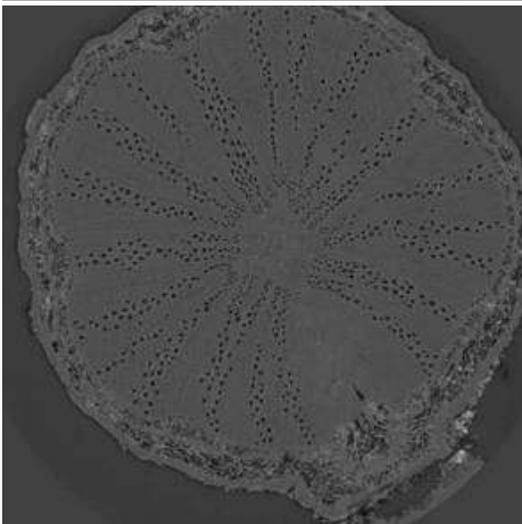


METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

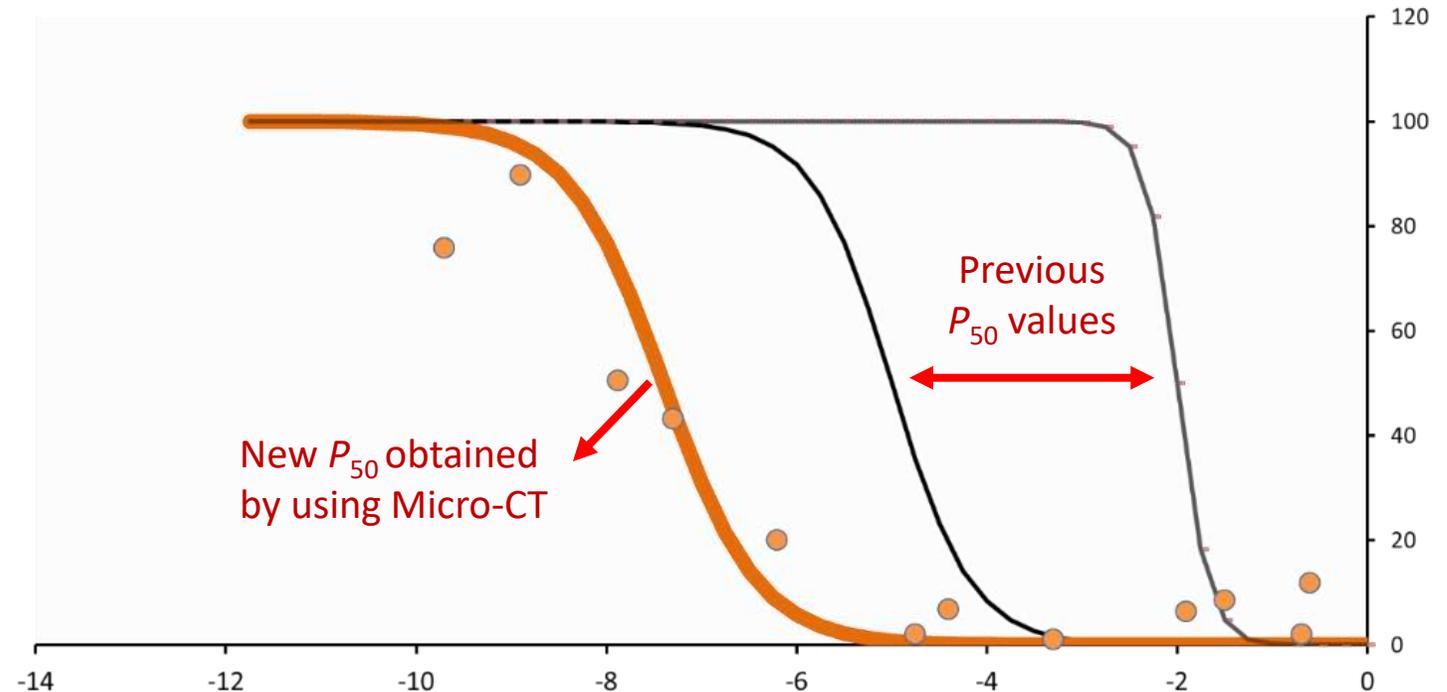
$\psi_{\text{xyl}} = -4.9 \text{ Mpa}$
PLC = 10%



$\psi_{\text{xyl}} = -8.7 \text{ Mpa}$
PLC = 90%



Quercus ilex



Martin-StPaul NK et al. 2015

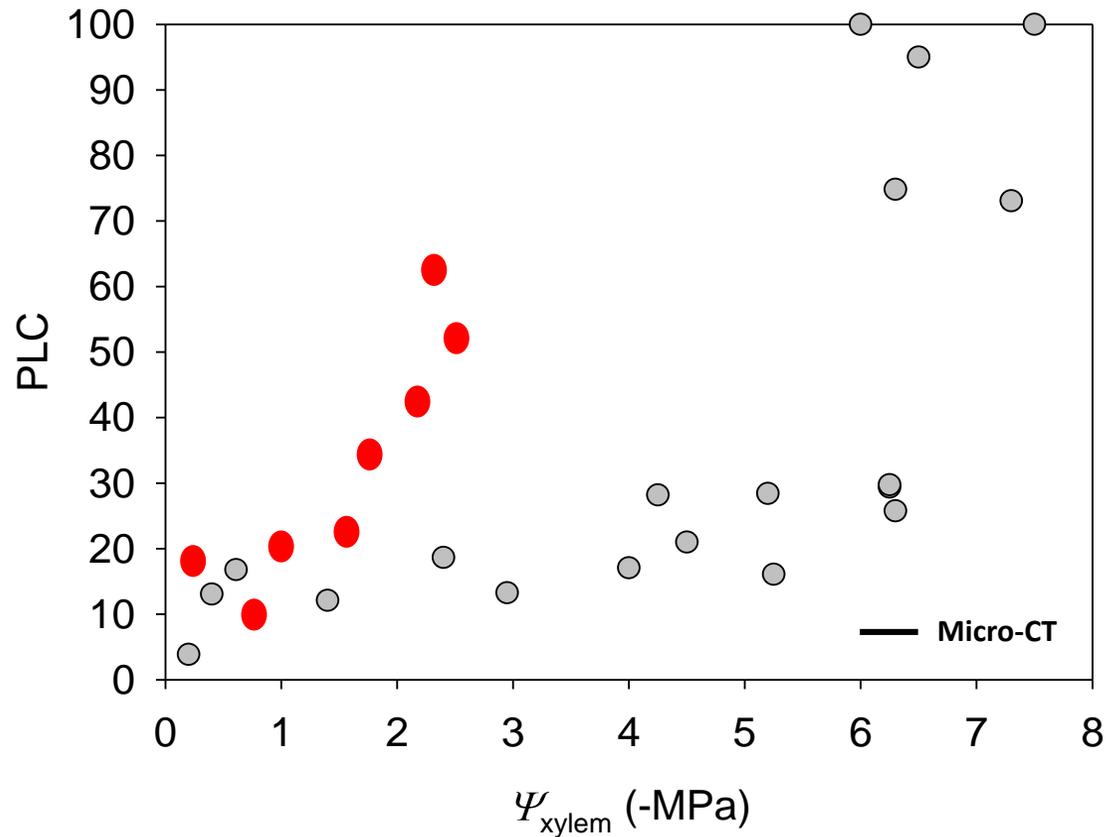
Previously published P_{50} values for this species ranged between -2.0 to -5.0 MPa. Micro-CT reported a P_{50} of -7.4MPa.



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

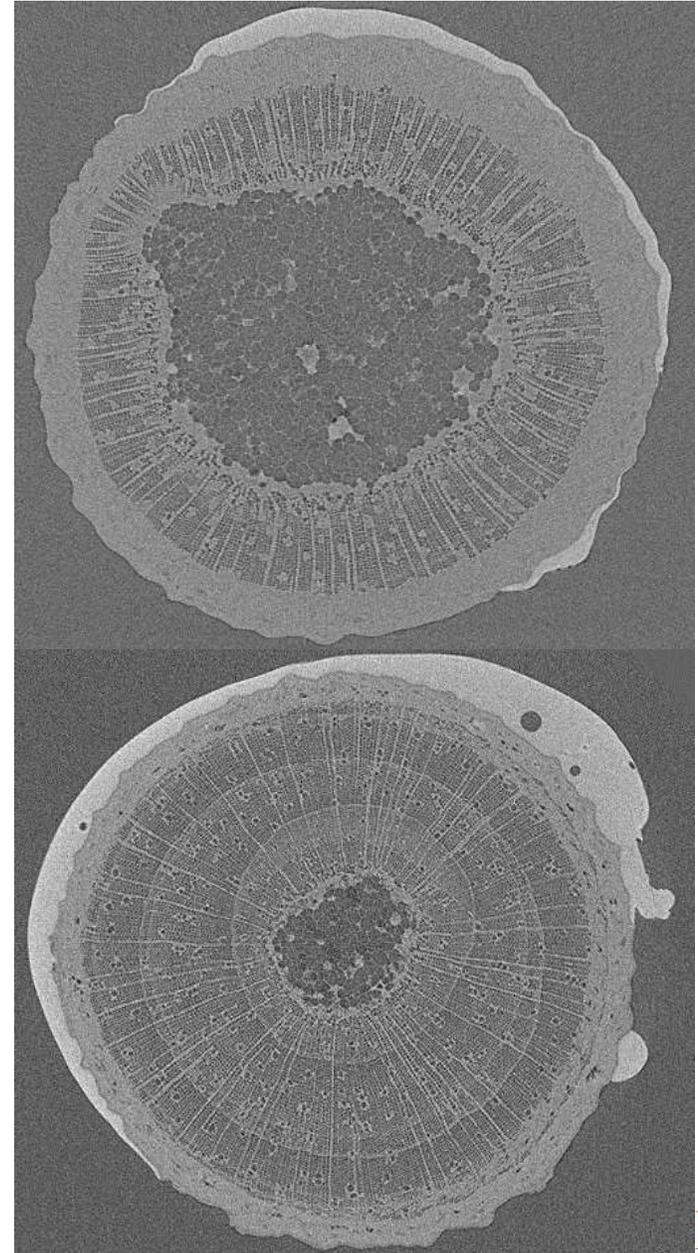


Laurus nobilis

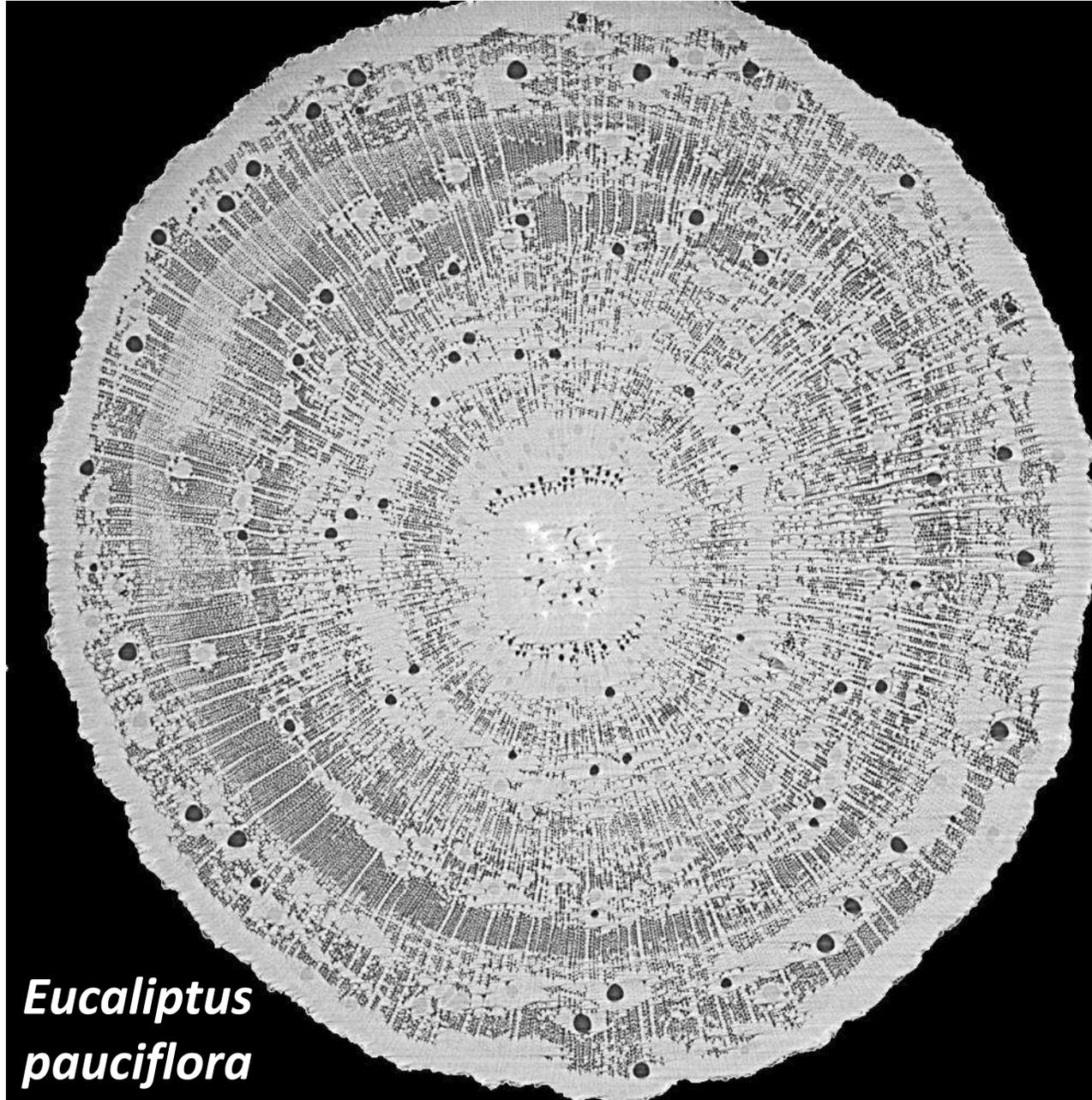


$\Psi_{\text{xylem}} = -4.5 \text{ MPa}$
PLC = 21%

$\Psi_{\text{xylem}} = -7.5 \text{ MPa}$
PLC = 100%



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS



*Eucalyptus
pauciflora*



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crodriguez@irnas.csic.es

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METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

3D images

Olea europea - Leaf



CSIC

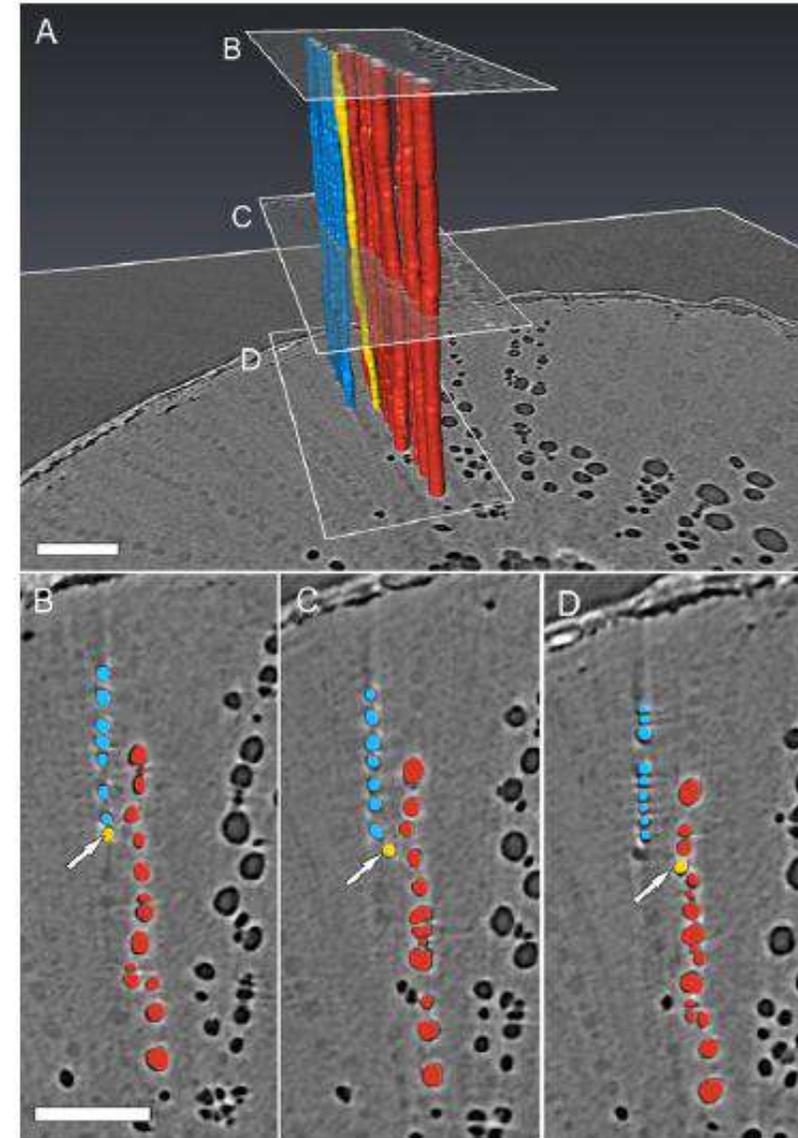
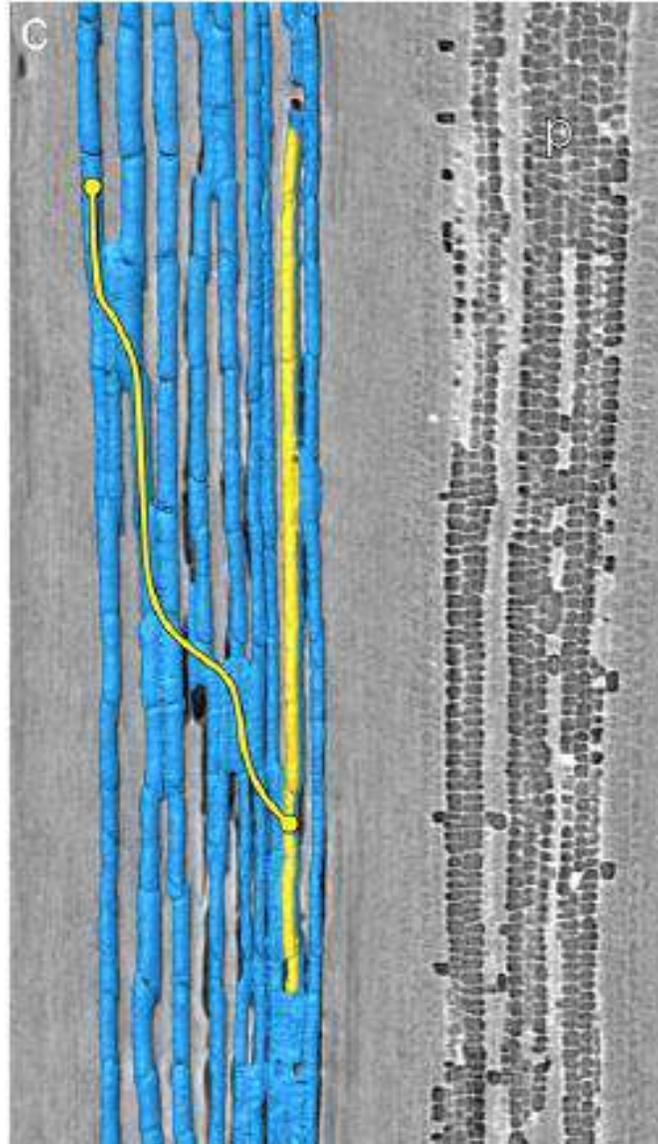
crodriguez@irnas.csic.es

Torres-Ruiz, Cochard & Badel

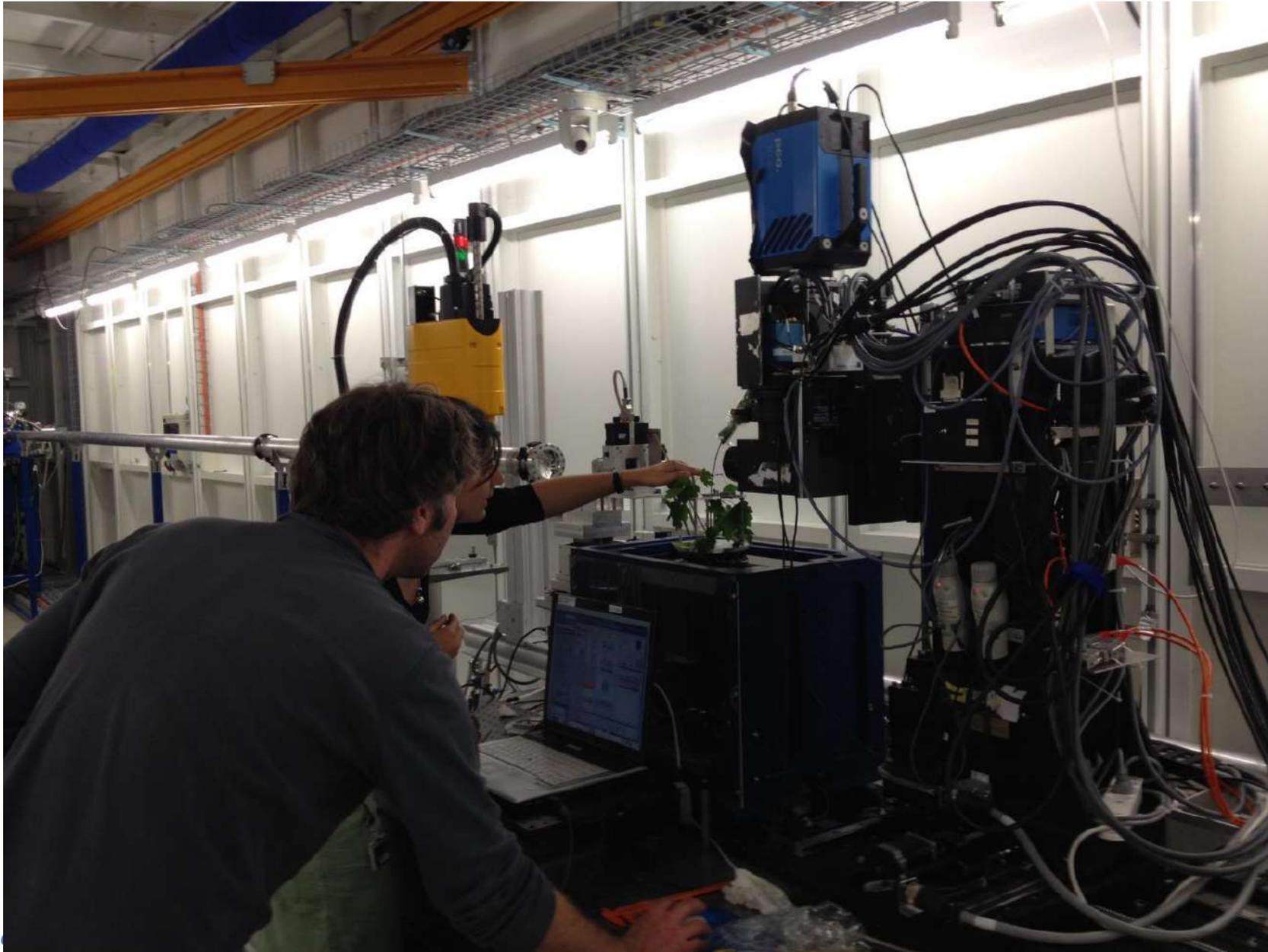
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METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

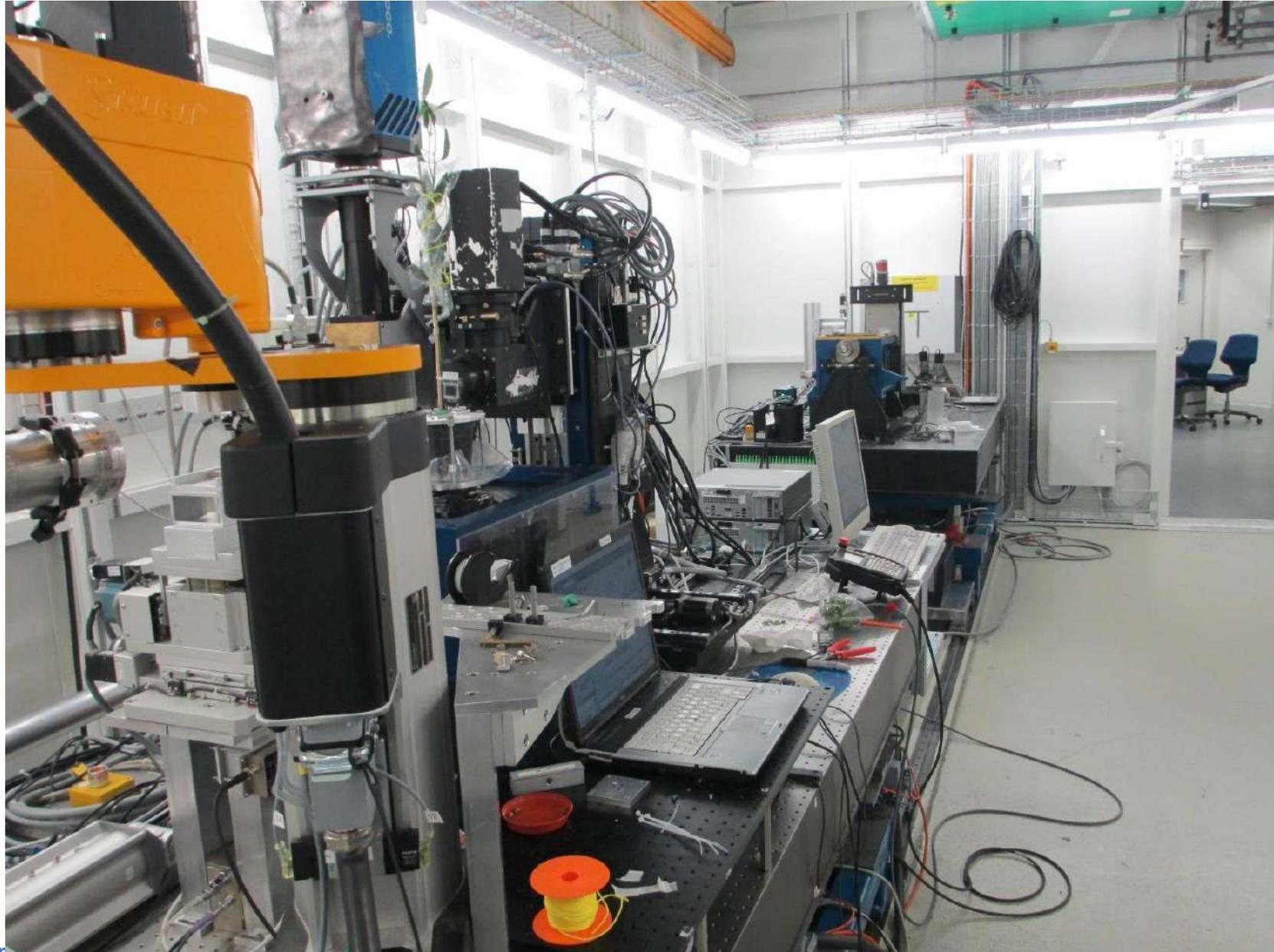
3D images



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS



METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS



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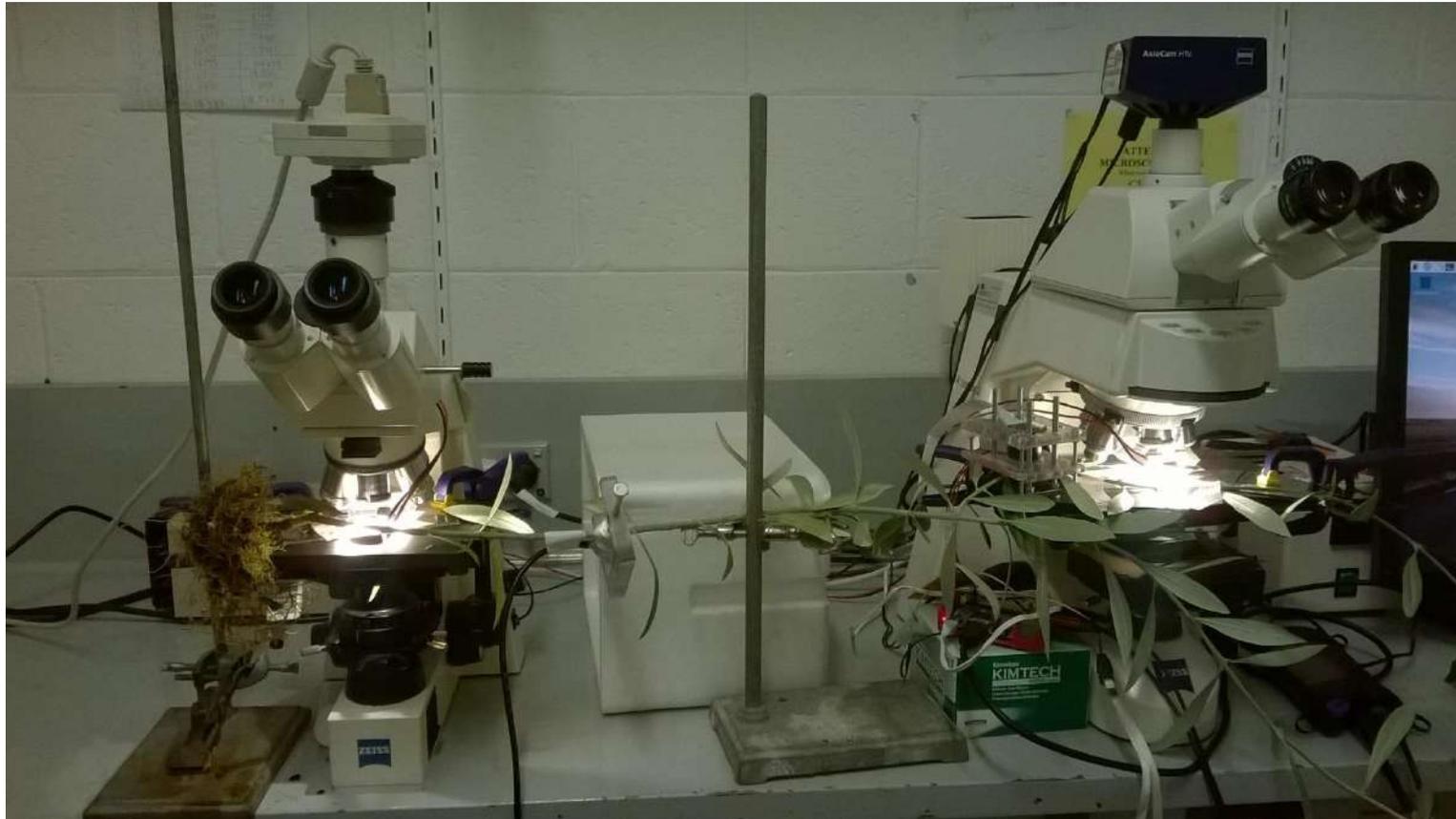
crodriguez@irnas.csic.es

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METHODS FOR MEASURING K AND GENERATING VCS: ACCURACY & PROGRESS

The Optical Method

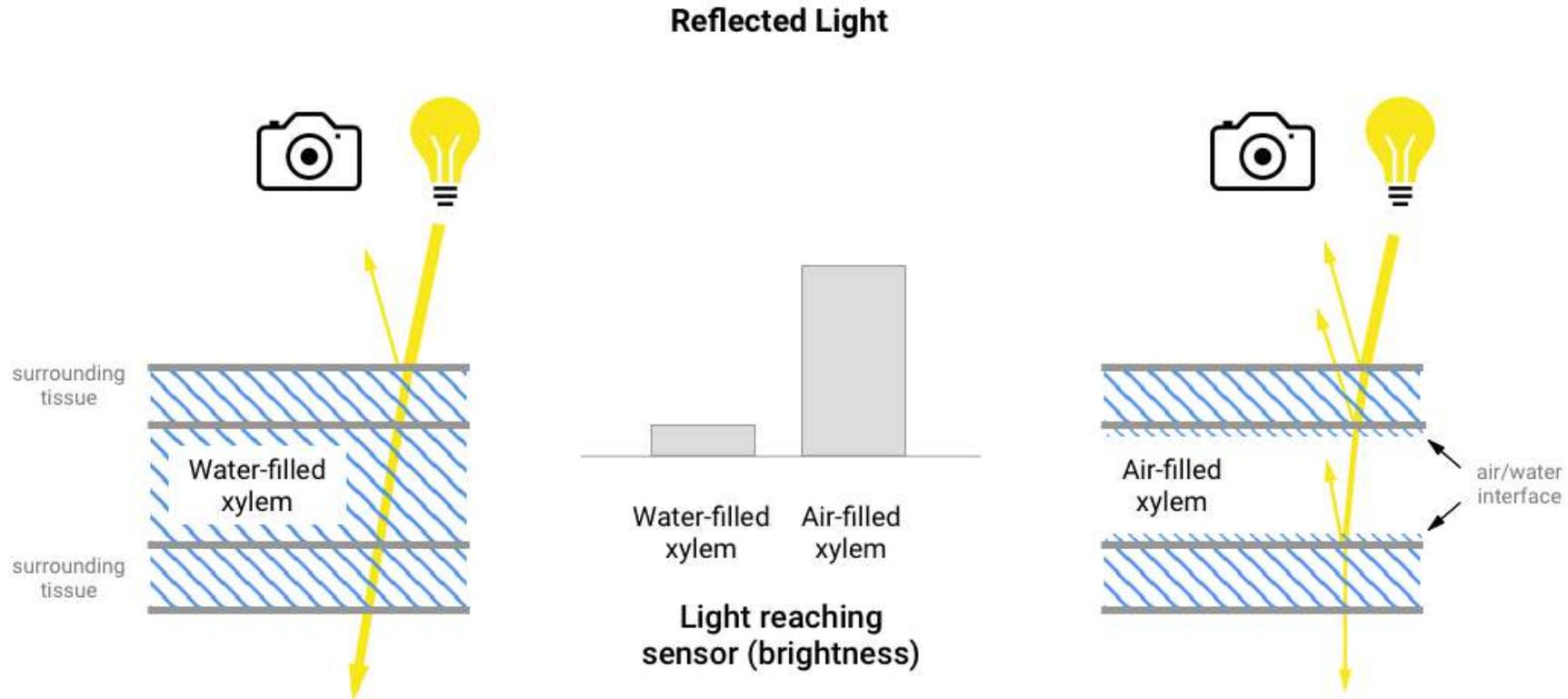
The optical method is based on the simple principle that light interacts differently with *xylem* that is water-filled vs air-filled



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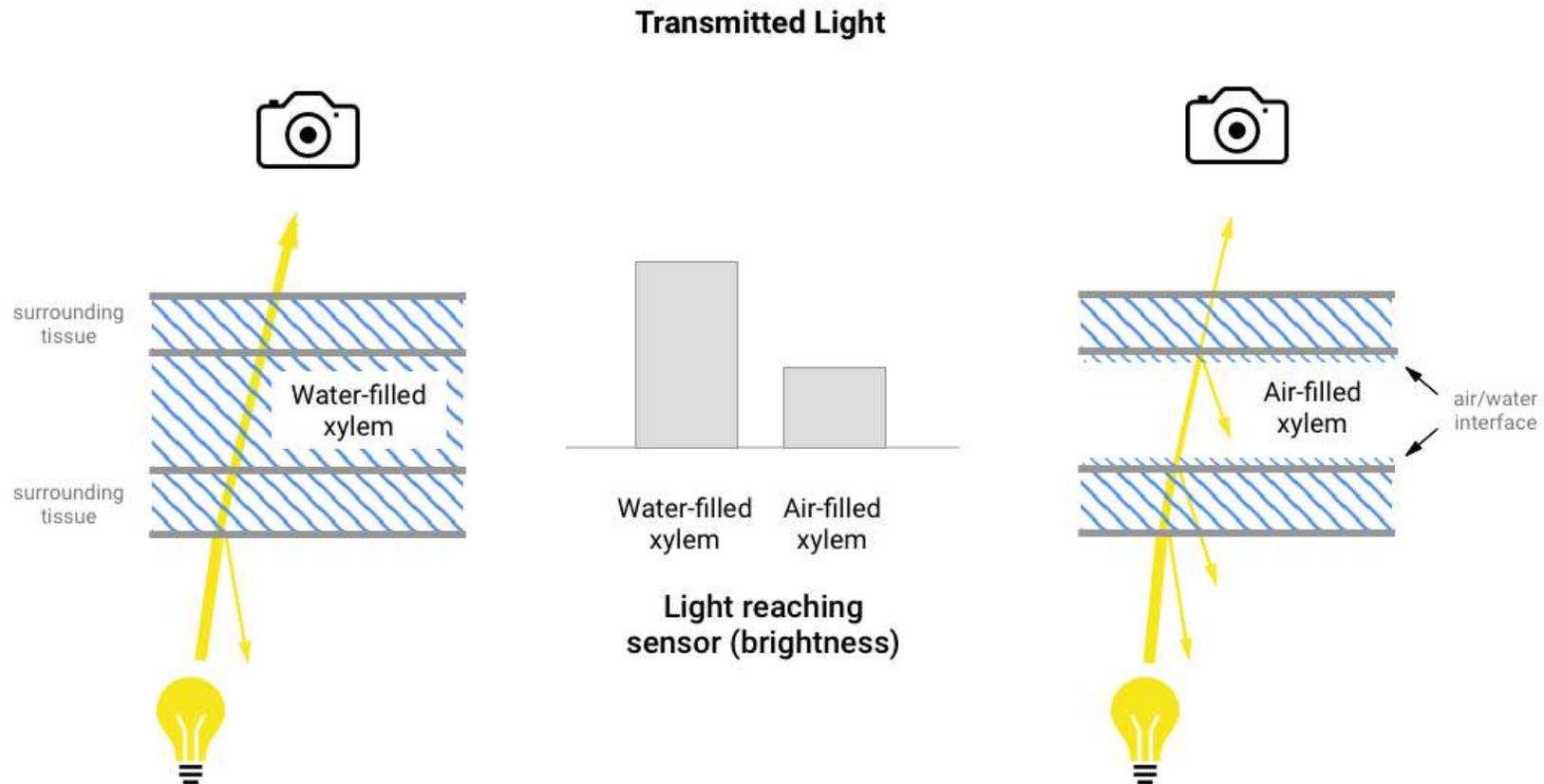
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<https://www.opensourceov.org/>

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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

Mapping xylem failure in disparate organs of whole plants reveals extreme resistance in olive roots

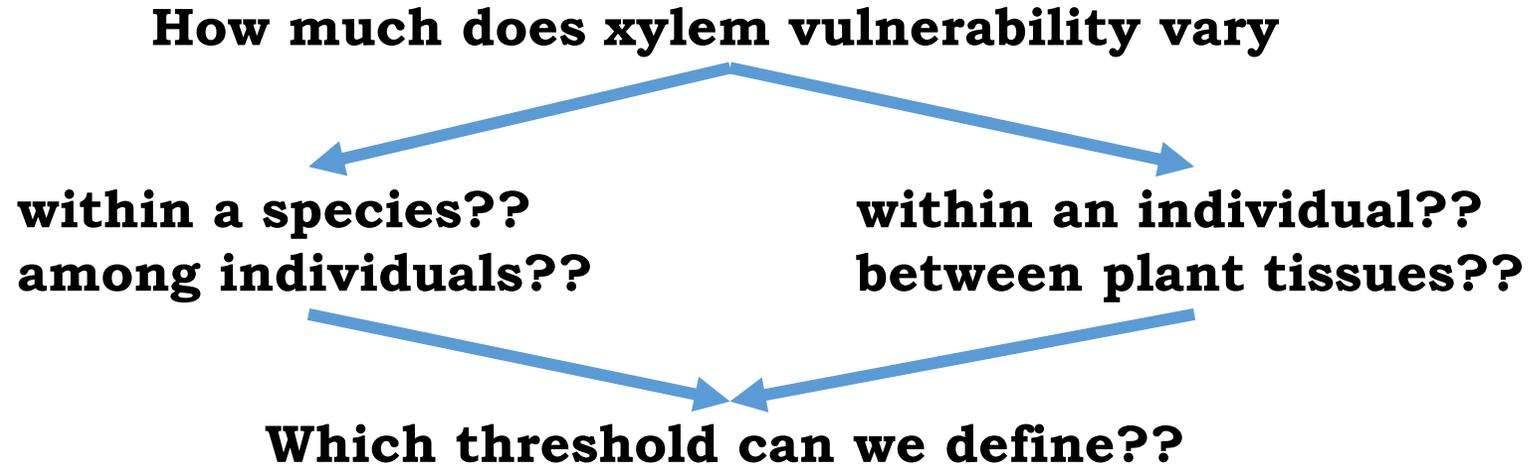


2018

Celia M. Rodriguez-Dominguez , Madeline R. Carins Murphy , Christopher Lucani  and Timothy J. Brodribb 



To measure vulnerability curves to embolisms *in vivo* and *simultaneously* in leaves, stems and roots of olive plants using the optical method



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

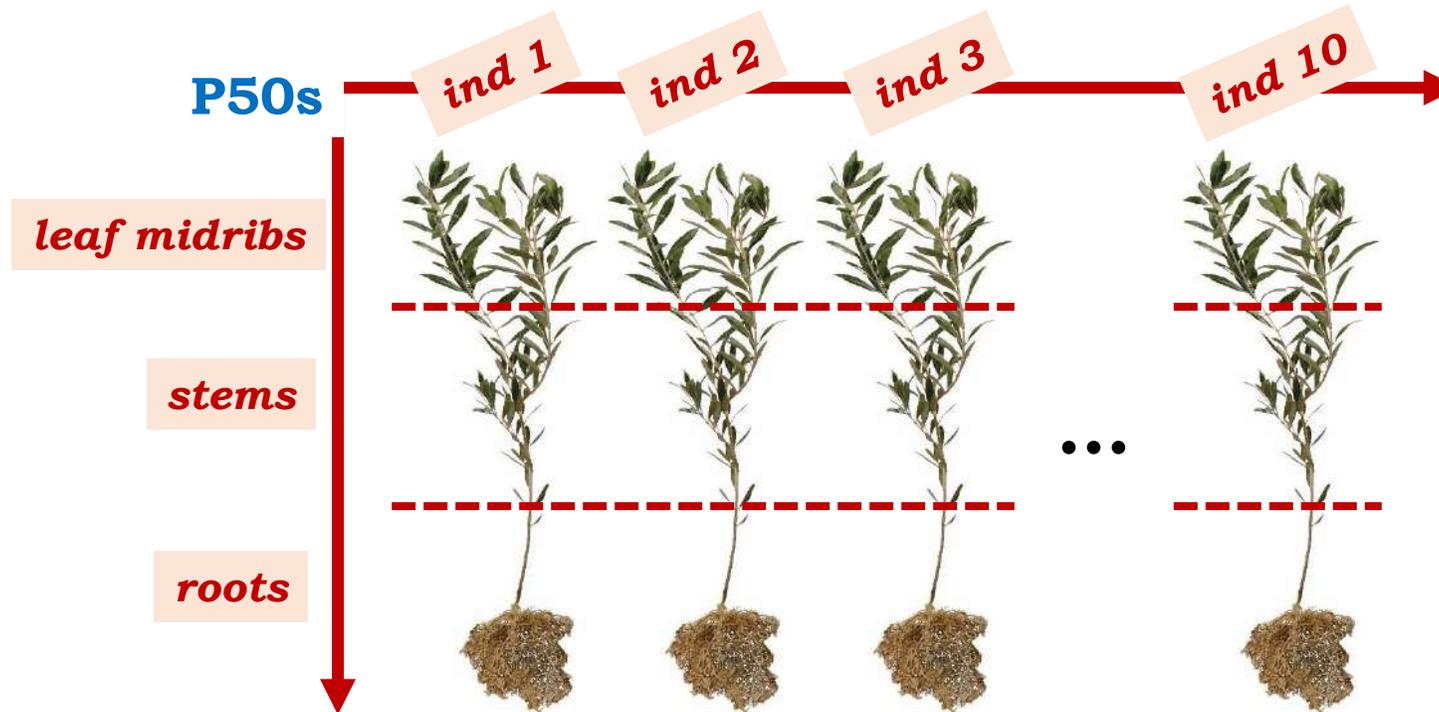
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2018

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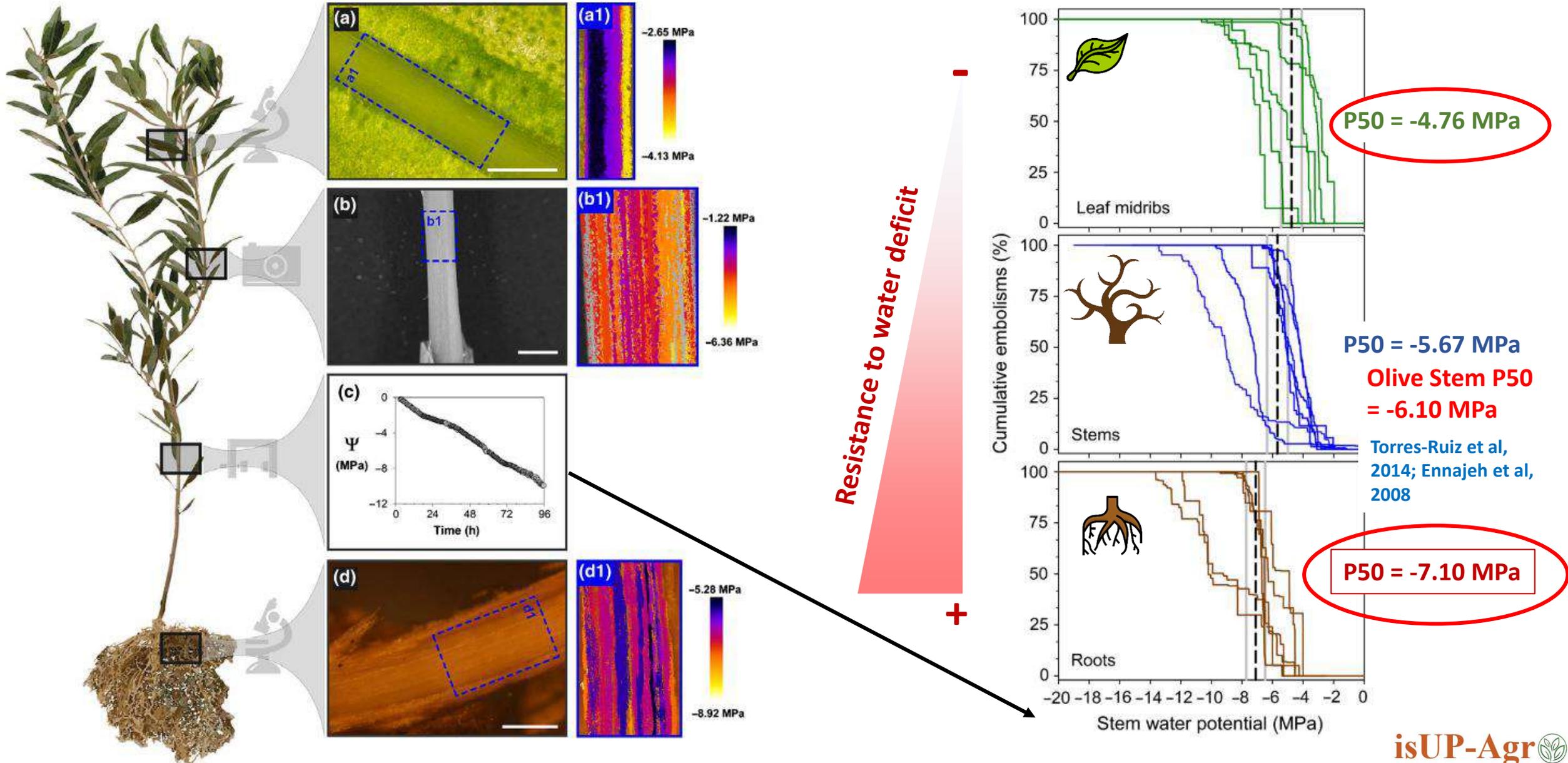


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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

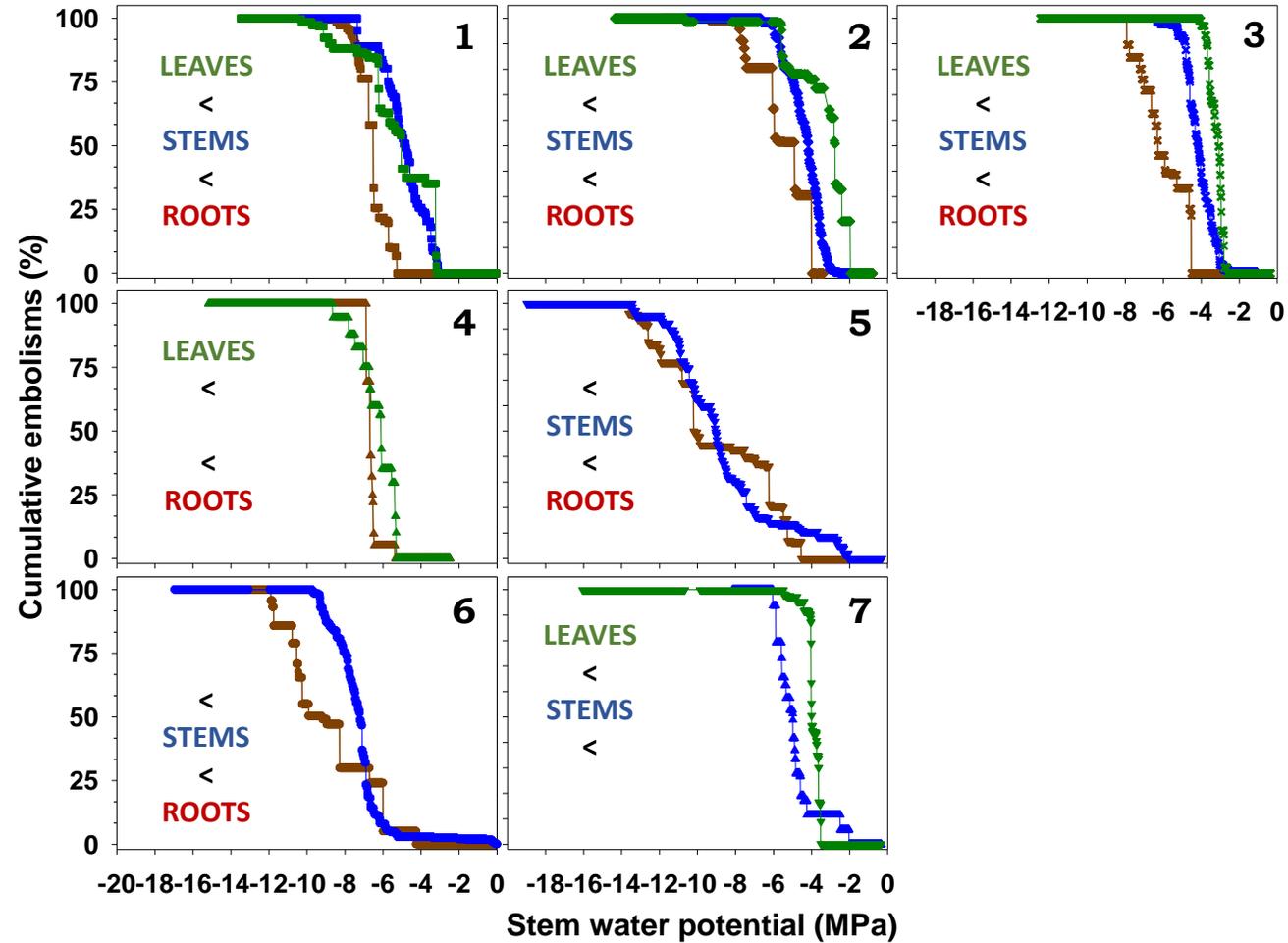
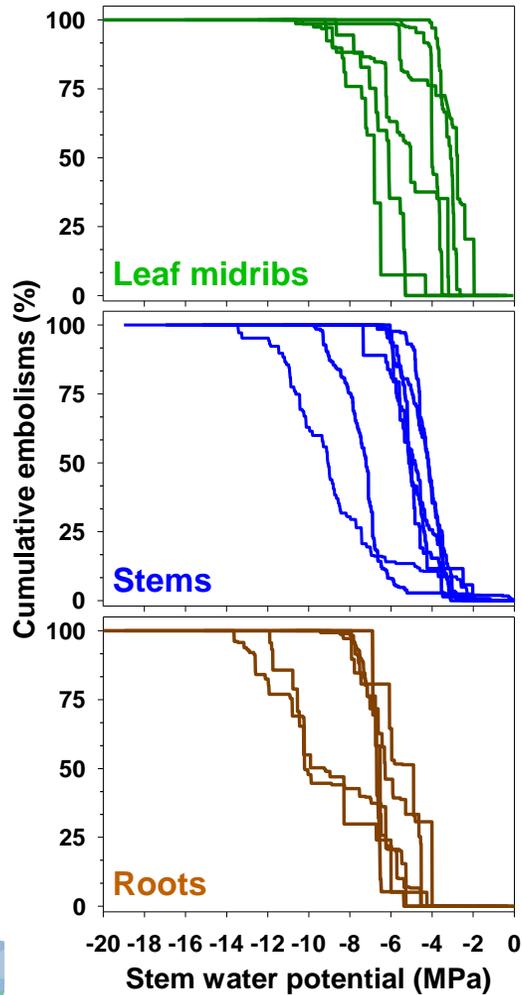
-- WITH PRACTICAL APPLICATIONS --



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

ROOTS > **STEMS** > **LEAVES**



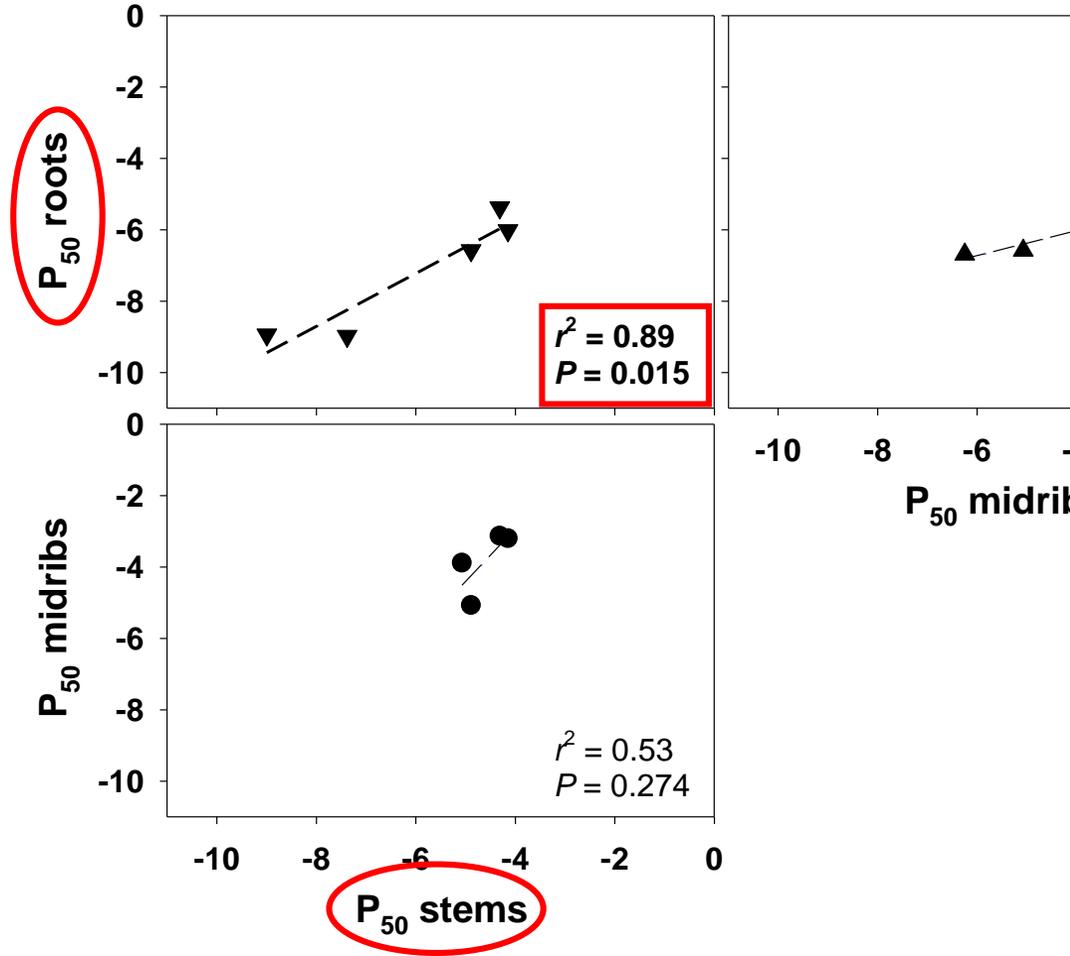
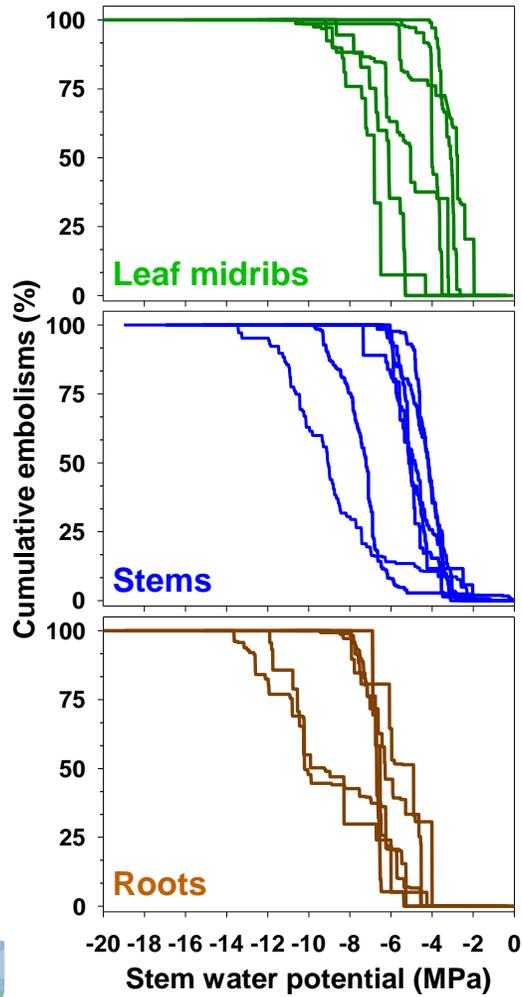
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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

ROOTS > STEMS > LEAVES

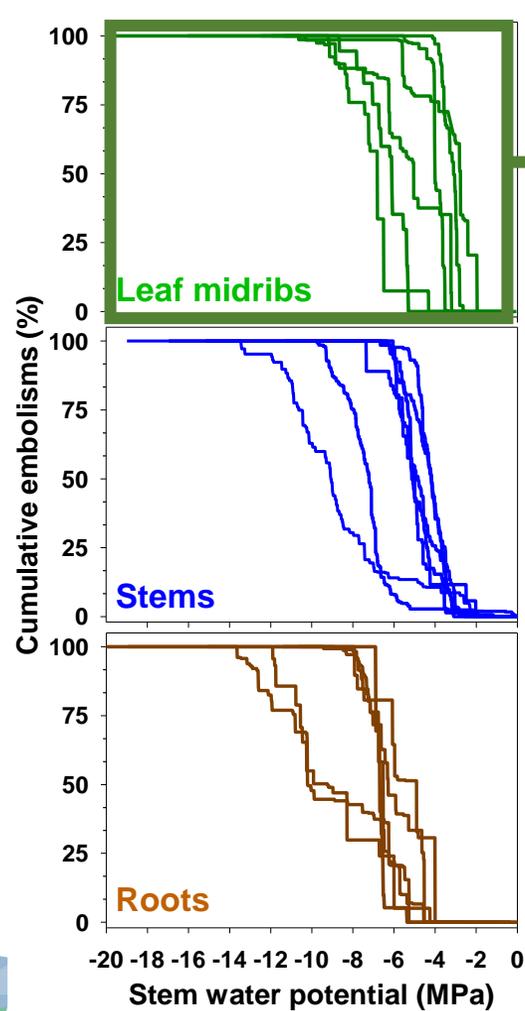


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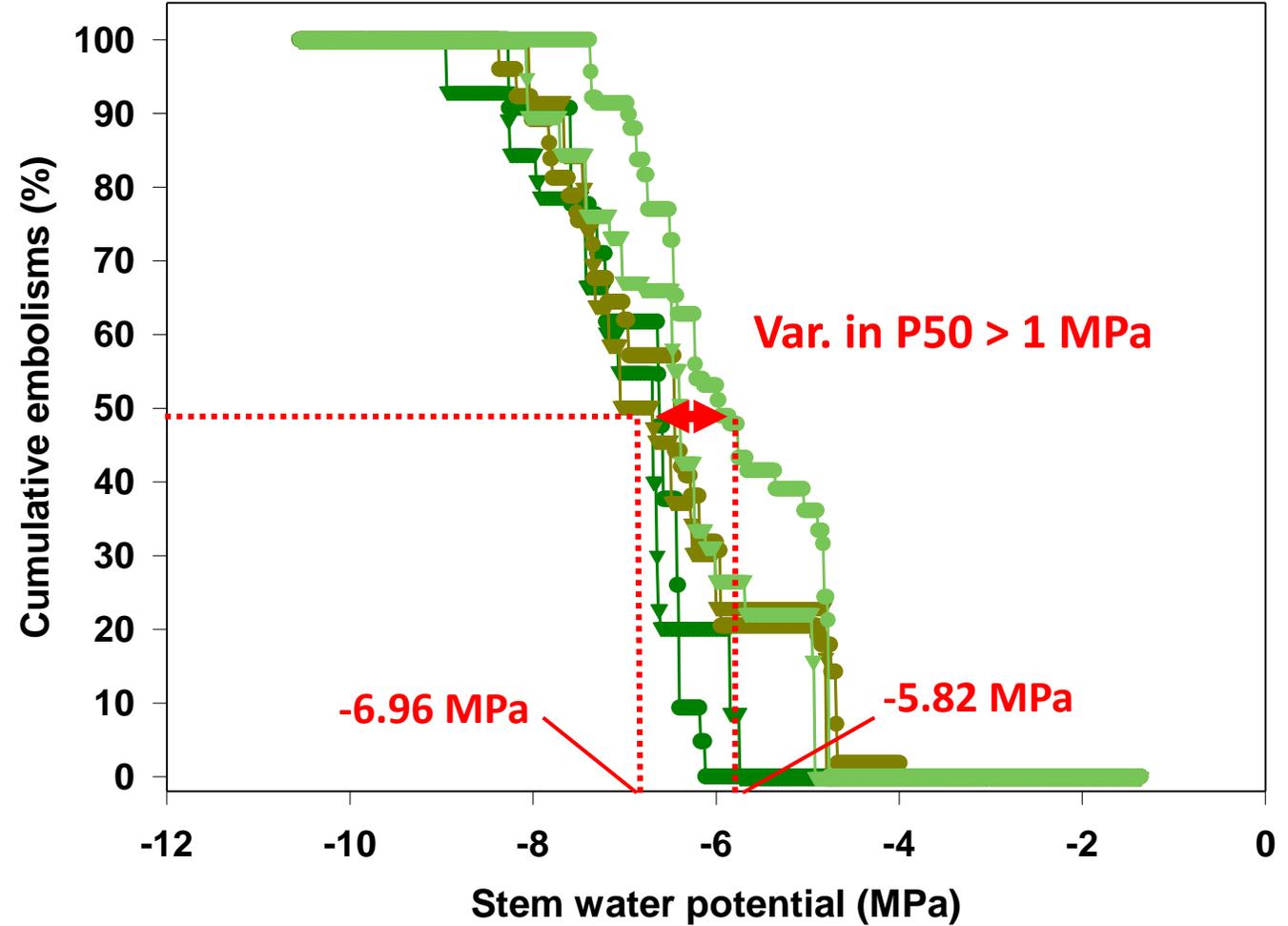
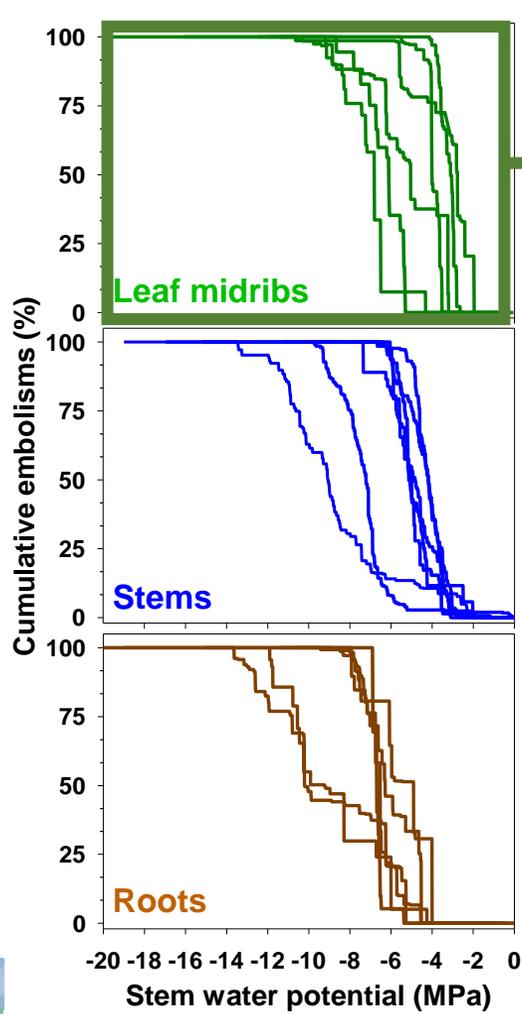
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

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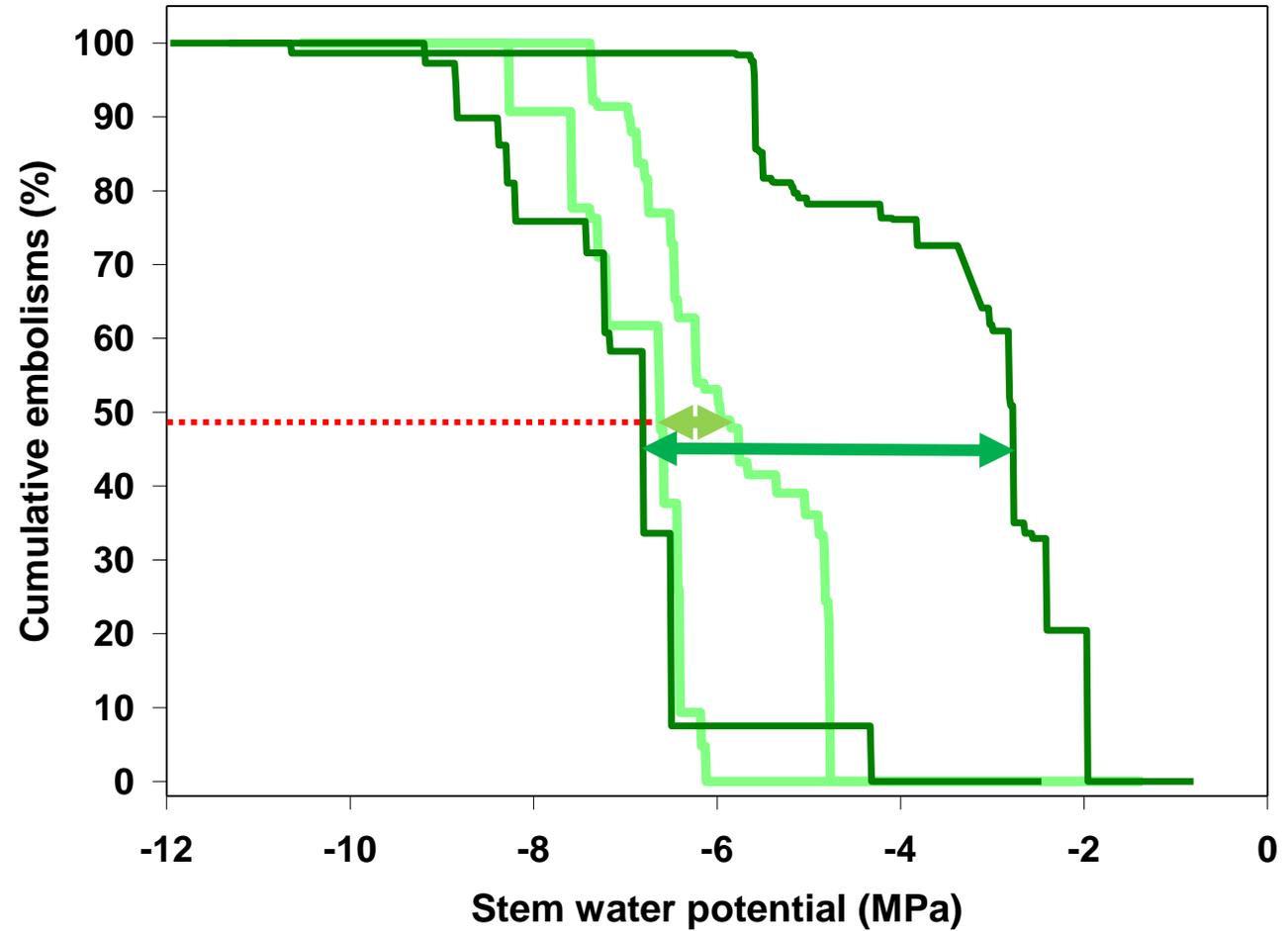
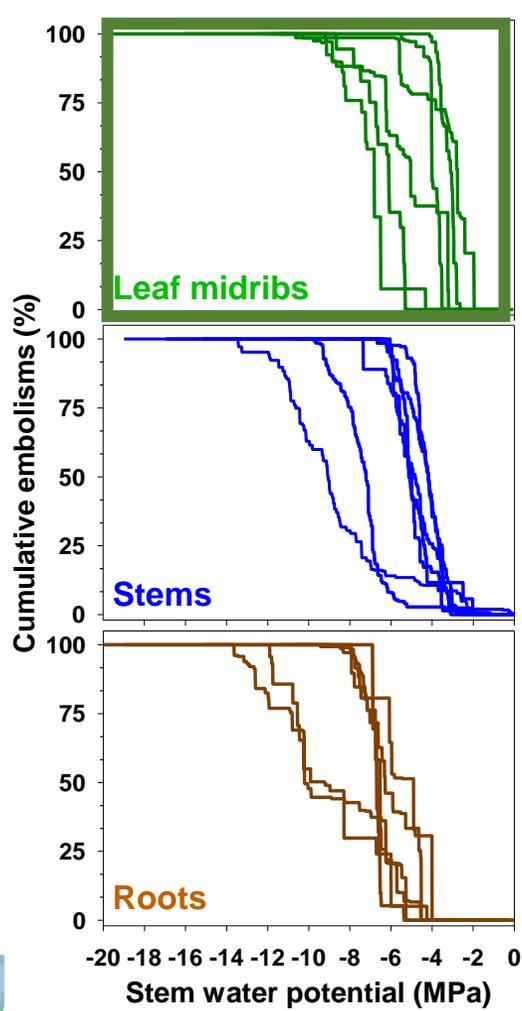


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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

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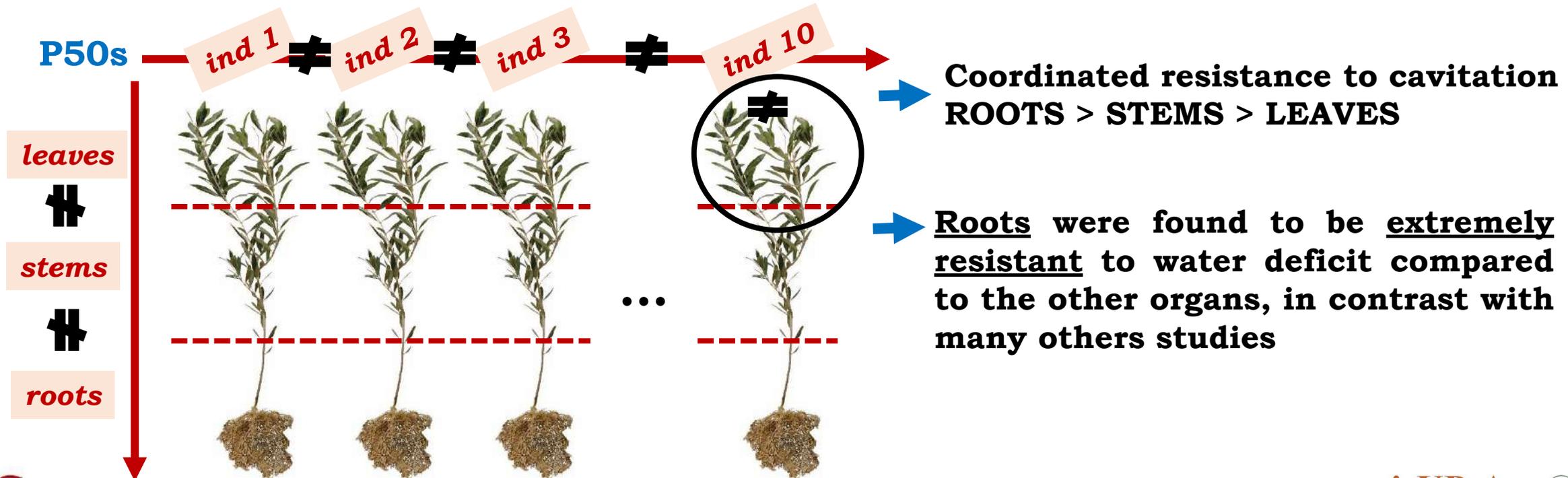
Mapping xylem failure in disparate organs of whole plants reveals extreme resistance in olive roots



2018

Celia M. Rodriguez-Dominguez , Madeline R. Carins Murphy , Christopher Lucani  and Timothy J. Brodrigg 

- ➔ Simultaneous monitoring of cavitation with OV method
- ➔ Variation between ind., between tissues and within tissues



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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

Declining root water transport drives stomatal closure in olive under moderate water stress

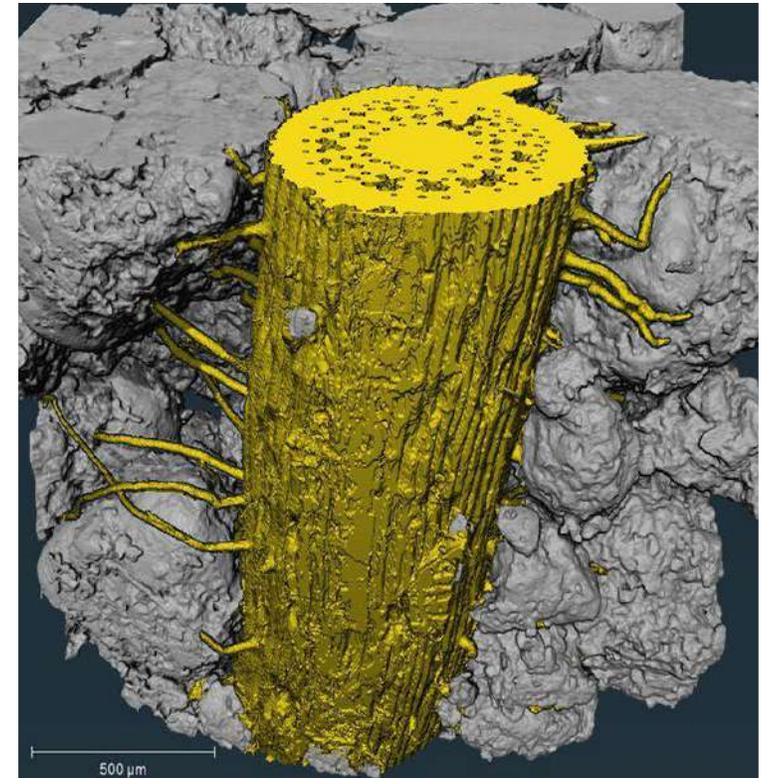
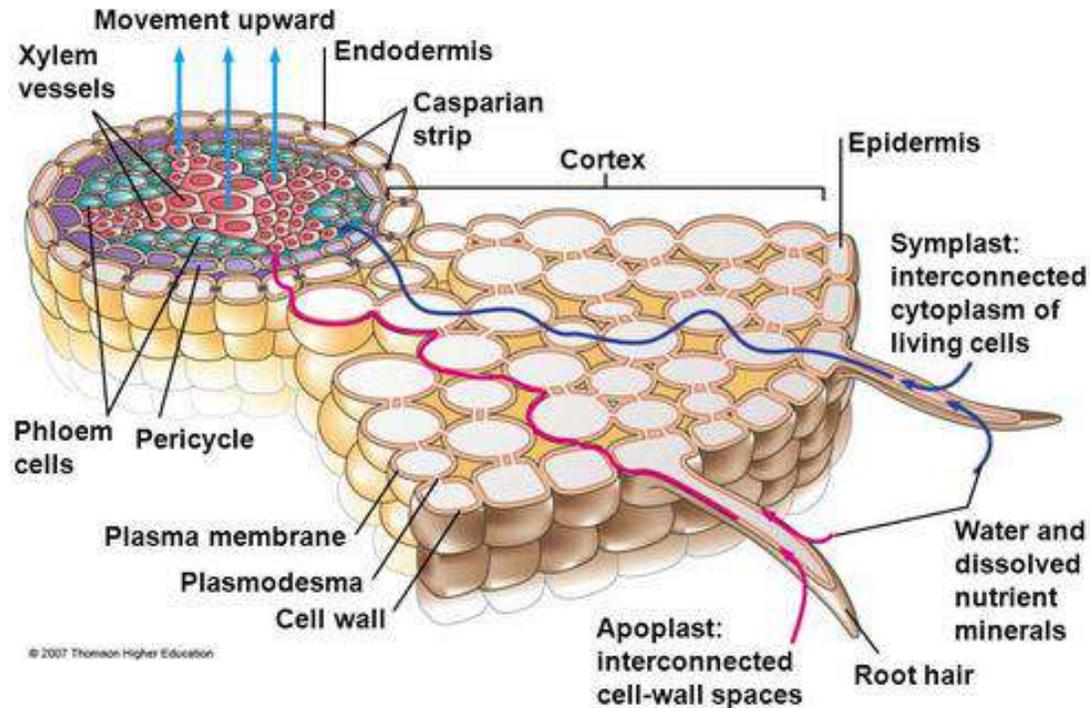
2020



Celia M. Rodriguez-Dominguez^{1,2} and Timothy J. Brodribb²



To quantify each hydraulic component *from the soil to the leaf* and assess its impact on the decrease of g_s in olive plants under water deficit



Duddek et al. 2022 *Plant Phys*



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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

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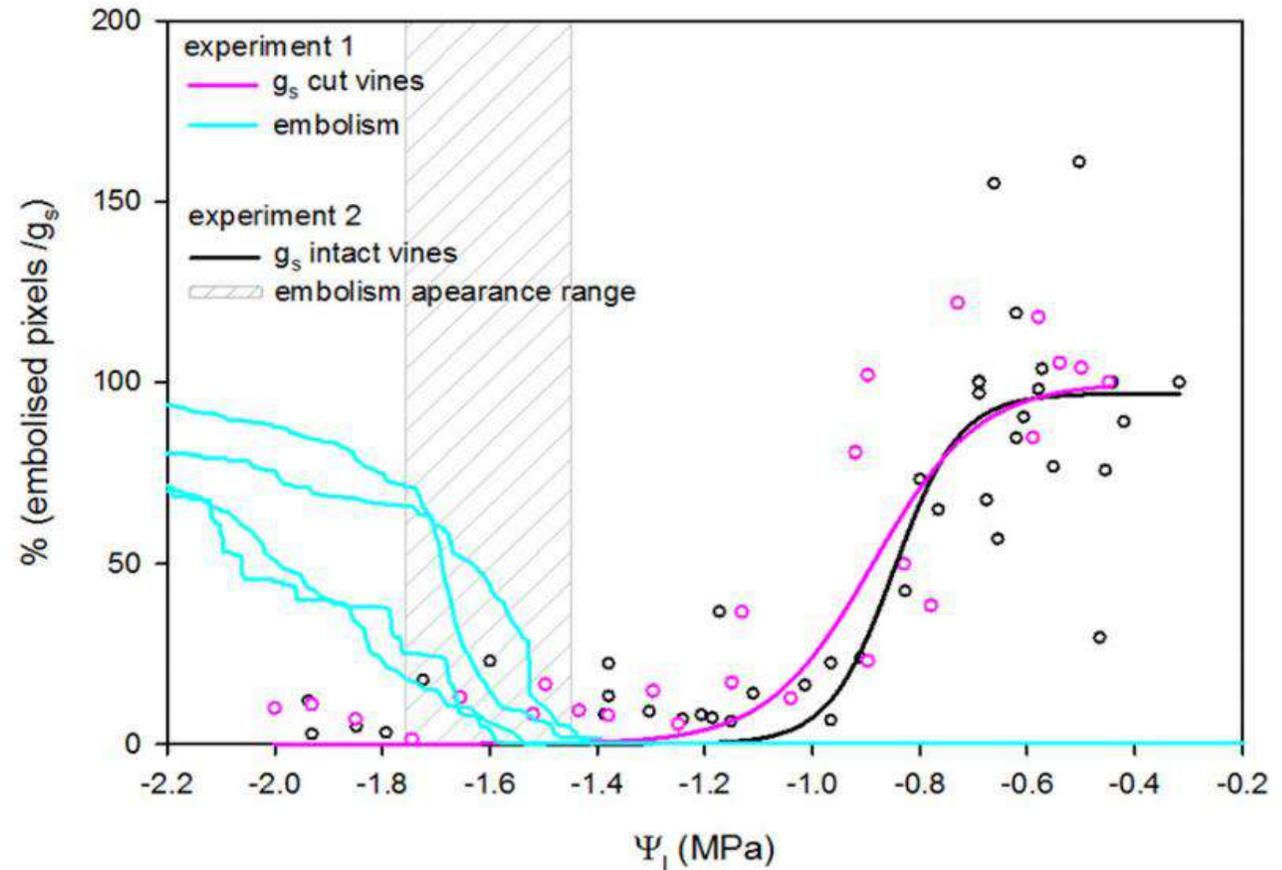
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Hochberg et al, 2017, *Plant Physiol*



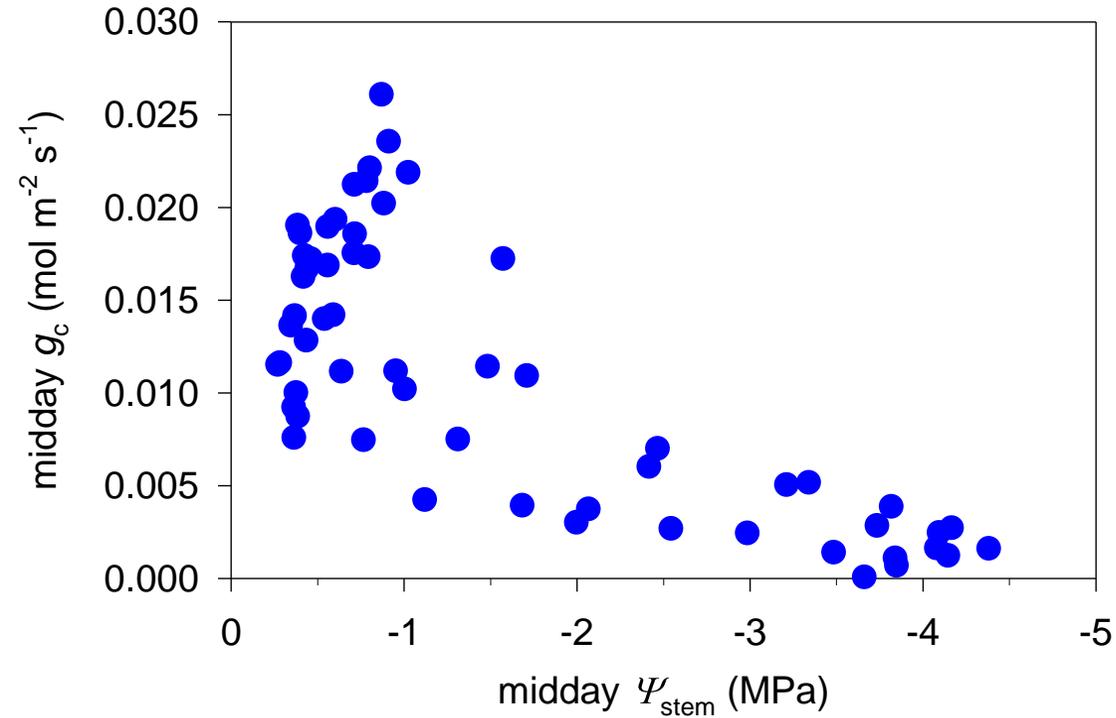
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NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

MONITORING PLANT WATER STATUS AND STOMATAL CONDUCTANCE DURING DROUGHT



~ -1 MPA

~ -2 MPA

~ -4 MPA

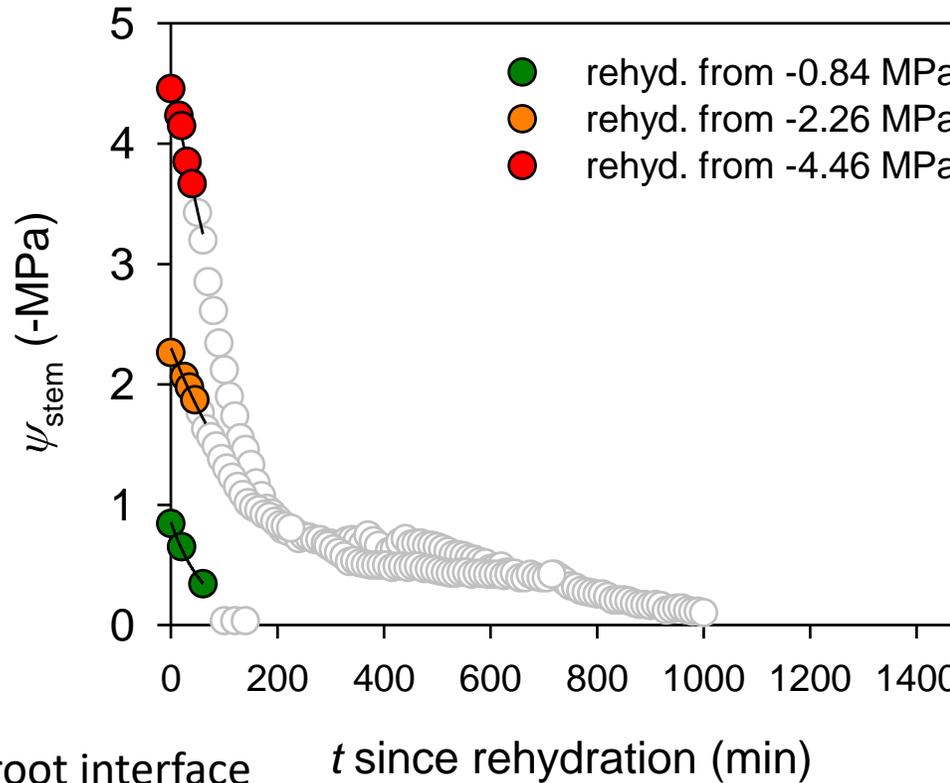
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

EXTERNAL REHYDRATION OF THE PLANT THROUGH THE SOIL

$$\Psi_{\text{stem}} = ae^{-bt}$$

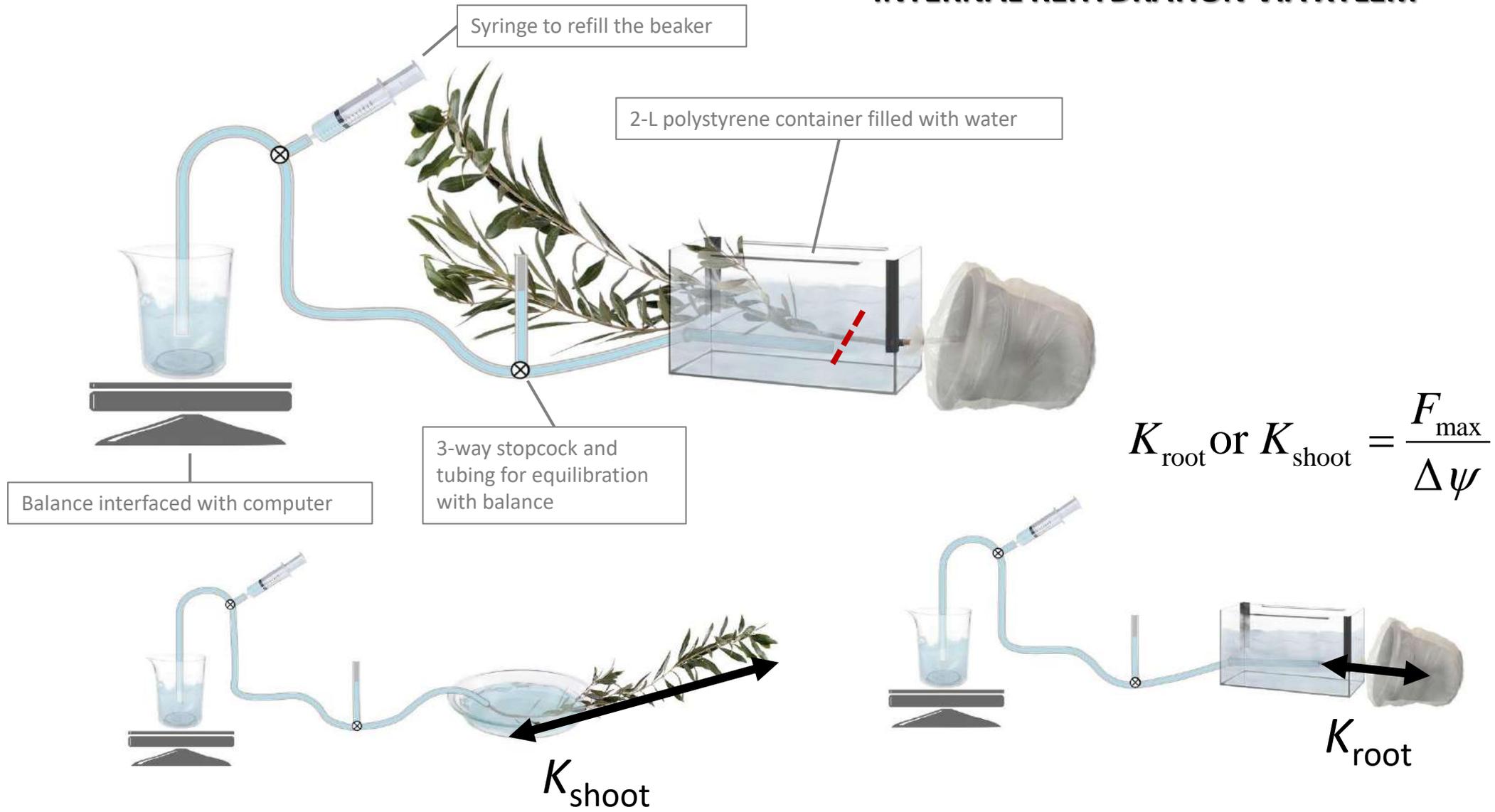
$$b = K/C$$



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

INTERNAL REHYDRATION VIA XYLEM



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

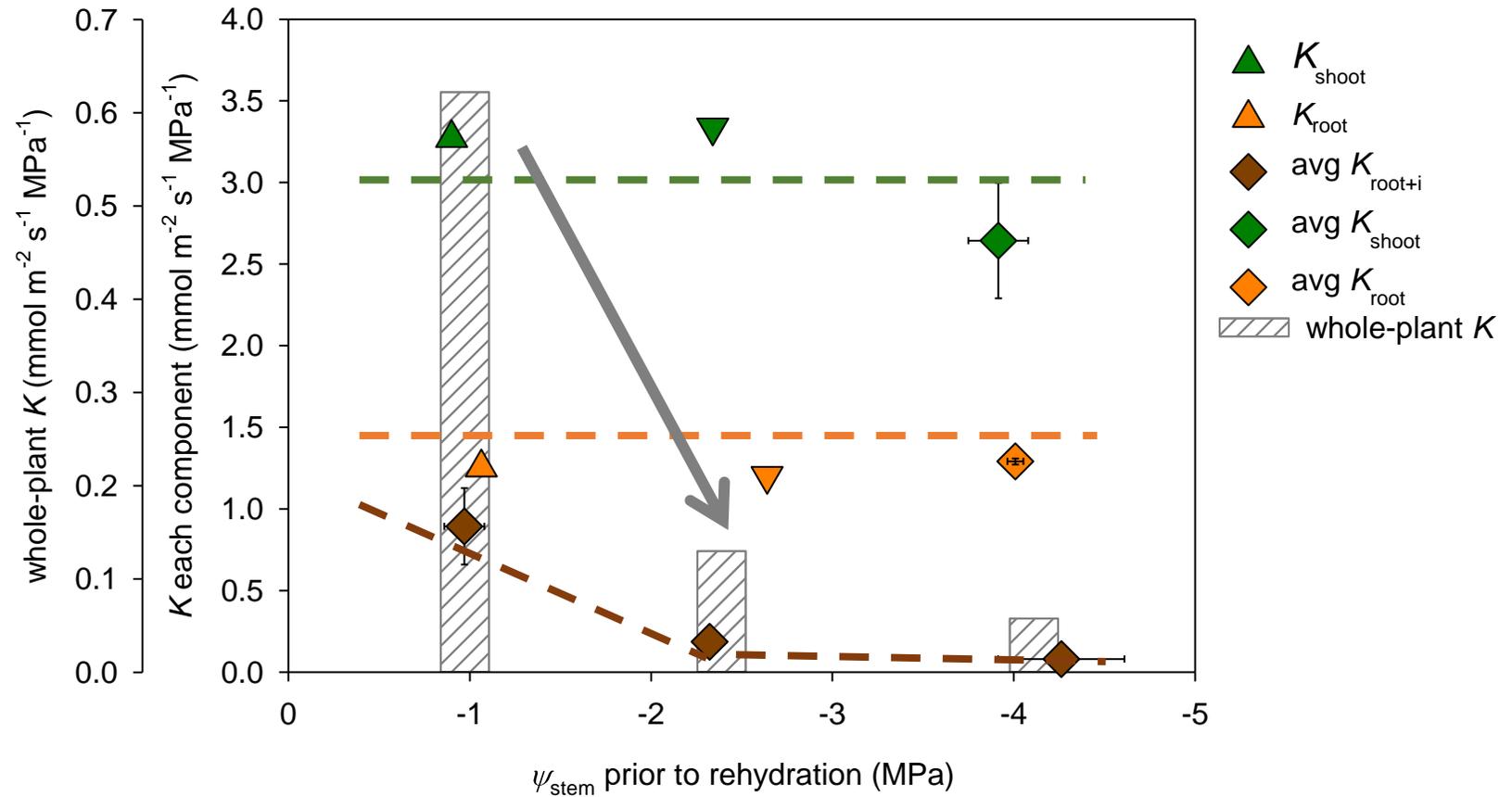
-- WITH PRACTICAL APPLICATIONS --

PARTITIONING HYDRAULIC PATHWAYS WITHIN THE PLANT



$$\frac{1}{K_{\text{plant}}} = \frac{1}{K_{\text{shoot}}} + \frac{1}{K_{\text{root+i}}}$$

$\begin{matrix} & \swarrow & \searrow & & \\ K_{\text{leaf}} & & K_{\text{stem}} & & \\ & \swarrow & \searrow & & \\ K_{\text{root}} & & K_i & & \end{matrix}$



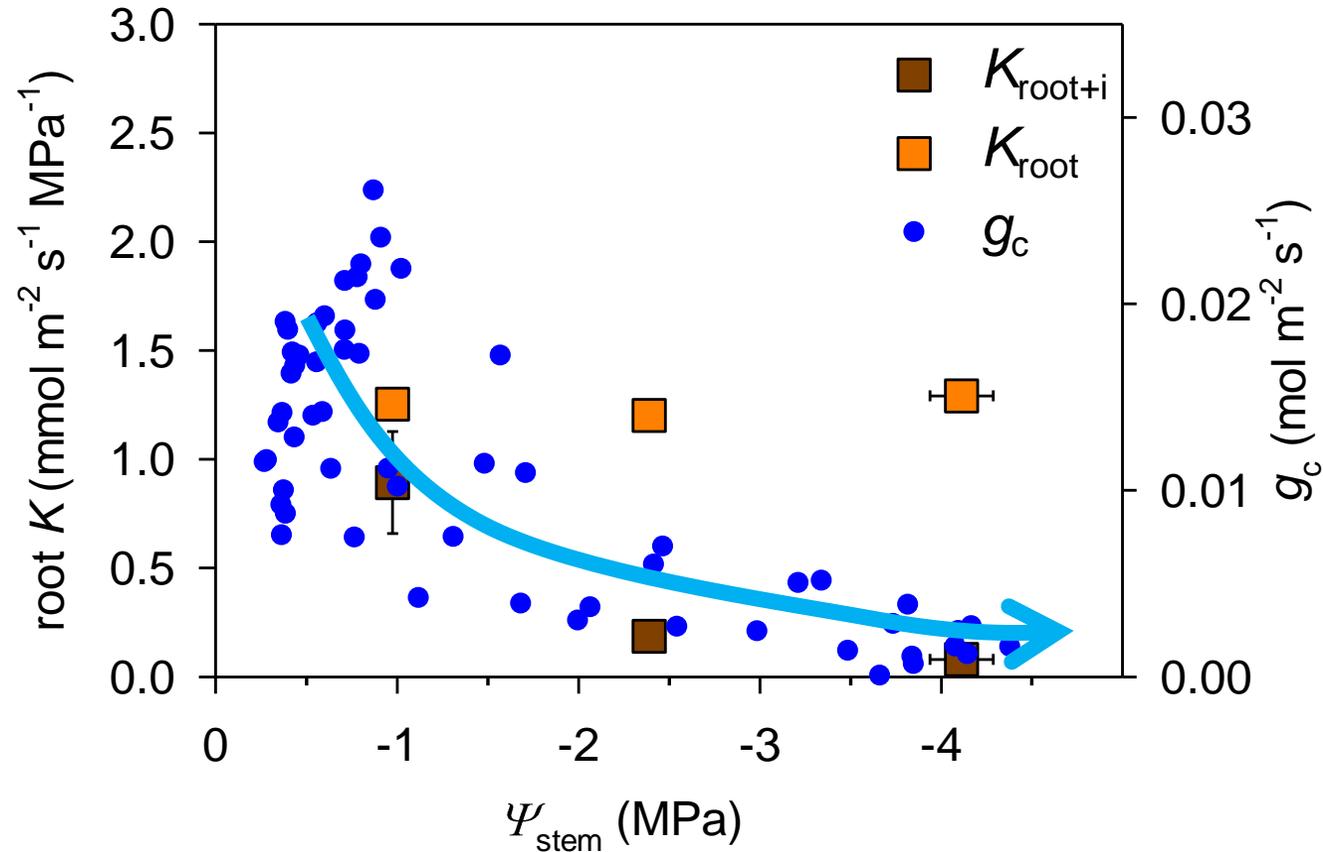
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

DECLINING IN $K_{\text{root+i}}$ DROVE STOMATAL CLOSURE

$$1/K_{\text{plant}} = 1/K_{\text{shoot}} + 1/K_{\text{root+i}}$$

K_{leaf} K_{stem} K_{root} K_i



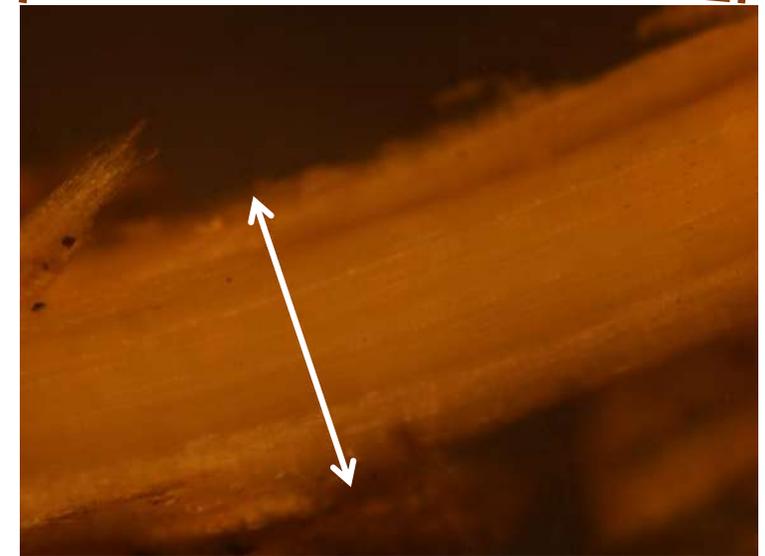
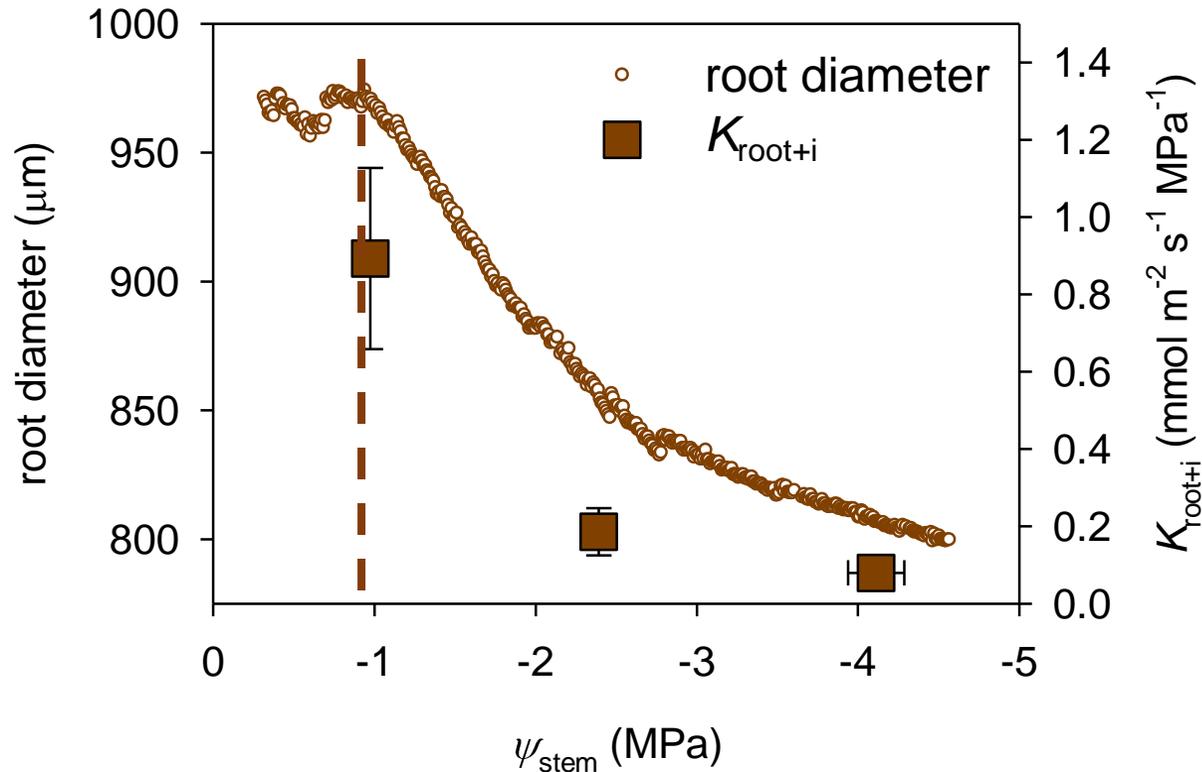
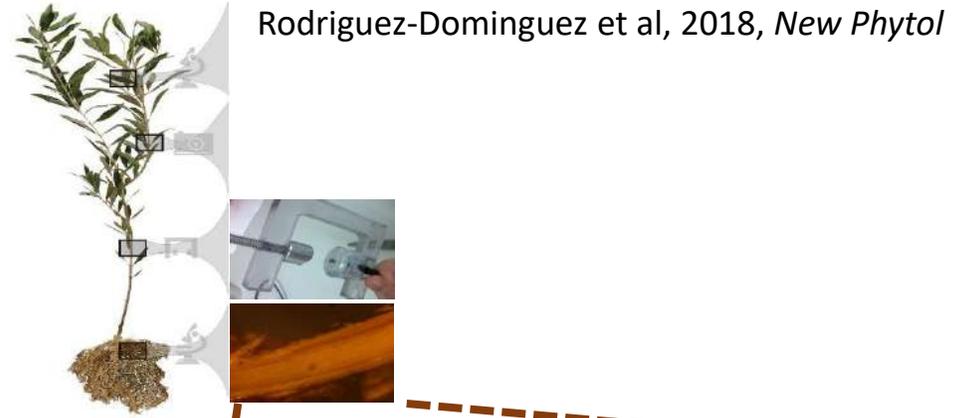
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

DISCONNECTING THE ROOT FROM THE SOIL

$$1/K_{\text{plant}} = 1/K_{\text{shoot}} + 1/K_{\text{root+i}}$$

K_{leaf} K_{stem} K_{root} K_i



$\psi_{\text{stem}} = -0.86 \text{ MPa}$
 $\phi = 971.06 \mu\text{m}$



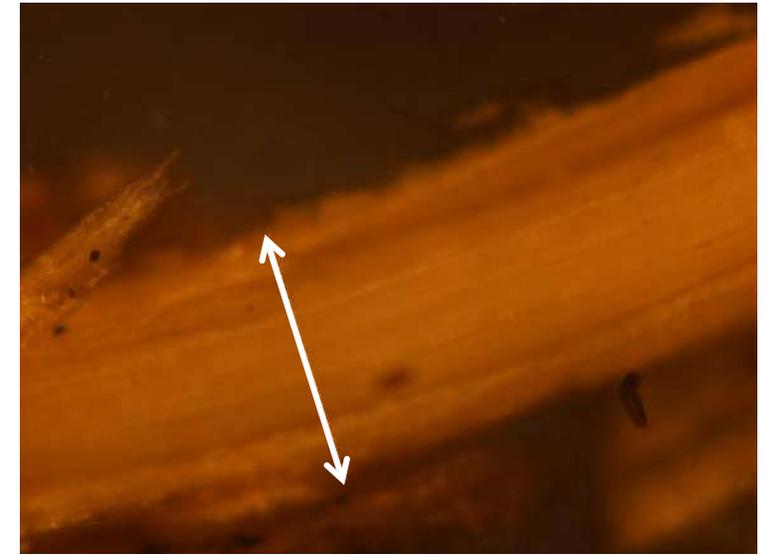
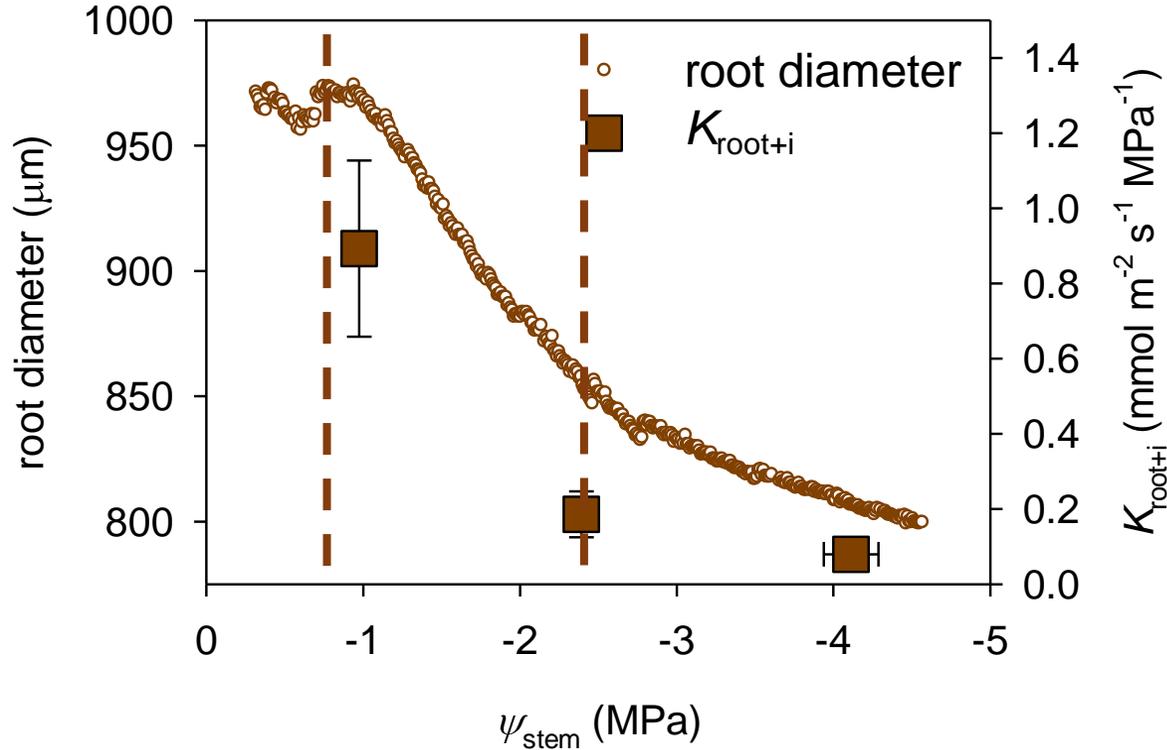
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

DISCONNECTING THE ROOT FROM THE SOIL

$$1/K_{\text{plant}} = 1/K_{\text{shoot}} + 1/K_{\text{root+i}}$$

$K_{\text{shoot}} = K_{\text{leaf}} + K_{\text{stem}}$
 $K_{\text{root+i}} = K_{\text{root}} + K_i$



$\Psi_{\text{stem}} = -2.38 \text{ MPa}$
 $\phi = 857.34 \text{ } \mu\text{m}$



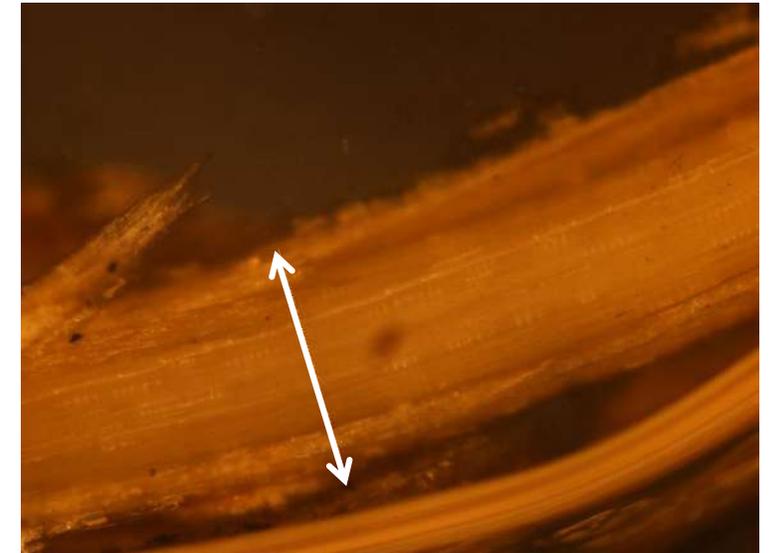
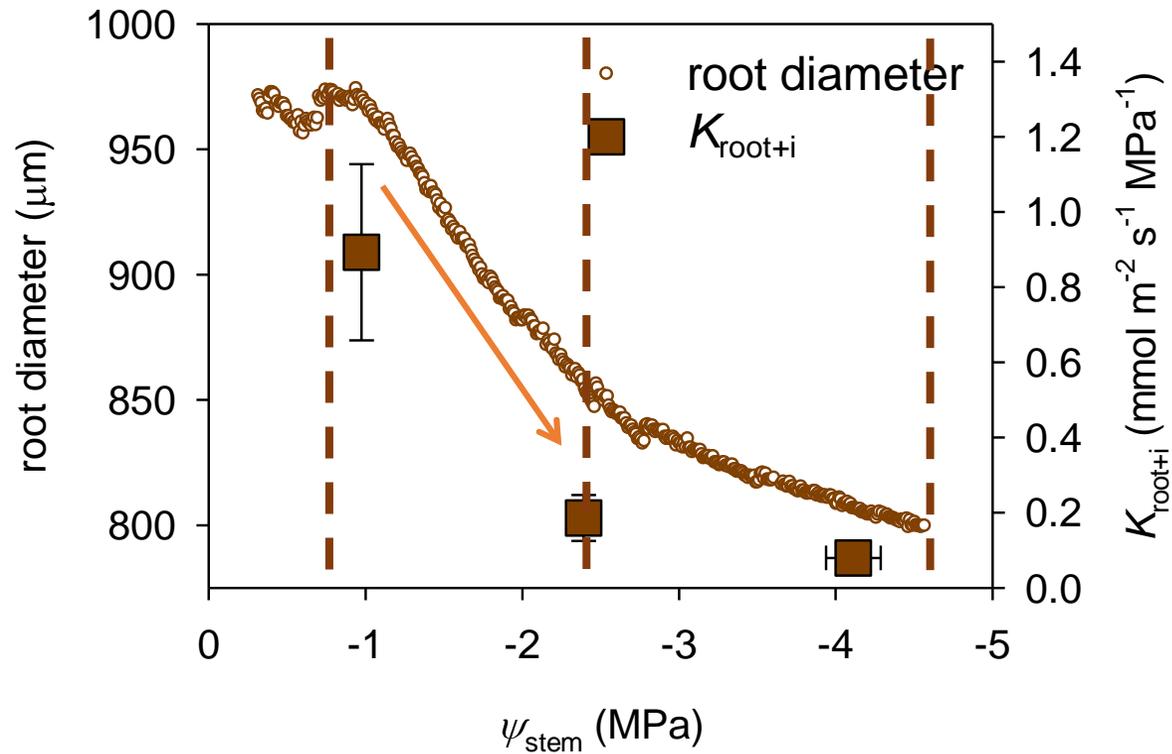
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

DISCONNECTING THE ROOT FROM THE SOIL

$$1/K_{\text{plant}} = 1/K_{\text{shoot}} + 1/K_{\text{root+i}}$$

$K_{\text{shoot}} = K_{\text{leaf}} + K_{\text{stem}}$
 $K_{\text{root+i}} = K_{\text{root}} + K_i$



$\Psi_{\text{stem}} = -4.73 \text{ MPa}$
 $\phi = 798.12 \mu\text{m}$



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --



Research

Rapid report

Declining root water transport drives stomatal closure in olive under moderate water stress

Celia M. Rodriguez-Dominguez^{1,2}  and Timothy J. Brodribb² 

$$1/K_{\text{plant}} = 1/K_{\text{shoot}} + 1/K_{\text{root+i}}$$

*K*_{leaf} *K*_{stem} *K*_{root} *K*_i

- ➔ Novel combination of rehydration techniques allowed us to partition hydraulic pathways within the plant
- ➔ A decrease of $K_{\text{root+i}}$ drove a decrease in K_{plant} ==> stomatal closure
- ➔ Our method provides new opportunities to study root behaviour, its connection with the soil and its impact on plant hydraulics

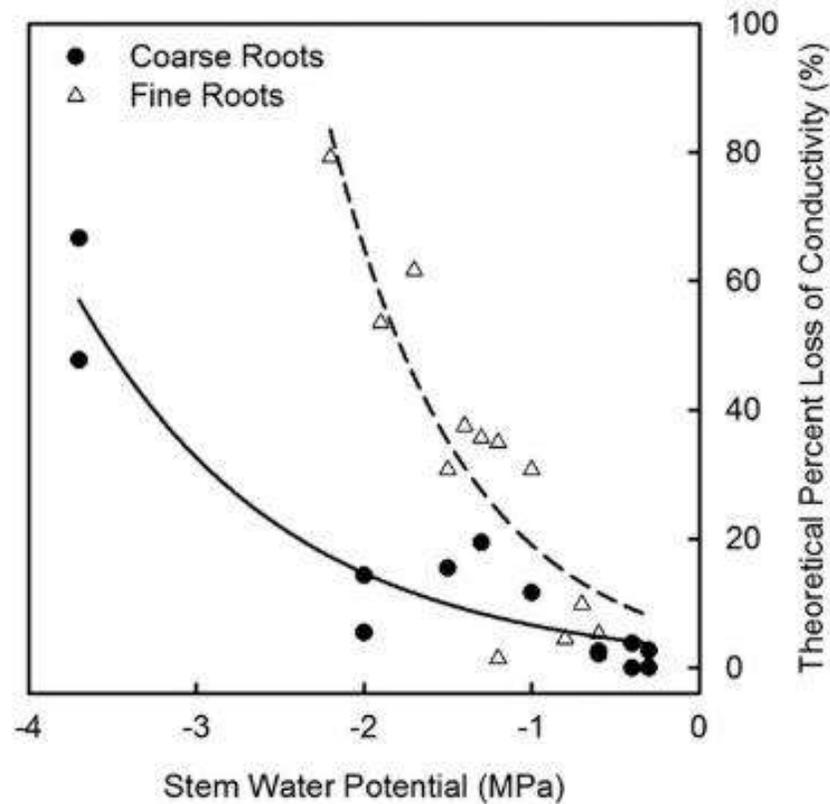


NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

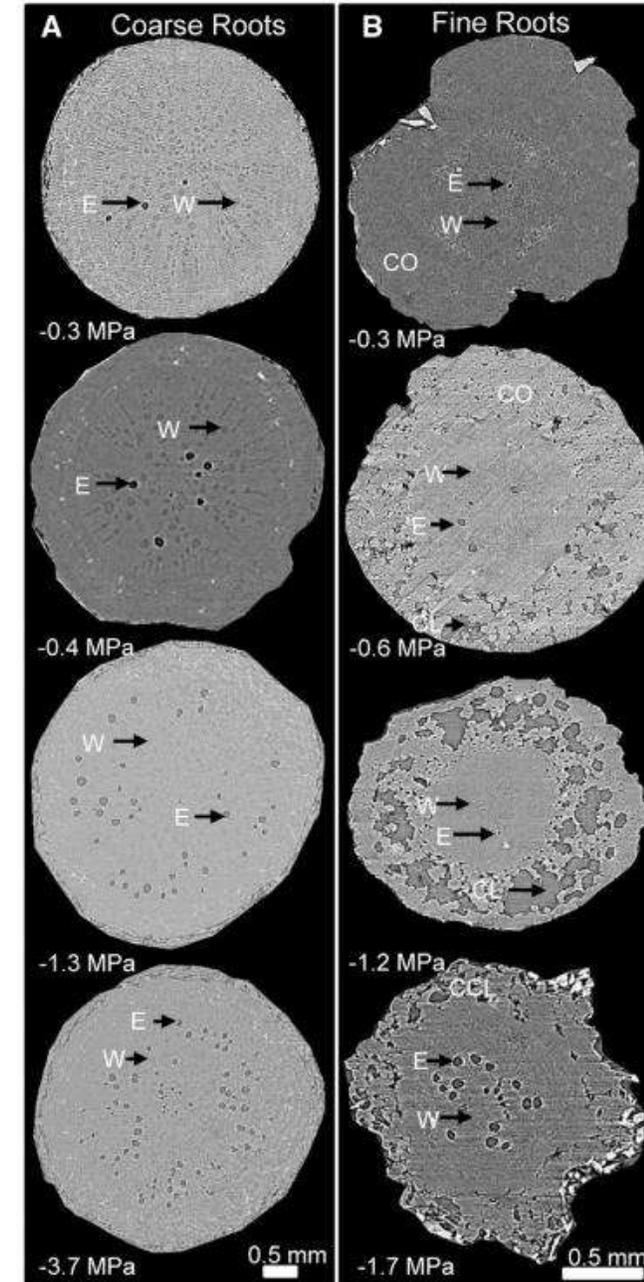
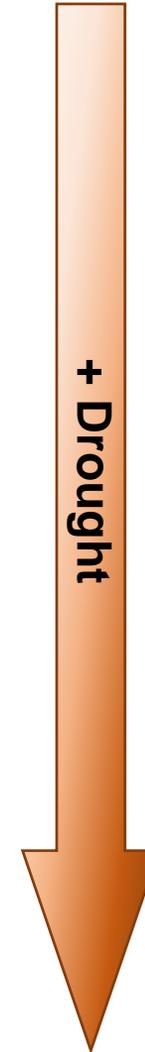
-- WITH PRACTICAL APPLICATIONS --

Mechanical Failure of Fine Root Cortical Cells Initiates Plant Hydraulic Decline during Drought^{1[OPEN]}

Italo F. Cuneo, Thorsten Knipfer, Craig R. Brodersen, and Andrew J. McElrone*



What about the soil and soil-root interactions????

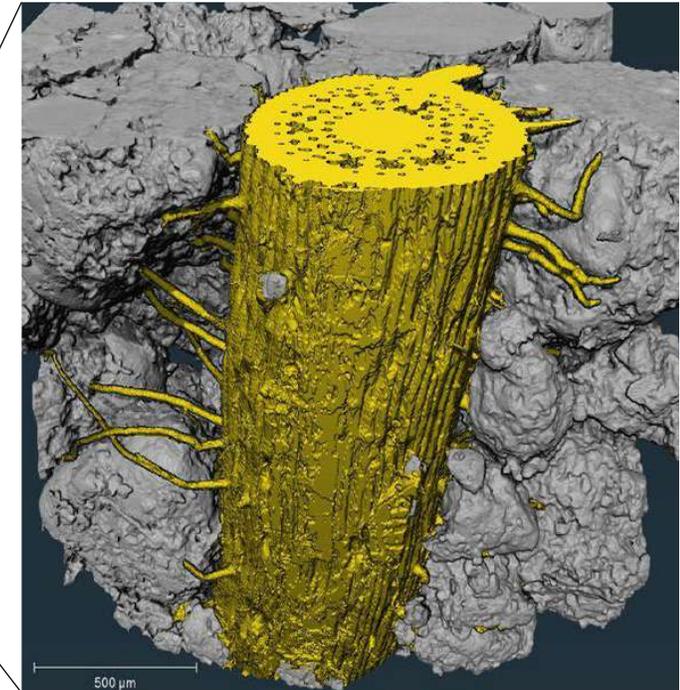
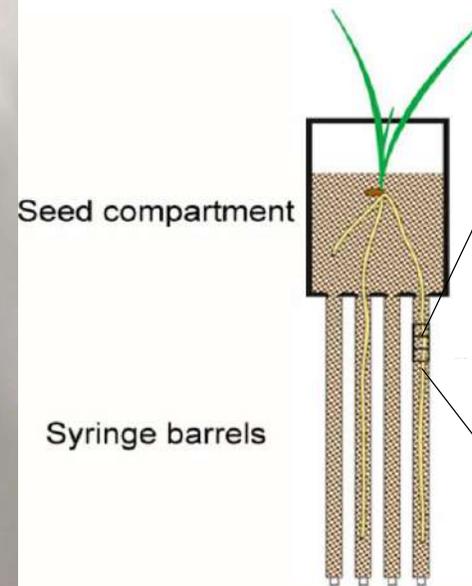


NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --



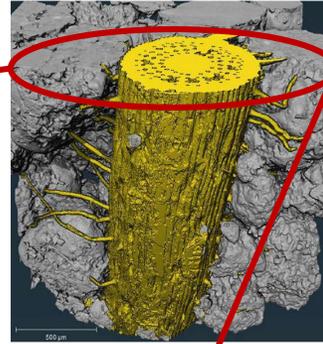
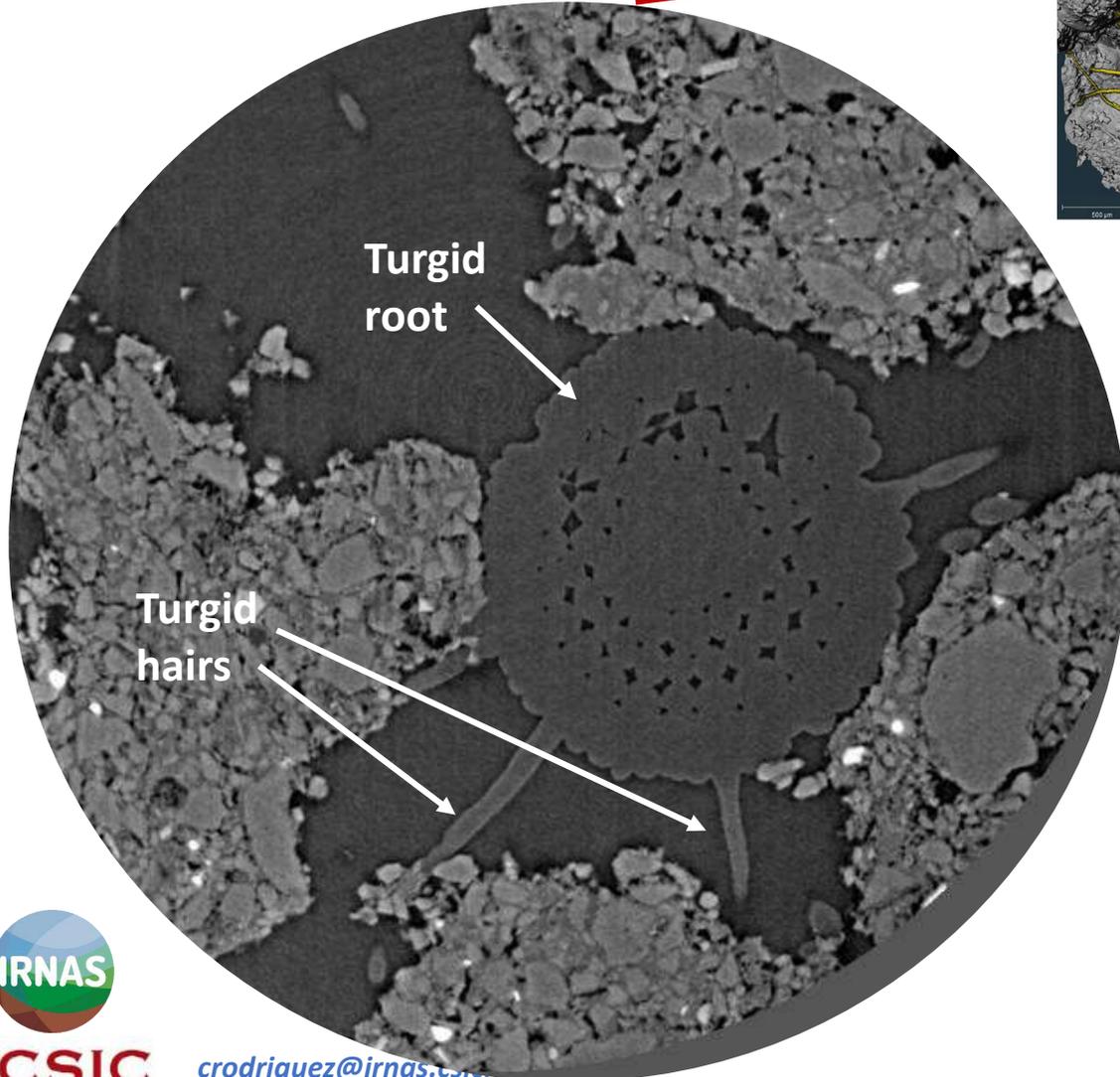
Duddek et al, 2022, *Plant Physiol*



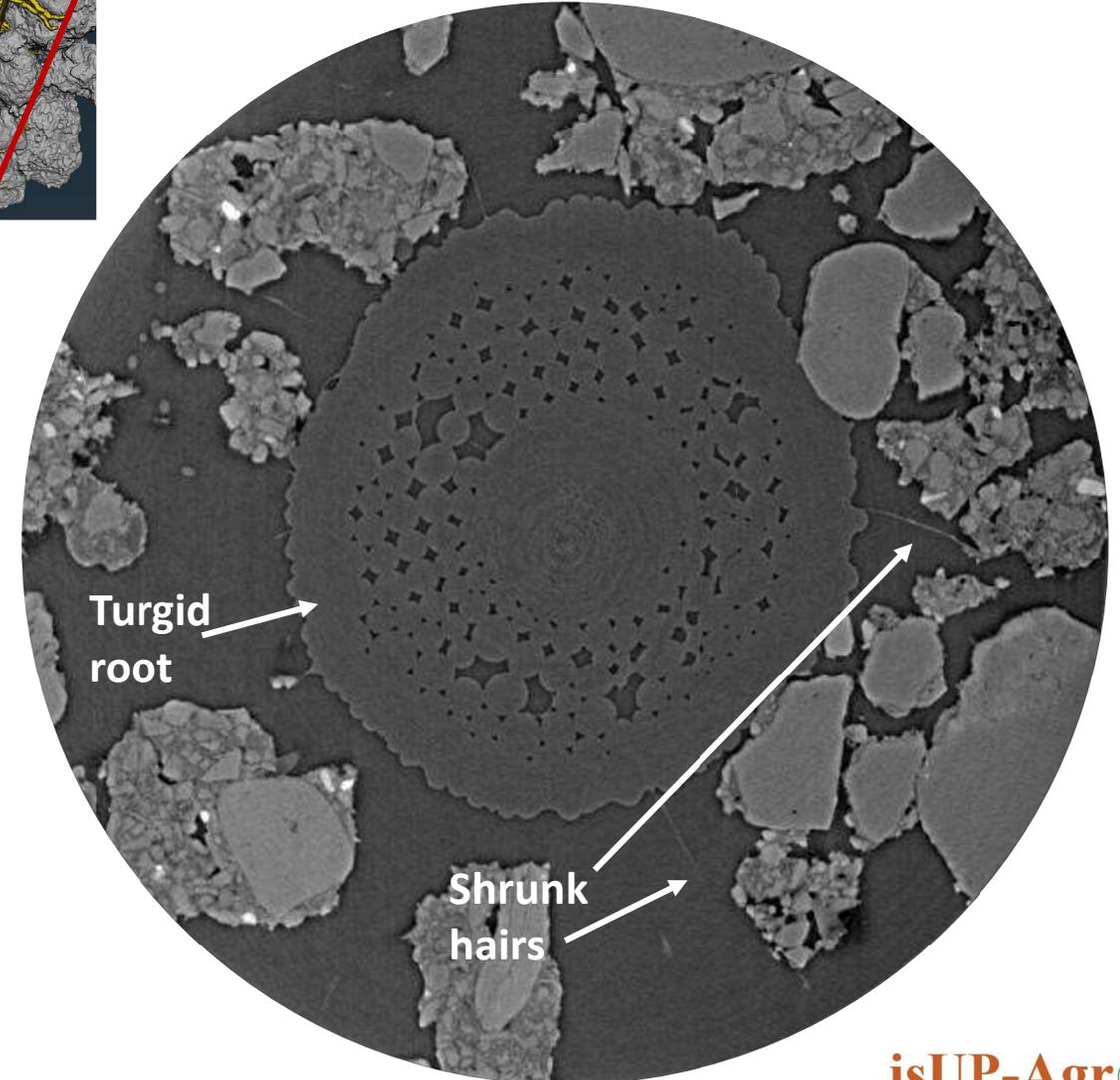
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

-0.01 to -0.1 MPa



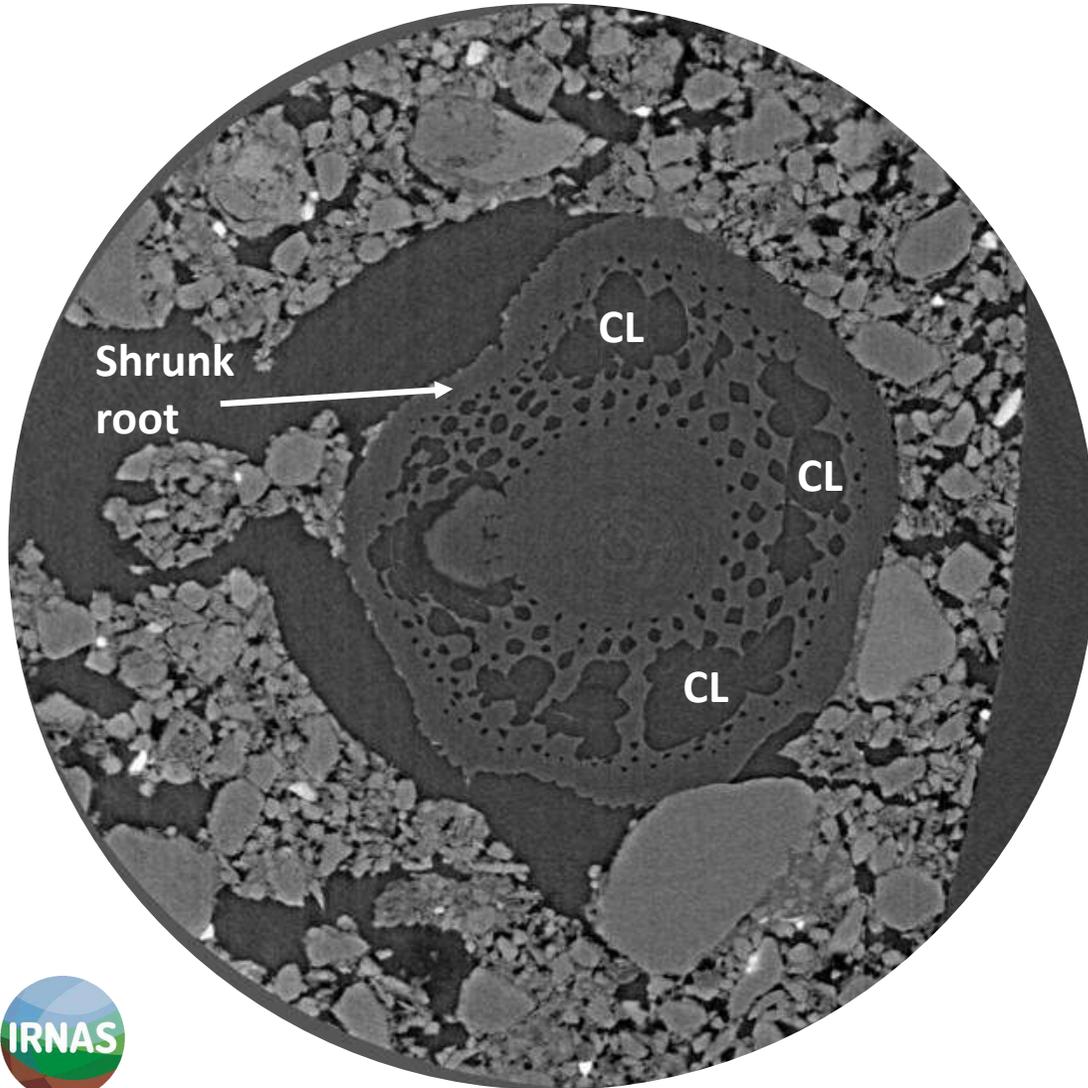
-0.1 to -0.3 MPa



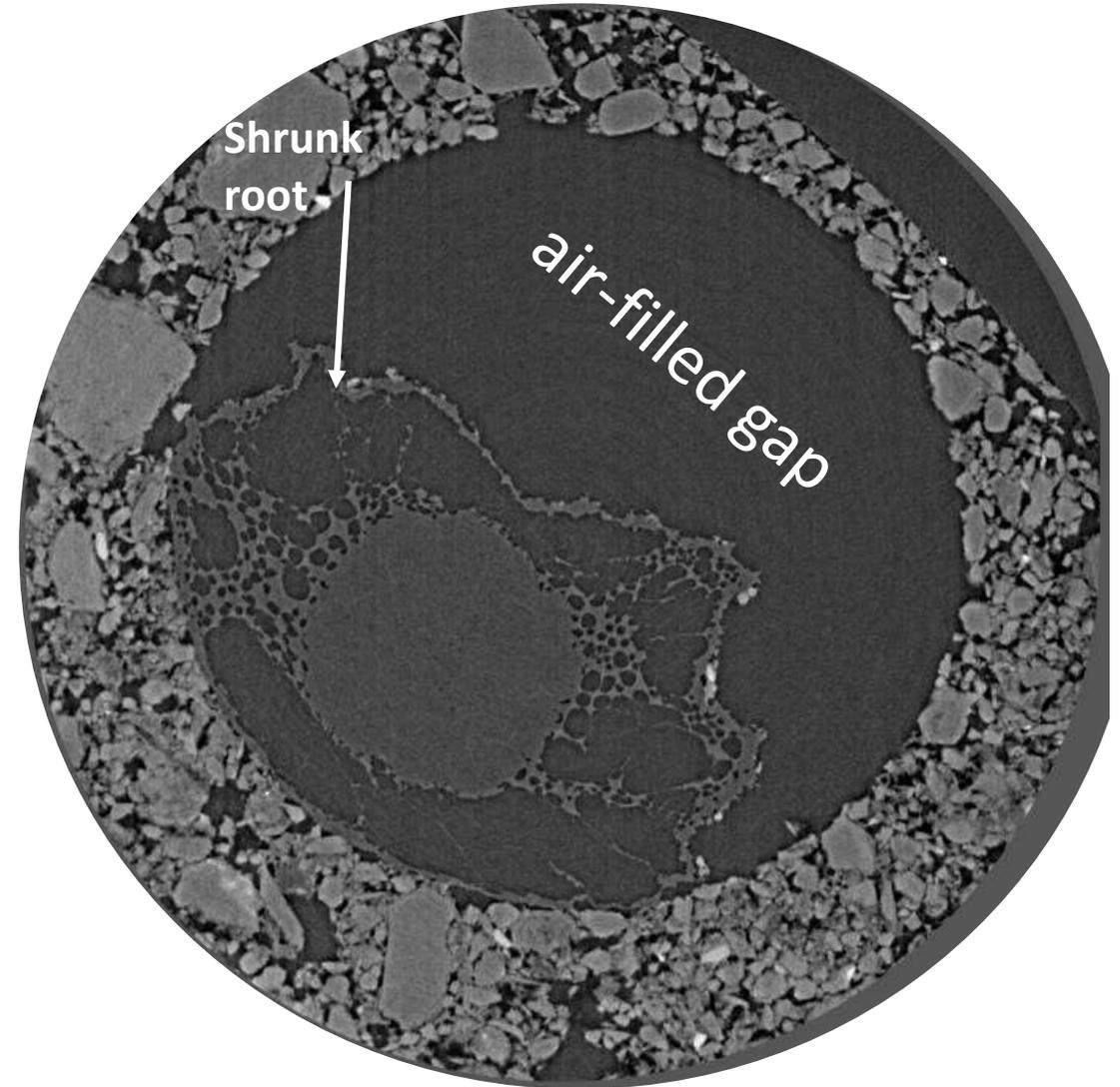
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

-0.6 to -1.5 MPa

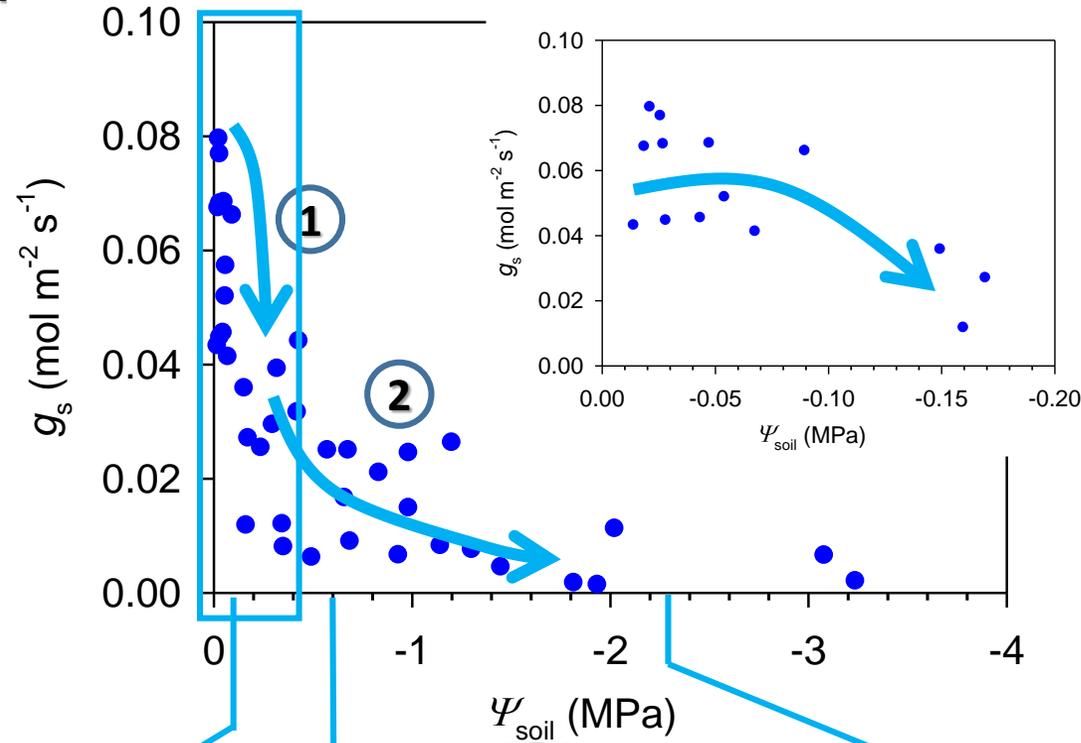
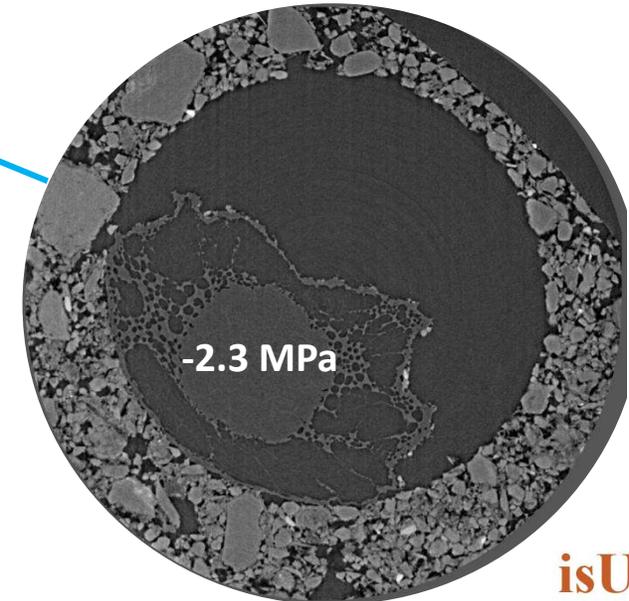
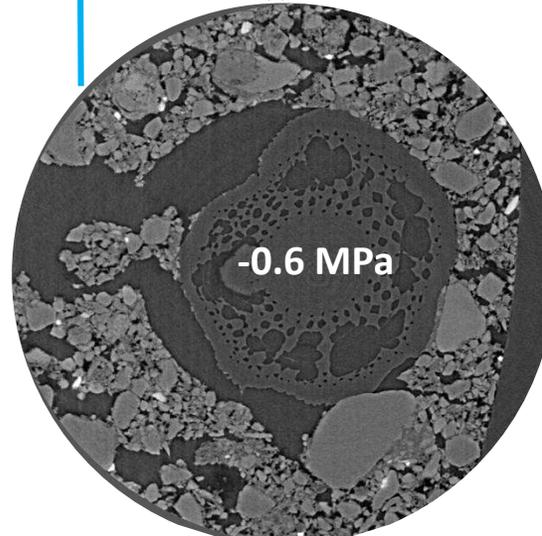
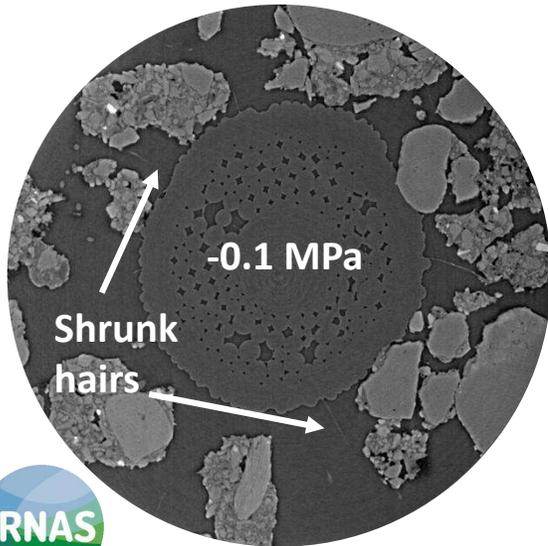
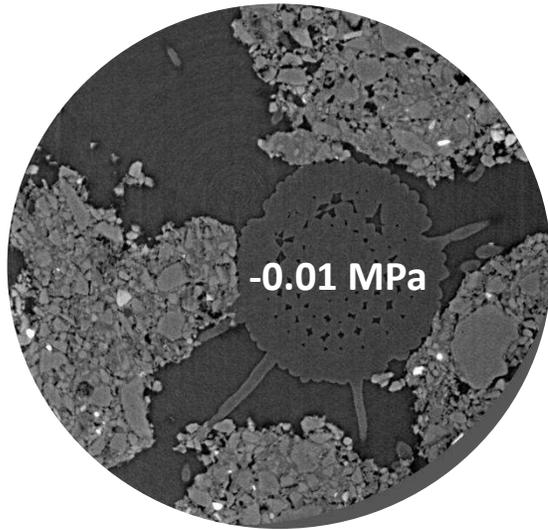


-2.0 to ... MPa



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICA



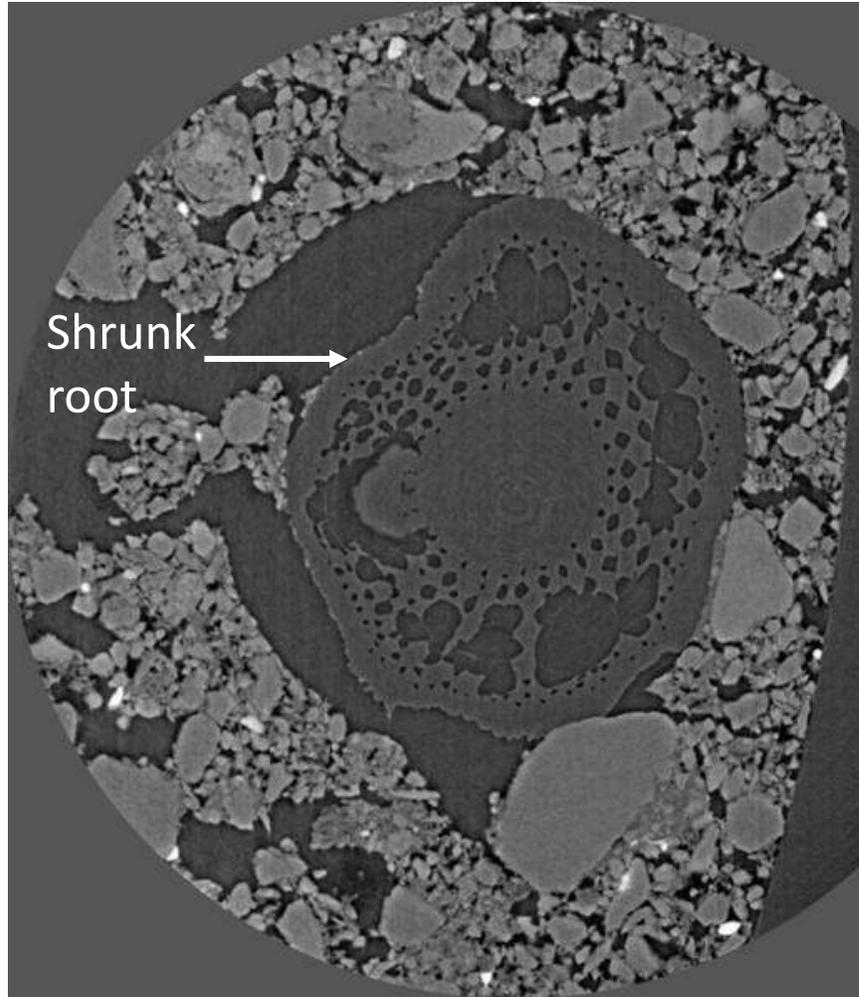
- 1 Steep g_s decline
Root hairs shrunk
- 2 Slower g_s decrease
Cortical lacunae formation,
collapse of cortical cells
Root shrinkage
No xylem embolisms!

Rodriguez-Dominguez et al, *in prep.*

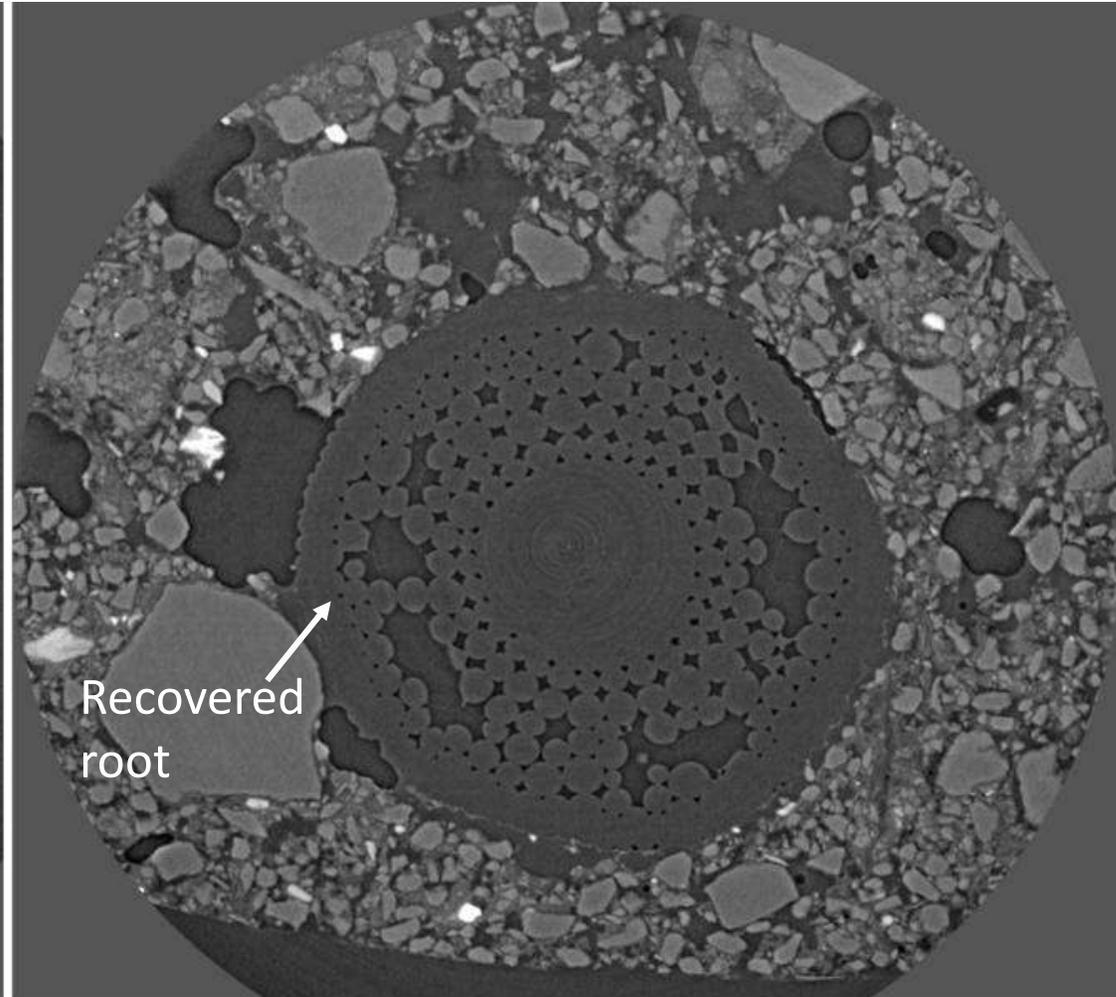
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --

BELOWGROUND PROCESSES – SOIL-ROOT CHANGES DURING SOIL DROUGHT AND RECOVERY



Before -0.6 MPa



After 3-4 hours



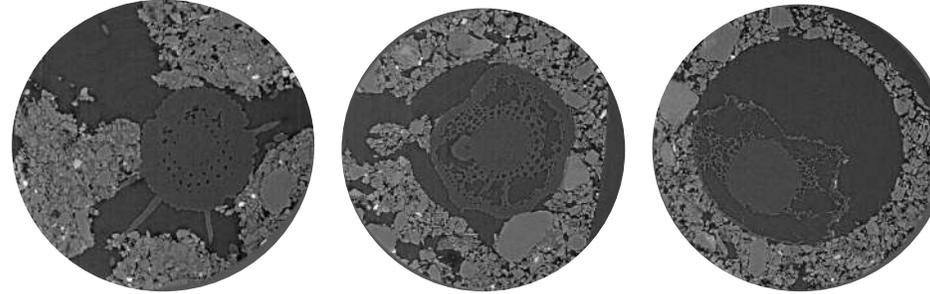
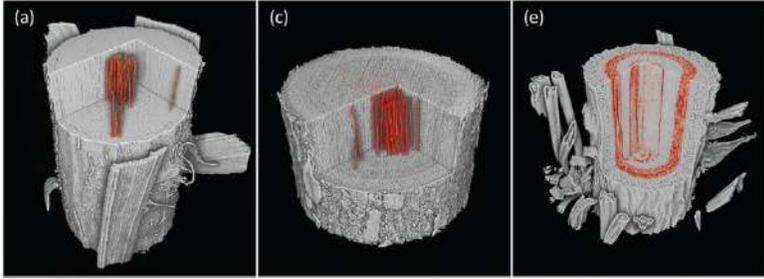
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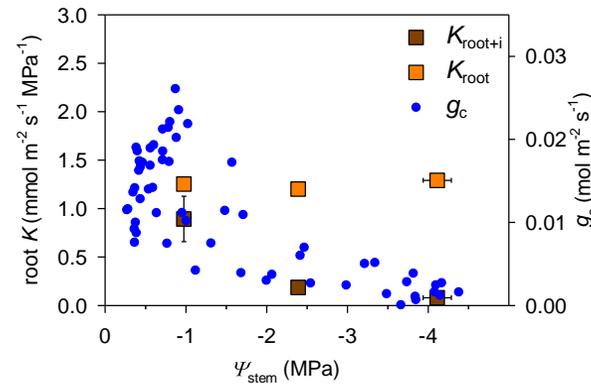
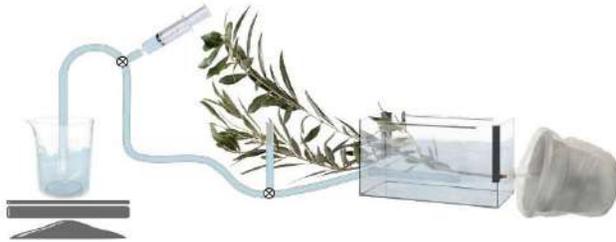
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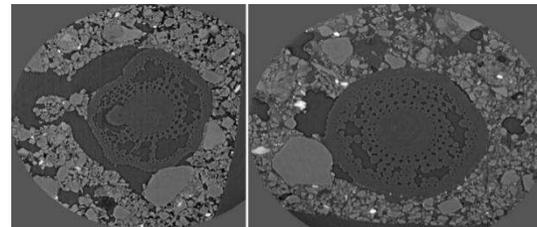
➔ New insights on root hydraulics have been generated from both advances on high resolution *in vivo* imaging...



...and new experimental approaches.



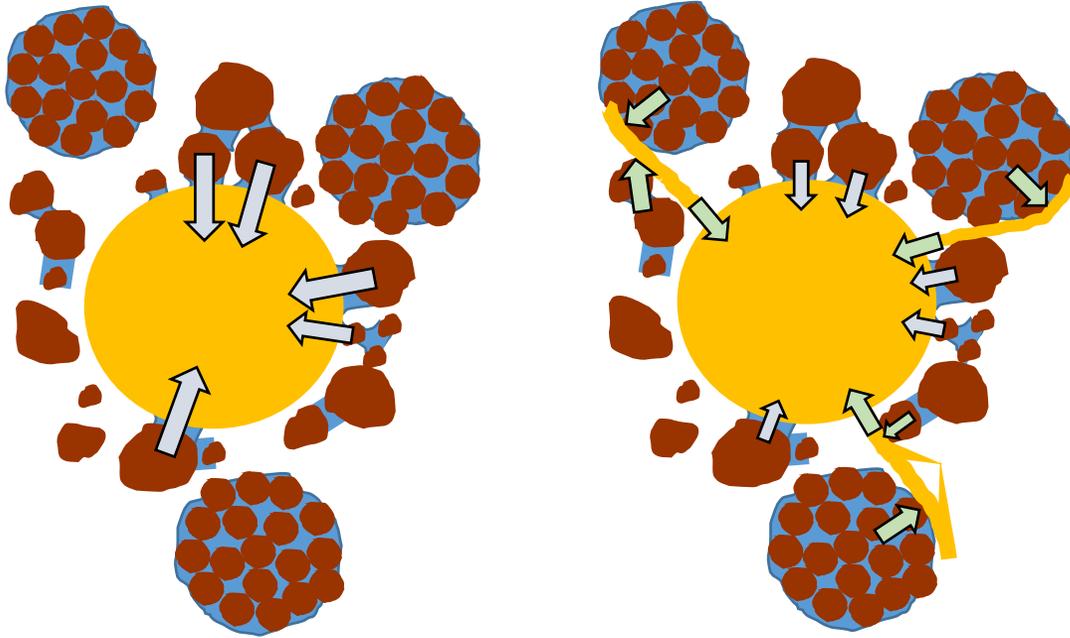
➔ We still need to apply these advances on different plant species and soil types to generalize these processes. In particular, to identify anatomical changes that modify soil-root interactions during soil drought, impact root water uptake, and recover both anatomical and functionally.



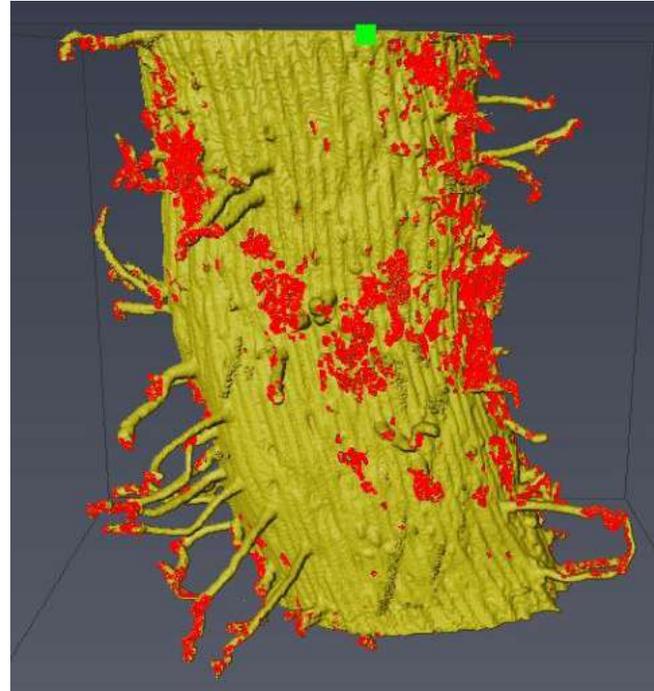
NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

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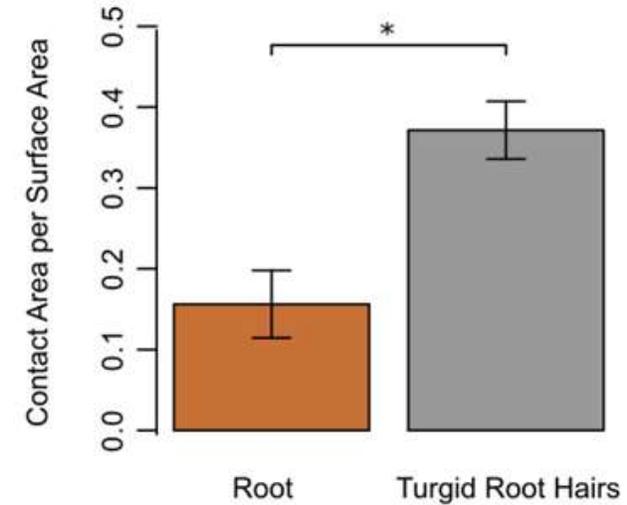
➔ Linking anatomical changes with their functional implications, both during drought and recovery, can help us to better understand belowground processes.



Red: root-soil contact



Root hairs facilitate water extraction by **increasing the root surface in contact with soil water**

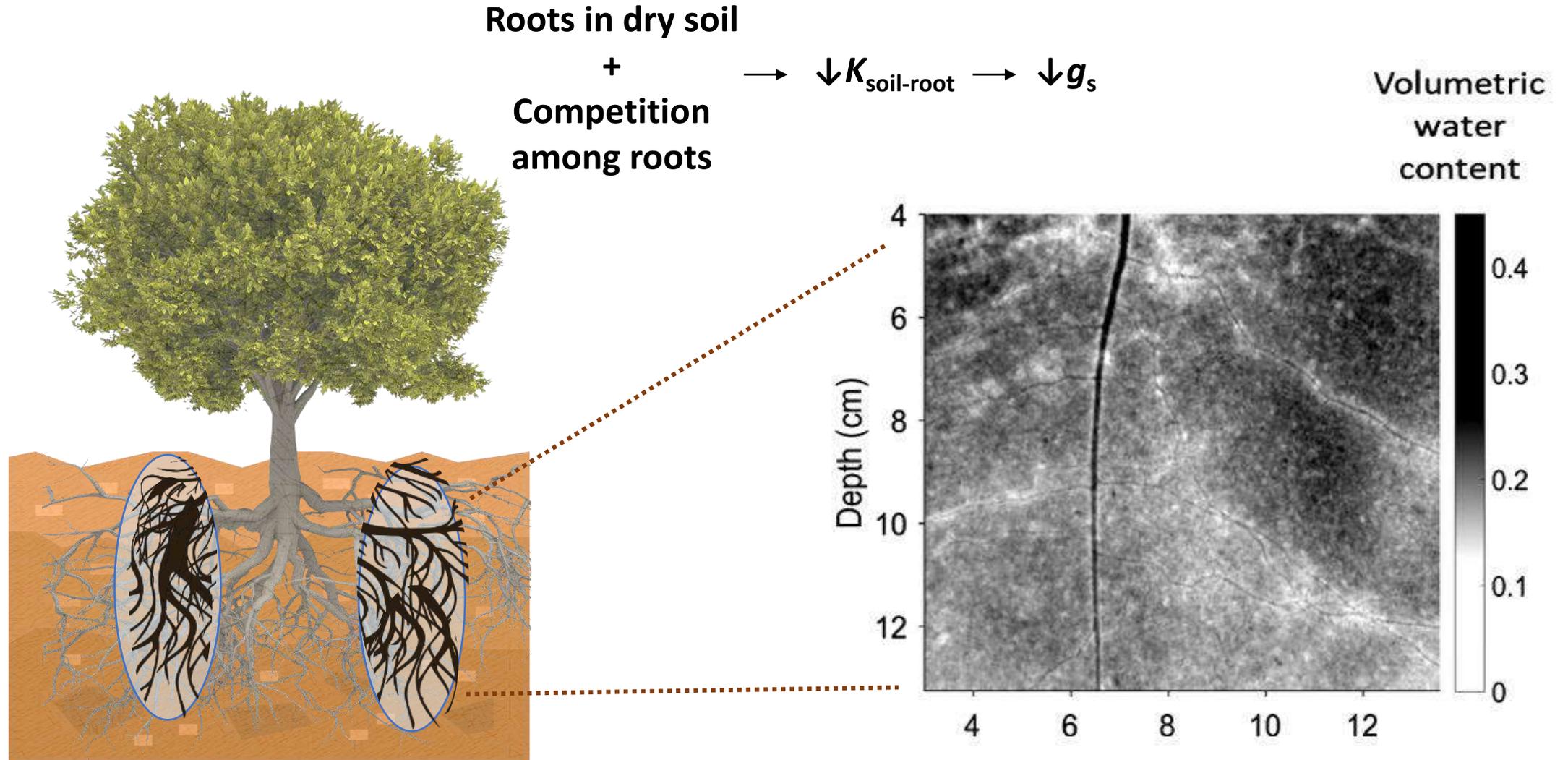


Duddek et al, 2022, *Plant Phys*



NEW INSIGHTS FROM NEW METHODS IN PLANT HYDRAULICS

-- WITH PRACTICAL APPLICATIONS --



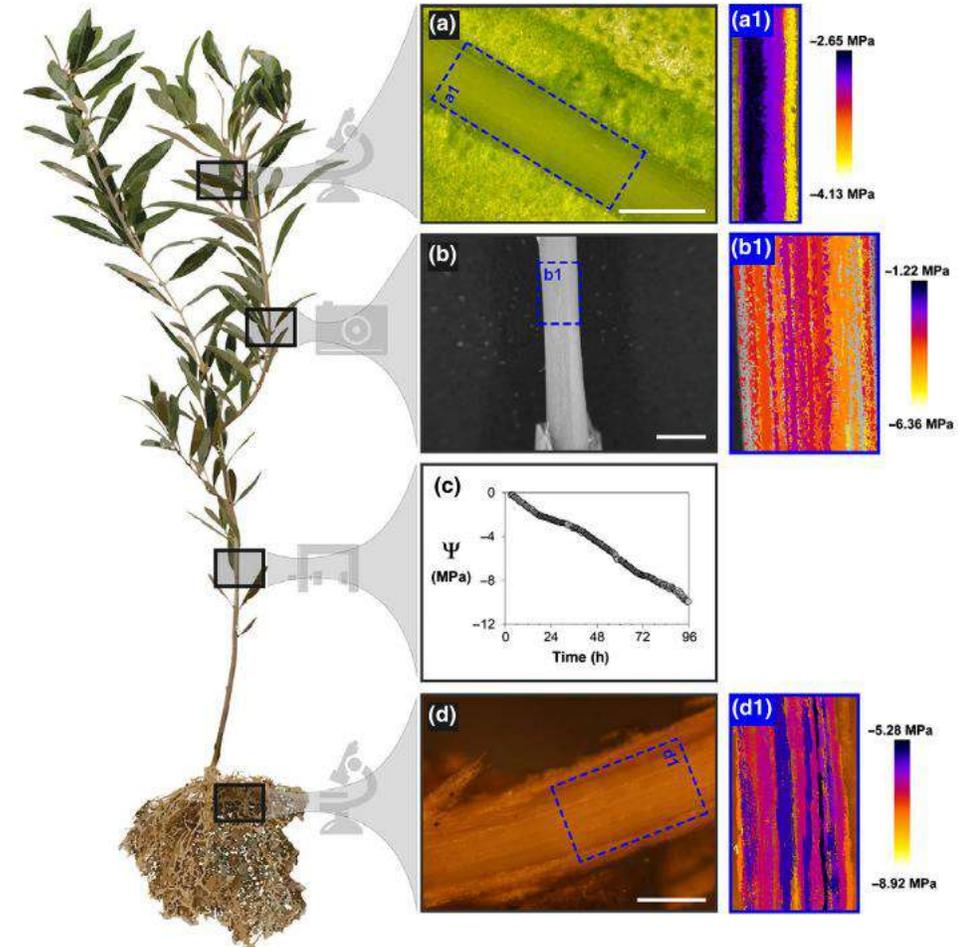
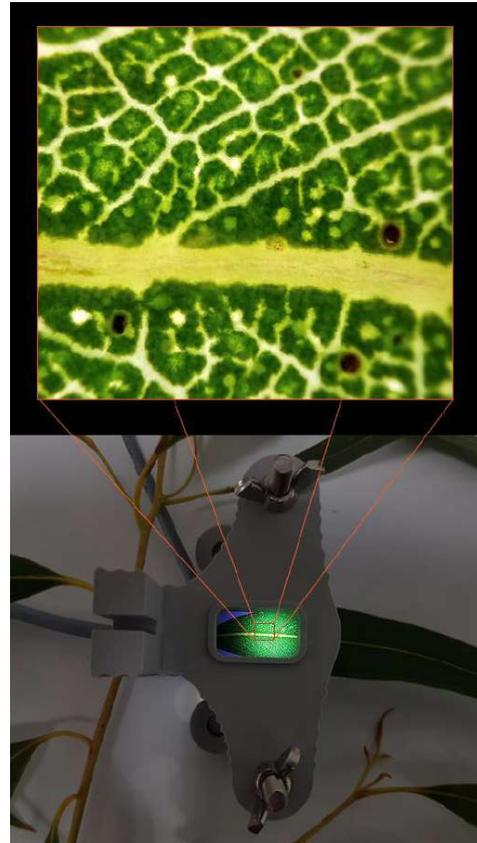
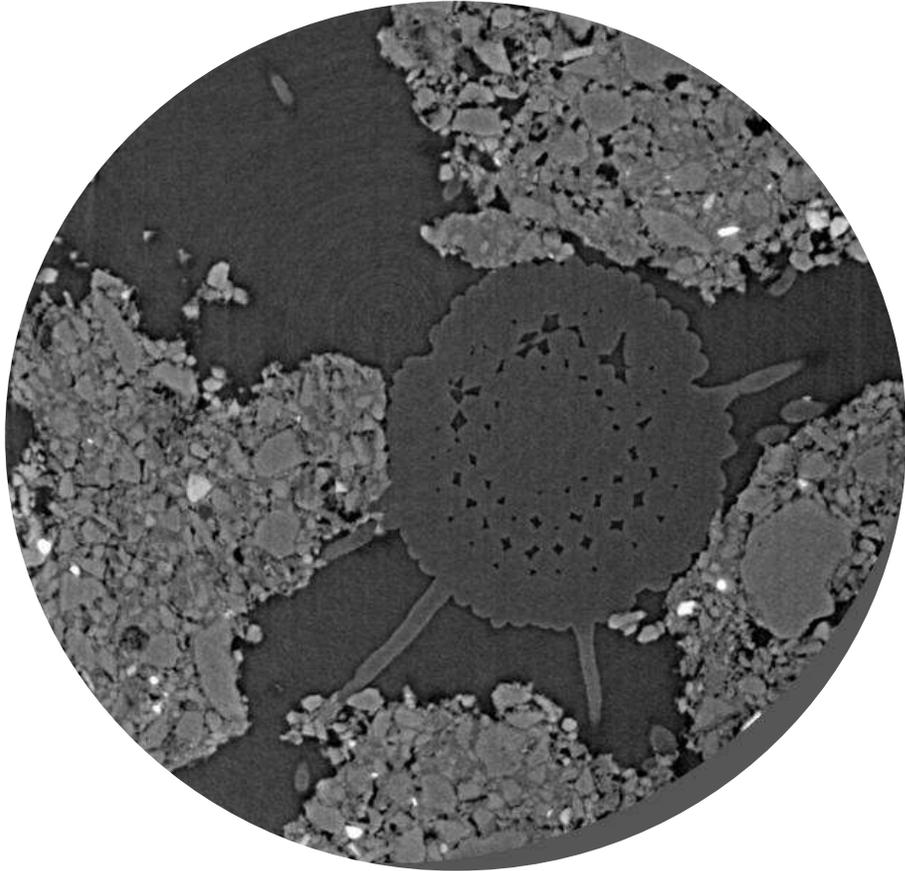
Carminati et al 2020 *New Phytol*



crodriguez@irnas.csic.es



THANK YOU!! QUESTIONS??



crodriguez@irnas.csic.es

2nd Training School - 27th January to 31st January 2025



Sensing Connected to Agriculture: Leaf Turgor Pressure-Related Sensor

Celia M. Rodríguez Domínguez

2nd Training School

27th January to 31st January 2025



CSIC

isUP-Agr 

UNLOCKING THE POTENTIAL FOR AGRICULTURAL
RESEARCH ON AN EU OUTMOST REGION:
BOOSTING ISOPLEXIS CENTRE



ISOPlexis
Centro de Agricultura Sustentável e Tecnologia Alimentar

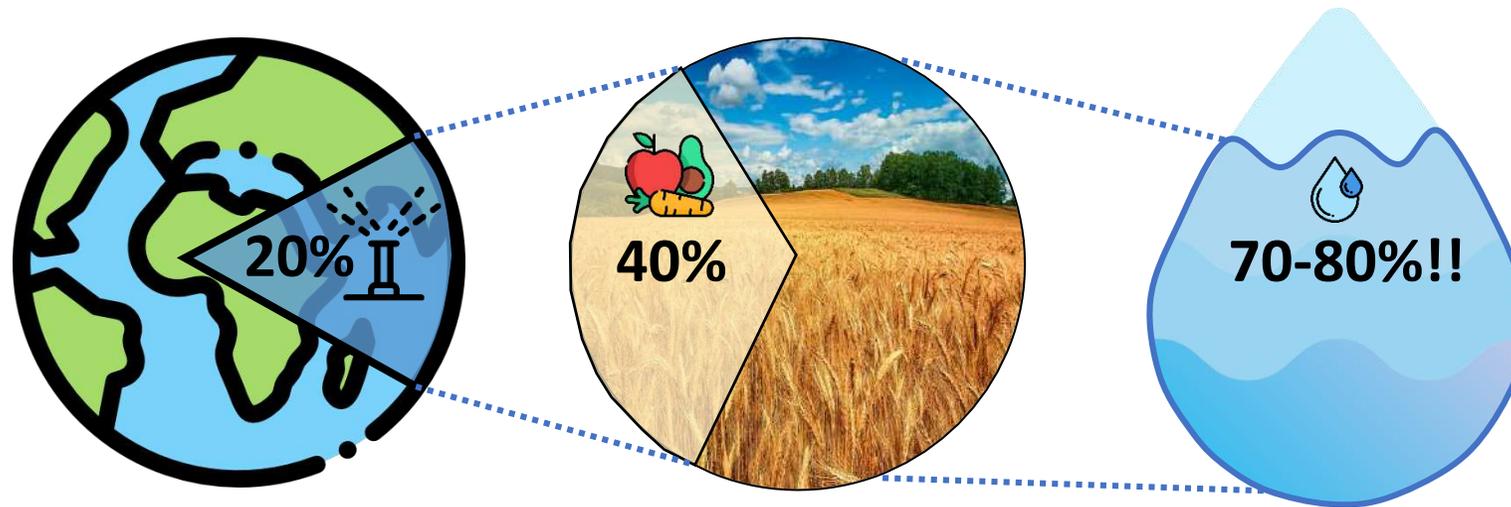
UNIVERSIDADE da MADEIRA

OBJECTIVES

- **To understand the principles of leaf turgor pressure and its importance in plant water status.**
- **To gain insights into the methodologies for using turgor pressure sensors effectively.**
- **To learn practical use of leaf turgor pressure sensors in precision irrigation practices to optimize water use.**



IMPORTANCE OF SENSING TECHNOLOGIES IN AGRICULTURE



FAOSTAT 2023

Fereres & Soriano 2007 *Journal Exp Bot*



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crodriguez@irnas.csic.es

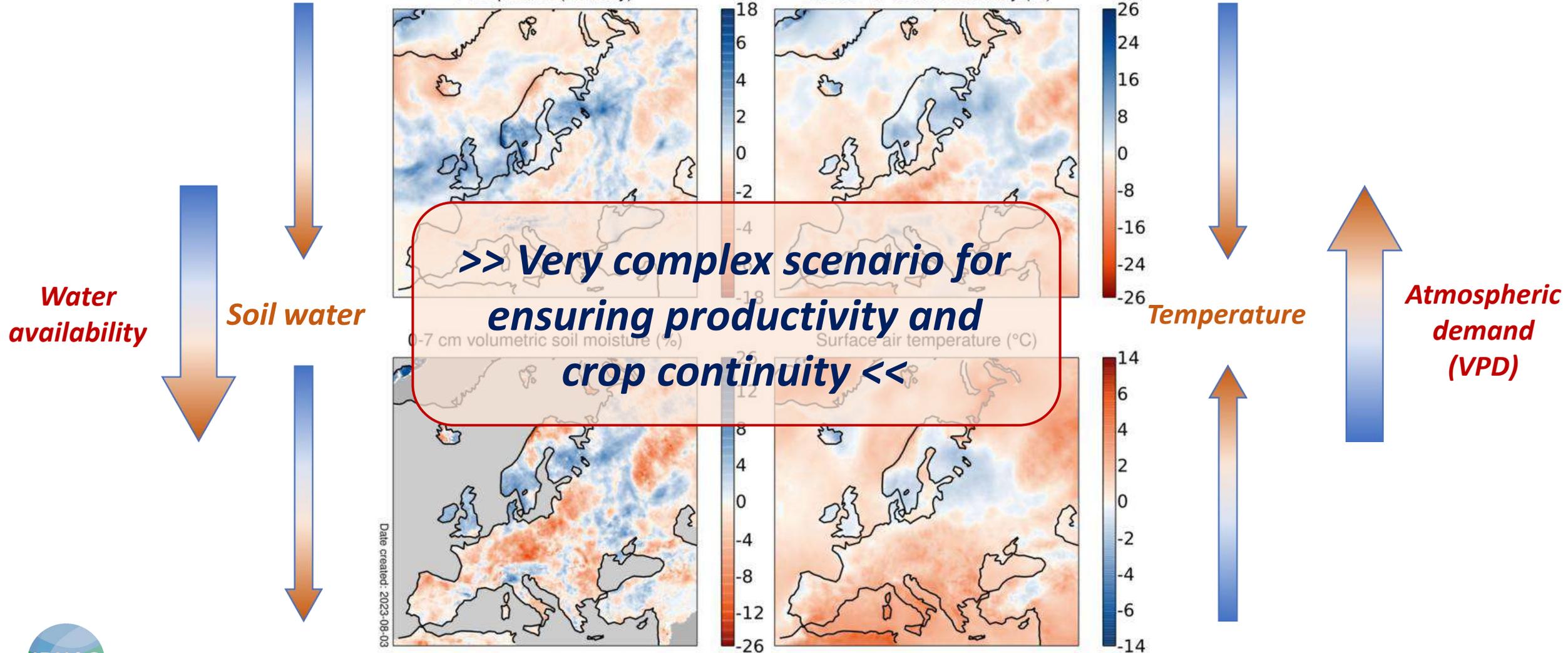
isUP-Agr 

IMPORTANCE OF SENSING TECHNOLOGIES IN AGRICULTURE

Precipitation

Anomalies for July 2023

Humidity



Date created: 2023-08-03

(Data: ERA5. Reference period: 1981-2010. Credit: C3S/ECMWF)



crodriguez@irnas.csic.es

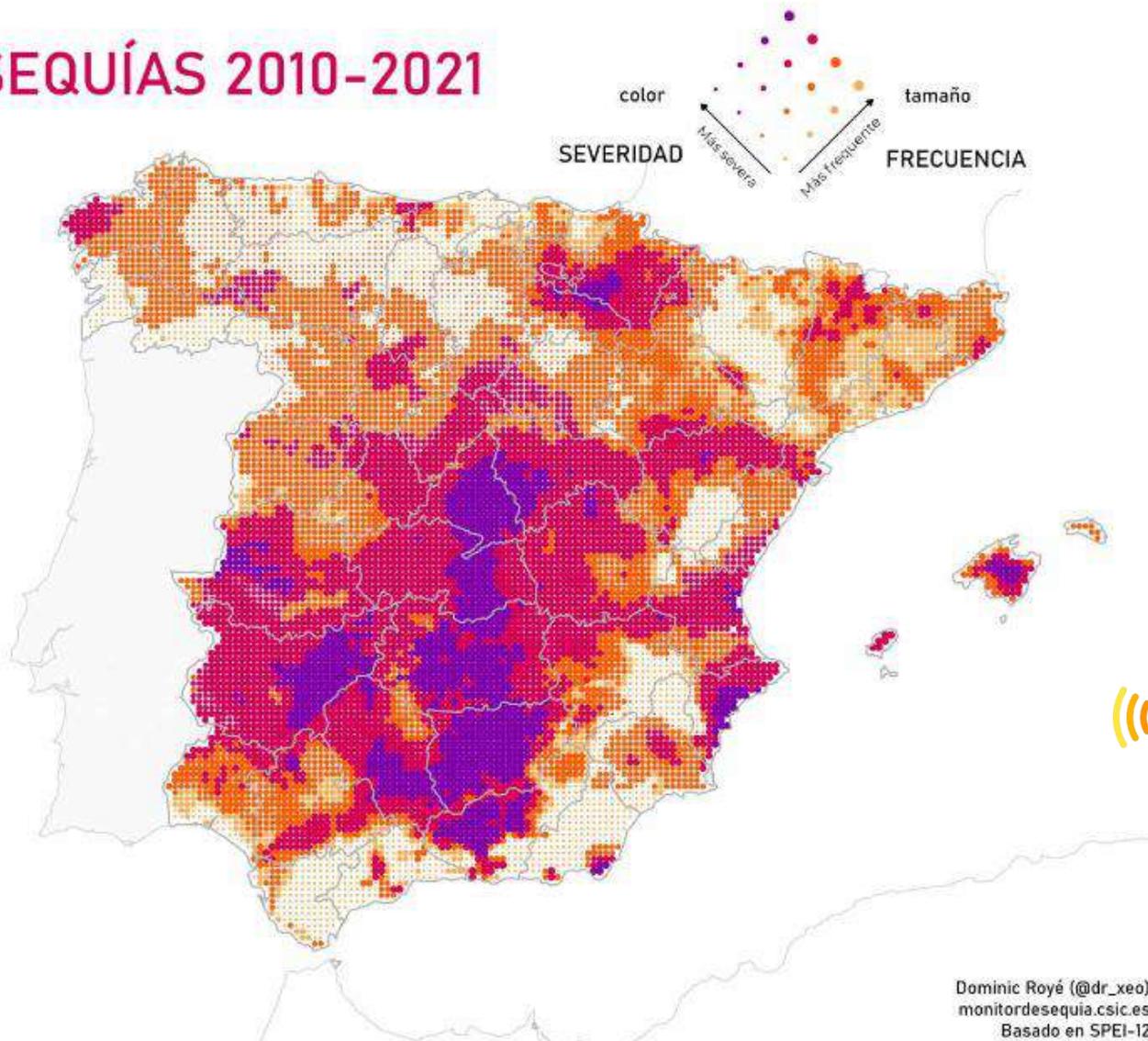


PROGRAMME OF THE EUROPEAN UNION

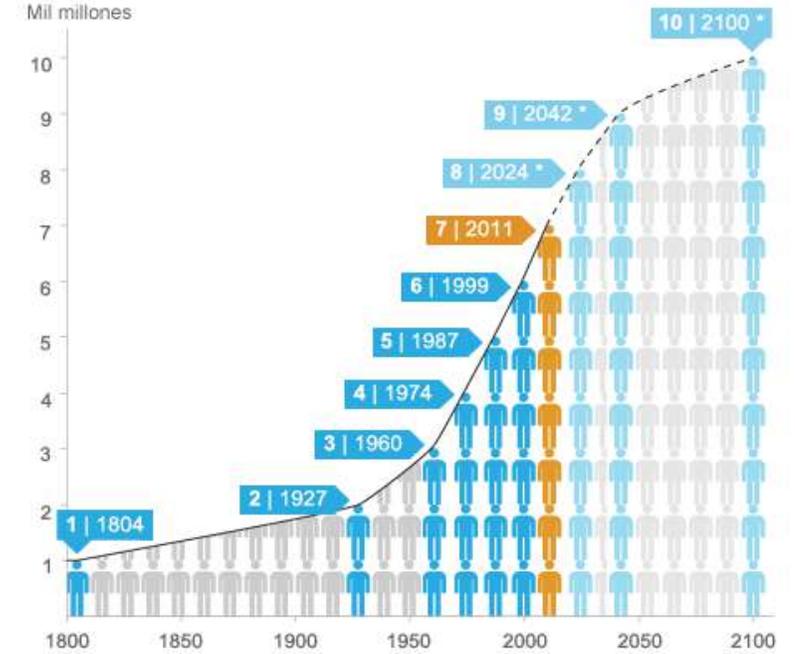


IMPORTANCE OF SENSING TECHNOLOGIES IN AGRICULTURE

SEQUÍAS 2010-2021



Dominic Royé (@dr_xeo)
monitordesequia.csic.es
Basado en SPEI-12



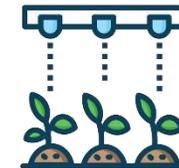
* Cifras poblacionales futuras basadas en las predicciones de la ONU con una variante media
FUENTE: Fondo de Población de la ONU



Agriculture
'under pressure'

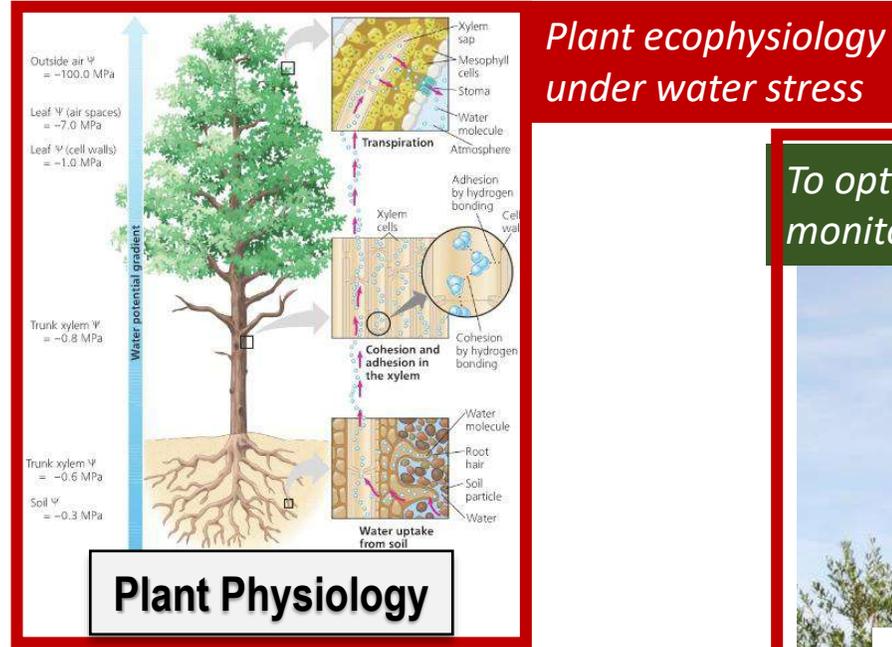


URGENT
Solutions

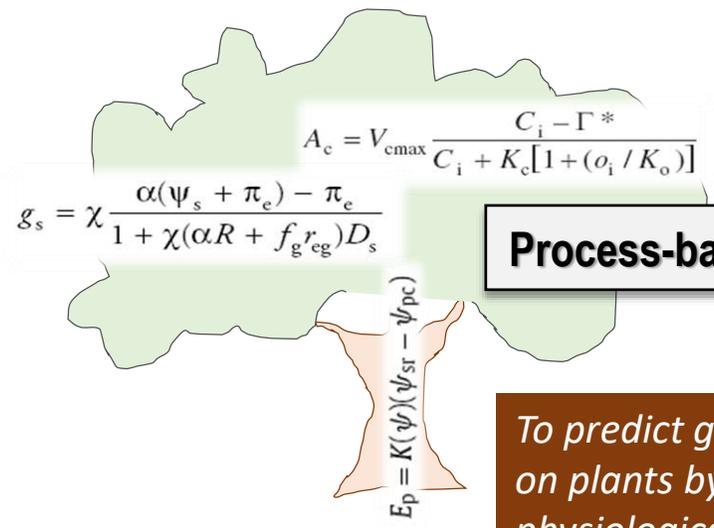


IMPORTANCE OF SENSING TECHNOLOGIES IN AGRICULTURE

- **Essential to optimize water use in Agriculture.**
- **To place plant sensor outputs within a physiological context.**
- **To simplify physiological models.**



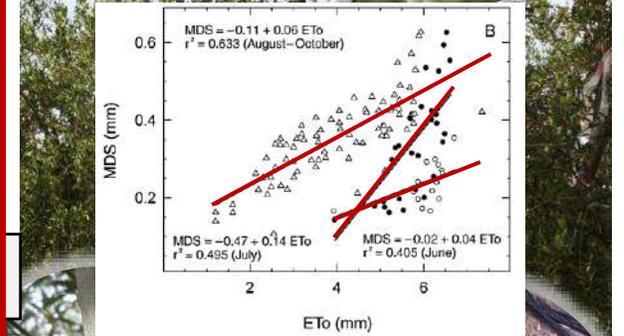
To optimize irrigation strategies by monitoring water stress in real time



Process-based model

Plant-based sensor

To predict global change impacts on plants by integrating disparate physiological responses

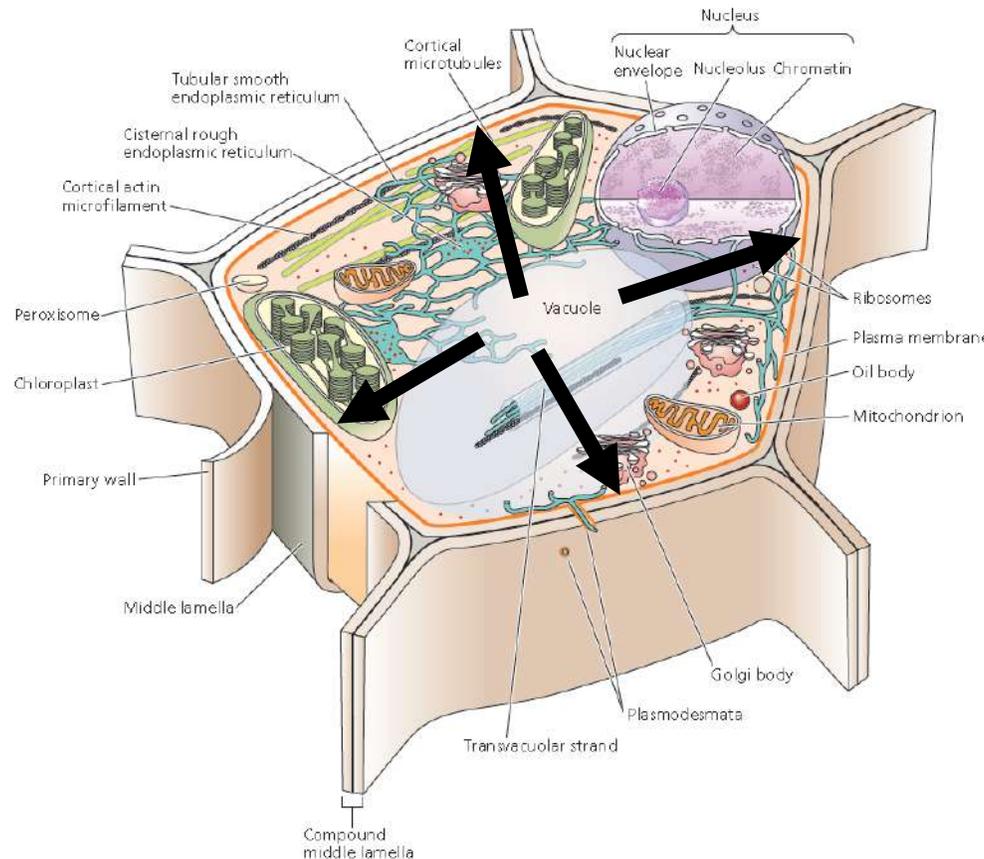


SAP FLOW

BASIC PRINCIPLES: LEAF TURGOR PRESSURE



$$\Psi = \Psi_s + \Psi_p \rightarrow \Psi_p = \Psi - \Psi_s$$



□ Hydrostatic pressure (+) within plant cells that maintains structure and drives growth.

□ Importance for:

- Cell expansion.
- Stomatal function and transpiration regulation.
- Overall plant water status.

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

Leaf Patch Clamp Pressure probe ZIM probe

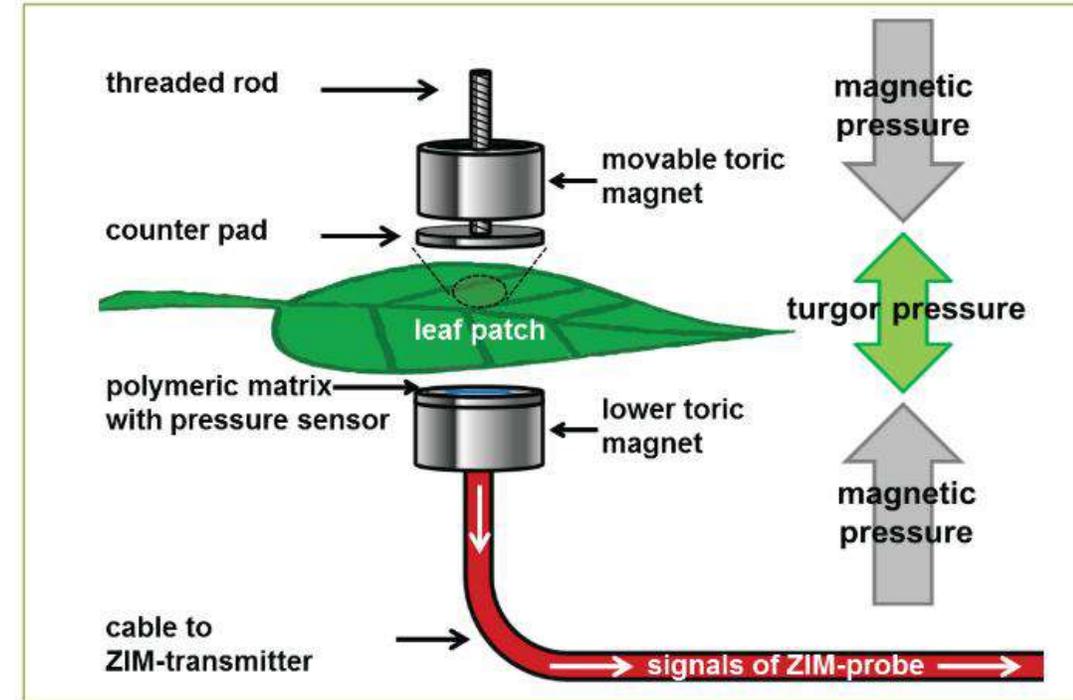


Ulrich Zimmermann
Biozentrum Universität Würzburg
Germany

ZIM probes record *relative changes* of leaf turgor pressure



Zimmermann et al. 2013 *Theor Exp Plant Phys*



BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

$$P_p = \left(\frac{b}{aP_c + b} \right)^{\frac{1}{a}} F_{a,\text{const}} P_{\text{clamp}} \quad P_p \text{ is a power function of } P_c$$

P_p = **Output signal** = Leaf reaction to the attractive force of the magnets

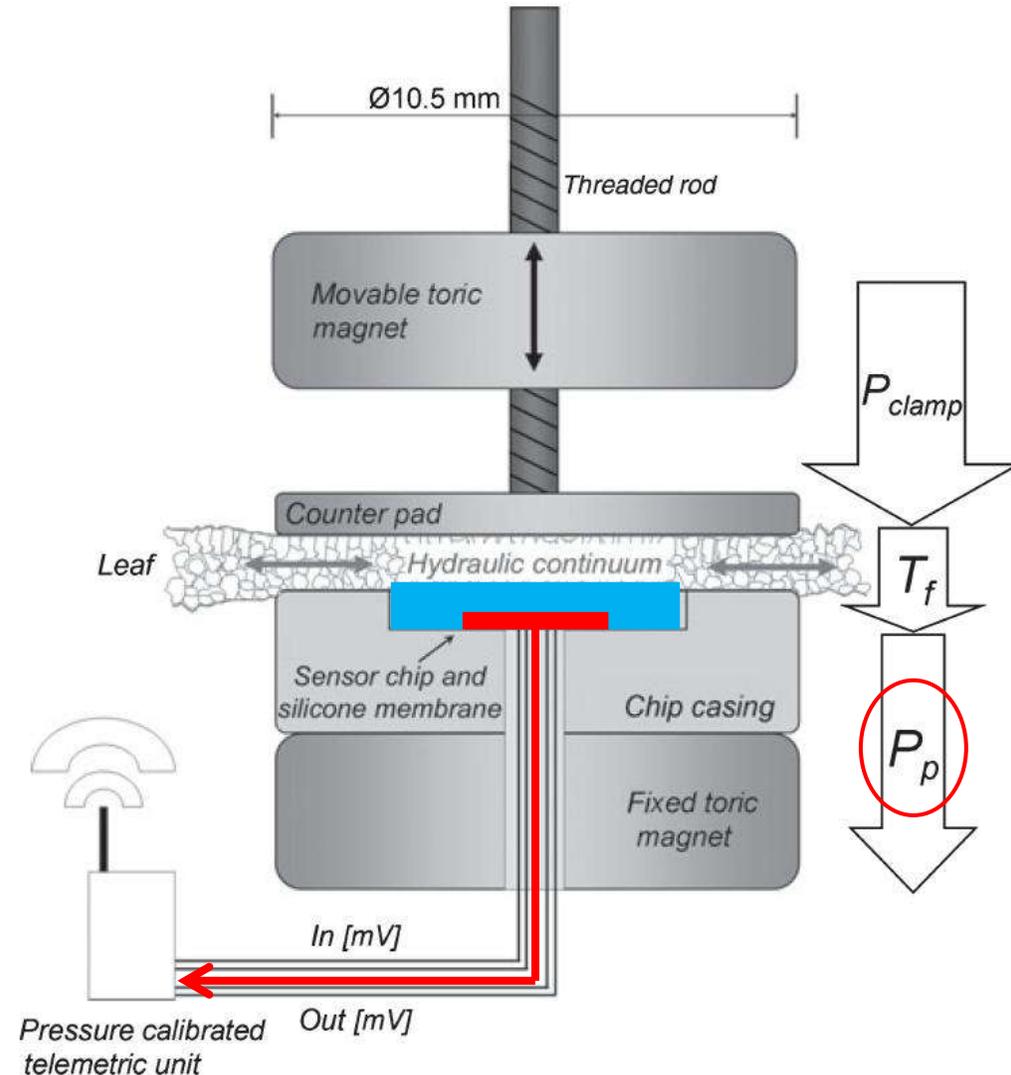
P_c = **Turgor pressure**

P_{clamp} = Pressure generated by the magnet

$F_{a,\text{const}}$ = Attenuation factor = Leaf-specific attenuation factor

** Only a fraction of P_{clamp} arrives at the cell level due to P_c -independent pressure losses [compressibility of the silicone and the leaf-specific structural elements]**

a and b are constants that depends on the species and are related to elastic properties



BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

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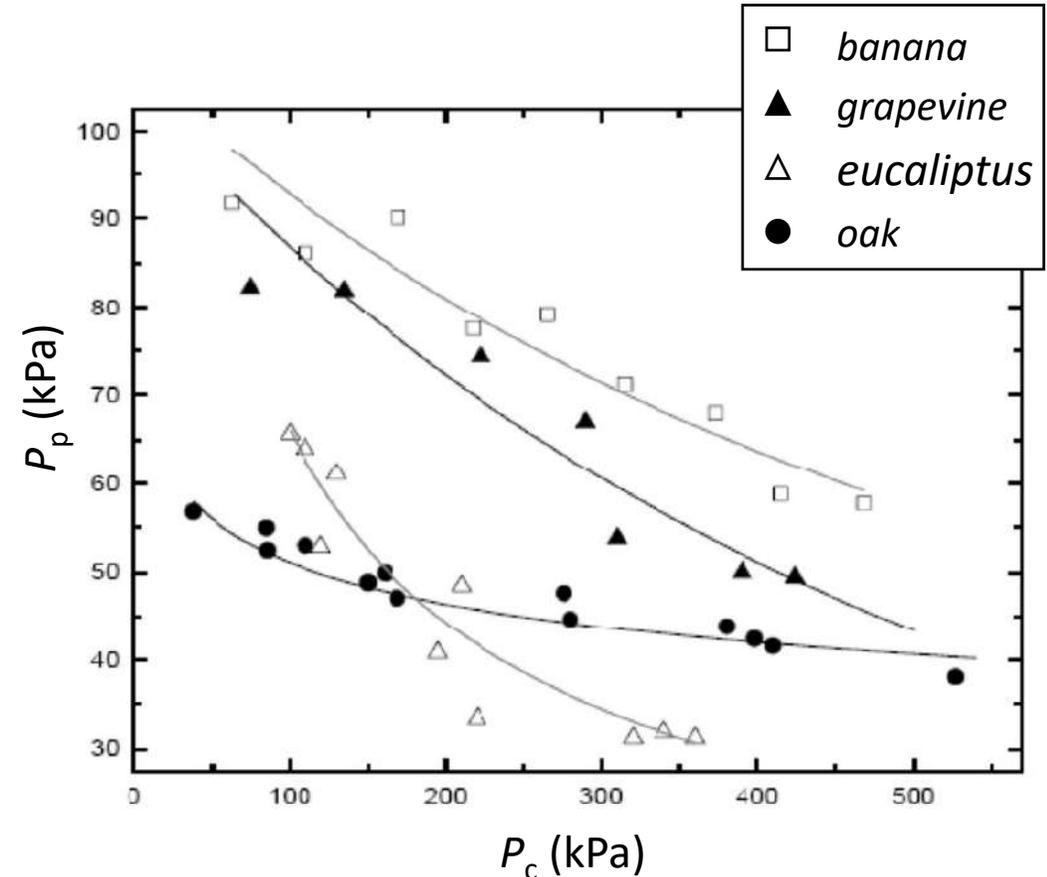
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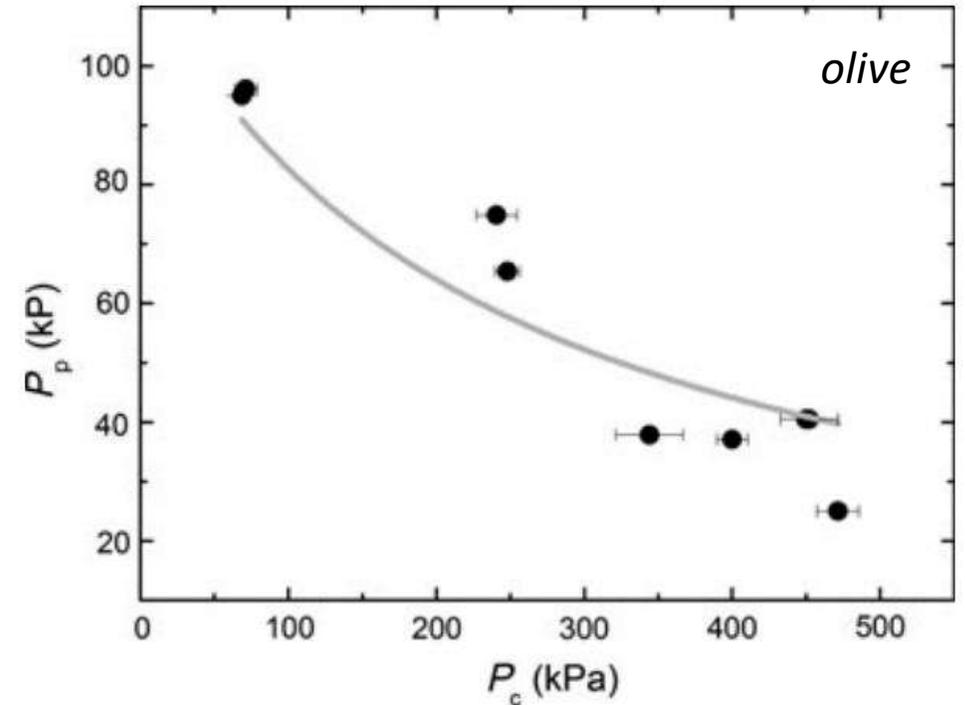
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** Only a fraction of P_{clamp} arrives at the cell level due to P_c -independent pressure [compressibility of the silicone and the leaf-specific structural elements]**

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F_a is constant when $P_c > 100$ kPa (**TURGID LEAVES**) and P_p increases linearly with decreasing P_c



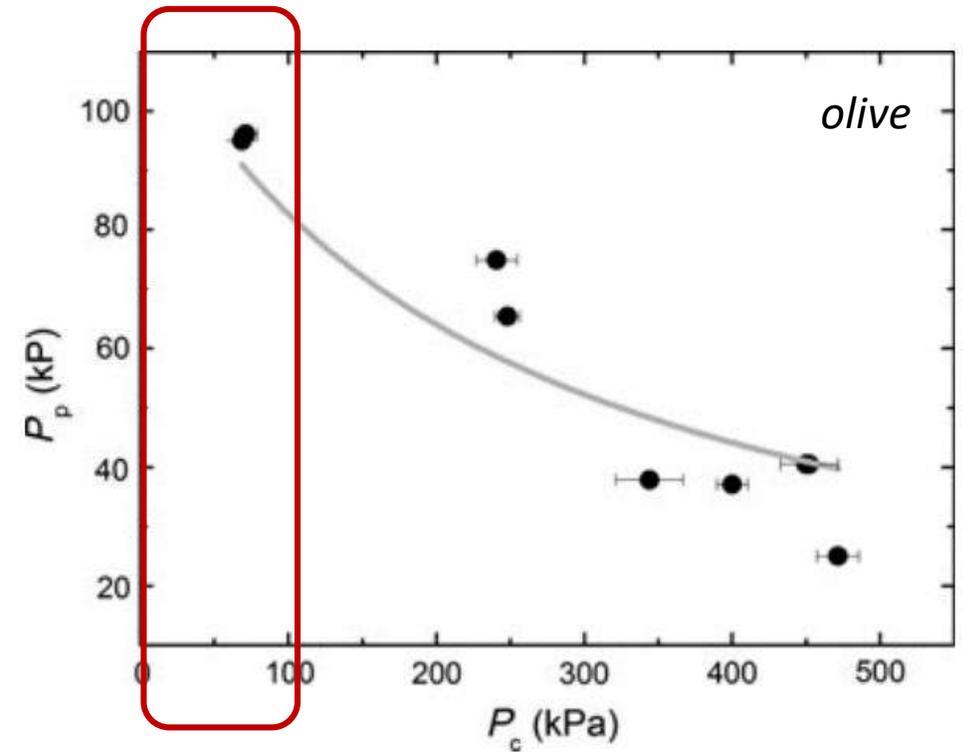
Ehrenberger et al. 2012 *Plant Biol*

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

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However, when leaves are **losing turgor**... and P_c becomes close to zero, the relationship between P_p and P_c is no longer linear...

$$P_p = F_{a,P_c \approx 0} \cdot P_{\text{clamp}}$$



Ehrenberger et al. 2012 *Plant Biol*

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

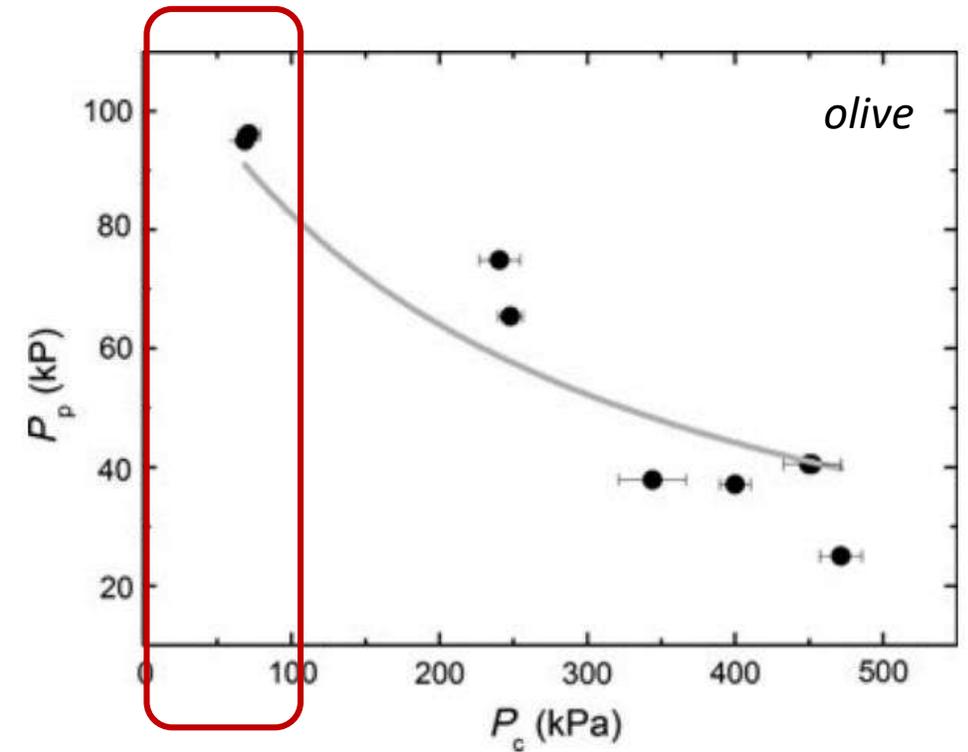
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LIMITATION??

[Application for this “limitation” in the next section]



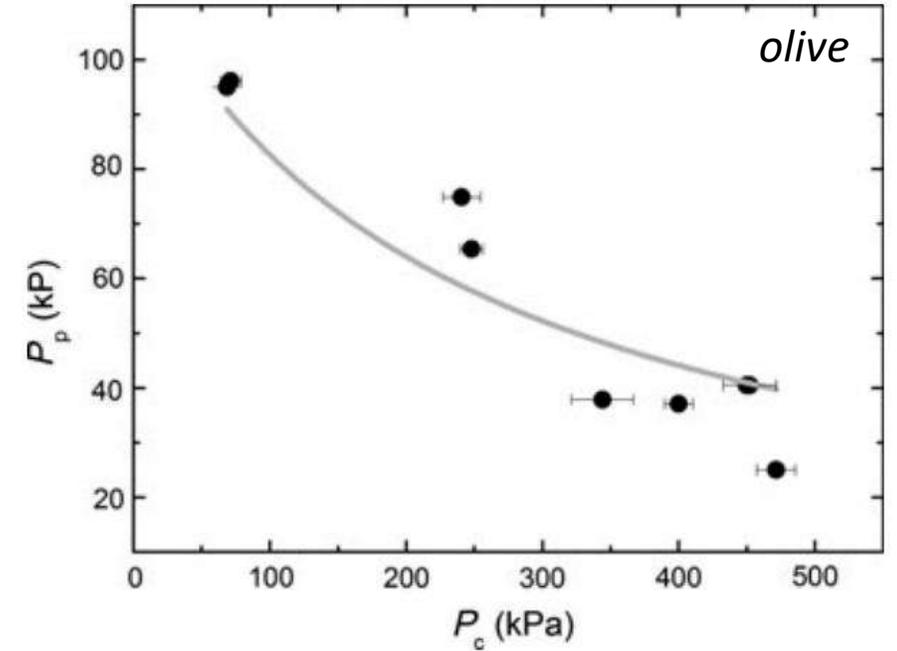
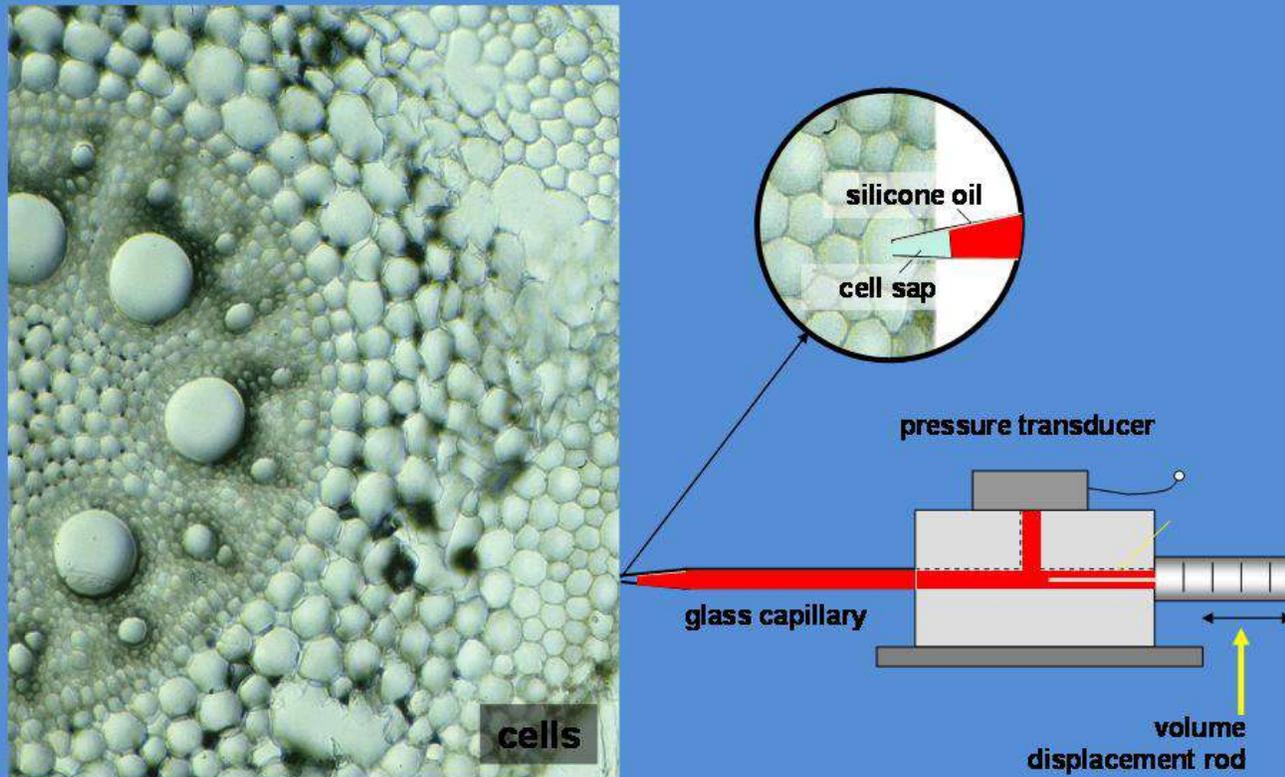
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BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

$$P_p = \left(\frac{b}{aP_c + b} \right)^{\frac{1}{a}} F_{a, \text{const}} P_{\text{clamp}} \quad P_p \text{ is a power function of } P_c$$

This relationship between P_p and P_c has been determined by using the “cell turgor pressure probe”

The turgor pressure probe



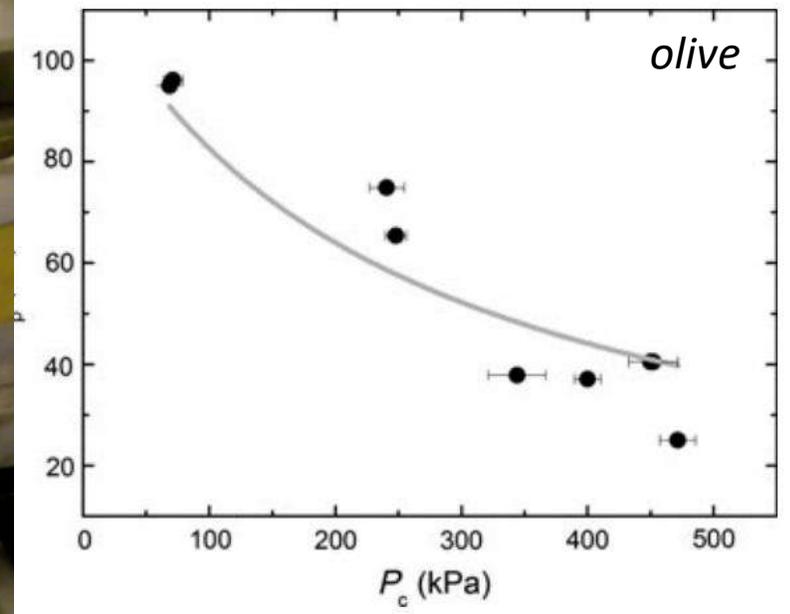
Ehrenberger et al. 2012 *Plant Biol*

Zimmermann et al. 2004 *New Phytol* 11:701-712

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR



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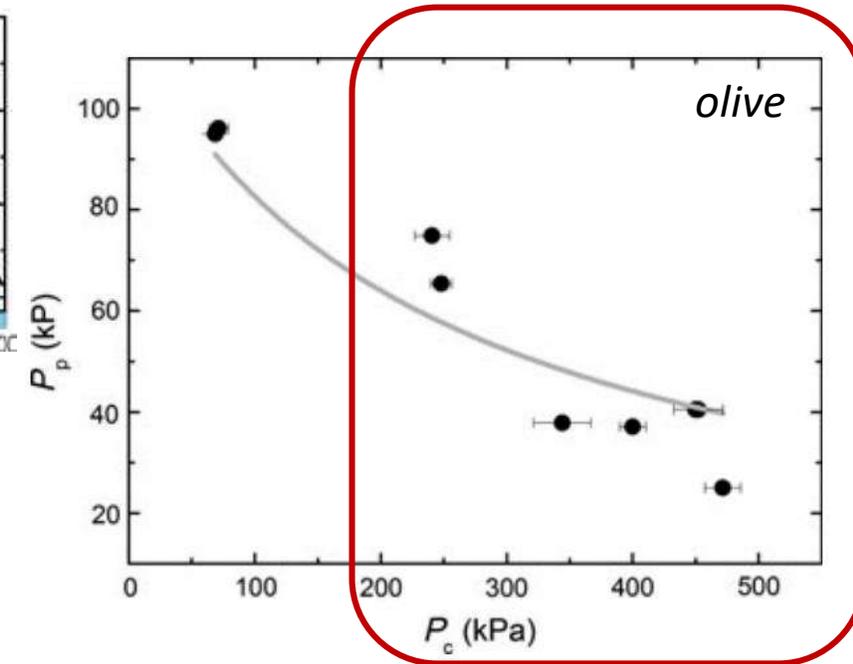
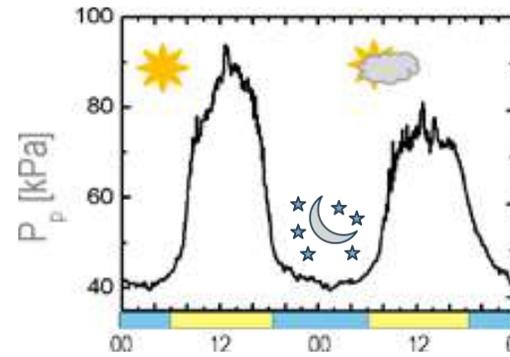


Ehrenberger et al. 2012 *Plant Biol*

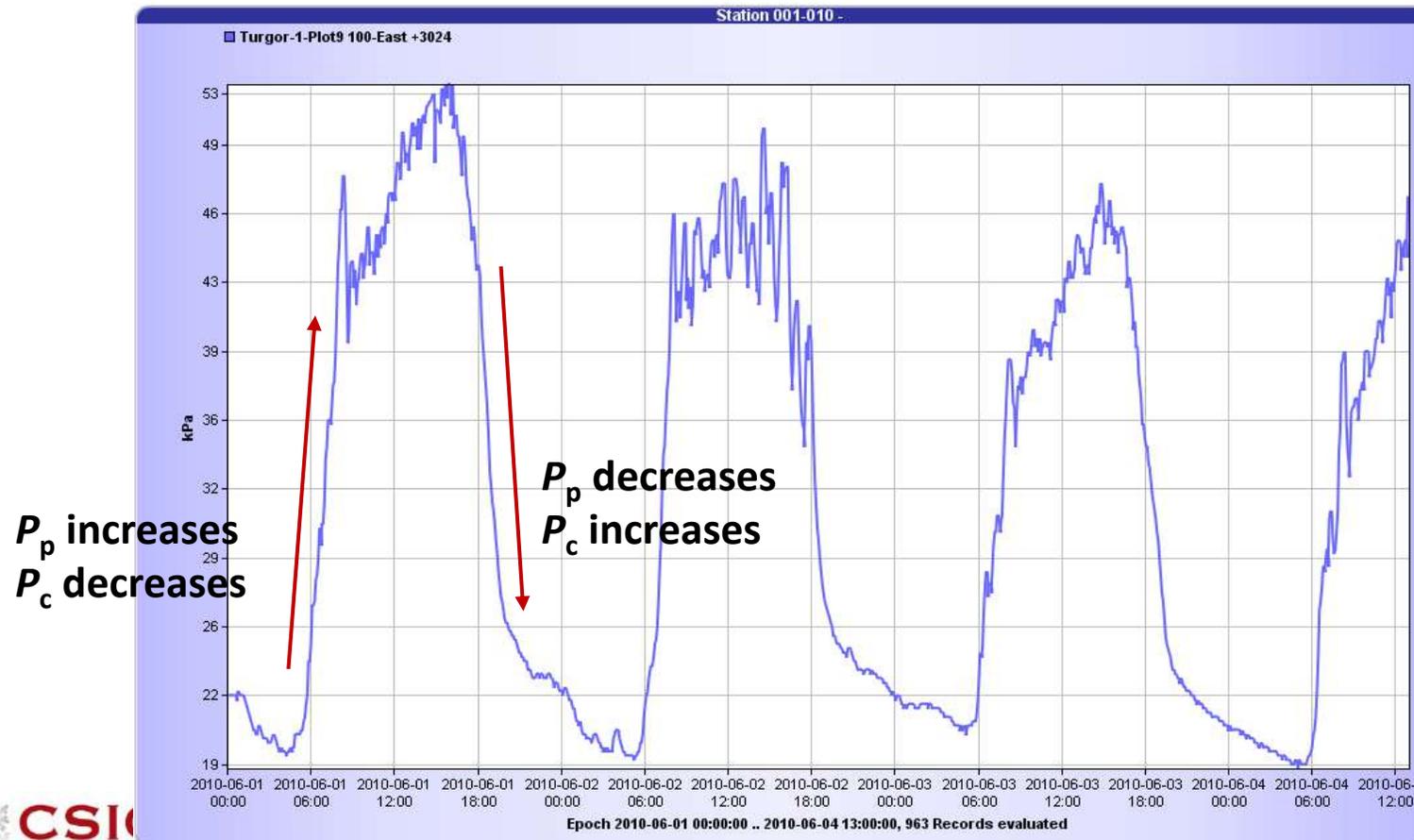
Photo: Christina Sann

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

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Ehrenberger et al. 2012 *Plant Biol*



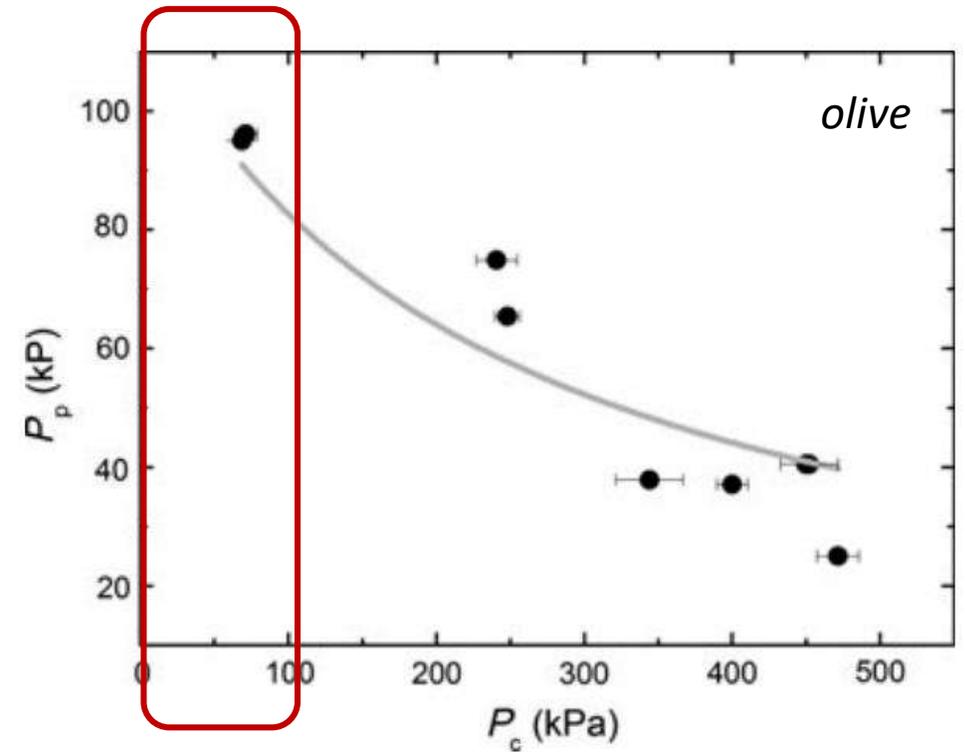
This is when leaves are *well hydrated, turgid*, and changes in P_p mainly depends in changes in P_c

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR

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However, when leaves are **losing turgor**... and P_c becomes close to zero, the relationship between P_p and P_c is no longer linear, and F_a becomes the main factor that influence P_p

$$P_p = F_{a, P_c \approx 0} \cdot P_{\text{clamp}}$$



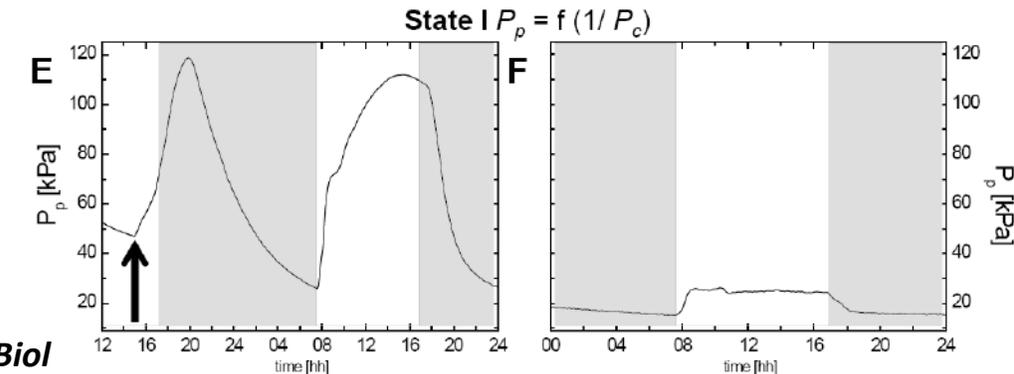
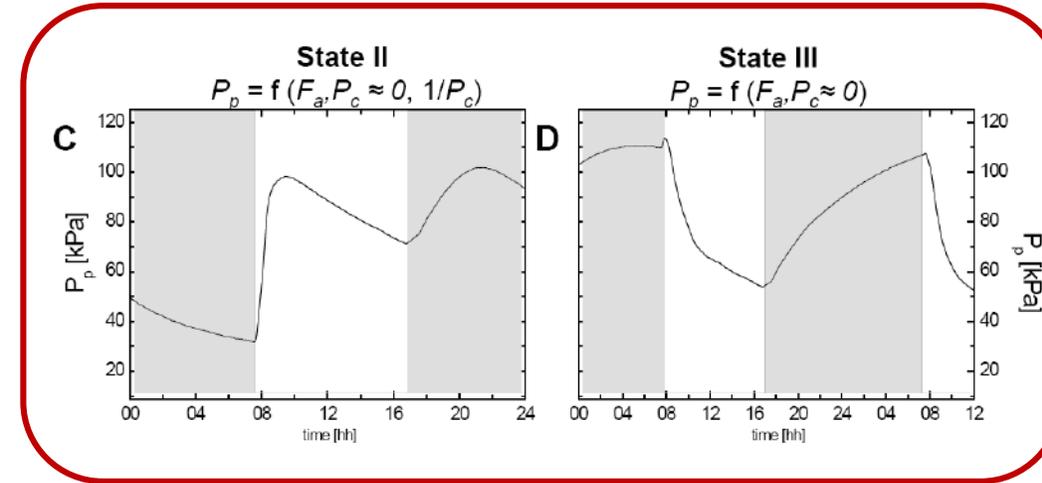
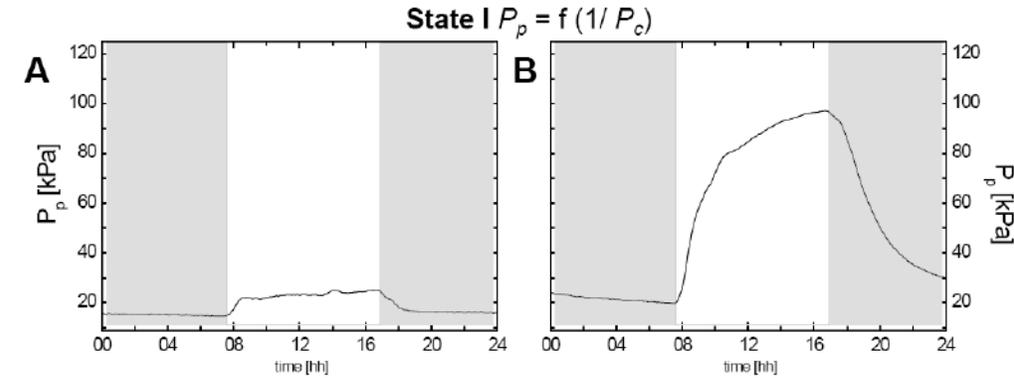
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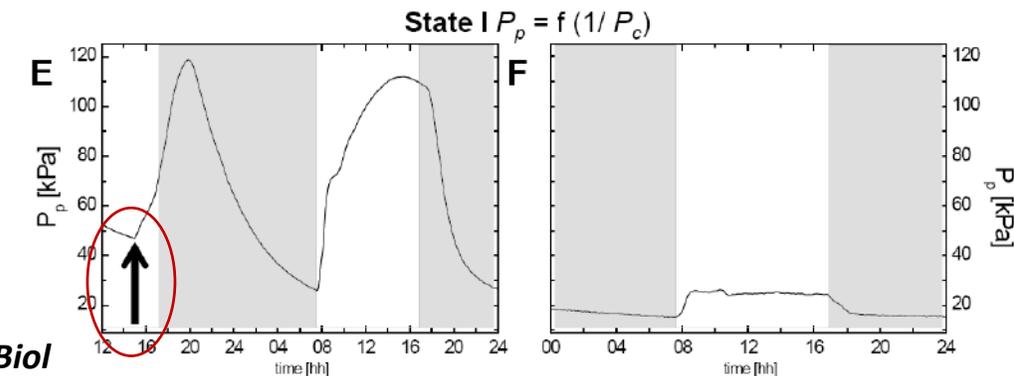
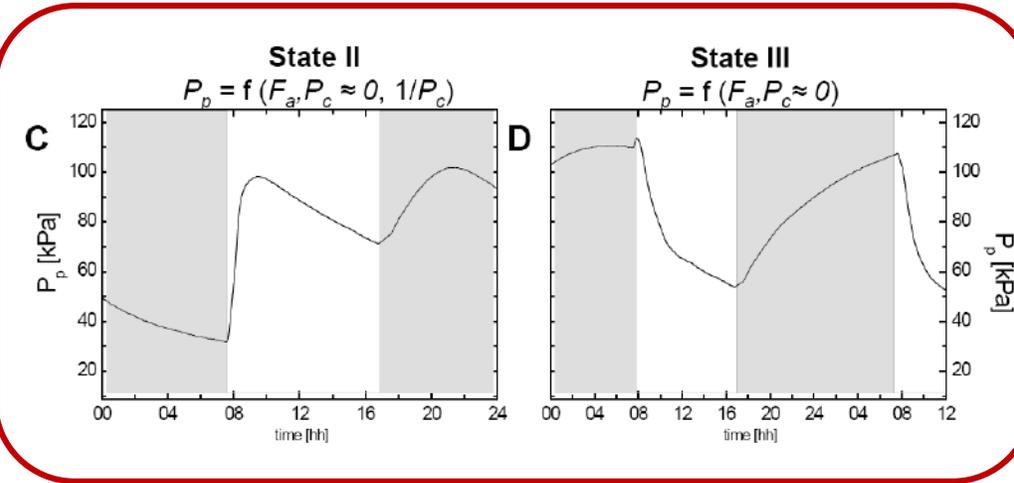
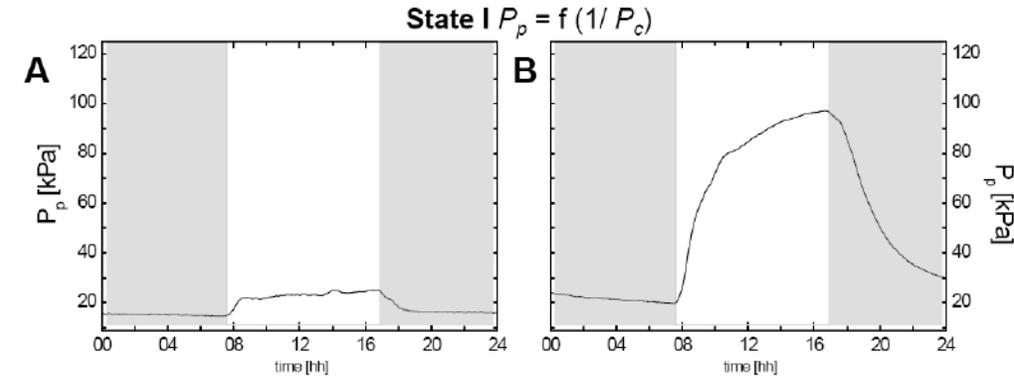
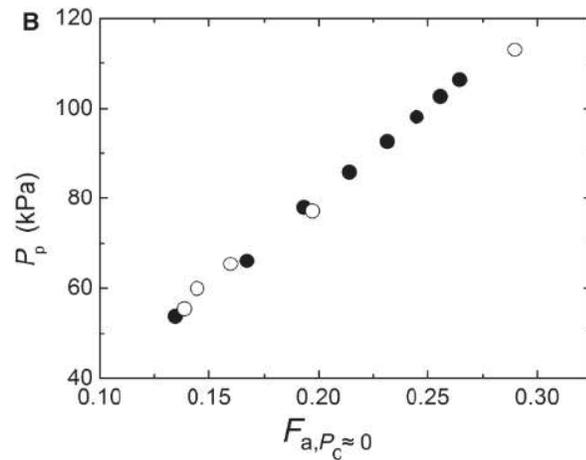
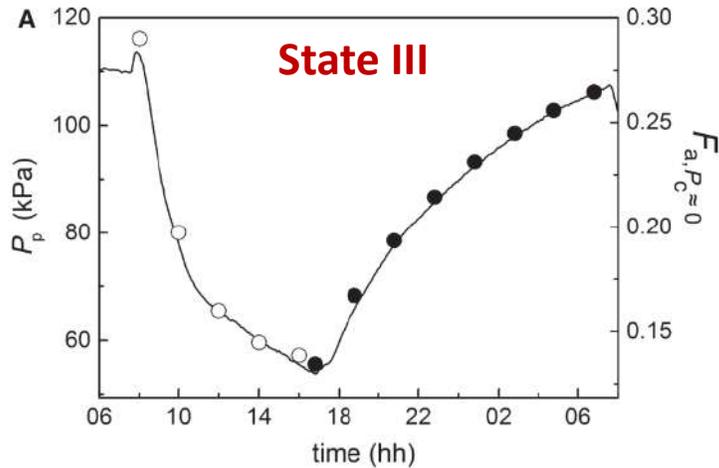


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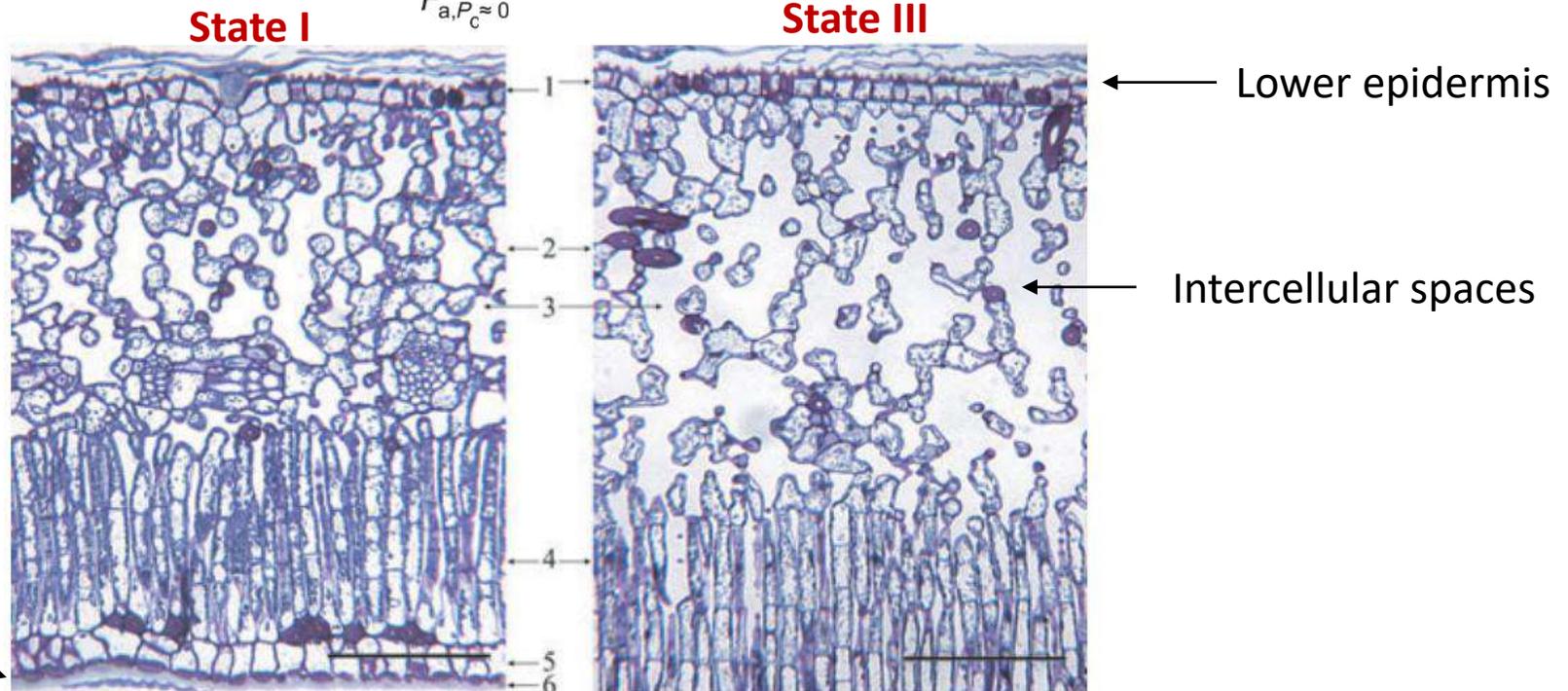
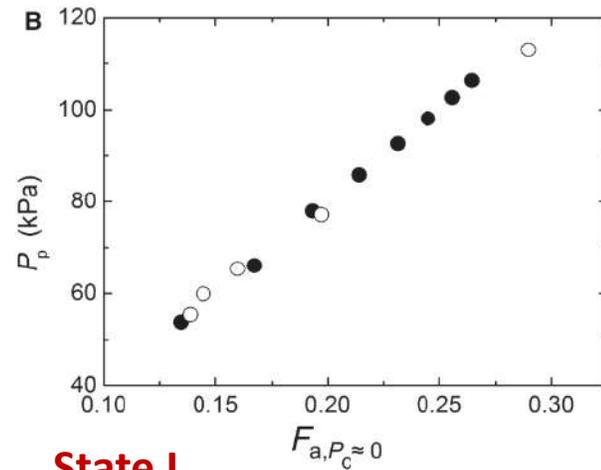
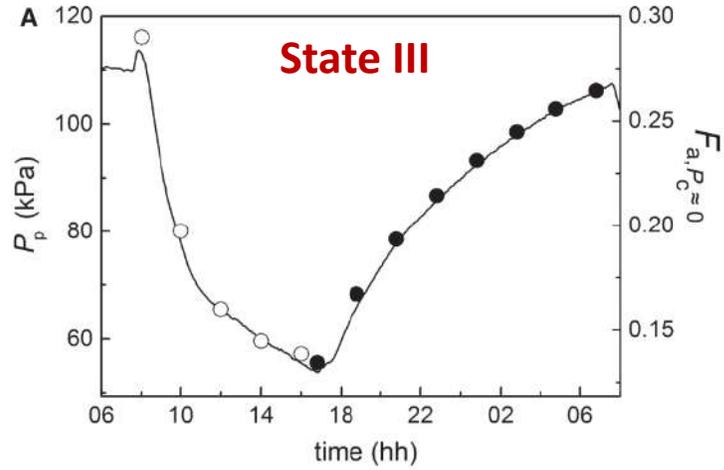
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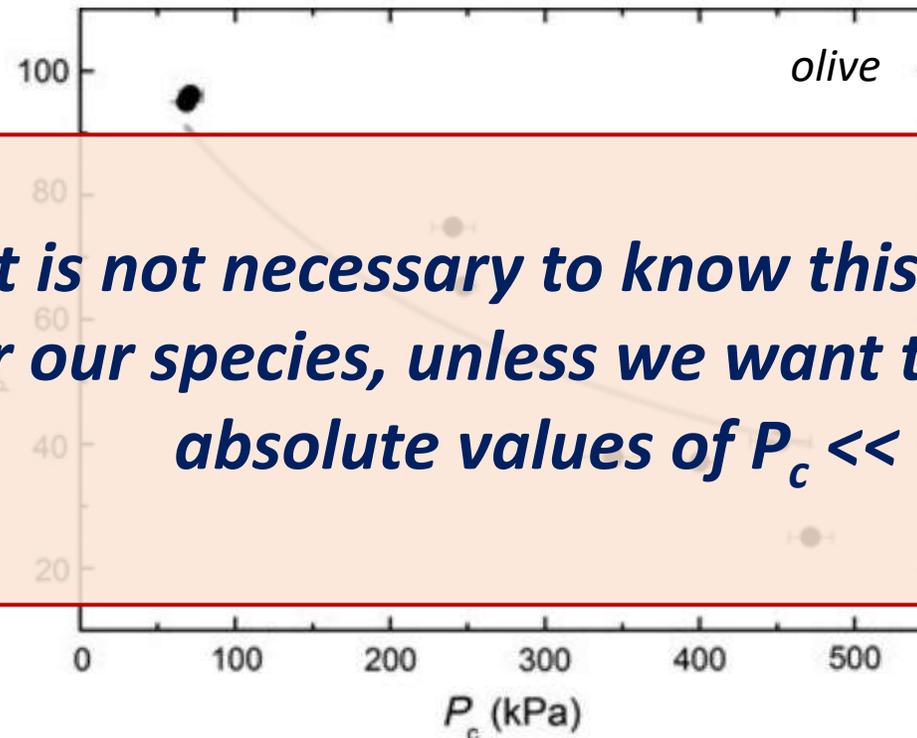
BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR



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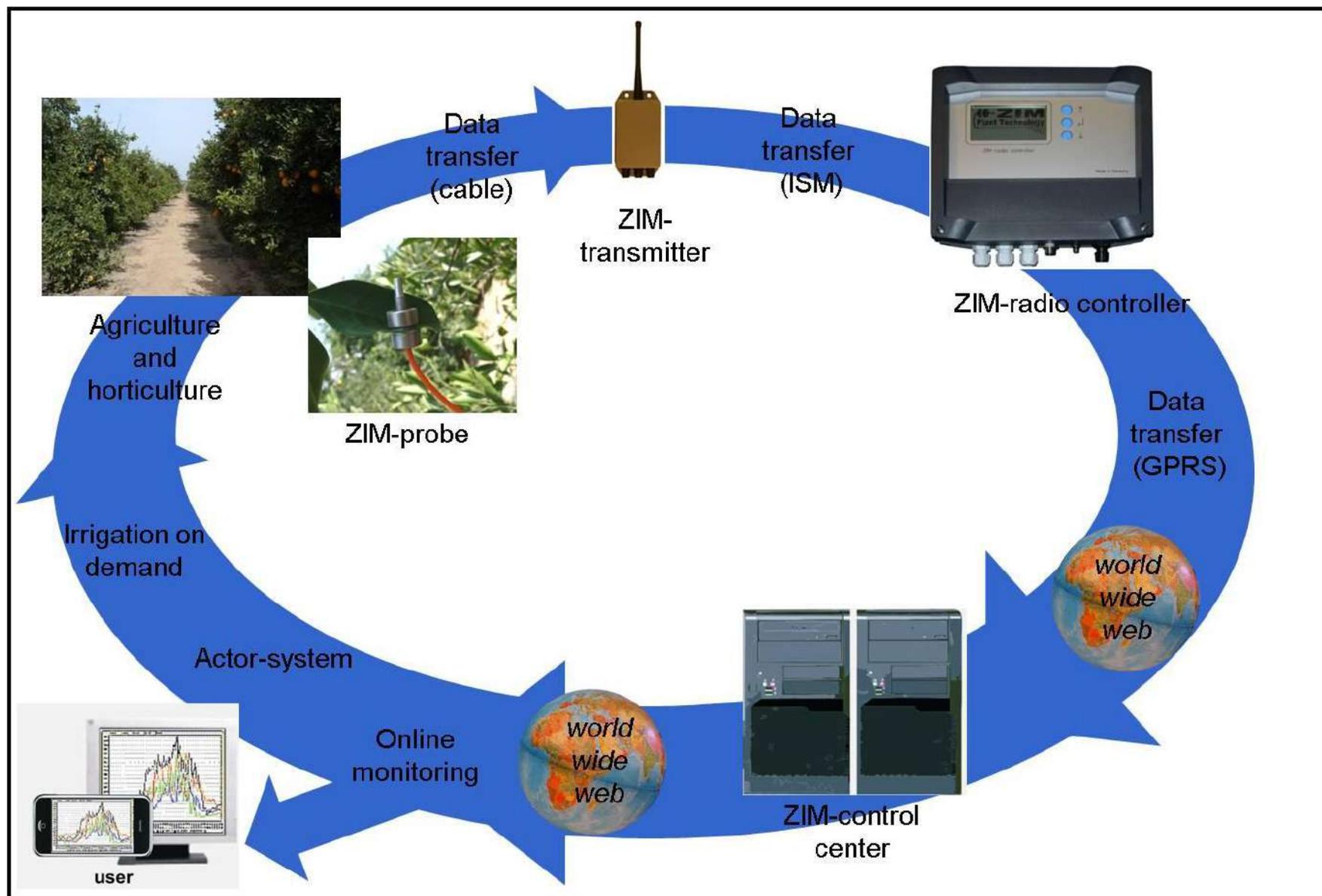
$$P_p = \left(\frac{b}{aP_c + b} \right)^{\frac{1}{a}} F_{a,\text{const}} P_{\text{clamp}} \quad P_p \text{ is a power function of } P_c$$

>> It is not necessary to know this function for our species, unless we want to know absolute values of P_c <<



Ehrenberger et al. 2012 *Plant Biol*

BASIC PRINCIPLES: LEAF TURGOR PRESSURE-RELATED SENSOR



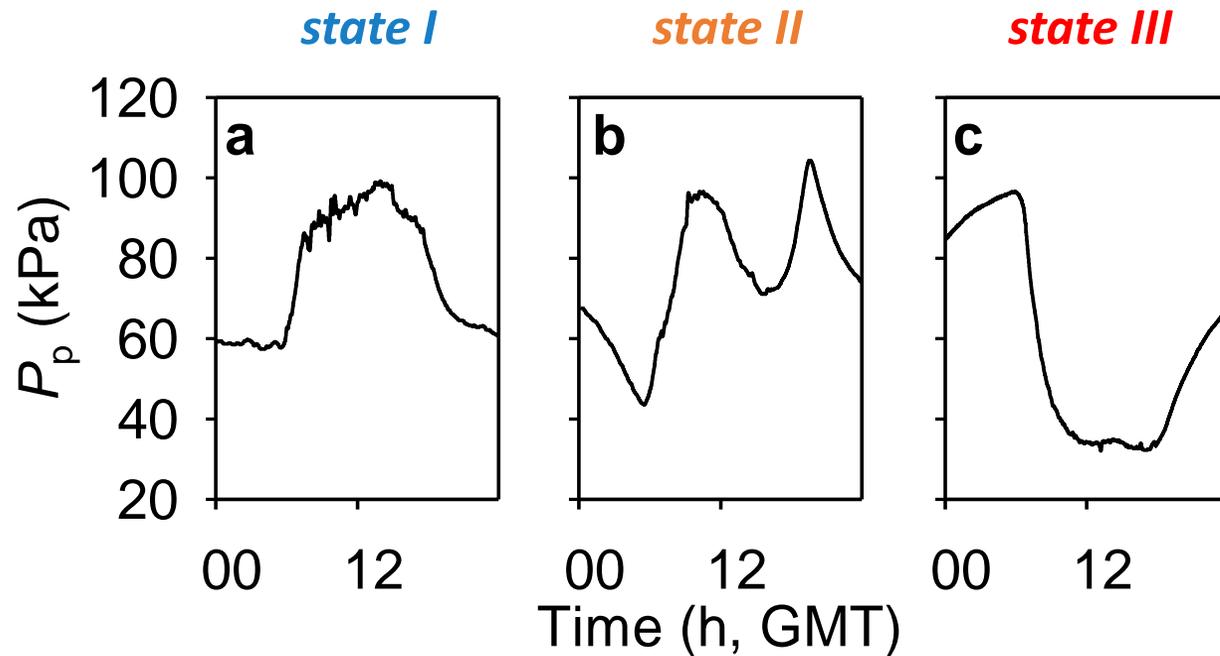
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE

Online-monitoring of tree water stress in a hedgerow olive orchard using the leaf patch clamp pressure probe

J.E. Fernández^{a,*}, C.M. Rodríguez-Dominguez^a, A. Perez-Martin^a, U. Zimmermann^b, S. Rüger^b,
M.J. Martín-Palomo^c, J.M. Torres-Ruiz^a, M.V. Cuevas^a, C. Sann^{b,d}, W. Ehrenberger^{b,d}, A. Diaz-Espejo^a

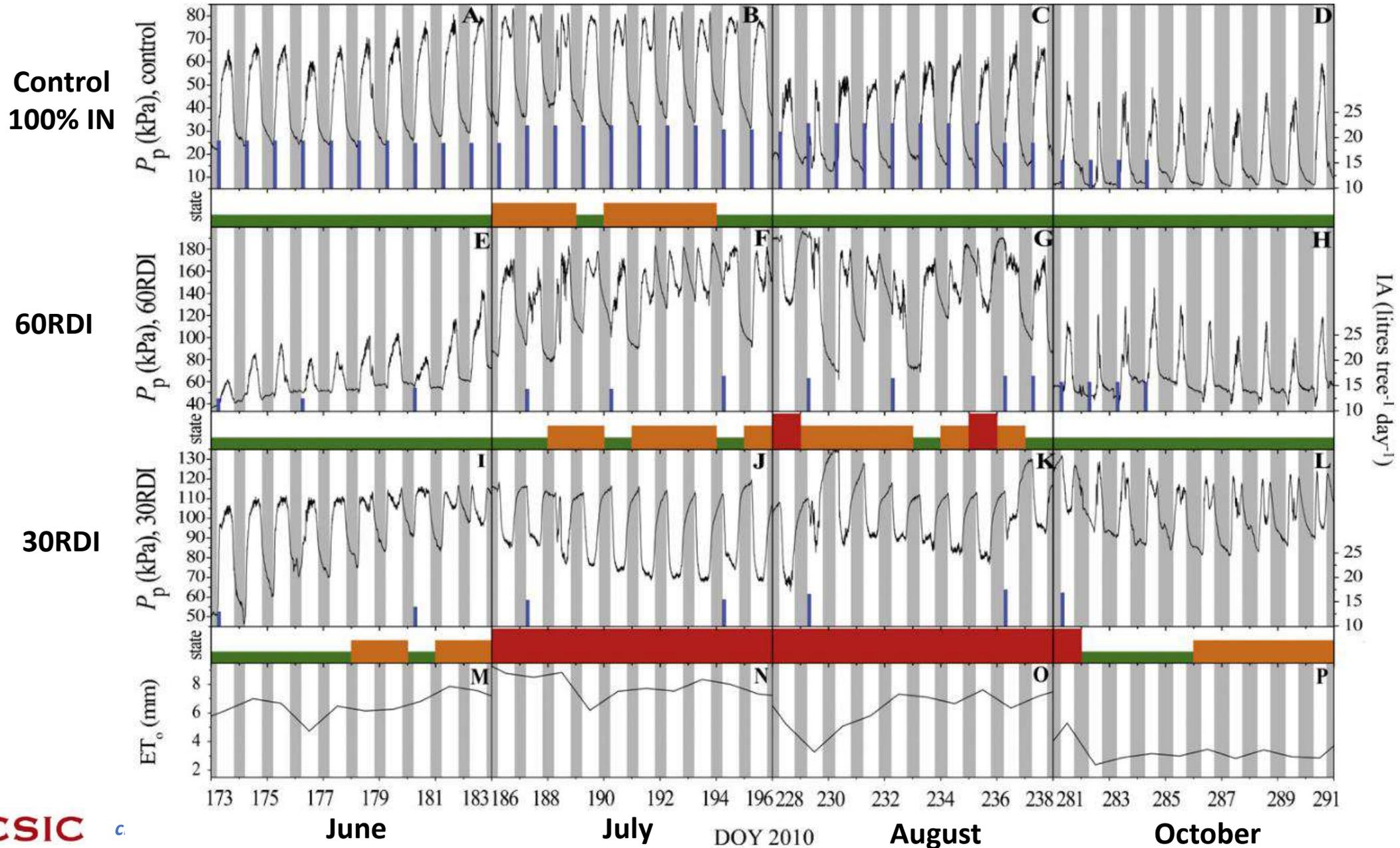


 To evaluate *leaf turgor sensors* for monitoring water stress in super-intensive olive orchard

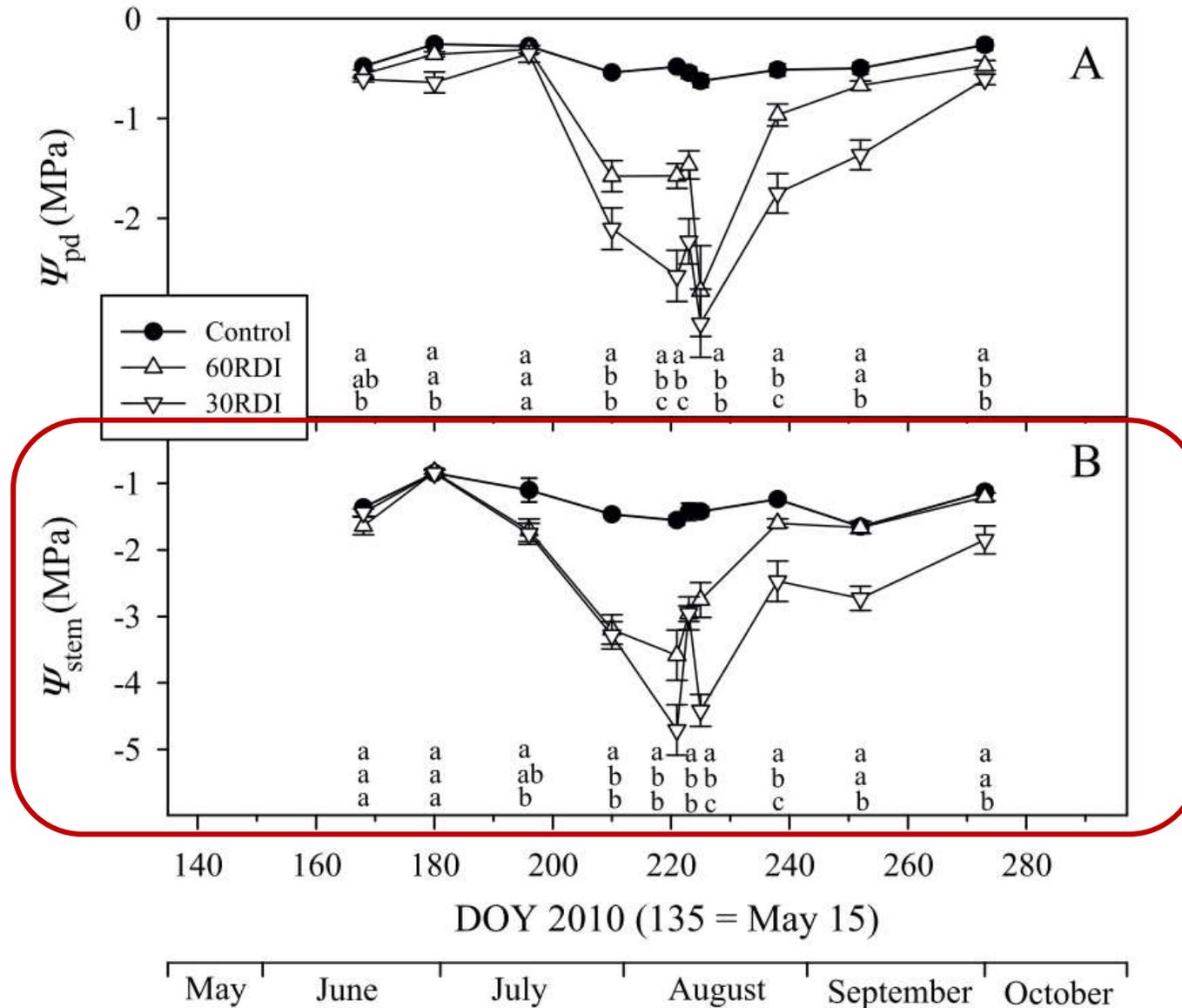


LIMITATION??

APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



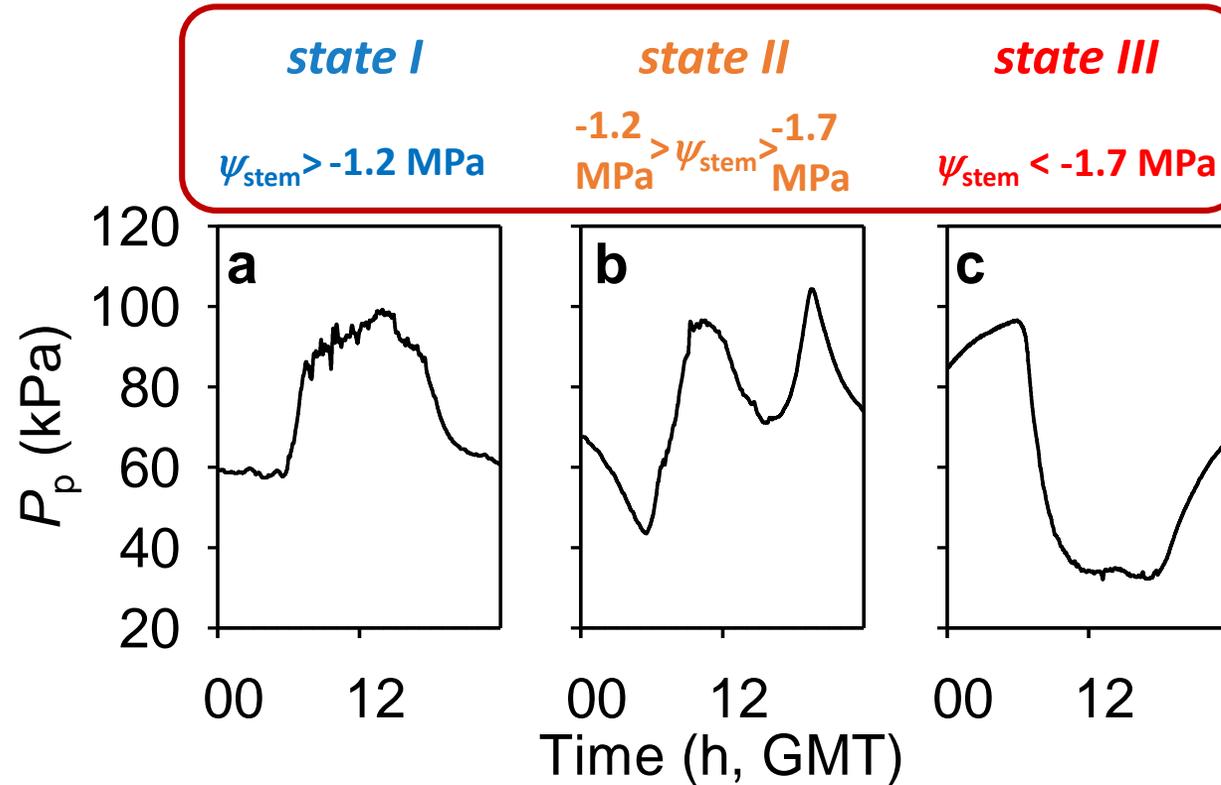
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



Padilla-Díaz et al. 2016
Martínez-Gimeno et al. 2017
Ballester et al. 2017

.....

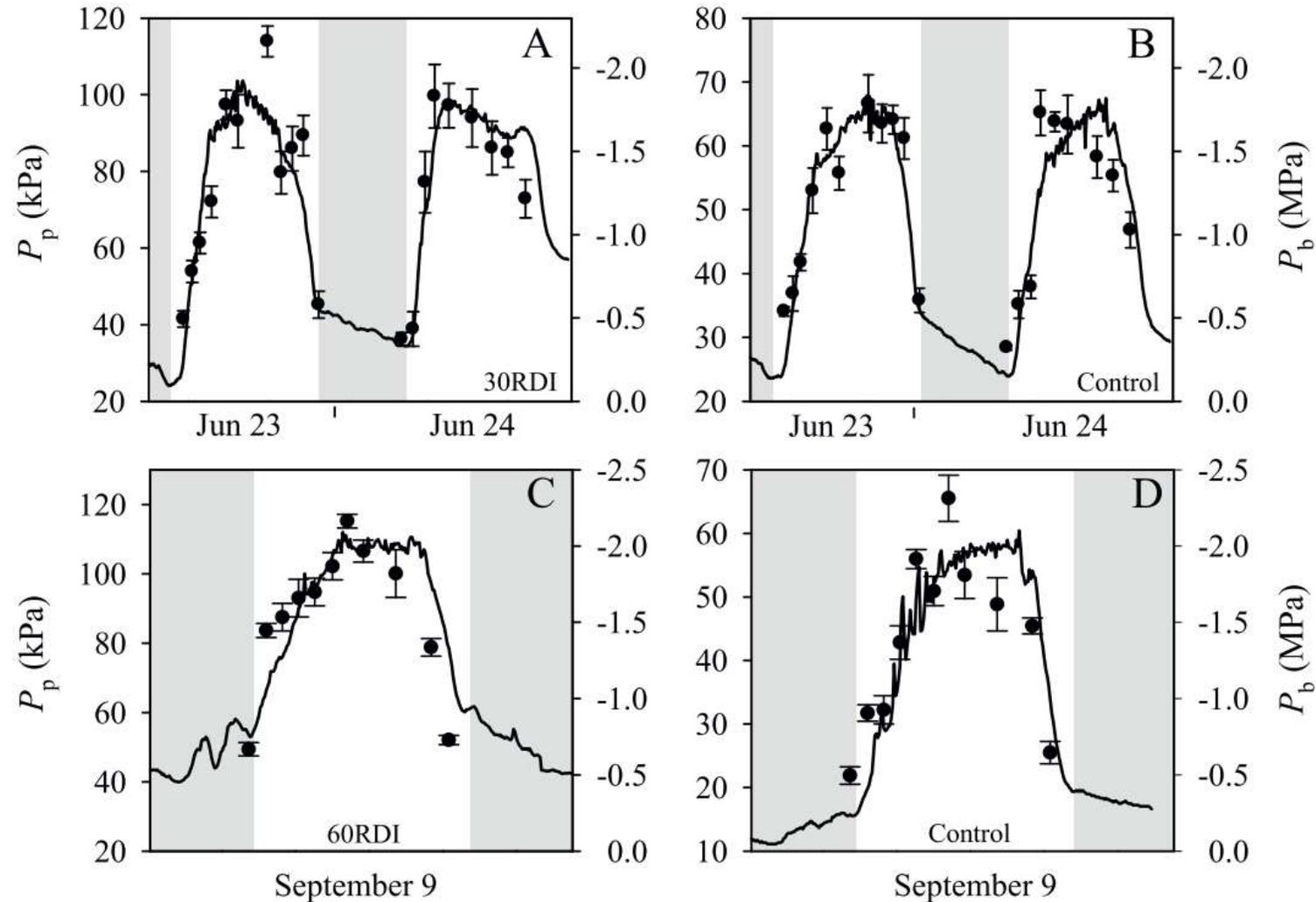
ZIM probe outputs VS pressure chamber (Ψ_{stem})



Characteristic diurnal curves coincided with **traditional water stress indicators** (Ψ_{stem})

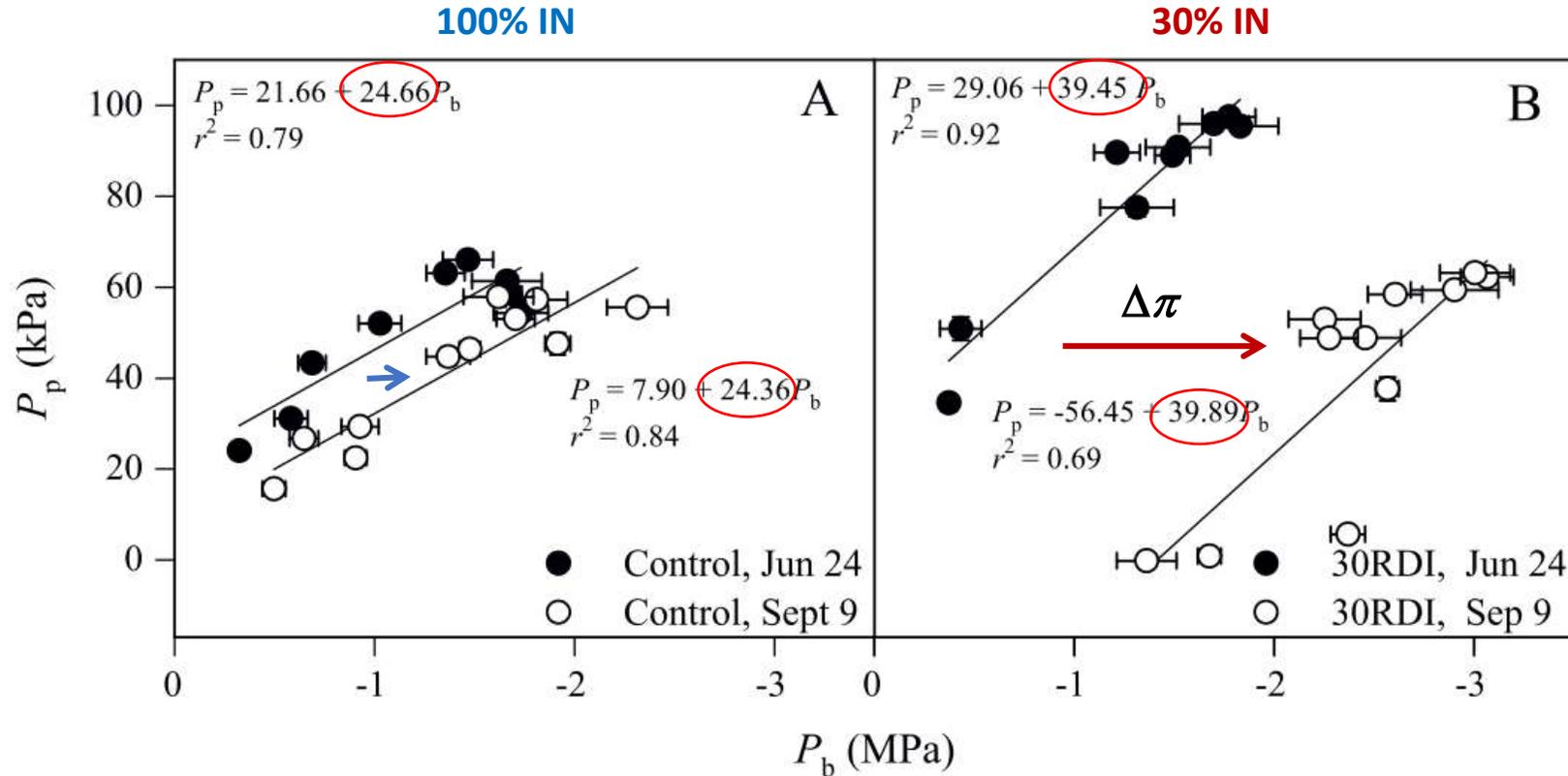
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE

ZIM probe outputs VS pressure chamber (Ψ_{leaf})



APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE

ZIM probe outputs VS pressure chamber (Ψ_{leaf})



$$\Psi_{\text{leaf}} = P_b$$

- slope did not change
- P_b decreased seasonally

$$\Psi = \Psi_s + \Psi_p$$

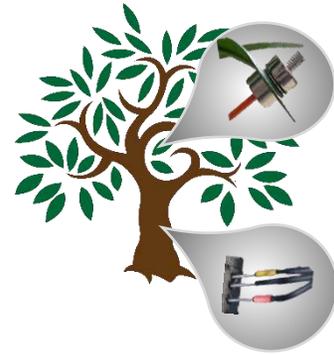
$\Delta\pi$ (MPa) -2.15 → -2.37

-1.74 → -2.35

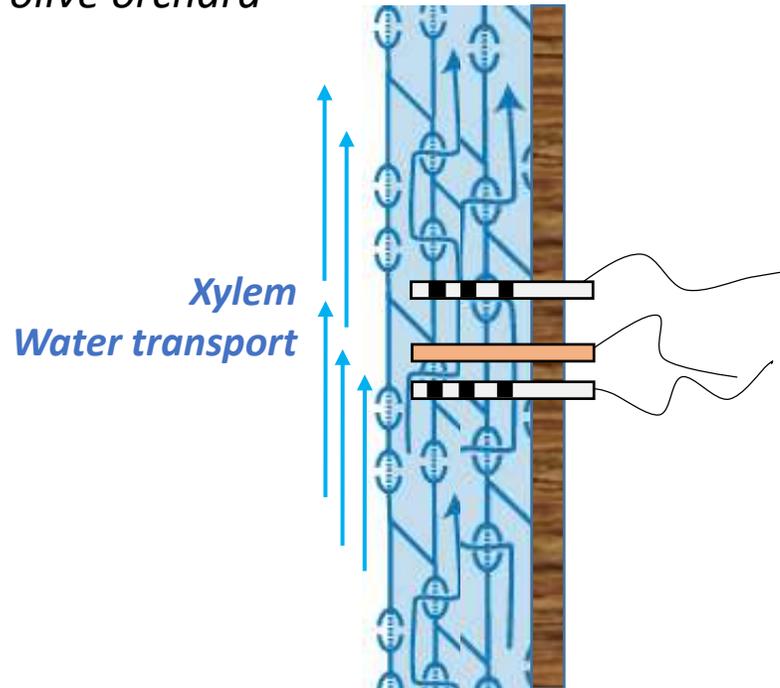
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE

Concomitant measurements of stem sap flow and leaf turgor pressure in olive trees using the leaf patch clamp pressure probe

C.M. Rodríguez-Domínguez^{a,1}, W. Ehrenberger^{b,c,1}, C. Sann^c, S. Rüger^c, V. Sukhorukov^b, M.J. Martín-Palomo^d, A. Díaz-Espejo^a, M.V. Cuevas^a, J.M. Torres-Ruiz^a, A. Pérez-Martín^a, U. Zimmermann^c, J.E. Fernández^{a,*}

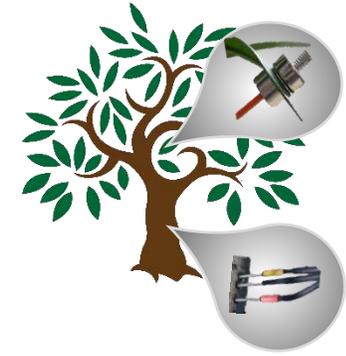


To combine **leaf turgor** and **sap flow** sensors to explore diurnal and seasonal dynamics of water relations in a super-intensive olive orchard

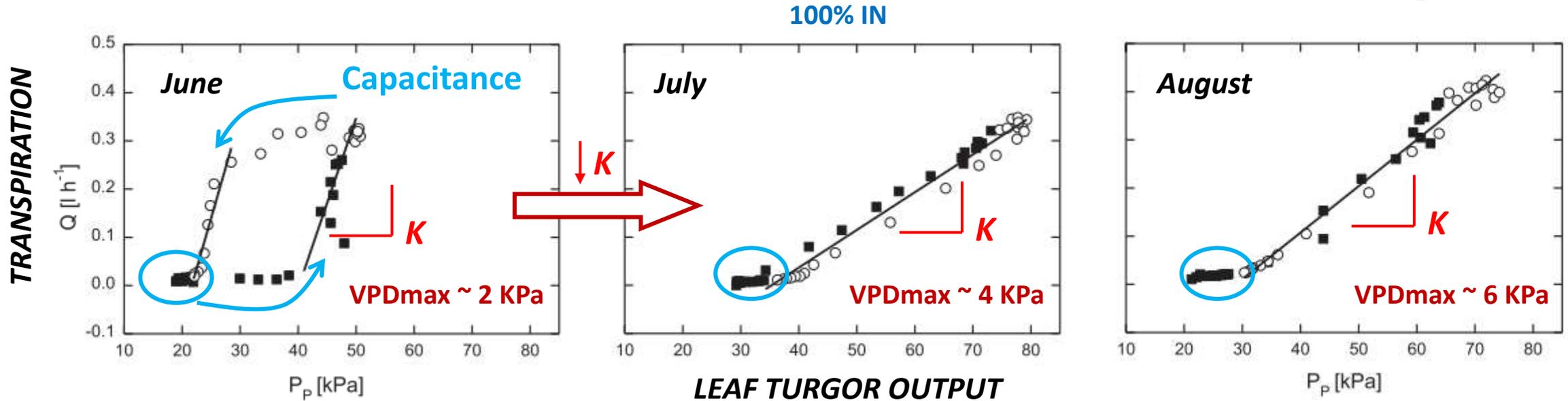


$$E_p = K (\psi_{\text{soil}} - \psi_{\text{leaf}})$$

APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



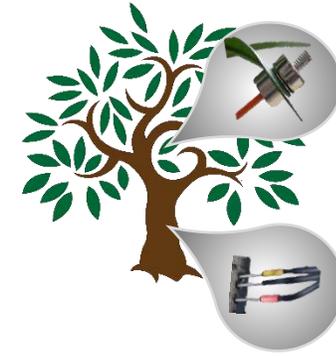
Identification of diurnal and seasonal changes in the use of water reserves (capacitance) and water transport efficiency (hydraulics)



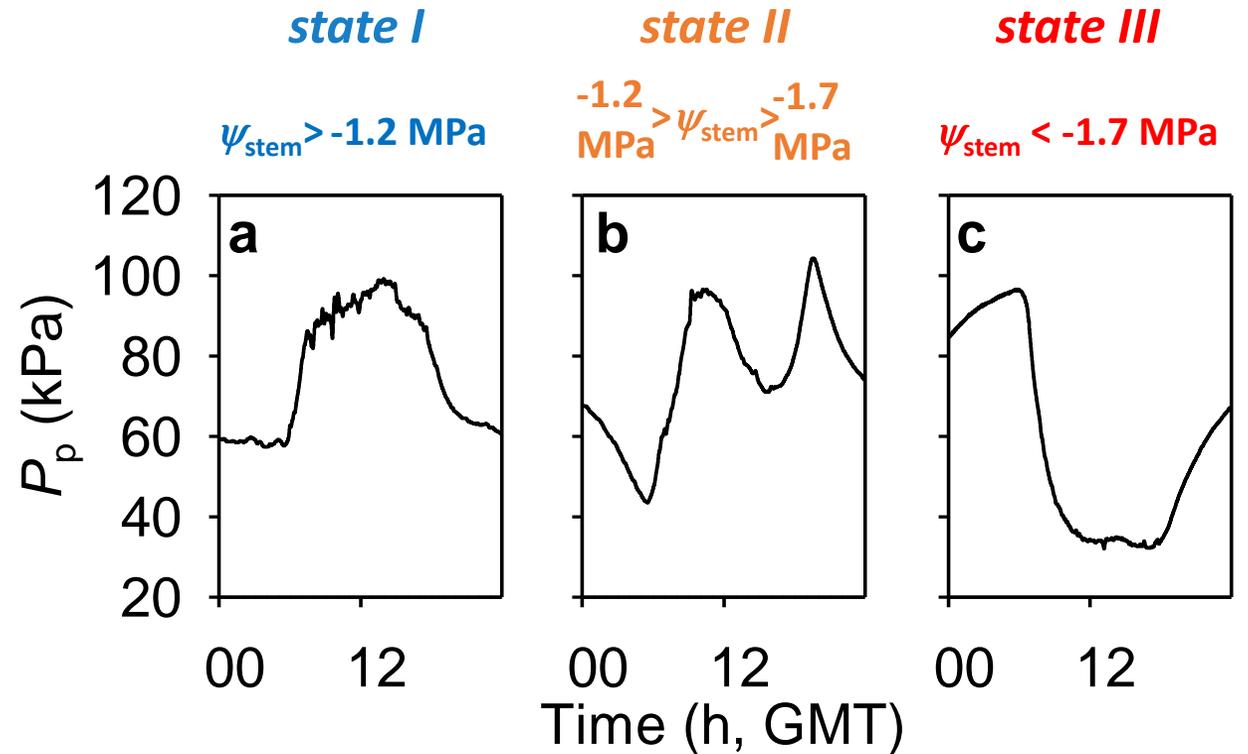
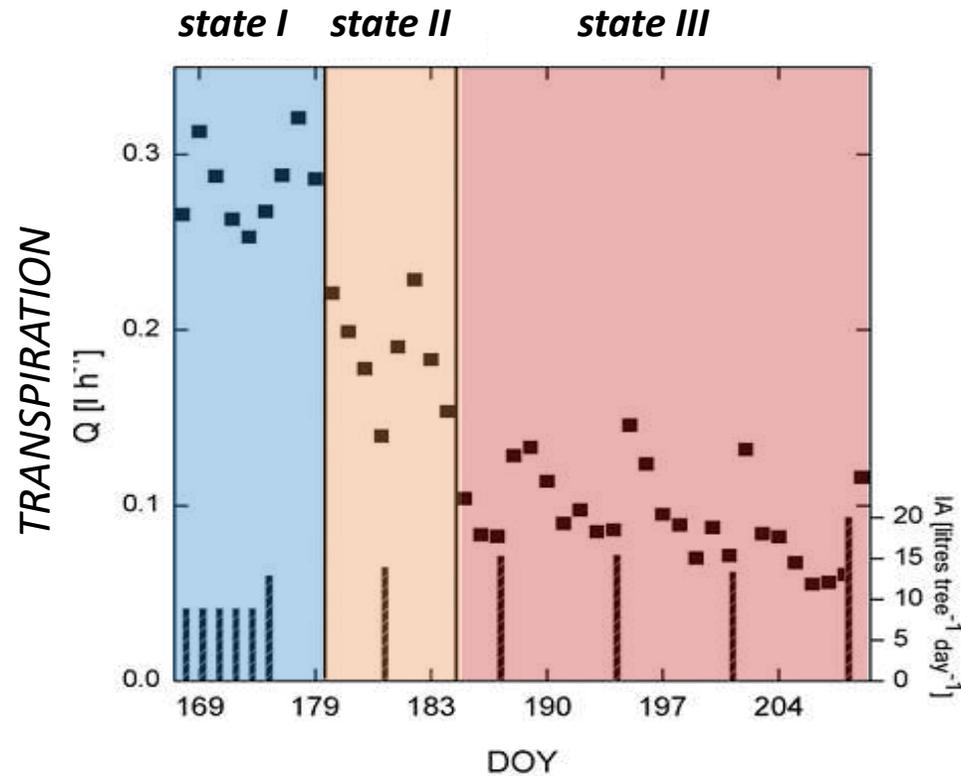
■ = dawn --> midday
○ = midday --> sunset

$$E_p = K (\psi_{\text{soil}} - \psi_{\text{leaf}})$$

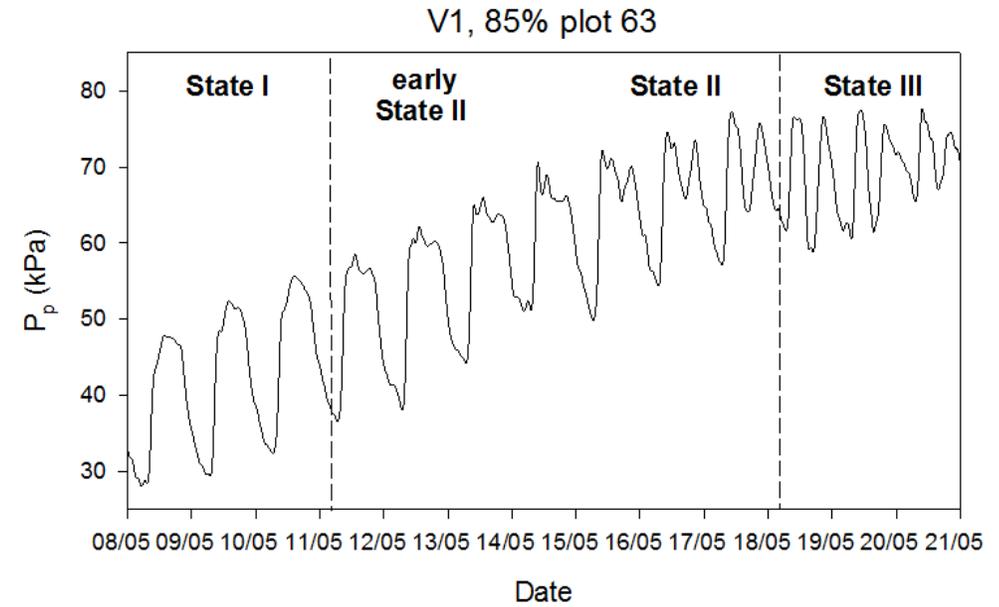
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



P_p states considered as **automatic water stress indicators**
easy to identify for irrigation scheduling



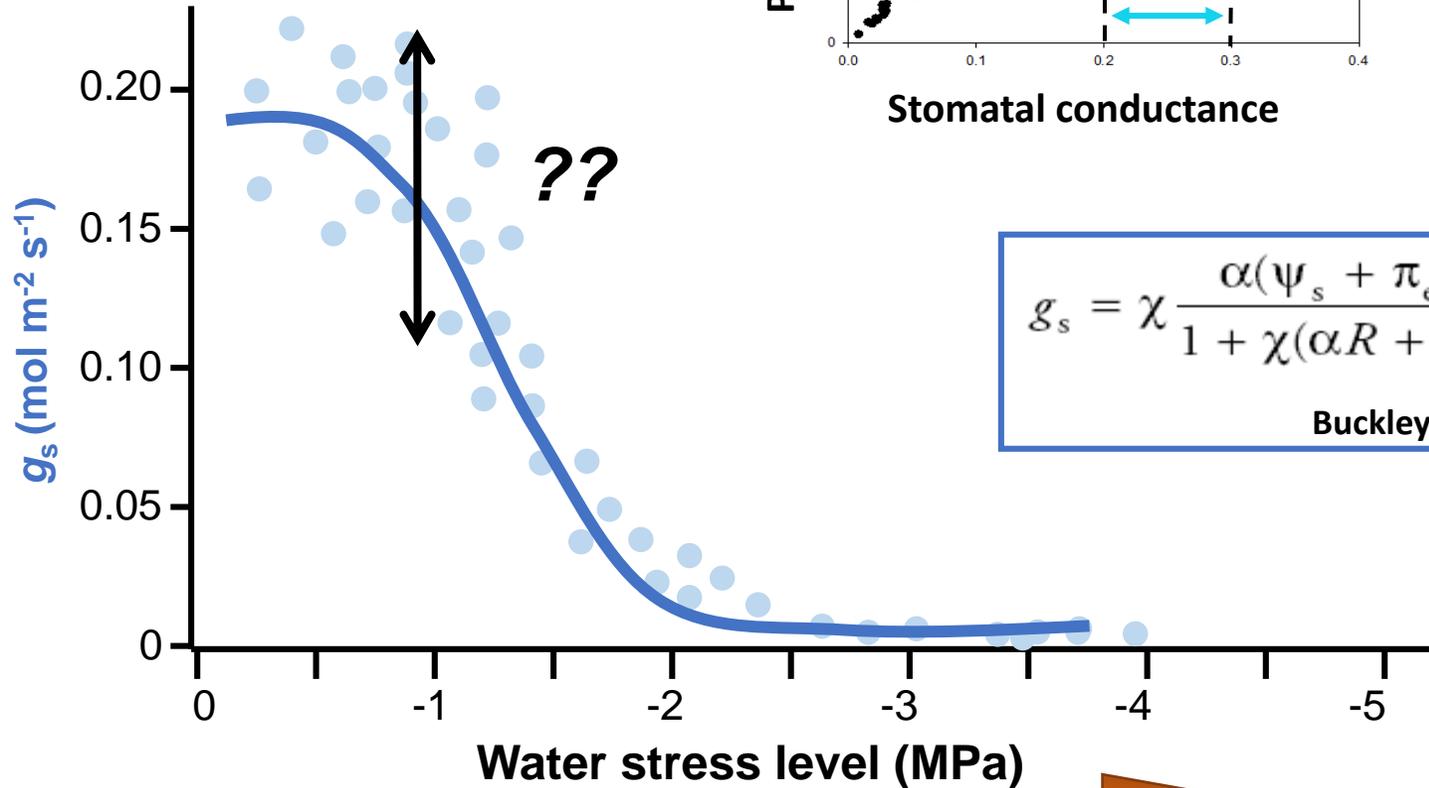
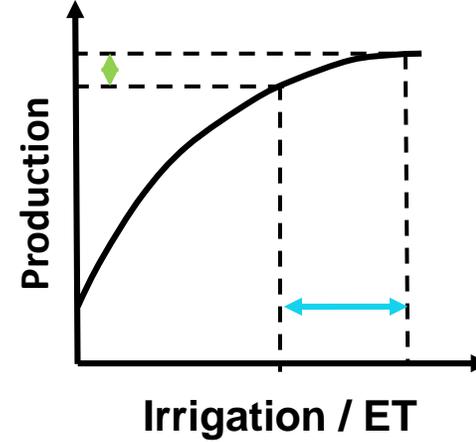
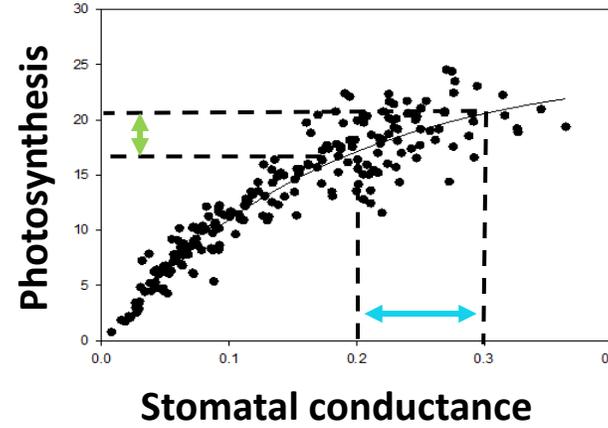
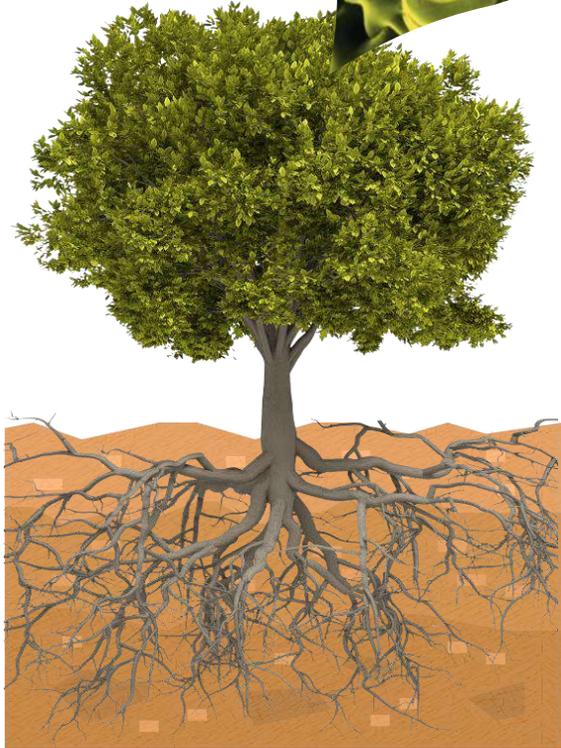
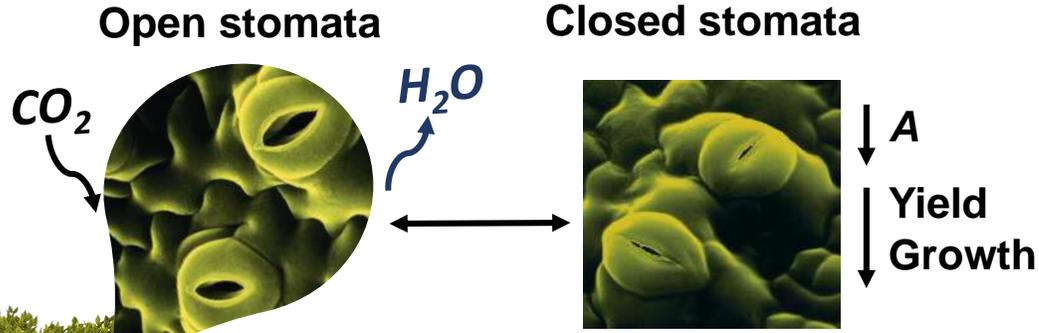
APPLICATION OF ZIM PROBES: A CASE STUDY IN OLIVE



- *High potential for irrigation scheduling*
- *However, the use that we gave to the ZIM probes was very empirical*

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

ECOVERs  STOMATA



$$g_s = \chi \frac{\alpha(\psi_s + \pi_e) - \pi_e}{1 + \chi(\alpha R + f_g r_{eg}) D_s}$$

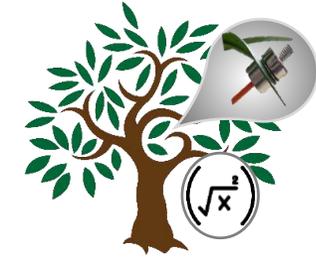
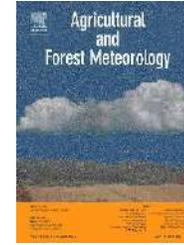
Buckley et al (2003)



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

Sensitivity of olive leaf turgor to air vapour pressure deficit correlates with diurnal maximum stomatal conductance

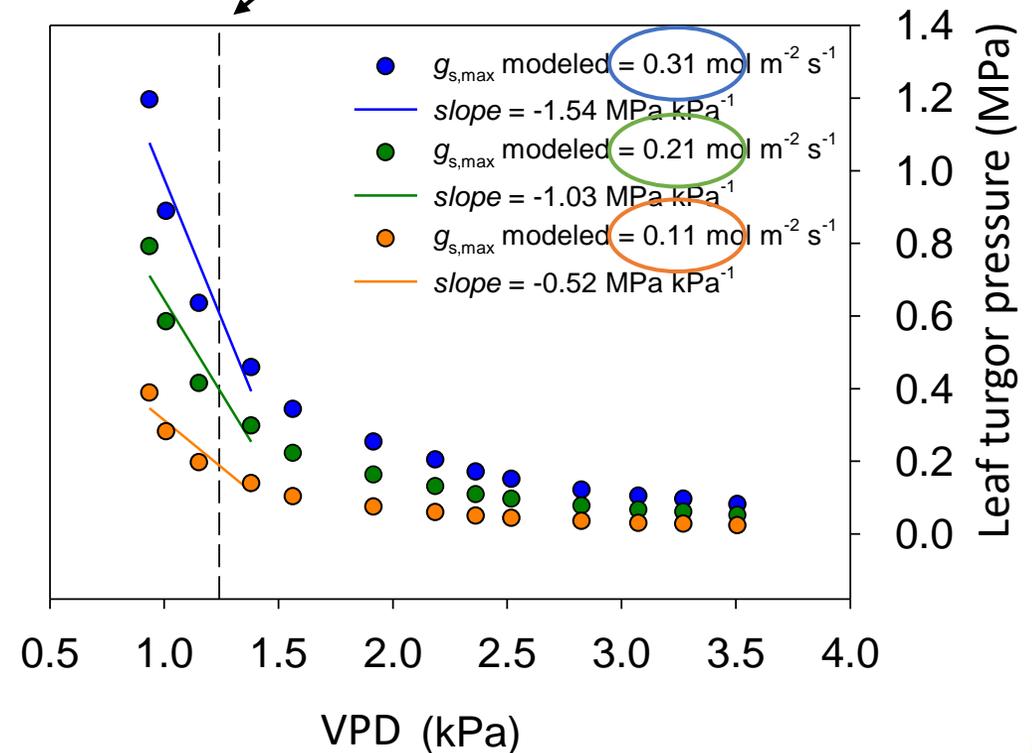
C.M. Rodriguez-Dominguez^{a,*}, V. Hernandez-Santana^a, T.N. Buckley^b, J.E. Fernández^a, A. Diaz-Espejo^a



To derive **continuous** g_s from the combination of a mechanistic model and leaf turgor and meteorological measurements in a super-intensive olive orchard



VPD at maximum stomatal conductance



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

Cell water relations

$$\Psi = \Psi_s + \Psi_p$$

$$P = \psi_{\text{leaf}} + \pi$$

Ohm's law analogy

$$g_s D = K (\psi_{\text{soil}} - \psi_{\text{leaf}})$$

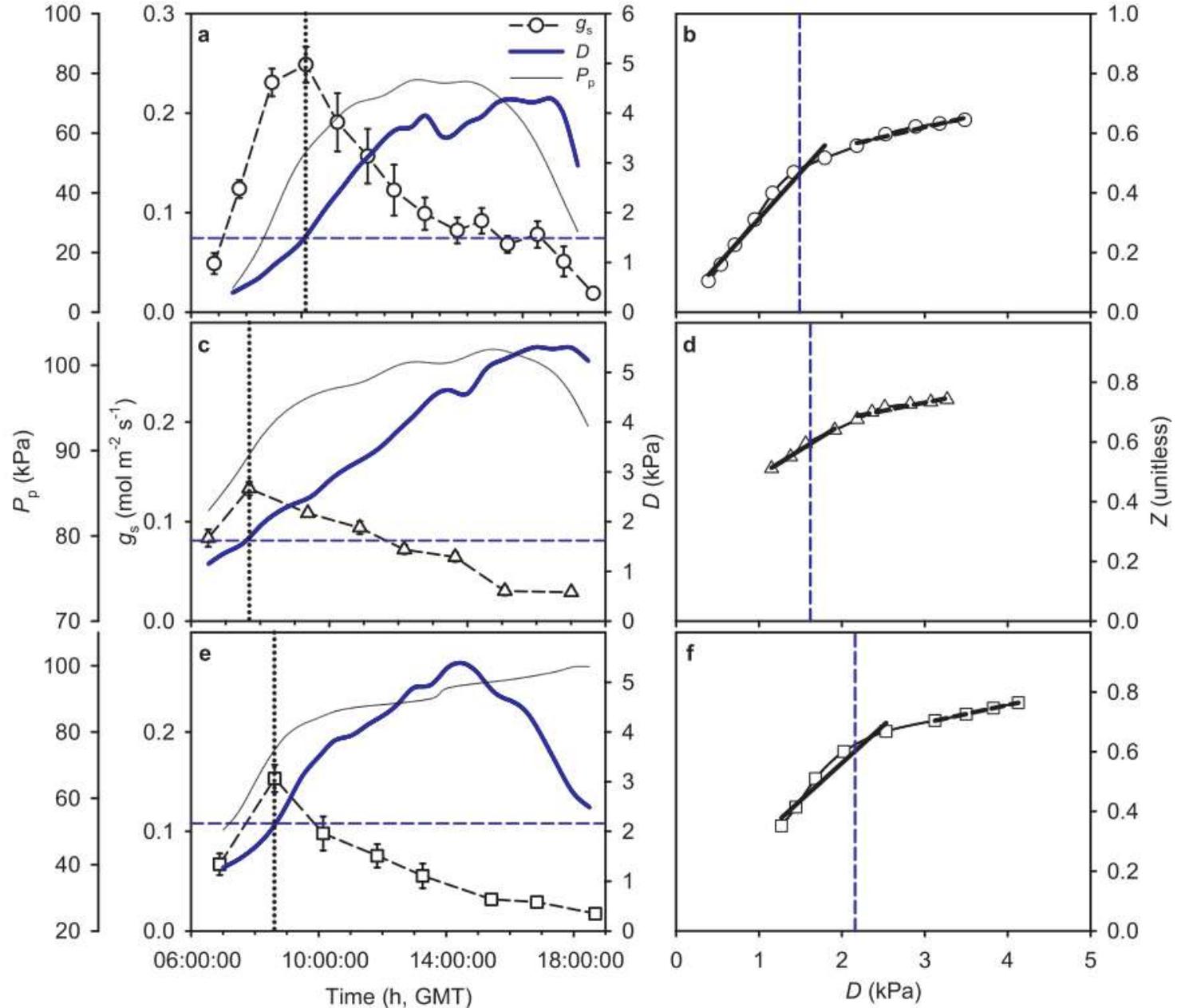
$$\psi_{\text{leaf}} = \psi_{\text{soil}} - \frac{g_s D}{K}$$

$D = \text{VPD}$

$$P = \psi_{\text{soil}} + \pi - \frac{g_s D}{K}$$

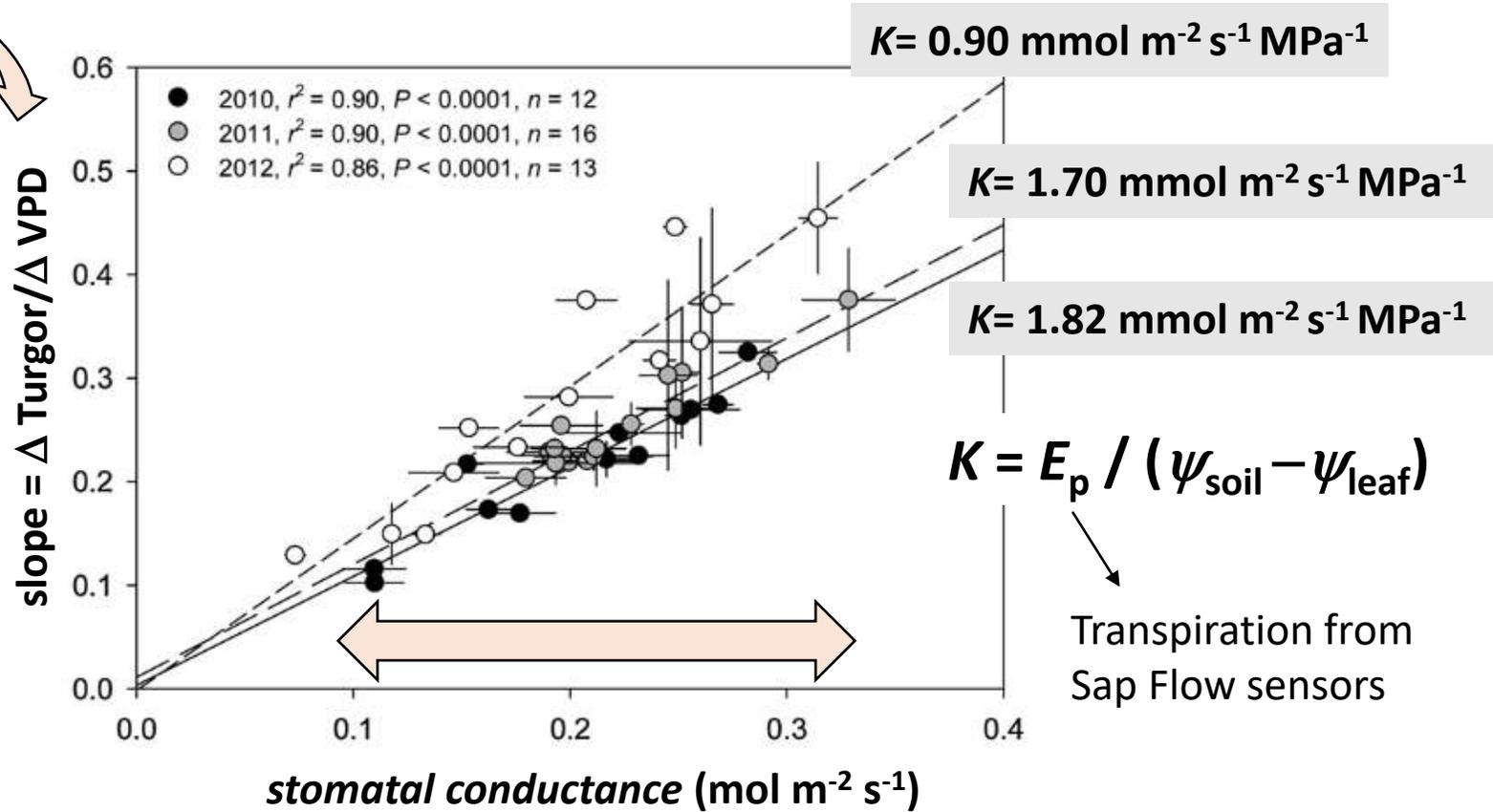
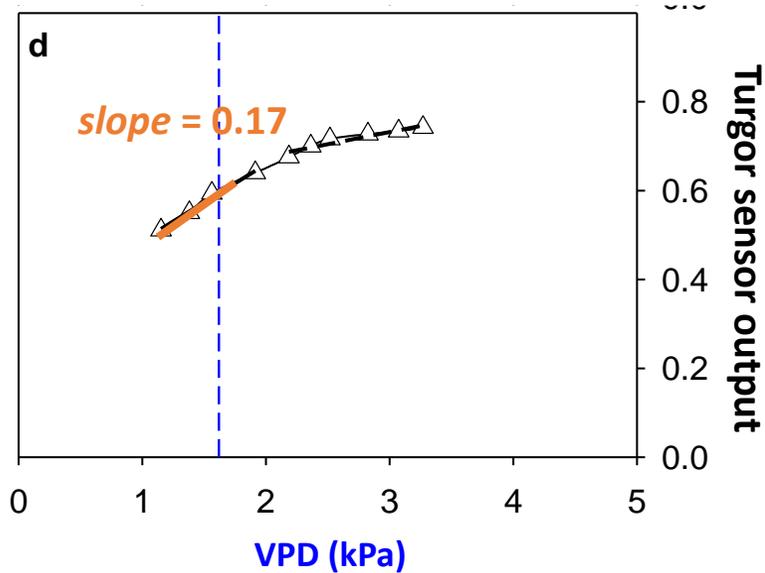
$$\frac{dP}{dD} = -\frac{g_s}{K} \left(1 + \frac{d \ln g_s}{d \ln D} \right)$$

$$\frac{dP}{dD} = -\frac{g_{s, \text{max}}}{E_{\text{max}}}$$



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

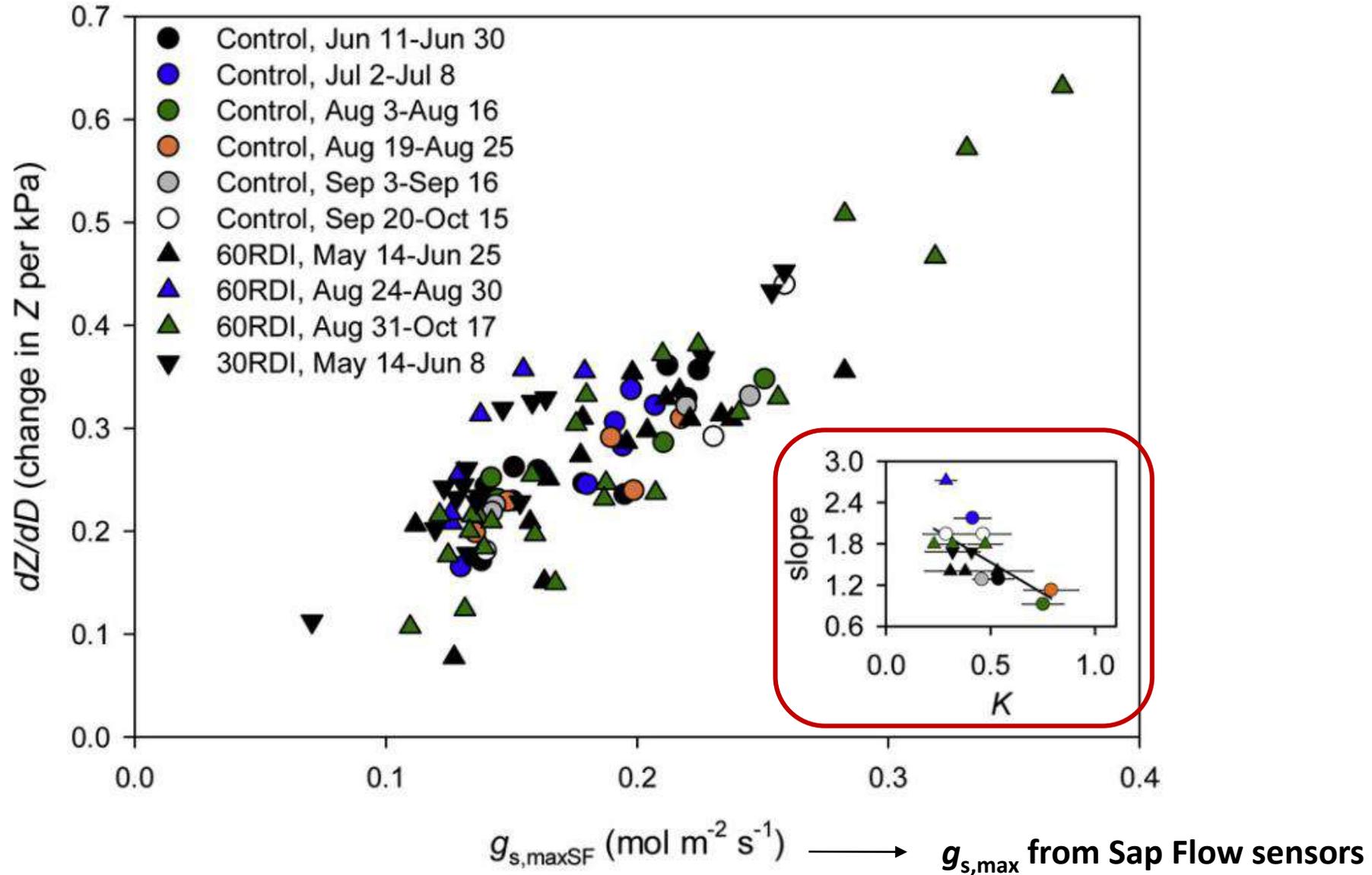
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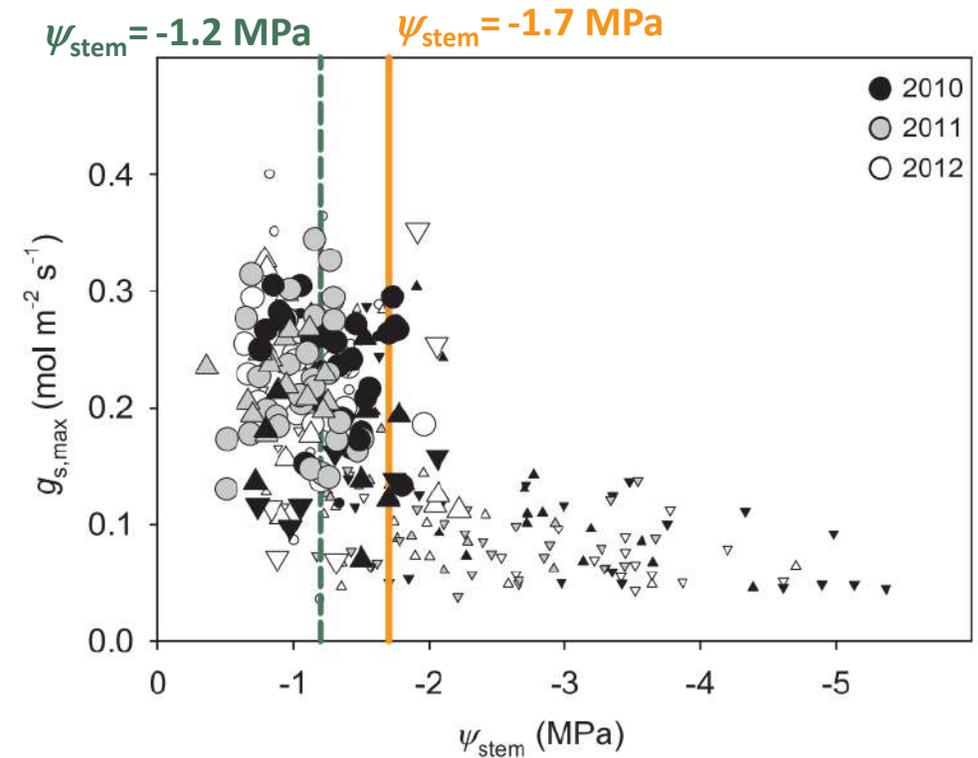
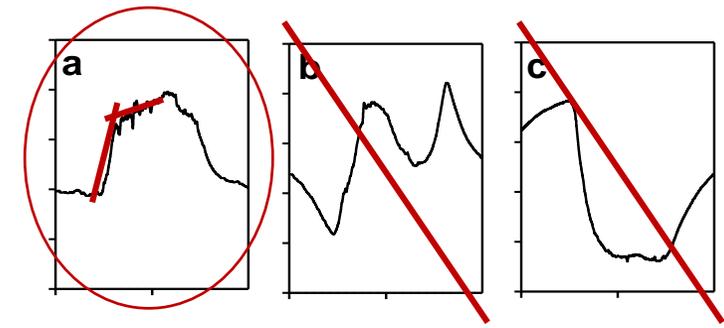
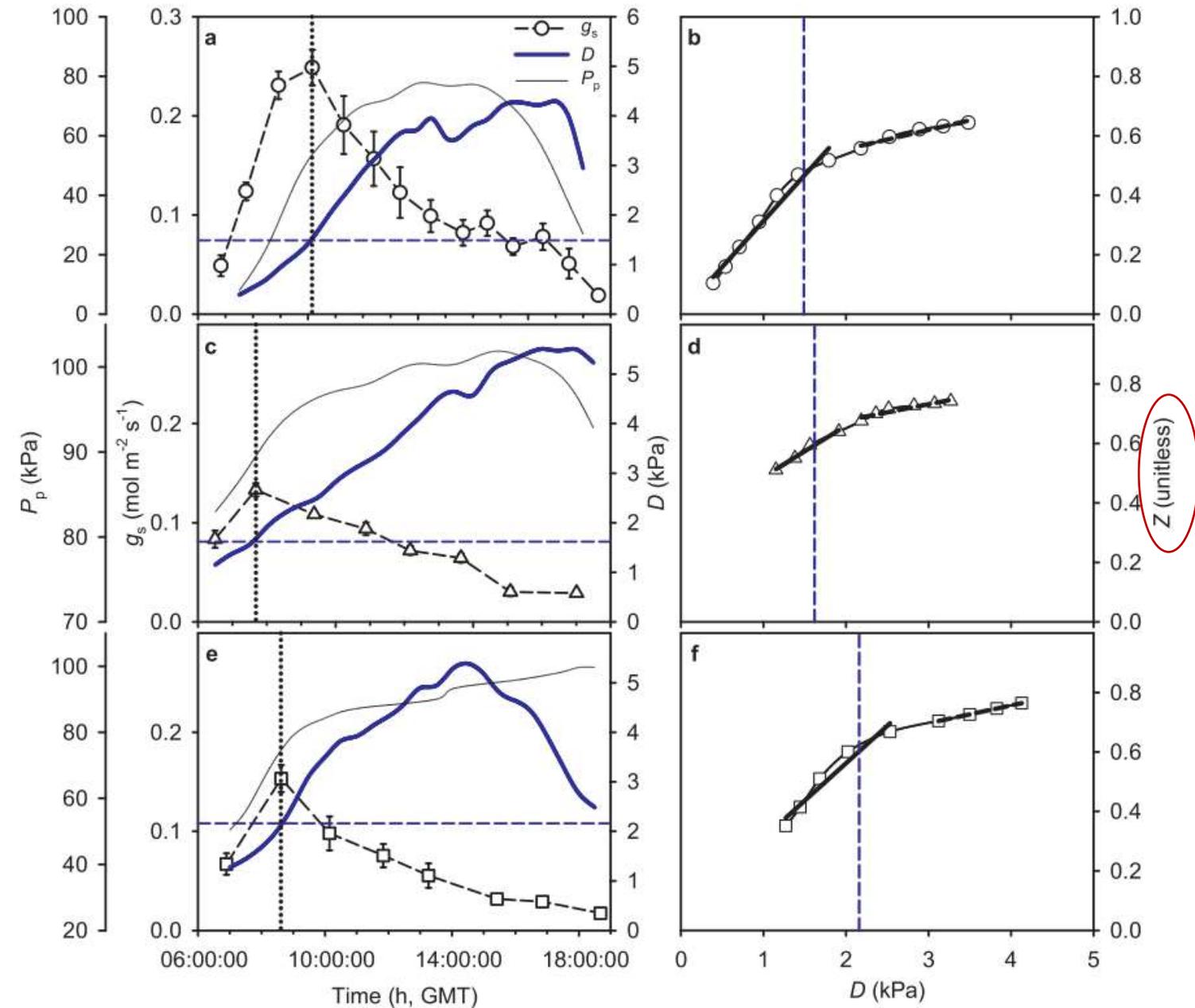
Continuous monitoring of $g_{s,\text{max}}$ from leaf turgor and vapor pressure deficit measurements

Increasing water stress

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



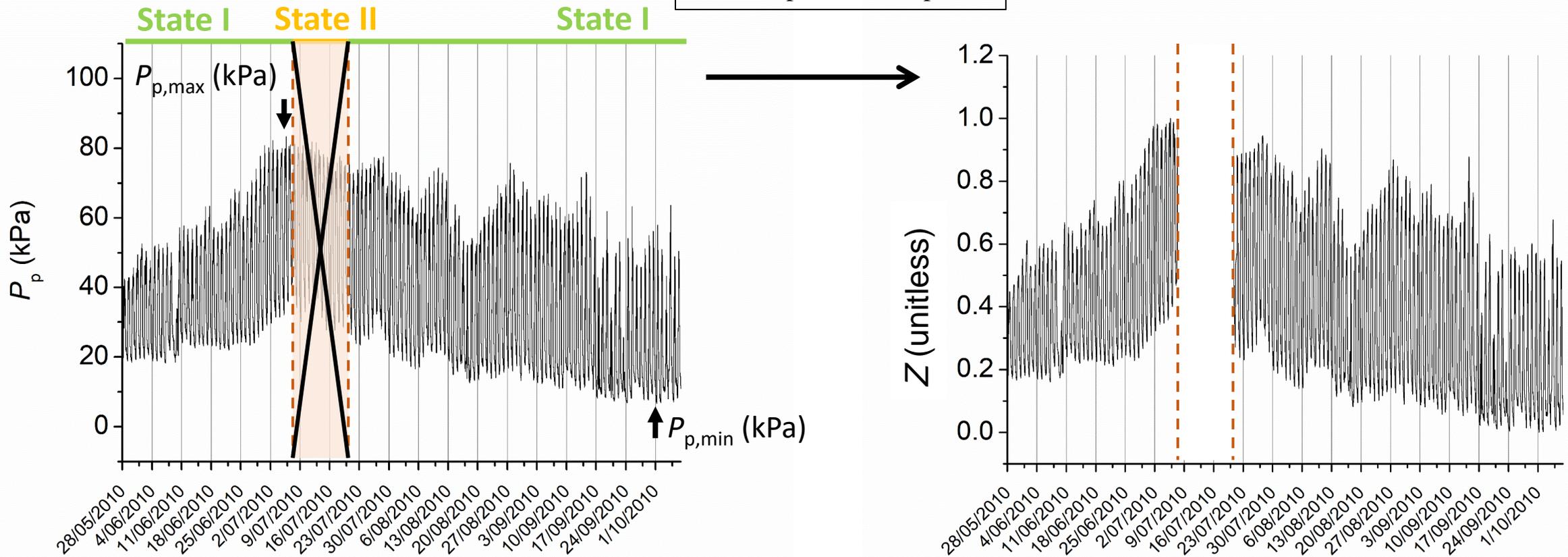
A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

Remember! ZIMs -> *Relative changes of P_c* -> Normalization is needed

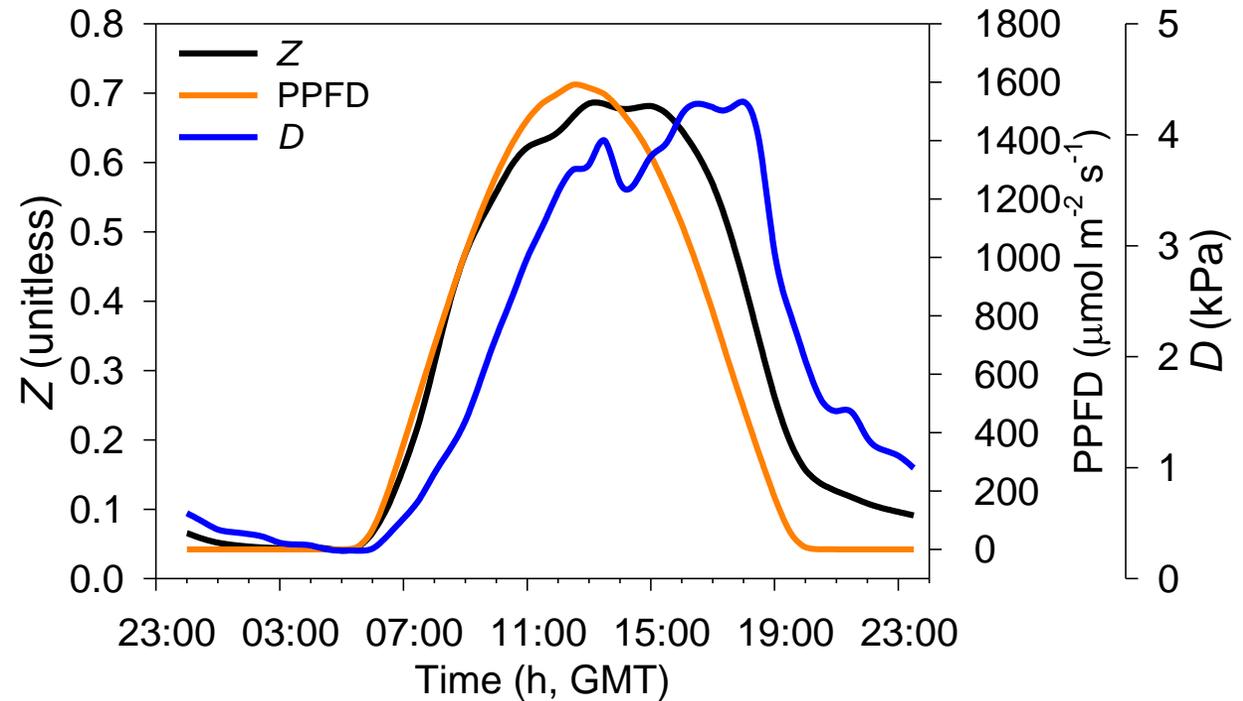
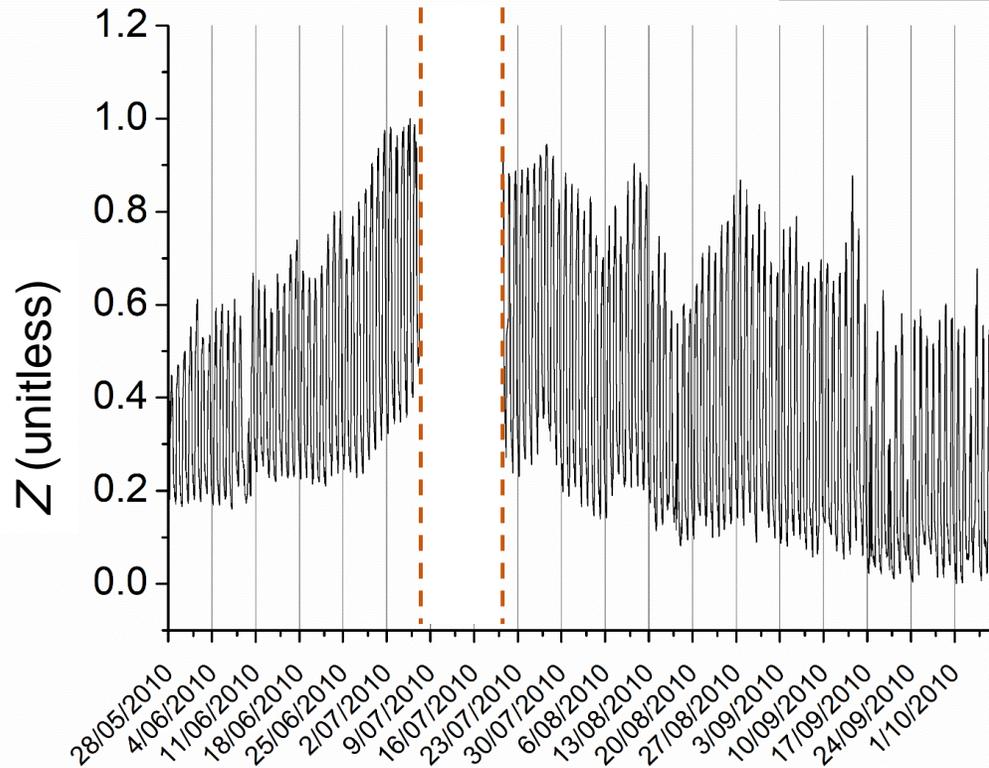
$$Z = \frac{P_p - P_{p,\min}}{P_{p,\max} - P_{p,\min}}$$



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

Remember! ZIMs -> *Relative changes of P_c* -> Normalization is needed

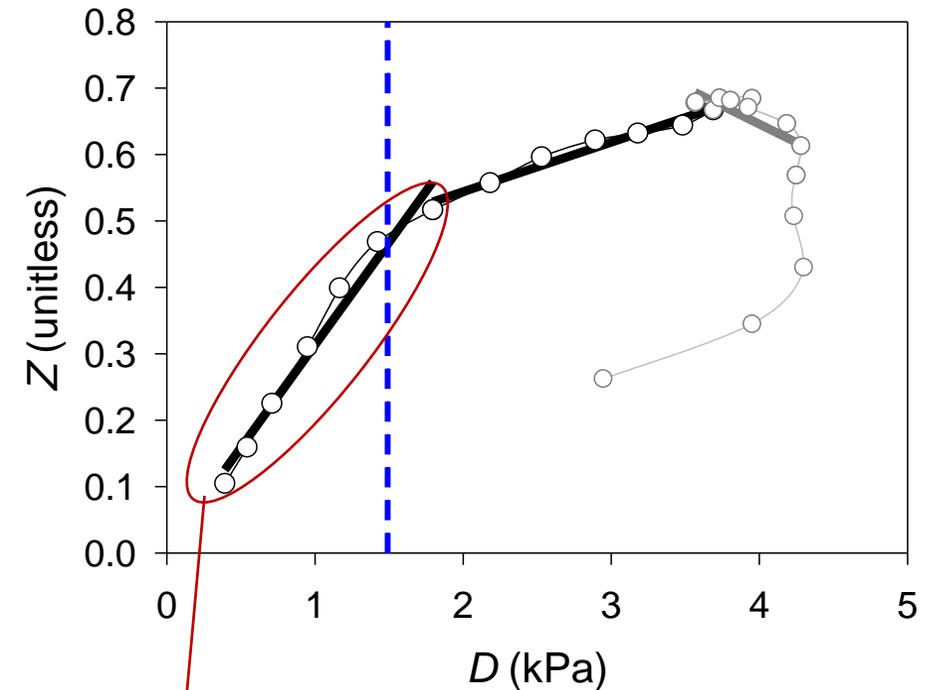
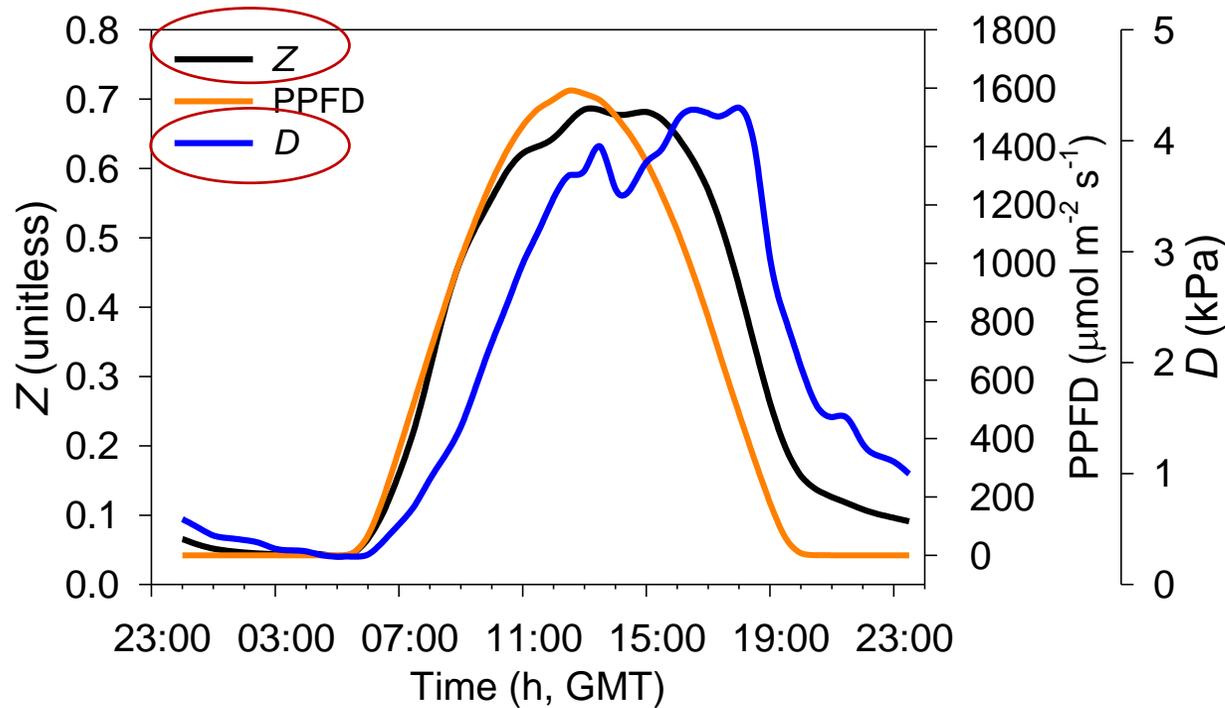
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A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

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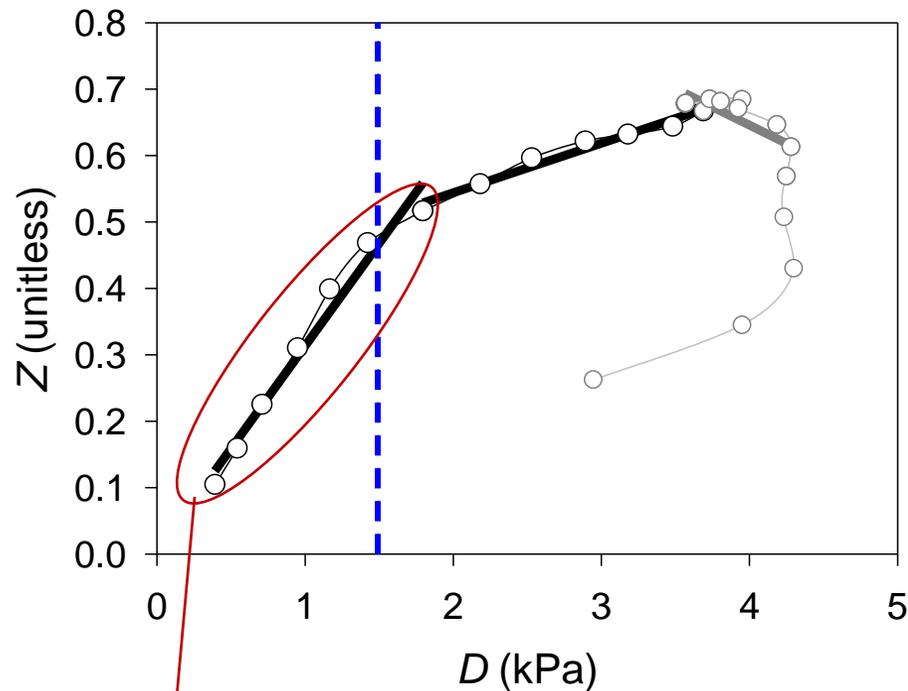


slope = $\Delta \text{Turgor} / \Delta \text{VPD}$

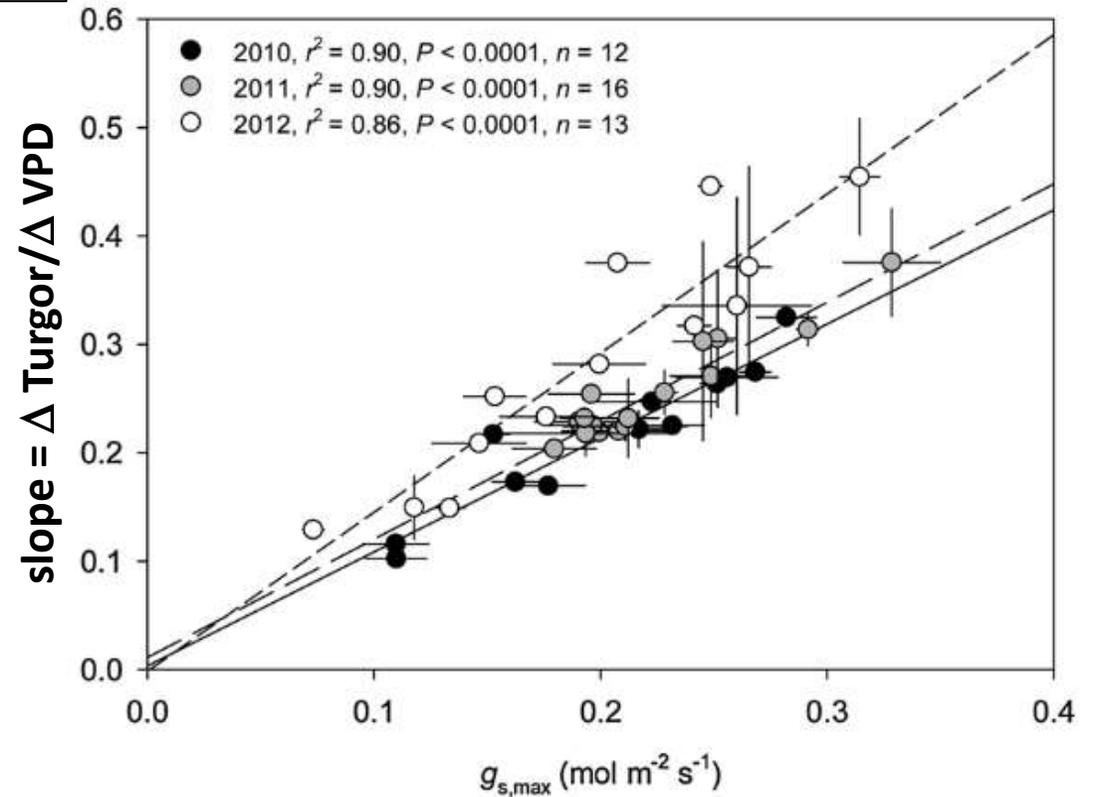
A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

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slope = Δ Turgor/ Δ VPD



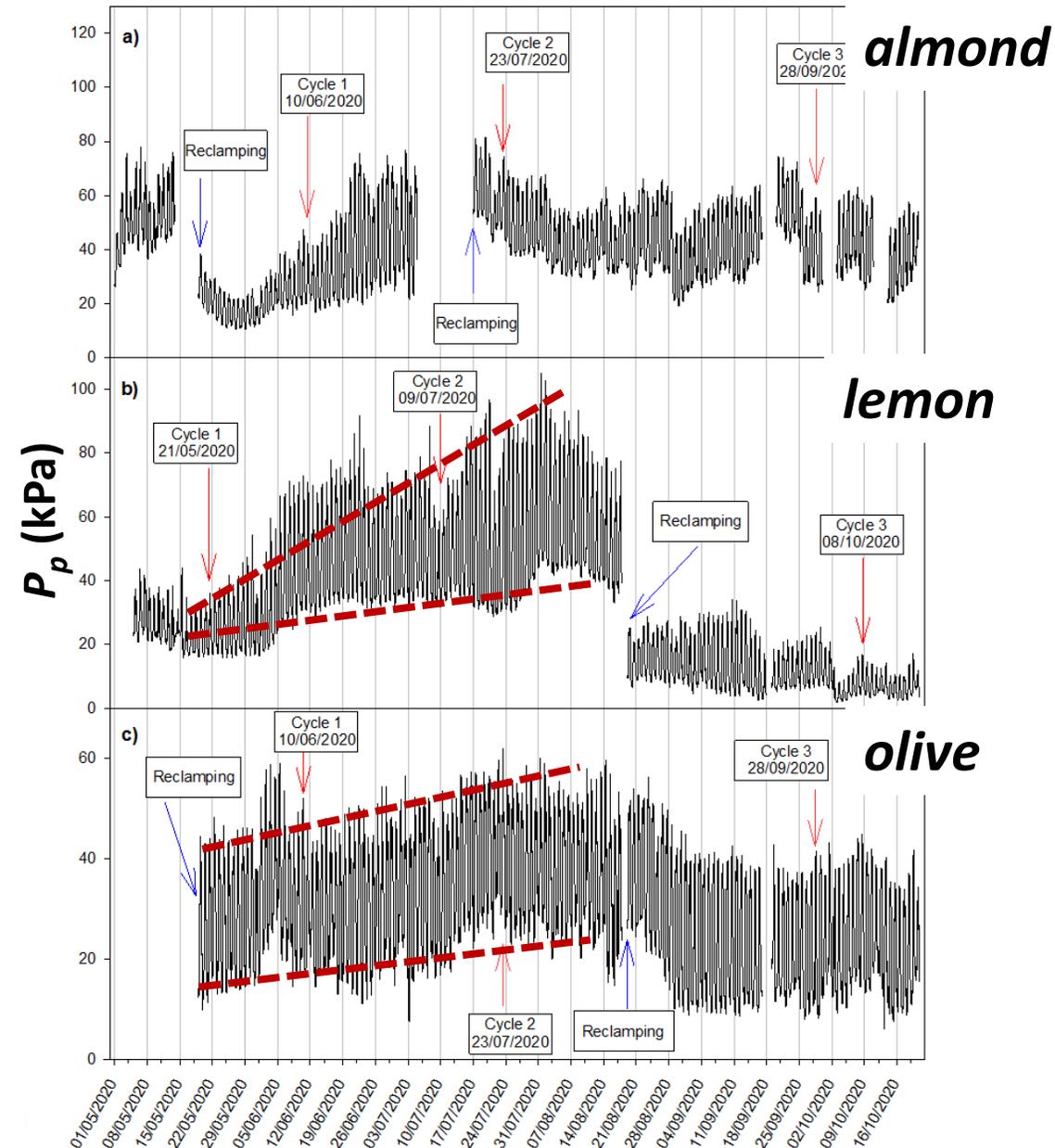
A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

However, Seasonal Normalization, depending on the application, may not be the best...



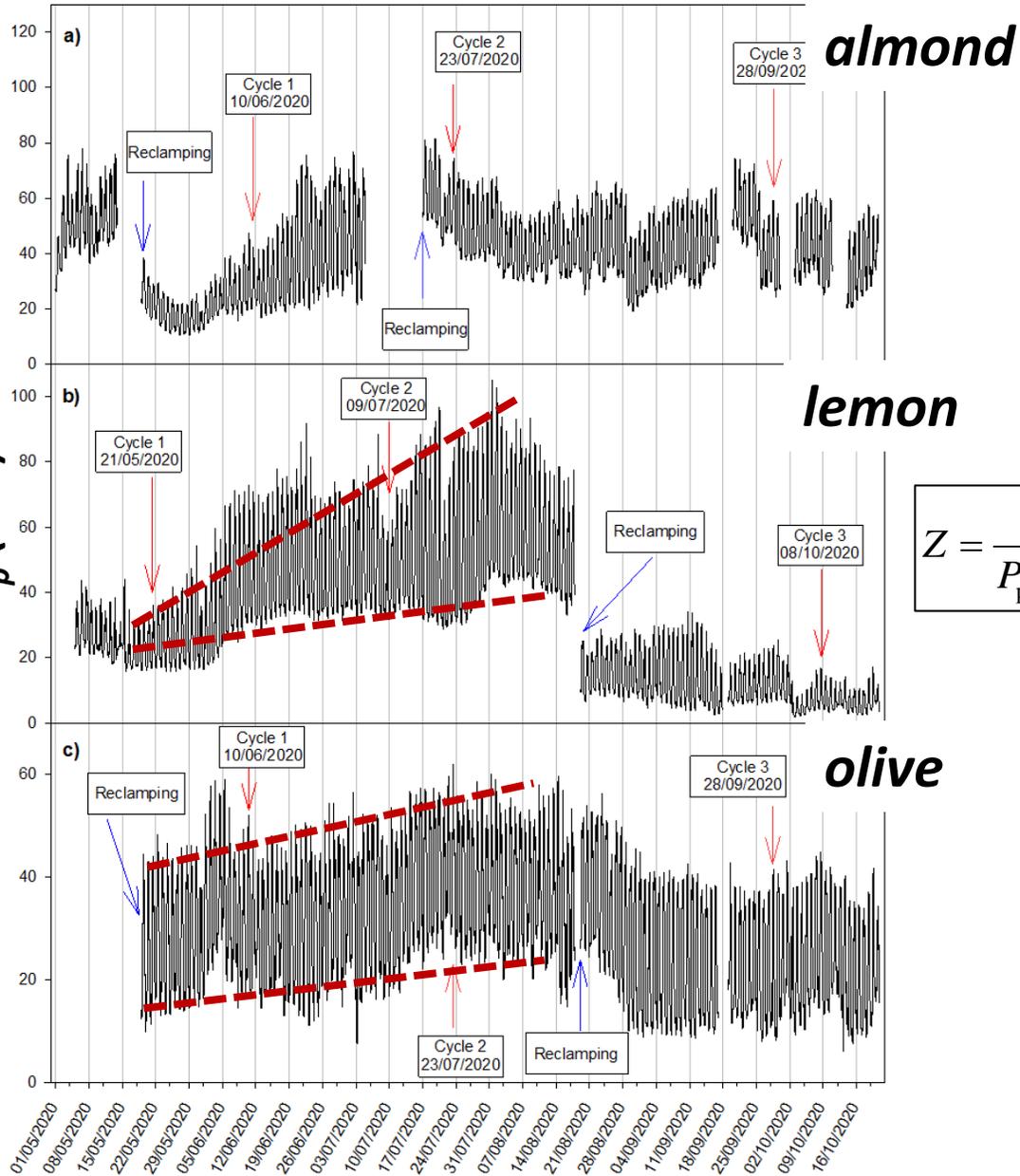
Luis Sánchez Fernández
Master Thesis

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



- *Almond* needed more reclampings
- P_p range in *lemon* was increasing
- P_p range in *olive* was more constant

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



almond

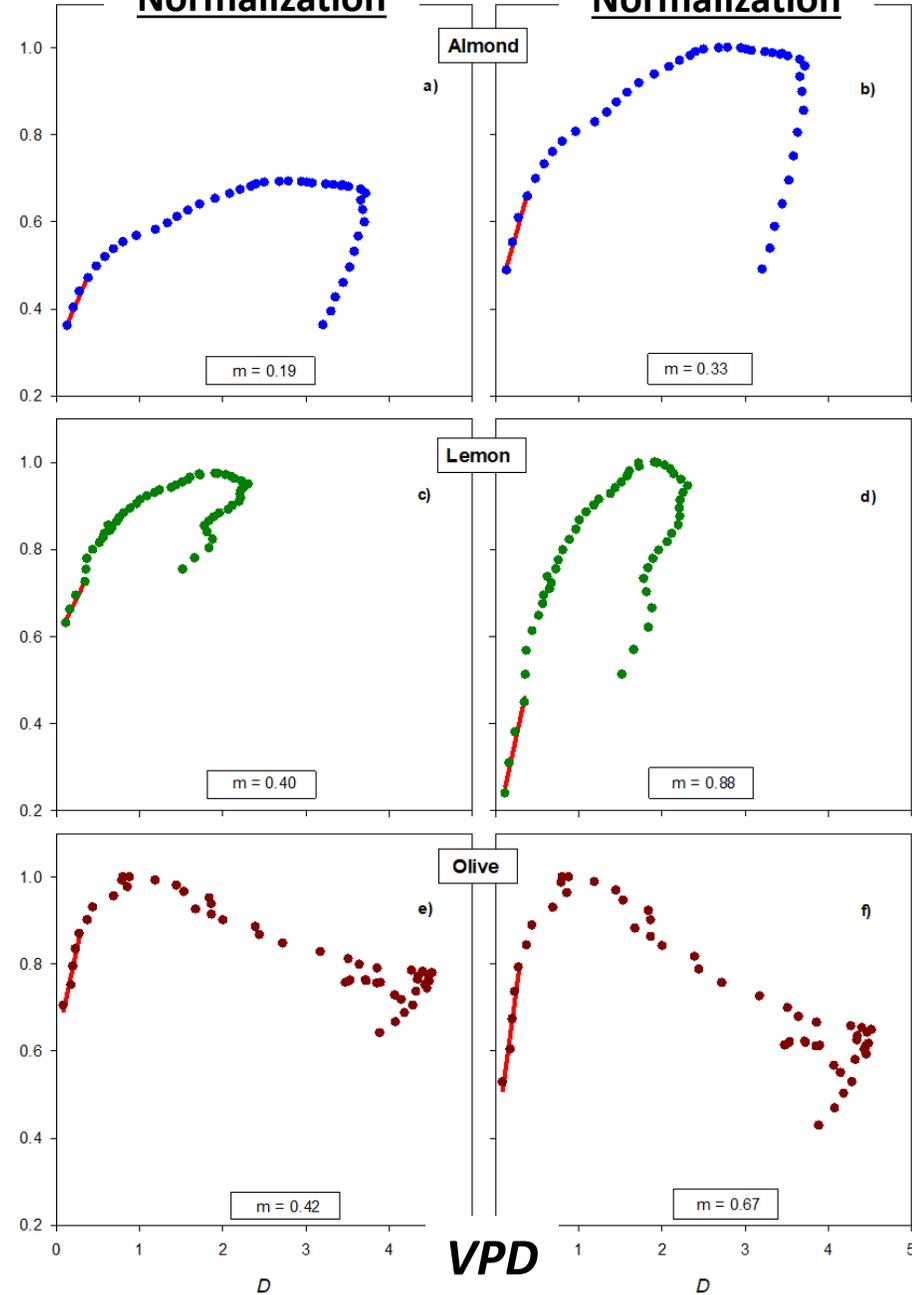
lemon

olive

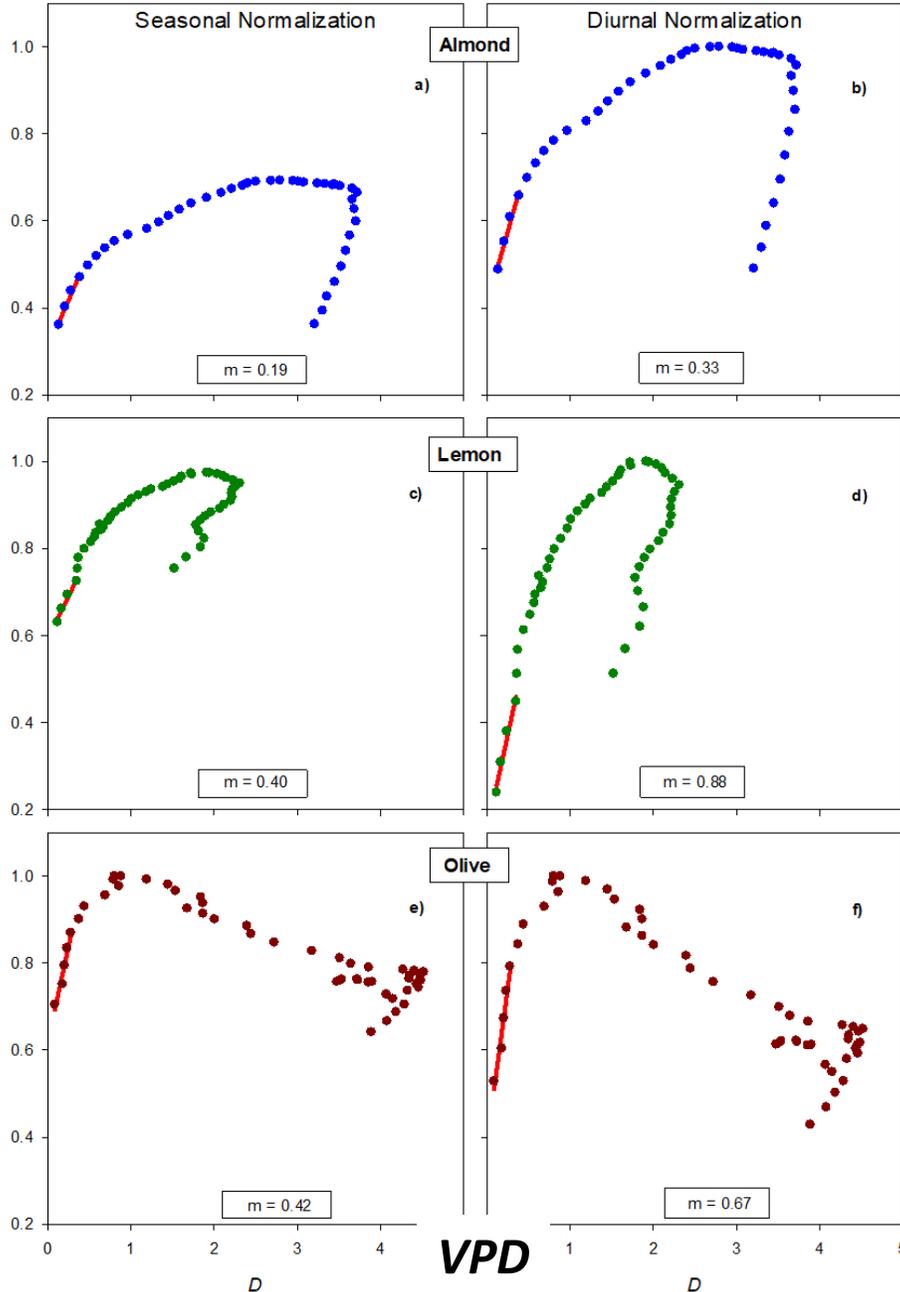
$$Z = \frac{P_p - P_{p,\min}}{P_{p,\max} - P_{p,\min}}$$

Seasonal Normalization

Diurnal Normalization



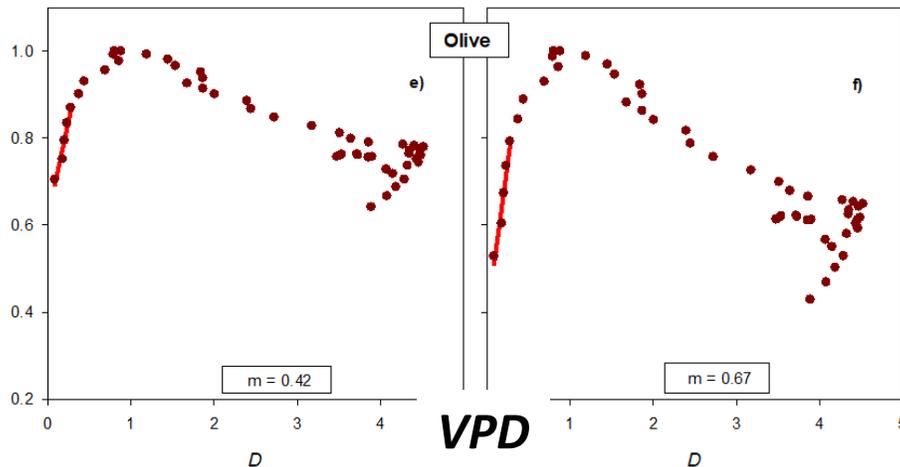
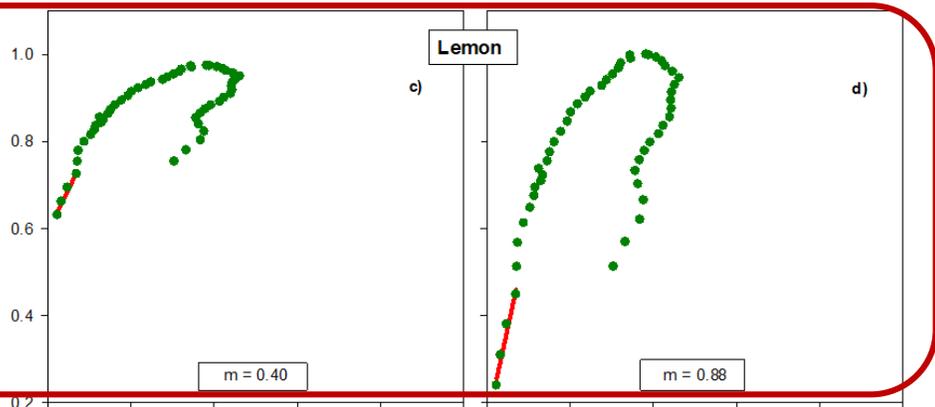
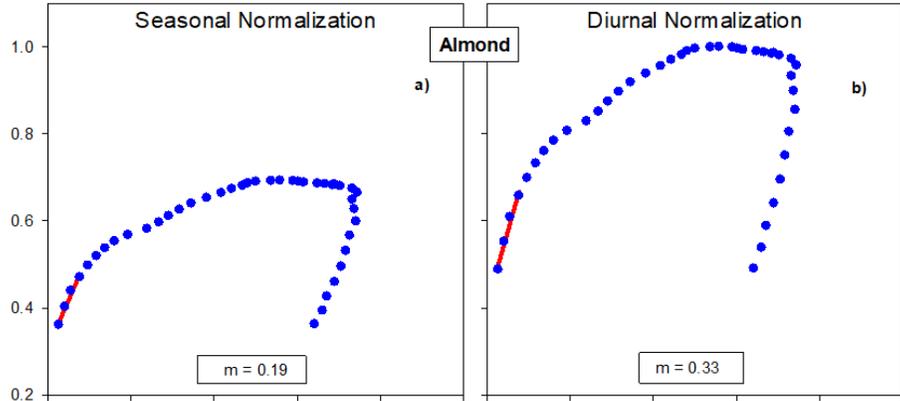
A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



- Diurnal normalization maintains the actual dynamics, as the increase in P_p ranges influences the normalized values.

$$Z = \frac{P_p - P_{p,\min}}{P_{p,\max} - P_{p,\min}}$$

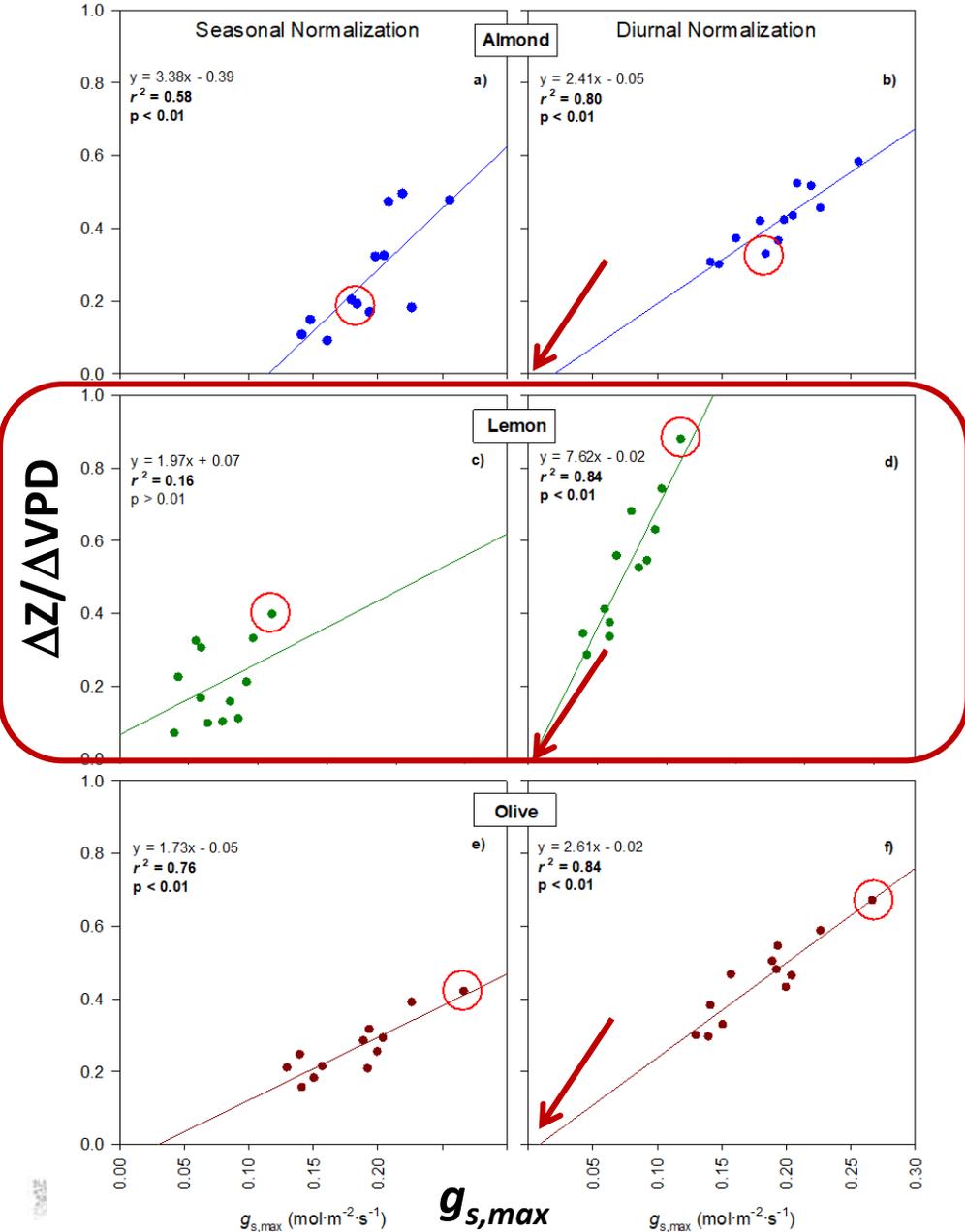
A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



- Diurnal normalization maintains the actual dynamics, as the increase in P_p ranges influences the normalized values.
- Considerable increase of $\Delta Z/\Delta VPD$ in lemon trees

$$Z = \frac{P_p - P_{p,\min}}{P_{p,\max} - P_{p,\min}}$$

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



- Diurnal normalization, especially in *lemon*, improved dZ/dD vs $g_{s,max}$ relationships.
- *Lemon* > *Almond* > *Olive*
- $r^2 > 0.80$ $P < 0.01$
- Intersection $dZ/dD - g_{s,max} \approx 0$

Does it mean seasonal normalization may be useless?

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

To explore why a diurnal normalization improved dZ/dD vs $g_{s,max}$ relationships, we had to derive seasonal Z

$$Z = \frac{P_p - P_{p,min}}{P_{p,max} - P_{p,min}}$$

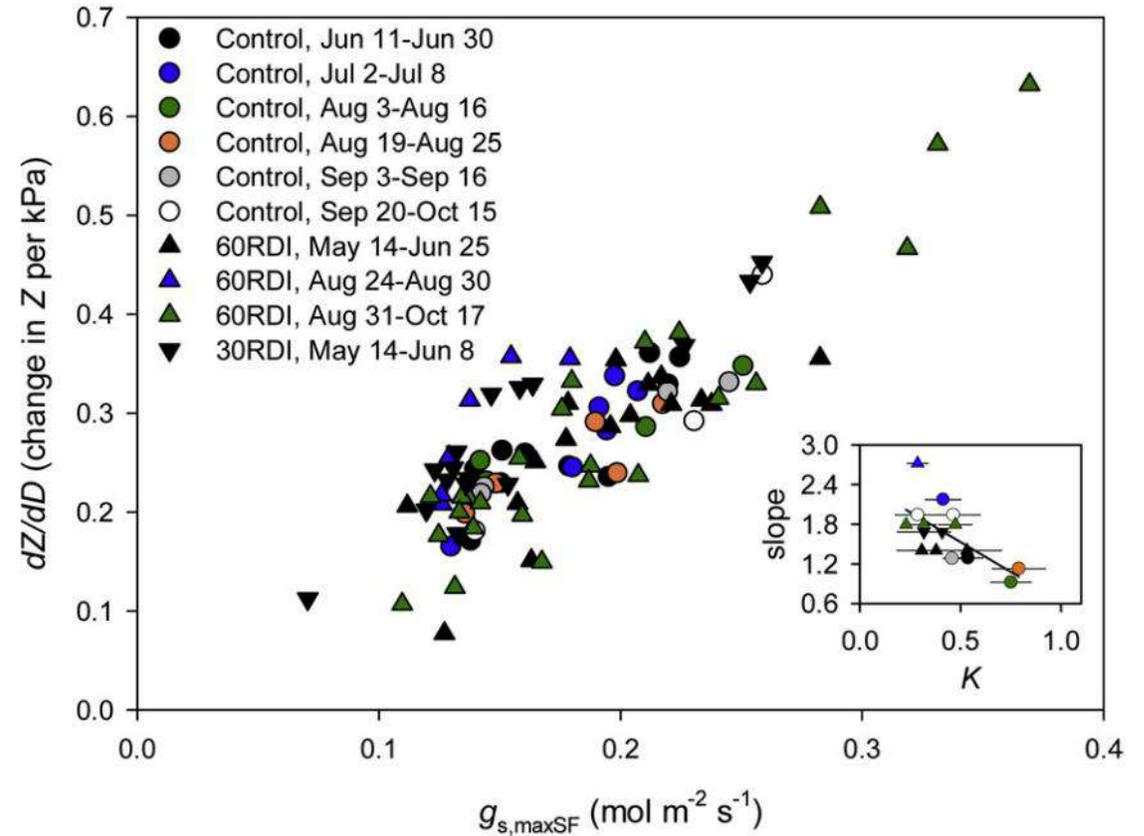
Why did it apply in olive from other orchard?

$$\frac{dZ}{dD} \propto \frac{g_{s,max}}{E_{max}}$$

$$E_{max} = K(\Psi_{soil} - \Psi_{leaf,min})$$

conservative within the season

Did not change over the growing season



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

To explore why a diurnal normalization improved dZ/dD vs $g_{s,max}$ relationships, we had to derive seasonal Z

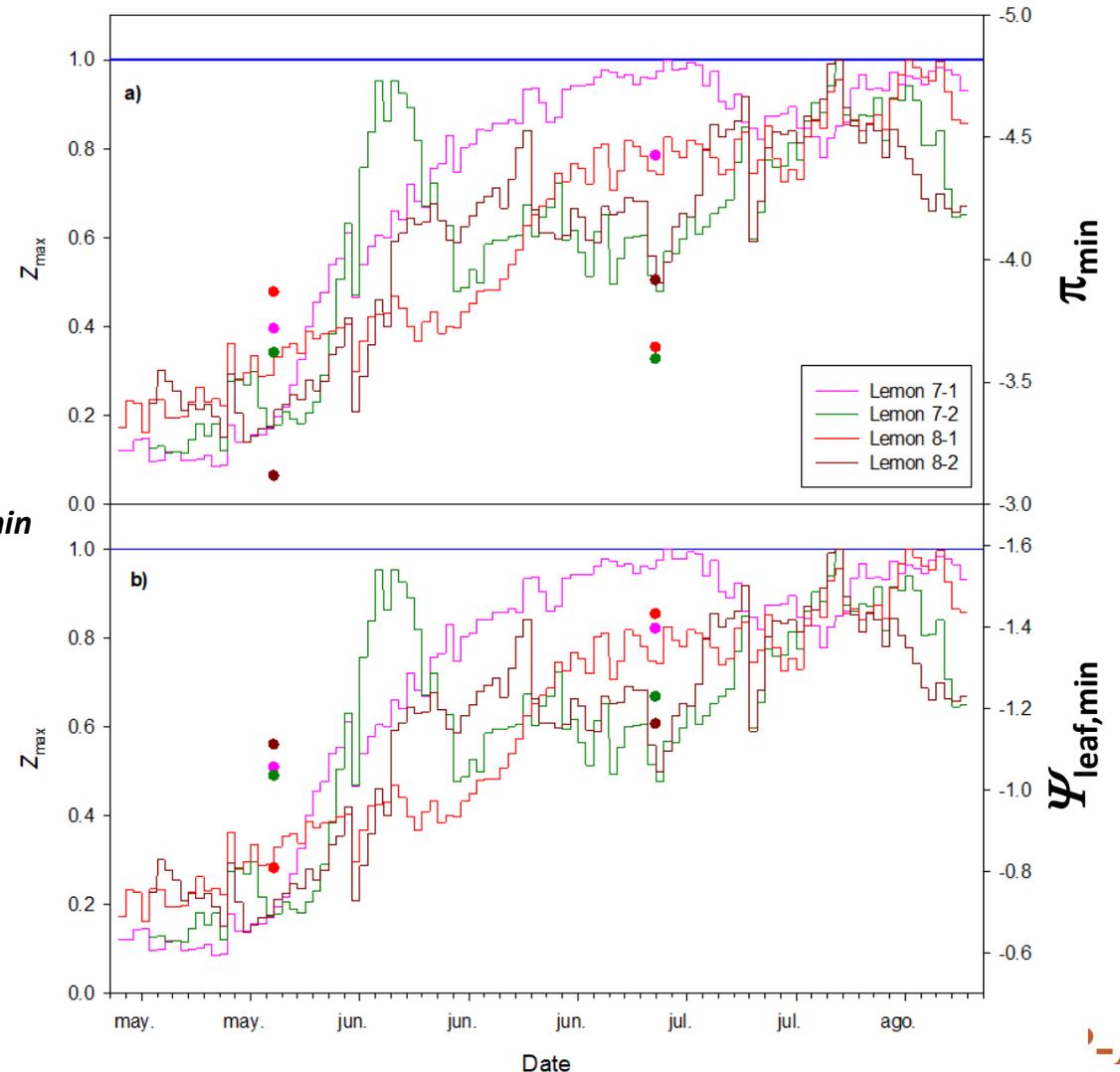
$$E_{max} = K(\Psi_{soil} - \Psi_{leaf,min})$$

- However, we did find a change in $\Psi_{leaf,min}$ in *lemon*, in parallel with a change in π_{min}

$$P_{c,min} = \Psi_{leaf,min} + \pi_{min}$$

$$Z_{max} \sim P_{c,min}$$

Lemon Z_{max} vs π_{min} and $\Psi_{leaf,min}$



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

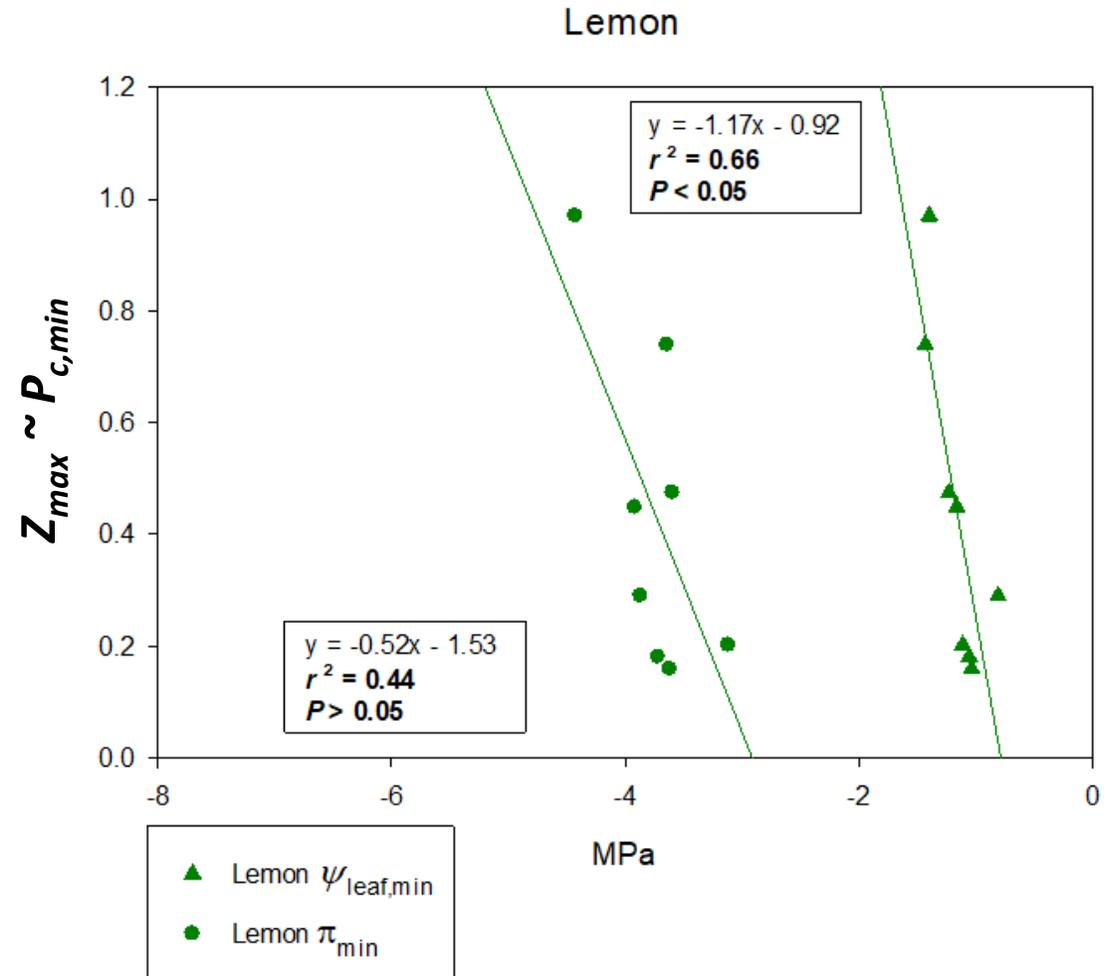
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$$P_{c,min} = \Psi_{leaf,min} + \pi_{min}$$

We did not find significant correlations neither between Z_{max} and $\Psi_{leaf,min}$ and π_{min} in *olive*, nor between Z_{min} and predawn leaf water potential ($\Psi_{leaf,pd}$) and predawn osmotic potential (π_{pd}) in both *lemon* and *olive* trees



A MORE PHYSIOLOGICAL USE OF ZIM PROBES?

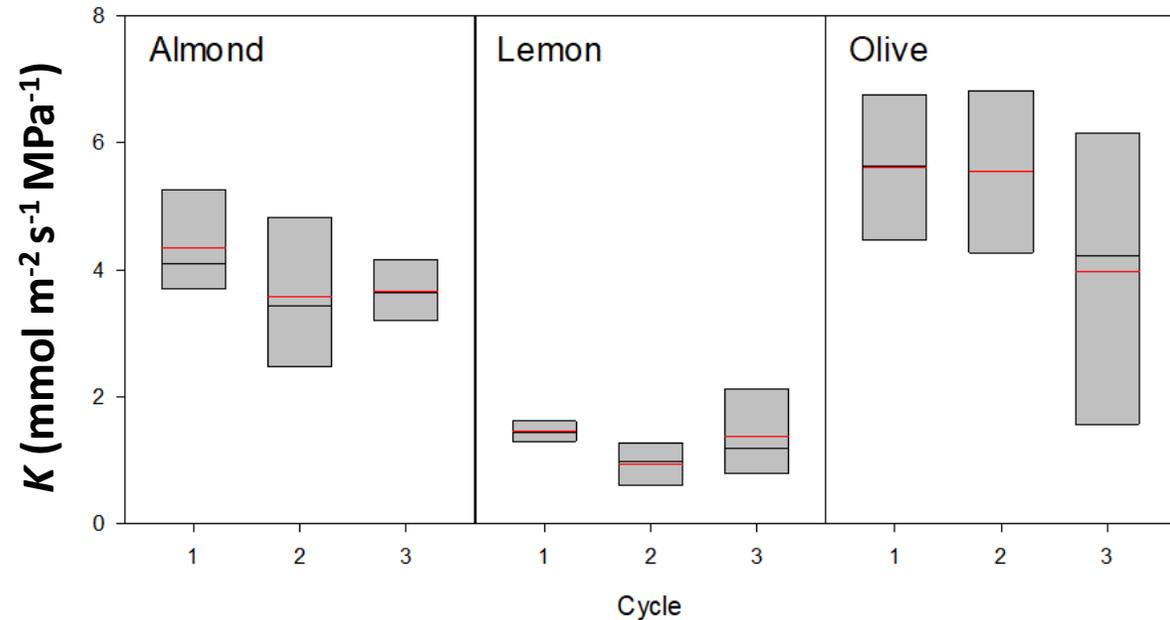
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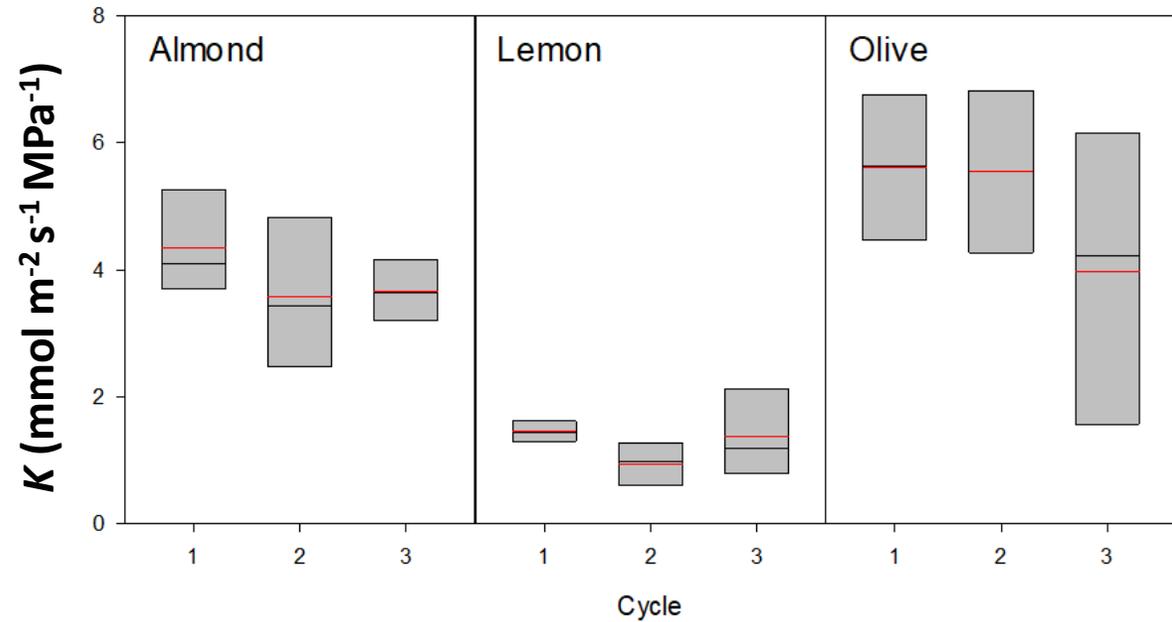
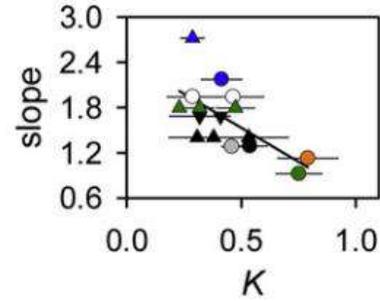
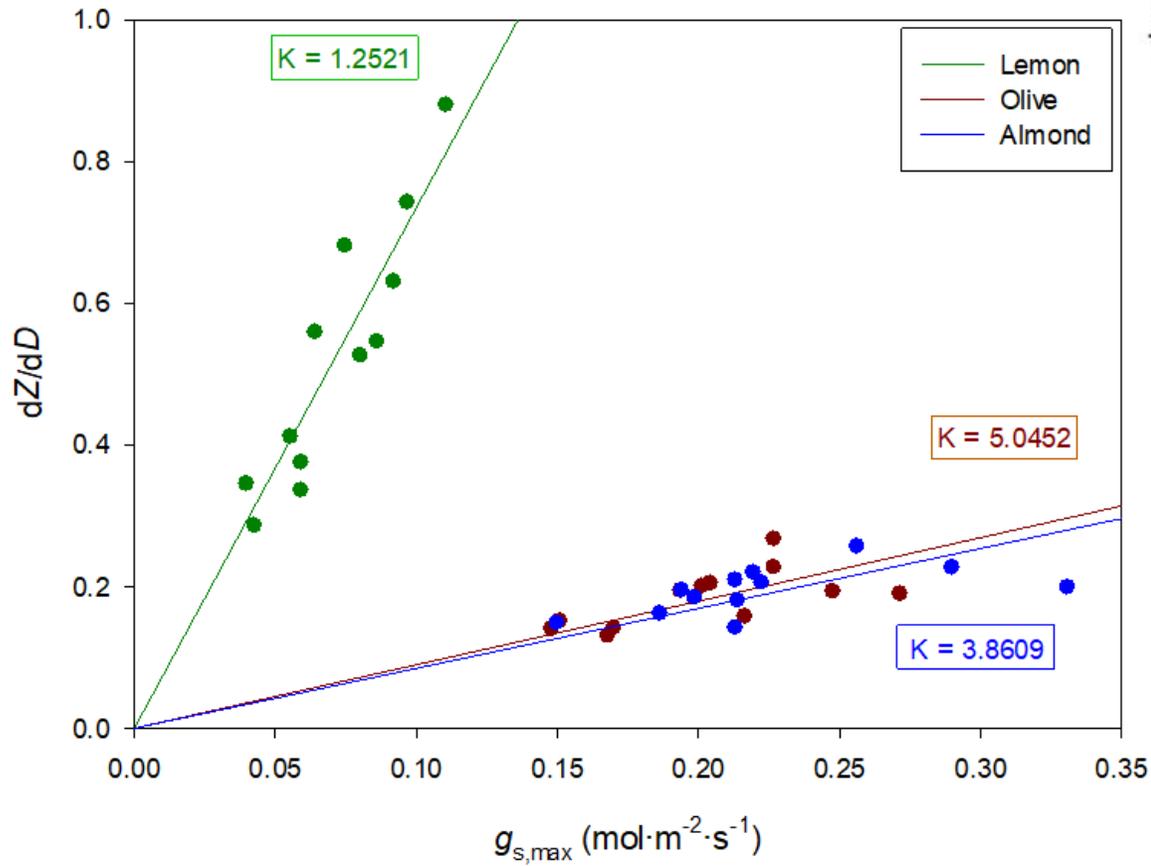
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- K did not differ within species' season

A MORE PHYSIOLOGICAL USE OF ZIM PROBES?



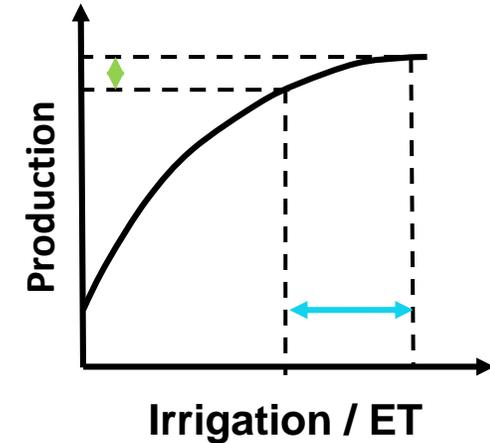
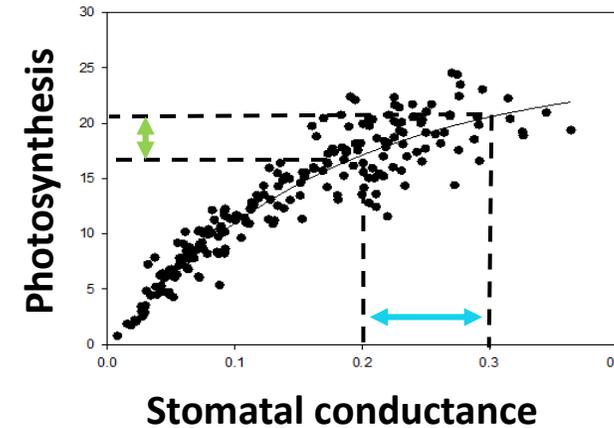
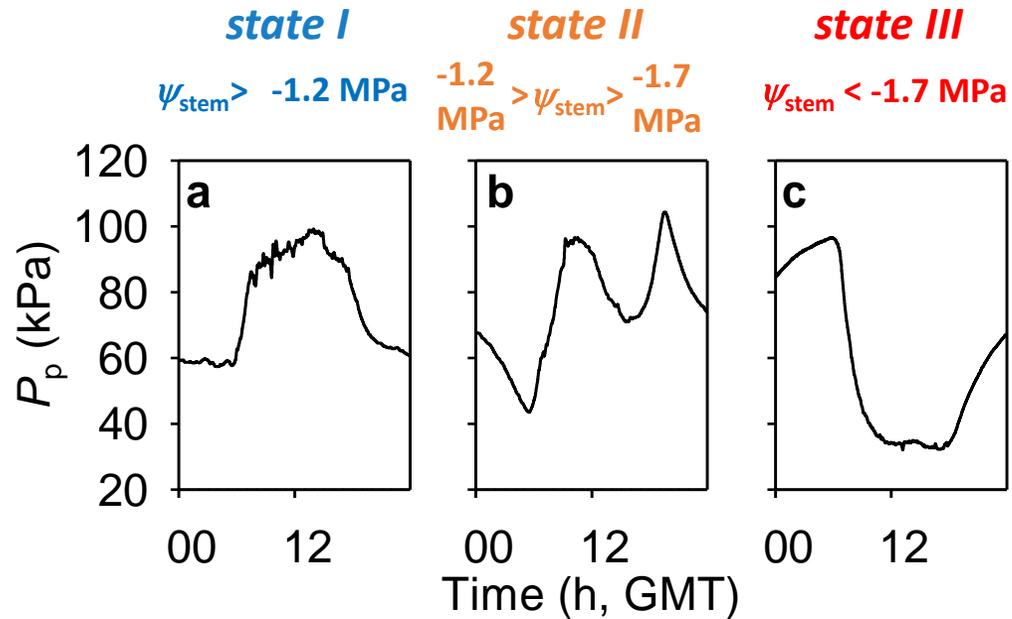
- The higher the slope dZ/dD vs $g_{s,max}$, the lower the K

- K did not differ within species' season

CONCLUSIONS FROM THE USE OF ZIM PROBES

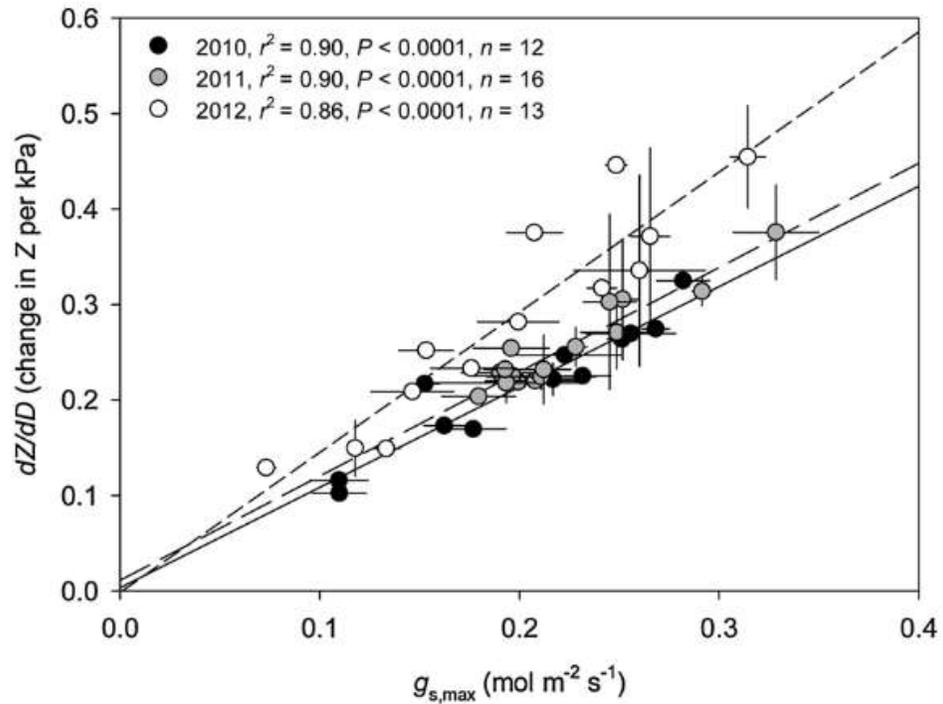
Two main Take-home messages:

- We can extract useful information for irrigation scheduling
- However, combining this sensor with physiological knowledge and mechanistic modelling is needed to better approximate irrigation needs



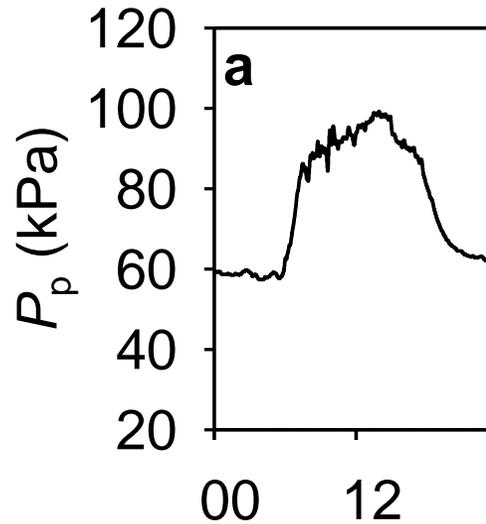


QUESTIONS??



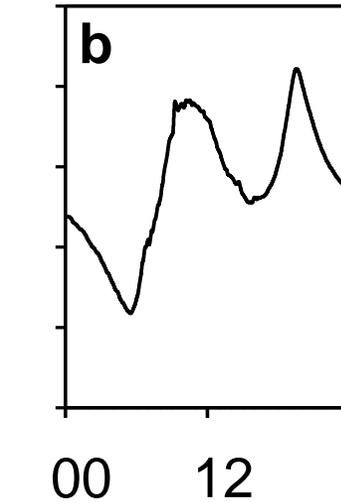
state I

$\psi_{stem} > -1.2 \text{ MPa}$



state II

$-1.2 \text{ MPa} > \psi_{stem} > -1.7 \text{ MPa}$



state III

$\psi_{stem} < -1.7 \text{ MPa}$

