ISHS SPWS 2016 - Sensing Plant Water Status - Methods and Applications in Horticultural Science

Hydraulic and stomatal factors affecting water transport

LIFE 14 CCA/GR/00389 - AgroClimaWater

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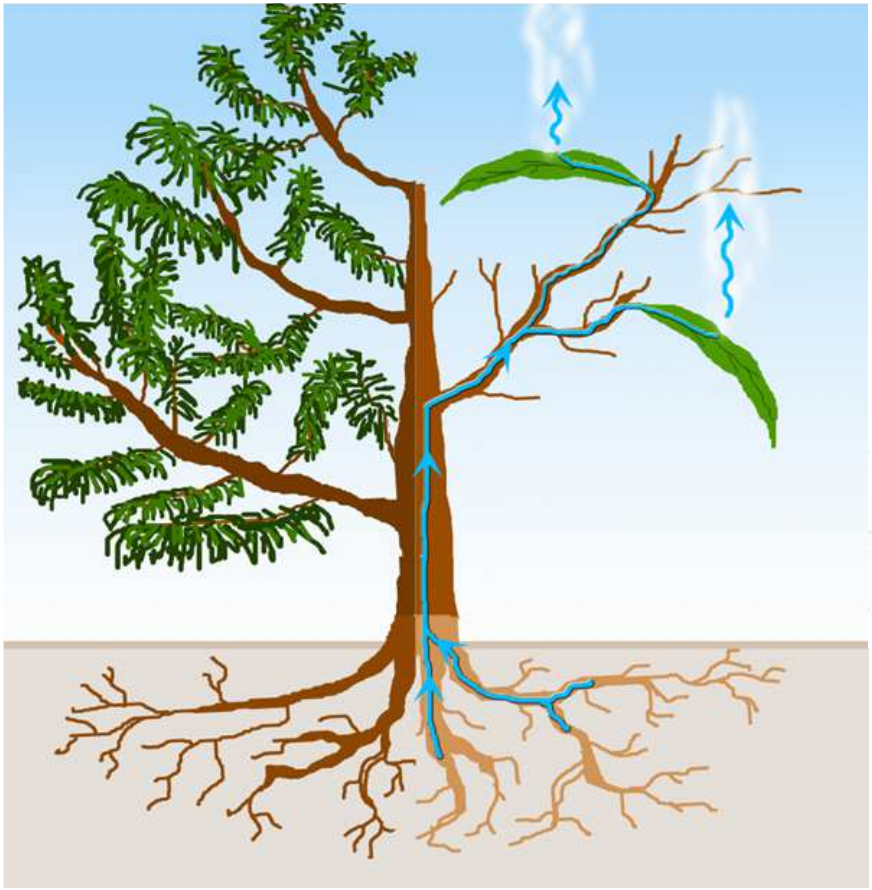
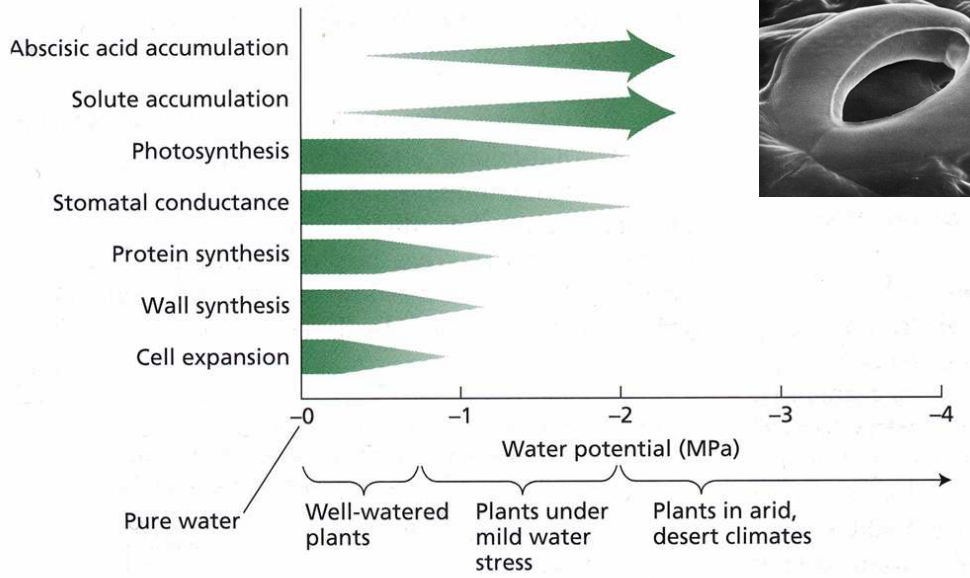


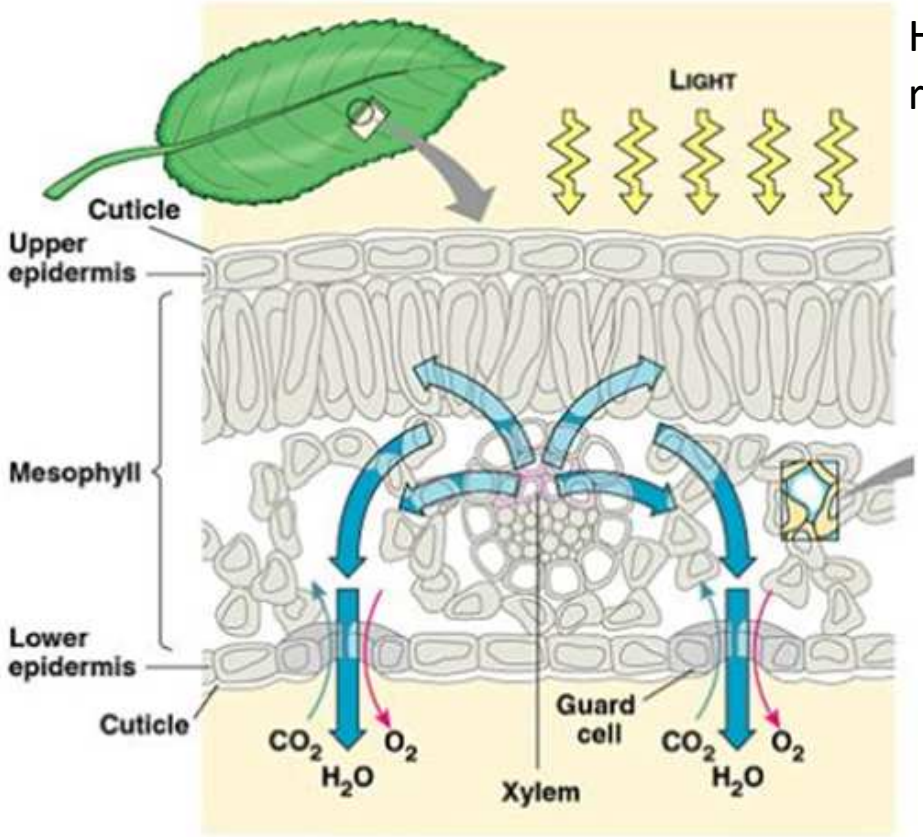


Many efforts have been made to understand the water balance of plants in terms of a regulation of transpiration, i.e. of how stomatal conductance would be affected by water status, light intensity, nutrition, and other factors.

Less efforts have been made to investigate the input side of the water balance, i.e. the acquisition of water from the soil.

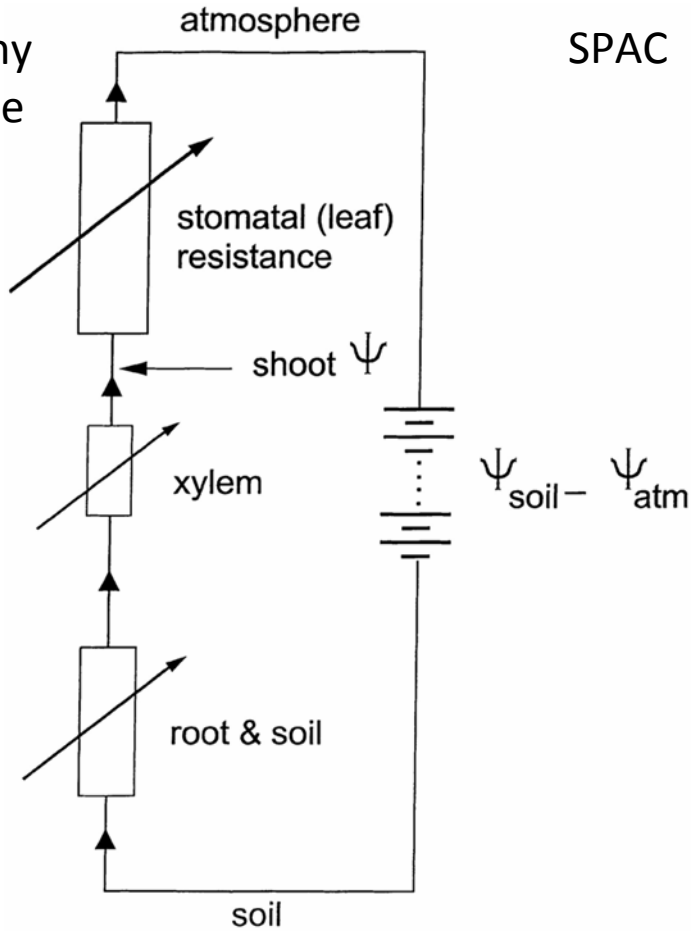
the knowledge of the characteristics of roots and its physiology helps to manage the orchard in a sustainable way

Water flow across the plant



Highest hy resistance

SPAC

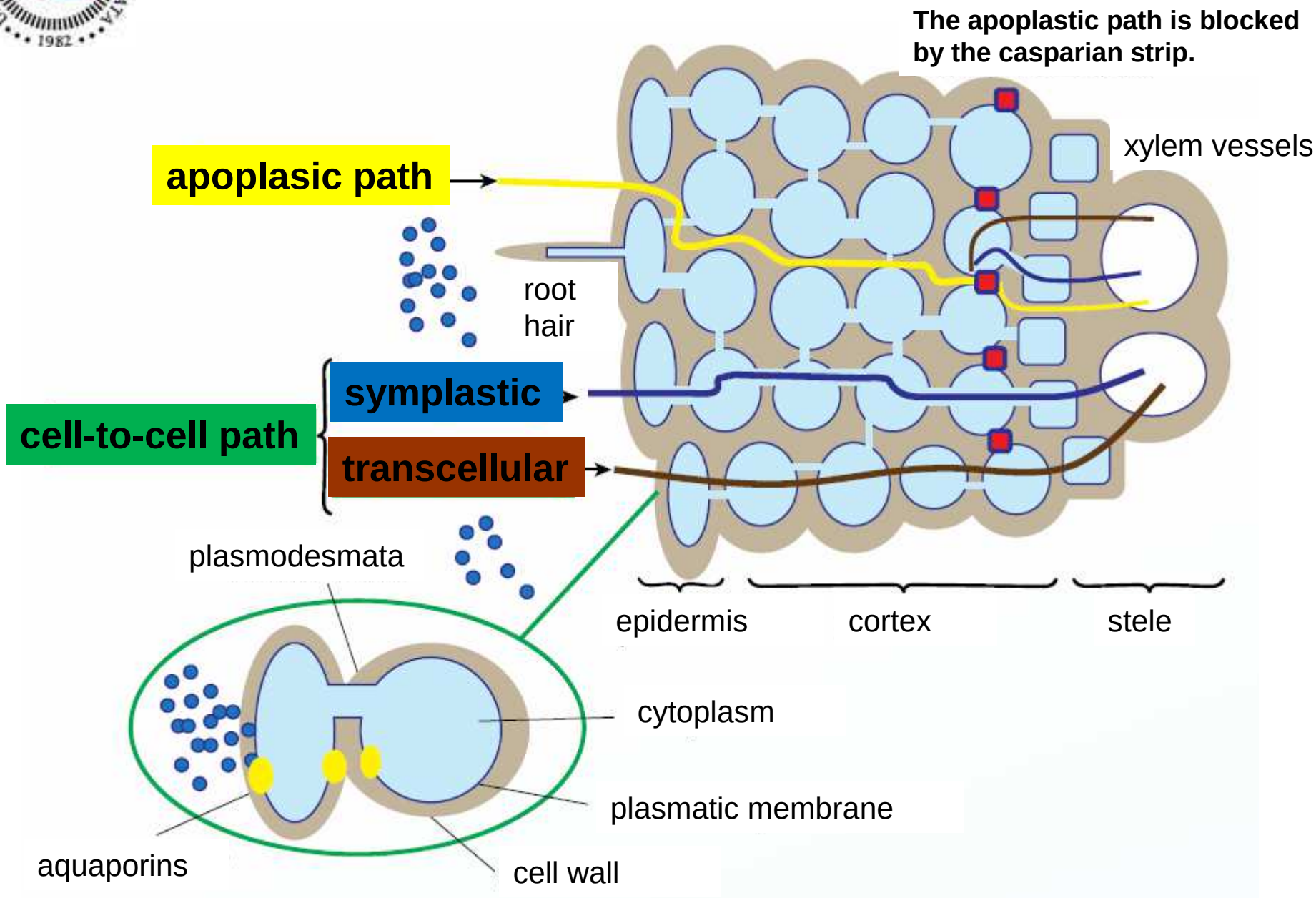


Ernst Steudle J. Exp. Bot. 2000;51:1531-1542

the variable root hydraulic resistance would contribute to the water potential (water status) of the shoot.

Radial water movement in roots

The radial water movement through the root is a complex process



What are the forces which drive the water flow?

Composite transport model (Steudle, 2000) predicts that root conductivity (Lp) differs depending on the nature of the forces which drive the water flow.

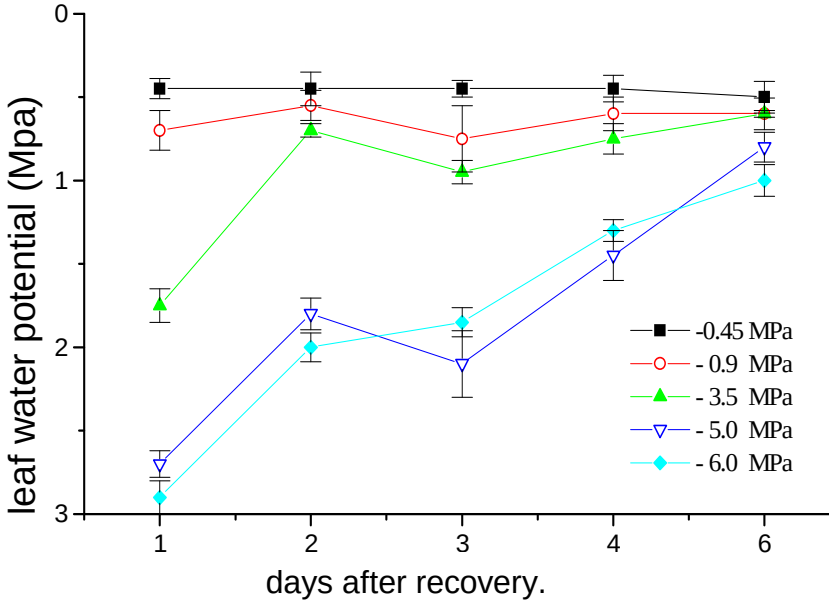
Hydrostatic pressure forces (due to transpiration) cause a much larger flow (root Lp) than **osmotic**. In tree roots, differences between hydraulic and osmotic water flow are up to three orders of magnitude.

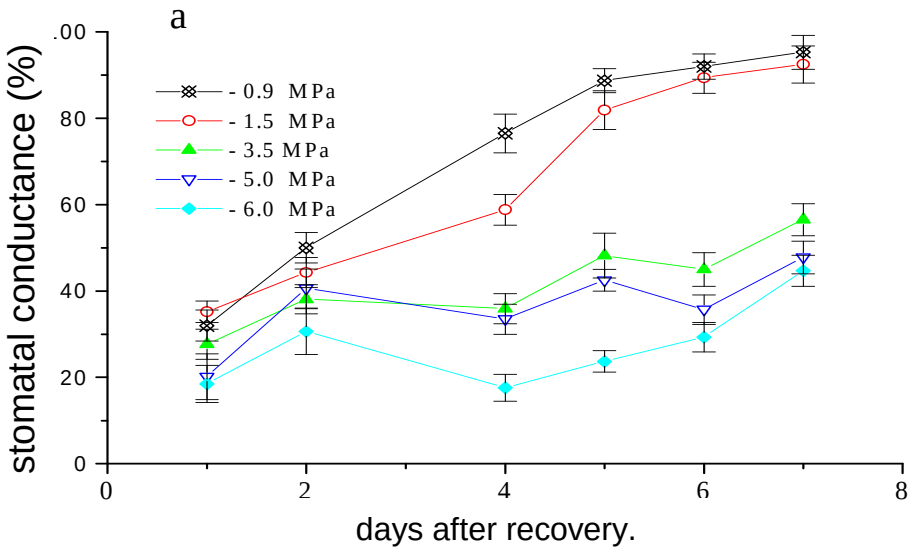
The hydraulic conductance of roots is influenced by plant physiology and environmental conditions.

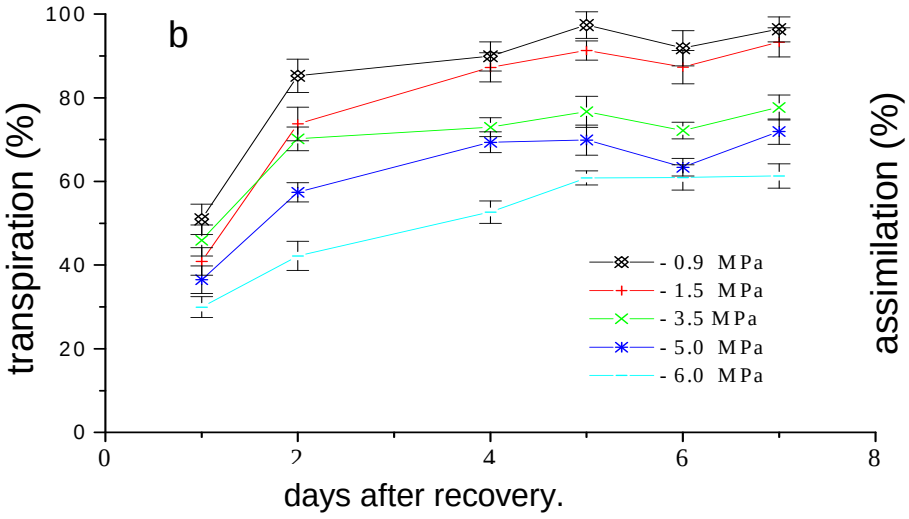
The apoplastic path, in fact, may be modified by **apoplastic barriers** (other than Casparian strips) in the exo- and endodermis, **suberin lamellae**.

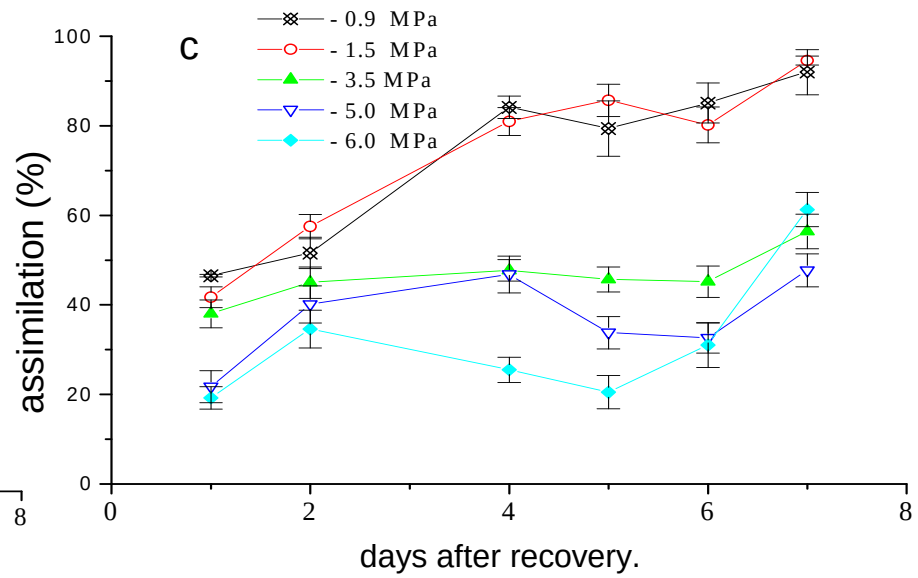
The cell-to-cell path may be affected by **water channel regulation**.

Physiologic inertia, during recovery phase, is a well known phenomena in literature (Dichio et al., 2006; Sofo et al., 2004; 2009)







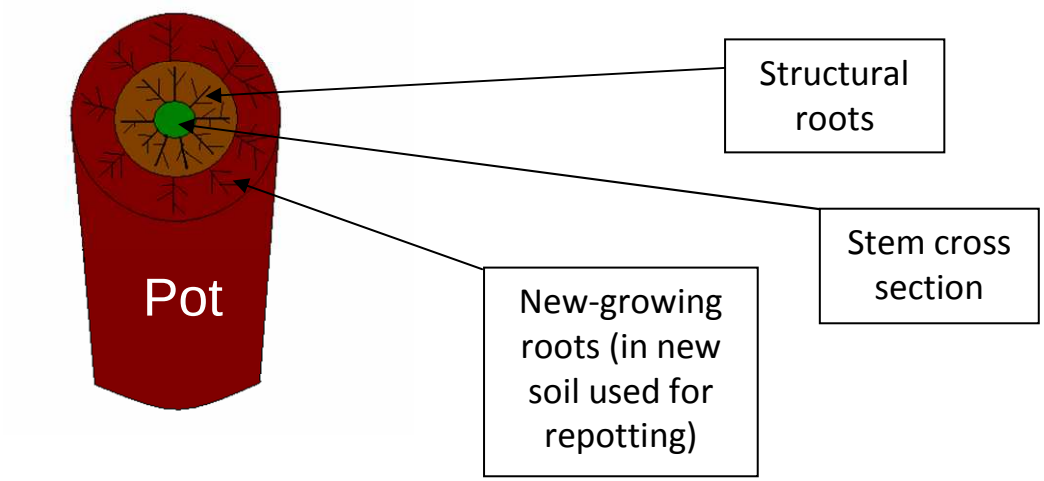


Experimentation

“apoplastic pathway and barriers” conductivity and morph-anatomical changes in olive roots under prolonged water deficit.

influence radial water uptake and transport through roots

Tataranni et al. 2015 - Tree Physilogy



White roots

Brown roots

The root was positioned inside the chamber after having cut the terminal portion in water and connected via a silicone tube to a needle in the chamber cap. The excised root system was immersed in a beaker containing pre-aerated pure water.

Root hydraulic conductivity (Lp m s^{-1} MPa^{-1}) *Nobel et al., 1990*

$$Lp = \frac{1}{A}\frac{Jv}{\Delta P}$$

where

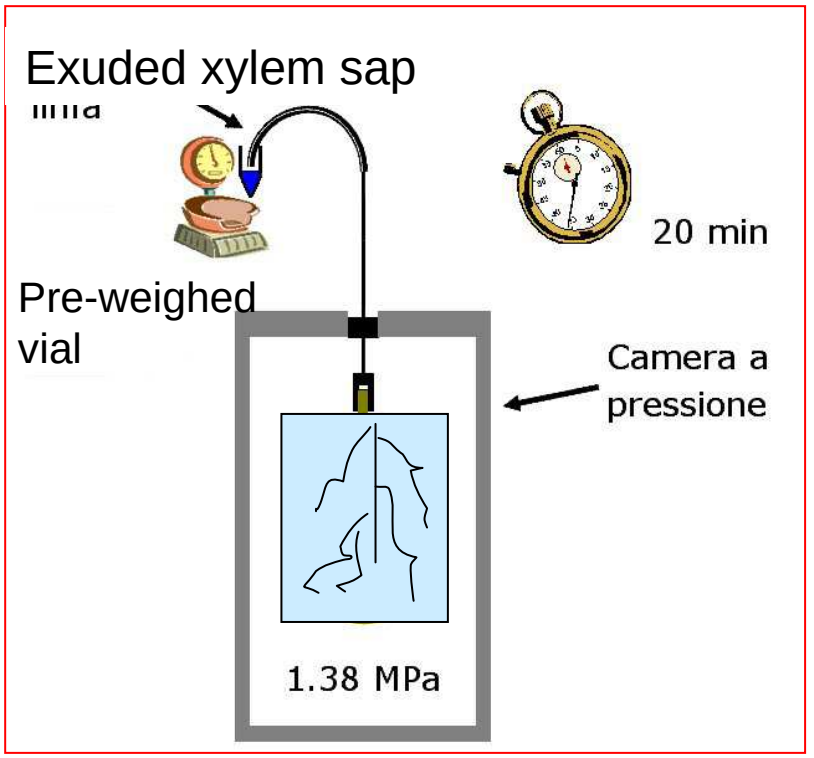
$$\text{Jv (the rate of water flow)} = \frac{\text{volume of water } (m^3)}{\text{time (s)}}$$

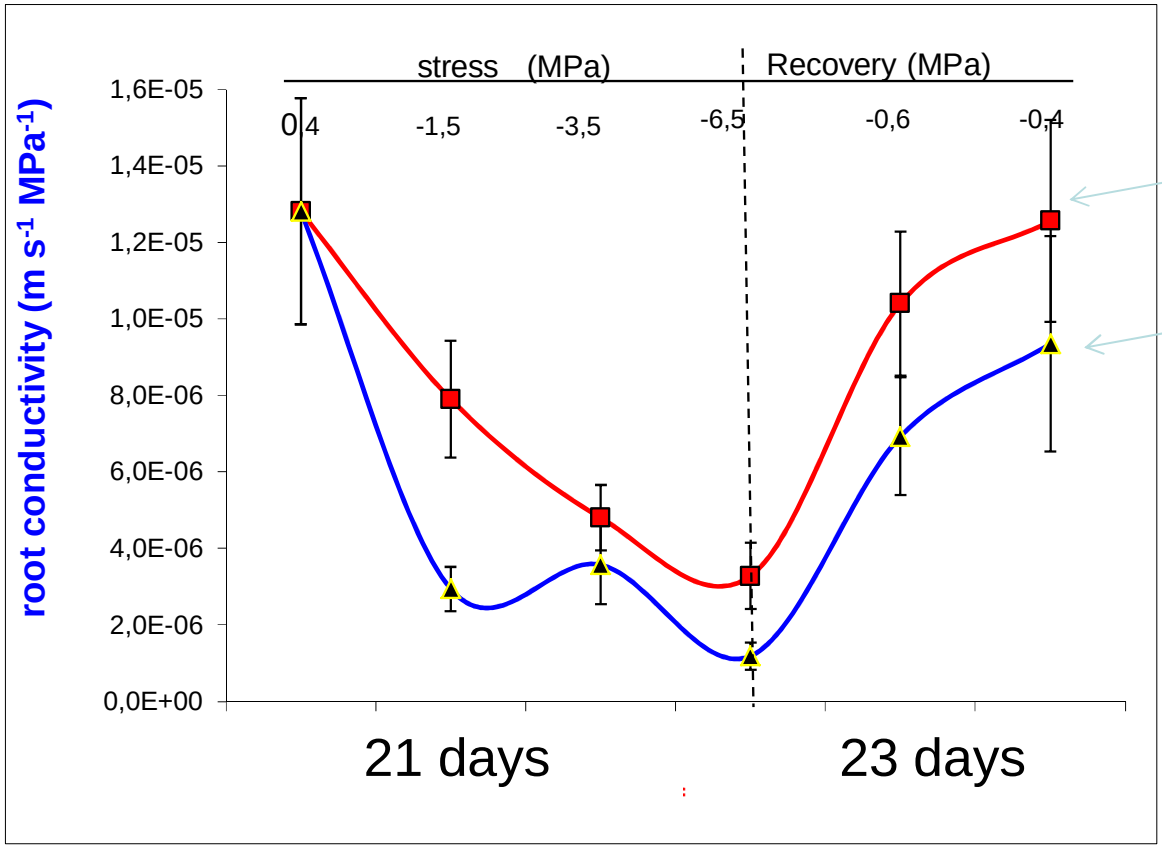
Where A Total root surface area (m^2)

Calculated taking in accont the average root radius

A = 2 π r * total root length

Pressure bomb method



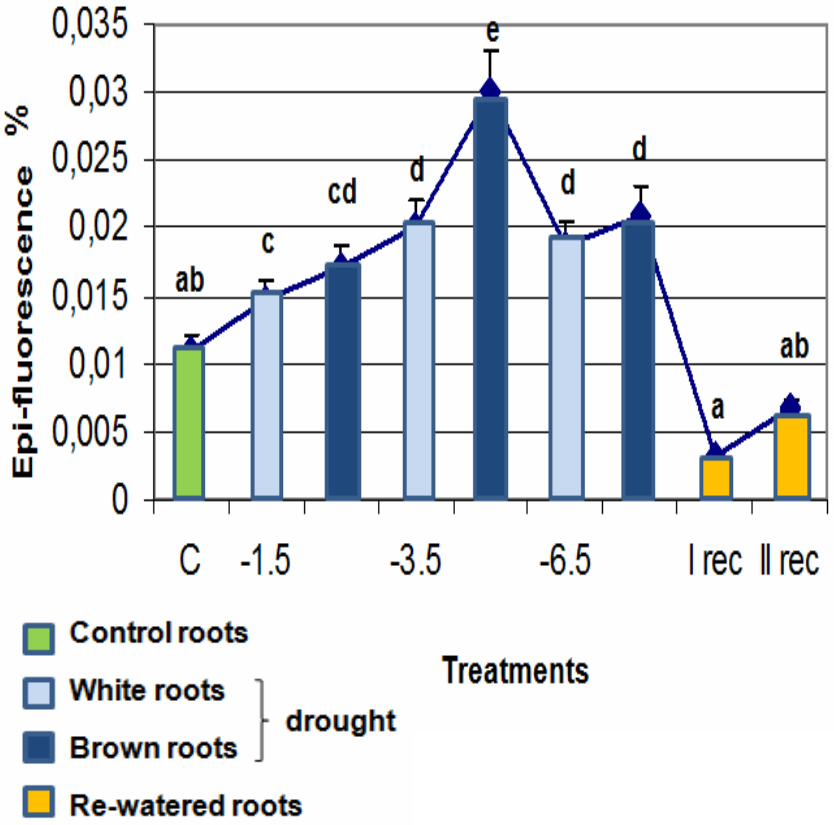


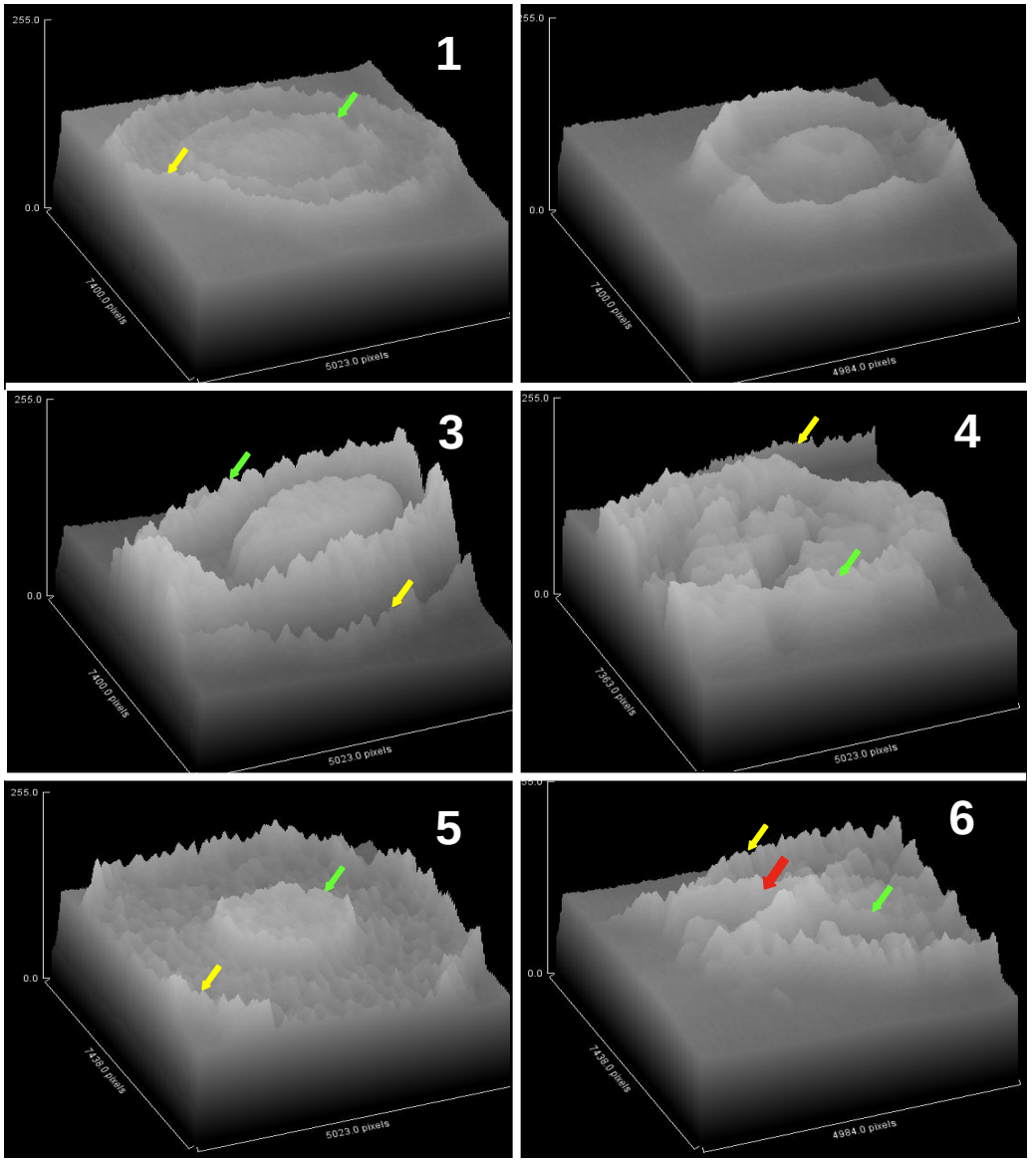
white roots

Brown roots

epi-fluorescence method

optical microscope - transmission light and a mercuric vapour lamp to check epi-fluorescence emissions by suberin. Quantification was done measuring 3D peaks





Green arrows: endodermis
Yellow arrows: exodermis
Red arrow: root primordia
1: control
2-4: stress levels
5-6: recovery

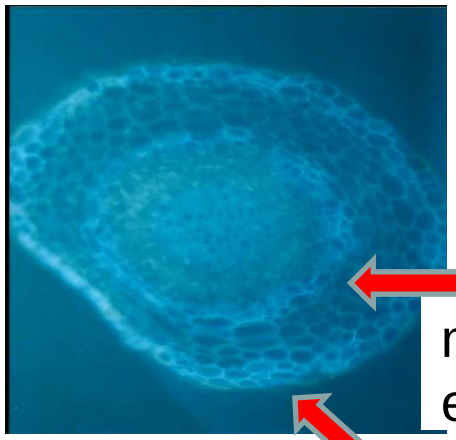
Suberin (hydrophobic) accumulation causes reduction in conductivity after a long term water stress. The trend of the suberification amount was inversely correlated to the conductivity.

“apoplastic pathway and barriers”

suberification of olive root (epi-fluorescence method)

Treatments

Control: -0,3 Mpa.
Water stress: -1,5 MPa; -3,5 MPa; -6,5 Mpa.
Recovery: -0,6 Mpa; -0,4 Mpa.

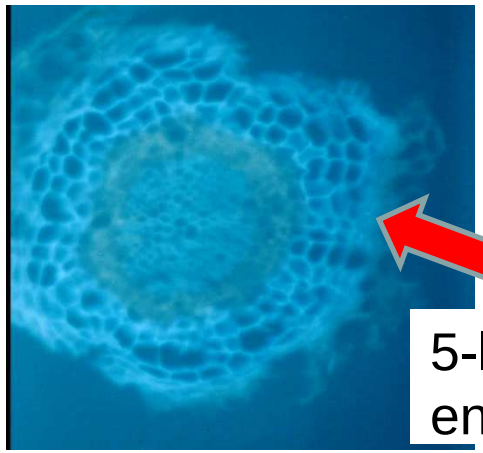


Well irrigated

monolyer endodermis

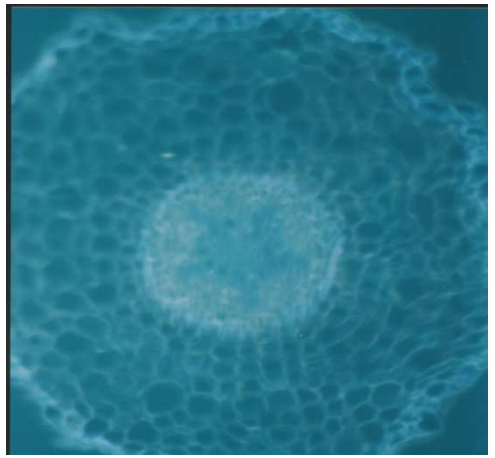
exodermis

Drought treatment
(-6.5 MPa predawn)



5-lyers endodermis

Recovery



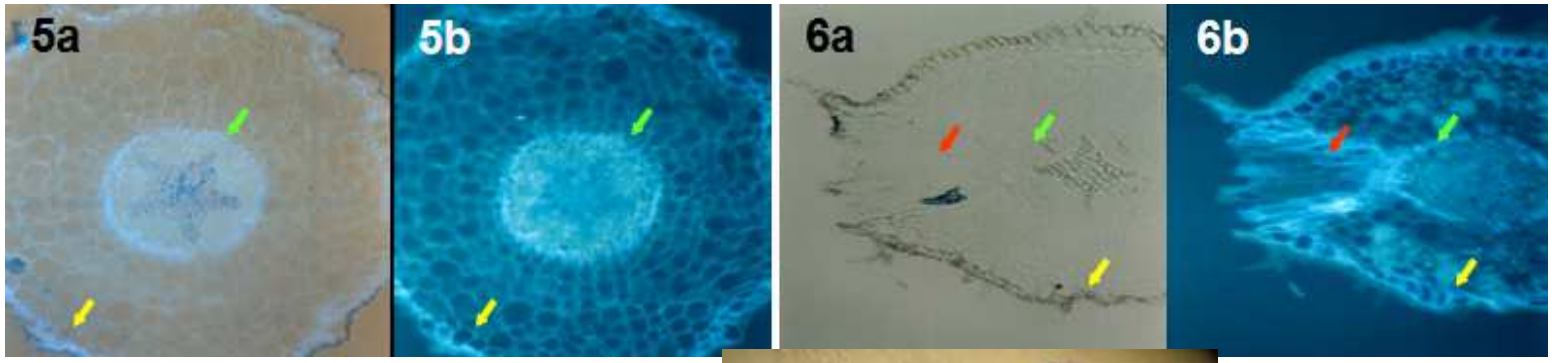
Suberification occurs at different stress levels

:

“apoplastic pathway and barriers”

Water status recovery

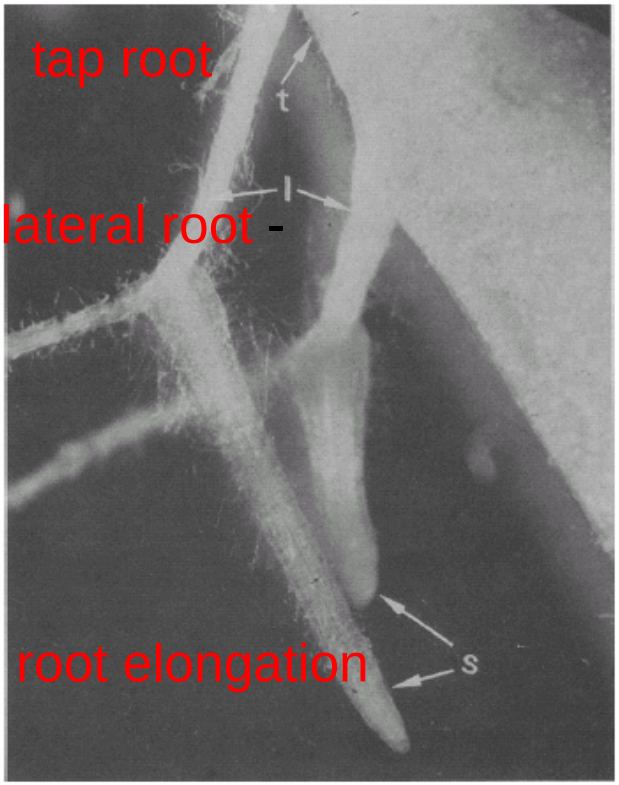
Suberin renders cell walls recalcitrant to biodegradation. So **new primordia,** that will form the completely active working roots, must physically emerge and break off the preexisting barriers, outside from the pericycle. The time necessary to obtain new mature roots probably accounts for the observed delay in complete restoration of the physiological processes.



New root *primordia*

Green arrows: endodermis
Yellow arrows: exodermis
Red arrows: root primordia
5-6: recovery

Formation of new roots i “ short tuberized roots” allows to recover after water stress period



Plants of mesophytic species *Sinapis alba*

Radici di olivo (*Olea europaea*)

Formazione di peli radicali e d allungamento delle “short tuberized roots” (x 25)

N. VARTANIAN Plant and Soil ***63, 83-92 (1981).***

Rewatering stage

“apoplastic pathway and barriers”

Summary #1

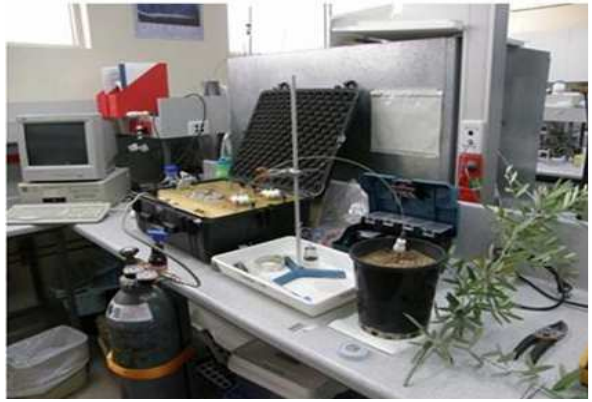
I) root hydraulic conductivity responds to water stress in olive;

II) the variation in root conductivity, **after a prolonged drought period**, is due to the **deposition of hydrophobic barriers**;

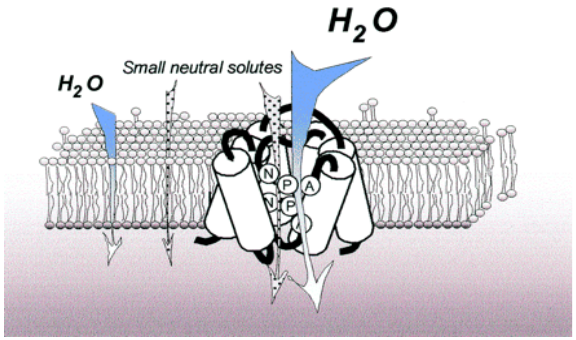
III) during soil water status recovery, **the recovery** in conductivity is due to the **physical break** of such barriers **due to the emergence of new roots.**

“cell-to-cell pathway and aquaporins”

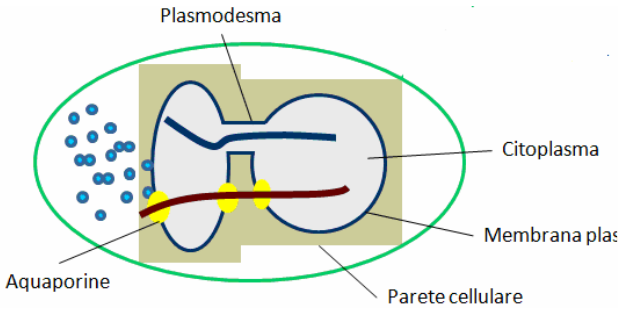
WATER UPTAKE AND TRANSPORT IN ROOTS (Olive)



Plants can also respond rapidly and reversibly to **short time environmental stimuli** (temperature, water deficit, low pH…), regulating activity of **aquaporins** (cell-to-cell path), channels through plasmatic membranes (modulation, expression, etc.).



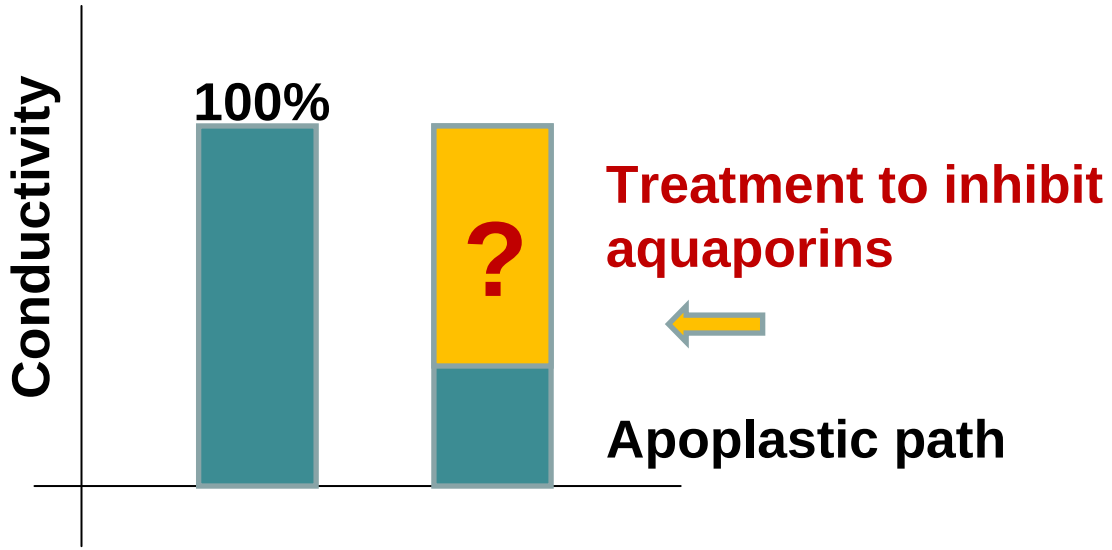
Aquaporins quantitatively play an important role **in flow regulations,** they control water exchanges between cell and environment and contribute to plant water status regulation.



“cell-to-cell pathway and aquaporins”

The experimentation was carried out at the University of Adelaide, South Australia, in the laboratories leaded by professor Stephen D. Tyerman.

What is the cell to cell pathway contribution to the total root conductivity



III part:

"cell-to-cell pathway and aquaporins"

Material and methods:

Plant material

- Five plants of ***Olea europaea***, per treatment in each experiment, were used after 6-8 weeks from the transplant.
- All the experiments, repeated three times, were carried out over spring-summer period in a controlled greenhouse.

Treatments

Water deficit: control plants remained well watered whereas water-stressed plants had water withheld until when their stem water potentials upgraded to -2.5 MPa approximately.
Considered stress is intense, but of short duration, that in order to avoid structural modifications and to estimate the effect exclusively on the regulation of aquaporins.

III part:
"cell-to-cell pathway and aquaporins"
Material and methods:

- Five plants of ***Olea europaea***, per treatment in each experiment, were used after 6-8 weeks from the transplant.
- All the experiments, repeated three times, were carried out over spring-summer period in a controlled greenhouse.

Further Treatments

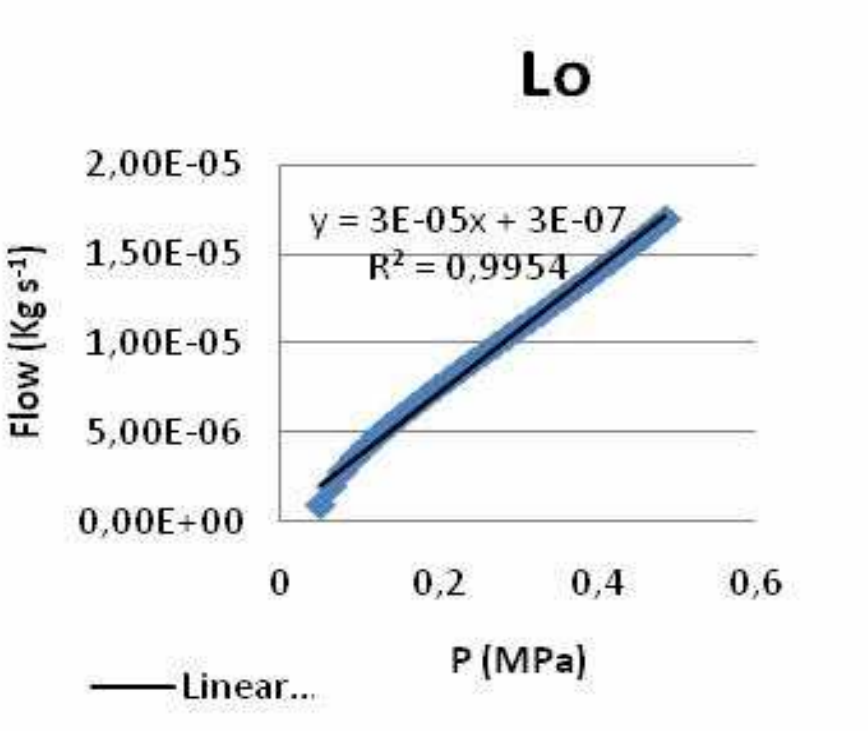
Acidic solutions: an hour prior to measurements, the plants, well watered, were treated, adding approximately 300 ml of solution per pot, with Na-Acetate 20 mM, pH 5.5. Treatment with a weak acid at low pH is known to inhibit aquaporins in roots, thereby giving a base level of conductivity. (Alleva *et al.*, 2006)

Light intensity: 24 hours prior to measurements, the plants, well watered, were shaded, reducing the light intensity (photosynthetic photon flux density, PPFD) of 80-90% than control.

“cell-to-cell pathway and aquaporins”
conductivity

High Pressure Flow Meter

This technique is destructive; water under pressure is forced to flow back into root system from the cut at the stem base,



the pressure ramped up to 0.5 MPa, at a rate of approximately 7 kPa*s-1,

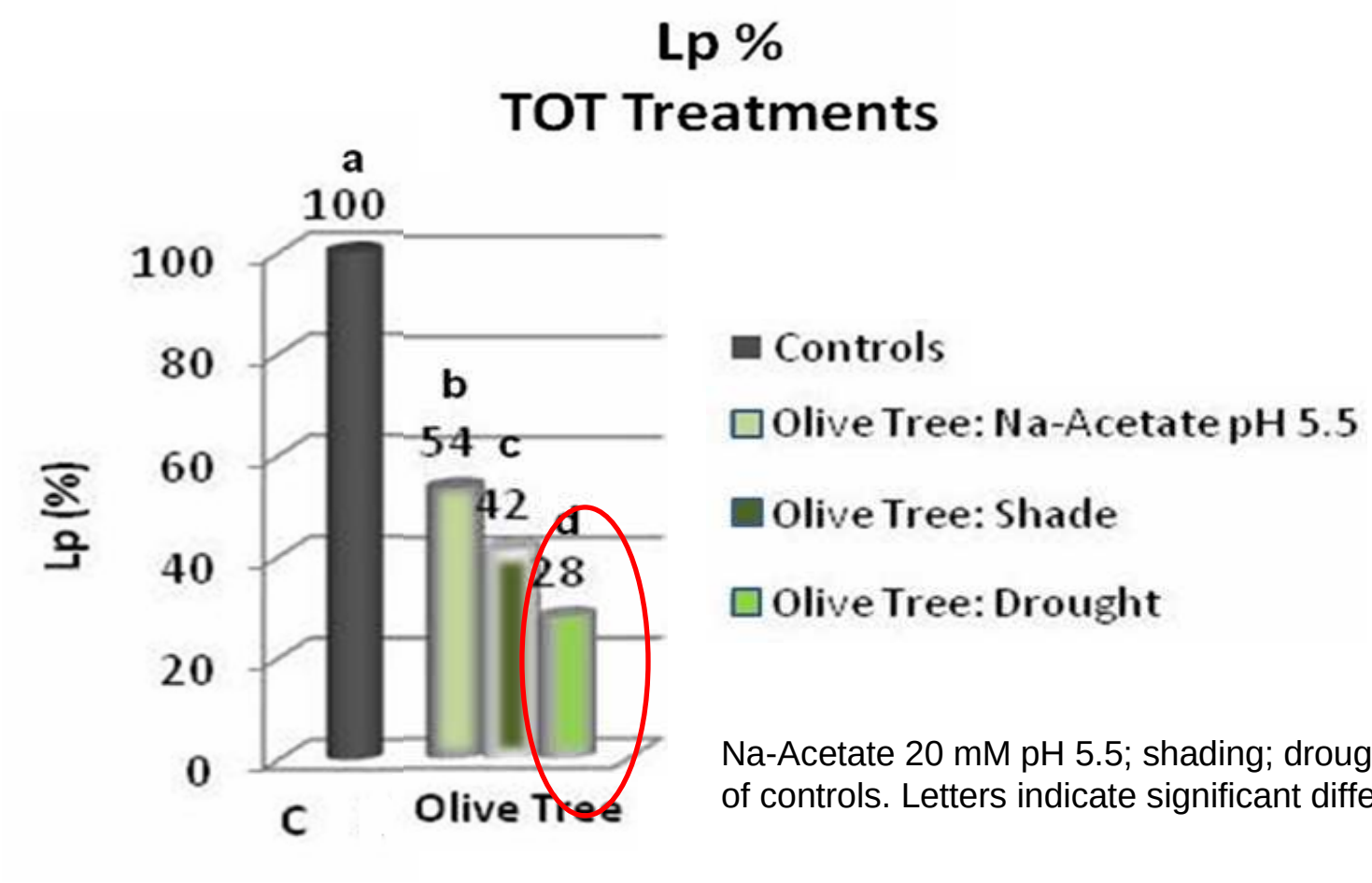
hydraulic conductance, Lo, as the slope of the plot of the water flow versus pressure

Root hydraulic conductivity $Lp = \frac{1}{r}\frac{Lo}{\Delta P}$

Nobel et al., 1990

r is the root dry weight, linearly correlated to root area.

Results
Hydraulic conductivity: single treatments



Na-Acetate 20 mM pH 5.5; shading; drought. Lp, in % of controls. Letters indicate significant differences.

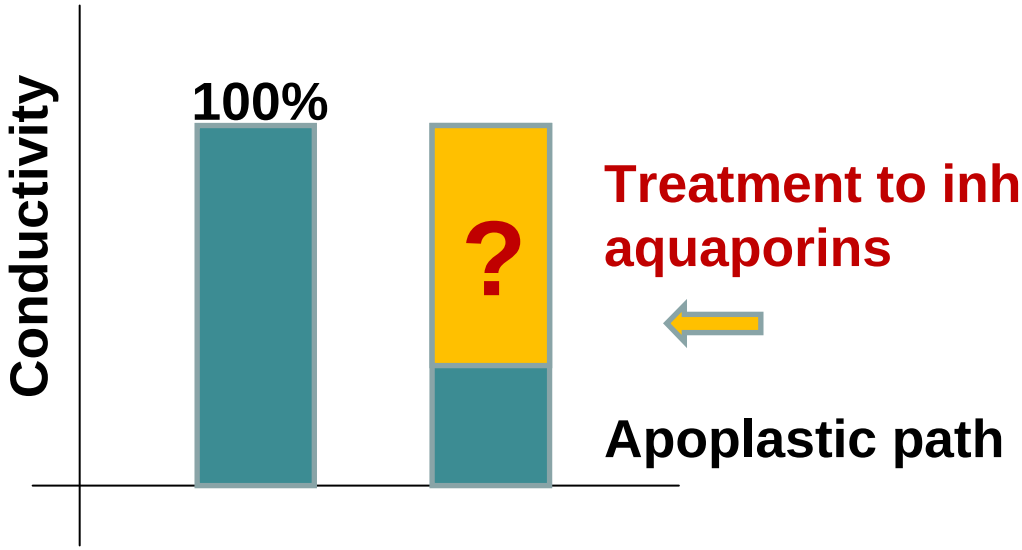
The biggest reduction in conductivity is due to drought.

We don't know if different treatments inhibit the same aquaporins

“cell-to-cell pathway and aquaporins”

Summary # 2

uptake and transport by the cell-to-cell pathway in Olive tree, Soybean and Maize.



70% Olive tree (DROUGHT)

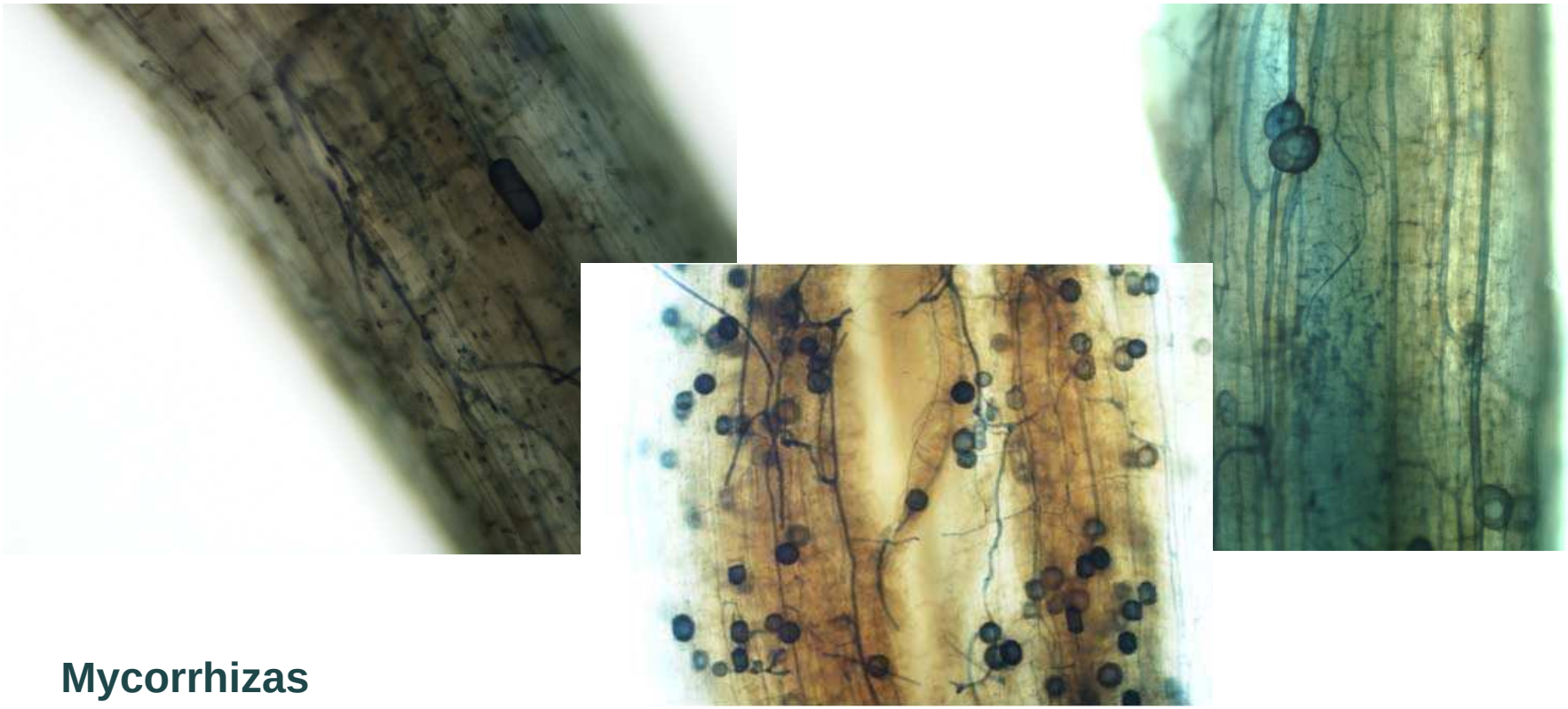
60% Soybean (DROUGHT AND SHADE)

50% Maize (LOW pH)

Similar results were seen in desert plants (North and Nobel, 1995, 1996; Martre et al., 2001; North et al., 2004)

Tataranni, 2010 (PhD dissertation)

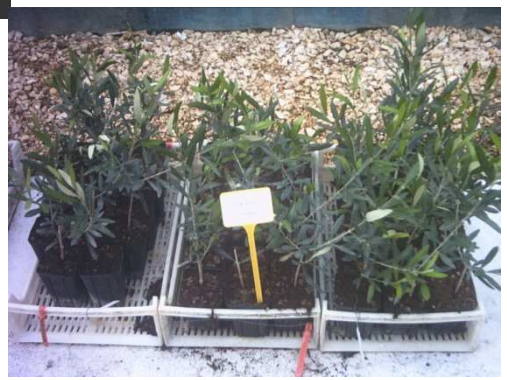
Improving water uptake and transport
“effects of mycorrhization”



Mycorrhizas

improve water flow potentially **available**, either directly, by fungal water uptake and transport, overcoming plant barriers, or indirectly by exploring a bigger soil volume.

Study of physiological parameters in function of mycorrhizae presence



Materials and Methods

Biological materials

- 120 plants of *Olea Europaea* (Frantoio);
- Sterilized soil (121°C; 20 minutes; P=1 bar)
- *Glomus intraradices* (Aegis tablet 5g/tablet)

Thesis	**WW**	**DS**
G	GxWW	GxDS
NOG	NOGxWW	NOGxDS

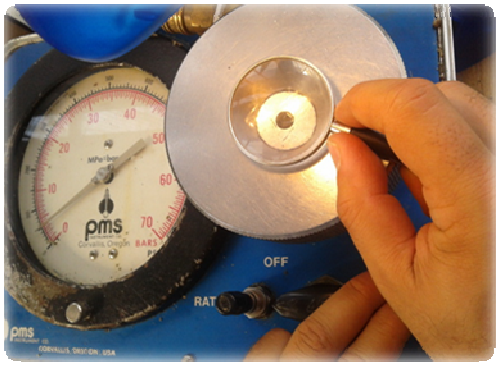
G= *Glomus intraradices*
NOG= No *Glomus Intraradices*

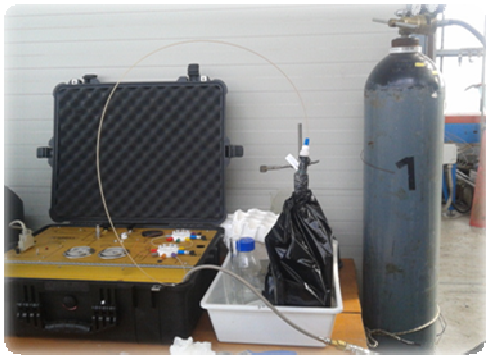
WW= well watered
DS= drought stress

Materials and Methods

Measurements

- Water leaf Potentials (sholander)
- Root Hydraulic conductance (HCFM)
- Gas excanges (Licor 6400-09)





Study of physiological parameters under drought in function of mycorrhizae presence

Materials and Methods

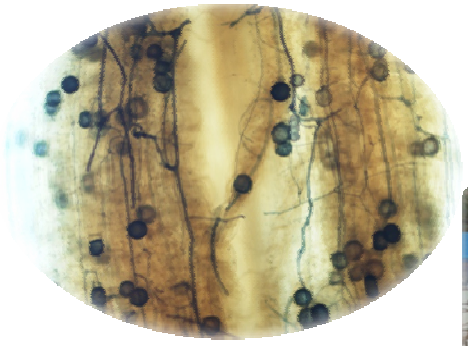
Treatments

All samples were treated with:

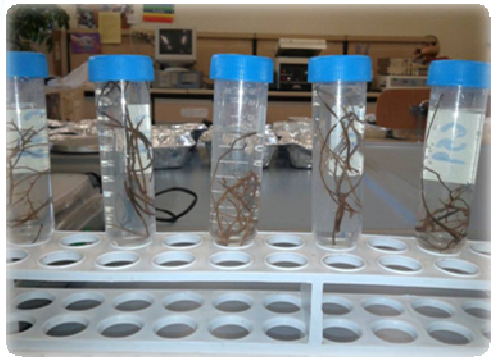
- KOH 10% (10 minutes 50°C)
- Washing : water
- Trypan blue 0.05% (20 minutes 50°C)

Observation:

- Microscope Nikon eclipse 80i objective 10x);
- Method Truvelot et al. 1986



Colonization



F%= (n°mycorrhizal root fragments/tot. Fragments)*100

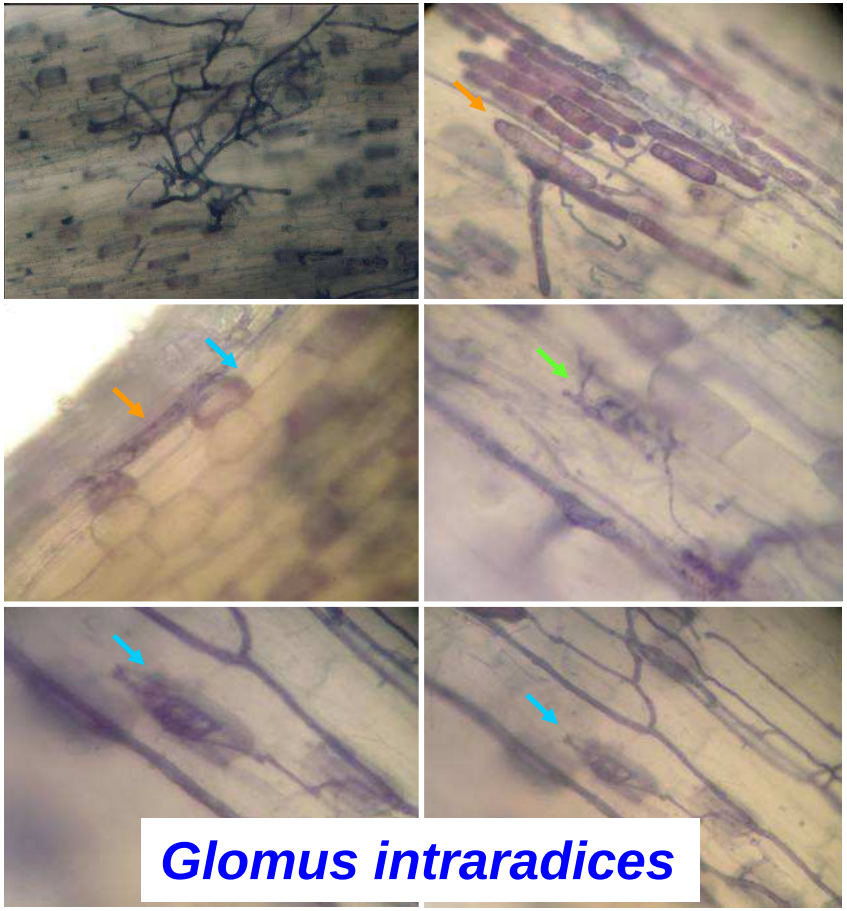
M%=(95n5+70n4+30n3+5n2+n1)/(tot fragments)

a%= (100mA3+50mA2+10mA1)/100

A%= a*(M/100)

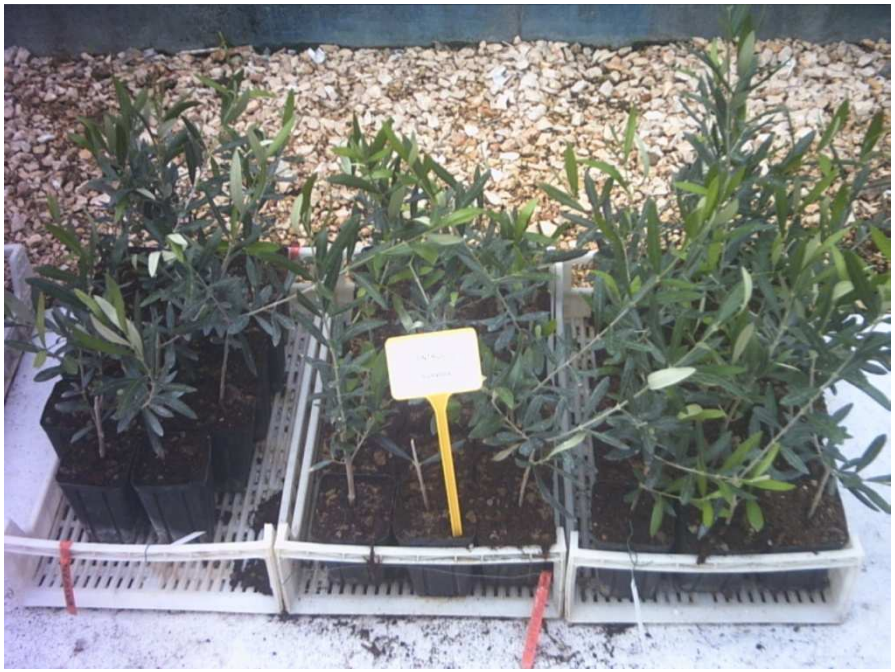
“effects of mycorrhization”
mycorrhizic colonization

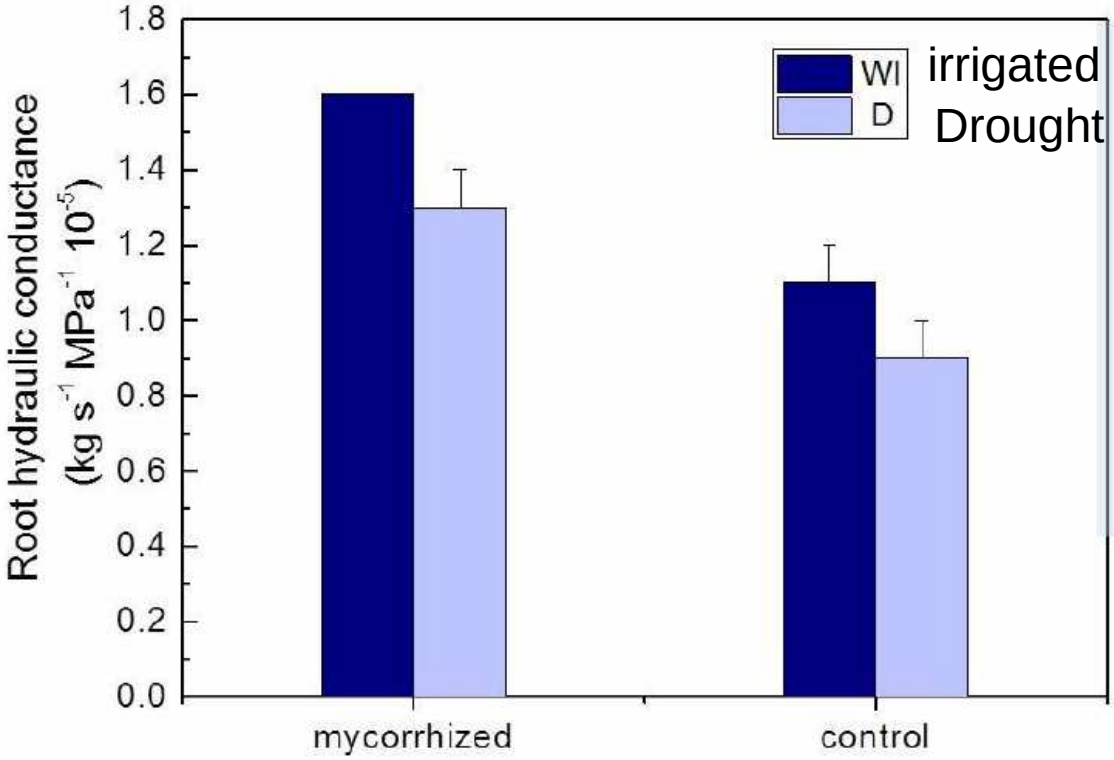
CV. Coratina	Control	*G. intraradices*
Myc. infection (%)	100,0	100,0
Intensity (%)	22,7	46,2
Arbuscular abundance (%)	0,6	19,3
Test F	a	b



Glomus intraradices

After six months mycorrhizic colonization





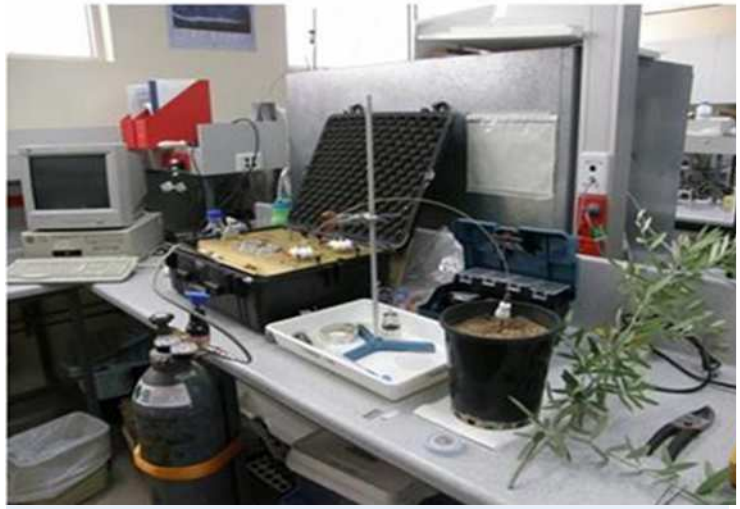
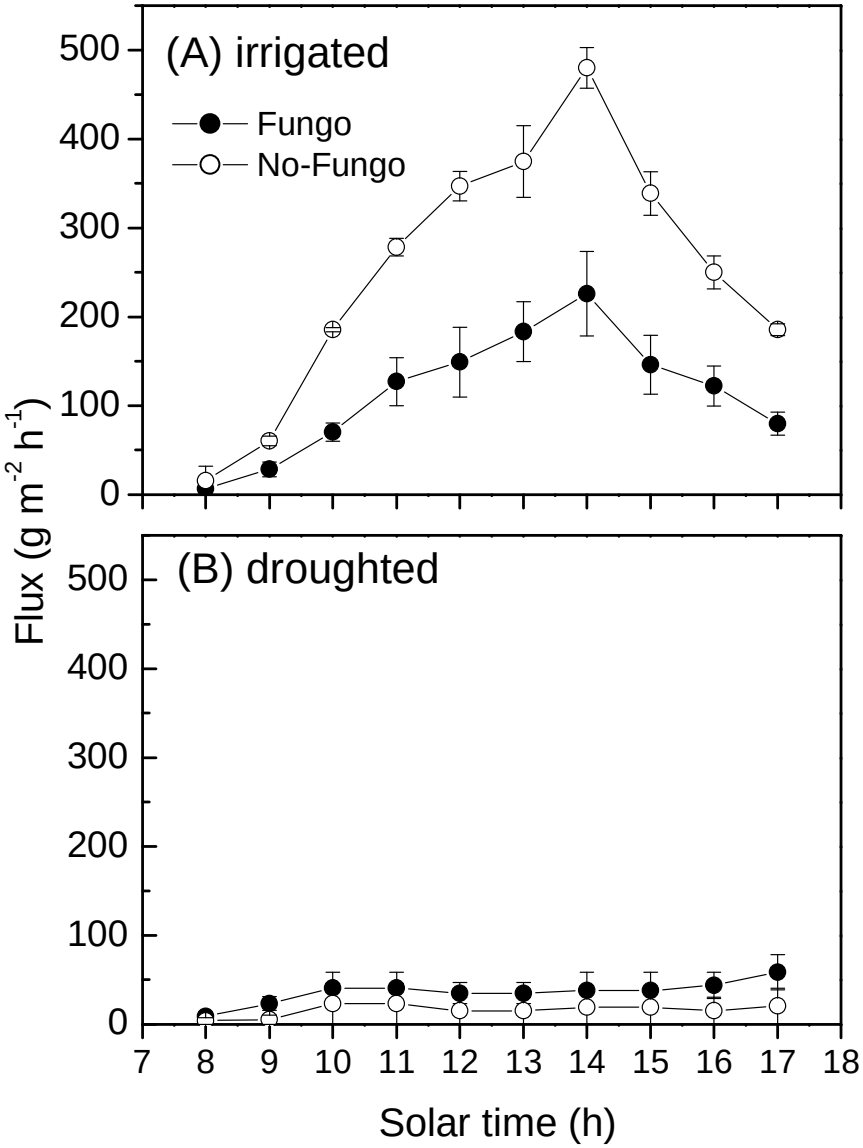
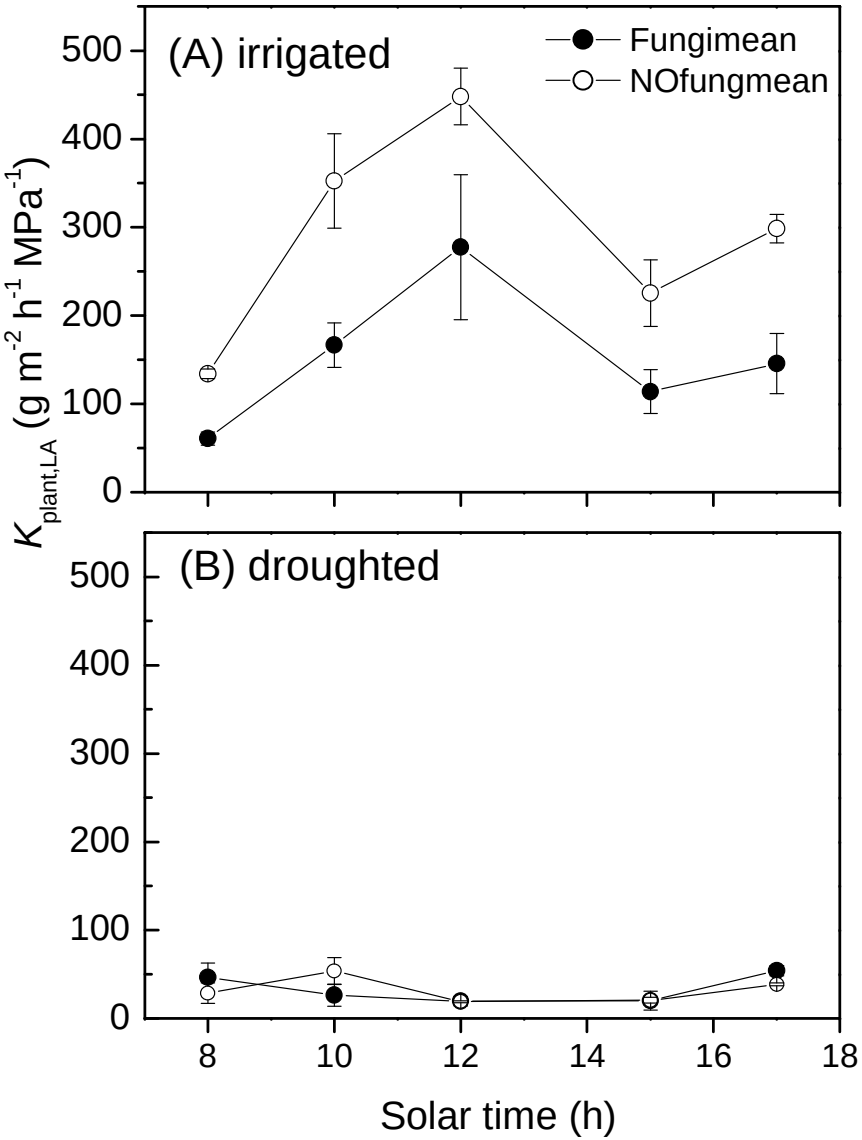


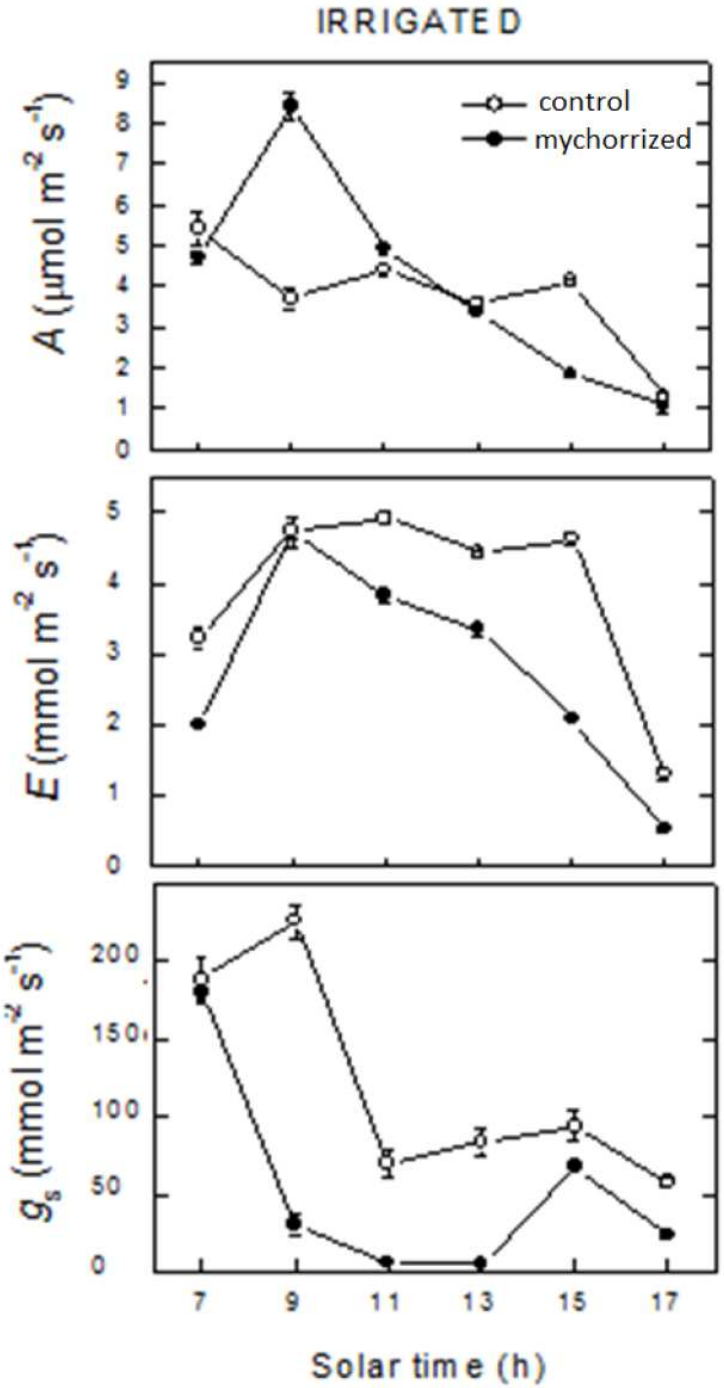
Figure 1 Mean root hydraulic conductance (±SE) measured in mycorrhized and control olive trees under well irrigation (WI) and drought (D) conditions.

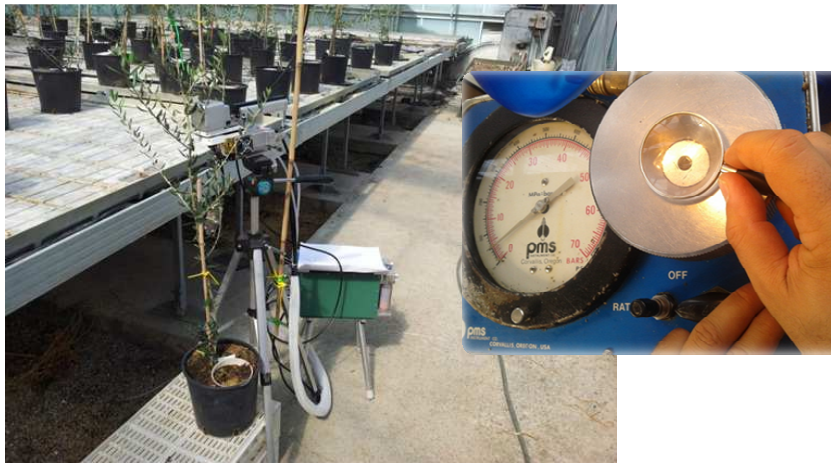
The root systems of olive trees showed ~45% increased *K* when colonised with *Glomus intraradices.* That positive effect was detected under both drought and well irrigation conditions (Fig. 1).

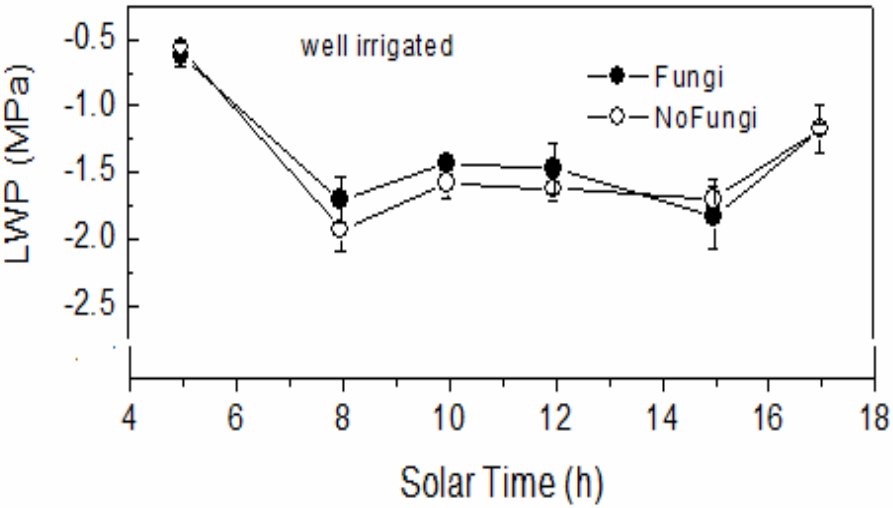
Kplant = E/ (Ψsoil-Ψleaf)





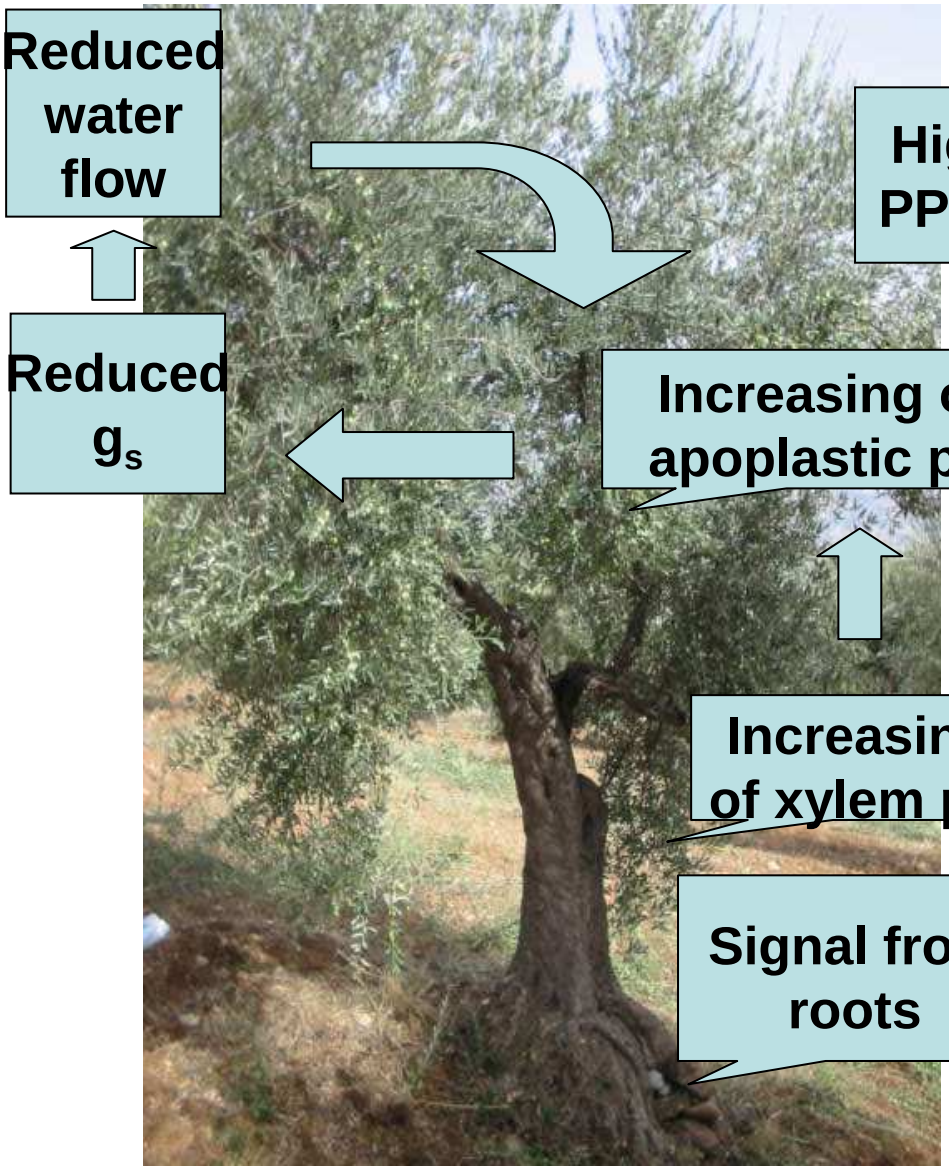


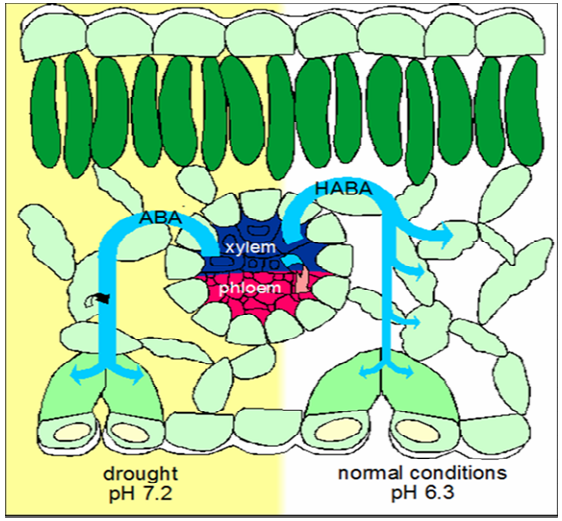




The mychorrized plant:
- reduces transpiration
- maintains the same Assimilation
- so induces an higher WUE

Hydraulic conductivity and stomata conductance





SHOOTS

ROOTS

Production of ABA and variation in pH, followed to root drying, influence shoot growth and stomatal conductance (Zhang and Davies, 1987; Gowing et al., 1990; Wilkinson and Davies, 1997.

Might an alternative signaling root-shoot, independent by water potentials, exist?

Bill Davies
Lancaster Environment Centre, Lancaster University, UK

aquaporins are involved in the regulation of stomatal (*g*s) and mesophyll (*g*m) conductance to CO2 During stress and recovering stage

This study supports the hypothesis that the regulation of mesophyll (*g*m) conductance to CO2 is regulated mainly by AQPs and that both ePIP1.1 and OePIP2.1 are likely to interact to exert a significant effect on gm.

Perez-Martin et al. 2014 JXB

the experiment in pots of the three treatments: C, control plants; S, stress plants; SP, stress-pruned plants. Values on each date are the means (±SE) between two different depths (0.05 and 0.20 m). Different letters indicate significant differences among treatments within each date (analysis of variance, Tukey: $P<0.05$). (b) VPD of air during the experiment in July and August of 2009. S and SP plants were last irrigated on day 0 a.w.i., with daily irrigation being applied again 13 d later (discontinuous line). a.w.i., after withholdong irrigation, a.r.i., after resuming irrigation.

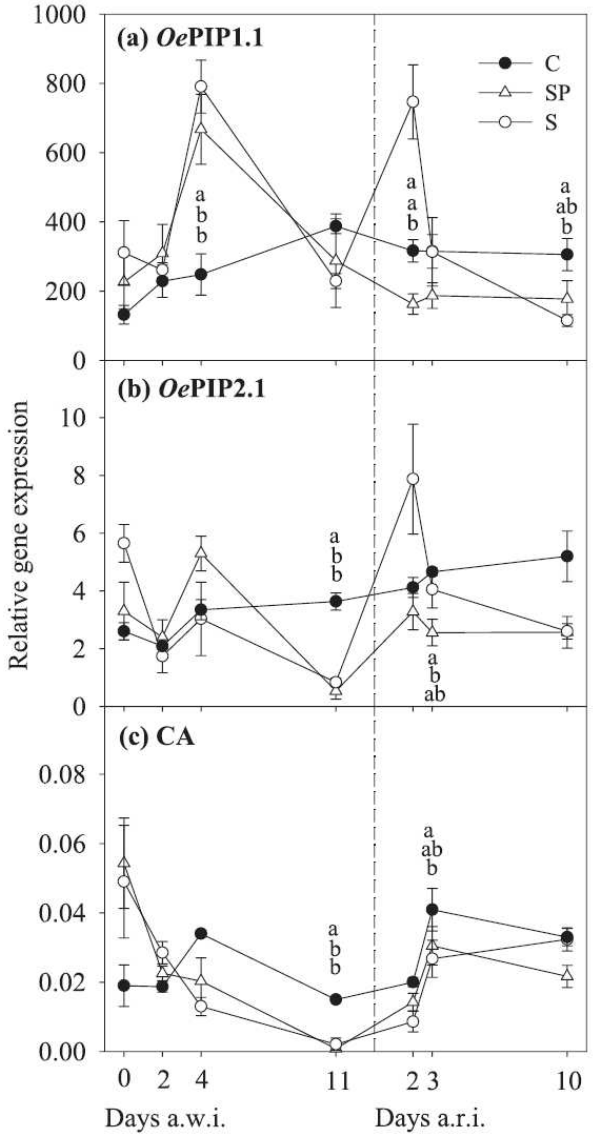


Fig. 5. Time course of the relative gene expression of aquaporins OePIP1.1 (a) and OePIP2.1 (b), and CA (c) in leaves of *O. europaea* throughout the experiment. Values are means (±SE) of three replicates per treatment and date. Treatments, letters, and the discontinuous line are as described in Fig. 1.

“cell-to-cell pathway and aquaporins”

aquaporin expression analyses in olive tree

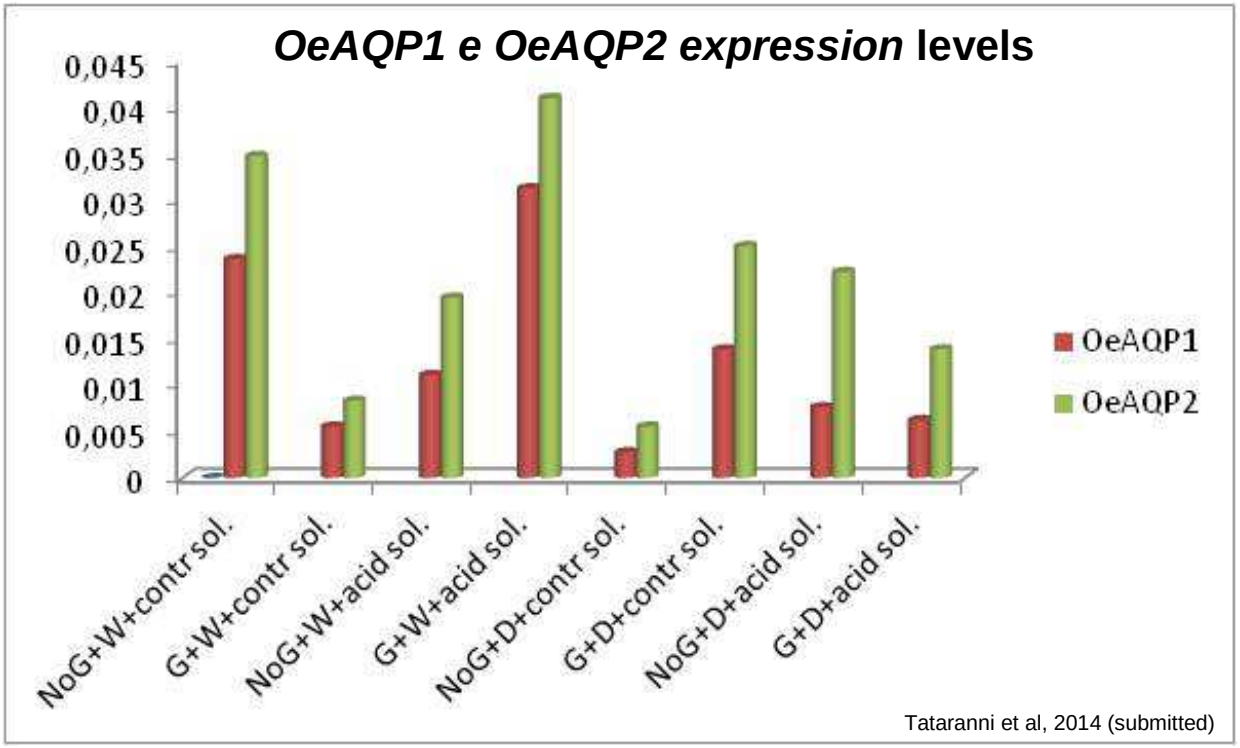
Real time primers on Olea europaea aquaporins:
OePIP1.1 (Olea europaea plasma membrane intrinsic protein (pip1) mRNA) and ***OePIP2.1*** (Olea europaea plasma membrane intrinsic protein (pip2) mRNA) (Li,T.et al. 2013)
and olive reference genes: ***PP2A1*** (http://140.164.45.140/oleaestdb/blast.php coding Serine/threonine-protein phosphatase 2A (PP2A)) (Ray and Johnson 2014) (Nonis et al., 2012) and ***18S*** (Olea europaea 18S ribosomal RNA gene, partial sequence) were designed and used for **quantitative real time**

Gene	Forward primer (5’-3’)	Reverse primer (5’-3’)	TaqMan probe (5’-3’)
OePP2A	GTGGGATTTTTTGACGATAAGCTT	GCAGCATCTCGAATAGAGTAAACCT	TGCCCTTTGCATGCAGTGGCTACA
OeAQP1	TGGCACGGCTGCTCAAA	CACCCGGCTCGAAAAGTG	AAGGACTACAAGGAACCACCACCGGC
OeAQP2	AATACTCCGCCAAGGACTACCA	GCTGCTCAGCTCATCAAAATCA	ATCCACCACCGGCGCCTCTCA
Olea18S	GAAACGGCTACCACATCCAAG	ACCCAAGGTCCAACTACGAG	AAGGCAGCAGGCGCGCAAA

“cell-to-cell pathway and aquaporins”
aquaporin expression analyses in olive tree

OeAQP1 e OeAQP2 transcription levels increase without Glomus or when water resultes less available, i.e. when plants were treated to block channels

Probably, in order to maintain a suitable water status under abiotic stress, both increased water transport via AQPs in some tissues and reduced in others are required (Secchi et al., 2007) because one of the main role of AQPs is to maintain homeostasis and water balance under water-stress conditions (Tyerman et al., 2002)



Tataranni et al, 2014 (submitted)

Summary # 3

Arbuscular mycorrhizal (AM) symbiosis can influence root hydraulic properties, including root hydraulic conductivity with effect on plant water balance

The presence of the AM fungus in the roots of the host plants was able to modulate the switching between apoplastic and cell-to-cell water transport pathways (Bárzana et al., 2012 in tomato and Maize) Calvo-Polanco et al. 2016 in olive)

The AM symbiosis regulated a wide number of aquaporins in the host plant, comprising members of the different aquaporin subfamilies. The regulation of these genes depends on the watering conditions and the severity of the drought stress imposed (Bárzana et al., 2014)

Expression (*OePIP2.1*) presented a clear link to water availability and affected the mesophyll conductance in olive leaf (Perez-Martin et al., 2014)

LIFE 14 CCA/GR/00389 - AgroClimaWater

LIFE 14 CCA/GR/00389 - AgroClimaWater

Promoting water efficiency and supporting the shift towards a climate resilient agriculture in Mediterranean countries

Project Beneficiaries:

SPYRIDIS A. - ROUTALOU V. G.P. ΥΕΤΟΣ (Coordinator) www.yetos.gr

Hellenic Agricultural Organization "Demeter" Directorate General of Agricultural Research - Institute for Olive Tree, Subtropical Plant and Viticulture. www.nagref-cha.gr - Soil and Water Resources Institute www.ini.gr

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INFORMATION

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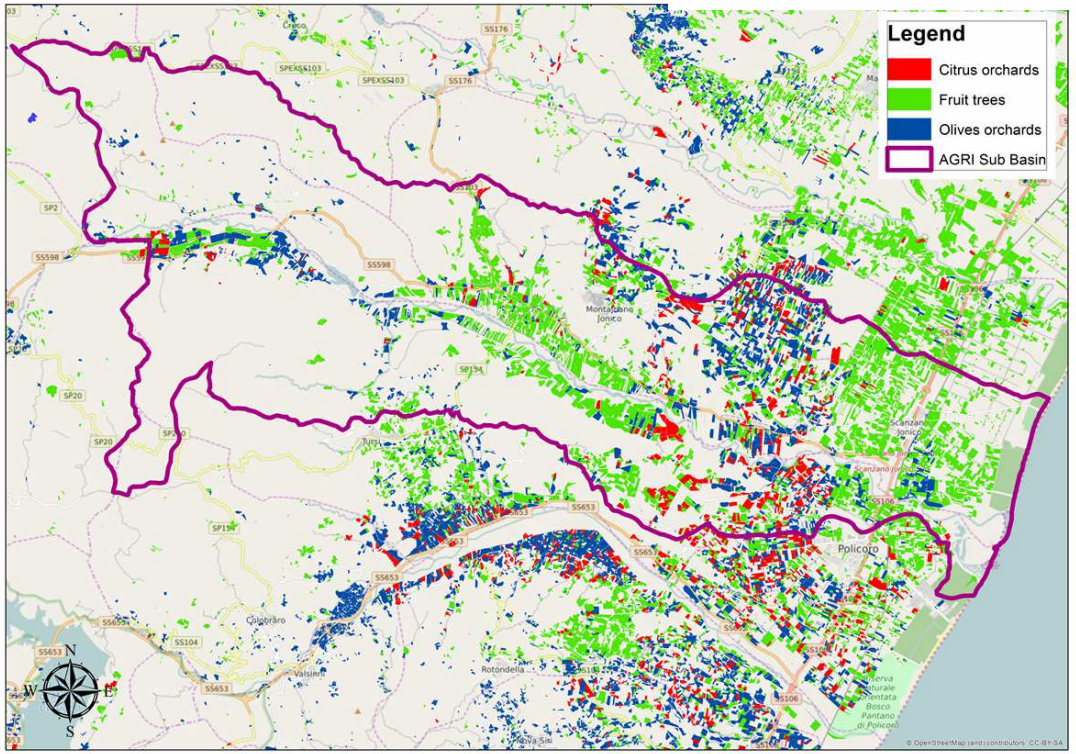

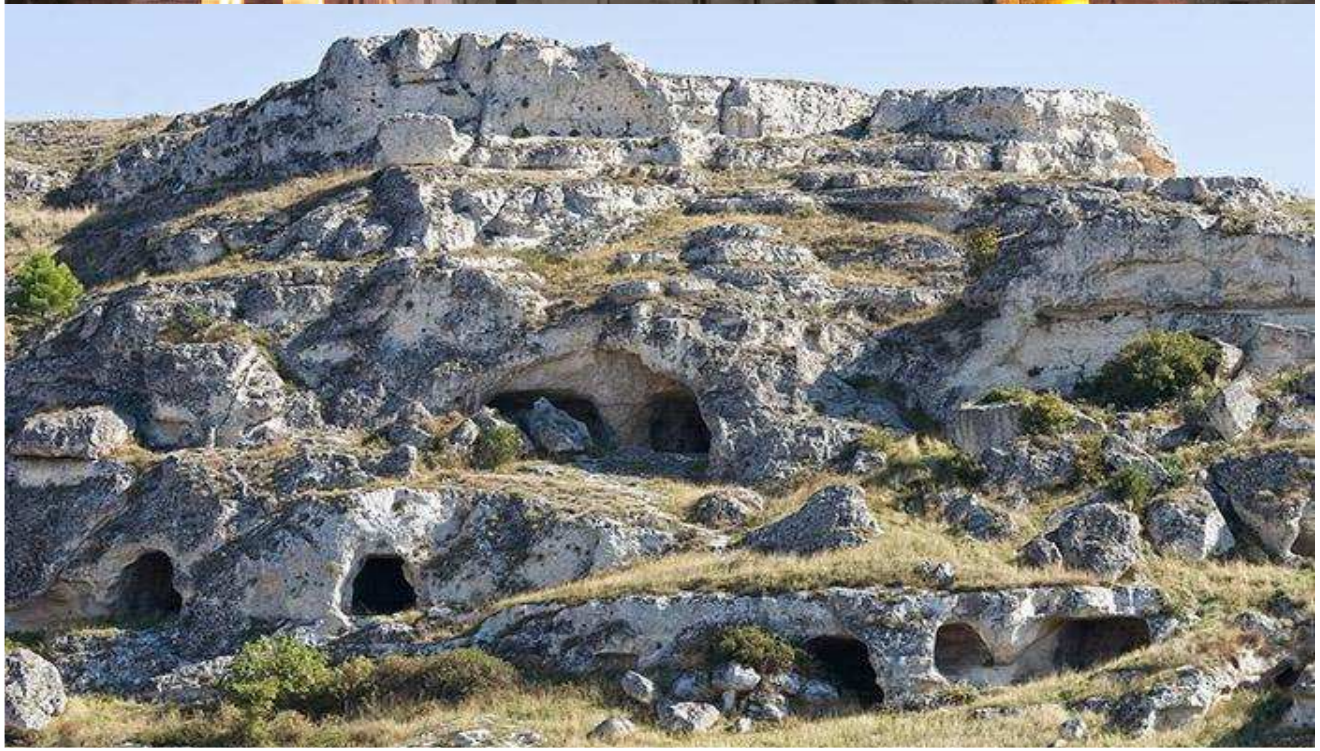

THANKS

MATERA 2019
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VENUE FOR

IX ISHS INTERNATIONAL SYMPOSIUM
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Conveners

Prof. Bartolomeo Dichio **Prof. Cristos Xiloyannis**

Direct effects of *Trichoderma harzianum* strain T-22 on micropropagated shoots of GiSeLa6® (*Prunus cerasus* × *Prunus canescens*) rootstock

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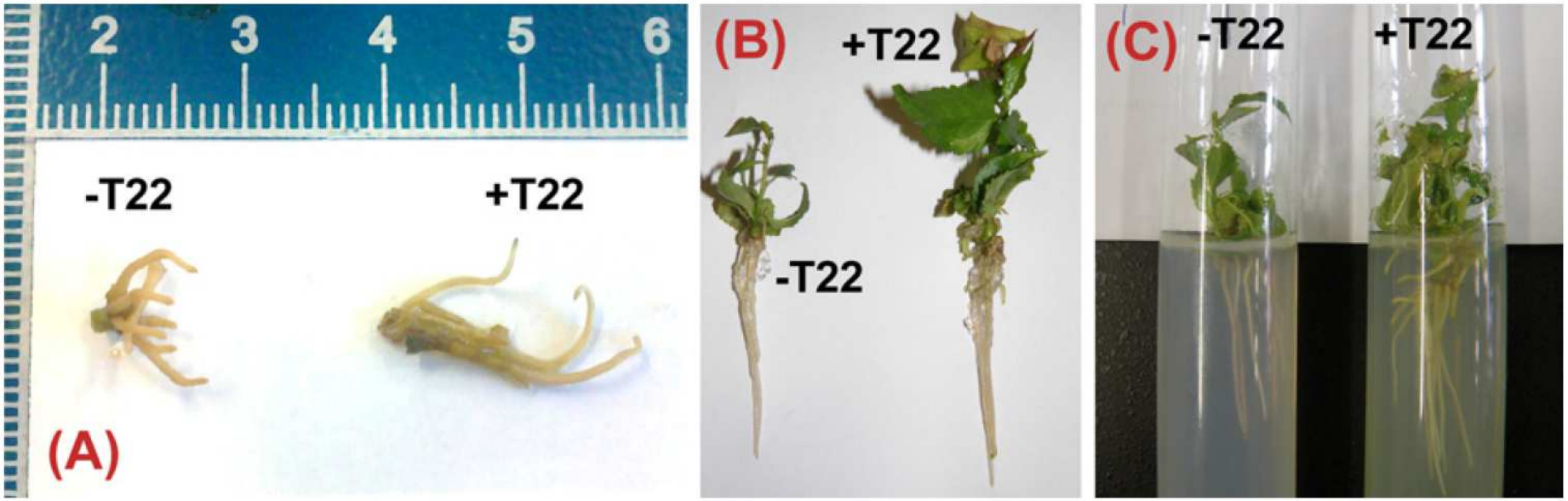

Table 1
Levels of indole-3-acetic acid (IAA), *trans*-zeatin riboside (*t*-ZR), dihydrozeatin riboside (DHZR) and auxin/cytokinins ratio (IAA/CK) in GiSeLa6® plants (*Prunus cerasus* × *P. canescens*) inoculated with *Trichoderma harzianum* strain T-22 and in un-inoculated plants. Values (±standard deviation) are means of 15 replicates ($n = 15$). For each column, values followed by a different letter are significantly different at $P \leq 0.05$, according to Fisher's LSD test.

Sample	Organ	IAA (ng g^{-1} fresh weight)	*t*-ZR (ng g^{-1} fresh weight)	DHZR (ng g^{-1} fresh weight)	IAA/CK (ng g^{-1} fresh weight)
Plant	Leaves	85.9 ± 6.5 b	1.7 ± 0.0 c	1.3 ± 0.0 a	28.5 b
	Roots	67.9 ± 4.1 c	3.2 ± 0.2 a	1.3 ± 0.1 a	15.0 d
Plant + T22	Leaves	127.4 ± 12.6 a	1.7 ± 0.0 c	1.1 ± 0.0 b	46.6 a
	Roots	82.6 ± 8.8 b	2.8 ± 0.1 b	1.1 ± 0.0 b	21.2 c

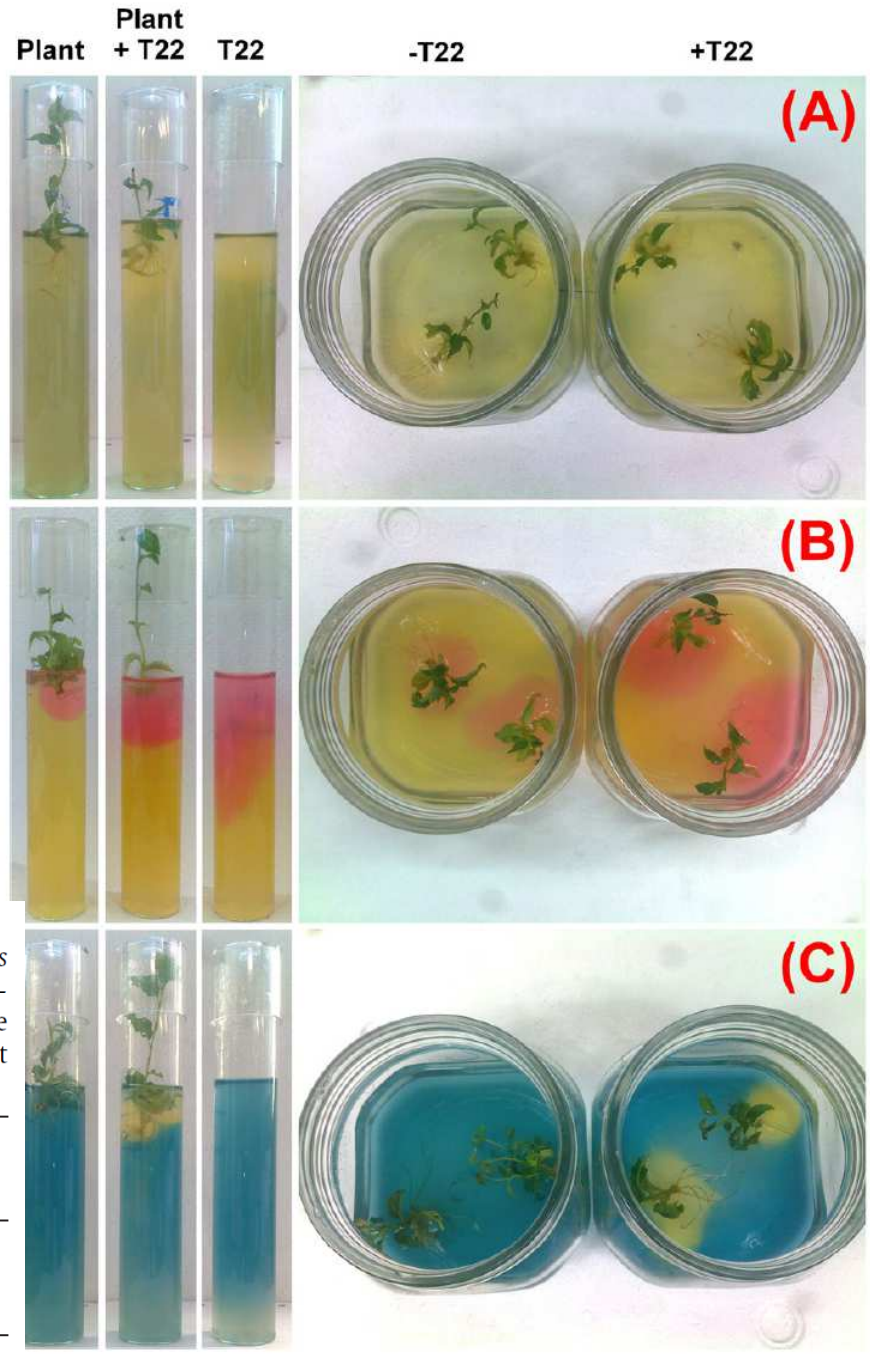

Fig. 2. Medium acidification by GiSeLa6® (Prunus cerasus ×P. canescens) un-inoculated plants, GiSeLa6® plants inoculated with Trichoderma harzianum strain T-22, and T22 alone, as assessed by (A) bromothymol blue (pH measuring range = 7.6–6.0, with colour change from pale green to yellow), (B) methyl red (pH measuring range = 6.2–4.2, with colour change from yellow to red), and (C) bromocresol green (pH measuring range = 5.4–3.8, with colour change from blue to yellow).

Table 2
Values of pH of the indicator media in the three treatments: GiSeLa6® (*Prunus cerasus* × *P. canescens*) un-inoculated plants, GiSeLa6® plants inoculated with *Trichoderma harzianum* strain T-22, and T22 alone. Values (±standard deviation) are means of eight replicates ($n = 8$). For each column, values followed by a different letter are significantly different at $P \leq 0.05$, according to Fisher's LSD test.

Sample	pH		
	Bromothymol blue	Methyl red	Bromocresol green
Only plant	6.2 ± 0.1 a	6.3 ± 0.1 a	6.2 ± 0.1 a
Plant + T22	4.0 ± 0.4 c	4.2 ± 0.5 c	4.0 ± 0.1 c
Only T22	5.0 ± 0.1 b	5.0 ± 0.4 b	4.9 ± 0.1 b