TESAF DEPARTMENT OF LAND, ENVIRONMENT, AGRICULTURE AND FORESTRY

DAFNAE Department of Agronomy, Food, Natural resources, Animals and Environment

INRA SCIENCE & IMPACT

UNIVERSITÀ DEGLI STUDI DI PADOVA

Fourth Xylem International Meeting

XIM4 Padua 2019

BOOK OF ABSTRACTS

New Phytologist

FESPB

S.I.B.V.

SISEF

PROGRAM

WEDNESDAY 25

9:00-9:30	Registration		
9:30-9:40	**Welcome and introduction to XIM4**		
9:40-10:00	**"A brief history of 'our' science: the origin of concepts and controversies over water flow in the past 125 years" by Mel Tyree**		
Session 1: METHODS AND CONCEPTS – Chair: Hervé Cochard			
10:00-10:15	S1.1	**Mayr Stefan**	Electrical Resistivity Tomography: New Insights
10:15-10:30	S1.2	**Blackman Chris**	Plant desiccation time to critical thresholds of hydraulic failure during drought is predictable across species of eucalyptus
10:30-10:45	S1.3	**Link Roman Mathias**	Estimating xylem vessel length distributions
10:45-11:00	S1.4	**Tyree Mel**	The reason why the Sperry centrifuge method is is incapable of measuring vulnerability curves of long-vesseled species
11:00-11:15	S1.5	**Yang Dongmei**	The quantitative relationship between water extraction curves and vulnerability curves
11:15-11:45	*SHORT COFFEE BREAK*		
Session 2: FROM XYLEM STRUCTURES TO HYDRAULIC PROPERTIES – Chairs: Steven Jansen and Anna Lintunen			
11:45-12:15	S2.1 (Keynote)	**Sean Gleason**	Hydraulics in agriculture and ecology: a personal perspective on unification and the work that lies ahead
12.15-12:30	S2.2	**Werner Julia**	Pit Membranes are not 2D
12:30-12:45	S2.3	**Carmesin Cora**	Down in the pits: challenges and questions from the pit membranophiles
12:45-14:45	*LUNCH*		
14:45-15:00	S2.4	**Robert Elisabeth**	Remarkable absence of within-species adjustments in conductive xylem, phloem and parenchyma structural traits along a water availability gradient
15:00-15:15	S2.5	**Lens Frederic**	The correlation between conduit diameter and drought-induced embolism resistance revisited: arguments pro and contra
15:15-15:30	S2.6	**Lemaire Cédric**	Anatomical determinants of plasticity of xylem resistance to cavitation in *Populus tremula x alba*
15:30-15:45	S2.7	**Heuret Patrick**	Allometric scaling of hydraulics and mechanics drive the leaf-stem size spectrum for 42 Neotropical tree species
15:45-16:00	S2.8	**Plavcová Lenka**	Anatomical and functional plasticity of xylem in young woody roots and branches
16:00-16:15	S2.9	**Lamarque Laurent**	The xylem embolism resistance spectrum of grapevine
16.15-17.15	*COFFEE BREAK AND POSTER SESSION*		
Session 2: FROM XYLEM STRUCTURES TO HYDRAULIC PROPERTIES – Chair: Missy Holbrook			
17:15-17:30	S2.10	**Windt Carel**	Old year rings may provide water storage capacity by cavitating first
17:30-17:45	S2.11	**Beikircher Barbara**	Hydraulic conductance in newly developing seedlings of *Acer pseudoplatanus*
17:45-18:00	S2.12	**McAdam Scott**	Exploring the link between embolism resistance and hydraulic failure in the earliest vascular plants
18:00-18:15	S2.13	**Sack Lawren**	Hydraulic conductance controls the dynamics of flux and productivity at ecosystem scale
18.15-18.30	S2.14	**Lopez Rosana**	Tree-ring density and C isotope data as early-warning signals of drought-induced mortality
18.30-18.45	S2.15	**Ziaco Emanuele**	Wood anatomy and cambial phenology in hyperarid environments: the key to understand past, present, and future of western US conifers

THURSDAY 26

Session 3: EXTREMITIES OF THE HYDRAULIC PATH, SEGMENTATION AND DROUGHT RECOVERY – Chairs: José Torres Ruiz and Tadeja Savi			
9:00-9.30	S3.1 (Keynote)	**Schreiber Lukas**	Structure and function of plant/environment interfaces: cutinzed leaf surfaces versus suberized root surfaces
9:30-9:45	S3.2	**Skelton Robert**	Patterns of local adaptation in xylem vulnerability to embolism vary between stems and leaves in a segmented North American oak
9:45-10:00	S3.3	**Martín Gómez Paula**	The isotopic signature of drought-induced embolism: from the leaf to the tree stem
10:00-10:15	S3.4	**Smith Christina Marie**	Determining the resistance of lianas and trees to drought using xylem pit membrane anatomy and visualization of embolisms
10:15-10:30	S3.5	**Levionnois Sébastien**	Hydraulic and vulnerability segmentations at the leaf-stem interface: Do they exist and are they coordinated across Neotropical trees?
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11:30-11:45	S3.7	**Padilla-Díaz Carmen M.**	Is the coordination between leaf and stem hydraulic capacitance the “power bank” to delay embolism in olive?
11:45-12:15	S3.8	**Creek Danielle**	Xylem embolism in leaves does not occur with open stomata: evidence from direct observations using the optical visualisation technique
12.15-12:30	S3.9	**Billon Lise-Marie**	Mind the cuticle conductance: When resistance to cavitation is not enough for evaluating resistance to drought
12:30-12:45	S3.10	**Bortolami Giovanni**	Leaf xylem occlusions in declining grapevine
12:45-14:30	*LUNCH*		
Session 3: EXTREMITIES OF THE HYDRAULIC PATH, SEGMENTATION AND DROUGHT RECOVERY – Chair: Sylvain Delzon			
14:30-15:00	S3.11 (Keynote)	**Carminati Andrea**	Soil hydraulic constraints on transpiration
15:00-15:15	S3.12	**Rodriguez-Dominguez Celia M.**	The importance of the hydraulic component connecting the roots to the soil
15:15-15:30	S3.13	**Schuldt Bernhard**	No differences in xylem safety between roots and branches in four temperate broad-leaved tree species
15:30-15:45	S3.14	**Cardoso Amanda**	The change of drought-induced leaf death is determined by the hydraulic vulnerability of individual leaves
15:45-16:00	S3.15	**Johnson Kate**	Wheat leaves embolized by water stress do not recover function upon rewatering
16:00-16:15	S3.16	**Salmon Yann**	Silver birch refilling of embolised xylem: evidence and mechanisms
16:15-17:00	*COFFEE BREAK AND POSTER SESSION*		
Session 4: MOLECULAR BIOLOGY OF PLANT HYDRAULICS – Chairs: Francesca Secchi and Uri Hochberg			
17:00-17:30	S4.1 (Keynote)	**Zwieniecki Maciej**	Biology and chemistry of xylem recovery from water stress
17:30-17:45	S4.2	**Brunetti Cecilia**	Dynamic changes in ABA content in water-stressed *Populus nigra*: effects on carbon fixation, soluble carbohydrates and hydraulic recovery
17:45-18:00	S4.3	**Gori Antonella**	Integrating metabolomic analysis into the physiological framework: possible information on the depletion of C and N storage compounds in droughted leaves
18.00-18.15	S4.4	**Casolo Valentino**	Soluble carbohydrates metabolism sustains energetics and xylem hydraulic functionality upon relief from drought in *Populus tremula x alba*
18.15-18.30	S4.5	**Tomasella Martina**	The relationships between vulnerability to xylem embolism and stem non-structural carbohydrates (NSC) in *Populus nigra* L.
18.30-18.45	S4.6	**John Grace**	Genetic control over leaf vascular anatomy in Switchgrass (*Panicum virgatum*)

FRIDAY 27

Session 5: PHYSIOLOGICAL RESPONSES TO THE ENVIRONMENT – Chairs: Georg von Arx and Rosana Lopez			
9:15-9.45	S3.1 (Keynote)	**Battipaglia Giovanna**	Multidisciplinary tree ring approach to gain insights into Mediterranean plant adaptation to drought
9:45-10:00	S3.2	**McDowell Nate**	On hydraulics and drought-associated mortality
10:00-10:15	S3.3	**Rehschuh Romy**	Recovery of *Pinus sylvestris* from severe drought: Impacts on leaf gas exchange and xylem embolisms
10:15-10:30	S3.4	**Gersony Jess**	Carbon export patterns in relation to diurnal and seasonal carbon and water dynamics in red oak leaves
10:30-10:45	S3.5	**Waite Pierre-André**	Xylem vulnerability to embolism in natural and transformed tropical systems in Sumatra, Indonesia
10:45-11.30	*COFFEE BREAK*		
11:30-11:45	S3.6	**Petruzzellis Francesco**	Water relations and hydraulic traits might explain the invasive potential of alien trees
11:45-12:00	S3.7	**Hochberg Uri Dodo**	To everything there is a season: seasonal hydraulic plasticity in grapevines
12:00-12:15	S3.8	**Lintunen Anna**	Winter embolism formation – new insights from freezing experiments
12.15-12:30	S3.9	**Gričar Jožica**	Post-fire effect on leaf development, radial growth and anatomy of secondary vascular tissues in *Quercus pubescens*
12:30-14:30	*LUNCH*		
14:30-17:00	*GUIDED TOUR OF THE BOTANICAL GARDEN, ITS LIBRARY AND COLLECTIONS*		
17:00-17:45	Bus transfer to Cinto Euganeo		
17:45-19:30	Final discussion with snacks and wine		
19:30-21:30	***XIM4 DINNER***		
21:30-22:15	Bus transfer to Padua		

INDEX OF ORAL CONTRIBUTIONS

Electrical Resistivity Tomography: New Insights

Stefan Mayr[1], Andreas Bär[1], Andrea Ganthaler[1] & Adriano Losso[1]

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Key message

Electrical resistivity tomography enables *in situ* analyses of tree stems. Hydraulic conclusions must consider species-specific patterns and variability as well as the influence of wood properties and temperature.

Electrical resistivity (ER) tomography enables the minimal-invasive, *in situ* analysis of tree stems. Based on measurements of the electrical conductance, it reflects the moisture, electrolyte content and density of wood. ER tomograms can thus give information on potential damage within the trunk as well as on hydraulic traits.

Based on ER measurements on several European forest species, inter- and intra-specific variation and patterns of tomograms were analysed and the influence of drought, electrolyte content, wood density, temperature and measurement setup tested. Furthermore, an annual course of ER tomograms was collected at the timberline.

All species under study showed relevant intra-specific variation in ER tomograms. ER patterns of conifers were more homogenous and concentric compared to the complex tomograms of angiosperms. Species-specific correlation between ER and wood moisture, electrolyte content and/or density were observed, whereby temperature and installation setup affected tomograms. Measurements in timberline trees revealed high ER over winter and ER tomograms in the vegetation period to mirror stem water relations.

Results demonstrate a high species-specificity of ER tomograms and of respective xylem traits. They underline the importance of reference measurements for a correct interpretation of tomograms and use of ER tomography in studies on plant hydraulics.

Plant desiccation time to critical thresholds of hydraulic failure during drought is predictable across species of eucalyptus

Chris J Blackman[1], Ximeng Li[1], Brendan Choat[1], David Tissue[1], Paul Rymer[1], Remko Duursma[1] and Belinda Medlyn[1]

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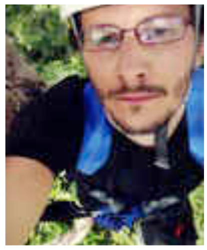
Key message

Knowing when trees die during extreme drought is essential for understanding the potential effects of climate-change on forests. We show that a trait-based model (t_{crit}) can be used to predict plant desiccaton time during drought.

Catastrophic failure of the water transport pathway in trees is a major mechanism of mortality during extreme drought, yet knowledge of when trees die of hydraulic failure and the traits that determine plant desiccation time remains limited. Here, we test for the first time a trait-based model (t_{crit}) of the time it takes trees to desiccate from the point of stomatal closure to critical levels of hydraulic dysfunction during drought. We grew plants of eight species of *Eucalyptus* from contrasting climates under well-watered conditions before allowing a subset to dehydrate to water potentials close to plant death. To parameterise the model we sourced published data and made new measurements of species cavitation resistance, allometry, minimum conductance, and leaf shedding. Plant desiccation time was highly predictable across species using t_{crit} in conjunction with a drought-induced leaf shedding function. Across species, plant desiccation times were longest in species with high cavitation resistance, wide stomatal-hydraulic safety margins, and high total plant water content to leaf area ratios. Importantly, the ranking of plant desiccation times across species was similar using t_{crit} parameterised with traits derived solely from excised branches, indicating potential for an achievable index of t_{crit} across diverse species globally.

Estimating xylem vessel length distributions



Roman M. Link[1], Bernhard Schuldt[1], Brendan Choat[2], Steven Jansen[3] & Alexander R. Cobb[4]

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Key message

We use probability theory and a simulation study to demonstrate that most published estimates of vessel length describe a size-biased subsample of vessels instead of the overall length distribution.

Vessel length is a highly important functional trait for plant hydraulics, because it determines the contribution of pit membranes to the total flow resistance of the xylem and therefore constitutes a central control variable for hydraulic efficiency. Additionally, there is a growing body of evidence indicating that several commonly used methods to determine plant vulnerability to embolism might be affected by vessel length related measurement artefacts. However, vessel length is under-studied compared to other wood anatomical traits as with currently available methods it cannot be measured directly, while indirect measurement based on silicon- or air-injection is rather time consuming and relies on estimation algorithms whose assumptions and validity are rarely assessed from a probability theoretic standpoint.

We present a brief introduction to the theory of vessel length estimation based on predictions from statistical theory and the outcome of a simulation study. In doing so, we provide evidence that most published estimates of average vessel length do not relate to the overall vessel length distribution, but to the size-biased subsample of vessels that are situated in a cross-section of the stem. We discuss their relationship to the population mean of vessel length and the implications of this finding for further studies.

The reason why the Sperry centrifuge method is incapable of measuring vulnerability curves of long-vesseled species

Mel Tyree[1], Dongmei Yang[1] & Guoquan Peng[1]

[1] College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, 321004 CHINA

Key message

The Sperry centrifuge method is incapable of measuring the correct vulnerability curve of long-vesseled species.

Vulnerability curves are a measure of how xylem tension impacts the efficiency of water transport in stems, roots or petioles of plants. Water transport is usually quantified as hydraulic conductivity, K_h, and tension is induced either by dehydrating large branches (much longer than the longest vessels) on a bench top while measuring xylem water potential (tension) or by spinning smaller stem segments in a centrifuge to induce tension quickly. After tension is induced in a centrifuge, the K_h is measured either while spinning the stem in the centrifuge or after removing the stem from the centrifuge. Many studies, including meta-analysis of previous studies, have suggested that all centrifuge methods disagree with bench-top dehydration methods in species in which mean vessel length is longer than the half-length of the stems spinning in the centrifuge rotor.

But the mechanims (reason) for this disagreement has never been firmly established. In this paper we will briefly review past evidence and present unpublished experiments that provides insights into the mechanims involved.

The presentation of Dr. Yang immediately following this paper will provide a solution to the problem.

The quantitative relationship between water extraction curves and vulnerability curves

Dongmei Yang[1], Guoquan Peng[1] & Melvin T. Tyree[1]

[1] College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, 321004 CHINA

Key message

The water extraction method is a quantitative centrifuge water-extraction protocol that is relatable to vulnerability cures in stem segments of long-vesseled species.

Significant improvements to the centrifuge, water-extraction method of measuring percentage loss water-volume, PLV, and vulnerability curves, VCs, are reported. The Cochard rotor (and maybe the Sperry rotor) is recognized as being incapable of measuring VCs of species with long vessels because of premature embolism induced by hypothetical nano-particles that can be drawn into the axis of rotation of stem segments during flow measurement. These nano-particles are presumed to induce anomalous embolisms; in contrast, water extraction presumably pushes nano-particles out of the stem. Hence, work towards establishing the validity of an alternative centrifuge method is desirable. This study focuses on a long-vessel species, *Robinia pseudoacacia* L., for which many VCs have been constructed by many different methods and the daily water relations has been quantified in previous papers. PLV extraction curves have dual Weibull curves and this paper focuses on the second Weibull curve because it involves extraction of water from vessels, as proven by staining methods. Using Poiseuille's law and the geometry of vessels we argue that $PLC=2PLV-PLV^2$ in the special case where all vessels regardless of size have the same vulnerability curve. In this special case, this equation predicts the data reasonably well.

Hydraulics in agriculture and ecology: a personal perspective on unification and the work that lies ahead

Sean M. Gleason[1,2]

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Key message

Recent advancements in hydraulic physiology hold promise for the improvement of agricultural species, but this will require an appreciation of water transport traits and a better understanding of hydraulic efficiency and safety.

Growth, reproduction, and survival of vascular plants depend critically on the delivery of large volumes of water to the sites of evaporation and photosynthesis within leaves. Structures and processes that influence access to water, its long-distance transport, or its regulation and exchange at the leaf surface, represent coordinated and costly solutions to a very old evolutionary challenge – invasion of earth's terrestrial habitats ca 430 Mya. The tradeoffs that have driven the divergence of vascular species should be viewed through this lens, i.e., engendered by natural selection to achieve this ambitious goal, however well- or poorly-suited they appear. Indeed, to study agriculture and ecology without understanding water, is to misunderstand plants and plant communities altogether. Although ecophysiology has largely embraced the cornerstone-importance of water transport, agriculture has yet to do so. Here, I will discuss four important challenges facing the improvement of agricultural species and how ecological physiology and crop physiology can both inform this process. These challenges are: 1) better research models are needed to quantify biological connections and complexity, 2) our understanding of the costs, risks, and proximate causes of hydraulic efficiency and safety requires much improvement, 3) hydraulic phenotyping is slow, indirect, and error-prone, and 4) there appear to be cultural and scientific obstacles that are thwarting the logical, and potentially synergistic, integration of ideas, methods, and objectives.

Pit Membranes are not 2D

Julia Werner[1], Cora Carmesin[1], Ana Diaz[2], Mirko Holler[2], Christine Kranz[3], Gregor Neusser[3], Christopher Parmenter[4], H. Jochen Schenk[5], Volker Schmidt[6], Paul Walther[7], Matthias Weber[6], Ya Zhang[1] & Steven Jansen[1]

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7 Institute of Electron Microscopy, Albert-Einstein-Alleen 11, Ulm University, D-89081 Ulm, Germany

Key message

Pit membranes between xylem conduits represent highly complex porous media, with a 3D micromorphology that is strongly affected by the methodology applied for their observation.

We study the morphology of angiosperm pit membranes to understand the functional importance of pits within segmented conduit networks. Traditionally, vulnerability of pit membrane pores to air seeding has been based on the assumption of cylindrical pore shapes, ignoring their complex porous medium characteristics.

We investigated wood samples that were fresh, dehydrated, dried-rehydrated, and high-pressure frozen freeze substituted, and conducted observations based on electron microscopy (TEM), confocal laser scanning microscopy (CLSM), and X-ray ptychographic tomography.

Pit membranes in never-dried samples under CLSM are coated with amphiphilic lipids, are cushion-shaped, and ca. 2 to 4 times thicker than freshly embedded TEM samples. Dehydrated pit membranes show irreversible shrinkage up to 66%. Moreover, dehydration results in reduced pore sizes (< 20 nm in diameter), and a reduced porosity of 62%, while fresh material has pores up to 50 nm and a mean porosity of 81%.

Our observations contribute to the development of a 3D pit membrane model, which is highly relevant to our mechanistic understanding of air-seeding. We hypothesise that the highly complex and tortuous pore volumes and pore throats in pit membranes function as a foam generating medium, avoiding embolism formation in xylem sap under tension.

Down in the pits: challenges and questions from the pit membranophiles

Cora Carmesin[1], Fabian Port[2], Lucian Kaack[1], H. Jochen Schenk[3] & Steven Jansen[1]

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[3] Department of Biological Science, California State University Fullerton, Fullerton, CA 92834-6850 U.S.A.

Key message

Based on various microscopy methods, I will present novel observations of the surface topography, nanomechanical properties, and chemical composition of interconduit pit membranes in *Clematis vitalba*.

Pit membranes between conduits in angiosperm xylem represent a key evolutionary invention that made it possible for plants to transport large amounts of water. Yet, our understanding of structure and function of interconduit pit membranes is incomplete, partly due to technical challenges of conducting ultrastructural observations without preparation artefacts. Based on atomic force microscopy (AFM), transmission electron microscopy, and confocal scanning microscopy, we will present detailed observations of the surface topography, nanomechanical properties and chemical composition of pit membranes in *Clematis vitalba*.

We compared cellulose-microfibrils and pore sizes between wet (i.e., never-dried) and dehydrated pit membranes using AFM, which offers a sensitive approach to measure detailed surface properties *in situ*. These data enable us to develop a 3D model of pit membranes.

Moreover, amphiphilic, insoluble lipids were found to be associated with inner conduit walls, pit borders, and pit membranes, affecting considerably their wettability. Our observations pave future possibilities for 3D flow simulations of metastable liquids, considering multiphase interactions between cellulose microfibrils, water, surfactants, and gas (air-bubbles). Unlike the classical air-seeding hypothesis, we hypothesize that pit membranes may function as foam generators, which keep infiltrating tiny gas bubbles safely, without causing embolism, into xylem sap that is (super)saturated with gas.

Remarkable absence of within-species adjustments in conductive xylem, phloem and parenchyma structural traits along a water availability gradient

E.M.R. Robert[1,2,3], M. Fonti[4], N. Kiorapostolou[5], G. von Arx[4], M. Mencuccini[1,6], T. Rosas[1], M. Ramírez Boixaderas[1], G. Petit[5], C. Nabais[7], & J. Martínez-Vilalta[1,8]

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4 Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
5 Department of Land, Environment, Agriculture and Forestry (TeSAF), University of Padua, Legnaro, Italy
6 ICREA, Barcelona, Spain
7 Centre for Functional Ecology, University of Coimbra, Coimbra, Portugal
8 Autonomous University of Barcelona, Cerdanyola del Vallès, Spain

Key message

Adjustments to water conditions at within-species level does not happen through proportions and design of xylem and phloem tissues and cells changing our view on how trees cope with their environment.

Structural characteristics of conductive xylem, phloem and xylem parenchyma differ between species, co-defining the environment species can thrive in. Along environmental gradients, we expect populations of the same species to also show differences in xylem and phloem structural traits that relate to the ability of trees to cope with the local environment. We quantified the variability in tissue fractions, conduit and parenchyma cell lumen area and cell density, and xylem conduit spatial organisation and cell wall thickness along species-specific gradients in water availability in Catalonia, NE Spain. From 450 trees of six species, tissue and cell structural traits were measured on branch micro-sections. Population-specific climate, soil and forest structure data were also obtained. Our results demonstrated clear differences between species in the studied traits. At within-species level, however, the variation in these traits were unrelated to water availability. This suggests that adjustment to water conditions at population level primarily takes place through physiological traits or other structural traits, while proportions and design of the xylem and phloem tissues and cells are conserved. Therefore, we might have to change our view on the underlying mechanisms of how trees of the same species cope with the local conditions they live in.

The correlation between conduit diameter and drought-induced embolism resistance revisited: arguments pro and contra

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Key message

Conduit diameter and drought-induced embolism resistance: arguments pro and contra.

Much of scientific understanding of the relationship between wood structure and function invokes the vulnerability-diameter link, with an entire body of hypotheses based on comparative anatomy, known as ecological wood anatomy, built around it. As a result, there is no other single assumption relating xylem structure and function that has so much depending on it. A vast amount of empirical evidence from various sources (wood anatomy, xylem physiology) is consistent with this structure-function link, but there is also compelling evidence that contradicts it. Even more baffling is that there is no entirely satisfactory theory linking drought induced vulnerability to conduit diameter, making it difficult for empirical workers to know exactly what patterns to search for in testing the link. To help in this effort, we review the current evidence consistent and inconsistent with the vulnerability-diameter link at various levels, i.e. within individuals, between individuals of the same species, and across species. Therefore, we summarize the main hypotheses that have been proposed to account for the diameter-vulnerability link, outline some of the contrasting empirical predictions they make, and encourage xylem workers to take all the data into account in building explanations of the vulnerability-diameter link.

Anatomical determinants of plasticity of xylem resistance to cavitation in *Populus tremula x alba*

Cédric Lemaire[1], Yann Quilichini[2], Nicole Brunel-Michac[1], Pierre Conchon[1], Romain Souchal[1], Julien Cartailler[1], Eric Badel[1], Jérémie Santini[2] & Stéphane Herbette[1]

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Key message

An assessment of structural determinants of plasticity of xylem resistance to cavitation.

Xylem resistance to cavitation is a key parameter in drought resistance of trees. Indeed, water stress tolerant species are less vulnerable to cavitation than water stress intolerant species. At the within species level, phenotypic variability was reported for the xylem cavitation resistance, mainly based on plasticity among environmental growth conditions. This raises the question of genetic and structural determinants of this plasticity. The objective of the present work is to identify the structural determinants controlling plasticity of xylem resistance to cavitation.

Young poplars trees were grown in contrasted water availability or light exposure in order to induce acclimation in hydraulic properties. We then characterized the structural modifications of their acclimated xylem at three levels: xylem organization, vessels dimensions and pits shape. We used different microscopy techniques including Transmission Electron Microscope (T.E.M.), Scanning Electron Microscope (M.E.B.) and Light Microscope – on the same individual trees. We specially investigated the intervessel pits that provide hydraulic connections between vessels while they prevent air embolism spreading.

Moreover, a local approach using direct observation of embolism spreading at the cellular level via X-ray micro-CT allowed us to explore the structural determinants of vessel resistance to cavitation.

Allometric scaling of hydraulics and mechanics drive the leaf-stem size spectrum for 42 Neotropical tree species

S. Levionnois[1*], C. Salmon[2], B. Clair[3], C. Ziegler[4], S. Coste[5], C. Stahl[6], P. Heuret[7*†]

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Key message

The leaf size-twig size relationship is allometric within a large range of leaf size on tropical species. Larger leaves display a higher leaf area : twig xylem area ratio and a higher xylem-specific conductivity.

The leaf economic spectrum is a powerful framework to understand the diversity of leaf structures and functions. However, this framework fails to integrate leaf size, especially leaf area, although this is subject to a variation of 6 orders of magnitude across species. Thus, we still do not know what are functional constraints prevailing on the shaping of leaf size diversity. We evaluated vasculature, hydraulic, and mechanical constraints on leaf size, by investigating shoots and leaves of 42 tropical tree species, covering a large range of blade surface area. Based on an anatomical approach, we measured tissue area, vasculature, and theoretical conductivity on shoots and leaves. We also measured stem modulus of elasticity and flexural stiffness. The leaf area-stem area relationship was allometric with higher leaf area per stem cross-sectional area on increasing leaf size. Large leave was characterized by (i) a higher leaf area-to-xylem area ratio and (ii) higher xylem-specific conductivity (K_s), driven by vessel size. We do not observe any loss of water supply per leaf area on large leaves thanks to the increasing K_s. Mechanical measurements suggest a more efficient way to achieve stiffness, due to geometry rather than the material, and driven by the pith proportion.

Anatomical and functional plasticity of xylem in young woody roots and branches

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Key message

The interplay between mechanical stiffness, nutrient storage, and hydraulic conductivity in xylem can be achieved by a plethora of anatomical strategies.

Differences in xylem structure between young woody roots and branches reflect not only great developmental plasticity of xylem but also contrasting adaptive emphasis on mechanical, storage, and hydraulic functions in these plant organs. To analyse the interplay between mechanical stiffness, carbohydrate storage, and hydraulic conductivity, we measured anatomical and functional parameters of woody roots and stems in a dozen of temperate tree species. Our results demonstrate that it is rare for xylem to compromise all three functions equally. Instead, one of the three functions is more pronounced, while the remaining two functions may or may not co-vary. In self-supporting stems, higher demands for mechanical stability put a major constraint on xylem structure, whereas root xylem can be designed with a greater emphasis on both storage and hydraulic functions. The functional trade-offs primarily originate from the division of labour between different cell types. However, plants can deploy a plethora of anatomical strategies to prioritize one function and to maintain partial multifunctionality of xylem. These strategies include variation in cell type relative fractions, variation in cell size and secondary wall reinforcement, and the use of specialized anatomical features, such as the presence of partially non-lignified rays and living fibres.

The xylem embolism resistance spectrum of grapevine

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Key message

Xylem embolism resistance significantly differs across 30 grapevine varieties phenotyped, suggesting that some would be better adapted than others to face ongoing climate warming.

Evaluating climate change impact on the currently cultivated grapevine varieties and determining which ones could be better adapted in the future are two current major challenges in viticulture. We assessed the underlying mechanisms that would allow this economically important crop species to cope with drought conditions using innovative methods such as *in situ* flow-centrifugation as well as microCT and optical technique observations. The intraspecific resistance to xylem embolism has been characterized for ca. 30 red and white grape varieties representing both mediterranean and temperate climatic conditions. The variability in Ψ_{50} was significant across varieties, with values ranging from -1.8 to -3.3 MPa. In addition, we found that embolisms in grapevine stems and leaves are irreversible under tension and embolism repair hardly manageable overwinter. Within-individual xylem embolism resistance differed between organs and is subject to ontogenic effects with increasing resistance (i.e. decreasing Ψ_{50} values) both throughout a growing season and over years. These results as a whole provide new insights into the ability of grapevine varieties to deal with increasing periods of water deficit.

Old year rings may provide water storage capacity by cavitating first

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Key message

An MRI study on beech and spruce reveals that old year rings may provide a limited amount of water storage capacity by cavitating before the current year ring.

The sapwood of temperate trees comprises many year rings. Conduits in the current year ring commonly are assumed to conduct most flow. Those in older rings, conversely, are assumed to be less efficiently connected to new leaves, and may have become more susceptible to cavitation. We hypothesize that older year rings thus will hold more dead-end conduits, conduct less flow and cavitate before the current year xylem does, releasing stored water.

To test this hypothesis we used MRI relaxometry and flowmetry, utilizing both a traditional high field imager and a mobile, battery driven one. MRI cannot always resolve singular xylem conduits, especially if it is a mobile device. We here propose and demonstrate MRI methods to nonetheless detect water filled conduits and quantify the flow conducting area – or loss thereof.

Large beech trees were exposed to drought. Initially, all xylem regions that contained water filled vessels also exhibited flow. Stagnant water in old or current year rings could not be detected. Cavitation did however start in the oldest year rings first, supporting our hypothesis. Observations in beech and spruce saplings confirmed this observation. However, release of water from old year rings did not appear to slow the spread of cavitation.

Hydraulic conductance in newly developing seedlings of *Acer pseudoplatanus*

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Key message

Structural and physiological changes during seedling establishment ensure well balanced water relations in young developmental stages.

Seedlings are particularly prone to drought-related death as the access to soil water is limited by the small root systems and water storage capacity. Hydraulic studies on early developmental plant stages though are rare as many classical hydraulic methods are not applicable to tiny and soft plantlets. In the present study, we used the evaporation flux method to (I) measure hydraulic conductance of six weeks old *Acer pseudoplatanus* seedlings emerging at different sites and under differing conditions and (II) to trace changes during further establishment. Hydraulic measurements were then related to various leaf and xylem anatomical traits. First results point to a high intraspecific variability and a pronounced acclimation potential of parameters related to hydraulic conductance at early development stages. Thereby, first leaves were particularly well supplied with water. During the following development, xylem and leaf anatomical changes led to an increase in hydraulic conductance and thus a constant water supply to the increasing leaf area. Outcomes indicate well-balanced hydraulics in newly developing tree seedlings and the ability to adjust to prevailing environmental conditions, which may be a prerequisite for successful establishment.

Exploring the link between embolism resistance and hydraulic failure in the earliest vascular plants

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Key message

Species of lycophyte, the oldest extant lineage of vascular plants, have xylem that is considerably resistant to embolism formation.

Selaginella is the most specious genus of lycophyte, the oldest extant lineage of vascular plants. Like many species in this clade the majority of *Selaginella* species are native to ever-wet environments. Here using two non-invasive imaging techniques, synchrotron-based X-ray micro-CT and the optical vulnerability method, we quantified the resistance to xylem embolism in three species of *Selaginella*, as well as determined the water potential at which leaf hydraulic conductance declined and stomata closed during drought. When imagining intact stems during dehydration the outer cortex was found to dramatically separate from the central stele containing the vascular tissue. This separation occurred before embolism formation in the xylem yet corresponded to a substantial reduction in leaf hydraulic conductance and stomatal closure. In *S. pulcherrima* and *S. haematodes* mean P50 was between -3.5 and -4 MPa, whereas *S. helvetica* had more vulnerable xylem with a mean P50 of -2.5 MPa. Our results suggest that resistant xylem has evolved multiple times in all lineages of vascular plants and is a universally rare event. The unique vascular anatomy and separation of the cortex from stele during drought in lycophytes may place a major physiological constraint on the ecological success of this group.

Hydraulic conductance controls the dynamics of flux and productivity at ecosystem scale

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Key message

We introduce K_{eco}, the hydraulic conductance of the forest ecosystem, calculated from canopy fluxes and water potential driving forces, and show that K_{eco} controls ecosystem water and carbon fluxes.

The dynamics of forest level flux and productivity are controlled by the above and below-ground environment and by the plants that constitute the majority of primary productivity and water use. The water transport system within the plant has been increasingly recognized as a major determinant of the dynamics of carbon and water fluxes of individual trees, shrubs and herbs. Thus, at organ or whole-plant scale, hydraulic conductance is generally recognized to limit overall transport and its dynamics diurnally and during drought and recovery after rainfall. However, at the scale of the whole ecosystem, hydraulic properties, while generally acknowledged to influence carbon and water fluxes, have never been clearly conceptualized or directly quantified, and often treated as constants, rather than dynamic properties. We introduce a scaled up version of hydraulic conductance, i.e., the efficiency of water transport of the forest ecosystem (K_{eco}), based on canopy fluxes and soil and leaf water potential, and show that K_{eco} exerts a major, and dynamic control on ecosystem water and carbon fluxes.

Tree-ring density and C isotope data as early-warning signals of drought-induced mortality

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Key message

Tree-ring density and C isotope data are useful traits to predict the susceptibility of trees to extreme droughts. Although pines suffered from C starvation during years, the ultimate cause of death after a severe drought was hydraulic failure.

Drought induced forest dieback and mortality have been observed across the globe in the last decades. We report drought-induced mortality of *Pinus canariensis* stands at the drier edge of its distribution limit. High mortality occurred after two consecutive extremely dry years with less than 100 mm of annual rainfall. We investigated the local climatic trend during the preceding 33 years of the mortality event and its relationship with radial growth, wood density, xylem anatomy and changes in water use efficiency (WUE) derived from C isotope discrimination. We compared these variables in dead and surviving neighbouring trees with similar age and size.

Extremely dry conditions resulted in low growth rates in both dead and surviving trees with no significant differences even in the years prior to death. However, surviving trees had significantly higher mean ring density, earlywood density and latewood density mainly as a result of forming tracheids with thicker walls. A decrease in latewood density in dead trees several years prior to death was found. Surviving trees showed an increase in WUE, whereas in dead trees WUE suddenly dropped in the years previous to death. Carbon isotope data and latewood density revealed either later stomatal closure with could have had a deleterious effect on the water conducting capacity due to generalized hydraulic failure or higher use of reserves to build up or to reinforce tracheids cell walls due to of the scarcity of recently photosynthesized carbohydrates.

Wood anatomy and cambial phenology in hyperarid environments: the key to understand past, present, and future of western US conifers

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Key message

In hyperarid environments wood anatomy closely reflects phenological plasticity of the cambium.

Xylem anatomical structure of conifer species from hyperarid environments in the Western US can change dramatically in relation to the seasonal patterns of cambial phenology. In this region, xylogenesis is predominantly driven by moisture conditions, and the cellular structure of woody tracheids records even sporadic events of moisture supply (i.e. summer rainstorms) as contractions of lumen diameter or as Intra Annual Density Fluctuations (IADFs). We present results from our studies on wood anatomy and cellular dendroclimatology of conifer species of the Western US, including *Pinus longaeva*, *Pinus ponderosa* and *Pseudotsuga menziesii*. Using different techniques of image acquisition and field monitoring of cambial phenology, we were able to capture linkages between intra-annual climatic variability, seasonal dynamics of xylogenesis, and their effect on tracheid features. Given the complexity of intra-annual processes of cellular differentiation under severe water limitations, we highlight the need to investigate short-term climate-anatomy relationships and to calibrate traditional tools of quantitative wood anatomy (i.e. tracheidograms) to account for species phenological plasticity. Bridging the gap between xylem structure, tree growth and plant physiology in western US conifers will improve our understanding of ecosystem response to present and future climate variability, ultimately boosting the paleoclimatic potential of these long-lived species.

Structure and function of plant / environment interfaces: cutinzed leaf surfaces versus suberized root surfaces

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Key message

Both leaf and root surfaces are covered by hydrophobic biopolymers forming the plant environment interface. However, hydraulic conductivities of suberized root surfaces are 2-4 orders of magnitude higher compared to cutinized leaf surfaces.

Living plant tissue is separated from the surrounding environment by extracellular biopolymers. The interface between the atmosphere and leaves is formed by the cuticle sealing outer epidermal cell walls. Soil/root interfaces are formed by exo- and endodermal tissue largely characterized by suberized cell walls. Since these polymers are lipophilic in nature they reduce free diffusion of water and dissolved molecules, thus forming barriers regulating water and solute transport at plant interfaces, and they significantly help to cope with abiotic and biotic stress. On the basis of permeances (diffusion velocity), which are independent from experimental boundary conditions (concentration gradients, duration of measurement, surface area, volume …), barrier properties of leaf and root surfaces can directly be compared. Different experimental approaches allowing the quantitative characterization of barrier properties of cutinized leaves and suberized roots will be introduced. Recent results of our ongoing research comparing barrier properties of cutinized leaf surfaces with suberized root surfaces and their response to abiotic environmental stress factors (water deficit) will be presented and discussed.

Patterns of local adaptation in xylem vulnerability to embolism vary between stems and leaves in a segmented North American oak

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Key message

Patterns of local adaptation in xylem vulnerability to embolism vary between stems and leaves in a segmented North American oak, with variation in vulnerability in leaves, but not stems, being driven by aridity.

Vulnerability to embolism varies considerably between con-generic species distributed along aridity gradients, yet little is known about intra-specific variation in vulnerability and its drivers. Less is known about intra-specific variation in tissues other than stems, despite results suggesting that roots, stems and leaves can differ in vulnerability. We hypothesized that intra-specific variation in vulnerability to embolism in leaves and stems is adaptive and driven by aridity.

We quantified vulnerability of leaves and stems of Californian blue oak using the optical technique. To assess the contribution of genetic variation and phenotypic plasticity to within-species variation, we quantified vulnerability of individuals from wild populations occurring along an aridity gradient as well as individuals sourced from the same wild populations, but grown in a common garden.

Variation in vulnerability was explained mostly by differences between individuals (>66% of total variance) and tissues (16%). Greater within-population than between-population variation suggests a lack of local adaptation to climate at the population level. However, leaves of individuals occurring in the garden, but originating from cooler, wetter sites were less vulnerable to embolism than leaves of individuals originating from drier sites. Intra-specific variation in vulnerability to embolism in leaves, but not stems, is driven by aridity.

The isotopic signature of drought-induced embolism: from the leaf to the tree stem

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Key message

The isotopic composition of stem water can unravel the link between the loss of hydraulic conductance in woody stems and plant dehydration causing tree mortality.

During drought, leaf water loss is regulated by stomatal closure, but afterwards, xylem embolism can still occur. To date, very little attention has been paid to the role of stem water loss in xylem embolism and ultimately tree mortality. However, some studies have measured significant stem transpiration providing evidence for evaporative losses through the bark when stomatal transpiration is minimal. As a result, gas exchange from stem surfaces can provide information about resistance to stem dehydration and hydraulic failure. Moreover, when sap flow is limited, stem water displays an evaporative-driven isotopic enrichment. Thus, xylem and stem surface stable isotope composition can provide information on the rate of stem evaporative losses when water fluxes are small providing an independent window on the dynamics of hydraulic failure in stems. In this context, we assessed the connection between non-stomatal water losses and xylem embolism to quantify the contribution of stem water losses during drought-induced mortality. To test this, a series of experiments in climate-controlled conditions have been designed with species of contrasting physiological and anatomical characteristics. We present here novel results of simultaneous and dynamic measurements of stem gas exchange, stem hydraulic conductance and the stable composition of stem water under prolonged drought.

Determining the resistance of lianas and trees to drought using xylem pit membrane anatomy and visualization of embolisms

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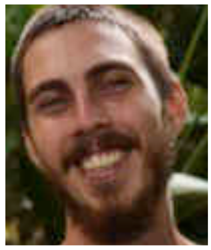
Key message

Our findings show that lianas and trees from drier tropical forests have thick pit membranes than ones from wetter forests, which supports the hypothesis that thicker pit membranes permit species from drier environments to withstand greater water deficit and be more resistant to drought.

Rising global temperature is likely to cause an increase in the frequency, duration, and severity of drought. This may result in widespread global plant mortality due to hydraulic failure. Xylem pit membranes play an important role in plant resistance to drought. The goal was to predict vulnerability to drought in growth forms that are common in tropical forests, i.e. trees, and lianas (woody vines). We measured xylem vulnerability to embolism with the optical method and pit membrane thickness of lianas and trees in a semi-deciduous dry forest and in an evergreen humid rainforest at the Smithsonian Tropical Research Institute in Panama. At each location, we measured 8 species of lianas and 8 species of trees for a total of 32 species. Species in the dry forest had thicker pit membranes than the ones in the humid forest ($F_{1,27}=15.03$, $P<0.001$). However, there was no difference in pit membrane thickness between lianas and trees within each forest ($F_{1,27}=1.06$, $P=0.313$). This is one of the first studies to measure pit membranes thickness in tropical lianas and trees and supports the hypothesis that thicker pit membranes permit species in drier forests to withstand greater water deficit and be more resistant to drought.

Hydraulic and vulnerability segmentations at the leaf-stem interface: Do they exist and are they coordinated across Neotropical trees?



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Key message

We investigated the relationship between the hydraulic segmentation and the vulnerability segmentation at the leaf-stem interface across 15 Neotropical tree species.

The hydraulic segmentation hypothesis predicts that hydraulic constriction occurs close to leaves, as "bottlenecks", in order to preserve lower water potentials, and promote drought-induced embolism containment in easy-to-renew organs. The vulnerability segmentation hypothesis predicts that leaves should be more vulnerable to the drought-induced embolism than stems. These two mechanisms should lead leaves to act as "safety valves" to protect perennials organs. With the increasing issue relative to drought resistance and climate change, the vulnerability segmentation has been intensively investigated in past years, although there is a lack of investigations for Neotropical trees. The hydraulic segmentation is more old-fashion and less investigated. Today, no study has investigated if these two sides of segmentation are related across species. We investigated across 15 Neotropical canopy tree species xylem vulnerability to embolism in stem (Cavi1000 method) and leaves (optical method) to assess an index of vulnerability segmentation. We used theoretical leaf-specific conductivity through an anatomical approach, to assess an index of hydraulic segmentation (Tyree & Ewers 1991). Our objectives were (i) to test the existence of vulnerability and hydraulic segmentations for Neotropical trees, and (ii) to test if these two mechanisms are related or not across species.

Drought tolerance in crops: understanding the declines of stomatal closure and leaf hydraulic conductivity in common wheat (*Triticum aestivum*) under water stress

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Key message

Declining leaf hydraulic conductance (K_{leaf}) in wheat leaves appears to occur during stomatal closure and before cavitation, but despite observing deformation of some xylem vessels under tension, this could not explain the magnitude of hydraulic dysfunction observed as wheat leaves become water stressed.

Decline of crop productions under dry conditions were reported all over the globe these past decades highlighting the urgent need to improve our knowledge on crops response to drought and especially on the physiological mechanisms involved in this response. Changes in stomatal conductance (*g*s), leaf hydraulic conductance (*K*leaf) and cell turgor during plant dehydration were investigated in common wheat (*Triticum aestivum*). The decline of stomatal conductance was concomitant with a marked reduction in *K*leaf during relatively mild water stress (leaf water potential >-1.5 MPa). This early decline of *K*leaf was not driven by xylem cavitation as first cavitation events were detected at Ψleaf = -1.5 Mpa. Xylem deformation was observed in some vessels in wheat leaves and stems, but the force driving changes in vessel shape seemed to originate from outside the xylem. Declining water potential was only weakly associated with an increase in xylem deformation; thus suggesting that the decline of *K*leaf under high water potential is driven by the outside-xylem conductance (*K*ox) in wheat. However the contribution of *K*ox decline to *g*s decline was negligible (<1%), while declining leaf turgor appeared to be clearly associated with stomatal closure.

Is the coordination between leaf and stem hydraulic capacitance the "power bank" to delay embolism in olive?

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Key message

Both stem and leaf tissues act as coordinated capacitors releasing water into the transpiration stream to delay xylem embolism.

The olive tree (*Olea europaea* L.) is becoming increasingly topical in plant hydraulics because of its physiological characteristics to cope with drought. Rodriguez-Dominguez et al. (2018) recently found that the coordination in olive of disparate organs like leaves and roots can significantly contribute to a marked stem xylem resistance reaching P_{50} values of ca. -6 MPa. Although such safety margin is well known, there is a lack of the knowledge about the effect of water that is released from surrounding tissues into the xylem (so-called hydraulic capacitance; kg MPa^{-1} m^{-3}), which contributes to guarantee plant hydraulic performance under drought stress. Moreover, the gradient of water potential that transpiration path creates from the leaves throughout the plant hydraulic system suggests a coordination between the hydraulic capacitance of leaf and stem tissues. To this end, we will present the results of drought-induced embolism in two-year old olive seedlings submitted to bench-top dehydration. The hydraulic capacitance of the stems, derived from desorption curves, and that of leaves, quantified from pressure-volume curves, were compared with xylem embolism formation, estimated from stem vulnerability curves.

Xylem embolism in leaves does not occur with open stomata: evidence from direct observations using the optical visualisation technique

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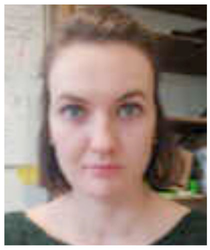
Key message

Plant close stomata early during water stress periods, preventing gas exchange but delaying irreversible leaf xylem embolism.

Drought represents a major abiotic constraint to plant growth and survival. On one hand, plants keep stomata open for efficient carbon assimilation, while on the other hand, they close them to prevent permanent hydraulic impairment from xylem embolism. The order of occurrence of these two processes (stomatal closure and the onset of leaf embolism) throughout plant dehydration has remained controversial, largely due to methodological limitations. However, the newly developed Optical Visualisation (OV) method now allows simultaneous monitoring of stomatal behaviour and leaf embolism formation in intact plants. We used this new approach directly by dehydrating intact saplings of three contrasting tree species and indirectly by conducting a literature survey across a greater range of plant taxa. Our results indicate that increasing water stress generates the onset of leaf embolism consistently after stomatal closure, and that the lag time between these processes (i.e. the safety margin) increases with increasing embolism resistance. This suggests that during water stress, embolism-mediated declines in leaf hydraulic conductivity are unlikely to act as a signal for stomatal down-regulation. Instead, plants converge towards a strategy of closing stomata early to prevent water loss and delay catastrophic xylem dysfunction.

Mind the cuticle conductance: When resistance to cavitation is not enough for evaluating resistance to drought



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Key message

It is important to detect which traits are implied in differences across and within species to understand why "more resistant" plants can die before "more sensitive" plants. This trait may be found in g_{min} and in leaf cuticular composition.

In the actual global warming context, drought events will tend to be more intense and more frequent, increasing the risk of dessication and death for plants induced by hydraulic failure. Under drought conditions, the higher atmospheric demand for water together with reduced water availability for plants increase the xylem tension until the xylem water columns break due to cavitation. To avoid reaching such risky xylem tensions, plants close their stomata to reduce the amount of water lost by transpiration and, therefore, keep the water potential relatively constant. However, despite stomatal closure, there are still some water losses through the plant cuticle (g_{min}) that keep the xylem tension decreasing. How efficient the plants are in reducing such water losses and how resistance are to cavitation (P50) will define the resistance of plant to drought. While the link between resistance to drought and P50 has been widely studied in the past, less is known about the link between drought resistance and variation in g_{min}. Indeed, g_{min} not only varies across species but also within species under different environmental conditions. We will evaluate here i) the link between g_{min} and cuticle composition; ii) the link between P50 and g_{min}, and iii) the role of g_{min} in plant resistance to drought.

Leaf xylem occlusions in declining grapevine

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Key message

Tyloses and gels form non-gaseous embolism inside leaf xylem vessels, suggesting a role of hydraulic dysfunction in *Esca* symptomatic leaves of declining grapevines.

Over the past twenty years, grapevine decline has increased in European vineyards, resulting in decreased yield and vine mortality. Trunk diseases, manly *Esca*, are one of the primary causes of plant decline. While many studies have focused their attention on wood decaying fungi in controlled environments, very few have try to identify the underlying physiological mechanisms of leaf symptom expression. Here we observed in real-time the vascular system of symptomatic and asymptomatic leaves in intact plants with non-invasive microCT technology, testing, for the very first time, the "hydraulic failure" theory on *Esca* symptom development.

Using synchrotron-based micro-CT technology we evidenced that: (i) symptomatic and asymptomatic leaves present the same level of native embolism; (ii) in symptomatic leaves, high percentages of xylem vessels are not functional; (iii) through histological analysis and the exploration of micro-CT 3D volumes, we found that gel and tyloses create non-gaseous embolism inside the vessel lumen of symptomatic leaves. These results stress the importance of hydraulic functioning in declining grapevine, opening new hypothesis on leaf symptoms, fungi development and plant death.

Soil hydraulic constraints on transpiration

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Key message

During drought soil hydraulic conductivity constrains transpiration. To avoid losses in leaf water potential and embolism, stomata promptly close when the soil-plant hydraulic conductance starts to drops.

When the soil dries out, its hydraulic conductivity decreases by several orders of magnitude and large gradients in water potential develop around the roots, which causes the xylem water potential to drop to very negative values, with increasing risks of cavitation. Although it is well accepted that stomata regulation allows for decreasing transpiration and preventing cavitation, the relation between soil drying and stomata conductance is difficult to predict. Here we propose that stomata close when the water potential at the root-soil interface drops and the soil-plant hydraulic conductance starts to decrease. In wet soils the relation between leaf water potential ψ_{leaf} and transpiration E is linear. In dry soils, the relation becomes nonlinear, with ψ_{leaf} rapidly and nonlinearly decreasing for small increases in E. We provide experimental evidence that stomata close when $d\psi_{leaf}/dE$ starts to decrease. Furthermore, we propose a mechanism, based on ABA production and dilution, that allows plants to respond to changes in soil-plant hydraulic conductance and to remain in the linear part of the $E(\psi_{leaf})$ relation. In summary, we show that soil-plant hydraulics constrains transpiration and that stomata regulation allows for maintaining $d\psi_{leaf}/dE$ constant. The proposed hydraulic framework places the focus on soil hydraulics for predicting transpiration response to soil drying.

The importance of the hydraulic component connecting the roots to the soil

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Key message

The magnitude of the decline in water transport efficiency between root xylem to the root surface had clear consequences for stomatal closure and regulation of transpiration in olive plants early during water stress.

Efficient water transport from soil to leaves sustains stomatal opening and steady-state photosynthesis. The above-ground portion of this pathway is well-described, yet the most important component lies below-ground and is poorly understood due to technical limitations. Here we used a novel rehydration technique to investigate changes in the hydraulic pathway between roots and soil and within the plant body as individual olive plants were subjected to a range of water stresses. Whole root hydraulic resistance (including the radial pathway from xylem to the root surface) constituted 82% of the whole-plant resistance in unstressed plants, increasing to >95% under a moderate level of water stress. The decline in root hydraulic conductance occurred in parallel with stomatal closure and contributed significantly to the reduction in canopy conductance according to a hydraulic model. Our results demonstrate that losses in root hydraulic conductance during moderate water stress in olive plants are profound and sufficient to induce stomatal closure before cavitation occurs. Future studies will determine whether this core regulatory role of root hydraulics exists more generally among diverse plant species.

No differences in xylem safety between roots and branches in four temperate broad-leaved tree species

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Key message

Although vessel tapering caused notable reductions in xylem hydraulic efficiency along the flow path in four temperate deciduous tree species, embolism resistance was not significantly lower in coarse roots than in branches.

Xylem hydraulic efficiency and safety are key mechanistic traits that will determine the fitness of trees in a warmer and drier world. While numerous studies assessed hydraulic properties of branches, our understanding of root hydraulic functioning remains limited although existing data suggest that overall roots are highly vulnerable to xylem embolism. We investigated 11 xylem traits related to xylem efficiency (water transport capacity) and safety (embolism resistance) along the flow path in four temperate broad-leaved tree species. Vessels tapered continuously from coarse roots to stem wood and branches causing considerable reductions in hydraulic efficiency. Wood density was always lowest in the roots, but did not decline linearly along the flow path. Contrastingly, xylem safety did not differ significantly between roots and branches, except for one species. The lack of variability in xylem safety between organs did not adequately reflect the corresponding reductions in vessel diameter (by ~70%) and hydraulic efficiency (by ~85%), which points to a missing relation between xylem safety and efficiency. We conclude that, at least in these temperate tree species, roots are not by far the most vulnerable organ along the hydraulic pathway, in contrast to general belief and despite low wood densities and large vessel diameters.

The change of drought-induced leaf death is determined by the hydraulic vulnerability of individual leaves

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Key message

Large variation in the resistance to embolism formation in the petioles of avocado leaves results in considerable heterogeneity in drought induced leaf damage across the canopy.

It is not entirely clear whether the formation of embolism in the xylem causes leaf death during drought. We utilized the unique species *Persea americana*, in which leaves across the canopy show varying degrees of death during drought, to study whether the degree of drought-induced xylem embolism is linked to the degree of leaf death in the canopy during drought stress. Plants were slowly droughted until leaves began to display regions of death. At this point plants were rewatered and maintained under well-watered conditions for two months. The percentage of embolized conduits in leaf petioles was then visualized in non-transpiring leaves that had experienced drought stress using cryo-SEM. A large range of embolized vessel areas were observed in the petioles of leaves across the canopy. This percentage of embolized vessels strongly correlated with degree of individual leaf damage caused by drought stress. The large variation in the percentage of embolized vessels in the petioles of leaves and the degree of leaf death across the canopy were consistent with a large inter-leaf variability in the xylem resistance to embolism observed using the optical method. The water potential at which 50% of the xylem in the leaf midrib of *P. americana* embolized ranged from -1.5 to -4.1 MPa. A strong correlation was also observed between the percentage of embolized vessels in the petiole and leaf gas exchange. Our study demonstrates how drought stress can induce heterogeneous canopy death due to a high inter-leaf variability resistance to xylem embolism.

Wheat leaves embolized by water stress do not recover function upon rewatering

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Key message

Drought-induced xylem cavitation in wheat leaves was associated with photosynthetic damage after 10-20% of leaf veins embolized. Rewatering did not induce visible refilling of xylem or full recovery of photosynthetic function.

New techniques make it possible to precisely and accurately determine the failure threshold of the plant vascular system during water-stress. This creates an opportunity to understand the vulnerability of species to drought, but first, it must be determined whether damage to leaf function associated with xylem cavitation is reparable or permanent. This question is particularly relevant in crop plants such as wheat, which may have the capacity to repair xylem embolism with positive root pressure. Using wheat (Triticum aestivum, Heron), we employed non-invasive imaging to find the water potential causing 50% xylem embolism (−2.87 ± 0.52 MPa) in leaves. Replicate plants were water-stressed to varying degrees to induce embolism ranging from minimal to substantial. Plants were then rewatered to determine the reversibility of xylem damage and photosynthetic inhibition in glasshouse conditions. Rewatering after drought-induced xylem cavitation did not induce visible refilling of embolized xylem, and embolized leaves showed photosynthetic impairment upon rewatering. This impairment was significant even after only 10–20% of leaf veins were embolized, and leaves accumulating >20% embolism died upon rewatering in 7/10 individuals. Photosynthetic damage and hydraulic decline occurred concurrently as wheat leaves dehydrated, and leaf shrinkage during drying was the best predictor of photosynthetic recovery.

Silver birch refilling of embolised xylem: evidence and mechanisms

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Key message

Silver birch can refilled fully embolised xylem. Evidence suggests that sugar to starch conversion facilitate the refilling process.

Silver Birch is one of the few species known to experience periods of positive pressure in the xylem. This takes place during the few weeks between soil thawing and bud-burst when the trees restore their hydraulic pathway. Hence, birch is susceptible to provide new insights in the on-going debate about the trees ability to refill embolised xylem conduits and the mechanisms potentially involved.

Combining field observations with controlled conditions studies of tree hydraulic, we tested the potential of silver birch branches to refill embolised xylem conduits, even under negative water potential. The hydraulic method suggests that refilling of embolised xylem can occur even under slight negative pressure. The successful ability of birch to refill xylem was confirmed by X-ray microtomography. Furthermore, we investigated the role of phloem and parenchyma cell in the refilling process, as well as the dynamic of sugar and starch conversions.

Our results show that in the field xylem pressure increases with temperature and is accompanied by a net sugar to starch turnover. Furthermore, refilling is unlikely to be driven osmotically given the small difference measured between the osmolality of the xylem sap and the source water.

Biology and chemistry of xylem recovery from water stress

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Key message

Priming xylem for stress recovery depends on the coordinated activity of sugar metabolic pathways and changes in xylem sap pH.

Most plants growing in terrestrial ecosystems undergo periods of drought followed by pulses of rain that allow for recovery from stress. The resulting impacts of water stress on plants largely depend on the severity and length of the dry period. Water stress induces a myriad of relatively well studied defense mechanisms, ranging from stomatal shutdown and osmotic adjustment to partial dieback and death. The effects that stress has on the transport system in addition to the subsequent mechanism(s) facilitating recovery remain unclear. However, despite controversies surrounding the effects of severe water stress on xylem function, including the extent to which transport is actually affected and whether xylem has the potential to recover its transport capacity, the fact of the matter is that many plant species survive and recover from severe stress without visible damage. Recent studies have shifted the focus of discussion from a purely physical aspect of recovery to a more holistic approach that simultaneously analyzes dynamic changes in stem biology and chemistry. This integrative approach offers new insight into an interlinked web of biological activities that prepare drought-stressed plants for successful recovery under favorable conditions. Amongst these activities, sugar metabolic pathways along with changes in xylem sap pH have been found to play a crucial role in facilitating transport recovery. This talk will encompass an overview of the current progress and discuss directions for future research in plant stress physiology.

Dynamic changes in ABA content in water-stressed *Populus nigra*: effects on carbon fixation, soluble carbohydrates and hydraulic recovery

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Key message

ABA is involved in optimizing water use efficiency and carbohydrate metabolism on a daily basis.

Drought compromises plants ability to replace transpired water vapour with water absorbed from the soil. Hydraulic and chemical signals operate in tandem to regulate systemic plant responses to drought to avoid extensive xylem dysfunction. The hormone abscisic acid (ABA) plays a major role in regulating stomatal closure and acts as a root-to-shoot signal under water deficit. *Populus nigra* seedlings were used as model systems to investigate: how hormonal and hydraulic signals contribute to optimize stomatal (g_s), mesophyll (g_m) and leaf hydraulic (K_{leaf}) conductance under well-watered (WW) and water-stressed conditions (WS); a possible role for ABA in mobilizing non-structural carbohydrates (NSC) within the leaf and stem; a possible relationship between NSC and xylem hydraulic function upon drought and re-watering. Our results demonstrate that ABA regulates the photosynthetic machinery on a daily basis in both WW and WS conditions to optimize water use efficiency (WUE). In addition, a possible relationship between ABA and NSC in the leaves and stem are reported, suggesting potential roles of this hormone in carbohydrate metabolism. We also hypothesize that the mobilization of stem carbohydrates might promote the restoration of xylem transport capacity.

Integrating metabolomic analysis into the physiological framework: possible information on the depletion of C and N storage compounds in droughted leaves

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Key message

Untargeted metabolomic profiling highlighted pools of primary metabolites affected by drought and related to physiological adjustments.

Plant responses to drought involve a complex interaction of physiological and biochemical processes. The elucidation of these processes as well as their integrative networks is critical to enhance drought tolerance in woody species. In the present study, black poplar (*Populus nigra*) plants subjected to water deficit were used as the model system to elucidate the effect of drought on leaf metabolic profile, and to investigate how the regulation of multiple biochemical pathways are involved in the control of leaf physiological homeostasis. Water stress was imposed by withholding water in order to obtain moderate- and severe-water-stressed plants, whereas well-watered plants were irrigated to pot capacity. Physiological parameters were monitored and water soluble metabolites were extracted from leaves and analyzed with NMR spectroscopy.

Metabolomic analysis highlighted pools of metabolites that affected the physiological adjustments of poplar under drought. In particolar, stress triggered changes in primary metabolism, especially increases in amino acids, tricarboxylic acids and non-structural carbohydrates, suggesting changes in resources allocation and possible mobilization of storage compounds.

These results provide a framework for better understanding multiple mechanisms in plant responses to drought. The use of metabolomic as a tool to monitor stress-responsive markers of C and N utilization is also discussed.

Soluble carbohydrates metabolism sustains energetics and xylem hydraulic functionality upon relief from drought in *Populus tremula x alba*

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Key message

Changes in NSC level in tissues and in xylem sap during drought and recovery are not only the result of an osmotic requirement, but rather a trait regulating the energetic status and the subsequent hydraulic restoration of xylem transport.

The role of non-structural carbohydrates (NSC) in tissues of woody plants during drought stress reveals different, sometimes contrasting, scenarios. A reduction of NSC level probably denotes that plant is undergoing a carbon starvation and consequently exposed to dieback. On the contrary, when soluble carbohydrates content increases, their possible role as osmotic compounds protecting from drought effects and trigging recovery is suggested. Our study focused on depicting the relationship between energetic metabolism and response to drought. Poplar trees were subjected to a drought treatment followed by a period of recovery (re-watering). Physiological measurements (water potential, growth parameters, PLC) were monitored in combination with analysis of hexose phosphate sugars, ATP and key-enzymes activity involved in glycolysis and starch-sugar metabolism. Drought treatment resulted in an alteration of carbohydrate metabolism, drawing a picture in which stem tissues (bark and wood) and xylem sap, differently responded to water stress and recovery, activating sugar oxidative pathways and using alternative sources of NSC. These mechanisms represent a natural trade-off between plant growth and energetic requirements. Our results pointed out that drought triggers an alteration of the energetic status aimed at regulating stress response and subsequent recovery.

The relationships between vulnerability to xylem embolism and stem non-structural carbohydrates (NSC) in *Populus nigra* L.

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Key message

Prolonged shading induced a decrease in stem and xylem sap NSC content and a contemporary increase in vulnerability to xylem embolism in *Populus nigra* saplings. Possible mechanistic connections between these two findings are discussed.

Recent evidence suggests that non-structural carbohydrates (NSC) are involved in the maintenance of water transport in trees, but it is unclear whether and how NSC availability may influence xylem vulnerability to embolism. *Populus nigra* L. saplings were exposed for two months to prolonged shading (S) or moderate drought stress (D). When S and D plants, as well as control well-watered plants (C) were exposed to a sudden drought of similar intensity for all groups (xylem water potentials between -1.2 and -1.7 MPa), S plants were more vulnerable to embolism formation. S plants also had lower NSC contents in bark, wood and xylem sap than C and D plants. Differences in stem xylem hydraulic conductivity as well as in xylem water potential were also recorded upon drought relief. Our findings suggest that higher stem and/or xylem sap NSC enhance the resistance to xylem embolism in the study species. NSC may be also involved in the recovery of xylem hydraulics during the recovery from drought. Different hypotheses possibly explaining these findings, such as the role of soluble carbohydrates in determining changes in xylem sap surface tension, are tested and discussed.

Genetic control over leaf vascular anatomy in Switchgrass (*Panicum virgatum*)

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Key message

The anatomical traits that characterize leaf hydraulic pathways strongly influence plant hydraulic efficiency and vulnerability. Variance in these traits is strongly controlled by underlying genetic architecture.

Leaves are the primary organ for photosynthesis and a major bottleneck in the plant hydraulic pathway. Both inside and outside the leaf xylem, hydraulic conductance and vulnerability to failure are fundamentally linked to anatomical structures. However, the genetic mechanisms driving the anatomical variance that constrains the trade-off between hydraulic safety and efficiency remain untested. We used *Panicum virgatum,* a C_4 grass for which adaptation to climate and growing season across a broad latitudinal gradient has driven divergence of resource acquisitive and conservative ecotypes, to evaluate the genetic architecture underlying leaf anatomy. We found significant genetic variance for anatomical traits across these ecotypes and used a genetic mapping population to identify quantitative trait loci (QTL) associated with vascular anatomy and patterns of trait integration. While shifts in high order traits, e.g. xylem conductance, are often hypothesized to result from many genetic changes of small effect, we found few QTL of large effect for sizes of veins and conduits in mid- and secondary veins and intervenal distance. Moreover, we found co-occurrence of cell size QTL across tissues and aim to relate these traits to leaf gas exchange. Our results show strong genetic control over ecotypic divergence in plant water use strategies.

Multidisciplinary tree ring approach to gain insights into Mediterranean plant adaptation to drought

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Key message

Mediterranean species cope with abrupt changes in climatic conditions, including summer drought, thanks to the high plasticity of their wood functional traits that allows to achieve a trade-off between the need to maintain high conductivity, when water is available, and to prevent phenomena of embolism during periods of severe drought.
Many wood traits within the tree rings (eg. size and thickness of the cell walls of the conductive elements and pits) are very sensitive to environmental variability and are modulated to contribute to the safety–efficiency trade off of water transport. Here, I analyse xylogenesis, quantify functional wood traits and differences in intrinsic water use efficiency (WUE_i) in long tree-ring series of Mediterranean species with intra-annual resolution, linking anatomical parameters with isotope signals and climate fluctuations. My results indicate consistent differences in WUE_i in the analyzed species, largely determined by leaf traits and differences in stomatal conductance control. Further I will discuss the different aptitude of the species to form Intra annual density fluctuation (IADF), considered as a strategy to adjust wood anatomical traits to short-term variations in temperature or precipitation patterns. The combination of different kinds of data offers new perspectives in the interpretation of physiological and ecological processes during wood formation.

On hydraulics and drought-associated mortality

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Key message

Strong evidence for a large role of hydraulics in drought-associated mortality of woody plants exists, yet the exact processes leading to death remain poorly known, yielding important challenges for hydraulic scientists.

A key role of plant hydraulics in woody plant mortality during drought has been theorized and subsequently validated through many experiments. However, the process details underlying drought-induced mortality remain challenging to test. Assuming that either hydraulic failure, carbon starvation, or their interaction kills plants remains premature based on the available evidence. I outline the state-of-the-knowledge regarding the role of hydraulic failure, and avoidance thereof, in drought-survival and mortality, and identify necessary next steps to enable a more thorough and accurate understanding and simulation of hydraulics and carbon metaboism in plant mortality.

Recovery of Pinus sylvestris from severe drought: Impacts on leaf gas exchange and xylem embolisms

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Key message

Both in vivo X-ray tomography and classical techniques show that Scots pine is not able to refill embolised stem xylem after a one-month recovery period from drought. The ability to recover is reduced once stem xylem embolisms occur.

Climate change increases the incidence of weather extremes with large implications for forest functioning. Scots pine (*Pinus sylvestris L.*) belongs to the most abundant conifers worldwide, but is generally susceptible to xylem embolism. To elucidate a potential refilling of xylem embolisms after drought release, we investigated the dynamics of leaf gas exchange, non-structural carbohydrate concentrations and hydraulic properties in Scots pine saplings. Irrigation was withhold until xylem water potential declined to -3.2 MPa (+/- 0.4), which indicated substantial loss of hydraulic conductivity (40-50%). Frequent measurements of xylem water potential, relative needle water content, and gas exchange were conducted. To assess the degree of embolised stem xylem we combined in vivo X-ray tomography with classical techniques (hydraulic conductivity measurements and dye staining). While xylem water potential recovered rapidly close to control values, both classical and X-ray tomography measurements revealed no recovery of hydraulic conductivity. In contrast, stomatal conductance and photosynthesis recovered to about 60% of control values. Our findings demonstrate that Scots pine is able to survive severe drought, although the ability to recover is reduced once embolism formation in stem xylem has occurred. We assume that growth of new wood in the next growing season might restore the hydraulic conductivity.

Carbon export patterns in relation to diurnal and seasonal carbon and water dynamics in red oak leaves

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Key message

Red oak trees maintain leaf carbon export throughout the day despite midday declines in leaf water potential. However, they also accumulate foliar NSCs, which contribute to diurnal turgor maintenance via osmotic adjustment.

Mature, temperate trees typically experience large depressions in water potential throughout the day. This potentially creates a problem for phloem functioning because of the interactions between xylem and phloem water dynamics. Data from the literature suggests that phloem sugar concentrations are not large enough to draw water from the xylem at midday. Therefore, we hypothesized that carbon export from the leaf into the phloem would be minimal at midday for red oak trees in Harvard Forest (Petersham, MA). To test this hypothesis, we measured diurnal and seasonal patterns of NSCs, photosynthesis, solute and water potentials, and estimated export in leaves of five red oak trees between June and September. Surprisingly, we found that export is occuring during the day (at equal or higher rates than at night), despite large depressions in water potential. Additionally, we observed that, despite the high daily export, there is an accumulation of NSCs over the course of the day (specifically, sucrose), which contributes approximately 50% to the diurnal osmotic adjustment of 5 bars. These findings illustrate the robustness of phloem functioning in the face of midday water potentials, the role of NSCs in leaf turgor maintenance, and the need to further study phloem dynamics in the context of water relations.

Xylem vulnerability to embolism in natural and transformed tropical systems in Sumatra, Indonesia

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Key message

While tropical rainforest tree species are characterized by a high diversity in branch embolism resistance influenced by tree height, oil palm monocultures have a vulnerable but highly plastic hydraulic system which response to soil water availability.

In investigations of plant sensitivity to drought, functional traits related to water transport such as the water potential at 50% loss of conductivity (P_{50}) are commonly used. While relationships between P_{50} and other plant traits are well documented for non-tropical trees, much less is known for tropical trees and palms. The present study examines several wood structural and anatomical, and hydraulic traits of oil palm (*Elaeis guineensis*) and rubber (*Hevea brasiliensis*) in monocultures, and of ten rainforest tree species in Sumatra, Indonesia. Branch embolism resistance (P_{50}) of the forest species ranged from -4.43 to -1.86 MPa, and was significantly negatively related to tree height, but not to wood density (WD). In rubber and oil palm, average P_{50} was -2.45 and -1.86 MPa, respectively. The plasticity in the hydraulic system in response to water availablity was particularly large in oil palm. The observed diversity in tree hydraulic strategies in the rainforest trees may increase the resistance and resilience of the natural forest to climatic changes. In contrast, oil palm, which widely replaces the forest, is more vulnerable to embolism. The high plasticity in the hydraulic system of oil palm may, however, reduces its vulnerability.

Water relations and hydraulic traits might explain the invasive potential of alien trees

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Key message

Higher vulnerability to drought (higher Y_{50} and Y_{tlp} values) coupled to lower leaf and wood construction costs are associated to higher productivity and invasiveness of alien tree species.

Our understanding of the ecophysiological mechanisms underlying invasion by alien tree species is still limited. The measurement of functional traits is a frequently used approach to highlight the features of successful invaders, although most previous studies have focused on traits relatively easy to measure, but often without clear mechanistic linkages with plant physiological performance. Specifically, hydraulic and water relation traits have been only seldom included in studies comparing native and invasive trees, and results are somehow contrasting. In this study, we compared several leaf and wood functional and mechanistic traits (i.e. traits clearly associated to a physiological process) in 9 native sub-mediterranean woody species and 3 invasive woody species (IAS) . IAS were more vulnerable to drought (higher Ψ_{50} and Ψ_{tlp}) than native species, and sustained lower carbon costs for leaves and wood production (lower leaf mass per unit area and wood density). Moreover, higher values of minor vein density (VLA_{min}) and leaf N content suggested that IAS had higher leaf water transport efficiency and higher photosynthetic rates than natives. Overall, our analysis suggested that mechanistic traits such as Ψ_{50}, Ψ_{tlp} and VLA_{min} might play a central role in determining the competitive advantage of IAS over native trees, providing stronger mechanistic linkages between drought vulnerability, construction costs, photosynthetic and growth rates.

To everything there is a season: seasonal hydraulic plasticity in grapevines

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Key message

Throuhgout the season grape leaves increase their osmotic content and resistance to embolism to maintain a constant safety margin.

Deciduous plants in Mediterranean climate normally budbreak when water is abundant and gradually deplete the soil water content toward fruit ripening. We hypothesized that leaf hydraulics correspondingly acclimates to low water availability conditions.

We measured osmotic content, xylem architecture and vulnerability to cavitation in a Cabarnet Sauvignon vineyard along the season in three degrees of water treatment: super well-watered (several irrigations every day), mild water deficit, and severe water deficit.

We found that during the season plants gradually accumulate osmolites and operate at lower water potential. Accordingly, leaves that emerged later in the season had smaller vessel and exhibited lower vulnerability to cavitation. Surprisingly, the super well-watered treatment that haven't suffered from any water shortage have also shifted their hydraulic parameters along the season, suggesting that changes could be driven by atmospheric differences or pre-programed development.

Winter embolism formation – new insights from freezing experiments

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Key message

Gas bursts, acoustic emissions and formation of gaseous volumes of various sizes were detected in xylem during ice propagation. This suggests that cavitation events would occur also during the freezing process.

Freezing can induce winter embolism, which affects the plants' hydraulic transport system. Winter embolism occurs when gases dissolved in xylem sap form bubbles during freezing. Upon thawing, a gas bubble can either expand and fill the conduit with air or collapse and redissolve, depending on its size and the tension in the surrounding xylem sap. In recent measurements, we have found that part of the gases dissolved in xylem sap are not trapped in ice but pushed out from the xylem during ice propagation. Peaks of acoustic emissions in the xylem were detected contemporaneously with gas. High resolution X-ray images revealed that not only spheric bubbles, but also gaseous volumes of various shapes and sizes are found from the frozen xylem sap. We hypothesize that winter embolism is induced i) via bubble trapping during ice propagation, leading to embolism on thawing if tension of the sap is high and/or bubbles are large, and ii) via cavitation of xylem sap prior to freezing due to low water potential at the ice–liquid interface, which locally increases tension in the sap inducing acoustic emissions. Gas bursts may be a result of volumetric expansion of water in xylem conduits due to water-ice transition and reduce the xylem gas content.

Post-fire effect on leaf development, radial growth and anatomy of secondary vascular tissues in *Quercus pubescens*

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Key message

Post-fire structural adjustments of xylem and phloem in *Quercus pubescens* are not coordinated; greater changes were found in the phloem conduit size and increment structure.

The increased frequency of fire events in the Slovenian Karst has been observed in the last two decades, therefore we studied the post-fire effect on leaf development, leaf carbon isotope composition (δ13C), radial growth patterns, and xylem and phloem anatomy of *Q. pubescens*, a dominant tree species in this region. We selected undamaged (H-trees) and fire-damaged trees (F-trees) in spring 2017, a growing season after a forest fire in August 2016. Leaves of F-trees showed higher LAI and more negative δ13C values than of H-trees. Cambial production stopped 3 weeks later in F-trees, resulting in 60% and 22% wider xylem and phloem increments, respectively. The study provides an evidence that exposure of *Q. pubescens* to fire is traceable in xylem and phloem anatomies in the next growing season. However, post-fire structural adjustments of the two vascular tissues were not coordinated; fire caused changes in conduit properties in the phloem but not in the xylem. In addition, late phloem was considerably wider in F-trees, suggesting that wider late phloem rich in axial parenchyma is likely to compensate for used reserves for resprouting following the fire. Nevertheless, the restoration of long-distance transport in phloem is crucial for tree survival.

POSTERS

SESSION 1: METHODS AND CONCEPTS

Application of pneumatic method to build vulnerability curves in solitary vesseled trees: Assessement of vulnerability to cavitation of three *Eucalyptus* species

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Forestry species should exhibit both high survival and fast growth rate. From this point of view, *Eucalyptus* is an interesting genus since it inhabits a wide diversity of climatic and environmental conditions and covers more than 20 million planted hectares worldwide. This genus presents solitary vessels, i.e. not directly connected to other vessels but surrounded by different imperforate tracheary elements and parenchyma that participate in a less known form in xylem safety and hydraulic efficiency. Thus, we carried out an experiment to check out whether a recently issued pneumatic device is relevant for establishing vulnerability curves for long or solitary vesseled plant-species, mainly in *E. camaldulensis*, *E. globulus* and *E. viminalis*. We obtained “S-shaped” curves and the results were similar to data already published. Moreover, we showed that there was no significant intraspecific difference between local *E. globulus* provenances in their water potential inducing 50% loss of hydraulic conductivity (Y_{50}). Considering the vulnerability to cavitation of these species, it came out that *E. camaldulensis* (-3.98 MPa) and *E. viminalis* (-4.26 MPa) were similar and that *E. globulus* (-4.60 MPa) was slightly more resistant than the previous ones ($P < 0.0001$). We concluded that the method was suitable for these species.

Quantifying plant water uptake: Application of the Soil Water Profiler

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Regulation of plant water balance involves highly interacting processes at leaf, stem and root level. To analyze functioning of the hydraulic system as a whole, quantitative data from all these levels are required. Especially measurement of root water uptake (RWU) is still challenging in terms of invasivity, spatial and temporal resolution or realistic plant growing conditions. We introduce the soil water profiler (SWaP), a user-friendly sensor for non-invasive soil water detection with 1 cm resolution in depth. Due to a precision of 3 µl, RWU rates resulting in soil water depletions can be quantified, even at early plant stages. We stimulate plant transpiration by precise modulation of light intensity via a custom made LED panel and present the resulting pattern of RWU rates measured with the SWaP. The depth profile of RWU rates is then related to the distribution of significant root system traits such as root length density which are derived by magnetic resonance imaging (MRI). Finally, we show data on the kinetics and interaction of stomatal conductance and RWU, measured with high temporal resolution after light stimulation. Here, we focus on the relation between stomatal sensitivity and RWU among different plant species.

Key message: We present different applications of the soil water profiler which dynamically measures root water uptake of plants in pots with high precision and spatial resolution.

Tree dieback in the Mediterranean region: a new monitoring system

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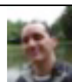
Mediterranean forests are subject to prolonged droughts (Gitlin et al., 2006): the lack of water entails in the plant's traumatic events (cavitation, carbon starvation, biotic attacks) that can lead to tree mortality (McDowell et al., 2008).

An increasing number of ecophysiological studies are currently trying to understand the responses of plants to water stress (eg Allen et al in 2010, Linares et al in 2010, Klein in 2015). However, most of these researches are based on a limited number of trees, sites and a reduced amount of time. Today, thanks to the development of a particular multi-sensor device called TreeTalker, able to continuously record multiple physiological functions of the tree and changes in the surrounding environmental conditions, it is possible to implement the necessary monitoring activity with extremely low costs and with a not obtainable resolution through the use of other remote sensing technologies.

Our study aims to monitor with the Tree Talker system, a large population of *Quercus Ilex* L. and *Pinus pinaster* Aiton population of Campania region, frequently exposed to climate-induced water stress and tree mortality. This system will be garantee real time biological monitoring of individual plant functions and will be able to relate, from sub-hourly to daily, seasonal and inter-annual frequency, the causal dependency of climate events with tree mortality and growth responses.

The Pneumatron: an automated pneumatic apparatus for estimating xylem embolism resistance at high resolution



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Xylem vulnerability to embolism represents an important functional trait to determine species distribution patterns and resistance to drought stress. Measuring xylem embolism resistance, however, generally relies on time-consuming measurements in the lab which may suffer from artefacts. Based on a recently developed pneumatic method, here we present and test the "Pneumatron", a device that generates high time-resolution and fully automated vulnerability curves when associated with a stem psychrometer. Validation of this device was performed by comparing Pneumatron measurements with two alternative methods. The Pneumatron greatly improved precision of the pneumatic data, detecting differences of the percent of air discharged (PAD) below 0.47%. It also allowed almost direct measurement of 50% PAD while embolism is induced by dehydration, similar to the bench dehydration method. We also described the Multiple-Pneumatron device, which can measure several branches at the same time. The Pneumatron is based on an open source platform and is therefore a low-cost and powerful tool that can significantly improve our understanding of plant water relations and the mechanisms behind embolism.

Key message: We present the "Pneumatron", a device that generates high time-resolution and fully automated vulnerability curves based on pneumatic method. It is a low-cost tool that uses an open source platform.

Post-harvest acoustic emission in angiosperm leaves and its relation to anatomy

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Knowledge about drought sensitivity of tree species is of high importance facing global change. Water is transported in a metastable state and the water columns are likely to break when drought stress increases above a certain level. Stress release after cavitation produces ultrasonic acoustic emission (AE). This process would result in high conductivity loss if trees would not react by closing their stomata. In that regard, leaves play a major role for tree survival because the hydraulic sensitivity to drought depends on timely closure of their stomata. Recent studies reveal that the water conducting system of leaves is less vulnerable than previously thought; a high proportion of leaf veins lose water merely after leaf wilting. In the present study, the AE hit rate and AE features of dehydrating angiosperm leaves of seven temperate angiosperm species were related to anatomical features of the leaf veins. Three AE clusters were detected, where the first showed the highest peak amplitudes. The cluster with the highest amplitudes comes presumably from cavitation in the midrib veins, whereas the following peaks were attributed to leaf wilting and cavitation in the minor leaf veins. The results are discussed regarding the species specific reaction to drought stress.

Key message: Ultrasonic acoustic emission rate clusters give information on distinctions in the hydraulic vulnerability of leaf veins.

The potential of Mid-Infrared spectroscopy for prediction of wood density and vulnerability to embolism in angiosperms

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Extreme climatic events resulted in increased tree dieback and forest mortality worldwide. Xylem resistance to embolism formation (P50) determines the species drought tolerance. We tested the use of Fourier Transform-Infrared (FTIR) spectroscopy as a method for estimating wood density (WD) and P50 in angiosperms. The studied species displayed different hydraulic resistance to drought stress, with higher WD correlated to more negative P50. Partial least squares regression applied to the models of spectra and reference data, showed a high predictive quality for WD, whereas the prediction of P50 was weaker. By including WD in the model as an additional factor influencing P50, its predictive power significantly increased. The wavenumber range didn't influence the model descriptors when the spectral range was narrowed (lignin, cellulose, hemicellulose), suggesting that for P50 prediction a broad spectral range is more informative than narrow regions reflecting spetial wood constituents. FTIR spectroscopy associated with WD measurements has proven to be a promising tool for screening of individual- or species-specific resistance to embolism.

Key message: FTIR spectroscopy associated with wood density measurements is a promising alternative to traditional methods for screening of individual- or species specific resistance to embolism in angiosperms.

SESSION 2: FROM XYLEM STRUCTURES TO HYDRAULIC PROPERTIES

Investigating Embolism Resistance in Maize and its Wild Relatives

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Maize (*Zea mays*) is one of the most important crops worldwide and is predominately non-irrigated. Water availability is at risk, with future predictions suggesting increased drought frequency and intensity through changing rainfall patterns and rising temperatures. Understanding the physiological processes of drought tolerance in maize is key to genetic selection and breeding of tolerant varieties. The resistance of embolism formation in the water conducting xylem is a critical threshold for plant survival under drought, as the integrity of the water column drives growth and productivity. Maize germplasm native to Mexico, the center of origin and domestication, span semiarid and humid agroclimatic environments, representing the plasticity of this crop to adapt across rainfall and soil moisture conditions. This project aims to characterize embolism resistance across the diversity of maize varieties using the nonintrusive optical vulnerability method. Other physiological responses to water stress including leaf ABA synthesis, timing of stomatal closure, and minimum stomatal conductance will be examined in closely related Teosinte grasses within *Zea* and the sister genus *Tripsacum.* Investigating the diversity within Tripsacinae offers insights into the selection of key drought tolerance traits during evolution, with the aim of improving maize to a changing climate.

Woody tissues and vulnerability to xylem embolism in angiosperms

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Plants frequently operate at leaf water potentials close to critical thresholds of conductivity loss by embolism formation. Therefore, it is expected that they evolved mechanisms either to limit embolism formation and spread, or to restore the hydraulic functionality. Species less vulnerable to embolism formation are commonly reported to have a higher wood density (*WD*). Nevertheless, the functional meaning of this relationship is still uncertain. We measured *P50*, *WD*, and the percentage of tissues composing wood (parenchyma cells, PA_{TOT}; vessel area, *VA*; fibre area, *FA*) on branches of angiosperm species.

A dataset of published data on anatomical and hydraulic traits, including information on non-structural carbohydrates content (*NSC*), was also compiled.

Less vulnerable species (i.e. more negative *P50*) had higher *WD*. This relationship was driven by either PA_{TOT} or *FA*, which were both significantly related to *P50* and inversely related to each other.

While remains uncertain why the amount of fibres relate to *P50*, the relationship between PA_{TOT} and *P50* would support the hypothesis that parenchyma has a fundamental role in embolism recovery, thus providing a functional explanation to the relationship between *WD* and *P50* in angiosperms.

Key message: In Angiosperms the relationship between *WD* and *P50* is driven by the amount of parenchyma, possibly having a key role in embolism recovery.

Implications of xylem network organization on the spread of *Xylella fatidiosa*

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Xylella fastidiosa (Xf) is a xylem-dwelling bacterium and the causal agent of a wide range of plant diseases, including grapevine, olive, and citrus. Once the Xf are introduced into the xylem, they spread systemically. This spread has been traditiolnaly associated with water transport dysfunction resulting in significant losses in crop productivity and plant death. This is because the xylem tissue is a network of pipe-like cells transporting water in a range of different anatomical pathways: axially within conduits and radially through the connections between these conduits. The radial movement is a critical factor in maintaining water transport by creating multiple pathways. However, greater xylem radial connectivity also increases the vulnerability to the spread of air-embolism and pathogens. Our objective is to understand the relationship between xylem network and Xf spread. We applied microCT imaging to identify and quantify xylem connections in diferent grapevines. *Vitis arizonica* (Xf-resistant) presented less vessel connections than the vinifera varieties, but the pit membranes air-seeding treshold pressure were not significantly different between *V. arizonica* -2.36 MPa (±1.41), Chardonnay -1.68MPa (±1.40), and Lenoir -2.10 MPa (±1.08). These data open avenues for future research in wood anatomy by providing detailed cellular ultrastructure organization of grapevine with different levels of Xf-tolerance.

Key message: Grapevine xylem network organization (radial connectivity) could influence on Xylella fatidiosa disease's tolerance.

Intra-annual dynamics of vessel formation and water fluxes in beech trees

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Plant stomata respond to climatic conditions, regulating sap flow and optimizing water loss relative to carbon gain. Xylem hydraulic conductivity (k_h) highly depends on the size and number of conduits per unit area. Therefore, even though the rate of water flux through and from the tree is largely determined by transpiration rates, it is also constrained by the xylem's anatomical features. However, much is still unknown about how trees adjust xylem anatomy to meet the canopy's transpiration needs. We analysed the intra-annual dynamics of transpiration and xylem formation in *Fagus sylvatica* L. during the period 2015-2018 using wood-formation-monitoring techniques, sap-flow sensors and an eddy covariance flux tower installed in a mature beech forest at Hesse (northeastern France). Vessels started to be potentially hydraulically active in mid-May and the maximal theoretical k_h was reached in mid- to late-July. Canopy transpiration (E_c) and sap flow started to increase in mid-April, thus relying on previous-year vessels for water transport, and peaked in June, before maximum current-year ring k_h was achieved. The inter-annual variation in maximum k_h and E_c were also unrelated. This uncoupling between vessel formation and transpiration dynamics may therefore indicate that xylogenesis processes in beech may not respond to current-year canopy transpiration needs.

Key message: Intra-annual dynamics of transpiration and xylem formation in beech are uncoupled, which could indicate that xylogenesis processes may not respond to current-year canopy transpiration needs.

Hydraulic patterns in the temperate liana *Hedera helix*

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Non self-supporting plants form long and flexible but disproportionally narrow stems and thus require particular strategies to keep the integrity of xylem water transport and ensure supply of large crown areas. It is largely unexplored if this requirements result in a particular within-plant coordination of hydraulic traits.

We show that in *Hedera helix*, a widespread and ecologically relevant liana in European temperate forests, high transport capacities allow to compensate for the narrow main stem, while resistance to drought-induced xylem dysfunction is limited and linked to conservative stomatal regulation. Main stems are significantly less embolism resistant but exhibit higher hydraulic conductivity compared to branches. In contrast, differences between juvenile and adult life phase were small. In branches, the cell turgor loss point of leaves decreased, while the xylem´s embolism resistance and conductivity as well as conduit diameters increased with height.

Patterns indicate a complex hydraulic architecture which is surprisingly similar to self-supporting trees and seems to be coordinated to comply with the higher evaporative demand of leaves in the upper canopy. The study highlights the necessity of studying whole-plant hydraulic architecture including varying growth forms to better understand the hydraulics of tall plants and underlying functional trade-offs.

Key message: Common Ivy ensures water supply of the crown by a well-balanced internal coordination of hydraulic efficiency and safety, with pronounced differences between main stem and branches and along the vertical axis.

Mechanisms of recovery from drought stress in *Eucalyptus saligna*: refilling or growth of new xylem?

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Xylem cavitation resistance sets critical thresholds of drought survivorship and is a key trait determining the distribution of plant species with regards to water availability and aridity. Recovery after drought is a crucial aspect of plant survival, but the mechanisms by which plants can restore hydraulic capacity are not well understood. Embolism repair and xylem growth have been proposed as the main mechanisms by which plants recover hydraulic capacity after drought stress. Embolism repair has been reported under positive and negative pressures, although refilling under tension is difficult to explain biophysically and the subject remains controversial. Additionally, many previous experiments have relied on indirect or destructive measurement techniques that may cause artefacts.

In this study, we used a non-invasive imaging technique (micro-computed tomography) to observe embolism dynamics in *Eucalyptus saligna* through a cycle of drought stress and rewatering. Changes in whole plant transpiration and leaf area were also monitored. We found that under medium and severe drought (30 to 80% of loss of conductivity), plants survived and restored hydraulic conductivity by growing new xylem tissue. No evidence of xylem refilling was observed in these plants over time periods ranging from 24 hrs to 6 months after rewatering.

Key message: We used micro CT to examine whether embolism repair occurs after a cycle of drought and rewatering. There was no evidence of xylem refilling during rehydration. Rather, plants recovered hydraulic capacity by growth of new xylem tissue.

The size matters: drought stress experiments with potted vines

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Several experiments studying water stress on grapevines was performed in pots. Although a pot effect (constrain) on plant growth and development has long been recognized, this confounding effect has generally been ignored. Here we aimed to study the differences on performing drought experiments on two different pot sizes.

Grapevines in small (7-L, S) or big (20-L, B) pots were subjected to drought stress treatments and recovery (re-watering). We monitored water status (g_s, Ψ_s) and photosynthetic rate (A_N) throughout the drought treatment and recovery phase. At the beginning of the drought, analyses of stem and petiole xylem anatomy, and total leaf (LA) area were performed.

Drought led to a similar stomatal closure and reduction of A_N in both pot categories. Data highlighted slightly different hydraulic strategies in S and B plants under drought, but significant differences after re-watering. S plants efficiently recovered after irrigation, while B plants were unable to fully recover g_s and A_N. Considering that LA was not different in drought-stressed S and B vines, the better recovery ability in S arise from a more conservative use of water and carbon, as they developed a more efficient water transport pathway (higher xylem hydraulic conductivity) beneficial during the recovery phase.

Key message: The size of the pot used for drought experiments in grapevines influence the xylem architecture and therefore the results obtained.

Linking wood anatomy with growth vigour and susceptibility to alternate bearing in composite apple and pear trees

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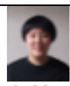
Composite fruit trees exhibit different growth vigour (GV) and fruit bearing characteristics. However, mechanisms underlying these processes are not fully understood. We examined if differences in GV and susceptibility to alternate bearing (AB) found across different scion/rootstock combinations of apple and pear trees are associated with vascular anatomy, namely anatomical traits related to water transport and nutrient storage. Roots of pear trees had greater hydraulic conductivity compared to apple trees mainly due to greater xylem proportions and vessel lumen fractions. Greater transport capacity was at the expense of carbohydrate storage because pear tree roots contained lower proportions of total parenchyma and less starch. We observed a negative correlation between proportions of total parenchyma in shoots and the AB index, suggesting that lower carbohydrate storage might be associated with increased susceptibility to AB. Furthermore, there was a clear tendency for greater hydraulic capacity in vigorously growing than in semi-dwarf trees, resulting in a positive correlation between mean vessel diameter of roots and annual shoot length. Vigorously growing trees also maintained less negative midday leaf water potentials and were less susceptible to drought-associated growth decline. Our findings shed more light on the controls of GV and AB in commercially important fruit trees.

Key message: In apple and pear trees, xylem transport capacity of rootstocks controls growth vigour while total carbohydrate storage capacity of shoots appears to be linked with susceptibility to alternate bearing.

The formation of earlywood vessels in deciduous ring-porous hardwoods in earlyspring



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Formation of earlywood vessels is crucial for growth of deciduous ring-porous hardwood trees, because earlywood vessles are the main pathways of water conduciton and they lose that function after only one year. The purpose of this study is to reveal the process in the formation of networks of earlywood vessels. We observed the process of diffrerentiation of vessel elements at different height positions of the stem along with leaf phenology and water distribution in the stem using light microscopy and cryo-SEM. Fomation of earlywood vessels started before bud swelling. At that time, the previous year's latewood vessels were filled with water. When bud growth was observed, the functional earlywood vessels were obsevred eariler at the top of the tree, however they were not observed at the middle and lower positions of the same stem. These results indicate that earlywood vessels start water conduction from top of the tree and the water supply from the networks in the previous year's xylem might be important for the start of water conduction via the networks of the current year's earlywood vessels.

Key message: In ring-porous hardwood trees, earlywood vessels became functional eariler near the top of the tree during bud break.

Ultrasonic acoustic emissions: daily courses in the xylem of mature *Picea abies*

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Ultrasonic acoustic emission (UAE) analysis is used to nondestructively monitor xylem embolism and estimate vulnerability to drought. UAE analysis normally is a laboratory-based method, while studies under field conditions are rare.

We thus monitored daily patterns of UAE in trunks of mature *Picea abies* trees growing in a mixed forest under drought (5-year-long through-fall exclusion; KROOF) and normal conditions (August 2018, continuous measurements over 3 days). UAE were also registered at different heights (from root to tip/branches) of a 33-m-tall specimen.

Similar daily courses were observed in all trees under study and within a tree. UAE activity started at around 8:00, showed its maximum intensities between 10:00 and 17:00 and decreased around 18:00. During the night, UAE activity was extremely low. Highest UAE activity was observed during the first day of observation with higher temperatures and lowest air humidity. UAE was higher in drought-stressed specimens than in control trees.

Higher UAE activity under drought compared to controls, on warm and dry days and during daytime indicate UAEs to be related to low xylem water potential. It is remarkable that UAEs similarly occur along the entire axes system but it remains to be analysed if their origin is embolism formation.

Key message: Ultrasonic acoustic emission activity was higher in drought-stressed than in control trees and exhibited daily courses in the xylem of main stem and branches.

High vulnerability to embolism in citrus species

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In commercial orchards, citrus trees combine two species in one plant. While the shoots (scions) are exposed to atmospheric conditions, the roots (rootstocks) are influenced by soil conditions. Interestingly, rootstocks modulate several shoot traits, such as leaf gas exchange and fruit yield. To date, there are no reports about how rootstocks affect shoot vulnerability to embolism or how the vulnerability to embolism differ among rootstock species. Herein, we evaluated the vulnerability to embolism in Valencia sweet orange scions grafted on Rangpur lime, Swingle citrumelo and Sunki mandarin rootstocks, considering both shoots and roots and using the pneumatic method. While P50 varied between -0.61±0.03 MPa (Swingle citrumelo) and -1.24±0.08 MPa (Sunki mandarin) among rootstocks, Valencia sweet orange shoots did not show significant variation due to rootstocks (-0.99±0.03 MPa, pooled data). As comparing to literature, our P50 estimates suggest high vulnerability to embolism in citrus species, regardless rootstocks and scions. This would justify leaf abscision around -2.8 MPa found in field-grown trees during the winter season in subtropical conditions.

Key message: Citrus species show high vulnerability to embolism in both roots and shoots. Citrus rootstocks did not affect the vulnerability to embolism in shoots of grafted plants.

Axial vessel traits variation in *Sedum rubrotinctum*

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The efficiency in moving water along stems is of key importance for plants. The efficiency of water uplift is due to the basipetal widening of conduits allowing constant hydraulic resistance while the pathway length increases with plant axial growth. Succulent plants evolved water storage tissue to keep their physiological activity during drought. Here we show a preliminary investigation of the axial variation of anatomical xylem traits in Sedum rubrotinctum, a leaf succulent. We investigated the axial vessel size variation and vessels cell-wall thickness in both primary and secondary xylem. We also quantified vessels grouping and within-group vessels number to parenchyma cell ratio. Our results show that primary vessels widen according to universal hydraulic model, while secondary vessels do not widen. We found a coherent variation of the within-group vessels number to associated parenchyma cells ratio. We conclude that the primary vessels are the only one providing water to leaves, while secondary vessels are not efficient in water transport and act as a water reservoir and contribute to plant mechanical support.

Key message: In Sedum rubrotinctorum primary vessels widen basipetally, while the thick-walled secondary vessels are cilindrical, suggesting only primary xylem provides water to the succulent leaves.

Upscaling plant anatomical traits to organ scale vulnerability curves

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Plant xylem response to drought is routinely represented by a vulnerability to embolism curve (VC). Despite the significance of VCs, the connection between anatomy and tissue-level hydraulic response to drought remains a subject of inquiry. A numerical model of water flow in diffuse-porous trees is presented that combines current knowledge on wood anatomy and embolism spread. The model produces xylem networks and uses mechanistic parameterizations of intervessel connection vulnerability to embolism spread: the Young–Laplace equation and pit membrane stretching. Its purpose is upscaling processes occurring on the microscopic length scales, such as embolism propagation through pit membranes, to obtain tissue- scale hydraulics. Branch VC was successfully reproduced based on measurements of xylem tissue anatomy, and the linkage between pit-scale and vessel-scale anatomical characters, along with xylem network topology shown to affect VCs significantly. The model is then complemented with an analytical upscaling exercise based on traits, geometry, and the air-seeding hypothesis so as to provide a novel perspective on controversies arising from VC shape.

Key message: It is possible to elevate the subject of plant hydraulics from a predominently experimental one by using a combination of analytical techniques based on probability and traits and numerical techniques. This makes the subject more amenable to hypothesis generation and testing.

Preliminary results on the relationship between xylem vessel formation dynamic and sap flow pattern across different forest tree species

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Effects of water regime on xylem anatomy are known to be imprinted in the resulting annual tree-ring. Despite recent studies bring new insights on the impact of climatic parameters on vessel development, underlying mechanisms driving the acclimation of wood formation processes to intra-annual changes in the water regime remain unclear. In addition, responses of vessel differentiation to water availability may change according to species and timing of water stress.

Sap flow is a physiological trait reflecting plant hydraulic functioning (transpiration, stomatal conductance) in regards to the environmental conditions (VPD, soil water potential). Cornerstone of tree water use, sap flow pattern appears to be a good candidate to explore how water regime impacts xylogenesis.

Here, we conducted an exploratory experiment to find potential external and internal drivers of xylem vessel formation under natural conditions. We focused on the coordination between intra-annual sap flow patterns and vessel development in three broadleaf tree species (*Quercus robur*, *Fraxinus angustifolia,* and *Fagus sylvatica*) showing contrasting water use strategies and wood structure (ring-porous vs. diffuse-porous).

Key message: Xylem vessel formation was investigated in relation to sap flow pattern. The main objective was to access how water regime impacts the dynamic of xylogenesis and vessel size in three contrasting broadleaf tree species.

Water potential distribution in aboveground part of sessile oak seedlings

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The flow of water through the plant's xylem is driven by water potential gradients. If the leaves and roots of both seedlings and older trees reach similar water potentials, the gradient of water potential should be by order higher in seedlings, because of their lower height. Considering the Ohm's law, it is interesting how the xylem resistance and water potential gradients are distributed along the seedlings body and how is it reflected in xylem anatomy. Measurements of transpiration, water potential, hydraulic resistance, and anatomy were performed on petioles and shoots of 3-years-old sessile oak seedlings (*Quercus petraea* (Matt.) Liebl.) and branches of 15-years-old trees. Maximum transpiration rate and minimum leaf water potential were similar in seedlings and older trees. The petiole anatomy of both seedlings and older branches was similar, suggesting no difference in water potential gradients in petioles. The total water potential gradient between petiole and shoot base was only slightly higher in seedlings than in branches of older trees, suggesting that higher hydraulic resistance resides in different organs of seedlings. In accordance with the segmentation hypothesis, the higher hydraulic resistances were observed in junctions of petioles as well as between shoots of different orders, which could serve as "safety valves" during drought.

Key message: Although seedlings have to withstand higher water potential gradients, leaf anatomy of seedlings was similar to that of older trees.

Hydraulic traits of *Juniperus communis* L. along an elevational and European transect

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Plant hydraulics play an important role in determining plant distribution and performance, by influencing their growth and productivity. Knowledge of the hydraulic amplitude and plasticity of species is thus a prerequisite for estimating future performance under climate change. We investigated hydraulic safety and efficiency in *Juniperus communis* L. to estimate its intra-specific hydraulic variability.

We analysed plants growing along an elevational transect (700-2000 m a.s.l., Tyrol, Austria) and plants grown in a common garden experiment from seeds collected in various European regions (France, Austria, Ireland, Germany and Sweden). Vulnerability to drought-induced embolism (i.e. hydraulic safety) was assessed via Cavitron and ultrasonic acoustic emission techniques while specific hydraulic conductivity (i.e. hydraulic efficiency) was measured with a flow meter.

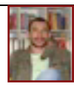
Hydraulic safety (water potentials inducing 12, 50 and 88% loss of conductivity) and efficiency did not differ significantly neither across elevations nor between European provenancies.

Common juniper proved to a be a species with high resistance to drouht stress and showed surprisingly homogenous hydraulic traits, despite sub-species are formed at higher elevation and plant morphology differed widely across provenancies. Due to its overall high hydraulic safety, this species can be considered as less susceptible to the effects of a warmer climate.

Key message: Hydraulic safety and efficiency of Common juniper were compared across elevations and European provenancies. All traits under study were similar indicating small hydraulic variability.

SESSION 3: EXTREMITIES OF THE HYDRAULIC PATH, SEGMENTATION AND DROUGHT RECOVERY

The cuticle takes over after stomata closure, but what does happen when it gets hot?



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Drought-stressed plants close their stomata to minimise water loss. Under such condition, the water permeability of the cuticle determines the minimum and inevitable water loss to the surrounding atmosphere. It has been proposed that, during heatwaves, excessive cuticular water loss and high leaf-to-air vapour pressure may lead to sudden xylem cavitation. Therefore, the efficient control of water loss at high temperatures is of fundamental importance for maintaining xylem hydraulic safety. We investigated whether the efficacy of the cuticular transpiration barrier and its susceptibility to thermal damage are ecophysiological traits differentially developed in the water-spender *Citrullus colocynthis* and the water-saver *Phoenix dactylifera*. We hypothesise that water-saver, as compared with water-spender, plants in hot deserts should have (i) a cuticular transpiration barrier with a lower water permeability and consequently a higher efficacy in reducing transpiration when stomata are closed, and (ii) a cuticular transpiration barrier that is less susceptible to adverse thermal effects. The minimum leaf conductance (g_{min}), as a proxy for cuticular permeability, was measured within the range of ecologically relevant temperatures. Also, we evaluated the qualitative and quantitative compositions of the cuticular waxes and cutin chromatographically. The melting behaviour of the cuticular waxes was investigated by Fourier transform infrared spectroscopy.

Key message: The cuticle of *P. dactylifera* (i) more efficiently reduces water loss, and (ii) does not experience thermal damage even at temperatures up to 50°C. Particular wax and cutin chemistry prevents the rise of its gmin at high temperatures.

Unravelling foliar water uptake mechanisms and physiological significance

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1 University of California Davis

Foliar water uptake, i.e. the absorption of liquid water by leaf surfaces, is a widely spread phenomenon across plants lineages nevertheless it has been overlooked for centuries. As such, the mechanisms and biological significance of foliar water uptake are poorly understood. To gain insight into this process, we carried out a series of experiments on intact leaves and whole plants. We recently developed a methodological framework that allows for the quantification of basic hydraulic parameters, including resistance to rehydration via the surface of fog-treated leaves. We applied this framework to evaluate the differential contribution of the cuticle and stomata to foliar water uptake. Preliminary results show that stomata can significantly contribute to water uptake and leaf rehydration in almond and pear. In another study, we found that foliar water uptake can recover hydraulic conductance of drought-stressed almond leaves and enhances whole-plant physiological recovery in terms of increased water potential, stomatal conductance and photosynthetic assimilation, and of decreased leaf abscisic acid content. This suggests that foliar water uptake may play a critical role in sustaining plant physiological function. Hence, more research efforts should be devoted to better understand this phenomenon, especially under the current global change scenarios.

Key message: The absorption of water via the leaf cuticle and, potentially, stomata enhances physiological recovery from drought in almond plants.

Capacitance in Clusia; Dissecting two succulent adaptaions in the leaves of tropical trees

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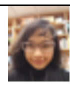
Leaf suculence is an adaptation thought to confer substantial hydraulic capacitance, thereby buffering plants against water loss. Intriguingly, in the genus *Clusia*, thicker, more visually succulent leaves do not always have higher capacitance values. To understand the basis for this apparent anomaly, we investigated different adaptations that contibute to the succulent syndrome. These included 'all-cell' and 'water-storage' succulence; the former describing larger photosynthetic chlorenchyma cells whilst the latter represents specialised, non-photosynthetic water storage tissue called hydrenchyma. 'All-cell' succulence is often accompanied by Crassulacean acid metabolism (CAM), a water-conserving mode of photosynthesis that allows stomata to open at night and close during the day. *Clusia* contains species with varying degrees of 'all-cell' succulence accompanied by changes to photosynthetic physiology. In addition, there is a great deal of diversity in hydrenchyma depth, which is independent of chlorenchyma size and CAM. Consequently, we investigated which succulent adaptation provides greater capacitance to leaves. Pressure-volume curves, and parallel anatomical measurments, determined that hydrenchyma contribute substantially more to capacitance than swollen chlorenchyma cells associated with CAM. Dissecting the two tissue layers indicated that this was due to hydrenchyma having higher saturated water content, which allows this tissue to deflate and buffer the chlorenchyma against water losses.

Key message: Specialised hydrenchyma tissue confers greater hydraulic capacitance to leaves than large, succulent chlorenchyma cells associated with CAM photosynthesis.

How do plants create openings in their epidermis?



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The stomatal mechanism opens and closes pores in the epidermis to allow CO2 uptake and prevent excessive water loss. In angiosperms, epidermal cells are thought to have a mechanical advantage over guard cells, such that changes in epidermal cell turgor can influence stomatal apertures and even lead to "wrong-way" responses. Yet in only a few species have the mechanical interactions between guard cells and the neighboring epidermal cells been examined. In this project we visualized the 3D structure of guard cells and adjacent epidermal cells of leaves flash frozen in liquid nitrogen under sunlight or dark conditions. Samples were imaged using a confocal microscope after freeze subsitution and fixation with 4% glutaraldehyde to reveal the three dimensional architecture of the stomatal complex (guard cells and neighboring epidermal cells).

Key message: 3D imaging of the stomatal complex captured under operational conditions provides insight into how changes in epidermal cell volume contribute to stomatal aperture.

Hydraulic response of European beech, Norway spruce and Douglas fir during an exceptionally dry summer

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Central European forests are increasingly affected by severe drought events, highlighting the need for adaptive forest management strategies. Norway Spruce, the commercially most important but potentially drought-sensitive species, might widely be replaced by Douglas fir in future in production forests. To confirm the species' higher drought-stress resistance, branch hydraulic and foliar characteristics of mature trees of the two conifers were related to stomatal regulation patterns, leaf water potential and sap flux density during the extreme summer drought of 2018, and compared to European beech. Although the three species differed in branch specific hydraulic conductivity, no species differences in embolism resistance and turgor loss point were observed. Douglas fir had the smallest hydraulic safety margin. Unexpectedly, sap flow in Douglas fir reached zero flux already in early August, while flux continuously declined in beech and spruce until mid of September. In beech, this was accompanied by a steady leaf water potential decline, indicating an anisohydric stomatal control strategy, while potentials remained constant at either high (spruce) or low (Douglas fir) level in the conifers. Stomatal conductance was sensitively regulated in response to air humidity in beech and spruce, but not in Douglas fir, which showed signs of an isohydric behaviour. This raises questions about the drought tolerance of this species.

Key message: In a field study on Douglas fir, Norway spruce and European beech, all three species did not differ in embolism resistance or turgor loss point, but beech followed an anisohydric and the two conifers an isohydric stomatal control strategy.

Embolism resistance in herbaceous and woody accessions of *Arabidopsis thaliana*

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The formation and spread of drought-induced embolism formation throughout the 3D vessel network could eventually lead to a lethal level hydraulic failure, resulting in branch sacrifice or even plant death. Stems of derived woody species have been shown to be more resistant to embolism formation compared to the stems of their herbaceous relatives, suggesting a functional link between increased woodiness and increased drought stress resistance in various lineages. In this study, the xylem vulnerability of stems and leaves in two woody *Arabidopsis mutants* (*soc1 ful* knock-out and *ahl15* overexpression) and three herbaceous wildtype accessions (Col, Cvi and Sha) will be investigated using the cavitron centrifuge method and the optical vulnerability technique. The hydraulic measurements in stems and leaves will be confronted with detailed anatomical observations using light and transmission electron microscopy to answer three questions: 1) are stems and leaves of woody mutant accessions more resistant to xylem embolism than those of herbaceous accessions; 2) are leaves more vulnerable than stems within the five accessions; and 3) what are the anatomical features underlying differences in P50 amongst different organs within accessions and between accessions?

Dynamic changes of leaf hydraulic conductance in response to dehydratation and re-watering under low vs high irradiance

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Leaf hydraulic conductance (K_L) is higly dynamic and typically responds to water status and irradiance. However, the relative contribution of vascular (K_x) and extra-vascular (K_{ox}) leaf water pathways to K_L changes in response to water potential decline and recovery, as a function of light conditions, remains poorly investigated.

We studied dynamic responses of leaf hydraulics in an evergreen (*Arbutus unedo* L.) and a deciduous (*Populus nigra* L.) tree species. We estimated the vulnerability of K_L, K_x and K_{ox} during dehydration, and the eventual recovery after re-watering leaves that experienced turgor loss. Measurements were performed at both low and high irradiance, and by using the vacuum chamber method (VCM) and the evaporative flux method (EFM).

K_L vulnerability was higher under high irradiance, as a result of marked changes in K_{ox}. Moreover, K_L recovery, as recorded in response to re-watering, was mostly due to K_{ox} recovery in both species.

Key message: Extra-vascular water pathway dominates leaf hydraulic decline under drought and recovery after rehydration.

Are root pressures used to refill cavitation by bamboos?

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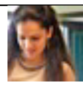
Bamboos generate root pressures every night, which is believed to refill xylem cavitation occurred during the day. Declines in leaf hydraulic conductance (Kleaf) of bamboo leaves were detected previously, while root pressures were proved to be high enough to refill xylem cavitation of leaves on the top of the canopy. By using bamboos in a common garden, here we first used the evapotranspiration method and rehydration method to generate leaf vulnerability curves. The results confirmed previous results that bamboo leaves were very vulnerable to declines in Kleaf, with minimum Kleaf reached at ~-1.8 MPa. Then we used the optical technique to detect cavitation spread in leaves during bench dehydration. Surprisingly, the first cavitation in most bamboo species occurred at water potentials ~-3 MPa. Therefore, the initial declines in Kleaf cannot be explained by xylem cavitation. Cryo-SEM images of desiccated leaves further proved that declines in Kleaf could not be explained by cavitation or conduit collapse. Additionally, the midday leaf water potentials of the studied species during the dry season were ~-2.5 MPa, suggesting xylem cavitation rarely happen in bamboo leaves. These results challenge the previous understanding that bamboos use root pressures to refill xylem cavitation on a regular basis.

Key message: Declines in Kleaf of bamboos during the day cannot be explained by xylem cavitation or collapse, challenging the understanding that root pressures are refilling cavitation regularly in bamboos.

SESSION 4: MOLECULAR BIOLOGY OF PLANT HYDRAULICS

Effects of a new protein hydrosilate-based biostimulant on responses to severe water stress and relief from drought in *Capsicum annum* L.



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Nowdays, water deficit is one of the most common environmental stresses capable of affecting plant growth, especially by impairing xylem water transport. Biostimulants are known to induce plant resistance to drought stress even by influencing plant hydraulic conductivity and stomata conductance (Van Oosten *et al.*, 2017). In the present study, a priming treatment with GHI_16_VHL, a plant protein hydrolisate-based biostimulant, was tested for its role in mitigating water stress responses and promoting a faster recovery on 3-month-old potted *Capsicum annum* L. plants grown in the greenhouse. GHI_16_VHL was applied by fertigation two times with a 7-day interval before the starting of the drought treatment. Once severe water stress levels were reached, two biostimulant treatments were applied with a 4-day interval during the re-watering recovery phase. In order to evaluate the biostimulant influence on plant water dynamics under stress and recovery conditions, shoot water potential, leaf gas exchange and percentage loss of hydraulic conductivity were measured throughout the entire experiment. Plant stress level was assessed by quantifying ABA level and ROS-scavenging activity, whereas plant growth was monitored by biometric measurements. Taken all together, our data suggest the stress mitigation role of GHI_16_VHL in pepper.

Key message: Priming treatments with a new biostimulant composed by plant protein hydrolisates affects the stress mitigation responses in pepper probably by modulating xylem water dynamics.

Investigating the role of ABA and embolism in leaf senescence and abscission

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Abscisic acid (ABA) is the most important plant hormone in plant drought responses, but it also has other major functions in plant growth and development. It appears that ABA and ethylene both play major roles in leaf senescence and abscission but almost all the work done on these processes in the last 15 years has been ethylene centric. My aim is to investigate the role of ABA in the processes of leaf senescence and abscission during drought and short days. Photosynthetic rate, stomatal conductance, foliar ABA levels, and leaf water potential were tracked in three winter deciduous tree species (*Quercus falcata, Phellodendron amurense, Ginkgo biloba)* into the autumn. In addition, similar data was collected in three drought deciduous species (*Erythrina sandwicensis, Cochlospermum fraseri, Shorea robusta*) exposed to a controlled drought. In all species leaf embolism resistance was determined using the optical method. We found that a major increase in foliar ABA levels is associated with leaf abscission during drought and under short days, however was not associated with the senescence of leaves during these events. This work provides major steps towards understanding the unknown underlying mechanisms that trigger arguably the most important plant processes affecting terrestrial productivity in the northern hemisphere and sub-tropics.

Key message: ABA plays a major role in leaf abscission in both drought and short day/cold deciduous species. The increase of foliar ABA appears to have no association with senescence and is entirely related to abscission.

Investigation of non-structural carbohydrates and xylem anatomy in petiole of grapevine varieties during water limitation and after re-irrigation

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Water shortage (WS) during growing of *Vitis vinifera* L. can limit shoot growth and affect yield and fruit quality, as well as allocation of carbon reserves into perennial organs for the upcoming years. Varietal anatomical differences, such as specific mean xylem vessel diameter in petiole, are expected to influence water transport in canes facing water limitation. Several authors have also evidenced that non-structural carbohydrates (NSC) of adjacent living parenchyma are involved in the repair mechanism of embolized vessels. In this work, we evaluated NSC level and xylem anatomy in petiole of Cabernet Sauvignon and Syrah varieties, subjected to WS and subsequent water refilling in the summer of 2017. The anatomical analysis highlighted that Syrah had high frequency of classes of large vessels, and that the xylem differentiation of vascular bundles was also affected by WS. Moreover, petiole NSC content was significantly influenced by WS and recovery, supporting the hypothesis that starch mobilization was associated to an elevated concentration in soluble NSC. This effect was determinant for Cabernet Sauvignon, whose stress response seemed to be based mainly on NSC metabolism. Finally, Syrah, differently to Cabernet Sauvignon, sustained the WS-induced increase in soluble NSC of petiole also 18 h after re-watering.

Key message: Petiole anatomy and NSC were compared in different grapevine varieties during water stress and after re-irrigation. The cultivar with the higher frequency in large vessels could maintain higher soluble NSC for recovery.

SESSION 5: PHYSIOLOGICAL RESPONSES TO THE ENVIRONMENT

Effects of mosses with contrasting functional traits on shrub growth and xylem anatomy under different precipitation regimes at the tundra

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In the Arctic tundra, bryophytes are the dominant growth form covering the soil surface of shrub communities. They can exert physical and chemical effects on the community through their capacity to retain moisture, their allelopathic compounds or the introduction of nutrients into the system. The study of the interaction between shrubs and bryophytes is essential to understand the functioning of these communities, which are expanding in the tundra due to global change. In this study, we collected *Betula nana* ramets growing on moss carpets dominated by the species *Hylocomium splendens*, *Pleurozium schreberi* or *Sphagnum* spp., which differ in their growth habit and the density of their carpets. We sampled three ramets per site in eight locations near Abisko, Sweden. Half of the sites correspond to low precipitation areas (571-755 mm) and the other half to high precipitation (811-1155 mm). We prepared microscopic sections of the shrubs stem base and measured growth rings and xylem anatomical parameters (vessel lumen area, vessel density and grouping, and theoretical hydraulic conductivity). Preliminary results indicate shrub growth differences depending on the dominating moss species. We discuss the importance of moss traits combined with the precipitation regime for the performance of tundra shrubs in the context of a changing climate.

Key message: Moss species with contrasting functional traits related to their growth habit differ in their influence on shrub performance at the tundra.physiology.

What is needed to live on the rocks? Three vascular plant species from Inselbergs reveal it

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Inselbergs are defined as xeric habitats because of its harsh environmental conditions markedly different from those of their surrounding environments, being thus an ideal system to study the effects of “environmental filtering” over species assembly and coexistence. In Colombia, their floristic diversity has been well-documented, but the ecophysiological characteristics that let plants live and coexist on Inselbergs are still understudied. Using a series of morpho-anatomical and physiological functional traits of three of the most common vascular plant species of an Inselberg found in Puerto Carreño, Vichada (Colombia), we answered two questions, i) what is the Grime’s CSR ecological strategy used by the studied species to live on Inselbergs? and, ii) is there a differential use of resources in terms of water and light among species? Based on Grime’s scheme we found two ecological strategies, stressors (S) and stressor/competitor (S/CS), and when morpho-anatomical and physiological traits are studied together we can see that each species have a different way to use water and light. Thus, the low water supply during the dry season, the high light intensity and temperatures and the low nutrient availability due to shallow soils, haven’t resulted in a reduced functional diversity on Inselbergs plant communities.

Key message: Despite the filter imposed by Inselbergs stressful abiotic conditions their huge floristic diversity and high number of endemism are the result of a differential use of resources that facilitates species coexistence.

Post-fire tree physiology: the role of xylem

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Fires of low and moderate intensity often do not constitute a direct lethal threat to mature tress, but rather may leave behind trees with a variety of heat injuries that can subsequently affect their physiology. Recent studies have made major steps forward in our understanding of mechanisms by which fire injuries impact whole-plant functioning and provided evidence that heat-induced xylem dysfunction plays a central role in post-fire tree physiology. Heat transfer to the xylem during fires can cause structural and non-structural impairments of the hydraulic system, which can interact with heat-induced carbon starvation processes and be amplified by biotic attacks.

Focusing on carbon and water as currencies of plant functioning, we integrated xylem dysfunction mechanisms into a conceptual framework that provides detailed decriptions of the involved processes, their interconnections and feedbacks. Evaluating the precise relationshiops between the physiological mechanisms will be crucial to fully understand how fires can affect tree functionality and to further improve models of post-fire tree mortality. Considering future climatedriven increases in fire frequency and intensity, knowledge of the physiolgical responses of trees is important to better estimate post-fire ecosystem dynamics and interactions with climate disturbances.

Key message: Fires can impair xylem hydraulics of surviving trees and consequently affect their physiology.

Intra-annual dendroanatomy can improve our understating of climate constraint on xylem functional traits

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Dendroanatomy is the study of xylem anatomical features in dated tree-ring series. This approach allows to get insights into growth – environment interactions, and inspecting xylem responses to past climate variability. While most studies have measured anatomical parameters such as cell lumen area, hydraulic diameter, cell wall thickness, with annual resolution (e.g. calculating the mean value in the ring), we applied dendro-anatomy at the intra-ring level, to retrieve information on growth response to intra-seasonal climate conditions. We present three investigations performed in the last years on different tree species and environments: (1) *Pinus cembra* at the altitudinal limit in the Italian Alps, (2) *Pinus pinea* at the sea level in southern Italy, and (3) *Quercus ithaburensis* and *Quercus boissieri* in dry lowland and mesic highlands in Israel.

Time series of intra-annual xylem anatomy proved useful to identify intra-seasonal patterns of climate influence on xylem structure. We believe that intra-annual dendroanatomy can considerably increase our understanding of the environmental influence on xylem functional traits, allowing to infer on xylem formation processes and growth strategies of different tree species.

Key message: Dendroanatomy at the intra-ring level was applied to different species and environmental conditions. This allowed to identify intra-seasonal patterns of climate influence on xylem structure.

Whole-tree xylem dynamics of long-lived *Fagus sylvatica* trees under contrasting environmental conditions

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Large old trees are among the most charismatic living beings on Earth, but concomitantly are highly exposed both to drought-related growth declines and size-dependent disturbance, especially when living at the southern edge of the specie distribution range.

We climbed with ropes and cored the main stem and selected branches of the oldest crossdated *Fagus sylvatica* L. (300-600 years according to site conditions) in Austria and Italy, in order to explore how their long term xylem dynamics change across the whole tree structure during its age/size development. The overall tree shape and productivity response of the oldest trees at each site have been analyzed among highly contrasting environmental conditions, i.e. low- to high-elevation, highly rainy to dry summer (eastern Alps to central Apennines). The variability in tree-ring growth dynamics at different tree heights have been assessed in terms of growth rates and productivity trends, response to growth-limiting climatic factors and extreme climatic events, and natural disturbances.

Key message: Trees approaching their maximum lifespan in different environments show high variability in tree-ring dynamics throughout their main stem and branches in response to climate and natural disturbance.

Effect of drought-stress on cambial activity and on morphology of newly formed tracheids in girdled *Pinus sylvestris* L. trees

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We studied how drought stress affects dividing cambial cells in girdled trees, specifically duration of differentiation phases and morphogenesis of forming tracheids. The xylem cells formation was analyzed by weekly microcore sampling of six control and six girdled Scots pine trees (girdled on 15th January 2014) during two growing seasons (2014 and 2015). Tracheid morphological parameters and timing of cell differentiation stages were analyzed in relation to soil water potential and sap flow rate. After girdling, cambium activated only above the girth. No significant difference in timing of differentiation and morphological parameters was found in 2014 but all measured parameters differed between control and girdled trees in 2015. Tracheids of girdled trees spend less time in differentiation phases, which resulted in their narrower diameter and thinner cell walls. Drought stress affected intensity of cambial cell division (initiation and reactivation) and thus the duration of xylem cells formation. Improvement of tree water status in July resulted in reactivation of the cambial zone and formation of intra-annual density fluctuations (IADF) in both monitored growing seasons. Finally, Mork's index was not an appropriate criteria to distinguish between earlywood and latewood tracheids.

Key message: Cambium immediatelly responds on fluctuations of water availability by reducing the number of dividing cells, affecting morphogenesis of differentiating xylem cells, and by IADF.

Hydraulic adaptations of secondary timber species along a precipitation gradient in central Germany

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Increasing summer drought in central and eastern Germany will drive *Fagus sylvatica* to its precipitation limit; forestry currently relies mostly on *Quercus petraea* as an alternative broad-leaved drought-tolerant timber species. In order to maintain diverse broad-leaved forests, four relatively drought-resistant secondary timber species (*Acer platanoides, Tilia cordata, Carpinus betulus* and *Fraxinus excelsior*) might be suited to replace *F. sylvatica* at the drought limit of its distribution range. We investigated a wide range of hydraulic parameters at leaf level (specific leaf area, leaf-to-sapwood area ratio, turgor loss point) and xylem level (specific conductivity, embolism resistance) in the four secondary timber species, and compared their response to that of *Q. petraea* along a steep precipitation gradient in Central Germany (490 - 880 mm y^{-1}). Although we observed pronounced differences between species in most studied traits, the climatic influence was weak, when considering precipitation only. Because other factors like tree size and soil physical properties often co-vary across climatic gradients, hydraulic plasticity cannot be captured fully by bivariate relationships alone. We present the results of multivariate models including the factors tree height and soil capacity for plant-available water as explanatory variables in addition to precipitation rates.

Key message: For studying hydraulic plasticity across climatic gradients, simultaneous changes in tree structure and soil physical properties must be addressed through multivariate analyses.

Effects of defoliation caused by pine processionary moth on xylem anatomy in Turkish pine

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Larvae of the pine processionary moth (PPM) *Thaumetopoeae wilkinsoni* Tams can cause severe degrees of defoliation and thus growth loss in *Pinus brutia* Ten (Turkish pine). Trees defoliated by PPM can become more vulnerable to biotic and abiotic stress factors. *P. brutia* is an important forest species of the eastern Mediterraneaen region, however only little is known about the effects of PPM defoliation on xylem anatomy. In 2018, twelve adult *P. brutia* trees were selected for wood formation and anatomical studies within a pine plantation in Antalya, Turkey. Six of these trees showed 50 – 60% defoliation caused by PPM, the other six trees (control) showed no defoliation. In February 2019, microcores were extracted from the stem of each tree to study xylem anatomical characteristics of the current (2018) and previous (2017) tree ring. Compared to 2017, increment in 2018 was 20% lower for the PPM group than for the control group. Furthermore, tracheids of the PPM group showed a significant decrease in lumen area (earlywood and latewood) in 2018. Tracheid dimensions of the control group showed no significant differences between the years. Preliminary results indicate hydraulic adjustments of the xylem anatomy due to PPM defoliation by forming narrower tracheids.

Key message: *Pinus brutia* trees subjected to defoliation by the pine processionary moth *Thaumetopoeae wilkinsoni* show decreased stem increment and xylem anatomical differences during the first year after defoliation.

Response of European beech and Norway spruce to long-term drought and re-irrigation in the KROOF experiment

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Throughfall had been excluded for five consecutive growing seasons (2014-2018) from six plots in a mixed forest of Fagus sylvatica and Picea abies (Kranzberger Forst, Bavaria, Germany) by a movable roof system whereas six other plots served as control (KROOF experiment). Water uptake into the soil was allowed during winter time. After an initial drought in spring the roofed plots are irrigated in a controlled manner to the soil moisture of control plots in June 2019. First results of the response of the relatively isohydric spruce in comparison to the more anisohydric beech on water consumption and lateral sap flow profiles in the xylem as well as leaf water potentials are reported.

Key message: Despite the xeromorph leaf type isohydric spruce suffers more from drought than the more anisohydric beech trees.

Temperature-induced xylem formation. An indirect effect of solar radiation on black locust cambial activity

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The study concerned effect of temperature on black locust (*Robinia pseudoacacia* L.) xylogenesis in natural conditions. Trees growing in the inner/peripheral zone of the woodlet were analyzed. We hypothesized that direct exposure of southern sides of peripheral zone tree-trunks to solar radiation will result in their stronger heating, thereby affecting xylogenesis. Temperature was measured at the northern and at southern sides of inner and peripheral zone tree-trunks. As expected temperature measured at the southern side of peripheral tree-trunk was higher than at the northern side. Collected tissues were analyzed in epifluorescence technique. We found that only at the southern side of tree-trunks of peripheral zone of the woodlet a vascular cambium deposited greater amount of xylem before full leaf development. After full leaf development no persistent predominance of deposition of xylem at either side of a trunk during the growth season were observed. We conclude that the difference in temperature before full leaf development between southern and northern side of a tree trunk has a positive effect on intensification of black locust radial growth of the vascular cambium and xylogenesis on the more heated side.

Key message: Temperature increase, caused by solar radiation, affects black locust xylogenesis before full leaf development.

Variations of the stem diameter as an indicator of plant mortality

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Continuous measurement for stem diameter variations allows to monitor the phenology, growth dynamic and related-physiology of plants. During a drought event, the decrease in stem diameter is due to a loss of water balance, mainly detected in the non-rigid bark. We have used a dedicated device (PepiPIAF system, Hydrasol) to monitor the response to extreme drought of lavender (*Lavandula angustifolia*). Lavender is a crop living under very dry conditions (Mediterranean biome) and barely studied. During a dehydration-rehydration experiment, physiological parameters such as water potential, stomatal and hydraulic conductances, cell damages and ultrasonic acoustic activity, were continuously monitored in relation with stem diameter.

Our results highlight the dehydration threshold (as a ratio of diameter loss) that significantly affect the resilience to drought and related mortality. We therefore developed a mortality index based on stem diameter monitoring that could help to anticipate lavander death. Based on the common mechanisms affecting plant, it should apply to many different species.

Key message: The variations of the stem diameter during a drought can be used to evaluate the dehydration threshold causing mortality in lavender.

Seasonal coordination of leaf hydraulics and gas exchange in the wintergreen fern *Polystichum acrostichoides*

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While some perennial species avoid winter exposure through deciduous leaves, the wintergreen fern *Polystichum acrostichoides* has fronds that are photosynthetically active all year, despite diurnal and seasonal changes in soil moisture, air temperature, and light availability. This species can fix the majority of its total annual carbon during periods when the deciduous canopy is open. Yet, remaining photosynthetically active during this window requires the maintenance of many physiological systems during freeze-thaw cycles that can lead to gas bubble formation in the xylem that block flow and reduce photosynthesis. We aimed to determine the anatomical and physiological strategies that *P. acrostichoides* uses to maintain positive carbon gain, and the degree of coordination between the hydraulic and photosynthetic systems. We measured hydraulic conductivity of fronds through the year and found that the percent loss of conductivity (PLC) increased 25% after the first frost, suggesting that freeze-thaw induced embolism was occurring. Maximum photosynthetic rate recovered in spring, despite PLC remaining high. The remaining hydraulic capacity was therefore sufficient to supply the leaves with water. The onset of colder temperatures also coincided with the development of a necrotic hinge zone at the base of the stipe, which allows fronds to overwinter lying prostrate, thereby minimizing the damaging effects of snow loading and maintaining a favorable energy balance. Our conductivity data show that the hinge zone did not affect leaf hydraulics because of the flexible nature of the vascular bundles. Collectively, these strategies allow *P. acrostichoides* to remain competitive and abundant in northeastern forests.

Key message: Despite experiencing freeze-thaw induced embolism in the winter, *Polystichum acrostichoides* fronds are able to recover photosynthetic activity in the spring in order to get a head start on carbon gain for the year.

Xylem vessel size of grapevine cultivars and Esca disease incidence in the field

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Esca is one of the most important grapevine trunk diseases of the crop, is considered one of the most relevant challenges to face for the modern viticulture. This disease seriously affects vineyard productivity and longevity, being the main cause of death of plants in most viticultural areas. Previous studies suggest that wide xylem vessel diameter favor the development of *Phaeomoniella chlamydospora*, one of the fungi involved in esca. The aim of this work was to determine the anatomical measurements (diameter and frequency) of xylem vessels in a list of grapevine cultivars with different susceptibilities to esca. In this study 27 white-berried and 24 red-berried grapevine cultivars, grown in the same experimental vineyard were analyzed and the vessel dimension characteristics were correlated with esca incidence previously detected. The cultivars showed significant differences in vessel parameters. However no linear relationship was detected among the vessel size and esca incidence in the field. Overall, white-berried cultivars showed wider vessel diameter than red-berried cultivars. In our investigations, it looks there is not a clear relationship between vessel size and esca incidence. Vessel anatomy profiles can be useful for further investigations on sensitivity of grapevine genotypes to grapevine trunk diseases.

Amplitude and velocity of response to drought are not directely linked to recovery capacities for tropical tree seedlings

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Climate models predict an increased severity and frequency of droughts. Tropical forests are known to be highly diverse both in terms of specific richness and functional diversity. The response of plants to water stress is also found to be highly diverging and species-specific within communities. Seedling establishment is a critical phase in plant development and understanding drought response at this stage is crucial for the prediction of tree growth and survival in tropical forests. In order to precise the drought response of seedlings and their recovery capacities, on both the hydraulic status and photosynthetic capacity, we realised a pot experiment on 5 tree species. During drought, we followed at each wilting stage (normal to severely wilted) a large set of mechanistic traits (Ψ_{leaf}, gs, Fv/Fm, ETR). All species decreased water potential and transpiration, accompanied by photosystem damage but with contrasting amplitude and velocity. After rewatering, all species showed efficient recovery capacities on all traits (stomatal and non-stomatal) with broad time lags among species. Our study underlines that the impact of drought and species' recovery capacities are not necessary linked regarding carbon- and water-use in tropical trees which will be discussed in relation to turgor loss point and cuticular conductance.

Key message: For tropical trees at the seedling stage, the impact of drought on hydraulic traits or stomatal and non-stomatal mechanisms are not necessary linked to recovery capacities after rewatering.

Anatomical and physiological drought adjustments of pubescent oak (*Q. pubescens*) from two adjacent sub-Mediterranean ecosites

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Ecosite characteristics, local soil water availability and microclimate, influence severity of drought. Anatomical and physiological drought stress related traits were studied in pubescent oak (*Quercus pubescens* Willd.) growing on adjacent sub-Mediterranean sites differing in bedrock and soil type (flysch with eutric cambisol, high water retention - F; limestone with rendzic leptosol, low water retention - L). In two years of the study, 2015 and 2016, in the part of the season with sufficient water supply, sap flow (sap flow density, SFD) was higher in oaks growing on L than those growing on F. With soil drying in July, SFD was reduced on both plots, most in trees growing on limestone. Trees on F were more buffered against drier hydroclimatic conditions due to higher availability of soil water. In the period of severe drought SFD in trees growing on L was decoupled from the changes of vapour pressure deficit (VPD), due to increased employment of water conservation strategies, such as decreases in stomatal conductance. This was further translated into stomatal limitation of photosynthesis and contributed to reduced radial growth. Anatomical studies revealed smaller yearly xylem increments in L trees and anatomical acclimations of conductive tissues, supporting/preserving hydraulic function of trees.

Key message: *Q. pubescens* from two ecosites employs different mechanisms to optimise and preserve hydraulic function in drought prone environment.

Embolism tolerance in Mediterranean tree species in response to drought

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Hydraulic failure, specifically embolism formation, is thought to have a key role in drought-induced tree mortality. Xylem resistance to embolism as well as embolism avoidance via stomatal regulation have long been studied as trees' strategies in face of drought. Our main goal was to examine another trait – 'embolism tolerance', i.e. the ability of trees to prevail under high levels of embolism, as a strategy trees adapt to cope with frequent and intense drought events. Here we present results from year-round measurements of leaf gas exchange and sap flux density in adult lemon (*Citrus limon*) and olive (*Olea europaea*) trees, together with vulnerability curves. Our results suggest that while lemon have higher hydraulic safety margins due to lower P_{50}, olive might possess the ability to survive extensive cavitation events, which allows it to grow under low water availability. These findings, along with MicroCT scans of other Mediterranean species that exhibited high degree of embolism, suggest that embolism tolerance might exist across a multiple tree species.

Key message: Embolism tolerance, i.e. the ability to prevail under high levels of embolism, might be a srategy in a tree's toolbox to cope with drought.

Effects of precipitation, soil and stand characteristics on branch hydraulic traits of European beech

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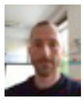
Recent and future climatic changes with increasing air temperature and more severe drought events likely are exposing plants to increasing drought stress. Although European beech (*Fagus sylvatica*) is the dominant broadleaved tree species in Central Europe, its future remains a matter of debate due to the anticipated drought-sensitivity of this species. For the present study, 30 evenly-aged mature beech stands on pleistocene soils were selected along a precipitation gradient (504 - 872 mm yr^{-1}) in northern Germany in order to disentangle the simultaneous effect of precipitation, tree height and soil water characteristics on hydraulic and foliar traits.

Along the gradient, embolism resistance significantly increased with increasing precipitation, accompanied by a decline in leaf size and an increase in sapwood-to-leaf area ratio, i.e. the same cross-sectional xylem area supported less leaf area in a drier climate. We assumed to find the opposite response and speculate that vertical within-tree gradients in hydraulic properties may overlay climatic signals because trees increase in height with increasing precipitation. This highlights the need for multivariate analyses along climatic gradients including precipitation, soil and stand characteristics in order to detect adjustments in hydraulic traits.

Key message: Along a climatic gradient, embolism resistance and the sapwood-to-leaf area ratio counterintuitively increased with increasing precipitation in European beech.

Drought hardiness of Douglas-fir progenies under early and late summer glasshouse conditions



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In Germany, growing of Douglas-fir (Pseudotsuga menziesii [MIRB.] FRANCO) may minimize risks of drought stress due to climate change. The objective of the study was to investigate drought hardiness of 11 progenies of Douglas-fir in a glasshouse trial descending from selected basic material in France, Germany and USA under the project FitForClim. The trial includes two moisture regimes over 6 weeks per season. Each progeny were represented by 60 trees. Foliage condition, tree height and diameter in both treatments were assessed. For the control treatment, hydraulic xylem conductivity of twig sections before and after a simulated drought stress was obtained. Opposite to needle colour, the initial state of seedlings characterized by photosynthetic vitality PI_{ABS} varied among progenies. With decreasing soil water content PI_{ABS}, chlorophyll- und anthocyanin concentrations decreased and showed significant differences among the progenies. Accordingly, visible needle damages increased and differed also significantly among a part of progenies as well as needle age. After re-watering PI_{ABS} of seedlings exposed to severe drought increased to the base level in different extent among the progenies. Loss of xylem hydraulic conductivity ranged from 25 to 77 % and was higher with progenies showing a relatively low PI_{ABS} level and a high mortality.

Key message: Douglas-fir progenies showed considerable differences in drought hardiness. A suitable rating scale should include serveral physiological and morphological characteristics.

Q How resistant are Neotropical canopy rainforest tree species to branch embolism and are they at risk of hydraulic failure during the dry season?

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Tropical rainforests play a predominant role in terrestrial biogeochemical cycles. To predict the fate of such ecosystems in the context of increasing frequency of drought severity in the Eastern Amazon, it is paramount to gain more knowledge on the drought resistance of canopy tree species. Xylem vulnerability to embolism, which has been associated to tree survival under extreme drought has been little studied in the tropics. Here, we assessed the range of xylem vulnerability to embolism and the risk of hydraulic failure during the dry season for the largest set ever of coexisting Neotropical rainforest canopy tree species. We measured key hydraulic traits on branches and leaves and estimated hydraulic safety margins. We provide evidence that rainforest canopy tree species are more resistant to embolism than what is presently known, have low variability in turgor loss point and midday leaf water potential and exhibit positive hydraulic safety margins. However, we found no relationship between hydraulic traits and species' bioclimatic niche distribution. We conclude that substantial hydraulic safety margins presently protect most Neotropical canopy rainforest tree species against hydraulic failure, but that interspecific differences may lead to a potential alteration in the composition of tree communities with contrasting effects of climate-change.

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