BOOK OF ABSTRACTS

12[TH] INTERNATIONAL WORKSHOP ON SAP FLOW

31 OCTOBER – 3 NOVEMBER 2023

ROTORUA, AOTEAROA NEW ZEALAND

#SAPFLOW2023

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Session 1 – Crop Management

Steve Green

Tree water use and irrigation management for salinity and drought

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Understanding the vadose-zone dynamics of root-water uptake functioning and tree water-use (TWU) is critical for establishing water requirements for irrigation. In this talk we present data from a number of field experimental that address different aspects of irrigation management of tree crops.

The first study is on avocados in the Highlands of Kenya where fruit quality and yields are often low because the trees have been poorly managed and without irrigation. Government-supported irrigation schemes are slowly coming to the Highlands and the farmers will then need to know when and how much water is needed to irrigate their trees. Sap flow data are presented to show how tree water responds to the local microclimate and soil water availability.

The second study on Date palms and Acacia trees comes from the United Arab Emirates where irrigation is essential due to very low rainfall (< 100 mm/y) and high rates of potential evapotranspiration (> 2400 mm/y). Sap flow data are used to determine irrigation allocation based on simple estimates of the tree's leaf canopy dimensions, combined with the salinity of the irrigation water and the local microclimate.

The third study is from kiwifruit in New Zealand, that grow in a moist maritime climate that has plenty of annual rainfall, but often experiences long dry-spells during summer. We show how the water balance of kiwifruit is being measured using a combination of time-domain reflectometry (TDR), drainage flux meters (DFMs) and run-off plots. Good data from detailed field experiments is essential for developing appropriate irrigation schedules that match irrigation supply with plant water demands.

Rafael Fernandes

Optimizing kiwifruit quality through estimation of leaf stomatal conductance from sap flux density: the KIWIQUALI project

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Most fruit crop irrigation is performed on empirical basis, with very little awareness of the consequences on yield and on water use efficiency. A model for leaf stomatal conductance from sap flux measurements in olive, which is a fruit crop that requires less water than kiwifruit vines. The main objective of the KIWIQUALI project is to estimate the leaf stomatal conductance (g_s) through models from sap flux density (J) measurement in *Actinidia chinensis*, allowing assessment of plant's water status continuously and improve irrigation management, while optimizing fruit quality. This goal will be reached through the following specific objectives: (*i*) to adjust models for estimation of kiwifruit trees g_s from J continuous measurements and atmospheric vapor pressure deficit (VPD); (*ii*) to understand which irrigation level optimizes fruit quality and productivity; (*iii*) to develop precision irrigation protocols based on g_s estimation. Aiming at obtaining measurements in different water status, kiwi vines in the Emilia Romagna region (Italy) will be submitted to four irrigation treatments, being one well-watered (100% of crop evapotranspiration ETc) and three deficit irrigation treatments (ca. 75%, 50% and 40% of ETc). The KIWIQUALI project will lead to innovative results as it will (*i*) provide a model to continuously estimate g_s on an isohydric, drought sensible crop (kiwifruit), improving the knowledge on the hydraulic limitations and physiological responses of one of the most water requiring fruit crops; (*ii*) improve understanding on how different plant water status and physiological performance at source level affects fruit yield and quality, and; (*iii*) provide a tool to optimize irrigation scheduling of *Actinidia chinensis*. The first season of measurements will happen during the European summer (from June to ca. October), therefore, this contribution will present the KIWIQUALI project and its preliminary results.

John Ji

Apple production management with new combinational plant sensor systems

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Abundant apple production in the Northwest USA sector has readily available water in many cases. In other areas of the USA, water is limited, and water use efficiency is paramount. However, the pumping cost, pump pressure to apply irrigation uphill, and the variation of slope and soil types make irrigation for consistent yield difficult to maintain and operate. The complex irrigation methods for apple orchard may incorporate drip irrigation, above canopy fog misting, overhead micro spray, and low impact spray irrigation from the ground surface. Irrigation is provided to cool plants above a temperature threshold. A variety of *Malus domestica* 'Honeycrisp' and the 'Honeycrunch' brand in New Zealand was developed by the Horticultural Research Center at the University of Minnesota. Both varieties are affected by bitter pit, and rather that producing 80% yield from harvest pack out, the yield is 60%, posing a difficult challenge to a management of low calcium disorders, from potentially too rapid tree growth, and low transpiration. Excessive fruit growth from overwatering is also an attribute of corking (an apple fluff). A new cloud IoT system is developed to measure the transpiration rate of a plant by sap flow sensors with the right combination of fruit and stem growth, soil moisture sensors, and flow meters to manage water. With a single system of plant tracking, water management, and environmental sensors we combine sensor readings allowing one to understand and control irrigation and manage both disorders. To make a myriad of sensor details available to growers, a new artificial intelligence (AI) platform will need to be developed to make this practical. Our electronics system combines cloud data integration which will be presented to AI technologists. New data shows the results of the new system invented for the application.

Junqi Zhu

Comparing common assimilate pool and phloem carbohydrate transport models for simulating biomass variability and carbohydrate partitioning

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Variability in fruit quality is a critical factor that can greatly impact the profitability of an orchard. Existing modeling techniques often fall short in identifying the causes of this variability, particularly concerning organ biomass. This study seeks to address this gap by comparing two models: the common assimilate pool (CP) and the phloem carbohydrate transport (CT) models, as applied to grapevines (*Vitis vinifera* L.). The CP model assumes that carbohydrates are attracted by developing plant organs based on sink strength alone. Conversely, the CT model adds complexity by considering the sink strength, topological position of the organs, and the carbohydrate concentration gradient. Both models were rigorously tested using a detailed potted experiment encompassing three levels of leaf area per vine. A statistical approach was designed for parameter optimization, involving large-scale simulations and emulation through the R package GPfit. Through global sensitivity analysis, it was found that carbohydrate allocation was affected by the amount of leaf area and the limiting factors for organ biomass development. When applied to a homogeneous canopy architecture where all grape bunches were equally close to carbohydrate sources, the CP and CT models produced similar results. However, under a heterogeneous canopy architecture, where distance between bunches and carbohydrate sources varied, the results were strikingly different. The coefficient of variation for fruit biomass increased from 0.01 to 0.17 as crop load rose, showing that carbohydrate allocation to fruits is influenced by both the size of the crop load and fruit distribution. This nuance is not adequately captured by the CP model. These results indicate that carbohydrate allocation to fruits is affected by both the size of crop load and fruit distribution, which is not adequately described by the common assimilate pool model.

Teruko Kaneko

Isohydric stomatal behaviour alters fruit vascular flows and minimizes fruit size reductions in drought-stressed avocado

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Plant water status is important for fruit development, as many fleshy fruits contain large amounts of water. The aims of this research were to explore the impact of drought stress on the water relations of the 'Hass' avocado plant, and its fruit growth, therefore, well-watered and water-stressed 'Hass' avocado plants were compared. Over four weeks, water flows through the shoot and fruit pedicel were monitored using external sap flow gauges. Fruit diameter was monitored using linear transducers (LTs), and stomatal conductance (g_s), photosynthesis (A) and leaf and stem water potential (Ψ_{leaf} and Ψ_{stem}) were measured to assess the plants' response to water supply. Under well-watered conditions, the average water inflow to the shoot was 72 g d^{-1} . Fruit water inflow was 2.72 g d^{-1}, but there was water loss of 0.37 g d^{-1} caused by the outflow (loss back into the tree) through the vascular tissues, and 1.06 g d^{-1} from the fruit skin. Overall, fruit volume increased by 1.4 cm^3 d^{-1}. In contrast, water flow into fruit of water-stressed plants decreased to 1.88 g d^{-1}, with the outflow increasing to 0.61 g d^{-1}. As a result, increases in fruit volume were reduced to 0.4 cm^3 d^{-1}. Following re-watering, a substantial recovery in growth rate was observed. A, g_s, and sap flow to shoots were also reduced during drought conditions. In summary, a reduction in growth of avocado fruit was observed with induced water deficit, but the isohydric stomatal behaviour of the leaves helped to minimize negative changes in water balance. Also, there was substantial recovery after re-watering, hence the short-term water stress did not decrease avocado fruit size. Negative impacts might appear if the drought treatment were prolonged. These results are important to hypothesise likely water movement within a planar cordon apple tree (a new orchard system).

Junghoon Lee

Internet of Vines

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Viticulture is a complex agricultural practice that requires a complete understanding of various factors that affect the growth of vines. These factors include water, soil, climate, and the plant's state. However, the direct monitoring of the plant's state has been incomplete so far because the lack of commercial-scale technology to measure the plant's biological responses to the factors mentioned above.

Telofarm, an agritech software company, has invented a new technology based on a semiconductor process to measure the plant's sap flow rate with minimum invasiveness to the plants. The sap flow rate represents the plant's response to the environment, similar to the blood pressure and heartbeat rate of a person. This signal is wirelessly collected together with other ones such as soil moisture and weather data, and integrated into our digital data network and software. We call this comprehensive system the Internet of Plants (IoP) that brings innovation to the viticulture.

With the IoP system, we are able to control the stress level of the vines precisely within the determined window for quality control and better yield. Various events such as the first rise of water, bud break, frost, pest, and irrigation can be monitored, analyzed, and critical recommendations such as irrigation timing and amount and various treatment protocols can be delivered to the field managers. In the level of ultimate service, the monitoring, irrigation, notice, and regular reports can be automated to meet the standards of the premium viticulture managements.

In 2022, we demonstrated the capabilities mentioned above in the Anderson Valley vineyards in California. We are now in the process of expanding our services to 30 vineyards in Napa, Sonoma, and Anderson valleys of California and New Zealand in 2023. We will present a detailed report at the XII International Workshop on Sap Flow.

Shinichi Takeuchi

Establishment of cultivation method of guava using unheated greenhouse only in winter, verification by sap flow measurement

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Guava is difficult to grow in Japan, except in sub-tropical region. This study is to introduce it as a substitute crop for the devastated tea garden, and cultivation has been attempted for three years in Shizuoka Prefecture, Japan. In 2018, ten seedlings were planted, and in the fall of 2020, a yield of 30.3 kg was obtained in fully open field cultivation. This is due to the relatively warm minimum temperature of -1.6°C during the winter of 2019-2020. In the following year, 2021, the canopies were covered with nonwoven fabric in the winter, but the yield was noting. Therefore, in 2022, individual sunlight greenhouses were introduced for 8 guava trees. The heat from the sun during the daytime is collected in a heat storage medium that utilizes pruned branch chips, etc. It is a non-heating system that mitigates the temperature drop by internal radiation. The utilization of this sunlight greenhouse supplemented the low cold resistance of guava in winter. The yield of 42.7 kg was obtained from the 7 plants due to the effect of the sunlight greenhouse and irrigation. These growth conditions were synchronized with the results of sap flow measurements, it shows significant sap flow during the winter months in 2022, while almost non sap flow in 2021. In addition, in the winter of 2022, we successfully overwintered 6 guavas in the tunnel-type sunlight greenhouse that included the existing stairs farm work. Although the outside temperature dropped to -5°C, the inside of the greenhouse was kept above 0°C without heating, and the leaves remained green. Sap flow in this tree indicates quite high level in May. From the above, it was shown that it is possible to expand the area where guava can be cultivated by using an unheated greenhouse that is effective only in winter.

Benye Xi

Evaporation-driven internal hydraulic redistribution alleviates root drought stress: mechanisms and modeling

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Maintaining the activity and function of the shallow root system of plants is essential for withstanding drought stress, but the associated mechanism is poorly understood. By investigating sap flow in 40 lateral roots randomly selected from trees in a Chinese white poplar (*Populus tomentosa*) plantation, an unknown root water transport phenomenon of simultaneous daytime bi-directional water flow was discovered in 18 lateral roots. On the long-term scale, the occurrence of bi-directional water flow was correlated with the soil water content. However, within the day, it was associated with transpiration. Our data demonstrated that bi-directional root sap flow was driven by evaporative demand. Thus, we named this phenomenon evaporation-driven hydraulic redistribution (EDHR). We further utilized micro-computed tomography technology to reconstruct the xylem network of the lateral roots and proposed new conceptual root water uptake models to verify EDHR. Our results indicated that the occurrence of EDHR is driven by the internal water potential gradient within the plant xylem network, which requires three conditions: high evaporation demand, soil water potential gradient, and special xylem structure of the root junction. The simulations demonstrated that during periods of extreme drought, EDHR could replenish water to dry roots and improve root water potential by about 38.9%-41.6%. This highlights the crucial eco-physiological significance of EDHR in drought tolerance. The new physiological phenomenon and the newly proposed models provide novel insights into the complex structure of root junctions and their impact on water movement within roots, enhancing our understanding of the relationship between xylem structure and plant hydraulics.

David Whitehead

Daily estimates of whole tree photosynthesis and water use efficiency demonstrate homeostasis between leaf and canopy scales for Dacrycarpus dacrydioides *and* Podocarpus totara *growing in a common garden experiment*

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Estimates of photosynthesis for forest ecosystems using eddy covariance are problematic for mixed species forests growing at sites with complex topography and models that scale from leaves to ecosystems require access for measurements distributed through canopies and inclusion of the non-linearity of driving variables. We tested an approach to estimate whole-tree values per unit ground area of net photosynthesis (*A*) from estimates of transpiration (*E*) using measurements of sapflow and water use (ω) efficiency. We estimated ω from the carbon isotopic discrimination of CO_2 evolved from shoot dark respiration.

We applied the approach over a 28 day summer period to two 20-year-old native conifers (Podocarpaceae) with similar stem volume but contrasting leaf area index, *Dacrycarpus dacrydioides* and *Podocarpus totara*, growing in a common garden in New Zealand.

Daily values of *A* ranged almost five-fold but there were no significant differences between species (mean 2.73 ± 1.02 g C m^{-2} day^{-1}). This was attributable to higher daily values of *E* (2.63 ± 0.83 mm day^{-1}) and lower ω (1.35 ± 0.53 g C kg^{-1} H_2O) for *D. dacrydioides* compared with lower *E* (1.88 ± 0.72 mm day^{-1})and higher ω (1.90 ± 0.77 g C kg^{-1} H_2O) for *P. totara*. *D. dacrydioides* exhibited similar values of *A*, greater *E* and lower ω compared to *P. totara*. These traits may help to explain why *D. dacrydioides* is restricted to swampy high rainfall areas while *P. totara* is widely distributed in New Zealand.

We showed that estimating carbon isotopic discrimination from dark-respired CO_2 is a feasible alternative to estimating ω with advantages in terms of allowing immediate calculations of whole tree *A* with daily temporal resolution and low costs for laboratory analysis and fieldwork.

Paulina Dukat

Employing Sap-flux Measurements in Research Spanning Tree to Forest Ecosystem Physiology Processes

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Sap flow observations can quantitatively describe and constrain key processes in individual trees and landscapes composed of different forest ecosystems. Here we present three such applications of sap flow measurements in boreal and temperate *Pinus sylvestris* (Scots pine) forests: (1) calculating the transport of dissolved CO_2 in xylem sap as a component of stem-surface net CO_2 efflux; (2), estimating stand-scale transpiration and water-use efficiency during drought conditions; and (3), estimating the impact of low severity wildfire (crowns unaffected; no tree mortality) on transpiration and tree growth. Key findings are:

1. The transport of dissolved CO_2 in xylem sap is the most difficult efflux component to estimate relative to the two other major fluxes n respiration and photosynthesis n accounting for 5-8 % of the total net stem-surface CO_2 efflux. The transport was calculated from the difference between respiration, estimated based on stem temperature, and daytime stem-surface net CO_2 efflux, measured in an opaque chamber thus preventing photosynthesis, and assuming the difference it is proportional to sap flow rate.

2. Transpiration upscaled from sap flow measurements may be used to diagnose the forest condition when all tree size-classes are monitored. Combined with latent-heat flux from eddy-covariance system, transpiration of unmonitored vegetation components and evaporation can also be estimated. This allows quantifying species and ecosystem component response to environmental variation, of particular importance when differential sensitivity to drought can be expected, especially among major tree species or size-classes.

3. Transpiration and growth of trees affected by low-severity fire decreased in the second post-fire growing-season compared to reference individuals. Because most fine roots in boreal Scots pine forests are near the surface, even low-severity fires may damage a large amount of surficial fine roots. This, coupled with growing-season precipitation composed mostly of light rains, would limit water uptake, canopy conductance and photosynthesis.

Donald White

Variation between clones of P. radiata *in the effect of weather and site on diurnal and seasonal patterns of diameter growth and sap velocity*

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The leaf (needle) water relations and stomatal response to environment are finely tuned and coordinated with xylem water relations to maximise carbon gain per unit water used with 'hydraulic safety'. This critical 'functional convergence' between leaf and xylem water relations is the basis for an active area of research linking diameter growth and diurnal patterns of growth and shrinkage with water relations and environment, and may provide a mechanistic basis for tree improvement and adaptation to climate change by linking value with vulnerability.

A set of clonal trials were established across an environmental gradient in New Zealand in 2015. One of these experiments was established on the Kaingaroa plateau. In 2018, sap flow sensors and point dendrometers were installed in three trees in each of four replicates and eight clones (64 trees in all) and measurements continued until March 2021. Generalised additive mixed effects models (GAMMs) were used to analyse the effect of climatic variables, genotype, and soil ECa on daily maximum diameter shrinkage (mm), daily maximum total water deficit (mm), and daily growth increment (mm). The climatic variables considered in the analysis were daily minimum temperature (°C), daily mean temperature (°C), daily maximum temperature (°C), daily minimum relative humidity (%), daily mean relative humidity (%), daily maximum relative humidity (%), daily total photosynthetically active radiation (□mol/m^2/s), daily total precipitation (mm), daily vapour pressure deficit (kPa).

After accounting for the effect of diameter at breast height as a covariate, temperature, relative humidity, daily precipitation and genotype were significant in the GAMM's for predicting diameter increment and maximum water deficit. Daily precipitation was not an important predictor of diurnal shrinkage. Diameter growth and daily shrinkage was maximized at temperatures of between 15 and 20 °C.

Bruce Dudley

Hydrological monitoring and modelling in New Zealand's 'Forest Flows' research catchments

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The Forest Flows project is a 5-year research programme examining the hydrologic processes that govern streamflow from plantation forests across New Zealand. Within this programme we are conducting a range of on-the-ground hydrological measurements, and hydrologic modelling approaches that surround and provide context to sapflow data. These include:

- Monitoring of climate, groundwater, throughfall, and water isotopes across the study catchments.
- Characterisation of geology in the near-subsurface using ground penetrating radar and soil cores.
- Development of vadose zone hydrologic models calibrated using soil moisture and soil water isotopes.
- Transpiration source water partitioning using stable isotopes.
- Measurement of streamflow using methods that protect migration pathways of New Zealand's native diadromous fish.
- Development of hydrological perceptual models for the test catchments.
- Implementation of hydrologic models to predict groundwater and stream flows under ch anging climate regimes.

I will present preliminary results from these efforts, and discuss potential next steps for forest hydrology research in New Zealand.

Remote Presentations

Brunella Morandi

The “FruitCREWS” network: a comprehensive analysis to define the best sensing technologies for irrigation scheduling

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Due to climate change, water scarcity and increased evapotranspiration requirements are serious challenges for agriculture worldwide and are jeopardizing the future supply of many crop productions. As perennials, fruit tree crops are particularly threatened by this risk and growers need rational strategies to improve their orchards water use efficiency. The FruitCREWS COST Action network (2022-2026) was recently funded by the EU research program and currently gathers more than 200 researchers from more than 40 countries. The Action aims at understanding the physiological behavior of fruit tree crops in response to drought stress, in different environments. Also, it will allow to identify the best tools to monitor plant water status in real time while allowing growers to precisely schedule irrigation through the adoption of new technologies. Existing datasets on the response of six different crops (apple, pear, kiwifruit, peach, olive and grapevine) in a range of different environments will be collected and merged in a unique database. The parameters will include, among others, soil water status, leaf and stem water potentials, sap flow, fruit growth, trunk diameter variations, radiometric indicators, etc. A joint, integrated analysis will allow to identify the most useful parameters to quantify drought stress while the most cost-effective and user-friendly monitoring tools will be identified. The physiological parameters will then serve as inputs of existing models to quantify plant water needs under drought, for possible implementation in decision support systems (DSSs). This contribution will report on the preliminary results derived from the database analysis and the identification of the most suitable parameter to be used as plant water status indicators for implementation in DSS for irrigation scheduling. Results from this network might provide relevant information for making a step forward towards a more sustainable future irrigation management of orchards and vineyards.

Salah Er-Raki

*Sap flow measurements in olive trees (*Olea europaea *L.) cultivar Menara under regulated and sustained deficit irrigation strategies*

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During the last decades, experimental methods based on sap flow measurements were developed in order to determine more precisely the amount of water consumed by trees. Trunk sap flow is a direct indicator of tree transpiration that reflects the physiological characteristics of trees and is essential for maintaining hydraulic transport between the soil and atmosphere.

The aim of this study is to evaluate the effect of sustained deficit irrigation (SDI) and regulated deficit irrigation (RDI) on measured sap flow. In this regard, measurements of sap flux were carried out during 2022 in the field on 12 year-old olive trees of cultivar Menara cultivated at 8 m x 8 m spacing with 156 trees/ha density in research station of Saada at INRA Marrakech, Morocco. Thermal dissipation probe method was used for Sap flow measurements. The irrigation regimes were studied according to the sensitivity of phenological phases to water stress with (SS) 'Sensitive Stage' and (SN) 'Normal Stage'. The control treatment T0 was fully irrigated (100% ETc) throughout the season. For RDI, four water regimes were tested: T1 (SS 100- SN 70% ETc), T2 (SS 100- SN 60% ETc), T3 (SS 80- SN 70% ETc), T4 (SS 80- SN 60% ETc). For SDI, a constant volume of water that is less than the evapotranspiration (ETc) during the entire irrigation season were evaluated: T5 (70% ETc) and T6 (60% ETc).

Relationships between the deficit irrigation treatments, sap flux measurements, stomatal conductance, and reference evapotranspiration (ETo) have been investigated. The results showed that sap flow values were affected by deficit irrigation treatments and climate demand. Higher values were obtained in the olive trees under control T0 than the stressed treatments. Treatment T6 of the SDI strategy showed that the soil moisture content is minimal, then the sap flow is minimal regardless of the meteorological factors driving it. Water availability was shown to noticeably influence measured stomatal conductance. High correlations and polynomial relationship were obtained between sap flow measurements and ETo, notably in control and RDI treatments with (R^2 =0.71; 0.67; 0.83; 0.78 and 0.75) under T0, T1, T2, T3 and T4, respectively. However, SDI treatments showed low correlations with (R^2 = 0.35 and 0.37) under T5 and T6 of SDI strategy. The results of the sap flow measurements provide a description of water use by olive trees monitored under different irrigation treatments and changing climatic conditions.

Melissa Venturi

Sap flow and fruit vascular relations under progressive water stress conditions

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Apricot is one of the most widely grown fruit crops in the world. Several studies have investigated the effects of deficit irrigation on apricot physiology as well as the fruit vascular flows characterization under no-limiting conditions. However, no information about the effect on sap flow, fruit vascular flows and fruit quality are available under progressive water stress conditions. This study assessed the effects of the lack of irrigation in the last weeks before harvest. Two irrigation treatments were imposed: "Stress", which completely stopped receiving irrigation during the last weeks before harvest, and "Control" that continued to receive 100% of ETc. During the growing seasons the following parameters were monitored: i) plant physiological parameters; ii) branch sap flow; iii) fruit vascular flows; iii) fruit quality; and iv) fruit nutraceutical quality on apricot cv. Farbela over two years. Trees were able to afford a long period in absence of irrigation without showing significant decrease in plant physiological performance as well as fruit growth over two years of study. Sap flow in the branch showed comparable values in plants subjected to different irrigation treatments. This means that leaves and fruit were able to maintain elevated transpiration rates thanks to the similar water volumes transported in the branches. Fruit vascular flows in "Stress" treatment presented higher values in the late evening or during the night. However, these differences were not found in the following year, probably because influenced by the different seasonal pattern. In addition, deficit irrigation seemed to improve fruit quality in terms of sugar content but the results shown in this study unfortunately do not provide strong demonstration of this relation. The lack of irrigation has also induced modifications in the quantity of some specialized metabolites, with 247 and 226 up- and down-regulated features in skin and flesh, respectively.

Costantino Sirca

Sap flow measurements for assessing water status in grapes

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Reliable methods for measuring sap flow (SF) on tree crops can help to diagnose water status of trees and then irrigation management. Previous works showed the potential of using a threshold or a sap flow pattern to manage irrigation. The main aim of this work was to evaluate the skills of SF sensors for assessing the water status in grapes (Vitis vinifera L.). We collected and analyzed continuous sap flow data and patterns for three consecutive growing seasons (2019-2021) in three different vineyards on the "Vermentino" variety. The experimental sites are on a classical Mediterranean landscape located in Sardinia, Italy. The hourly SF rate showed a midday peak in well watered conditions and clear differences with respect to night values. On the opposite, not significant differences in SF rates between daily and night values during high water stress conditions was observed. Daily SF averages and SF values recorded during midday are less correlated with the water stress degree. Preliminary results confirm that monitoring based on SF sensors can help for irrigation management.

Session 3 – Vascular Functioning

Christine Scoffoni

The dynamic multi-functionality of leaf water transport outside the xylem

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Understanding plant's physiological responses to drought is more critical than ever under our changing climate. While most studies have focused on severe and prolonged drought and their impact on plant mortality in natural systems, an equally important aspect to consider is that of mild drought in limiting growth, particularly in crop systems. While a surge of papers have reported low leaf vulnerability to xylem embolism during drought, I will here focus on the less studied, and more sensitive, outside-xylem leaf hydraulic responses to multiple internal and external conditions. Studies of 34 species have resolved substantial vulnerability to dehydration of the outside-xylem pathways, and studies of leaf hydraulic responses to light also implicate dynamic outside-xylem responses. Detailed experiments suggest these dynamic responses arise at least in part from strong control of radial water movement across the vein bundle sheath. While leaf xylem vulnerability may influence leaf and plant survival during extreme drought, outside-xylem dynamic responses are important for the control and resilience of water transport and leaf water status for gas exchange and growth. I will present some recent insights and experiments from my lab highlighting the dynamic hydraulic responses to mild drought at the leaf level, and how these can scale up to influencing plant growth.

Zuosinan Chen

High-resolution in-situ water flux and water isotope measurements in northern environments: do boreal trees use more summer rainfall or less when the winter snowfall is reduced?

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In boreal forests, the second largest biome worldwide, 65% of the water being 'pumped' from soil to the atmosphere (evapotranspiration) is done so through tree water use (transpiration). While boreal forests have evolved under highly seasonaly precipitation, the snow-rain seasonality in (pan-)Arctic regions is currently being altered with more apparent warming at high latitudes, including warming-induced shifts from snowfall to rainfall, more frequent rain-on-snow events, and less summer rainfall. These do not only directly change the amount and the timing of the water inputs to boreal forests with less early-spring snowmelt water and more variable summer rainfall, but could also alter the early-spring soil thermal profile, root water uptake function, early growing season tree growth, and ultimately affect growing season tree water use. However, it is still unclear how boreal tree water use (TWU) strategies respond to ongoing rapid changes in snow and rain at high latitudes. For estimating the amount, detecting the direction, and tracing the source of tree water use at high-resolution scales, the combination bi-directional sap flow measurements and the in-situ stable water isotope technique based on the direct vapor equilibrium method is the best way for ecohydrologists to elucidate tree water use strategies, especially to understand the process-based mechanisms. Therefore, for the first time in northern environments, we are applying in-situ continuous measurements of water fluxes (heat pulse sap flow sensors, soil moisture sensors, etc.) and stable water isotopes (non-destructive Water Isotope Probes and Picarro L2140-i isotope analyzer) along the entire water movement pathway of TWU, i.e., snow (snowmelt water), event-based rain (throughfall and stem flow), soil (vertical and horizontal), roots, and stems. These measurements are carried out in typical boreal forests in Finland around the Arctic Circle.

Timo Vesala

The Hidden Role of Gases in Trees

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We report on our efforts to understand the transport and physico-biological reactions of the different gases in trees. First, we have investigated the ability of trees to intake O_2 in stress conditions in a laboratory and in the field. Birch and oak (without aerenchyma tissues) resistance to lack of oxygen were investigated. Anoxic stress conditions were organized by flooding water and covering stem by parafilm which strongly reduces O_2 radial diffusion and uptake by lenticels. The results revealed effective axial diffusion of O_2 . The axial O_2 diffusion was sufficient to maintain trees life functions. Field measurements have been established in the pine-dominated wetland (bog). Sap flow, oxygen concentration in peat, CO_2 concentration in stem were placed for the locations with varying soil water content and water table. Second, we have investigated numerically the formation and stability of nanobubbles in the sap related. Phospholipids and glycolipids likely coat the surfaces of nanobubbles within sap, stabilizing them in several key respects: Crucially, phosphate groups are negatively charged, preventing bubble coalescence. Lipid coatings are somewhat elastic, stabilizing the bubbles with respect to embolism. We have used molecular dynamics simulations to investigate biologically relevant mixed monolayers, pulled at mild negative pressures. Increased surface tensions are observed with increasing area and decreasing temperature. Finally, the experiments and molecular dynamic simulations are aimed to be linked by parameterizations of the processes to be used in network simulations. On a centimeter scale at the xylem tissue level, we attempt to establish an analytical linkage between vessel network structure and fluid transport processes in the xylem. We use a combination of a vessel network topology model and a pore network modelling tool to simulate the transport of gases and sap in the xylem.

Luciano de Melo Silva

Gas diffusion kinetics in relation to embolism formation and propagation in angiosperm xylem: a mini-review of the latest experimental and modelling evidence

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Embolism formation in xylem conduits has been given considerable attention in the past as it relates to sap flow efficiency in vascular plants. Once embolism reduces the number of functional conduits in xylem, the sap flow to leaves decreases and reduces the plant's ability to supply water to leaves, ultimately affecting gas exchange. Although we do not fully understand the mechanisms behind embolism formation, gas, and especially gas-liquid interfaces, are shown to play an essential role. The gas dynamics in sap-filled and gas-filled vessels indicate that embolism spreading may represent a relatively slow process, and is largely but not exclusively pressure-driven. In flow-centrifuge experiments, xylem pressure is the most important determinant in initiating a given level of embolism in xylem conduits, but embolism propagation proved to be dynamic, involving multiphase interactions in conduits. Embolism spreads from an embolised conduit to a neighbouring, sap-filled one based on the pressure difference, but is also determined by bulk gas movement and gas diffusion, which is affected by temperature, time, and the proximity to large volumes of atmospheric gas. Moreover, there is evidence for diurnal patterns in the gas concentration of sap, with an oversaturation during warm periods. These findings raise questions on how plants can simultaneously deal with high levels of dissolved gas in sap and negative pressure. One potential explanation is the presence of lipid-coated nanobubbles in xylem sap, which function as a tiny gas reservoir, and may prevent dissolved gas from coming out of the solution and disrupt bonds between water molecules that are necessary for water transport under negative pressure. In this mini-review, we discuss the functional importance of gas diffusion kinetics in embolism formation and spreading based on a correlation between experimental and modelling evidence.

Willem Goossens

Foliar water uptake dynamics in shade and sun leaves of Fagus sylvatica *L*

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Climate change impacts various natural vegetation systems, as current predictions indicate an increase in frequency, duration, and intensity of erratic weather conditions. Prolonged drought episodes intensify stress responses of natural vegetation systems across many regions worldwide. Drought-sensitive tree species, such as European beech (*Fagus sylvatica* L.), could, therefore, lose their viability and dominant role to other co-existing trees. Fortunately, beech shows a high capacity for foliar water uptake (FWU), a process that enables the absorption of water through its leaves. These seemingly small water inputs, originating from leaf wetting events that occur on average at least 100 days a year across all ecoregions, may be the holy grail to beech's resilience and survival in the future. This phenomenon not only mitigates drought-induced stress at the plant level but also serves to partially compensate for annual transpiration in ecosystems. As only few reports deal with FWU in broadleaves, and often on saplings, we investigated the FWU dynamics in shade and sun leaves of mature European beech trees. FWU capacity, which can be defined as the maximum amount of water an area of leaf tissue can absorb, is a useful plant characteristic to estimate the benefit from leaf wetting events. However, this assumes that the tree canopy consists of uniform leaves with similar morpho-chemical characteristics . Therefore, submersion experiments were performed in sun and shade leaves of European beech, revealing a different uptake pattern in terms of rate and maximum FWU. To address this discrepancy, a model was proposed that normalizes FWU by leaf water potential, as it serves as the driving force behind the water uptake process.

Kris Kramer-Walter

Xylem uptake and mobilisation of exogenous sucrose in dormant kiwifruit canes

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During dormancy in kiwifruit there is a hiatus of xylem sap flow between leaf senescence in autumn and shortly before spring bud break. During winter chilling, starch in wood and bark tissues is hydrolysed back into soluble sugars. It is possible that under low chilling conditions there is a paucity of soluble sugar availability to support the metabolism of dormant buds. Xylem vessels and their neighbouring vessel associated cells release and absorb sugars from the apoplasm under regular cell functioning during active growth and have been shown to uptake sugars even during dormancy of deciduous species. We hypothesised that exogenous sucrose delivered via the xylem may support kiwifruit bud development under low chilling conditions. We excised canes in early winter after approximately 70 hours of chill accumulation. Treatment canes and low chill control canes received no additional chill, while high chill control canes received 1200 hours total chill. Control canes were not perfused, while treatment canes were perfused three time over four weeks with a range of sucrose concentrations (0 - 15%). Bark and wood carbohydrate concentrations were quantified before and after treatments, and canes were assessed for bud break and flower production. Canes perfused with sucrose solutions had increased wood and bark starch and sucrose concentrations but had lower concentrations of raffinose and stachyose in comparison to high-chill control canes. Spring bud break and flower production were not increased in perfused canes relative to low-chill controls. These results demonstrate that kiwifruit canes are capable of metabolising exogenous sugar delivered via xylem, but that the increased availability of sugar during dormancy is not a sufficient substitute for winter chilling to drive higher productivity in spring.

Kaat De Boeck

Impact of woody tissue photosynthesis on the hydraulic function of Platanus x acerifolia

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In green stem components such as bark, xylem rays and pith tissues, locally respired CO 2 is recaptured through woody tissue photosynthesis. The input of photosynthates resulting from this process into the trees' carbon balance is known to be particularly significant when leaf photosynthesis is compromised. However, apart from its role as local energy source, the inherent connection between phloem and xylem hints at a plausible role of green stem tissues in maintaining the hydraulic integrity of the tree. This might be especially important for urban trees that are facing more frequent and intense drought events in a changing climate. For this study, plane tree (*Platanus x acerifolia*) was chosen as the study species because of its prevalence in cities and its characteristic peeling bark, thereby revealing deeper and greener tissues. The aim of this research was to better understand whether woody tissue photosynthesis enables plane trees to maintain hydraulic function for an extended period during drought stress. To achieve this purpose, three plane trees (with a stem diameter of 4 cm at breast height) were subjected to a light-exclusion treatment while three other trees remained uncovered. Bench-top dehydration experiments were performed to establish acoustic vulnerability, desorption and stress-strain curves. These curves allow to link patterns of stem weight loss and stem shrinkage to the loss in hydraulic conductivity. Moreover, comparisons were made between estimates of P x (the water potential at which x% conductivity is lost), hydraulic capacitance and apparent elastic moduli of light-excluded and control trees. The findings of this study offer valuable perspectives on the importance of green woody tissues in maintaining hydraulic integrity longer during drought stress.

Louise Comas

Do lags in hydraulic time constants of sap flow through maize stems correspond to size of capacitance tissues?

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Lags in hydraulic time constants have been routinely observed across multiple species and sap flow sensor types but their interpretation has stimulated controversy. These lags have been construed as occurrences when water uptake through root systems was insufficient and stored water from plant capacitance tissues downstream of sap flow sensors was required to supplement transpiration. We show consistent 20-30-minute lags in hydraulic time constants in maize sap flow compared to continuous gravimetric transpiration measurements in young plants in pots in the greenhouse. Differences in transpiration from sap flow lags during the late morning were similar to those in the afternoon such that daily estimates of plant water use from sap flow sensors matched gravimetric measurements (R^2 = 0.89). In the field, we found larger lags in sap flow in mature plants with bigger stalks, on days with greater vapor pressure deficit (VPD) and solar radiation, on days prior to irrigation, and in deficit compared to full irrigation treatments. These patterns are consistent with an interpretation of lags in sap flow hydraulic time constants as the functioning of capacitance tissues during periods of high VPD and irradiance, and suggests that capacitance tissues are crucial for maintaining daily plant transpiration rates. Being able to identify genotypes with greater hydraulic capacitance tissues and breed for this trait could provide valuable new avenues for improving crop productivity.

Session 4 – Stress and Climate Change

Brendan Choat

Physiological mechanisms of drought-induced mortality in woody plants

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Severe droughts have caused widespread tree mortality across many forest biomes with profound impacts on ecosystem function and carbon balance. Climate change is expected to intensify regional scale droughts via the effects of higher temperatures and evaporative demand, with evidence suggesting that amplification of drought stress by anomalously high temperatures is already occurring. Recent extreme droughts in south-east Australia, the USA and Europe have killed hundreds of millions of trees over short time scales. These events have focused attention on the physiological underpinnings of drought-induced tree death. Catastrophic failure of the plant hydraulic system is now recognised as a principal mechanism associated with tree mortality during drought. Plant hydraulic failure is caused by the formation of gas emboli in the xylem conduits of leaves, roots and stems. Recent work has advanced our understanding of this process and identified species-specific physiological thresholds for tree death. I will explore our current understanding of tree response to drought, including the application of hydraulic failure thresholds to process-based models predicting mortality and developments in methodology used to evaluate the impacts of water stress on plant hydraulic function. In particular, the use of non-invasive imaging techniques such as micro-computed tomography has allowed novel insights into hydraulic failure and mechanisms of recovery after drought.

Phumudzo Tharaga

Detecting period of water stress among sweet cherry trees under rainfed conditions using sap flow during the fruit development stages

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Plant stress is one of the factors which have negative impacts on the development of and growth rate of sweet cherry trees which are not irrigated. Therefore, detecting the period at which stress occurs every season will help in putting precautionary measures on how to deal with stress in the future. Tree crops requires timely and accurate determination of crop water status for making decisions to either irrigate or not irrigation. However, commonly used techniques for measuring tree water status, such as stem water potential measured with pressure chambers or leaf stomatal conductance measured with porometers, are manually operated and lack the timeliness for making decisions in real-time. The objective of this research was to determine the period which trees experience water stress during the fruit development stage over three growing seasons. Replicated sensors were installed in experimental orchards planted with sweet cherry trees. Six trees were installed with thermal dissipation sap flow sensors. The data was only analysed for the months of September and October. Trees where usually stressed during the Schulk split and schulk fall fruit development stages of sweet cherry fruits. During the 2017, 2028 and 2019 seasons, low water availability was recorded. The transpiration rates decreased with a depletion in soil moisture as this is the period of very low rainfall and the hourly sap flow rate dropped drastically. During the flowering stage transpiration rates where 12 l/hour and during the Schulk split and fall stages transpiration rates were 6 l/hour, half of what the trees where using during the early stages of fruit development. Water stress during this stage led to loss of fruit, as shrinking was observed in most of the fruits and they fell without full development. Irrigation will be required in this area during the intensive fruit development stage in order to prevent fruit losses.

Zeshan Zhang

Comparing AquaCrop simulated transpirations with sap flow measurements in cotton under drip irrigation and plastic film cover condition

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Moisture-driven crop simulation models are commonly employed to evaluate crop yields and irrigation management strategies to improve agricultural water productivity. The objective of this study was to assess the capability of the AquaCrop model to simulate the performance of cotton under different row spacing and topping methods under plastic film cover and drip irrigation in northern Xinjiang, China. The performance of the model for simulating transpiration was assessed by comparing it to transpiration data measured using a heat ratio sap flow meter in a field condition. The findings of the two-year experiment (2020-2021) demonstrated that the model could adequately simulate canopy cover with a normalized root mean square error (nRMSE) of less than 2 and a model efficiency (EF) close to 1. The model simulation of transpiration obtained a good agreement with a 4.4 of nRMSE across all years and treatments. However, the simulated transpiration was higher than that measured using sap flow meter with an EF of 0.3 and a coefficient of residual mass (CRM) of -32%. That might be due to the 20% of overestimation of the crop coefficient (KC) in the model under plastic film cover condition, when soil evaporation was strongly limited. In addition, air temperature, vapor pressure difference, and radiation had positive effects on cotton transpiration while humidity had negative effects. The model could capture the trends between simulated transpiration and climate factors, but stronger than the field sap flow measurements. The overestimation of model simulation might be caused by poor performance of the model under plastic film cover and drip irrigation condition. Across all years and treatments, the model simulated water use efficiency (WUE, 4.70 g m^{-2} mm^{-1}) and water productivity (WP, 22.9 g m^{-2}) of cotton were lower than those calculated from actual measurements, with WUE of 5.42 g m^{-2} mm^{-1} and WP of 25.9 g m^{-2} . In conclusion, the AquaCrop model could be useful in evaluating the effectiveness of cotton irrigation strategies in arid zones. However, further refinement is necessary to improve the simulation accuracy under plastic film cover and drip irrigation conditions.

Wakana Azuma

The daily use of stem-water storage in two clonal cultivars of Japanese cedar estimated from sap flow and dendrometer

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There is concern that Japanese cedar (*Cryptomeria japonica*), which accounts for about 40% of plantation forest in Japan, will become unsuitable for future growth due to air drying caused by climate change. On the other hand, since trees cannot move and are long-lived, they are thought to acclimate to their environment and routinely use not only sap flow through the conducting tissues of their trunks but also water stored in their living tissues to regulate water relations from roots to leaves. To examine whole-tree water use in relation to internal stem-water storage, we measured the crown and basal sap flow using Granier sensors and point dendrometer in two clonal cultivars of Japanese cedars. The study was conducted from July, 2021 to March, 2023 at the Tano Field Science Station in Miyazaki, southern Japan. If a time lag in the diurnal peak of sap flow between the crown and the base of tree trunks is observed, it can be assumed a proxy for the use of stem-water storage that could complement transpiration. Although a time lag in sap flow was observed for both cultivars, the time lag values differed between them, suggesting that the contribution of stem-stored water was different even for the same species. Stem radial expansion and contraction during the daily cycle was observed in both cultivars, suggesting the contribution of stored water in the inner bark. The amplitude of diurnal variation was the same at the crown and the base of one cultivar, while the canopy was greater than the base in the other. When a heating experiment was conducted at the base of the trunk, the amplitude of diurnal variation was greater for both cultivars. This suggests that higher temperature may increase the daily use of stem-water storage in Japanese cedar.

Robert Skelton

Root stratification and small leaf sizes promote function of co-occurring shrubs during dry summer periods in a diverse shrubland

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Shrublands contain high species richness, but low above-ground growth form diversity, which is often attributed to convergent evolution driven by functional convergence. For example, it is thought that shrub species in more arid climates tend to have smaller leaves to drive sensible heat loss during drier periods. Yet, species with similar aboveground growth forms (e.g., leaf size) often have contrasting below-ground traits (e.g., varying rooting depth), raising the possibility that we do not fully understand the relationship between form and function. Here, we use a diverse shrubland community where species have similar aboveground growth forms, but contrasting below-ground traits, to examine the hypothesis that small leaf size is an adaptation to dry summer periods and that root stratification might promote separation of soil moisture resources.

To do so, we examined the dynamics of sap movement within unrelated plants of the same above-ground growth form (similar leaf sizes) but with dissimilar rooting depths. We tracked *in situ* physiological changes in five sample species, representing dominant species of the renosterveld shrubland using miniature external (i.e., non-invasive) sap flow technology. Sensors placed on roots and small branches allowed us to explore within individual sap flow dynamics. *Elytropappus gnaphaloides* showed moderate xylem water potentials despite high conductances, suggesting that individuals have deep roots with access to deep soil water. *Anthospermum* and *Diosma* showed low xylem water potentials and low conductances, suggesting that individuals have shallow roots and are highly reliant upon regular summer rainfall events. Future reductions in rainfall amounts - or changes in rainfall seasonality that are associated with less summer rainfall - are most likely to negatively impact growth in shallow-rooted species, while deeper-rooted small leaved shrubs are likely to be more resilient to the impacts of climate change.

Session 5 – Methodology

Josef Urban

Remembering Professor Jan Čermák

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Josef has kindly agreed to lead a reflection on the contributions of Professor Jan Čermák to the discipline of tree physiology. Professor Čermák, who was well known to many who have attended this workshop series, passed away on December 23, 2021.

Georgianne Moore

Developing a robust tree-specific in situ calibration for thermal dissipation sap flow sensors

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There is a growing recognition that uncalibrated use of thermal dissipation (TD) sap flow sensors impacts measurement accuracy. Thus, species-specific corrections are becoming more common to better leverage this method's advantages for use in forest transpiration studies. However, there is a need to explain which species-specific attributes (e.g., vessel arrangement and other anatomical traits) or more generalizable attributes (e.g., age, size, and radial variation) cause discrepancies in measurements, since these factors affect the physics of heat dissipation within the wood. Particularly for trees with steep radial gradients in sap flux across the length of the sensor, the characterization of fine-scale radial variability could lead to the development of a robust tree-specific calibration. The goal of this study was to develop a novel field methodology using a movable TD sensor to construct characteristic curves that correct estimates of sap flux density (J_s) *in situ*. We tested the hypothesis that radial profile shapes are systematically linked to deviations from Granier's original empirical relationship using diffuse porous *Liquidambar styraciflua* trees (n=10) in East Texas, USA. Using a mobile 10-mm TD heater sensor, the radial profile shape was characterized for individual trees by inserting and moving the probe at various 1-mm depth increments, repeating trials to validate the method. Although the gaussian radial profile shape was repeatable across replicates, the depth to peak flow differed greatly between trees, indicating that knowledge of depth to peak is critical for sensor placement in replicate trees of the same species. We also found evidence to support our hypothesis that steeper gradients led to greater measurement errors and that radial profile shape may at least partially explain whether the original Granier relationship under- or over-estimated flows. This novel field approach demonstrates high potential for improving J_s estimates through precision placement of TD sensors.

Matthew Rennie

Flexible external heat-pulse sap flow sensor for bi-directional measurement of sap-flow in small-diameter stems of Populus alba and Betula pendula

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As sap flow research expands new challenges arise when examining the dynamics of saplings, or plant parts with small dimensions. Coupled with species that in response to air temperatures will display bi-directional flows, the need for non-invasive, low-power sap flow sensors becomes more important. At present, most sap flow sensors are probe-based and are therefore inappropriate for the direct measurement of sap flow in saplings. While external non-invasive sap flow sensors have been shown to measure bi-directional flow in trees with diameters less than 10 mm, they were based on the heat ratio method derived from a line heat source. This presents the potential to create localized heating around the heat source, disrupting the natural flow of sap within the tree. Therefore, in this study, we propose a non-invasive sap flow sensor consisting of a ring heat source heating the circumference of the stem along with a modified heat ratio method. Calibration of the measured heat pulse velocity was conducted by gravimetric measurement of an imposed flow through sections of stem. Two species were examined - white poplar (*Populus alba*), and paper birch (*Betula pendula*). Preliminary results of the sap flow sensor show linear agreement of both species, with their imposed flow, ranging between -20 to 20 g h^{-1}.

Venkatraman Srinivasan

The role of noise on the accuracy of different sap-flow measurements using heat pulse techniques

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Heat-pulse (HP) methods such as; i) the compensation heat pulse method (CHP), ii) the T-max method, iii) the heat ratio method (HR) method, and other variations of these methods have been widely used in sap flow meters to measure plant water. Although all these methods are based on Marshall's theory, in practice, their accuracy varies depending on the rates of sap flow. While the CHP method is accurate under moderate-high sap flow rates, the HR method performs better under low and reverse sap flow rates, and the T-max method is accurate under high sap flow rates. Here we use mathematical model simulations to show that the presence of noise in temperature measurements plays a critical role in determining the accuracy of HP methods. We show that in the absence of noise, all HP methods perform well under a range of sap flow velocities. However, in the presence of noise, the accuracy of the different HP methods suffers depending on the sap flow rates, the thermal diffusivity of the sapwood, and the magnitude of the noise. While conventional HP measurements log temperature data every second for about 3 minutes at upstream and/or downstream locations following an HP, most HP methods only use a few chosen data points to estimate sap flow velocity. When the signal-to-noise ratio of these chosen data points is small, or when the model sensitivity to these temperature inputs is large, the prediction accuracy suffers. Using an approach similar to the Sapflow+ method that uses all the logged temperature data, we show that the errors in sap flow estimation due to noise can be virtually eliminated over the entire parameter space. The stochastic framework presented in this paper can be used to provide quantitative error estimates of HP methods under different parameter conditions.

Steve Green

Optimization of heat-pulse methods to measure sap flow in kiwifruit

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Actinidia (Kiwifruit) is a diverse genus of dioecious, deciduous, climbing plants. The vines tend to have large leaf areas compared to relatively small trunk cross section, and this leads to very high sap flux densities (>300 $cm^3/cm^2/h$) during the middle part of the day. Furthermore, the sapwood also has large and sparse xylem vessels that could disrupt the flow of heat around the measurement probes. These factors present a practical challenge to measuring sap flow using heat-pulse methods. This paper summarises a series of calibration tests that were set up to verify the accuracy of the Tmax heat-pulse method. The tests were done under controlled conditions, in the laboratory, by forcing water at known rates through excised sections of kiwifruit stems. A range of flow rates were established by varying the pressure gradient across the stem sections. Results are compared against theoretical calibrations from a 2-d numerical model for heat and water flow. This study confirms a number of factors related to the Tmax heat-pulse method.

- The Tmax method is accurate (+/- 10%) across a wide range of sap flux densities, from almost zero up to more than 1000 cm/hr. We are not aware of any other heat-pulse method that can resolve such high flow rates.
- The appropriate wound width for the 1.6 mm stainless-steel sensors, was calculated to be in the vicinity of 2.0 to 2.4 mm, which seems realistic for this 'physical parameter'.
- Two-channel sensors are sufficient to obtain reliable and accurate measurements of tru nk sap flow in kiwifruit stems of < 10 cm diameter.
- Experience shows the only other practical factors to consider are variability in the stem and carefu l installation of sensors in the field situation.

Session 6 – Vascular Functioning

Cate Macinnis-Ng

Saving some for later: Seasonal variations in sap flow, withdrawal and elastic storage in large trees under throughfall exclusion

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Transpiration is influenced by vapour pressure deficit, solar radiation and soil moisture but these factors fluctuate at different time scales ranging from hours to days to weeks and months. Furthermore, when using sap flow to infer transpiration, withdrawal from stem water stores complicates estimates of timing and amounts of transpiration, particularly in large trees. Kauri (*Agathis australis*) is one of the largest and longest-lived conifers in the world with sapwood depths of up to 20 cm. Published P50 values indicate stems and roots are highly vulnerable to xylem embolism and regular summer droughts are increasing in intensity and frequency with climate change. We established a thoughfall exclusion experiment in kauri forest in West Auckland, Aotearoa New Zealand to explore responses of water relations to drying soils in mature trees. Our three year experiment indicated sap flow was responsive to soil moisture at seasonal time-steps.

Seasonal sap flow significantly increased as seasonal soil moisture increased but the slope of the curve was steeper for control compared to drought trees. Stem water withdrawal was a higher proportion of daily water use for drought trees (8-28%) compared to control trees (5-12%) but the volume of withdrawal (in L) increased as seasonal tree water use (per unit sapwood area) increased. Elastic storage increased as stem water withdrawal increased.

These results indicate the importance of withdrawal and elastic storage in large trees during drought conditions and is likely reducing the incidence of embolism in these iconic trees. Analysing relationships between seasonal soil moisture and seasonal sap flow provides a clearer picture of the influence of soil moisture on sap flow patterns.

Sicong Gao

Evaluating the relationship between sun-induced chlorophyll fluorescence and transpiration in sparse Australian floodplain woodlands using sap flow measurements

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Floodplain vegetation is experiencing more severe drought and extreme weather events due to climate change. Ensuring vegetation has a sufficient water supply is of considerable concern to sustain ecosystem vitality, condition and function. Transpiration serves as an effective factor to monitor tree water use, offering insights into vegetation function under various environmental pressures, including water and heat stress. Sun-induced chlorophyll fluorescence (SIF), emitted during photosynthesis, plays a pivotal role in indicating carbon assimilation and water uptake. It could also be used as a surrogate to transpiration measurements to understand tree plant water relations. Previous studies have demonstrated a strong correlation between SIF and transpiration in densely forested regions, however, the sparse structure of floodplain woodlands introduces uncertainties into this relationship by increasing noise in the SIF measurement. To evaluate this relationship further, three open woodland sites with varying fractional vegetation cover (sparse, moderate-sparse and normal) were chosen. Ten sap flow loggers were installed on six trees to measure transpiration within a 50 m^2 plot for each site. A three-dimensional radiative transfer model, FluorFLiES, was applied to simulate full spectrum SIF by incorporating LiDAR data to accurately model woodland vegetation structure. Five specific SIF bands, ranging from red to near-infrared, were selected to quantify their correlation with field measured transpiration. The coefficient of determination of the simulated far-red SIF and field measured transpiration was 0.85 and 0.93 at hourly and monthly scales, respectively. Across all SIF bands, the correlation between SIF and transpiration improved with increasing fractional vegetation cover. However, performance of red SIF for prediction of transpiration decreased 24% when assessing red SIF emissions from leaf scale to canopy scale. In summary, this study can be applied regionally to predict transpiration using remotely sensed SIF datasets in the absence of *in-situ* data or in areas of low data availability or accessibility.

Katrien Schaepdryver

Quantification of sap flux density and stem water content of oak and beech by using the Sapflow+ method

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The accurate determination of sap flow and stem water content is essential for understanding plant-water relations. Advancing our knowledge of tree transpiration, encompassing various tree species and environmental conditions, is crucial, particularly in the face of increased drought events driven by climate change. In this study, we used the nonempirical heat-pulse-based Sapflow+ method to quantify sap flux density and stem water content in two ecologically and economically important tree species in temperate climates: *Fagus sylvatica* L., characterized by diffuse porous wood, and *Quercus robur* L., characterized by ring porous wood. The Sapflow+ method relies on measuring temperature changes around a linear heater in both axial and tangential directions following the application of a heat pulse. By fitting the appropriate heat conduction-convection equation to the measured temperature profiles, sap flow densities can be accurately determined. Originally, this method requires a labour-intensive, sensor-specific calibration step to precisely determine the heat input using a medium with known thermal characteristics. In this study, we propose an improvement to the Sapflow+ method that eliminates the need for heat input in the model, thereby bypassing the time-consuming sensor calibration step. To validate this model improvement, we collected data on beech and oak trees and compared sap flow and stem water content between the two species. These results contribute to our understanding of plant-water relations and provide valuable insights for further research.

James Robinson

Examining the effect of sap sugar concentration on fibre embolism in sugar maple (Acer saccharum)

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Sugar maple (*Acer saccharum*) develops elevated pressures in response to repeated cycles of freezing and thawing. This pressure is theorised to develop due to compression of gas present within fibres. Due to surface tension the pressurised gas within fibres should rapidly dissolve. That the gas persists over time is believed to be due to a supposed osmotic barrier present between fibres and vessels that prevents sucrose from diffusing into fibres. This creates sufficient osmotic pressure to prevent gas dissolution and so maintain fibre embolisms. In our work we examine this hypothesis using synchrotron based microCT to produce high-resolution three-dimensional images of sectioned stem segments. Using this technique, we directly resolved the gas present in the fibres. Subsequently we perfused stem segments with either water or 2% sucrose and re-imaged them to examine any changes in fibre embolisms. Additionally, we also looked at samples that were frozen for 2-3 months to promote fibre embolism development, and for contrast we look at paper birch (*Betula pendula*), a species which develops elevated stem pressures through an entirely different mechanism than *Acer*. From the fresh stem segments, we observed fibre embolisms are indeed present, and that when perfused with sucrose solution there is little to no change in fibre embolisms, whereas in almost all cases perfusing with water led to partial or complete refilling of fibre embolisms, consistent with the presence of an osmotic gradient. The frozen samples did not display complete xylem embolization, in contrast to expectations, and showed complete refilling upon perfusion with either solution, suggesting cell damage had occurred. The birch samples also showed fibre embolisms. These embolisms remained after perfusing with sucrose solution, and there was some evidence the fibre embolisms refilled upon perfusion with water, however more samples are required to confirm these observations.

David Moore

Modeling Winter-Dormant-Season Sap Flow and Sap Pressurization With Wood Temperature in Deciduous, Woody Angiosperms in New England

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During winter dormancy, when deciduous trees are leafless, sap often still flows even though there are no stomates to provide an interface between trees' vascular systems and the atmosphere. Furthermore, some of these trees have methods for repairing embolisms (gas bubbles in the xylem vessels and tracheids that restrict sap flow) whereby the pressure in the xylem increases substantially to cause the redissolution of gas bubbles into the sap, and this process also occurs in winter dormancy in a handful of deciduous, woody angiosperms. Industries such as the maple syrup industry in northeastern North America and the birch sap industry in parts of Europe rely on this winter-dormant-season pressurization phenomenon, as it allows for sap harvesting. In the absence of stomatal transpiration, other biological or physical mechanisms cause sap to flow and to pressurize. During winter dormancy in 2019, 2020, and 2021, species from 17 different genera of deciduous, woody angiosperms (*Acer*, *Actinidia*, *Alnus*, *Betula*, *Carpinus*, *Carya*, *Fagus*, *Fraxinus*, *Liriodendron*, *Ostrya*, *Platanus*, *Populus*, *Robinia*, *Salix*, *Sassafras*, *Tilia*, and *Ulmus*) were monitored continuously for sap flow and wood temperature, and a subset of these genera (*Acer*, *Alnus*, *Betula*, *Carpinus*, *Fagus*, *Platanus*, and *Tilia*) were monitored for sap pressure concurrently. Wood temperature is a very strong predictor of winter-dormant-season sap flow and sap pressurization in some species of deciduous, woody angiosperms, whereas variables other than temperature may be stronger predictors of winter-dormant-season sap flow and sap pressurization in other species. While the underlying anatomical and physiological mechanisms for this winter-dormant-season sap activity are at least somewhat understood for some genera (*Acer*, *Betula*, and *Juglans*), they are still relatively unknown for most of these other genera, and this research increases the understanding about these economically important processes.

Christopher Vincent

How dynamic is phloem speed in trees?

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Dynamic behavior of vascular transport is important in predicting responses to climate change or hypothesizing the impacts of crop production practices or genetic gains through specific traits. Phloem transport speeds in particular have been difficult to measure. Few environmental or other dynamic responses have been measured. Radiolabeling offers the opportunity to measure transport speeds. Although data are still sparse and concentrated in a relatively small number of species, most experiments have failed to find dynamic shifts in phloem transport speeds. In various ^{14}C-based experiments related to source-sink dynamics of *Citrus* spp., we have shown important shifts in direction or total photosynthate availability, but have not found differences in stem transport speeds, except in the case of a phloem-limiting disease (Citrus greening, aka. huanglongbing). Only under very specific conditions have we been able to find changes in speeds of longleaf pine needles. Other researchers have found similar results in girdling studies of *Quercus rubus*. Some models of phloem function suggest that phloem transport speed may not be highly variable, at least in the short term. There are still important unanswered questions such as the variation in speed ranges measured in different experiments. However, the lack of short-term manipulability of woody plant transport speeds suggests an important role of intermediate storage organs in maintaining photosynthate supply to sinks and the possibility that structural traits may be the limiting factor in transport speeds of trees.

Damien Sellier

A numerical model of coupled phloem-xylem flows for dynamic long-distance transport in trees

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In trees, the vascular system is dual in structure and function. Sap flows upwards in the xylem to hydrate tissues and refill reserves from transpiration loss. In the leaves, some of the water recirculates into the phloem and sap, loaded with photoassimilates, flows downwards to supply tissues with carbohydrates. Flow and counter-flow occur in physically separated but hydraulically connected pathways. Water exchanges take place all along them. Understanding the entire system and its subprocesses is essential to precisely quantify the carbon-water fluxes at the soil-atmosphere interface. That understanding is also key to predict the functional limits of vascular transport and, when dysfunction occurs, how it will impact the vitality of plant communities subject to drought events and foliar pathogen outbreaks. Here we present an integrated, spatially explicit model of phloem-xylem transport. The model implements Münch's osmo-regulated pressure flow hypothesis for phloem transport and cohesion-tension for xylem transport. The changes of phloem pressure, carbohydrate concentration and xylem pressure over time are governed by three coupled nonlinear partial differential equations. Sap flow velocity, volume flow and the amount of shrinkage and swelling are calculated as derived variables. The model uses a special-purpose numerical scheme and can simulate response to dynamic forcing such as the diurnal patterns of phloem loading and transpiration. Unlike in other models, transport equations are solved for a surface and account for tangential movement of water and carbohydrates. Phloem and xylem are treated as elasto-porous media with distributed hydraulic and mechanical properties. We also present a semi-automated image processing method to calculate the theoretical hydraulic conductivity of phloem and xylem tissues based on their anatomy. Key hydraulic characteristics are given for the phloem of juvenile *Pinus radiata* D. Don.

Session 7 – Stress and Climate Change

Rafael Poyatos

Sap flow monitoring in environmental research networks: lessons learned from SAPFLUXNET and challenges for the future

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In only four years since its first publication, the SAPFLUXNET global database of sap flow measurements has opened the door to a new, biogeographical perspective of tree water use for ecologists, modellers and the remote sensing community. Here, we will summarise what we have learnt from SAPFLUXNET and how this initiative can continue improving our understanding of tree water use and drought responses globally. Across forest biomes, we have shown that vapour pressure deficit (VPD) is the major determinant of tree water use regulation. The interspecific diversity of such regulation can be partly explained by climate, water relations traits and tree size. Within species, we have observed that stand structure overshadows climatic and soil factors in determining tree water use responses to VPD. When looking at specific drought events, SAPFLUXNET also provides the opportunity to investigate the patterns and underlying mechanisms of tree water use resilience to drought. At the ecosystem scale and beyond, SAPFLUXNET has been used to benchmark transpiration algorithms and models, including those using eddy covariance or remote sensing data. Looking into the future, SAPFLUXNET needs to update its data infrastructure to allow continuous contribution of new datasets and additional variables, to refine the data and metadata requirements to maximise the potential of data syntheses and to improve the integration with existing and new ecophysiological networks. More efforts should be devoted to gradually turn SAPFLUXNET into a real grass-roots network to share methodological innovations, guide and promote collaborative data syntheses and overcome the lack of long-term funding. We argue that the International Workshop on Sap Flow can become one of the key components of this strategy of scientific community building towards a more decentralised and resilient network.

Josef Urban

Effect of forest canopy density on water relations and carbon assimilation of understory herbs during drought

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Forest canopy creates a living environment for understory plants. Trees manipulate the availability of light and water for herbaceous species. A dense canopy may lower the surface temperatures of herbs' leaves and help them to survive drought and heat waves. To test this hypothesis, we conducted an in-situ experiment on 13 species of herbs from the temperate oak forest with various degrees of canopy density. We found, that predawn leaf water potential was less negative in the sparse than in the dense forest. In other words, the water availability was better in the sparse forest. The difference between predawn and midday water potential was usually larger in the sparse than in the dense forest. It was a result of higher transpiration due to higher leaf irradiance in a sparse forest. Still, the stomatal conductance and photosynthesis were also higher in the sparse that in the dense forest. To get a better mechanistic understanding, we conducted a greenhouse experiment on a subset of species. The plants better survived a dry heatwave in the simulated sparse forest than in the dense forest. The reason for lower mortality in a simulated sparse forest was probably the ability to maintain high levels of soluble carbohydrates. They increased the osmotic water potential and maintained plants' metabolism when the stomata were closed. Especially in the sparse forest, the herb layer represents a significant portion of a leaf area. Any changes in its composition and cover may therefore affect transpiration from a whole forest stand.

Rafael Poyatos

Water use strategies in pines and oaks across the globe are modulated by soil water availability

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Temperate forests and Mediterranean woodlands of the northern hemisphere are dominated by the tree species in the Pinaceae and Fagaceae families. A recent increase in the dominance of Fagaceae has been reported worldwide, related to changes in management and disturbance regimes. Contrasting ecological strategies between the two families can also mediate these biogeographical shifts, particularly in response to the increasing drought conditions associated to climate change. Within these families, *Pinus* and *Quercus* species are the most abundant and probably the most economically and ecologically relevant. Pines and oaks frequently co-occur and display contrasting water use strategies, linked to different functional traits related to water use. However, water use responses to drought of pines and oaks has never been compared at the global scale. We use the global sap flow database SAPFLUXNET to test whether pines show a stricter control of water use in response to drought compared to oaks. The final data set consisted of almost 500 trees belonging to 16 oak and 11 pine species, from 47 study sites across Europe, Asia, and America. A linear mixed-effects model was fitted, with daytime-aggregated sap flow per basal area as the response variable and air vapor pressure deficit (VPD), soil water content (SWC) and genus as the interacting explanatory variables. When soils were dry, oak species showed higher sap flow at a given VPD but this pattern reversed under wetter soil conditions, when pines showed higher sap flow. This shift in water use responses remained when a subset of the data with co-existing pines and oaks was analysed and the SWC threshold for the shift was at around 10%. Our results show that oaks have a competitive advantage over pines under drier soils, which can be mediating the increased abundance of oaks in many temperate regions of the World.

Kazuhiro Nishioka

Exploring the potential of acetic acid as a biostimulant to reduce water loss in grapevines in dry condition using advanced sap flow sensors

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As the severity of global climate change intensifies, agricultural farmers are confronted with the pressing need to identify adaptive strategies. A significant challenge is the escalating water stress experienced by crops in dry farming, posing a threat to agricultural sustainability and productivity on a global scale. Recent studies have demonstrated that acetic acid can confer drought tolerance to plants by influencing plant hormone metabolism, leading to stomatal closure on the undersides of leaves where water loss occurs. This effect appears to be more potent than that of other substances. If confirmed, the efficacy of acetic acid in dry farming could offer an innovative approach to mitigating the declared impacts of climate change on agriculture. Consequently, this study focuses on wine grapes, often cultivated using irrigation technology in arid or semi-arid regions, to investigate the effectiveness of acetic acid as a biostimulant in reducing water loss from grapevines. We irrigated vines under dry conditions in a vineyard with a diluted acetic acid solution, targeting the base of vines, and evaluated the impact of acetic acid on stomatal responses to environmental factors. The stomatal response was assessed by monitoring gas conductance behavior, utilizing an advanced thin-film sap flow sensor that we designed and fabricated. This research represents a significant advancement in the exploration of adaptation strategies for viticulture in the face of climate change. It not only provides insights into the effectiveness of acetic acid as a biostimulant but also contributes to water conservation in valuable agricultural water resources.

Eduardo Barragan

Pit membrane thickness variation across vein orders and species: Impact on drought tolerance

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Intervessel pit membranes are important components of a plant's vascular tissue, providing both a pathway for water movement between xylem conduits, and acting as a barrier preventing the spread of emboli or pathogens. While past studies have focused on the role and variation of pit membranes in stem physiology, little is known of how these vary across vein orders in leaves. Here, we investigate for the first time how pit membrane thickness (T_{PM}) varies across vein orders of diverse species, and its relation to leaf function and drought tolerance. We quantified pit membrane thickness, xylem conduit size, secondary wall thickness and vein density for the midrib, tertiary and minor veins of eight diverse species. We constructed pressure-volume curves, leaf hydraulic and stomatal vulnerability curves to dehydration. We found significant variation in T_{PM} across species, and vein orders. While midrib T_{PM} was significantly thicker than that of tertiary or minor veins, no significant differences were found between the tertiary and minor T_{PM}. Additionally, T_{PM} scaled across vein orders. The helicoidal structure of the vein conduits for three species was so pronounced that secondary walls (and pit membranes) were not visible in our samples. We could not resolve a link between T_{PM} and anatomy or physiology. However, our data revealed a strong coordination of turgor loss point and TPM for all vein orders, pointing to a role of pit membrane anatomy in shaping the drought tolerance of leaves. Our results are in accordance with previous studies showing a link between petiole or midrib T_{PM} and resistance to embolism formation. Together, our data demonstrate the importance of pit membrane diversity across species and vein orders towards enabling drought tolerance across species. Future studies should explore the role of the exposed primary walls on hydraulic transport and embolism, which appear predominant in some species.

Yi Chen

In vivo measurement of potassium ions in pine xylem sap with implanted bioelectronics

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Plant growth is the sum of the response to environmental stimuli and resource availability. The challenge in monitoring the tree growth status of individual trees is the continuous measurement of real-time internal physiological parameters over the long-term. The field of bioelectronics, such as organic electrochemical transistor (OECT) has witnessed the rapid development of miniaturised in vivo implantable sensors minimising wound damage. These OECT-based sensors have been used to provide physiological data for human health. Thanks to a recently funded Smart Idea project by the Ministry of Business, Innovation and Employment (MBIE), we have started to explore the possibility to adapt these OECT sensors to monitor tree physiological parameters. Our first sensor targets the detection of potassium cation [K+], the major cation in plant cytoplasm and sap. Potassium is crucial for xylogenesis (wood formation), fruit yield and post stress recovery. Our team successfully implanted an OECT sensor, monitoring the potassium cation concentration in softwood tree (radiata pine) xylem. This sensor provided real-time (every 10 s) in vivo measurement of xylem's sap potassium ions concentration on the long-term (over one month). It revealed a pattern of daily sap [K+] fluctuations. Furthermore, to improve the long-term integration of the sensor we are working on the improving wound healing as well as the biocompatibility of the sensors. With this purpose we are now working on tree wound response mitigation and development of bio-based materials for the implantable sensor. We anticipate that the outcome of this work will help to address fundamental plant physiology questions related to the cycles of this cation between foliage and root. We foresee the impact of this research will be to enable new tools to monitor plant health for the digitalisation of forest nurseries, precision agro-forestry and horticulture.

Yongfan Chen

Crop transpiration reveals opportunities to reduce yield risks when applying harvest aids to cotton for efficient mechanical harvesting

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Chemical defoliation is an essential pre-harvest practice in machine-harvested cotton production in China. It can effectively improve the quality of cotton by reducing the remaining green leaves and unopened bolls at harvest time. Due to the unstable climate at harvest time, it is difficult to accurately determine the spraying time of chemical defoliants (harvest aids) in machine-harvested cotton. To be able to optimize the time for rapid leaf abscission in cotton without loss of yield, we investigated th e time from the formation of the cotton abscission zone (AZ) to leaf abscission in response to harvest aids using sap flow measurements, and the consequences for yield. Field experiments were carried out in 2020-2021 in Xinjiang, China. The treatments consisted of a defoliant treatment with harvest aids (a mixture of 10% thidiazuron and 40% ethephon) sprayed in mid-September and water as a control, under four typical sowing patterns. Sap flow was measured using a Heat Ratio Method (HRM) during the defoliation period and then calculated as transpiration per unit ground area and per unit leaf area. At harvest time, the boll opening percentage in the defoliation treatment (84.9%) was high er than the control (77.3%), resulting in a 14.4% higher cotton lint yield. The application of harvest aids accelerated the defoliation process, resulting in a final defoliation rate 33.5% higher than the con trol over two years. Row spacing and plant density did not affect cotton lint yield, defoliation rate, and boll opening rate. Daily transpiration per unit ground area under the harvest aids treatment was 1.26 mm d^{-1} and 64.3 % lower than in the control (2.07 mm d^{-1}), and the daily transpiration per unit leaf area was 11% lower than the control. The daily transpiration per unit ground area did not ch ange significantly in the first four to five days after spraying, and then started to decrease rapidly. Th is is the time needed from the petiole abscission layer form to leaf drop after spraying. The results of this study would promote cotton mechanical harvesting by applying harvest aids earlier to avoid th e unstable climate and ensure timely leaf drop without loss of yield.

Taketo Kogire

Diurnal and seasonal changes in acoustic emissions and sap flow in living tree trunks

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In living tree trunks, cavitation occurs in the water-conducting tissue, leading to embolism. The occurrence of embolism can be evaluated by detecting Acoustic emission (AE), which is emitted when cavitation occurs, but long-term measurements have rarely been conducted to understand the factors and patterns of embolism. As tree sap is essential for the transport of nutrients and phytohormones within the tree, elucidating its variation is fundamental to understanding the physiological survival strategies of trees. The aim of this study is to infer variation of sap flow cavitation in saplings and mature trees. We used an olive sapling and a mature ash tree in our experiment, which was conducted at Kobe University, Japan from May to December 2021. The number of AE events was recorded at 10-minute intervals, and the sap flow velocity was measured using a heat flux sensor, both non-destructive measurements. In the olive sapling, few AE events were observed after the first irrigation, and the number of AE events gradually increased after 27 days. When irrigation was applied during this period of AE generations, AE events decreased temporarily, but then reappeared soon after. As described above, we confirmed that the number of AE events in an olive sapling changes in response to irrigation. In a mature ash tree, the daily cycle of AE events was synchronized with the sap flow rate during the summer months of June-August, but in September AE events began to occur at night, and in November-December AE became more irregular.

Renee Prokopavicius

The mysterious lives of urban street trees: How does drought affect tree function and performance?

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Cities have hotter, drier air than the surrounding landscape, and as climate change intensifies, the health of urban trees will be increasingly impacted. We found extensive crown dieback in 13 of 23 urban tree and shrub species in Sydney, Australia during the record-breaking austral 2019-2020 summer. To identify the short- and longer-term effects of drought stress on urban trees, we measured tree ecophysiology, recovery, and growth in the extreme year and the following three wet summers of 2020 to 2023. Heat stress, rather than drought stress, was a larger threat to urban tree survival. Evidence of catastrophic hydraulic failure was limited to four of 148 trees, but it was unclear why certain individual trees were more prone to drought stress. We found that 11 species had significantly lower predawn leaf water potentials (Ψ_{pre}) in the extreme summer, relative to subsequent wet years, whereas Ψ_{pre} was relatively stable across years for 12 species. Responses were not always consistent within species, however. Urban trees with less water access in the extreme summer were more likely to surpass their turgor loss point and reach the point of stomatal closure. Drought-stressed trees had modest levels of chronic photoinhibition and lower Ψ_{pre} than unstressed trees in the extreme year, plus lower relative height growth rates over the first two years of recovery. Recovery in terms of reduction in crown dieback (mean < 25%) required one year for four species but two wet years for nine other vulnerable species. Trees with high levels of crown dieback were less likely to achieve positive height growth within a two-year recovery period. Drought stress clearly affects the life and death of urban trees, but plant water availability in urban environments is heterogeneous in space and time, requiring repeated measurement of leaf water potentials to reliably detect urban tree water status.

Muthianzhele Ravuluma

Sap flow dynamics of young and mature pomegranate orchards under semi-arid conditions

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Accurate quantitative information on the transpiration rates of pomegranate orchards is essential for irrigation scheduling, especially in semi-arid countries like South Africa. Water resources in major fruit-producing catchments in South Africa are almost entirely allocated, and water scarcity is exacerbated by climate change and climate variability. Currently, there is no accurate quantitative information on the water requirements of pomegranate orchards in South Africa. This study seeks to close this important information gap using data from a young and mature pomegranate orchard under drip irrigation. The study was conducted in the Western Cape Province in South Africa in both young and mature orchards on commercial farms in the Porterville and Drakenstein district, respectively. Transpiration was measured using the Heat Ratio Method (HRM) method. Weather data was recorded using an automatic weather station. Sap flow measurements were conducted for seven months (October to April) during the 2022/2023 growing season for three trees that varied in stem diameter (45 mm, 72 mm and 90 mm). The average daily sap flow rate for the young pomegranate tree (45 mm) ranged from 1.10 to 4.97 L tree^{-1} day^{-1}. The mature trees (72mm and 90mm) showed higher sap flow rates ranging from 1.10 to 14.17 L tree^{-1} day^{-1}.The highest transpiration rates were observed in February during the middle of the summer season. The major drivers of the transpiration rates for pomegranate trees are radiation and vapour pressure deficit.

Spandan Sogala Balaram

Review of heat pulse based sap flow measurement techniques

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Heat pulse (HP) based methods are widely used to estimate sap flow velocities in plants. Some of the conventional HP methods include; i) compensation heat pulse (CHP) method, ii) TMax method, and iii) heat ratio (HR) method. The advantages and limitations of these conventional models have been well-characterized. More recently, modifications to these conventional methods have been proposed to improve their performance over a broader range of parameter and sap flow conditions. These include i) dual heat pulse (DHP) method, ii) dual method approach (DMA), iii) calibrated average gradient (CAG) method, iv) maximum heat ratio (MHR) method, v) double ratio method (DRM), and vi) Sapflow+ (SFP) method. In this study, we employ numerical model simulations to compare the performance of these newer methods and assess their relative advantages and limitations.

Shinichi Takeuchi

Long term sapflow measurements to verify avocado outdoor cultivation in Japan

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Avocado outdoor cultivation in Japan is economically limited because of overwintering issues, except in the subtropical regions of the southwest. In this study, two cold-tolerant cultivars of avocados, *Bacon* and *Mexicola*, were introduced and examined under unique microclimatic conditions peculiar to the slope. Moreover, sap flow measurements were applied from the initial stage to verify the cultivation method. In November 2018, the heat ratio method was used to measure the base of the trunk on the *Bacon* cultivar, which had a diameter of 5.3 cm and grew up to 12.4 cm by June 2023. Notably, the detection velocity at a depth of 12.5 mm continuously exhibited convex changes with time; it reached the maximum value in the second year and significantly decreased thereafter. Similarly, the detection velocity at a depth of 27.5 mm exhibited convex changes annually; the convex annual change reached the maximum value in the third year and decreased thereafter. The sap flow rate was calculated over 4 years based on the depth changes of 12.5 mm and 27.5 mm, and there was a difference of 36%, which corresponded to 1.2 and 0.8 times the average value of both, respectively. Regarding Mexicola, the difference was 76%, which corresponded to 1.6 and 0.4 times the average value of both, respectively. The calculation results varied depending on the handling of the detection point. Based on the comparison of the relationship between vapor pressure deficit and sap flow rate in terms of the year, plot variation was found to be smaller in 2021 and 2022 than in 2020, suggesting that active irrigation based on soil moisture content was effective. However, this study revealed that the number of avocados was unstable throughout the past 3 years, which indicates persistent challenges in avocado cultivation.

Xiao Tao

Mechanisms of different effects of nitrogen deposition on soil respiration and its components in urban-rural gradient forests

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Understanding the effects of N deposition on soil C flux is essential for predicting forest ecosystem C cycling processes; however, few studies have investigated the effects of N deposition on soil respiration (Rs) and its components (autotrophic respiration [Ra] and heterotrophic respiration [Rh]) in urban-rural gradient forests. This study established experimental plots in urban, suburban and rural forests with similar stand conditions and vegetation growth, and conducted a four-year field experiment to simulate elevated N deposition (0 kg N ha^{-1} $year^{-1}$, 50 kg N ha^{-1} $year^{-1}$, and 100 kg N ha^{-1} $year^{-1}$) to investigate the effects of N deposition on forest Rs and its components. The study indicated that N addition did not significantly increase Rs and had different influencing mechanisms. In urban forest N deposition contributed to Rh by increasing soil microbial biomass nitrogen and inhibited Ra by increasing soil NO3--N, while in suburban and rural forests the positive effects of climatic factors were antagonistic to the negative effects of soil microbes and soil properties. Two-factor models of soil temperature and soil moisture content explain Rs changes better than single-factor fitting models, and soil temperature sensitivity was significantly suppressed under 100 kg N ha^{-1} $year^{-1}$ of N only in term of urban forest Rs and Ra, and the rural forest Ra. In summary, N addition did not significantly affect soil C release to the atmosphere in urban-rural gradient forests and offset the effect of climatic factors, soil microbes and soil properties on forest soil C release. This study identified the mechanisms through which N affects Rs, the components of Rs, and soil temperature sensitivity. The findings provide a basis for modeling and predicting the response of forest ecosystem soil C cycling and storage to climate change in the context of urbanization.

Sarah Verbeke

Validating wheat sap velocity measured with a heat pulse sensor with PET imaging

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True sap velocity is hard to determine. Sap flow sensors can measure the velocity or flow in plant stems by measuring the drag of a heat pulse or continuous heating by the sap. Temperature sensors above and below a resistor measure the change in temperature of the sap which was heated by the resistor. In case of a heat pulse, the time it takes to arrive at the temperature sensors is used to calculate heat pulse velocity. The heat pulse velocity is linearly related to the sap velocity through thermal conductivity. This parameter can be determined unequivocally for trees, where the different tissues consist of uniform concentric cylinders. For smaller plants, like wheat, the vascular bundles are dispersed in parenchyma tissue, which means that heat is conducted through different tissues with each their unique thermal conductivity before it is measured by the sensor. The hollow stem of wheat increases the complexity of the stem cross section.

Calibration of the heat pulse sensor was performed in a previous experiment by gravimetrical measurements of transpiring plants and comparing the loss of water to the cumulative measured sap flow. The objective of the current experiment was to validate the previous calibration with measurements of sap velocity through PET (positron emission tomography) imaging. To this end, radioactive tracer (FDG; fluorodeoxyglucose) dissolved in water was applied to the cut stem of ten wheat (*Triticum aestivum* L. cv. Viking) plants (Zadoks' scale Z7), after which they were subjected to a dynamic PET scan for 2 hours. Above the field of view, two ExoBeat sap flow sensors were installed on each plant. By measuring the progression of the FDG signal in the dynamic PET images, the velocity of unhindered sap can be compared to the measured velocity by the sap flow sensors. This will allow us to determine not only if the two methods measure similar velocities, but also if FDG can represent water flow in plants, thereby opening up a new method to visualize water flow in small plants.

Christopher Vincent

Chronic versus acute impacts of tropical storms on vascular function and canopy recovery

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Trees growing in subtropical and other wind-prone environments must often endure and recover from effects of high-wind speeds to continue to thrive in their environment. Although windsheer is expected to damage vascular tissue, we do not know he nature of this damage, nor long it takes to recover. Understanding recovery time would allow us to predict the effects of storms on agricultural trees or natural forest ecosystems. Hurricane Ian directly impacted the orange production region of Florida with sustained winds ranging from 110-220 km h^{-1}. Subsequently we endeavored to survey the severity and duration of damage across and outside the impacted region. We sought to 1) determine the recovery time of citrus trees through the path of Hurricane Ian and to 2) determine the effects of wind damage on xylem function. Our initial observations of low Fv/Fm measured by dark acclimated chlorophyll fluorescence more than 6-months after the hurricane are that areas with more moderate damage (eg. 110 km h^{-1} winds) have faced chronic stress to photochemistry, even though more foliage remains on these trees than those in more severely impacted areas. Observations of xylem function via active xylem staining and measurements of hydraulic conductance suggest that limited xylem function may be responsible for this chronic stress. Results suggest that understanding the impacts of hurricanes requires consideration of both the acute, visible damage of severe storms and the chronic less visible damage to vascular function induced by more moderate windstorms.

Moari West

Water use characteristics of planted indigenous and exotic tree species

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Interest is growing in utilising native trees in a range of afforestation projects across New Zealand. However, little is known about the potential impact of native species on catchment hydrology, or how their effects might differ in comparison to more commonly planted exotic plantation species. Five native (*Fuscospora fusca*, *F. solandri*, *Lophozonia menziesii*, *Prumnopitys taxifolia*, and *Podocarpus totara*), and three exotic (*Eucalyptus fastigata*, *Pinus radiata*, and *Sequoia sempervirens*) tree species were selected at our main study site, Rewanui Forest Park. Sap flux density was obtained using heat-ratio sap flow meters. The relationships between sap flux density, soil moisture and meteorological conditions were explored. Preliminary results showed that the larger exotic trees had higher sap flow and stronger seasonal variation in sap flow compared to the smaller native trees. Of the meteorological variables measured, vapour pressure deficit and solar radiation were the strongest drivers of transpiration. A lack of difference in the maximum rate of photosynthesis and stomatal conductance between species suggests that differences in transpiration rate are more likely to be driven by differences in leaf area and sapwood area between species, rather than transpiration rates per unit leaf area, as seen in previous studies.

Xiaoning Zhao

Spatial variations of trunk sap flux density in Populus tomentosa *and their influencing factors*

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The thermal dissipation probe (TDP) has been widely used in the study of whole-tree and stand-level transpiration, but its successful application depends on the full understanding of the spatial variation of sap flux density (SFD). It is well known that there is a spatial SFD variation, and ignoring this variation will be detrimental to the accurate estimation of stand transpiration. However, what factors drive and how they drive the spatial variation of SFD are still not fully understood. Therefore, this study used *Populus tomentosa* as the experimental material to investigate the radial and azimuthal variations of SFD by TDP probes. The results showed that the SFD of *P. tomentosa* had a significant radial variation, and the variation pattern changed greatly among individual trees. The difference in SFD between inner and outer sapwood was unrelated to the trunk size ($P > 0.05$). However, it was correlated with the leaf area index ($P < 0.05$, R^2 = 0.18-0.51) and the potential evapotranspiration (ETo) ($P < 0.05$, R^2 = 0.06-0.56), with its value increasing with leaf area index or ETo in most cases. There was also an azimuthal variation in SFD, which could be explained by the leaf area index to some extent ($P < 0.05$, R^2 = 0.18-0.40), and the degree of explanation depending on the azimuthal direction. But the azimuthal SFD variation was not influenced by tree height, diameter, and vapor pressure deficit. The whole tree transpiration estimation showed a great variation ($P < 0.05$) when different azimuthal directions were chosen to measure SFD, with more measurement directions leading to a more accurate estimate. Among the four azimuthal directions, the SFD measurement in the western direction was most close to the average value of all directions. According to the above results, two calibration models were finally established to correct the radial and azimuthal SFD variations. The findings of this study will be helpful for accurately estimating the stand water use in pure plantations of different tree species.

Xin Zhuang

Effects of sink limitation on Betula pendula *carbon translocation*

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In plants, photoassimilates are primarily produced by leaf mesophyll, and translocated through phloem under an osmotically generated pressure difference between the sources and sinks. Impaired sink strength may limit canopy photosynthesis and phloem transport. We aim to study how sink limitation regulates phloem loading-transport-unloading process, and whether it causes non-stomatal limitation of photosynthesis. We hypothesize that sugar accumulation causing increasing mesophyll resistance is the bottleneck for photosynthesis and for phloem loading when plants have a restricted carbon sink.

Under controlled greenhouse condition, we applied 4°C cooling treatment to 2.5m tall silver birch (*Betula pendula*) sapling soil and roots to reduce their sink activity, with untreated saplings as controls. We kept monitoring plant hydraulic status by measuring sap flow, soil water content and leaf water potential. When the treatment caused leaf A/Ci ratio to drop by half after 4-7 days, we performed pulse-chase labelling to the plants' canopy with a 30-minute exposure to ^{13}C-enriched CO_2 and tracking the photoassimilate translocation through stem respiration and from phloem exudate. By calculating soil to leaf hydraulic conductance, we were able to separate the effect of sink limitation on leaf gas exchange from the effect of decreased water uptake capacity of the roots due to the reduced temperature. Our preliminary results show that the soil-cooling treatment causes an impaired carbon sink of saplings, leading to delayed phloem loading and decreased carbon transport velocity. The treatment also led to canopy gas exchange and mesophyll conductance decrease. Additionally, we captured a considerable lag of isotopic signal appearing in phloem sap and in stem respired CO_2, suggesting the time for stem metabolic respiration to be considered in calculating phloem transport velocity.