# **Proceedings of the 2024 Australian Society of Plant Scientists Conference**



#### Hubs in Auckland, Palmerston North & **Christchurch**

28th & 29th November 2024

**NSW/ACT node Western Sydney University South Parramatta Campus 28 November 2024**

# <span id="page-1-0"></span>Acknowledgement of Country

We are meeting on *Dharug* Aboriginal land. The ASPS and Western Sydney University acknowledges, with deep respect the traditional owners of this land, the *Dharug* people, on which we meet. We acknowledge that it is a privilege to stand on Country and walk in the footsteps of those before us.

Present day Parramatta is occupied by the *Burramattagal* people, a clan of the *Dharug*, who first settled along the upper reaches of the Parramatta River. The *Burramattagal* have a close connection with the river and the word *Burramattagal* is likely derived from the Aboriginal word for 'place where the eels lie down' to breed within the Parramatta River.

The *Dharug* people still populate the areas of Parramatta, Greater Western Sydney and the Blue Mountains. We pay our respect to elders in the past, present and emerging and to the Aboriginal community that continues to care for Country.

# <span id="page-2-0"></span>Thank you to our Sponsors

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# <span id="page-4-0"></span>Message from Functional Plant Biology

Good Afternoon ASPS Membership!

CSIRO Publishing is proud to publish **Functional Plant Biology** (FPB), now the official journal of the Australian Society of Plant Scientists! FPB is a hybrid journal offering open access options, publishing peer-reviewed contributions on new and significant information on the functional biology of plants at all scales from the molecular through whole plant to community.

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Enjoy the conference!

From,

The CSIRO Publishing Team

# <span id="page-5-0"></span>Program overview Thursday 28<sup>th</sup> of November 2024



This meeting was organised by Drs. Kristine Crous, Rob Sharwood and Nijat Imin from Western Sydney University for the NSW/ACT mode as part of the 2024 annual ASPS meeting.

# <span id="page-6-0"></span>Detailed Program Thursday 28<sup>th</sup> of November 2024



10.30 am Morning tea and poster session Atrium



### **Plant Function and Biotechnology** EA. G.19

### Chairs: Rob Sharwood & Garima Dubey





### **Plant Development and Structure** EA. G.19

### Chairs: Nijat Imin & Baber Ali





# <span id="page-10-0"></span>Poster numbers



### **Plant Development and Structure**





# <span id="page-12-0"></span>Meeting Venue and Transport

### Western Sydney University's Parramatta South Campus

Building EA, rooms G.18 and G.19

Address: Western Sydney University, Parramatta South campus *Parramatta South, Building EA, Ground floor, Rooms 18 and 19 Cnr James Ruse Drive & Victoria Rd Rydalmere, NSW 2116 Australia* 

#### **Campus map**

#### **<https://www.westernsydney.edu.au/future/our-campuses/parramatta-south-campus>**

[https://www.westernsydney.edu.au/content/dam/digital/pdf/campus](https://www.westernsydney.edu.au/content/dam/digital/pdf/campus-maps/Parramatta_Campus_General.pdf)[maps/Parramatta\\_Campus\\_General.pdf](https://www.westernsydney.edu.au/content/dam/digital/pdf/campus-maps/Parramatta_Campus_General.pdf)



#### **By bus**

Buses stop right outside our campus on Victoria Road, Rydalmere (opposite Rippon Avenue). Bus routes include M52, 520, 521, 523, 524 and 525.

#### **By train**

Catch the train to Parramatta station. Then catch a bus (see By bus, just above). Or walk 21 min from Parramatta station to Western Sydney University.

#### **By car**

From M4, take the James Ruse Drive exit off the M4.

The University Entry is via Victoria Road (171 Victoria Road). If you are driving Eastbound on Victoria Road and are in the left-hand lane, a safer way to access campus is to turn left at Bridge Street and use the overpass to enter Parramatta South campus. There is ample parking available at Parramatta North, however parking at Parramatta South is in high demand, particularly at the start of semester. Nearest parking lots are P5, P6. P16 and P7. Parking fees apply.

### Close-up of campus map



For our concurrent sessions, we have two adjacent rooms each with tiered seating as in the pictures below.



#### **Location for the reception with canapes from 5:30 pm (on campus)**

The Boilerhouse is located in **building EKb (with the smoke stack)**



# <span id="page-14-0"></span>WiFi access at Western Sydney

Visitors to Western Sydney University can use "eduroam*"* with your university credentials.

Alternatively, you can use the "Western Events" from the list of wifi networks

Password is "Western@2024"

*If Login page is not loading automatically please visi[t http://wsucha.connectmy.net](https://url.au.m.mimecastprotect.com/s/gdmYCL7ExGcRXA6LxHBfgHyQ-fi?domain=wsucha.connectmy.net) manually on your browser.*

# <span id="page-15-0"></span>ASPS Award winners of 2024

### *Peter Goldacre Award*

#### **The DNA hypomethylome: unlocking crop epigenomics to uncover and engineer hidden diversity**

#### **Peter Crisp1**

<sup>1</sup> School of Agriculture and Food Sustainability, The University of Queensland, Brisbane, Old, 4072, Australia

*Bio: Dr Peter Crisp is a Group Leader and Senior Lecturer in the School of Agriculture and Food Sustainability at The University of Queensland. Peter's research program is focused on crop functional genomics, epigenetics and biotechnology, and has significantly advanced our understanding of the contribution of epigenetics to heritable phenotypic variation in plants. His group has invented groundbreaking technologies for harnessing (epi)genetic variation and their discoveries have led to exciting new avenues for decoding genomes and for the rational engineering of gene regulation for trait improvement in plants. Peter is a former recipient of an ARC DECRA Fellowship and a UQ Amplify Fellowship. Having benefited immensely from brilliant mentors, Peter is passionate about training. He leads a budding group of talented students and researchers and is a Chief Investigator in the ARC Training Centre in Predictive Breeding and the International Research Training Group for Accelerating Crop Genetic Gain. Peter is also an affiliate of the Queensland Alliance for Agriculture and Food Innovation and the ARC Centre of Excellence for Plant Success in Nature and Agriculture.*

Decoding the information stored in nucleic acids has been transformative to our understanding of life and inheritance. However, beyond the sequence of genes, it has been more challenging to understand the rules of the DNA regulatory code in the non-coding portion of plant genomes, particularly in the vast genomes of many crop species. In addition to the DNA bases A, T, G and C, heritable information can also be stored using modified bases, such as 5-methylcytosine, commonly known as DNA methylation. Profiling the patterns of DNA methylation now enables us to rapidly distil a genome down to the relatively small fraction of regions that are functionally most valuable for trait variation. Genomic regions that lack DNA methylation, named Unmethylated Regions (UMRs), provide very useful information for decoding a plant genome because they can predict loci enriched for cis-regulatory elements. We have developed approaches that use DNA methylation profiling of a single tissue (e.g. a leaf) to discover and characterise plant UMRs, which collectively comprise the 'hypomethylome' of a species. We are using this approach in multiple plant species, in particular cereals such as sorghum, wheat, barley and maize to annotate the regulatory portion of these genomes and to investigate natural epigenetic variation. Using the new hypomethylome annotations we have also selected novel gene regulatory regions as targets for gene editing to engineer gene expression variation for trait improvement.

### *ASPS Education Award*

#### **Genomics for all: a cross-sector effort to make cutting-edge genomics a cornerstone of education, industry, government, and public health.**

Ashley Jones<sup>1</sup> and **Benjamin Schwessinger<sup>1</sup>** 

<sup>1</sup> Australian National University, Canberra, ACT, 2601, Australia

*Bio: Over the past seven years, we have continuously innovated our teaching of the latest genomic analysis approaches in plant sciences, spearheading the establishment of Oxford Nanopore sequencing across Australia and in the classroom. Our impact in plant science teaching and beyond has been significant, influencing multiple sectors including universities, industries, government, and communities. Key to these achievements, is that we pursue a highly integrative approach to teaching from wet lab protocol development to dry lab analysis pipeline sharing. In addition, we focus on development of wider scientific communities, as we share teaching materials and protocols online via Protocols.io.*

Rapid advancements in long-read DNA sequencing technologies offer unprecedented potential to transform genomic research. However, the complexity of these technologies can hinder widespread adoption. Over the past seven years, we have been at the forefront of integrating long-read sequencing into various sectors, including academia, industry, government, and public health. Through hands-on workshops, open-source resources, and collaborative networks, we have facilitated knowledge sharing, skill development, and the application of genomic technologies across diverse fields. Our efforts have led to significant advancements in student education, empowering them with practical skills, leading to improved engagement and learning outcomes. In the community, we have applied genomics to develop valuable genome resources for Australia's native flora, enhance biodiversity conservation and improve agricultural genotyping. Our collaboration with government agencies has enhanced biosecurity practices to rapidly detect invasive pathogens, while partnerships with public health organisations have contributed to addressing pressing issues like the COVID-19 pandemic. We believe our collaborative approach to education and outreach fosters a scientifically skilled workforce that empowers individuals to utilise the full potential of genomics for innovative advancements.

### *Jan Anderson Award*

#### **Sweet green tales: efforts to unravel the complexities of plant polysaccharides**

#### **Jenny Mortimer**<sup>1</sup>

<sup>1</sup> School of Agriculture, Food and Wine, & Waite Research Institute, University of Adelaide, 5064, Australia

<sup>2</sup> ARC Centre of Excellence in Plants for Space, University of Adelaide, 5064, Australia

- <sup>3</sup> ARC Training Centre in Future Crops Development, University of Adelaide, 5064, Australia
- <sup>4</sup> Joint BioEnergy Institute & Lawrence Berkeley National Laboratory, Berkeley, CA 94608, USA

*Bio: After completing her PhD at Cambridge University, UK, she began exploring how engineering the plant cell wall could deliver sustainable and economically viable biofuels: first as a postdoc in Cambridge, then as a research fellow at RIKEN Japan, before joining Berkeley Lab in 2014, and Adelaide in 2021. Her team's research focuses on understanding and manipulating plant cell metabolism, with a focus on complex glycosylation. The goal is to develop knowledge and crops* 



*which contribute to a sustainable and renewable bioeconomy. Her group is using synthetic biology to develop new crops for food and materials production in controlled growth environments, including for Space settlement (P4S), applying new agricultural biotechnologies to develop resilient field crops as a CI in the ARC Training Centre for Future Crops Development, and developing Australian feedstocks for sustainable jet fuel as a CI in the ARC Research Hub for Engineering Plants to Replace Fossil Carbon. She collaborates extensively internationally, and projects include a UK Space Agency funded project to develop a plant growth facility for Axiom Station, and a NASA funded project to develop a payload for Artemis III, the mission that will return humans to the surface of the moon. She was selected as a World Economic Forum Young Scientist (2016/17), where she contributed to the WEF Code of Ethics for Researchers [\(widgets.weforum.org/coe\)](https://widgets.weforum.org/coe/), and she is an editor for the society journals Plant Cell Physiology and Plant Journal. You can find out more information on the lab here: [mortimerlab.org/](https://mortimerlab.org/)*

Plant glycosylation is a highly complex and essential biological process, yet it is still poorly understood. From glycan modifications of proteins and metabolites, to storage polysaccharides such as starch, to the structural complexities of cell wall polysaccharides, these shape how plants grow and respond to the environment. My research has focused on understanding how these complex glycans are formed, how their structure relates to their function, and how we can harness them to support a transition away from fossil-fuel based technologies.

Here, I will highlight our work in identifying key enzymes involved in cell wall and sphingolipid biosynthesis, and our progress in linking glycan structure to function. I will also explore how we are engineering these glycans to enhance biomass traits for biorefinery applications, extending their utility beyond serving as a carbon source for microbial conversion to a broader range of sustainable uses. Finally, I will discuss how understanding the regulation of plant cell wall biosynthesis is important when growing plants in a controlled environment, whether in a vertical farm on earth, or on the lunar surface.

### *JG Wood Lecture*

#### **Cell-based phenotyping for breeding crops for future climates**

#### **Sergey Shabala1**

<sup>1</sup> School of Biological Sciences, University of Western Australia, Crawley WA6009, Australia

*Bio: I was trained as an electrical engineer (B Eng Hon 1984) but then became fascinated by living systems and moved into biology, receiving a PhD in Plant Physiology in 1989 from the Institute of Experimental Botany in Minsk (former USSR). In 1995 I came to University of Tasmania as a post-doc in biophysics. In 1998 I got my tenure in the School of Agricultural Science where I have been working until 2023. During this time, I have built a highly productive laboratory focusing on stress physiology and membrane transport in plants, exploring mechanisms of plant sensing and adaptation to harsh environmental conditions such as drought, salinity, waterlogging, oxidative stress, and nutritional disorders. After working at UTAS for 28 years, I have moved to Western Australia to become a UWA Chair in Plant Physiology in June 2023. Over my research career, I have published over 460 peerreviewed papers and supervised to completion 58 PhD students.*

Agriculture is vulnerable to climate change, and sustainable agricultural food production will be not achievable by the current agronomical and breeding practices, due to impact of climate changes and associated abiotic stresses on crop performance. At the same time, tolerance to key abiotic stresses (such as drought; heat; salinity; flooding) is conferred by multiple mechanisms. Each of them operates in a specific tissue/cell type and is regulated by multiple genes. In this context, the (empirical) whole-plant phenotyping (regardless of whether it is hyperspectral imaging, or ionomics, or any other whole-plant based trait) will be always critical for a final validation of genetic material (e.g., in field trails) but is unlikely reveal the role of a specific mechanism/gene, amongst others. To be more effective, breeding targets can be directed towards specific mechanisms. In this talk, I will argue for a need for a paradigm shift from whole-plant to cell-based phenotyping approach and discuss its current prospects and limitations. Using salinity stress as an example I will show the pitfalls of the whole-plant phenotyping approach for crop breeding, and then illustrate how using cell-based phenotyping platforms allow to overcome this problem. I will then demonstrate how combining novel electrophysiological and imaging techniques can be used for discovery of the candidate genes and/or QTLs conferring not only salinity but also tolerance to other abiotic and biotic stresses. I will also argue for a need for a broader use of wild relatives, to regain abiotic stress tolerance that was lost during domestication process.

# <span id="page-19-0"></span>NZSPB Award winners of 2024

### *Roger Slack Award*

### **Genomics for restoring a critically threatened tree species in the rohe of Rangitāne o Manawatū**

Colan Balkwill<sup>1</sup>, Keith Funnell<sup>2</sup>, Emily Koot<sup>2</sup>, Julie Deslippe<sup>1</sup>, Alana Nuku<sup>3</sup>, Paul Horton<sup>3</sup>, Wayne Blissett<sup>3</sup>, **David Chagné**<sup>2,4</sup>

- <sup>1</sup> Victoria University of Wellington, Wellington
- <sup>2</sup> Plant & Food Research, Papaioea
- <sup>3</sup> Rangitāne o Manawatu, Papaioea
- <sup>4</sup> Genomics Aotearoa

Swamp maire (*Syzygium maire*; maire tawake) is an endemic tree species of Aotearoa's swamp forests that is currently listed as nationally critical due to habitat loss and, most recently, infection by myrtle rust. With fewer than twenty mature trees of swamp maire remaining within the Rangitāne o Manawatū rohe, including a remnant population under threat from the construction of Te Ahu a Turanga Manawatū Tararua, a Mana Whenua-led project was set up for conserving the species in the rohe, in accordance with Rangitānenuiarawa (Rangitāne o Manawatū tikanga). Genome sequencing of naturally occurring trees and seedlings from within the rohe was performed to generate knowledge of genetic diversity. A high-quality reference genome was assembled for the species, becoming the first genome sequence to be named by an indigenous group (Ngā Hua o te Ia Whenua). This genomics-based mahi focused on understanding the past and current population structure, how much inbreeding has occurred and how related trees are to each other and to other populations in Aotearoa. This research contributed to developing a restoration plan integrating Mātauranga Māori, genetic diversity and habitat suitability for replanting.

### *NZSPB Elected Fellow to the NZ Royal Society*

#### **Fast flowering as a tool for gene discovery in woody perennials**

#### **Allan, A.C.1,2**

1 Plant & Food Research, Mt Albert, Auckland, New Zealand

2 School of Biological Sciences, University of Auckland, Auckland, New Zealand

Plants should be considered as a third of the solution to the climate crisis, as they fix  $CO<sub>2</sub>$ and make all our food (directly or indirectly). Moving to a more plant-based economy requires both new crops and enhanced climate-resistance of existing crops. New Zealand's horticultural sector is based on temperate perennials. Breeding woody perennials requires a very long-term program. However, can genetic gain be quick enough in crops which have long generation cycles (seed-plant-seed)? New Breeding Technologies (NBTs) use molecular methods that quickly provide step changes in traits. We are using NBTs to make novel crosses with plants that are more floral. The question remains of how NZ will respond to such plants, which have no additional DNA and harbour only new variants of genes which are identical to "natural" variants already in the environment. In most countries (but not NZ) these resulting plants are not regulated. NZ must quickly decide if NBTs will play a part in our response to a changing climate.

#### **The Evolution of Flavonoid Biosynthesis**

Kevin M. Davies<sup>1</sup>, Nick W. Albert<sup>1</sup>, Yanfei Zhou1, Samarth Kulshrestha<sup>1</sup>, Rubina Jibran<sup>2</sup>, John W. van Klink<sup>3</sup>, David Chagné<sup>1</sup>, Marco Landi<sup>4</sup>, Peter Schafran<sup>5</sup>, Fay-Wei Li<sup>5</sup>, Stefan J. Hill<sup>6</sup>, John L. Bowman<sup>6</sup>

- <sup>1</sup> Plant and Food Research, Private Bag 11600, Palmerston North 4442, NZ
- <sup>2</sup> Plant and Food Research, Private Bag 92169, Auckland Mail Centre, Auckland 1142, NZ
- <sup>3</sup> Plant and Food Research, Department of Chemistry, Otago University, Dunedin 9054, NZ
- <sup>4</sup> Department of Agriculture, Food and Environment, University of Pisa, Italy
- <sup>5</sup> Boyce Thompson Institute, Ithaca, NY 14853, USA
- <sup>6</sup> Scion, Private Bag 3020, Rotorua 3046, NZ
- <sup>7</sup> School of Biological Sciences, Monash University, Melbourne, VIC 3800, Australia

The flavonoid pathway is characteristic of land plants and a central biosynthetic component enabling life in a terrestrial environment. It is one of the most studied plant characters, and the subject of >15,000 journal articles each year. However, it is only with the recent advent of model systems for non-seed plants, that we have started to understand how and why the pathway may have evolved. The phylogenetic and functional data on non-seed plants challenge the idea of a canonical flavonoid pathway inherited from the Last Common

Ancestor (LCA) of all land plants. Rather, it suggests extensive gene losses and gains within each lineage.

Some flavonoid pathway branches are well conserved and may have been present in the LCA. In particular, the UVR8/HY5-mediated induction of colourless flavonoids for tolerance of UVB-light is strongly conserved between Arabidopsis and the liverwort Marchantia polymorpha. In contrast, the stress-related red pigments have striking biosynthetic and functional diversity. Notably, the red pigments of liverworts are a previously unreported flavonoid type 'auronidins' that are cell-wall located polymers that provide protection against abiotic and biotic stresses. One lineage, the hornworts, has lost flavonoid biosynthesis entirely. Yet genome sequencing for eight hornwort genera found a single 'canonical' flavonoid biosynthetic gene in the phylogenetic outlier hornwort species. Thus, the hornwort ancestor may have inherited the flavonoid pathway but the biosynthetic and regulatory genes were lost during lineage-specific evolution. The results illustrate the importance of extending studies out from the usual suspects of plant models and across the embryophyte diversity.

# <span id="page-22-0"></span>Presentation abstracts

#### *Keynote 1: Associate Professor Brent Kaiser*

#### **AMF transporters are important for cellular ammonium management and nitrogen delivery to developing seeds**

Wenjing Li<sup>1,2</sup>, Apriadi Situmorang<sup>3</sup>, Kamal Uddin<sup>1</sup>, Danielle Mazurkiewicz<sup>3</sup>, Tongtong Liu2,4, Guangda Ding2,4, Megan Shelden3, Stephen Tyerman3, Chuang Wang2,4, **Brent Kaiser1**

- <sup>1.</sup> The Sydney Institute of Agriculture, The Faculty of Science, The University of Sydney, 380 Werombi Road, Brownlow Hill, NSW 2570 Australia
- <sup>2.</sup> Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), MOA, Huazhong Agricultural University, Wuhan 430070, P. R. China
- <sup>3.</sup> University of Adelaide, School of Agriculture Food and Wine, 2B Hartley Grove, Urrbrae, SA 5064 Australia
- <sup>4.</sup> Microelement Research Center, College of Resources & Environment, Huazhong Agricultural University, Wuhan 430070, P. R. China

The ammonium facilitator (AMF) transport proteins are related to DHA2 Drug: H<sup>+</sup> antiporters, linked to multidrug resistance and H<sup>+</sup>-dependent antiport of amino acids, cations, siderophores and glutathione in yeast<sup>1</sup>. We have characterized the functional activities of three AMF genes in Arabidopsis and the single AMF homolog in Rice for their role in NH<sub>4</sub><sup>+</sup> transport, cellular NH4+ homeostasis, growth and nitrogen redistribution. AMF proteins are exclusively found at the endoplasmic reticulum (AtAMF1;1), the tonoplast (AtAMF1;2) and the plasma membrane (ATAMF1;3, OsAMF1). Using yeast cell lines with variable NH<sub>4</sub><sup>+</sup> sensitivities (31019b and CY162), AtAMF1;2 and AtAMF1;3 were found to transport and/or retain cellular NH4+ in 31019b cells. The tonoplast localized AtAMF1;2 increased yeast tolerance to toxic concentrations of NH<sub>4</sub><sup>+</sup> (>100 mM) when expressed in a plasma membrane K+ transport and NH4+ sensitive mutant (CY162). A disruption of AMF activity through T-DNA gene silencing, initiated an early senescence phenotype in Arabidopsis leaves (chlorosis) and a reduction in seed size, seed yield and seed nitrogen accumulation. A similar trait of leaf senescence and necrosis, reduced seed size and compromised seed nitrogen was observed in Osamf1 mutants. AMF overexpression (Arabidopsis or Rice) could recover the loss AMF activity in AMF KO plants. The distinct cellular membrane localisation of the AMF protein family suggests a role in the intercellular management of NH<sub>4</sub><sup>+</sup> that when disrupted increases NH<sub>4</sub><sup>+</sup> sensitivities in plants and disrupts the recycling and redistribution of shoot nitrogen to developing reproductive tissues.

#### *Keynote 2: Distinguished Professor Belinda Medlyn*

#### **Putting ecophysiology to the test: predicting climate change impacts on Australian forests and woodlands**

#### **Belinda Medlyn<sup>1</sup>**

<sup>1.</sup> Hawkesbury Institute for the Environment, Western Sydney University

Climate change, driven by rising atmospheric  $CO<sub>2</sub>$  concentrations, is well under way. In Australia we are seeing rising temperatures, increased heat extremes, and hotter (and therefore more severe)

droughts. Predicting the likely impacts of these changes in the near-term, so that we can proactively manage for them, as opposed to reacting as they occur, has become a pressing need. Making such predictions for Australian forests and woodlands requires a quantitative understanding of the effects of these environmental factors on the ecophysiology of our woody species. My team is currently developing the DAVE – Dynamics of Australian Vegetation – model, with the aim of predicting distribution, function and dynamics of Australian vegetation types based on their physiology and ecology. In this talk I will describe how we are drawing on plant ecophysiological research to inform this model. I will survey some recent results on the effects of  $CO<sub>2</sub>$  enrichment, warming, heatwaves and drought on Australian forest species, and highlight some critical gaps in our current knowledge base.

#### *9.30 am Concurrent session: Climate Change and Ecology*

#### **A general framework explaining variation in economic traits with environment and through ontogeny**

**Daniel Falster<sup>1</sup>**, Isaac Towers<sup>1</sup>, Peter Vesk<sup>2</sup>, Mark Westoby<sup>3</sup>

- <sup>1.</sup> University of New South Wales Sydney
- 2. University of Melbourne
- <sup>3.</sup> Macquarie University

Plant economic traits, such as leaf mass per unit leaf area (LMA) and stem specific density (SSD), capture diversity among plant species in how common tissues (leaf, wood, root) are constructed. Such traits have emerged as some of the most important descriptors of plant strategy, yet it has proven difficult to explain this variation with process-based models. In this talk, I reveal a potentially general explanation on why economics traits vary with environment, through ontogeny, and with other plant traits. This explanation relies on two core assumptions: a trade-off between the efficiency of tissue construction and increased turnover rate, and that plants seek to maximise leaf area growth. Moreover, I show how the optimal value of an economic trait varies with the plant's biomass production rate. This general effect means economic traits should naturally covary with both the abiotic environment, competitive context, and other features of the plant. Specific implementations of this model for LMA and SSD capture a variety of empirical phenomena, providing broad support for the theory. Broadly, our results help explain how the form for a wide diversity of species is shaped to suit their environment.

#### **Forest responses to climate change: scaling from seedlings to the continent**

#### **Danielle Way1**,2,3

- 1. The Australian National University
- <sup>2.</sup> The University of Western Ontario
- <sup>3.</sup> Duke University

Rising atmospheric  $CO<sub>2</sub>$  concentrations could reach 1000 ppm by 2100, increasing global temperatures 3-4  $\degree$ C. Both elevated CO<sub>2</sub> and warming affect photosynthesis, tree growth and survival. Climate change-induced shifts in photosynthesis and forest growth also affect the global carbon cycle, mitigating or accelerating further climate change. Understanding how forests acclimate to future climate conditions is therefore critical for accurately predicting the trajectory of future climate change, as well as for estimating plant productivity in a warmer, high  $CO<sub>2</sub>$  world. I'll discuss how elevated temperatures, high  $CO<sub>2</sub>$  concentrations and vapor pressure deficit impact forest performance using results from my lab from seedlings through to continent-wide analyses of northern forest species.

#### **Understanding links between plant allometry, functional traits and the environment using first principles**

**Shubham Chhajed<sup>1,2,3</sup>, Andrea Westerband<sup>4</sup>, Lily Dun<sup>5</sup>, Mark Westoby<sup>2</sup>, Ian Wright<sup>1,2,3</sup>** 

- <sup>1.</sup> Hawkesbury Institute for the Environment, Western Sydney University, NSW 2753, Australia.
- <sup>2.</sup> School of Natural Sciences, Macquarie University, NSW 2109, Australia.
- <sup>3.</sup> ARC Centre for Plant Success in Nature & Agriculture, Western Sydney University, NSW 2753, Australia.
- 4. University of Louisiana, Lafayette, LA 70503, The United States of America.
- <sup>5.</sup> The University of Queensland, Brisbane, QLD 4072, Australia

Plants use the same resources (carbon, water, nutrients) for their growth and survival, yet they show vast differences in how they allocate these resources towards tissues that reflect distinct functions, including photosynthesis, hydraulics, and growth. We predicted that tissue-level traits would be coordinated with biomass allocation to different tissue types along climate and soil gradients, and with plant size. Using an Australia-wide dataset encompassing 176 woody species from 11 sites, we found that species from colder or drier sites deploy a greater total leaf mass per sapwood mass, considered at whole-plant scale. Leaf: sapwood allocation was coordinated with plant size and tissue-level traits such as leaf mass per area (LMA) and sapwood density. Plants with relatively more leaf mass amount per unit sapwood mass tended to have expensive leaves (high LMA). Additionally, species became relatively woodier with height within and across sites. This knowledge coupled with further analysis of whole-plant growth strategies has clear potential to enrich models used to predict vegetation shifts under future climate and land-use change scenarios.

#### **Efficient or proficient? Leaf phosphorus recycling among species in a nutrientpoor, species-rich community**

**Sushmita Dhakal<sup>1</sup>**, Kazi Mehnaz<sup>1</sup>, Erin Rogers<sup>1</sup>, Ian Wright<sup>1</sup>, David Ellsworth<sup>1</sup>

<sup>1.</sup> University of Western Sydney, Hawkesbury Institute for the Environment, Australia

Internal nutrient recycling mechanisms, particularly leaf nutrient resorption, serve as an important strategy for plants to optimize their growth and survival on nutrient-poor soils. Evidence suggests there is considerable interspecific variation (20-90%) in phosphorus (P) resorption efficiency (PRE) among species living on P-poor soils. However, the key driver behind this variation remains unknown. We hypothesized that covariation across species in leaf lifespan (LL) and percent P allocated to the labile leaf P fraction (P) will determine how efficiently these species can resorb and recycle P on a site characterised by P-poor soil. To test this, we evaluated inter-specific variation in LL, P including its components inorganic phosphates (Pi) and soluble phosphorylated metabolites (Pmet), and total concentrations of N and P in green and senesced leaves for 14 common woody species in a speciesrich but nutrient-poor sclerophyll woodland community at Davies Park in the Blue Mountains, NSW, Australia. We computed N and P resorption efficiency (NRE and PRE, respectively) for these species.

LL explained a substantial portion of the variation (55%) in leaf PRE and NRE among species. Additionally, PRE was strongly associated with higher allocation of leaf total P to Pliable, and a lower concentration of P in senesced leaves (Psen). However, PRE appeared largely independent of P concentration in green leaves (Pgr) and local site-related variability in soil P availability. Overall, we provide improved understanding of the underlying ways species vary in PRE, a key strategy plants employ to cope with P-limitation across P-poor but species-rich ecosystems.

#### *9.30 am Concurrent session: Plant Development and structure*

#### **Targeting chloroplast ion transport to improve salt tolerance in plants**

Jayakumar Bose<sup>1</sup>, Yogendra Kalenahalli<sup>2</sup>, Apriadi Situmorang<sup>3</sup>, Sergey Shabala<sup>4</sup>, Matthew Gilliham<sup>3</sup>, Steve Tyerman<sup>3</sup>

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Yield losses in crop plants due to increasingly saline soils are linked to the effects of salt on chloroplasts and their photosynthetic function. The classical view is that photosynthetic enzymes in both glycophytes and halophytes are equally sensitive to salt; hence, there is no difference in chloroplast sensitivity to salt stress expected between halophytes and glycophytes. In this respect, assessing the salt sensitivity of halophyte and glycophyte chloroplasts is difficult because the cytoplasmic salt concentrations to which chloroplasts are exposed in the halophytes and glycophytes can differ markedly because of their different capacity for ion exclusion and vacuolar sequestration, which are as-yet unknown quantitatively. To overcome this limitation, chloroplasts were isolated from glycophytes (*Arabidopsis thaliana, Pisum sativum, Phaseolus vulgaris*) and halophytes (*Eutrema salsugineum, Chenopodium quinoa, Carpobrotus glaucescens*) and the cytosolic salt concentrations assumed to surround chloroplasts during salt stress were applied. A new approach was developed to study ion-transport processes across the chloroplast envelope in response to salt stress, using the non-invasive microelectrode ion flux estimation (MIFE) technique. The results showed that halophytes are able to maintain higher relative electron transport rate (rETR), maximum quantum yield (Fv/Fm), steady-state fluorescence (Ft), and oxygen evolution in comparison with glycophytes at the same cytosolic salt concentrations by regulating sodium and potassium concentrations inside chloroplasts. However, the molecular identity of ion transport systems involved in sodium and potassium homeostasis in chloroplasts during salt stress is not fully understood. A pyruvate (BASS2- bile acid: sodium symporter2) transporter and a sodium-proton exchanger (NHD1) have been suggested to move sodium in and out of chloroplasts. Using the MIFE technique, we studied the sodium, potassium, and proton transport processes across the isolated chloroplasts of Arabidopsis thaliana BASS2 and NHD1 knockout mutants under salt stress. Chloroplasts from the bass2 mutant had reduced sodium entry and potassium loss leading to salt tolerance. The opposite was true for the NHD1 knockout mutant. Further characterization of AtBASS2 and EsBASS2 in Xenopus oocytes and yeast revealed that the halophyte EsBASS2 can transport protons in addition to sodium – allowing EsBASS2 to maintain its pyruvate import function into the chloroplasts using proton gradients. This work forms the framework to improve the salt tolerance of chloroplasts from glycophytic crops.

#### **Comparative Analysis of Homeodomain Transcription Factor Function in Leaf Shape**

Gabriella Jessica<sup>1</sup>, Elizabeth Tay<sup>1</sup>, Eleanor Imlay<sup>1</sup>, Mary Byrne<sup>1</sup>

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The leaf is an essential organ within a plant, serving as a site for physiological processes like photosynthesis. Leaf morphology varies widely from simple leaves (e.g. *Arabidopsis thaliana*) to compound leaves with distinct leaflets (e.g. *Cardamine hirsuta*), affecting plant productivity and survival. TALE (Three Amino Acid Loop Extension) homeodomain transcription factors, which consist of KNOX (Knotted1-like homeobox), BLH (BEL1-like homeodomain) proteins, and the closely related protein, KNATM, play a key role in leaf development. These proteins are conserved across plant species and vital in regulating cell differentiation, shoot apical meristem function, and organ morphogenesis. Notably, some TALE are expressed only in compound leaves, driving leaflet formation, which highlights the need for comparative studies on their functions in species with varying leaf morphologies. Among these proteins, KNATM, BEL1, and BLH11 remain relatively unexplored, prompting further research. We are investigating the function of these genes in A. thaliana and *C. hirsuta* to understand their contribution to leaf development and morphology. KNATM, a novel KNOX gene lacking a homeodomain, is thought to regulate the function of BLH genes, and we found this gene is required for leaf serrations in A. thaliana. In *C. hirsuta*, KNATM is expected to affect leaf complexity. Conversely, BEL1 was found to not regulate serrations by itself or alongside other BLH proteins in the simple leaves of *A. thaliana*, but its role in complex leaf development requires further investigation. BLH11 was found to redundantly regulate marginal serrations in simple leaves, while its orthologue appears to increase leaflet number in complex leaves, warranting further exploration. To better understand these genes, gene editing techniques are being employed to interrogate and compare the functions of KNATM, BEL1, and BLH11 in *A. thaliana* and *C. hirsuta*. These findings will progress our knowledge regarding the role of TALE homeodomain proteins in leaf morphology diversification.

#### **Bioengineering a better plant root - do phi thickenings make plant roots stronger?**

#### **David Collings1**

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Is it possible that a highly prominent anatomical feature, found in more than twenty plant families including important crop species, can lack demonstrated function(s) despite having been discovered 150 years ago? With phi thickenings, the surprising answer is yes!

Phi thickenings are narrow bands of stiff, secondary cell wall that loop around the radial walls of root cortical cells, a location that textbooks suggest should only have thin, primary walls. Because thickening formation is coordinated between adjacent cells, thickening networks form a reinforcing scaffold surrounding the stele. Confocal microscopy demonstrates strong similarities between thickening networks present in diverse angiosperm and gymnosperm species. In *Brassica* roots, phi thickening networks develop rapidly following treatments with the stress hormone jasmonic acid (JA) (Aleamotuʻa *et al*., 2022, J. Exp. Bot. 73, 756) and JA regulation of thickening development occurs in at least three further plant families (*Fabaceae, Gerianiaceae, Resedaceae*) with tests ongoing in additional families. This apparent conservation of developmental pathways raises the question as to whether phi thickenings are a conserved structure or result from convergent evolution. Elucidation

of the molecular pathways leading to phi thickening development, which has commenced using *Brassica* breeding populations, may resolve this question. Phi thickenings have been suggested to mechanically strengthen the growing root tip, aiding root penetration through the soil. We tested this hypothesis modifying a two-phase agar plate system and asking whether roots with phi thickenings penetrate a hard substrate more effectively than roots lacking thickenings. Preliminary data suggest that a cultivar which induces thickenings in response to JA shows increased penetration whereas a non-inducing cultivar showed little difference in penetration. As Australian farmers need crop varieties whose roots can penetrate deep into the soil to access available water and nutrients, understanding how phi thickenings increase root strength and improve root penetration is fundamentally important.

#### **Sonic-induced cellular vibrations unzip intertwined anther cone trichomes to trigger floral self-pollination and boost tomato fruit size**

Sidra Anwar<sup>1</sup>, Happy Happy<sup>1</sup>, Angus Dingley<sup>2</sup>, Thailammai Vinoth<sup>3</sup>, Weiguang Liang<sup>1</sup>, Brian Sindel<sup>2</sup>, Laurel George<sup>1</sup>, Chun Wang<sup>3</sup>, Christopher Cazzonelli<sup>1</sup>

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Artificial tomato pollination methods rely on cellular vibrations from air displacement, electric vibration wands and trellis tapping, which have potential to spread pathogens. Bioacoustic frequencies emitted from buzzing bees to ultrasonication can vibrate plant cells without physical contact. The effects of frequency-dependent sonication on the poricidal anther cone structure, floral self-pollination, seed set, and fruit size remains unclear. We engineered devices to investigate the frequency-dependent power-law behaviour of floral living cells from greenhouse-grown tomato varieties - contrasting contact-induced oscillations from a vibrating wand and mechanical shaker arm with precision non-contact sonication emitted by a subwoofer speaker. The velocity and acceleration of vibrating flowers and impact on poricidal anther cone cellular structures, selfpollination, and fruit development were assessed. Sonic frequencies ranging from 50 to 10,000 Hz increased pollination, fruit size, weight, and seed set in Sweetelle and Endeavour commercial varieties. Scanning electron microscopy revealed sonication separated the intertwined trichomes and unzipped their meshed network that locks the poricidal anther cone lobes together thereby releasing pollen grains. Near ultra-sonic frequencies boosted fruit size, whereas seed set remained constant thereby challenging the floral cell power-law rheological characteristics under different frequency scales. Tomato flowers displayed a low power-law cell behaviour to frequency-dependent sonication enabling its effectiveness as a precision non-contact technology to boost pollination and tomato fruit size without a substrate-borne component.

#### *11.00 am Concurrent session: Climate Change and Ecology*

#### **How are native plants on low-phosphorus sites phosphorus-use efficient?**

Erin Rogers<sup>1</sup>, Sushmita Dhakal<sup>1</sup>, Kazi Mehnaz<sup>1</sup>, **David Ellsworth<sup>1</sup>** 

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When leaf phosphorus (P) is deficient, individual crop and model species show strong reductions in electron transport rates and photosynthetic capacity. However, across species there is less of a reduction in photosynthesis at low-P conditions, and for our native species on soils with very low available-P, photosynthetic capacity can be maintained quite well. We undertook a large-scale study of Australia's native plant photosynthetic phosphorus-use efficiency (PPUE) across soils ranging in total P concentration ([P]) to establish how species on low [P] soils can sustain photosynthetic capacity. Across nine sites from eastern and western Australia with 85 species, species on low [P] soils (total soil [P] < 100 ppm) diverged strongly in terms of photosynthetic electron transport capacity (Jmax) with respect to total leaf [P] compared to all other species. As a result, these low-Padapted species, and prominently those from the Proteaceae, Fabaceae, and Myrtaceae families, showed higher PPUE than species from soils with higher total [P] but similar leaf [P]. We show evidence that the higher PPUE in these species was achieved by both the allocation of some leaf P away from phospholipids, and the efficient use of leaf orthophosphate pools for photosynthesis. Both phenomena lead to higher-than-expected Jmax at low leaf [P] for these specific plant lineages adapted to low soil [P]. Our results demonstrate the adaptations that these species use to be photosynthetically competent even when growing with extremely poor P supply from the soil. By gaining further insight into the mechanisms that underpin the PPUE of species across Australia, we can better understand the selective pressure that low-P may have on photosynthetic physiology and hence on species diversity in these systems. We also call for further work to understand how P is partitioned in leaves to facilitate high PPUE, both spatially and across different chemical types, to enhance vegetation models for how productivity is regulated by nutrient availability.

#### **Climate niche and functional traits facilitate the expansion of Eucalypts in cities worldwide**

**Manuel Esperon-Rodriguez**<sup>1,2</sup>, Mark G. Tjoelker<sup>1</sup>, Jonathan Lenoir<sup>3</sup>, Bree Laugier<sup>1</sup>, Rachael V. Gallagher<sup>1</sup>

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Climate plays a key role in determining the geographic space that a species can occupy across the landscape, where range limits are established by the species' intrinsic tolerance. Cities, however, are unique environments where human preferences and management define the species composition. Therefore, cities often harbour species outside the limits of their climatic tolerance inferred from their native range. Eucalypts are popular urban tree species worldwide, but little is known about which aspects of their climatic niche and which functional traits predict their success, and vulnerability, to current climate change. We assessed the relationship between the climatic tolerance limits of 50 eucalypt species and key plant functional traits to classify species resilience

and vulnerability in 67 cities in 21 countries. We used the concept of species safety margin - an index of potential climatic tolerance, to determine the cities where eucalypt species are planted outside their native climatic tolerance. Then, we assessed which functional traits (leaf  $\delta^{13}C$ , leaf dry mass, leaf length, leaf N per dry mass, wood density) were correlated with species' safety margins. Fortytwo species planted in 40 cities exceeded their thermal safety margins for temperature, while 43 species in 38 cities exceeded their hydraulic safety margins for precipitation. In general, species with small leaves, high  $\delta^{13}$ C, high leaf N per dry mass and high wood density were more likely to be planted in cities outside their native climate tolerance. Eucalypts exhibit broad adaptability in terms of occurrence in cities outside their native climatic ranges displaying functional trait characteristics of heat and drought tolerance.

#### **Assessing Thermal and Drought Tolerance in Urban Vegetation: A Comparative Study of Trees and Shrubs**

**Davide Siclari**<sup>1</sup>, Renee Prokopavicius<sup>1</sup>, Sebastian Pfautsch<sup>1</sup>, Mark Tjoelker<sup>1</sup>, Paul Rymer<sup>1</sup>

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Cities trap more heat than the natural environment, so urban vegetation is crucial for mitigating urban heat. As climate change intensifies, a greater risk of desiccation and mortality for urban green infrastructure is expected in many cities. Trees and shrubs may differ in heat tolerance due to contrasting morphology and water use strategies. Yet, their thermal tolerance and responses to extreme heat remain poorly quantified in urban settings. To achieve this aim, we monitored plant water relations and leaf thermoregulation mechanisms in four tree and four shrub species that are commonly planted in Australian cities. Research occurred during the 2023/2024 austral summer in a hotter, drier suburb of western Sydney. Trees showed less water stress and lower crown dieback than shrubs in general, but responses were species-specific. The two shrub species exhibiting the highest crown dieback and mortality had the lowest pre-dawn leaf water potentials (Ψ) and midday Ψ exceeding the turgor loss point. Thermal safety margins (TSM), defined as the difference between the critical temperature ( $T_{\text{crit}}$ ) of chlorophyll fluorescence and leaf temperature ( $T_{\text{leaf}}$ ), did not differ between trees and shrubs. As future climates will impose longer, hotter, and more frequent heat extremes, species' ability to maintain  $T_{\text{leaf}}$  lower than  $T_{\text{crit}}$  to avoid canopy damage is critical for urban species health and survival. Our results highlight that even a moderately warm and wet summer can significantly impact urban vegetation. To create more climate-resilient cities, it is a priority to build green areas with thermally tolerant species.

#### **Unexpected strong physiological thermal plasticity of montane Andean trees**

**Mirindi Eric Dusenge** <sup>1,2</sup>, Sebastian González-Caro <sup>2</sup>, Zorayda Restrepo <sup>3</sup>, Anna Gardner <sup>2,4</sup>, Patrick Meir<sup>5</sup>, Iain P. Hartley <sup>2</sup>, Stephen Sitch <sup>2</sup>, Adriana Sanchez <sup>6</sup>, Juan Camilo Villegas<sup>7</sup>, Lina M. Mercado<sup>2,8</sup>

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Global warming in the Andes is gradually altering the composition of tree species with a relative increase in the abundance of lowland species in historically cooler, higher-elevation montane species sites, a phenomenon known as thermophilization. It has been hypothesised that weaker physiological adjustment to warming in montane species, compared to lowland counterparts, is a key driver of this thermophilization, but this theory remains largely untested. To address this uncertainty, we used a field experiment with 5-year-old juvenile trees of 12 naturally co-occurring montane and lowland species growing in three common gardens established along an elevation gradient differing in temperature in the tropical Andes. Here, we show that montane tree species can thermally acclimate net  $CO<sub>2</sub>$  assimilation through photosynthesis to achieve similar carbon uptake rates to their lowland counterparts with rising temperatures. Combining our findings with evidence of widespread thermal acclimation of leaf respiration of tropical montane tree species, we demonstrate that physiological plasticity in leaf carbon metabolism to warming is not the primary mechanism driving thermophilization. Our results suggest that the strong physiological acclimation of photosynthesis to warming among montane Andean tree species should be considered when predicting future impacts of warming on Andean plant communities.

#### **Declining growth and changes in biomass allocation with warming in rainforest trees from temperate to tropical climates**

**Zineb Choury**<sup>1</sup>, Yainsa Olararont<sup>1,2</sup>, Belinda Medlyn<sup>1</sup>, Mark Tjoelker<sup>1</sup>, Kristine Crous<sup>1,3</sup>

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Australian rainforests occur from temperate to tropical latitudes, but how climate warming will affect tree growth along this climate gradient remains poorly understood. We examined how changes in biomass allocation, leaf area, and photosynthetic capacity were linked to the capacity of these species to maintain growth rates with +3.5°, +7° and +10.5°C warming in seedlings of tropical, subtropical and temperate rainforest trees. Temperate species maintained or increased final biomass (+14%), leaf area (LA) and leaf area ratio (LAR, the ratio of leaf area to plant dry mass) with warming along with similar or increased leaf mass fraction (+14%). Subtropical species increased biomass with +3.5° (+28%) and +7 °C (+17%) warming but were negatively impacted with 10 °C warming (- 31%). Tropical species reduced LA, LAR, photosynthetic capacity and leaf nitrogen, along with 14% increased root allocation in response to warming, resulting in large biomass reductions with +3.5 °C (-20%) and +7 °C (-53%). Tropical species were more susceptible to climate warming with reduced photosynthetic capacity and reduced biomass which can lead to a reduced carbon sink in the future, especially in late-successional tree species which are the most abundant with a large role in carbon assimilation of tropical rainforests.

#### **Tissue-Specific Leaf Dark Respiration and Bivariate Scaling Across Plant Functional Types**

**Xuan Hu<sup>1</sup>**, Yuzhen Fan<sup>1</sup>, Daniel Cowan-Turner<sup>1</sup>, Ellie Jordan<sup>1</sup>, Andrew P. Scafaro<sup>1</sup>, Owen K. Atkin<sup>1</sup>

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Leaf dark respiration (R<sub>dark</sub>) plays a crucial role in terrestrial carbon cycling, contributing 60 Gt of photosynthetically fixed  $CO<sub>2</sub>$  back to the atmosphere. Current models predicting  $R<sub>dark</sub>$  at 25°C (R25  $d_{\text{ark}}$ ) based on Rubisco carboxylation capacity ( $V_{\text{cmax}}$ ) and leaf nitrogen content assume a homogenised R<sub>dark</sub> across the entire leaf surface. This assumption overlooks the increased energy demands associated with phloem loading in vascular tissues compared to mesophyll tissues. Here, we coupled high-resolution imaged-based sensing of  $R_{dark}$  across leaf surfaces with high throughput R25 dark rate quantification in 45 tree species from four sampling sites in Australia and America. We explored three main questions: (1) whether R25 dark is higher in vascular tissues versus mesophyll tissues, (2) how R25 dark-leaf dry mass per area (LMA) relationship differs by tissue type, (3) how plant functional type (PFTs) influence R25 dark-LMA scaling. Results reveal that area-based R25 dark is significantly higher in vascular tissue. Vascular tissues display a higher intercept in R25 dark-LMA scaling. Deciduous trees, characterized by lower LMA and rapid growth rates, exhibited a steeper area-based R25 dark-LMA relationship. Conversely, evergreen trees, with greater structural investment, showed a steeper dry mass-based R25 dark-LMA scaling. These findings underscore the importance of incorporating tissue-specific respiration rates into model predictions, especially considering the distinct metabolic demands of vascular tissues.

#### *11.00 am Concurrent session: Plant Function and Biotechnology*

#### **Understanding and engineering cyanobacterial CO2 concentrating mechanism components for functional targeting to C3 plant chloroplasts**

Sarah Rottet<sup>1</sup>, Loraine Rourke<sup>1</sup>, Sacha Pulsford<sup>1</sup>, Isaiah Pabuayon<sup>2</sup>, Nghiem Nguyen<sup>1</sup>, Simon Williams<sup>1</sup>, Colin Jackson<sup>1</sup>, **Ben Long<sup>3</sup>**, James Moroney<sup>2</sup>, G. Dean Price<sup>1</sup>

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- 
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Cyanobacterial  $CO<sub>2</sub>$  concentrating mechanisms (CCMs) are bipartite systems consisting of active membrane bicarbonate transporters and protein-bound Rubisco microcompartments called carboxysomes. As part of a program to enhance  $CO<sub>2</sub>$  capture in plants using cyanobacterial CCMs, we studied the ATP-driven bicarbonate transporter 1 (BCT1) and the α-carboxysomal carbonic anhydrase, CsoSCA. BCT1 from *Synechococcus* is a four-component membrane transporter with potential to boost photosynthetic CO<sub>2</sub> assimilation in plant chloroplasts. However, targeting its subunits (CmpA, CmpB, CmpC, and CmpD) to three chloroplast sub-compartments is complex. We explored BCT1 integration into *Nicotiana benthamiana* chloroplasts and found promising strategies that enable correct targeting of each component to the desired location at the chloroplast envelope. Despite successful targeting, expression in CO<sub>2</sub> dependent *E. coli* did not show bicarbonate uptake. Using rational design and directed evolution, we created new, constitutively active BCT1 forms.

Several mutants, including a CmpCD fusion, were recovered and characterized in Arabidopsis. In parallel, our analysis of the carboxysomal carbonic anhydrase CsoSCA from *Cyanobium* sp. PCC7001 showed that this enzyme is allosterically activated by the Rubisco substrate ribulose-1,5 bisphosphate and forms a hexameric trimer of dimers. Phylogenetic and mutational analyses (again using rational design and directed evolution) suggest this regulation is unique to cyanobacterial αcarboxysome CAs, and that this characteristic is likely required to modulate pH inside carboxysomes to maximise Rubisco function under fluctuating RuBP. This understanding affects our engineering strategies to build carboxysomes in C3 plant chloroplasts. Taken together our findings advance the understanding of CCM components in cyanobacteria and suggest strategies for modifying CCM modules for improved plant photosynthetic efficiency.

#### **Hydraulic safety and stomatal control underpin heat and drought adaptation in the widespread grass,** *Themeda triandra* **(kangaroo grass)**

**Vinod Jacob<sup>1</sup>**, Ian Wright<sup>1,2</sup>, Brian Atwell<sup>2</sup>, Brendan Choat<sup>1</sup>, Luke Yates<sup>3</sup>, Eloise Foo<sup>3</sup>, Alejandro Correa Lozano3

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Kangaroo grass (*Themeda triandra*) is the most widespread species in Australia, thriving in some of the countries' driest environments. This makes it an ideal species for understanding drought adaptation and tolerance mechanisms. While the ability of woody species to survive intense drought is strongly related to xylem vulnerability to cavitation, the traits and processes that enable herbaceous species to survive extreme drought are less understood. Additionally, intraspecific variation in xylem vulnerability is poorly characterized in woody species and virtually unknown in herbaceous species. To address this knowledge gap, we conducted a drought manipulation experiment wherein we grew eight populations of kangaroo grass, from across a rainfall gradient, under varying levels of water stress. Our aim was to identify the key traits and trait constellations that enable kangaroo grass to thrive in dry environments and answer two key questions:

- At what level of drought stress is the xylem damaged in plants from wet vs dry environments?
- How do plants from wet vs dry environments regulate water loss through stomatal control and turgor loss?

We find that xylem vulnerability is not clearly linked to precipitation at climate of origin but that plants from drier environments survive by losing turgor and closing their stomata early during water stress, thereby limiting water loss. Our work provides valuable insights into the traits that underpin the success of herbaceous plants in dry environments and enhances our understanding of how Australian grasslands will respond in a drier future.

#### **Photosynthetic capacity in C4 plants varies with extrinsic environmental, but not intrinsic biological, conditions**

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Plants using the C4 photosynthetic pathway (C4 plants) have better photosynthetic performance in hot and arid regions compared to their C3 counterparts. However, our understanding of how C4 photosynthesis varies among species and across growth and measurement conditions remains limited. Here, we collated 1,696 CO<sub>2</sub> response curves of net  $CO<sub>2</sub>$  assimilation rate (A/Ci curves) from C4 species grown and measured at a range of conditions. We then used these data to estimate the apparent maximum carboxylation activity of phosphoenolpyruvate carboxylase (VpmaxA) and  $CO<sub>2</sub>$ saturated net photosynthetic rate (Amax), the two most common parameters used to describe C4 photosynthetic capacity. We examined to what extent VpmaxA and Amax vary with species-specific traits (e.g. C4 biochemical subtypes and growth form), growth environments and measurement conditions. We showed that VpmaxA and Amax do not differ between C4 biochemical subtypes or growth forms. We found a positive correlation between VpmaxA and Amax and highlighted that growth and measurement conditions are major factors determining photosynthetic capacity. Specifically, plants grown indoors exhibit higher VpmaxA and Amax when compared to those grown outdoors, with VpmaxA and Amax decreasing with mean maximum growth temperature (Tmax). Regardless of growth conditions, VpmaxA and Amax increase with increasing measurement temperature (Tleaf) and irradiance (PPFD), and Amax is more temperature sensitive in cool-grown plants compared to warm-grown plants. We found no evidence that common C4 model species (e.g. *Zea mays, Sorghum bicolor and Setaria viridi*s) have different photosynthetic capacity than nondomesticated C4 grasses and eudicots, implying that data from model species can be used to model native C4 vegetation. Overall, our model accounts for 47-51% of the variation reported in VpmaxA and Amax across our studies and we argue that environmental conditions have a greater influence on C4 photosynthetic capacity than inherent biochemical subtypes or growth forms.

#### **The Impact of PEPC Overexpression on photosynthetic performance and growth of** *Setaria viridis*

**Sarah Fletcher<sup>1</sup>**, Rowarne Leith<sup>2</sup>, Maria Ermakova<sup>3</sup>, Robert Furbank<sup>2</sup>, Oula Ghannoum<sup>1</sup>, Robert Sharwood1

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Climate change, food security, and limited agricultural space are three key concerns which will require a sustained research effort to reduce detrimental impacts on our global population. The focus of this project is on increasing the carbon concentrating capacity of *Setaria viridis* by overexpressing a Sorghum-derived phosphoenolpyruvate carboxylase (PEPC). PEPC is a key part of the C4 carbon concentrating mechanism (CCM) and catalyses the reaction which converts HCO $_3$ and PEP into oxaloacetate. PEPC is the primary carboxylase of C4 photosynthesis and is responsible for the carbon input to the CCM which results in higher  $CO<sub>2</sub>$  concentrations around Rubisco. Due to this enzymes' role in C4 photosynthesis, we aim to characterise how overexpression of PEPC influences CO<sub>2</sub> assimilation in *S. viridis*. We have overexpressed a codon modified version of sorghum PEPC in *S. viridis* and transgenic plants have been assessed for changes in leaf physiology through leaf gas exchange measurements (A-Ci), phenotypic data and qPCR. Three Independent transgenic lines - 7B, 18 and 32 had significantly higher carboxylation rates than the wildtype accession MEO34V as well as a positive log fold change which indicates upregulation of the sorghum-derived PEPC shown through qPCR data. Based on these results, lines 7B, 18 and 32 will be used for further study to generate a deeper understanding of how the overexpression of a Sorghum-derived PEPC affects photosynthesis in the C4 species *S. viridis*.

#### **Screening for heat tolerance using automated high-throughput measurements of photosynthesis and respiration**

**John Mackenzie<sup>1</sup>**, Andrew Scafaro<sup>1</sup>, Owen Atkin<sup>1</sup>

<sup>1.</sup> Australian National University

The temperature response of leaf net photosynthetic carbon assimilation during the day and dark respiration at night is crucial in determining the sensitivity of crop yields to heat. Heat stress reduces photosynthesis while increasing CO<sub>2</sub> release by dark respiration, which limits the availability of carbon for biomass accumulation and grain development. Both processes can adjust/acclimate to sustained increases in growth temperature by increasing photosynthesis and lowering respiration. Targeting for germplasm which exhibits strong acclimation through high rates of photosynthesis combined with low rates of respiration under high temperatures is a promising target for improving heat tolerance of major crops like wheat. Screening for these traits requires high-throughput techniques to be effective. We have developed a high-throughput fluorophore-based approach using a robotic system capable of measuring oxygen-based  $O<sub>2</sub>$  respiration and photosynthesis in a closed system. We have deployed it in multiple ongoing field trials across Australia, screening 200 varieties of wheat to assess their response to heat stress.

#### **Neo-domestication of Salicornia as an oilseed crop for seawater-based agriculture**

**Melino Vanessa<sup>1</sup>**, Wang Yunchuan<sup>2</sup>, Laxman Adhikari<sup>2</sup>, Nataliya Kovalchuk<sup>2</sup>, Lina Leal<sup>2</sup>, Jana Čížková<sup>3</sup>, Denisa Beránková<sup>3</sup>, Eva Hřibová<sup>3</sup>, Mark Tester<sup>2</sup>

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Water security and resilience has reached the point of crisis in many more regions globally; twentyone aquifers have exceeded sustainability tipping points. Threats to water security, in turn, limit agricultural production and jeopardize food security. Saline agriculture is an innovative and sustainable farming approach that harnesses seawater or brackish water to cultivate halophytes. *Salicornia* species are halophytes edible as either an asparagus-like vegetable or a vegetable oil crop. We have collected diverse germplasm and developed the genomic and genetic resources required for the neo-domestication of *Salicornia*. Domestication traits of interest include increasing the seed size, improving the phenology, reducing seed saponin content, improving seed oil content and removing photoperiod sensitivity. Global populations of several *Salicornia* species were collected from wild populations or seed banks. The relatedness of 300 accessions was determined by whole genome phylogenetic clustering using 1.7 million filtered SNPs (Minor Allele Frequency; 0.05; 20%) derived from sequence read (5-10 x coverage) mapping to a synthetic octaploid genome (a combination of four diploid genomes). Whole genome phylogenetic clustering revealed distinct clades from similar geographic origins; one Southern European tetraploid group, one Middle Eastern diploid group and another MENA group of varying ploidy. A representative of each Salicornia species was selected for whole genome sequencing. Sequence reads were produced using PacBio HiFi, scaffolded using Hi-C mapping and/or bionano optical mapping and de novo assembled. Four diploid genomes showed high collinearity at the nucleotide level with each-other and with subgenome B of the tetraploid genomes. These resources will support the worlds' first modern *Salicornia* breeding program. We envisage a sustainable saline cropping system for growing oilseeds that does not use freshwater resources or arable land, contributing to food security and conservation of freshwater.

#### *4.30 pm Concurrent session: Plant Function and Biotechnology*

#### **Unravelling the components of mesophyll conductance in Gossypium species to improve tolerance to heat and drought stress**

**Demi Sargent<sup>1, 2</sup>, Warren Conaty<sup>2</sup>, Robert Sharwood<sup>1</sup>** 

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Cotton yields are predicted to decline with the increasing frequency and severity of heat and drought events due to climate change. However, improving photosynthetic efficiency and resilience to abiotic stresses are promising solutions to developing climate-adapted cotton germplasm. A poorly understood avenue of photosynthetic enhancement is improving the temperature sensitivity of mesophyll conductance. This process can be understood as the 'gatekeeper' to photosynthesis, controlling the entry of CO<sub>2</sub> inside the leaf, thereby regulating photosynthetic carbon assimilation and growth. New findings have uncovered native Australian cotton species with greater photosynthetic thermotolerance and higher propensity to facilitate the entry of CO₂ into the chloroplasts. However, the mechanisms behind variation in mesophyll conductance in cotton are unknown. Our research uncovers interspecies variation in the temperature sensitivity of mesophyll conductance, its response to a combination of heat and drought stress treatments and potential links to anatomical characteristics. An update on this research will be presented.

#### **The kinetic basis of photosynthetic heat tolerance**

### **Nicole Bison<sup>1,2</sup>, Sean Michaletz<sup>1,2</sup>**

- <sup>1.</sup> Department of Botany, The University of British Columbia
- <sup>2.</sup> Biodiversity Research Centre, The University of British Columbia

Under climate change, it is important to understand how changes in extreme and average temperatures will impact ecosystems. One of the most temperature-sensitive biological processes is photosynthesis, and evidence suggests that temperatures may soon approach or exceed the heat tolerance of photosynthesis. Thus, characterizing photosynthetic heat tolerance has generated significant interest, especially for predicting how future climate scenarios may impact primary production. However, current estimates of photosynthetic heat tolerance are controversial because assay durations vary among studies and are typically very short (e.g. 15 min) and do not reflect realistic climate regimes. Indeed, all extant estimates of photosynthetic heat tolerance reflect the assay duration used in the study, due to the strong exponential relation between assay temperature and duration. Here we generate scale invariant estimates of photosynthetic heat tolerance using rate process theory for thermal denaturation of proteins and cells. We evaluate the theory with new data from 15 plant species in a common garden. Our analysis reveals remarkable consistency of heat tolerance across a diverse set of plant taxa.

#### Contrasting sensitivities of O<sub>2</sub>-based respiration and photosynthesis to high **temperatures in wheat leaves and spikes**

**Joy Ojo<sup>1</sup>**, Onoriode Coast<sup>1</sup>, Rebecca Thistlethwaite<sup>2</sup>, Owen Atkin<sup>3</sup>

- <sup>1.</sup> University of New England, Armidale, NSW, Australia
- <sup>2.</sup> University of Sydney University, Narrabri, NSW, Australia
- 3. Australian National University, Canberra, ACT, Australia

Increasing wheat yields to meet future demand in the face of climate change will require intensification of efforts to identify traits that enhance heat-tolerance in future cultivars of cereals such as wheat. Most efforts to-date have largely focused on leaf  $CO<sub>2</sub>$  exchange, in particularly photosynthetic carbon assimilation. There is opportunity to further increase stress tolerance and yield by exploiting sensitivities of exchange (i.e. respiratory  $O<sub>2</sub>$  consumption in the dark and photosystem II release [PSII]) in leaves and spikes. We conducted a series of controlled environment and field experiments with 10-50 wheat genotypes to assess the sensitivity of leaf and spike and PSII to high temperatures (warming and heatwaves). Under warming, rates of at 25 °C were higher in leaves (4.3 µmol m<sup>-2</sup> s<sup>-1</sup>) than in spikes (3.8 µmol m<sup>-2</sup> s<sup>-1</sup> at 25 °C), as was PSII (66.1 µmol m<sup>-2</sup> s<sup>-1</sup> for leaves 31.3 µmol & gt; 0.05). However, in spikes showed greater sensitivity to instantaneous temperature increases, with a Q (proportional change per 10 °C increase in temperature) being 33% higher than in leaves. Imposition of a 3-day heatwave at anthesis reduced rates of flag leaf (measured at 25 °C) to similar extent (19-21%) in leaves and spikes. The impact of heatwaves was more pronounced on PSII in leaves (28% reduction) than in spikes (12% reduction), suggesting greater heat tolerance in spikes. However, estimates of energy use efficiency (PSII) measured at 25 °C was higher in leaves than in spikes across all treatments. The implications of these findings will be discussed, along with the importance of considering spike respiratory and photosynthetic traits in the development of heat-tolerant wheat.

#### **Response of wild rice to short- and long-term heat stress**

#### Arindam Deb<sup>1</sup>, Demi Sargent<sup>1,2</sup>, Robert Sharwood<sup>1</sup>

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- <sup>2.</sup> CSIRO, Agriculture and Food, Narrabri 2390 NSW, Australia

Rice is a staple food for over 3.5 billion people worldwide. Climate change poses a serious threat to global rice production sustainability. Rising ambient temperatures present a significant abiotic constraint, severely impacting rice productivity. Unfortunately, the full range of biotechnological tools have not yet been developed and/or utilised to address this serious challenge. To enhance rice resilience, exploring natural variation in wild and domesticated rice species has become a promising approach. This study examines the photosynthetic temperature response and thermal optimum (Topt) of three native Australian wild rice species, *Oryza australiensis, O. meridionalis,* and *O. rufipogon* as well as *O. nivara*, a potential wild progenitor of cultivated rice. Two cultivated rice varieties, Kitaake and VL163, were used as commercial controls. Our results indicated that *O. nivara* displayed the highest Topt relative to *O. meridionalis*, Kitaake, and VL163. In contrast, *O. australiensis* exhibited the lowest Topt but maintained the highest photosynthetic rate across the temperature range among native wild species. To assess the impact of long-term heat stress, all species were subjected to a 5-day heatwave (45/35°C day/night). *O. australiensis* uniquely maintained its photosynthetic rate without significant decline, unlike other native wild species, which showed marked decreases and lack of recovery post-stress.

#### *4.30 pm Concurrent session: Plant Development and Structure*

#### **Genome Evolution and Functional Analysis of Abiotic Stress Response in Ferns**

#### **Zhonghua Chen1**,2

- <sup>1.</sup> School of Science, Western Sydney University, Penrith, New South Wales, Australia
- <sup>2.</sup> Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia

The large size and complexity of most fern genomes have hampered efforts to elucidate fundamental aspects of fern biology and land plant evolution through genome-enabled research. We present a chromosomal genome assembly and associated methylome, transcriptome and metabolome analyses for the model fern species *Ceratopteris richardii*. The assembly reveals a history of remarkably dynamic genome evolution including rapid changes in genome content and structure following the most recent whole-genome duplication approximately 60 million years ago. These changes include massive gene loss, rampant tandem duplications, and multiple horizontal gene transfers from bacteria, contributing to the diversification of defence-related gene families. We demonstrate that members of the largest subclade of leptosporangiate ferns, Polypodiales, have significantly faster stomatal response to blue light than more ancient fern lineages and a representative angiosperm. We link this higher sensitivity to levels of differentially expressed genes in blue light signalling, particularly gene duplication events in the cryptochrome (CRY) signalling pathway. Also, we show that key elements of the chloroplast retrograde signalling process, the nucleotide phosphatase (SAL1) and 3′-phosphoadenosine-5′-phosphate (PAP) metabolism, evolved in streptophyte algae. We discover an early evolution of SAL1-PAP chloroplast retrograde signalling in stomatal regulation based on conserved gene and protein structure, function, and enzyme activity

and transit peptides of SAL1s in species including flowering plants and *C. richardii*. We established a protocol for gametophyte-based screening single-guide RNAs (sgRNAs) with high efficiency for CRISPR/Cas9-mediated gene knockout in *C. richardii*. We successfully edit a few genes, such as CrSAL1, CrCRY4, and Phytoene Desaturase (CrPDS) that resulted in an albino phenotype in *C. richardii*. Knockout of CrSAL1 led to significantly reduced stomatal conductance, leaf transpiration rate, stomatal pore length, and abscisic acid-induced reactive oxygen species accumulation in guard cells. Taken together, *C. richardii* genome, optimized CRISPR/Cas9 system, and evolutionary analysis provide useful tools for functional genomics, allowing further exploration of fern gene functions. monitors plant stress by measuring the duration in hours (stress-hours) that Tc exceeds cotton's optimal 28°C. The speed and number of stress-hours accumulated is directly related to plant water stress and therefore will influence yield and can be used to determine irrigation. A successful forecast must predict Tc, hotter Tc > 28°C and accumulated stress-hours accurately. Current models lack modern time-series techniques, particularly in predicting high-temperature and accumulated stress-hours. We test the performance of modern models (lightGBM, auto-ARIMA) against current models (periodiCT and periodiCTS) in predicting across three field-year combinations at Narrabri (2016, 2018) and Carrathool (2021). We find that lightGBM improves hightemperature predictions by 13% (0.2°C) and stress-hour predictions by 18% (12 hrs a fortnight) over the current best methods. Particularly for Carrathool, lightGBM and auto-ARIMA improve forecasting by 30%, for Tc > 28°C (0.5°C) accuracy and stress-hours (30 hrs a fortnight) respectively. Models had equivalent forecasting ability with just air temperature and time covariates over additional biologically relevant solar information (radiation, relative humidity, wind speed), providing practical and accurate forecasts with minimal data. Additionally, we suggest a sensor transmission frequency reduction to hourly can conserve battery life without compromising accuracy. This study establishes optimal forecasting methodology for Tc, enabling precise BIOTIC-based management, and shows the power of data-driven models to enhance water efficiency and improved canopy monitoring.

#### **Novel resources to investigate leaf plasmodesmata formation in C3 and C4 grasses**

Hong Ting Tsang<sup>1</sup>, Diep Ray Ganguly<sup>2</sup>, Robert Furbank<sup>1</sup>, Susanne von Caemmerer<sup>1</sup>, **Florence Danila**<sup>1</sup>

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- <sup>2.</sup> University of Pennsylvania, Philadelphia, USA

Plasmodesmata (PD) are nanochannels that facilitate cell-to-cell transport in plants. More productive and photosynthetically efficient C4 plants form more PD at the mesophyll (M)- bundle sheath (BS) interface in their leaves than their less efficient C3 relatives. In C4 leaves, PD play an essential role in facilitating the rapid metabolite exchange between the M and BS cells to operate a biochemical  $CO<sub>2</sub>$  concentrating mechanism, which increases the  $CO<sub>2</sub>$  partial pressure at the site of Rubisco in the BS cells and hence photosynthetic efficiency. The genetic mechanism controlling PD formation in C3 and C4 leaves is largely unknown, especially in monocot crops, due to the technical challenge of quantifying these nanostructures with electron microscopy. To address this issue, we have generated stably transformed lines of *Oryza sativa* (rice, C3) and *Setaria viridis* (*setaria*, C4) with fluorescent protein-tagged PD to build the first spatiotemporal atlas of leaf pit field (cluster of PD) density in monocots without the need for electron microscopy. Across leaf development, *setaria* had consistently more PD connections at the M-BS wall interface than rice while the difference in M-M pit field density varied. While light was a critical trigger of PD formation, cell type and function determined leaf pit field density. Complementary temporal mRNA sequencing and gene coexpression network analysis revealed that the pattern of pit field density correlated with differentially expressed PD-associated genes and photosynthesis-related genes. PD-associated genes identified from our co-expression network analysis are related to cell wall expansion, translation, and chloroplast signaling.

#### **Controlling sodium accumulation within chloroplasts underpins leaf tissue tolerance to salinity in wheat**

**Nivethitha Baluchamy**<sup>1</sup>, Nishanth Baluvanahalli Manjunath<sup>1</sup>, Qui Fang<sup>1</sup>, Niharika Sharma<sup>2</sup>, Yogendra Kalenahalli<sup>3</sup>, Michelle Mak<sup>1</sup>, Jayakumar Bose<sup>1</sup>

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Wheat is the major cereal crop providing 20% of global dietary energy and protein intake. Rapidly increasing human population demands a doubling of wheat production by 2050. However, arable lands are becoming increasingly saline and posing a major threat to wheat production by causing up to 60 per cent yield reduction. Hence, improving wheat salt tolerance is critical to ensure global food security. Salt exclusion from the photosynthetic tissue has been considered a common salt-tolerant mechanism in wheat, however, some genotypes showed higher salt accumulation while maintaining comparable photosynthetic performance. This observation led us to test how salt loads are managed within the mesophyll cells, in particular vacuole and chloroplasts of contrasting wheat genotypes. We treated nine genotypes of *Triticum aestivum* with AABBDD genome, and its relatives *T. durum* with AABB, *Aegilos tauschii* with DD genomes to 150mM NaCl stress and observed their leaf tissue tolerance. We measured chlorophyll content, photosynthetic rate, stomatal conductance and chlorophyll fluorescence parameters such as *Fv/Fm* ratio, *PhiNO* and *PhiPSII* up to three weeks of salt treatment from the fourth leaf. Sodium and potassium accumulation in leaves after three weeks of salt treatment was quantified using ICP-OES. Vacuolar and chloroplast Na accumulation within leaf mesophyll cells were quantified using the Na-selective fluorescent dye CoroNa green. We also measured the gene expression of key transporters implicated in vacuolar sequestration (NHX), and chloroplast ion homeostasis (MSL2/3, BASS2, NHD1 and KEA1) to understand the molecular mechanism involved in leaf tissue tolerance. Our results suggested that controlling Na accumulation within the mesophyll chloroplasts through regulated expression of transport proteins is a key determinant to maintaining photosynthetic function during salt stress.

#### **Deciphering the Impact of Electrical Conductivity on Greenhouse Cucumber Quality: Insights from Physiological Responses and Potassium Transporter Genes**

**Sonali Koundal**<sup>1</sup>, Jing He, Jay kumar Bose<sup>1</sup>, Michelle Donovan Mak<sup>1</sup>, Samsul Huda<sup>1</sup>, Vijay Jayasena<sup>1</sup>, Zhong-Hua Chen<sup>1</sup>, Weiguang Liang<sup>2</sup>, Talaat Ahmed<sup>3</sup>, David T. Tissue<sup>4</sup>

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The rising global population and increased per capita income are expected to drive greater demand for agricultural products by the mid-21st century. To meet this demand, extensive use of fertilisers and pesticides has become prevalent, impacting climate change both directly and indirectly. Greenhouse horticulture is a viable, resource-efficient alternative for vegetable production, maximising resource utilisation and returns. This study evaluated the productivity and quality of two cucumber varieties, Lebanese, and Continental, grown under three EC levels (1.0, 2.5, and 3.5 dS/m) in high-tech greenhouses. The EC 2.5 fertigation led to the highest cucumber yields, with improvements in water use efficiency (WUE) and fruit quality. Key physiological responses, such as net photosynthesis, stomatal conductance, and transpiration rates, remained stable across EC levels, while electron transport rates were highest at EC 2.5. The study also showed significant differential expression of potassium transporter genes, which correlated with improved yield and WUE, linking nutrient management to improved plant responses. To obtain response of potassium transporter genes at different EC levels relative expression analysis on cucumber leaves and fruits revealed interesting participation of genes like *KEA3, KUP5, KEA5, KEA4, HAK5, KUP11, KAT3* affecting fruit quality and overall morphology of crop. Post-harvest results revealed the highest percentage of weight loss in EC 1.0 of Lebanese and Continental at 2°C and 7°C. Primary metabolites such as carbohydrates, sugars, and amino acids played a crucial role across all EC levels in sustaining cucumber fruit quality through metabolomic analysis. These results can be taken as recommendations for better quality and yield enhancement by using low fertigation levels to achieve more resource-sustainable and profitable cucumber production in greenhouses.

# <span id="page-41-0"></span>Poster abstracts

#### **#1 - Mass Mortality in Mulga: Patterns, Reasons, and Predictions**

Aaranya Gayathri Sekaran<sup>1</sup>, Belinda Medlyn<sup>1</sup>, Rachael Nolan<sup>1</sup>, Brendan Choat<sup>1</sup>, Laura Williams<sup>1</sup>, Katherine Tuft<sup>2</sup>, Graeme Finlayson<sup>3,4</sup>

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Mulga (*Acacia aneura*) is an ecologically, economically, and culturally significant tree species that dominates Australia's arid and semi-arid zones. Mulga are highly drought-resistant; however, severe droughts that exceed thresholds cause mass mortality. If recruitment does not replace high mortality, population crashes will ensue in mulga, which in turn will jeopardize the landscape. My study aims to evaluate droughts' impacts on mulga populations and assess their survival under the expected hot and frequent droughts. I aim to investigate the patterns and drivers of mortality, recruitment, and growth in mulga and then parametrize a process-based model for mulga to forecast its population under future climate change scenarios. To quantify patterns and identify the drivers of mortality, recruitment, and growth, we collected demographic and physiological data on mulga from two sites in SA that experienced the 2017-2019 drought. From the plots surveyed (n=59, 0.5 ha each), we recorded an average mortality of 25% in mulga (range 0-95%, 368 dead out of 1458 adult trees). We recorded several seedlings (n=658); however, their distribution was highly patchy (68% of plots had <10 seedlings). Besides, more than half the trees that showed dieback signs in 2020 (n=87) did not recover as of 2024, despite rains. Physiology measurements conducted on 21 healthy and 15 unhealthy trees further corroborated the findings. Mulga was found to be operating at low water potentials that were lower in unhealthy trees, although with similar stomatal conductance. Additionally, the sugar-to-starch fraction was relatively lower in the bark of unhealthy trees, indicating the use of carbon reserves. The demographic and physiological findings indicate that severe droughts can cause significant irreplaceable population losses in mulga. Future work evaluating the role of hypothesized factors and population simulation and forecasting exercises will aid in predicting what the future holds for mulga and the landscape.

#### **#2 - Paddock trees promote pasture biomass accumulation and improves soil properties in grazing systems**

Abigail Addo-Danso<sup>1</sup>, Paul Kristiansen<sup>1</sup>, Brian Wilson<sup>1</sup>, Onoriode Coast<sup>1</sup>

1 University of New England, Armidale, Australia

Pasture systems occupy about three billion hectares and contribute almost \$21 trillion to the economy globally. They are important for animal-based foods, carbon storage, water catchment reserves, biodiversity maintenance and cultural and recreational needs. However, the productivity of pasture systems may be reduced with increasing climate hazards, such as heat stress and drought, due to global warming. Therefore, it is imperative to make pasture systems more climate resilient and productive. To evaluate if trees in pasture systems are favourable for pasture performance, we conducted field experiments in the New England Tablelands and assessed soil properties and leaf functional traits (net carbon assimilation, photosynthetic heat tolerance, specific leaf area and leaf nitrogen content), under tree canopies, at tree canopy edges and beyond tree canopies (i.e. in open fields). Aboveground and belowground pasture biomass and soil chemical properties were significantly higher under paddock tree canopies compared with canopy edges and in open fields. Higher under-canopy pasture biomass was not associated with leaf functional traits. This study demonstrates the significant role of paddock trees within tree-pasture systems in driving pasture productivity, particularly through improved soil fertility. These results emphasize that paddock trees can contribute to climate change resilience of pastures in grazing systems.

#### **#3 - Pushing the Envelope: Does Range Size Limit Eucalypt Tolerance to Warming?**

**Bree Laugier<sup>1</sup>**, Mark Tjoelker<sup>1</sup>, Rachael Gallagher<sup>1</sup>, Manuel Esperon-Rodriguez<sup>1</sup>

<sup>1.</sup> Hawkesbury Institute for the Environment, Richmond, Australia

This study investigates whether geographic range size influences eucalypt tolerance to warming by examining the relationship between range size and temperature tolerance in over 50 species from six defined biomes. It is hypothesized that species with wider geographic ranges will exhibit broader thermal niches due to exposure to varying climates. Eucalypts (*Eucalyptus* and *Corymbia*) were grown at temperatures from 16°C to 35°C to assess growth traits and temperature optima across Australian summer growing climates. The findings reveal no correlation between fundamental thermal niche breadth and range size or realized climatic niche breadth. However, temperature optima were significantly associated with temperature predictors, with notable differences between temperate and tropical species. These results highlight that while fundamental thermal niche breadths are uncoupled from realized niche breadths, variations in temperature optima may drive eucalypt responses to climate warming.

#### **#4 - Realtime sensor networks for measurement of plant water use and water stress.**

#### **Brendan Choat1**

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Improved long term monitoring of ecosystem function is desperately needed to understand the impacts of environmental change and land management on Australian ecosystems. Realtime sensor networks are invaluable for monitoring plant function, providing continuous and remote measurement of plant water use and water stress. These systems deliver high-resolution data on soil moisture, transpiration, water potential and microclimatic variations, enabling the greater insight into plant response to stress events than is possible with snapshots from manual measurements at discrete timepoints. In the face of extreme climate events, such networks help identify early signs of stress, supporting timely management actions to enhance resilience. Fine grained data also contribute to understanding how plant communities are impacted by environmental stress and are essential to improve process-based models and machine leaning approaches aimed at predicting the response of plants to major disturbance events. We are establishing a smart sensor network in forest and woodland sites across a broad environmental gradient in the Greater Blue Mountains World Heritage Area. In this seminar, I will describe the structure of the network, target variables, sensor types, and wireless interface.

#### **#5 - Identifying mechanisms driving the temperature response of growth for rainforests and wet sclerophyll forests along the Australian east coast**

Camille Sicangco<sup>1</sup>, Kristine Crous<sup>2</sup>, Belinda Medlyn<sup>1</sup>

- <sup>1.</sup> Hawkesbury Institute for the Environment, Western Sydney University, Richmond NSW
- <sup>2.</sup> School of Science, Western Sydney University, Richmond NSW

Dynamic global vegetation models (DGVMs) are powerful tools for predicting impacts of climate change but are not free of limitations. A key weakness of current DGVMs is that they lack welldeveloped, mechanistic representations of the temperature response of forest growth. This exclusion contradicts empirical evidence that shows a peaked response of growth to temperature (i.e., that growth increases as temperatures rise to a thermal optimum, then declines for temperatures beyond that optimum). In addition, it can lead to inaccuracies in predicted impacts of warming on carbon uptake and losses from forests. Our aim is to identify physiological mechanisms driving the response of forest growth to temperature. We focus on east coast Australian rainforests and wet sclerophyll forests, which provide an ideal study system as they are seldom water-limited and are distributed along a long latitudinal (and thus, temperature) gradient. We synthesized studies of physiological and growth temperature responses of Australian rainforest and wet sclerophyll species in both controlled environments and field settings. In addition, we measured photosynthetic temperature responses for a Tasmanian forest to extend the latitudinal gradient of physiological temperature responses measured in the field, which will enhance our capacity to predict growth along the latitudinal gradient based on physiological mechanisms. We present our Tasmanian fieldwork as a case study illustrating the diverse strategies by which rainforest and wet sclerophyll taxa cope with a range of temperatures, as well as preliminary results from our data synthesis. Our future work will integrate these empirical findings into a dynamic global vegetation model, LPJ-GUESS, to improve predictions of rainforest and wet sclerophyll growth in a warming climate.

#### **#6 - Assaying leaf respiratory response to instantaneous and sustained increased temperatures across thermally contrasting biomes**

**Daniel Cowan-Turner<sup>1</sup>**, Xuan Hu<sup>1</sup>, Ellie Jordan<sup>1</sup>, Peter Reich<sup>2</sup>, Kevin Griffin<sup>3</sup>, Matthew Turnbull<sup>4</sup>, Andrew Scafaro<sup>1</sup>, Owen Atkin<sup>1</sup>

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Understanding leaf respiration is key to modelling planetary carbon fluxes, with Terrestrial ecosystems contributing ~106–130 Pg C yr<sup>-1</sup> into the atmosphere through respiration. As an enzymecatalysed process, respiration (R) increases near-exponentially with warming on a time scale of minutes to hours. In recent decades, efforts have been made to describe leaf respiratory responses to temperature (T). A key method used in studying plants has been the use of high-resolution shortterm T-response curves to characterise the impact of changes in the thermal environment on rates of respiratory  $CO<sub>2</sub>$  release in darkness (R–T curves). Such curves provide evidence that leaf respiration (R) adjusts to sustained changes in growth T, with the transcontinental coverage of R–T curves increasing our understanding of global patterns of leaf R. But the short measurements (holding temperature for a few minutes) do not assess the sustained effect of leaf heating (over hours). We measured the response of night-time leaf respiration to temperatures (19-45˚C) using high-throughput oxygen  $O<sub>2</sub>$ -florophore measurements on leaf discs. These measurements were made to determine the instantaneous respiration at dusk (over 2 hrs at dusk) and the sustained effect of temperatures on leaf respiration (over the following 16hrs). An analysis across tree and shrub species along a latitudinal gradient of thermally contrasting biomes across the United States and Australia allowed us to explore the effect of temperature on the biochemical, enzymatic and morphological temperature limits of respiration in the absence of any cooling via transpiration. These data could be used to improve global models of leaf respiratory response to instantaneous and sustained changes in temperature.

#### **#7 - Present and Future Distributions of C3 and C4 Vegetation: Insights from a Dynamic Vegetation Model**

**Jürgen Knauer**<sup>1</sup>, Drew Holzworth<sup>1</sup>, Assaf Inbar<sup>1</sup>, Clare Stephens<sup>1</sup>, Laura Williams<sup>1</sup>, Belinda Medlyn<sup>1</sup>

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Grasslands are one of the most widespread vegetation cover types worldwide and provide crucial ecosystem services such as carbon sequestration and climate regulation. The response of grassland ecosystems to climate change is expected to vary depending on the photosynthetic pathway of their vegetation. C3 grasses typically show stronger responses to increasing  $CO<sub>2</sub>$  concentrations but are less well adapted to hot climates, where they are less efficient in their water- and nutrient use compared to C4 grasses. However, the dominant drivers of the C3/C4 distribution, and the expected changes under future climates, remain debated. In this context, we present the functionality and first results of the DAVE-Grass model, a novel dynamic and process-based model of herbaceous vegetation function. This model integrates processes considered to be critical for predicting the

response of C3 and C4 vegetation to changes in climate - such as photosynthesis, growth, carbon allocation, phenology, establishment, persistence, and water and nutrient dynamics — processes that are often highly simplified or ignored in global models. The model has been evaluated against data from manipulative experiments, land-atmosphere fluxes, biomass observations, and phenology inferred from phenocams and satellites. We demonstrate how this new framework simulates C3/C4 vegetation distribution and how it compares to the latest data-driven products across Australia. We also identify the most important environmental factors and processes driving this distribution. Finally, we predict how these two vegetation types are expected to compete under climate change scenarios and how changes in their distribution affect ecosystem function and land surface fluxes.

#### **#8 - Coordination between whole-shoot properties and wood anatomy in diverse angiosperm species set**

Kasia Zieminska<sup>1</sup>, Camille Ziegler<sup>2</sup>, Olivier Martin<sup>2</sup>, Claire Fortunel<sup>2</sup>, Raphael Pelissier<sup>2</sup>

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On the macroscopic scale, tree shoots come in all shapes and sizes: from unbranched, chubby stems deploying large leaves to highly branched, slender ones with small leaves. But how is this macroscopic diversity coordinated with the underlying microscopic structure? We hypothesised that microscopic structure would be counterbalanced at the macroscopic scale. For example, species with narrower vessels—and so less efficient per-vessel water transport—would have larger wood areas to contain more vessels.

We sampled terminal shoots from 50 trees representing 18 species growing in the rainforest in French Guiana. All shoots were 0.7 m long. We measured their macroscopic traits (e.g., stem diameter, volume, leaf area to wood area ratio, conductive and living cross-sectional wood area), anatomical traits (vessel traits, tissue fractions), and calculated whole-stem properties (e.g., potential conductivity of the entire conductive wood).

We found mixed support for our hypothesis. Species with smaller vessels tended to have smaller stems (i.e., narrower stem diameter) and smaller conductive wood areas, contrary to our hypothesis. Whole-stem potential conductivity was driven by both conductive wood area and wood-specific conductivity, in similar proportions. The total parenchyma amount was primarily driven by stem diameter, not by parenchyma fraction. Parenchyma fraction was not correlated with living wood area, but it did trade off with conductive wood thickness, such that species with thinner conductive wood layers had larger parenchyma fractions, supporting our hypothesis. Overall, our results show that macroscopic and microscopic stem properties coordinate with each other and together influence whole-stem functions, but with various strengths depending on the function.

#### **#9 - P-availability controls soil respiration in a mature eucalypt woodland under now and future rising CO2**

#### **Min Zhao1**

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Phosphorus (P) limitation may reduce the capacity of ecosystems to remove  $CO<sub>2</sub>$  from the atmosphere under rising atmospheric  $CO<sub>2</sub>$  concentration (Ca) because plants allocate extra carbon fixed belowground in to access P. In this study, we studied the effect of P-availability on soil respiration (SR) under ambient and future elevated Ca conditions. In a FACE experiment on low-P soil, we measured SR before and after adding P. We found that P addition decreased SR on average by 17.6%. Elevated Ca did not increase SR significantly both before and after adding P and the effect of eCa on SR increased after adding P. At our sites, both the proportion of autotrophic respiration (*R*a) and the effect of P addition or eCa increased with decreasing soil moisture. We interpreted this phenomenon as P addition and eCa mostly influencing *R*a, that P addition decreased *R*a while eCa increased *R*a. In addition, the eCa effect on *R*a remained unchanged while eCa effect on increasing fine root biomass emerged after adding P, which suggests that plants may be able to utilise belowground allocation effectively to absorb P and support growth after adding P. Our findings revealed the great impact of P limitation in preventing the forestry ecosystem from fixing more carbon under eCa.

### **#10 - An Eco-cultural Study of Culturally Significant Species on Wangkumarra Country**

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An Eco-cultural Study of Culturally Significant Species on Wangkumarra Country Wangkumarra Country is located on the Cooper Basin, in south-west Queensland. Native Title determination was granted to Wangkumarra people in July 2024. This PhD project is an eco-cultural assessment of my Grandfather's country. Understanding the ecological condition of our ancestral lands will encourage my mob to return to Country to resume our cultural practices. Minnarityi (*Acacia cyperophylla* a.k.a. red mulga) is a culturally significant species found only in arid and semi-arid areas of central Australia. Wangkumarra people used it for tools, weapons, shelter, ceremony, and food acquisition. Very little is currently recorded in the literature about the ecology of minnarityi on Wangkumarra Country. This study will compare the population dynamics of minnarityi across land use types using two-way science, including contemporary quantitative and qualitative ecological assessment tools and Aboriginal traditional ecological knowledge (TEK). The anticipated outcomes of the project include: the development of culture-based conservation strategies educational resources that will enable Wangkumarra people to reconnect with Country and to understand their cultural responsibilities in caring for Country recommendations for collaborative conservation to protect ecosystems on Wangkumarra Country. This project is also a platform for revitalising Wangkumarra language and will preserve cultural knowledge for future generations. With greater two-way understanding, the results of the project will help develop strategies that empower Wangkumarra people and improve the condition of our ancestral lands.

#### **#11 - The diverse heat sensitivity of photosynthetic components of Australian native plants**

**Sabrina De Zen<sup>1</sup>**, Rosalie Harris<sup>1</sup>, Danielle Way<sup>1</sup>, Andrea Leigh<sup>2</sup>, Adrienne Nicotra<sup>1</sup>, Pieter Arnold<sup>1</sup>

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To understand how future thermal regimes will affect the photosynthetic capacity of plants, we first need to accurately describe the effects of increasing temperatures on the processes that underlie photosynthesis. Photosystem II (PSII) is one of the most heat-sensitive components of the electron transport chain, and chlorophyll fluorescence has provided reliable measurements of changes in PSII functionality under heat stress. The carbon-fixing enzyme Rubisco, on the other hand, is largely thermostable, and its deactivation under high temperatures is attributed to the thermal lability of its regulatory chaperone, Rubisco activase (Rca). Most studies on the thermal sensitivity of both Rubisco and Rca are however crop-focused and confined to tests at few temperatures, which limits our understanding of the diversity of effects of heat stress on carbon-fixing reactions. We conducted a comprehensive assessment of the variability of thermal sensitivity of PSII on ~70 Australian native species of different growth forms (forbs, shrubs, trees) originating from distinct biomes (arid, alpine, temperate, tropical), all growing at the Australian National Botanic Gardens in Canberra. Results for the thermal sensitivity of PSII by pulse-amplitude modulated fluorimetry highlight substantial variation in both outright heat tolerance and sensitivity to heat among species, even within genera, growth form, and biome of origin. Next, we will analyse changes in Rubisco and Rca content following exposure to progressively increasing temperatures, which will be paired with these PSII data. Our findings will provide crucial information for improving understanding and modelling photosynthesis responses of plants under a changing climate.

#### **#12 - High-throughput phenotyping of wheat grown under current and future climates**

#### **Zixiong Zhuang<sup>1</sup>**, Dani Way<sup>1</sup>

#### <sup>1</sup> Australian National University

Following current emission trajectories, most climate scenarios predict an atmospheric  $CO<sub>2</sub>$ concentration of 800 ppm and a 5°C warming for wheat-growing regions of NSW by 2100. Despite this, experiments manipulating both temperature and  $CO<sub>2</sub>$  concentrations are scarce in wheat research, leading to uncertainty regarding how climate change will impact Australian wheat production. However, a diverse set of wheat genotypes are available to assess how to breed wheat to better cope with future climate conditions. Therefore, we grew 30 Australian wheat lines under current and future (+5 $\degree$ C warming and +375 ppm CO<sub>2</sub>) climate conditions to assess their ability to maintain yield under future  $CO<sub>2</sub>$  and temperature conditions. Here, we provide insights into how the future climate treatment alters wheat phenology, as well as structural traits and spectral traits that could be used to identify climate-resilient lines for breeders, with the longer-term goal of identifying traits that support higher yields and seed quality in the coming decades.

#### **#13 - Molecular and physiological assessment of stay green trait for enhanced heat and drought stress tolerance in wheat**

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Wheat productivity is increasingly threatened by rising temperatures and prolonged droughts caused by climate change. The stay-green phenotype during abiotic stresses results in extending photosynthesis and grain filling, contributing to stress tolerance. Four commercial Australian wheat cultivars (Coota, Catapult, Beckom, and Sunmaster) were evaluated in a greenhouse experiment for their physiological, biochemical, and agronomic traits in response to heat and combined heat and drought stress. The differences under these stress conditions was further evaluated by analyzing the expression of key ion channel genes involved in stomatal regulation, and key genes involved in chlorophyll degradation, and heat shock-responses. We have identified Beckom as a stay-green variety, showing significantly higher photosynthesis (15.02 and 12.05  $\mu$ mol m $^{-2}$  s $^{-1}$ ) and transpiration rates (10.66 and 8.64  $\mu$ mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) under heat and combined heat-drought stress. Beckom also exhibited significantly higher expression (1.9 and 1.5 fold) of *TaGORK* gene, a voltage gated ion channel critical for stomatal regulation under the two stresses. In contrast, Coota showed significantly higher expression of *TaHPX* (5 and 2 fold) and *TaLipo* (3.5 and 1.5 fold), indicating increased reactive oxygen species (ROS) production and chlorophyll degradation, leading to leaf senescence and stress susceptibility. Upon harvest, Beckom produced 1.5 times more grains per spike and 4 times more grain weight compared to the other three cultivars. This study underscores the complexity of physiology underlying the stay-green trait and suggest the involvement of multiple pathways including stomatal regulation and chlorophyll degradation. Also, we identified key physiological, biochemical, and genetic traits that can be used for marker-assisted breeding to select heat and drought tolerant wheat varieties, ensuring sustainable food production in the future.

#### **# 14 - Transient and Stable Transformation of Wheat Immortalized Callus to Analyze Plasmodesmata-Targeted Proteins**

#### **Christian Paolo Balahadia1**, Florence Danila1

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Plasmodesmata (PD) are microscopic cytoplasmic bridges that connect plant cells through the symplast. These nanostructures are crucial for plant growth, development, and environmental responses. To study genes responsible for PD formation and function, we developed a transient expression system using immortalized callus from wheat, an agronomically important crop. This species-specific approach offers distinct advantages over heterologous expression systems. In wheat, immature embryos are the most amenable tissues for callus induction and regeneration. However, maintaining a consistent supply of immature embryos for transformation is both costly and time-consuming. While maintaining immortalized callus presents an alternative, these cultures

typically lose totipotency through subsequent subcultures, resulting in poor regeneration efficiency. Recent discoveries of morphogenic genes that enhance embryogenesis in calli offer a promising solution to this challenge. Here, we demonstrate a proof-of-concept system using wheat immortalized callus to study PD-targeted proteins. We successfully transformed the immortalized callus with a known PD-targeted protein fused to yellow fluorescent protein, confirming the viability of this expression system. Current efforts are focused on optimizing the selection of morphogenic genes and refining protocols for efficient callus regeneration. This system provides a reliable platform for analyzing PD-related gene function in wheat, offering insights into symplastic transport mechanisms in cereal crops.

#### **# 15 - Elucidating the role of BLH homeodomain transcription factors in leaf development**

**Eleanor Imlay<sup>1</sup>**, Andy Wilson<sup>1</sup>, Bevan Uzun<sup>1</sup>, Mary Byrne<sup>1</sup>

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The observable spectrum of leaf morphology in plants is extensive, ranging from leaves that are simple, with undivided lamina, to complex, with blades that are partitioned into leaflets. Leaves are further distinguished by features like serrations or lobes, the shape of which is determined by defined regions of growth along the leaf margin. A key class of regulatory proteins involved in leaf morphogenesis are the three amino acid loop extension homeodomain transcription factors, including KNOTTED1-LIKE and BEL1-LIKE (BLH) proteins. The BLH transcription factor family features 13 members in *Arabidopsis thaliana*. The *BLH* genes *SAW1* and *SAW2* are known to have a role in the leaf margin given *saw1 saw2* has more prominent serrations than wild-type leaves. To date, the function of many other BLH genes remains elusive. We are investigating the activity of a clade of BLH genes which includes *BLH1, BLH3, BLH5, BLH6, BLH7, BLH10,* and *BLH11*. Using GUS transcriptional reporters, we found these genes are expressed in the leaf. Genetic analysis in Arabidopsis confirmed there is significant redundancy between these *BLH3, BLH6, BLH7, BLH10, BLH11* genes. However, when these genes are mutated in a *saw1 saw2* background there is a significant enhancement in the serration profile of leaves. Using CRISPR/Cas9 mediated mutagenesis, we query whether loss-of-function mutations in *BLH1* and *BLH5* will further enhance this phenotype. By determining the extent of redundancy between these genes it may be possible increase our understanding of genetic plasticity and resilience to change in developing leaves.

#### **# 16 - Elucidating the role of plasmodesmata in wheat stripe rust infection**

Haoran Li<sup>1</sup>, John Rathjen<sup>1</sup>, Florence Danila<sup>1</sup>

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Stripe rust caused by the pathogen *Puccinia striiformis f. sp. tritici* (*Pst*) can lead to 60-80% yield loss in wheat resulting in 1.5 billion annual loss globally. Developing *Pst*-resistant wheat varieties is essential, but a deeper understanding of the infection mechanism of *Pst* is needed to achieve this. Plasmodesmata (PD) are nanochannels embedded within plant cell walls, linking cytoplasm between neighbouring cells. In some pathosystems, PD act as battle ground between host plant and pathogen, hence a potential target to build plant immunity. In this study, we aim to elucidate the role PD play in wheat-stripe rust infection and the potential proteins involved. Complementary macroscopic, microscopic and molecular analyses were applied comparing susceptible and resistant wheat cultivars. Phenotyping revealed delayed sporulation and lower spore production in resistant cultivars compared to the susceptible cultivars. Confocal fluorescence microscopy showed restricted hyphal growth in resistant cultivars with increased callose deposition at infection sites. Electron microscopy suggested that *Pst* degrade host cell walls where pit fields or clusters of PD were located. Preliminary proteomics study identified unique PD-associated proteins in the resistant cultivar that are related to translation, energy metabolism and oxidative stress. In contrast, unique proteins found in the susceptible cultivar were linked to redox balance and signal transduction. Overall, this study highlights the role of PD in wheat-stripe rust infection, the differences in *Pst* growth across wheat cultivars, and the identification of PD-associated proteins potentially conferring *Pst* resistance or susceptibility in wheat. Further functional validation of these proteins is required to inform strategies towards breeding *Pst*-resistant wheat or developing stripe rust-specific fungicides.

#### **# 17 - Characterisation of capsaicinoids from capsicum and chilli fruits and plant waste**

Mursleen Yasin<sup>1</sup>, Michelle Mak<sup>1</sup>, Zhonghua Chen<sup>1</sup>, Sunil Panchal<sup>1</sup>

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Capsaicinoids are groups of alkaloids synthesised in plants through a chain of metabolic reactions and source of pungency. Capsaicin and dihydrocapsaicin are the major members comprising almost 90% of this group. The major production and accumulation site is the non-edible part of fruit i.e., placenta. Other parts of the plant like stem, leaves, pericarp and seeds also contain these pungent compounds. However, it is evident that significant amounts of capsaicinoids can be extracted from parts of the plant which are ultimately treated as waste biomass, or waste materials. Capsaicinoids are enriched with properties like analgesic, anti-inflammatory, antimicrobial, anti-carcinogenic, and anti-diabetic, etc. The study examined 3 different nutrient treatments for growth, yield and capsaicinoids concentration in capsicum and chilli fruits and their plant waste. For research 3 capsicum (Royston, Groote and Owen) and 2 chilli varieties (Jet and Caysan) were grown in a hightech glass house facility in Sydney, Australia. Plants were treated with three levels of nutrient treatments i.e., EC 1.8, EC 2.8 and EC 3.8 (dS/m). Samples were further separated by edible and nonedible (seed and waste) parts. Solvent extraction procedure is used to extract the secondary metabolites followed by UPLC. Untargeted metabolomics is performed to identify the essential compound sand pathways of the plant. Anthocyanin profile of the capsicum samples is also being observed by targeted metabolomic analysis. The results showed that on average Royston variety had higher fruit and capsaicin yield. Capsaicinoids profile was dominated by dihydrocapsaicin in capsicum fruits and seeds (non-edible) samples and by capsaicin in chilli samples. The research findings endorse that capsicum and chilli have great bioactive potential and crop waste needs further investigation to be functionalised as a sustainable source of capsaicinoids and for future development of health beneficial product.

#### **# 18 - Trade-offs between nitrogen-fixing symbiosis and nematode parasitism in**  *Medicago truncatula*

### **Chia-Han Jeffrey<sup>1</sup>, Ulrike Mathesius<sup>1</sup>**

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*Medicago truncatula* is both a forage legume as well as a model for research into plant-microbe interactions. In addition to forming mutually symbiosis with nitrogen fixation bacteria (rhizobia), legume plants are also easily infested by root-knot nematodes (RKN), which cause severe root galls and yield loss. Some studies have suggested that symbiotic rhizobia have potential activity of disease control against RKN due to partially overlapping response pathways. However, the underlying mechanisms of the trade-offs between nodulation and nematode parasitism are currently unknown (Costa et al., 2021). In this study, I will try to investigate the rhizobia traits that contribute to the repression of RKN (*Meloidogyne javanica* infection, as well as identify whether there is a link between efficiency of symbiosis and susceptibility to rhizobia and RKN and what some of the underlying mechanisms of interaction are. Initial experiments suggested that effective nodulation was essential for protection of plants from RKN parasitism by rhizobia (Costa et al., 2020). First, 18 rhizobia strains were used to characterize the traits of carbon and nitrogen utilisation, biochemical and plant growth promoting (PGP) traits. Afterwards, these strains, which included specific mutants of *Sinorhizobium meliloti* were screened for individual nodulation on *M. truncatula* cv. Jemalong A17. I also measured the effect on galls and RKN egg formation upon concurrent inoculation of rhizobia and RKN. There was significant variation in nodulation and other traits between rhizobia strains, as well as a significantly variable effect of the different rhizobial strains on RKN parasitism. Future experiments will test the plant traits that determine the effect of rhizobial strains on RKN parasitism. This will pave the way for strategies aimed at RKN control in legumes.

#### **# 19 - Exploring the relationship between plants and their microbial partners in the rhizosphere: Insights from** *Themeda triandra*

**Dhruthi Somesh<sup>1</sup>**, Uffe Nielsen<sup>1</sup>, Brajesh Singh<sup>1</sup>, Eleonora Egidi<sup>1</sup>

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This study aimed to investigate the role of rhizosphere microbial communities in *Themeda triandra*  (Kangaroo grass) growth. Soil samples were collected from the rhizosphere of *T. triandra* plants in three locations across New South Wales, Australia, with varying climatic conditions and geological substrates, and a pot experiment was carried out in which seeds of *T. triandra* were cultivated in sterilised soil that had been amended with five different microbial inocula, such as individual inocula sourced from three different sites, a mixed inoculum, and a sterile inoculum, to determine the specificity of rhizosphere-plant relationships in *T. triandra*, other common, co-occurring grass species were grown under the same conditions. The addition of inocula resulted in higher *T. triandra*  growth (shoot/root biomass) compared with the sterile inoculum. The impact was influenced by the source of the microbial community, exhibiting a more significant effect when inoculated with the rhizosphere soil from temperate sites where *T. triandra* thrives. Conversely, we found that the positive influence of *T. triandra* rhizosphere microbes on plant growth was not apparent in other grasses. These findings suggest that *T. triandra* may engineer its rhizosphere microbial community to support its growth and that this effect may be related to microbial community complexity. Further research should focus on identifying specific microbes associated with increased growth and isolating

potentially "beneficial" microbes for plant growth promotion. This study highlights the importance of studying soil microbial communities to better understand the ecology of keystone species such as *T. triandra*, particularly in the context of changing environments.

#### **# 20 - FieldDino: High-throughput stomatal phenotyping to advance plant breeding research**

**Edward Chaplin<sup>1</sup>**, Guy Coleman<sup>2</sup>, Andrew Merchant<sup>1</sup>, Richard Trethowan<sup>1</sup>, William Salter<sup>1,3</sup>

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Wheat is the world's most widely grown cereal and a vital crop for Australia. Climate predictions indicate we are going to face more frequent, intense, and prolonged heatwaves and droughts in the future, particularly across the Australian Grain Belt. Stomata, responsible for regulation of  $CO<sub>2</sub>$ availability for photosynthesis and plant water-use, significantly impact plant productivity and resource use efficiency. However, despite their importance for yield and dynamic responses to abiotic stress, conventional methods for measuring stomatal traits are low-throughput and timeconsuming, limiting their adoption in plant breeding programs. Recent advancements in instrumentation and data analytics offer opportunities to realise the potential of stomatal traits at scales relevant to plant breeding. We have developed FieldDino, an open-source phenotyping approach to screen stomatal physiology and anatomy traits at scale. This low-cost method includes: (1) *in situ* stomatal conductance data collection using a handheld porometer; (2) streamlined collection of stomatal anatomy images with a handheld digital microscope, 3D printed microscope mount and custom Python-based image capture app; and (3) automated analysis of stomatal features using deep learning. FieldDino was deployed and validated across two seasons of field trials containing 200 diverse wheat genotypes and two irrigation treatments at two times of sowing, capturing a wide diversity in stomatal traits driven by both the environment and genetics. Stomatal conductance tended to be higher under timely sown and irrigated conditions while stomatal density was greater in later sown and rainfed plants. Both stomatal conductance and density were greater on the adaxial surface. Variation in stomatal traits across treatments highlights that environmental stress influences stomatal development and behaviour, but variability across genotypes and high heritability values suggests there is an opportunity for improvement via targeted plant breeding. Further analyses should uncover genetic bases of stomatal traits and more detailed phenomic assessment will uncover desirable stomatal traits.

#### **# 21 - Are wild Australian cotton relatives especially heat tolerant?**

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Rising global temperatures will impinge on the performance of crops, including cotton. A genetically diverse panel of wild Australian cotton species was investigated as a source of traits for improved thermotolerance. Early vegetative performance was assessed in four Australian Gossypium species endemic to northern Australia (*G. australe, G. bickii, G. robinsonii and G. sturtianum*) and compared with *G. hirsutum* (commercial cotton). Plants were grown in glasshouses at daytime maxima of 30°C (optimal) or 38°C for 25 d from the four-leaf stage. Aboveground traits (biomass and leaf area), relative rates of growth and specific leaf areas are reported along with leaf gas exchange variables. Individual leaf area and shape, and electron micrographs of leaf-surfaces were assessed as putative contributors to thermotolerance. When growth at 38°C was compared with 30°C, the four wild species were significantly more tolerant than *G. hirsutum*, with two of the wild species substantially larger (*P*< 0.001) at 38°C than at 30°C. Areas of individual leaves and leaf numbers both contributed to these contrasting growth responses, with fewer and smaller leaves when *G. hirsutum* was grown at 38°C. Carbon dioxide assimilation, transpiration rates, electron transport rate and carboxylation efficiency were substantially reduced in *G. hirsutum* at 38°C, in contrast with the wild species. Leaf morphology and anatomy also appeared to contribute to thermotolerance in the wild species. We speculate that leaf shape, as assessed by invaginations of leaf margins, leaf size and surface decorations contributed differentially to heat dispersal. For example, highly complex trichomes and cuticles characterised the four wild species but not *G. hirsutum*. This study provides the first body of evidence that Australian wild cotton species have evolved superior resilience to very hot conditions.

#### **# 22 - Finding the genetic control of leaf plasmodesmata formation in C3 and C4 crops**

Hafiz Sabah-Ud-Din Mazhar<sup>1</sup>, Julian Greenwood<sup>1</sup>, Ulrike Mathesius<sup>1</sup>, Florence Danila<sup>1</sup>

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Photosynthesis in C3 plants, such as wheat, barley, and rice, is limited by the enzyme Ribulose-1,5- Bisphosphate carboxylase/oxygenase (Rubisco) which in addition to  $CO<sub>2</sub>$  can also fix  $O<sub>2</sub>$  leading to energy-wasteful photorespiration. In contrast, C4 plants like corn and sugarcane have evolved a unique  $CO<sub>2</sub>$  concentrating mechanism leading to a more efficient two-cell C4 photosynthesis involving mesophyll (M) and bundle sheath (BS) cells. This efficiency is partly due to the higher plasmodesmata (PD) density in C4 leaves, which is crucial for cell-to-cell communication and photosynthetic carbon flux. Despite the importance of PD in plant development and photosynthesis, the genetic mechanisms behind PD formation in C3 and C4 leaves, especially in monocots, remain poorly understood. My PhD project aims to develop a spatiotemporal atlas of PD density throughout the leaf development across different cell interphases via confocal microscopy using YFP-tagged PD lines. A multi-omics approach to study the genetic mechanism of leaf PD formation in Oryza sativa (C3), *Setaria viridis* (C4 NADP-ME subtype), and *Panicum milaceum* (C4 NAD-ME subtype) will be done. The initial list of candidate genes for PD formation has been identified, and functional validation of these genes using CRISPR/Cas9 is underway. This research will contribute significantly

to the understanding of cell communication during leaf development, its impact on photosynthesis, and C4 evolution.

### **# 23 - Shining the light on photosystem II heat tolerance (Tcrit): predicting Tcrit from hyperspectral reflectance**

**Hanna Amoanimaa-Dede<sup>1</sup>**, Rebecca Thistlethwaite<sup>2</sup>, Robert Cope<sup>1</sup>, Owen Atkin<sup>3</sup>, Onoriode Coast<sup>1</sup>

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Measures to scale up estimates of leaf photosynthetic heat tolerance could benefit research in crop science and ecophysiology. Here, we show the possibility of non-invasive, high-throughput methods for estimating leaf T<sub>crit</sub> – the temperature at which photosystem II (PSII) function is compromised. We used 2899 paired leaf T<sub>crit</sub> and hyperspectral reflectance datasets to develop models for predicting Tcrit in wheat (*Triticum aestivum*; 214 genotypes), barley (*Hordeum vulgare*; 213 genotypes), and Eucalyptus (*Eucalyptus viminalis*; 64 genotypes). Leaf T<sub>crit</sub> was highest for wheat (44.6 °C) while barley (40.9 °C) and Eucalyptus (41.6 °C) had similar values. Using support vector machine regression (SVMR), model predictions of  $T_{\text{crit}}$  across species based on hyperspectral reflectance across the full light spectrum (390–2500 nm) was promising ( $r^2$  = 0.60; root mean square error [RMSE] = 1.38). However, within-species predictions were poor  $(r^2 = 0.09 - 0.28)$ ; RMSE = 2.35-5.38). Improvements in within-species predictions would likely be achieved with greater diversity in the range of paired  $T_{\text{crit}}$  and reflectance data. Models developed using wavelengths from the visible light region (390–750 nm) or the short-wave infrared region (1300–2500 nm) had a slightly lower prediction accuracy (r2) and higher RMSE than models built from the full wavelength. These region-specific predictions suggest that leaf pigments in the palisade mesophyll, associated with chlorophyll absorption (e.g. chlorophyll a and b, β-carotene) and leaf water content may play significant roles in estimating leaf T<sub>crit</sub> from reflectance spectra. This study provides opportunities to support the breeding of heat-tolerant crops and improve understanding of plant adaptation to climate change.

#### **# 24 - Investigating the biochemistry of PEPC in the Paniceae tribe**

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Climate change and anthropogenic pressures currently affect global food production. A potential strategy to increase crop production and global productivity is by utilising the C4 photosynthetic machinery. C4 plants are adapted to perform in harsh environments relative to C3 plants. C4 plants have increased water and nutrient efficiency and have evolved a carbon-concentrating mechanism that results in high  $CO<sub>2</sub>$  around Rubisco. Phosphoenolpyruvate carboxylase (PEPC) catalyses the first committed step in C4 photosynthesis. PEPC facilitates the conversion of phosphoenolpyruvate (PEP) and bicarbonate into oxaloacetate and inorganic phosphate to enhance the carbon flux entering the C4 cycle. PEPC (EC 4.1.1.31) is a homotetrameric enzyme that contains four identical monomeric subunits. This PhD aims to examine the structure-function relationships of PEPC catalysis and develop novel isoforms with enhanced catalytic activity. The key targets of this thesis will focus on engineering an *Escherichia coli* strain to be dependent on PEPC for growth to provide a platform to explore and screen PEPC variants. Utilising directed evolution to generate catalytically superior isoforms of PEPC will focus on optimising allosteric inhibition constraints and increase catalytic efficiency under suboptimal conditions. This thesis will also utilise site-directed mutagenesis to mutate key residues involved in various tetramer assembly and unravelling the structural features impacted by allosteric regulators.. The residues being targeted are involved; in the binding site of the allosteric activator Glucose-6-Phosphate, in tetramer stabilisation, and key residues that involved in the evolution of the C4 PEPC protein. Another critical area of research is to transform model C4 grass *Setaria viridis* with superior PEPC isoforms derived from *Urochloa panicoides*. The aim is to improve photosynthetic efficiency by translating this knowledge into crop breeding programs. This research will lay the groundwork for future metabolic engineering efforts to increase crop resilience and sustainability in an era of environmental uncertainty.

#### **# 25 - Automated and high throughput measurement of leaf stomatal traits in canola**

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Automating stomatal trait measurement has gained popularity because of their inherent importance for field phenotyping application as stomata are critical for both carbon capture and water use efficiency in plants. Such tool has been reported for rice, wheat, tomato, barley and oil palm. However, none exist yet for canola, which is an important economic and agronomic crop globally. We developed a new toolkit called Stomatal Comprehensive Automated Neural Network or SCAN by combining the use of high-resolution portable digital microscopy with machine learning based on You Only Look Once algorithm (YOLOv8). Digital micrographs of leaf surfaces enter the SCAN pipeline, which includes stomata detection, stomata segmentation and stomatal pore segmentation models, to measure stomatal density, stomatal size and stomatal pore size, respectively. In addition to SCAN's ability to measure leaf stomatal traits in canola at 91 to 94% accuracy, we also showed that SCAN can be used to predict stomatal density even in species not included in the training set such as Arabidopsis, tobacco, rice, wheat, maize and proso millet. SCAN was designed for the biological science community with the premise that users are not required to possess advanced programming capabilities to manage dependency prerequisites, execute the models, and integrate the analysis. This was achieved by packaging the models into a desktop application system that can be accessed offline. Overall, SCAN provides a non-destructive, real-time, portable, and highthroughput measurement of leaf stomatal traits in canola. The minimised hardware requirement and user-friendly desktop application system make SCAN suitable for field phenotyping application.

### **#26 - Role of Annexin 8 in Stomatal Regulation of Triticeae under Salinity and Heat Stress**

**Seidat Oluwadamilola Dauda<sup>1</sup>**, Tao Tong<sup>4</sup>, Mohammad Babla<sup>1</sup>, Michelle Mak<sup>1</sup>, Jayakumar Bose<sup>1,2</sup>, Fanrong Zeng<sup>4</sup>, ZhongHua Chen<sup>1,2,3</sup>

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Annexins are a multigene family linked to calcium ion  $(Ca<sup>2+</sup>)$  regulation under abiotic stress in plants. These genes bind to negatively charged phospholipids in membrane walls and are upregulated to signal calcium production under stress. Annexins have been studied under salinity, oxidative, drought, cold and abscisic acid stress conditions in diverse plants. However, abiotic stresses occur simultaneously, and independent stress application is not enough to gauge plants response to these conditions. Thus, the role of annexins in combined salinity and heat stress in wheat is unknown. In barley, we found *HvANN8* confers salt tolerance in barley via the regulation of Ca<sup>2+</sup> and mediated reactive oxidative species signalling. We also identified 25 annexins in wheat and this includes three annexin 8 gene (*TaANN8s*) in the A, B and D sub genomes. This study evaluates the role of calcium-signalling annexin 8 gene in salinity and heat stress tolerance in wheat/barley crop (*Triticeae*). Here, we employed diverse approaches to study the role of annexin in combined stress tolerance in wheat. Localisation analysis has shown diverse expression of this gene in wheat leaves and roots. This study screened 319 wheat varieties including wild types for heat tolerance in the field at Hawkesbury River farm and greenhouse at Western Sydney University Hawkesbury campus (S35) to identify sensitive and tolerant cultivars by subjecting them to heat stress of 36 °C for 5 days at the flowering stage to identify 16 heat tolerant and sensitive varieties. The heat tolerant varieties were screened for salinity stress against common bread wheat variety (fielder) by subjecting to salinity stress of 200 mM NaCl at the seedling stage to identify two salinity tolerant cultivars. The results showed that H-199 and H-031 were more tolerant to salt stress than the other cultivars and they were screened for comparative combined stress tolerance with Fielder.

#### **# 27 - Developing a low cost, scalable irrigation management system through precise forecasting of canopy temperature**

**Stephen Rogers<sup>1</sup>**, Warren Jin<sup>2</sup>, Rose Roche<sup>2</sup>, Dani Way<sup>1</sup>

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Accurate forecasting of cotton canopy temperature (Tc) can effectively predict water stress, informing timely irrigation decisions. The BIOTIC (stress-time) approach monitors plant stress by measuring the duration in hours (stress-hours) that Tc exceeds cotton's optimal 28°C. The speed and number of stress-hours accumulated is directly related to plant water stress and therefore will influence yield and can be used to determine irrigation. A successful forecast must predict Tc, hotter Tc > 28°C and accumulated stress-hours accurately. Current models lack modern time-series techniques, particularly in predicting high-temperature and accumulated stress-hours. We test the performance of modern models (lightGBM, auto-ARIMA) against current models (periodiCT and

periodiCTS) in predicting across three field-year combinations at Narrabri (2016, 2018) and Carrathool (2021). We find that lightGBM improves high-temperature predictions by 13% (0.2°C) and stress-hour predictions by 18% (12 hrs a fortnight) over the current best methods. Particularly for Carrathool, lightGBM and auto-ARIMA improve forecasting by 30%, for Tc > 28°C (0.5°C) accuracy and stress-hours (30 hrs a fortnight) respectively. Models had equivalent forecasting ability with just air temperature and time covariates over additional biologically-relevant solar information (radiation, relative humidity, wind speed), providing practical and accurate forecasts with minimal data. Additionally, we suggest a sensor transmission frequency reduction to hourly can conserve battery life without compromising accuracy. This study establishes optimal forecasting methodology for Tc, enabling precise BIOTIC-based management, and shows the power of data-driven models to enhance water efficiency and improved canopy monitoring.

#### **# 28 - Identification of Paired Wheat Resistance and Leaf Rust Avirulence Genes Contributing to Lr41 Resistance**

**Yi Ding**<sup>1</sup>, Peng Zhang<sup>1</sup>, Bayantes Dagvadorj<sup>2</sup>, Benjamin Schwessinger<sup>2</sup>, Simon G Krattinger<sup>3</sup>, Brande Wulff<sup>3,</sup> Ann Kwan<sup>1</sup>, Evans Lagudah<sup>4</sup>, Li Huang<sup>5</sup>, Robert Park<sup>1</sup>

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Leaf rust, caused by the fungus *Puccinia triticina*, continues to be the most damaging disease of wheat globally. The resistance (R) - avirulence (Avr) interaction model is widely exploited in wheat breeding of leaf rust resistance because this simple mode of action can be selected based on clear phenotypes in specific wheat cultivars and *Pt*isolates and mostly offers resistance at all plant growth stages. The lack of knowledge of resistance mechanisms and the host and pathogen *R* and *Avr* genes that modulate pathogen ingress has often led to "blind" selection of disease resistance, and due to fast adaptation of pathogens in one-on-one relationships, rapid breakdown of resistance. Till now, more than 80 loci conferring resistance to Pt ("Lr" genes) have been designated in wheat, however, few of the actual Lr genes have been identified and no corresponding Avr gene has been isolated from Pt. This study identified a race-specific all-stage wheat R gene Lr41, which is among the first known alien introgression from *Aegilops tauschii* on chromosome 2DS. The presence of *Lr41* as a non-NLR gene was confirmed with comparative genomics and transcriptomics, mutagenesis and functional validation. We further showed that Pt virulence on Lr41 was due to haplotype sequence variations in a specific genomics region affecting AvrLr41. Experimentally confirmed with native- and hetero-expression, this study provided the first model illustrating direct and unconventional R-Avr interaction in wheat leaf rust resistance. We anticipate the current findings would help understand how wheat recognises Pt or even other pathogens through race-specific modes. Identification of the paired Lr41 and AvrLr41 may be directly fed into wheat breeding programs for engineering disease resistance.

#### **# 29 – Comparative Analysis of Homeodomain Transcription Factor Function in Leaf Shape**

Gabriella Jessica<sup>1</sup>, Elizabeth Tay<sup>1</sup>, Eleanor Imlay<sup>1</sup>, Mary Byrne<sup>1</sup>

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The leaf is an essential organ within a plant, serving as a site for physiological processes like photosynthesis. Leaf morphology varies widely from simple leaves (e.g. *Arabidopsis thaliana*) to compound leaves with distinct leaflets (e.g. *Cardamine hirsuta*), affecting plant productivity and survival. TALE (Three Amino Acid Loop Extension) homeodomain transcription factors, which consist of KNOX (Knotted1-like homeobox), BLH (BEL1-like homeodomain) proteins, and the closely related protein, KNATM, play a key role in leaf development. These proteins are conserved across plant species and vital in regulating cell differentiation, shoot apical meristem function, and organ morphogenesis. Notably, some TALE are expressed only in compound leaves, driving leaflet formation, which highlights the need for comparative studies on their functions in species with varying leaf morphologies. Among these proteins, KNATM, BEL1, and BLH11 remain relatively unexplored, prompting further research. We are investigating the function of these genes in A. thaliana and *C. hirsuta* to understand their contribution to leaf development and morphology. KNATM, a novel KNOX gene lacking a homeodomain, is thought to regulate the function of BLH genes, and we found this gene is required for leaf serrations in A. thaliana. In *C. hirsuta*, KNATM is expected to affect leaf complexity. Conversely, BEL1 was found to not regulate serrations by itself or alongside other BLH proteins in the simple leaves of *A. thaliana*, but its role in complex leaf development requires further investigation. BLH11 was found to redundantly regulate marginal serrations in simple leaves, while its orthologue appears to increase leaflet number in complex leaves, warranting further exploration. To better understand these genes, gene editing techniques are being employed to interrogate and compare the functions of KNATM, BEL1, and BLH11 in *A. thaliana* and *C. hirsuta*. These findings will progress our knowledge regarding the role of TALE homeodomain proteins in leaf morphology diversification.

#### **# 30 – Understanding the Role of Peptide-Receptor Pathways in Nitrogen Acquisition and Plant Development**

**Imin N**<sup>1,2</sup>, Banda S<sup>2</sup>, Leung KP<sup>2</sup>, Taleski M<sup>3</sup>, Djordjevic MA<sup>3</sup>

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Nitrogen acquisition, allocation, and root system architecture are critical for plant growth and crop yield. Small signalling peptides, particularly CEPs (C-Terminally Encoded Peptides) and CLEs, play crucial roles in regulating these processes. CEP peptides are induced under nitrogen starvation and travel from roots to shoots, activating receptors that generate rootward systemic signals to regulate nitrogen uptake and root proliferation. In legumes, the formation of nitrogen-fixing root nodules is controlled by a negative feedback mechanism called Autoregulation of Nodulation (AON), mediated by CLEs, and positive feedback mediated by CEPs. The AON process in legumes involves rhizobial infection triggering CLE peptide expression, which activates the SUNN receptor in the shoot, leading to leading to control of root nodulation. We have identified key downstream components of the

nitrogen demand signalling and AON mechanism, including novel transcription factors. Our work provides insights into how plant peptide signalling modulates root, shoot, and seed development in response to nitrogen availability, linking nitrogen-demand signalling to developmental programs and growth. Our study of these signalling peptides reveals their importance as regulators of plant development, connecting nitrogen-demand signalling to developmental programs and growth. We propose a unified model for growth regulation in response to nutrient availability, particularly nitrogen. Understanding these molecular processes can contribute to developing crops with improved nitrogen efficiency and higher yields. By unravelling the intricate mechanisms of nitrogenresponsive peptide signalling and its effects on plant development, we aim to pave the way for innovative approaches to crop improvement and agricultural sustainability.

#### **# 31 – APPN Field Phenomics - high resolution, high throughput phenotyping on the ground and in the sky**

#### **Salter William1**

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The Australian Plant Phenomics Network now operates a coordinated network of common field phenomics infrastructure across six nodes at institutions spanning Australia. With standardised equipment, skilled staff, common data collection protocols and FAIR data analysis workflows, we can support your research from project inception to contextual interpretation of results. We provide access to novel drone and ground-based platforms and can deploy a broad variety of sensors, from LiDAR to RGB to hyperspectral, to enable you to answer the research questions that are important to you.

#### **# 32 – Supercharging the carbon concentrating mechanism in C4 plants**

Robert Sharwood<sup>1</sup>, Grant Pearce<sup>2</sup>, Maria Ermakova<sup>3</sup>, Robert T Furbank<sup>4</sup> and Oula Ghannoum1

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An escalating challenge facing Australia's grain crop production is sustaining the supply of nutritious food within water limited environments. Extreme climate events and decline in available water are threatening crop yield and quality necessitating new solutions to ensure optimal water use within future food systems. We have sought to identify natural biochemical solutions within  $C_4$ plants that confer improved responses to carbon assimilation and plant productivity under future climates. We have taken a transgenic approach to overexpress the primary carboxylase of  $C_4$ photosynthesis - phosphoenolpyruvate carboxylase (PEPC) in *S. viridis* and a biochemical approach to investigate the *in vitro* biochemistry of PEPC across biochemical subtypes within the Paniceae tribe of  $C_4$  grasses. We have successfully expressed recombinant  $C_4$  PEPC isoforms within *E. coli* and investigated their catalytic properties including the K<sub>m</sub> for PEP and bicarbonate,

 $V_c^{max}$  and inhibition by feedback inhibition by malate and aspartate. In addition, we discovered that the PEPC isoform from the PCK subtype were catalytically superior and contained an amino acid signature that corresponds to decreased malate inhibition which is crucial for increasing flux through the CCM. Therefore, we have identified a number of new avenues to improve carbon in C<sub>4</sub> plants particularly under abiotic stress conditions. An update on the current progress of this research will be presented.

# <span id="page-61-0"></span>List of NSW participants and delegates



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