Development of suberized barrier is critical for ion partitioning between senescent and non-senescent tissues in a succulent halophyte Sarcocornia quinqueflora

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Senescence of plant tissues is a physiologically synchronized process that enables an evergreen or a perennial plant to retrieve, recycle and remobilize nutrients from elder to younger tissues or upcoming seeds. The succulent perennial halophyte *S. quinqueflora* utilizes this process to discard excess salt being accumulated in outer tissues of their leafy stems. The exact mechanism for salt shedding in this plant, however, remains elusive. In this work we show that the plant develops two distinct types of tissues - an endodermis-like layer (suberized layer, ED), and an additional internal photosynthetic layer (IP) - to enable this process. Their potential roles toward salt-coping strategy were investigated in this study. We show that elevated salinity leads to an accelerated development of the ED, and that its development strongly affected ion partitioning between outer (senescent) and inner (non-senescent) tissues. A positive correlation between the ratio of ED to a bead diameter and the outer to inner concentration of Na^+ was observed. These ratios were highest in older (basipetally-located) beads and progressively decreased towards the tip. Furthermore, the Na^+/ K^+ ratio in inner tissues of bottom beads at highest salinity treatments (800 and 1000 mM NaCl) that showed clear senescence symptoms was ~1.0, indicative of complete separation of the outer and inner tissues at late developmental stage due to the fully suberized ED multilayer. A dual-sources : dual-sinks model explaining the role of the IP layer in plant adaptation to salinity is presented.

Xylem cavitation isolates leaky flowers during water stress in pyrethrum

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Flowers underpin plant evolution, genetic legacy and global food supply. They are exposed to similar evaporative conditions as leaves, yet floral physiology is a product of different selective forces. We used Tanacetum cinerariifolium, a perennial daisy, to examine the response of flowers to whole-plant water stress, determining if flowers constitute a liability during drought, and how this species has adapted to minimize risk associated with reproduction.

We determined the relative transpiration cost of flowers and leaves and confirmed that flowers in this species are xylem-hydrated. The relative water stress tolerance of leaves and flowers then was compared using xylem vulnerability measurements linked with observed tissue damage during an acute drought treatment.

Flowers were a major source of water loss during drought but the xylem supplying them was much more vulnerable to cavitation than leaves. This xylem vulnerability segmentation was confirmed by observations that most flowers died whereas leaves were minimally affected during drought.

Early cavitation and hydraulic isolation of flowers during drought benefits the plant by slowing the dehydration of perennial vegetative organs and delaying systemic xylem damage. Our results highlight the need to understand flower xylem vulnerability as a means of predicting plant reproductive failure under future drought.

Nitrogen fertiliser increases LAI but creates water costs in *Eucalyptus nitens*

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Leaf area index (LAI) is an important driver of primary productivity, and affects water and nutrient cycling. Extra leaves have both a cost and a benefit to a plant in terms of carbon and water balance and nutrient economics. Greater leaf area increases photosynthetic area, but also incurs a respiratory cost to the plant in terms of leaf construction and maintenance. Optimal leaf area is therefore influenced by the trade-off between carbon gains through photosynthesis and carbon loss through respiration, but is also influenced by transpirational demands. Furthermore, optimal leaf area responds to environmental factors such as nutrition, temperature and water supply. Using three field experiments across a rainfall and temperature gradient in Tasmania, I investigated the way in which nutrient supply influences the optimal leaf area of the globally-important plantation tree, *Eucalyptus nitens*.

Results show that the costs and benefits of extra leaf area depend on nutrient supply as well as site characteristics. Specifically, LAI was highest at intermediate nitrogen levels over the first growing season, with associated changes to maximum net photosynthetic rate, dark respiration and stomatal conductance. Thus, leaf area response to nutrition is decidedly non-linear in this system with corresponding influences on plant water use and physiology. These results will contribute to the development of efficient nutrition management of production forests through an improved ability to predict and model the impact of fertiliser on productivity.

Canopy damage during a natural drought depends on species identity, physiology and stand composition.

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Vulnerability to xylem cavitation is a strong predictor of drought-induced damage in forest communities. However, biotic features of the community itself can influence water availability at the individual tree-level, thereby modifying patterns of drought damage. Using an experimental forest in Tasmania, Australia, we determined the vulnerability to cavitation (leaf P_{50}) of four tree species and assessed drought-induced canopy damage of 2944 six-year-old trees after an extreme natural drought episode. We examined how individual damage was related to their size and the density and species identity of neighbouring trees. The two co-occurring dominant tree species, *Eucalyptus delegatensis* and *E. regnans* were most vulnerable to drought-induced xylem cavitation and both species suffered significantly greater damage than neighbouring, sub-dominant species *Pomaderris apetala* and *Acacia dealbata*. While the two eucalypts had similar leaf P_{50} values, *E. delegatensis* suffered significantly greater damage which was strongly related to the density of neighbouring *P. apetala*. Damage in *E. regnans* was less impacted by neighbouring plants and smaller trees of both eucalypts sustained significantly more damage than larger trees. Our findings demonstrate that natural drought damage is influenced by individual plant physiology as well as the composition, physiology and density of the surrounding stand.

Temporal and spatial linkage between xylem failure and leaf damage

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Global warming is expected to dramatically accelerate forest mortality as temperature and drought intensity increase. Predicting the magnitude of this impact urgently requires an understanding of the process connecting atmospheric drying to plant tissue damage. Recent episodes of forest mortality worldwide have been widely attributed to dry conditions causing acute damage to plant vascular systems. Under this scenario vascular embolisms produced by water stress are thought to cause plant death, yet this hypothetical trajectory has never been empirically demonstrated. Here we provide foundational evidence connecting failure in the vascular network of leaves with tissue damage caused during water stress. We observe a catastrophic sequence initiated by water column breakage under tension in leaf veins which severs local leaf tissue water supply, immediately causing acute cellular dehydration and irreversible damage. By highlighting the primacy of vascular network failure in the death of

leaves exposed to drought or evaporative stress our results provide a strong mechanistic foundation upon which models of plant damage in response to dehydration can be confidently structured

Reproductive water supply is prioritised during drought in tomato.

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Reproductive success largely defines the fitness of plant species. Understanding how heat and drought affect plant reproduction is thus key to predicting future plant fitness under rising global temperatures. Recent work suggests reproductive tissues are highly vulnerable to water stress in perennial plants where reproductive sacrifice could preserve plant survival. However, most crop species are annuals where such a strategy would theoretically reduce fitness. We examined the reproductive strategy of tomato (*Solanum lycopersicum* var. Rheinlands Ruhm) to determine whether water supply to fruits is prioritized above vegetative tissues during drought. Using optical methods, we map xylem cavitation and tissue shrinkage in vegetative and reproductive organs during dehydration to determine the priority of water flow under acute water stress. Stems and peduncles of tomato showed significantly greater xylem cavitation resistance than leaves. This maintenance of intact water supply enabled tomato fruit to continue expanding during acute water stress, utilising xylem water made available by tissue collapse and early cavitation of leaves. Here, tomato plants prioritize water supply to reproductive tissues, maintaining fruit development under drought conditions. These results emphasize the critical role of water transport in shaping life history and suggest a broad relevance of hydraulic prioritization in plant ecology.

***In vivo* monitoring of drought-induced embolism in *Callitris rhomboidea* trees reveals wide variation in branchlet vulnerability and high resistance to tissue death**

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Damage to the plant water transport system through xylem cavitation is known to be a driver of plant death in drought conditions. However, a lack of techniques to continuously monitor xylem embolism in whole plants *in vivo* has hampered our ability to investigate both how this damage propagates, and the possible mechanistic link between xylem damage and tissue death. Using optical and fluorescence sensors we monitored drought-induced xylem embolism accumulation and photosynthetic damage in vivo throughout the canopy of a drought resistant conifer, *Callitris rhomboidea*, during drought treatments of approximately 1 month duration. We show that drought-induced damage to the xylem can be monitored *in vivo* in whole trees during extended periods of water stress. Under these conditions, vulnerability of the xylem to cavitation varied widely among branchlets, with photosynthetic damage only recorded once >90% of the xylem was cavitated. The variation in branchlet vulnerability has important implications for understanding how trees like *Callitris rhomboidea* survive drought and the high resistance of branchlets to tissue damage points to runaway cavitation as a likely driver of tissue death in *C. rhomboidea* branch-tips.

Ancestral state reconstruction of functional characteristics

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This talk explores some basic ideas about ancestral state reconstruction, and also presents a shameless plug for a quantitative study of the accuracy some representative types. Ancestral state reconstruction is extraordinarily widely used, especially when implicit reconstructions are included – cases in which there is no formal analysis.

The talk focuses on methods in which we take an existing tree and reconstruct states of nodes. It will briefly consider assumptions underlying ancestral state reconstruction, and how some important methods relate to these

assumptions, especially for characters under natural selection. This leads to his paper – Holland BR, Ketelaar-Jones SK, O'Mara AR, Woodhams M, Jordan GJ (2020) Accuracy of ancestral state reconstruction for non-neutral traits. *Scientific Reports* **10**, 7644 – which simultaneously evolved synthetic (simulated) phylogenetic trees and binary characters under a range of evolutionary conditions (different rates of character evolution, state-dependent speciation and extinction) and tested how well ancestral state reconstruction matched the true evolution of the character. The take home messages are perhaps intuitive, but the literature indicates that many studies ignore them. In decreasing order of their impact, errors are more likely when the character is labile, deep nodes are considerably harder to reconstruct than nodes closer to the tips, selection on the character (in the form of impacts on extinction, speciation or change in character state) increases the error. These errors are largely in addition to those implied by confidence intervals of likelihood based models and ambiguity in parsimony methods.

Biofortification of zinc in barley for human and animal health

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Zinc (Zn) deficiency is a global health concern affecting 31% of the entire population, which are found to increase up to 73% in regions with high cereal consumption. Deficiency of Zn is also a major problem in agricultural animals and causes significant losses to the livestock industry. Poor Zn levels in both shoots and grains are attributed to cultivation on marginal Zn soils. This research demonstrates the role of both soil and plant (genotypic) factors to achieve the target Zn concentrations in aboveground parts of barley plant (*Hordeum vulgare* L.). A meta-analysis was conducted by collecting all peer-reviewed literature between 1945-2020 to assess Zn concentrations in barley. Our results show that substrate Zn undoubtedly has a significant positive correlation with Zn concentrations in both barley shoots and grains. Additionally, genotypic variation in barley Zn levels was found to produce a difference of 3.5-fold in shoots to 4.5-fold in grains between low and high Zn accumulating cultivars under similar conditions. Significant positive correlations were determined between Zn concentrations of roots and shoots, and shoots and grains of barley. This indicates that shoot concentrations could also be a selection criterion during screening of cultivars for grain Zn. These findings also suggest that the Zn concentrations in barley shoots and grains could be improved by use of optimal Zn fertiliser and high Zn accumulating cultivars. There is a need to further investigate and validate the candidate genes involved in efficient Zn uptake and translocation mechanisms in barley through use of marker-assisted breeding and transgenic approaches.

Investigating pollen compatibility of commercial sweet cherry cultivars by DNA analysis

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Sweet cherries (*Prunus avium* L.) are largely self-incompatible, which is determined by a gametophytic self-incompatibility system that is controlled by a multi-allelic *S*-locus. Commercial cherry orchards select cross-compatible cultivars based on synchronous flowering and cross-compatible *S*-alleles to maximise pollination success. This study explored the use of microsatellites and *S*-locus genes to investigate pollen interactions in commercial cultivars (e.g. Kordia and Regina) that are notorious for below average fruit set (<20%). These molecular techniques have been tested in other plant models, however, there exists limited examples in commercial sweet cherry orchards. This study used primers targeting specific alleles of the *S* haplotype-specific F-box (*SFB*) gene (S_1, S_2, S_3, S_4, S_4', S_6, S_9, S_{12}, S_{13} and S_{36}), and 13 microsatellite primers, to assess the performance of common pollinisers for Kordia and Regina. *S*-allele analysis revealed the pollen donor candidates for Kordia and Regina seeds in the open-pollinated orchard; however, further analysis was required to discriminate between pollen donor candidates with the same *S*-alleles. Subsequently, we tested 13 microsatellites to identify those that vary in size among all pollen donors by separating microsatellite products on agarose gel. While microsatellite size variation was insufficient to provide reliable discrimination on an agarose gel, this study identified loci that have this potential if run on a DNA sequencer. *S*-genotyping of seeds is a simple method to determine the performance of pollinisers in commercial cherry orchards where pollen donors do not share *S*-alleles. The *S*-allele specific *SFB* primers that we report can be used in other

important cherry cultivars. Developing a multiplex PCR would require little additional investment to make this technique even faster and cheaper.

I love the smell of nodules in the morning: Using a cheap electronic nose to smell clover-rhizobia communication

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Legumes form symbiotic relationships with rhizobial bacteria when the soil nitrogen is too low to meet their requirements. To encourage the relationship, the plants communicate to free-living rhizobial bacterial via chemical signals, though these signals can be difficult to measure in the field. This research aimed to evaluate whether a cheap electronic nose (e-nose) containing an array of off-the-shelf sensors can be used *in situ* to smell volatile organic compounds released by the legume roots, and whether it could differentiate from legumes with and without rhizobial associations.

A 2x2 factorial design with two legume species; subterranean clover (*Trifolium subterraneum*) and lucerne (*Medicago sativa*), and two rhizobia inoculation treatments (inoculated and non-inoculated) were conducted in a glasshouse for six weeks. Six e-noses were added after two weeks and were rotated between replicates. Above and belowground biomass, nodule score and plant nitrogen concentration were measured at the end of the experiment.

The e-nose did show different readings between inoculated and non-inoculated subterranean clover plants. These signals appeared well before visible nodules were expected to form. No significant differences were detected between inoculation treatments in the lucerne plants, though nodulation was poor in this species. Daily peaks were also detected in all treatments, which are possibly related to diurnal biological processes.

In conclusion, results suggest that the e-nose could be used to detect legume-rhizobia communication, potentially well before nodulation can be visually assessed. This suggests e-noses could be used on-farm as an early indicator of successful nodulation.

The e-nose development has been supported by the Cooperative Research Centre for High Performance Soils whose activities are funded by the Australian Government's Cooperative Research Centre Program [Project ID: 2.1.004]

The rare *Eucalyptus risdonii* is expanding its range under climate change through hybridisation despite hybrid inferiority

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The genus *Eucalyptus* is renowned for its high level of hybridisation between species. One well-studied hybrid system involves *Eucalyptus risdonii* and *E. amygdalina*. These species are endemic to Tasmania (Australia) where small disjunct populations of *E. risdonii* occur within a matrix of *E. amygdalina*. Phenotypes representing a continuum between the pure form of each species occur in localised hybrid swarms. Intermediate phenotypes are also found as isolated individuals or patches of varying size scattered within the range of *E. amygdalina*. Studies in 1980s suggested that *E. risdonii* was invading the surrounding *E. amygdalina* by pollen dispersal followed by resurrection. We extend these studies by integrating results from genomics, common-garden field trials, and environmental modelling to genetically characterise pure and intermediate phenotypes and evaluate the later-age fitness of pure and hybrid products. Analysis of 3,362 SNPs showed the hybrid zone had a complex genetic structure comprising multiple generations of hybridisation. Consistent with expectations, isolated intermediate phenotypes within the range of *E. amygdalina* generally matched F_1, and hybrids well-separated from *E. risdonii* but tending towards its phenotype matched simulated backcrosses. Selection acting in favour of *E. risdonii* phenotypes was shown to have persisted, as evidenced by greater mortality of *E. amygdalina* compared with *E. risdonii*. The current seedling cohort contained more *E. risdonii*-like phenotypes than expected based on adult frequencies. A genetic basis to the fitness differentials was demonstrated in the common garden trial, in which hybrids had significantly poorer fitness than either pure species, consistent with hybrid inferiority. However, these differences were not

manifested until later age. This study contributes to the increasing number of reports of hybrid inferiority but argues that despite strong post-zygotic barriers hybridisation is an important evolutionary mechanism, in the present case appearing to contribute to the range expansion of *E. risdonii* in response to climate change.

GABA in regulating plant adaptive responses to hostile soils

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In mammalian systems, a broad class of chemical substances termed "neurotransmitters" operate upstream of receptors and membrane transporters. One of them is g-aminobutyric acid (GABA). In this work, we demonstrate that GABA also play a major role in mediating plant adaptive responses to hostile soils, namely soil salinity and flooding. Using Arabidopsis *pop2-5* and *gad1,2* mutants we show that *gad1,2* mutant lacking an ability for conversion of glutamate to GABA possessed over-sensitivity to salinity while the *pop2-5* mutant that was capable to over-accumulate GABA in its roots showed a salt-tolerant phenotype. The above differential salinity tolerance between two lines was explained by GABA's operation upstream of H^+-ATPase that resulted in better membrane potential maintenance and reduced extent of stress-induced K^+ leak from roots. Also reduced was the rate of net Na^+ uptake in *pop2-5* roots and rate of H_2O_2 production in salinity-exposed roots in GABA over-accumulating plants. The second case study revealed the role of GABA in plant adaptive responses to waterlogging. While the GABA content in WL-grown plants increases several orders of magnitude, the physiological rationale behind this elevation remains largely unanswered. By combining genetic and electrophysiological approach, we show that hypoxia-induced increase in GABA content is essential for restoration of membrane potential and preventing ROS-induced disturbance to cytosolic K^+ homeostasis and Ca^{2+} signaling. We show that reduced O_2 availability affects H^+-ATPase pumping activity, leading to membrane depolarization and K^+ loss via outward-rectifying GORK channels. Hypoxia stress also results in H_2O_2 accumulation in the cell that activates ROS-inducible Ca^{2+}-uptake channels and triggers self-amplifying "ROS-Ca hub", further exacerbating K^+ loss via non-selective cation channels that results in the loss of the cell's viability. Hypoxia-induced elevation in the GABA level may restore membrane potential by pH-dependent regulation of H^+-ATPase and/or by generating more energy through the activation of the GABA shunt pathway and TCA cycle. Elevated GABA can also provide better control of "ROS-Ca^{2+} hub" by transcriptional control of RBOH genes thus preventing over-excessive H_2O_2 accumulation. Finally, GABA can operate as a ligand directly controlling the open probability and conductance of K^+-efflux GORK channels, thus enabling plants adaptation to hypoxic conditions.

Triggered at the first sign of stress – examining the evolutionary history of *NCED* genes and abscisic acid biosynthesis in land plants

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Diverse land plants synthesise the stress hormone abscisic acid (ABA) in response to dehydration, with the rate-limiting step in ABA biosynthesis controlled by 9-*cis*-epoxycarotenoid dioxygenase (NCED) enzymes. In flowering plants (angiosperms), ABA can be triggered rapidly in response to the first sign of dry conditions including a decrease in air humidity/increase in the vapour pressure deficit (VPD) between the leaf and the atmosphere, via transcriptional induction of key *NCED* genes in the leaf. ABA signalling pathways are known to prompt closure of stomatal pores in angiosperms, preventing further loss of water from the plant via transpiration. ABA also activates slower plant responses that enhance desiccation tolerance, as well as other diverse roles in growth and development. This talk will examine the evolutionary history of *NCED* genes in plants, what we know so far about how these genes differ, and how we can use this information to make predictions for less well-studied plant species.

Water transport failure in minor veins triggers rapid dehydration and damage in Eucalyptus viminalis leaves

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Cavitation is known as a major driver of tree mortality, but the mechanistic link between hydraulic failure and plant damage in trees is still missing. We used *Eucalyptus viminalis*, a woody tree species which has been recently impacted by extensive dieback in Australia to determine whether water transport dysfunction is directly responsible for triggering lethal leaf desiccation during water stress. Examining two scenarios that could sever water supply to leaf tissue, either runaway xylem cavitation in the leaf vein network or local failure of minor veins, we compared the timing of a decrease in chlorophyll fluorescence (F_v/F_m) with key metrics associated with plant hydration and xylem cavitation. We also calculated the theoretical water potential at which runaway cavitation is expected to occur in leaves. The water potential at which F_v/F_m was observed to decline corresponded to a rapid acceleration in the rate of leaf dehydration, suggesting that damage was triggered by truncated water supply in the leaf. The decline in F_v/F_m corresponded also to early (20%) cavitation in the minor veins order which suggested a causal link between damage and reduction in hydraulic supply in the tissue supplied by minor veins. Our results underlie the clear connection between tissue damage and cavitation in the non-redundant rank of leaf minor veins. This highlights the importance of minor vein cavitation for understanding and modelling canopy desiccation.

Right place, right time; exploring the action of the plant hormone gibberellin during nodulation

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Plants form beneficial associations with microorganisms to obtain out of reach nutrients in exchange for reduced carbon. For example, nitrogen is one of the most limiting elements in agriculture and plants form symbiotic associations with nitrogen fixing bacteria to gain access to fixed nitrogen from the atmosphere. Legumes have evolved the ability to form nodules, root organs that host nitrogen-fixing bacteria collectively known as rhizobia and provide the ideal environment for the symbiosis and nutrient exchange. Plants tightly regulate this symbiosis to control the extent of the infection and to carefully regulate the complex process of nodulation. Plant hormones play an important role in legume-rhizobia interaction and nodule formation, but the exact place, time, and mechanism of action of each hormone is not completely understood. We work with pea as a model to understand how bioactive gibberellins control nodulation. We will discuss the distinct roles of gibberellin in rhizobia infection at the epidermis and the activation of the cell division and nodule organogenesis in the cortex. We will also report on the crosstalk of gibberellin with other plant hormones involved with nodulation including cytokinin and ethylene. We have used a range of approaches to examine these questions, including hormone biosynthesis mutants, the use of *Agrobacterium*-mediated root transformation of specific root cell layers, the evaluation of distinct aspects of nodule development, gene expression, hormone levels and the interaction with root architecture. Our results provide evidence of an important role for gibberellins in limiting infection in the epidermis while stimulating cell division and nodule development in the cortex.

Investigating the AON and AOM pathway in the novel mutant *K301*

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Plant symbiosis such as nodule forming interactions between legumes and rhizobia and more widespread associations between plants and mycorrhizal fungi play an essential role in nutrient uptake, development and health. For symbiosis to be beneficial rather than parasitic, the plant must tightly regulate this process through pathways such as the autoregulation of nodulation (AON) pathway or autoregulation of mycorrhizae (AOM) pathway. While the AON and AOM genetic pathways are not completely understood it is thought that the AON has evolved from the

AOM and shares many key elements. Through studies we are investigating various components of the AON and AOM pathways. This includes characterising the *K301* mutant created with the chemical mutagen nitrosoethylurea. This mutant displays excessive nodulation and mycorrhizal symbioses, suggesting it is disrupted in the AON and AOM pathways. We are undertaking a range of investigations to characterise the role of this gene including investigating shoot and root morphology; vascular anatomy; whether the gene affected is shoot or root acting; how the mutant acts under different nutrient conditions; and, through genotyping, whether the mutated gene in question may be one known to cause similar phenotypic responses in other species.