



PHYTOGEN

A
NEWSLETTER
FOR
AUSTRALIAN
PLANT SCIENTISTS

Volume 14 Number 2
August 2012

PHYTOGEN

Volume 14: Number 2

In This Issue

President's Report August 2012

Message from the President Elect

ASPS Office Bearers 2012

A Message from the Editor

International Fascination of Plants Day – A Great Success

Science Meets Policymakers Summit

A report by Chris Cazzonelli

State of Affairs – Western Australia

A wealth of information about what's happening in research in WA coordinated by Tim Colmer

Update for:

Functional Plant Biology

Rana Munns

From Our New PhDs

The research of 6 recently-graduated PhD students from Queensland, Tasmania, South Australia, Western Australia and New Zealand covering an eclectic mix of topics

ComBio 2012, Adelaide

2013 Conferences

Were you aware that?

Information about ASPS activities

Thanks to all the contributors for being prepared to give their time to provide informative articles for this issue of Phytogen.

It takes something extra to make these contributions.



AUSTRALIAN SOCIETY OF PLANT SCIENTISTS

President's Report August 2012

The next big thing on the ASPS agenda is ComBio2012 24-27th September in Adelaide. We have a great line up of speakers in the plant sessions. The informal ASPS dinner will again be held on Tuesday 25th Sept in the evening – thank you to Education Rep Kathy Soole for taking the lead on this, helped by postgrads Gordon Wellman and Sandra Schmoeckel. This is always great fun, so please put it in your diary. Our AGM is on Wednesday 26th, 5 pm (time TBC). Please think now about who you would like to nominate for the various positions becoming vacant (see listing elsewhere), or whether you would like to serve the Society in this way. There will be a full council meeting on Sunday 23rd September in the afternoon (about 4pm TBC).

This year ComBio will have a record seven societies participating, the most since its inception in 1999. Particularly relevant to us are symposia organised by the Australian Society of Plant Pathologists (Molecular and Physiological Special Interest Group), the annual meeting of the Australasian Genomic Technologies Association (AMATA) and the New Zealand Society of Plant Biology, so there will be plenty of good talks to choose from. Thank you to Rachel Burton and Steve Tyerman for representing the ASPS on the organising committee and Conference Chair Stuart Pitson.

The new ASPS website will go live at the AGM. Rob Shepherd (postgrad and ASPS executive assistant) has been working with web master Michael Major to revitalise it, especially updating the back end. You'll be able to post job advertisements directly on line, and with live news feeds and links to social media it will be much more dynamic.

The Australian Academy of Science has a series of national committees that act as a conduit up and down to the various scientific societies. There is also the sense that such committees provide a different perspective on their discipline areas. I serve as our representative on the National Committee for Plant and Animal Sciences, having taken over from David Day a year or so ago. The committee covers just about all biology that isn't medicine or biochemistry (including genetics, ecology, systematics, marine biology etc.). There are two things to report. First, we are in the early stages of developing a decadal plan for Agricultural Sciences in Australia (strengths, weaknesses, opportunities and threats). Second, as part of the Academy's review of their national committees, we have been asked to comment on our links with various international unions. NCPAS is joined to the International Union of Biologist.

The ASPS Facebook page now has 60 "Likes" (that means those people get our posts automatically) and we have 64 people "following" us on Twitter. This is not a lot, but it's a start. It is increasingly the way people are communicating, so why not join up and find out what it's about?

I look forward to seeing as many of you as possible at the upcoming ComBio.

Cheers,
Ros Gleadow

Message from the President Elect

I look forward to catching up with many of you at ComBio2012 in Adelaide, 24th – 27th September. Although we are all focused on the conference in Adelaide, for which the program looks excellent, some arrangements have already commenced for ComBio2013 in Perth, 29th September to 3rd October. Please pencil in those dates to join us in Perth – you might consider also staying a few extra days to see the world-famous wildflowers of Western Australia?

ComBio2013 will have two plant streams – ‘Plant Biology’ and ‘Plant Ecophysiology’. Symposia within the ‘Plant Biology’ stream will include: Photosynthesis and respiration, Membrane transport, Symbiotic associations, Secondary metabolites, Metabolism. Symposia within the ‘Plant Ecophysiology’ stream will include: Water relations/water stress, Seed germination, Phosphorus physiology, Abiotic stress, Global change physiology.

In addition to the two plant streams, we will also have plant symposia within some of the other streams. Namely, Plant systems biology (in the stream ‘Genomics and Bioinformatics’), Regulation of leaf and root development (in the stream ‘Developmental Biology’), and Signalling responses to biotic and abiotic stresses in plants (in the stream ‘Cell Signalling’). This arrangement promises greater opportunities for interactions by us plant scientists with members of the other societies, and we hope some very interesting interdisciplinary discussions will result! So, with plenty of plant content, as well as the biodiverse plant communities for you to see within a comfortable drive from Perth (and some other nearby plant-based attractions, e.g. wineries, Kings Park, etc.), we are looking forward to many plant scientists from Australia and overseas joining us for ComBio2013.

Hans Lambers, Martha Ludwig, and I, are the ASPS members on the combined organising committee – so feel free to please contact one of us with any suggestions or questions regarding ComBio2013. We will keep you informed of further development of the conference program in future issues of Phytogen.

Best wishes,

Tim Colmer.

ASPS COUNCIL MEMBERS – 2012***Executive:***

| | | |
|---------------------------|----------------------------|--|
| President | Ros Gleadow | Monash University |
| President Elect | Tim Colmer | University of Western Australia |
| Honorary Secretary | Christine Beveridge | University of Queensland |
| Honorary Treasurer | Helen Irving | Monash University |

Discipline Representatives:

| | | |
|---|-------------------------|---------------------------------------|
| Genetics & Molecular Biology | Oliver Berkowitz | Murdoch University |
| Cell Biology | Zhonghua Chen | University of Western Sydney |
| Plant Microbe Interactions | Uli Mathesius | Australian National University |
| Whole Plants | Tim Cavagnaro | Monash University |
| Plant Development | Jim Reid | University of Tasmania |
| Environmental & Ecophysiology, | | |
| Global Climate Change | Belinda Medlyn | Macquarie University |
| Plant Science Education | Kathleen Soole | Flinders University |
| <i>Student Representative</i> | Jessica Bovill | ACPF, Adelaide |
| <i>Public Officer</i> | John Evans | Australian National University |

ASPS SUB-COMMITTEES

| | | |
|---|---------------------|---------------------------------------|
| <i>FASTS</i> Representative & Global plant Council | Barry Pogson | Australian National University |
| <i>Phytogen</i> Editor | Tina Offler | University of Newcastle |
| <i>Functional plant biology</i> Editor in Chief | Rana Munns | Functional plant Biology |

SUPPORT SERVICES

| | | |
|-------------------------|----------------------|--|
| <i>Webmaster</i> | Michael Major | Michael Major Media Pty Ltd, Adelaide |
|-------------------------|----------------------|--|

A message from the editor

Dear Fellow ASPS Members,

This issue is packed full of reading, you will need more than one night to do it justice. As always we wouldn't have a newsletter without the generosity of those who have given their time to put together their articles. The society, and I as editor, are indebted to all contributors. Thank you.

The research, or rather some of the research, happening in Western Australia presented under the "State of Affairs" banner is the work of many, ably collated by Tim Colmer. Six newly-graduated PhD society members responded to my call for articles and are representing work done in their states and New Zealand – a very varied collection of topics presented with refreshing enthusiasm.

The first 'Fascination of Plants Day' has happened and Arwen Cross has put together the details of how Australian plant scientists contributed to making the day a success. When children compose songs and produce videos our message about plant science is getting "out there". But it didn't happen without considerable effort and commitment. Thanks to all those committed people and perhaps next year will see them joined by more members of our society.

In this issue we have messages from our President, Ros Gleadow and President-elect, Tim Colmer, and an update on the activities and plans for Functional Plant Biology from the Editor-in-Chief, Rana Munns. There is also what I consider a very timely report on the "*Science Meets Policymakers Summit*" by Chris Cazzonelli who attended the meeting in February this year. Within his report entitled "*Science meets Policymakers: promoting evidence-based policy development*" are a number of key action points arising from the proceedings worthy of consideration by all of us.

Hope to see many of you at ComBio2012 in Adelaide.

Tina Offler

Remember 18 May this year was:

International Fascination of Plants Day

Here is a report on the Australian activities

Fascination of Plants Day 2012 Roundup

by Arwen Cross

The world celebrated Fascination of Plants Day for the first time on 18 May 2012. Events were run in plant research institutions and botanic gardens in 39 countries. The day was organised by the European Plant Science Organisation (EPSO) to promote the importance of plants and plant science worldwide.

In Australia 14 institutions ran 22 events including a tour of the Aboriginal Garden at Monash University, plant experiments in the Q-lab at Questacon, a public lecture by Julian Cribb on global food security at the University of Queensland, and an online video competition. For a full list of events and a few photos see <http://www.plantday12.eu/australia.htm>

Radio interviews brought Fascination of Plants Day to Australians that couldn't attend live events. ABC Brisbane interviewed Dr Paul Scott from the University of Queensland about why plants are so important in our daily lives. He went on to describe his research in developing biofuels from tree legumes. You can listen to the podcast here <http://blogs.abc.net.au/queensland/2012/05/world-fascination-of-plants-day.html>

In SA, year 11 student Kathryn Law sang her song Plants are Fascinating on the SA Country Hour. Her song was the winning senior entry in the Fascination of Plants Day Video Competition run by the Australian Centre for Plant Functional Genomics. It was an adaptation of a pop song with original plant science lyrics including, "The leaves sunlight doesn't miss, Will have photosynthesis, I wasn't looking for this!". The winning student in the junior category used an emotional Beethoven soundtrack for his video showing how he imagines a world without plants. His food and furniture disappeared, and the trees outside faded to black and white before vanishing. It was a poignant reminder of how important plant products are in our everyday lives. These winners were selected from about 40 entries in the Fascination of Plants Day Video Competition. One teacher said that the competition "helped students contextualise their knowledge of plants and combine their love of biology with talents in the arts". Several plant themes came up in many of the videos. Many students introduced the science of photosynthesis and the importance of plants in producing oxygen in the atmosphere. Plants as food providers for people and animals, and the visual beauty of plants were also important to students. You can watch the videos at the videos tab of the completion website <http://www.acpfg.com.au/videocomp/>

Dr Monica Ogierman, the event co-ordinator, spoke at the Plant Biology Conference in Freiburg in August about Australia's involvement in this event. She was invited to showcase photographs from our events and videos made by Australian high school students about why they find plants and plant science fascinating.

Fascination of Plants Day will be run again in 2013, and we hope that earlier notice will allow even more Australian institutions to participate. Live or online events, articles, competitions, open days and public lectures held in the month around Fascination of Plants Day can all be registered as Australian events celebrating this day.



Artist Sophie Munns presented the exhibition *Homage to the Seed* at the University of Queensland.



Beth Gott gave tours of the Aboriginal Garden at Monash University



Staff at the Australian Centre for Plant Functional Genomics wore green to celebrate the day.

The Australian Fascination of Plants Day committee is Dr Monica Ogierman, Ms Alison Hay and Dr Arwen Cross. Many thanks to them for their efforts in making Australia's contribution so successful.

Tina Offler

Science meets Policymakers Summit

Science meets Policymakers: promoting evidence-based policy development

A report prepared by Chris Cazzonelli

On the 24th of February 2012 The Science meets Policymakers Summit brought together over 300 of Australia's top scientific researchers and policymakers to Canberra to investigate the intersection between the evidence base and government policy development. The theme for this year's summit was: Evidence-based policy development.

ASPS member Dr. Christopher Cazzonelli attended this unique event and commented "The Science meets Policymakers Summit was a real eye opener on the depth of challenges faced when attempting to instigate evidence-based policy development". Australia will be able to better address complex and demanding policy changes by improving the communication ties between scientists and policymakers. Issues such as our aging population, natural resource availability and environmental sustainability could become compromised unless the links between science and policy are streamlined.

A general theme was that good science is just not enough without well developed and sound public policy. It was deemed important we don't ignore the scientific evidence base when developing public policy. The fisheries management policy was highlighted as an outstanding example of where science has integrated well into policy and practice and as a result the community is better for it. But it didn't take long to identify areas where public policy is driven by short-term politics and vested interests rather than scientific evidence and perhaps the Murray-Darling Basin Plan was the best example discussed where there is some division between policy and science letting the country down. To find answers to complex policy questions we do rely on good science and experts who can communicate with influence and take action when necessary. It was understood that to ignore scientific evidence when establishing public policy could set Australia up to fail in the long term.

This year's event hosted by Science and Technology Australia focussed on finding practical solutions to improve the links between science and government policy development. As one might expect there were frustrations in the room, felt by scientists and policymakers alike. Policy development is not a linear process, nor are research outputs likely to be picked off the supermarket shelf. So in consideration of the reality that politics and community sentiment play a part in policy development, there was a strong consensus that evidence-based policy development should be a rule in policy development and not the exception.

This year's summit aimed to:

- To shape a forward agenda between the research community and government that will allow us to move beyond identifying the need to improve evidence-based policy development and focus on what we need to do to achieve it.
- Better understand the barriers to effective evidence-based policy development.
- To inform and enable policymakers and scientists.
- To improve science communication.
- Generate key action points that can be put into practice.

Key Action Points include:

- 1) Produce short issues summaries (maximum of 4 pages) detailing what the scientific evidence base says about relevant issues.** While an accurate and current four page brief will never replace ongoing and important dialogues and collaborations with discipline-specific researchers, it will certainly replace Google as a first port of call. This action seeks to make available to policymakers readable, engaging and accessible research reports based on a successful model utilised by the UK's Parliamentary Office for Science and Technology (POST).
- 2) Drive options for the Australian equivalent of fellowships, such as those that exist at the AAAS and similar schemes in order to broaden skills and raise understanding about the policy making process.** Aimed at addressing the poor engagement between scientists and policy makers by broadening skills in both groups and is one way forward based on a successful model used in the USA.
- 3) Remove disincentives for policymakers who wish to engage more with the scientific process and for scientists who wish to know more about policy development and engage with policy formulators.** Consideration should be given to include into academic reward structures recognition of 'national service' (e.g. contribution to policy development). Funding bodies (e.g. ARC, NHMRC) could recognise, or at the very least not compromise, participation in activities that improve policy formulation and science communication outside of an academic or highly specialist audience.
- 4) Formalise scientific advice models within government organisations.** It was proposed that an independent scientific adviser or dedicated internal science advisors might be appointed to every government department as is the case in the UK.
- 5) Find mechanisms for Government to identify, recognise and value scientific skills within the Australian Political System.** Tertiary science and mathematics education provides individuals with problem-solving, analytical and decision-making skills. Better use of public servants harbouring these skills should be adopted and mechanisms established to identify officers with unique problem solving and analytical skills so they can offer scientific advice, in particular, to improve rigour in policy development.
- 6) Strive to make research reports readable, engaging and accessible and best practice should be applied in their preparation and presentation.** More efficient reporting could be adopted to assist researcher and policymakers present their work in a readable, engaging and accessible way. For example, a layered model where detailed information appears in appendices or attachments on their website. In addition, reports could include chapters targeting interested persons, an overview that's more accessible to a wider policy interested audience, and a one-page box for political leaders to distil everything down to key points.

Science & Technology Australia is committed to raising outcomes from this Summit with the relevant authorities and stakeholders by advocating for change that will improve evidence-based policy development and public policy outcomes. This will be achieved by exploring stakeholders' willingness to convene and participate in small forums or workshops to progress the action items as well as working with the Department of Industry, Innovation, Science, Research and Tertiary Education to explore the possibility of a second or annual Science meets Policymakers Forum.

Disclaimer. This report was prepared with the kind assistance from Anna-Maria Arabia from Science and Technology Australia.

State of Affairs – Western Australia

SOME OF THE PLANT SCIENCE AT THE UNIVERSITY OF WESTERN AUSTRALIA

Exploring genetic diversity in phosphorus acquisition and conservation strategies in Australian native plants

Ricarda Jost, Hans Lambers and Patrick M. Finnegan
School of Plant Biology, The University of Western Australia



The plant family *Proteaceae* shows high diversification in the South-West of Western Australia and the Cape Floristic Region of South Africa, regions that both feature ancient infertile landscapes (Hopper, 2009). Plant growth on these soils is largely limited by phosphorus (P) availability (Lambers et al., 2011). Unravelling the molecular mechanisms that have evolved in plants adapted to these harsh environments will expand our understanding of P homeostasis away from current model species with very limited plasticity in the underlying genetic traits. This project involves researchers from UWA (Bahram Mirfakhraei, Robert Pontre) and collaborators Wolf-Rüdiger Scheible at the Noble Foundation (USA), Mark Stütt and others at the Max-Planck Institute of Molecular Plant Physiology (Germany), Maheshi Dassanayake and John Cheeseman (University of Illinois), Austin R. Mast (Florida State University) and Gane Ka-Shu Wong from the 1kp transcriptome project (University of Alberta, Canada).

Proteaceae species that flourish in these ancient landscapes share two major physiological adaptations that allow them to cope with the extremely low levels of soluble soil P: 1) They form clusters of determinate lateral roots (so called proteoid or cluster roots) to enhance root surface area for the exudation of phosphatases, ribonucleases and carboxylates that help to release bound phosphate (P_i) into the soil solution, and for subsequent P_i uptake. 2) These plants have a very high internal P-use efficiency and are extremely proficient at remobilising P from senescing tissues.

We are developing Harsh Hakea (*Hakea prostrata*) into a molecular model species for the *Proteaceae* to be able to identify the molecular determinants of their 'extremophile life style' on nutrient-impooverished soils. Particular focus points are the size of organic P pools, ribosome numbers for protein synthesis, adjustments in carbon, nitrogen and sulfur assimilation, changes in the regulatory networks that govern P_i homeostasis as well as root architectural features that control cluster-root development.

Using clonal propagation we have generated genetically uniform material of this diploid out-crossing species that has been grown hydroponically at four P_i supply levels. Tissues harvested from these plants are being used for metabolite profiling and RNA sequencing.

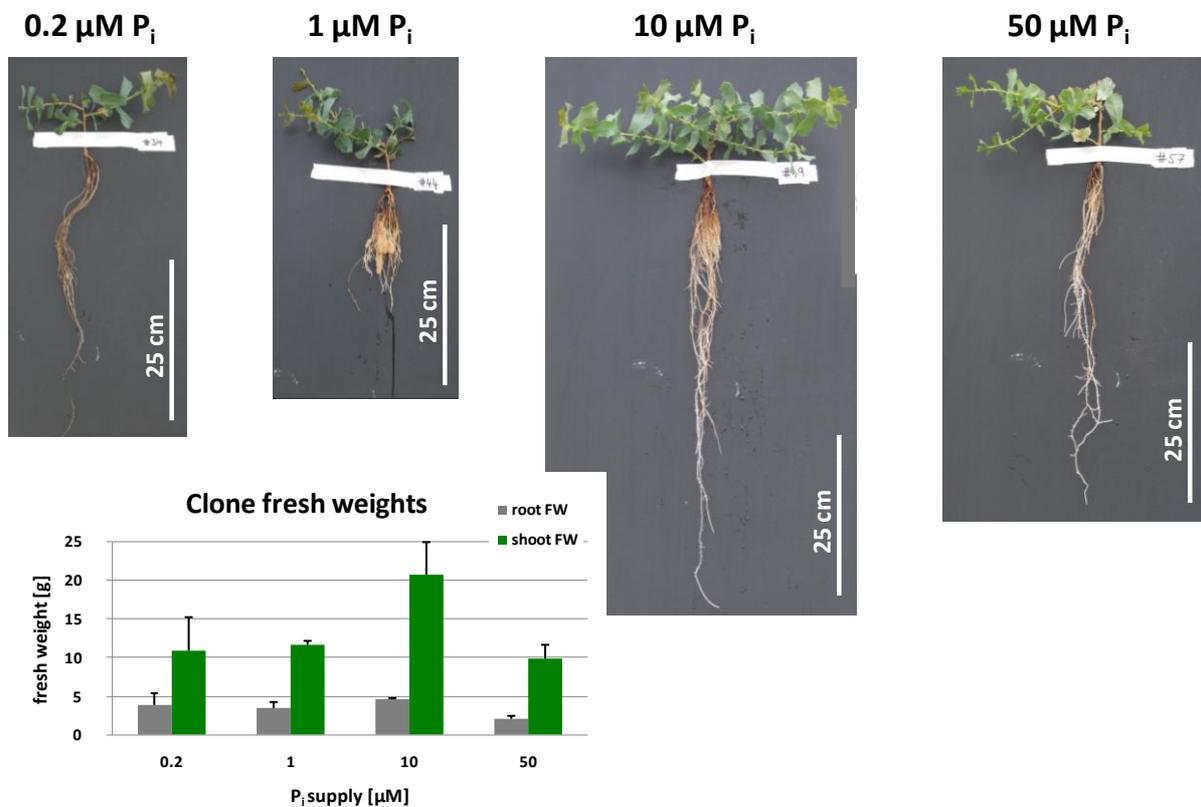


Figure: Growth habit and biomass accumulation of *Hakea prostrata* clones grown for 8 weeks in root-cooling tanks with solutions containing the indicated phosphate concentrations. Aerated nutrient solutions were replaced daily. Optimal growth was observed at 10 $\mu\text{M } P_i$ in the nutrient solution, while cluster-root formation was most prominent at 1 $\mu\text{M } P_i$. Plants grown at 50 $\mu\text{M } P_i$ showed stunted growth due to P_i hyper-accumulation resulting in P_i toxicity symptoms.

This project is funded by the ARC discovery program.

References

- Hopper, S.D. (2009). OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil* **322**, 49-86.
- Lambers, H., Brundrett, M.C., Raven, J.A., and Hopper, S.D. (2011). Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* **348**, 7-27.

The Molecular Evolution of C_4 Photosynthesis

Martha Ludwig

School of Chemistry and Biochemistry, The University of Western Australia

The C_4 photosynthetic pathway evolved more than 60 times in the angiosperms, representing a remarkable example of convergent evolution. The C_4 syndrome is a CO_2 concentrating mechanism, and gives plants with this biochemistry growth advantages over plants using the ancestral C_3 photosynthetic pathway in arid, high light and/or saline environments. Orthologues of all the genes encoding the enzymes in the C_4 pathway exist in C_3 plants; however, in C_3 species, they are not typically involved in photosynthesis, but instead have anapleurotic roles. The work in my lab focuses on the changes that

occurred in these ancestral C_3 proteins and their cognate genes that resulted in the C_4 enzymes. We are using plants in the dicot genus *Flaveria* and the grass subtribe Neurachninae as both of these groups contain closely related species that use different photosynthetic biochemistries. In addition to C_3 and C_4 species, both taxa contain C_3 - C_4 photosynthetic intermediate species, allowing us to dissect the molecular steps along the evolutionary path from the ancestral C_3 condition to the more derived C_4 state.



Collecting Neurachninae species. Eleven species of the Neurachninae are endemic to Australia. One species is found in the temperate zone while six species are found in the arid and semi-arid regions of Western Australia.

Clockwise from left: Terry Macfarlane (Western Australian Herbarium, Dept of Environment & Conservation); Martha Ludwig; Pat Finnegan (UWA); John Cheeseman and Bette Chapman (Univ Illinois at Urbana-Champaign); Harmony Clayton (UWA). Photo by Rowan Sage (Univ Toronto)

Terry Macfarlane (Western Australian Herbarium, Dept of Environment & Conservation) and Martha with *Neurachne minor*, a C_3 - C_4 intermediate. Photo by Rowan Sage (Univ Toronto).



Martha with several *Flaveria* species while on study leave at the Max Planck Institute of Molecular Plant Physiology.



Martha can be contacted at: martha.ludwig@uwa.edu.au

Phosphorus nutrition of Proteaceae: strategies in phosphorus-impooverished landscapes

Hans Lambers

School of Plant Biology and Institute of Agriculture, The University of Western Australia

Australia was once part of Gondwanaland and some of the most ancient parts of the Earth' crust can be found here. South-western Australia has been above water since sea levels were at their highest known, 90 million years ago. Moreover, the landscape has not been glaciated for over 200 million years, and the climate has been oceanically-buffered since the early Cretaceous, 140 million years ago (Hopper, 2009). South-western Australia is also one of only 25 global biodiversity hotspots; it is a region that is rich in higher plant species of which many are endemic and endangered (Myers et al., 2000). Therefore, this environment offers a unique opportunity to study plant endemism and adaptations to nutrient-poor conditions (Fig. 1).

A relatively large proportion of the species from the very phosphorus-poor soils in Western Australia cannot produce a symbiotic association with a mycorrhizal fungus (Lambers et al., 2010). This appears paradoxical, as mycorrhizas are considered an adaptation to phosphorus-impooverished soils. Instead, many species in these families (*e.g.*, Proteaceae, Cyperaceae) produce root clusters, which release large amounts of carboxylates (*e.g.*, citrate, malate), whose role is that of mobilisation of phosphorus (P) and micronutrients (Lambers et al., 2006). Mycorrhizas can enhance a plant's P acquisition when the amount of P is too low for roots to acquire sufficient amounts; however, when the amount of P is even lower, most of the soil P is sorbed onto soil particles, and hardly or not available for mycorrhizal fungi. Carboxylates in high concentrations compete with P for binding sites in the soil, and thus solubilise P, making it available for uptake by roots. The cluster-root strategy is effectively a "mining" strategy, as opposed to the "scavenging" strategy of mycorrhizas (Lambers et al., 2008). The same strategy is also effective on much younger volcanic, acidic soils, which contain plenty of P, but where it is tightly sorbed, *e.g.*, in Chile (Lambers et al., 2012a).



Figure 1. Habitat of investigated *Banksia* and *Hakea* species in Lesueur National Park, near Jurien Bay in Western Australia (Photos: Marion Cambridge).

Proteaceae are not only very good at acquiring P, but also excellent at moderately high rates of photosynthesis at very low leaf P concentrations (Denton et al., 2007). Low leaf P concentrations are only partly due to large amounts of sclerenchymatic tissues (Fig. 2). We recently started a collaboration with Mark Stütt and colleagues (Max Planck Institute of Molecular Plant Physiology in Golm, Germany), Wolf-Rüdiger Scheible (Samuel Roberts Noble Foundation, USA) and Ben Turner (Smithsonian Tropical Research Institute Panama). In the first paper based on our collaboration, we describe that mature leaves of *Banksia* and *Hakea* species (Proteaceae) have replaced their phospholipids by galactolipids (Lambers et al., 2012b). This offers a partial explanation for their very high photosynthetic P-use efficiency. With the same team, and with Peta Clode at UWA's Centre for

Microscopy and Microanalysis, we are exploring other pivotal aspects of the very high photosynthetic P-use efficiency of Proteaceae.

Our detailed ecophysiological approach, in which we frequently use molecular techniques, is allowing us to gain insight into the functioning of very biodiverse ecosystems. Evolutionary biologists can enlighten us about when the different species in our global biodiversity evolved (Hopper, 2009). To understand how that biodiversity functions requires the type of approach we are pursuing, in interactions with these evolutionary biologists as well as other scientists.

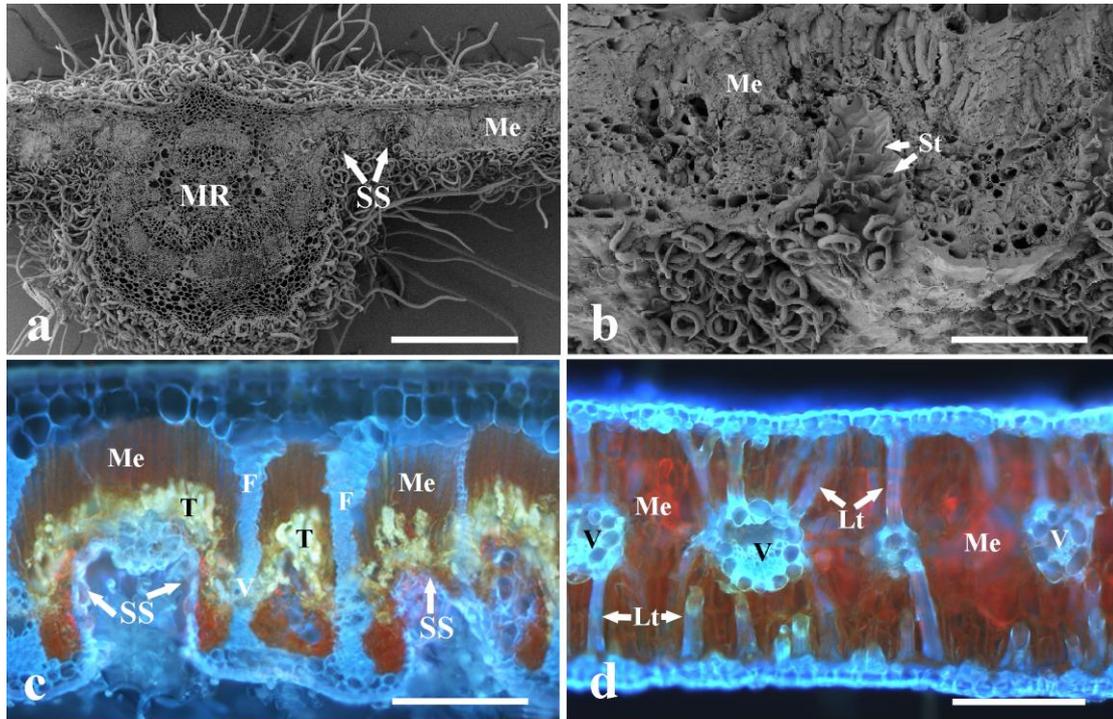


Figure 2. (A) Scanning electron microscopy (SEM) image of a cross section of a young *Banksia attenuata* leaf blade showing a midrib (MR), dense trichomes covering both surfaces and sunken stomata (SS) on the abaxial surface; scale bar = 500 μm . (B) SEM image of a group of stomata (St) in a stomatal crypt of a *Banksia menziesii* leaf; scale bar = 200 μm . (C) Fluorescent image of a cross section of a *Banksia menziesii* leaf blade showing sunken stomata (SS), tannin-rich materials (T) appearing as yellow in mesophyll tissues (Me). Note that lignified and thick-walled fibrous bundles (F) are dividing mesophyll tissue (Me) into segments; scale bar = 200 μm . (D) Fluorescent image of a cross section of a leaf blade of *Hakea prostrata* showing laticifer-like structures (Lt) distributed among mesophyll (Me), which appear to be connecting vascular bundles (V) and the epidermis; scale bar = 200 μm (Photos: John Kuo).

References:

- Denton MD, Veneklaas EJ, Freimoser FM, Lambers H. 2007.** *Banksia* species (Proteaceae) from severely phosphorus-impooverished soils exhibit extreme efficiency in the use and re-mobilization of phosphorus. *Plant, Cell and Environment* **30**: 1557-65.
- Hopper SD. 2009.** OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil* **322**: 49-86.
- Lambers H, Bishop JG, Hopper SD, Laliberté E, Zúñiga-Feest A. 2012a.** Phosphorus-mobilisation ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. *Annals of Botany* **110**: 329-348.
- Lambers H, Brundrett MC, Raven JA, Hopper SD. 2010.** Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* **334**: 11-31.
- Lambers H, Cawthray GR, Giavalisco P, Kuo J, Laliberté E, Pearse SJ, Scheible W-R, Stitt M, Teste F, Turner BL. 2012b.** Proteaceae from severely phosphorus-impooverished soils extensively replace phospholipids by galactolipids and sulfolipids during leaf development to achieve a high photosynthetic phosphorus-use efficiency. *New Phytologist*, in press.
- Lambers H, Raven JA, Shaver GR, Smith SE. 2008.** Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution* **23**: 95-103.
- Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006.** Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**: 693-713.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000.** Biodiversity hotspots for conservation priorities. *Nature* **403**: 853-858.

Proteomic and epigenetic changes in *Brassica rapa* responding to low soil phosphate availability

John P. Hammond

School of Plant Biology and Institute of Agriculture, The University of Western Australia

John has recently arrived in the School of Plant Biology, supported by an ARC Future Fellowship, to investigate proteomic and epigenetic changes in *Brassica rapa* responding to low soil phosphate availability. John has previously used transcriptional profiling approaches to characterise plant responses to low P availability in *Arabidopsis*, *Brassica* species and potato, extending the application in the latter to demonstrate a proof of concept model for using transcriptional profiling as a crop diagnostic tool for phosphate fertiliser requirements [1]. Recent use of expression QTL (eQTL) approaches has also led to the identification of regions of the *B. rapa* genome enriched with trans-eQTL associated with phosphate availability [2]. The genetic factors associated with these regions will be identified as part of John's fellowship and the relationships between transcriptional and proteomic responses will be explored.

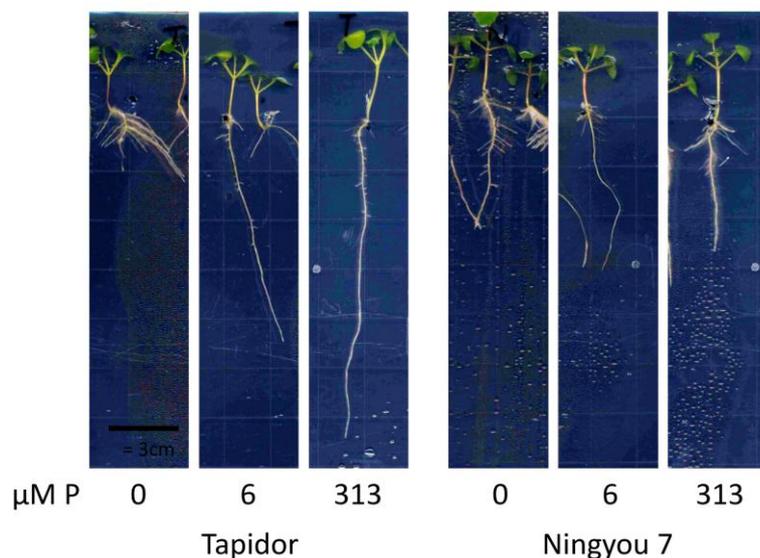


Figure: Root architectural responses to low phosphate availability in the parents of the *Brassica napus* Tapidor x Ningyou-7 double haploid mapping population. Part of an on-going collaboration with Dr. Lei Shi (Huazhong Agricultural University, Wuhan, China).

Aspects of John's research also explore the natural genetic variation present in crop traits important for the acquisition and utilisation of mineral nutrients, in collaboration

with Prof. Philip White (James Hutton Institute, UK) and Dr Martin Broadley (University of Nottingham, UK). These include root adaptations to low P availability [3, see Figure] and the acquisition of calcium and magnesium in edible plant parts [4, 5]. Genetic loci associated with these traits are currently being explored in *B. oleracea* and *B. rapa*.

References:

1. Hammond JP, Broadley MR, Bowen HC, Spracklen WP, Hayden RM, White PJ (2011) Gene expression changes in phosphorus deficient potato (*Solanum tuberosum* L.) leaves and the potential for diagnostic gene expression markers. *PLoS One* **6**(9), e24606. doi:10.1371/journal.pone.0024606.
2. Hammond JP, Mayes S, Bowen HC, Graham NS, Hayden RM, Love CG, Spracklen WP, Wang J, Welham SJ, White PJ, King GJ, Broadley MR (2011) Regulatory hotspots control plant gene expression under varying soil phosphorus (P) supply in *Brassica rapa*. *Plant Physiology* **156**, 1230-1241.
3. Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architectural traits. *Journal of Experimental Botany* **60**, 1953-1968.
4. Rios JJ, Ó Lochlainn S, Devonshire J, Graham NS, Hammond JP, King GJ, White PJ, Kurup S, Broadley MR (2012) Distribution of calcium (Ca) and magnesium (Mg) in the leaves of *Brassica rapa* under varying exogenous Ca and Mg supply. *Annals of Botany* **109**, 1081-1089.
5. Broadley MR, Hammond JP, King GJ, Astley D, Bowen HC, Meacham MC, Mead A, Pink DAC, Teakle GR, Hayden RM, Spracklen WP, White PJ (2008) Shoot calcium (Ca) and magnesium (Mg) concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in *Brassica oleracea*. *Plant Physiology* **146**, 1707-1720.

Oxidative signalling, acclimation and phenology of perennial plants

Michael Considine

School of Plant Biology and the Institute of Agriculture, The University of Western Australia; Irrigated Agriculture and Diversification, Dept. of Agriculture and Food WA

michael.considine@uwa.edu.au

I hold a joint position between UWA and DAFWA and maximise my opportunities to work collaboratively to span pure science through to industry development of perennial fruit. I'm driven by **translational research** that is **responsive to industry** needs, based on a **good physiological understanding** and effective communication. My research spans disciplines of plant physiology and molecular biology through to pharmacology.

The **signalling and regulation of plant development and acclimation** are my key interests. **Redox and oxygen** signalling are at the hub of GxExM control in both optimal and stressed conditions. I use a range of plant models to explore the role of these processes in a horticultural context; grapevine and apple are major crops of interest. These fundamental features mediate multiple levels of cellular and ontogenic control and are of central importance to the perennial plant habit, which is finely tuned to seasonal change.

A recent paper demonstrated that SO₂ elicits defence metabolism in grape berries, suggesting that inducible defences may contribute significantly to the preservative effect of SO₂. This is a step-change in our thinking towards safer alternatives for horticulture (Giraud et al., 2012). Other research investigates molecular signalling and control of dormancy and flowering, particularly in the context of suboptimal climate zones where inflorescence abortion limits productivity. I'm particularly interested in further research in perennial meristem regulation. Yet other studies emphasise opportunities for crop improvement in apple breeding.

I work with colleagues in the Centre for Plant Sciences, University of Leeds, UK, the ARC Centre of Excellence in Plant Energy Biology (UWA), School of Medicine and Pharmacology (UWA) and the Institute for Grape and Wine Sciences (ISVV), France.

I supervise five postgraduate students and seek further students interested in molecular signalling and development.



Recent papers:

Giraud et al. (2012) Sulphur dioxide evokes a large scale reprogramming of the grape berry transcriptome associated with oxidative signalling and biotic defence responses. *Plant Cell Environ* 35: 405-417, 2012.

Considine et al. (2012) Molecular genetic features of polyploidization and aneuploidization reveal unique patterns for genome duplication in diploid *Malus*. *PLoS ONE* 7: e29449

Bondonno et al. (2012) Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: a randomised controlled trial. *Free Rad Biol Med* 52: 95

Climate ready crops

Kadambot Siddique

The UWA Institute of Agriculture, The University of Western Australia

Kadambot Siddique is the Hackett Professor of Agriculture Chair and Director of the UWA Institute of Agriculture, The University of Western Australia.



Figure: L to R: Kadambot Siddique, Jairo Palta, Sam Henty, Renu Saradadevi, Eduardo de Oliveira, Alan Robson and Helen Bramley in a climate ready wheat experiment at UWA.

Kadambot Siddique's research involves crop physiology, production agronomy, farming systems, genetic resources, breeding research in cereal, grain and pasture legumes and oilseed crops. His pioneering research on chickpea has contributed enormously to the Australian chickpea industry that is currently valued at more than \$300 million per annum.

More recently Kadambot Siddique and his team has been focussing on the response of wheat and other annual crops to elevated CO₂ concentration, temperature and terminal drought and their interaction on growth, development and yield.

Key research areas include:

- The interaction between elevated CO₂, high temperature and terminal drought in wheat
- The impact of climate change on hydraulic mechanisms controlling water uptake in wheat
- Abscisic Acid (ABA) – mediated stomatal response in wheat under drought
- Assessing the role of leaf traits and transpiration in ameliorating leaf temperature
- The role of root architecture and function in water and nutrient uptake in wheat, lupins and other annual crops.

The above research will generate new information relevant to wheat physiology and breeding strategies for the development of future water efficient crop varieties. Early results from the above studies support the hypothesis that the effect of elevated CO₂ combined with high temperature (2° C higher than ambient) can ameliorate the severe effects of terminal drought on biomass accumulation and grain yield of wheat.



Figure: Example of root systems of wild *L. angustifolius* (genotypes #085, #060, #071, #004 and #044). Images are presented in groups of three: left image is a photograph from the large semi-hydroponic phenotyping experiment; central image is the root system modelled by ROOTMAP; and right image is the root system modelled by *SimRoot*.

In the lupin root studies we used new screening techniques and advanced computer modelling to understand lupin root systems variability. We screened the world's largest lupin genetic resource collection and identified tremendous genetic variation in lupin root systems. Our findings may be used in breeding new varieties of lupins with modified root systems and function that may produce higher yields in soils with relatively limited water and nutrient resources.

Relevant recent publications:

Fang, X., Turner, N.C., Yan, G., Li, F and Siddique, K.H.M. (2010). Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. *Journal of Experimental Botany* **61**: 335–345.

Farooq, M., Bramley, H., Palta, J.A, and Siddique, K.H.M. (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences* **30**:491-507.

Chen, Y.L., Dunbabin, V.M., Postma, J., Diggle, A.J., Palta, J.A., Lynch, J.P., Siddique, K.H.M. and Rengel, Z. (2011). Phenotypic variability and modelling of root structure of wild *Lupinus angustifolius* genotypes. *Plant and Soil* **348**: 345-364.

Xia, Y., Ning, Z., Bai, G., Li, R., Yan, G., Siddique, K.H.M., Baum, M. and Guo, P. (2012). Allelic variation of a light harvesting chlorophyll II A/B-binding protein gene (*Lhcb1*) associated with agronomic traits in barley. *PLoS One*, **7** (5): e37573. doi:10.1371/journal.pone.0037573.

Kadambot Siddique's email address: kadambot.siddique@uwa.edu.au

The ‘fungicide’ phosphite – its interplay with phosphate signalling pathways and boosting effect on plant defence responses

Ricarda Jost¹ and Oliver Berkowitz^{1,2}

¹*School of Plant Biology, The University of Western Australia.*

²*School of Biological Sciences and Biotechnology, Centre for Phytophthora Science and Management, Murdoch University.*



Our research focuses on the effect of phosphite (H_2PO_3^-) on plant physiology and defence mechanisms. The molecular mechanism of phosphite-induced resistance is analysed in the model species *Arabidopsis thaliana*. Due to its close resemblance with phosphate, phosphite is also used as a tool to investigate phosphate sensing and signalling to further the understanding of phosphate acquisition and homeostasis. This project involves researchers from UWA (Msc. Marina Borges Osorio, Prof. Patrick Finnegan, Prof. Hans Lambers), Murdoch University (Daniel Kollehn, Dr. Philip O’Brien, Prof. Giles Hardy) and collaborators Wolf-Rüdiger Scheible at the Noble Foundation (USA), Mark Stitt and others at the Max-Planck Institute of Molecular Plant Physiology (Germany) and Jurriaan Ton at the University of Sheffield (UK).



Many plant species endemic to the South West Botanical Province of Western Australia, one of the world’s biodiversity hotspots, are currently under threat by the ‘dieback disease’ caused by the oomycete pathogen *Phytophthora cinnamomi* Rands. In this region 40% of the species are susceptible, while 14% are highly susceptible to this pathogen. First isolated from cinnamon trees in Sumatra in 1922, this pathogen was reported in Australia in 1935 and can now be found in all the higher rainfall areas across the Australian continent and Tasmania (O’Gara et al., 2005). It has a host range of over 4,000 species, including important crop species such as avocado, macadamia and pine.

The only effective and economically viable treatment option against *Phytophthora cinnamomi* on an ecosystems scale is the biostatic agent phosphite (Ouimette and Coffey, 1985), which is also used widely for crop protection around the world. It acts both directly on spore viability and hyphal growth as well as indirectly by the activation of plant defence responses (Smillie et al., 1989). Phosphite triggers the salicylic acid-dependent induction of defence genes such as *PR1*, *EDS1*, *PAD4* and primes plants for a stronger and faster response dependent on the mitogen-activated protein kinase MPK4 (Molina et al., 1998; Friedrich et al., 2001; Eshraghi et al., 2011; Massoud et al., 2012).

Levels of the macronutrient phosphate inside of plant cells are tightly controlled due to its involvement in many essential enzymatic processes and plants have acquired complex regulatory networks to maintain these (Chiou and Lin, 2011). Phosphite is a more reduced form of phosphorus than phosphate with an associated valence change from +5 to +3. Phosphite is taken up by plants through high-affinity phosphate transporters and is transported in both acropetal and basipetal fashion throughout the plant (Darcylameta and Bompeix, 1991). Unlike phosphate, phosphite is not a source of phosphorus for plants and there are no plant enzymes known that can oxidize phosphite into phosphate (Guest and Grant, 1991). The addition of phosphite to phosphate-limited plants exacerbates phosphate starvation symptoms that are reversible by phosphate fertilisation. However, phosphite application may lead to P fertilisation of sensitive ecosystems as phosphite is converted to phosphate by soil microbes.

We are currently establishing pathosystems for several root pathogens apart from *P. cinnamomi*, for which *Arabidopsis* is a non-host, such as *P. capsici* and *P. parasitica/nicotianae*. We have also established a high-throughput system to quantify tissue phosphite accumulation (Berkowitz et al., 2011). In collaboration with Wolf-Rüdiger Scheible and Mark Stitt as well as several other groups at the Max-

Planck Institute for Molecular Plant Physiology in Golm, Germany, we have recently analysed metabolite and gene expression profiles of phosphate-limited plants in response to equimolar tissue concentrations of either phosphate or phosphite. Preliminary results suggest that phosphite induces the expression of an array of defence-related genes. Together with identified changes in metabolite levels these observations offer first insights for the broad effectiveness of this ‘fungicide’ against a wide range of necrotrophic, hemi- and biotrophic oomycetes.

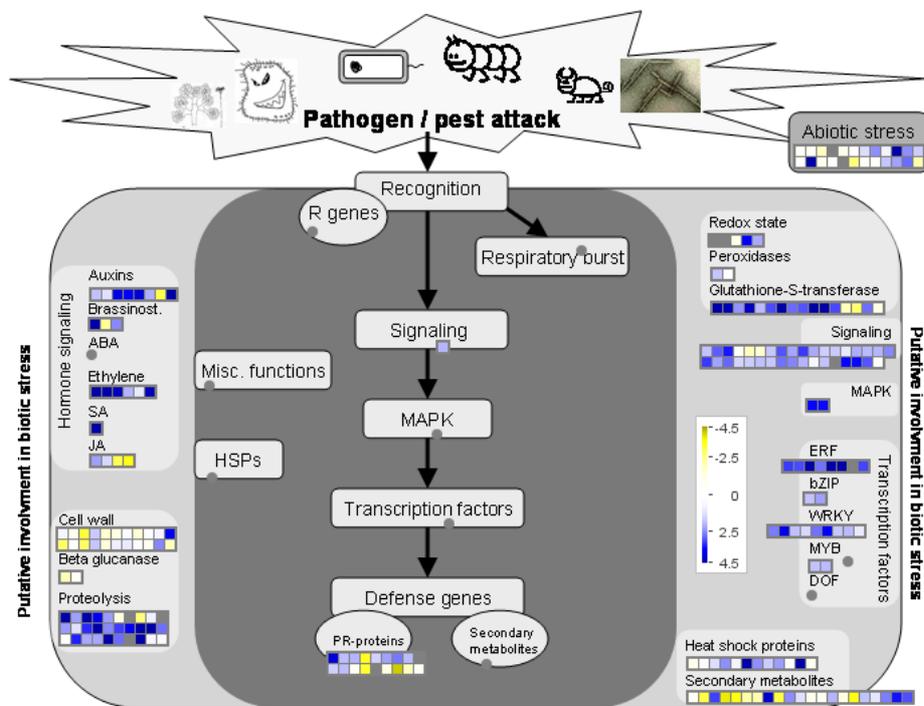


Figure 2: Transcript profile of defence-related genes in response to an 8 h treatment of phosphate-limited Arabidopsis seedlings with 1 mM phosphite visualized with the MapMan tool (Thimm et al., 2004). –Fold changes relative to phosphate-limited control seedlings are shown on a log₂ scale. Phosphate treated plants show very little response in this panel of genes (data not shown).

This project is funded by the ARC, the Department of Environment and Conservation, MERIWA and industry partners ALCOA, BHP Billiton, TRONOX and WESTERNPOWER.

References

- Berkowitz, O., Jost, R., Pearse, S.J., Lambers, H., Finnegan, P.M., Hardy, G.E., and O'Brien, P.A. (2011). An enzymatic fluorescent assay for the quantification of phosphite in a microtiter plate format. *Anal Biochem* 412, 74-78.
- Chiou, T.-J., and Lin, S.-I. (2011). Signaling Network in Sensing Phosphate Availability in Plants. *Annual Review of Plant Biology* 62, 185-206.
- Darcylameta, A., and Bompeix, G. (1991). Systemic Transport of Tritiated Phosphonate in Tomato Plantlets (*Lycopersicon-Esculentum* Mill). *Pesticide Science* 32, 7-14.
- Eshraghi, L., Anderson, J., Aryamanesh, N., Shearer, B., McComb, J., Hardy, G.E.S., and O'Brien, P.A. (2011). Phosphite primed defence responses and enhanced expression of defence genes in Arabidopsis thaliana infected with *Phytophthora cinnamomi*. *Plant Pathology* 60, 1086-1095.
- Friedrich, L., Lawton, K., Dietrich, R., Willits, M., Cade, R., and Ryals, J. (2001). NIM1 overexpression in Arabidopsis potentiates plant disease resistance and results in enhanced effectiveness of fungicides. *Mol. Plant-Microbe Interact.* 14, 1114-1124.
- Guest, D., and Grant, B. (1991). The Complex Action of Phosphonates as Antifungal Agents. *Biological Reviews of the Cambridge Philosophical Society* 66, 159-187.
- Massoud, K., Barchietto, T., Le Rudulier, T., Pallandre, L., Didierlaurent, L., Garmier, M., Ambard-Bretteville, F., Seng, J.M., and Saindrenan, P. (2012). Dissecting Phosphite-Induced Priming in Arabidopsis Infected with *Hyaloperonospora arabidopsidis*. *Plant Physiology* 159, 286-298.
- Molina, A., Hunt, M.D., and Ryals, J.A. (1998). Impaired fungicide activity in plants blocked in disease resistance signal transduction. *Plant Cell* 10, 1903-1914.
- O'Gara, E., Howard, K., Wilson, B., and Hardy, G.E.S.J. (2005). Management of *Phytophthora cinnamomi* for Biodiversity Conservation in Australia: Part 1 – A Review of Current Management. (A report funded by the Commonwealth Government Department of Environment and Heritage and the Centre for Phytophthora Science and Management, Murdoch University, Western Australia).
- Ouimette, D., and Coffey, M.D. (1985). *In vivo* efficacy of 5 phosphite compounds against *Phytophthora capsici* on pepper plants. *Phytopathology* 75, 1330-1330.
- Smillie, R., Grant, B.R., and Guest, D. (1989). The Mode of Action of Phosphite - Evidence for Both Direct and Indirect Modes of Action on 3 *Phytophthora* Spp in Plants. *Phytopathology* 79, 921-926.
- Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y., and Stitt, M. (2004). MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant Journal* 37, 914-939.

Plant tolerance of flooding and salinity

Tim Colmer

School of Plant Biology and Institute of Agriculture, The University of Western Australia

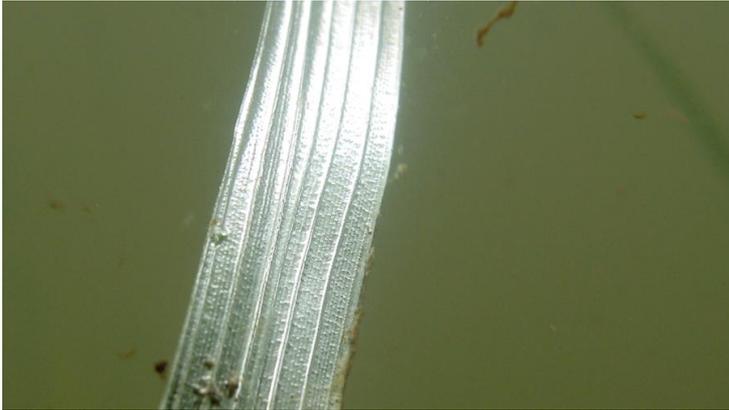
Flooding stress research has focused on anoxia tolerance using rice coleoptiles as a model system, and on O₂ movement in the aerenchyma in roots of crops (especially rice and wheat), pasture species, and wetland plants (including ‘wild’ Triticeae). Research on salt tolerance focuses on the regulation of tissue concentrations of organic solutes and Na⁺ and Cl⁻ in wheat and its wild relatives, chickpea, pasture species, and some Australian halophytes (*Tecticornia* species; e.g. English and Colmer, 2011). Much of our research deals with plant responses to the combined effects of flooding and salinity.

Some of our recent work on submergence tolerance, in collaboration with Ole Pedersen at the University of Copenhagen, has been on photosynthesis and oxygen transport by plants when completely submerged. Underwater photosynthesis by submerged terrestrial plants is of importance for survival, since oxygen produced enhances internal aeration of the plant body and provides sugars to sustain metabolism. Of particular recent interest has been the role of leaf gas films on submerged plants. Leaves of rice, and of several other emergent wetland species, retain a thin film of gas on their hydrophobic surfaces when submerged. Leaf gas films facilitate oxygen and carbon-dioxide exchange with floodwaters. Leaves with gas films present, as compared to those with the gas films experimentally removed, had: (i) up to 5-fold higher oxygen uptake rates in darkness, and (ii) up to 6-fold higher carbon-dioxide uptake during light periods (i.e. underwater net photosynthesis). Improved oxygen entry during darkness and higher net photosynthesis during light periods, both enhanced internal oxygen concentrations within shoots, and oxygen moved via aerenchyma into roots in an anoxic medium. When gas films were removed from leaves of submerged plants, tissue oxygen declined, sugar concentrations became lower after a few days, and shoot and root growth were reduced, when compared to submerged plants with gas films. Our experiments compared field collected leaves of several wetland species (Colmer and Pedersen, 2008), as well as elucidation of the detailed oxygen dynamics for rice plants under various manipulations in controlled conditions (Pedersen et al., 2009). Recently, we demonstrated the role of leaf gas films for internal aeration of rhizomes of *Spartina alterniflora* during tidal submergence in the field (Winkel et al., 2011). The data support the conclusion that gas films on leaves contribute to submergence tolerance of wetland plants.

We also continue our research on root aeration (e.g. including photosynthesis by aquatic roots; Rich et al., 2001), and regulation of sodium entry during saline waterlogged conditions (e.g. Munns et al., 2011). A PhD student, Budiastuti Kurniasih (Tuti) from Indonesia, has also been working on combined anoxia and salinity tolerance at germination and early seedling growth of rice (also with Hank Greenway) – she will soon submit her PhD thesis. Tuti’s project has expanded our understanding of salinity-hypoxia interactions from roots, to consider also the responses of the anoxia-tolerant coleoptile of rice seedlings, when faced with salinity in addition to oxygen deficiency.



Setting up oxygen microelectrodes for measurements in a rice field. Ole Pedersen (kneeling) positions an electrode with a micromanipulator, while Anders Winkel (standing) gives the thumbs up for skilled placement without breakage! (Photo by Anja).



Gas film on the surface of a submerged leaf of rice, in a flooded field. The silvery appearance results from the reflection of sunlight (photo by O. Pedersen).

References:

- Colmer TD, Pedersen O. 2008.** Underwater photosynthesis and respiration in leaves of submerged wetland plants: gas films improve CO₂ and O₂ exchange. *New Phytologist* **177**: 918-926.
- English JP, Colmer TD. 2011.** Salinity and waterlogging tolerances in three stem-succulent halophytes (*Tecticornia* species) from the margins of ephemeral salt lakes. *Plant and Soil* **348**, 379-396.
- Pedersen O, Rich SM, Colmer TD. 2009.** Surviving floods: leaf gas films improve O₂ and CO₂ exchange, root aeration, and growth of completely submerged rice. *Plant Journal* **58**: 147-156.
- Munns R, James RA, Islam AKMR, Colmer TD. 2011.** *Hordeum marinum*-wheat amphiploids maintain higher leaf K⁺:Na⁺ and suffer less leaf injury than wheat parents in saline conditions. *Plant and Soil* **348**, 365-377.
- Rich SM, Ludwig M, Colmer TD. 2011.** Aquatic adventitious roots of the wetland plant *Meionectes brownii* can photosynthesize: implications for root function during flooding. *New Phytologist* **190**: 311-319.
- Winkel A, Colmer TD, Pedersen O. 2011.** Leaf gas films of *Spartina anglica* enhance rhizome and root oxygen during tidal submergence. *Plant, Cell & Environment* **34**, 2083-2092.

Understanding tree decline in Kings Park

Jason Stevens¹, Ben Miller¹, Steve Easton¹, Martin Bader² and Jerome Chopard²

¹Kings Park and Botanic Garden; ²Centre of Excellence for Climate Change, Woodland & Forest Health, The University of Western Australia

Rainfall in southwest Australia has decreased dramatically over the past few decades and this trend is predicted to continue under current climate change scenarios. At the same time, many tree species of the woodlands and forests in this region have started to decline. In Kings Park for example, Banksias have declined or vanished from many areas with catalogued losses of up to 85% in the three previously dominant species – *B. attenuata*, *B. menziesii* and *B. grandis* over 60 years.

This is particularly alarming as Banksia species are not just a major component of Kings Park bushland overstorey but they are also regionally important as keystone species supporting native nectar-feeding animals such as honeyeaters, honey-possums and many insects, and seed eating birds such as Carnaby's cockatoo. Their nectar production provides an important resource for the honey industry, and of course banksias are iconic and much loved parts of the bushlands of Kings Park and the Southwest.

Despite the general link between climate and plant health, the exact causes of Banksia tree decline have remained unresolved.

New technology now exists allowing plant response mechanisms to drying and warming events to be understood, and Kings Park is now using it to understand tree decline. This will allow, for the first time, real time and online monitoring of water relations and water stress in native plants.

How does the technology work?

“The magnetic, non-invasive ZIM-probe measures very small leaf turgor changes in real time”



Individual leaves of Banksia or Eucalyptus trees are placed between an upper, movable and a lower magnet in which a highly sensitive pressure sensor is integrated.

The pressure exerted by the magnets on the leaf patch is kept constant during the measurements.

The turgor (or water content/pressure) in the leaf patch is opposed to the magnetic pressure. The ZIM-probe measures the difference between magnetic pressure and the relative turgor value.

The signal is then relayed from the leaf sensor to a local station through the wireless network before being beamed direct to the control centre in Germany for analysis. Leaf turgor responses are then related to environmental variables such

as ambient temperature, relative humidity, light irradiation and soil moisture.

Results are uploaded every few minutes onto the web and can be accessed through a standard computer or smart phone, so you always know how the plants are responding to their environment.

Depending on the plant species only a slight degradation of chlorophyll is observed which will not affect turgor or water potential of the leaves. Cell damage and/or necrosis are not observed in many species even after 2 to 3 months of leaf patch clamping meaning this method is not destructive. This is a massive advantage compared to traditional monitoring techniques.

Bringing new plant monitoring technology to the Park through collaboration

To bring this new technology to Western Australia scientists from Kings Park and the University of Western Australia have established an international collaboration with ZIM plant Technology (recipient of the SENSOR Innovation Award 2011, Germany).

Recently Professor Zimmerman, the inventor of this technology visited Kings Park as part of the distinguished scholars program to develop research capacity that will allow researchers to

- Estimate tree water consumption and water stress in native trees over the course of days and seasons
- Potentially identify physiological tipping points related to dry conditions and other climatic drivers, and
- Develop management strategies to combat tree decline across southwest Australia.

The implemented research

The research now being conducted in Kings Park is focussed on understanding the mechanisms (hydraulic models) of tree water use. The resulting tree hydraulic models will aid our understanding of current tree health problems that are occurring throughout Kings Park and across Western Australia, and allow predictions of tree responses to different climatic drivers.

To derive these models Kings Park and UWA researchers have installed sensors in one tree each of *Banksia menziesii* (firewood banksia) *Eucalyptus gomphocephala* (tuart) and *Eucalyptus marginata* (jarrah) in an attempt to begin to understand plant responses to a drying climate. The crowns of these trees have been equipped with a wireless sensor network comprising a large number of magnetic leaf turgor probes as well as microclimate sensors measuring temperature and humidity. Additional probes measure stem diameter fluctuations (representing both growth and water content) and sap flow, while

other devices assess soil temperature and soil moisture content (to 1m depth). These data are complemented by measurements of leaf fluorescence (light stress) and rates of water transpiration and carbon uptake – each requiring direct canopy access via a cherry picker, which means they are only recorded during specific measurement campaigns experiments.

This research is the first time that such a comprehensive suite of complementary measurements using state-of-the-art technology will be applied to study water relations in Eucalypt and Banksia trees.

The approach will enable scientists to understand the degree to which water stress through drought can lead to tree decline, ultimately leading to a better understanding of how to manage Perth's important urban woodlands.

The future

Recent funding from the Friends of Kings Park will allow for more plants to be monitored and allow scientists to build a more robust understanding of how mature overstorey species (Banksia, Eucalyptus and Allocasuarina) respond to a changing climate.

In a world first, this technology will also be used by Kings Park Scientists to assess restoration success, helping scientists to understand restoration failure across Western Australia. This work will be conducted in collaboration with Rocla Quarry Products.

SOME OF THE PLANT SCIENCE AT CURTIN UNIVERSITY

Pulse Pathology & Genetics Group at Curtin University



From left to right: Chala J. Turo (Aus-Aid PhD student), Judith, Kingyin Lui (Y3 undergraduate, E&A, CU), Lina M. Farfan-Caceres (lab assistant), Julie Lawrence (lab officer), Caris Ottenhof (Y4/Hns at Chemical Eng., CU). Absent: Melissa Padgett (Y3 undergraduate, E&A, CU).

The Pulse Pathology and Genetics (PPG) group, led by Dr Judith Lichtenzveig, was established in July 2011 at the Department of Environment and Agriculture (E&A), Curtin University.

We are particularly interested in major diseases of winter pulse crops such as field pea, chickpea, lentil and faba bean. Our projects cover a wide range of topics, including: pathogenomics, molecular aspects of host-pathogen interactions, pathogen population genetics, host-pathogen evolution, and resistance breeding.

Our current main interest is on Ascochyta blight (AB), one of the major factors affecting gross profits and yield stability of pulses in Australia and

worldwide. The disease is caused by closely related necrotrophic fungal Didymellaceae species, in the order of the Pleosporales. *Didymella pinodes* is the predominant causal agent of pea-AB or Blackspot (other causal fungi include: *Peyronellaea pinodella* [syn. *Phoma pinodella*], *D. pisi* and *P. koolunga*); *D. rabiei* causes AB in chickpea, *D. lentis* in lentil, and *D. fabae* in faba bean.



Funds are provided by the Grains Research and Development Corporation (GRDC) and Curtin University. For more information contact: Judith.Lichtenzveig@curtin.edu.au

SOME OF THE PLANT SCIENCE AT MURDOCH UNIVERSITY

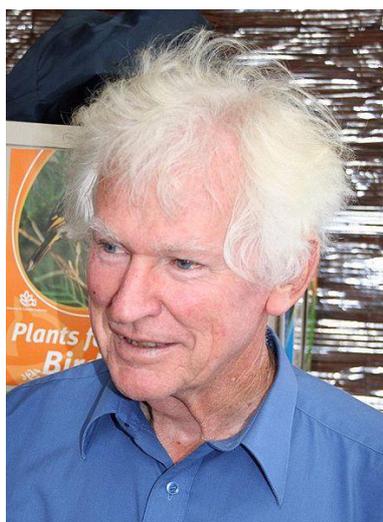
Two Murdoch University plant scientists honoured in the June 2012 Awards



Murdoch Emeritus Professor Jen McComb has been appointed a Member of the Order of Australia for her work in education, research and public service.

Over four decades, Jen, who is one of Murdoch's founding staff members, has made major contributions to research in plant breeding, reproductive biology and plant physiology, authoring or contributing to over 170 publications. Her most notable achievements include propagating die-back resistant jarrah and salt tolerant eucalypts and helping to establish sandalwood plantations in WA.

"There's no more exciting place to be a botanist than Western Australia. I've been very lucky," Jen said. "I hope all the people who have worked with me share in the pleasure of my recognition. Nothing in teaching or research is done solo these days – it is teamwork – and I've had hosts of people collaborate with me, from technicians to post-docs and fellow academics"



Murdoch Emeritus Adjunct Professor Alex George has also been appointed a Member of the Order of Australia for "service to conservation and the environment as a botanist, historian and author, particularly in the area of Australian flora, and through roles with national and international professional organisations".

From 1981 to 1993, Alex lived in Canberra and worked as Executive Editor for the *Flora of Australia* series. His extensive revision of the genus *Verticordia*, an arrangement which included new taxa, was published in *Nuytsia* in 1991. He now lives in Perth again, and works as a botanical and editorial consultant. He is also an Honorary Research Associate with the Western Australian Herbarium, and an Adjunct Associate Professor with the School of Biological Sciences, Murdoch University.

Mike Jones, WA State Agricultural Biotechnology Centre, Murdoch University

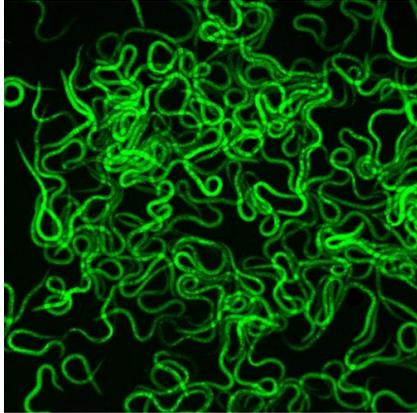


The WA State Agricultural Biotechnology Centre (SABC) is a multi-user research facility based at Murdoch University, dedicated to research in and solving problems for crop plants and animals using the genomics-proteomics-metabolomics- bioinformatics continuum. The SABC operates as a 'Research Hotel', in which different research groups from universities, state government and private companies can access high quality bench space and advanced facilities to undertake molecular research in a cost-effective manner.

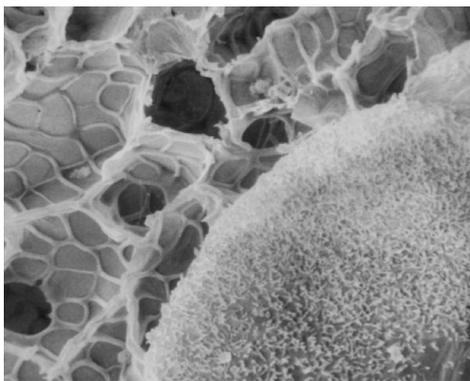
Murdoch has a strong tradition in plant sciences ranging from ecology, conservation and plant biosecurity, to plant pathogenomics, crop and algal biotechnology. Examples of this mix are given below.

Plant Biotechnology Research Group – Controlling nematodes in crop plants

Mike Jones, John Fosu-Nyarko and team



Fluorescent juvenile (J2) root-knot nematode (photo by Derek Goto)



SEM of cell walls of root knot nematode induced giant cell with wall ingrowths adjacent to abnormal xylem elements (cell contents removed – photo Mike Jones)



Transgenic wheat plants challenged with root lesion nematodes in PC2 glasshouse (photo Mike Jones)

Eight out of ten of all multicellular organisms are nematodes – they range from free living species such as *Caenorhabditis elegans* to parasites of insects, domestic animals, humans and plants. Plant parasites, such as root-knot, cyst- and root lesion nematodes, cause global crop losses estimated at \$120 billion p.a. They do this by damaging plant roots, reducing uptake of water and nutrients, and pre-disposing roots to infection by other soil pathogens. The focus of the molecular plant nematology group is on understanding the molecular basis of nematode-plant interactions, and applying this knowledge to develop new forms of host resistance to economically important nematodes.

The group is studying both changes that occur in host plants as a result of nematode infection, and pathways in sequences from host plants when nematodes ingest cell contents. It is important to study both the host and the pathogen in any plant-pathogen interaction, and although *C. elegans* is an excellent model, from using ‘Next Gen’ sequencing of plant nematode transcriptomes and comparative genomic analyses, we have found many differences between *C. elegans* and plant parasites. These include genes for ‘effectors’ that have enabled nematodes to parasitise plants, including genes that encode wall degrading enzymes, genes that lead to modification of host plant cells and that enable nematodes to evade host defences.

We have used this information for *in planta* delivered silencing of target genes in nematodes, to confer host resistance. This has been achieved both in model plants such as *Arabidopsis*, and in crop plants such as wheat and sugarcane.

This research has an international flavour, with membership of the EU COST Action 872 (Nematode Genomics, Chair Dr John Jones, James Hutton Institute, Scotland), Australia India Strategic Research Fund (with the Indian Agricultural Research Institute, New Delhi), and collaboration with A/Prof Derek Goto, Hokkaido University, Japan. Derek has recently been awarded a ‘Distinguished Collaborator’ status at Murdoch University.

For more information, contact Mike Jones on (08) 9360 2424, or m.jones@murdoch.edu.au

Plant Biotechnology Research Group – High throughput sequencing to identify indigenous and exotic viruses in Australian plants

Stephen J. Wylie, M. Saqib, Hua Li, Jamie Ong, Michael G.K. Jones (Murdoch University)

Until about three years ago, the principal technologies available for identifying new plant viruses were group-specific antibodies and degenerate primer sets, such as those with activity towards members of the genus *Potyvirus*, family *Potyviridae*, the second largest genus of plant viruses, numbering 146 described species. Consequently, research on Australian plant viruses was restricted largely to identifying viruses closely related to well-studied exotic species, and almost all of the viruses discovered from Australian indigenous plants using these methods were novel potyviruses or exotic viruses. It was noted that the ‘Australian potyviruses’ formed a closely-related group within the *Bean common mosaic virus* (BCMV) subgroup of the *Potyviridae*. It was estimated from nucleotide substitution rates that these BCMV-like potyviruses were, in fact, quite recent arrivals, possibly transported by Austronesians on their crop plants as they colonised the region about 2000 years ago.

Given the great age of the Australian continent, high endemism amongst its plant flora, and the varied ecosystems present, we hypothesized that the indigenous viral flora would be much richer than that described. We also hypothesized that the indigenous flora would be suffering invasion by aggressive exotic viruses. New high-throughput nucleotide sequencing technologies coupled with bioinformatics provided the tools needed to test these hypotheses. A range of indigenous and exotic plant species from southern Australia was tested. As expected, more BCMV-like potyviruses were identified, but surprisingly, non-potyviral members of the *Potyviridae* were also present, indicating a more complex, and possible more ancient association with Australian flora than previously recognised. Members of a group of families collectively known as flexiviruses were identified from monocot and dicot native plants—some within recognized genera and others separate from them. Also present were members of the family *Secoviridae*. Other viruses found do not fit readily into existing higher order taxa. Most of the indigenous viruses found induce no apparent symptoms in their hosts, although this is not always the case when inoculated onto experimental host plants.

Exotic viruses found for the first time in Australia in garden plants, imported bulbs and naturalized weeds include allexiviruses, various potyviruses, *Japanese iris necrotic ring virus*, poleroviruses and carlaviruses. Most of these exotic viruses induce symptoms of infection in their hosts.

The results gained so far indicate that there is indeed a large indigenous viral flora in Australia. Their existence in asymptomatic native host plants challenges the prejudice that viruses are necessarily pathogenic. Examples of long-term coexistence of plants and viruses with minimal effects on fitness are beginning to appear in the literature, and our future research will aim to quantify the impacts and roles of ancient plant/virus associations.



Figure: Wild *Diuris laxiflora* (Bee orchid) plant asymptotically infected with a novel potyvirus

For more information, contact Steve Wylie on (08) 9360 6600/ s.wylie@murdoch.edu.au, or Mike Jones on (08) 9360 2424/m.jones@murdoch.edu.au



Giles Hardy and colleagues - State Centre of Excellence for Climate Change, Woodland and Forest Health

We've seen dramatic forest changes due to the significant decline of tuart, wandoo, jarrah and peppermint. As a 'keystone species', the continuing collapse of marri trees across the South-West will likely have dramatic consequences for South-West ecosystems.

Biodiversity loss, dangerously high fuel loads and increased wildfires are just some of the outcomes to be expected in the future. Deciphering the complex processes involved in tree decline is difficult because of the manner in which stress factors interact and interrelate. Decline isn't a straight line and can't always be traced to one factor. Though decline process descriptions require a great deal of time and research, they are an invaluable tool in finding sustainable management solutions.

Giles says that protecting marri has many underappreciated benefits. People have to understand how tree decline affects their everyday lives, from the costs incurred by shires and councils for tree and limb removal to impacts on secondary industries, such as the apiary and honey industry. Honey production from marri has declined approximately 70 per cent since 2008.

Even more, we need to consider the positive social impacts conservation brings. Seeing WA's iconic trees dying can be devastating for communities.



Treana Burgess leads research into the impact of fungal pathogens on the biodiversity and biosecurity of Australia's eucalypts, both in plantations and native forests. These projects are managed through extensive collaboration with other researchers in Asia, Africa and South America. The molecular taxonomy and population genetics of pathogens is used to study introduction patterns, global movement and landscape genetics. Dr Burgess is also recognised in Europe as an expert in forest biosecurity and has been an expert member of several EU COST projects the most recent being PERMIT (Pathway Evaluation and Pest Risk Management in Transport). Dr Burgess together with Prof Giles Hardy

champions the Centre for Phytophthora Science and Management (CPSM) one of the world-leading groups in Phytophthora research. Research focus on the biodiversity, epidemiology, diagnosis and management of Phytophthora –related diseases in the natural ecosystems.



John Howieson, Graham O'Hara, Wayne Reeve and colleagues of the Centre for Rhizobium Studies (CRS) have released six strains of root-nodule bacteria to commerce, which have been widely 'sown' across southern Australia. These fix nitrogen that forms a substantial portion of this \$2 billion asset. The CRS has been very influential in the improvement of inoculant carrier technologies that deliver these elite strains in good condition to their end users. The CRS is currently strongly involved in selecting and breeding new perennial legumes that are adapted to acidic and infertile soils, as well as developing appropriate rhizobia for them. The CRS has a very strong molecular group that assists in understanding the

response of rhizobia to stress, which is very relevant to our agriculture.

Current exciting research includes the sequencing of 25 rhizobium genomes, with 10 published so far, and 95 more to do this year!

A major activity of John Howieson's at the moment is in assisting the Gates Foundation with legume development in sub-Saharan Africa.



Richard Bell (left) is an expert in soil fertility and land management. He specialises in soil management in highly weathered soils, fertility and management constraints associated with acid, salt-affected and degraded soils, fertiliser requirements for field cropping, rehabilitation of degraded land, the physiology and agronomy of food legumes, canola, wheat and rice, and mine rehabilitation.



Bernie Dell (right) has interests that overlap with those of Richard Bell, and **focus on plant nutrition, the selection of mycorrhizal fungi and bacteria** for improving plant growth, health of natural and man-made ecosystems, animal-fungal interactions, edible mycorrhizal and other fungi, and new plantation tree crops.

Agricultural biotechnology, gene discovery and breeding in wheat and barley



Associate Professor Mehmet Cakir is nationally and internationally recognised in the agricultural biotechnology area with a particular expertise in gene discovery and breeding for biotic and abiotic stresses in wheat and barley.

His research interests include breeding for biosecurity threats to Australia, such as Stem rust strain Ug99 and Russian Wheat Aphid.

He is currently leading the global Russian Wheat Aphid Project with research partners from the USA, France, Turkey, Syria, Iran, South Africa, Kenya, Ethiopia and Argentina.

Rudi Appels (right) is a leading researcher on wheat quality and genomics, and is currently involved in a major project with Genomics Australia (Framework Data set) to sequence the wheat genome. He works closely with other researchers such as Wujun Ma and Jingjuan Zhang on cereal quality and abiotic stress.



Plant Biosecurity



Kirsty Bayliss is responsible for the education program of the Plant Biosecurity CRC. She is responsible for coordinating the PhD training program within the CRC. Kirsty also develops various workshops and training days for staff and students working in plant biosecurity, and oversee the running of a successful school education program.



She is also a member of the international QUADs education program, aimed at developing international standards for teaching biosecurity, and presents lectures in biosecurity, plant pathology and plant biotechnology in her role as Academic Chair for the postgraduate courses in plant biosecurity. Kirsty is currently involved in several plant pathology/biosecurity research projects including postharvest pathology of stored grains, disease spread via nurseries and other networks, and plant pathogens in irrigated agriculture.

Algal Biotechnology

The Algae R&D Centre at Murdoch University under Michael Borowitzka continues the work of developing microalgae and microalgae culture systems for products such as biofuels and high-value chemicals in a range of indoor and outdoor culture systems (raceway ponds, plate and tubular photobioreactors, LED reactors, etc) ranging from 100 mL to over 60,000 L.



A wide variety of algae species are being studied and the isolation and characterisation of new strains is ongoing. Studies of the factors which limit photosynthetic efficiency and primary productivity in these cultures, especially under the highly variable outdoor conditions of irradiance, temperature, pO₂, carbon supply, and how these limitations may be overcome are an important part of our work, as is the study of lipid and long-chain hydrocarbon synthesis.



Mike Bunce, is an ARC 'Future Fellow' who specialised in the recovery of **ancient and degraded plant and animal DNA** from a variety of biological substrates. One example of his research was the use of the SABC's Roche 454 second generation platform to conduct a detailed molecular audit of Traditional Chinese Medicines, which don't always contain what they say! (see <http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1002657>). A further example, is the use of ancient DNA extracted from archaeological cave sediments

from south west WA. His team has an ARC discovery grant to use high-throughput sequencing to characterise the ancient plant DNA contained in the sediments over the past 50,000 years - the aim is to investigate how the flora of this biodiversity hotspot has changed over time.

Update on:

Functional Plant Biology

Editor-in-Chief: Dr Rana Munns

We are pleased to announce that the Impact Factor for 2011 released last month has increased to 2.93. Submissions have increased, as have requests for special issues on conference proceedings, and research fronts.

Just published is a Research Front "From Genome to Phenome in cereals", with emphasis on drought resistance at the reproductive stage (<http://www.publish.csiro.au/nid/102.htm>). Coming up soon is a large Special Issue on plant phenomics.

Special issues for next year include papers arising from two conferences:

- Physiology and Biochemistry of Halophytes, Hannover, Germany, August 2012
- 6th Asia & Oceania Conference on Photobiology, Sydney, November 2013.

FPB will continue to publish reviews from the winners of the society's Goldacre Award, and on a regular basis the winners of the NZ Society of Plant Biologist's annual Roger Slack Award. The current issue of FPB contains a beautiful review by Kevin Davies (Roger Slack Award for 2011) "The molecular regulation of flower colouration and mechanisms for pigmentation patterning" (<http://dx.doi.org/10.1071/FP12195>).

Editor-in-Chief, Rana Munns

From Our New PhDs

For this issue I had the idea of trying to have the research of a representative from each state and territory contribute to this section of Phytogen. Below are articles covering a wide range of research topics from our recently graduated PhDs from Queensland, Tasmania, South Australia, Western Australia and New Zealand. Perhaps we can find another group of contributors for the next issue,

Tina Offler

QUEENSLAND

Structure and function of wood in mangroves

Nadia S. Santini

Supervisor Prof. Catherine E. Lovelock
Co Supervisor Dr. Nele Schmitz

University of Queensland

Wood maintains the structure of the trees by providing resistance to breakage and flexibility when strong winds and other environmental forces act upon trees. The wood of angiosperms is comprised of different cell types that arise from the secondary growth of the vascular cambium. These cell types include: the xylem vessels that provide water transport; the phloem, which transports nutrients and other organic materials; the fibres, thick-walled cells that offer support to the plant; and the rays, linking the xylem and the phloem and acting as nutrient storage tissue.

During my PhD, I investigated the wood structure and its associated functions of mechanical strength and growth rates in mangrove species. In addition, I used the abundance of ^{18}O isotopes in xylem sap water to identify the relative contribution of different water sources used by the mangrove *A. marina* in different hydrological settings and I assessed whether mangrove growth, measured as increments in stem circumference, is sensitive to variation in rainfall availability.

Wood density and mechanical strength in mangrove branches

The results of my study indicate that mechanical strength varies with wood density in branches of mangrove species from South East Queensland. I found mechanical strength and wood density in mangrove branches to be mainly explained by reductions in xylem vessel lumen areas, which may have costs by reducing water transport and growth. In addition, higher mechanical strength and wood density were associated with increases in fibre wall thickness and with lower pith content, where storage of water and synthesis of hormones and enzymes occur. Given mangroves provide coastal protection from tidal and wind action as well as from cyclones and tsunamis, these results have implications for predicting mangrove ecosystems function and their responses to disturbance. Species with higher wood density and mechanical strength which are less vulnerable to damage, may survive and maintain ecosystem services after cyclones or other natural forces damage species with lower wood density.

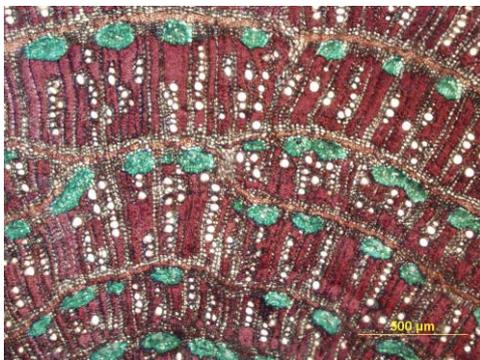
Dependence on fresh and seawater sources in mangroves

Wood characteristics of mangroves, such as xylem vessel size and growth rates, are affected by a range of environmental factors, including water availability. Although mangroves are remarkably salt tolerant, high salinity can have negative effects on mangrove growth rates. Firstly, salt increases the water potential of the soil. This exerts similar effects as drought stress. Consequently, plant growth is reduced because low soil water potentials reduce the capacity for water uptake and transport; thereby reducing cell turgor, cell expansion and growth. Due to the energetic costs associated with maintaining water

uptake under saline conditions mangroves have been observed to preferentially utilize water sources of lower salinity to support their growth. It is possible to determine the use of different water sources by mangroves by investigating the natural oxygen (O) stable isotopes within their tissues. The abundances of ^{18}O and ^{16}O stable isotopes can vary between water sources. The $^{18}\text{O}/^{16}\text{O}$ ratio ($d^{18}\text{O}$) of the xylem water reflects the $d^{18}\text{O}$ of the water sources taken up by the plant. I found that the $d^{18}\text{O}$ isotopic composition of xylem water was similar to more than one source of water indicating that mangroves used a mix of fresh and saline water sources for metabolic processes.

Overall, the results of my PhD project provide an insight of the wood characteristics that determine tree function in mangrove species. By understanding responses of xylem vessel diameter, phloem in wood, wood density and wood growth to climatic factors my project provides a valuable tool informing mangrove forest management in the future. Additionally, by determining the dependence of mangrove growth to freshwater availability, including how and when mangroves use different water sources, we can increase the knowledge needed to manage and preserve ecosystem function in Australia with changing rainfall patterns.

After my PhD I would like to pursue a postdoctoral fellowship in Mexico to investigate wood structure and water use by mangroves and coastal plants of the Yucatan Peninsula.



Transverse wood section from the widespread mangrove species *Avicennia marina* with successive cambia, which are defined as a complete band of xylem (in red) and phloem (in green).

For more information,

Santini NS, Schmitz N, Lovelock CE (2012) Variation in wood density and anatomy in a widespread mangrove species. *Trees* DOI: [10.1007/s00468-012-0729-0](https://doi.org/10.1007/s00468-012-0729-0).

Santini NS, Schmitz N, Bennion V, Lovelock CE. (2012) The anatomical basis of the link between wood density and mechanical strength in mangrove branches. In preparation.

Santini NS, Reef R, Lockington D, Lovelock CE. (2012) Dependence on fresh and seawater sources in the *Avicennia marina* mangrove. In preparation.

Acknowledgements,

I thank The National Council for Science and Technology (CONACYT, Mexico), The Secretary of Public Education (SEP, Mexico), The School of Biological Sciences (UQ, Australia), The National Centre for Groundwater Research and Training (NCGRT, Australia) and The Australian Nuclear Science and Technology Organisation (ANSTO, Australia) for financial support. It is a pleasure to thank my generous and stimulating supervisors, Prof. Catherine Lovelock and Dr. Nele Schmitz and all the people that in one or another way helped with the completion of my PhD project: Vicki Bennion, Dr. Ruth Reef, Dr. Fernanda Adame, Frida Sidik, Matt Hayes, Prof. Susanne Schmidt, Prof. David Lockington, Mitchell Zischke, Dr. María Gómez-Cabrera, Dr. Tim R. Mercer, Lucy Hurrey, Robert Gould, Richard Webb, Robyn Webb, Wendy Armstrong, and Kay Hodge from the University of Queensland. Jennifer and Denver Blake from Giralia Station. Dr. Greg Skrzypek and Douglas Ford from the University of Western Australia. Dr. Julieta Rosell from the National Autonomous University of Mexico (UNAM). Prof. Marilyn Ball and Jack Egerton from the Australian National University.

QUEENSLAND

Postharvest browning of cut *Backhousia myrtifolia* stems

Joseph Eyre

Agri-Science Queensland
Department of Agriculture, Fisheries and Forestry



Backhousia myrtifolia Hook. & Harv. is a canopy tree species endemic to the eastern coastal region of Australia. It is typically found in the transition zone between open eucalypt forest and rain forest from Bega, New South Wales to Bundaberg, Queensland. Selections from a particularly floriferous and attractive wild population are grown commercially in northern NSW and southern Queensland for cut flower production (Fig. 1). The canopy of these cultivated selections is managed to produce a dense shrub that, during spring and summer, bears numerous small florets with prominent white sepals and glossy deep-green foliage. *B. myrtifolia* is harvested either when tight white buds are present in the centre of the star-shaped sepals or following bud burst, after the petals and stamens have abscised to leave only the sepals. However, cut stems can suffer from unsightly browning that was observed for the first time during the 2004 season.



Figure 1. Bouquet of flowering *B. myrtifolia* stems. (Photo courtesy of Lily Lim-Camacho)

Vase life simulation experiments indicated that browning was a common symptom of stressed or injured *B. myrtifolia*, but a group of leaf and flower symptoms collectively termed the 'post-harvest browning syndrome' were associated with low temperature storage (Eyre *et al* 2011). The postharvest browning syndrome symptoms most closely resembled photographs and descriptions of the symptoms observed by growers and marketers.

Brown lesions developed on *B. myrtifolia* leaves stored at 0 to 10 °C, but symptom appearance was highly variable (Eckman *et al* 2008). The critical storage temperature below, and the period beyond which symptoms develop, were also highly variable. Brown lesions frequently developed on stems stored at ≤5 °C for 1 week and less frequently developed on stems stored at >5 to ≤10 °C for 1 week. Symptoms on stems stored at >5 to ≤10 °C were only very slight, rarely caused vase life termination and required up to 2 weeks storage to develop. If browning symptoms developed, then both the depth of colour and distribution of leaf symptoms were highly variable.

Attempts to objectively measure and predict low temperature injury with chlorophyll fluorescence and respiration measurements were unsuccessful, possibly because of difficulties reproducing symptoms, non-uniformly affected tissues (i.e. isolated lesions), and/or very rapid browning following injury.

Pre-harvest conditions during tissue growth and development influenced *B. myrtifolia* susceptibility to low temperature injury and therefore contributed to the observed symptom variability. Leaves that developed during mild pre-harvest temperatures (20 to 30 °C) with non-limiting water and nutrients had proportionally larger brown lesions than those that developed at more extreme temperatures (>20 to <30 °C). However, there was no relationship between pre-harvest temperatures and postharvest low temperature injury for commercially grown stems. Instead, it is hypothesised that unknown pre-harvest factor(s) that are influenced by temperature, such as growth rate, determined susceptibility to postharvest low temperature injury.

Leaf age also influenced the colour of low temperature injured leaves. Freeze injured young mature leaves became glossy black upon thawing whereas freeze injured older mature leaves turned a lighter greyish-black. The number of young mature leaves per stem was highly variable within and between plants (coefficient of variation 0.6). A model system was developed to further investigate the influence of leaf age and wilting on the tissues potential for discolouration caused by phenolic compound oxidation. The model system involved a freeze-thaw cycle to more reproducibly disrupt leaf cells than low temperature storage. The system also modified atmospheres to induce multiple levels of wilt in young and old mature leaves. The model system successfully modified wilt levels, disrupted cells and induced leaf discolouration similar to that observed on stems with the postharvest browning disorder. That is, freeze injured older mature leaves became light-tan-brown to brown, whereas young mature leaves became dark-brown to black. Furthermore, freeze injured and wilted leaves appeared lighter coloured than did freeze injured and non-wilted leaves.

Biochemical analysis suggests that dark pigments derived from the oxidation of phenolic compounds potentially caused *B. myrtifolia* leaf browning. Total phenolic compounds were reduced by ca. 33%, and browning indexes 357 and 418 increased ca.1.36 and 4.25 fold, respectively, for freeze injured young mature leaves. Young mature leaves had an ellagic acid content of 45.6 - 45.8 mg/g DW which declined to 25.2 - 28.5 mg/g following freeze injury, accounting for up to 73% of the observed decline in total phenolic compounds. Reduced phenolic compound oxidation in freeze injured old mature leaves appeared to account for the effect of leaf age on symptom colour variation. However, the effects of leaf water content on symptom colour appeared to be independent of changes in phenolic compounds and chlorophylls. The effects of wilt may alternatively be due to physical properties such as air ingress or altered cell structure.

The postharvest browning syndrome is a low temperature storage disorder that manifests as a highly variable group of flower and leaf discolourations. These symptoms represent a continuum of susceptibility to low temperature injury, biochemical browning potential and leaf reflectance. Pre-harvest factor(s), influenced by temperature, determine cut *B. myrtifolia* stems susceptibility to low temperature injury. Susceptible stems stored at ≤ 5 °C became discoloured during, or upon removal, from storage and the appearance of this discolouration was influenced by leaf age and water content (Fig. 2).

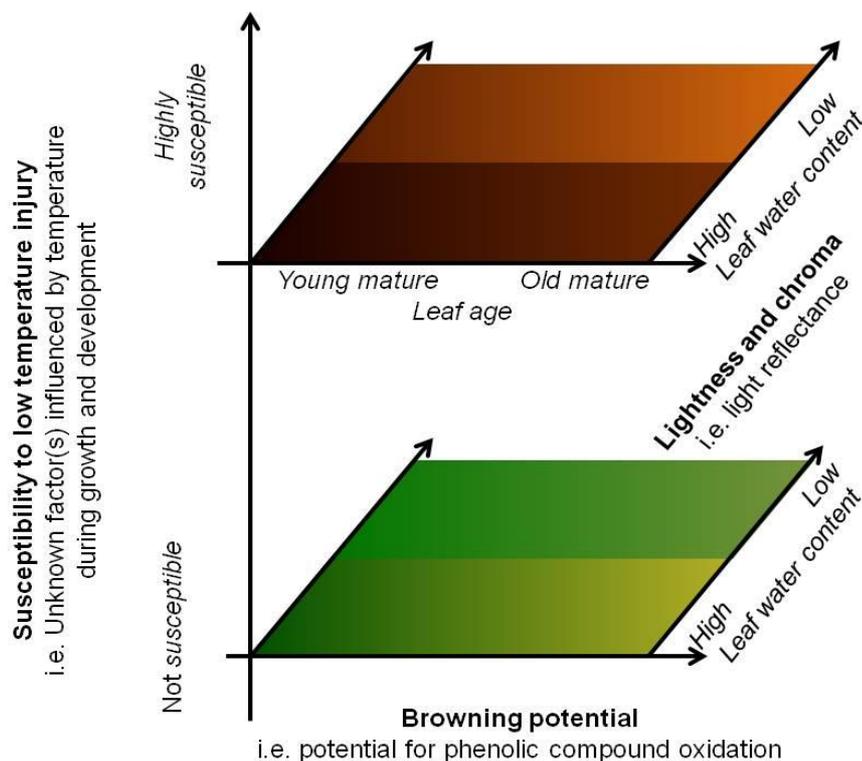


Figure 2. Schematic diagram for the *B. myrtifolia* postharvest browning syndrome demonstrating the proposed biophysical mechanisms responsible for the effects of leaf age and wilt on leaf colour.

I gratefully appreciate the guidance, enthusiasm, and tireless support from my advisors Professor Daryl Joyce and Dr Donald Irving. I appreciate their constructive criticism, assistance with developing the research projects and preparation of manuscripts associated with this thesis. I would also like to acknowledge the support and help from my fellow post-grads, research, technical and administrative staff. A special thanks to my wife and family for their support.

References

- Ekman J Eyre JX Joyce DC 2008 Flowers by Sea. Improving market access for Australian wildflowers RIRDC Publication No 07/181
- Eyre JX Joyce DC Irving DE 2011 Post-harvest browning syndrome and other qualities and defects in *Backhousia myrtifolia* *The Journal of Horticultural Science & Biotechnology* **86**, 225-229.

TASMANIA

Auxin biosynthesis in Pisum sativum: a physico-chemical approach

Dr. Laura Quittenden

University of Tasmania

The biosynthesis of the key plant growth hormone auxin has long remained enigmatic. Despite decades of research, only recently have the pathways responsible for indole-3-acetic acid (IAA; the main plant auxin) synthesis started to become clear. For some time there has been good evidence that the amino acid tryptophan is an early IAA precursor, but for any given species the downstream pathways to IAA remained elusive. From tryptophan, the suggested pathways to auxin become diverse: proceeding via indole-3-pyruvic acid (IPyA), tryptamine, indole-3-acetaldoxime (IAOx), or indole-3-acetamide (IAM), although none of these is fully characterised in terms of the isolation of the genes encoding the appropriate enzymes, nor the identification and quantification of precursor compounds.

Indeed, the more IAA biosynthesis was studied, the more the suggested pathways appeared to diversify. On the basis of models presented, there are not only species-related differences in the pathway used to produce the hormone, but also redundancies occurring in a single species (with more than one pathway operating), or multiple genes for single steps in a pathway. Plant species as diverse as rice, tomato, tobacco, and *Arabidopsis* have been studied, and gene families are generally responsible for each biosynthesis pathway step, making the production of IAA biosynthesis mutants very difficult. Furthermore, the pathway used by a particular plant species can vary depending on its environmental conditions, developmental stage, and tissue type (Ljung et al., 2005).

My PhD project involved dissecting out the possible IAA biosynthetic pathways within the roots of pea (*Pisum sativum*), an important legume species, using a physico-chemical approach. I used GC-MS and UPLC-MS to monitor pathway intermediates, and conducted metabolism experiments to show the presence or absence of pathway function. Many of the techniques used (including metabolism experiments and derivatisation of intermediates) were developed throughout my PhD. I also used a low auxin (although not a biosynthetic mutant) mutant, *bushy*, as a tool in order to better understand the regulation of auxin synthesis in peas.

Recent studies have highlighted the importance of the IPyA, and to a lesser extent, the IAM pathway to auxin in higher plants. However, this was not the case at my PhD initiation: the tryptamine pathway was favoured in the literature. The IAOx pathway was already proposed to be restricted to certain plant

families, however one of its constituents, indole-3-acetonitrile (IAN), was presumed to be more widespread.

I used GC-MS to monitor for the pathway intermediates mentioned, and found good evidence for the presence of indole-3-acetaldehyde (IAAld; a joint IPyA and tryptamine pathway intermediate), and tryptamine in pea roots, with no evidence for IAM, IAOx or IAN. On conducting whole root metabolism experiments using IAM, there was no conversion to IAA (Quittenden et al., under review). Likewise, deuterium labelled tryptophan was not converted to IAM, IAOx or IAN. However, tryptophan was converted to IAA, tryptamine, IAAld, and the IAAld storage product indole-3-ethanol (IEt). It is possible that the label found in IAA could be attributable to the IPyA pathway functioning; however the fact that label was recovered within tryptamine is good evidence that this pathway is operative (Quittenden et al., 2009).

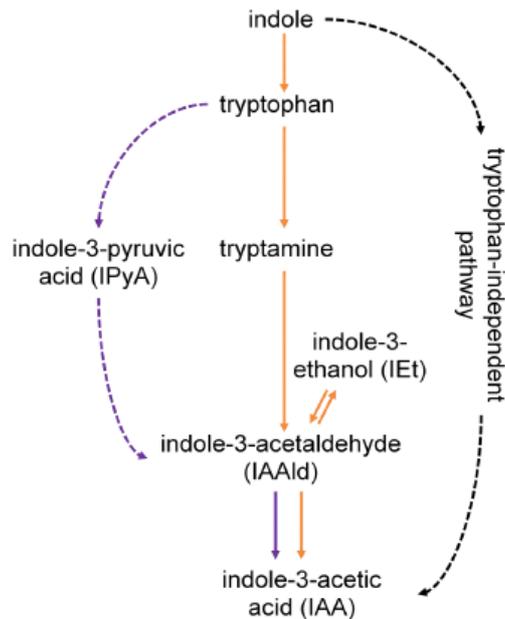
Complementing these findings, the low auxin mutant *bushy* was found to accumulate tryptamine, IAAld and IEt. It is important to note that although *bushy* is not an auxin biosynthesis mutant, it can be used here to study the regulation of auxin synthesis: as auxin levels are reduced in the mutant, it is plausible that the *bushy* mutation is either directly or indirectly blocking a biosynthetic step. Therefore, if an intermediate accumulates when auxin levels are reduced, this is evidence for its involvement in the active biosynthesis pathway. Although this was the case with the intermediates IAAld and tryptamine, the intermediates IAN, IAM and IAOx were still not identified within *bushy* mutant tissue, strengthening the argument that they are not involved in auxin synthesis in pea.

It is probable that the level of IAA in any one tissue type of any one species at any given time is under strict regulatory control. It was suggested previously that a negative feedback system operates to regulate IAA biosynthesis in *Arabidopsis*, where an initial increase of IAA level after treatment with naphthylphthalamic acid (an auxin transport inhibitor) induced an IAA feedback inhibition of IAA synthesis (Ljung et al., 2001). Conversely, Strader et al. (2011) suggest that a positive feedback system occurs in *Arabidopsis*, in agreement with the finding that in carrot cell cultures, synthetic auxin application stimulates an increase in the level of IAA (Michalczyk et al., 1992). However, there are few reports documenting the response of IAA biosynthesis pathway intermediates to exogenous auxin application. I studied the regulation of auxin biosynthesis in pea by incubating pea roots with the synthetic auxin, NAA. In comparison to control plants, those treated with NAA had lower IAA levels, and, again, higher IAAld levels. These data are consistent with a role for IAAld in auxin synthesis. In terms of the regulation of IAA, I found that a negative feedback system is operative in pea roots; the production of IAA is reduced by slowing the conversion of intermediates to the hormone when auxin levels are high (such as when auxin is applied). This finding is in agreement with Ribnicky et al. (1996) and Ljung et al. (2001), in carrot and *Arabidopsis*, respectively.

The research carried out in my PhD allowed the biosynthetic pathway to auxin in pea vegetative tissues to be simplified dramatically (Figure 1), eliminating the prospect of the intermediates IAM, IAOx and IAN having involvement. The next step in this species is to determine the relative importance of the tryptamine, IPyA and tryptophan-independent pathways to auxin synthesis, along with investigating other tissues in the same species (Tivendale et al., 2012). My PhD project was undertaken at the School of Plant Science, University of Tasmania, with Associate Professor John Ross, and our investigation into this area continues now.

- Ljung K, Bhalerao RP, Sandberg G (2001) Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *The Plant Journal* 28: 465-474.
- Ljung K, Hull AK, Celenza J, Yamada K, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. *The Plant Cell* 17: 1090-1104.
- Michalczyk L, Ribnicky DM, Cooke TJ, Cohen JD (1992) Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures. *Plant Physiology* 100: 1346-1353.
- Quittenden LJ, Davies NW, Ross JJ (2012) Indole-3-acetic acid is not synthesized via indole-3-acetamide in pea roots. *Journal of Plant Growth Regulation*: under review.
- Quittenden LJ, Davies NW, Smith JA, Molesworth PP, Tivendale ND, Ross JJ (2009) Auxin biosynthesis in pea: Characterization of the tryptamine pathway. *Plant Physiology* 151: 1130-1138.

- Ribnicky DM, Ilić N, Cohen JD, Cooke TJ (1996) The effects of exogenous auxins on endogenous indole-3-acetic acid metabolism. *Plant Physiology* 112: 549-558.
- Strader LC, Wheeler DL, Christensen SE, Berens JC, Cohen JD, Rampey RA, Bartel B (2011) Multiple facets of *Arabidopsis* seedling development require indole-3-butyric acid-derived auxin. *The Plant Cell* 23: 984-999.
- Tivendale ND, Davidson SE, Davies NW, Smith JA, Dalmais M, Bendahmane AI, Quittenden LJ, Sutton L, Bala RK, Le Signor C, Thompson R, Horne J, Reid JB, Ross JJ (2012) Biosynthesis of the Halogenated Auxin, 4-Chloroindole-3-Acetic Acid. *Plant Physiology* 159: 1055-1063.



Me, in the glasshouse at the University of Tasmania.

Figure 1. The three possible pathways for IAA synthesis in pea vegetative tissues. Shown are the tryptamine (orange) and IPyA (purple) pathways. The tryptophan-dependent pathway (black) may also operate. The dashes represent steps that have not been studied here, but which could occur in pea vegetative tissues.

SOUTH AUSTRALIA

Counting the cost of environmental pollution: Dealing with cadmium in Chinese rice

Matthew Rodda

School of Earth and Environmental Sciences, University of Adelaide

I have recently successfully completed a PhD in Associate Professor Rob Reid's laboratory of Plant Physiology and Plant Nutrition at the University of Adelaide. My thesis, which was recently examined and accepted, was titled, 'The Physiological Basis for Variable Cadmium Accumulation in Rice: Interaction of Environmental and Genetic Factors.' This research was funded through an ARC Discovery Project in Rob's lab on, 'New strategies for reducing the concentrations of cadmium and arsenic in crop plants.'

In addition to the research I conducted in Adelaide, I was also fortunate to be awarded a Prime Minister's Australia Asia Endeavour Award, which enabled me to conduct a year of my research at the Chinese Academy of Sciences, Xiamen, China, with an international collaborator in this project, Prof. Yong-guan Zhu, director of the Institute for Urban Environment (previously a post-doctoral researcher at the Waite Institute, SA). This large new research institute in Xiamen is part of an initiative by the Chinese Academy of Sciences to study and find solutions to environmental problems which impact on China's increasingly urban population.

My time in China allowed me to pursue areas of research that were not possible in Australia. It also meant that my PhD included a thorough cross-cultural immersion as well as providing broad grounding in scientific research. The only reason I applied for the Endeavour Award was because of the encouragement of a member of the academic staff, so supervisors should note that it is worth encouraging your students to stretch themselves and apply for additional scholarships and grants for travel.

In addition to a number of international meetings held at IUE while I was there, during my studies I was also able to attend and present a poster at the 15th International Workshop on Plant Membrane Biology, in Adelaide. This was a great eye-opener for me and a chance to experience the international nature of plant science research.

I am now employed as a research scientist in the field of molecular plant breeding and germplasm enhancement at the Department of Primary Industries, Horsham, Victoria. The most important things I gained during my PhD were skills in independent research and scientific communication; these and the varied experiences I had were crucial for me winning this position.

Synopsis

Cd is a toxic heavy metal element that occurs in some agricultural soils and is known to be taken up by plants, including the edible portions of crops. Human exposure to elevated levels of Cd in the environment is known to lead to accumulative toxicity and rice is a key pathway of entry for communities exposed to elevated levels of Cd in Asia. In terms of the risk of Cd to human health, the consumption of contaminated rice as a part of subsistence diets is of particular concern because of the relatively low levels of Zn, Fe and Ca in rice grains, a nutrient imbalance that increases the absorption of Cd.

Rice plants vary in the degree to which they accumulate Cd in their grain, but the nature of genotypic variation in Cd accumulation and the molecular mechanisms responsible are not fully understood. Recently, significant progress has been made in rice with the discovery of a Zn membrane transporter,

OsHMA3, which is critical for the root vacuolar storage of Cd. Nevertheless, this gene is not the basis of all genotypic variation in rice grain Cd accumulation, and there is also significant genotype by environmental interaction in the Cd accumulation of rice genotypes, the reasons for which have not been fully elucidated.

My PhD research was fundamentally a study of the physiological mechanisms involved in Cd accumulation in rice and also the factors responsible for genotypic differences in Cd accumulation between rice cultivars. This included, firstly, a study of the timing of Cd loading into the grain over the rice lifecycle, comparing accumulation before and after flowering. Post-flowering Cd was found to contribute 40% of grain Cd in hydroponically grown rice plants, showing that grain Cd is not just the product of shoot accumulation of Cd prior to flowering. Secondly, I attempted to contrast naturally occurring variation in Cd uptake with genes thought to be involved in plant Cd uptake. A selection of germplasm, which represented the large degree of the diversity in Cd accumulation in modern rice varieties, was used in analysis of the genotypic expression of membrane transporters putatively involved in Cd uptake and translocation. These varieties were characterised for Cd accumulation under different conditions, including under hydroponics and flooded paddy soil, and this was compared with patterns in gene expression. A significant aim was to apply much of the published research on Cd transport to the question of why rice cultivars differ in Cd uptake. There was not a consistent pattern between expression of these candidate genes and Cd accumulation characteristics, and it is likely that the molecular bases of Cd accumulation differences are not the same in all cultivars. There were some general trends that received further study, including the higher root expression of the Fe/Cd transporter OsNRAMP1 in high Cd accumulating varieties. Particularly there were similarities between varieties of the *indica* and *japonica* subspecies of rice in terms of gene expression.

Other nutrition factors, including Fe and Si, were examined alongside this work for their role in influencing Cd uptake. Silicon supply was confirmed to decrease the accumulation of Cd in rice, and this seemed to be because of a mechanism associated with the accumulation of Si in shoot tissues. Growing rice without Fe led to a great increase in the accumulation of Cd, showing evidence of the link between Cd uptake and Fe nutrition. Iron deficiency response in other plants has been seen to have a large effect on Cd uptake, and so its role in the accumulation of Cd was examined. The regulation of Fe/Cd membrane transporters during the development of Fe deficiency was studied and compared with the influx of Cd accumulation that occurred concomitantly. Surprisingly, the effect of Fe deficiency (approximate 20% increase in shoot Cd) was found to be smaller than the effect of competition with Fe²⁺ ions in solution, but this was not seen for Fe(III) supplied in a chelated form. It has previously been postulated that Fe deficiency could play a role in Cd accumulation in field-grown plants, and so this hypothesis was tested with rice grown under variable irrigation regimes, including continuous and intermittent flooding over the growing season. Analysis of the availability of Cd, Fe and Mn under these conditions, led to novel observations of the fluctuation in the availability of these metals with changes in soil redox conditions. Changes in redox conditions and Cd availability were contrasted with Fe deficiency response during the growth of the plants and importantly during the development of the rice grain. Upregulation of iron-deficiency responsive genes was observed in some plants grown in aerobic soils, especially in *indica* varieties, but this was not found to be associated with a specific increase in Cd accumulation.

Finally, on the basis of results of earlier experiments and reports in the recent literature, OsNRAMP1 and its effect of Cd translocation were further studied using transgenic rice varieties with manipulated expression of this membrane transporter (sourced from Prof. Nishizawa's lab at the University of Tokyo). Despite reports to the contrary, no evidence could be found for a role for OsNRAMP1 in the accumulation of Cd by rice plants. Over-expression produced large differences in the number of OsNRAMP1 transcripts but this did not result in a significant increase in Cd accumulation. The main observable effect of OsNRAMP1 was increased shoot Fe content relative to WT plants, but this was only found to occur with the co-upregulation of other iron-deficiency responsive genes under minus Fe conditions. OsNRAMP1, therefore, seems to play a role in the pathway of movement of Fe from root to shoot, but not for Cd at physiologically relevant concentrations.

This research was a step towards a better understanding of the physiological and molecular regulation of Cd uptake in rice, and higher plants in general. Specifically it has provided a better understanding of the way in which the nutritional factors Fe and Si influence Cd uptake. It has also given clarification of the role of the Fe transporter OsNRAMP1, showing its limited effect on Cd accumulation in rice.



Matthew (far right-back row) with his colleagues from the Chinese Academy of Sciences, Xiamen, China

Acknowledgements

I would like to thank my principal supervisor, Assoc. Prof. Rob Reid, who was always generous with his time and was greatly supportive while I carried out my studies. This research was supported under Australian Research Council's *Discovery Projects* funding scheme (project number DP0773638) and a University of Adelaide - Faculty of Sciences divisional PhD scholarship. Some experimental work was also supported by the Chinese Academy of Sciences, International Collaboration Fund (GJHZ200828). My travel, living and study allowance in China were funded by a Prime Minister's Australia Asia Endeavour Award (2010).

WESTERN AUSTRALIA

Biom mineralisation in Australian desert *Acacia* species: unexplored potential for phytoremediation

Honghua He

School of Plant Biology, The University of Western Australia

Biom mineralisation, the formation of minerals by living organisms, is a widespread phenomenon displayed by most forms of life on Earth, including plants. Calcium, an alkaline earth element, is the predominant cation associated with biom mineralisation for most organisms, and calcium-bearing minerals represent about 50% of known biom minerals. In the plant kingdom, the most common type of crystals is calcium oxalate. Some proposed functions of biom mineralisation in plants include bulk element (mainly calcium) regulation, detoxification of aluminium and heavy metals, mechanical support, protection against herbivory, and light redistribution. As an important part of my PhD research, the biom mineralisation in four *Acacia* species native to the Telfer region in the Great Sandy Desert in north-western Australia was studied.

The four species were *Acacia stipuligera* F. Muell. (subgenus *Phyllodineae*, section *Juliflorae*; evergreen, bushy shrub), *Acacia ancistrocarpa* Maiden & Blakely (subgenus *Phyllodineae*, section *Juliflorae*; evergreen, multi-stemmed shrub), *Acacia stellaticeps* Kodala, Tindale & D. Keith (subgenus *Phyllodineae*, section *Plurinerves*; evergreen, flat-topped shrub) and *Acacia robeorum* Maslin (subgenus *Phyllodineae*, section *Phyllodineae*; evergreen, diffuse shrub). Phyllodes (modified petioles that function as leaves) and branchlets of mature plants grown in their natural habitat and two-year seedlings grown in potting mix in a glasshouse were studied by means of optical and scanning electron microscopy (SEM), together with energy-dispersive X-ray spectroscopy (EDS) to investigate the crystal distributional patterns, morphologies and elemental compositions. Crystals of various morphologies and distributional patterns were observed in the four *Acacia* species studied. Magnesium, strontium, barium, and potassium were precipitated together with calcium, mainly in phyllodes of the four *Acacia* species, and sometimes in branchlets. These elements were most likely precipitated in forms of oxalate and sulfate in various tissues, including epidermis, mesophyll, parenchyma, sclerenchyma (fibre cells), pith, pith ray and cortex. Elements precipitated differed between soil types, plant species, and tissues within an individual plant; the precipitation was also related to tissue age, so I surmise that, in most cases, precipitation of calcium, magnesium, strontium and barium is biologically induced for these species.

Among the four species, *A. robeorum* was a striking example of a species that formed numerous crystals of various morphologies in multiple tissues. It had many more crystals containing calcium and magnesium than the other three species, and its foliar calcium, magnesium and sulfur concentrations were significantly higher than those of the other three species. *A. robeorum* can be identified as a thiophore (plant that can accumulate sulfur) because the sulfur concentration in the phyllodes of its mature plants in their natural habitat was up to 42 mg g⁻¹ dry mass.

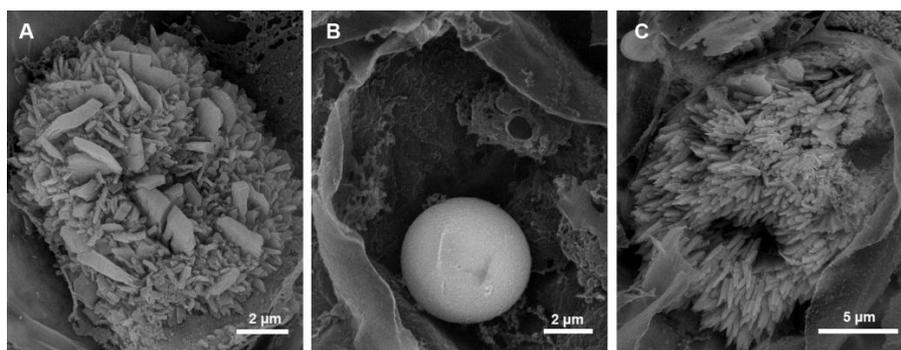


Figure Typical crystals formed in phyllodes of *Acacia robeorum*.

When seedlings of *A. stipuligera* and *A. robeorum* were grown in sandy topsoil or a mixture of sandy topsoil and siltstone (a proposed rehabilitation substrate) collected from the Telfer gold mine, a series of elements, including calcium, magnesium, strontium, barium, sodium, potassium, aluminium, iron, manganese, copper, titanium, vanadium and sulfur were sequestered in phyllodes of the two species, most likely in forms of oxalate, sulfate, silicate, silica, and metal oxides.

Mining is a major economic activity in the Australian arid region, and it can severely disturb land surfaces and destroy biodiversity within natural ecosystems. The Telfer gold mine, which is operated in the Great Sandy Desert, will generate 1,100 Mt of waste material over the next 20 years, 25% of which is potentially acid-generating, and there is a potential risk of contamination of soils with toxic metals, which could have negative effects on the environment. In addition, sulfate concentrations are generally high where acid mine drainage occurs. Mine-site rehabilitation is a fundamental part of the mining operation. The rehabilitation objective of most Australian mine sites is to restore the pre-mining ecosystem and make it self-sustaining in the long term. Therefore, native plant species, which are adapted to the local conditions, including climate, soil and hydrology, are the first choice. *Acacia* is a large and diverse genus that is dominant in the vegetation of arid Australia, including the Telfer region, and plays an important role in maintaining desert ecosystem stability. Many Australian *Acacia* species occur on a range of soil types, and they are important components of rehabilitated ecosystems. It is not known whether the biomineralisation processes in the current *Acacia* species really played a role in detoxifying metals or not; if they did, the *Acacia* species studied in my PhD project may offer promise for phytoremediation (a technology that uses plants to remove pollutants from contaminated environments) on metal-contaminated and sulfur-rich substrates such as tailing-disposal areas with acid mine drainage, although these species are not hyperaccumulators of heavy metals, as indicated by their only moderately high metal concentrations.

The results of this project enriched the present knowledge on biomineralisation in plants, and opens up several research areas in terms of the biochemistry in biomineralisation and the roles of biominerals in plant functioning. *Acacia robeorum*, in particular, can serve as a model plant in studying calcium, magnesium and sulfur nutrition of plants, and biomineralisation as well.

I greatly appreciated the input of all my supervisors, Prof. Hans Lambers, Prof. Erik Veneklaas and Dr Timothy Bleby in the School of Plant Biology at The University of Western Australia (UWA) for their contribution to this project. I received great scientific and technical assistance from Prof. John Kuo and Mrs Yaowanuj Kirilak at the Centre for Microscopy, Characterisation & Analysis, UWA, a facility funded by The University, and State and Commonwealth Governments. This work was supported by Australian Research Council, Mineral and Energy Research Institute of Western Australia, Newcrest Mining Ltd. (Telfer Gold Mine), and the School of Plant Biology, UWA. China Scholarship Council and UWA provided a scholarship for my PhD.

References:

- He H., Bleby T.M., Veneklaas E.J., Lambers H. (2011) Dinitrogen-fixing *Acacia* species from phosphorus-impooverished soils resorb leaf phosphorus efficiently. *Plant, Cell & Environment* 34, 2060-2070.
- He H., Bleby T.M., Veneklaas E.J., Lambers H. (2012) Arid-zone *Acacia* species can access poorly soluble iron phosphate but show limited growth response. *Plant and Soil*. doi: 10.1007/s11104-011-1103-5.
- He H., Bleby T.M., Veneklaas E.J., Lambers H., Kuo J. (2012) Morphologies and elemental compositions of calcium crystals in phyllodes and branchlets of *Acacia robeorum* (Leguminosae: Mimosoideae). *Annals of Botany* 109, 887-896.
- He H., Bleby T.M., Veneklaas E.J., Lambers H., Kuo J (2012). Precipitation of calcium, magnesium, strontium and barium in tissues of four *Acacia* species (Leguminosae: Mimosoideae). *PLoS ONE* 7, e41563.
- He H., Kirilak Y., Kuo J., Bleby T.M., Veneklaas E.J., Lambers H. Sequestration of metals and sulfur in two north-western Australian desert *Acacia* species. SUBMITTED.

NEW ZEALAND / NEW SOUTH WALES

Novel markers for drought resistance in white clover

Wouter L. Ballizany

Department of Wine, Food & Molecular Biosciences
Agriculture & Life Sciences
Lincoln University, New Zealand



Summary. White clover is an important forage legume in temperate pastures, but does not have sufficient resistance against drought stress. Quercetin is a flavonol conferring plant sunscreen and anti-oxidant properties under UV-B stress with a trade-off of reduced biomass. An F₁ full-sib cross between stress tolerant and stress sensitive white clover genotypes showed the ability to increase quercetin glycoside accumulation associated to maintenance of dry matter yield under a near wilting drought.

Introduction. White clover (*Trifolium repens* L.) is an out-crossing allotetraploid ($2n=4x=32$), and at meiosis bivalents ($n=16$) (a pair of associated homologous chromosomes) are formed, i.e. white clover is an allotetraploid that acts like a diploid (Majumdar *et al.* 2004), also called *amphidiploid*. White clover is a forage crop with a high level of genetic heterogeneity, which allows it to adapt to changing environments. However, growth of white clover is often impaired by summer drought (Hutchinson *et al.* 1995). New cultivars need to adapt to higher levels of abiotic stress to survive and persist (Jahufer *et al.* 1999). Recent research in white clover populations demonstrated that levels of a secondary metabolite, glycosides of the phenolic compound quercetin (Q), were positively associated with abiotic stress resistance, and inversely related to dry matter (DM) production (Hofmann *et al.* 2000; Hofmann and Jahufer 2011). Molecular plant breeding methods using DNA markers and quantitative trait loci (QTL) analysis can increase breeding efficiency using information on genotype to complement phenotype, and identify genes controlling complex traits (Barrett *et al.* 2004).

Aims. The objectives of my Ph.D. study were (i) to investigate genetic control of the complex traits Q glycosides and DM production in contrasting environments, (ii) to test the inverse correlation between Q glycosides and DM production, and (iii) to identify DNA markers associated with quantitative trait loci (QTL) for use in marker-assisted selection (MAS).

Materials and methods. An F₁ mapping population ($n = 190$) created from a paired cross between two highly heterozygous white clover genotypes sampled from New Zealand cultivar “Kopu II” and Chinese ecotype “Tienshan” was used for phenotyping and genotyping. Phenotyping consisted of three parts 1) in pots under outside conditions in separate pilot and phenotyping experiments, 2) in pots outside under imposed water deficit, and 3) in the field in two environments contrasting in soil moisture. Important traits were measured, including morphological, e.g. root DM, shoot DM, total DM, root-to-shoot DM ratio, stolon density, leaf size; biochemical, glycoside levels of the flavonols quercetin and kaempferol (K), Q:K glycoside ratio (QKR); as well as physiological, e.g. water potential, stomatal conductance and carbon isotope discrimination. Genotyping was investigated by means of single sequence repeat (SSR) markers and diversity array technology (DArT) markers from the genomes of *Trifolium repens*, *Trifolium occidentale* and *Medicago truncatula*.

Results. Phenotyping in pot studies revealed sufficient genetic variation in the population, and only a weak albeit significant ($P < 0.001$) correlation between dry matter yield and Q, with levels of one explaining less than 10% of variation in the other. Imposed water deficit decreased leaf water potential by more than half overall, Q glycosides increased more than twofold and the ratio of quercetin to kaempferol glycosides increased preferentially towards Q. Furthermore, the most productive genotypes in the controls showed the greatest proportional reduction, the root:shoot ratio increased by half and individuals with high QKR levels reduced their biomass least under water deficit, and in turn increased their Q glycosides and QKR most. In the field studies, water deficit significantly reduced carbon isotope discrimination, Q glycosides were preferentially synthesised over K, accumulation of Q glycosides was related to retaining biomass, and stolon density was inversely related to stolon numbers. Genotyping resulted in a large number of DNA markers associated with genome regions influencing traits in the population. QTL effects were consistent over pot- and field experiments in some cases. Suitable markers were used to build a partial linkage map to better test for chromosomal locations containing QTLs associated with the traits of interest. Interaction between Q glycosides and DM QTLs was analysed.



Wouter L. Ballizany taking measurements on white clover in the field at Ashley Dene dryland farm, Lincoln University, New Zealand.

Discussion and conclusions. The data show, for the first time, that at the individual genotype level increased Q glycoside accumulation in response to water deficit is associated with retaining higher levels of biomass production (Ballizany *et al.*

2012b, 2012a). These findings are the first indication that forage populations that are both high yielding and show high Q glycoside levels are possible. The single locus discoveries of marker-trait associations provide a basis for enhanced plant breeding efficiency for these traits, and specific sources of new variation for economically significant traits. Overall, the findings can be used to better understand the physiological underpinnings of water stress relations in forage plants, and improve gain from selection in white clover by increasing the precision with which improved plants and populations are identified.

- Ballizany, WL, Hofmann, RW, Jahufer, MZZ, Barrett, BA (2012a) Genotype \times environment analysis of flavonoid accumulation and morphology in white clover under contrasting field conditions. *Field Crops Research* **128**, 156-166.
- Ballizany, WL, Hofmann, RW, Jahufer, MZZ, Barrett, BA (2012b) Multivariate associations of flavonoid and biomass accumulation in white clover (*Trifolium repens*) under drought *Functional Plant Biology* **39**, 167-177.
- Barrett, B, Griffiths, A, Schreiber, M, Ellison, N, Mercer, C, Bouton, J, Ong, B, Forster, J, Sawbridge, T, Spangenberg, G, Bryan, G, Woodfield, D (2004) A microsatellite map of white clover. *Theoretical and Applied Genetics* **109**, 596-608.
- Hofmann, RW, Jahufer, MZZ (2011) Tradeoff between biomass and flavonoid accumulation in white clover reflects contrasting plant strategies. *Plos One* **6**, DOI: 10.1371/journal.pone.0018949.
- Hofmann, RW, Swinny, EE, Bloor, SJ, Markham, KR, Ryan, KG, Campbell, BD, Jordan, BR, Fountain, DW (2000) Responses of nine *Trifolium repens* L. populations to ultraviolet-B radiation: Differential flavonol glycoside accumulation and biomass production. *Annals of Botany* **86**, 527-537.
- Hutchinson, KJ, King, KL, Wilkinson, DR (1995) Effects of rainfall, moisture stress, and stocking rate on the persistence of white clover over 30 years. *Australian Journal of Experimental Agriculture* **35**, 1039-1047.
- Jahufer, MZZ, Cooper, M, Bray, RA, Ayres, JF (1999) Evaluation of white clover (*Trifolium repens* L.) populations for summer moisture stress adaptation in Australia. *Australian Journal of Agricultural Research* **50**, 561-574.
- Majumdar, S, Banerjee, S, De, KK (2004) Meiotic behaviour of chromosomes in PMCs and karyotype of *Trifolium repens* L. from Darjeeling Himalaya. *Acta Biologica Cracoviensia Series Botanica* **46**, 217-220.

ComBio 2012, Adelaide

Adelaide Convention Centre, 23 – 27 September 2012

ComBio 2012, in Adelaide is shaping up to be a great meeting with plenty of plant options together with excellent Plenaries from other disciplines. We look forward to seeing you there!!

There are a record seven societies participating in the meeting including the New Zealand Society of Plant Biologists and for the first time the Australasian Plant Pathology Society - Molecular and Physiological Plant Pathology Special Interest Group.

The plant “people on the ground” are Steve Tyerman, David Day, Brent Kaiser and Geoff Fincher with Rachel Burton and Margaret Barbour as joint coordinators for the Plant Stream.

The **streams and stream co-ordinators** are:

- Cell biology, Architecture and Trafficking (Yeesim Khew-Goodall / Alpha Yap)
- Developmental Biology (Paul Thomas / Patrick Tam)
- Gene Regulation, Genomics and Bioinformatics (Greg Goodall / Philip Gregory)
- Plant Biology (Rachel Burton / Margaret Barbour)
- Molecular and Physiological Plant Pathology (Klaus Oldach / Richard Oliver)
- Protein structure, function and proteomics (Grant Booker / Michael Parker)
- Signalling (Angel Lopez / Roger Daly)

For the **Plant Biology Stream** Rachel and Margaret are organizing Chairs and Co-chairs for the nine Symposia and a Colloquium. The topics are:

- Plant Cell Walls
- Plant Genomics and Epigenetics
- Photosynthesis
- Plant Ecophysiology
- Plants and Human Nutrition
- Plants and Climate Change
- Water and Solute Transport in Plants
- Plant Phenomics and Imaging
- Plant Development
- Colloquium session for students and ECRs

For a full list of the **Plenary Speakers** see: <http://www.asbmb.org.au/combio2012/>

Some of particular interest for ASPS members will be:

Ryan Lister - UWA, formerly Salk Institute – Plant biology - DNA methylation, genomics

Susan McCouch - Cornell University, USA - Rice genetics, GWAS, SNPs

Anne Osbourn - John Innes Centre UK - Crop and model plants - genetics, genomics, computational biology, cell biology, protein and small molecule biochemistry

Dale Sanders - John Innes Centre, UK - plant Ca signalling/transport

Richard Dixon - Samuel Roberts Noble Foundation, USA - plant cell walls.

The RN Robertson Lecture will be delivered by **John Patrick** – University of Newcastle

2013 Conferences

International Plant Nutrition Colloquium

Istanbul, Turkey 19 - 22 August 2013

<http://www.ipnc-istanbul.org>

The colloquium will focus on research topics dealing with agro-ecological, environmental, physiological, genetic and molecular aspects of plant mineral nutrition. Major attention will be paid to i) soil nutrient dynamics, ii) root biology and microbe interactions, iii) nutrient management, iv) ion toxicity and remediation, v) nutrient uptake, transport and remobilisation, vi) nutrient functions and vii) mitigating impacts of mineral nutrition on various environmental stress factors such as drought, salinity and diseases. The main theme of the 17th IPNC will be "Plant nutrition for nutrient and food security".

International Conference on Arabidopsis Research

Sydney, Australia 24 - 28 June 2013

<http://www.icar2013.com.au>

We warmly invite you to be a part of the 24th International Conference on Arabidopsis Research (ICAR), the largest annual international scientific conference devoted to *Arabidopsis thaliana* – a model plant worked with by an estimated 16,000 labs around the world. Experiments performed in *Arabidopsis* often underpin plant research in general and *Arabidopsis*-driven research leads the way with technologies and concepts.

International Conference on Plant Vascular Biology

Helsinki, Finland 26 – 30 July 2013

<http://pvb2013.org/>

The conference will focus on recent progress in vascular plant biology, including development, evolution, structure, function and regulation, environmental influences, metabolism and nutrition, transport systems, local and long-distance communication, insect and pathogen challenge, proteomics and metabolomics, and agricultural and biotechnological applications.



Were you aware that....?

- ✚ **ASPS Website.** The ASPS website has been thoroughly revamped and is being continuously upgraded.

 - Membership dues can now be paid on line.
 - You can advertise jobs, PhD scholarships, conferences, books by contacting Rob Shepherd via advertise@asps.org.au. To cover the costs involved, the society has introduced a small charge of \$34 for members and \$74 for non-members **FOR EMPLOYMENT ADS ONLY**. Advertising conferences and books (edited by society members or containing chapters written by society members) are **FREE OF CHARGE**.

- ✚ **RN Robertson Travelling Fellowship.** This named Fellowship recognises and celebrates the sustained contribution made by RN Robertson (Sir Bob) in nurturing young plant scientists in Australia spanning across four decades from the 1950's. The Australian Society of Plant Scientists is indebted to Hank Greenway and Joe Wiskich who generated and championed the early development of the RN Roberston Travelling Fellowship.

- ✚ **Student Travel Funds.** Funds are set aside each year to sponsor student travel to our annual conference (2012 ComBio, Adelaide), and contribute to their professional development in plant science. Support will vary from year to year depending on the Society finances, location of meeting and number of applications. The Treasurer will apply a formula in calculating individual entitlements and takes these factors into account. Applicants must be financial members of ASPS and presenting a paper or poster at the ComBio meeting.

- ✚ **Postgraduate Section.** We are proud to announce that student members who have recently completed their PhD and had their thesis passed can submit a summary that features in Phytogen. Members of the Council feel that this is an important opportunity for our postgraduate students to showcase their research. Such successful student members are advised that the summary can be accompanied by a key image in suitable format and that they should submit their items to the editor of Phytogen at any time for inclusion in the next issue.

- ✚ **Society Funding for Workshops and Conferences.** The society has a total of \$10,000 available each year to provide seeding money and sponsorship for up to four conferences organised by members. The amount available to assist each conference will be about \$2,500. For more details see the website: <http://www.asps.org.au> and take the link to conferences.

- ✚ **Corresponding and Life Memberships.** Life Membership recognises an outstanding and sustained contribution to the Society by a long-standing ASPS member who, through their professional activities, has substantially enhanced the international profile of Australian plant science research. Corresponding Members are high profile overseas colleagues who have contributed substantially to plant science research within Australia. If you know of a deserving recipient for Life or Corresponding Membership, please consider putting a nomination forward. The procedure to follow is outlined on the ASPS website (see: <http://www.asps.org.au> and click on "About ASPS" where there is also a list of Life and Corresponding members).