PHYTOGEN

A
NEWSLETTER
FOR
AUSTRALIAN
PLANT SCIENTISTS

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PHYTOGEN

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In This Issue

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

AUSTRALIAN SOCIETY OF PLANT SCIENTISTS

News from the President

Phytogen, April 2011

Dear Member,

One of the roles of the ASPS is to promote the Plant Sciences in Australia. According to our web site "The Society can be broadly described as:

- A group of individuals who are deeply interested in how plants function.
- Providing a forum for sharing of knowledge so that the membership can build both the depth and breadth of knowledge of plant functions.
- Providing mutual support and collective mentorship.
- Recognising and rewarding excellence at all levels of scientific career development without fear or favour.
- Nurturing the next generation of plant scientists.
- Working on behalf of members to protect their ability to do research and to educate others in plant sciences.
- Supporting a journal, Functional Plant Biology, that reflects the broad interests of the members."

In order to be able to be true to this, we need to keep a watchful eye on the way that the plant sciences are perceived through the Excellence in Research Australia (ERA) process and future funding models. Thank you to all those, especially Council members, who provided feedback on the FoR (Field of Research) codes and rankings for journals in the plant sciences last month. The next item on the agenda needing attention is comments on the Strategic Roadmap for Australian Research Infrastructure, which will articulate Australia's national research infrastructure priority areas (comments due 4th May).

One way we recognise excellence in our community is through our awards. Nominations are now open for the ASPS Teaching award and the prestigious Goldacre Award for research by someone within 10 years of completing their PhD. The 10 years is equivalent full time and takes into account interruptions to a person's research career. Submissions should be made to the secretary John Evans by 22 May 2011. (For details see: www.asps.org.au/awards/). The ASPS-FPB Best Paper Award is given for the best paper published in the Journal in each calendar year by an early-mid career researcher (i.e. within 10 years of receiving their PhD), nominated by Functional Plant Biology reviewers. Please consider nominating papers for this award next time you review for FPB.

The Society supports students through the RN Robertson Travelling fellowships (to support research) and the ASPS Travel awards to support attendance at the annual ComBio conference. This year the travel awards are also available for student members presenting papers at the International Botanical Congress in Melbourne in July.

Our future is with our younger members. In order to better connect with the next generation of plant

scientists I am in the process of setting up Facebook and Twitter for the ASPS, having played with them a bit over the past year to get the hang of it. Details will be emailed to members at a later date. (I am told email is something 'young' people use to communicate with 'old' people.)

Finally I would like to encourage you to think about how you could contribute to the community of plant scientists in Australia. Several positions on the Council will become open at the annual meeting in September in Cairns. An important role is that of Secretary, with John Evans' term expiring. The role of President-elect will also be coming up, allowing a one-year overlap to learn the ropes before taking on the position of President in 2012. We now have Jen Price acting as executive officer to take on some of the more routine administrative tasks, which really helps, so please think now about putting your hand up.

Best wishes,
Ros Gleadow

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A message from the editor

Dear Fellow ASPS Members,

In my message at the end of 2010 I indicated that one aim for 2011 was to hear from those states that haven't reported on their research activities for a few years - Tasmania, Victoria and Queensland. So in this issue *State of Affairs* features an interesting account of the research interests of some of Tasmania's plant scientists with thanks to Sergey Shabala. In seeking articles for the *Historical Perspectives* section, I am delighted to thank Hal Hatch for putting some of his experiences under the title *"Some Reminiscences and Eureka Moments",* the former reminding us about the history of plant biochemistry research and the latter focused on C_4 photosynthesis. The older members of our society provide extraordinary achievements coupled with much wisdom that it would be a shame to lose. To this end I have also reproduced Bob Robertson's *"A Life Of Surprises in Plant Physiology"* first published in 1996. This article provides great reading and you will find many of Bob's experiences have a resonance today – the human condition never changes!! To balance our history with the new frontiers of Australian plant science research I also attempted to stir our youngest members into action to report their exciting PhD research. Unfortunately this endeavour has been without success. However, in this issue you will find an interesting report from Cara Griffiths a recipient of the RN Robertson Travelling Award. Sadly this year has seen the death of another of our members Andy Netting. You will find a tribute to him written by Rana Munns and Robert Willows.

Of course the International Botanical Congress, Melbourne 2011 is fast approaching (July 23 - 30) to be followed by Combio in Cairns (September 25 - 29). And, beyond our shores, the International Conference on Plant Metabolism is being convened in China, June 30 – July 3, 2011. In this issue you will find a somewhat belated but I hope informative précis of the International Conference on Plant Vascular Biology 2010, Columbus, Ohio, USA. I am attempting to include accounts of conferences in Phytogen so hope to find a champion to report some aspects of the IBC and Combio for subsequent issues. For the next issue it is Victoria's turn for "*State of Affairs".* Any other suggestions, and of course contributions – book reviews, significant issues for plant science, education issues, are most welcome. As editor I would like to stress that *Phytogen* is your newsletter.

As always, thanks to those who have contributed to this issue. I hope you will all enjoy reading it.

Tina Offler

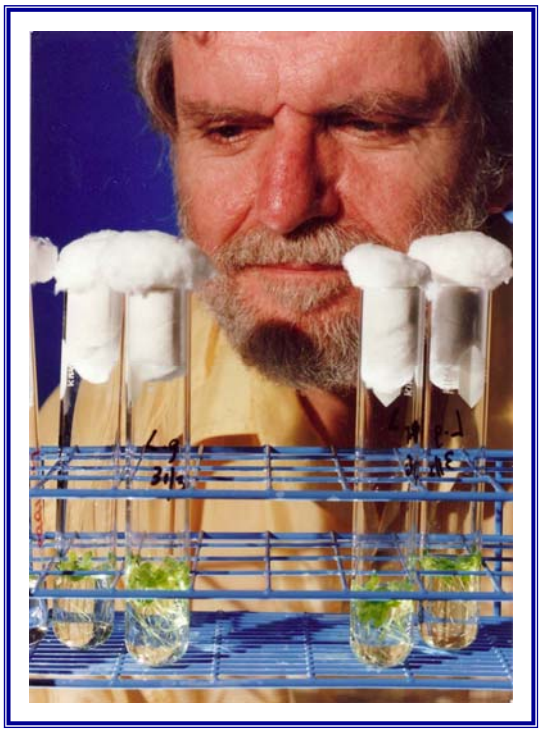
An apology from the editor:

In the last issue (Phytogen v 12, number 3) I incorrectly formatted a section of the report on the International Workshop on Plant Membrane Biology, Adelaide prepared by Steve Tyerman. The omission of a number of lines has been rectified in the pdf on the ASPS Website. Sorry Steve.

Tina

A Tribute to Andy Netting

Andy Netting: Passed away on 13 February 2011, Sydney, aged 69.



Andy Netting was active in the Society and a current member when he suffered cancer and died this year, still writing scientific notes and papers. He was a strong supporter of ASPP/ASPS and a regular participant at ComBios. For some years he helped Helen Irving as editor of Phytogen.

Andy's early work in the 1970s was on plant lipids and waxes, with Penny and Diter von Wettstein at the Carlsberg laboratories in Copenhagen. Back in Sydney, he worked in Barry Milborrow's lab at the University of New South Wales on fatty acid analysis and HPLC separation theory. Over the last 25 years he turned to the metabolism of abscisic acid and its precursors, which was a long-running theme involving trips to Canberra and Copenhagen. He supervised PhD students at the University of NSW, Robert Willows, Pat Duffield and Martin Windsor, and a host of short term intern students from Denmark. He had several projects over this time, but the long-standing objective was to identify the immediate precursors to ABA involved in long-distance signalling, and to establish how they were regulated, especially by pH. Andy made original discoveries and developed novel theories on the nature of root-shoot signalling. He called the putative ABA precursors "ABA-adduct", and made great efforts to characterise and understand what the ABA-Adduct is. He found out much about this Adduct but like a lot of things in science there is always more to learn.

He spent his retirement travelling to Antarctica and also did a retro trip to the Grose Valley and produced a picture story "In the steps of Robert Brown". His two trips to the Antarctic Peninsula are recorded in a wonderfully illustrated blog: www.travelblog.org/Bloggers/AndrewGeorgeNetting . But over the last 10 years or so he also went to Lancaster to visit Bill Davies and Ian Dodd for several months, and last year to Michigan to work in Jerry Cohen's laboratory. His time in Michigan was spent learning new methods and he wrote copious notes about how to use these to get the structure of the Adduct. The last few weeks before he died Robert Willows and I collected his hand written notes together with some draft manuscripts to distribute to his Lancaster and Michigan collaborators so they can carry on or complete this work.

Rana Munns and Robert Willows

State of Affairs -- Tasmania

Research in the School of Agricultural Science

Professor Sergey Shabala (Stress Physiology Research Group Leader)

The last two years were highly successful for the group. I have managed to secure some substantial funding for the lab by obtaining two ARC Discovery, one ARC Linkage and one GRDC grants totalling ca $1.7M. Also, University of Tasmania has contributed close to $200,000 for the renovation of the new laboratory to host the group, and we have celebrated our move in early 2011. There are currently three post-doctoral research fellows and ten PhD students in the lab. The current research focus is on mechanisms of salinity and oxidative stress perception and signalling.

One of my Discovery projects deals with the role of ***membrane transporters in oxidative stress signalling and tolerance in plants.*** Reactive oxygen species (ROS) are ubiquitous second messengers in plants. At the same time, stress-induced elevation in ROS production is detrimental to crop performance. Thus, the ability of a plant to maintain the fine balance between ROS production and scavenging is critical to growth, development and adaptation. Specific physiological mechanisms controlling this balance remain obscure. This project will reveal the identity of key membrane transporters mediating ROS signalling and tolerance in plants, and link them with plant adaptive responses to salinity and drought. The results obtained will be used by plant breeders to improve plant stress tolerance and minimise detrimental effects of salinity and drought on crop production. The project aims to (i) reveal the stress specificity of ROS production in plants under drought and salinity; (ii) quantify the effects of various ROS species (H_2O_2 and $^{\bullet}OH^-$) on activity of the key membrane transporters potentially contributing to cytosolic K^+, Na^+, and Ca^{2+} homeostasis; and (iii) to reveal the molecular identity of Ca^{2+} efflux systems contributing to cytosolic Ca^{2+} homeostasis, and their role in ROS stress signalling and adaptation.

Understanding ***mechanisms of Na^+ uptake, transport and compartmentation between various plant tissues*** and organelles is paramount to overcoming salinity problems and breeding plants for salinity tolerance. Until now, the progress in this area was significantly handicapped by the lack of appropriate techniques allowing *in planta* measurements of rapid Na^+ flux kinetics across plant membranes. The very poor selectivity of all existing Na^+ liquid ion exchangers prevents the application of Na^+ sensing microelectrodes in salinity research. Another **Discovery** project is aimed to resolve this issue. Two major pathways are currently being explored: (1) by using a lab-on-a-chip and (2) by designing the new (more selective) Na^+ ionophore. The project utilises most recent advances in microtechnology and separation science and is aimed to produce a highly selective system for Na^+ measurements with high spatial and temporal resolution, which then can be applied to address a range of fundamental physiological questions related to mechanisms of salt tolerance in plants.

Large volumes of saline wastewater are generated by industry and municipal water treatment processes every year. To be used for conventional crop irrigation, this water needs to be desalinised,

at a very high cost. An economically viable alternative is to use (naturally salt tolerant) halophytes as an alternative cash crop species. My current **ARC Linkage** project entitled "***Halophytes for high saline agriculture***" is aimed to investigate the suitability and optimise the performance of selected halophytes for a variety of soil types and environmental conditions. The project will also provide deep insights into the cellular and ionic mechanisms underlying salinity tolerance in halophytes. Specifically, we will (i) reveal identity of Na^+ transport systems in various halophyte families; (ii) understand the role of specific K^+ transport systems contributing to maintenance of K^+ homeostasis in halophyte roots and leaves; (iii) characterise functional properties and gating modes of vacuolar channels mediating Na^+ and K^+ transport across the tonoplast in different halophyte families; and (iv) reveal mechanisms regulating Na^+ and K^+ transport in salt gland cells.

In 2010 we have received $500K funding from **GRDC** for a project aimed to ***quantify the relative contribution of several key physiological mechanisms conferring plant salinity tolerance***. Over the past decades, breeding for salinity tolerance both in Australia and worldwide was essentially empirically-driven and relied mainly on plant phenotyping under field or glasshouse conditions. However, given the complexity and plethora of physiological and genetics mechanisms conferring salinity tolerance in plants, the practical outcomes of such approaches are disappointingly low. It has become evident that there is an urgent need to quantify a specific contribution of each of these diverse (and often controversial) mechanisms towards salinity tolerance. This project is designed to fulfil this aim and provide the first comprehensive assessment of the relative contributions of several key physiological mechanisms contributing towards plant salinity tolerance, namely sodium exclusion from uptake, potassium retention, vacuolar sequestration of sodium, control of xylem loading, and osmotic tolerance. A large number of barley and wheat genotypes will be ranked for each of the above traits and then recommended to breeders as "tolerance gene donors" allowing the "pyramiding" of beneficial physiological traits in order to create truly salt tolerant genotypes. This project involves TIAR barley breeders (A/Prof Meixue Zhou) and is complementary to the phenotyping work conducted at ACPFG by Prof Tester's group.

A large cohort of new PhD students was taken on-board over the last two years (see below):

Will Percey	*Sodium transport and compartmentation in cereal crops under saline conditions*
Jingyi Zhang	*Age- and tissue-specific aspects of salinity tolerance in barley*
Alex Mackay	*Mechanisms for salinity tolerance in halophytes*
Adam Pirie	*The potential of Carpobrotus rossii plant extracts as a source of highly concentrated antioxidant & novel flavonoid compounds*
Suresh Panta	*Halophytes for high saline agriculture*
Naina Marks	*Understanding apoptosis and programmed cell death in plants*
Maheswari Jayakannan	*The role of salicylic acid in inducing salinity tolerance in plants*
Min Zhu	*Physiological traits conferring osmotic adjustment in plants and their contribution to differential salinity tolerance in wheat and barley*
Avril Lam	*Quantifying the relative contribution of cytosolic sodium exclusion and potassium retention to salinity tolerance in barley and wheat*
Tamilarasan Thangavel	*Broad spectrum resistance to tuber invading diseases of potato*

The group maintains very strong international collaboration. Just over the last two years we have hosted 19 visitors from 13 countries, typically between 2 and 6 months each. Highlights on some of these collaborative projects, as well as a brief synopsis of the key personnel and some visitors in my laboratory, are given below.

Dr Tracey Cuin (post-doctoral Research Fellow between 2004 and 2010)

I moved to the University of Tasmania in 2004, and spent six very happy and highly productive years working in Sergey's group. My interest is in ion transport mechanisms in plants and the role that such transporters have in plant processes. In Hobart, the focus of my work was the role of K^+ homeostasis in salinity tolerance. Using the MIFE system, I investigated the massive efflux of cellular K^+ that occurs during abiotic stress. I explored the significance of this loss to the ability of a plant to withstand salinity. I deciphered a role for compatible solutes in reducing this loss by protecting membrane ion transporters from damage by ROS, so increasing salinity tolerance.

I attempted to elucidate the ion transport systems that play a role in K^+ loss, acquisition and retention under adverse conditions and provided evidence that linked salinity, ROS production and cellular K^+ loss via the K^+ efflux channel GORK1 to programmed cell death. This work has highlighted the critical role that K^+ plays in salinity tolerance, providing targets and approaches for plant breeders in their attempts to produce more salinity tolerant crop varieties. I also looked at Na^+ exclusion in wheat. I found evidence for a SOS1-like Na^+/H^+ exchanger at the plasma membrane alongside a NHX1-like exchanger at the tonoplast; transporters that would contribute towards low cytosolic Na^+ levels and consequently, to salinity tolerance in this important crop species.

I moved to France in October 2010 on a Marie Curie International Reintegration Fellowship, a fellowship set-up to lure European scientists back to Europe. I joined the Electrical and Calcium Signalling Team of Dr. Jean-Baptiste Thibaud at BPMP-IBIP, Montpellier, embarking on research entitled: Disclosing the Molecular Bases of Electrical Signalling in Plants. Although electrical signals such as action potentials are the fastest ubiquitous signalling mechanism in plants, little is known about their underlying mechanisms. Their generation and transmission involves cellular fluxes of ions through channels, but the molecular identity and regulation of these channels is unclear. Using a range of electrophysiological techniques, I aim to discover, decipher and describe the ion channels involved in initiating and propagating electrical signals, with the final goal of producing a mathematical model of electrical signalling in plants. This work will provide valuable information about a signalling mechanism that could be vital in informing the whole plant of any external threat or environmental stress. Plus, it follows my primary interest of the role of ion channels in plant physiology.

Dr Jayakumar Bose (post-doctoral Research Fellow)

I was born in India in 1981. Being a lad from a farming family, opportunities to improve farms and farmers through innovation have always inspired me. My postgraduate (MSc in Soil Science and Agricultural Chemistry) research work in Rice rhizosphere chemistry and my work experience in the field of remote sensing and ground water recharge at the Tamil Nadu Agricultural University (Coimbatore, India) have strengthened my conviction to enter into the research environment where I can find intellectual stimulation. I was awarded the Endeavour International Postgraduate Research Scholarship to do my PhD in Agriculture at the University of Western Australia (Perth, Australia) and received my PhD in 2010.

My research interests are (i) nutrient acquisition and long-distance transport in plants, and (ii) plant signalling and adaptations to a wide range of environmental stresses (soil acidity, salinity, extreme

temperatures, water logging, drought, biotic stresses). The approaches and techniques I have used range from; root growth image analysis, photosynthetic efficiency, plasma membrane ion transport mechanisms using the non-invasive Microelectrode Ion Flux Estimation (MIFE) technique, intracellular ion imaging using Fluorescence Life Time Imaging (FLIM) technique, enzyme assays, chemical analysis of root exudates and aerial image interpretation for soil groups and groundwater. Since 2005, I have published nine research papers, four extension papers and given oral/poster presentation at seven conferences.

I came to the University of Tasmania in October 2010 to do a post-doc in Sergey's lab. Over the next two years I'll be working on a variety of projects including (i) studying the transport mechanisms of polyamines in pea roots, (ii) tissue specific (epidermal vs. stele) membrane potential regulation under salt stress in pea roots, (iii) developing and testing sodium-ion selective liquid ion exchangers (LIX), (iv) studying ion-transport across chloroplast membranes under anoxia and salinity stress conditions, (v) mechanisms of ion transport in halophyte salt bladders, and (vi) interactions between salinity and ROS in Arabidopsis mutants. It is my strong belief that these projects will make a significant contribution towards breeding salt tolerant crop species.

Dr Edgar Bonales (post-doctoral Research Fellow)

I was born in Colima, Mexico where I completed my PhD in Physiological Science under the supervision of Dr. Igor Pottosin and Dr. Oxana Dobrovinskaya. My thesis work was dealing with lipid rafts in Jurkat T Lymphocytes and their modulation of the function of potassium channels. This study was conducted using techniques such as patch clamp, cell cultures, spectrophotometry and immunofluorescence.

I then spent 18 months as a postdoctoral fellow in the Laboratory of Neurophysiology in the University of Guadalajara, Mexico, where I was working on the characterization of chloride ion currents and the immuno-detection of the proteins that evoke them, using patch clamp in combination with confocal and fluorescence microscopy in *Giardia lamblia.*

In January 2010 I started a five-month stay in Igor Pottosin's lab, where I had the opportunity to learn and perform the MIFE (Micro-electrode Ion Flux Estimation) technique in Jurkat T lymphocytes. Finally, in December 2010 I started a 18-month Postdoctoral appointment in Sergey's laboratory at the University of Tasmania. I will be working with the patch clamp technique in halophyte species such as *Chenopodium quinoa* and *Disphyma crassifolium* that have two different morphological strategies for handling salt stress. I will also be working in age-related effects on channel properties in barley root; in the characterization of the major ion channels present in quinoa root epidermis and in the electrophysiological properties of salt bladders in quinoa. All of these patch clamp experiments will be an integral part of other group's projects, providing a mechanistic explanation of observed phenomena and complementing other data obtained by MIFE and other up to date plant physiological techniques.

Dr Lana Shabala (post-doctoral Research Fellow)

I am a cell biologist with 12 years expertise and about 30 papers published in the area of membrane transport and intracellular signalling in living organisms. I've received my PhD in 2002 from UTas for pioneering the application of the microelectrode ion flux estimation (MIFE) technique in bacterial physiology. I have been working as a post-doc since then in a Food Safety Centre, School of Mathematics and Physics, and Menzies Research Institute (all at Univ. Tasmania) applying this

technique to study adaptive responses of bacterial, plant, and mammalian cells to a range of abiotic stresses. The range of organisms studied included food-born bacteria such as *Listeria* or *E. coli*, marine protist *Thraustochytrium*, T cell lymphocytes, neurons, cardiomyocytes, yeasts, as well as a wide range of plant systems. I have joined Sergey's group in February 2011 to work on a range of projects related to oxidative and salinity stress signalling in plants. While my primary focus will be on mechanisms of programmed cell death and its control by ROS and intracellular potassium levels, I will also supervise several PhD projects elucidating the causal relationship between oxidative and salinity stress tolerance, quantifying key traits conferring salinity stress tolerance in wheat and barley, and looking at the interaction between salinity and waterlogging. I am also involved in a few collaborative projects on other systems such as yeasts (with Univ. Bonn) and cardiomyocytes (with Univ. Colima).

Professor Igor Pottosin (Visiting Professorial Fellow)

From 1996 I am working at the University of Colima as a Professor of Physiology, since 1998 also accredited by SNI as a Federal Research Fellow. I am internationally recognized for the pioneering studies of the intracellular channels in plants. My current project, supported by the Mexican Research Council (CONACyT) is devoted to the role of polyamines in the control of plasma membrane ion transport under salt stress.

Polyamines are unique polycationic metabolites, which are known to control multiple cation channels in animal cells, some plant intracellular channels, and more recently, also several ones of plant cell membranes. The latter, however, turned out to be a much more complicated issue compared to that of channels in animal cells, as we found out that polyamine effects on plasma membrane ion channels in plants were indirect, thus, not easily reproducible on isolated membranes. This forced us to look up what happened in intact living tissues, by applying non-invasive MIFE technique and microelectrode impalements.

During my 4-months stay in Sergey´s lab supported by the Tasmanian University Visiting Fellowship, we were able to demonstrate that, in a species- and concentration-dependent manner, polyamines were able to provoke pronounced changes in the resting membrane potential (MP). Such changes will inevitably affect all electrogenic events and transport occurring at plasma membrane. It was very curious to see, that depending on its concentration within a physiological range, a natural tetra-amine spermine could evoke either negative or positive MP changes. Even more curiously, at higher achievable range of concentrations spermine provoked a sustained membrane depolarization. In contrast, the same plant, pea, which is considered to be salt-sensitive, although displaying a similar by magnitude initial depolarization while challenged by high external salinity, was able to restore the initial level of the MP within a few minutes! Increase of polyamine levels is a common component of stress (e.g. salt) responses in plants, but obviously polyamines exert differential effects on ion transport as compared to the salt itself, which may be due to the activation of additional cation channels and/or suppression of the ion pumps activity. Both alternatives are intriguing, because the classical view is that polyamines are normally the channel blockers and positive regulators of the pumps. We have shown also by a combination of MIFE and patch-clamp techniques that polyamines are able to potentiate the ionotropic effects of other stress molecules, in particular reactive oxygen species. This synergism implies that plant stress responses need to be investigated by a systemic approach, assuming the simultaneous and interactive effects of different key factors, rather than exploring the effects of a single isolated factor - an important lecture to be learned!

Dr Lars Wegner (Visiting Academic)

I am a "green" electrophysiologist mostly working on higher plants and plant cells. A focus of my work is on long-distance ion and water transport processes in the xylem of higher plants, e.g. under conditions of salinity and drought stress, and on long-distance signalling. I am also interested in electrophysiological techniques and their use in biotechnology; this includes monitoring of phosphate in sewage water by using phosphate electrodes, screening of membrane transport processes by combining electrical and optical techniques, as well as basic research into the molecular processes induced by the exposure of cells to strong electric fields used, e.g., for the introduction of DNA (electroporation).

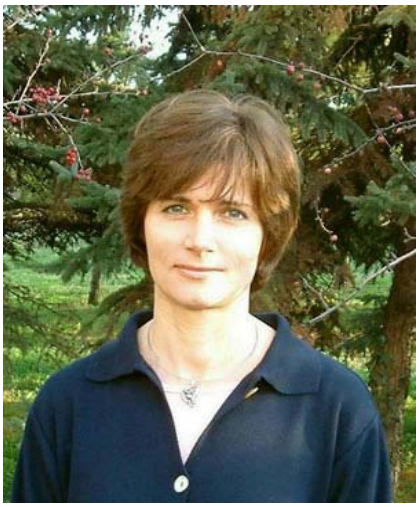
I have been collaborating with Sergey and his group since 2007, and this has been a very stimulating and fruitful cooperation indeed. Sergey has visited my lab twice in 2007 and 2008 for joint experiments. These visits resulted in three joint publications that were well received by the scientific community. In October 2010, I stayed at the UTAS as a guest scientist in order to make use of the MIFE technique to study changes in membrane permeability induced by nanosecond pulsed electric fields (nsPEFs). Protoplasts derived from maize roots were exposed to nsPEFs (field strength ranging between 4-40 kV/cm) for 500 ns using a Blumlein-line type pulse generator (Kolb et al. 2006). Subsequent K^+ efflux from the protoplasts induced by these pulses could be monitored with the MIFE technique, when tailor-made cuvettes were employed. Monitoring of net K^+ fluxes upon exposure to nsPEFs revealed that a train of 5 rectangular pulses induced a strong, transient efflux that decayed within minutes following an exponential time course. Frequently, we observed strong superimposed flux oscillations that persisted beyond the transient K^+ efflux component. A joint publication summarizing these observations is under preparation. Currently, there is much excitement about the use of nsPEFs in medicine and biotechnology, e.g. in skin cancer therapy (e.g. Nuccitelli et al. 2009). We have demonstrated that the MIFE technique is a powerful diagnostic tool with unrivalled temporal resolution for monitoring the effect of electric field pulses on the integrity of cellular membranes. Future work will focus, among other things, on the introduction of MIFE to medical diagnostics.

Dr Camilla Pandolfi (Visiting Academic)

I was born in Italy and am currently working at the ACT Team of the European Space Agency. After the graduation, I started my research career at the University of Florence, in Italy. I was so fascinated by my research experience and electrophysiology that I started my PhD in plant gravitational physiology and plant adaptation. During this period, I spent five months at the University of Tasmania where I had the opportunity to learn the MIFE technique, a non-invasive microelectrode technique for measurements of net ion fluxes from plant cells and tissues, as well as other advanced electrophysiological methods.

After this useful experience, I successfully applied for an Endeavour Research Award of the Australian Department of Education and I had the opportunity to come back to UTas for another 6 months term. The focus of my research during this second term was the mechanisms of plant acclimation to salt stress and, specifically, involvement of root and leaf ion transporters in this process. Two papers are currently being prepared as an outcome of this visit.

Dr Branka Živanović (Visiting Academic)



I am working at the Institute for Multidisciplinary Research (University of Belgrade, Serbia). My research interests are in biophysical and biochemical study of plant growth. I spent 1.5 years working as a post-doc in Sergey's lab in 2001-2003. We then collaborated on a range of projects such as the study of anti-oxidative enzyme activity in plant roots exposed to salt stress, as well as on mechanisms of the circadian clock in plants. Now I have come for another brief (2 months) visit to extend our research on mechanisms of the ionic basis for light driven leaf growth.

Using the MIFE technique, we are measuring kinetics of net ion fluxes from various leaf tissues (e.g. mesophyll and epidermis) with different growth history (basal-growing and tip-non-growing leaf zone). It appears that light-induced flux kinetics of all ions measured are strikingly different between the two leaf regions. Data implicates that light-induced ion flux changes are associated with both leaf growth and photosynthesis. Another set of measurements was performed to assess the spectral and dose dependence of light-induced ion flux responses from maize leaves and relate them to leaf growth. Growing and non-growing leaf tissue showed different patterns of light-induced K^+ flux kinetics. Moreover BL- and RL-induced K^+ fluxes measured from the epidermis are probably directly involved in turgor-driven leaf expansion growth, while these fluxes recorded from mesophyll tissue might have a charge-balancing role. Ca^{2+} fluxes were more affected by RL compared to BL which argues in favour of an important role for RL in Ca^{2+} signalling during leaf growth. We are currently focusing on a pharmacological study of components of the Ca^{2+} signal transduction pathway involved in leaf expansion growth.

Ana Rodrigo Moreno (visiting academic)

I am from Málaga, a warm place at the south of Spain. There I started working on electrophysiology at the last year of my Degree in Biology. Then I got a grant in Barcelona, where I am living now, doing the PhD. I decided to visit UTAs and learn the basics of the MIFE technique in Sergey's group during six months, from April to September 2010. The focus of our research was the tolerance to salinity and oxidative stress in halophyte species such as *Atriplex* and quinoa. We discovered that different root zones show remarkably different responses to applied stresses. We also measured, for the first time, ion flux kinetics from halophyte salt bladders. The research line continued after my return back to Barcelona, by mapping ROS production and doing viability studies in stressed roots.

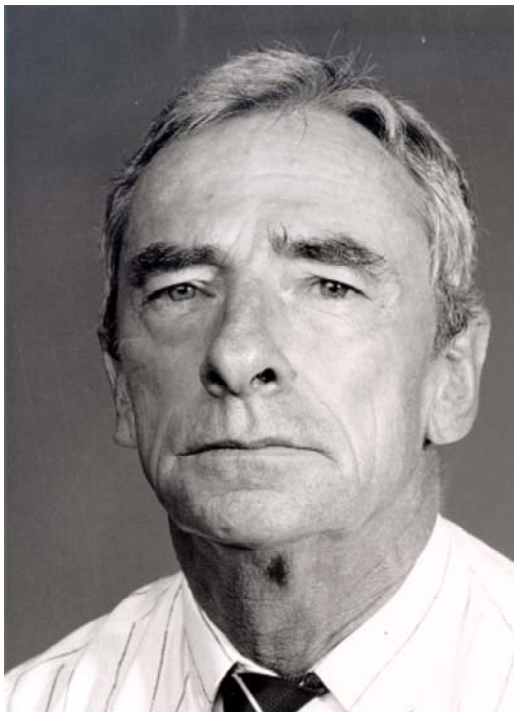
OUR SOCIETY
AN HISTORICAL PERSPECTIVE

SOME REMINISCENCES AND EUREKA MOMENTS

by

MD (Hal) Hatch

Retired - Division of Plant Industry, CSIRO, Canberra



When Tina asked whether I would consider writing something for Phytogen my first reaction was what could I possibly say that might be remotely interesting to your readers. Tina wasn't really helpful but remained insistent so here is a random selection of reminiscences.

In the early 1950's James Bonner authored a book entitled *Plant Biochemistry*. It catalogued the chemical structures of various plant constituents, described some catabolic enzymes and what evidence there was for a few metabolic processes such as glycolysis. There was much speculation about what might be and whether the very modest amount known about metabolism in the more popular animal and microorganism systems might also operate in plants. Lacking the medically driven impetus of these other systems, plants were also unpopular amongst biochemists because of their low levels of enzymes but high content of extraneous substances damaging in extracts to enzymes and organelles. In the preface to that book Bonner made the hardly prophetic comments that "—there is much work to be done in plant biochemistry" and "---our understanding of metabolic pathways in higher plants is lamentably fragmentary".

It was about this time in the early 1950's that I was introduced to undergraduate biochemistry. Even by my mid-1950's Honours year one could claim to know pretty well all there was to know about plant metabolism. And it was not uncommon to read a new issue of Plant Physiology from cover to cover.

By the time the third edition of Bonner's *Plant Biochemistry* appeared in the 1970's (now edited with J. Varner) it was not possible to fit all that was known in a thousand pages. There followed in 1981 the eight volume series *The Biochemistry of Plants* edited by P.K.Stumpf and E.E. Conn. By the late 1980's updates extended this series to 16 volumes. I guess you would now need 16 volumes to cover plant

molecular biology/genetics alone. The 60 years from 1950 has certainly been an interesting time for someone interested in plant biochemistry!

During my Post-doc stay at the University of California, Davis in1959-61 an English colleague use to cheer us up by quoting his Cambridge PhD supervisor. His advice was "Inure yourself to drudgery and disappointment in scientific research". I am sure you know what he was getting at. Testing ideas that came to nothing or experiments that came to grief for no obvious reason. And, of course, there are those mundane but necessary data collecting exercises, like surveys or determining the kinetic characteristics of an enzyme.

But then occasionally, following an insightful idea or by pure chance, there comes a seminal discovery which compensates for all the drudgery; you know, those 'eureka!' moments that change the course of events. I thought it might be of some interest to briefly mention some of these discoveries that came up during our initial studies on C_4 photosynthesis. In particular, those findings in the first 8-9 years that allowed us to formulate schemes for the three options for C_4 photosynthesis. This is not the place for a lot of references. For those interested, these can be found in two summarizing publications (MD Hatch (1999) In: *C4 Plant Biology*, RF Sage and RK Monson Editors, pp.17-46, Academic Press, and also MD Hatch(1992) Photosynthesis Research, 33; 1-14).

In our first paper (published in 1966 with Roger Slack), we confirmed the observations of Hawaiian workers that $^{14}CO_2$ was initially fixed predominantly into C_4 acids rather than 3-phosphoglycerate (3-PGA) during photosynthesis by sugarcane leaves. Critical additional information was that a small pool of oxaloacetate was labelled as well as malate and aspartate and that these acids were initially labelled in the C-4 carboxyl. Also, a pulse-chase experiment showed that this C-4 carbon was transferred to the C-1 of 3-PGA and then to the other intermediates of the Photosynthetic Carbon Reduction (PCR) cycle (Calvin cycle). These observations allowed us to construct a simple working model for the path of carbon in sugarcane.

In the following paper we surveyed a variety of plants for the pattern of labelling from $^{14}CO_2$. Most showed the conventional 3-PGA dominated labelling typical of the PCR cycle (C_3 plants). The exciting observation was that a dozen or so grass species showed the C_4 acid-dominated labelling first seen in sugarcane. Included amongst these were maize and sorghum. Significantly, the one sedge we looked at also showed this pattern. So this novel labelling pattern occurred widely in the grass family and in at least one species of the sedge family.

The model developed on the basis of our initial studies allowed us to make a number of important predictions. One was that phosoenolpyruvate (PEP) carboxylase may be responsible for the primary assimilation of CO_2. This led to the discovery that species showing this C_4 acid labelling contained more than 20 times the PEP carboxylase activity found in C_3 plants.

It followed that these C_4-type plants would probably needed to rapidly convert pyruvate to PEP. We found that the activity of the only known enzyme capable of this conversion, pyruvate kinase, was far too low in the PEP direction. We went on to discover an entirely new enzyme that catalysed the following reaction:

$$\text{Pyruvate} + \text{ATP} + \text{Pi} \leftrightarrow \text{PEP} + \text{AMP} + \text{PPi}$$

We could not detect this activity in C_3 plants.

This enzyme (pyruvate, Pi dikinase) turned out to have an almost unbelievable number of unique features both in respect to its mechanism and its regulation. But that is another story. On the way we showed that these C_4-type plants had activities of adenylate kinase and pyrophosphatase at least 40 times those detected in C_3 plants. These enzymes had a critical role in recycling the AMP and PPi produced in the above reaction.

With oxaloacetate (OAA) as the primary product, it followed that these C_4 plants should have a high capacity to reduce OAA to malate. Since photosynthetically-generated NADPH was the most likely reductant, we naively set about looking for a malate dehydrogenase that could use NADPH.

It turned out that the C_4 plants we were studying at that time all contained high activities of such an NADP-specific dehydrogenase. This was an entirely novel activity but, to our surprise, it was also detected in C_3 leaves but at much lower levels.

To complete the cycle we then needed an enzyme that would decarboxylate malate at a sufficient rate. During an earlier search for an enzyme to synthesise C_4 acids we noted that sugarcane and some related C_4 plants had very high activities of an NADP-specific malic enzyme. Although this activity was more than 20 times the activity seen in C_3 plants it was not sufficient to account for malate synthesis. However, its activity in the malate decarboxylating direction was more than adequate.

So, for the C_4 plants like sugarcane, maize and sorghum at least it all fell into place, with NADP-malic enzyme decarboxylating malate and the CO_2 being refixed by the PCR cycle. Meanwhile, the developing view that the C_4 process was a CO_2-concentrating mechanism was supported by our demonstration of a large pool of CO_2 developing in C_4 plants during steady-state photosynthesis.

But there was a problem. Several species showing the early labelling of C_4 acids from $^{14}CO_2$ did not show the unusual pattern of enzyme activities seen in sugarcane and related species. They lacked the high levels of NADP malate dehydrogenase and NADP malic enzyme. But, notably, we found that they contained exceptionally high activities of aspartate aminotransferase and alanine aminotransferase (see Table). So how were C_4 acids decarboxylated in these species?

ACTIVITY RANGES OF SOME KEY ENZYMES IN RELATION TO C_4 SUB-TYPE
(Relative to average for C_3 species given the arbitrary value of unity)

Enzyme	C_4 Type			
	NADP-ME	PCK	NAD-ME	C_3 plants
NADP malic enzyme	20-25	0.5	1	1
PEP carboxykinase	1	50-70	1	1
NAD malic enzyme	<1	<1	21-50	1
NADP malate dehydrogenase	10-16	1-3	1-2	1
Aspartate aminotransferase	2-3	20-30	13-24	1
Alanine aminotransferase	1-2	19-21	13-33	1

Soon after, this problem was partly resolved with a report from Clanton Black's laboratory that some of these species lacking NADP malic enzyme showed very high activities of PEP carboxykinase. So we now had a PEP carboxykinase (PCK)- type option for C_4 photosynthesis. The problem was that there remained a residue of species lacking **both** NADP malic enzyme and PEP carboxykinase (see Table). Could there be three options for C_4 photosynthesis?

This question was resolved when I happened to sit in a presentation of a paper at the annual ASPP (now ASPS) meeting. There was mention in that paper of mitochondria from various non-leaf plant tissues containing significant activities of an NAD-specific malic enzyme. A search for such an activity in those species lacking the other decarboxylases soon revealed high activities of this enzyme. Activities were 20 to 50 times those observed in the other groups of C_4 species or in the leaves of C_3 species (see Table). So, mitochondria were directly implicated in a photosynthetic pathway for the first time.

Subsequent studies in the early 1970's, both in our laboratory and in Gerry Edwards' lab, showed that a wide selection of these C_4 species all fell into one or the other of these three types now identified,

NADP-ME-type, PCK-type, or NAD-ME-type (see Table). Combined with further radiotracer kinetic studies and information on the inter- and intracellular location of enzymes, it was possible by then to construct reasonably detailed schemes for photosynthesis in these three types of C_4 plants.

However, it was several years before the mechanism in PCK-type species was more precisely resolved. And, of course, there followed over the next decades all kinds of critical studies on the regulation of enzymes, transport of metabolites and a wide range of studies on the physiological aspects of C_4 photosynthesis. During this time more than 10,000 C_4 species were identified in 2 monocot and 14 dicot families.

Until 1970 these studies were conducted in laboratory of CSR Co. Ltd located in Brisbane and after that in Canberra at the CSIRO Division of Pant Industry. Colleagues in these pioneering studies of the first 9 years or so were Roger Slack, supported by PhD students from the University of Queensland, Hilary Johnson (Warren) and John Andrews and later at CSIRO, Shaio-lim Mau and a Post-doc, Tak Kagawa.

A LIFE OF SURPRISES IN PLANT PHYSIOLOGY.

By

R. N. (Bob) Robertson

An invited essay first published in the

1996 ASPP Membership Directory.

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When our Hon. Secretary, Paul Kriedemann, following up a suggestion of our President-elect, Joe Wiskich, asked me whether I would like to contribute some recollections of the early days of Australian plant physiology, my first reaction was to decline because I wondered whether I had enough of interest. What would an old bloke of nearly eighty-three say to those who are in full swing advancing the plant physiological, indeed the whole biological world? Then I thought that an opportunity to record my thanks to those who helped me to have such an interesting and rewarding life was reason enough. Some of the history of Australian plant physiology is reflected in my experiences.

Of course, I cannot thank everyone so I am recording some of my surprises. Some of these came through my teachers, some through my students and some through *Nature,* like the recent ones (Abrahams *et al.* 1994 ; Cross 1994) which described the fascinating structure of the membrane-bound F_0F_1ATPase or ATP synthase which appears to be a molecular electric motor with a positive current (does it really rotate at about 200 times a second?).

Knobs and ATP synthase

My thanks here go back to a surprise of about forty years ago when Marjorie Wilkins and I, both of CSIRO Food Preservation and Transport, were collaborating with John Farrant, CSIRO Industrial Chemistry. With his electron microscope, we had seen what we then called 'knobs' protruding from the inner surfaces of plant mitochondria (Farrant *et al.* 1956). A bigger surprise is that the molecular structure of such bodies is now so well understood, thanks to the experiments of many workers around the world, that we have a fairly full understanding of their function at a molecular level. As a retired plant physiologist, I have become specially interested in the membrane-bound F_0F_1ATPase of *E. coli,* also an ATP synthase. Here my thanks are due to the Frank Gibson (1991) and Graham Cox Group in JCSMR, ANU, whose work led to my being able to understand the hypothesis of its structure and function and also to the very elegant working model made by Istvan (Steve) Zaveczky of the RSBS workshop, ANU (Figure 1). I find it fascinating that the 'knobs' attached to a membrane-bound portion, are thought to be motors, which, rotating about 200 times per second, pump protons across the membrane. Proton pumping in one direction results in formation of ATP from ADP and phosphate; in the other direction, ATP is hydrolysed to ADP, liberating hydrogen ions, to which the movement of other ions is linked. Thanks to the techniques of molecular biology and to many workers, much of the structure is now understood and is explained in the *Nature* papers. The 'knobs' in mitochondrial and chloroplast membranes are similar in structure, but as they are hell-bent on making ATP, they do not seem to rotate! (Abrahams *et al.* 1994).

Figure 1. Scale model of ATPase: suggestion, Graeme Cox; craftmanship, Istvan Zaveczky (left); presented to Bob Robertson (right). Wooden stand = cell exterior; clear plastic cylinder = bilayer membrane (c. 5 nm thick) with F_0, hydrophobic portion of 'knob', visible inside; hydrophilic portion of 'knob' $F_,$ with six massive units in cell. Protons entering F_0 cause rotation and produce ATP from ADP and inorganic phosphate in knob. Photograph, Jeff Wilson RSBS ANU.

Becoming a plant physiologist.

When you think that in my student days, some senior biologists were saying that proteins were such complicated structures that it was improbable that we should ever be able to elucidate their structure, it really is a surprise that I have lived long enough to appreciate and get enjoyment from the intricate structure and complex functions of an ATPase/ATP synthase. There have been many surprises and many people to thank, including my parents who, recognising my intense interest in science from about age ten, encouraged it.

One of my early surprises was as a student at Sydney University where I fully intended to go on with chemistry, but found the way organic chemistry was taught, depending on masses of memory work, exceedingly dull. Practical physical chemistry, which I enjoyed, gave me a surprise and taught me a valuable lesson. It was supervised by Tom Iredale whom we respected because it was said that he was brilliant at research but never did any. When asked about this by the Professor, it was said that he replied "You can't do research with a test tube and a piss-pot". When it was my turn to do the experiment on the depression of the freezing point, I did not get the 'right' answer. I repeated the experiment a number of times, getting further and further behind because I should have moved on to the next experiment. As we knew what solute and solvent we were using, we knew what the result should be and I found my fellow students had just written it in and gone on to the next experiment. They thought I was stupid. Exasperated, I consulted Iredale who did the experiment himself: he got the same result as I did! The solvent which we were using was not pure. My results needed no 'fixing' of the type my classmates had used but our reagents did! Iredale and I became friends.

Botany, which had been my second subject became increasingly attractive, and I finished up taking the honours degree in Botany. There I was fortunate to be under the influence of T. G. B. Osborn, subsequently Professor of Botany in Oxford, and an impressive personality. At the end of my third year in Botany and Chemistry I went with my parents for a holiday in Adelaide. There I was lucky because a friend of my father's introduced me to the Waite Agricultural Institute staff who were good in showing me the different research activities. The most striking personality as far as I was concerned was the plant physiologist, A.H.K. Petrie, a Sydney graduate. When I told him I was about to start an honours year in Sydney, in his forthright manner he said something like "You must do plant physiology; all other branches of Botany are impossible in Sydney!" Incidentally, it was at the Waite that I began friendships with Les Ballard and Bob Willams, two of the very few graduate students in plant physiology in Australia then.

I started plant physiology although there was no plant physiologist in Sydney and my decision to try to become one at that time was more foolhardy than sensible, though in retrospect I am glad I did. I had tried to examine the $O_2/C0_2$ concentrations in intercellular spaces of leaves and relate that to the degree of stomatal aperture. It was a surprise that this earned me a Scholarship of the Royal Exhibition of 1851 and enabled me to go to Cambridge University for post-graduate training.

A most welcome surprise was the plant physiology lab in the Botany School, where my supervisor for a PhD was G.E. Briggs. I was lucky because Les Ballard was there before me and was able to warn me about what to expect viz. conversations almost every day with Briggs, so stimulating with such a lively critical mind, treating us as equals in areas of physiology in which we were trying to run to keep up with his sprinting. He did not do experiments but led us into thinking out the next one, sometimes arriving with a new idea spouted out before he was half way across the large lab in which four of us worked, sometimes forgetting that the experiment we agreed upon yesterday would take at least a week before asking "have you done that experiment?" The daily conversations to which we were expected to contribute proved to be the proper way to education, i.e. we learned to think for ourselves.

Return to Australia; teaching and learning

My next important surprise came with my association with Eric Ashby, a plant physiologist and a most outstanding man: after a remarkable career, he became Master of Clare College, Cambridge and a Member of the House of Lords. At the time I speak about, he had gone from England, where we had met, to be Professor of Botany in Sydney University, and to my great pleasure, he offered me a job there. Mary and I returned to Australia in January, 1939. Eric discussed my responsibilities with me. After explaining my share in the first year practical classes, he asked me to take charge of the second and third year practical plant physiology. This was great, because Ashby was an especially brilliant lecturer, and bright people who had taken Botany I as a fill-in subject, were coming on to Botany II because of his lectures. In those days too, Agriculture II and Forestry II went on with us.

You can imagine my pleasure when he said that the practicals were our opportunity to lead young men and women into thinking for themselves and to do that in the way that a research scientist carries out his or her investigation. Our students were encouraged to define a clear aim, preferably in the form of an hypothesis to be tested, deciding on what quantitative results would be necessary and, with the apparatus to hand; getting on with it; writing up discussions of whether their results supported or negated the hypothesis and why. In other words, behaving like scientists.

I especially enjoyed the transition when individuals changed from wanting to ask me everything to where they would ask only if really stuck; so often I could say “you ought to be able to think that out for yourself” or “if I gave you this hint can you think it out?” The look of satisfaction as they did so was the reward of teaching in the “think for yourself manner”. Soon people were not coming back to ask me. I just happen to remember that our ASPP President-elect was one of those brighter ones, so I hardly knew he was there until he brought his book for final checking or had something he wanted to disagree with. I repeat the quotation from *The King and I* with which I began one of my books:

“It's a very ancient saying but a true and honest thought,
That if you become a teacher, by your pupils you'll be taught”.

One pleasant surprise during my time in Sydney, was finding a few outstanding scientists in hospital laboratories in Sydney, especially Rudi Lemberg a biochemist at Royal North Shore; and the physiologists, Jack Eccles and Bernard Katz in the Molteno Institute, Sydney Hospital, both to become Nobel Prize winners. We had a weekly discussion group including Jim Vincent, Adrien Albert and others which helped in broadening my biological horizons. This group did not continue during the worst phases of the War. Bernard Katz went off to become a RAAF Pilot-Officer (Radar) and was sent close enough to the action to be picking up the Japanese survivors of the battle of the Coral Sea. The discussion group resumed at the end of the War and as far as I was concerned continued until about 1961 when I left Sydney. I owe much to the stimulating discussion with the variety of men and women who joined that group from time to time. I don't know whether the atmosphere in scientific circles has changed much with the present emphasis on 'output' but we certainly had time to enjoy broadening our knowledge and understanding.

Heat and wheat

From August 1939, when Australia was among the nations at war with Germany, the lives of all of us were very much affected. Most teachers in universities were classified into reserved occupations and not allowed to enlist in the services. The atmosphere was strongly conducive to wanting to do something additional that would be useful in the war effort. What could a plant physiologist do?

Due to the diversion of ships to other more urgent things, export of wheat almost ceased and every single silo in Australia was full. Departments of Agriculture around Australia were experimenting with the simple structures, now commonly seen in wheat growing areas at harvest time, but then quite new, of spreading huge piles of wheat on waterproof sheeting on the ground and covering it with more waterproof sheeting. These hills of dry wheat seemed to keep satisfactorily except that the temperature went up to about 40° C and remained there. All around the edge of the wheat stack to a depth of about 50 cm., was a thick infestation of grain-eating insects. At the time, working with CSIR Division of Entomology, Joan Milthorpe and I were asked to find out whether the respiration of the dry wheat caused the rise in temperature which attracted the insects and led to the damage. We measured the respiration of the dry grain which, not surprisingly, was only marginally different from zero and concluded that it could not be responsible for the temperature rise. However, we went on to show that only one insect per hundred grains would easily produce enough heat to take the temperature up and as it rose, the insects moved out to the edges, doing little damage on the way, but there eating the wheat and providing an edge of heat production which kept the whole mass hot (1948 a, b). A surprise for plant physiologists to be mixed up with bulk wheat but the problem turned out to be one for entomologists !

Fruit storage; CSIR and the Plant Physiology Unit

The same shortage of shipping which affected wheat's transport meant that fresh fruit could not be exported; storage became important This brought me into contact with the CSIR Division of Food Preservation and Transport as it was then called, and began my long and happy association with that Organisation and with Jim Vickery, the Chief of that Division. After the War when I changed my job from Sydney University to that Division of CSIR (later CSIRO), Frank Mercer was in charge of the Botany School plant physiology and he and I decided that, with the limited resources and staff numbers in Sydney plant physiology, it was foolish to try to run two different research establishments. The University and CSIRO were persuaded to let us combine as a Plant Physiology Unit, which flourished for a number of years, later being moved to Macquarie University. Among those associated with the Unit in my time were John and Donella Turner, Hugh McKee, Alex Hope, Lydia Nesztel, Gerda Urbach, Jaros Smydzuk, Jeanette Gregory, Ken Glasziou, Hal Hatch, Shigeru Honda (USA), Judith Pearson, Marjorie Wilkins, Kingsley Rowan (Melbourne), David Weeks (Melbourne) and Harlan Pratt (U. Cal., Davis). I ceased to be associated with the Unit when I became a member of the CSIRO Executive in 1959.

Joys of research; a bright idea on a bright day.

During the War, I managed to continue some work on the relation of plant respiration to active transport, i.e. cellular absorption of ions against a concentration gradient. I was chewing over my results on the afternoon of a perfect day in the Easter holidays 1945 when I had a pleasant surprise. It was one of those occasions when one can remember the actual moment of having the good idea. I was gazing out the window of our house which overlooked one of Sydney's surf beaches, with bright sunlight on golden sand, blue water with white breakers. My experimental results with tissue from carrot roots had shown that the process of active transport was dependent on a cytochrome-mediated respiration. Since cytochrome was known to change from its neutral ferrous form to a ferric form, it might then have a positive charge and could pick up a negative ion. If this complex could then move through a barrier impermeable to free ions, the anion liberated when the cytochrome was reduced by the next electron would be trapped behind the barrier. If a cytochrome could take an electron in one directon, it might take an anion in the opposite. I suddenly realised that if the mechanism depended on cytochrome as an anion carrier, then there should be a stoichiometric relation between the amount of oxygen absorbed and the amount of salt accumulated. To my delight, results to hand on that lovely

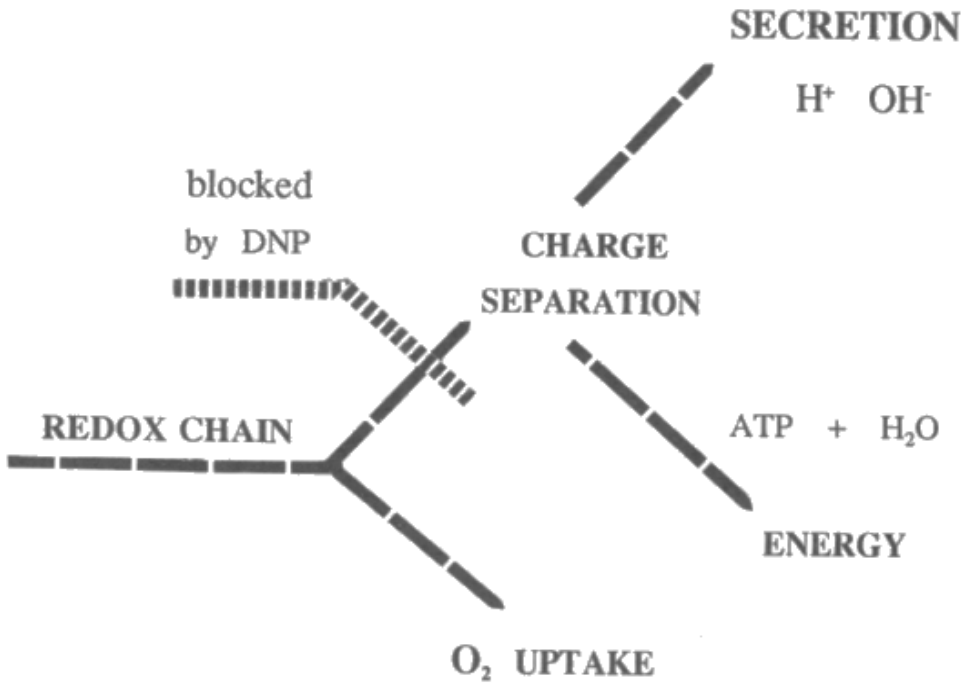
afternoon showed that the two rates were of the same order of magnitude. That afternoon became even lovelier !

During that time I was in correspondence with Professor Henrik Lundegardh, who, apart from members of his laboratory in the University of Lund in Sweden, was about the only person in the world who believed that there was a special cytochrome component of the respiration (he called it the anion respiration) associated with the active "transport of ions". His data on the respiration of wheat roots associated with ion absorption gave us the confidence to go ahead with experiments to look for the exact quantitative relationship which would be necessary to support our hypothesis. This work, with Marjorie Wilkins, gave us the quantitatively consistent results, a great deal of satisfaction, and two papers (Robertson and Wilkins 1947, 1948). To have an hypothesis which makes a quantitative prediction and then to confirm the expected figures by experiment is one of the most pleasing experiences of research, especially if a new paradigm follows.

Carrot roots and frogs' stomachs.

One of my surprises of this time was Crane and Davies's finding (1948 a, b) that the amount of g equiv HCl secreted into the lumen of frog's stomach was about four times the stimulated amount of g mol of oxygen, and was accompanied by an equivalent amount of $HC0_3^-$ passing to the exterior of the stomach This was consistent with the idea that the protons were derived by their separation from the electrons which form hydroxyls at the cytochrome system and then get converted to bicarbonate by carbonic anhydrase, active in the gastric mucosa. Furthermore it was becoming apparent that both these processes in carrot and in gastric mucosa were related in some way to the formation of ATP from ADP and inorganic phosphate. Following a thorough review of the published evidence and the confused ideas about the relations of H^+ secretion, ion transport and phosphorylation, I was able to suggest in 1960 (and I think I was the first to do so) that the separation of positive and negative charges was probably the fundamental process in electron transport and that it might *precede both the movement of ions and the phosphorylation of ADP to ATP;*

thus:-



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I did not have enough insight to realise that the reactions connecting the H^+ and O^- were reversible but Peter Mitchell did, and soon after this he gave me one of the pleasant surprises of the times when he published his chemiosmotic hypothesis in *Nature* (Mitchell, 1961). This work, which was to lead to our lasting friendship, despite some differences of interpretation at times, resulted in his very well deserved Nobel Prize in 1978. The other surprise of those times was how long it took the biochemical community to accept the essential principles of his hypothesis, particularly as Williams (1961, 1963), independently reached a somewhat similar interpretation.

Proteins and DNA

For me, the greatest surprises of my lifetime were in the early fifties when, thanks largely to Fred Sanger, the work which has led to our understanding of protein structure, began. Then on top of that, and at about the same time, the improbable Watson and Crick deduction of the three dimensional structure of DNA and their immediately inferring its mechanism of replication. Sanger has said that this "brilliant accomplishment ranks as one of the most significant in the history of biology". I do not need to spell out the consequences of these 'surprises' to members of this Society where, according to the 1994 Directory of Members about 16% list 'molecular biology' as among their interests, and many others are increasingly dependent on the consequences of this knowledge. Protein structure, essential to our understanding of enzymes and carriers and other cellular organs, is dependent on our understanding of DNA with gene expression and all the theory and techniques of molecular biology. No wonder that Jim Peacock gave me a surprise a few years ago when I had not been watching what had been happening in his Division of CSIRO and he said that they did not appoint plant physiologists any more, they appointed molecular biologists; I soon found out why!

Friendships

I suppose that a 'surprise' in the sense of not anticipating it when I began on a science degree in 1930, is the number of friends that I have made in the 66 years since, not only nationally but also internationally. Many of the people involved have helped me and sometimes I have had the pleasure of being able to help them. The pleasing success of this Society has contributed greatly to my circle of friends. To all of those people and especially to my wife, Mary, my great gratitude.

The Future

My faith in the coming discoveries of plant scientists, so essential for the future feeding of our increasing population, for the conservation of land and marine communities and for the maintenance of this planet, is undiminished; my imagination insists that the unknown to be revealed will be as exciting as I have seen in my 66 adult years; my hope is that the results will be used more wisely!

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RN Robertson Travelling Fellowships

The objective of the Professor R.N. Robertson Fund is to encourage young Plant Scientists to participate in mechanistically (functionally) orientated research in a discipline that differs from their own. The Fund has been used to set up The RN Robertson Travelling Fellowship to recognise and celebrate the sustained contribution made by RN Robertson (Sir Bob) in nurturing plant scientists in Australia spanning across four decades from the 1950s.

The Travelling Fellowship is focused on support for graduate students and recent PhD graduates to undertake research aimed at elucidating plant function and should enhance the current research of the applicant by providing access to expertise and facilities outside of that currently available to them. The fellowship should be undertaken at another institution outside of the Australian state in which their current research institution resides. For overseas applicants, the proposed host research institution must be within Australia.

Report by:
Cara Griffith, 2010 Recipient

I was fortunate enough to be granted a R.N. Robertson travelling fellowship from the Australian Society of Plant Scientists for my visit to the University of Queensland (UQ) for two weeks in February 2010. I am a PhD student at Monash University studying the function of a dehydration responsive gene in the resurrection grass *Sporobolus stapfianus* and as part of my project I am to develop a method for the transformation and regeneration of *Sorghum bicolor*. Through a visit to Monash University by Prof. Ian Godwin from UQ, I was offered the opportunity to learn from his lab members Guoquan Liu, Rajnesh Sant, Norazlina Ahmed and Ed Gilding, the current method they use to produce transgenic Sorghum.

During my visit I was involved in every step of the process. I started off learning the basics, how to grow Sorghum optimally in the field and in the green house. From this I was shown how to induce embryogenic callus cultures from immature embryos and how to identify what embryos from developing seeds are suitable from field and greenhouse grown plants. Choosing the right time of embryo development for callus induction is perhaps the most important part of producing transgenic Sorghum as the health and growth of callus susceptible to transformation and regeneration is a difficult element to achieve. I was able to monitor the growth of callus, and first hand was able to see how to take care of proliferating callus so it is at optimal health prior to transformation. After this, I was involved in the transformation of Sorghum using particle bombardment and an *Agrobacterium* mediated method. Generally, the particle bombardment method is most commonly used at UQ and I was able to conduct transformations and learn what parameters are important for successful transformation of callus. Fortunately, I was also able to visualise the success of these transformations through the use of a GFP expression vector.

After callus transformation and selection of transformed callus I was able to regenerate whole plants from callus which will ultimately produce transgenic seed. Keeping the regenerating plantlets at optimal health is imperative at this time as Sorghum is sensitive to *in vitro* culture and I was able to learn how to best take care of the plantlets at this time. As well as gaining knowledge in the method of producing transgenic Sorghum at UQ I was also educated on the importance of the constructs used for foreign protein expression especially how different promoters can show different expression levels in Sorghum plants.

I cannot express how grateful I am to all staff and students who I met and who offered me guidance and knowledge during my visit and my supervisors at Monash University: Dr. Alan Neale, Prof. John Hamill and A/Prof. Ros Gleadow for allowing me to expand my knowledge in this area. Of course, I show great appreciation to the ASPS for awarding me the R.N. Robertson travelling fellowship.

Cara Griffiths

Conference Report

Meeting reports provided by members from around the country

International Conference on Plant Vascular Biology

PVB 2010

The Ohio State University, Columbus, Ohio, USA
July 24 -28

One hundred and eighty participants, including three Australian and two New Zealanders enjoyed a stimulating journey into and through the plant vascular highway.

The single-sessioned oral programme and associated posters on display throughout the five days eliminated the stress of making those difficult decisions of which session to attend and which posters to read. On the spot food and beverages also added to create a very relaxed atmosphere. The Co-Chairs Biao Ding (Ohio State University) and David Hannapel (Iowa State University) and their organizing committee had done a great job. Here are some snippets of the programme.

The conference commenced on Saturday afternoon following a welcome lunch. Bill Lucas started off the conference by expounding on the central role of the plant vascular system in holding the promise for sustainable food, fibre and energy requirements for the planet – big ideas and a big ask. A brain stimulator!!

The vascular system was then duly developed (Hiroo Fukuda with a transcriptome analysis of xylem formation) and control of vascular patterning and differentiation explored in apices (Rebecca Lamb) and leaves (Thomas Berleth; Makoto Shirakawa). Xylem featured with an analysis of signalling by cytokinin and auxin interaction exercising spatial control on proto- and metaxylem differentiation (Yka Helariutta). Aspects of the evolution and development of the vascular cambium provided considerable breadth (girth) (Andrew Groover) together with insights into the molecular regulation of secondary growth (Thomas Greb; Xia-Qiang He). The phloem did get a look in with Karl Oparka providing exciting images of phloem plasmodesmata made possible by using "super-resolution" imaging (3D-Structured Illumination Microscopy – 3D-SIM)) and field-emission scanning electron microscopy (FESEM).

Then to transport and distribution of water and nutrients within the vascular pipelines. Missy Holbrook's message was that "a major challenge to understanding xylem transport is to establish how air embolisms are repaired". Norbet Sauer presented some interesting findings on reminding us about inositol transporters in the phloem and their evolutionary conservation with animal equivalents and Michael Thorpe provided some elegant electrophysiology demonstrating that stopping flow in sieve elements in response to pathway chilling in legumes is consistent with florisome dispersal. Then the phloem got loaded with sucrose (Bob Turgeon) and amino acids (Mechthild Tegeder) with Rainer Hedrich exploring sucrose- and H^+-dependent gating of SUC 2 and at the other end of the pipeline unloaded in developing seeds via John Patrick's "Push-me, Pull-me System". David Braun presented his reverse genetic approach to determine biological functions of SUTs in maize to provide a deeper

understanding of the genes regulating sucrose entry into the phloem and Michael Grusak discussed microelement trafficking in plants.

Sessions on vascular trafficking and development uncovered a wealth of recent findings about phloem RNAs (Julia Kehr), long-distance trafficking of mRNA and miRNA regulating tuber induction and development in potato (David Hannapel; Paula Suarez-Lopez) and P-proteins in sieve elements (Sylvie Dinant). Then plasmodesmata got a turn starting with an "Omics" approach to defining the plasmodesmal proteome very ably presented by Andy Maule and followed by genetic regulation of plasmodesmatal structure and function (Pat Zambryski) and the role of callose synthase in fine tuning movement of signalling molecules through plasmodesmata (Jae-Yean Kim).

This brought us to lunch on Day 4. From lunch through to 9.30 pm the conference was entirely devoted to poster viewing time and then two poster sessions with presenters in attendance. The latter sessions were cleverly programmed either side of a very adequate dinner. One might have imagined a poor showing after dinner – not so and some participants were still there at 9.30 pm! (see photos) – a measure of the enthusiasm for the science on offer.

A couple of readily recognizable people in these images – Karl Oparka (bottom left) just listening in on the discussion ready to add his comments and John Patrick (top right) waiting patiently to be given an opportunity to get his message across.

On the last day attention turned to biotic plant interactions – plant defenses to invasion by phloem-feeding whitefly (Linda Walling); virus transport (Richard Nelson), long-distance chemical signalling and auto-regulation of nodulation in legumes (Peter Gressoff) and the fascinating *de novo* vascular development at nematode-induced feeding sites (Ulrich Hammes). A final session on "Integrative Plant Vascular Biology" introduced participants to new technologies to deal with such questions as the velocity of phloem sap flow (Carel Windt using MRI imaging) and the structure of protein subunits (Denis Renard) together with noninvasive imaging of cadmium (Shu Fujimaki) and immunological tools to study vascular development (Gary Thompson). A fitting conclusion to a stimulating programme.

In concluding, a striking feature of the conference was the large number of young plant scientists – post-docs and postgraduate students participating both with oral and poster presentations. A group of them are seen below enjoying the dinner that marked the end of a great conference.

Tina Offler

Birgit Absmanner (Germany), Caitlin Byrt (Australia), Ralf Metzner (Germany), Thomas Slewinski (USA) and Johannes Liesche (Denmark).

CONFERENCES 2011

The 2nd International Conference on Plant Metabolism

June 30—July 3, 2011 Qingdao, China

Theme: Plant Metabolism & Modern agriculture

Topics: 1) Primary metabolism; 2) Secondary metabolism; 3) Metabolomics; 4) Metabolic engineering & signalling; 5) Yield & nutrition.

Invited Speakers:

Wilhelm Gruissem ETH Zurich, Switzerland
Yuji Kamiya RIKEN Plant Science Center, Japan
Anne Osbourn John Innes Centre, UK
Cathie Martin John Innes Centre, UK
Danièle Werck INSB, CNRS, France
Natalia Dudareva Purdue University, USA
Kazuki Saito Chiba University, Japan
Harry Klee University of Florida, USA
Peter Beyer University of Freiburg, Germany
Xuemin Wang University of Missouri, USA
Wataru Sakamoto Okayama University, Japan
Toshiaki Umezawa Kyoto University, Japan
Li Li Cornell University, USA
Sheng Luan University of California, Berkley, USA
Shuqun Zhang University of Missouri, USA
Susheng Gan Cornell University, USA
Yongling Ruan University of Newcastle, Australia
Kexuan Tang Jiaotong University, China
Shan Lu Nanjing University, China

Richard Dixon Samuel Roberts Noble Foundation, USA
Xiaoya Chen Institute of Plant Physiology and Ecology, SIBS, China
Qifa Zhang Huazhong Agricultural University, China
Xu Lin Institute for Nutritional Sciences, SIBS, China
Lothar Willmitzer Max Planck Institute of Molecular Plant Physiology
Wolf B. Frommer Carnegie Institution for Science, USA
Yi-Fang Tsay Institute of Molecular Biology, Academia Sinica, Taiwan
Harro Bouwmeester Wageningen University, Netherlands
Changjun Liu Brookhaven National Laboratory, USA
Alisdair Fernie Max Planck Institute of Molecular Plant Physiology
Vincent Bulone Swedish Centre for Biomimetic Fibre Engineering
Laigeng Li Institute of Plant Physiology and Ecology, SIBS, China
Toni Kutchan Donald Danforth Plant Science Center, USA
Barry Pogson Australian National University, Australia
Jan Jaworski Donald Danforth Plant Science Center, USA
Arthur Grossman Carnegie Institution for Science, USA
Xiaoquan Qi Beijing Botanical Institute, CAS, China
Hongwei Xue Institute of Plant Physiology and Ecology, SIBS, China
Chunyi Zhang Chinese Agricultural Academy, China

Organizers

- Institute of Plant Physiology & Ecology (SIPPE), SIBS, CAS
- Chinese Society for Plant Physiologists
- Qingdao Institute of Bionergy and Bioprocess Technology, CAS

Sponsors

- Chinese Academy of Sciences (CAS)
- Shanghai Institutes for Biological Sciences (SIBS)
- State Basic Research Program (973)
- Shanghai Association for Sciences & Technology
- SIPPE, SIBS, CAS

Deadlines: Abstract submission, May 15, 2011 Travel Awards, April 30, 2011
Early Registration, March 31, 2011 Registration, June 15, 2011

http://www.cspp.cn/2icpm/eindex.asp

IBC2011
XVIII international botanical congress
MELBOURNE AUSTRALIA | 23-30 JULY 2011

The IBC is fast approaching. I encourage you to visit the website at: **www.ibc2011.com**
In particular, have you booked your accommodation yet?

We have something to suit every budget - from economy priced rooms and self-catering apartments to standard, superior and first-class hotels.
If you have not already secured accommodation, now is the time to take action as rooms are filling fast. Simply complete the "Rest Easy" form and fax back to the XVIII IBC 2011 Congress Secretariat.

We look forward to seeing you in Melbourne!

ComBio 2011 Cairns

Cairns Convention Centre

25 - 29 September 2011

Registration and Call for Abstracts now open:

Abstract and Early Registration Deadline, Friday, 24 June 2011

ComBio2011 is the combined conference of the Australian Society for Biochemistry and Molecular Biology, the Australian Society of Plant Scientists and the Australia and New Zealand Society for Cell and Developmental Biology.

The ComBio2011 online registration and abstract submission pages can now be accessed at:
http://www.asbmb.org.au/combio2011/registration.html and
http://www.asbmb.org.au/combio2011/abstracts.html respectively.

Program information (including provisional symposium schedule and conference timetable) can be downloaded from: http://www.asbmb.org.au/combio2011/program.html

A Message from the ASPS Organizers:

Organization for the up coming ComBio conference in Cairns is progressing.

The plenary speakers include:
Julian Hibberd
Carroll Vance (Annals of Botany Lecture)
Susanne von Caemmerer (JG Wood lecture)
Lauren Sack
Glaucia Souza

Whilst we are sorting out the last few Symposia those already developed include:
Linking Plant Form and Function
Plant Microbe Interactions
Polyploidy
Molecular Solutions to Plant Nutrient Acquisition
Carbon Transport Metabolism and Signalling
Plant Animal Interactions,
Plant Phenomics - Imaging Plant Performance
Nitrogen/Water Use Efficiencies

In addition there will be a colloquium session showcasing our students and younger scientists.
Of course there will also be the society dinner at a local restaurant - organizing this is my most important task. So we hope to see many of you in Cairns, the city is amenable for playing and working hard (and of course an extended stay).

Best Wishes *Graham Bonnett*

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 Kiran Sarfaraz via advertise@asps.org.au. To cover the costs involved, the society has introduced a small charge of $30 for members and $70 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 (2011, IBC, Melbourne; ComBio, Cairns), 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).