29th New Phytologist Symposium

Stomata 2012

2–4 July 2012
Manchester Conference Centre, Manchester, UK

Programme, abstracts and participants

New Phytologist

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Acknowledgements

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Programme, abstracts and participant list compiled by Jill Brooke
'Stomata 2012' illustration by A.P.P.S., Lancaster, UK

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Information for Delegates

Meeting Location
The 29th New Phytologist Symposium will be held at the Manchester Conference Centre, Sackville Street, Manchester, M1 3BB.

Catering
Coffee breaks and lunch will be served in the Weston Restaurant of the Manchester Conference Centre.

A **Tour and Conference dinner** will be held on Tuesday evening at Manchester Museum, Oxford Road, Manchester, M13 9PL. At 18:00 there will be a drinks reception and tour of the 'Living Worlds Gallery' followed by dinner at 19:30 in the 'Fossils Gallery'. The tour and conference dinner are included in the registration fee for all delegates attending the symposium. If you require transport to Manchester Museum where the tour and conference dinner will take place (note it is a 15 minute walk from Manchester Conference Centre) please speak to Helen or Jill before Tuesday lunch time. Please see the map at the back of this booklet for directions.

Posters
If you have submitted a poster abstract to share your research with the community this should be A0 in size and portrait in orientation. Please display your poster as soon as possible on the 2nd July, on the numbered board which corresponds with the number your poster abstract has been allocated in the abstract book. Please remove all posters by 16:00 on Wednesday 4th July.

Posters will be open for viewing throughout the symposium and will be located in the foyer and Weston Restaurant of the conference centre. A dedicated poster session will be held on Monday from 17:00 please stand by your poster and remember there will be prizes! Talks selected from submitted posters will be given during Session 3 on Tuesday 3rd July.

Map
A map showing the location of the conference centre, Piccadilly train station and Manchester Museum is provided at the back of this booklet. Please note 24-hour parking is available in the NCP car park on Charles Street, adjacent to Manchester Conference Centre.

Internet Access
Manchester Conference Centre offers free internet access throughout (including within The Days Inn Hotel).

Meeting Programme

Monday 2 July

8:00–9:15	Registration
9:15–9:30	Welcome and Introductions
Session 1:	**Guard cell function** ***Chair: Neil Baker***
9:30–10:00	**Metabolomics of Arabidopsis guard cells** *Sally Assmann*
10:00–10:30	**Guard cell CO_2 and abscisic acid signal transduction network** *Julian Schroeder*
10:30–11:00	Tea/coffee break
11:00–11:30	**Guard cell plasma membrane H^+- ATPases: highly regulated proton pumps to control gas exchange** *Nathalie Leonhardt*
11:30–12:00	**The involvement of the cytoskeleton and lipids in the regulation of stomatal aperture** *Alistair Hetherington*
12:00–12:30	**Signaling in stomatal guard cells in response to blue light** *Ken-ichiro Shimazaki*
12:30–13:30	Lunch
13:30–14:00	**Apoplastic ROS and stomatal signaling – processes and components involved** *Jaakko Kangasjärvi*
14:00–14:30	**Biology of guard cell anion channels** *Rainer Hedrich*
14:30–15:00	**The MPK6-ERF-ROSE7/GCC-box complex modulates oxidative gene transcription and ROS signaling in *Arabidopsis thaliana*** *Chun-Peng Song*
15:00–15:30	Tea/coffee break
15:30–16:00	**Guarding the gates: Stomatal responses to pathogens** *Silke Robatzek*
16:00–16:30	**Quantitative systems modelling of the stomatal guard cell yields unexpected and emergent behaviours** *Mike Blatt*
16:30–17:00	Discussion
17:00–18:30	Poster session with drinks reception and canapés

Meeting Programme

Tuesday 3 July

8.50–9:00	Announcements
Session 2:	**Stomatal development and evolution** ***Chair: Janice Lake***
9:00–9:30	**Here for the long haul: Organizing principles and innovations in stomatal development** *Dominique Bergmann*
9:30–10:00	**Stomatal development and evolution** *Julie Gray*
10:00–10:30	**Evolution of stomatal function: New perspectives and application to the fossil record** *Jennifer McElwain*
10:30–11:00	Tea/ Coffee break
11:00–11:30	**The significance of C_4 photosynthesis for stomatal patterning and behaviour** *Colin Osborne*
11:30–12:00	**Passive valves or metabolic mouths? The evolution of stomatal physiology** *Tim Brodribb*
12:00–12:30	**Cell-cell-communication and stomatal patterning** *Keiko Torii*
12:30–13:00	Discussion
13:00–14:00	Lunch
Session 3:	**Selected poster talks** ***Chair: Colin Osborne***
14:00–14:20	**Deep evolutionary origins of stomatal development (P6)** *Casper Chater*
14:20–14:40	**The physiological consequences of stomatal patterning and density in *Arabidopsis thaliana* (P10)** *Graham Dow*
14:40–15:00	**Phylogenetic analysis of SLAC1 protein family structure, predicted function and conservation in land plants (P12)** *Sanna Ehonen*
15:00–15:30	Tea/coffee break
15:30–15:50	**CO_2 regulation of stomatal development by carbonic anhydrases (P13)** *Cawas Engineer*
15:50–16:10	**Hydraulic limits to maximum transpiration (P33)** *Stefano Manzoni*

Meeting Programme

16:10–16:30	**Ontogenetic priming of stomatal control in *Arabidopsis* leaves through gradual exposure to low humidity (P43)** *Florent Pantin*
16:30–17:00	Discussion
18:00	Drinks reception and tour of the 'Living Worlds' Gallery, Manchester Museum (see the map at the end of this booklet for directions).
19:30	Conference Dinner in the 'Fossils Gallery', Manchester Museum.

Meeting Programme

Wednesday 4 July

9:20–9:30	Announcements
Session 4:	**Stomata at the whole plant/canopy level and in crops** ***Chair: Jennifer McElwain***
9:30–10:00	**Genome size as a constraint on productivity and water-use efficiency** *Peter Franks*
10:00–10:30	**Stomata – a global view** *Ian Woodward*
10:30–11:00	Tea/coffee break
11:00–11:30	**Fluctuations in stomatal behaviour: impacts on carbon gain and water use efficiency** *Tracy Lawson*
11:30–12:00	**Using soil drying as a regulative tool to enhance crop water use efficiency** *Jianhua Zhang*
12:00–13:00	Lunch
13:00–13:30	**Stomatal responses to humidity and temperature are consistent with a vapor-phase mechanism** *Keith Mott*
13:30–14:00	**From roots to stomata** *Josette Masle*
14:00–14:30	Tea/coffee break
14:30–15:00	**Optimisation of maximal stomatal conductance in subtropical vegetation under rising CO_2** *Hugo Jan de Boer*
15:00–15:30	**A new stomatal response: stress and recovery** *Janice Lake*
15:30–16:00	Discussion and summary
16:00	Depart

Speaker Abstracts

** S=speaker abstract; P=poster abstract*

Session 1: Guard cell function
Chair: Neil Baker

Metabolomics of Arabidopsis guard cells

S1.1

XIAOFEN JIN[1], RUISHENG WANG[2], REKA ALBERT[2], SIXUE CHEN[3], SARAH M. ASSMANN[1]

9:30–10:00

sma3@psu.edu

[1]Biology Department and [2]Physics Department, Penn State University, University Park, PA, 16802, USA; [3]Department of Biology, University of Florida, Gainesville, FL, USA

Plants synthesize a vast array of primary and secondary metabolites with a diversity of functions. We are interested in characterizing the guard cell metabolome, its response to abscisic acid (ABA), and its regulation by heterotrimeric G proteins. We performed targeted metabolite analysis using RP-LC-MS/MS on Arabidopsis wild-type Col and *Gα* (*gpa1*) mutant guard cell protoplasts treated with 50 μM ABA (or solvent control) for 2, 10, 30 or 60 minutes. Each sample contained 3-6 million guard cell protoplasts and four replicates were obtained for each treatment and timepoint. A total of 85 metabolites were reliably identified and quantified. Metabolome profiles differed significantly between treatments and genotypes, and metabolites clustered into different temporal profiles. Fewer metabolites changed in response to ABA treatment in *gpa1* than in Col guard cells, consistent with ABA hyposensitivity in inhibition of stomatal opening previously observed in *gpa1* mutants.

Guard cell CO_2 and abscisic acid signal transduction network

S1.2

JULIAN I. SCHROEDER, SHINTARO MUNEMASA, FELIX HAUSER, HONGHONG HU, TAE-HOUN KIM, BENJAMIN BRANDT, NORIYUKI NISHIMURA, MARIA ISRAELSSON-NORDSTROM, AURELIEN BOISSON-DERNIER, DENNIS BRODSKY

10:00–10:30

jischroeder@ucsd.edu

Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093-0116, USA

The continuing rise in atmospheric CO_2, and the daily increase in CO_2 concentration in leaves due to respiration in the dark, cause closing of stomatal pores and regulate stomatal development and thus affect CO_2 influx into plants and transpiration. Under drought conditions, the plant hormone abscisic acid triggers stomatal closing, thus reducing plant water loss. Guard cells have been developed as a model system for dissecting time-resolved early signal transduction mechanisms and ion channel functions. Elevations in atmospheric CO_2 and the hormone abscisic acid (ABA) both trigger signaling cascades in guard cells that converge resulting in ion channel regulation, stomatal closing and plant water loss reduction. However, the CO_2 signal transduction mechanisms remained largely unknown. We have recently characterized CO2-binding carbonic anhydrases, the SLAC1 anion channel and calcium signaling mechanisms that are essential for triggering early CO_2 signal transduction in guard cells (Hu et al., 2010 Nature Cell Biol.; Vahisalu et al., 2008 Nature; Negi et al., 2008 Nature; Xue et al., 2011 EMBO J.). Genetic loci and mechanisms that mediate CO_2 control of plant gas exchange will be presented. The PYR/RCAR proteins were identified as ABA receptors (Park et al., Science 2009; Ma et al., Science 2009). We have reconstituted multiple signaling components enabling functional reconstitution of ABA activation of SLAC1 anion channels. This research further reveals a branched role for type 2C protein phosphatases in ABA regulation of SLAC1 anion channels, a new phosphorylation site required for Ca^{2+}-dependent activation of SLAC1 and an alternative functional ABA signal transduction core in the absence of a SnRK2(OST1) protein kinase.

Guard cell plasma membrane H^+- ATPases: highly regulated proton pumps to control gas exchange

S1.3

LUDOVIC MARTIN, HELENE JACQUET, JEANNE RENAUD, VALERIE COTELLE, CÉCILE GIACALONE, ALAIN VAVASSEUR, NATHALIE LEONHARDT

11:00–11:30

nathalie.leonhardt@cea.fr

Laboratoire de Biologie du Développement des Plantes (LBDP), Institut de Biologie Environnementale et de Biotechnologie (IBEB), Service de Biologie Végétale et de Microbiologie Environnementales (SBVME) UMR 7265 CNRS/CEA/Université Aix-Marseille CEA Cadarache Bat 156, 13108 St Paul Lez Durance, France

Guard cells provide an ideal system to elucidate early events in higher plant signal transduction. Up today, several key guard cell ion channels have been proposed to function as important signal transducers and mediators of stomatal movements. During stomatal opening under favourable conditions, activation of plasma-membrane-localized proton pump, H^+-ATPase, establishes a negative membrane voltage that drives the uptake of K^+. For stomatal closure, anion channels that depolarize the membrane are activated, setting conditions for long-term K^+ and anion efflux. The question of whether deactivation of the proton pump is needed for stomatal closure, or whether the activation of anion channels is sufficient to sustain the membrane depolarization necessary to drive K^+ efflux, remains largely unsolved. In this context, using a multidisciplinary approach involving physiology, electrophysiology, molecular biology, biochemistry and molecular genetics, the roles of the three major isoforms of the plasma membrane H^+-ATPases expressed in guard cell, AHA1, AHA2 and AHA5, are currently investigated in the control of the membrane potential in response to environmental stimuli and abiotic stress. Our recent data on the functions of these 3 isoforms in the stomatal movement regulation will be presented and their role in guard cell signalling will be discussed.

The involvement of the cytoskeleton and lipids in the regulation of stomatal aperture

S1.4

ALISTAIR M. HETHERINGTON

11:30–12:00

Alistair.Hetherington@bristol.ac.uk

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK

Guard cell actin reorganisation has been observed in stomatal responses to a wide array of stimuli. However, how the guard cell signalling machinery controls actin dynamics is poorly understood. The presentation will report the identification of a new allele of the Arabidopsis ARPC2 locus (encoding the ARPC2 subunit of the ARP2/3 complex) designated hsr3. When treated with ABA, guard cell actin filaments underwent fast disruption in wild-type plants, whereas those in hsr3 remained largely bundled. Our recent work indicates that control of actin reassembly through ARP2/3 complex activity is crucial for stomatal regulation. Recent unpublished data pointing to a role for lipid species during stomatal opening will also be presented.

Signaling in stomatal guard cells in response to blue light

S1.5

KEN-ICHIRO SHIMAZAKI[1], NAOYUKI SUGIYAMA[2], ATSUSHI TAKEMIYA[1]

12:00–12:30

kenrcb@kyushu-u.org

[1]Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan; [2]Institute for Advanced Biosciences, Keio University, Tsuruoka 997-0017, Japan

We have been investigating blue light-dependent stomatal opening in higher plants. We demonstrated that phototropins initiate the light signaling and activate the plasma membrane H+-ATPase that drives stomatal opening. The signaling between phototropins and the H+-ATPase is mediated by type 1 protein phosphatase (PP1). We showed the type 1 protein phosphatase (catalytic subunit; PP1c) acts as a cross talk point between signalings of blue light and ABA, and identified the regulatory subunit (PPr) for PP1c in guard cells. The ppr mutant showed partial impairment in blue light response of stomata. To identify other signaling components, we developed a method for screening Arabidopsis plants impaired in blue light-dependent stomatal opening by infrared thermography. A weak blue light superimposed on red light decreased the leaf temperature in wild-type by stimulated transpiration, but the light did not affect the temperature in *phot1 phot2* double mutant. On the basis of this response, we obtained several mutants. We characterized these mutants and selected a typical one that exhibited the complete loss of blue light-dependent stomatal opening. The mutant showed the normal phototropin-mediated responses, including phototropism, chloroplast movement, and leaf flattening. We identified the gene responsible for this phenotype as a novel protein kinase.

Apoplastic ROS and stomatal signaling – processes and components involved

S1.6

JAAKKO KANGASJÄRVI

13:30–14:00

Jaakko.kangasjarvi@helsinki.fi

Division of Plant Biology, Department of Biosciences, University of Helsinki. POB 65, Helsinki, FIN-00014, Finland

Effective responses to external and internal stimuli will ensure optimal growth and survival in an environment where productivity and product quality are adversely affected by stresses. Plants must have effective means of adapting to changes in their environment. The main features of such defense strategies involve early recognition and perception of the developing stress, and subsequent activation of induced adaptive and defensive responses. Stomata are an important component in adaptation and defense and reactive oxygen species (ROS) formed in guard cells by several stresses are one of the factors that contribute to, and regulate stomatal responses. Strong evidence has accumulated that ROS play an important role in the signaling resulting in stomatal regulation under both abiotic and biotic stresses. The air pollutant ozone generates reactive oxygen species (ROS) in the apoplast. Consequently, ozone has been used as a tool to unravel *in planta* ROS-induced processes and apoplastic ROS sensing. Examples of identification of the molecular identity of some components, and interaction between multiple regulatory cascades in guard cells involving apoplastic ROS, discovered with this approach are presented.

Biology of guard cell anion channels

S1.7

RAINER HEDRICH[1], DIETMAR GEIGER[1], PETER ACHE[1], KHALED AL-RASHEID[2]

14:00–14:30

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[1]Lehrstuhl für molekulare Pflanzenphysiologie und Biophysik, Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, Julius-von-Sachs-Platz 2, 97082 Würzburg, Germany; [2]Zoology Department, College of Science, King Saud University, P.O.Box 2455, Riyadh 11451, Saudi Arabia

Guard cell anion transport is mediated via channels of the S(Slow)- and R(Rapid/QUAC)-type. These anion channels are encoded by different genes and characterized by different voltage dependencies, kinetics, and susceptibility towards blocker. The *Slow Anion Channel 1 gene* (SLAC1 gene) encodes a plasma membrane protein that in planta. Following heterologous expression in *Xenopus laevis* oocytes SLAC1 function was shown to be under the control of distinct protein kinase-phosphatase pair. Upon kinase activation SLAC1 currents were reminiscent to S-type anion currents in guard cell. In addition to SLAC1, Arabidopsis guard cells operate the SLAC1 homolog SLAH3. SLAH3 conducts NO_3^- in a feed forward reaction to increasing external nitrate levels.
Rapid QUAC anion channels: Recently, ALMT12 was found to be expressed in guard cells, and its loss-of-function resulted in impaired stomatal closure. This member of the ALMT family was named QUAC1 (QUickly activating Anion Channel1) because it reflected the hallmark properties of the first described R-type guard cell anion channel and references therein). R-type channels represent strongly voltage-dependent plasma membrane anion channels.
At the meeting new insights into the structure, function, and physiology of guard cell anion channels will be presented.

The MPK6-ERF-ROSE7/GCC-box complex modulates oxidative gene transcription and ROS signaling in *Arabidopsis thaliana*

S1.8

PENGCHENG WANG, YANYAN DU, XIAOLIANG ZHAO, YUCHEN MIAO, CHUN-PENG SONG

14:30–15:00

songcp@henu.edu.cn

Institute of Plant Stress Biology, State Key Laboratory of Cotton Biology, Department of Biology, Henan University, Kaifeng 475001, China

Reactive oxygen species (ROS) have been characterized as both important signaling molecules in guard cells and universal stressors that mediate many developmental and physiological responses. So far, details of the transcriptional mechanism of ROS-responsive genes are still largely unknown. In the study reported herein, we identified eight potential ROS-responsive cis-acting elements (ROSEs) from the promoters of genes upregulated by ROS. We also found that the APETALA2 (AP2/EREBP)-type transcription factor ERF could bind specifically to the ROSE7/GCC box. Co-expression of ERF enhanced luciferase activity driven by ROSE7. ERF interacted physically with mitogen-activated protein kinase 6 (MPK6), and also served as a substrate of MPK6. MPK6-mediated ERF phosphorylation at both Ser 266 and Ser 269 affected the dynamic alternation of ERF protein, which resulted in changes in ROS-responsive gene transcription. These data might provide new insight into the mechanisms that regulate ROS-responsive gene transcription via a complex of MPK6, ERF, and the ROSE7/GCC box.

Guarding the gates: Stomatal responses to pathogens

S1.9

SILKE ROBATZEK, ROSA LOZANO-DURAN, GILDAS BOURDAIS, JI ZHOU

15:30–16:00

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The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

Pathogens exploit different infection strategies to infect plants and stomata represent a direct pathway by which microbes can enter the plant. To counter this, guard cells have evolved the ability to detect conserved microbial molecules referred to as pathogen-associated molecular patterns (PAMPs), and PAMP-induced closure of stomata is an essential layer of the plant's immune system. Pathogens need to overcome stomatal immunity, and for this purpose they use effector molecules that are delivered into the plant cell. We found that the bacterial effector HopM1 suppresses stomatal closure, in a proteasome-dependent manner. Interestingly, this effect can be mimicked by chemical disruption of 14-3-3 interactions with their client proteins, which also restores virulence of a HopM1 deletion P. *syringae* mutant.

Despite the importance of stomatal immunity, the pathways underlying stomatal behavior and their interaction with immunity control remain largely unknown. To address this we have developed a novel imaging method allowing us to measure stomatal apertures in high throughput, and we can recapitulate responses to PAMPs, as well as the ABA-induced stomatal closure. I will present first data on the genetic dissection of stomata response pathways highlighting *tmm* and *bak1* mutants, and discuss exciting evidences on the biophysical control of stomatal closure.

Quantitative systems modelling of the stomatal guard cell yields unexpected and emergent behaviours

S1.10

MICHAEL R. BLATT, YIZHOU WANG, MARIA PAPANASTIOU, CORNELIA EISENACH, RUCHA KARNIK, VIRGILIO L. LEW[1], ZHONGHUA CHEN[2], ULRIKE BAETZ, ANNA AMTMANN, ADRIAN HILLS

16:00–16:30

Michael.Blatt@glasgow.ac.uk

Laboratory of Plant Physiology and Biophysics, University of Glasgow, Bower Building, Glasgow, G12 8QQ; [1]The Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, CB2 3EG, UK; 2School of Science and Health, University of Western Sydney, Hawkesbury Campus, Richmond, NSW 2753, Australia

The dynamics of stomatal movements have long been incorporated into mathematical models, but few of these have been developed from the 'bottom-up' and none are sufficiently generalised to be widely applicable in predicting stomatal behaviour at a cellular level. We have developed a dynamic computational model for cellular physiology building on the wealth of biophysical and kinetic knowledge available for guard cell transport, signalling and homeostasis. The Onguard software and model incorporate explicitly all of the fundamental properties for transporters at the plasma membrane and tonoplast, the salient features of osmolite metabolism, and the major controls of cytosolic-free Ca^{2+} concentration and pH. The model integrates the kinetics of these processes and has proven remarkably robust in recapitulating physiologically complex behaviours, including those of oscillations in membrane voltage and cytosolic-free Ca^{2+} concentration previously reported in vivo. The predictive power of the OnGuard model is evident in its ability to generate a number of unexpected and counterintuitive outputs of physiological relevance and without ad hoc assumptions or additional regulatory networks. Several predictions now fuel substantive research projects in their own right. Thus, the OnGuard model sets out a framework for the systems biological analysis of stomatal guard cells, and a flexible, mathematical modelling environment that should find application in exploring similar physiological and related problems in stomatal biology.

Session 2: Stomatal development and evolution
Chair: Janice Lake

Here for the long haul: Organizing principles and innovations in stomatal development

S2.1

DOMINIQUE BERGMANN, EMILY ABRASH, KELLI DAVIES, TIE LIU, CORA MACALISTER, JULIANA MATOS, KYOKO OHASHI-ITO

9:00–9:30

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Biology, Stanford University/ HHMI-GBMF, 371 Serra Mall, Stanford, CA 94305, USA

Plant development has a significant postembryonic phase that is guided heavily by interaction between the plant and the outside environment. This interplay is particularly evidence in the development, pattern and function of stomata. Stomata have been found in fossils dating from more than 400 million years ago; strikingly the morphology of the individual stomatal complex is largely unchanged, but the sizes, numbers and arrangements of stomata and their surrounding cells have diversified tremendously. In many plants, stomata arise from specialized and transient stem-cell like compartments on the leaf. I will discuss some of our studies in the flowering plant Arabidopsis thaliana that established a basic molecular framework for the acquisition of cell fate and generation of cell polarity in these compartments, as well as describing some of the key signals and receptors required to produce stomata in organized patterns and in environmentally optimized numbers. I will then present progress from recent genetic and molecular forays into representatives of plant groups in which there have been striking innovations in stomatal morphology, pattern or behavior.

Stomatal development and evolution

S2.2

JULIE GRAY

9:30–10:00

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Molecular Biology & Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, UK

The mosses represent the most ancient lineage of plants that have stomata but the function of their stomata which are present on the spore capsule, remains unclear. Stomata of two moss species tested closed in response to darkness, exogenously added ABA and in response to elevated CO_2 levels. Stomata of *Physcomitrella* patens genetically manipulated to be deficient in OST1, a component that is essential for Arabidopsis ABA and CO_2-induced stomatal closure, were impaired in their response to closure stimuli. These results indicate that basic signalling mechanisms regulating stomatal closure most probably evolved shortly after stomatal pores themselves, over 400 million years ago.

Evolution of stomatal function: New perspectives and application to the fossil record

S2.3

JENNIFER C. McELWAIN

9:30–10:00

jennifer.mcelwain@ucd.ie

School of Biology and Environmental Science, University College Dublin, Belfield, Dublin, Ireland

Vascular plants have evolved a suite of different strategies to optimize carbon uptake against water loss via developmental, physiological and cellular level control of stomatal function. Alterations to the development of stomatal number and size in response to atmospheric CO_2 concentration enable some species to regulate stomatal conductance on time-scales of weeks to decades. Other species demonstrate physiological control of stomatal aperture allowing for rapid control of stomatal function in response to abiotic factors on time scales of seconds to minutes. Competing hypotheses have been proposed for the evolution of stomatal function in land plants with suggestions on one side that stomatal function is highly conserved across the plant phylogeny and the alternative view that stomatal function has increased incrementally across vascular plants from monilophytes to spermatophytes. Observations of stomatal response to both instantaneous and long-term exposure to elevated atmospheric CO_2 in UCD PÉAC (Programme for Experimental Atmospheres and Climate) do not completely support either competing hypothesis. Rather they suggest that the evolution of stomatal function is more complex and does not display a strong phylogenetic signature. We show that loss of one aspect of stomatal function in response to an external stimulus can be readily compensated by improved functionality in another. Experiments also show that there may be a trade-off between physiological control of stomatal conductance via stomatal aperture opening/closing and morphological control of conductance through developmental alteration to stomatal density and pore size. This "stomatal trade- off hypothesis" is supported by the observation of a significant negative correlation between the magnitude of response of conductance to instantaneous change in CO_2 and the magnitude and sign of response of stomatal density to long term elevated CO_2 exposure. In other words, species which show rapid instantaneous changes in stomatal conductance do not alter stomatal density inversely under elevated CO_2 and vice versa. This has obvious and important implications for future development and application of the stomatal-CO_2 proxy method which uses the inverse relationship between SD and CO_2 to reconstruct palaeo-CO_2 concentration in the geological past. This will be discussed in relation to new estimates of palaeo-CO_2 spanning the Eocene-Oligocene boundary between 40 and 25 million years ago when the Earth transitioned between an ice-free (greenhouse) and glaciated (icehouse) state.

The significance of C_4 photosynthesis for stomatal patterning and behaviour

S2.4

COLIN OSBORNE[1], SAM TAYLOR[1], PETER FRANKS[2], BRAD RIPLEY[3], JESSICA PASQUET-KOK[4], CHRISTINE SCOFFONI[4], LAWREN SACK[4], BETH SPRIGGS[5], PASCAL-ANTOINE CHRISTIN[5], ERIKA EDWARDS[5], IAN WOODWARD[1]

11:00–11:30

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[1]University of Sheffield, UK; [2]University of Sydney, Australia; [3]Rhodes University, South Africa; [4]UCLA, USA; [5]Brown University, USA

C_4 photosynthesis ranks among the most important evolutionary innovations in plants, with multiple origins spanning a diverse range of independent plant lineages. We interpret the functional significance of the C_4 syndrome within its evolutionary context. Our comparative experiments test the general hypothesis that lower stomatal conductance for a given rate of photosynthesis in C_4 than C_3 plants is associated with changes in stomatal patterning and behaviour. Under mesic conditions, lower stomatal conductance in C_4 than C_3 grasses causes a smaller soil-leaf water potential gradient. Maximum stomatal conductance is consistently lower in C_4 species than their C_3 relatives, because of a shift towards smaller stomata at a given density. Under drought, stomatal conductance declines more dramatically in the C_3 than C_4 species, although the water-use efficiency advantage held by C_4 species under control conditions is diminished. These results are consistent with a hydraulic advantage for the C_4 pathway mediated by a greater ratio of hydraulic to stomatal conductance. Protection of the hydraulic system allows stomata to remain open and photosynthesis to be sustained for longer under drying atmospheric and soil conditions. Comparative physiological and ecological data suggest that water relations played key roles in the origins and diversification of C_4 grasses.

Passive valves or metabolic mouths? The evolution of stomatal physiology

S2.5

TIM BRODRIBB

11:30–12:00

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Plant Science, University of Tasmania, Bag 55, Hobart, Tasmania 7001, Australia

The stomata of "modern" seed plants respond to a diverse array of signals, allowing them to efficiently regulate the use of water by leaves while protecting the plant against catastrophic failure of the water transport system. As might be expected by these complicated demands, the signaling and response physiology of stomata is complex and poorly understood. Here I discuss some results from an evolutionary approach that is providing new insight into why and how stomata of the dominant seed-plant lineage behave the way they do. By making detailed measurements of stomatal behavior in species spanning the phylogeny of vascular plants we show that key components of the stomatal response network are absent in early-branching clades. Based on observations of lycophytes, ferns, gymnosperms and angiosperms we propose that the ancestral functional state of stomata in vascular plants was a simple hydraulic valve, opened by light and regulated passively by leaf hydration. Key innovations such as stomatal responses to metabolic rate or hormone levels are proposed to have evolved at the base of the seed plant clade sometime after the divergence of ferns and lycophytes. The profound ecological, atmospheric and physiological implications of this stomatal "evolutionary model" are discussed.

Cell-cell-communication and stomatal patterning S2.6

KEIKO U. TORII **12:00–12:30**

ktorii@u.washington.edu

Howard Hughes Medical Institute and Department of Biology, University of Washington, Seattle, WA 98195, USA

During development of photosynthetic organs, a selected population of undifferentiated protodermal cells undergoes asymmetric cell divisions that initiate the stomatal cell lineage. A stomatal precursor cell reiterates asymmetric cell division and eventually differentiates into guard cells. Recent progress by our group and others has led to the discovery of key molecules and pathways controlling stomatal patterning and differentiation: (1) Sequential and combinatory actions of five bHLH transcription factors specifying stomatal precursor cell state transitions; (2) A ligand-receptor system enforcing frequency and orientation of asymmetric cell division; (3) Other intrinsic polarity and cellular constituents required for creating and maintaining asymmetry.
Genetic studies have revealed putative ligands and receptors controlling proper stomatal patterning and differentiation. It has been proposed that two candidate ligands, EPF1 and EPF2, are secreted from stomatal precursor cells to enforce stomatal patterning. Their putative receptors are ERECTA-family receptor kinases and TMM receptor-like proteins. However, nothing was known about their molecular associations or signal specificity. By taking *in planta* biochemical co-immunoprecipitation assays and newly developed receptor biosensor chips, we demonstrate that bioactive EPF1/2 peptides robustly bind to ERECTA-family with saturable kinetics. We further show that ERECTA-family receptor kinases constitute receptor homomers in vivo. TMM, on the other hand, associates with ERECTA-family but not with itself. Finally, using bioactive EPF peptides that elicit unique developmental responses and dominant-negative receptors that specifically block each ERECTA-family signaling in vivo, we delineated the in vivo specificity for each ligand-receptor pair. Our results place ERECTA-family as the primary receptors for EPFs with TMM as a signal modulator, and establish EPF2-ERECTA and EPF1-ERL1 as ligand-receptor pairs specifying two critical steps of stomatal development: initiation and spacing divisions. Combining high-resolution live imaging approaches and biochemistry, we seek to unravel how each ligand-receptor pair elicits specific developmental responses, and how this ligand-receptor signaling system regulates transcription factor control of stomatal differentiation in real time and space.

Session 3: Selected poster talks
Chair: Colin Osborne

Six talks, which have been selected from the poster abstracts, will be presented in this session. The poster numbers for these talks are listed below to enable you to locate the abstract within this book.

Deep evolutionary origins of stomatal development (P6)
CASPER CHATER

The physiological consequences of stomatal patterning and density in *Arabidopsis thaliana* (P10)
GRAHAM DOW

Phylogenetic analysis of SLAC1 protein family structure, predicted function and conservation in land plants (P12)
SANNA EHONEN

CO_2 regulation of stomatal development by carbonic anhydrases (P13)
CAWAS ENGINEER

Hydraulic limits to maximum transpiration (P33)
STEFANO MANZONI

Ontogenetic priming of stomatal control in *Arabidopsis* leaves through gradual exposure to low humidity (P43)
FLORENT PANTIN

Session 4: Stomata at the whole plant/canopy level and in crops
Chair: Jennifer McElwain

Genome size as a constraint on productivity and water-use efficiency

S4.1

PETER FRANKS

9:30–10:00

peter.franks@sydney.edu.au

Faculty of Agriculture, University of Sydney, Sydney, NSW 2006, Australia

Guard cell size and nuclear genome size appear to be positively correlated across a broad range of taxa and within the plastic range of developmental adaptation to growth conditions. The underlying mechanism is unknown, exacerbated by deep divisions in the field of genome size evolution and controversial views on genome plasticity. The observation poses important questions about genome size as a constraint on plant productivity and water-use efficiency because of the physical limitation that stomatal size places on leaf diffusive conductance. Natural and artificial selection for stomatal conductance to optimise plant performance under different climatic conditions depend upon variation in genome size as a phenotypic trait.

Stomata – a global view

S4.2

IAN WOODWARD

10:00–10:30

f.i.woodward@sheffield.ac.uk

Animal & Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK

Vegetation is an essential component of global climate through its impact on the global cycles of carbon and water. The interface of these two cycles is strongly influenced by the activity of stomata; however the operation of stomata in global vegetation and climate models is not well defined. At the leaf level stomatal conductance can be nicely defined by the physics of diffusion but this requires information on stomatal size and density. Such information could be the basis of a mechanistic base for simulating the stomatal controls of gaseous diffusion in vegetation, at the global scale. This presentation describes attempts to map stomatal size and density at the global scale, based on a large data base of field observations and climate, to provide the necessary underpinning data for simulating stomatal conductance. Further analysis, at the Angiosperm family level, indicates that stomatal size in arborescent species is strongly determined by temperature but also the evolutionary age of the family.

Fluctuations in stomatal behaviour: impacts on carbon gain and water use efficiency

S4.3

L. MCAUSLAND, A. DUMBRELL, N. R. BAKER, T. LAWSON

11:00–11:30

tlawson@essex.ac.uk

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Stomata control the rate of CO_2 diffusion into the leaf, as well as water loss through transpiration, and can limit photosynthetic rates by up to 50% depending upon species and water availability. Stomatal conductance (gs) has been directly correlated with yield in wheat (Richards, 2002) and manipulation of stomatal traits identified as a potential area for yield improvements. In order for plants to use water efficiently, stomata must ensure an appropriate balance between CO_2 demands for photosynthesis and water loss through transpiration by correlating stomatal conductance with mesophyll photosynthetic rates. In a fluctuating environment, both mesophyll carbon assimilation and stomatal conductance respond to a number of environmental cues such as light, temperature and humidity in a hierarchical manner. However, responses are often not synchronized, with stomatal movements often unable to keep up with the more rapid photosynthetic adjustments. This lack of coordination between stomatal conductance and photosynthetic rate means that under naturally fluctuating environmental conditions carbon assimilation and water use efficiency can be far from optimal. Selecting genetic traits that result in phenotypic differences in the speed of stomatal responses would reduce the time periods in which plants lose water unnecessarily due to a greater stomatal conductance than is required for the potential carbon gain at that moment in time (e.g. during a shade fleck). In additional this would also enable greater carbon gain (through faster opening) when light conditions dramatically increase and A is initially limited by CO_2 diffusion. Here we present data examining the impact of a step change in irradiance on stomatal limitation, carbon assimilation and plant water use efficiency using traditional gas exchange measurements and a combination of imaging techniques. Examining the dynamics of photosynthetic carbon assimilation and stomatal conductance responses revealed different patterns across crop species. Further analysis of the relationship between properties of the stomatal response relative to the photosynthetic responses will provide a mechanistic understanding of species specific responses and the impact on plant water use efficiency.

Using soil drying as a regulative tool to enhance crop water use efficiency

S4.4

JIANHUA ZHANG

11:30–12:00

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School of Life Sciences and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, Hong Kong

We have been using soil drying as a regulative tool to improve crop water use efficiency at two fronts. The first is to exploit stomatal responses to soil drying when part of the plant root system is irrigated while the rest part is left drying. We have developed several irrigation practices such that partial root drying is maintained in the field for long term such that plant transpiration efficiency, termed as plant physiological water use efficiency, can be improved. In an extreme case of cotton production where all most all watering is delivered by irrigation, water use can be effectively reduced by 30%.

At another front, we used soil drying to enhance rice grain filling such that plant harvest index can be substantially improved and crop water productivity, termed as agronomic crop water use efficiency, is enhanced. Harvest index has been shown as a variable factor in rice production, especially in cases where whole plant senescence is unfavourably delayed. Such delayed senescence can delay the remobilisation of pre-stored carbon reserves in the straw and results in lower harvest index. A controlled soil drying can enhance whole plant senescence and therefore improve the remobilisation of pre-stored carbon reserve.

Stomatal responses to humidity and temperature are consistent with a vapor-phase mechanism

S4.5

KEITH MOTT

13:00–13:30

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Biology Department, Utah State University, 5305 Old Main Hill, Logan, Utah 84322-5305, USA

We recently proposed a new mechanism for stomatal responses to humidity and temperature that is based on vapor phase water transport to the guard cells. A simple closed-form expression containing three variables---gs^0, Θ, and Z---was derived based on this mechanism. The mechanism is unique because the values of Θ and Z are based largely on anatomical characteristics of the stomata and the leaf, and should therefore be approximately constant for leaves of the same species grown under the same conditions. The value gs^0 is dependent on environmental parameters such as CO_2 and light intensity and does not change with humidity. This suggests two testable hypotheses concerning the mechanism, First, all stomatal responses to humidity for leaves of a single species grown under similar conditions should be predicted by the model using the same values for Θ and Z, with only the value of gs^0 varying among leaves and with other environmental conditions. Second, the values of Θ and Z should vary in predictable ways with leaf and stomatal anatomy; specifically leaves with sunken stomata or stomata in crypts should show higher Z/Θ ratios than leaves with normally positioned stomata. Both of these predictions were supported by data for stomatal responses to humidity and temperature in leaves of *Nerium oleander*, *Pastinaca sativa*, and *Xanthium strumarium*.

From roots to stomata

S4.6

JOSETTE MASLE

13:30–14:00

josette.masle@anu.edu.au

Research School of Biology, College of Medicine, Biology and the Environment, The Australian National University, Canberra, 0200, Australia

Stomata act as gate-keepers for plants, allowing CO_2 entry while restricting water loss. Their number, aperture and distribution on the leaf surfaces are developmentally regulated but also extremely plastic in the face of environmental variations. Stomata do not develop nor respond to environmental variation in isolation. There is evidence of some stomata-mesophyll coordination within leaves at the developmental and biochemical levels, and the central role of hydraulic effects on stomatal dynamics is well known. Beyond changes in soil water content, stomata are extremely sensitive to changes in the physical and biological properties of the root environment. Our earlier work uncovered a direct signaling to leaves and stomata of mechanical stress at the root tip, which triggers conservative growth and water conservation, even in the absence of water stress. We have recently been investigating the underlying genetic pathways. We will discuss some of the results that identify novel networks for the concerted responses to stress, of roots, shoot apical meristems and stomata.

Optimisation of maximal stomatal conductance in subtropical vegetation under rising CO_2

S4.7

HUGO J. DE BOER[a], EMMY I. LAMMERTSMA[b], FRIEDERIKE WAGNER-CREMER[b], MARTIN J. WASSEN[a], ANDY F. LOTTER[b], DAVID L. DILCHER[c], STEFAN C. DEKKER[a]

14:30–15:00

h.j.deboer@uu.nl

[a]Department of Environmental Sciences, Utrecht University, Utrecht, the Netherlands; [b]Department of Physical Geography, Utrecht University, Utrecht, the Netherlands; [c]Department of Biology, Indiana University, Bloomington, USA

Data series are presented that show a consistent and significant reduction in maximal stomatal conductance (g_{smax}) of ~34% (+/- 12%) in nine Florida C3 species over the 100 ppm increase in atmospheric CO_2 concentrations [CO_2] of the past century. The studied species are common to subtropical ecotypes in Florida and included 5 angiosperms, 3 conifers and one fern. The cuticle material analysed originates from subfossil leaf fragments retrieved from well-dated young peat deposits, leaves stored in herbaria and modern leaves collected at various sites in Florida. Despite species specific strategies in adaptation of stomatal densities and geometries, all species displayed highly similar reductions of g_{smax} in response to rising [CO_2]. Based on these similar responses we hypothesised that the Florida species reduced g_{smax} in order to optimise carbon gain under the constraint of a physiological cost of water loss. To test our hypothesis, we used a model that simulates the optimisation of photosynthesis with minimal water loss by adjustments of g_{smax} under rising [CO_2]. Our model reproduced the observed stomatal adaptations of the Florida species and predicted that these adaptations will continue with further rising [CO_2]. These model results are discussed in relation to potential limits to (phenotypic) stomatal responses. The consequences of stomatal adaptations on transpiration and the hydrological cycle are indicated.

A new stomatal response: stress and recovery

S4.8

J. A. LAKE, M. STEVEN, K. SMITH, B. H. LOMAX

15:00–15:30

janice.lake@nottingham.ac.uk

The School of Biosciences, Division of Agricultural and Environmental Sciences, The University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK

Stomata respond to numerous abiotic stresses including drought, anoxia, ozone, salinity, UV, chilling: all of which have been known for decades. Here, we present the first new whole-plant stomatal response for over 20 years; resulting from ultra-high CO_2 concentrations in the soil environment. CO_2 (>25%) in the soil occurs in the context of potential pipeline leakage scenarios for the deployment of CCS pipelines to transport power-generated CO_2 to offshore reservoirs in the UK. However, high levels of CO_2 are concomitant with a reduction in available O_2. The experimental control suppresses O_2 by supplementing the rhizosphere with N_2, indicating that this root-to-shoot response is CO_2-specific. Extremely rapid (within 30 mins) wilting is the primary response. Furthermore, recovery from wilting is initiated without alleviation of the stress. The plant remains under stress despite recovery, with g_s and E lower than controls while photosynthesis is unaffected. These observations set the response apart from drought and anoxia which require alleviation of the stress or specialised cell types for tolerance. A new response carries huge implications for the possibility of manipulating WUE, one of the most pressing targets for future food security. Preliminary characterisation of the stomatal response to elevated soil CO_2 is presented.

Poster Abstracts

** S=speaker abstract; P=poster abstract*

Poster Abstracts

Poster abstracts are listed alphabetically by first author, presenting author is underlined.

P1 Stomatal functioning of fava bean in response to different closing stimuli influenced by preceding relative air humidity

S. ALI NIAEI FARD, U. VAN MEETEREN

Horticultural Supply Chains Group, Dept. Plant Sciences, Wageningen University, P.O. Box 630, 6700 AP Wageningen, The Netherlands

The guard cells signalling pathways are changed after prolonged exposure of plants to high relative air humidities (RH). Using *Vicia faba*, we have identified that exposure of leaves for 5 days to high RH generate a 'memory effect' in the guard cells, resulting in a higher rate of water loss during rapid desiccation, as compared to leaves not exposed to high RH. The rate of water loss was less sensitive to a decrease in relative water content (RWC) in high RH-grown plants. At a RWC of 90%, high RHgrown leaves kept 6 times higher water loss rate compared to moderate RH-grown leaves. The rate of water loss in high RH-grown leaves was not affected by exposure to light or darkness. We used average value of PSII efficiency (Φ_{PSII}) under non-photorespiratory conditions (2% O_2) as an indicator of stomatal closure. Φ_{PSII} was not decreased in high RH-grown leaves by exogenous application of ABA, but it tremendously decreased in moderate RH-grown leaves. Moreover, higher Φ_{PSII} was found in high RH-grown leaves after 90 min SNP (nitric oxide donor) application. Using epidermal strips in stomatal opening medium confirmed that stomata of high RH-grown leaves are less responsive to ABA, SNP and H_2O_2 compared to moderate-RH grown leaves. It is concluded that high RH disturb major parts in the broad bean guard cells signalling pathway.

P2 Guard cell autonomous ABA synthesis rescues *Arabidopsis thaliana* from drought stress

H. BAUER, P. ACHE, S. LAUTNER, J. FROMM, W. HARTUNG, K. A. S. AL-RASHEID, S. SONNEWALD, U. SONNEWALD, S. KNEITZ, N. LACHMANN, R. R. MENDEL, F. BITTNER, A. M. HETHERINGTON, R. HEDRICH

Molecular Plant-Physiology and Biophysics, Julius von Sachs Institute for Biosciences, Julius-von Sachs-Platz 2, 97082 Würzburg, Germany

Stomata are able to sense atmospheric humidity changes directly. During this process the stress hormone ABA opens SLAC1-type anion channels via the protein kinase OST1. Although we got a picture of fast ABA signaling, the mechanism behind guard cell humidity adaptation and regulation of ABA metabolism in response to dry air remains unknown. To search for guard cell genes associated with humidity-sensitive stomatal movement, Arabidopsis microarrays were used. In guard cells challenged with dry air or exogenous ABA, distinct mRNAs involved in ABA signaling were recognized. Comparing the OST1 and SLAC1 loss of function-affected guard cell transcriptome with low humidity and ABA-regulated genes in wild type plants (wt), a set of dry air response master genes was found. We then focused on the guard cell ABA biosynthesis. The molybdenum cofactor sulfurase ABA3 activates aldehyde oxidase AAO3, promoting the last step in ABA synthesis. When stressed by dry air, leaves of Arabidopsis aba3-1 mutants (impaired in ABA synthesis) wilted, while leaves of wt- and aba3-1-plants with ABA synthesis rescued in guard cells only, did not. This behavior indicates that guard cell autonomous ABA synthesis is required and sufficient to integrate the drop of humidity into ABA-triggered stomatal closure.

P3 Signalling in stomatal responses to biotic and abiotic stresses

G. BOURDAIS, S. ROBATZEK

The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

Stomata allow plants to balance gas exchanges necessary for photosynthesis, and water loss by regulating width of the pore. But, as natural opening, stomata are also entry points for bacterial invasion. Even if the importance of stomata in innate immunity against bacterial invasion have been described, the mechanism of guard cell signaling upon biotic stress remains unclear. We developed a high-throughput microscopy approach to analyse stomata response in large populations of genotypes upon flagellin and ABA treatment in order to build biotic and abiotic signalling networks. The principle of using a close system by looking at guard cells responses allows us to reveal new members of flagellin induced plant triggered immunity signalling pathway by avoiding gene redundancy that can occur at a plant level while analysing growth inhibition or ROS production. As an example, 4 out of the 44 members of the CRK family (Cysteine Rich Receptor-Like Kinases) which have been suggested to play important roles in the regulation of pathogen defence and programmed cell death show interesting results. In fact, *crks* impaired in flagellin stomata closure response were also more susceptible to *Pseudomonas* infection bringing new insight in both flagellin signalling pathway and CRK characterization.

P4 Hygroscopic leaf surface particles and the hydraulic activation of stomata

J. BURKHARDT[1], H. KAISER[2], S. PARIYAR[1], M. HUNSCHE[1]

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Hygroscopic particles on leaves are strong water vapour sinks close to stomata; they are ubiquitous and may reach the amount of leaf waxes. The influence of added or excluded particles on stomatal functioning was assessed. The apertures of 50 to 75 stomata of elder and bean leaves were recorded together with gas exchange during opening and closing reactions, before and after treatment with $NaNO_3$ or KH_2PO_4 particles, respectively. Both salts caused higher leaf conductance at the same degree of stomatal opening. Bean and sunflower plants were grown in ventilated greenhouses supplied with either ambient (AA) or filtered air (FA). Transpiration of FA plants was about 20-40% lower compared to AA plants. When CO_2 was doubled to 800 ppm, FA plants stabilized transpiration and increased photosynthesis, while AA plants stabilized photosynthesis and decreased transpiration. On hydrophobic tomato cuticles, NaCl and $NaClO_3$ particles underwent repeated deliquescence/efflorescence cycles within an environmental scanning electron microscope and spread out in dentritic form. This mechanism may eventually establish the 'hydraulic activation of stomata', i.e. continuous thin water connections linking apoplast and leaf surface along the stomatal walls and enabling bidirectional stomatal transport of liquid water, solutes, and hydraulic signals. Aerosols may therefore have important ecophysiological functions.

P5 Stomatal response of *Camellia sinensis* (L.) O. Kuntze to elevated carbon dioxide

H. I. U. CALDERA[1,2], W. A. J. M. DE COSTA[3], F. I. WOODWARD[1], J. A. LAKE[4], S. M. W. RANWALA[2]

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Camellia sinensis leaves are used to produce tea. Limited research has been conducted on the stomatal response of *C. sinensis* to elevated CO_2. This study determined the stomatal and related physiological responses of *C. sinensis* to elevated CO_2. Plants were grown under elevated (800ppm) and ambient (400ppm) CO_2 concentrations in controlled environment chambers for nine months. Stomatal density and stomatal index did not respond to elevated CO_2 and field-based studies confirmed the relative insensitivity of *C. sinensis* stomatal frequency to environmental change. However, stomatal conductance (g_s) decreased by 25%, suggesting an aperture level response. Guard cell length (GCL) showed a significant 3% decrease, implying that stomatal dimensions were more responsive to CO_2 than stomatal numbers in *C. sinensis*. There was no significant relationship between stomatal density and GCL. Net photosynthetic rate increased by 57% showing that CO_2 supply was not a limiting factor despite the lower g_s. A 15% reduction in leaf N at elevated CO_2 indicated photosynthetic down-regulation. Decreased g_s led to an increase in instantaneous water use efficiency (WUE). Palisade and spongy parenchyma thickness increased thereby facilitating higher photosynthesis. Thus, *C. sinensis* responds to predicted future increases in CO2 by adaptations in stomatal size and leaf anatomy leading to increased photosynthesis and WUE.

P6 Deep evolutionary origins of stomatal development

C. CHATER, A. CUMING, C. MCALISTER, D. BERGMANN, S. WALLACE, A. FLEMING, J. GRAY, D. BEERLING

Department of Molecular Biology and Biotechnology, Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK

Stomata in angiosperms are formed by asymmetric and symmetric divisions controlled by a suite of positive and negative regulators of cell development. Stoma-like complexes are found on ancient plant cuticle fossils and on the sporophytes of extant mosses and hornworts. It is not known, however, whether these are orthologous structures to tracheophyte stomata. Recent evidence suggests angiosperm-like molecular signalling and physiological responses in the stomata of the model moss *Physcomitrella*. To determine whether stomata are monophyletic, we investigated the role of several moss gene homologues to those involved in *Arabidopsis* stomatal development. Expression of moss genes homologous to SPEECHLESS, MUTE, and FAMA complement mutant Arabidopsis plants defective in those genes (McAlister & Bergmann, 2011). Here we investigate whether knocking out these genes in the moss will affect stomatal development. Furthermore, we explore the roles of moss genes similar to epidermal patterning factors 1 (EPF1 & 2) and the TOO MANY MOUTHS (TMM) receptor-like protein. Our data suggest that the mechanism of stomatal development has been broadly conserved across land plant evolution.

P7 The role of ethylene in regulating leaf age-dependent stomatal responses to ABA and soil drying

L. CHEN, S. WILKINSON, J. THEOBALD, I. DODD, A. BELIMOV, W. J. DAVIES

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Abscisic acid (ABA) is a key signal which regulates plant response to stress, particularly in regulating stomatal responses to drought. Early findings by Atkinson et al. (1989) suggest that older leaves lose stomatal sensitivity to ABA. Ethylene is produced in increasing amounts when plants exposed to abiotic stress. Recent studies indicate that ethylene can close stomata when the ABA levels are relatively low; but antagonize ABA induced stomatal closure such that they can remain open when ABA levels increase (Wilkinson and Davies, 2010). The work described here showed that older, more mature leaves lost their ability to close stomata in response to ABA treatments and soil drying followed by rehydration, while young mature leaves closed stomata more fully. Plants were pretreated with 1-methylcyclopropene (1-MCP) which can antagonize ethylene receptors, or soil inoculation with the rhizobacterium *Variovorax paradoxus* (which use ACC - the precursor of ethylene - as a carbon and nitrogen source) restored the ability to close stomata after soil drying-rehydration treatments, indicating ethylene is involved in the sluggish stomatal response to ABA in older leaves. Further work suggests that stomata of older leaves are more sensitive to ethylene compared to young leaves, explaining the relative insensitivity of stomatal closure to both ABA and drought/rehydration in older leaves.

ATKINSON, C. J., DAVIES, W. J. & MANSFIELD, T. A. 1989. Changes in Stomatal Conductance in Intact Ageing Wheat Leaves in Response to Abscisic Acid. *J. Exp. Bot.,* 40**,** 1021-1028.
WILKINSON, S. & DAVIES, W. J. 2010. Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant Cell and Environment,* 33**,** 510-525.

P8 Stomatal closure is associated with high *AtHXK1* expression in *Arabidopsis*

R. DAVID-SCHWARTZ[1], G. KELLY[1], N. SADE[2], M. MOSHELION[2], A. LEVI[3], V. ALCHANATIS[3], D. GRANOT[1]

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Arabidopsis hexokinase (*AtHXK1*) is a dual function enzyme mediating sugar sensing in addition to its enzymatic hexose phosphoryaltion activity. *AtHXK1*-mediated sugar sensing effects in Arabidopsis were obtained only in presence of high (5%-6%) exogenous glucose, thus limiting most studies to seed germination and seedlings development on sterile agar media. To overcome this obstacle, we re-transformed Arabidopsis plants with *AtHXK1* and searched for transgenic lines with high expression of *AtHXK1*. These lines exhibit sugar sensing effects when grown on soil under natural growth conditions without any external sugar, thus allowing analysis of adult plants. High expression of AtHXK1 reduced stomatal conductivity leading to an increase in leaf temperature. These results indicate a role for *AtHXK1* in the regulation of *Arabidopsis* stomata closure.

P9 Process-based model of stomatal conductance and its application to deficit irrigation in fruit trees

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Over the last decade it has been very demanded by scientists working on ecophysiology of leaf gas exchange a processes based model of stomatal conductance, similarly to the biochemical model of photosynthesis proposed by Farquhar et al. (1980). Buckley et al. (2003) proposed a hydromechanical model of stomata functioning which has been now adapted to be friendly applicable. The great advantage of this model is that his parameters have full physiological meaning: osmotic pressure, plant hydraulic resistance and sensitivity of guard cell to turgor (potentially ABA-related). The seasonal evolution of these parameters in an olive orchard under well-watered and deficit irrigation conditions have been estimated, and it was found to correlate well with independent estimations of these variables. Furthermore, it is proposed a quantitative analysis of the main limitations to stomatal conductance by hydraulic and non-hydraulic signals in a similar fashion to that proposed by Grassi and Magnani (2005).

P10 The physiological consequences of stomatal patterning and density in *Arabidopsis thaliana*

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[1]Department of Biology, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA; [2]Department of Global Ecology, Carnegie Institution of Washington, 260 Panama Street, Stanford, CA 94305, US A; [3]Howard Hughes Medical Institute

Genetic and cell biological mechanisms that regulate stomatal development are necessary to generate an appropriate number of stomata and enforce a minimum of one epidermal cell spacing between them. The ability to manipulate these processes in a model plant system allows us to investigate the physiological importance of stomatal patterning and changes in density, therein testing underlying theories about stomatal biology. Twelve *Arabidopsis thaliana* genotypes that have varied stomatal characteristics due to mutations or transgenes were analyzed in this study. Stomatal traits were used to categorize the lines and predict maximum tomatal conductance to water vapor (g_{max}) for individuals. Leaf-level gas-exchange measurements determined experimental g_{max} in addition to parameters for carbon assimilation, water-use efficiency (WUE), and stomatal responses to increasing [CO_2]. Genotypes with proper spacing (<5% of stomata in clusters) achieved experimental gmax values comparable to predicted g_{max} across a ten-fold increase in stomatal density, while lines with sufficient clustering (>5%) did not. Genotypes with clustering also had reduced photosynthetic activity and impaired stomatal pore closure. Intrinsic WUE had a negative log correlation with stomatal density, irrespective of clustering. Consequently, optimum stomatal function is dependent on maintaining proper spacing requirements and the physiological parameters controlled by stomata are strongly correlated with density.

P11 Ozone effects on stomatal responses to environmental parameters (blue light, red light, CO_2 and VPD) in three poplar genotypes and the study of gene expression on guard cells.

J. DUMONT, F. SPICHER, D. COHEN, N. NINGRE, P. MONTPIED, Y. JOLIVET, P. DIZENGREMEL, D. LE THIEC

INRA, Université de Lorraine, UMR1137 Ecologie et Ecophysiologie Forestières, IFR110 EFABA, F-54280 Champenoux, France

Tropospheric ozone acts as a phytotoxin which produces an oxidative stress in plants. Two ways of defense are used, either by preventing ozone input through the regulation of stomatal conductance, or by detoxifying ozone and ROS in cells. It is known that stomatal movements are altered by ozone. We performed fumigation experiment on three euramerican poplar genotypes (*Populus deltoides x Populus Nigra : 'Carpaccio', 'Cima' and 'Robusta'*), cultivated in pots in phytotronic chambers submitted to 120 ppb ozone or filtered air. We explored the effects of ozone on stomatal responses to four environmental parameters (blue light, red light, CO_2 and VPD) using Licor6400. We also find out using a porometer that the decrease of stomatal conductance due to ozone is earlier on the adaxial face than on abaxial face. Finally, to better understand these impacts, we studied ultrastructure of guard cells by TEM, stomatal density and size of stomata by SEM, and we performed X-ray microanalysis of guard cells mineral content. These approaches are coupled with the study on microdissected stomata of expression of genes involved in regulation of stomatal movements.

P12 Phylogenetic analysis of SLAC1 protein family structure, predicted function and conservation in land plants

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The *Arabidopsis thaliana* S-type anion channel SLAC1 has been shown to be responsible for rapid stomatal closure in response to various environmental factors. The molecular mechanisms involved in SLAC1 regulation have been extensive elucidated but to our knowledge there has been no attempt to study its role from ecological and ecophysiological perspectives outside laboratory conditions. In order to yield insight into the significance of SLAC1-dependent regulation in the stomatal responses under natural conditions and especially in trees, we have started to elucidate the presence and structure of SLAC1 protein family and related stomatal function in several plant species for which genome sequence information is available. The sequence information available suggests that *Populus* species may have lost the functionally important regulatory and structural features of SLAC1 and thus have altered ability to regulate their stomatal aperture, which is evident in measurements of stomatal function. We have conducted a phylogenetic analysis of SLAC1 family proteins in different plant species and the goal is to link the variation in protein structure and conservation to the differences in stomatal regulation between various plant species.

P13 CO_2 regulation of stomatal development by carbonic anhydrases

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How signals are perceived and transduced during the regulation of stomatal development by atmospheric carbon dioxide (CO_2) levels is not known. Currently one mutant, *hic*[1], has been demonstrated to show a de-regulation of CO_2-controlled stomatal development. We have isolated Arabidopsis thaliana carbonic anhydrase mutants which show an impaired stomatal movement response to shifts in atmospheric CO_2 levels[2]. Data will be presented showing that these plants exhibit, relative to wild type plants, a disruption of CO_2 control of stomatal development. We investigated the molecular and genetic mechanisms mediating CO_2-regulated stomatal development in these mutants. We used cell lineage-specific markers, confocal microscopy and mutants in the stomatal development machinery, to characterize the CO_2-controlled stomatal development phenotype in Arabidopsis. Complementation studies with heterologous carbonic anhydrase expression in our mutants indicate that CO_2 control of stomatal development functions via cell-cell signaling mechanisms occurs during a defined phase of stomatal cell lineage specification. We conducted CO_2-dependent systems experiments in an attempt to capture cell-cell signaling candidates. The results of these analyses and phenotypes of candidate genes involved in the CO_2-control of stomatal development will be presented along with a model for signaling during organ development and cell fate specification.

References

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P14 Characterization of drought tolerance in the rice G-protein α subunit mutant, *d1*

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Rice is one of the three major cereal crops and is a staple food for more than a third of the world's population. The rice dwarf mutant, *d1*, contains a non-functional RGA1 gene, encoding the GTP-binding α-subunit of the heterotrimeric G protein. This mutant was originally isolated as a spontaneous mutant with reduced height and shorter, erect, thicker, broad, dark green leaves, compact panicles, and short, round grains. We have examined the physiological responses of the *d1* mutant to mild and severe water limitation during both vegetative and reproductive development in comparison with its background Nipponbare line. The *d1* plants present higher photosynthetic rates, stomatal conductance, and ψleaf than wild type during both mild and severe water limitation, with resulting higher reproductive yield.

Through quantification of water use efficiency, A-Ci and light response curves, chlorophyll fluorescence to discern between photochemical and non-photochemical quenching, stomatal densities and other leaf anatomical features, and evaluation of stomatal and non-stomatal limitations, we are dissecting the parameters that confer drought tolerance on the d1 α-subunit mutant.

P15 Effects of overirrigation on stomatal behaviour and plant hormone balance of tomato (*Solanum lycopersicum* cv. Ailsa Craig)

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Although the effects of soil inundation on stomatal behaviour have been well, effects of suboptimal soil aeration caused by overrirrigating pot plants has not, despite likely commercial significance. By automatically scheduling irrigation according to soil moisture thresholds (Delta-T SM200 soil moisture sensors connected to a GP1 datalogger), this work explored the possibility that overirrigating the rootzone could alter root-to-shoot signalling to induce stomatal closure. Tomatoes were grown in a peat-based substrate, and exposed to different irrigation treatments by varying the number of drippers in the pot (1 deficit irrigated, 2 control, 3 overirrigated). Three weeks of overirrigation decreased growth (biomass, height and leaf area). Stomatal conductance (gs) of the 2nd leaf (from the plant base) decreased, but gs increased and was even higher than the control for younger leaves. Slightly higher levels of abscisic acid (ABA) were found in xylem sap and 3rd leaf of overirrigated plants when compared to the control. Changes in leaf hormonal balance and/or delivery of root-synthesised signals might be responsible for stomatal closure and recent developments in ethylene measurement technology (fluid gas analyser to detect rootzone ethylene production) will help understand those mechanisms.

We thank the Horticulture Development Council (HDC) for support of this work.

P16 Do veins and stomata patterns in angiosperms obey general rules?

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Plants have evolved a huge variety of leaf forms, vein structures, stomata dimensions and behavior. Here we investigate whether general principles might be evoked to explain the distribution of leaf veins and stomata across species. We analyzed 20 angiosperms grown in different environments (alpine, middle latitude, tropics). We took 5-15 mm^2 samples from the proximal, middle and distal part of a mature leaf of each species. Stomata imprints were collected using acrylic varnish on transparent tape; veins were highlighted by chemical treatment using NaOH. With a semi-automatic GIS-based procedure we identified the smallest leaf area completely enclosed by veins as a loop and we measured area, contour, stomata number, pore lengths for each loop. About 60-700 loops were measured for each species. Stomata density was species-specific (ranging 50-350 pore/mm^2) and seemed not to change with position on the leaf nor with the order of surrounding veins. Notably, in each loop the number of stomata scaled almost isometrically with loop area (exponents ranging 0.9-1.0) (i.e. $N_s \sim A^1$) and loop contour (0.9-1.2) (i.e. $N_s \sim L^1$) in all measured species; moreover the scaling coefficients appeared related to pore length. This would suggest that a simple scale-free pattern has evolved regardless of species or environment.

P17 Identifying genetic variation in crop water use efficiency in durum wheat and Maize

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Crops are often subjected to multiple periods of soil and atmospheric water deficit during their life cycle which reduce plant growth and yield and decrease food security. Climate change predictions suggest an increasing scarcity of water for agriculture in the future and so it is becoming increasingly important to understand genetic and environmental determinants of crop water use efficiency (WUE). One of the first responses of a plant to water stress is to close stomata to limit water loss to the atmosphere, but this restricts CO_2 uptake by the leaf, thus limiting photosynthesis and biomass accumulation. The EU **DROPS (Drought Tolerant Yielding PlantS)** project is developing novel methods and strategies aimed at yield maintenance under fluctuating water deficit and enhanced plant water use efficiency. Panels of 100 durum wheat and maize genotypes were grown on a phenotyping platform in a controlled environment glasshouse to investigate relationships between WUE, phytohormone (ABA, ethylene, IAA) concentrations and their interactions, and environmental variables. Heritabilities of WUE and its components were calculated, as a prelude to association genetics to identify QTL for WUE.

P18 Effects of mesophyll apoplastic solution on stomatal responses to CO_2

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We modified the microscopic observation system of Mott et al. (2008) to observe stomata under controlled conditions. Our study using *Commelina communis* revealed that responses of stomata in the epidermal strip placed on the buffer-containing gel were not sensitive to CO_2. When the epidermal strip was placed on the exposed mesophyll, however, the CO_2 sensitivity became comparable to that in the leaf segment. We also revealed that mesophyll photosynthesis played the main role for inducing the stomatal opening by red light at low CO_2. These results suggest that the "mesophyll signals" control stomatal responses to CO_2. We tested whether mesophyll signals were gaseous. When the contact between the epidermis and the mesophyll was blocked by inserting a donut-shaped polyethylene spacer (thickness 50 μm), the responses of stomata just above the donut hall were not sensitive to CO_2. When inserting a donut-shaped cellophane spacer (thickness 50 μm), CO_2 responses just above the donut hall became comparable to those in the leaf segment. It is suggested that mesophyll signals move to the epidermis via the aqueous phase in the apoplast. We are now trying to extract the apoplastic solution from the mesophyll for identifying the mesophyll signals.

P19 Stomatal responses to soil water deficit in annual and perennial crops

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The relationship between stomatal conductance (g_s) and leaf water potential (LWP) was determined for several crop species grown under different levels of water deficit. Experiments usually consisted in subjecting plants of kiwifruit (*Actinidia deliciosa*, cv. Hayward), tobacco (*Nicotiana tabacum*, Burley cv. 104), olive (*Olea europaea* L., cv. *Frantoio and Leccino),* apricot *(Prunus armeniaca,* cv. Amabile Vecchioni)*, grapevine (*Vitis vinifera, cv. Grenache) to cycles of water stress followed by relief of stress under conditions of Mediterranean climate. The leaf water potential was measured by the pressure chamber technique, g_s by infrared gas analysis. Apricot and kiwifruit showed a rapid decrease in g_s to decreasing soil moisture and their stomata were virtually closed at pre-dawn LWP less than –1 MPa. Grapevine also showed a drastic decrease in g_s when LWP decreased, whereas g_s of tobacco and olive remained relatively high over a wide range of LWP values. Varietal differences in the stomatal response to water deficit were evident in olive, apricot and grapevine plants. The different response of these five species cannot be attributed to differences in stomatal density. Differences in stomatal behaviour are important features of resistance strategies developed by these species to cope with periods of drought.

P20 Brassinosteroids regulate stomatal development through inhibition of BIN2- mediated phosphorylation of SPEECHLESS

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Stomatal formation is regulated by multiple developmental and environmental signals, but how these signals are integrated to control this process is not fully understood. The activity and stability of the transcription factor SPEECHLESS, essential for stomatal development, is negatively by ERECTA family and TMM receptors through MAP kinase (MAPK)-mediated phosphorylation. We have found that SPCH activity is also modulated by the GSK3/SHAGGY-like kinase BIN2, which functions as a negative regulator of brassinosteroid signaling. BIN2 phosphorylates SPEECHLESS in residues overlapping those targeted by MAPKs, as well as in four residues in the N-terminal region of the protein outside the MAPK target domain. These phosphorylation events antagonise SPCH activity and limit epidermal cell proliferation. Conversely, brassinosteroid-mediated inhibition of BIN2 activity stabilizes SPCH and triggers excessive stomata and nonstomatal cell formation. Thus, SPEECHLESS serves as an integration node for the ERECTA/TMM and BR signalling pathways to control stomatal and epidermal development.

P21 Genetic screening of stomatal mutants from SALK population using a novel infrared imaging system

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A Precisely Controlled Environmental Chamber (PECC) was developed specially for screening stomatal mutants, which consists of three parts including treatment chamber, infrared camera system and environmental control unit. A 150mm diameter window made from optical Germanium was embedded on the treatment chamber so that infrared ray of plants could reach the infrared camera outside the chamber with high transmittance. The environmental control unit maintains humidity, CO_2 concentration and temperature in the treatment chamberi.e 10~85%±5% relative humidity, 100~1000ppm (5%FS) and 10~50±0.2 degree Celsius. SALK mutants have been extensively used for the functional analysis of *Arabidopsis* genes, but there is no application in genetic screening of stomatal mutants. Using this newly-invented PECC, we carried out a series of genetic screening on SALK population and identified some new humidity and CO_2 stomatal mutants. Currently, vigorous functional studies are underway.

P22 Immunohistochemical analysis of activation status of the guard-cell plasma membrane H^+-ATPase in response to blue light

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Blue light receptor phototropins (phot1 and phot2) activate the guard-cell plasma membrane H^+-ATPase through phosphorylation of a penultimate threonine (Thr) in C-terminus, and mediate stomatal opening. So far, activation status of the plasma membrane H^+-ATPase has been examined using the guard cell protoplasts (GCPs). However, preparation of GCPs from Arabidopsis for this purpose requires over 5,000 rosette leaves and takes over 8 hours. In this study, we established the immunohistochemical method using a specific antibody against phosphorylated penultimate Thr of the H^+-ATPase to visualize activation status of the guard-cell plasma membrane H^+-ATPase in the epidermis. Blue light-induced phosphorylation of the guard-cell plasma membrane H^+-ATPase was detected in wild type, but not in phototropin double mutant (*phot1 phot2*). We also found that physiological concentrations of phytohormone ABA completely inhibit phosphorylation of the H^+-ATPase. To clarify inhibitory mechanism of the H^+-ATPase phosphorylation by ABA, we examined phosphorylation status of the guard-cell H^+-ATPase in ABA insensitive mutants, such as *abi1-1*, *abi2-1*, and *ost1-2*. The results revealed that inhibition of the H^+-ATPase phosphorylation by ABA is mediated by an early ABA signalling pathway including PYR/PYL/RCAR-PP2Cs-SnRK2s.

P23 Stomatal response of *Nothofagus fusca* (New Zealand red beech) to CO_2 availability and nutrient status

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In order to validate models of plant response to CO_2 change over the Pleistocene, it is necessary to quantify plant structural response, especially leaf cuticle micro-phenology, to CO_2 variability. Quantification of such response necessitates a three-pronged approach: herbarium studies to quantify changes from c. 315-400 ppm, the study of sub-fossil leaf material, and growth experiments with Pleistocene flora at extreme CO_2 levels (e.g., < 200 ppm). As CO_2 level rises, stomatal conductance decreases in order to limit water loss and plant water-use efficiency increases. At low atmospheric CO_2 levels stomatal conductance decreases to increase CO_2 absorption. Variability in transpiration rates may have a significant impact on plant physiology, vegetation community structure, and the local hydrology. The response limits of C3 vegetation to glacial or future CO_2 levels, however, are not yet well known. Results from a growth study with *Nothofagus fusca* at low, ambient, and high CO_2 levels will be presented. We demonstrate a clear trend in stomatal conductance response at low, ambient, and high CO_2 levels, and postulate that the response limits for stomatal parameters in C3 plants may be reached earlier than current models suggest.

P24 Stomatal responses to various stimuli are affected in plants with modulated ERD15 Expression

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Recently, we have found that ERD15, a protein earlier shown to affect both abiotic and biotic stress responses, is also centrally implicated in plant stomatal regulation. Plants overexpressing ERD15 and plants where ERD15 expression is driven by a strong guard cell specific promoter have constitutively high stomatal conductance and impaired stomatal closure in response to darkness, elevated CO_2, ozone treatment and ABA. Plants with decreased ERD15 expression show increased stomatal closure in response to these stimuli.
ERD15 has a PAM2 motif and we have shown strong interaction with Arabidopsis polyA-binding proteins PAB2 and PAB4, suggesting a role for ERD15 in RNA metabolism and/or translational regulation. This is further supported by our observation of similar impairment in stomatal responses in *pab2/4* double mutants when compared to ERD15 overexpressors. In order to elucidate the function of ERD15 in stomatal signalling, we are currently characterizing changes in gene expression in plants with modulated ERD15 expression. Unravelling the molecular mechanisms of ERD15 in guard cells could reveal novel counterparts in stomatal regulation and would contribute to current understanding of plant stomatal regulation.

P25 The identification of novel guard cell transcripts by tiling array

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Transcriptomics has been a useful tool to identify genes involved in guard cell signalling and development. Previous microarray experiments have used epidermal fragments, protoplasts or the use of Arabidopsis mutants that have much increased or reduced numbers of stomata, to enrich for stomatal transcripts. This has allowed the characterisation of genes involved in both guard cell development and aperture control by the isolation of mutations. These experiments relied on previously known expression information either from EST data or gene prediction programs. We have used a whole genome tiling array to provide an unbiased approached to the identification of new guard cell genes. We used RNA from the *epf2* mutant, which has increased stomatal lineage cells and compared them to wild type controls and also compared wild-type to EPF2 overexpressors, the latter of which have reduced numbers of stomata. This has identified potential new protein coding genes that were not on previous arrays, new unannotated transcripts, non-coding RNAs, pseudogenes and natural antisense genes.

P26 A closer look at stomatal oscillations

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Stomatal control of transpiration and CO_2-exchange relies on feedback-loops formed by guard cells being sensitive to water loss and intercellular CO_2-concentration. Oscillations of stomatal aperture indicate instabilities of these feedback loops. Direct aperture observations on *Sambucus nigra, Vicia faba, Helianthus annus, Avena sativa* and *Rhoeo discolor* showed that during oscillations in all species stomata cycled between the closed and slightly opened state. Guard cells intermittently deflated beyond the threshold of complete stomatal closure. It is suggested, that the discontinuous change in feedback gain at the point of opening/closing together with hydropassive acceleration of turgor movements promotes oscillatory responses. The divergence between oscillations of individual stomata in dependence on the distance was measured. While immediately adjacent stomata were tightly coordinated, phase-shifts and difference in frequency led to increasing discoordination at a spacing above 1 mm. This agrees well with earlier findings that a pores transpiration may trigger closure of other stomata in a distance up to 0.5 mm, thereby spatially coordinating movements. Measurements of changes in leaf extension as a proxy for epidermal turgor fluctuations confirmed that the detailed timing of opening and closing and hydraulic events agrees with the assumed causal role of hydraulic disturbances in the development of stomatal oscillations.

P27 Sugar signals within guard cells stimulate stomatal closure via hexokinase and abscisic acid

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Water is the major factor limiting the growth and development of many land plants and stomata, composed of two guard cells, are the chief gates controlling plants water loss. Many environmental and physiological stimuli control stomatal opening, but they all function through the regulation of guard-cells osmolarity. Increased guard-cells osmolarity leads to the opening of the stomata and decreased osmolarity causes the stomata to close. The prevailing paradigm is that sucrose acts as an osmoticum in the guard cells, thereby contributing to the opening of the stomata. In contrast, we show here that sucrose closes stomata via a nonosmotic mechanism. Furthermore, our results show that the guard cells' response to sucrose is dependent on the sugar-sensing enzyme hexokinase, which triggers the abscisic acidsignaling pathway within the guard cells, leading to stomatal closure. These findings reveal a sugar-signaling mechanism within guard cells and support the existence of a feedbackinhibition mechanism that is mediated by a product of photosynthesis - sucrose.

P28 Analysis of quantitative trait loci for stomatal density of flag leaf and its relationship to stomatal conductance and photosynthesis rate in rice

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Photosynthesis is a fundamental trait for crop improvement and is directly related to stomata function. However, little is known about genetic basis of stomata development in rice. In this study, we conducted genetic analysis of stomatal density, which is expected to become an index of photosynthetic ability. Firstly, we made panoramic images of flag leaf surface from captured scanning electron microscope (SEM) images, and secondly, counted total number of stomata and average number of stomata between small vascular bundles using newly developed software. The high-yielding *indica* rice cultivar Takanari has a higher stomatal density than the *japonica* rice cultivar Koshihikari. Quantitative trait locus (QTL) analysis derived from a cross between Koshihikari and Takanari revealed that the stomatal density QTL with the largest effect was located on chromosome 6. To confirm the effect of this QTL, we surveyed stomatal density in a series of chromosomal segment substitution lines (CSSLs) with the corresponding segment form Takanari. By using a series of CSSLs, this QTL was mapped within a region of about 3.3 Mbp, and showed increased stomatal density as well as stomatal conductance compared to those in Koshihikari. These results imply a causal association between stomatal density and leaf photosynthesis rate.

P29 The *gaping stomata* mutant in *Arabidopsis thaliana*

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The *gaping stomata* (*gps*) mutant was selected during a random mutagenesis screen designed to isolate mutants with a stomatal phenotype. The *gps* mutant is characterized by having significantly increased stomatal apertures under standard light conditions. This is coupled with increased water loss as determined by water loss assays, whilst thermal imaging results show that *gps* mutants are cooler under both day and night conditions compared to control plants. The responsiveness of the mutant stomata to various stimuli was investigated in a bioassay setup using epidermal peels. The stimuli tested were light, darkness, abscisic acid (ABA), external calcium (Ca^{2+}), carbon dioxide (CO_2), auxins and a nitric oxide (NO) scavenger (cPTIO). Stomata of the mutant showed normal responses to most stimuli, with the exception of auxins. Addition of either indole-3-acetic acid (IAA) or α-naphthalene acetic acid (NAA) was unable to partially inhibit closure during a light to dark transition even at high concentrations (100μM). Interestingly, the mutant develops longer root hairs, which might compensate for the increased water loss observed.

P30 Dark and Disturbed or Sunny and Bright: A new approach to determine early angiosperm habitat

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Whether the earliest angiosperms functioned with high or low photosynthetic gas exchange capabilities remains a key debate in the on-going research into angiosperm origins. The high gas exchange hypothesis supports the notion that the first angiosperms existed as opportunistic weedy species favoured in exposed sunny environments. Conversely, the low gas exchange hypothesis would infer that the earliest angiosperms existed in understory forest canopies with low transpirational demands. We have devised a novel method to test these competing hypotheses through developing a leaf energy balance model capable of predicting leaf temperature as a function of air temperature, radiation load and stomatal conductance. The modelled solution of leaf temperature will help to determine the environmental tolerance of the early angiosperms and whether – given that their low stomatal conductance would limit their transpirational cooling capacity - the earliest angiosperms could have survived the exposed sunny environments of the Aptian when temperatures would have ranged from 38-43^{0}C. Furthermore, full stomatal closure would likely occur at midday so regardless of baseline conductance, whether the small size of early angiosperms leaves was enough to avoid heating to lethal temperatures is fundamental as to whether the angiosperms could have survived out of the shade.

P31 Reconstructing the genome size of early angiosperms.

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Darwin in his often quoted letter, dated 22nd July 1879 to JD Hooker remarked that "…*the rapid development, so far as we can judge, of all the higher plants* [angiosperms] *within recent geological times is an abominable mystery*". Today some 130 years later scientists are still grappling with this key question. Recent sequencing work suggest that the angiosperm diversification event was accompanied by successive whole genome duplication events, suggesting that polyploidy may have played an important role in this diversification event. The relationship between cell size and genome size is well known and has been used to reconstruct the palaeogenome size of a range of disparate fossil animal groups. Recent experimental work using *A. thaliana* has shown that the relationship between guard cell length (GCL) and genome size is independent of environment, with GCL showing minimal variation when exposed to a wide range of ecologically and geologically relevant environmental perturbations (e.g. CO_2, drought, UV-B radiation).
This presentation will outline how through the integration of fossils and experimental data advances are being made in this area, leading to the reconstruction of angiosperm genome size as they radiated.

P32 Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1

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S-type anion channels are direct targets of abscisic acid signaling and contribute to chloride and nitrate release from guard cells, which in turn initiates stomatal closure. SLAC1 was the first component of the guard cell S-type anion channel identified. However, conductance in guard cells of *Arabidopsis* SLAC1 mutants still exhibited S-type anion channels that predominantly permeate nitrate anions. We found that the SLAC1 homologue 3, SLAH3, was also present in guard cells and that co-expression of SLAH3 with the Ca^{2+}-dependent kinase CPK21 in *Xenopus* oocytes, mediated nitrate-induced anion currents. CPK21- dependent SLAH3 phosphorylation and activation was blocked by ABI1, a PP2C protein phosphatase negatively regulating the ABA-signaling pathway in guard cells. Using in vitro kinase assays we could reconstitute the fast ABA signaling pathway leading to SLAH3 activation. Therein ABA perception by the complex between ABA receptors of the RCAR/PYR/PYL family and ABI1 leads to the release of CPK21 from inhibition by ABI1. An increase of the cytosolic Ca^{2+} concentration further promotes the phosphorylation of SLAH3 by CPK21. Thus, the identification of SLAH3 as the nitrate-, calcium- and ABAsensitive guard cell anion channel provides new insights into the nature and relationship between stomatal drought stress control and nitrate metabolism and -signaling.

P33 Hydraulic limits to maximum transpiration

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At the leaf level, transpiration is controlled by stomatal aperture, but the water supply to the leaves depends on the xylem hydraulic conductivity and is driven by the water potential gradient between the soil and the leaves. Large gradients provide a stronger driving force stimulating transpiration, but as xylem water potential becomes more negative, the hydraulic conductivity decreases due to cavitation. Hence, there is a tradeoff between hydraulic efficiency and driving force, resulting in maximum transpiration rates at intermediate values of water potential. On the one hand, reaching this theoretical limit would allow fully exploiting available water resources, maintaining leaves hydrated, and sustaining carbon uptake. On the other hand, stomatal regulation may prevent plants from reaching this limit, and save water. Accordingly, whether plants across the globe actually transpire at the maximum theoretical rate is explored. We address this question using a minimalist model of plant hydraulics, and show that indeed maximum transpiration rates are reached across biomes and over a wide range of plant sizes. These results suggest that plant xylem and stomata co-evolved to meet peak transpiration, thus sustaining carbon uptake while avoiding tissue damage. These results also explain the observed strong coordination between liquid- and gas-phase conductances.

P34 High transpiration mutant *bdg* identified in a screen showes increased cuticular conductance and permeability

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We developed a screen to find genes involved in the regulation of transpiration. Stomata and cuticle are key players in water loss from the plant to the atmosphere. Therefore genes involved in the development and regulation of stomata and cuticle are of high importance in plant transpiration. We screened for mutants with low leaf temperature, fast wilting and high water loss. A mutant allele of BODYGUARD (BDG) was identified with this method. BDG is a putative hydrolase involved in the biosynthesis of the cuticle. *bdg* showed increased cuticular conductance and cuticular permeability to toluidine blue. Full-length *BDG* promoter expressing GUS and GFP was found active in developing cuticle-covered tissues (nodes, apical buds, siliques), but unexpectedly also in embryo, leaf vasculature and roots. *bdg* mutant seeds revealed a decrease in an embryo-specific fatty acid monomer.
Finally, this study confirmed that BDG is expressed in cuticle-specific tissues, but it is also expressed in other plant tissues such as embryo, vasculature and roots. This suggests BDG role in suberin biosynthesis.

P35 Guard cell plasma membrane H^+- ATPases: highly regulated proton pumps to control gas exchange

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Guard cells provide an ideal system to elucidate early events in higher plant signal transduction. Up today, several key guard cell ion channels have been proposed to function as important signal transducers and mediators of stomatal movements. During stomatal opening under favourable conditions, activation of plasma-membrane-localized proton pump, H+-ATPase, establishes a negative membrane voltage that drives the uptake of K+. For stomatal closure, anion channels that depolarize the membrane are activated, setting conditions for long-term K+ and anion efflux. The question of whether deactivation of the proton pump is needed for stomatal closure, or whether the activation of anion channels is sufficient to sustain the membrane depolarization necessary to drive K+ efflux, remains largely unsolved. In this context, using a multidisciplinary approach involving physiology, electrophysiology, molecular biology, biochemistry and molecular genetics, the roles of the three major isoforms of the plasma membrane H+-ATPases expressed in guard cell, AHA1, AHA2 and AHA5, are currently investigated in the control of the membrane potential in response to environmental stimuli and abiotic stress. Our recent data on the functions of these 3 isoforms in the stomatal movement regulation will be presented and their role in guard cell signalling will be discussed.

P36 Differences in the responses of guard cells to ABA and water stress across vascular plant lineages

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Central to the control of seed-plant stomatal movement is the phytohormone abscisic acid (ABA); however differences in the sensitivity of guard cells to this ubiquitous chemical have been reported across land plant lineages. Using a phylogenetic approach to investigate guard cell control we examined the diversity of stomatal responses to endogenous ABA and leaf water potential during water-stress. We show that although all species respond similarly to leaf water deficit in terms of enhanced levels of ABA and closed stomata, the function of fern and lycophyte stomata diverged strongly from seed-plant species upon rehydration. When instantaneously rehydrated from a waterstressed state, fern and lycophyte stomata rapidly reopened to pre-droughted levels despite the high levels of endogenous ABA in the leaf. In seed-plants under the same conditions, high levels of ABA in the leaf prevented rapid reopening of stomata. We conclude that endogenous ABA synthesised by ferns and lycophytes plays little role in the regulation of transpiration, with stomata passively responsive to leaf water potential. These results support a gradualistic model of stomatal control evolution, offering new opportunities for molecular and guard cell biochemical studies to gain further insights into stomatal control.

P37 Combined chlorophyll fluorescence and thermal imaging system for assessment of stomatal responses that promote water use efficiency

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Changing climate and a growing global population emphasise the importance of selecting crops that are more water use efficient; maximising photosynthetic carbon gain while minimising water lost via the stomata. Combined chlorophyll *a* fluorescence and thermal imaging allows a rapid, non-invasive assessment of photosynthetic performance and stomatal behaviour to be studied spatially through time. Using the data from these two approaches provides a novel technique for imaging plant water use efficiency.

P38 Dynamic cells need dependable energy

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One of the first steps in stomatal opening is activation of plasma membrane proton pumps. These proton pumps require energy in the form of ATP and it is thought that this energy requirement is met by oxidative phosphorylation of carbohydrate-derived organic acids in the mitochondria and photophosphorylation in the chloroplasts. Oxidative phophorylation appears to be the dominant pathway and high respiration rates have led to descriptions of 'heterotrophic features' in guard cells. However, flexibility means that photophosphorylation can easily compensate when oxidative phosphorylation is disabled, and guard cells have also been shown to exhibit disproportionately high rates of photophosphorylation relative to carbon fixation. Here we report on a previously unsuspected guard cell metabolic pathway that helps supply an 'energy boost' for speedy opening in the early morning when energy stores are low.

P39 *Arabidopsis thaliana* single mutants in proteins involved in calcium sensing and ABA transport generally show intact stomatal closure responses to environmental stimuli

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Key endogenous factor triggering stomatal closure is the plant hormone abscisic acid (ABA), whereas second messengers as Ca^{2+} also function in ABA signaling. The exact role of cytoplasmic Ca^{2+} in stomatal reactions is, however, still obscure. Here we present the stomatal responses to environmental stimuli of mutants defective in 1) different proteins involved in Ca^{2+} sensing: calcium-dependent protein kinases CPK21 and CPK23, regulating the activity of guard cell slow anion channel; SnRK2-interacting Calcium Sensors SCS1 and SCS2 and calmodulinelike Ca^{2+}-binding proteins CML23/CML24; 2) ABC transporters involved in ABA transport (AtABCG25 and AtABCG40). The stomatal reactions were studied using a whole-plant gas exchange system. The steady state stomatal conductance of *atabcg25* and *cml23/24* was lower compared to wildtype Col-0. The stomatal closure, in response to darkness, elevated CO_2, reduced air humidity and O_3-pulse, was generally not impaired in these mutants. Only CPK23 was to a minor extent involved in stomatal responses to elevated CO_2 and humidity and AtABCG40 and CML23/CML24 in O_3 response. Due to a multitude of plant proteins involved in Ca^{2+} signalling and also in ABA transport (other ABC transporters, e.g. AtABCG22), it is likely that single mutants do not show different *in vivo* stomatal responses compared to wildtype.

P40 Requirement of ABA-PYR/PYL-PP2C-OST1-SLAC1 signaling module for stomatal closure triggered by environmental cues

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Timely stomatal closure is crucial for plant survival during drought and air pollution episodes. Rapid stomatal closure induced by environmental stimuli, such as elevated CO_2, reduced air humidity, darkness and ozone requires phosphorylation of guard cell plasma membrane anion channel SLAC1 by protein kinase OST1. Activation of OST1, in turn, requires sequestration of PP2C protein phosphatases by the PYR/PYL receptor proteins. To address the role of this signaling mechanism for environmental stimuli-induced stomatal closure we have used plants with deregulated PP2Cs, triple knock-out mutant of PP2Cs and quadruple, pentuple and sextuple knock-out mutants of PYR/PYLs. These experiments suggest that PYR/PYL-dependent inhibition of PP2Cs resulting in OST1 and SLAC1 activation is essential for rapid stomatal regulation in response to ozone and reduced air humidity, whereas PYR/PYL proteins seem to function in a dose-dependent manner. There is also a clear inhibition of darkness and CO_2-induced stomatal signaling However, most of analyzed mutants, except only *slac1* and *ost1*, showed weak but clear CO_2- and darkness induced responses, suggesting the presence of alternative, PYR/PYLindependent activation of OST1. Also preliminary characterization of two new mutants with severely impaired CO_2- and ozone-induced stomatal closure will be presented.

P41 Evolution of Stomatal Responses to ABA and CO_2

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Stomata regulate CO_2 uptake and water loss in vascular plants by controlling the aperture of microscopic pores. The most ancient, and non-vascular, plant lineage to form stomata is the mosses which have a ring of stomata around the base of their sporophyte structures. We have recently demonstrated that the apertures of these moss stomata close in response to ABA and use a similar mechanism to that of vascular plants, involving the protein kinase OST1. Recent work by others has shown that the vascular plant stomatal aperture response to CO_2 also requires OST1. Here we present evidence suggesting that signalling components similar to OST1 and ABI1 are functional in ancient plant stomatal responses to both ABA and CO_2. Expression of moss genes homologous to vascular plant *OST1* or *ABI1* was able to restore ABA- and CO_2-induced stomatal aperture responses to vascular plants lacking these components. Our experimental evidence suggests that stomatal ABA and CO_2 signalling responses both evolved before the last common ancestor of mosses and flowering plants over 410 million years ago.

P42 Insect-induced stomata attenuate sink strength and enhance parasite fitness

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We observed that parasitism of grape leaves by phylloxera induced adaxial stomata where only abaxial stomata typically occur. For plant hosts, parasites reduce productivity by altering cell fate, changing sink/source identity, and reducing defenses through dramatic modifications of host behavior, physiology, and morphology. Because these changes are often linked to parasite fitness, they are hypothesized to serve as an extension of the parasite's phenotype. We tested the hypothesis that induced stomata enhance parasite fitness, and then examined the gall transcriptome to test if manipulation of carbon metabolism was regulated globally at the gene level. Induced stomata assimilated labeled CO_2 and the adjacent gall sequestered the labelled assimilate. This occurred concurrent with global reconfiguration of gene expression from an autotrophic to a heterotrophic profile and corresponding shifts in downstream secondary metabolism transcription. As a result, we provide physiological and genomic evidence in support of the extended phenotype hypothesis that phylloxera induces stomata to enhance fitness, and globally manipulates plant genetics to enhance sink strength. To our knowledge this is the first account of functional, insect induced stomata in nature.

P43 Ontogenetic priming of stomatal control in *Arabidopsis* leaves through gradual exposure to low humidity

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In plants, stomata form flexible pores at the leaf surface which facilitate CO_2 entry for photosynthesis at the expense of water loss. To save water, plants evolved complex signalling mechanisms that close stomata at night when photosynthesis is impossible and in response to abscisic acid (ABA), a major drought hormone. Using reverse genetics, electron microscopy, and physiological assays in isolated epidermis and *in planta*, we demonstrate that stomata of *Arabidopsis thaliana* are unexpectedly passive in the young leaves and acquire sensitivity to ABA and darkness during leaf ontogeny. On the other hand, stomata of adult leaves remain insensitive to ABA when leaves are entertained at high atmospheric humidity, and recover ABA sensitivity when exogenous ABA is applied on the leaves the day prior to the assay. This suggests that lower humidity increases ABA level to a threshold necessary to prime stomatal functioning. Interestingly, an oxygen isotope analysis of leaf water supported the conclusion that adult leaves are exposed to a lower humidity than young leaves. We propose that the microclimate within the Arabidopsis rosette leads to an ontogenetic hardening of stomata, through a gradual exposure of the leaf to low humidity which triggers an activation of ABA metabolism and signalling.

P44 The dual effect of abscisic acid on stomatal conductance

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The classical view that the drought-related hormone abscisic acid (ABA) simply acts at the guard cell level to induce stomatal closure is questioned by discrepancies in stomatal response to ABA between isolated epidermis and intact plants. We tested the hypothesis that ABA mediates an additional effect *in planta* by changing hydraulic regulation in the leaf upstream the stomata. By gravimetry, porometry to water vapour and argon, and psychrometry, we investigated the effect of exogenous ABA on transpiration, stomatal conductance and leaf hydraulic conductance of mutants described as ABAinsensitive in epidermal peels. We show that stomatal transpiration of ABA-insensitive mutants does respond to ABA *in folio*. We then demonstrate that ABA decreases stomatal conductance by downregulating leaf hydraulic conductance in both the wild-type Col-0 and the ABA-insensitive *ost2*. We propose that ABA promotes stomatal closure both via its biochemical effect on guard cells and via an indirect hydraulic effect through a decrease in leaf water permeability, triggered within vascular tissues by distinct signalling components. Variability in sensitivity of leaf hydraulic conductance to ABA among species could provide a physiological basis to the isohydric or anisohydric behaviour.

P45 Stomatal adaptation in *Begonia*

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Begonia is one of the largest genera of angiosperms with more than 1500 species inhabiting tropics and sub-tropics and each species is restricted to a very small geographic niche described as shady and humid. Adaptation to such very confined niches may be reflected in the development of specific physiological traits in stomatal behaviour and their distribution. *Begonia* species show a variation in stomatal development with several *Begonia* species exhibiting stomatal clusters. We are investigating the physiological context of stomatal patterns in *Begonia*, characterising species with single stomata and with stomatal clusters, focusing on *B. coccinea* and *B. glabra*, exhibiting single stomata and stomatal clusters respectively. The results of initial studies, including gas exchange and electrophysiological measurements are summarised.

P46 Using a process-based model to predict water use in olive

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Improvements in water use efficiency and precise irrigation scheduling are crucial in a scenario of global change and increasing human population. This becomes more important in fruit trees orchards in arid and semi-arid environments where the water resource is scarce. Thus, there is a raising need to predict the consequences of drought on crop plants with the objective to design optimal irrigation strategies and to improve water use. Modelling stomatal conductance with a mechanistic basis appears as a powerful tool for simulation and prediction purpose, and is required for modelling plant transpiration. The aim of this work was to apply a process-based stomatal model (Buckley et al., 2003) in a hedgerow olive orchard under deficit irrigation for understanding the mechanisms behind the control of transpiration and, thus, for predicting water use of the crop. We estimated the canopy conductance (g_c) from sap flow measurements. The hydromechanical model fitted our canopy conductance data satisfactorily and allowed us to analyze the physiological parameters obtained. The role played by hydraulic and non-hydraulic signals, as well as the intensity of osmotic adjustment, was evaluated.

P47 Guard cells meet microbes

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Guard cells are not only sensing abiotic signals, but also enhance the fitness of plants by responding to micro-organisms. We examined early responses of guard cells to pathogenic fungi, by placing conidia of powdery mildew in close proximity of stomata. Life cell imaging showed that fungal growth and development inhibits stomatal opening. Using intracellular micro electrodes, we could link the inability of stomata to open, to an increased activity of guard cell anion channels. The high activity of anion channels impairs light-induced hyperpolarization of the guard cell plasma membrane. Because of the likely role of Microbe Associated Molecular Patterns (MAMPs), a major elicitor derived from fungal cell walls was applied by nano infusion through open stomata. Stimulation with chitosan activated guard cell anion channels and induced stomatal closure. We are currently testing the ability of guard cells to discriminate between MAMPs of different micro-organisms. On the meeting new insights gained with these studies will be presented and implemented in a model that links MAMPreceptors to S-type anion channels.

Koers S, Guzel-Deger A, Marten I, Roelfsema MRG (2011) Barley mildew and its elicitor chitosan promote closed stomata by stimulating guard-cell S-type anion channels. Plant J. 68: 670-680

P48 Regulation by Calcium-dependent protein kinases of Skaker channels expressed in guard cells

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Stomatal movements result from osmotically-driven fluxes of water, which follow massive exchanges of solutes, including K^+ ions, between guard cells and surrounding tissues. These exchanges involve a set of dedicated transport systems, controlled through signalling pathways, including Ca^{2+}-dependent ones. For example, Ca^{2+} signals have been reported to lead to stomatal closure through the inhibition of inward and activation of outward K^+ channels. This could be mediated in particular by Ca^{2+}-dependent kinases targeting these channels.
Here, we addressed the post-translational regulation of three K^+-selective voltage-gated channels expressed in *Arabidopsis* guard cells (inwardly-rectifying KAT1 and KAT2, and outwardly-rectifying GORK) by Ca^{2+}-dependent protein kinases (CPKs). Of the 34 *CPK* genes in *Arabidopsis thaliana*, some CPK are thought to be expressed in guard cells. Seven of these CPKs were co-expressed in Xenopus oocytes with the three studied Shaker channels, and their influence on K^+ transport was examined with a classical voltage-clamp technique. Pinpointed interactions were checked by on-chip phosphorylation assays on peptide arrays designed from the channel primary sequence. This enabled us to list a number of candidate phosphorylation sites and, subsequently, to design mutant channels expected to display CPK-insensitive of CPK-phosphorylated phenotype. The functional characterisation of these mutant channels and the phenotype of plants overexpressing CPKs will be presented.

P49 Recommended liming of an acidic soil decreases stomatal conductance of pea (*Pisum sativum* L. cv. Alderman)

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Soil pH is traditionally managed by applying lime (calcium carbonate) to optimise nutrient availability and maximise plant yield. Over-liming can induce stomatal closure through lime-induced iron chlorosis. Peas (*Pisum sativum* L. cv. Alderman) were grown in a low pH (pH 5.5) agricultural soil or soil amended with lime to target pH 6.5 (recommended rate) or over-limed (twice recommended rate). Stomatal conductance (gs) was significantly reduced by 27% in recommended and over-limed plants when compared to un-limed plants. However, foliar applications of 54 umol NaFeEDTA solution to restore shoot Fe levels failed to reinstate gs in limed plants, suggesting that lime induced chlorosis was not responsible for observed gs reductions. Alternative mechanisms to explain stomatal closure include elevated xylem sap calcium concentration (which could directly close stomata) and/or increased soil bicarbonate levels, resulting in higher xylem sap malate concentrations which would alkalinise xylem sap. Since alkaline xylem sap decreases gs via an ABAdependent mechanism (Wilkinson et al. 1998; Plant Physiology 117, 703-9) future investigations will measure gs of wild-type and ABA-deficient peas grown in limed soil.

P50 Closing the gaps between genomes and phenomes: High-throughput analysis of the plant epidermis

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The epidermis of mature *Arabidopsis thaliana* leaves is a jigsaw puzzle of pavement cells, perforated non-randomly with stomatal pores defined by guard cell pairs. We are interested in the underlying genetic mechanisms that mediate environmental control of epidermal cell type distribution in the leaf epidermis of *Arabidopsis* and other species. In order to carry out these studies, large scale, accurate and quantitative collection of *Arabidopsis* epidermal cell parameters is essential. We will present the results of applying a novel technology to capturing the epidermal cell characteristics of *Arabidopsis* and a variety of other plants. The method is very fast, non-destructive, and generates very large and highly precise data sets on living tissues, rendering a variety of their characteristics available for statistical and structural analysis. The data sets are amenable to analysis by pattern recognition software, with the objective of automating the determination of cell type and size distributions. Our initial efforts with this technology are focused on the effects of varying carbon dioxide levels of the epidermal phenotype while at the same time casting as wide a net as possible in exploring the technology's analytical potential.

P51 An investigation of the responses of barley stomata to abiotic stresses

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The mechanisms responsible for the regulation of stomatal opening and closure are well characterized in broad-leaved non-crop plants. However, the majority of the world's main agricultural crops are cereals the stomata of which are morphologically distinct from those found in broad-leaved species – the stomata are dumbbell-shaped rather than kidney bean shaped. Consequently, to date we know very little about the mechanisms by which stomatal opening and closure are regulated in cereals. I have developed a stomatal bioassay in barley which I have used to investigate the responses of barley stomata as a model monocot to abscisic acid (ABA), H_2O_2, CO_2 and temperature both individually and in multifactorial experiments in order to assess interacting abiotic stresses modulate the control of stomatal aperture in monocots. My data suggests possible differences in the signalling mechanisms by which the stomata of barley and broad-leaved model species respond to ABA. I will subsequently explore whether the induction of barley homologues of guard cell ABA induced genes from Arabidopsis exhibit similar differences. I will use my barley bioassay data to construct a simple model to simulate and predict barley responses to interacting abiotic stresses.

P52 Perception and downstream signaling of apoplastic reactive oxygen species

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Reactive oxygen species (ROS) have important roles as signaling molecules in plant stress responses and development. Ozone (O_3), which degrades to ROS in the extracellular space of plant cells, can be used as a tool to unravel in planta processes induced by ROS. O_3 exposure initiates ROS signaling pathways leading to stomatal closure. The aim of this project is to identify novel components in early stomatal signalling downstream of apoplastic ROS. This study focuses on characterization of two novel O_3 sensitive mutants*, rcd7* and *suu1*. The RCD7 candidate gene, identified by genome resequencing, appears to encode a novel receptor-like kinase involved in ROS perception/signaling. SUU1, which is currently being mapped, regulates both O_3 and CO_2-induced stomatal closure. A mutant screen is also being conducted to discover components involved in stomatal Ca^{2+} signaling, and to elucidate how they are involved in ROS-dependent regulation of stomatal movements. Understanding the regulation of stress responses and stomatal movements by ROS has important implications for agriculture and applied research towards improving crop quality.

P53 Epidermal impact on stomatal sensitivity in six deciduous tree species

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Stomatal reactions to multiple signals are not always additive and hydraulic signals often dominate. Over metabolic signals (The mechanisms, causing this hydraulic priority are not known. Suppression of metabolic signal or amplification of hydraylic signal can occur. The impact of epidermal turgor on guard cells during stomatal reactions (often called as mechanical advantage of epidermal cells over guard cells) is one possible mechanism to create positive feedback and to amplify hydraulic signal. We described the relative epidermal impact on stomatal conductance and sensitivity in six temperate deciduous tree species, using two methods (impact was calculated from dynamics of stomatal conductance during rapid desiccation and from anatomical parameters of leaves).The epidermal impact on stomata was more pronounced in slow-growing species with low hydraulic conductance and sensitive stomata, but the relative impact was bigger in the case of fast-growing species with high hydraulic conductance and lower stomatal sensitivity. Thus, epidermal cell turgor needs to be taken into account if comparing stomatal conductance and sensitivity in different species.

P54 Intra-canopy variation in epidermal morphology in *Sequoia sempervirens* and *Sequoiadendron giganteum*

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Climatic and physiological data collected throughout the canopies of coast redwood (*Sequoia sempervirens*) and giant sequoia (S*equoiadendron giganteum*) reveal a wide range of microclimate variation. This provides a unique opportunity to study the large variation in leaf shape and particularly epidermal features for the same genotype in very different levels of drought stress and light in their natural environment. We have used cuticular analysis to quantify changes in epidermal features in the canopies of both species, including stomatal patterning on the abaxial and adaxial side of the leaves. Our analysis shows that in *S. sempervirens* an increase in height is correlated with a change from hypostomatous to amphistomatous leaves, higher stomatal densities, lower stomatal pore areas, and more random distribution of the stomatal angles. Leaves of *S. giganteum* are amphistomatous throughout the canopy, though from higher up have higher stomatal density, lower stomatal pore areas, and a less obvious increases in the distribution of stomatal angles. These differences in adaptive syndromes in leaf and epidermal morphology to height and microclimatic conditions might be explained by differences in physiological drought.

P55 Trait of low stomatal density confers drought tolerance in rice

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Water shortage is a major constraint for crop production. Up to 97% of water absorbed by roots is lost into the atmosphere through transpiration via stomata. Stomata are key to plant physiology and can respond to environmental signals such as drought and elevated CO_2 concentration in the atmosphere by adjusting stomatal opening, stomatal density and stomatal size. Accumulating evidence suggest that there exists a negative correlation between stomatal size and stomatal density. In this study, we assessed the variation of stomatal density among different lines of rice and demonstrated improved plant growth tolerance to limited water availability in rice is often associated with low stomatal density. We are exploring the possibility to improve crop drought tolerance and water use efficiency by engineering the beneficial trait of low stomatal density into rice via traditional hybrid rice breeding programme.

Key words: rice, low stomatal density, stress tolerance, sustainable agriculture, water use efficiency.

P56 A novel protein kinase BLUS1 is essential for stomatal opening in response to blue light

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Blue light perceived by phototropins mediates stomatal opening through the activation of the plasma membrane H^+-ATPase via protein phosphatase 1 (PP1). However, the signal transduction between phototropins and the H^+-ATPase remains largely unknown. In this study, we developed a screen system that obtains mutants deficient in blue light-dependent stomatal opening by infrared thermography, and identified a novel protein kinase *BLUS1* (*BLUS LIGHT SIGNALING 1*) in guard cells. The *blus1* mutation impaired blue light-dependent stomatal opening, H^+ pumping, and phosphorylation of the H^+-ATPase, but showed normal activity of phototropins. The BLUS1 was localized in the cytoplasm and the gene was preferentially expressed in guard cells. Phosphoproteome analysis identified a Ser residue in the C terminus of BLUS1 as a blue light-dependent phosphorylation site. Site-directed mutagenesis revealed that the identified Ser residue and the kinase activity were absolutely required for the response. Further biochemical analysis demonstrated that both phot1 and phot2 mediated this phosphorylation, and the PP1 inhibitor tautomycin did not affect the reaction. These results suggest that BLUS1 mediates the signaling from phototropins to the H^+-ATPase and is located upstream of PP1 in the pathway.

P57 ROOT PHOTOTROPISM2 (RPT2) is not involved in phototropin-mediated stomatal opening

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Blue light perceived by phototropins (phot1 and phot2) mediates stomatal opening through the activation of the plasma membrane H^+-ATPase via unknown signaling. A recent study has suggested the possible involvement of ROOT PHOTOTROPISM2 (RPT2), a BTB/POZ family protein functioning for phototropism, in the stomatal opening in response to blue light. However, conclusive evidence for the requirement of RPT2 in the activation process of the H^+-ATPase and the stomatal opening has not been obtained so far. In this study, we have generated double mutants of RPT2 and phototropins, and investigated the blue light-specific stomatal responses in the mutants. We first confirmed the impaired hypocotyl phototropic response in *rpt2* mutants. However, blue light-dependent H^+ pumping from guard cell protoplasts and binding of a 14-3-3 protein to the H^+-ATPase were not affected both in *rpt2* single mutant and *phot1 rpt2* and *phot2 rpt2* double mutants. In accord with these results, the stomata of all *rpt2* mutants opened in response to blue light in epidermal peels and intact leaves, suggesting that RPT2 is neither required for the activation of H^+-ATPase nor stomatal opening.

P58 Natural variation as a tool to identify stomatal regulators

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Natural variation in *Arabidopsis thaliana* has been extensively used to study developmental processes (e.g. flowering) and stress responses. Since stomata are crucial regulators of gas exchange and water loss it is likely that regulators of stomatal aperture are also under natural selection. Hence, the large collection of genetic tools in Arabidopsis can be exploited to better understand stomatal function. Ozone is an air pollutant that enters through stomata and elicits cell death and we have previously shown that stomatal function explains a large part of ozone induced cell death. We are using four different screens to find stomatal regulators: (1) Ozone induced cell death in three quantitative trait loci (QTL) mapping populations (Col×Cvi, Te×C24, MAGIC). (2) Genome wide association mapping for ozone induced cell death in 400 Arabidopsis accessions. (3) Waterloss in Col×Cvi, Te×C24 and MAGIC QTL mapping populations. (4) Stomatal conductance in the MAGIC QTL mapping population. Candidate genes for altered stomatal responses in Cvi have been identified.

P59 Steady state and dynamic responses of stomatal conductance to irradiance: characterization and modelling

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During a daily time course, stomatal conductance to water vapour (g_s) undergoes a succession of transitory regimes, due to continuous changes of the environment. A hysteresis of g_s is often observed, e.g. stomatal conductance is smaller in the afternoon compared to the morning under equivalent temperature and irradiance. We propose a new dynamic model of stomatal conductance using an asymmetric sigmoidal function which has already been used to describe the relationship between stomatal aperture and turgor pressure in guard cells. This model reproduces the observed hysteresis by describing the differences in speed between stomatal opening and closing and by providing parameters related to steady-state as well as to dynamic changes. The model was first tested on defined red and blue light stimulations on leaves of different oak species. A close fit was reached and the adjusted parameters differed among species. Further, the model was adjusted to data obtained with simulated diurnal time-courses of irradiance. Adjustments were of high quality, and the model was able to predict the observed hysteresis. A probable role of this hysteresis in observed differences in intrinsic water use efficiency between the species is discussed.

P60 Cell-type specific networks in *Brassica napus* guard cell responses to drought

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Desiccation of crops during drought causes severe damage and lost yields. Fresh water scarcity is one of the major global problems in this century. As global temperatures rise, we will encounter increased variability in amounts and distribution of precipitation, increased water demand, and enhanced susceptibility to drought. This will result in profound impacts on global fresh water resources, 65% of which are used for agriculture. Guard cells regulate the stomatal pore size, controlling water loss and CO_2 assimilation in response to fluctuations in the local environment. Under drought conditions, plants increase water use efficiency by closing stomata, which is mediated by the plant hormone abscisic acid (ABA). Not surprisingly, a high level of functional redundancy in plant genomes as well as high ploidy in some species hampers conventional and functional genomic investigations of these complex processes. To circumvent limitations imposed by this redundancy, we have been generating cell-type specific analyses of mRNA, proteins, and metabolites. Using this information we are elucidating networks of guard cell signaling pathways and genes regulated in response to ABA and drought. We are currently developing a user-friendly web site and graphic tools for -omic data to make these data easily accessible by the scientific community and the public. A recent progress will be presented.

P61 Quantitative kinetic modelling of stomatal guard cells uncovers counter-intuitive connections in ionic homeostasis

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Stomatal guard cells play a key role in gas exchange for photosynthesis and in foliar water conservation under stress. Key information has come from the analysis of the mutants of several ion transporters and their regulatory components. In many cases, these studies have yielded a number of unexplained (sometimes contradictory) observations, often seemingly unrelated to the function(s) of the transporter. For example, the *tpk1* mutant has been reported to have a moderate effect on plant K^+ homeostasis, yet it profoundly alters stomatal movements under some conditions [Gobert, et al. (2007) PNAS 104,10726]. We recently constructed a set of computational models of the guard cell around the OnGuard software, building on the wealth of biophysical and kinetic knowledge available for guard cell transport, signalling and homeostasis. The models incorporate explicitly all of the fundamental properties for transporters at the plasma membrane and tonoplast, the salient features of osmolite metabolism, and the major controls of cytosolic-free Ca^{2+} concentration and pH. We previously demonstrated that the models recapitulate all of the major behavioural characteristics of guard cells in response to a number of environmental variables and incorporate substantial predictive power in generating unexpected and counterintuitive outputs which are nonetheless documented in the literature. We now provide direct confirmations of – and an accounting for – the predictions that selected ion channel mutants have seemingly inexplicable effects on the ionic homeostasis of the guard cell and, hence, on ion flux through unrelated transporters.

P62 Modulation of reactive oxygen species signaling by antioxidants in guard cells of *Arabidopsis thaliana*

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This study investigates whether flavonols accumulate in guard cells as a means to regulate reactive oxygen species (ROS)-driven stomatal movement. ROS have been shown to act as signaling molecules in guard cells mediating ABA-dependent stomatal closing. ROS are produced in transient bursts and their accumulation must be balanced to keep concentrations from reaching damaging levels within the cell. If flavonols accumulate in guard cells, they may buffer ROS concentrations and thereby modulate stomatal movement. Using a flavonol-specific dye and confocal microscopy, we observed flavonol accumulation in the guard cells, but not in surrounding pavement cells of wild type plants. ROS accumulation was monitored with DCF and increased levels of ROS were found in guard cells of *tt4*, a mutant that synthesizes no flavonols. Additionally in *tt4* mutants, stomata are more closed than WT under optimal opening conditions, and have a more rapid ABA- induced stomatal closure, suggesting a functional role for flavonols. We are also currently investigating whether hormones that induce flavonol accumulation, such as ethylene, affect stomatal closure through elevated flavonol accumulation. Together these results suggest that the striking flavonoid accumulation in guard cells may modulate ROS levels and thereby regulate the signaling pathways that control stomata aperture.

P63 How well do stomatal conductance models perform on closing plant carbon budgets? A test using seedlings grown under current and elevated air temperatures

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Future carbon and water fluxes within terrestrial ecosystems will be determined by stomatal conductance (g_s) responses to rising atmospheric CO_2 and temperature. CO_2 effects on g_s have been studied, but g_s acclimation to warming is less certain. Six *gs* models were parameterized using gas exchange data from black spruce grown at 22/16 °C (day/night) or 30/24 °C temperatures. Models were assessed by how well carbon gain reproduced carbon costs to close the seedlings' seasonal carbon budgets, a 'long-term' success indicator. A model holding a constant intercellular to ambient CO_2 ratio and the Ball-Berry model (based on stomatal responses to relative humidity) could not close the carbon balance for either treatment; the

Jarvis-Oren model (based on stomatal responses to vapor pressure deficit, *D*) and a model assuming a constant g_s each closed the carbon balance for one treatment. Two models based on g_s responses to *D* estimated carbon uptake within 10% of carbon costs for both treatments: the Leuning model and an optimization model that maximizes carbon gain per unit water loss. Since g_s responses in the optimization model are not *a priori* assumed, this approach can be used in modeling land-atmosphere exchange of CO_2 and water in future climates.

P64 Is there a role for nitric oxide in stomatal 'lock-up'?

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The phenomenon of stomatal 'lock-up' was first described by Prats et.al., whilst working on the barley powdery mildew pathosystem. They noticed that stomata of cultivar P01, showing HR mediated resistance, failed to close in the dark when infected with the pathogen. Stomata on these plants continued to lock-open even with the application of ABA and after the onset of drought. Such stomatal dysfunction is likely to affect plant physiological processes and the plants ability to cope with water stress and therefore highlights a potentially significant cost of resistance. Nitric oxide (NO) is an important signalling molecule in plants, involved in both plant defence responses and ABA induced stomatal closure. Stomatal conductance was measured on both nitrate reductase deficient mutants and plant Hb over-expression lines to determine whether it also plays a role in 'lock-up'. The stomata of these NO deficient mutants continued to lock-open when infected with powdery mildew suggesting that NO is not a key signalling molecule involved in locking. However, visualisation of both NO and H_2O_2 using confocal microscopy showed an increase in H_2O_2 in the NO deficient plants, which could provide an alternative mechanism for triggering 'lock-up'.

P65 Nitric oxide inhibits blue light-induced stomatal opening through a pathway involving Ca^{2+}-blocked K^+ influx in Guard Cells

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Our previous studies showed that nitric oxide (NO) functioned in the downstream of H_2O_2 and was involved in ABA-inhibited blue light (BL)-dependent stomatal opening. For gaining further insights into NO function in mediating BL- induced stomatal opening, guard cell protoplasts (GCPs) were patch-clamped in a whole-cell configuration. The results showed that twice BL pulses effectively activated inward rectifying K^+ channels by 67% and 20% in *Vicia* GCPs, respectively, but red light (RL) showed little effect. In accord with this, BL also increased inward K^+ current by 54% in *Arabidopsis thaliana* wild type *gl1*, but not in *phot1-5 phot2-1* (BL receptor phototropin deletion mutant). SNP (a NO donor), blocked K^+ influx and inhibited BL-induced stomatal opening, which were abolished by c-PTIO (a specific NO scavenger). Interestingly, both NO and BL effectively activated the plasma membrane Ca^{2+} channels, but the extent of activation was different, which result in the cytosolic Ca^{2+} accumulation at different levels. Furthermore, cytosolic Ca^{2+} promoted K^+ influx at below 0.5 μM, and significantly inhibited K^+ influx at 10 μM or above. These results indicated that NO inhibits BL-induced K^+ influx to modulate stomatal aperture of plants maybe by adjusting the cytosolic Ca^{2+} concentration.

P66 *PHO1* expression in guard cells mediates the stomatal response to abscisic acid in *Arabidopsis*

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We present evidence that stomatal responses to ABA are regulated by *PHO1* expression in guard cells of Arabidopsis thaliana. *PHO1* is involved in the export of phosphate into the root xylem vessels. In leaves, *PHO1* was found expressed in guard cells and up-regulated following treatment with ABA. The *pho1* mutant was unaffected in ROS production following ABA treatment, and in stomatal movements in response to light cues, high extracellular calcium, auxin, and fusicoccin. However, stomatal movements in response to ABA treatment were severely impaired, both in terms of induction of closure and inhibition of opening. Micro-grafting a *pho1* shoot scion onto wild-type rootstock resulted in plants with normal shoot growth and Pi content, but failed to restore normal stomatal response to ABA treatment. *PHO1* knockdown using RNAi specifically in guard cells of wild-type plants caused a reduced stomatal response to ABA. In agreement, specific expression of *PHO1* in guard cells of *pho1* plants complemented the mutant guard cell phenotype and re-established ABA sensitivity, although full functional complementation was dependent on shoot Pi sufficiency. Together, these data reveal an important role for phosphate and *PHO1* action in the stomatal response to ABA.

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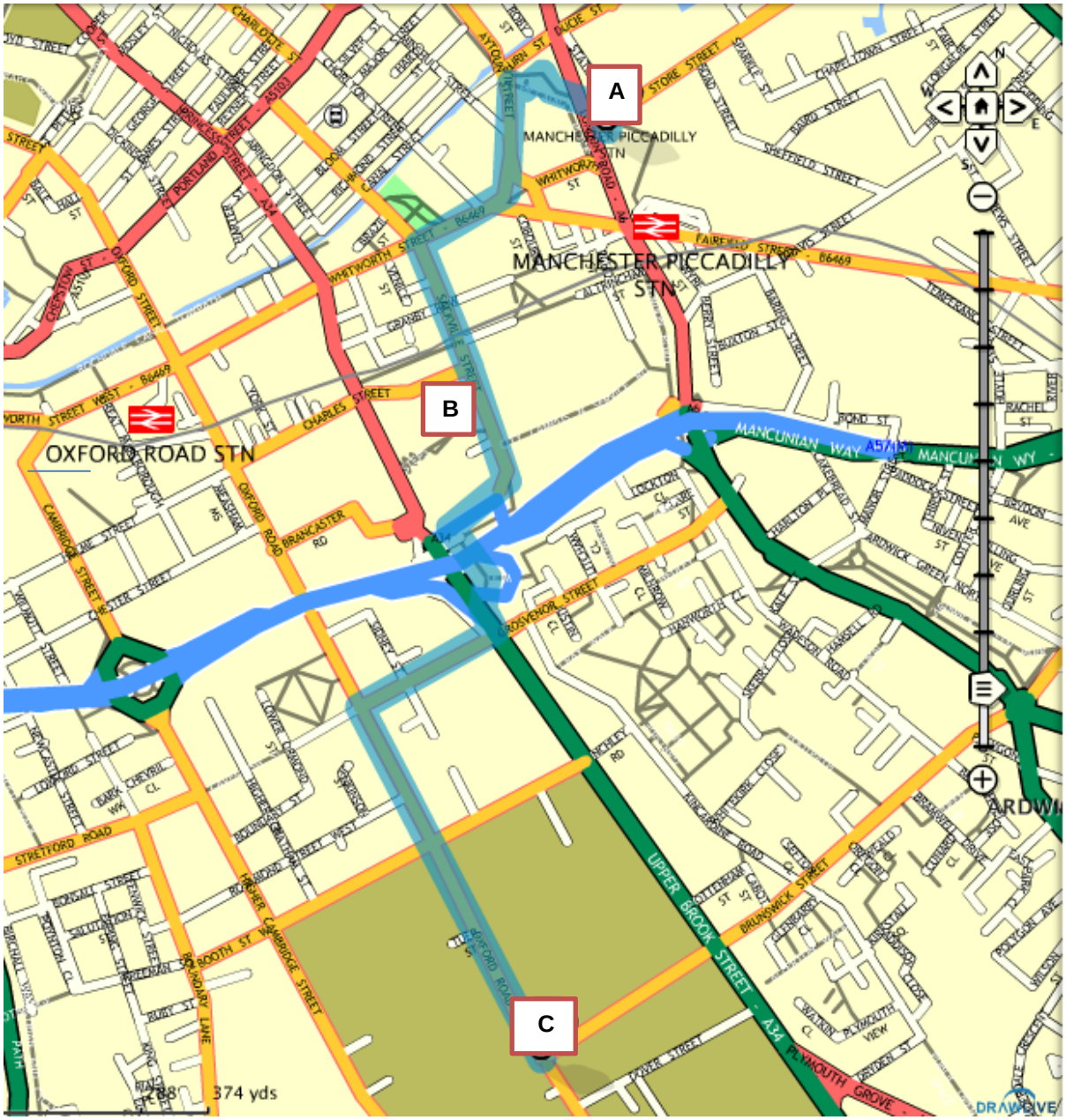
Map

A Piccadilly Train Station
B Manchester Conference Centre, Sackville Street, M1 3BB.
C Manchester Museum, Oxford Road, M13 9PL



To walk from A to B takes approx. 8 minutes.

To walk from B to C takes approx. 14 minutes.