The Canadian Society of Plant Biologists Eastern Regional Meeting 2013

Délibérations du Congrès de la Société Canadienne de Biologie Végétale Congrès de l'Est 2013

December 6th and 7th, 2013

University of Toronto Mississauga, Mississauga ON CANADA



Organizing Committee

Thomas Berleth, Ingo Ensminger (Chair), George Espie, Herbert Kronzucker, Nick Provart, Rowan Sage, Deep Saini

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Carol Solonenko, Antonia Maughn, Jennifer Lee, Jenny Hu, Mikael Koza, Keith Nablo, Carolyn Moon and our student and post doc volunteers Maryam Moazami-goudarzi, Omar El-Ansari; Charlotte de Araujo, Emmanuelle Frechette, Laura Junker, Christine Chang, Thomas Braukmann, Ina Anreiter, Sarzana Hasin Zafar, Janola Jeyachandra, Alex Zubilewich.

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Schedule Overview

Friday, December 6, 2013; UTM Instructional Building (IB)

5:00 – 7:00 pm	Registration, Poster set-up and Viewing
7:00 – 7:10 pm	Welcome
7:10 – 8:00 pm	Plenary Lecture Dr John MacKay , University of Laval – IB120 Conifer giga-genomics: evolution, diversity and functional adaptations
8:00 – 10: 00 pm	CSPB Mixer: light food, light and hearty beverages Poster viewing & discussion

Saturday, December 7, 2013; UTM Instructional Building (IB)

7:30 – 8:30 am	Registration & Poster set-up and Viewing
8:30 – 8:40 am	Welcoming Remarks
8:40 – 9:30 am	Plenary Lecture Dr Jennifer Baltzer, Wilfred Laurier University – IB120 Boreal forests on permafrost: biotic and abiotic drivers of vegetation composition and function.
9:30 – 10:00 am	Refreshment break – Poster viewing
10:00 – 12:00 pm	Concurrent oral presentations – Sessions 1, 2, 3 & 4 Locations: IB 140, IB 150, IB 235& IB 245
12:00 – 1:30 pm	Buffet Lunch; CSPB executive meeting & lunch – Poster viewing UTM student centre; Upper conference room
1:30 – 2:20 pm	Plenary Lecture Dr. Herbert Kronzucker, University of Toronto – IB120 Agroecology and the challenge of hunger
2:20 – 2:30 pm	Moving time
2:20 – 2:30 pm 2:30 – 3:40 pm	Moving time Concurrent oral presentations – Sessions 5, 6 & 7 Locations: IB 120, IB 140 & IB 150
-	Concurrent oral presentations – Sessions 5, 6 & 7
2:30 – 3:40 pm	Concurrent oral presentations – Sessions 5, 6 & 7 Locations: IB 120, IB 140 & IB 150

Concurrent Oral Sessions

Session 1 Cell Biology

Chair: Vincenzo De Luca

#	TIME	PRESENTER	TITLE
1.1	10:00 – 10:15 am	Rex S. Chiu, Sonia Gazzarrini	Regulation of Proteasomal Activity by Abscisic Acid and High Temperature Stress during germination in Arabidopsis
1.2	10:15 – 10:30 am	Katie Woolfson, Fang Yu, Vincenzo De Luca	ATP-binding cassette transporter from Vinca minor is involved in alkaloid secretion
1.3	10:30 – 10:45 am	Michael Wozny, Neeta Mathur, Dr. Jaideep Mathur	Phosphate deprivation increases the frequency of membrane extensions from plastids
1.4	10:45 – 11:00 am	Davood Emami Meybodi, Anthony Percival-Smith, Abdelali Hannoufa	Uncovering the molecular link between miR156/SPL15 and carotenoid accumulation in <i>Arabidopsis thaliana</i>
1.5	11:00 – 11:15 am	Surinder Singh, Chi-Kang Tsai, Jaswinder Singh	Transposon-mediated epigenetic control of seed germination in cereals
1.6	11:15 – 11:30 am	Alena M. Mammone, Kiah A. Barton, Michael Wozny, Neeta Mathur, Dr. Jaideep Mathur	Characterization Of Plastid Localized Lipases As Regulators of Stromule Formation Through Membrane Contact Sites With The ER
1.7	11:30 – 11:45 am	Ashley Jaipargas, Firas Bou Daher, Nigel Griffiths, Jaideep Mathur	Cytosolic sugar levels regulate mitochondrial morphology in plants
1.8	11:45 – 12:00 pm	Joan Laur, Uwe G. Hacke	Needle water uptake facilitates xylem refilling through aquaporin regulation in <i>Picea glauca</i>

Session 2 Climate Change

Chair: Ingo Ensminger

#	TIME	PRESENTER	TITLE
2.1	10:00 – 10:15 am	Gang Li Christopher M. Brown, Natalie Donaher, Avery McCarthy, Douglas A. Campbell	The Nitrogen Costs of Photosynthesis in a Diatom under Current and Future pCO ₂
2.2	10:15 – 10:30 am	Danielle A. Way, Wataru Yamori	Thermal acclimation of photosynthesis: on the importance of definitions and considering respiration
2.3	10:30 – 10:45 am	Christine Chang, Emmanuelle Fréchette, Ingo Ensminger	Sensitivity of autumn cold acclimation to elevated temperature in <i>Pinus</i> strobus
2.4	10:45 – 11:00 am	Joseph R. Stinziano, Danielle A. Way	Canada's Forests in a Changing Climate: Impacts, Responses, and Unanswered Questions
2.5	11:00 – 11:15 am	Emmanuelle Fréchette, Christine Chang, Ingo Ensminger	The photochemical reflectance index (PRI) as an indicator of photochemical efficiency in Pinus strobus during the autumn.
2.6	11:15 – 11:30 am	Holly Croft, Jing M. Chen, Yongqin Zhang, Anita Simic, Thomas L. Noland, Nadine Nesbitt	Modeling spatial and temporal variations in leaf chlorophyll content from remotely-sensed satellite data
2.7	11:30 – 11:45 am	Laura Verena Junker, Henning Wildhagen, Ingo Ensminger	Field grown Douglas-fir provenances differ in photosynthetic performance under conditions of limited water availability
2.8	11:45 – 12:00 pm		

Session 3 Metabolism I

Chair: Gale Bozzo Room: IB 235

#	TIME	PRESENTER	TITLE
3.1	10:00 – 10:15 am	Avery McCarthy, Michelle Chung, Norman P. A. Hüner	Sugar insensitivity in a model Arabidopsis thaliana cell suspension culture
3.2	10:15 – 10:30 am	Zaheer Ahmed, Ian J. Tetlow, Duane E. Falk, Michael J. Emes	Post-translational Interactions Among Enzymes of Starch Biosynthesis and Their Affect on Physical Properties of Starch in High- amylose Barley Genotypes.
3.3	10:30 – 10:45 am	Jonathan S. Griffiths, Allen Tsai, Gillian Dean, Catalin Voiniciuc, George W. Haughn	Cellulose, pectin and an AGP facilitate rapid expansion of seed coat mucilage.
3.4	10:45 – 11:00 am	Jenelle Patterson, Ian J. Tetlow, Michael J. Emes	Post-Translational Regulation of Starch Synthase II in Arabidopsis thaliana
3.5	11:00 – 11:15 am	Lily Nasanovsky, Ian Tetlow	Branching Enzymes as Agents for Modifying Glucan Structure in Industrial Processing
3.6	11:15 – 11:30 am	Qianru Zhao Ian Tetlow, Michael Emes	Starch biosynthesis in Arabidopsis thaliana
3.7	11:30 – 11:45 am	Geoffrey B. Lum, Carolyne J. Brikis, Kristen L. Deyman, Gordon J. Hoover, Jennifer R. DeEll, Barry J. Shelp, Gale G. Bozzo	Oxidative stress metabolism is associated with flesh browning of 'Honeycrisp' apples
3.8	11:45 – 12:00 pm	Thomas Braukmann Saša Stefanović	Extensive gene loss and plastome rearrangements in mycoheterotrophic Ericaceae

Session 4 Plant Development

Chair: Thomas Berleth

#	TIME	PRESENTER	TITLE
4.1	10:00 – 10:15 am	Katharina Bräutigam, Sherosha Raj, Sabrina Chamberland, Stefan Schreiber, Barb Thomas, Uwe Hacke, Malcolm M. Campbell	Genetic and epigenetic impacts on growth performance and stress response in <i>Populus</i>
4.2	10:15 – 10:30 am	Gregory S. Downs, Christophe Liseron-Monfils, Lewis N. Lukens	Regulatory motifs identified from a maize developmental coexpression network.
4.3	10:30 – 10:45 am	Rodney Savidge	New Insight into the Biogenesis of Bordered Pits and Cellulose
4.4	10:45 – 11:00 am	Emily Indriolo, Darya Safavian, Daphne Goring	The role of ARC1 in the self- incompatibility pathway in Arabidopsis spp.
4.5	11:00 – 11:15 am	Duncan Holbrook-Smith, Dr. Shigeo Toh, Dr. Yuichiro Tsuchiya, Dr. Peter McCourt	A small-molecule screen in Arabidopsis and yeast uncovers selective hormone mimics
4.6	11:15 – 11:30 am	Wenzi Ckurshumova, Tatiana Smirnova, Danielle Marcos, Adriana E. Caragea, Yara Zayed, Thomas Berleth	A defined role for an Auxin Response Factor in <i>de novo</i> shoot formation
4.7	11:30 – 11:45 am	Madiha Khan, Paul Tabb, Huasong Xu, Bhaswati Devi, Michael Bush, Shelley R. Hepworth	BLADE-ON-PETIOLE genes: setting boundaries in inflorescence development
4.8	11:45 – 12:00 pm	Julia Nowak, Joseph Skaf, Malcolm Campbell	Differential expression between male and female flowers of balsam poplar

Session 5 Technology

Chair: Katharina Bräutigam

#	TIME	PRESENTER	TITLE
5.1	14:30 – 14:45	Won-Sik Kim, Yousef Haj-Ahmad	The Importance of Plant total RNA purification method for down stream applications
5.2	14:45 – 15:00	Meyer, Ann; Downs, Gregory; Lukens, Lewis	Estimating allele-specific expression levels from RNA-Seq data for <i>Zea mays</i>
5.3	15:00 – 15:15	Behnaz Saatian, Gary Tian, Ryan S. Austin, Edward W. T. Tsang, Susanne Kohalmi, Yuhai Cui	Next-generation mapping of seed storage protein gene repressors in <i>Arabidopsis</i>
5.4	15:15 – 15:30	Shaowei Dong, Andrew Doxey, William Willats, Nicholas Provart	Molecular-binding Exploration Based On Proteome-wide Tertiary Structure Prediction: <i>in vitro</i> Validation

Session 6 Photosynthesis

Chair: Rowan Sage

#	TIME	PRESENTER	TITLE
6.1	14:30 – 14:45	Charlotte de Araujo, Matthew S. Kimber, George S. Espie	A novel γ-carbonic anhydrase essential for carboxysome function in cyanobacteria
6.2	14:45 – 15:00	Jackie Zorz, Amanda Cockshutt	Cross-Taxon Analyses Of The Photosynthetic Protein Complex Allocations In The Picocyanobacteria
6.3	15:00 – 15:15	Stefanie D. Sultmanis, Nathan I. Warkentin, Amy Xue Jin, Corey R. Stinson, Yannay Khaikin, Rowan F. Sage, Tammy L. Sage	A blast from the past: Reestablishment and characterization of hybrids between C ₃ and C ₄ species of <i>Atriplex</i> to dissect the control of differentiation of C ₄ Kranz anatomy
6.4	15:15 – 15:30	Lauren E. Hollis, Norman P.A. Huner	Redox state of the plastoquinone pool and chlorophyll biosynthesis are coupled to regulate greening in <i>Chlorella vulgaris</i>

Session 7 Metabolism II

Chair: Allison McDonald

#	TIME	PRESENTER	TITLE
7.1	14:30 – 14:45	Karina I. Neimanis, Allison E. McDonald	Characterization of the Alternative Oxidase from the moss <i>Physcomitrella</i> patens
7.2	14:45 – 15:00	Reynald Tremblay, Andrew Hand, Joseph Colasanti	Using the INDETERMINATE DOMAIN family as window into carbon metabolism
7.3	15:00 – 15:15	Adel Zarei, Christopher P. Trobacher, Alison Cooke, Barry J. Shelp	Biochemical characterization, expression and subcellular localization of two apple fruit diamine oxidases with differing substrate specificity
7.4	15:15 – 15:30	Megan Smith-Uffen, Susanne E. Kohalmi	Dissecting substrate specificity of arogenate dehydratases (ADTs) from Arabidopsis thaliana

Session 8 Abiotic Interactions

Chair: Elizabeth Weretilnyk

#	TIME	PRESENTER	TITLE
8.1	16:00 – 16:15	Çağdaş Kera Yücel, Melike Bor, Peter Ryser	Interspecific variation in root antioxidant enzyme systems reflects root turnover strategies and preferred habitats in wetland graminoids
8.2	16:15 – 16:30	Devrim Coskun, Dev T. Britto, Mingyuan Li, Alexander Becker, Herbert J. Kronzucker	Rapid ammonia gas fluxes underlie NH ₃ /NH ₄ ⁺ toxicity in roots of higher plants
8.3	16:30 – 16:45	Dominique Gagnon, Peter Ryser	Root characteristics of contrasting functional types of wetland plants
8.4	16:45 – 17:00	Mitchell MacLeod, Elizabeth Weretilnyk	Coping with water deficits: contrasting drought response strategies for two Eutrema salsugineum accessions
8.5	17:00 – 17:15	Katrina Hiiback, Michael Stokes, Malcolm Campbell	Exploring the plant priming response to low temperature exposure with small molecules

Session 9 Biotic Interactions

Chair: Martin Turcotte

#	TIME	PRESENTER	TITLE
9.1	16:00 – 16:15	Martin M. Turcotte, Nash E. Turley, Amaneet Lochab, Marc T.J. Johnson	Evolution by domestication drives the ecology and evolution of plant-herbivore interactions
9.2	16:15 – 16:30	Shailu Lakshminarayan, Mark Bernards, Lining Tian, Tawfiq Qubbaj, Luis Caceres' Abdelali Hannoufa	Analysis of <i>Arabidopsis</i> plants overexpressing carotenoid cleavage dioxygenases for volatile emissions and insect resistance
9.3	16:30 – 16:45	Nash E. Turley, T. Jonathan Davies, Hanno Schaefer, Michael J. Crawley	Grazing and Nitrogen Interact to Shape Plant Species and Phylogenetic Diversity
9.4	16:45 – 17:00	Kristie Bruinsma, Vojislava Grbic	Arabidopsis response to spider mite feeding: perception, signaling, and response
9.5	17:00 – 17:15	Connor R. Fitzpatrick, Michael D. Preston, Nathan Basiliko, Marc T.J. Johnson	Effects of herbivory, intraspecific genetic variation and rapid evolution in plants on ecosystem processes.

Session 10 Metabolism III

Chair: Barry Shelp Room: IB 120

#	TIME	PRESENTER	TITLE
10.1	16:00 – 16:15	Kathleen L. Hefferon	Plant-based Expression of
			Pharmaceutical Proteins to Improve
			Global Health
10.2	16:15 – 16:30	Greta Chiu,	Metabolite and expression analysis of
		Adel Zarei,	the 4-aminobutyrate (GABA)
		Gordon J Hoover,	pathway in Arabidopsis mutants
		Barry J. Shelp	subjected to salinity and chilling
			stresses
10.3	16:30 – 16:45	Anja Geitmann	Intracellular transport logistics
			during plant cell morphogenesis
10.4	16:45 - 17:00	Banyar Aung,	Overexpression of microRNA156
		Margie Gruber,	leads to enhanced forage yield in
		Lisa Amyot,	alfalfa
		Nusha Keyghobadi,	
		Abdelali Hannoufa	
10.5	17:00 – 17:15	Hemanta Raj Mainali	Genome-wide Analysis of Cyclophilin
		Sangeeta Dhaubhadel	Gene Family in Soybean and
			Characterization of GmCYP1

List of Posters

P1 Bioavailability of iron bound to strong chelators

Harold G. Weger¹, Carlyn J. Matz¹, Crystal N. Walker¹, Michael B. Fink¹ and Ron G. Treble²

P2 Improvement of Recombinant IL-10 Production by Suppression of *Cysteine Protease* Gene Expression in Transgenic Tobacco Plants

<u>Kishor Duwadi</u>*1, Ling Chen², Angelo Kaldis², Rima Menassa^{1, 2}, Sangeeta Dhaubhadel^{1, 2}

P3 Expression analysis of histone acetyltransferases in rice under drought stress

Hui Fang 1,2, Xia Liu², Greg Thorn¹, Lining Tian^{1,2}

P4 Autofocusing of transcription factor expression – a case study in plant pattern formation

James Colapinto and Thomas Berleth

Department of Cell and Systems Biology, University of Toronto

P5 Antitumorigenic and Antioxidant activity of methanolic extract of Gloriosa superba leaves

Saradha devi K.M¹, A. Poongothai¹, K. Ashokkumar^{2*} and S. Annapoorani ¹,

P6 HPLC analysis of flavonoids profile from Indian Curry leaf (Murraya koenigii. L)

<u>Kaliyaperumal Ashokkumar^{1*}</u>, Kumarakurubaran Selvaraj², Saradha Devi, K.M³

P7 Breeding potato varieties for chip processing and nutritional qualities – evaluation for central Canada conditions

Reena G. Pinhero¹, Qiang Liu², J. Alan Sullivan³, Vanessa Currie³ Benoit Bizimungu⁴ and Rickey Y. Yada¹

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⁴ Agriculture and Agri-Food Canada, Fredericton, New Brunswick E3B 4Z7

P8 Screening potato varieties for canning and nutritional qualities

Reena G. Pinhero¹, Qiang Liu², Rickey Y. Yada¹

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P9

P10 Ecological and evolutionary patterns of herbivory across vascular plants

Martin M. Turcotte¹, T. Jonathan Davies², Christina J.M. Thomsen³, and Marc T.J. Johnson¹

¹Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

P11 The potential of willows for phytoremediation of arsenic polluted sites: physiological results and molecular perspectives.

Aymeric Yanitch¹*, Frederic Pitre^{1,2}, Simon Joly^{1,2} and Michel Labrecque^{1,2}

P12 Complexitites of analysing bacterial hormone production with respect to growth characteristics of different species and strains

Anna Kisiala, R.J. Neil Emery

Department of Biology, Trent University, Peterborough, ON, Canada

P13 Live imaging of plant cells suggests a major role for the ER in creating organelle pleomorphy

<u>Kiah Barton</u>*, Ashley Jaipargas, Michael Wozny, Alena Mammone, Neeta Mathur, Nigel Griffiths & Jaideep Mathur

Laboratory of Plant development & Interactions, Department of Molecular & Cellular Biology, University of Guelph, Guelph, ON, Canada. N1G2W1.

P14 Toward the characterization of the two-spotted spider mite plant feeding pattern and associated damage.

Nicolas Bensoussan*¹, E. Santamaria¹, V. Zhurov¹, V. Grbic¹

P15 Metabolite and expression analysis of the 4-aminobutyrate (GABA) pathway in apple fruit stored under controlled atmosphere

<u>Carolyne J. Brikis^{1*}</u>, Adel Zarei¹, Kristen L. Deyman, Jingyun Liu, Greta Chiu, Christopher P. Trobacher, Gordon J. Hoover, Gale G. Bozzo and Barry J. Shelp¹

P16 A highly charged region in the middle domain of plant ER-localized HSP90 is involved in tunicamycin-induced ER stress resistance and ATP hydrolysis

Lisa P. Chong*^{1,2}, Yao Wang, Nathaniel Anderson, Rongmin Zhao^{1,2}

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P17 Polarization-dependent second and third harmonic generation imaging of *Haematococcus pluvialis*

Danielle Tokarz^{1,2}, Richard Cisek^{1,3}, Omar El-Ansari^{4,5}, George Espie^{4,5}, Ulrich Fekl^{1,2}, and Virginijus Barzda^{1,2,3}

P18 Investigating the role of *BLADE-ON-PETIOLE* genes in secondary wall formation in *Arabidopsis* and Poplar

Bhaswati Devi*, Eryang Li, Shabnam Gholoobi, Madiha Khan, and Shelley R. Hepworth *Department of Biology, Carleton University, Ottawa, ON, Canada*

P19 Investigating the role of a protein kinase during Arabidopsis pollen-pistil interactions

Jennifer Doucet* and Daphne Goring

Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada

P20 Transient expression of β-glucuronidase in soybean somatic embryos using cell penetrating peptide transfection

Mimmie Lu¹, Atiyyah Ferouz*^{1,2}, François Eudes³, Danielle Way², Lining Tian^{1,2}

P21 Biochemical characterization of anthocyanidin reductases from the seed coat of darkening cranberry beans (*Phaseolus vulgaris*)

José A. Freixas-Coutin*, K. Peter Pauls and Gale G. Bozzo

Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada

P22 Two spotted spider mite (Tetranychus urticae) adaptation to Arabidopsis thaliana

<u>Huzefa Ratlamwala</u>*¹, Vladimir Zhurov¹ and Vojislava Grbic¹

P23 Further defining the roles of ARC1 and Exo70A1 in pollen-pistil interactions in the Brassicaceae

<u>Daniel C Johnson¹</u>, and Daphne R Goring¹

P24 C₄ plants optimize chloroplast number and position in mesophyll cells

Roxana Khoshravesh, Matt Stata, Troy D. Rennie, Tammy L. Sage, Yannay Khaikin, Stefanie Sultmanis and Rowan F. Sage

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Department of Biology, The University of Western Ontario, London, ON, Canada

¹Department of Cell and Systems Biology, University of Toronto

P25 Investigation of the effect of high temperature stress on reproduction in *Arabidopsis thaliana* Vanessa Lundsgaard-Nielsen and Tammy L Sage

Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

P26 Characterization of the *Arabidopsis thaliana INDETERMINATE DOMAIN2* gene suggests a role in controlling early seedling growth behaviour

Tylar Meeks*1, Reynald Tremblay1 and Joseph Colasanti1

P27 Transgenic anti-florigen system to study long-distance movement of small RNA

Mark Minow^{1*}, Viktoriya Coneva¹ and Joseph Colasanti¹

P28 Spatial variation in aboveground herbivory due to biocontrol agents of the noxious weed *Cirsium arvense*

Krystal A.M. Nunes¹, Peter M. Kotanen¹

P29 The role of exocyst complex in the early stages of compatible pollen-pistil interactions in *Arabidopsis*

<u>Darya Safavian</u>, Yara Zayed, Emily Indriolo, Laura Chapman, Abdalla Ahmed, Daphne R. Goring Department of Cell & Systems Biology, University of Toronto

P30 Fungal endophyte benefits host plant only in the absence of grazing

James S. Santangelo*¹, Nash E. Turley¹, Marc T. J. Johnson¹

P31 Colonization of *Gluconacetobacter diazotrophicus* in *Brachypodium distachyon* for Studying Nitrogen Fixation in Monocot Plant

Xuan Yang^{1,2}, Gang (Gary) Tian², Kathleen Hill¹, Kevin Vessey³, Lining Tian²

P32 The second hyperpolarizability of LHCII

Danielle Tokarz^{1,2}, Richard Cisek^{1,3}, Ulrich Fekl^{1,2}, and Virginijus Barzda^{1,2,3}

P33 MicroRNA156 regulates plant development and nodulation in Lotus japonicus.

Ying Wang*^{1,2,3}, Lisa Amyot², Ziqin Xu³, Lining Tian^{1,2} and Abdelali Hannoufa^{1,2}

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P34 The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid

Yue Wu*, Charles Després

Department of Biological Sciences, Brock University, St. Catharines, ON, Canada

P35 Evaluating the roles of root suberin constituents in the adaptive response to drought

Nayana de Silva*¹, Isabel Molina², and Owen Rowland¹

P36 A New Suite of Pathogenesis-Related Gene Markers in Arabidopsis thaliana

Matthew Ierullo*, Nicholas Provart, and Darrell Desveaux

Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada

P37 Effects of elevated CO₂ and growth temperature on respiration rates in Norway spruce (*Picea abies*).

Yulia Kroner¹ and Danielle A. Way¹

P38 Reverse genetics using phospho-mutations at the AKIN10 target site in FUSCA3 reveals differential regulation of heat stress response during germination as well as seedling leaf and root development

Allen Yi-Lun Tsai², Aaron Chan², Areej Adil¹, Jinyuang Wang¹, Hardy Lou¹, Sonia Gazzarrini^{1,2}

P39 Shandong and Yukon accessions of the extremophile model plant *Eutrema salsugineum* exhibit different levels of resistance to *Pseudomonas syringae*

May Yeo*1, Philip Carella1, Elizabeth Weretilnyk1, Robin Cameron1

P40 Investigation on the mechanism of chloroplast positioning in single-cell C4 Bienertia sinuspersici

Sarah Schoor*¹, Dustin Sigurdson¹, Simon Chuong¹

P41 Root death of perennial herbaceous plants as a winter survival strategy in cool temperate wetlands.

Susara Marcotte*1, 2 and Peter Ryser 1, 2

P42 Predicting the catalytic function of the carboxysomal γ-carbonic anhydrase CcmM

Maryam Moazami-Goudarzi¹, Charlotte de Araujo¹, and George S. Espie^{1, 2}

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P43 An F-Box from Arabidopsis is a negative regulator of salt tolerance

Qingfang Lin, Jie Liu, Xuanjun Feng and Xuejun Hua

The key laboratory of plant resource, institute of botany, Chinese Academy of Science, Beijing, PR China

P44 Maintaining the Carbon Sink Function of a Peatland Margin After Hydrological Disturbance Ellie Goud*¹, Tim Moore¹ and Nigel Roulet¹

P45 Detecting transcriptome responses to environmental stimuli in field-grown, adult conifer trees Moritz Hess^{1,2}, Henning Wildhagen^{1,#} and Ingo Ensminger^{1,3,4}

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List of Registrants

FIRST NAME	SURNAME	PRESENTATIONS	AFFILIATION
Zaheer	Ahmed	3.2	University of Guelph
Banyar	Aung	10.4	University of Western Ontario
Jennifer	Baltzer	Plenary 2	Wilfrid Laurier University
Kiah	Barton	1.6, P13	University of Guelph
Laura	Boyer		
Gale	Bozzo	3.7, P15, P21,	University of Guelph
Katharina	Braeutigam	4.1	University of Toronto
Carolyne	Brikis	3.7, P15	University of Guelph
Kristie	Bruinsma	9.4	University of Western Ontario
Robin	Cameron	P39	McMaster University
Douglas	Campbell	2.1	Mount Allison University
Aaron	Chan	P38	University of Toronto
Jean-Benoit	Charron		McGill University
Chen	Chen		Western University
Rex	Chiu	1.1	University of Toronto, Scarborough
Ewa	Cholewa		Nippising University
Wenzi	Ckurshumova	4.6	University of Toronto
Amanda	Cockshutt	6.2	Mount Allison University
James	Colapinto	P4	University of Toronto
Emel	Con		University of Guelph
Alison	Cooke	7.3	University of Guelph
Katherine	Cornelius		McMaster University
Devrim	Coskun	8.2	University of Toronto, Scarborough
Charlotte	de Araujo	6.1, P42	University of Toronto, Mississauga
Vincenzo	De Luca	1.2	Brock University
Nayana	De Silva	P35	Carleton University
Bhaswati	Devi	4.7, P18	Carleton University
Shaowei	Dong	5.4	University of Toronto
Jennifer	Doucet	P19	University of Toronto
Gregory	Downs	4.2, 5.2	University of Guelph
Kishor	Duwadi	P2	University of Western Ontario
Davood	Emami Meybodi	1.4	Agriculture Agri -Food Canada
Michael	Emes	3.2, 3.4, 3.6	University of Guelph
Ingo	Ensminger	2.3, 2.5, 2.7, P45	University of Toronto, Mississauga

FIRST NAME	SURNAME	PRESENTATIONS	AFFILIATION
George	Espie	6.1, P17, P42	University of Toronto, Mississauga
Hui	Fang	Р3	University of Western Ontario
Atiyyah	Ferouz	P20	Agriculture and Agri-Food Canada
Jose	Freixas Coutin	P21	University of Guelph
Dominique	Gagnon	8.3	Laurentian University
Sonia	Gazzarrini	1.1, P38	University of Toronto
Anja	Geitmann		University of Montreal
Daphne	Goring	4.4, P19, P23, P29	University of Toronto
Elissa	Goud	P44	McGill University
Jonathan	Griffiths	3.3	University of British Columbia
Reza	Habibi		
Kathleen	Hefferon	10.1	Cornell University
Shelley	Hepworth	4.7, P18	Carleton University
Katrina	Hiiback	8.5	University of Toronto
Allyson	Hill		Queen's University
Duncan	Holbrook-Smith	4.5	University of Toronto
Lauren	Hollis	6.4, P9	Western University
Xuejun	Hua	P43	Chinese Academy of Science
Norman	Huner	6.4, P9	Western University
Matthew	Ierullo	P36	University of Toronto
Emily	Indriolo	4.4, P29	University of Toronto
Ashley	Jaipargas	1.7, P13	University of Guelph
Tim	Jiang		University of Toronto
Daniel	Johnson	P23	University of Toronto
Ashokkumar	Kaliyaperumal	P6	University of Saskatchewan
Roxana	Khoshravesh	P24	University of Toronto
Anna	Kisiala	P12	Trent University
Yulia	Kroner	P37	University of Western Ontario
Herbert	Kronzucker	Plenary 3, 8.2	University of Toronto, Scarborough
Shailu	Lakshminarayan	9.2	Agriculture and Agri-Food Canada
Chenlong	Li		Western University
Fushan	Liu		University of Guelph
Geoffrey	Lum	3.7	University of Guelph
John	MacKay	Plenary 1	University of Laval
Mitchell	MacLeod	8.4	McMaster University
Hemanta Raj	Mainali	10.5	University of Western Ontario
Shavkat	Mallaev	10.3, P42	Samarkand State University
Alena	Mammone	1.6, P13	University of Guelph

FIRST NAME	SURNAME	PRESENTATIONS	AFFILIATION
Susara	Marcotte	P41	Laurentian University
Jaideep	Mathur	1.3, 1.6, 1.7, P13	University of Guelph
Avery	McCarthy	2.1, 3.1	Western University
Allison	McDonald	7.1	Wilfrid Laurier University
Tylar	Meeks	P26	University of Guelph
Fatemeh	Mehrpooyan		
Ann	Meyer	5.2	University of Guelph
Mark	Minow	P27	University of Guelph
Maryam	Moazami-Goudarzi	P42	University of Toronto, Mississauga
Eshan	Naik		University of Toronto
Julia	Nowak	4.8	University of Toronto, Scarborough
Krystal	Nunes	P28	University of Toronto, Mississauga
Jenelle	Patterson	3.4	University of Guelph
Reena	Pinhero	P7, P8	University of Guelph
Kosala	Ranathunge		
Huzefa	Ratlamwala		
Jonathon	Roepke		University of Guelph
Owen	Rowland	P35	Carleton University
Peter	Ryser	8.1, 8.3, P41	Laurentian University
Behnaz	Saatian	5.3	Western University
Darya	Safavian	4.4, P29	University of Toronto
Deep	Saini		University of Toronto, Mississauga
James	Santangelo	P30	University of Toronto, Mississauga
Rodney	Savidge	4.3	University of New Brunswick
Barry	Shelp	3.7, 7.3, 10.2, P15	University of Guelph
Inder	Sheoran		University of Toronto, Mississauga
Michael	Stasiak		University of Guelph
Joseph	Stinziano	2.4	Western University
Michael	Stokes	8.5	University of Toronto
Jason	Stout		
Stefanie	Sultmanis	6.3, P24	University of Toronto
Howard	Teresinski		University of Guelph
Gang	Tian	P31	Agriculture and Agri-Food Canada
Martin	Turcotte	9.1, P10	University of Toronto, Mississauga
Nash	Turley	9.1, 9.3, P30	University of Toronto, Mississauga
Vera Marjorie	Velasco		McMaster University
Ying	Wang	P33	Western University
Danielle	Way	2.2, 2.4, P20, P37	University of Western Ontario

FIRST NAME	SURNAME	PRESENTATIONS	AFFILIATION
Harold	Weger	P1	University of Regina
Daniel	Wilson		McMaster University
Katie	Woolfson	1.2	Brock University
Michael	Wozny	1.3, 1.6, P13	University of Guelph
Yue	Wu	P34	Brock University
May	Yeo	P39	McMaster University
Adel	Zarei	7.3, 10.2, P15	University of Guelph
Shuhua	Zhan		University of Guelph
Rongmin	Zhao	P16	University of Toronto Scarborough
Qianru	Zhao	3.6	University of Guelph
Sihui	Zhong		University of Guelph
Ina	Anreiter	volunteer	University of Toronto, Mississauga
Thomas	Braukmann	volunteer	University of Toronto, Mississauga
Christine	Chang	volunteer	University of Toronto, Mississauga
Omar	El-Ansari	volunteer	University of Toronto, Mississauga
Emmanuelle	Frechette	volunteer	University of Toronto, Mississauga
Janola	Jeyachandra	volunteer	University of Toronto, Mississauga
Laura	Junker	volunteer	University of Toronto, Mississauga
Maryam	Moazami-Goudarzi	volunteer	University of Toronto, Mississauga
Sarzana Hasin	Zafar	volunteer	University of Toronto, Mississauga

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Plenary Lectures

Plenary 1

Conifer giga-genomes: sequencing, evolution and diversity

John MacKay*^{1,2,3}, Jukka-Pekka Verta^{1,2}, Elie Raherison^{1,2}, Gaby Germanos^{1,2}, Isabelle Giguère^{1,2}, Sébastien Caron ^{1,2}, Nathalie Delvas¹, Eric Bauce¹, Nathalie Pavy¹, Christian Landry¹, Jean Bousquet^{1,2}, Jean Beaulieu⁴ Department of wood and forest sciences and ² Institute for integrative and systems biology, Université Laval, Québec, Québec, Canada, GIV 0A6

Despite their very large size of 20-24 gigabases, drafts of the Norway spruce and white spruce genomes have recently been published (Nystedt et al. 2013 Nature 497: 579; Birol et al. 2013 Bioinformatics 29: 1492) and a draft of the loblolly pine genome has been released. An overview of the strategies and outcomes of the spruce genome sequencing initiatives will be presented, and recent findings pertaining to the evolution of conifer genomes will be discussed. Results show that conifers genomes have become so large because of uncontrolled mass replication of two major classes retrotransponsons, dating back tens of millions of years. Although the phenomenon is ubiquitous among conifers, it shows significant variations between families. Recent results will also be presented relating to genetic diversity in white spruce covering three aspects: (i) a large-scale analysis of the distribution of intraspecific sequence polymorphisms in regard to protein families, gene expression and natural selection signatures; (ii) genetic diversity affecting gene expression in regard to evolution and adaptation (Verta et al. 2013 Molecular Ecology 22: 2369) and; (iii) diversity in a novel biotic defence mechanism against the spruce budworm. Findings relating to spruce budworm resistance are uncovering what appears to be the first known enzyme in the synthesis of acetophenones, and indicate that glucosylation may be an adaptation to facilitate their accumulation to high levels.

Plenary 2

Boreal forests on permafrost: responses to climate warming

Jennifer Baltzer

Department of Biology, Wilfrid Laurier University

Boreal forests occupy latitudes that are expected to warm most dramatically over the coming decades, and evidence indicates that changes are already underway in these systems. Much of the boreal is underlain by permafrost, which can be expected have important consequences for boreal forests as the climate warms. The southern margin of permafrost is especially susceptible to warming, since in this region, the permafrost is discontinuous, relatively thin, warm and ice-rich. In the zone of discontinuous permafrost, permafrost forms the physical foundation on which trees develop, forming tree-covered peat plateaus where trees are likely to contribute to permafrost maintenance and aggradation processes through reductions in radiation load and changes in snow accumulation. Forests are restricted to peat plateaus while wetland communities characterize the permafrost-free areas. The structure and composition of these communities and transitions among them are strongly influenced by hydrological inputs and connectivity. Evidence suggests that warming is leading to permafrost thaw and ground surface subsidence, which decreases forest cover while increasing wetland hydrological connectivity. Ongoing research regarding the role of permafrost in subarctic boreal forest structure and function will be presented.

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Plenary 3

Agroecology and the challenge of hunger

Herbert Kronzucker

Biological Science, University of Toronto, Scarbourgh

Agricultural activity places greater demands on land and water than any other human activity on the planet, and leads to the emission of more greenhouse gases than all the world's traffic combined. At the same time, agricultural systems are experiencing unprecedented stresses, such as drought, salinity, soil degradation, and a changing climate. I will explore our role as plant biologists in addressing the challenge of feeding a growing human population, with these environmental issues in mind. A few examples from my own team's research in plant nutrition and ion transport will accompany the discussion.

Concurrent Oral Session Abstracts

1.1

Regulation of Proteasomal Activity by Abscisic Acid and High Temperature Stress during germination in Arabidopsis

Rex S. Chiu^{1,2}, and Sonia Gazzarrini^{1,2}

¹Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON Canada ²Department of Cell and Systems Biology, University of Toronto, Toronto, ON Canada

Dormancy is defined as the inability of a seed to germinate under viable growth conditions. Primary dormancy is introduced during the final stages of embryogenesis and is accompanied by a rise in abscisic acid (ABA). Over time, the dormancy of freshly-harvested seeds decreases due to a slow catabolism of, and decreased sensitivity to, ABA. The dormant seed integrates endogenous (hormonal) and environment signals, such as light, temperature, and nutrient availability, to govern germination. Many abiotic stresses, such as high temperature, increase the de novo synthesis of ABA to activate the ABA signalling pathway. This causes global changes in the transcriptome and proteome to maintain dormancy, delay germination, and elicit protective responses. Changes in ABA levels alter the stability and levels of many proteins involved in ABA signalling and dormancy regulation through the proteasome pathway. However, the role and activity of the proteasome during germination and in response to hormonal and environments signals is poorly understood. FUSCA3, a transcription factor involved in desiccation tolerance and dormancy establishment during lateembryogenesis, is induced during germination at high temperature to reinstate dormancy, partially by increasing de novo synthesis of ABA. FUSCA3 is an unstable protein, rapidly degraded by the proteasome and stabilized in the presence of ABA. Here, we characterize the activity of the proteasome during early germination at high temperature and in the presence of ABA, by measuring its ability to degrade FUSCA3 and a synthetic peptide in-vitro. Our results indicate an inactive proteasome during early germination that gradually increases in activity as optimal germination progresses. High temperature stress and exogenous ABA exhibits an inhibitory effect on proteasome activity during germination. In conclusion, our data suggests that a combination of environmental and hormonal signals contribute to regulate the activity of the proteasome during germination.

1.2

ATP-binding cassette transporter from Vinca minor is involved in alkaloid secretion

Katie Woolfson*¹, Fang Yu¹, Vincenzo De Luca¹

The various steps of monoterpene indole alkaloid (MIA) biosynthesis are known to occur in specialized cell types and subcellular compartments. Numerous MIAs display powerful biological activities that have led to their use as pharmaceutical treatments for cancer, hypertension and malaria. Many of these compounds are suspected to accumulate on the leaf surface of medicinally important Apocynaceae plants, which led to the recent discovery and characterization of an ABC transporter (CrTPT2) that was shown to mobilize catharanthine from its site of biosynthesis in epidermal cells to the leaf surface of *Catharanthus roseus* [PNAS (2013) 110: 15830-15835]. Bioinformatic analysis of transcriptomes from several geographically distant MIA-producing species led to the identification of proteins with high amino acid sequence identity to CrTPT2. This study describes the molecular cloning and functional identification of a similar transporter (VmTPT2) from

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Vinca minor. The MIA transport ability of VmTPT2 confirms the hypothesis that this class of transporter may be common to geographically distant members of the Apocynaceae family. The study also suggests that the TPT2 class of transporter evolved by gene duplication and neofunctionalization in this family of plants in order to secrete MIAs onto their leaf surfaces. Details of gene expression related to MIA biosynthesis and accumulation *in planta*, and transport activity in recombinant yeast expressing VmTPT2 will be presented.

1.3

Phosphate deprivation increases the frequency of membrane extensions from plastids

Michael Wozny¹, Neeta Mathur¹ & Dr. Jaideep Mathur¹

¹ Laboratory of Plant development & Interactions, Department of Molecular & Cellular Biology, University of Guelph, Guelph, ON, Canada. N1G2W1.

Stromules are stroma filled tubular extensions of the plastid membrane whose function in the plant cell has remained elusive since their description in the late 19th century. The advent of green fluorescent protein (GFP) based highlighting of stromules has led to recent observations that stromule extension is greatly increased upon exogenous sugar application as well as in response to light in a diurnal cycle. Whereas stromule extension has been proposed as a means of increasing the interactive surface area between the plastid and the rest of the cell it is unclear which specific ions or metabolites might be exchanged through this interaction. In this context an important metabolite for plastid functions is phosphate that plays integral roles in the light and dark reactions within plastids, sugar trafficking to and from the plastid and in fatty acid synthesis and its distribution. Notably plastid membrane alterations are highly dependent upon the availability of phosphate. Given the fundamental importance of phosphate in relation to plastidial functions we manipulated the availability of phosphate to the plant cell and observed the responses of plastids through live imaging using confocal microscopy. Our observations reveal a significant increase in stromule induction frequency under phosphate limiting conditions in both *Arabidopsis* and tobacco. Detailed observations and their implications for understanding the function of stromules will be presented.

14

Uncovering the molecular link between miR156/SPL15 and carotenoid accumulation in Arabidopsis thaliana

Davood Emami Meybodi*^{1,2}, Anthony Percival-Smith² and Abdelali Hannoufa^{1,2}

Carotenoid Cleavage Dioxygenases (*CCDs*) are an enzyme family that cleaves specific double bonds in carotenoids. *MicroR156* in Arabidopsis regulates a network of genes by repressing 10 *SPL* genes, among which, *SPL15* was found to regulate shoot branching and carotenoid accumulation. The expression of *CCD1*, *CCD4*, *CCD7*, *CCD8*, *NCED2*, *NCED3*, *NCED5*, *NCED6*, *NCED9* and *SPL15* was evaluated in siliques at 10 days post anthesis and in 10-day-old roots in Arabidopsis wild type, *sk*156 (miR156 overexpression mutant), *RS105* (*miR156* overexpression line), *spl15* (SPL15 knockout mutant) and two 35S:SPL15 lines. Results showed that most of *CCD/NCED*, genes were affected both in the roots and siliques by function of miR156. In addition transcript levels of four *CCD/NCED* genes were up regulated in 35S:SPL15 lines. To test binding of *SPL15* to affected *CCD/NCED* genes, 35S:SPL15-GFP transgenic lines were generated and three independent lines were subjected to ChIP-qPCR. The results revealed strong and selective binding of SPL15 on the promoter regions of candidate *CCD/NCED* genes. In addition, *sk156* and *RS105* had larger size of elaioplasts and plastoglobules compared to wild type. My results indicate that miR156/SPL15 control carotenoid

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accumulation by regulating expression of CCD/NCED genes and by increasing the cell's capacity to store carotenoids.

1.5

Transposon-mediated epigenetic control of seed germination in cereals

<u>Surinder Singh</u>*, Chi-Kang Tsai, Jaswinder Singh Department of Plant Science, McGill University, Montreal, Canada

Among various functional genomic tools to characterize genes in plants, transposon-based approach offers great potential, especially in barley and wheat, which possess large genomes and genetic transformation is not a routine. Transposons also served as building block for epigenetic gene control due to their ability to recruit the silencing machinery. Using transposon vehicles, we are exploring cereal genes involved in various stages of plant development especially seed dormancy and pre-harvest sprouting. Our barley-rice synteny approach detected 24 candidate ESTs, two of which show polymorphism and differential gene expression in different barley genotype. These genes have indicated their role in malting process. In addition, a key gene of RdDM pathway, *ARGONAUTE4_9* has been found to be associated with Pre-harvest Sprouting in barley and wheat. Our data indicate that *AGO4_9* class acts as an epigenetic switch to determine how a particular plant responds to high humidity and excess rainfall in order to avoid precocious grain germination on the spike. Taken together, this will allow us to further understand the epigenetic role in seed development and germination.

1.6

Characterization Of Plastid Localized Lipases As Regulators of Stromule Formation Through Membrane Contact Sites With The ER

Alena M. Mammone¹, Kiah A. Barton¹, Michael Wozny¹, Neeta Mathur¹ & Dr. Jaideep Mathur¹ Laboratory of Plant development & Interactions, Department of Molecular & Cellular Biology, University of Guelph, Guelph, ON, Canada. N1G2W1.

Targeting of green fluorescent protein to the plastid stroma revealed the presence of dynamic tubules called stromules, that extend and retract from the organelle. Stromules are induced in response to sugar, exhibit a diurnal cycle in response to light and are aligned with tubules making up the endoplasmic reticulum (ER). The mechanism underlying stromule formation and retraction is poorly understood. We speculated that their extension might result from local lipid modifications in the plastid envelope and involve pulling force from the ER at membrane contact sites (MCSs). Recently, a *Brassica napus* chloroplast lipase protein 1 (BnCLIP1) was identified as a putative plastid-ER MCS localizing protein. We used confocal microscopy based live-imaging of transgenic plants of Arabidopsis to evaluate the localization of BnCLIP1:GFP and its physical relation to extending stromules. Our observations show a unique polar localization pattern of BnCLIP1 on the plastid envelope at a point that frequently becomes the base of an extending stromule. Multiple BnCLIP1:GFP punctae are found on very long stromules such as those observed in the accumulation and replication of chloroplasts 6 (arc6) mutants. The Arabidopsis thaliana homolog of BnCLIP1 called AtCLIP has been cloned and exhibits a sub-cellular localization pattern similar to BnCLIP1. Our observations support a clear role for the lipases BnCLIP1 and AtCLIP in local modulation of membrane lipids to facilitate the extension of stromules.

Cytosolic sugar levels regulate mitochondrial morphology in plants

Ashley Jaipargas*, Firas Bou Daher, Nigel Griffiths and Jaideep Mathur

Laboratory of Plant Development and Interactions, Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

Dynamic mitochondrial activity in living cells includes rapid fusion and fission. Whereas mitochondria in animal cells appear elongated and tubular under normal conditions of growth and development, in plants they are usually spherical to ovate with diameters ranging from 0.7 to 1.5 μm. Interestingly, mitochondria in animal cells suffering from high sugar levels display a spherical morphology similar to plant cells. On the basis of these disparate observations of this highly conserved eukaryotic organelle, we hypothesized that sugar availability within a cell is a major regulator of mitochondrial morphology in plants as well. Confocal laser scanning based live imaging of mitochondria in *Arabidopsis thaliana* plants expressing a mitochondrial targeting signal sequence from the beta-ATPase subunit fused to GFP or to photo-convertible mEosFP was used for analyzing mitochondrial dynamics under varying levels of exogenously supplied sugar. In plants since cytosolic sugars increase during photosynthesis our follow up experiments included light and dark growth conditions. We found that removal of light and sugar results in tubular mitochondria whereas increasing sugar levels result in progressively smaller and spherical mitochondria. In addition hypoxic conditions induced giant, plate like mitochondria irrespective of the sugar levels in a cell. We conclude that the response of mitochondria in plant cells is very similar to that observed in animal cells and both cell types rely on the same sugar based regulatory machinery for shaping their mitochondria.

1.8

$Needle\ water\ up take\ facilitates\ xylem\ refilling\ through\ aquaporin\ regulation\ in\ \textit{Picea\ glauca}$

<u>Joan Laur</u>*¹ and Uwe G. Hacke¹

University of Alberta, Department of Renewable Resources, 442 Earth Sciences Building, Edmonton, AB T6G 2E3, Canada

Despite several evidences for foliar water uptake in different plant taxa and notably conifers, the molecular mechanisms underlying this alternative water pathway and its significance are barely considered. Our aim was therefore to monitor the role of needle-source water in stem xylem refilling under control conditions. When aerial parts of drought-stressed *Picea glauca* saplings were exposed to a water-saturated atmosphere, not only stomatal conductance quickly rose to Control level but we also observed an overnight recovery of xylem conductivity in stem. In addition to those physiological inputs, we provide a detailed analysis of aquaporin possible involvement in such mechanisms. Genomic and transcriptomic database analysis revealed that out of 31 Major Intrinsic Protein expressed genes present in *Picea sp.*, 11 are expressed in needles, 7 belonging to the Plasma membrane Intrinsic Protein subfamily. Specifically located on the cell plasmalemma and extensively characterized as true water channels, needle expressed PIPs may be involved in water movement regulation across tissues. Interestingly, protein pattern of PIPs aquaporins is tightly associated with overall plant-water status improvement. Moreover, the 7 PIP genes were monitored at the expressional level. Both the regulation of *PgPIP1;1*, *PgPIP1;2*, *PgPIP2;1* and *PgPIP2;2* along the experiment and their specific localization into endodermal and/or central cylinder cell layers evokes some similarities with the role of aquaporins in the control of the symplastic water path in roots.

The Nitrogen Costs of Photosynthesis in a Diatom under Current and Future pCO₂

Gang Li, Christopher M. Brown, Natalie Donaher, Avery McCarthy & Douglas A. Campbell* *Department of Biology, Mount Allison University, Sackville, NB Canada*

Phytoplankton accumulate large protein pools to mediate photosynthesis, and must also counter the light-dependent photoinactivation of Photosystem II, through a repair cycle. We measured the metabolic cost to accumulate the photosynthetic system and maintaining Photosystem II function in the diatom Thalassiosira pseudonana, growing across a range of light and pCO2. The photosynthetic system contains ~12 to 20% of total cellular nitrogen. Under low growth light Photosystem II enjoys a long functional life, comparable to the generation time of the diatom, and nitrogen (re)cycling through Photosystem II repair is only ~ 1% of the cellular nitrogen assimilation rate. As growth light increases to inhibitory levels, nitrogen cycling through Photosystem II repair increases to ~14% of the nitrogen assimilation rate. We hypothesize this light-dependent burden upon nitrogen metabolism limits diatom exploitation of increasing growth light. Cells growing under the future 750 ppmv pCO2 show higher growth rates under optimal light, coinciding with a lowered metabolic cost to maintain photosynthesis, but then suffer increased photoinhibition of growth, coincident with sharply rising costs to maintain their photosynthesis under combined excess light and higher pCO2. We are now analyzing light-dependent nitrogen cycling costs across other phytoplankton groups.

2.2

Thermal acclimation of photosynthesis: on the importance of definitions and considering respiration Danielle A. Way¹ and Wataru Yamori²

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While interest in photosynthetic thermal acclimation has been stimulated by climate warming, comparing results across studies requires consistent terminology. We identify five types of photosynthetic adjustments in warming experiments: photosynthesis as measured at the high growth temperature, the growth temperature, and the thermal optimum; the photosynthetic thermal optimum; and leaf-level photosynthetic capacity. Adjustments of any one of these variables need not mean a concurrent adjustment in others, which may resolve apparently contradictory results in papers using different indicators of photosynthetic acclimation. We argue that photosynthetic thermal acclimation (i.e. that benefits a plant in its new growth environment) should include adjustments of both the photosynthetic thermal optimum (Tont) and photosynthetic rates at the growth temperature (Agrowth), a combination termed constructive adjustment. However, many species show reduced photosynthesis when grown at elevated temperatures, despite adjustment of some photosynthetic variables, a phenomenon we term detractive adjustment. An analysis of 70 studies on 103 species shows that adjustment of Topt and Agrowth are more common than adjustment of other photosynthetic variables, but only half of the data demonstrate constructive adjustment. No systematic differences in these patterns were found between different plant functional groups. We also discuss the importance of thermal acclimation of respiration for net photosynthesis measurements, as respiratory temperature acclimation can generate apparent acclimation of photosynthetic processes, even if photosynthesis is unaltered. We show that while dark respiration is often used to estimate light respiration, the ratio of light to dark respiration shifts in a non-predictable manner with a change in leaf temperature.

Sensitivity of autumn cold acclimation to elevated temperature in Pinus strobus

Christine Chang¹, Emmanuelle Fréchette¹, Ingo Ensminger¹

Autumn phenology is driven by decreasing temperature and photoperiod. These signals induce evergreen conifers to prepare defenses against winter stressors, including downregulating photosynthesis to reduce photooxidative damage, and cold hardening to protect overwintering needles from freezing. However, elevated temperatures associated with climate change may adversely affect timing of such phenological events. Here we aim to assess the impact of elevated autumn temperature on photosynthesis and cold hardening in Pinus strobus. We hypothesized that increasing temperatures will extend the growing season, delaying downregulation of photosynthesis and cold hardening. In a temperature free-air-controlled enhancement (T-FACE) experiment at Koffler Scientific Reserve in King City, Ontario, 3-year-old seedlings were arranged in 5 control and 5 elevated temperature (eIT) plots heated +1.5°C day/+3°C night. Photosynthesis was assessed monthly from August to December using gas exchange and chlorophyll fluorescence; freezing tolerance was assessed in August and December. Treatments showed similar downregulation in photosystem II dynamics over the five months. However, elT plants experienced a 50% reduction of stomatal conductance and 10% reduction of carbon assimilation during August-October. During August-December, we also observed higher nonphotochemical quenching (NPQ) in elT plants, indicating that these plants experience increased impairment of photochemical reactions. There was no significant difference in freezing tolerance between treatments after cold hardening. We conclude that elevated autumn temperatures will not significantly delay photosynthetic downregulation and does not impair cold hardening in P. strobus. However, elevated autumn temperatures may induce additional mechanisms of temperature-stress mitigation during photosynthetic downregulation and cold hardening.

2.4

Canada's Forests in a Changing Climate: Impacts, Responses, and Unanswered Questions Joseph R. Stinziano^{1,*} and Danielle A. Way^{1,2}

Air temperatures are expected to increase to a greater extent at high latitudes this century, mainly due to anthropogenically-driven increases in CO₂. Warming is expected to positively influence Canada's boreal forests by lengthening the growing season, advancing the treeline, and increasing productivity and growth. Combined with the expected effects of CO₂ fertilization on tree growth, Canada's forests are expected to become a greater carbon sink this century. However there are other environmental factors such as photoperiod, edaphic features, water, and nutrient availability that may constrain tree responses to global change. Further, the interactive effect of increased temperature and CO₂ on Canadian boreal species as a whole is relatively unknown. Using a meta-analysis of studies involving temperature and CO₂ manipulations on Canadian boreal tree species, we find that Canadian boreal forest species as a whole show a decline in biomass with increasing growth temperatures, which is ameliorated by elevated CO₂. Photosynthetic CO₂ assimilation tends to respond positively to increased temperatures and CO₂, while there is insufficient data on the effects of temperature on photosynthetic capacity in Canadian boreal species to draw conclusions. The constraint that photoperiod imposes on seasonal changes in photosynthesis in Canadian boreal evergreen species is currently unknown, but my on-going work aims to elucidate whether photoperiod impacts the autumnal decline in photosynthetic capacity in a dominant Canadian tree species, white spruce (*Picea glauca*).

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The photochemical reflectance index (PRI) as an indicator of photochemical efficiency in *Pinus strobus* during the autumn.

Emmanuelle Fréchette*¹, Christine Chang¹ and Ingo Ensminger¹

In summer, dissipation of excess light energy in evergreen conifers is facilitated by dynamic changes in the deepoxidation state (DEPS) of the xanthophyll-cycle, a group of photoprotective pigments. This quickly reversible process allows for rapid adjustment of non-photochemically quenched energy (NPQ). Changes in xanthophylls are reflected in leaf spectral signatures, and are closely related to the photochemical reflectance index (PRI). In autumn, as conifers transition to winter dormancy, flexible energy dissipation via the xanthophyll-cycle (q_E) is gradually replaced by sustained energy quenching (q_I) as the photosynthetic apparatus reorganizes. Our objectives were to investigate 1) the relationship between xanthophyll-cycle dynamics, PRI, and NPQ during the autumn transition from the q_E to the q_I energy quenching mode, and 2) how elevated autumn temperature might affect this relationship. In climate-controlled experiments, summeradapted 3-year-old Pinus strobus seedlings were subjected to either low temperature control autumn (12°C) or elevated temperature autumn (22°C) conditions. Over 36 days, we followed PRI, needle photosynthetic pigment content, chlorophyll fluorescence and carbon assimilation. We observed that low temperature exposure is necessary for q_I induction, and that shortened daylength alone is insufficient to trigger photosynthetic downregulation in Pinus strobus. We also observed a strong positive correlation between PRI and photosynthetic efficiency, and a strong negative correlation between PRI and DEPS under warm autumn conditions. Both relationships weakened with exposure to low autumn temperature. In both autumn treatments, PRI appeared to be controlled primarily by chlorophyll and xanthophyll pigment content, suggesting that for evergreen conifers PRI is controlled by adjustments in total pigment pools, rather than DEPS at a seasonal scale.

2.6

Modeling spatial and temporal variations in leaf chlorophyll content from remotely-sensed satellite data Holly Croft¹, Jing M. Chen¹, Yongqin Zhang², Anita Simic³, Thomas L. Noland⁴ and Nadine Nesbitt¹

Leaf chlorophyll content is a paramount ecological variable that plays a central role in plant photosynthesis, indicates vegetation stress and provides inputs to plant productivity and carbon-cycle models. However, retrieving leaf chlorophyll from remotely-sensed data is complex, as canopy reflectance in visible and near-infrared wavelengths is also affected by leaf area index (LAI), canopy architecture, illumination/viewing geometry and understory vegetation. Whilst considerable research has focused on developing statistical relationships between spectral indices and chlorophyll content, these algorithms are often ecosystem or location specific. By contrast, process-based models use physical laws to account for confounding canopy reflectance variables, which could provide more accurate leaf chlorophyll estimates.

This research investigates the retrieval of leaf chlorophyll content using empirical spectral indices and a coupled canopy (4-Scale) and leaf (PROSPECT) model. Over thirty sites were selected in Ontario, Canada representing a range of dominant vegetation species (Picea mariana, Pinus banksiana, Populus tremuloides and Acer saccharum) and canopy architectures. Canopy reflectance data were acquired from satellite remotesensing platforms (Landsat-5 TM and MERIS). Ground measurements included LAI, leaf reflectance spectra

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and laboratory chlorophyll content. Leaf chlorophyll ranged from 16-78 $\mu g/cm^2$ and LAI from 1.14-7.15, providing a large dynamic range. Process-modeled results showed strong relationships with measured chlorophyll content for satellite products (e.g. Landsat-5, $R^2 = 0.78$). Empirical methods performed well under homogenous canopy conditions, and where LAI = >3. However, for coniferous species, understory and non-photosynthetic materials played a perturbing role; requiring site calibration for accurate chlorophyll retrieval and limiting their application over larger spatial extents.

2.7

Field grown Douglas-fir provenances differ in photosynthetic performance under conditions of limited water availability

Laura Verena Junker*^{1,2}, Henning Wildhagen ^{1,3}, Ingo Ensminger ^{1,2}

Douglas-fir (Pseudotsuga menziesii) is grown as valuable commercial timber throughout North America and Europe, but climate change and associated summer droughts might limit its economic value. In its original North American habitat two subspecies evolved under dry and humid conditions, Interior (var. glauca) and Coastal Douglas-fir (var. menziesii), respectively. Drought tolerance is often associated with include increased ability for photoprotective mechanisms mediated by photoprotective isoprenoids, in particular thermal dissipation of excess energy via non-photochemical quenching in the xanthophyll cycle. We hypothesize that drought adaptation involves increased amounts of photoprotective isoprenoids, since drought limits photosynthesis and increases the need for photoprotective non-photochemical quenching. 50-year-old trees of one Interior and three Coastal provenances from contrasting habitats grown at two common garden field sites in Southern Germany were studied under varying conditions of water availability to assess the impact of isoprenoid-mediated photoprotective mechanisms on drought tolerance. Gas exchange and chlorophyll fluorescence measurements were linked to isoprenoid content analyses of needle samples. As expected, seasonal and site specific constrains in water availability limit photosynthetic metabolism by decreased stomatal conductance and consequently reduced CO2 assimilation rate. Provenance specific differences in assimilation rate reveal superior performance of the Interior provenance Salmon Arm and Coastal provenance Conrad Creek. While Salmon Arm can maintain twice as high stomatal conductance and assimilation rates under drought, Conrad Creek shows slightly higher xanthophyll cycle pool sizes at all times indicating that drought tolerance may be linked to improved photoprotective potential. We conclude that provenances vary in their physiological response to drought due to local adaptation.

3.1

Sugar insensitivity in a model Arabidopsis thaliana cell suspension culture

Avery McCarthy*¹, Michelle Chung¹ and Norman P. A. Hüner¹

Sugars are a major end-product of photosynthesis, and they have important roles as both nutrients and signaling molecules. Normally, when sugars accumulate to high levels in vegetative tissue, photosynthetic gene expression is repressed. However, we have identified a cell suspension culture of *Arabidopsis thaliana* that is insensitive to sugar. When grown in media containing 3-15% sucrose, this cell culture has a dark green phenotype and expresses the major photosynthetic proteins. Both light- and dark-grown cells had comparable growth rates when grown in a medium containing 3% sucrose, thus the cells can import ample sugar from the medium. Why are the cells expressing the photosynthetic machinery when it is not necessary for growth?

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Insufficient intracellular sugar accumulation could have explained the sugar insensitive phenotype. Since the major sugar sensors in *Arabidopsis* cells are localized to the cytoplasm, intracellular sugar concentrations largely contribute to sugar signaling. Therefore, the cell suspension culture was grown in media containing 0-9% sucrose, and intracellular sugar content was quantified with enzymatic assays. Large changes in the sugar content of the medium had no effect on cellular sugar concentrations in the cell suspension culture. Therefore, intracellular sugar concentrations in these cells are regulated by sugar transport. Remarkably, the sugar concentration in the cells was comparable to levels in non-green leaves of seedlings grown on a high sucrose medium. Therefore there is substantial sugar accumulation in the cells, suggesting that dysfunctional sugar sensing or sugar signaling could be responsible for the sugar insensitivity.

3.2

Post-translational Interactions Among Enzymes of Starch Biosynthesis and Their Affect on Physical Properties of Starch in High-amylose Barley Genotypes.

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The present study investigated the role of protein phosphorylation, and protein complex formation between key enzymes of amylopectin synthesis, in a range of barley mutants exhibiting "high amylose" phenotypes. The genotypes used in this study were mutants of starch branching enzyme (sbeiia and sbeiib), starch synthase SSII (ssii, sex6) and SSIII (ssiii, amo1). Mutation in either starch branching enzyme (SBE) IIa or IIb caused pleiotropic effects on the activities/ amounts of starch synthases (SSs) and starch phosphorylase (SP). Mutation in either of SBEIIa or SBEIIb resulted in formation of novel protein complexes in which the missing SBEII isoform appears to be substituted by SBEI and SP. In the sbeiib mutant there was a > 95 % loss of measurable, soluble SP activity. None the less, SP was incorporated into a heteromeric protein complex with SBEI and SBEIIa. Accumulation of starch in the sbeiib endosperm was reduced compared with sbeiia and the reference genotype. Evidence suggests that in sbeiia and sbeiib mutants, different protein complexes are involved in the synthesis of A- and B-granules reflected in alteration of the granule proteome. The amol mutation resulted in pleiotropic effects on SS activities. Soluble activity of SSI was increased compare to the reference genotype. In the sex6 mutant no protein complexes involving SBEIIa or SBEIIb were detected in amyloplasts. Studies with Pro-Q Diamond revealed that GBSS, SSI, SSIIa, SBEIIb and SP are phosphorylated in their granule bound state. A higher amylose content was associated with altered thermal properties of starch, and significantly reduced onset temperature (To) and (ΔH) of gelatinization compared to the reference genotype. This study adds to our understanding of the mechanisms by which mutations in the biochemical pathway of starch biosynthesis give rise to the resulting starch phenotype.

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Cellulose, pectin and an AGP facilitate rapid expansion of seed coat mucilage.

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Primary plant cell walls are composed of three types of carbohydrates (cellulose, pectins and hemicellulose) and structural proteins. Interactions between these components are complex and poorly understood. Arabidopsis seed mucilage, composed primarily of pectins, but with smaller amounts of cellulose and hemicellulose, is a unique type of cell wall that represents an excellent system to study interactions between cell wall components. Hydration of seeds induces the rapid expansion of mucilage that then separates into two distinct layers: an adherent layer that remains attached to the seed and a non-adherent layer that can be removed by gentle shaking. Two genes have been shown to be required for mucilage adherence: CELLULOSE SYNTHASE 5 (CESA5) and the arabinogalactan protein, SALT-OVERLY SENSITIVE 5 (SOS5). Mutations in both genes have nearly identical phenotypes, leading to the hypothesis that SOS5 mediates adherence through CESA5. Here we examine the genetic interactions between CESA5 and SOS5, and demonstrate that these two genes function independently to mediate mucilage adherence. The cesa5-1 sos5-2 double mutant is more severely affected than either single mutant. Biochemical analysis of cell wall composition in cesa5 and sos5 mutants indicates that SOS5 is not involved in cellulose biosynthesis. Further genetic interactions between CESA5/SOS5 and FLYI, a pectin-related gene, indicate that SOS5 is actually required for pectin-mediated adherence. By combining observations from the localization of pectins and cellulose in mucilage with functional data on CESA5 and SOS5, we have developed a model of interactions between cellulose, pectin and AGPs that explains how mucilage expands.

3.4

Post-Translational Regulation of Starch Synthase II in Arabidopsis thaliana

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Starch is the most important caloric energy source to humans, and is produced in plants in both storage tissues, such as tubers and seed endosperm, and in leaves to transiently store excess photosynthates, which are degraded at night and support respiration. Starch is composed of two glucan polymers: branched amylopectin and linear amylose, the relative quantity of each determines the granule's crystallinity and biochemical properties. The maize sugary-2 (su2) mutant granule phenotype has a higher freeze-thaw tolerance without significantly decreasing carbohydrate content or granule size, thus is widely used for both food and industrial applications. A su2 allele is caused by a single amino acid substitution (Gly522>Arg) in a glucan-binding domain of the biosynthetic enzyme, starch synthase II (SSII). SSII forms the core of a heteromeric protein complex, involved in amylopectin cluster formation; the mutation in its glucan-binding domain not only affects SSII catalytic activity, but also prevents the trafficking of all associated enzymes into the granule, causing the altered granule phenotype. SSII is also phosphorylated within the starch granule, but the site and effects of this modification on catalytic activity and complex assembly remain unknown. The objectives for this project are to identify and characterize post-translational modifications of SSII in Arabidopsis thaliana in vitro, using recombinant proteins, and in vivo, by complementing SSII-null mutants using Agrobacteriummediated transformation. Given that SSII is necessary for amylopectin synthesis and multi-protein complex trafficking, it is important to understand the mechanisms underpinning protein complex formation, and the role protein phosphorylation in mediating this process.

Branching Enzymes as Agents for Modifying Glucan Structure in Industrial Processing

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Starch is used as a cheap, renewable, chemically reactive matrix in many industrial processes. During processing, access to chemically reactive groups on starch is essential and largely depends on their exposure, which is a factor of the branching frequency within starch.

The ability to manipulate glucan branching in starch and other polyglucans offers many industrial end users (e.g. paints/inks, coatings, adhesives sectors) with superior performance bio-products. Branching enzymes (BE) introduce α -1,6 branch points in starch, consequently increasing the number of non-reducing reactive chemical ends, thus making starch more reactive to synthesis and digestion, and facilitating its solubility. Although existing starch-based crops with desirable structural traits are available (e.g. waxy maize), they are more costly than normal starches, and immediate product improvement is currently only possible through post-harvest modification of starch using environmentally hazardous chemical agents. A number of thermotolerant recombinant BEs suited to a range of industrial processing conditions have been developed and their kinetics characterized through various activity assays. The branching patterns introduced by recombinant BEs in a number of test glucans have been analyzed and compared. The BEs developed throughout the course of this project offer an important new tool and platform technology for bio-product development, which will circumvent the use of environmentally hazardous chemical agents. Additionally, the ability to modify the branching of normal starches will potentially open the market for locally grown starch-based crops as a raw material for biopolymer industries.

3.6

Starch biosynthesis in Arabidopsis thaliana

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Starch is an important carbohydrate in higher plants and widely used in food and non-food industries. Starch synthesized in different plastids can be divided into two groups, transient starch in chloroplasts and storage starch in amyloplasts, transient starch turns over in a day/night cycle. Previous studies in amyloplasts suggest that protein phosphorylation-dependent assembly of multi-enzyme complexes is involved in storage starch biosynthesis. The present study investigated the post-translational regulation of starch synthases (SS), and starch branching enzymes (SBE) in leaf chloroplasts of Arabidopsis thaliana including protein phosphorylation and protein-protein interactions. The sub-organelle distribution of SS and SBE isoforms was investigated in wild-type and several mutant lines. In the wild type, SS2 and SBE2.2 were found to be trapped in the starch granule at the end of the day in 16/8 h light/dark photoperiod. Starch granules of ss2 mutants also lacked SBE2.2 which suggests that SS2 traffics other biosynthetic enzymes into the starch granule. ss3 mutants lacked SS2 and SBE2.2. The cDNA sequences of SBE isoforms were cloned into commercially available vectors and expressed with an affinity S-tag in Escherichia coli. y- [32P] -ATP labelling of the recombinant proteins showed that recombinant SBE 2.1 and SBE2.2 can be phosphorylated by chloroplast lysates extracted during the light period. SBE activity was reduced by dephosphorylation using a non-specific bacterial alkaline phosphatase. This study provides evidence that enzymes of starch biosynthesis in leaves are regulated by post-translational modification and provides insight for understanding starch synthesis and turnover in crops.

Oxidative stress metabolism is associated with flesh browning of 'Honeycrisp' apples

<u>Geoffrey B. Lum</u>*¹, Carolyne J. Brikis¹, Kristen L. Deyman¹, Gordon J. Hoover¹, Jennifer R. DeEll², Barry J. Shelp¹ and Gale G. Bozzo¹

1-Methylcyclopropene (1-MCP, ethylene antagonist) application and controlled atmosphere (CA) storage are practices used to maintain quality and minimize senescence of apples. CA consists of low temperature in combination with low O₂ and elevated CO₂ partial pressure. A drawback is 1-MCP tends to exacerbate flesh browning during elevated CO₂ storage. Recent reports suggest that pre-conditioning apples at 10°C for up to 7 days before CA storage reduces the incidence of physiological disorders. However, no such strategy exists for 'Honeycrisp' fruit. Furthermore, limited information exists regarding the early biochemical mechanisms promoting flesh browning in 'Honeycrisp' apples, but may involve alterations in oxidative stress metabolite levels. In this study, we investigated the effect of pre-conditioning and 1-MCP on CA-related flesh browning development in 'Honeycrisp' apples and the change in ascorbate/dehydroascorbate and glutathione/glutathione disulphide balance, as well as γ-aminobutyrate, which were quantified by enzyme-coupled spectrophotometric and HPLC assays, respectively. During CA, 1-MCP treated apples developed an 80% incidence of flesh browning, which was coincident with a 4-fold increase in γ-aminobutyrate, as well as 65% and 70 % declines in glutathione/glutathione disulphide and ascorbate/dehydroascorbate ratios, respectively. By comparison, preconditioning apples for 5 days at 10°C decreased the impact of 1-MCP on the incidence of flesh browning by nearly 80%, and this was associated with 70% lower γ-aminobutyrate concentration. Moreover, a transient 3fold increase in ascorbate/dehydroascorbate ratios occurred during pre-conditioning, and an 80% decline in glutathione/glutathione disulphide ratio was apparent within 2 weeks of storage. This project may provide diagnostic information on storage disorders of apples.

3.8

Extensive gene loss and plastome rearrangements in mycoheterotrophic Ericaceae

Thomas Braukmann*¹, and Saša Stefanović¹

Heterotrophic plants exhibit a wide range of evolutionary degradation of photosynthetic ability and rely entirely or partially on their hosts to supply water and nutrients. These plants are divided into two distinct, but evolutionary artificial groups, parasitic and mycoheterotrophic plants. Haustorial parasitism has evolved at least 11 times independently and there are at least 10 independent origins of mycoheterotrophy in angiosperms. Ericaceae, the heather family, is a large and diverse group of plants with elaborate symbiotic relationships with mycorrhizal fungi, including several mycoheterotrophic lineages. Grounded within a phylogenetic framework and broad taxonomic sampling, a comparative investigation of plastid genomes was conducted in the family using a slot-blot Southern hybridization approach. This survey contained lineages within Ericaceae with different life histories and trophic levels, including multiple representatives from the hemi-mycoheterotrophic pyroloids and holo-mycoheterotrophic monotropoids. A number of fully photosynthetic (autotrophic) members were included to best represent the other major clades within Ericaceae. This survey used 55 probes derived from all categories of protein-coding genes typically found in the plastomes of photosynthetic plants. Based on the hybridization results, the plastomes of eight representative Ericaceae species from different trophic levels were using the nextgen sequencing approach (Illumina). Preliminary results indicate an unusually high number of plastome rearrangements within Ericaceae. Consistent with the hybridization results, the plastomes

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of monotropoids exhibit extensive loss off genes relating to photosynthetic function and retain genes with possible function outside photosynthesis. Furthermore, hemi-mycoheterotrophic plants retain most genes relating to photosynthesis but are polymorphic for the plastid 'ndh' genes. Our results extend previous inferences that plastid gene losses occur prior to becoming holo-heterotrophic and that mycoheterotrophic Ericaceae exhibit gene loss similar in pattern to parasitic plants.

4.1

Genetic and epigenetic impacts on growth performance and stress response in Populus

<u>Katharina Bräutigam</u>^{1,3}, Sherosha Raj¹, Sabrina Chamberland², Stefan Schreiber², Barb Thomas², Uwe Hacke², and Malcolm M. Campbell^{1,3}

For plants, it is of key importance to adapt to local environments. This might be especially true for long-lived clonally propagated forest trees, like poplar. While largely determined by the genetic makeup, growth and performance of plants also retain a degree of plasticity which is crucial under ever-changing environmental conditions. To assess the persistent influence of past experiences on plant performance, five economically genotypes were compared. For each genotype, vegetatively propagated plants from different geographic locations (i.e. genetically identical individuals with different clone histories) were grown and studied in a common environment. The analyses of growth performance, physiological responses, and hydraulic traits uncovered genotype-specific differences, as well as differences based on geographic origin of popular clones with identical genotypes. Modifications in resource allocation and physiological parameters revealed variation in the strategies employed to contend with an abiotic stress, drought, between genotypes. Moreover, differences between genetically identical plants with different clone histories revealed within-genotype plasticity. More recently, in addition to genetic factors, the importance of epigenetic patterns for plant performance has been reported. Differences in drought transcriptome responses in genetically identical genotypes were found to be paralleled by differences in DNA methylation indicating a possible mechanism for adding an additional layer of plasticity in long-lived organisms. The work contributes to our understanding of plant-environment interactions, with applications related to nursery source effects in poplar clones and their impacts on future clone performance in plantations.

4.2

Regulatory motifs identified from a maize developmental coexpression network.

Gregory S. Downs*¹; Christophe Liseron-Monfils²; Lewis N. Lukens¹

Transcriptional control is an important determinant of plant development, and distinct modules of coordinated genes characterize plant developmental transcriptomes. Upstream regulatory sequences are often the primary factors that control gene expression pattern and abundance. Recently, we reported at set of 24 gene expression modules constructed from transcript abundances of 34,876 *Zea mays* (maize) gene models from embryogenesis to senescence. Here, we present 244 regulatory motifs that are significantly enriched within these coexpression modules. We identify motifs that have not been characterized. In addition, we identify motifs similar to experimentally verified motifs, and the functions of these motifs overlap with predicted module functions. This

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work demonstrates the power of transcript-level coexpression modules to identify both variants of known regulatory motifs and novel motifs that control a species' developmental transcriptome.

4.3

New Insight into the Biogenesis of Bordered Pits and Cellulose

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Introduction: Developmental competence for bordered-pit formation had been achieved by Late Silurian (~420 Ma). Bordered pits have frequently been described; however, the cell biology underlying how, when and where they form has remained a mystery.

Objective: The aim was to elucidate how bordered pits develop in cambial derivatives of *Abies balsamea* (L.) Mill.

Methods: Light and electron microscopy investigations were done at sequential phenological stages, beginning with late-winter dormancy extending into springtime earlywood formation.

Results: The precisely opposed locations of a pit pair between adjoining cells first appear as localized modifications to the opposing plasma membranes, creating a circular bordered-pit template in each. The template's centre evidently controls subsequent events. A spherical vesicle ca. 2 µm in diameter is conveyed through cytoplasm to the template centre. At contact, the vesicle enlarges to approximately 4 µm in diameter and transforms into a bordered-pit organelle (BPO) displaying fine thread-like material on its surface. The spherical BPO becomes anchored in the template by radiating microfilaments, whereupon the BPO collapses into a disk appressed against the template. A cellulosic ring, the developing pit border, arises at the perimeter of the BPO disk. After the template border has been reinforced with microfibrils, the primary-wall areas beneath the template are hydrolyzed, and the original bordered-pit template emerges as the pit membrane with central torus.

Discussion: The overall process of bordered-pit formation is highly complex and wanting of more intensive investigation. Production of cellulose microfibrils by the BPO requires reassessment of all current thinking about cellulose biogenesis

4.4

The role of ARC1 in the self-incompatibility pathway in Arabidopsis spp.

Emily Indriolo¹, Darya Safavian¹ and Daphne Goring¹

In some species of flowering plants, genetic diversity is driven by preventing self-fertilization, a trait known as self-incompatibility. In the Brassicaceae, this process is regulated by a signalling pathway activated by the stigma-specific, *S* Receptor Kinase (SRK), following binding of a pollen-specific ligand, SCR/SP11. In *Brassica* species, downstream signalling components of the pathway have been identified such as the *M* Locus Protein Kinase, the ARC1 E3 ubiquitin ligase, and the Exo70A1 subunit of the exocyst complex. While the functions of SCR/SP11 and SRK are known to be conserved in the *Arabidopsis* species, nothing is known about the downstream signalling pathway. We performed a genome wide survey of numerous species in the Brassicaceae and determined that ARC1 is frequently deleted in self-compatible species, indicating that ARC1 may have a conserved role in self-incompatibility signalling in the Brassicaceae. We identified an *A. lyrata ARC1* homologue to *Brassica ARC1*, and investigated if the role of ARC1 is conserved in regulating pollen rejection in the naturally occurring *Arabidopsis lyrata* self-incompatibility system. We demonstrated that *ARC1* was required for self-incompatibility in *A. lyrata* and have shifted focus to an artificial self-

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incompatibility system in *A. thaliana*. These results lead us to investigate what would happen when *ARC1* was expressed in *A. thaliana* with *SRK* and *SCR* and we are currently examining plants expressing all three genes. We also are in the process of confirming the conservation of the proposed protein-protein interactions of ARC1 with SRK and Exo70A1, to further study the conservation of this pathway.

4.5

A small-molecule screen in Arabidopsis and yeast uncovers selective hormone mimics

<u>Duncan Holbrook-Smith</u>*¹, Dr. Shigeo Toh¹, Dr. Yuichiro Tsuchiya², Dr. Peter McCourt¹

Strigolactones are a newly discovered class of plant hormones that are responsible for suppressing axillary branching, but they also play a role as interspecies signaling molecules. Strigolactones are exuded into the rhizosphere to encourage symbiotic fungal associations, but certain parasitic plants have evolved the ability to use host strigolactones as a cue for germination and parasitism. These parasitic plants, most notably *Striga hermonthica*, cause 7 billion dollars worth of crop damage in the developing world, and thus understanding how strigolactones are perceived is of great humanitarian importance. Over the past year a small family of α/β hydrolases have been identified as the receptors for strigolactones in a variety of plant species. Genetic evidence suggests that one of the receptors known as HTL/KAI2/D14L(HTL) is required for germination in response to strigolactone in Arabidopsis. This has lead to the question of how signal transduction through HTL works on a mechanistic level in both Arabidopsis and *Striga*. In the past, selective hormone signaling agonists have been used to understand receptor function and elucidate signaling mechanisms. In order to develop selective agonists for strigolactone signal transduction, a trio of screens of up to 14200 small-molecules have been conducted to identify strigolactone mimics that are selective for specific molecular targets of HTL.

4.6

A defined role for an Auxin Response Factor in de novo shoot formation

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In vitro regeneration of complete organisms from diverse cell types is a spectacular property of plant cells. Despite the great importance of plant regeneration for plant breeding and biotechnology, its molecular basis is still largely unclear and many important crop plants have remained recalcitrant to regeneration. Hormone-exposure protocols to trigger the *de novo* formation of either roots or shoots from callus tissue demonstrate the importance of auxin and cytokinin signaling pathways, and genetic differences in these pathways may contribute to the highly divergent responsiveness of plant species to regeneration protocols. Using loss-of function mutant, mis/overexpressor and gain-of-function genotypes we define the role of *MONOPTEROS* (MP)/AUXIN RESPONSE FACTOR 5 in establishing the signaling landscape during stages of *in vitro* organogenesis. Our data suggests that ARF5 has profound impact on regeneration properties in Arabidopsis and presumably in a wide variety of plant species. Moreover, MP exerts its role in *de novo* shoot formation through known positive regulators of shoot stem cell formation, such as SHOOTMERISTEMLESS/(STM) and the cytokinin signaling pathway component, CYTOKININ RESPONSE FACTOR2/ (CRF2).

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BLADE-ON-PETIOLE genes: setting boundaries in inflorescence development

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BLADE-ON-PETIOLE (BOP) genes encode an ancient and conserved subclade of BTB-ankryin transcriptional co-activators, divergent in the NPR1 family of plant defense regulators. Loss-of-function studies in a variety of land plants show that BOPs modulate growth, differentiation, and determinacy at lateral organ boundaries in control of leaf, flower, and inflorescence architecture and abscission. Our recent work in Arabidopsis shows that BOP expression is excluded from the apical meristem by a pair of BEL1-like homeodomain proteins, PENNYWISE (PNY) and POUNDFOOLISH (PNF), whose products function with SHOOT MERISTEMLESS in conferring meristem cell fate. Loss-of-function pny pnf mutants are an interesting case because meristem perception of floral inductive signals is blocked, resulting in a non-flowering phenotype. Our work shows that misexpression of BOPs in the shoot apical meristem of pny pnf mutants activate a set of lateral organ boundary genes whose products block meristem acquisition of inflorescence fate. We further show that activity of this pathway is dependent on Clade I TGA bZIP transcription factors, TGA1 and TGA4, whose expression overlaps with BOPs at lateral organ boundaries. Identifying the transcriptional targets of this pathway will shed light on how the meristem responds to floral inductive signals in making the inflorescence.

4.8

Differential expression between male and female flowers of balsam poplar

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The organisation and function of plant organs is shaped by the transcriptional regulation of genes in those organs. This study interrogated patterns of transcript abundance in *Populus balsamifera* (balsam poplar) and *Arabidopsis thaliana* organs, including leaves, roots, xylem, and flowers, to identify developmentally-specified transcription modules. Male and female catkins in balsam poplar showed highest degree of similarity in transcript abundance across the subset of the examined transcriptome. A similar pattern was observed in young and mature leaves in *A. thaliana*, while 34% of genes analysed showed a significant and highest degree of difference between the male and female tissues (stamens and carpels, respectively). Although transcript abundance patterns in male and female balsam poplar catkins were virtually identical, a set of 36 genes (0.47% of total genes analysed) showed a significant difference between males and females. These genes were further examined in an attempt to identify gene(s) significant to male or female development in poplar and to investigate their homology in *A. thaliana*.

5.1

The Importance of Plant total RNA purification method for down stream applications Won-Sik ${\rm Kim}^{*1}$ and Yousef Haj-Ahmad $^{1,\,2}$

MicroRNAs are endogenous 20 to 24 nucleotide noncoding RNAs that play crucial posttranscriptional regulatory roles in plant and animals. Tremendous efforts are currently being undertaken to understand the profile of the entire miRNA population of a biological sample. Many miRNA discovery tools, including micro

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arrays and Next-gen-based sequencing, have made it possible to comprehensively and accurately assess the entire miRNA repertoire. A prerequisite for obtaining successful results from these approaches is an efficient method for total RNA purification without bias. The choice of the method of RNA purification is critical to the outcome of downstream analysis. This is made more significant in variations of the plant specimens and the high phenolics, starch and other inhibitors co-isolating with the RNA. The three most popular RNA purification methods (spin columns using Silicon Carbide, spin columns employing silica membrane and phenol/chloroform extraction) are compared in terms of quality, quantity and small RNA recovery from difficult and moderately challenging plant samples and the examples of the result will be discussed.

5.2

Estimating allele-specific expression levels from RNA-Seq data for Zea mays

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RNA-Seq analyses have the potential to estimate the transcript abundances of different alleles of the same gene. However, estimates of allelic abundances may be biased when reads are mapped to an assembled reference genome. Reads of transcripts with high similarity to the reference allele map more efficiently than reads with low similarity to the reference allele. Mapping bias is a particular problem in maize because of its highly polymorphic genome. We describe the magnitude and prevalence of this bias in an analysis of RNA-Seq data from reciprocal B73 x Mo17 hybrids mapped to the B73 reference genome. To reduce mapping bias within the hybrids, we describe a multi-step data processing strategy that utilizes RNA-Seq data from the inbred parents. The data processing strategy improves estimates of allele-specific expression levels, thereby strengthening downstream analyses.

5.3

Next-generation mapping of seed storage protein gene repressors in *Arabidopsis*

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Seed maturation is a highly regulated developmental phase when the embryos accumulate high quantities of storage compounds, including seed storage proteins (SSPs). Genes encoding SSPs are specifically and highly expressed in the seed during maturation. However, the mechanisms that repress the expression of these genes in vegetative tissues are not well understood. To identify the repressors of SSP in vegetative organs, transgenic Arabidopsis plants expressing the GUS reporter gene under the control of a SSP gene promoter were generated. Homozygous transgenic plants were mutagenized and the progeny of mutated plants were screened for individuals displaying ectopic expression of seed storage protein genes (essp). Two mutant lines, essp7 and essp8, were used in this study. Genetic analysis revealed that essp7 and essp8 mutations are both recessive. Bulked-segregant analysis with simple sequence length polymorphism (SSLP) markers, located the mutations on chromosome 1 and chromosome 3 in essp7, and on chromosome 2 in essp8. Next-generation mapping (NGM) was used to detect the genes responsible for the observed mutant phenotypes in the mapped regions. essp7 and essp8 plants were sequenced using 100nt paired-end sequencing on the Illumina HiSeq and 300nt paired-end sequencing on the Illumina MiSeq, respectively. The NGM results were consistent with prior

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mapping results for both mutants. All non-synonymous mutations identified within the candidate regions were detected and used for further validation. NGM could provide high-resolution genotypic information that can be used as a complement for traditional map-based cloning by direct identification of mutations in a fast and cost-effective way.

5.4

Molecular-binding Exploration Based On Proteome-wide Tertiary Structure Prediction: in vitro Validation

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There are current 27,416 protein coding genes in *Arabidopsis thaliana* released by TAIR10, but only around 600 protein structures have been elucidated and deposited in Protein Data Bank (PDB). Computational prediction is a good method to gain more insight of the unknown protein structures, and in our lab, we have a protein structure-ome containing 67,275 predicted protein structure models covering around 70% of the Arabidopsis proteome (Fucile et al., 2011). My project aims to (1) prove this database is a reliable tool in protein structure and function studies, and (2) explore the relationship between protein structure (like aromatic side chain structures) and protein-ligand (like protein-carbohydrate and protein-protein) binding ability.

6.1

A novel γ -carbonic anhydrase essential for carboxysome function in cyanobacteria

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The CO₂ concentrating mechanism (CCM) is an integral part of the photosynthetic apparatus of cyanobacteria and essential to their photoautotrophic growth. Every carbon that is fixed by Rubisco in the Calvin cycle must first pass through the CCM. Therefore understanding the flux of carbon through the CCM is vital to understanding the factors that control primary productivity. The carboxysome is the terminal component of the CCM and mediates the acquisition of inorganic carbon from the cytosol and the concentration of CO₂ at the Rubisco active site. Here we demonstrate that CcmM is a carboxysome-localized, redox-regulated γ carbonic anhydrase essential to carboxysome function in Nostoc PCC 7120. Biochemical analysis indicated that the γ -CA activity resided in the N-terminal 201 amino acids that included a Cys194-Cys200 disulfide bridge. Deletion of the Cys residues resulted in 95% loss in catalytic activity. Similarly, protein variants with Cys substituted residues displayed between 20 – 90% of wildtype activity indicating that the Cys residues played no direct role in the catalytic mechanism. Phosphine and thiol reducing agents (TCEP, THP, DTT, β-ME and glutathione) inhibited the y-CA activity of CcmM201. The inhibitory effect was reversed by the thiol oxidizing agent diamide and molecular O2, indicating that the Cys194-Cys200 disulfide bond is critical for the oxidative activation of the enzyme. Redox-mediated structural changes in CcmM209 were observed using intrinsic Trp fluorescence and circular dichroism spectroscopy indicating that CcmM adopts two states; an active state and a catalytically inactive state. Thus, the supply of CO₂ to Rubisco is dependent upon the redox state of the carboxysome.

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Cross-Taxon Analyses Of The Photosynthetic Protein Complex Allocations In The Picocyanobacteria Jackie Zorz & Amanda Cockshutt

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Marine *Synechococcus* and *Prochlorococcus* are the nondiazotrophic picocyanobacteria that predominate in subtropical, oligotrophic marine environments. Some models of climate change predict a decline of total phytoplankton biomass with a shift towards picophytoplankters. We used quantitative immunoblotting to measure the allocation of resources to the major complexes of photosynthesis in *Synechococcus* WH 8102, *Prochlorococcus* MED4 and *Prochlorococcus* MIT 9313 grown under common conditions. When normalized to PSII content, WH 8102 and MED4 show similar Cytochrome b6f and PSI contents, while MIT 9313 has twice the Cytochrome b6f content and 4 times the PSI content of these strains. Interestingly the *Prochlorococcus* strains have only one third to one half of the Rubisco catalytic subunits that the marine *Synechococcus* strain has. Thus, these strains show markedly different photosynthetic apparatus stoichiometries despite very similar growth conditions. The abundance of Cytochrome b6f and PSI in MIT 9313 and the relatively low level of Rubisco are consistent with cyclic electron flow around PSI in this strain.

6.3

A blast from the past: Re-establishment and characterization of hybrids between C_3 and C_4 species of *Atriplex* to dissect the control of differentiation of C_4 Kranz anatomy

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There is currently much interest in using hybrids of C₃ and C₄ species to identify genes controlling the development of C₄ Kranz anatomy using the high-throughput tools of the "omics" era. We recently generated hybrids between *Atriplex prostrata* (C₃) and *Atriplex rosea* (C₄) to study the genetic control over C₄ Kranz anatomy. Compared with parental lines, mature leaves of F2 hybrids are characterized by either reduced or enhanced (i) vein density, (ii) bundle sheath (BS) to mesophyll ratio, (iii) stomatal density, and (iv) mesophyll adaxial/abaxial cell layers. BS ultrastructure of the F2 hybrids resembles that of C3-C4 intermediate species with respect to organelle distribution, numbers and chloroplast size. The F2 hybrids have abnormalities in vascular patterning, BS cell division and enlargement reminiscent of maize with mutations in the *Scarecrow* gene recently proposed to function in Kranz anatomy development. Finally, tissue differentiation along a leaf developmental gradient is delayed in the F2 hybrids relative to the C₃ and C₄ parents. Combined, the developmental features of the *Atriplex* hybrids should facilitate the identification of the genes controlling various components of Kranz anatomy.

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Redox state of the plastoquinone pool and chlorophyll biosynthesis are coupled to regulate greening in *Chlorella vulgaris*

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Photoautotrophs balance light energy absorbed through fast, temperature-independent photochemistry with the capacity for energy consumption through slower, temperature-dependent biochemistry. Imbalances in energy flow are sensed as changes in excitation pressure (EP), a measure of the relative redox state of Q_A. Chlorella vulgaris acclimated to high EP (HEP) exhibits a yellow-green phenotype characterized by reduced chlorophyll per cell, higher chlorophyll a/b ratios and reduced light-harvesting complex abundance compared to the green phenotype of cultures acclimated to low EP (LEP). If EP is the sole regulator of phenotype, exposure to darkness should cause the reversion of the HEP to the LEP phenotype. However, exposure of HEP cells to darkness did not induce greening. The reversion of the HEP to the LEP phenotype is light dependent with a maximum rate of reversion occurring at 112 µmol photons m⁻² s⁻¹. When HEP cultures were shifted to optimal light intensities, the cultures visibly greened; this phenotypic adjustment was associated with increases in chlorophyll per cell, and decreases in chlorophyll a/b ratio with increases in light-harvesting polypeptide abundance. This greening was inhibited by DBMIB but not by DCMU indicating that the redox state of the plastoquinone pool regulates greening. At suboptimal light intensities, the cultures failed to green and maintained the HEP phenotype despite a measurably LEP. Thus, the regulation of phenotypic reversal appeared to be uncoupled from EP. In addition to redox regulation of nuclear encoded Lhcb genes, lightdependent limitations at the level of the chlorophyll biosynthesis must also contribute to the regulation of greening.

7.1

Characterization of the Alternative Oxidase from the moss Physcomitrella patens

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The electron transport system (ETS) of plant mitochondria generates energy in the form of ATP through the process of oxidative phosphorylation. All plants investigated to date also contain the enzyme alternative oxidase (AOX) which is a second terminal oxidase present in the respiratory system that creates a branch-point at the level of ubiquinol. Electrons that are passed directly to AOX bypass Complexes III and IV of the ETS and therefore less ATP is produced per oxygen consumed in respiration. The physiological role of this "energetically wasteful" pathway in most plants remains to be verified. The AOXs of angiosperm (flowering) plants are encoded by a multigene family of 5-6 members which makes gene knock-out experiments using anti-sense or RNAi technologies challenging. We recently discovered that the moss Physcomitella patens contains only one AOX gene which makes it an attractive model system in which to study AOX transcript expression and the post-translational regulation of the AOX protein. As a first step, the moss AOX cDNA was amplified by reverse-transcriptase PCR from a total RNA extraction from moss gametophyte tissues. The moss AOX cDNA was modified for insertion into the pYES 2.1 vector (Life Technologies) for expression in the yeast Saccharomyces cerevisiae. We will present data indicating that the yeast system can be used to successfully express AOX from moss and that it is correctly targeted to mitochondria. Our preliminary results indicate that moss AOX may be present as oxidized and reduced dimers similar to the situation found in angiosperms.

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Using the INDETERMINATE DOMAIN family as window into carbon metabolism

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The fixation of atmospheric carbon dioxide into carbohydrates is one of the most critical processes required to support all life on the planet. Despite the importance of carbon assimilation, the genetic pathways that modulate carbon assimilation, partitioning and allocation in plants are poorly understood. One possible player in these regulatory networks is the *INDETERMINATE DOMAIN* family of plant-specific zinc finger transcription factors. *IDD* genes are known to be involved in a variety of developmental events, including flowering, seed and root development, as well as gravitropism. Further analysis on this family has also identified potential role in carbon metabolism. Recent genomic and transcriptomic analysis combined with carbon metabolite profiling of 3 *idd* mutants in *Arabidopsis thaliana* has found a strong association of these genes with carbon allocation in leaves under different environmental conditions. Illuminating these regulatory networks and understanding how carbon flow regulates plant development is of vital importance, as meeting the nutritional demands of expanding populations and the challenges of climate change may not be met with traditional breeding approaches.

7.3

Biochemical characterization, expression and subcellular localization of two apple fruit diamine oxidases with differing substrate specificity

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4-Aminobutyrate (GABA) accumulates in apple fruit during controlled atmosphere (CA) storage. A potential source of GABA is the polyamine putrescine, which can be oxidized via diamine oxidase (DAO), resulting in the production 4-aminobutyraldehyde or Δ^{-1} -pyrroline, with the consumption of O_2 and release of H_2O_2 and ammonia. Five putative DAO isoforms were cloned from apple (Malus domestica Borkh. cv. Empire) fruit and found to contain the active sites typically conserved in copper amine oxidases. Genes encoding two of these enzymes, MdDAO863012 and MdDAO197462, were highly expressed in apple fruit and responsive to CA. Amino acid sequence analysis predicted the presence of a signal peptide and three N-glycosylation sites in MdDAO197462 and a C-terminal SKL peroxisomal tripeptide in MdDAO863012. Transient expression of GFP-fusion proteins in Arabidopsis protoplasts revealed a peroxisomal localization for MdDAO863012 and localization in cell wall and intercellular space for MdDAO197462. We were not able to express MdDAO197462 in Escherichia coli perhaps due to lack of N-glycosylation, but transient expression of MdDAO197462 in tobacco is now underway. MdDAO86301 was successfully expressed in E. coli and the activity of the purified recombinant protein measured as H₂O₂ production using a coupled reaction. Maximal activity was obtained with putrescine, followed by 1,3-diaminopropane, cadaverine and agmatine as substrates. The enzyme did not use monoamines or polyamines. The pH optimum was 8.4, which differs from the pH optimum of approximately 7 reported for other plant DAOs. Thus, apple DAO may contribute to GABA production from putrescine in apple fruit under CA conditions.

$\textbf{Dissecting substrate specificity of arogenate dehydratases (ADTs) from \textit{Arabidopsis thaliana} \\$

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Phenylalanine (Phe) is an essential aromatic amino acid that serves as a precursor for protein synthesis and a wide range of secondary metabolites in plants. Two pathways have been described for the last two steps of Phe biosynthesis: the prephenate and the arogenate pathway. In the first pathway, prephenate dehydratases (PDTs) convert prephenate to phenylpyruvate, which is then transaminated by an aminotransferase to Phe. In the second, prephenate is first transaminated to arogenate, which is subsequently converted to Phe by arogenate dehydratases (ADTs). ADTs and PDTs are structurally very similar, as are their substrates. In Arabidopsis thaliana six sequence-related proteins were identified that use arogenate as a substrate and hence were named ADTs (ADT1-ADT6). Two of these enzymes, ADT1 and ADT2, can also use prephenate as a substrate according to biochemical and yeast complementation assays. Since the six ADTs share a high degree of sequence similarity, we hypothesize that PDT function can be introduced into an arogenate-only ADT (ADT3-ADT6) through few mutations. We chose a two-part approach to test this hypothesis. First, we created several ADT2/ADT4 chimeras through PCR mediated recombination, to identify the domain(s) required for PDT function. These constructs were tested for PDT function by complementation using a yeast PDT knockout mutant strain. Second, the required domain(s) will be randomly mutated through error-prone PCR to identify specific amino acids required for PDT function. This research represents the first identification of amino acids that discriminate an ADT from a PDT.

8.1

Interspecific variation in root antioxidant enzyme systems reflects root turnover strategies and preferred habitats in wetland graminoids

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Fine roots of most wetland graminoids in Northern Ontario survive the winter, but in some perennial species the whole root system dies back in the fall. This root death is obviously triggered by environmental cues. In the present work we investigate whether roots with these two contrasting turnover strategies differ in their antioxidant enzyme activities over the growing season. In animals and plant leaves variation in antioxidant enzyme activities is associated with stress tolerance, senescence and longevity, but also with metabolic activity in general. We hypothesized that roots dying in the fall, and roots preparing for the winter would show marked differences in their antioxidant activity.

Two species with annual roots systems – *Sparganium androcladum* and *Rhynchospora alba* – and two species with roots surviving the winter – *Scirpus microcarpus* and *Carex exilis* – were investigated. The first-mentioned species of each pair is characteristic of productive wetlands, the second one of nutrient-poor ones. Root SOD, POX, APX, CAT and GR contents were analysed for field-collected plants in May, August and September, and for garden grown plants in June.

The results indicate higher antioxidant activities and a lower degree of lipid peroxidation in the annual roots compared to species with perennial roots. Individual antioxidant enzymes varied in their response, but when all five studied enzymes were analysed together using a discriminant analysis, clear and consistent distinctions between species with different root turnover strategies and with different habitat preferences emerged, emphasizing the ecological relevance of interspecific variation in antioxidant enzyme systems.

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Rapid ammonia gas fluxes underlie NH₃/NH₄⁺ toxicity in roots of higher plants

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Ammonia/ammonium (NH₃/NH₄⁺) toxicity in higher plants is a major environmental disturbance globally and is primarily brought on by the industrialized production and overuse of N fertilizers. This has resulted in crop reduction, forest decline, and biodiversity loss. Key towards our understanding of the mechanistic underpinnings of toxicity is the phenomenon of futile transmembrane cycling, whereby extremely rapid fluxes and high efflux-to-influx ratios of NH₃/NH₄⁺ in roots have been successfully linked to toxicity. Surprisingly, however, the fundamental question of which species of the conjugate pair (NH₃ or NH₄⁺) participates in such fluxes remained unresolved until recently. Using flux analyses with the short-lived, positron-emitting, radioisotope, ¹³N, coupled with electrophysiological, respiratory, and histochemical measurements, we show that futile cycling in roots of intact barley (*Hordeum vulgare* L.) is predominately of the gaseous NH₃ species, rather than the NH₄⁺ ion. Preliminary (pharmacological) evidence suggests that rapid NH₃ fluxes are mediated by aquaporins, consistent with earlier demonstrations in heterologous expression systems. We also discuss the environmentally important aspect of K⁺-induced alleviation of NH₃/NH₄⁺ toxicity and its link to aquaporinmediated NH₃ fluxes *in planta*. Our work fundamentally revises the model of futile transmembrane cycling by demonstrating unequivocally that rapid NH₃ gas fluxes predominate and contribute to NH₃/NH₄⁺ toxicity in higher plants.

8.3

Root characteristics of contrasting functional types of wetland plants

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Plants with different ecological strategies possess different methods of resource allocation. The above-ground characteristics can be aligned along a gradient of leaf economic spectrum, but the concurrent belowground relationships remain relatively unknown.

Fine roots of most herbaceous wetland plants in northern Ontario survive the winter, but in some perennial species the roots senesce in the fall. This study is a part of investigations aiming to understand the ecological constraints determining adaptive advantages of these two root turnover strategies, focusing on root characteristics and growth, and relevant above-ground traits.

Fine roots of *Dulichium arundinaceum*, *Carex stricta*, *Scirpus microcarpus*, *Carex oligosperma*, *Carex lasiocarpa*, *Eleocharis palustris*, and *Schoenoplectus acutus* survive the winter, whereas roots of *Alisma triviale*, *Sparganium androcladum*, *Rhynchospora alba*, *Calla palustris*, *Sagittaria latifolia*, and *Pontederia cordata* senesce in the fall. We hypothesised that the result in resource allocation of the opposing ecological strategies translates to differing root characteristics, similar to that of the above-ground strategies. The plants were garden grown for one season, and harvested in two separate pot experiments.

Preliminary results demonstrate that the species with annual root systems had a greater specific root length and a lower root dry matter content than species with perennial root systems. Plants with annual root systems have a lower leaf dry matter content and higher specific leaf area. This indicates that the strategy of perennial plants to produce new roots for each growing season is associated with root and leaf characteristics to be expected from the known economics spectrum.

Coping with water deficits: contrasting drought response strategies for two Eutrema salsugineum accessions

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Eutrema salsugineum (Thellungiella salsuginea) is an extremophile crucifer with an innate tolerance to salinity and freezing temperatures but its tolerance to water deficits remains largely uncharacterized. Plants originating from the Yukon Territory, Canada were compared to an accession from Shandong, China. Cut rosette water loss assays show that leaves of Yukon plants conserve water at early stages of water deficits, the leaves remain turgid at a low leaf water content for a prolonged period, and water loss is accompanied by a decrease in leaf solute potential indicating solute accumulation. This physiological response is consistent with the Yukon accession employing drought tolerance strategies when challenged by water deficit. Contrastingly, Shandong plants exposed to the same drought conditions show no differences in cut rosette water loss or leaf water content until late time points in drought treatment and solute accumulation only occurs when turgor is lost. These changes are indicators of drought avoidance by the Shandong accession. After a two day recovery period, a second drought treatment was imposed. Yukon plants maintain higher leaf water content levels than at the same time point in the first drought exposure while Shandong plants respond in a manner similar to their first drought exposure. The temporal expression of drought-responsive genes also differs with Yukon plants tending towards a higher baseline and earlier increase in transcripts. Thus Yukon plants show distinct properties with respect to dynamic leaf water content as well as drought responsive gene expression yielding a beneficial outcome of increased tolerance to water deficits.

8.5

Exploring the plant priming response to low temperature exposure with small molecules

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As sessile organisms, plants must contend with frequent fluctuations in environmental conditions. Some of these conditions limit plant growth and productivity, and may impinge upon survival. Priming is a mechanism in which exposure to one environmental condition results in more rapid or vigorous acclimation when the plant is exposed to subsequent challenges. This preparation therefore enhances plant tolerance to environmental conditions, and can minimize losses in productivity. We have undertaken a high-throughput approach that tests the capacity of thousands of "chemical environments" to prime *Arabidopsis thaliana* seedlings for subsequent exposure to extreme abiotic conditions. With this approach, we aim to identify compounds capable of priming for enhanced tolerance to abiotic challenges. To date, this approach has suggested that a class of compounds, called cryoximes, can alleviate the impact of subsequent exposure of seedlings to low temperatures. Cryoximes are novel compounds that had a hitherto unknown role in plant cold tolerance. Further investigation will confirm the priming ability of cryoximes against cold and other abiotic conditions. Their mode of action will be characterised through dose-response experiments and functional genomics-based dissection of the plant cryoxime response pathway. Screening for additional compounds able to prime for resistance to low temperature exposure and other abiotic stress environments will be continued.

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Evolution by domestication drives the ecology and evolution of plant-herbivore interactions

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Theories of plant defense often depend on strong trade-offs between investment in defense and non-defensive traits (e.g. growth), but these can be difficult to identify. Here we use the domestication of crops, which can be viewed as a massive evolution experiment, replicated hundreds of times, to test whether domestication interferes with mechanisms of plant defense against herbivores. To maximize the generality of our experiment, we studied 30 independent domestication events, each represented by a crop species and its closest wild progenitor species. We tested the resistance of each species to two generalist herbivores (a phloem feeding aphid and a leaf chewing caterpillar) and measured putative chemical and morphological defensive traits. Unexpectedly, domestication has increased susceptibility to caterpillars but not to aphids. Yet, crop-progenitor pairs show a variety of patterns suggesting a lack of generality in the effect of domestication on resistance. We also identified a number of resistance plant traits and tested whether their effect is dependent on plant evolutionary history. Moreover, changes in plant resistance caused by domestication could in theory alter selection on pest populations and thus drive current pest evolutionary dynamics. We will also present preliminary results from high-throughput genotyping that tracked the genetic changes occurring in the aphid populations growing on each plant. This research will help us understand how plants deal with strong tradeoffs in investment (i.e. growth versus defense) and could help explain why different plants use such a diverse array of defense mechanisms against herbivores.

9.2

Analysis of *Arabidopsis* plants overexpressing carotenoid cleavage dioxygenases for volatile emissions and insect resistance

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Carotenoid degradation by enzymatic oxidative cleavage produces an array of terpenoid products that are collectively known as apocarotenoids, which include volatile and non-volatile compounds. In *Arabidopsis thaliana*, a family of nine genes known collectively as the carotenoid cleavage dioxygenases are involved in the production of apocarotenoids. Of these, four encode the carotenoid cleavage dioxygenases (*CCDs – CCD1*, *CCD4*, *CCD7*, and *CCD8*) and five encode the nine-cis-epoxycarotenoid dioxygenases (*NCEDs – NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9*). A previous study by Wei et al. (2011) showed that *CCD1* overexpression in Arabidopsis resulted in enhanced emission of β-ionone and improved insect deterrence. As a follow up study, we generated *CCD4* and *CCD8* Arabidopsis overexpression lines, and we characterized these lines on the basis of their carotenoid contents and volatile profiles. Surprisingly, enhanced accumulation of key carotenoids in the leaves was observed in both CCD4 and CCD8 plants. In addition, the CCD4 and CCD8 lines emitted volatile apocarotenoids and other volatile compounds, including sesquiterpenes and monoterpenes. Further, CCD4 lines showed the highest feeding deterrence towards flea beetles insects suggesting that the volatile compounds released from these plants may possess anti-feeding properties towards these insects.

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Grazing and Nitrogen Interact to Shape Plant Species and Phylogenetic Diversity

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A major goal in biology is to understand what ecological factors generate, maintain, and erode diversity within communities. Diversity is measured at multiple levels including species diversity and phylogenetic diversity (PD) – a measure of the evolutionary history represented within a community – and both have significant ecological impacts on communities and ecosystems. In this study we ask: what ecological factors are most important in shaping plant species diversity and phylogenetic diversity? In the acid grasslands at Silwood Park, England, we manipulated presence-absence of rabbits, insects, mollusks, nutrients, and lime in a fully factorial design and recorded the relative abundance of all plant species after 22 years. We determined PD using community composition data and a dated molecular phylogeny of all species in the community. We found that nitrogen was by far the most important factor shaping species diversity with nitrogen addition decreasing species diversity by 26%. However, for PD rabbit grazing had the largest effects, although there was a strong interaction with nitrogen. When nitrogen was added the removal of rabbits decreases PD by 74% however in nitrogen control plots the effect size of rabbits on PD dropped to 16%. Together these results show that different biotic and abiotic factors are important in shaping species and phylogenetic diversity and that anthropogenic inputs into systems, like nitrogen, can play a dominant role in shaping diversity.

9.4

Arabidopsis response to spider mite feeding: perception, signaling, and response

Kristie Bruinsma*¹ and Vojislava Grbic¹

Herbivores derive nutrients at the expense of a plant host, representing a major biotic factor in both natural ecosystems and agricultural settings. Much research has been pursued in the attempt to unravel the factors affecting the interaction between arthropod herbivores and their plant hosts. From the plant's perspective, this interaction begins with perception of attack, which initiates a signalling cascade involving phytohormones and ultimately resulting in transcriptomic and metabolic defence responses. To date, most molecular-genetic studies of plant-arthropod interaction have focused on insects; however, herbivorous mites feed on a variety of plant species. The two-spotted spider mite, Tetranychus urticae, feeds on over 1000 plant species, one of which is Arabidopsis thaliana, a model plant species previously used in studies of plant-arthropod interaction. Using these two model organisms, my research focuses on using microarray data from two Arabidopsis accessions with drastic differences in susceptibility to spider mite feeding to identify how Arabidopsis defends itself against spider mites using pre-existing differences in defensive states as well as induced plant defences. I used this microarray data to: a) study how Arabidopsis perceives attack, utilizing damage associated molecular pattern receptors, PEPR1 and PEPR2, to aid in perceiving plant tissue damage; b) identify jasmonic acid as the key phytohormone involved in orchestrating the defence response following perception; and c) identify secondary metabolic compounds, indole glucosinolates, that affect mite performance and development, leading to enhanced plant resistance. Thus, my findings provide insight into how plants defend themselves against this major class of arthropod herbivores.

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Effects of herbivory, intraspecific genetic variation and rapid evolution in plants on ecosystem processes.

Connor R. Fitzpatrick ^{1,2}, Michael D. Preston ³, Nathan Basiliko ⁴, Marc T.J. Johnson ^{1,2}

Terrestrial ecosystems are shaped by complex interactions between biotic and abiotic factors operating above and belowground. Plants provide the energy source for, and thus link, the above and belowground communities, which in turn drive ecosystem processes such as energy and nutrient cycling. Identifying factors that influence the quality or quantity of plant-derived material will aid in our understanding of the biotic control on ecosystem process rates. We used plots from a long-term field experiment that were either exposed or protected from ambient insect herbivory. This manipulation caused rapid evolution in *Oenothera biennis* populations and altered plant communities. Our specific research questions were: 1) What are the relative contributions of herbivory, genetic variation, and rapid evolution in plant populations to ecosystem-level processes? 2) Can these ecosystem-level effects feedback to alter the performance of O. biennis? In each plot we quantified leaf decomposition and net N mineralization rates, as well as soil microbial activity under additions of natural and synthetic substrates. Additionally, we examined the effect of intraspecific genetic variation in O. biennis on these ecosystem-level responses. We also quantified seedling performance of the experimental O. biennis genotypes grown in soil from all plots to assess the possibility of a feedback between the soil ecosystem and the performance of evening primrose genotypes. Overall we found that genetic variation explained the largest proportion of variance across our ecosystem-level processes. However, herbivores had a positive influence on seedling performance unrelated to nutrient availability across the treatment.

10.1

Plant-based Expression of Pharmaceutical Proteins to Improve Global Health

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Plants offer tremendous advantages as cost-effective, safe and efficacious platforms for the large-scale production of vaccines and other therapeutic proteins. Plant-derived vaccines provide a way by which to enhance vaccine coverage for children in developing countries, and have the potential via oral administration to elicit a mucosal immune response. Plants have the added advantage of simultaneously acting as an antigen delivery vehicle to the mucosal immune system while preventing the antigen from degradation as it passes through the gastrointestinal tract. Both transgenic plants, transplastomic plants and plant virus expression vectors have been designed to express vaccine epitopes as well as full therapeutic proteins in plant tissue. This presentation describes the use of different strategies, with a particular emphasis on a novel virus expression vector system to produce vaccines and therapeutic proteins in plants as a suitable means by which to improve global health.

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Metabolite and expression analysis of the 4-aminobutyrate (GABA) pathway in *Arabidopsis* mutants subjected to salinity and chilling stresses

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4-Aminobutyrate (GABA) is a ubiquitous 4-C, non-proteinaceous amino acid that can be synthesized from glutamate via glutamate decarboxylase (GAD) and then is catabolized to succinic semialdehyde (SSA) via GABA transaminase (GABA-T). SSA is oxidized to succinate by SSA dehydrogenase, or alternatively, reduced to 4-hydroxybutyrate (GHB) via SSA reductase (GLYR/SSAR for glyoxylate/SSA reductase). The physiological role(s) of GABA in plants is not yet firmly established, but there is extensive evidence linking it to stress. It is becoming clear that the regulation of GABA metabolism involves both gene-dependent and independent processes. In this research, we utilized double homozygous $glyr_1/glyr_2$ T-DNA knockout mutants of Arabidopsis, as well as gaba-t knockout, GABA-T overexpression (GABA- T_{Ox}) and GLYR₁ overexpression $(GLYR_{1OX})$ mutants. The $glyr_1/glyr_2$ mutants were more sensitive to SSA than the wild-type (WT) during culture on solid agar, whereas their sensitivity to GHB did not differ. In liquid culture, salinity stress (150 mM NaCl) rapidly enhanced GABA (as measured by HPLC) levels in WT and gaba-t within 2-8 h; GHB (as measured by GC-MS-MS) levels were also enhanced in WT, $GABA-T_{Ox}$ and $GLYR_{IOx}$, but much less so in gaba-t and glyr₁glyr₂. The expression of GAD_4 was consistently, albeit transiently, enhanced. Similarly, GABA and GHB levels were transiently increased in WT, glyr₁glyr₂ and GLYR_{1Ox} with chilling (4 °C), but this was accompanied by elevated expression of GAD3 and GAD5, but not GAD4. These data are interpreted as evidence for stress-specific induction of GAD expression, and for multiple sources of GHB in plants subjected to abiotic stress.

10.3

Intracellular transport logistics during plant cell morphogenesis

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Cellular growth in plants implicates the assembly of new cell wall surface. The targeted deposition of the material necessary for the assembly of new surface - cell wall polymers and membrane - is therefore a crucial regulatory feature in plant development. The intracellular delivery of this material is generally performed by vesicles, but how is the logistics of this transport process regulated in space and time? Monitoring and quantifying the dynamics of these vesicles in the living cell is severely challenged by the small size of these organelles which is below the diffraction limit of the optical microscope. Particularly in situations when vesicles move rapidly and/or in dense clouds, conventional particle tracking methods are therefore powerless. By combining high temporal and spatial resolution confocal laser scanning microscopy with advanced imaging techniques originally developed for the analysis of molecular movements (STICS, spatio-temporal image correlation spectroscopy), we monitored the intracellular dynamics of vesicles in the growing pollen tube. We used these motion data to generate dynamic profiles mathematically model the principal mechanism governing intracellular trafficking.

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Overexpression of microRNA156 leads to enhanced forage yield in alfalfa

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Abstract: MicroRNAs (miRNAs) are a class of regulatory small RNAs that play crucial roles in almost all biological and metabolic processes in plants. In particular, miR156 is known as a novel regulator of plant biomass production through regulation of members of *Squamosa Promoter-Binding Protein-Like* (*SPL*) genes. Here we conducted a study to investigate the role of miR156 in *Medicago sativa* (alfalfa), a forage and potential bioenergy crop. To determine the number of loci encoding miR156 paralogs in alfalfa, an *in silico* search was conducted using publicly available sequences. Alfalfa plants overexpressing *M. sativa* miR156 were generated, and the miR156 cleavage targets were validated using a modified 5'-RACE technique. Of the five predicted target *SPLs* genes, three (*SPL6*, *SPL*12 and *SPL*13) contained miR156 cleavage sites and their expression was downregulated in transgenic alfalfa overexpressing miR156. These transgenic alfalfa plants had reduced internode length, more nodes per stem, as well as enhanced shoot branching, trichome production, and plant height, but no effect on root length and nodulation. Furthermore, overexpression of miR156 had a minor effect on floral transition stage by delaying flowering by two to five days. Our observations imply that miR156 could be employed as potential tool to enhance forage yield and quality in alfalfa.

10.5

Genome-wide Analysis of *Cyclophilin* Gene Family in Soybean and Characterization of *GmCYP1* Hemanta Raj Mainali^{*1}, Sangeeta Dhaubhadel^{1, 2}

Cyclophilins (CYPs) belong to the immunophilin superfamily with peptidyl-prolyl *cis-trans* isomerase (PPIase) activity. PPIase catalyzes the interconversion of the *cis-* and *trans*-rotamers of the peptidyl-prolyl amide bond of peptides. By *in silico* analysis, we identified 62 *CYP* genes in soybean (*GmCYP1* to *GmCYP62*) of which 8 are multi-domain and 54 are single-domain proteins. At least 25% of the *GmCYP* genes are expressed in soybean, as they have 99-100% sequence identity with soybean Expressed Sequence Tags. The expansion of *GmCYP* genes in soybean and their distribution pattern on the chromosomes strongly suggested a genome-wide segmental and tandem duplication.

GmCYP1 is a single domain CYP, localizes both in the nucleus and the cytoplasm and interacts with an R1 MYB transcription factor, GmMYB176, in the nucleus. GmMYB176 binds with 14-3-3 protein that affects its localization and isoflavonoid biosynthesis in soybean. We recently discovered that GmCYP1 is a new client of soybean 14-3-3. We speculate that interaction of GmCYP1 with 14-3-3 and GmMYB176 may possibly be associated with isoflavonoid biosynthesis in soybean. Furthermore, a loss-of-function mutation in *Arabidopsis* ortholog of *GmCYP1* (*ROC1*, *ROC3*, *ROC5*) resulted into sensitivity towards salt during seed germination, but no differential response was observed when salt stress was applied to mature plants. Additionally, *roc1*, *roc3*, and *roc5* displayed early flowering phenotype under both long-day and short-day light regimes.

Overall, we have identified the largest *CYP* gene family known to date. Our findings suggest that GmCYP1 may be involved in a variety of biological processes in soybean.

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Poster Abstracts

P1

Bioavailability of iron bound to strong chelators

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HBED is a very strong Fe³⁺ chelator. Strategy I vascular plants, which use a reductive system for iron acquisition, similar to many green algae, are able to access iron from. However, iron-limited cells of the Strategy I green alga Chlamydomonas reinhardtii were unable to access iron present as Fe³⁺-HBED. In contrast, Fe3+ chelated with HEDTA (a weaker chelator) was rapidly taken up by iron-limited Chlamydomonas cells. Chlamydomonas ferric reduction rates with Fe³⁺-HBED was approximately 15% of the rate observed with Fe³⁺-HEDTA, suggesting that low reduction rates with Fe³⁺-HBED might be one factor in the low rate of iron acquisition. By contrast, iron-limited cells of the Strategy I green alga Chlorella kessleri were able to rapidly assimilate Fe³⁺ chelated by HBED, although ferric reduction rates with Fe³⁺-HBED were approximately 38% the rate of activity with Fe³⁺-HEDTA. Similar differential iron uptake rates for the two algal species were obtained using the strong Fe³⁺ chelator (and siderophore analogue) DFB and the cyanobacterial siderophore schizokinen. These results suggest that there are differences among Strategy I green algae in their abilities to acquire Fe³⁺ from various ferric chelates: not all Strategy I algae can equally access tightly complexed Fe³⁺. Chlamydomonas appears to be the first documented Strategy I organism that is unable to acquire iron from Fe³⁺-HBED. These results also suggest that green algal iron acquisition from siderophores is species-dependent. Finally, we suggest that iron acquisition from Fe³⁺-HBED might serve as an assay for an organisms' ability to access tightly complexed iron.

P2

Improvement of Recombinant IL-10 Production by Suppression of *Cysteine Protease* Gene Expression in Transgenic Tobacco Plants

Kishor Duwadi*1, Ling Chen², Angelo Kaldis², Rima Menassa¹,², Sangeeta Dhaubhadel¹,²

Plants are an attractive host system for pharmaceutical protein production. Many therapeutic proteins have been produced and scaled up in plants at a low cost compared to the conventional production systems. The main technical challenge during this process is to produce sufficient level of protein in plants. Low yield is generally caused by proteolytic degradation during expression and downstream processing of recombinant proteins. One approach to overcome proteolytic degradation involves creation of stable transgenic lines with reduced proteolytic activity. Recently, it has been found that cysteine protease (CysP) inhibitors show protective effect on human immune-regulatory interleukin-10 (IL-10) produced in transgenic tobacco plants. To identify *CysP* gene(s) involved in IL-10 accumulation, the DFCI *Nicotiana tabacum* gene index was searched using "cysteine protease" as the keyword. This search revealed a total of 55 putative *CysPs*, out of which 32 are tentative contigs (TC) and 23 are singletons. Based on their expression in leaf tissue, 10 candidate *CysPs* were selected for further characterization. Overexpression and silencing constructs were made for all 10 candidate *CysPs* in order to study the effect of the selected *CysP* genes in tobacco lines that

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overexpresses IL-10 protein (WT-IL-10). Agrobacteria-mediated plant transformation technology was utilized to generate transgenic lines with reduced CysP and increased IL-10 accumulation. Using Enzyme linked immunosorbent assay it was found that recombinant protein yield could be increased up to 1.6 fold in T₀ CysP silenced plants in comparison to the level present in WT-IL-10 tobacco plants.

P3

Expression analysis of histone acetyltransferases in rice under drought stress

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Histone acetylation is one of the vital reversible modifications that regulate the chromatin structure to modify genomic DNA accessibility in eukaryotes. Histone acetylatransferases (HATs) and histone deacetylases (HDACs) maintain the homeostasis of histone acetylation. Studies in *Arabidopsis* revealed that HATs involve in plant responses to various stress including light, temperature, salt and ABA stress. Drought stress, as a very common stress, could cause a range of physiological and biochemical responses in plants. Eight *HATs* which belong to four different families (CBP, GNAT, MYST, and TAFII250 family) have been identified in rice. In this research, four *HATs*, one from each family, were chosen based on *in silico* domain and promoter analysis. The real-time quantitative polymerase chain reaction analysis demonstrated that drought stress causes a significant increase in the expression of these four *HATs* (*OsHAC703*, *OsHAG703*, *OsHAF701* and *OsHAM701*) in rice plants. Additionally, the western-blot analysis showed that the acetylation level on certain lysine sites of H3 (lysine 9, lysine 18 and lysine 27) and H4 (lysine 5) increased accordingly. These results indicated that drought stress induced a significant increase in the transcript levels of *HATs* and the acetylation levels on lysine residues of Histone H3 and H4. These significant increases indicated that *HATs* are involved in drought stress responses in rice.

P4

Autofocusing of transcription factor expression – a case study in plant pattern formation

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Localized developmental decisions (patterning), for example, the localization of plant stem cell niches (meristems) regulate plant growth and regeneration properties, and are thus key areas of plant biotechnology. In the past ten years, it has become increasingly clear that feedback-mechanisms regulating the distribution of the plant hormone auxin provide the positional information underlying the most fundamental patterning mechanisms. Auxin influences gene expression through 22 Auxin Response Factors (ARFs), which in turn are regulated by physical interaction with 29 Aux/IAA co-regulator proteins. The expression pattern of MONOPTEROS(MP)/ARF5 is highly dynamic, with a tendency to become progressively restricted to lines or small spots of expression. Interestingly, this process is promoted by the function of MP itself and distorted in mp mutants, as well as in a recently developed new analytical tool, $MP \Box$, (New Phytologist (2012) 194(2): 391-401), an irrepressible variant of MP that can no longer interact with negatively regulating Aux/IAA proteins. By building on a number of specifically designed genotypes and creating another set of genotypes in the model plant Arabidopsis thaliana, this project will address how MP controls its own expression. To explore a direct feedback mechanism, known binding sites of MP to its own promoter will be mutated and, conversely, multimerized in the absence of interfering influences from a clade of five related ARFs. Parallel constructs with a constitutively active $MP \Box$ variant will explore how auxin distribution and Aux/IAA activities feed into

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this process. Further readouts will include the expression patterns of genes in auxin transport, as they participate through dynamic redistrubution of auxin in feedback regulations on the organismal level.

P5

Antitumorigenic and Antioxidant activity of methanolic extract of Gloriosa superba leaves

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The aim of the present study was carried out the in *vitro* and *in vivo* potential of methanolic leaf extract of *Gloriosa superba* as a natural antitumour and antioxidant. The cytotoxic effect of methanolic extract of *Gloriosa superba* (MEGS) to DLA tumour cells was found to be dose dependent and the ED₅₀ value was found to be 52µg. This 52µg was administered in the following *in vivo* study. Evaluation of the antioxidative and antitumorigenic potential of MEGS of Swiss albino mice were divided into six treatment groups such as water control, DMSO, PBS, methanol extract and methanol extract + DLA tumor cells. The experiment was carried out for 21 days. The mice liver homogenate of each treatment groups were taken and assessed the activities of enzymic and the levels of non enzymic antioxidants. The enzymic antioxidants catalase, superoxide dismutase, and glutathione reductase and the levels of non enzymic antioxidants vitamin A, vitamin C, and reduced glutathione were found to be significantly increased and the lipid peroxidation was found to be significantly decreased by the administration of MEGS individually and to DLA induced mice.

P6

HPLC analysis of flavonoids profile from Indian Curry leaf (Murraya koenigii. L)

<u>Kaliyaperumal Ashokkumar^{1*}</u>, Kumarakurubaran Selvaraj², Saradha Devi, K.M³

Murraya koenigii, L. Spreng, a medicinally important herb of Indian origin, it has used for centuries in the Ayurvedic system of traditional Indian medicine and very popularly used in Indian cuisine are the daily basis. To evaluate the quality of *M. koenigii*, L. leaves, a sensitive, simple and precise reversed-phase high performance liquid chromatography method was developed for assessment of four major bioactive flavonoids: Rutin, quercetin, myricetin and kaempferol. Separation was achieved on a reversed phase column (ZORBAX, Eclipse plus-C₁₈, 5 μm, 4.6 x 150 mm, Agilent, USA) and methanol–acetonitrile–water-acetic acid (40:20:39:1, v/v/v/v) was employed as the isocratic eluent. Sample was eluted at 0.8 ml/minutes and peaks were simultaneously identified using UV/Vis absorbance at 350 nm for kaempferol and 254 nm for rutin, myrcetin and quercetin. The authenticated four analytes were used to construct linear standard curves by injecting range of 20 - 200 ng. The correlation coefficients of the calibration curve for all the analytes was higher than 0.999. Isolation of the four compounds in *M. koenigii*, leaves was achieved by the HPLC method above and results showed that mean value of rutin (924.25mg/kg) and quercetin (85.88 mg/kg) accumulated greater concentration. Lowest flavonoid concentration 5.88 mg/kg and 0.20 mg/kg were found to possess in myricetin and kaempferol, respectively. Total flavonoids concentration was observed 1015.50 mg/kg. Present

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study suggested accumulation of the greater amount of bioactive components; rutin and quercetin in *M. koenigii* leaves will be more useful information for further pharmacological investigations.

P7

Breeding potato varieties for chip processing and nutritional qualities – evaluation for central Canada conditions

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Chips constitute one of the two major processed potato products in the food industry. Numerous factors affect chip processing, nutritional qualities and safety such as sugars, asparagine, polyphenol content and antioxidant potential, which are influenced by the genotype and the environment during growth and storage. This study evaluated thirteen elite lines including two colored lines from the potato breeding program of Agriculture and Agri-Food Canada, Fredericton and University of Wisconsin along with five promising varieties suitable for growing under Ontario conditions. These varieties were grown at the Elora Research Station, Elora, ON. Two varieties were also evaluated in Alliston, Ontario to measure regional influence. A one-year storage study at a commercial storage facility was also conducted. The colored potato lines F06053, F06058 had higher glucose content throughout the analysis period before and after storage. Comparatively lower glucose contents were observed in Tundra, W2715-15, Waneta, Beacon Chipper, W8867-5, and W2438-3Y before storage. Waneta consistently had low glucose content throughout the storage periods. Glucose content was greatly influenced by the environment as evidenced by Beacon Chipper grown at Alliston which had a lower glucose as compared to the Elora location. A general trend of lower sucrose content was observed in F06058, W2438-3Y, W5955-1 and Nicolet whereas W8641-4, F06058, F06053, and W5051-12 showed consistently lower asparagine content, a precursor for acrylamide production, both before and after storage. F06053, F06058, and W2438-3Y consistently had the highest total phenolic contents and antioxidant potential in potato dry matter and chips.

P8

Screening potato varieties for canning and nutritional qualities

Reena G. Pinhero¹, Qiang Liu², Rickey Y. Yada¹

Potatoes rank fourth in world for food production and are the world's number one non-grain food commodity. In today's fast-paced life, processed foods such as canned potatoes are convenient, used in soups, for mashed potatoes, hash browns or as an accompaniment to a main course. The major defect in canned potatoes is sloughing (disintegration/cracking of the outer surface) for which the determination of specific gravity has been the major predictor; however, this has not always ensured consistent high quality canned potatoes. Recently, potatoes have been shown to have both good nutritional and economic value. The present study evaluated four potato varieties which fell within the specific gravity range normally used for canning along with a control, Atlantic, which has a higher specific gravity, in order to determine the starch characteristics which may contribute to sloughing. In addition, the nutritional qualities of these varieties were determined. Sloughing was assessed visually and by total solid leakage using turbidity analysis and showed the highest sloughing in Russet Norkotah, followed by Vivaldi, Superior and Sifra. Significant differences were also

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observed in total starch and amylopectin contents. Light microscopy of starch granules showed differences in the size of starch granules. These varieties also showed significant differences in total protein, total phenolics and antioxidant potential. The above results are discussed in relation to selection of potato varieties for canning and nutritional quality.

P9

P10

Ecological and evolutionary patterns of herbivory across vascular plants

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The consumption of plants by animals underlies some of the most important evolutionary and ecological processes in nature. Arthropod herbivory evolved approximately 415 mya and the ensuing coevolution between plants and herbivores is credited with giving rise to much of the macroscopic diversity on Earth. In contemporary ecosystems, herbivory provides the major conduit of energy from primary producers to consumers. Here we show that when averaged across all major lineages of vascular plants, herbivores consume 5.3% of the tissue produced annually by plants, whereas previous estimates are up to 3.8x higher. This result suggests that herbivory plays a smaller role in ecosystems than currently thought. Our analyses of a phylogenetically diverse global sample of 1058 species studied across 2085 populations also shows that stabilizing selection has caused rates of leaf consumption to vary substantially within and among major plant lineages. A key determinant of this variation is plant growth form, where woody plant species experience 64% higher herbivory than non-woody plants. Higher herbivory in woody species supports a key prediction of the long-standing but often overlooked Plant Apparency Theory. Our study provides insight into how a long history of coevolution has shaped the ecological and evolutionary relationships between plants and herbivores.

P11

The potential of willows for phytoremediation of arsenic polluted sites: physiological results and molecular perspectives.

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Arsenic (As) is one of the most hazardous elements in the environment coming both from geologic and anthropogenic activities such as mining or other industrial processes. Its toxicity in plants is mainly mediated by the competition between arsenate (AsV) and phosphate in metabolic processes resulting to enzymatic disruption. Regardless of As negative effect, several plant species have shown the capacity to extract, degrade, or immobilize As contaminants.

Despite of a continuously growing number of As contaminated sites, limited financial resources are available for environmental remediation. In this study, we propose the use of phytoremediation as an economic and environmental-friendly alternative for As polluted sites. By using physiological and transcriptomic approaches, our goals are to show the ability of willows as well as different plant species to tolerate and accumulate As in their tissues, in order to determine which species could be the most efficient for detoxifying polluted sites.

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Results from a four weeks hydroponic study with *Salix purpurea 'Fish Creek'* saplings showed that these shrubs are able to support up to 5 ppm As without showing any significant symptoms while accumulating As in its tissues. Physiological measurements, including photosynthesis, transpiration, and biomass production, were measured in plants exposed to 0, 5, 30 and 100 ppm of As. Molecular analyses (Next Generation Sequencing and qRT-PCR) are currently in process in order to compare As specific transcripts levels accumulation in leaves, stems and roots. In addition, a field trial experiment will be used for validating these results.

P12

Complexitites of analysing bacterial hormone production with respect to growth characteristics of different species and strains

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One of the important characteristics of Plant Growth Promoting Bacteria (PGPB) is their ability to synthesize plant hormones. Microbial growth regulators can increase total pool of endogenous plant hormones and have been previously observed to enhance the effect of these compounds in plants inoculated with beneficial strains. In vitro bacterial production of growth regulators is commonly standardized to the level of a single cell. Estimation of bacterial cell number in liquid culture is important especially when the tested species produce high amounts of secondary metabolites (e.g. exopolysaccharides secreted by Rhizobia), as this significantly increases bacterial biomass and confounds measurements normalized to fresh or dry mass. On the other hand, in case of microbes from the *Methylobacterium* genus, precise determination of cell number in liquid culture can be very difficult. Methylobacteria are beneficial plant endophytes that effectively produce cytokinins. They are ubiquitous in the environment and often drought resistant, which significantly improves their competitiveness among other plant microsymbionts. However, drought resistance necessitates adaptations in their growth character, some of which results in the tendency to form aggregates in liquid culture. In this work, CK levels produced in vitro by two WT strains and two exo- mutants of Sinorhizobium meliloti were analysed by HPLC-MS/MS, and the results expressed both normalized to pellet weight and cell number. Additionally, the attempt has been made to standardize growth curves for a variety of aggregating and non-aggregating Methylobacterium strains by combining the results of direct cell counts on agar plates with optical density readings and pellet weight measurements.

P13

Live imaging of plant cells suggests a major role for the ER in creating organelle pleomorphy

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The concept of an organelle in the eukaryotic cell recognizes the sequestration of metabolic functions and their attendant enzymatic machinery within a membrane bound compartment. Whereas organelle shape does not form a part of its definition, over time, largely through the influence of diagrammatic depictions of electron photomicrographs of fixed, processed sections we have started associating each organelle with a recognizable shape and size. Interestingly the visualization of living plant cells expressing genetically encoded fluorescent proteins targeted to different cellular components shows a very different picture. A new appreciation of the pleomorphy and extremely dynamic behaviour of organelles comes through upon observing that plastids, mitochondria, peroxisomes, the nucleus and vacuoles morph rapidly and extensively, produce tubules and

membrane extensions, and appear to interact constantly with each other. The endoplasmic reticulum, a continuous system comprising of tubules and cisternae, encompasses other organelles and appears to govern their pleomorphy through membrane contact sites (MCS). Here we present a video poster showing the dynamic nature of the living plant cell including the formation of organelle extensions such as stromules and peroxules and the integral role played by the ER.

P14

Toward the characterization of the two- spotted spider mite plant feeding pattern and associated damage.

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The two-spotted spider mite, *Tetranychus urticae* is one of the most polyphagous Arthropods and is a major agricultural pest worldwide. The spider mite has a rapid life cycle and feeds on over 1000 plant species belonging to more than 150 different plant families, including many agriculturally important crops such as tomatoes, peppers, strawberries, grapes and citrus. Damage to crops as a result of spider mite feeding is estimated to be ~1 billion (USD) a year. Therefore, understanding the molecular mechanism underlying plant's ability to recognize and respond to spider mites is essential for addressing this problem. Presently, the primary effect of spider mite feeding on plant tissue is largely unknown. The objective of this study is to further characterize the feeding mode of T. urticae, its pattern and associated damage. Analyses of histological leaf cross section from Arabidopsis and beans infested with mites were performed to identify the feeding site and to reconstitute the stylet penetration through the cortical parenchyma. Microscopic analysis of these sections reveals for the first time that stylets insert between epidermal cells and follow a straight route through the tissue. A selective dye that stains only dead cells (trypan blue) was used to determine the pattern of tissue damage. According to preliminary results, it seems that spider mite feeding causes the death of individual/small number of mesophyll cells. Understanding the immediate effects of T. urticae feeding will help us better understand Plant-Arthropod interactions at the cellular level and will ultimately lead to novel crop protection strategies.

P15

Metabolite and expression analysis of the 4-aminobutyrate (GABA) pathway in apple fruit stored under controlled atmosphere

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4-Aminobutyrate (GABA) is primarily synthesized from glutamate via glutamate decarboxylase (GAD) and then is catabolized to succinic semialdehyde (SSA) via GABA transaminase (GABA-T). SSA is oxidized to succinate by SSA dehydrogenase (SSADH), or alternatively, reduced to 4-hydroxybutyrate (GHB) via SSA reductase (GLYR for glyoxylate/SSA reductase). GABA can also be synthesized from putrescine and possibly spermidine (polyamines) via reactions involving diamine oxidase (DAO), and NAD-dependent 4-aminobutyraldehde dehydrogenase (ABALDH). The physiological role(s) of GABA in plants is not yet firmly established, but there is extensive evidence linking it to stress. For example, GABA is known to accumulate in apple fruit subjected to controlled atmosphere (CA) storage. We recently demonstrated that 'Empire' apple fruit contain: (i) two cytosolic calmodulin-dependent GADs, as well as a novel CaM-independent GAD that

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does not possess a C-terminal autoinhibitory domain; and, (ii) two mitochondrial pyruvate/glyoxylate-dependent GABA-Ts. Here, we describe the metabolite (GABA, succinate, GHB and polyamines [free, soluble conjugated, and insoluble conjugated forms of putrescine, spermidine and spermine]) and expression (GAD_{1-3} , $GABA-T_{1,2}$, $GLYR_{1-2}$, $SSADH_{1,2}$, DAO_{1-5} , $ABALDH_{1,2}$ and alanine transaminase as a positive indicator of O_2 deficiency) analysis of 'Empire' apples stored for up to 16 weeks at 0 or 3 °C, 2.5 kPa O_2 , and 0.03 or 5 kPa CO_2 . This work will be complemented by measurements of the pyridine nucleotide (NAD[P][H]) pools. These findings are interpreted as support for the involvement of both gene-independent and –dependent processes in GABA accumulation in CA-stored apple fruit.

P16

A highly charged region in the middle domain of plant ER-localized HSP90 is involved in tunicamycininduced ER stress resistance and ATP hydrolysis

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Heat shock protein HSP90 is a highly conserved molecular chaperone that is involved in modulating a multitude of cellular processes under both physiological and stress conditions. In Arabidopsis, there are seven isoforms (AtHSP90.1 to AtHSP90.7) that are localized to the cytoplasm/nucleus, mitochondria, chloroplast and endoplasmic reticulum (ER). AtHSP90.7 is previously reported to help the folding of CLAVATA (CLV) proteins responsible for shoot meristem maintenance in plants. After analyzing the sequence of AtHSP90.7 and other ER GRP94 proteins from plants and animals, we identified a short, charged region that is only present in the middle domain of plant-derived GRP94 proteins. We analyzed transgenic plants that overexpress a mutant protein HSP90.7^{A22}, which has the charged region in the HSP90.7 middle domain deleted. We showed that seedlings overexpressing HSP90.7^{A22} have enhanced sensitivity to tunicamycin-induced ER stress. We also found that HSP90.7^{\Delta 22} has an ATP hydrolysis activity similar to prokaryote-originated HSP90 orthologues, which was higher than canine GRP94 and cytosolic HSP90s from higher organisms. Increased catalytic efficiency of HSP90.7^{\text{\text{A22}}} may interfere with CLV protein synthesis, folding and/or signalling activity, and could affect the WUSCHEL (WUS)-CLV pathway. Developmental characteristics were examined in the progeny of HSP90.7^{\text{\text{\text{MSP}}}2} transgenic plants crossed to wus-1 and clv3-2 mutants, as well as in wus-1 and clv3-2 mutants that overexpress HSP90.7 $^{\Delta 22}$. Since plants expressing HSP90.7 $^{\Delta 22}$ were phenotypically indistinguishable from plants that did not, we propose that any possible enhancement or inhibition of the WUS-CLV pathway by HSP90.7 $^{\Delta22}$ overexpression did not reach a threshold necessary to trigger observable phenotypes.

P17

Polarization-dependent second and third harmonic generation imaging of *Haematococcus pluvialis* Danielle Tokarz^{1,2}, Richard Cisek^{1,3}, Omar El-Ansari^{4,5}, George Espie^{4,5}, Ulrich Fekl^{1,2}, and Virginijus Barzda^{1,2,3}

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Haematococcus pluvialis is a freshwater species of green algae which is known to accumulate astaxanthin when faced with unfavourable environmental conditions such as high or low light intensity, high salinity, higher temperatures and starvation. Little research has been done to spatially locate astaxanthin in Haematococcus pluvialis during stress. In order to study the accumulation of astaxanthin, imaging of Haematococcus pluvialis was performed with multiphoton excitation fluorescence (MPF), second harmonic generation (SHG), and third harmonic generation (THG) simultaneously. MPF signal was observed at the cell periphery due to chlorophyll fluorescence. Strong SHG signal was observed from starch granules. Weak THG signal was seen around the edges of the green non-induced palmelloid cell and more THG signal was observed in the middle of the induced palmelloid cell. In red aplanospores, strong THG signal was observed at the periphery where cytosolic lipid vesicles are believed to be located. The current study demonstrates that nonlinear optical microscopy enables one to follow closely the structural dynamics of green algae during adaptation conditions to environmental stress conditions.

P18

Investigating the role of BLADE-ON-PETIOLE genes in secondary wall formation in Arabidopsis and Poplar

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The BLADE-ON-PETIOLE1 (BOP1) and BOP2 genes of Arabidopsis thaliana comprise a conserved subclade of BTB-ankyrin transcriptional co-regulators whose activities at lateral organ boundaries regulate leaf, flower, fruit, and inflorescence architecture. Transgenic plants overexpressing BOPs are late flowering with short internodes containing an overabundance of lignin including ectopic deposition of lignin in the pith. Several lignin biosynthetic genes are up-regulated in the stem of BOP-overexpressing lines including a class III cell wall peroxidase gene PRXR9GE whose activity is predicted to catalyse polymerization of monolignols. These data suggest a role for BOPs in developmental regulation of lignin biosynthesis. Microarray data in poplar tree indicated that BOP-like (BPL) genes are expressed in the stem where they may promote differentiation of lignified cell-types for wood development. To examine this hypothesis, we are characterizing BOP orthologs in poplar, PtrBPL1 and PtrBPL2. Alignment of Arabidopsis and poplar BOPs show that the proteins are highly conserved at the amino acid level. We also show that poplar BOPs complement the bop1 bop2 Arabidopsis mutant phenotype and produce a similar overexpression phenotype. Quantitative RT-PCR analysis of poplar tissues shows that BPL expression in abundant in the leaf petiole and in vascular tissues of the stem (xylem and phloem). We are currently examining the detailed expression pattern of PtrBPL1 and PtrBPL2 in poplar tree apex and stem and will use an RNAi approach to generate loss-of-function mutants in poplar. Our data support a conserved role for *BOP*s in tree development.

P19

Investigating the role of a protein kinase during Arabidopsis pollen-pistil interactions

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In the Brassicaceae (mustard family), the acceptance of compatible pollen is tightly regulated as the pollen grain is dependent on the dry-type stigma for the initial stages of pollen hydration and germination and pollen tube penetration through the stigmatic surface. Upon recognition of the pollen grain by the stigma, secretory vesicles are proposed to transport factors from the stigmatic papilla to the pollen grain to allow pollen

hydration and the subsequent pollen tube penetration for fertilization. The physiological events following compatible pollen recognition are well known, but the signalling events which trigger this response remain unknown. Proteins kinases are well-known signaling proteins involved in a wide range of plant signaling processes, and we are currently using a reverse genetics approach to identify candidate genes that may have a role in compatible pollen acceptance signalling. Mutants are being generated by knocking-down the expression of candidate genes through RNA interference with artificial microRNA constructs. Available SALK T-DNA mutant lines for the candidate genes are also being investigated. These lines are being tested for reductions in pollen adhesion, pollen tube penetration and seed set. Through this study, we hope to identify protein kinases that are important for compatible pollen acceptance signalling.

P20

Transient expression of β -glucuronidase in soybean somatic embryos using cell penetrating peptide transfection

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P21

Biochemical characterization of anthocyanidin reductases from the seed coat of darkening cranberry beans (*Phaseolus vulgaris*)

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Cranberry bean (*Phaseolus vulgaris*) seed coat darkening occurs during postharvest storage and culminates in lower aesthetic quality, effectively decreasing shelf life and economic value. Seed coat darkening is associated with the biosynthesis and availability of proanthocyanidins, and their subsequent oxidation. Anthocyanidin reductase (ANR) is a key enzyme known to catalyze the NADPH-dependent reduction of anthocyanidins yielding flavan-3-ols (e.g. epicatechin), precursors of proanthocyanidins. Seed coat specific isozymes are known to occur in legume plants, however no information exists for *P. vulgaris* ANR(s). In the present study, recombinant inbred lines from an 'Etna' (darkening) by 'Wit-Rood' (non-darkening) cranberry bean cross

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The genetic transformation of plants is essential to the alteration of plant properties. However, many of the
plant transformation technologies available have demonstrated low levels of transfection efficiency. There are
a number of plants that are particularly resistant to transformation using the current methods. Soybean is an
agriculturally important crop that provides a protein source for people's diets. Soybean exhibits very low
transfection levels using more conventional transformation methods. Cell penetrating peptide transfection is a
transformation method that has only recently been investigated for use in plants. Cell penetrating peptides
(CPP) are short amino acid sequences that possess the ability to cross over membranes carrying
macromolecular cargo up to 100 times their size. Many CPPs are based on invasive proteins, such as the
Trans-activator of transcription (Tat) protein from HIV-1. In the current study we examine the application of
this novel transformation method in soybean somatic embryos. Embryos were incubated with CPP-plasmid
complexes consisting of a Tat dimer protein and a linearized plasmid containing a β-glucuronidase (GUS)
reporter gene. A GUS histochemical assay was performed and transient expression of β-glucuronidase was
observed in the somatic embryo clusters. Our results indicate potential for the future use of CPP technology in
soybean transformation. This study represents the first assessment of CPP use in soybean.

were greenhouse cultivated; and seed coats were collected from mature beans. Two ANR genes, *PvANR1* and *PvANR2*, have been cloned from the seed coat cDNA of a darkening cranberry bean recombinant inbred line. A phylogenetic analysis of *P. vulgaris* ANRs revealed genes that cluster with previously biochemically characterized ANRs from various legume species, including *Glycine max* and *Medicago truncatula*. Moreover, *PvANR1* is more closely related to *GmANR1*, a gene encoding an enzyme associated with browning of *G. max* seed coats. Biochemical characterization of both recombinant cranberry bean ANRs purified from a bacterial expression system is on-going. Thus far, HPLC analysis of *in vitro* assays revealed that an N-terminal hexahistidine tagged recombinant PvANR1 catalyzed the NADPH-dependent reduction of cyanidin into epicatechin. This project may provide important information for breeding efforts aimed at preserving the visual appeal and marketability of cranberry beans.

P22

Two spotted spider mite (Tetranychus urticae) adaptation to Arabidopsis thaliana

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The two-spotted spider mite, *Tetranychus urticae*, is one of the most polyphagous arthropods and a major agricultural pest worldwide. Notorious for its ability to develop resistance to pesticides and its broad host plant range, it feeds on over 1,100 plant species belonging to more than 140 plant families, including many agriculturally important crops. T. urticae also feeds on the model plant Arabidopsis thaliana; upon mite feeding, A. thaliana initiates the accumulation of indole glucosinolates (IGs) which are plant secondary metabolites that affect mite development and mortality. In this study, I will determine the adaptability of T. urticae to new hosts containing different levels of IGs and will focus on identifying the potential mechanisms that spider mites evolve during adaptation to IGs using transcriptome analysis. The experimental design is to propagate bean-adapted spider mites on A. thaliana strains that contain different levels of IGs for 25 generations: cyp79b2/b3 (lacks IGs), Col-0 (normal IG levels) and artD1 (over-accumulates IGs). Spider mite adaptation to the new host will be established by measuring several performance factors including adult mortality, fecundity and developmental time relative to the ancestral mites. Next, spider mite transcriptome analysis using qRT-PCR will be performed on several candidate genes whose expression associated with mite responses to different levels of IGs. Changes in gene expression levels of adapted mites to different IG levels will then be related to gene expression of the progenitor strain. In doing so, a defined set of genes and gene families of potential adaptive relevance will be identified.

P23

Further defining the roles of ARC1 and Exo70A1 in pollen-pistil interactions in the Brassicaceae

<u>Daniel C Johnson¹</u>, and Daphne R Goring¹

Monecious flowering plants have both male (pollen) and female (pistil) reproductive organs within their flowers. Some species reject their own pollen to prevent inbreeding depression and produce offspring with greater genetic diversity. In the Brassicaceae, this self-incompatibility response is controlled by the pistil through the arrest of resources required for pollen hydration and germination. Signalling events responsible for this are initiated by the allelic binding of the pollen SCR/SP11 ligand to the pistil *S* Receptor Kinase (SRK). From interaction studies, *in vitro* assays and transgenic plant studies in *Brassica*, the downstream signalling events are proposed to include the ARC1 E3 ubiquitin ligase which, following phosphorylation by SRK, ubiquitinates and inhibits Exo70A1. With compatible pollen, Exo70A1, as part of the exocyst complex, is

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normally proposed to be responsible for directing cargo-filled secretory vesicles to the stigmatic papillar plasma membrane. With the inhibition of Exo70A1, resources are not delivered to the self-incompatible pollen grains, resulting in self-pollen rejection. Recent work from our research group indicates this self-incompatibility signalling pathway is conserved in naturally occurring self-incompatible *Arabidopsis lyrata*. However, there are still many aspects of the self-incompatibility and compatibility pollen response pathways that need to be further defined at the cellular level in these Brassicaceae species. We are now generating and using tagged ARC1 and Eo70A1 constructs to further explore how these components functions at cellular level in regulating the rejection of self-incompatible pollen and the acceptance of compatible pollen.

P24

C₄ plants optimize chloroplast number and position in mesophyll cells

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The convergent evolution of C_4 photosynthesis over 65 times in 18 families of flowering plants enable us to identify novel traits essential to the function of the C4 pathway by comparing closely related C_3 , C_4 and C_3 - C_4 intermediate species from each lineage. We used 11 independent lineages of eudicots and grasses to characterize the mesophyll chloroplast number and position during the evolutionary transition from C_3 to C_4 photosynthesis. In all 11 lineages, the average chloroplast number and chloroplast coverage of the mesophyll cell periphery of C_4 species is reduced to half the values of the closest C_3 species. Additionally, the amount of cytoplasm between the chloroplasts and the cell periphery in C_4 plants is higher than their C_3 relatives. These patterns are interpreted to reflect the different site of initial carboxylation in C_3 and C_4 species. C_3 plants by having high chloroplast numbers and coverage of the mesophyll cell periphery, enhance CO_2 diffusive conductance into the chloroplast, the site of initial carbon fixation. In contrast, lower chloroplast coverage in mesophyll cells of C_4 plants enhances the diffusion of CO_2 into cytosol where PEP carboxylase performs the primary CO_2 fixation. In conclusion, high number of chloroplasts in mesophyll cells of C_4 plants would create a barrier to CO_2 influx into the cytosol, and would shade the bundle sheath chloroplasts where net photosynthetic carbon reduction largely occurs.

P25

Investigation of the effect of high temperature stress on reproduction in *Arabidopsis thaliana*

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High temperatures can severely reduce the number of viable pollen available for fertilization. Abiotic stress, to include high temperature, can induce the production of reactive oxygen species (ROS) in plant cells, and has been associated with a reduction in pollen viability in some species. Reactive carbonyl species (RCS), formed downstream of ROS can oxidatively modify proteins, thereby rendering them nonfunctional. We have identified a gene in *Arabidopsis*, High Temperature Tolerance (*HTT*) that encodes a plastid-targeted protein that functions in pollen to remove RCS. In the high temperature-tolerant Landsberg Erecta (Ler) accession, the gene is induced in the anther upon heat stress and pollen remain viable. In contrast, the gene is not induced in the temperature-sensitive Cape Verdi Island (Cvi) accession and pollen is nonviable. Consistent with a role for *HTT* in conferring temperature tolerance in pollen by removal of RCS, carbonylated protein levels were significantly lower in Ler than Cvi at high temperature. The lack of pollen viability is associated with (i) disruption of the pollen plastid envelope, (ii) failure to degrade starch grains during pollen maturation, (iii)

swelling of mitochondria, (iv) autophagy of plastids and mitochondria, and (iv) abnormal lipid body morphology and accumulation. Finally, nonviable pollen grains accumulate high levels of H_2O_2 in plastids and mitochondria as indicated by the presence of cerium perhydroxide precipitate in these organelles following incubation with cerium chloride. Results indicate that HTT is essential for tolerance of Arabidopsis pollen to high temperature stress and underscore the importance of plastid function for pollen maturation.

P26

Characterization of the *Arabidopsis thaliana INDETERMINATE DOMAIN2* gene suggests a role in controlling early seedling growth behaviour

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The Zea mays flowering time regulator INDETERMINATE1 (ID1) is the founding member of a large gene family characterized by a conserved zinc finger region called the INDETERMINATE DOMAIN (IDD). IDD genes are found in the genomes of all land plants studied thus far. The model plant, Arabidopsis thaliana, has sixteen IDD genes. Only a few of these genes have been characterized and they have diverse roles in different aspects of plant development. My research aims to determine the function of Arabidopsis IDD2 (IDD2). The function of IDD2 is being elucidated through analysis of IDD2 expression patterns, loss-of-function knock out idd2 mutants, and IDD2 over-expression. An IDD2 promoter-driven GUS:GPF fusion reporter plasmid has been constructed to follow IDD2 expression in all stages of development and a 35S::IDD2 transgenic line will be analyzed to determine the effects of over-expression. In initial experiments, germination behaviour and root growth rates were compared between wild type and idd2 plants. idd2 plants have similar germination times but slower root growth on media containing various amounts of sucrose or glucose compared to wild type plants. Hypocotyl elongation is also slower for idd2 mutants compared to wild type plants. Preliminary analysis suggests that IDD2 may have a role in transporting sucrose in the developing seedling and/or in controlling the processes of division or elongation.

P27

Transgenic anti-florigen system to study long-distance movement of small RNA

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Small RNA (sRNA) molecules play key roles regulating processes including plant development, pathogen defense and response to abiotic stress. sRNAs can move long distances via the phloem, but not all types of sRNA are transported systemically. The machinery behind this selective movement remains unknown. The transition from vegetative to reproductive growth is an important developmental switch; in Arabidopsis the floral transition is regulated by the transcription factor FLOWERING LOCUS D (FD), which becomes activated when a leaf-derived florigen protein, encoded by the *FLOWERING LOCUS T (FT)* gene, is translocated from the leaves to the shoot apical meristem (SAM). A transgenic system was created that mimics the leaf to the SAM movement of FT. This uses a synthetic sRNA based anti-florigen to study the long-distance translocation of sRNAs. In our system, expression of an RNA interference signal in leaf vasculature that targets FD should result in a flowering time delay, if this signal is transported to the shoot apical meristem. In preliminary studies, transgenic Arabidopsis lines with this construct show a significant delay in flowering time. The creation of a phenotype reliant upon the movement of a long distance sRNA signal will allow us to use this system in a suppressor/enhancer screen to elucidate novel components of the sRNA transport mechanism in plants.

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Spatial variation in aboveground herbivory due to biocontrol agents of the noxious weed *Cirsium arvense*

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Canada thistle (*Cirsium arvense*) is a Eurasian weed invasive across North America. Biological control has been attempted, but with very mixed results. To describe variation in attack by its herbivores and determine the potential consequences for plant performance, an 815 km latitudinal transect was sampled from agricultural southern Ontario to boreal James Bay. Herbivory was documented by quantifying leaf damage, stem galls, and attack by seed parasitizing insects. Number and thickness of shoots, plant height, number of flowers, and seed production were quantified as estimates of performance. Initial data suggest that (1) plants in southern Ontario are heavily damaged by biocontrol insects, (2) leaf and stem damage are greatly reduced at northern and isolated sites, and (3) seed herbivory shows great spatial variation related to land use type and population isolation. The most southerly and northerly sites exhibited high rates of seed herbivory, but the causal organism differed between these regions. Isolated mid-latitude populations showed no evidence of aboveground herbivory, likely because populations were very sparsely distributed. These results suggest that there is a critical host abundance required to support *C. arvense*'s natural enemies, which are often absent from range margins and/or isolated sites.

P29

The role of exocyst complex in the early stages of compatible pollen-pistil interactions in *Arabidopsis* Darya Safavian, Yara Zayed, Emily Indriolo, Laura Chapman, Abdalla Ahmed, Daphne R. Goring Department of Cell & Systems Biology, University of Toronto

The initial events of pollen-pistil interactions are fundamentally important in flowering plants as they will influence successful fertilization. Of particular interest is the exocyst complex, known to be necessary for tethering exocytic vesicles to specific sites at the plasma membrane in yeast and animals. Previously, we have provided evidence that Exo70A1, a putative subunit of the plant exocyst complex, is essential in the stigma for compatible pollen-pistil interactions in Arabidopsis thaliana and Brassica napus. Based on this discovery, we hypothesize that Exo70A1 functions as part of the exocyst complex to tether secretory vesicles to the plasma membrane under the pollen attachment site to deliver essential stigmatic resources for the compatible pollen to undergo hydration and germination. In transmission electron microscope images taken at different time points following pollination, we have observed targeted exocytosis to the stigmatic papillar plasma membrane under the compatible pollen grain. To provide further support for the role of the exocyst complex in compatible pollen acceptance, the roles of the remaining seven subunits of the exocyst complex, Sec3, Sec5, Sec6, Sec8, Sec10, Sec15 and Exo84 were investigated in Arabidopsis. Stigma-specific knock-down constructs were used to suppress the expression of each exocyst subunits individually. The early post-pollination stages of pollen hydration, pollen grain adhesion, pollen tube penetration and overall fertility are currently being analyzed to determine the requirement of each subunit. Thus from this study, we will determine whether all eight exocyst subunits are required for the early stages of compatible pollen-pistil interactions.

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Fungal endophyte benefits host plant only in the absence of grazing

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Symbiotic interactions in nature can be context dependant, ranging from parasitic to mutualistic under varying conditions. Fungal endophytes of grasses are often thought to be mutualists due have frequently been regarded as such due to their herbivore deterrent properties but studies showing such effects have focused primarily on agronomically important crops and thus the roles these symbionts play in native grasses are less clear. We studied grass-endophyte interactions at Silwood Park, UK, a sandy and acidic grassland with intense grazing by rabbits (Oryctolagus cuniculus L.). To test the prediction that infection frequencies would decrease following removal of herbivores, we examined seeds of red fescue (Festuca rubra) collected from 15 rabbit exclosures ranging in age from 4 months to 21 years in age. Suprisingly, the frequency of endophytes increased by 34.5% over time since rabbit exclusion. To better understand the mechanism driving this increase in frequency, we conducted a greenhouse experiment where both infected and uninfected F. rubra plants were either subjected to simulated grazing or left undamaged. In the ungrazed treatment we found that endophyte infected plants had 13.1% higher growth rates and produced 86.1% more aboveground biomass but there were no differences in growth in the grazed treatment. However, infection status of F. rubra did not influence tolerance to herbivory suggesting endophytes change plant growth but not compensatory responses to damage. Together, these results suggest that endophytes may act as mutualists in this system, but only in the absence of herbivores. This work demonstrates context dependency of mutualisms and underscores the need to measure costs and benefits of symbiotic interactions under multiple environmental conditions.

P31

Colonization of *Gluconacetobacter diazotrophicus* in *Brachypodium distachyon* for Studying Nitrogen Fixation in Monocot Plant

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Gluconacetobacter diazotrophicus is an endophytic nitrogen-fixing bacterium that was originally found inside of sugar cane plant and the bacterium provides significant amounts of fixed nitrogen to sugar cane plants. As an emerging model research plant for monocots, Brachypodium distachyon has a small genome, small physical structure, short lifecycle and is related to most cereal crops. These advantages make B. distachyon an excellent choice for studying and exploring the nitrogen fixation of G. diazotrophicus in different monocot crops. In our study, the colonization of G. diazotrophicus in B. distachyon was studied by using several inoculation methods to compare the inoculation efficiency. The colonization was confirmed by microscopic observation, species-specific PCR and bacteria re-isolation. The highest inoculation success rate was found among seedlings grew under hydroponic condition, in which over 80% of the inoculated plants showed colonization. G. diazotrophicus were found mainly inside the roots, while much fewer bacteria were observed in the aerial tissues. The colonization displayed a "crack entry" pattern, which means G. diazotrophicus colonize the root through the latter root emergence sites. Colonization can be detected after 21 days post inoculation. The G. diazotrophicus population inside the root tissue is being estimated by MPN/Colony counting.

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The second hyperpolarizability of LHCII

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Imaging photosynthetic structures with nonlinear optical microscopy gives rise to high intensity third harmonic generation (THG) signal due to the presence of chlorophylls and xanthophylls which have large second hyperpolarizability (γ) values. The second hyperpolarizability of trimers of the light-harvesting chlorophyll a/b pigment-protein complex of photosystem II (LHCII) isolated from pea (*Pisum sativum*) plants was investigated by the THG ratio technique at 1028 nm wavelength. The γ value was found to be (-1600 \pm 400)×10⁻⁴¹ m²V⁻². This large negative γ value is due to the presence of chlorophyll a and chlorophyll b which also have large negative γ values, while positive γ values of xanthophylls reduce the magnitude of the THG signal. Variation was observed between the measured γ value of LHCII and the approximated γ value of LHCII obtained by adding individual γ values of xanthophylls and chlorophylls. This difference can be attributed to the differing inter-pigment interactions of oriented xanthophylls and chlorophylls in LHCII as compared to randomly oriented non-interacting pigments in solution and the differing dielectric environment within the protein complex versus the surrounding of organic solvent.

P33

MicroRNA156 regulates plant development and nodulation in Lotus japonicus.

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MicroRNAs (miRNAs) are non-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level by base pairing to complementary target mRNA. In *Arabidopsis thaliana*, *miR156* regulates a network of genes by targeting *SPL* (*SQUAMOSA PROMOTER BINDING PROTEIN LIKE*) transcriptional factors. These in turn regulate plant growth and development, including the rate of leaf initiation and transition from vegetative growth to reproductive growth. We have cloned a *miR156* homolog from *Lotus japonicus*, *LjmiR156*, and partially investigated its biological function in this model legume species. *LjmiR156* was overexpressed in *Lotus japonicus* under the control of CaMV35S promoter. Transgenic plants harboring this construct exhibited a dramatically altered morphological phenotype, including enhanced shoot branching, smaller but more numerous leaves, varied inflorescences and siliques, and delayed flowering compared to wild type plants. *In planta* and expression analyses revealed that transcripts of two *SPL* genes were targeted for cleavage by *LjmiR156*. Furthermore, reduced nodulation and delayed flowering in transgenic *LjmiR156* overexpression plants were further validated by analyzing effects of *LjmiR156* on expression of key nodulation- and flowering-related genes. Results will be discussed in relation to the role of *LjmiR156* in plant development and symbiosis.

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The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid

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Salicylic acid (SA) is an essential hormone in plant immunity, but its receptor has remained elusive for decades. The transcriptional coregulator NPR1 is central to the activation of SA-dependent defense genes, and we previously found that Cys⁵²¹ and Cys⁵²⁹ of *Arabidopsis* NPR1's transactivation domain are critical for coactivator function. Here, we demonstrate that NPR1 directly binds SA, but not inactive structural analogs, with an affinity similar to that of other hormone-receptor interactions and consistent with in vivo *Arabidopsis* SA concentrations. Binding of SA occurs through Cys^{521/529} via the transition metal copper. Mechanistically, our results suggest that binding of SA causes a conformational change in NPR1 that is accompanied by the release of the C-terminal transactivation domain from the N-terminal autoinhibitory BTB/POZ domain. While NPR1 is already known as a link between the SA signaling molecule and defensegene activation, we now show that NPR1 is the receptor for SA.

P35

Evaluating the roles of root suberin constituents in the adaptive response to drought

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Suberin, a complex aliphatic and aromatic heteropolymer deposited in specific root cell walls, plays important roles in controlling water and ion movement as well as in protection against pathogens. Typical aliphatic suberin monomers are C16-C24 chain-length α, ω -dicarboxylic acids, ω -hydroxy fatty acids, *unsubstituted fatty* acids, and primary fatty alcohols, while the main aromatic component is ferulic acid. Glycerol is also part of the suberin polymer. The roles of individual constituents in the protective functions of suberin are presently unknown. To address this gap in our understanding, we are using a set of *Arabidopsis thaliana* mutants that each have unique alterations in suberin amount and/or composition (*esb1*, *gpat5*, *cyp86a1*, *cyp86b1*, *asft*, *far1*, *far4*, and *far5*). We focused on drought response in this study. *Arabidopsis* wild type and suberin mutants were subjected to control moisture levels (20% v/v) or drought stress levels (5% v/v). Total biomass production in plants was measured as a function of available water through the root system. Plant responses to drought stress were analyzed using several physiological traits of biomass partitioning, including the ratio of root to shoot biomass as an indicator of drought responsiveness. Suberin load and composition were also measured comparing control (non-drought stressed) and drought-stressed plants. We found that suberin mutants were differentially affected in their adaptive response to drought stress. We will report on the specific relationships between suberin composition and drought responsiveness, which likely reflect altered water relations.

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A New Suite of Pathogenesis-Related Gene Markers in Arabidopsis thaliana

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Understanding plant-pathogen interactions is fundamental to protecting the agriculture industry from pathogens. The current plant defence model indicates PAMP/MAMP-Triggered Immunity (PTI) as the first line of defense for plants; recognizing slowly evolving portions of pathogens in order to induce a defense response. Pathogens have responded with effectors to suppress or hide from the PTI. These effectors can be recognized by R proteins as the plant's second line of defence to induce Effector Triggered Immunity (ETI) followed by a stronger defence response. Currently, gene markers for specific infection conditions are poorly defined. The aim of this project is to identify genes that are highly expressed under specific pathogenesis-related conditions (eg. during PTI or ETI responses) in order to provide a novel set of markers for the study of host-pathogen interactions. By analyzing publically available microarray data, 19 gene candidates were identified in *Arabidopsis thaliana* that show strong expression with specific strains of *Pseudomonas syringae* at specific time points. The role of these genes in plant immunity will be determined based on the expression of *A. thaliana* plants unable to express genes related to defence.

P37

Effects of elevated CO₂ and growth temperature on respiration rates in Norway spruce (*Picea abies*).

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Future climate change scenarios will include carbon dioxide-based increases in temperature. Boreal forest ecosystem located in circumpolar region will experience the most dramatic change in temperature. Plants have the ability to acclimate to changes in temperatures and rising carbon dioxide and these physiological responses will likely change global carbon fluxes. For proper modeling of future carbon fluxes from vegetation, major parameters affecting the carbon cycle, such as autotrophic day and night respiration, have to be better characterized. My study investigates the response of respiration rates and light-saturated photosynthesis in the dominant boreal evergreen species, Norway spruce, to predicted climate change scenarios (higher carbon dioxide levels and rising temperatures), and therefore provides essential information required for accurate modeling of forest carbon fluxes. Physiological measurements for current growth season show lower respiration rates in trees grown in elevated than in ambient temperatures, however additional research and analysis of other parameters are required for further examination.

P38

Reverse genetics using phospho-mutations at the AKIN10 target site in FUSCA3 reveals differential regulation of heat stress response during germination as well as seedling leaf and root development AllenYi-LunTsai², Aaron Chan², AreejAdil¹, Jinyuang Wang¹, Hardy Lou¹, Sonia Gazzarrini^{1,2}

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FUSCA3 has been identified to regulate various developmental processes such as embryo maturation, germination and leaf maturation in Arabidopsis. Recent work has identified that FUSCA3 is phosphorylated by SNF1 KINASE HOMOLOG 10 (AKIN10). AKIN10 overexpression was demonstrated to delay degradation of FUSCA3 in cell free extracts suggesting that AKIN10 could indirectly enhance FUSCA3 activity. AKIN10 and FUSCA3 overexpression has previously been shown to similarly retardation of phase development in

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germination, leaf maturation and flowering. However the specific effects of phosphorylation at potential AKIN10 target sites have not been directly examined. A reverse genetics approach using phospho-mutated *FUSCA3* has been employed to probe for developmental phenotypes. Current results suggest that phosphorylation at the AKIN10 target site (Serine 55-57) may regulate heat stress response during germination, seedling leaf development speed and root growth.

P39

Shandong and Yukon accessions of the extremophile model plant *Eutrema salsugineum* exhibit different levels of resistance to *Pseudomonas syringae*

May Yeo*¹, Philip Carella¹, Elizabeth Weretilnyk¹, Robin Cameron¹

The extremophile plant *Eutrema salsugineum* is highly tolerant of abiotic stresses such as saline soils, drought and freezing temperatures. While abiotic stress responses are extensively studied in *Eutrema*, information on the response to biotic stress in this model is limited. In this study, we examined the disease resistance responses of two *Eutrema* accessions, Shandong and Yukon, to the hemibiotrophic plant pathogen *Pseudomonas syringae*. In comparison to *Arabidopsis*, both accessions exhibit increased resistance to *P. syringae pv tomato* DC3000 (*Pst*), with Shandong *Eutrema* exhibiting greater resistance than Yukon *Eutrema*. Effector-triggered immunity (ETI) and the age-related resistance (ARR) responses were also investigated and a differential capacity to employ these mechanisms was observed in these accessions. We tested *Eutrema* resistance to *P. syringae* pv. *maculicola* (*Psm*), observing extensive bacterial growth limitation in both accessions. Publically available RNA-Seq data of Yukon and Shandong *Eutrema* showed an overrepresentation of putative defense genes in healthy untreated Shandong plants, which included the resistance marker *pathogenesis-related1* (*PR1*). RT-PCR confirmed that Shandong plants constitutively express *EsPR1* in a manner similar to defense-primed plants. Constitutive *PR1* expression alongside heightened resistance to both *Pst* and *Psm* suggest that Shandong *Eutrema* exists in a primed-like state of defense-preparedness.

P40

Investigation on the mechanism of chloroplast positioning in single-cell C4 *Bienertia sinuspersici* Sarah Schoor*¹, Dustin Sigurdson¹, Simon Chuong¹

In *Bienertia sinuspersici*, C4 photosynthesis is achieved without the presence of Kranz anatomy by partitioning dimorphic chloroplasts into two distinct cytoplasmic compartments within a single cell type. Such a unique chloroplast arrangement is not always present however, as early developing *Bienertia* cells simply perform C3 fixation. As the chlorenchyma cell develops, chloroplasts differentiate and move into their respective cytoplasmic compartments via the cytoskeleton. One of our research foci is to identify the components which control chloroplast movement and organization during this C3-C4 transition. To further this goal, genes previously characterized in Arabidopsis were identified and sequenced in *Bienertia*: chloroplast unusual positioning protein 1 (CHUP1) a chloroplast bound membrane protein that interacts with actin and aides in plastid movement (Oikawa et al., 2003) and clumped chloroplasts 1 (CLMP1) which has been linked to chloroplast separation and distribution (Yang et al., 2011). Preliminary studies indicate a developmentally dependent role for both genes in *Bienertia* as comparison of transcript levels between shoot tips and mature leaves shows a decrease in expression as the leaf matures. To further assess the role of CHUP1 and CLMP1 in chloroplast arrangement, future work will focus on the characterization and localization of these proteins throughout leaf development in *Bienertia*.

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Root death of perennial herbaceous plants as a winter survival strategy in cool temperate wetlands.

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Winter survival strategy varies for plant species in cool-temperate biomes. Two well-known strategies are that of annual and perennial growth forms. However, not all perennial monocots preserve the root system during the winter months. A group of perennials have evolved to let their roots die for the winter.

The aim of this study is to understand how the autumnal root death of some perennial plants is related to above-ground senescence, environmental conditions and species' ecological strategies.

Six wetland monocots were cultivated in an experimental artificial wetland system in Sudbury, Ontario, from June to end November, and harvested from mid-August: *Rhynchospora alba*, *Eriophorum vaginatum*, *Sparganium androcladum Alisma triviale*, *Calla palustris*, and *Sagittaria latifolia*. The progression of root death was analyzed by harvesting the roots bi-weekly and staining them with 2,3,5 triphenyltetrazolium chloride (TTC). Soil temperature, water temperature and above-ground senescence were recorded. In addition, the re-allocation of nutrients (nitrogen and phosphorous) from dying roots and nutrient availability in the natural habitats of these species are also being investigated.

Preliminary results indicate interspecific differences in the progression of root death. Roots of A. *triviale*, C. *palustris*, and S. *latifolia* showed earlier mortality than S. *androcladum*, R. *alba*, and E. *vaginatum*.

The preliminary results indicate that the six species differ with respect to the timing of root death in preparation for cold temperature survival. This correlates with phylogeny and growth form, roots of broadleaved species from the order Alismatales dying earlier than those of the graminoid Cyperales and Typhales.

P42

Predicting the catalytic function of the carboxysomal γ -carbonic anhydrase CcmM

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Carboxysomes are complex biochemical compartments essential for photosynthesis and the CO₂ concentrating mechanism in cyanobacteria. These prokaryotic organelles house most of the cellular complement of ribulose 1, 5- bisphosphate carboxylase (Rubisco) within a protein shell. CO2 is delivered to Rubisco by one of a variety of strain-specific HCO₃ dehydration complexes (BDC). CcmM is a universal component of the BDC in β-cyanobacteria comprised of an N-terminal γ-carbonic anhydrase (CA) like domain and 3 or 4 repeats of an RbcS-like domain. This protein plays a key role in the structural organization and assembly of the βcarboxysome. Amino acid sequence analysis of the γ-CA like domain of CcmM from 95 cyanobacterial strains revealed that it can be categorized into two distinct groups: one that encodes a catalytically active γ-CA and the other which encodes an inactive protein. Experimentally, we show that the first group, exemplified by CcmM from Thermosynechococcus elongatus and Nostoc sp PCC7120, are in fact catalytically active enzymes as determined by ¹⁸O exchange assays. Using truncated forms of CcmM, biochemical analysis and homology modeling we defined the boundary of the γ-CA catalytic domain to reside within the N-terminal 201 amino acids. We also identified several important structural motifs which are unique to the γ -CA domain of the cyanobacterial enzyme. These included the A(7)APPTPWS(14) motif found in the β 1 - β 1 loop, a small α helix (αC) between residues 197 and 207 and a disulfide bond that spans αB- αC between C194 and C200. Reducing agents, DTT, TCEP and THP inhibited the γ-CA activity of recombinant Nostoc PCC7120 CcmM209 through

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a mechanism that was reversed by the thiol oxidizing agent diamide. These data suggest that the C194-C200 disulfide bond was critical for the oxidative activation and stabilization of enzyme structure (Peña, K.L., et al., PNAS 2010. **107**: 2455-2460). The second group of CcmM proteins, including CcmM from *Synechocystis* PCC 6803 and *Synechococcus* PCC 7002 have a divergent γ -CA like domain and are believed to be catalytically inactive. PCC7002 CcmM209 contains all amino acid sequences elements known to be required for catalysis, save C194 and C200. The recombinant protein proved to be catalytically inactive. We attempted to reconstitute enzyme activity in PCC7002 CcmM209 by making site directed mutations to restore C194 and C200 along with 3 other mutation to restructure α C. Homology models for the PCC7002 CcmM209 variants were created using Phyre2 and the coordinates from the X-ray crystal structure (3KWC) to the *T. elongatus* BP-1 enzyme as template. Root mean square deviation of the models improved from 2.39 Å over 1384 atoms for the wild-type to 0.91 Å over 1431 atoms for the V191G-S192Y-G194C-S200C variant. However, no CA activity has yet been detected in these variants. This suggest that other, unidentified residues, within the *Synechococcus* PCC7002 CcmM209 structure are critical to enzymatic activity despite 78% identity and 90% similarity to its closet active homolog.

P43

An F-Box from Arabidopsis is a negative regulator of salt tolerance

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F-Box protein, the building block of SCF complex, is functioning in substrate recognition of SCF subtype of E3 ubiquitin ligase. However, the role of F-Box protein in plant salt response is not very well understood. Here, we describe the characterization of an Arabidopsis salt-tolerant mutant *est1* resulting from a T-DNA insertion. *est1* has demonstrated significantly reduced sodium and enhanced potassium level after NaCl treatment compared with wild-type. Tail-PCR revealed that the T-DNA was inserted in a gene encoding a putative F-Box protein and the complementation experiment further supported that this F-Box gene is indeed *EST1*. On the other hand, over-expression of *EST1* resulted in increased sensitivity to salt stress, suggesting that *EST1* may act as a negative regulator for plant salt tolerance. In addition, *EST1* is probably also involved in reactive oxygen species accumulation and cell death. Apart from the salt tolerant phenotype, *est1* also displayed hyper-sensitivity to abscisic acid (ABA) and lower temperature. Taken together, our results suggest that F-Box protein can negatively regulate salt tolerance in Arabidopsis by modulating ion homeostasis via ABA dependent manner.

Maintaining the Carbon Sink Function of a Peatland Margin After Hydrological Disturbance Ellie Goud*¹, Tim Moore¹ and Nigel Roulet¹

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Peatlands store one-third of the global carbon pool even though they represent less than 3% of the Earth's surface. As net carbon sinks, they play a mitigating role in climate warming induced by increased greenhouse gases. However, the release of this large carbon stock back into the atmosphere could accelerate climate warming. Understanding the dynamics of carbon cycling is essential for assessing the carbon sink function of a peatland, and is critical in the face of a warming climate which may alter the structure and function of peatlands. An ecosystem-scale manipulation was used to lower the water table at a peatland in Ontario to test the hypothesis that water table is the primary driver of carbon dioxide (CO₂) exchange. Objectives were to evaluate the plant functional responses at the margin before and in the first season of the drainage. We compared field measurements of CO₂ fluxes across 29 sites and 9 species assemblages. We hypothesized that a drastic reduction in water table would result in a weakened carbon sink due to increased respiration and decreased photosynthesis. However, the net ecosystem CO₂ exchange did not differ significantly between years across all sites. Rates of change differed between species assemblages; many sites had lower rates of photosynthesis and respiration. Peatland species were able to maintain a similar net CO₂ exchange between years suggesting that the carbon sink function of the peatland margin may not be as sensitive to hydrological disturbance, potentially due to species-level adaptations and regulation of photosynthetic mechanisms.

P45

Detecting transcriptome responses to environmental stimuli in field-grown, adult conifer trees Moritz Hess^{1,2}, Henning Wildhagen^{1,#} and Ingo Ensminger^{1,3,4}

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Climate change is expected to challenge the adaptive potential of plant populations. It is therefore of great interest to assess the interactions between differentially shaped genotypes and the fluctuating conditions in a natural growing environment. We are investigating these interactions on the level of global gene expression in the economically important conifer tree species *Pseudotsuga menziesii* (Douglas-fir). Using deep mRNA sequencing, we performed an in-depth analysis of the Douglas-fir needle transcriptome. We used mixed linear regression models to investigate the correlation of transcript expression levels with environmental conditions in two Douglas-fir subspecies. Here we show results of the modelling approach and demonstrate the challenges of genome-wide expression analysis conducted in a semi-controlled experimental setting.

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