
The Canadian Society of Plant Biologists/ La Societé Canadienne de Biologie Végétale
Theobroma cacao flower
Eastern Regional Meeting
November 19, 2016
McMaster University
@ Royal Botanical Gardens
Photo by Jackie Patrick

Cover: Photo by Hamilton photographer Jackie Patrick of a *Theobroma cacao* flower.

The cocoa tree that bore this flower is in the McMaster University Greenhouse on the main campus. Cocao flowers form clusters, each flower is 1 to 2 cm in diameter, quite unremarkable compared to the cocoa pod that can be up to 30 cm long. The greenhouse is home to the McMaster Biodiversity Collection comprised of 271 species of tropical plants, many specimens are not found in any other collection in Canada. The McMaster Biodiversity Collection was in the news for supplying fresh bamboo to the pandas in the Toronto Zoo in the middle of winter and for the flowering of several *Amorphophallus titanum* (Titan arum or corpse flower) plants.

For more on the McMaster Greenhouse:
http://www.macbiogreenhouse.ca/

For more artwork by Jackie Patrick: (www.JackiePatrick.ca)

Royal Botanical Gardens is pleased to welcome members of the Canadian Society of Plant Biologists, Eastern Region, for your meeting on 19 November 2016. Since the early years of formal program development in the 1940s, Royal Botanical Gardens has been undertaking field botany and plant taxonomy research, and for many years was actively engaged in ornamental plant breeding. Developed as both a centre for expertise in and demonstration of ornamental horticulture for Ontario, and as a significant complex of protected nature sanctuaries, Royal Botanical Gardens today is a leader in terrestrial and wetland restoration ecology, and in outreach and education about botany, ecology, and horticulture. Each year we welcome visiting researchers from across Ontario and further afield undertaking projects in our nature sanctuaries, herbarium, cultivated plant collections, and extensive archives. Royal Botanical Gardens serves several important roles for the scientific and conservation communities. We are the International Registration Authority for Lilacs under the International Society for Horticultural Science, the National Focal Point for Canada for the UNEP's Global Strategy for Plant Conservation, and a member of the Sentinel Plant Network and the Ecological Restoration Alliance of Botanic Gardens. For more information about RBG please feel free to contact Dr. David Galbraith, Head of Science at RBG (dgalbraith@rbg.ca).

Acknowledgments:

The organizers for the 2016 CSPB-SCBV ERM meeting would like to thank the many sponsors and volunteers who contributed their resources and time to helping us put this meeting together.

Special thanks go to Michael Stasiak, University of Guelph, who is a webmaster wizard and someone who found solutions before we knew there were problems. To our undergraduate student volunteers from McMaster- thank you for giving up a Saturday in November when we know the end-of-term exams loom large. Good luck on those exams!!! A thank you to Art Dunham for helping us organize the conference fees and making it easier for those attending and those organizing to keep on-track. Finally, a special thanks to Daphne Goring who provided great advice from her experience with the 2015 ERM and who was quick to respond to any questions we had.

A meeting cannot happen in a vacuum. To our sponsors, the CSPB-SCBV Executives, and those attending our meeting, thank you for participating and supporting our efforts. To the Session Chairs, Judges of the Poster and Oral Presentations, and our invited speakers, we greatly appreciate your enthusiasm and participation at the 2016 CSPB-SCBV ERM meeting hosted by McMaster University.

Your Local Organizing Committee,

Robin Cameron, Peter Summers, and Elizabeth Weretilnyk

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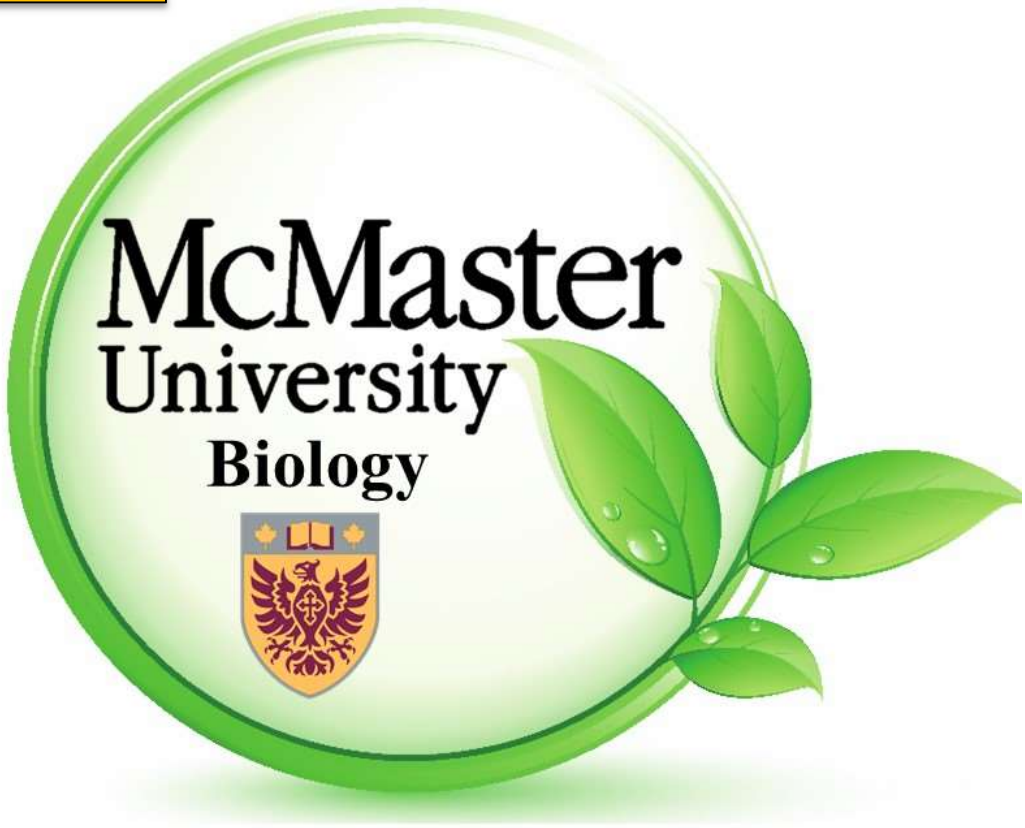

Welcome from Biology

Welcome to the Eastern Regional Meeting for the Canadian Society of Plant Biologists hosted by McMaster University. McMaster is the smallest Canadian University ranked in the top 100 in the world by the Times Educational Supplement- and this is reflected in McMaster's Biology department. We are a relatively small department – and hence our experts across the broad disciplines of Biology interact and collaborate extensively, both in our research and in our teaching. Our collaborations include strong ties with the Royal Botanical Gardens where your conference is being held, those links include research on their managed lands.

Biology provides outstanding in-lab training opportunities for motivated undergraduates, graduate students, and postdoctoral fellows. Our strengths include Plant Biology, Bioinformatics and Functional Genomics, Cell & Developmental Biology, Ecology & Evolution, Environmental Physiology, Genetics & Molecular Biology, and Microbiology.

For those interested in advanced research training at the graduate (M.Sc. or Ph.D.) or postdoctoral level, the Department hosts a large student cohort and very diverse research interests under one roof. Our well-equipped laboratories, together with facilities for more specialized work, such as aquatic animal, molecular biology, cell culture, microscopy, and plant growth facilities, a plant biodiversity collection, computational infrastructure, and a microscopy suite, ensures a state-of-the-art research experience.

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2016 CSPB/SCBV ERM Program

Friday, November 18

6:30 - 8:00 PM **Registration: 30 *Pine St., Hamilton***

6:30 - 9:00 PM **Opening Mixer: 30 *Pine St.*** *Delegates are invited to a mixer where a variety of hors d'oeuvres and refreshments will be served.*

Saturday, November 19 Royal Botanical Gardens, Main Building, Plains Road, Burlington

7:30 - 9:30 AM **Registration, Coffee, Pastries** (Atrium)

7:30 - 8:50 **Poster Setup** (Atrium)

8:50 - 9:00 **Conference Welcome** (Auditorium A)

9:00 - 9:45 Plenary Lecture I (*Auditorium A*). **Maheshi Dassanayake**, Louisana State University. **Structural genomic variation in stress adaptation: insights from extremophyte genomics.**

9:45 - 10:30 Plenary Lecture II (*Auditorium A*). **Jean Greenberg**, University of Chicago. **Flagellin peptide flg22 is transported to distal tissues in Arabidopsis.**

10:30 - 10:45 **Refreshment Break** *(Auditorium B)*

Concurrent Session 1: Physiology and Biochemistry (Auditorium A, Left Side)

Chair: **Ingo Ensminger, University of Toronto-Mississauga**

1.1 10:45 -11:00 **The consumer focused apple breeding program at Vineland.** Beatrice Amyotte*, A. Bowen, T. Banks, I. Rajcan, D. Somers.

1.2 11:00 -11:15 **Investigation of ICE protein-protein interactions involved with freezing tolerance and stomatal development in grapevine.** Alison Edge*, A. Nassuth.

1.3 11:15 -11:30 **Irrigation scheduling algorithms to reduce the environmental impact of Ontario's ornamental nurseries.** Jared A. Stoochnoff*, N. Tran, T. Graham, M.A. Dixon.

1.4 11:30 -11:45 **Nitrogen source and availability alter the effect of low CO_2 on growth, photosynthesis, and N dynamics.** Andre G. Duarte*, F.J. Longstaffe, D.A. Way.

1.5 11:45 -12:00 **Monitoring photosynthetic phenology in evergreen conifers and deciduous trees using spectral reflectance.** Christopher Y.S. Wong, A. Arain, Ingo Ensminger.

1.6 12:00 -12:10 **C_3-C_4 intermediacy in grasses: organelle enrichment and distribution, glycine decarboxylase expression, and the rise of C2 photosynthesis.** Roxana Khoshravesh*, M. Stata, R.F. Sage, T.L. Sage.

1.7 12:10 -12:20 **Intercellular signalling is dysregulated in a loss-of-function mutant of *GLUCAN SYNTHASE-LIKE 8* in Arabidopsis.** Behnaz Saatian*, S. Kohalmi, Y. Cui.

1.8 12:20 -12:30 **Investigating the role of plant shikimate kinase-like 1 in chloroplast development.** Michael Kanaris*, D. Christendat.

Concurrent Session 2: Biotic Interactions (Rooms 1-2, 2nd Floor above Gift Shop)

Chair: Darrel Desveaux, University of Toronto

2.1 10:45 -11:00 **Probing for novel *Pseudomonas syringae* recognition using natural variation in *Arabidopsis thaliana*.** Timothy Lo*, P. W. Wang, D. S. Guttman, D. Desveaux.

2.2 11:00 -11:15 **The diverse roles of Arabidopsis triphosphate tunnel metalloenzymes in senescence and in pathogen defense and their potential for agricultural applications.** Purva Karia[1*], H. Ung, W. Moeder, K. Ebine, A. Poleatewich, M. Pautler, T. Banks, D. Somers, T. Ueda, K. Yoshioka.

2.3 11:15 -11:30 **The *dnd1*-suppressor, *repressor of defense, no death (rdd1)*, suggests a role for *CNGC2* in defense and auxin signaling.** Sonhita Chakraborty*, W. Moeder, K. Chin, A. Fortuna, M.J. Champigny, E. Nambara, K. Yoshioka.

2.4 11:30 -11:45 **Development of a type III effector compendium for the plant pathogen *Pseudomonas syringae*.** Bradley Laflamme*, R.N. De Almeida, M. Dillon, D.S. Guttman, D. Desveaux.

2.5 11:45 -12:00 **Expanded Type III Effector Recognition by the Arabidopsis ZAR1 Resistance Protein Using ZED1-Related Kinases.** Derek Seto*, N. Koulena, T. Lo, D.S. Guttman, D. Desveaux.

2.6 12:00 -12:10 **Assessing the role of CYP71A12 and CYP71A13 in the Arabidopsis Age-Related Resistance response against *Pseudomonas syringae*.** Christine J. Kempthorne*, D.C. Wilson, D. Liscombe, R.K. Cameron.

2.7 12:10 -12:20 **Proteomic characterization of plant immune complexes using proximity-based BioID.** Madiha Khan*, D. Desveaux, R. Subramaniam.

2.8 12:20 -12:30 **Network analysis of the interactome of plant cell-surface receptors identifies functional modules and stabilizing sites for plant defence and development.** Glen A. Mott*, E. Smakowska, M. Layeghifard, Y. Belkhadir, D. Desveaux, D.S. Guttman.

Concurrent Session 3: Metabolism (Café Annex, First Floor)

Chair: Michael Phillips,University of Toronto-Mississauga

3.1 10:45 -11:00 **Phosphorylation of FUSCA3 by SnRK1 regulates seed development and seed vigor in Arabidopsis.** Aaron Chan*, C. Carianopol, A. Tsai, K. Varatharajah, S. Gazzarrini.

3.2 11:00 -11:15 **Biochemical and molecular characterization of AtPAP17, a novel cell wall-localized purple acid phosphatase upregulated by phosphate-starved *Arabidopsis thaliana*.** Mina Ghahremani[1]*, K. Stigter, M. Pyc, R. Mullen, W. Plaxton.

3.3 11:15 -11:30 **Investigating the calcium dependent regulation of plant myosins.** Howard Teresinski*, W. Snedden.

3.4 11:30 -11:45 **Analysis of bacterial-type phosphoenolpyruvate carboxylase expression in vascular plants indicates a widespread anaplerotic role in diverse sink tissues.** Michael K.Y. Ting*, W.C. Plaxton.

3.5 11:45 -12:00 **Carbon fixation, partitioning, and export in tomato source leaf exposed to LED of spectral quality.** Jason Lanoue* [1,2], D. Leonardos, S. Khosla, X. Hao, B. Grodzinski.

3.6 12:00 -12:10 **The study of bifunctional dehydroquinate dehydratases-quinate dehydrogenases in *Solanacea and Brassicacea* species.** Artyom Gritsunov*, J. Peek, D. Christendat.

3.7 12:10 -12:20 **Photosynthesis in the cold: characterization of photosynthetic ferredoxin from the Antarctic alga *Chlamydomonas sp.* UWO241 reveals novel features of cold adaptation.** Marina Cvetkovska[1*], B. Szyszka-Mroz, M. Possmayer, R. Morgan-Kiss, D.R. Smith, N.P.A. Hüner.

3.8 12:20 -12:30 **Kinetic labeling of whole plants to study rates of terpenoid biosynthesis.** Michael A. Phillips*, D. Gonzalez-Cabanelas, A. Chavez, J. Rohwer, L.P. Wright.

12:30 – 1:30 PM	**LUNCH** provided	Atrium
	CSPB Exec. Meeting	RBG Boardroom, above Auditorium
1:30 – 2:30	**Poster Session**	Atrium –Dessert and Coffee available

Concurrent Session 4: Molecular Signalling (Auditorium A, Left Side)

Chair: David Liscombe, Vineland Research and Innovation Centre

4.1	2:30 -2:45	**Evidence for multiple roles of intercellular salicylic acid during the Age-Related Resistance response of *Arabidopsis thaliana* to *Pseudomonas syringae.*** Daniel C. Wilson*, C.J. Kempthorne, P. Carella, R.K. Cameron.
4.2	2:45 -3:00	**The Arabidopsis transcription factor ETHYLENE RESPONSE FACTOR 8 (AtERF8) is involved in pathogen defense and ABA signaling.** Feng Yi Cao*, T. DeFalco, H. Ung, W. Moeder, S. Lumba, D. Desveaux, B. Ellis, K. Yoshioka.
4.3	3:00 -3:15	**Investigating the regulation of drought responsive genes via the activity of histone deacetylase BdHD1 in response to drought stress in *Brachypodium distachyon.*** Gabe Song*, H.A.L. Henry, L. Tian.
4.4	3:15 -3:30	**Versatile tools to study *in planta* calcium signaling dynamics in *Nicotiana*.** Thomas A. DeFalco*, M. Toyota, V. Phan, W. Moeder, S. Gilroy, K. Yoshioka.
4.5	3:30 -3:45	**SnRK1-ABA interactome in *Arabidopsis thaliana*.** Carina S. Carianopol*, S. Lumba, P. McCourt[2], S. Gazzarrini[1,2].
4.6	3:45 -3:55	**An Interactome map of *Fusarium graminearum* mycotoxin related proteins.** Armand Mirmiran*, C. Tsai, C. Mogg, G. Subramaniam, D. Desveaux.
4.7	3:55 -4:05	**Introgression of chemical-mediated pest resistance in greenhouse tomato.** David Liscombe*, R. Buitenhuis, M. Goertz, D. Pearson, V. Primomo, A. Summerfield, R. Zielinski-Lawrence.
4.8	4:05 -4:15	**Mapping and marker development for black spot disease (*Diplocarpon rosae* W.) resistance in tetraploid landscape roses (*Rosa* spp.).** C. Rouet*, D. Somers, E. Lee, T. Banks, A. Poleatewich.
4.9	4:15 -4:25	**How *Striga* wakes up: Elucidating its germination code.** S. Toh, Shelley Lumba*.

Concurrent Session 5: Metabolism and Genetics (Rooms 1-2, 2nd Floor above Gift Shop)

Chair: Olivia Wilkins, McGill University

5.1	2:30 -2:45	**The mystery of ADT5 nuclear localization.** Sara A. Rad∗, S. Kohalmi. Dept. of Biology,
5.2	2:45 -3:00	**Characterization of the anatomy, development, and transcriptome of C_3 and C_4 *Atriplex* species.** Stefanie Sultmanis*[1], M. Stata, U. Gowik, A. Bräutigam, A. Weber, P. Westhoff, R.F. Sage, T.L. Sage.
5.3	3:00 -3:15	**ADT activity? Let me check: A novel assay to detect arogenate deydratase activity *in vivo.*** Emily J. Clayton*, S.E. Kohalmi.
5.4	3:15 -3:30	**Diverse C_3-C_4 intermediates make Blepharis (Acanthaceae) a powerful new system for C_4 evolutionary research.** Matt Stata*, R.F. Sage.
5.5	3:30 -3:45	**Promoter sequence diversity and its roles in differential expression of six *Arabidopsis AROGENATE DEHYDRATASE* genes.** Emily J. Cornelius*, S.E. Kohalmi.
5.6	3:45 -3:55	**Identification of C_4 genetic determinants – forward genetic approaches.** Kumari Billakurthi*, F. Döring, U. Gowik, S. Das Gupta, S. Sultmanis, R. Khoshravesh, T. Sage, P. Westhoff.
5.7	3:55 -4:05	**An improved method for long non-coding RNA prediction that includes small ORF coding probabilities.** Caitlin Simopoulos*, G.B. Golding, E.A. Weretilnyk.
5.8	4:05 -4:15	**A method for comparing allele specific expression differences in *Zea mays* hybrids.** Shuhua Zhan*, K. Feys, H. Nelissen, D. Inze, L. Lukens.
5.9	4:15 -4:25	**How convergent is convergent evolution? Case studies from the evolution of C_4 photosynthesis.** Rowan F. Sage*, M. Stata, T.L. Sage.

Concurrent Session 6: Cell to Whole Plant Responses (Café Annex, First Floor)

Chair: Danielle Way, Western University

6.1	2:30 -2:45	**Investigating the role of XERICO in GA-ABA crosstalk during stress response**. Eliana Vonapartis*, D. Mohamed, C. Carianopol, S. Gazzarrini.
6.2	2:45 -3:00	**Identification and molecular characterization of *Brachypodium distachyon* NRT2 family, with a putative essential role of BdNRT2.1 on nitrogen use efficiency.** Jiang Wang*, N.P.A. Hüner, L. Tian.
6.3	3:00 -3:15	**Acclimation capacity of photosynthesis and respiration to temperature in dominant mature boreal conifer tree species.** M. Eric Dusenge*, J.R. Stinziano, J.M. Warren, E. Ward, S.D. Wullschleger, D.A. Way.
6.4	3:15 -3:30	**The effect of allantoin on Arabidopsis seedlings tolerance in response to salt stress.** Solmaz Irani*, C.D. Todd.
6.5	3:30 -3:45	**Identification and characterization of a novel lipid droplet protein in *Arabidopsis.*** Michal Pyc*, O. Yurchenko, Y. Cai, S.K. Gidda, K.D. Chapman, J.M. Dyer, R.T. Mullen.
6.6	3:45 -3:55	**Temperature stress and reproduction in the emerging biofuel crop *Camelina sativa.*** Vanessa Lundsgaard-Nielsen*, C. So, T.L Sage.
6.7	3:55 -4:05	**Carbon fluxes acclimate more strongly to elevated growth temperatures than to elevated CO_2 in a northern conifer.** Y. Kroner, Danielle Way*.

4:25 - 4:45 PM	Break, cold beverages *Auditorium B*
4:45 – 5:30	Plenary Lecture III. (*Auditorium* A) **Daphne Goring**, University of Toronto. **Pollen acceptance or rejection? Intersecting signaling pathways in the Brassicaceae stigma**
5:30	Awards and Closing

ABSTRACTS

Plenary Lectures.

Structural genomic variation in stress adaptation: insights from extremophyte genomics.

Maheshi Dassanayake, Dept. of Biological Sciences, Louisiana State University.
9:00 AM, Auditorium A.

Extremophyte genomes present an emerging genetic resource to understand genome reorganization leading to environmental stress adaptation. Despite high genomewide macrosynteny between closely related stress-sensitive and stress-adapted genomes, genome structural variations, including gene duplications and translocations lead to transcriptome profiles that exemplify gene expression enriched for stress adapted biological processes in extremophytes.

Flagellin peptide flg22 is transported to distal tissues in Arabidopsis.

Joanna Jelenska[1], Sandra M. Davern[2], Robert F. Standaert[2,3,4], Saed Mirzadeh[5], **Jean T. Greenberg**[1]. [1] Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637, USA; [2] Biosciences, [3] Biology & Soft Matter and [5] Nuclear Security & Isotope Technology Divisions, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA; [4] Department of Biochemistry and Cellular & Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA. *9:45 AM Auditorium A.*

Plants often deploy secreted peptide ligands to control defense and developmental responses *via* receptor kinases. They also respond to pathogen-derived peptides to elaborate both local and systemic defense responses. A key gap in knowledge is the fate of such ligands: whether and by what mechanism they might enter cells and their potential to be mobile in plants. We used biologically active fluorophore and radiolabeled peptides to establish that flg22 is trafficked to distal organs with the closest vascular connections. Remarkably, entry into the plant cell *via* endocytosis together with the FLS2 receptor is needed for delivery to vascular tissue and long-distance transport of flg22. This contrasts with known routes of long-distance transport of other non-cell permeable molecules in plants, which require membrane-localized transporters for entry to vascular tissue. Thus, a plasma membrane receptor acts as a transporter to enable access of its ligand to distal trafficking routes.

Pollen acceptance or rejection? Intersecting signaling pathways in the Brassicaceae stigma.

Daphne R. Goring. Dept. of Cell and Systems Biology, University of Toronto.
4:45 PM Auditorium A.

In the Brassicaceae, a tight control of pollen-pistil interactions is rapidly initiated following contact between a pollen grain and a stigmatic papilla at the top of the pistil. This is the first of several barriers in the pistil regulating successful fertilization and seed production. My research program investigates intracellular signaling pathways that regulate the recognition and rejection of self-pollen to prevent inbreeding (self-incompatibility pathway), and the recognition and allocation of resources to compatible pollen to promote germination (basal compatible pollen response pathway). While key signaling proteins regulating self-incompatibility are well-characterized, relatively little is known about the factors regulating stigmatic papillar cellular responses to compatible pollen. We have discovered that the exocyst complex is required in *Brassica* and *Arabidopsis* stigmas for vesicle secretion to the compatible pollen grain, and we are actively searching for signaling proteins that regulate this process. As well, autophagy is activated in the self-incompatibility pathway, and we are currently exploring how this is connected to the self-pollen rejection response. Finally, our studies suggest that two competing cellular responses can be in play in the stigmatic papilla following pollination: the basal compatible pollen response pathway and the self-incompatibility signaling pathway. We are currently examining the interplay between these two pathways to determine how self-pollen rejection overrides the basal compatible pollen response.

Session 1. Physiology and Biochemistry.
Auditorium A

1.1 The consumer focused apple breeding program at Vineland. Beatrice Amyotte*[1,2], A. Bowen[1], T. Banks[1], I. Rajcan[2], D. Somers[1]. [1]Applied Genomics, Vineland Research and Innovation Centre and [2] Plant Agriculture, University of Guelph.

Apples are one of the most economically important temperate fruit crops in the world, and represent a $200 million industry in Canada. Canadian growers and industry stakeholders have expressed a need for new high quality apple cultivars in order to remain competitive on the global market. To address this need, the Vineland Research and Innovation Centre began an apple breeding program in 2011. This presentation will focus on the research components of breeding apples at Vineland. Briefly, our breeding program aims to develop regionally adapted cultivars with outstanding consumer appeal. We have therefore adopted an integrated consumer insights and applied genomics research strategy. Recently we conducted a genome wide association study of apple taste, texture and flavour to understand the genetic basis of fruit quality in apple. A diverse collection of apple cultivars was examined through descriptive sensory evaluation by a trained sensory panel and subjected to genotyping by sequencing for marker discovery. We identified genomic regions associated with several sensory traits including juiciness and crispness. We conducted a consumer preference study which indicated that these traits were critical components of consumer liking. We are currently evaluating the potential of these genomic associations to be used in DNA marker assisted selection. The results of this study will be integrated into our existing marker assisted breeding efforts which include selecting for improved firmness over time. Over the next few years, we will conduct advanced screening of 20,000 apple seedlings in order to identify superior potential cultivars for the Canadian apple industry.

1.2 Investigation of ICE protein-protein interactions involved with freezing tolerance and stomatal development in grapevine. Alison Edge*, A. Nassuth. Dept. of Molecular and Cellular Biology, University of Guelph.

Although Ontario's grape and wine industry generates $3.3 billion annually, the high-quality wine grape *Vitis vinifera* is frost-sensitive, and damage from Canadian winters can reduce crop yield as much as 54%. Leaves of *Vitis riparia*, which is more frost-tolerant than *V. vinifera*, have more stomata and the number of stomata also increases in both species when exposed to low temperature. ICE transcription factors are basic helix-loop-helix (bHLH) proteins and central regulators of both freezing tolerance and stomatal development in *Arabidopsis*. Freezing tolerance is imparted through the activation of ICE via post-translational modifications, which in turn induces *CBF* gene expression, whereas stomatal differentiation is regulated via dimerizations of ICE with SPCH, MUTE, and FAMA. While dimerization of bHLH proteins is a vital factor in determining DNA-binding and protein activity, it is not known whether freezing tolerance requires ICE to form dimers, and if so, whether the stomatal proteins SPCH, MUTE, and FAMA may be involved. This project aims to investigate protein-protein interactions between each of the four *Vitis* ICEs and SPCH, MUTE, and FAMA in order to determine which proteins can form dimers. Preliminary results obtained using bimolecular fluorescence complementation suggest that SPCH, MUTE, and FAMA can form both homodimers and heterodimers with ICE1-3, with no dimerizations observed with ICE4. To verify the results, pull-down assays utilizing epitope-tagged proteins will be used as a second approach. Assays will be extended to include proteins with mutations in amino acid residues within the bHLH domain that are predicted to be involved in dimerization.

1.3 Irrigation Scheduling Algorithms to Reduce the Environmental Impact of Ontario's Ornamental Nurseries. Jared A. Stoochnoff*, N. Tran, T. Graham, M.A. Dixon, School of Environmental Science, University of Guelph.

In typical ornamental nursery operations, irrigation schedules are predominantly determined by the nursery manager's subjective assessment of crops water requirements. In lieu of quantitative assessment strategies, managers tend to err on the side of caution and water to excess. This results in poor water use efficiency and significant fertilizer leaching that negatively impacts local watersheds. Using innovative water potential sensors that *directly* measure plant water stress/status in near real time, we are characterizing the relationships between crop water stress levels, prevailing environmental (weather) conditions, and species-specific water stress tolerance thresholds. Irrigation scheduling algorithms that predict plant water status thresholds based on cumulative daily environmental conditions (i.e., cumulative Vapour Pressure Deficit – cVPD) were tested on Chanticleer Pear *(Pyrus chanticleer)* trees grown in a pot-in-pot production system equipped with drip irrigation. Three water restriction treatments were applied: 1) Control (nursery irrigation schedule), 2) Moderate restriction/stress (irrigation triggered at 24 kPa•hrs cVPD), and 3) High restriction/stress (irrigation triggered at 36 kPa•hrs cVPD). The moderate and high stress treatments resulted in a 46% and 63% water savings relative to the control. Trees grown under the moderate treatment showed no significant difference in growth compared to the control trees. Trees grown under high water stress did experience reduced growth, as determined by caliper diameter differentials, but otherwise appeared healthy. Irrigation scheduling algorithms that predict the crops physiological water requirements will dramatically reduce water consumption and environmental impact of nursery operations without compromising plant productivity or nursery profitability.

1.4 Nitrogen source and availability alter the effect of low CO_2 on growth, photosynthesis, and N dynamics. Andre G. Duarte*, F.J. Longstaffe, D.A. Way. Dept. of Biology, Western University.

Low atmospheric CO_2 concentrations dominated the recent evolutionary history of plants. However, our knowledge of plant performance in low CO_2 environments is poor, and that of how low CO_2 interacts with nitrogen source and availability is even poorer. Here, we investigated growth, photosynthetic traits and nitrogen isotopic signature in *Elymus canadensis*, grown from seed at either current CO_2 concentrations (400 ppm) or values representative of the last glacial maximum (180-190 ppm), in combination with different concentrations of N (full or half-strength) and various forms of inorganic nitrogen (nitrate, ammonium, and a mix of both). Low CO_2 reduced total biomass by almost half, except when nitrogen was limited, where growth was unaffected by CO_2. N limitation also diminished the effect of low CO_2 on photosynthesis. Plants grown with nitrate or ammonium up-regulated photosynthesis when grown at low CO_2, presenting photosynthetic rates that were higher than in plants grown at ambient CO_2. Growth at low CO_2 increased nitrogen content and shifted the N isotopic signature in leaves and roots. Compared to plants from

ambient CO_2, low CO_2 plants were more enriched in ^{15}N (1.18‰) under nitrate fertilization, but 0.79‰ depleted if nitrogen availability was limited, while root $\delta^{15}N$ decreased 0.77‰ under ammonium fertilization in low CO_2 plants. Our work demonstrates that the effects of low CO_2 on plants varies depending on nitrogen source and availability, responses that should be considered when inferring data about plant responses to past climates.

1.5 Monitoring photosynthetic phenology in evergreen conifers and deciduous trees using spectral reflectance. Christopher Y.S. Wong[1,2], A. Arain[3], I. Ensminger[1,2,4]. [1]Dept. of Biology, University of Toronto Mississauga. [2]Graduate Program in Ecology and Evolutionary Biology, University of Toronto, [3]School of Geography & Earth Sciences, McMaster University and [4]Graduate Program in Cells and Systems Biology, University of Toronto.

Evergreen conifers in boreal and temperate regions undergo strong seasonal changes in photoperiod and temperatures, which determines their phenology of high photosynthetic activity in the growing season and downregulation during the winter. Monitoring the timing of the transition between summer activity and winter downregulation in evergreens is difficult since this is a largely invisible process, unlike in deciduous trees that have a visible budding and a sequence of leaf unfolding in the spring and leaf abscission in the fall. The light-use efficiency (LUE) model estimates gross primary productivity (GPP) and may be parameterized using remotely sensed vegetation indices. Using spectral reflectance data, we derived the normalized difference vegetation index (NDVI), a measure of leaf "greenness", and the photochemical reflectance index (PRI), a proxy for chlorophyll:carotenoid ratios which is related to photosynthetic activity. To better understand the relationship between these vegetation indices and photosynthetic activity and to contrast this relationship between plant functional types, the phenology of NDVI, PRI and photosynthesis was monitored in an evergreen forest and a mixed deciduous forest at the leaf and canopy scale. Our data indicates that the LUE model can be parameterized by NDVI and PRI to track forest phenology. Differences in the sensitivity of PRI and NDVI will be discussed. These findings have implications to address the phenology of evergreen conifers by using PRI to complement NDVI in the LUE model, potentially improving model productivity estimates in northern hemisphere forests, that are dominated by conifers.

1.6 C_3-C_4 intermediacy in grasses: organelle enrichment and distribution, glycine decarboxylase expression, and the rise of C2 photosynthesis. Roxana Khoshravesh*, M. Stata, R.F. Sage, T.L. Sage. Dept. of Ecology & Evolutionary Biology, University of Toronto.

Photorespiratory glycine shuttling and decarboxylation in bundle sheath (BS) cells of C_2 eudicots has been demonstrated to be the evolutionary bridge to C_4 photosynthesis. To evaluate this in grasses, we compare anatomy, cellular localization of glycine decarboxylase (GDC), and photosynthetic physiology of a suspected C_2 grass, *Homolepis aturensis*, with these traits in the C_2 grass, *Steinchisma hians* and C_3 *S. laxum* that is sister to *S. hians*. We also use publicly available genome/RNA-seq data to examine the evolution of GDC subunits and enhance our understanding of the evolution of BS-specific GDC expression in C_2 and C_4 grasses. We confirm *H. aturensis* as a C_2 species; GDC is confined to the organelle-enriched BS cells in *H. aturensis*. Phylogenetic analyses and immunodetection of the P-subunit of GDC is consistent with the hypothesis that BS dominant levels of GDC in C_2 and C_4 grass species is due to changes in expression of a single GLDP gene in M and BS cells. This is in contrast to molecular mechanisms confining GDC to the BS in the eudicot *Flaveria* that results from the pseudogenization of one of two GLDP genes. All BS mitochondria and peroxisomes and most chloroplasts in *H. aturensis* and *S. hians* are situated centripetally in a pattern identical to eudicots. In *S. laxum*, which has C_3-like gas exchange patterns, mitochondria and peroxisomes are positioned centripetally as they are in *S. hians*. This cellular phenotype, also present in eudicots, is posited to initiate a facilitation cascade leading to C_2 and C_4 photosynthesis.

1.7 Intercellular signalling is dysregulated in a loss-of-function mutant of *GLUCAN SYNTHASE-LIKE 8* in Arabidopsis. Behnaz Saatian[1,2]*, S. Kohalmi[1], Y. Cui[1,2]. [1]Dept. of Biology, Western University and [2] London Research and Development Centre, Agriculture and Agri-Food Canada, London.

Plant cell walls have unique chemical composition and features which enable them to play essential roles during plant development as shaping the cells and providing intercellular communication between adjacent cells. Polysaccharides, including callose, and glycoproteins are known as the main constituents of the cell wall. Although callose exists at a very low level in the cell wall, it plays critical roles. Callose is accumulated at the cell plate, at plasmodesmata and in male and female gametophytes. *CALLOSE SYNTHASE* (*CALS*), also known as *GLUCAN SYNTHASE-LIKE* (*GSL*) genes in Arabidopsis comprise a family of 12 members. A new allele of *GSL8*, *essp8*, was identified as having seedling-lethal phenotype. *essp8* seedlings exhibit pleiotropic phenotypic defects, including disruption of root tissue patterning, dwarfism and ectopic proliferation. Histochemical assays showed reduction of callose deposition at plasmodesmata and an increase in the size exclusion limit in *essp8* seedlings. Further investigation showed that the increase in size exclusion limit leads to dysregulation of symplastic trafficking in the primary root of *essp8* seedlings. Attempts to identify the components of a hypothetical callose synthase complex revealed the interaction of GSL8 with two plasmodesmata-associated proteins and SUCROSE SYNTHASE 1 suggesting that they all might be parts of a single complex allowing a concerted regulation of callose deposition at plasmodesmata. Our findings suggest that GSL8 is required for plasmodesmata regulation during early seedling development in Arabidopsis.

1.8 Investigating the Role of Plant Shikimate Kinase-like 1 in Chloroplast Development. Michael Kanaris*, D. Christendat. Dept. of Cell and Systems Biology, University of Toronto.

Plastid biogenesis in plants is a complex process involving the differentiation of precursor proplastids that develop into one of several types, including that of the photosynthetic chloroplasts. Chloroplast biology is a highly researched topic however there remains many uncharacterized players involved in chloroplast biogenesis with functions that are not well understood. Shikimate kinase-like 1 (SKL1), an ancient gene homolog of shikimate kinase, has been implicated in chloroplast biogenesis; the *Arabidopsis thaliana SKL1* T-DNA insertional mutants (*skl1-8)* lack properly developed chloroplasts and present an albino phenotype**.** Co-expression analysis of *A. thaliana SKL1* with phylogenetically related plants including *Glycine max*, *Zea mays*, and *Oryza sativa* has led to the identification of a set of highly correlated genes that are specifically involved in chloroplast biogenesis, including that of thylakoid membrane formation and

photosystem I or II (PSI/PSII) establishment. Generation of transgenic *skl1-8* mutants containing a dexamethasone (DEX) inducible expression of SKL1 have shown partial functional complementation. DEX controlled expression of SKL1 in *skl1-8* background *A. thaliana* produces green cotyledons however this effect does not propagate to the true leaves which remains white or partially variegated. Lipid analysis of these tissue types has shown that the white or variegated true leaves contain predominantly lyso-forms of the major lipid types whereas the green cotyledon lipid profiles are comparable to that of wildtype, containing intact lipids. Together, these results reinforce previous notions about SKL1's role in development of chloroplasts and suggest its involvement in lipid synthesis however still remains to be investigated.

Session 2. Physiology and Biochemistry.
Rooms 1-2

2.1 Probing for novel *Pseudomonas syringae* recognition using natural variation in *Arabidopsis thaliana*. Timothy Lo*[1], P. W. Wang[1,2], D. S. Guttman[1,2], D. Desveaux[1,2]. [1]Dept. of Cell and Systems Biology, University of Toronto and [2]Centre for the Analysis of Genome Evolution and Function (CAGEF), University of Toronto.

The recognition of pathogens is vital to the survival of all living organisms including plants. *Pseudomonas syringae* is a Gram-negative bacterial pathogen that can infect a wide range of crop plants including tomatoes, soybean and the model plant organism *Arabidopsis thaliana*. Utilizing a needle-like secretion system, *P. syringae* injects virulence proteins termed effectors directly into plant host cells to suppress immunity. Fortunately, plants have evolved resistance (R) proteins that can monitor for the presence of specific effectors to trigger a rapid and robust immune response termed effector-triggered immunity (ETI). Though hundreds of effector proteins and R proteins have been identified in *P. syringae* and *A. thaliana* respectively, there are but a handful of effectors that have had their cognate R protein elucidated. Thus novel effector-R protein pairings were sought in a forward screen using the well-characterized tomato *P. syringae* pathovar DC3000 (*Pto*DC3000) and a collection of *Arabidopsis* ecotypes. Ecotypes showing resistance to *Pto*DC3000 were identified and one ecotype was selected for further characterization. This project aims to identify the *Pto*DC3000 effector conferring *Arabidopsis* resistance and use Next-Generation Mapping methods to identify the corresponding *Arabidopsis* R protein. Uncovering novel effector-R protein combinations will allow further insight into plant immunity and the goal of generating more resistant crop plants.

2.2 The diverse roles of Arabidopsis triphosphate tunnel metalloenzymes in senescence and in pathogen defense and their potential for agricultural applications. Purva Karia[1*], H. Ung[1], W. Moeder[1], K. Ebine[2], A. Poleatewich[3], M. Pautler[3], T. Banks[3], D. Somers[3], T. Ueda[2], K. Yoshioka[1]. [1]Dept. of Cell &Systems Biology, University of Toronto. [2]Dept. of Biological Sciences, Graduate School of Sciences, The University of Tokyo. [3]Vineland Research and Innovation Centre.

Triphosphate tunnel metalloenzymes (TTMs) comprise a superfamily of enzymes that hydrolyze organophosphate substrates. Members of this superfamily are found across taxa, yet the biological function of most family members is unclear. Three *TTM* genes (*AtTTMs*) are present in the *Arabidopsis thaliana* genome. Both AtTTM1 and AtTTM2 localize at the mitochondrial outer membrane. Interestingly, AtTTM1 and 2 hydrolyze pyrophosphate *in vitro*, making them the only TTMs characterized so far to possess pyrophosphatase activity. However, despite their high sequence identity, knockout mutant analysis revealed different biological functions for both genes. *AtTTM1* is involved in senescence whereas *AtTTM2* plays a role in pathogen resistance. Knockout mutants of *AtTTM1* exhibit delayed developmental, dark- and ABA-induced senescence, indicating that *AtTTM1* acts as a positive regulator of senescence. The *ttm1 ttm2* double mutants displayed the same degree of delayed senescence and enhanced pathogen resistance as their respective single mutants, further confirming their roles in distinct biological processes. However, promoter swap analyses revealed that they both can functionally complement each other, indicating that the observed functional differences are governed by their transcriptional regulation. Overall, these data suggest a novel connection in regulating programmed cell death (PCD) during senescence and pathogen defense through their mitochondrial membrane-localized phosphatase activity. Similar transcriptional regulation of *TTM2* paralogs in various crop plants indicates a conserved role of *TTM2* in pathogen resistance, which suggests potential utilization for agricultural application. Thus, EMS-mutagenized populations of petunia, tomato, pepper, and soybean are being utilized to generate and characterize *TTM2* mutants with increased pathogen resistance.

2.3 The *dnd1*-suppressor, *repressor of defense, no death (rdd1)*, suggests a role for *CNGC2* in defense and auxin signaling. Sonhita Chakraborty[1*], W. Moeder[1], K. Chin[1], A. Fortuna[1], M.J. Champigny[2], E. Nambara[1], and K. Yoshioka[1]. [1]Dept. of Cell and Systems Biology, University of Toronto and [2]Dept. of Molecular and Cellular Biology, University of Guelph.

Cyclic Nucleotide-Gated Channels (CNGCs) are non-selective cation channels that are involved in plant immunity signalling. The Arabidopsis *CNGC2* null mutant, *defense, no death1* (*dnd1*), exhibits autoimmune phenotypes and has been studied intensively for its role in pathogen defense. However, so far no signaling component has been identified in the *CNGC2*-mediated signaling cascade. To better understand *CNGC2*-mediated signal transduction, the first *dnd1* suppressor mutant, *repressor of defense, no death1* (*rdd1*), which suppresses most *dnd1*-associated phenotypes, was identified. Conventional map based cloning methods revealed that *RDD1* is located at an 800kb genetic interval in the upper arm of chromosome 5 and through whole genome sequencing, four potential causative point mutations had been identified. One of these mutations occurs in the open reading frame of a gene involved in auxin biosynthesis. Current genetic data strongly support the notion that *RDD1* is in fact this auxin biosynthesis gene. Auxin is a plant hormone that is involved in a wide range of plant physiology such as development, flowering and gravitropism. Our current data suggests that *dnd1* seedlings exhibit statistically lower levels of endogenous auxin in its roots than wildtype Columbia. The expression pattern of the DR5:GUS reporter construct suggest reduced auxin sensitivity in *dnd1*. Furthermore, *dnd1* exhibits an impaired gravitropic response and this response is partially rescued in *rdd1 dnd1*. Taken together, these data indicate that *CNGC2* is involved in auxin signaling. This suggests that *CNGC2* may mediate auxin and defense signaling.

2.4 Development of a type III effector compendium for the plant pathogen *Pseudomonas syringae*. Bradley Laflamme[1]*, R.N. De Almeida[1], M. Dillon[1], D.S. Guttman[1,2], D. Desveaux.[1,2] [1]Dept. of Cell & Systems Biology, University of Toronto and [2]Centre for the Analysis of Genome Evolution and Function, University of Toronto.

Pseudomonas syringae infects many plant species of major economic importance. The virulence of this pathogen requires its Type III secretion system, which is used to inject "effector" virulence proteins into host cells. Type III effectors make up roughly 60 protein families in *P. syringae* and are associated with a broad range of effects that diminish plant fitness. Though classical studies of *P. syringae* effectors usually focused on examining single alleles from these families, next-generation sequencing and deeper phylogenetic analyses have revealed that there are often hundreds of structurally and functionally distinct alleles within each *P. syringae* effector family. We believe that taking a family-centered approach to studying *P. syringae* effectors – one which aims to screen the diversity within each family – will allow us to better understand the evolutionary and functional diversity that governs both phytopathogen virulence and plant immunity. Towards this goal, we have optimized a high-throughput infection assay to screen effector functions and developed an algorithm, PIDIQ (Plant Immunity and Disease Image-based Quantification), which assesses the promotion or suppression of disease symptoms by individual effectors. Our goal is to use these technologies to functionally survey the phylogenetic diversity of the *P. syringae* type III effector repertoire in the model plant *Arabidopsis thaliana*. Results of our initial screens will be presented. This project will improve our general understanding of plant pathogenesis, in particular bringing attention to how phylogenetic diversity within effector families contributes to the arms race between virulence mechanisms and plant immunity.

2.5 Expanded Type III Effector Recognition by the Arabidopsis ZAR1 Resistance Protein Using ZED1-Related Kinases. Derek Seto[1*], N. Koulena[1], T. Lo[1], D.S. Guttman[1,2], D. Desveaux[1,2]. Dept. of Cell & Systems Biology, University of Toronto and [2]Centre for the Analysis of Genome Evolution & Function, University of Toronto.

Pseudomonas syringae is a Gram-negative bacterial pathogen capable of infecting a wide range of plant species, including important crop species such as wheat, bean, and tomato. *P. syringae* possesses a needle-like type III secretion system, which is required for injecting effector proteins into plant cells. These effectors can disrupt the signaling components of the plant immune system, thereby promoting pathogen virulence. However, plants have evolved nucleotide-binding leucine rich repeat (NLR) proteins to recognize effectors and activate effector-triggered immunity (ETI) to counter pathogenesis. Effector recognition by NLR proteins can occur through direct interaction or indirectly, by monitoring for effector-induced modifications on specific host proteins. For example the NLR protein ZAR1 indirectly recognizes the effectors HopZ1a and AvrAC by monitoring the host kinases that they target; ZED1 and PBL2, respectively. To avoid recognition by the plant immune system, *P. syringae* has diversified its collection of effectors to the point where ~60 families have been identified, each containing many allelic variants. The type III secreted effector HopF2 from *P. syringae* pv. *Pto*DC3000 has previously been demonstrated to target kinases and promote *P. syringae* virulence in Arabidopsis. We present a novel HopF allele that elicits an ETI response in *Arabidopsis*. We show that this ETI response requires the ZAR1 NLR gene as well as a ZED1-related kinase (ZRK). These results emphasize that the ZAR1 NLR protein has evolved to monitor multiple host kinases for effector-induced perturbations thereby broadening its recognition specificity and providing an effective guardian of the plant kinome.

2.6 Assessing the role of CYP71A12 and CYP71A13 in the Arabidopsis Age-Related Resistance response against *Pseudomonas syringae*. Christine J. Kempthorne*[1], D.C. Wilson[1], D. Liscombe[2], R.K. Cameron[1]. [1]Dept. of Biology, McMaster University and [2]Vineland Research and Innovation Centre.
Age-Related Resistance (ARR) is a defense response whereby mature plants become resistant to pathogens that they were susceptible to at an earlier age. ARR does not require previous pathogen exposure and has been observed in economically important crop plants. Although the mechanisms by which this occurs are currently unclear, previous work suggests mature Arabidopsis secretes antimicrobial compounds into the intercellular space where pathogenic *Pseudomonas syringae* bacteria reproduce. We present data demonstrating that CYP71A12 and CYP71A13 contribute to ARR in the *Arabidopsis thaliana-Pseudomonas syringae* pathosystem. Recent evidence suggests that these two cytochrome P450s are at a metabolic branch point in the biosynthesis of several indolic compounds. Current work is focused on characterizing the ARR phenotype of genetic biosynthesis mutants downstream of CYP71A12/A13, analyzing accumulation of indolic compounds in the intercellular and intracellular spaces using UPLC-MS/MS, and examining antimicrobial activity of relevant indolic compounds *in vitro*. Understanding Age-Related Resistance will contribute to efforts to improve disease resistance and enhance crop yields.

2.7 Proteomic characterization of plant immune complexes using proximity-based BioID. Madiha Khan*[1,2], D. Desveaux[1,3], R. Subramaniam[1, 2]. [1]Dept. of Cell and Systems Biology, University of Toronto, [2]Agriculture and Agri-Food Canada, Ottawa, and [3]Centre for the Analysis of Genome Function and Evolution, University of Toronto.

Pathogens employ effectors to target host immunity and promote infections. Plants in turn have evolved nucleotide binding leucine-rich repeat (NLR) proteins to detect effectors and initiate effector-triggered immunity (ETI). NLRs monitor effector "sensors" that can represent a domain of the NLR protein resulting in direct effector recognition, or alternatively sensors can represent autonomous proteins that provide indirect recognition. The *Pseudomonas syringae* type III effector HopZ1a is an acetyltransferase that targets the Arabidopsis effector sensor ZED1. Acetylation of ZED1 by HopZ1a activates the NLR protein ZAR1, resulting in ETI. Although we know that ZED1 and ZAR1 form a HopZ1a recognition complex, we know very little about additional proteins in this complex or their downstream signaling components. We aim to advance this knowledge by applying proximity dependent biotin identification (BioID) to characterize the ZAR1 immune complex. We have produced functional transgenic Arabidopsis lines of the effector HopZ1a, the sensor ZED1 and the NLR ZAR1, all fused to the promiscuous biotinylating protein (BirA*). We have expressed these proteins and demonstrated promiscuous biotinylation of proteins *in planta*. We will present our latest efforts to optimize *in planta* BioID and identify biotinylated proteins by mass spectrometry. This approach will allow us to build a proximity-based ZAR1 network to identify additional components of this immune complex that contribute to ETI.

2.8 Network analysis of the interactome of plant cell-surface receptors identifies functional modules and stabilizing sites for plant defence and development. Glen A. Mott[1]*, E. Smakowska[2], M. Layeghifard[1], Y. Belkhadir[2], D. Desveaux[1,3], D.S. Guttman[1,3]. [1]Dept. of Cell & Systems Biology, University of Toronto. [2]Gregor Mendel Institute, Austrian Academy of Sciences, Vienna Biocenter, [3]Centre for the Analysis of Genome Evolution & Function, University of Toronto.

Plants use a large family of cell-surface receptors to detect environmental signals and use this information to guide decisions involving immunity and growth. The leucine-rich repeat, receptor-like kinases (LRR-RLKs) are a family of over 220 cell surface proteins comprised of an extracellular domain to bind to small molecule ligands, a single trans-membrane domain, and an intracellular kinase domain to begin a signaling cascade within the cell. Detection of an extracellular signal by these LRR-RLKs can trigger homo- and hetero-dimerization of receptors, ultimately resulting in the formation of signaling competent structures able to integrate complex signals to guide plant defence and growth. In order to study the formation of these structures and how they control these processes we have conducted a high-throughput interaction screen to determine the interactions between 200 LRR-RLKs. Using network analysis and community detection we have detected six distinct, but interconnected, subnetworks that show evidence of specialization of biological activity. We also show that the network is an excellent tool for identifying functionally relevant interactions to assign biological functions to previously unidentified regulators of LRR-RLKs known to function in plant growth and immune response.

Session 3. Metabolism. *Café Annex.*

3.1 Phosphorylation of FUSCA3 by SnRK1 Regulates Seed Development and Seed Vigor in Arabidopsis. Aaron Chan*, C. Carianopol, A. Tsai, K. Varatharajah, S. Gazzarrini. Dept. of Biological Sciences, University of Toronto-Scarborough and Dept. of Cell and Systems Biology, University of Toronto.

The transcription factor *FUSCA3* (*FUS3*) acts as a major regulator of seed maturation in Arabidopsis. FUS3 is phosphorylated by the SnRK1 catalytic subunit AKIN10, which belongs to a conserved eukaryotic kinase complex involved in energy **homeostasis**. Here we show that AKIN10 and FUS3 share overlapping expression patterns during embryogenesis, and that FUS3 is phosphorylated by AKIN10 in cell extracts from early embryos. To understand the role of FUS3 phosphorylation by SnRK1, we generated FUS3 phosphorylation null (FUS3 S>A) and mimic (FUS3 S>D) variants. Both variants were able to rescue most of the fus3-3 seed maturation defects, suggesting that phosphorylation is not required for late embryogenesis **under optimal growth conditions**. However, phospho-null variants showed increased frequency of **polycotyledon** embryos and caused a novel delayed embryo development phenotype. Furthermore, phospho-null variants had reduced seed yield, which correlated with increased seed abortion. Interestingly, reciprocal crosses (between the *FUS3* complementation and phospho-null lines) suggest that seed abortion is due to maternal FUS3 S>A and appeared to be caused by unfertilized ovules. Most phenotypes were exaggerated when plants were grown at elevated temperature. Interestingly, both *akin10* and *akin11* mutants displayed a frequency of seed abortion and polycotyledons similar to *fus3-3*. Taken together, these results suggest that FUS3 phosphorylation by SnRK1 regulates embryo growth rate and seed development under optimal and adverse environmental conditions. These findings uncover novel roles for two major regulators of development and metabolism in plants.

3.2 Biochemical and molecular characterization of AtPAP17, a novel cell wall-localized purple acid phosphatase upregulated by phosphate-starved *Arabidopsis thaliana*. Mina Ghahremani[1]*, K. Stigter[1], M. Pyc[2], R. Mullen[2], W. Plaxton[1]. [1]Dept. of Biology, Queen's University and [2]Dept. of Molecular and Cellular Biology, University of Guelph.

Purple acid phosphatases (**PAP**s) function in the production and recycling of Pi, a limiting macronutrient that roots must assimilate from the soil as soluble inorganic phosphate (**Pi**). A 35-kDa PAP was purified to homogeneity from cell wall (**CW**) extracts of Pi-starved (**–Pi**) *Arabidopsis* suspension cells and identified by mass spectrometry as AtPAP17 (At3g17790), one of 29 predicted PAP isozymes. AtPAP17 had been previously implicated to play a role in both Pi acquisition- and use-efficiency owing to its marked transcriptional induction during Pi deprivation or leaf senescence. In contrast to all other characterized PAPs, AtPAP17 displayed a remarkably broad pH-phosphatase activity profile, exhibiting maximal activity between pH 5.5 - 7.5 (!). Although AtPAP17 exhibited non-specific substrate selectivity (K_m [phosphoenolpyruvate] = 100 µM), it showed near maximal activity with 5 mM phytic acid. This is notable since phytic acid is the predominant organic-P component of many soils, but other well characterized secreted PAPs upregulated by –Pi *Arabidopsis* (*i.e.,* AtPAP12 and AtPAP26) do not exhibit phytase activity. Non-denaturing PAGE followed by in-gel phosphatase activity staining together with immunoblotting using anti-PAP17-IgG indicated that AtPAP17 was *de novo* synthesized and targeted to both CW and intracellular fraction (*i.e.* cell vacuole) of the –Pi cell cultures. Following its transient expression in Arabidopsis and tobacco cells, AtPAP17-GFP was localized to punctuate structures that likely represent lytic vacuoles. Thus, AtPAP17 appears to be dual-targeted to both CW and cell vacuole during Pi deprivation, as previously demonstrated for AtPAP26.

3.3 Investigating the Calcium Dependent Regulation of Plant Myosins. Howard Teresinski[1]*, W. Snedden[1]. [1]Dept. of Biology, Queen's University.

Calcium is a ubiquitous second messenger in signal transduction pathways of eukaryotic cells. Our lab is primarily interested in understanding how plants use calcium signals to regulate various cellular events. In animal cells, links between calcium signalling and cytoskeletal activity have been well documented. A major component of the animal cytoskeleton is the acto-myosin complex, where myosin motor-domain proteins "walk" processively along actin filaments and function in a range of important events including muscle contraction, chromosomal rearrangements, and many other processes. Calcium signalling is linked to animal myosin function through the binding of light chains, specifically, the canonical calcium sensor calmodulin, to myosin neck regions where it functions as part of the 'lever' mechanism in myosin walking. In plants, much less in known about the biochemical properties and physiological roles of myosins. Arabidopsis possesses 17 myosin isoforms divisible into two classes based upon predicted structural differences; class VIII and XI. Although most plant myosins have not been well studied, emerging evidence suggests roles in events such as organelle remodeling and movement, gravotropic response, immune

response, and cell expansion. Thus, myosins represent an interesting but unstudied link between cytoskeletal activity and calcium signaling in plants. I will present preliminary biochemical data showing plant myosins interacting with calmodulin and several novel light chains. This research aims to explore questions addressing the mechanisms through which plant myosins use calcium sensors to regulate their activity

3.4 Analysis of bacterial-type phosphoenolpyruvate carboxylase expression in vascular plants indicates a widespread anaplerotic role in diverse sink tissues.
Michael K.Y. Ting*, W.C. Plaxton. Dept. of Biology, Queen's University.

Two distinct phosphoenolpyruvate carboxylase (PEPC) isozymes occur in vascular plants and green algae: plant-type PEPC (PTPC) and bacterial-type PEPC (BTPC). PTPC polypeptides typically form a tightly regulated cytosolic Class-1 PEPC homotetramer. BTPCs, however, appear to be less widely expressed and to exist only as catalytic and regulatory subunits that physically interact with co-expressed PTPC subunits to form hetero-octameric Class-2 PEPC complexes that are highly desensitized to Class-1 PEPC allosteric effectors. Recent RNA-seq and microarray data were analyzed to obtain a better understanding of *BTPC* expression patterns in different tissues of various plant species. High levels of *BTPC* transcripts, polypeptides and Class-2 PEPC complexes were originally discovered in developing castor seeds, but the analysis revealed a broad range of diverse tissues where abundant *BTPC* transcripts are also expressed, such as the developing fruits of cucumber, grape, and tomato. Marked *BTPC* expression correlated well with the presence of Class-2 PEPC complexes in the immature fruit of cucumbers and tomatoes and is characteristic of tissues accumulating high levels of malate. It is therefore hypothesized that in vascular plants BTPC and thus Class-2 PEPC complexes maintain anaplerotic PEP flux in tissues with elevated malate levels that would potently inhibit 'housekeeping' Class-1 PEPCs. Elevated levels of malate can be used by biosynthetically active sink tissues such as immature tomatoes and cucumbers for rapid cell expansion, drought or salt stressed roots for osmoregulation, and developing seeds and pollen as a precursor for storage lipid and protein biosynthesis.

3.5 Carbon Fixation, Partitioning, and Export in Tomato Source Leaf Exposed to LED of Spectral Quality.
Jason Lanoue*[1,2], D. Leonardos[1], S. Khosla[3], X. Hao[2], B. Grodzinski[1]. [1]Dept. of Plant Agriculture, University of Guelph, [2]Agriculture and Agri-food Canada, Harrow, and [3]Ontario Ministry of Agriculture Food and Rural Affairs, Harrow.

In Canada, controlled environment growth facilities, such as greenhouses equipped with supplemental lighting are essential for optimizing year-round production in both vegetable and ornamental crops. The addition of supplemental lighting allows for the increase in daily light integral (DLI) via the lengthening of the photoperiod or by increasing the amount of photosynthetically active radiation (PAR) the plant is exposed to. This increase in DLI can lead to higher yields during the light limiting months. Historically, commercial greenhouses have used high intensity discharge (HID) lighting such as high pressure sodium (HPS) lights. However, there is a growing trend towards the refinement of light emitting diodes (LEDs) as a more energy efficient alternative.
An increase in quality and yield in high value crops, including tomatoes, from the addition of supplemental lighting has forced us to re-examine how light interacts with the plants. The ability to produce wavelength specific LED-luminaries has allowed us to examine the effects of spectral quality on photosynthesis, C-partitioning, and export rates of photo-assimilates during production. We compare existing HPS lighting to commercially available LED-luminaries to determine their effects on whole plant net carbon exchange (NCER). We also present new evidence for a direct effect on ^{14}C-export kinetics with wavelength specific LEDs. The implications of these findings for the future design of LEDs for commercial production will be discussed including their potential role in inner canopy lighting in greenhouses.

3.6 The study of bifunctional dehydroquinate dehydratases-quinate dehydrogenases in *Solanacea and Brassicacea* species.
Artyom Gritsunov*, J. Peek, D. Christendat. Dept. of Cells and Systems Biology, University of Toronto.

Bifunctional dehydroquinate dehydratase (DHQD)-quinate/shikimate dehydrogenase (QDH) is an enzyme capable of directing dehydroquinate either to shikimate pathway or to the chlorogenic acid biosynthesis. Removal of water from dehydroquinate diverts it towards shikimate pathway which leads to the synthesis of aromatic compounds including phenylalanine, tryptophan, and tyrosine amino acids. Additionally, by catalyzing the dehydroquinate-quinate conversion with NADP/NAD cofactors, dehydroquinate can be diverted to other anabolic/catabolic processes. Quinate is important for the biosynthesis of chlorogenic acids, lignin, phenylpropanoid-derivatives and other central metabolites some of which can function as as feeding deterrents. We have identified plants quinate dehydrogenases and are working towards establishing the role of these compounds in plant development and defence mechanisms. The advancement in bioinformatics allows us to conduct modelling studies to determine the relative 3D structures and to determine the expression patterns of these putative quinate dehydrogenases. Concurrently, we are conducting classical biochemical assay to determine the in *vivo* enzymatic activities of both the quinate dehydrogenase and dehydroquinate dehydratase domains. This presentation will therefore focus on the biochemical and biological role of these putative quinate producing enzymes in plants and also on the implications in modulating the activities of these functional domains in plants.

3.7 Photosynthesis in the cold: characterization of photosynthetic ferredoxin from the Antarctic alga *Chlamydomonas sp.* UWO241 reveals novel features of cold adaptation.
Marina Cvetkovska[1*], B. Szyszka-Mroz[1], M. Possmayer[1], R. Morgan-Kiss[2], D.R. Smith[1], N.P.A. Hüner[1]. [1]Dept. of Biology, Western University and [2]Dept. of Microbiology, Miami University

Chlamydomonas sp. UWO241 is a unique, psychrophilic green alga isolated from Lake Bonney, Antarctica. Despite the importance of polar algae as the primary source of organic carbon in cold environments, relatively little is known about how physiological processes function at low temperatures. The objective of this work is to characterize chloroplastic ferredoxin, a key enzyme involved in the distribution of photosynthetic reducing power. Typically, cold-adapted enzymes posses a range of features that confer a high level of structural flexibility and high activity at low temperatures. This is usually accompanied by low stability at moderate temperatures. By isolating ferredoxin from UWO241 and observing its biochemical and structural properties, we show that this protein has both high activity at low temperatures and high stability at moderate

temperatures. Thus, it represents a novel class of cold-adapted enzymes. Photosynthetic organisms encode multiple ferredoxin isoforms, where typically only one is highly abundant and involved in photosynthetic electron transport. The primary protein sequence of photosynthetic ferredoxin is highly conserved among algae, and we determine that subtle differences in sequence can lead to significant changes in activity at low temperatures. We also show evidence for a duplication of the main ferredoxin gene in the UWO241 genome, which results in the presence of two highly similar and functional photosynthetic ferredoxins. The presence of two proteins in UWO241 could provide an adaptive advantage for survival at cold temperatures. In conclusion, our study on ferredoxin from the psychrophile UWO241 reveals novel insights on the functioning of photosynthesis in the cold.

3.8 Kinetic labeling of whole plants to study rates of terpenoid biosynthesis. Michael A. Phillips[1]*, D. Gonzalez-Cabanelas[2], A. Chavez[3], J. Rohwer[4], L.P. Wright[2]. [1]Dept. of Biology, University of Toronto, [2]Dept. of Biochemistry, Max Planck Institute for Chemical Ecology (Germany), [3]Program for Plant Metabolism and Metabolic Engineering, Center for Research in Agricultural Genomics (Spain), [4]Dept. of Biochemistry, Stellenbosch University (South Africa)

The study of plant metabolism has entered a new era in which steady state ^{13}C labeling of whole plants under physiological conditions merges with metabolomics analysis to provide kinetic rate data on metabolic pathways. We have applied this strategy to the biosynthesis of terpenoids (also known as isoprenoids). Most plant terpenoids are derived from the plastid-localized methylerythritol phosphate (MEP) pathway, which supplies isopentenyl and dimethylallyl diphosphate needed to synthesize photosynthetic pigments, redox cofactors, and several phytohormones. In addition, many volatile terpenoids are likewise made from MEP pathway precursors. Using an isotopic labeling system, we applied the principles of metabolic control analysis to investigate the regulation of this pathway in Arabidopsis and found that its first enzyme, deoxyxylulose phosphate synthase, controls the majority of flux through this pathway. A downstream intermediate, methylerythritol cyclodiphosphate, plays a secondary role outside the chloroplast as a retrograde signal. The export of this metabolic intermediate from its normal environment in the chloroplast is triggered by certain types of stress, leading to a series of novel metabolic transformations separate from the MEP pathway sequence. This same labeling strategy was applied to study the kinetics of monoterpenoid formation in *Pelargonium*, a genus of the Geraniaceae known for copious terpenoid oil accumulation. Here we describe whole plant kinetic labeling as a quantitative method to study both primary and secondary plant metabolism.

Session 4. Molecular Signalling. *Auditorium A*

4.1 Evidence for multiple roles of intercellular salicylic acid during the Age-Related Resistance response of *Arabidopsis thaliana* to *Pseudomonas syringae.* Daniel C. Wilson[1]*, C.J. Kempthorne[1], P. Carella[2], R.K. Cameron[1]. [1]Dept of Biology, McMaster University and [2]Sainsbury Laboratory, University of Cambridge, United Kingdom.

Some plants become more resistant to pathogens as they reach later developmental stages. In *Arabidopsis thaliana* for example, mature plants are more resistant than young plants to the bacterial pathogen *Pseudomonas syringae*. This defense response is known as Age-Related Resistance (ARR), and involves the accumulation of salicylic acid, specifically in the intercellular space of leaves. Study of the ARR-defective *short vegetative phase* (*svp*) mutant has provided further evidence that intercellular salicylic acid is a major contributor to the arabidopsis ARR response. While it has long been hypothesized that salicylic acid acts as an antimicrobial compound in the intercellular space, recent evidence will be presented that suggests that during ARR SA may also act to limit bacterial biofilm formation.

4.2 The Arabidopsis transcription factor ETHYLENE RESPONSE FACTOR 8 (AtERF8) is involved in pathogen defense and ABA signaling. Feng Yi Cao[1*], T. DeFalco[1], H. Ung[1], W. Moeder[1], S. Lumba[1], D. Desveaux[1,2], B. Ellis[3], K. Yoshioka[1,2]. [1] Dept. of Cell and Systems Biology, University of Toronto, [2] Center for the Analysis of Genome Evolution and Function and [3] Dept. of Botany, University of British Columbia.

Phytohormones mediate complex signaling networks in plants, allowing them to respond to various external stimuli while ensuring survival and optimal growth under different conditions. Extensive crosstalk occurs between phytohormone signaling pathways to ensure rapid and effective stress responses. The phytohormone abscisic acid (ABA) plays a prominent role in responses against abiotic stresses such as drought and cold, however, increasing number of studies also indicate the importance of ABA in pathogen defense. Recently, we have found that two immunity-related mitogen activated protein kinases MPK4 and MPK11 interact with the ABA-inducible Arabidopsis transcription factor, ETHYLENE RESPONSE FACTOR 8 (AtERF8). The knockdown mutant of *AtERF8* exhibited enhanced susceptibility against the hemibiotrophic pathogen *Pseudomonas syringae* and the biotrophic pathogen *Hyaloperonospora arabidopsidis*, whereas *AtERF8* overexpression conferred enhance resistance against these pathogens. Moreover, overexpression of *AtERF8* is sufficient to elicit hypersensitive response (HR)-like programmed cell death in *Arabidopsis* and *Nicotiana benthamiana*. These findings support a positive role of AtERF8 in plant immunity. We have further demonstrated that MPK4 and MPK11 phosphorylate AtERF8, indicating the regulation of AtERF8 by these kinases. In addition, AtERF8 negatively regulates ABA-mediated responses such as germination. Thus, our findings support the dual role of AtERF8 in ABA-mediated responses as well as plant immunity.

4.3 Investigating the regulation of drought responsive genes via the activity of histone deacetylase BdHD1 in response to drought stress in *Brachypodium distachyon.* Gabe Song[1, 2]*, H.A.L. Henry[1], L. Tian[1, 2]. [1]Dept. of Biology, Western University and [2]London Research and Development Centre, Agriculture and Agri-Food Canada.

Plants are frequently exposed to environmental stresses, such as drought, during their growth and development. At the molecular level, plant response to drought is based on the activation and regulation of specific drought-responsive genes. Gene expression repression by histone deacetylase (HDAC) is associated with decreased histone acetylation levels of specific histone lysine residues. The acetylation levels in a number of lysine residues, such as H3K9 (histone 3 lysine 9), are affected under abiotic stresses. This research aims at elucidating the role of BdHD1, which shares high levels of similarities with Arabidopsis HDAC1, in transcriptional regulatory networks in response to drought stress in monocots. *Brachypodium* (*Brachypodium distachyon*) is used as a model monocot species in the research. Analysis of gene expression of *Brachypodium*

indicated that transcription level of BdHD1 was down regulated by drought stress. Comparing to wild type, BdHD1-overexpression plants were more tolerant to drought stress. Meanwhile, BdHD1 RNAi plants in which the expression of BdHD1 was repressed were hypersensitive to drought stress. Drought responsive genes with changes of acetylation levels enriched on histone lysine residue H3K9 were sequenced and analyzed using chromatin immunoprecipitation followed by sequencing (ChIP-seq). Eleven drought responsive genes have been identified and showed increased level of H3K9 acetylation under drought stress. Preliminary results indicated that one ABA transporter gene (BdAT1) and one hydrophobic protein (BdHP1) showed higher expression in RNAi plants and lower expression in BdHD1-overexpression plants comparing to wild type plants. Research is being done to reveal how BdAT1 and BdHP1are regulated by BdHD1 in response to drought stress.

4.4 Versatile tools to study *in planta* calcium signaling dynamics in *Nicotiana*. Thomas A. DeFalco[1]*, M. Toyota[2,3], V. Phan[1], W. Moeder[1], S. Gilroy[2], K. Yoshioka[1,4]. [1]Dept. of Cell & Systems Biology, University of Toronto, [2]Dept. of Botany, University of Wisconsin, [3]PRESTO, JST, Japan, and [4]CAGEF, University of Toronto.

Ca^{2+} signaling is a central component of plant biology, however, direct analysis of *in vivo* Ca^{2+} levels is experimentally challenging. In recent years the use of genetically-encoded Ca^{2+} indicators as Ca^{2+} visualization tools have revolutionized the study of plant Ca^{2+} signaling, though such studies have been largely restricted to the model plant Arabidopsis. We have developed stable transgenic *Nicotiana benthamiana* and *Nicotiana tabacum* lines expressing single-molecule fluorescent Ca^{2+} indicators of the GCaMP or cameleon family. Ca^{2+} levels in these plants can be imaged *in situ* using fluorescence microscopy, and these plants can be used to both qualitatively and quantitatively evaluate Ca^{2+} signals in response to a broad array of both biotic and abiotic stimuli. Furthermore, these tools can be used in conjunction with well-established *Nicotiana* techniques such as virus-induced gene silencing or transient heterologous expression to assay the effects of loss- or gain-of-function on Ca^{2+} signals. Using these techniques, along with chemical inhibitor treatments, we will show how these plants can be used to elucidate the molecular components governing Ca^{2+} signaling in response to specific stimuli.

4.5 SnRK1-ABA interactome in *Arabidopsis thaliana*. Carina S. Carianopol[1,2]*, S. Lumba[2], P. McCourt[2], S. Gazzarrini[1,2]. [1]Dept. of Biological Sciences, University of Toronto at Scarborough and [2]Dept. of Cell and Systems Biology, University of Toronto.

Plants have developed intricate mechanisms to enable survival under non-optimal conditions. The plant SnRK1 (Sucrose non-fermenting Related Kinase 1), yeast Snf1 and mammalian AMPK are conserved energy sensors activated under metabolic stress to achieve energy homeostasis. The SnRK1 heterotrimeric kinase complex comprises 2 catalytic (SnRK1α), 3 regulatory β and 2 γ subunits, one of which is plant-specific ($\beta\gamma$). The catalytic SnRK1α1/AKIN10 subunit has been proposed to link stress, sugar and developmental signals to regulate plant metabolism and energy balance and therefore survival of the plant under stress. Recent work indicates that the SnRK1 and ABA signaling pathways share common transcriptional targets during stress response, although the mechanism of this crosstalk is unclear. The aim of this project is to investigate how SnRK1 and ABA interact to regulate growth, development and stress responses in *Arabidopsis thaliana*. A high-throughput yeast two-hybrid (Y2H) screen using all SnRK1 subunits against a library of 258 ABA-regulated genes was performed, resulting in 363 total interactions among 157 unique interactors. 47 putative SnRK1α and $\beta/\beta\gamma$ partners were identified in the ABA-SnRK1 interactome, 30 of which involve the most abundant catalytic subunit, AKIN10. Potential targets include metabolic, regulatory and stress-related proteins. Bioinformatics analysis suggests that a subset of 17 proteins may interact with the SnRK1 complex and participate in the plant response to salt and osmotic stress. Three of these interactions have so far been confirmed *in planta*. The possible role of selected SnRK1 interactors in plant development and stress response will be discussed.

4.6 An Interactome map of *Fusarium graminearum* Mycotoxin Related Proteins. Armand Mirmiran[1,2*], C. Tsai[1], C. Mogg[1,2], G. Subramaniam[1,2], D. Desveaux[1,3]. [1]Dept. of Cell & Systems Biology, University of Toronto. [2]Agriculture and Agri-Food Canada, Ottawa, [3]Centre for the Analysis of Genome Evolution and Function.

Fusarium Head Blight (FHB) is a disease affecting wheat (*Triticum aestivum*) and other cereal crop worldwide resulting in the loss of over $2 billion to the North American wheat industry. This disease, results in the loss of yield, quality and food safety in wheat due to production of trichothecene mycotoxin. The trichothecene, deoxynivalenol (DON), is both responsible for the spread of the disease within the host as well as the inhibition of protein synthesis when consumed resulting in symptoms such as anorexia, vomiting and immune response alteration. *F. graminearum*, a plant fungal pathogen, is the primary agent responsible for FHB in wheat and forms its toxin while under stress conditions such as low pH, light and nutrient limiting conditions. The trichothecene biosynthetic pathway has been established to be expressed through a cluster of genes called the TRI genes. These genes have also been shown to be regulated by two of its members: TRI6 and TRI10. Studies have shown that disruption of either regulators result in no DON production rendering the fungus non-pathogenic. Our interests lie in further exploring the regulation of DON synthesis to uncover novel regulatory pathways that contribute to mycotoxin production and *F. graminearum* virulence. To address this, we have created a protein-protein interaction map of genes that are co-regulated during DON production. We will discuss the hypotheses we have generated from our preliminary analysis of this network and our efforts to functionally test them.

4.7 Introgression of chemical-mediated pest resistance in greenhouse tomato. David Liscombe*, R. Buitenhuis, M. Goertz, D. Pearson, V. Primomo, A. Summerfield, R. Zielinski-Lawrence. Vineland Research and Innovation Centre.

Insect pests are of major concern to the Canadian greenhouse vegetable sector as these organisms cause significant crop damage and product losses. Moreover, these pests are vectors for other diseases such as tomato spotted wilt virus, which leads to further losses. Growers typically control these pests using predatory insects as part of integrated pest management (IPM) strategies, but outbreaks must be treated with pesticides. Several wild relatives of cultivated tomato are well-documented for their abilities to produce specialized metabolites that exhibit deterrent and even lethal effects against arthropod pests. These chemical-mediated resistance traits could help provide a natural, plant-derived protection for cultivated tomatoes, against a number of key greenhouse pests. Some wild relatives of tomato

produce methyl ketones that could be very beneficial for cultivated varieties due to their effects on greenhouse pests such as whitefly and spider mite. Our multidisciplinary team is using chemical markers to breed for pest resistance. We have generated an F2 mapping population derived from a prolific methyl-ketone producing *Solanum hirsutum f. glabratum* accession and a greenhouse tomato inbred. Methyl ketones were quantified by GC-MS and pest assays with selected F2 individuals demonstrate a substantial deterrent effect with spider mites and whiteflies that is positively correlated with methyl ketone content.

4.8 Mapping and marker development for black spot disease (*Diplocarpon rosae* W.) resistance in tetraploid landscape roses (*Rosa* spp.). C. Rouet[1]*, D. Somers[1], E. Lee[2], T. Banks[1], A. Poleatewich[1]. [1]Dept. of Applied Genomics, Vineland Research and Innovation Centre and [2] Dept. of Plant Agriculture, University of Guelph.

Combining the knowledge of genetic inheritance of important traits with molecular tools is necessary for optimizing breeding strategies. Therefore, we have initiated research to modernize the Canadian hardy rose breeding program by introducing genetic mapping. Black spot disease (*Diplocarpon rosae* W.) is the most deadly foliar disease of field grown roses which reduces the ornamental value and marketability of the plants. The objective of this research was to develop molecular markers associated with black spot disease resistance for application in rose breeding. Race 8 black spot resistance was measured in a segregating population of 150 individuals using a detached leaf assay. In addition, genotype- by-sequencing (GBS) was used to simultaneously generate SNP markers and a genetic map of tetraploid rose. This work generated the first high-density integrated SNP-based map of all 56 rose chromosomes of tetraploid rose and mapped the race 8 black spot resistance locus. We have also used GBS to examine and differentiate additional single spore isolates of black spot combined with classic host differential analysis to identify new prevalent races. We aim to map more race-specific black spot resistance genes in segregating populations as well as additional traits of interest for rose breeding such as cold hardiness and flower quality features. Developing these innovative tools used in variety development will advance rose breeding and improve the competitiveness of Canadian bred roses.

4.9 How *Striga* wakes up: Elucidating its germination code. S. Toh[1], Shelley Lumba[2]*. [1]Division of Biological Science, Nagoya University and [2]Dept. of Cell and Systems Biology, University of Toronto.

In Africa, the parasitic plant, *Striga hermonthica* infects major food crops causing 30 to 100% yield losses and affecting over 100 million subsistence farmers. *Striga* infestations represent one of the major impediments to food security in Africa. Because of its obligate nature, it is essential for *Striga* to germinate with a host present. As crop plants grow, their roots exude the hormone, strigolactones (SLs), which are then used by *Striga* to indicate that a host is nearby. Because *Striga* is not amenable to genetics studies, it has been challenging to elucidate the molecular mechanisms underlying its germination. In higher plants, various signals like hormones, light and nutrients are transduced through signaling pathways into combinations of gene expression comprising a “germination code”. *Striga*, however, has lost sensitivity to signals that promote germination such as the hormone, gibberellic acid (GA) and light. Instead, *Striga* has evolved high sensitivity to SLs. To determine how *Striga* has ‘rewired’ the germination code, we are combining bioinformatics, transcriptomics and large-scale protein interaction approaches to begin construction of the first interactome in *Striga*. My group will compare *Striga* networks with those from Arabidopsis to determine the extent of evolutionary conservation of germination pathways between parasitic and non-parasitic plants. These comparisons will lead to insight about the contribution of germination mechanisms in the evolution of parasitic lifestyles and yield potential solutions in combating *Striga*.

Session 5. Metabolism and Genetics. *Rooms 1-2*

5.1 The mystery of ADT5 nuclear localization. Sara A. Rad∗, S. Kohalmi. Dept. of Biology, Western University.

Arogenate dehydratases (ADTs) are a family of six enzymes in *Arabidopsis thaliana* that catalyze the last step of phenylalanine (Phe) biosynthesis. During our localization studies of these enzymes we found that all the six members of this family localize in the stromules (stroma filled tubules) of chloroplasts. Surprisingly one member of this family, ADT5, also localized to the nucleus in addition to stromules. This unique localization pattern led us to the hypothesis that ADT5 is a moonlighting protein and has an unknown role in the nucleus aside from its enzymatic role in the chloroplasts. Many moonlighting proteins function in a complex network of interactions with other proteins. An *in silico* search identified few putative interactors of ADT5 and among them IMPA-6, the only with a known nuclear role. It is one of the carrier proteins that import proteins from the cytosol to the nucleus through the nuclear pores. We performed Y2H and BiFC assays and our results confirmed an interaction between IMPA-6 and ADT5. However, IMPA-6 was also able to interact with all other ADTs that were never observed in the nucleus. Since the online databases only listed a handful of putative ADT5 interactors we performed an Y2H screen in the hope to identify additional interactors of ADT5 in an *Arabidopsis* cDNA library. Our selection identified 54 colonies expressing interacting proteins that represented 18 unique sequences, and among these 8 proteins with a predicted nuclear localization. We will present the results for our ADT5 screen and follow up experiments.

5.2 Characterization of the anatomy, development, and transcriptome of C_3 and C_4 *Atriplex* species. Stefanie Sultmanis*[1], M. Stata[1], U. Gowik[2], A. Bräutigam[3], A. Weber[4], P. Westhoff[2], R.F. Sage[1], T.L. Sage[1]. [1]Dept. of Ecology and Evolutionary Biology, University of Toronto, [2]Dept. of Development and Molecular Biology of Plants, Heinrich Heine University, [3]Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) and [4]Institute for Plant Biochemistry, Heinrich Heine University.

C_4 photosynthesis is a carbon concentrating mechanism (CCM) that arose during low atmospheric CO_2 concentrations around 23 million years ago. An essential feature of C_4 is the spatial separation of the initial fixation of atmospheric carbon by phosphoenolpyruvate carboxylase (PEPC) from the secondary refixation of CO_2 by RuBP carboxylase/oxygenase. This relationship is present in ~8100 C_4 species whether the reactions are occurring within single cells or between two cell types, mesophyll (M) and bundle sheath cells (BS) surrounding the vascular tissue (Kranz-anatomy). Numerous variants of Kranz-anatomy have evolved. In each instance, one PEPC-containing M cell is associated with one BS cell. This cellular relationship results from increased vein density (VD) and a reduction in M cell

numbers between veins. High plasmodesmata numbers between the two cell types have also evolved to facilitate rapid flux of metabolites between the two compartments in C_4. The present study analyzed a *de novo*-assembled transcriptome generated along a leaf developmental gradient in *Atriplex prostrata* (C_3) and *A. rosea* (C_4) to identify key genes involved in the generation of high VD and plasmodesmata in *A. rosea*. High VD results from accelerated vascular tissue initiation and addition of one extra vein order associated with prolonged up-regulation of MONOPTEROS and TARGET OF MONOPTEROS 5. Changes in transcript levels of genes involved in secondary plasmodesmata formation and permeability correspond to formation of secondary plasmodesmata in C_4. These findings facilitate identification of gene candidates required for establishing C_4 photosynthesis in C_3 crops – a key strategy in the C_4 Rice Project.

5.3 ADT activity? Let me check: A novel assay to detect arogenate deydratase activity *in vivo.* Emily J. Clayton*, S.E. Kohalmi. Dept. of Biology, Western University.

In *Arabidopsis thaliana,* six arogenate dehydratases (ADTs) synthesize Phe via the arogenate pathway, in which prephenate is transaminated to arogenate by a prephenate aminotransferase (PAT), followed by a decarboxylation/dehydration of arogenate to Phe by an ADT. Of the *A. thaliana* ADT family, ADT1 and ADT2 can also participate in the prephenate pathway of Phe biosynthesis, acting as prephenate dehydratases (PDTs). This involves converting prephenate to phenylpyruvate in a decarboxylation/dehydration reaction by a PDT, then the transamination of phenylpyruvate to Phe by a phenylpyruvate aminotransferase (PPAT). Enzymatic assays are an important aspect of characterizing an unknown protein/enzyme. However *in vitro* assays are expensive, and in some cases even not possible if the substrate is not commercially available. As part of characterizing this ADT family, we can test PDT activity *in vivo* using a yeast complementation test in the *Saccharomyces cerevisiae pha2* strain, and we have developed a method to determine ADT activity *in vivo* that is fast and efficient. *ARO8* and *ARO9* encode the two *S. cerevisiae* PPATs required for Phe synthesis via the prephenate pathway. We have generated a double knockout strain of the *S. cerevisiae* PPATs. As predicted, the Δ*aro8aro9* knockout is Phe requiring. This yeast strain was transformed with the *AtPAT* to introduce the arogenate pathway. In the presence of the PAT, an ADT will complete the pathway to synthesize Phe in this yeast strain. This assay will be the only *in vivo* test of ADT activity that can distinguish between an enzyme with ADT/PDT activity, or only ADT activity.

5.4 Diverse C_3-C_4 Intermediates Make Blepharis (Acanthaceae) a Powerful New System for C_4 Evolutionary Research. Matt Stata, R.F. Sage. Dept. of Ecology and Evolutionary Biology, University of Toronto.

C_4 photosynthesis has independently evolved from C_3 ancestors in numerous Angiosperm lineages in response to a fundamental limitation of Rubisco: in an oxygen-rich, low-CO_2 atmosphere Rubisco is prone to a wasteful oxygenase side-reaction where O_2 is fixed instead of CO_2, resulting in photorespiration, which competes with photosynthesis and reduces productivity. C_4 plants suppress photorespiration by restricting Rubisco expression to the bundle sheath and activating a series of enzymatic steps which fix CO_2 via PEP carboxylase in the mesophyll and "pump" it into the bundle sheath, saturating Rubisco. Flaveria (Asteraceae) is considered a model genus for C4 evolutionary studies due to the presence of related C_3 and C_4 species as well as a large number of extant evolutionary intermediates (11 species) . We have identified Blepharis (Acanthaceae) as a powerful parallel system: the Acanthodium subgroup contains 14-15 C_4 species, at least two C_3 species, and potentially as many as 36 intermediates. This would make Blepharis the most diverse C_3-C_4 transitional lineage known and represents a goldmine for the study of C_4 evolution.

5.5 Promoter sequence diversity and its roles in differential expression of six *Arabidopsis AROGENATE DEHYDRATASE* genes. Emily J. Cornelius*, S.E. Kohalmi. Dept. of Biology, Western University.

The phenylpropanoid pathway is among the most important secondary metabolic pathways in plants, as it allows the synthesis of lignins, pigments and defense compounds. The pathway precursor is the aromatic amino acid phenylalanine (Phe). The final step of Phe synthesis in plants is catalyzed by the enzyme arogenate dehydratase (ADT). The *Arabidopsis thaliana* genome encodes six ADTs which are similar in amino acid sequence and subcellular localization, but have very different gene expression levels during development and under stressful growth conditions. Different gene expression levels suggest that *ADT*s are transcriptionally regulated, and since promoters play a key role in initiating transcription, this research focuses on *ADT* promoters. The first objective was to identify known regulatory motifs present in each *ADT* promoter and categorize them based on their predicted function. Not surprisingly, a large number of motifs are recognized by transcription factors related to stress response and development, complementing the evidence that *ADT*s are differentially regulated during these conditions. The second objective is to clone *ADT* promoters into a vector 5' to e*GFP* and *GUS* to create *ADT-GFP/GUS* promoter sequences to be introduced into the *Arabidopsis* genome using *Agrobacterium*-mediated stable transformation. This will allow the comparison of *in planta* expression profiles with promoter sequence patterns to determine which motifs are responsible for the differential regulation of *ADT* genes. Not only is this research an excellent opportunity to study eukaryotic gene regulation in a small enzyme family, but also offers a chance to tailor plants to specific environmental needs.

5.6 Identification of C_4 genetic determinants – forward genetic approaches. Kumari Billakurthi*[1], F. Döring[1], U. Gowik[1], S. Das Gupta[1], S. Sultmanis[2], R. Khoshravesh[2], T. Sage[2], P. Westhoff[1]. [1]The Developmental and Molecular Biology of Plants, Heinrich Heine University – Düsseldorf and [2]Dept. of Ecology and Evolutionary Biology, University of Toronto.

Numerous international initiatives are focusing on enhancing photosynthesis to include the introduction of C_4 photosynthesis into C_3 plants. This later effort requires an understanding of the photosynthetic activation of bundle sheath (BS) cells that occurs during the evolution of C_4 from C_3. Gene regulatory systems operating in BS cells of C_4 and C_3 of monocots and eudicots are partly conserved. Thus, C_3 model species can be used for the discovery of conserved genes that are required for BS development. Here, we describe two different forward genetic screens using *Arabidopsis thaliana*, a chemical mutagenesis approach (Ethyl methanesulfonate) and an activation tagging approach, designed to find genes involved in the development of BS cells. Since BS cells are only visible at the microscopic level, changes in their properties cannot be easily visualised in a non-destructive way. Therefore, we

have labelled BS cells of *Arabidopsis thaliana* by expressing a chloroplast-located GFP under the control of a known BS-specific promoter from the glycine decarboxylase P-protein gene of the C_4 species *F. trinervia*. In both genetic screens we were able to obtain aberrant phenotypes that alter cell division and expansion in the BS and vascular tissue and identify candidate genes for the activation of the BS during synthetic engineering of C_4 into C_3 crops.

5.7 An improved method for long non-coding RNA prediction that includes small ORF coding probabilities. Caitlin Simopoulos*, G.B. Golding, E.A. Weretilnyk. Dept. of Biology, McMaster University.

Long non-protein coding RNAs (lncRNAs) represent a diverse and functionally important classification of RNA transcripts. Although research on these transcripts is in its infancy, and most plant lncRNAs lack putative functions, recent studies have indicated the importance of these transcripts in proper plant development and for stress tolerance. Multi-species lncRNA databases can help facilitate functional identification by making lncRNA sequences available to researchers. The recently developed lncRNA database GreeNC is currently the largest lncRNA database of its kind, with over 20,000 predicted lncRNA from 45 plant species. However, the alignment-based methodology used in the GreeNC prediction protocol may not be best suited to lncRNA identification. We propose a similar, but more powerful, method that uses logistic regression models to calculate protein coding potential (using Coding Potential Assessment Tool), which also is used in the calculation of small peptide translation probabilities of putative lncRNAs. Further, our method uses the Diamond blastx algorithm for an improvement in computational speed over the GreeNC method. Preliminary work suggests this method predicts over 94% of the same lncRNA transcripts as GreeNC in *Arabidopsis thaliana*, *Oryza sativa,* and *Eutrema salsugineum* including previously empirically validated lncRNAs such as IPS1, indicating that the two methods are comparable. It also succeeds in predicting thousands more un-annotated putative lncRNA that meet all lncRNA criteria. This new method will allow researchers to quickly obtain putative lncRNAs from transcript sequences, as well as predict small coding ORFs to facilitate a continued functional annotation of lncRNAs in plants.

5.8 A method for comparing allele specific expression differences in *Zea mays* hybrids. Shuhua Zhan*[1], K. Feys[2], H. Nelissen[2], D. Inze[2], L. Lukens[1]. [1]Dept. of Plant Agriculture, University of Guelph and [2]Dept. of Plant Systems Biology, VIB, Ghent University.

Allele specific expression (ASE) analysis evaluates expression differences between parental alleles in the F1 hybrid. Allele specific expression may be affected by many factors. It has been used to determine the role of cis-regulatory variation in gene expression and studied for heterosis, where the hybrid has improved performance over the parents for one or more traits. RNA-Seq data allows for an unprecedented number of transcripts to be surveyed for allele specific expression. However, genetic diversity is known to bias estimates of ASE. It is common for estimates of one allele's transcript abundance to be over-estimated relative to another allele's because the former preferentially maps to a reference genome. Here, we report a novel method to compare ASE between maize hybrids subjected to different treatments. First, to account for sequence variation, we construct polymorphism-aware reference genomes to map RNA-Seq reads. Second, we measure differences in ASE between two treatments using logistic regression. This study provides an unbiased method and an integrated pipeline for identifying ASE differences between plants. The method can be applied to any population which is comprised of highly similar, heterozygous individuals, such as horticultural plants.

5.9 How Convergent is Convergent Evolution? Case Studies from the Evolution of C_4 Photosynthesis. Rowan F. Sage*, M. Stata, T. L. Sage. Dept. of Ecology and Evolutionary Biology, University of Toronto.

The C_4 photosynthetic pathway represents one of the most convergent of complex traits in the biosphere, having evolved independently over 65 times in the last 35 million years. Despite the universal ability to concentrate CO_2 around Rubisco via a metabolic pump that originates with PEP carboxylation, there is substantial variation in the details of the C_4 mechanism between the different lineages. Different C_4 subtypes exist, utilizing different decarboxylating enzymes and transfer metabolites, and the nature of the Kranz anatomy which confers the required compartmentalization of enzymes in the C_4 pathway can also vary, such that each lineage is unique in some way. With high throughput sequencing, we have been able to examine patterns of convergence and divergence at the molecular level. In the case of PEP carboxylase, the one enzyme upregulated in all C_4 lineages, the same gene copies are repeatedly used in most lineages; however, in the case of multigenic controls, for example over organelle development, evolution appears to haphazardly target different genes in a developmental pathway to effect a common phenotypic outcome. This will be demonstrated using examples of chloroplast division, where evolution reduced the number of M cell chloroplasts in different C_4 lineages by targeting different sets of genes controlling chloroplast division. In summary, C_4 photosynthesis is an excellent case study to demonstrate that phenotypic convergence is the end result of convergent and divergent phenomena within the molecular and developmental hierarchy that controls whole plant identity.

Session 6. Metabolism and Genetics. *Rooms 1-2*

6.1 Investigating the role of XERICO in GA-ABA crosstalk during germination and stress response. Eliana Vonapartis*, C. Carianopol, D. Mohamed, S. Gazzarrini. Dept of Biological Sciences, University of Toronto Scarborough and Dept. of Cell and Systems Biology, University of Toronto.

Plant hormones are small molecules whose signaling pathways interact, together forming a complex crosstalk network that modulates the plant's response to developmental signals and environmental stressors. Although hormone crosstalk is central to various aspects of plant biology, it is a phenomenon that is poorly understood despite much recent progress. XERICO (XER) is a DELLA-induced putative RING E3 ubiquitin ligase that has been found to play a role in increasing intracellular abscisic acid (ABA) levels, thereby enhancing tolerance to drought. Since its precise mode of action remains unexplored to date, we aim to understand the role of XER in GA-ABA crosstalk during plant development and stress response. As a first step in characterizing XER function, transient expression assays of its RING-mutated form in *Nicotiana benthamiana* show that it localizes to the chloroplast envelope and cell periphery. In contrast, the wild-type XER protein shows very low expression in transient assays, suggesting that it may be

subjected to fast turnover. To shed light on XER involvement in hormone signaling at the molecular level, yeast one-hybrid (Y1H) and two-hybrid (Y2H) approaches were conducted. Six potential XER upstream regulators, as well as 40 potential downstream targets have been isolated, most of which are transcriptionally regulated by stress. Overexpression lines and *xer* hypomorphic mutants have been characterized to study its *in planta* function and its subcellular localization *in vivo*. Notably, results of the yeast screens as well as phenotypic analysis of mutant lines suggest that XER may also regulate stomatal development and patterning.

6.2 Identification and molecular characterization of *Brachypodium distachyon* NRT2 family, with a putative essential role of BdNRT2.1 on nitrogen use efficiency. Jiang Wang[1*], N.P.A. Hüner[1], L. Tian[1, 2]. [1]Dept. of Biology, Western University and [2]London Research and Development Centre, Agriculture and Agri-Food Canada.

Agricultural productivity relies heavily on nitrogenous fertilizers. Excess N fertilizer application leads to lower nitrogen use efficiency (NUE) along with energy waste and environmental problems. Therefore, improving NUE in plants is of key importance. The small monocot plant *Brachypodium distachyon* (Brachypodium) is rapidly emerging as a powerful model system to study questions unique to the monocot crops (wheat, maize, rice, etc.). Six genes encoding Brachypodium putative high affinity nitrate transporters (BdNRT2s) were identified and were named *BdNRT2.1, BdNRT2.2, BdNRT2.3, BdNRT2.4, BdNRT2.5,* and *BdNRT2.6*. Analysis of individual BdNRT2 gene expression under different nitrogen sources and concentrations showed that *BdNRT2.1* and *BdNRT2.2* were strongly induced by low nitrate concentrations and were classified as inducible genes, whereas other members were constitutively expressed. On the other hand, *BdNRT2.5* was found to be repressed by higher concentration of both nitrate and ammonium. Furthermore, the high affinity transporter system (HATS) was reduced by 36% in *bdnrt2.1* mutant and its overall NUE was also decreased by 37% and both HATS and NUE could be recovered in *bdnrt2.1* rescue lines. Interestingly, *BdNRT2.2* lost its nitrogen inducible property in *bdnrt2.1*, suggesting *BdNRT2.1* regulates *BdNRT2.2* expression. Additionally, *BdNRT2.1* over-expression lines showed a 24% NUE increase. The research finding implicates the essential role of BdNRT2.1 in NUE related metabolism and its potential application for breeding crops with more efficient use of fertilizer.

6.3 Acclimation capacity of photosynthesis and respiration to temperature in dominant mature boreal conifer tree species. M. Eric Dusenge[1]*, J.R. Stinziano[1], J.M. Warren[2], E. Ward[2], S.D. Wullschleger[2], D.A. Way[1,3]. [1]Dept. of Biology, Western University, [2]Oak Ridge National Laboratory and [3]Nicholas School of the Environment, Duke University.

Boreal forests are often assumed to be temperature-limited, and warming is therefore expected to stimulate their carbon uptake. However, much of our information on the ability of boreal conifers to thermally acclimate photosynthesis and respiration comes from seedlings. We measured net CO_2 assimilation rates (A) and dark respiration (R) measured at 25 °C (A25 and R25) and at prevailing growth temperatures (Agrowth and Rgrowth) in mature *Picea mariana* (spruce) and *Larix laricina* (tamarack) exposed to ambient, +2.25, +4.5, +6.75 and +9 °C warming treamtents at the SPRUCE experiment. In spruce, R25 showed little thermal acclimation, leading to higher Rgrowth in the warmest treatments, especially in August. In May, A25 was similar across plots, such that Agrowth was higher in warmer treatments. Later in the summer, warmer treatments had higher A25, an effect that was offset by warmer leaf temperatures in the Agrowth data. In tamarack, respiration thermally acclimated (i.e. R25 was similar across plots), but while this generated homeostatic Rgrowth in June, Rgrowth in August was still stimulated at the warmer treatments. A25 was stimulated by warming in June, an effect that was mainly offset by leaf temperature when Agrowth was assessed. In August, A25 was slightly suppressed by warming, a result that was exacerbated by direct leaf temperatures (measured as Agrowth). Our work suggests that the capacity for thermal acclimation in both photosynthesis and respiration varies among boreal tree species, which may lead to shifts in the community composition of northern forests as the climate warms.

6.4 The effect of allantoin on Arabidopsis seedlings tolerance in response to salt stress. Solmaz Irani*, C.D. Todd. Dept. of Biology, University of Saskatchewan.

Allantoin is a nitrogenous compound derived from purine catabolism that contributes to nitrogen recycling in plants. It has been reported that allantoin is involved in activation of abscisic acid (ABA) metabolism and protection of plant in response to abiotic stress conditions. Recently, we showed that allantoin concentration increases in response to NaCl and mannitol in Arabidopsis seedlings. Moreover, Arabidopsis allantoinase-negative (*aln3*) seedlings, that contain constitutively high allantoin levels, exhibited better performance when germinated in the presence of NaCl compared to the wild-type. The present study shows that exogenous application of allantoin improves wild-type seedlings' tolerance to NaCl. To aid in understanding the molecular mechanism, the effect of exogenous allantoin treatment on expression of several stress-related genes was investigated. In presence of allantoin, expression of *RD26* (Response to Dehydration), a transcription factor involved in ABA dependent stress signalling, was upregulated. In addition, allantoin increased the expression of *SOS1* (Salt Overly Sensitive 1) and *RCD1* (Radical-induced Cell Death) in seedlings. Both SOS1 and RCD1 proteins had been reported to contribute in oxidative stress tolerance and detoxification pathway in Arabidopsis. Our results suggest that allantoin might participate in the salinity stress response by affecting the salt sensitive pathway and transcriptional activation of stress-responsive genes. These findings provide more evidence for the role of allantoin in enhancing plants tolerance to oxidative stress.

6.5 Identification and characterization of a novel lipid droplet protein in *Arabidopsis.* Michal Pyc[1]*, O. Yurchenko[2], Y. Cai[3], S.K. Gidda[1], K.D. Chapman[3], J.M. Dyer[2], R.T. Mullen[1]. [1] Dept. of Molecular and Cellular Biology, University of Guelph. [2] U.S. Department of Agriculture, Agricultural Research Service, U.S. Arid-Land Agricultural Research Center, Maricopa, AZ. [3]Dept. of Biological Sciences, Center for Plant Lipid Research, University of North Texas.

Cytosolic lipid droplets (LDs) are evolutionarily conserved organelles found in a wide range of unicellular and multicellular organisms. Uniquely delineated by a single phospholipid monolayer and coated with a diverse set of proteins, LDs function primarily in the storage of energy-rich neutral lipids, such as triacylglycerols. In plants, LDs have been studied mostly in oilseeds, although all plant cell types, including vegetative tissues, have the machinery required for accumulating and storing lipids in LDs. We recently identified a class of proteins in *Arabidopsis* called the LD-associated

proteins (LDAPs) that are abundant components of LDs in non-seed cell types and are required for their proper compartmentation. In an effort to identify other novel LD proteins, the LDAPs were used as 'bait' proteins in yeast two-hybrid screens against an *Arabidopsis* cDNA 'prey' library. Here we describe one of the candidate LDAP3 interactors, termed *Arabidopsis* mycobacterial-like protein (AMLP), which is homologous to members of a *M. tuberculosis* protein family involved in lipid transport. We show that, similar to the LDAPs, AMLP is expressed in *Arabidopsis* in a variety of tissues and at various developmental stages, and is localized to the LD surface by a discrete targeting signal motif that appears to be conserved among other plant AMPLs. Furthermore, analyses of an *Arabidopsis amlp* mutant revealed conspicuously enlarged LDs in both leaves and mature seeds, suggesting that AMLP plays a role in regulating LD size in plant cells. Potential mechanisms by which AMLP functions in LD-related processes in plants will be discussed.

6.6 Temperature stress and reproduction in the emerging biofuel crop *Camelina sativa*
Vanessa Lundsgaard-Nielsen*, C. So, T.L Sage. Dept. of Ecology and Evolutionary Biology, University of Toronto.

Camelina sativa is an oilseed crop in the Brassicaceae family that has significant potential as a crop for biofuels and industrial oils. *Camelina* has recently garnered attention for its potential in Canadian biotechnology research due to its short growing season, ease of transformation, and published genome. *Camelina* is resistant to drought, cold, salt, nutrient-poor soil, pathogens, and insects. However, *Camelina* is not tolerant to high temperature (HT) such that yields are reduced under even moderate heat stress (>32°C). High temperatures are well known to reduce yields through a negative effect on male reproductive development, although this has not been examined in *Camelina*. Our microscopic studies indicate that the cause of low yields during HT stress in *Camelina* results from an adverse effect of HT on pollen development and pollen tube growth within the ovary that results in reduced ovule fertilization. Carbonylation of proteins is a well known indicator of cellular stress and HT stress resulted in an increase in the levels of carbonylated proteins in mature anthers, pollinated stigmas, styles, and ovaries in *Camelina*. A positive correlation between the accumulation of carbonylated proteins and stress-induced damage in pollen and pollen tubes indicates a cause-effect relationship between carbonyls and the reduction in pollen tube efficacy required for fertilization and hence seed production.

6.7 Carbon fluxes acclimate more strongly to elevated growth temperatures than to elevated CO_2 in a northern conifer.
Y. Kroner[1], Danielle Way[1,2*]. [1]Dept. of Biology, Western University and [2]Nicholas School of the Environment, Duke University.

Increasing temperatures and CO_2 concentrations will affect tree carbon fluxes, with potential feedbacks on global climate. We studied how elevated temperatures and CO_2 impacted carbon dynamics in Norway spruce (*Picea abies*), a dominant boreal tree, to improve predictions of future photosynthetic and respiratory fluxes from northern conifers. Seedlings were grown under ambient (AC, ~435 µmol mol^{-1}) or elevated (EC, 750 µmol mol^{-1}) CO_2 concentrations at ambient, ambient +4 °C, or ambient +8 °C temperatures. Photosynthetic rates were high in +4 °C/EC seedlings and lowest in +8 °C spruce, implying that moderate, but not extreme, climate change may stimulate carbon uptake. Photosynthesis, dark respiration (R_{dark}) and light respiration (R_{light}) rates acclimated to temperature but not CO_2: the thermal optimum of photosynthesis increased, and respiration was suppressed under warming. The Q_{10} of R_{light} (the relative increase in respiration for a 10 °C increase in temperature) was 35% higher than the Q_{10} of R_{dark}, so the ratio of R_{light}:R_{dark} increased with rising leaf temperature. Across all treatments and measurement temperatures, a consistent relationship between R_{light} and R_{dark} was found, which could be used to model R_{light} in future climates. Acclimation reduced modeled respiratory losses from warm-grown seedlings by 22-56%. When R_{light} was modeled as a constant fraction of R_{dark}, modeled respiratory losses were 11-65% greater than when using measured values of R_{light}. We highlight the impact of acclimation on predictions of carbon fluxes in northern trees, particularly the need to model daytime respiration from direct measurements of R_{light} or appropriate relationships with R_{dark}.

POSTER Abstracts. *Atrium*

P1. Investigating a conserved role for *BLADE-ON-PETIOLE* genes during secondary growth in *Arabidopsis thaliana* and *Populus trichocarpa.* Gamalat Allam*, B. Devi, E. Li, M. Khan, S.R. Hepworth. Dept. of Biology, Carleton University.

Plant growth and development relies on the activity of meristems. The vascular cambium is a lateral meristem that directs radial thickening of plant organs through the production of secondary xylem (wood) and secondary phloem (inner bark). *BLADE-ON-PETIOLE1* (*BOP1)* and *BOP2* genes in *Arabidopsis thaliana* (Arabidopsis) are members of a conserved subclade of BTB-ankyrin transcriptional co-regulators in land plants. Studies in Arabidopsis show that these factors are enriched at meristem-organ boundaries and interact with KNOX-BELL homeodomain proteins in controlling meristem activity and cell fate determination. Recent studies show that spatial regulation of *BOP1/2* by KNOX factors plays an essential role in modulating the differentiation of lignified cell types during secondary growth in the hypocotyl-root and the inflorescence stem. How these findings apply to wood development in trees is unknown. The genome of *Populus trichocarpa* (Poplar) contains two *BOP-LIKE* genes, designated *PtrBPL1* and *PtrBPL2*. We show here that *PtrBPL1* or *PtrBPL2* complement Arabidopsis *bop1 bop2* mutant phenotypes. Furthermore, Arabidopsis plants overexpressing *PtrBPL1* or *PtrBPL2* have defects in stem growth and lignification similar to *AtBOP1/2* overexpressing lines. Quantitative RT-PCR analysis of dissected poplar tissues shows that *PtrBPL1/2* transcripts are enriched in vascular tissues of the woody stem. Construction of *PtrBPL1/2* loss and gain-of-function lines in poplar is currently underway. Analysis of these lines will shed light on mechanisms controlling secondary growth in trees.

P2. How do plants deal with low phosphate? Getting to the root of the problem.
Anna Axakova*, V. Velasco, and E.A. Weretilnyk. Dept. of Biology, McMaster University.

Eutrema salsugineum is a halophilic extremophile plant, a member of the Brassicaceae family and hence a relative of *Arabidopsis thaliana*. The Yukon ecotype of *Eutrema* is adapted to extreme environmental conditions including a soil low in phosphate. Plants produce and can exude extracellular purple acid phosphatases (PAPs) to scavenge organic phosphates in the rhizosphere when phosphate-deprived. PAP activity was visualized in *Eutrema* and *Arabidopsis* seedling roots using 5-bromo-4-chloro-3-indolyl phosphate (BCIP) that produces a blue precipitate when hydrolyzed by a phosphatase. PAP activity was observed in the entire root system of *Eutrema* whether plants were grown with or without phosphate. In *Arabidopsis*, PAP activity was mostly localized to the root tips and activity was induced by low phosphate. Growing seedlings on nutrient gel plates containing bromocresol purple pH indicator showed minimal acidification around *Eutrema* roots and prominent acidification around *Arabidopsis* roots. Acidification of the rhizosphere suggests that *Arabidopsis* has a greater capacity for secreting PAPs into the soil where activity is promoted by acid conditions. Incubating seedling roots with ELF-97 leads to a fluorescent precipitate when hydrolyzed by a phosphatase. Using ELF-97, PAP activity was detected across *Eutrema* roots but largely absent from root hairs whereas PAP activity was largely localized to the epidermal cells and root hairs of *Arabidopsis*. The propensity of *Eutrema* to flourish under low phosphate conditions may reside with *Eutrema* being equipped to constitutively express PAPs that are not secreted into soil. These findings could inform future studies leading to improved phosphate use efficiency in crops.

P3. Characterization of the feeding apparatus and salivary glands associated with feeding in the two spotted spider mite, *Tetranychus urticae*. Nicolas Bensoussan*, V. Zhurov, M. Grbić, V. Grbić. Dept. of Biology, Western University.

The two-spotted spider mite (TSSM), *Tetranyhus urticae,* is one of the most polyphagous arthropods, feeding on over 1,000 plant species. TSSM is known for its ability to develop resistance to pesticides making its control challenging and prompting the development of environment-friendly pest control strategies. Initially focusing on understanding the pattern of mite feeding, I have examined the plant damage associated with mite feeding, as well as the stylet penetration into the leaf (presumably TSSM's feeding organ that comes in contact with plant tissue). The stylet is inserted between epidermal cells without causing damage to these cells and inserts following a straight route into a single mesophyll cell. However, the mechanism of the plant cell content acquisition is still unknown and might involve the secretion of an enzymatic cocktail for pre-oral digestion. Additionally, emerging evidence suggests that spider mites might have evolved a powerful strategy to interact with plant immunity toward its attenuation, most likely mediated by secretion of salivary effectors. Recently, we identified a family of salivary proteins using a trancriptomic and proteomic approach. *In situ* hybdization using DIG-labeled antisense RNA probes was successful in localizing the salivary protein expression domain, in fixed adult mite tissues. A series of tools for discovery and validation of the effect of mite salivary proteins *in planta* will be further developed. The identification of the basic mechanisms at play, between spider mites and the plants they feed on, can be exploited to develop novel non-chemical approaches to crop protection.

P4. Seasonal and interspecies dynamics of foliar nitrogen and chlorophyll at Turkey Point, Ontario. Yazad F. Bhathena[1]*, C. Wong[1,2], A. Arain[3], I. Ensminger[1,2,4]. [1]Dept. of Biology, University of Toronto Mississauga; [2]Graduate Program in Ecology and Evolutionary Biology, University of Toronto; [3]School of Geography and Earth Science, McMaster University and [4]Graduate Program in Cells and System Biology, University of Toronto.

Leaf nitrogen and chlorophyll pigment concentration are fundamental to the understanding of plant physiology as they can act as proxies for photosynthesis. Consequently, it is not unexpected that leaf nitrogen and chlorophyll concentration are often used as parameters for gross primary productivity (GPP) models such as the Joint UK Land Environment Simulator (JULES). As leaf nitrogen and chlorophyll pigment dynamics vary across the course of the season and across species, accurate model estimates of GPP rely on having a thorough understanding of the variability and the relationship that exists between leaf nitrogen and chlorophyll content across species and seasons. This study examines the seasonal relationship between chlorophyll concentration and nitrogen concentration in leaves of 3 different tree species representative of the Great Lakes-St. Lawrence forest region: *Acer rubrum*, *Quercus alba*, and *Pinus strobus*, at two field sites at Turkey Point, Ontario. Leaf samples were collected for nitrogen and chlorophyll pigment analysis from two temperate forests in Southern Ontario, from August 2015 till August 2016. From both the nitrogen and chlorophyll pigment data, differences in onset of winter downregulation and spring recovery were seen between the evergreens and

deciduous trees and a strong correlation between leaf nitrogen concentration and chlorophyll concentration was observed for all three species. Given the seasonal and species specific variation in both nitrogen and chlorophyll pigment concentration found in this study, accurate estimates of GPP based on model calculations depend on species and season specific nitrogen and chlorophyll data.

P5. Compatible pollen signalling in Arabidopsis thaliana: the proposed role of stigma-expressed RLCKs. Jennifer Doucet, N. Udugama, D.R Goring. Dept. of Cell and Systems Biology, University of Toronto.

The characteristic dry stigmas in the Brassicaceae means that compatible pollen must first be recognized by stigma papillae for successful germination, but relatively little is known about cellular responses to compatible pollen. In our working model, upon compatible pollen recognition, vesicle trafficking is initiated in the stigmatic papilla towards the plasma membrane under the pollen contact site. This regulated secretion allows for water release and cell wall modifications to mediated pollen hydration, germination, and subsequent pollen tube growth. Previously, we have shown that the exocyst complex which functions in tethering secretory vesicles to target membranes during exocytosis, is required in the stigma for accepting compatible pollen. We have used a reverse genetics approach to identify candidate signalling proteins for activating this regulated secretion. We identified two stigma-expressed Receptor-Like Cytoplasmic Kinases (RLCKs) which are conserved within the Brassicaceae, supporting their possible involvement in a family-wide compatible pollen recognition pathway. We hypothesize that these RLCKs, as part of an early pollen recognition signalling pathway, function upstream of exocyst-mediated vesicle trafficking. This project represents an exciting first step towards understanding the basal compatible pollen response pathway in the stigmatic papillae.

P6. Possible roles for a cytochrome p450 and ABC transporter in deoxynivalenol tolerance in the biocontrol agent *Clonostachys rosea* strain ACM941. Zerihun A. Demissie*, M.C. Loewen. Aquatic and Crop Research and Development, National Research Council of Canada.

Clonostachys rosea strain ACM941 is a fungal bio-control agent developed and patented in Canada against the FHB disease causative agent *Fusarium graminearum*. Although the molecular and biochemical basis of its tolerance are yet to be resolved, one peculiar feature of *C. rosea* is its ability to tolerate high levels of the *Fusarium* mycotoxin deoxynivalenol (DON). Based on available EST databases arising from mycotoxin treated *C. rosea* strain IK726, a cytochrome p450 and two ABC-transporter homologs (abcg5 and abcg29) were identified as potential targets of interest in *C. rosea* strain ACM941. The transcriptional activities of the abcg29 were not significantly affected by treatments with either DON or spun *F. graminearum* strain Fg3639 growth media. In contrast, the Fg3639 spun media did result in up-regulation of abcg5 and the cytochrome p450 homolog was transcriptionally up-regulated by both DON and Fg3639 growth media. Furthermore, yeast cells transformed with vector containing the CYP450 showed improved growth characteristics compared to control yeast in the presence of DON. Together these data imply potential roles for the cytochrome p450 and the abcg5 homologs in DON tolerance. We are in the process of testing the abcg5 in a recombinant yeast system and determining the reaction catalyzed by the CYP450 toward understanding their roles in *C. rosea*'s antagonistic property against Fg3639.

P7. A family of Arabidopsis MYB transcription factors that control the regulation of suberin deposition. Hefeng Hu*, D. Klein, J. Murmu, O. Rowland. Dept. of Biology and Institute of Biochemistry, Carleton University.

Suberin is a cell wall-associated polymer consisting of glycerol, phenolics, and various chain-length fatty acids and fatty alcohols. Suberin is deposited in diverse plant tissues including root exodermis and endodermis, aerial and underground periderms, and seed coats. While suberin is produced constitutively in these specialized tissues, suberization of cell walls also occurs in these and other tissues under stresses such as wounding. Suberin plays important roles in controlling water and solute movement, especially in roots. It also helps plants to defend against various stressors, including drought, high salinity, toxic metals, insects, and microbial pathogens. Although much progress has been made in identifying genes encoding suberin biosynthetic enzymes, the molecular mechanisms governing the regulated deposition of suberin are currently unclear. We provide evidence that Arabidopsis transcription factors MYB53, MYB92, and MYB93 are important regulators of suberin during root development. We first identified these MYB proteins as positive suberin regulators in a transient assay screen using leaves of *Nicotiana benthamiana*. Analysis of promoter::GUS transgenic lines revealed that *MYB53*, *MYB92*, and *MYB93* have overlapping gene expression patterns in root endodermis when suberin is being deposited. Loss-of-function mutants of the three transcription factors exhibited major reductions of suberin in the endodermis of young roots. We also characterized an Arabidopsis steroid-inducible overexpressing MYB53 line and found that suberin can be rapidly and ectopically induced in both roots and leaves. The identification of master regulators of suberin provides the means to generate crops that are more stress resistant via enhancement of their suberized cell walls.

P8. Natural variation of disease resistance to the *Pseudomonas syringae* effector HopX1 in *Arabidopsis thaliana*. N. Hoffmann[1]*, B. Laflamme[1], D. Desveaux[1,2]. [1]Dept. of Cell and Systems Biology, University of Toronto and [2]Centre for the Analysis of Genome Evolution and Function, University of Toronto.

Pseudomonas syringae is a Gram-negative bacterial pathogen that causes disease in many plant species, including the model plant *Arabidopsis thaliana*. *P. syringae* is able to directly inject virulence proteins called effectors into plant cells through a type III secretion system to suppress plant immunity and cause disease. Plant species have evolved to recognize effectors using a diverse array of *Resistance* (*R*-) genes, which activate a robust immune response. HopX1, a member of the HopX family of effectors, is a cysteine protease that targets the jasmonic acid plant hormone pathway and suppresses immunity in susceptible plants. However, mechanisms of HopX1 detection in resistant plants remain largely uncharacterized. In order to better define the genetic and molecular basis of HopX1 recognition, we will catalog the natural variation of HopX1 resistance in an infection assay of global *A. thaliana* accessions collected by the 1001 Genomes Consortium. We hypothesize *R*-gene diversity between accessions will lead to altered disease susceptibility to *P. syringae* expressing HopX1. Isolation and genetic analysis of *A. thaliana* accessions with enhanced resistance to HopX1 will aid in further understanding of *P. syringae* recognition and in the creation of more pathogen resistant crops.

P9. An updated model of how plant growth-promoting rhizobacteria mitigate metal stress in plants. Joshua J. Frank*, S.M. Macfie. Dept. of Biology, Western University.

Stressed plants produce ethylene, which leads to stunted growth and senescence. *Pseudomonas fluorescens*, a plant growth-promoting rhizobacterium, promotes plant growth under stress conditions. The proposed mechanisms for this involve (1) metabolizing the precursor to ethylene, 1-amino-cyclopropane-1carboxylate (ACC) by the bacterial enzyme ACC deaminase (AcdS) and (2) synthesising the plant growth hormone indole-3-acetic acid (IAA). Wildtype *Pseudomonas fluorescens* UW4 and a mutant, *P. fluorescens* UW4-a*cdS*$^-$ that lacks AcdS, were used to test the hypothesis that, under cadmium and copper stress, *Arabidopsis thaliana* (Col-0) inoculated with wildtype *P. fluorescens* UW4 will have reduced ethylene and larger size than both non-inoculated control plants and those inoculated with the mutant bacterium. Furthermore, plant abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) concentrations were measured to determine whether *P. fluorescens* UW4's ethylene-lowering and IAA-producing capabilities affect other plant hormone pathways. When grown in the presence of cadmium or copper, plants inoculated with the wildtype bacterium produced 80-90% less ethylene compared to plants inoculated with the mutant bacterium and non-inoculated plants. In addition, we confirmed that *P. fluorescens* UW4 can produce IAA. While inoculation had no significant effects on plant ABA, JA and SA concentrations, the trend for plants inoculated with wildtype bacterium was to have higher concentrations of these hormones. Therefore, there is potential for hormones other than ethylene and IAA to play a role in the bacterium-plant interaction and thus the existing model has been updated accordingly.

P10. Combined transcriptomic and metabolomic approaches provide new insights into C/N partitioning in roots of *Arabidopsis thaliana.* Shrikaar Kambhampati*[1,2], S. Pandurangan[1,2], J.B. Renaud[2], R.S. Austin[2], M.W. Sumarah[2], F. Marsolais[1,2]. [1]Dept. of Biology, Western University and [2]London Research and Development Center, Agriculture and Agri-Food Canada.

Balance between carbon and nitrogen metabolism is a requirement for the sustained growth of organisms. In plant leaves, this balance is achieved by inter-relationships between photosynthesis, respiration and amino acid metabolism in a photoperiod dependent manner. The GS/GOGAT cycle is a well understood mechanism in plants known to serve as a cross-road between carbon and nitrogen metabolism. Non-photosynthetic tissues (e.g., roots, germinating seeds), however, lack a sufficient supply of carbon skeletons under high nitrogen conditions and hence may resort to other mechanisms, along with the GS/GOGAT cycle, to achieve the desired carbon/nitrogen balance. Considering the importance of asparagine as a major storage form of nitrogen, this study elucidates carbon and nitrogen partitioning within Arabidopsis roots upon asparagine treatment. Here, we propose a potential role for the GAT1_2.1 enzyme in hydrolyzing glutamine to glutamate which can serve as a carbon skeleton for channeling carbon to the TCA cycle, under high nitrogen conditions. Transcriptome analysis revealed a 4.3 fold upregulation of a class I glutamine amidotransferase, GAT1_2.1; GAT1_2.1 was shown to be highly responsive to nitrogen levels and has a root specific expression in Arabidopsis. Metabolite profiling data further strengthened the transcriptome data and suggest a major reprogramming of C and N metabolites to sustain TCA cycle.

P11. Uptake and phytotoxic effect of benzalkonium chlorides in *Lepidium sativum* and *Lactuca sativa*. Sheila M. Macfie1*, A.H. Khan[2], M. Libby[1], D. Winnick[1], J. Palmer[1], M. Sumarah[3], M.B. Ray[2]. [1]Dept. of Biology, Western University, [2]Dept. of Chemical and Biochemical Engineering, Western University and [3]Agriculture and Agri-Food Canada, London.

Extensive use of cationic surfactants such as benzalkonium chlorides (BACs), as biocides in hospitals, food processing industries, and personal care products, leads to their significant presence in natural environments, wastewater, sewage sludge and sediments. In this study, the potential for uptake and toxicity of the two most commonly used BACs to lettuce *(Lactuca sativa)* and garden cress (*Lepidium sativum)* was tested in a hydroponic system. Individual and mixed BACs at concentrations up to 100 mg L^{-1} did not affect germination; however, emergent seedlings were sensitive at 1 mg L^{-1} for lettuce and 5 mg L^{-1} for garden cress. After 12 d exposure to 0.25 mg L^{-1} BACs, plant dry weight was reduced by 68% for lettuce and 75% for garden cress, and symptoms of toxicity (necrosis, chlorosis, wilting, etc.) were visible, although plants took up less than 2% of the available BACs. Concentrations of 12 plant nutrients were measured using inductively coupled plasma-mass spectroscopy. High performance liquid chromatography-mass spectroscopy analysis showed the presence of BACs in the roots and shoots of both plant species. No conclusive relationship was established between growth inhibition, nutrient content, or BAC uptake but BAC-treated lettuce had 50% lower N and Mg concentrations, indicating that BACs might induce nutrient deficiency. Although bioavailability of BACs in hydroponics is significantly higher than that in soil, these results confirm the potential of BACs to harm vascular plants.

P12. Elucidating putative protein interactors of Pollen Acceptor Stigma Kinases (PASKs). Hyun Kyung Lee*, D.R. Goring. Dept. of Cell and Systems Biology. University of Toronto.

Plants have evolved large number of receptor-like cytoplasmic kinases (RLCKs) that interact with membrane bound receptors to modulate signalling cascades. The focus of this research is on putative RLCKs regulating the initial stages of pollen-stigma interactions in plant reproduction. The Brassicaceae family of plants have dry stigmas and employ tight regulation in accepting pollen as desiccated pollen grain requires delivery of necessary cellular components from the stigma to become metabolically active. Once pollen is captured, the stigmatic papillae initiates signalling cascades to deliver water to the pollen grain for hydration and loosen the papillar cell wall for pollen tube entry. However, key signalling components which govern these molecular changes are currently unknown. PASK1 and 2 were previously identified in the Goring lab as potential signalling RLCKs in pollen-stigma interactions as knockdown transgenic lines displayed reduced fertility. The overall goal of this project is to elucidate putative interactors of PASK1/2 as they are pseudokinases and may require other proteins to function in the stigma. Yeast two-hybrid screening of a flower bud cDNA library and yeast mating assay with potential membrane bound receptor kinases were conducted and several putative interactors were identified. To further investigate the specificity of these interactions, *in planta* interactions and knockdown and/or knockout lines will be characterized for reduced compatible pollen acceptance. Overall, this project will provide insights into the roles of PASK1/2 and their putative interactors in the stigmatic papillar signaling pathway regulating the acceptance of pollen in *A. thaliana*.

P13. Response of photosynthesis and respiration to elevated temperature and CO_2 in two boreal conifers. Sasha Madhavji*[1] and D. Way[1,2]. [1]Dept. of Biology, University of Western Ontario and [2]Nicholas School of the Environment, Duke University, Durham, NC, USA.

Global temperatures and atmospheric levels of carbon dioxide (CO_2) have been increasing steadily since the industrial revolution. The effects of climate change are expected to affect higher latitudes disproportionately, such that the Canadian boreal forest will encounter a higher-than-average temperature increase. These changing conditions will affect the mechanisms of plant mitochondrial respiration and photosynthesis. This study examined the effects of elevated temperature and CO_2 on dominant boreal tree species *Picea mariana* (black spruce) and *Larix laricina* (tamarack). Trees were grown from seed in London, Ontario in three different temperature treatments ranging from ambient to ambient +8°C, at either 400 ppm or 750 ppm CO_2. Planting was in May 2016 and data collection began in September. Photosynthesis (A) and dark respiration (R_{dark}) were measured in 5°C increments from 10°C to 40°C using a Li-Cor 6400XT.

I n both species, the T_{opt} of photosynthesis increased with growth temperature though the effect was less pronounced in tamarack. Spruce, however, showed a considerably sharper decline in A_{max} at increased growth temperatures than did tamarack. Spruce also showed a more profound suppression of photosynthesis at high growth temperatures. Both species acclimated R_{dark} at increased growth temperatures, however spruce did so much more effectively. Mortality was also markedly increased in both species in the +8°C temperature treatments. Contrasting needle strategies (spruce being evergreen, tamarack being deciduous) perhaps influence the differing mechanistic responses to global change in these two dominant boreal conifers.

P14. Anatomy, ultrastructure, and anisotropic growth of the nectar-secreting petal spur of *Centranthus ruber* (Valerianaceae). J.L.K. Mack, Art R. Davis*. Dept. of Biology, University of Saskatchewan.

About 21 angiosperm families contain some species whose flowers form spurs. Attaining a defined length per species, spurs are hollow, tubular outgrowths of the calyx or corolla and typically contain nectar. In this way, a mutualistic relationship has co-evolved between plants that form spurred flowers, and their primary pollinators (e.g., bee, butterfly, bird), typically based on tongue length. Surprisingly, few spurs have been examined for their patterns of growth and for their nectar-secreting tissues. In the ornamental species, C*entranthus ruber*, each flower bears a short spur (4.5 mm) that initiates from a zone of cells at the abaxial base of the corolla tube. After an inaugural period of cell division, cellular elongation (anisotropic growth) almost exclusively accounts for the spur's final size. Although the spur's external surface is smooth - lacking trichomes and stomata - internally the spur possesses both secretory and non-secretory trichomes. The former are unicellular and secrete nectar along the abaxial surface of the spur's entire length, whereas the non-secretory hairs may help ward off small, nectar-robbing insects. The unicellular secretory trichomes, plus the companion cells of the spur's phloem supply, exhibit wall ingrowths typical of transfer cells, but of a different type from each other. Nectar secretion begins at anthesis. Nectar-carbohydrate composition is almost 70% sucrose, and remains constant as the flower ages, wherein any uncollected nectar is eventually reabsorbed by the spur.

P15. **Plant derived RNA interference to control two-spotted spider mite (*Tetranychus urticae* Koch).** Hooman H. Namin[1*], I. Scott[2], M. Grbic[1], V. Grbic[1]. [1]Dept. of Biology, Western University and [2]Agriculture and Agri-Food Canada, London Research and Development Centre.

RNA interference (RNAi) is a natural mechanism of post-transcriptional gene regulation that results in the down-regulation of a target gene. This regulatory mechanism has been exploited to silence arthropod pest genes encoding proteins with essential functions for survival and reproduction. Among several RNAi methods, RNAi-expressing plants produce a continuous supply of double-stranded RNAs (dsRNAs) which are processed into small interfering RNAs (siRNA) upon feeding, triggering an RNAi response in herbivorous pests. This method holds a promise for the plant protection and may eventually replace chemical insecticides that are currently used.

The two-spotted spider mite, *Tetranychus urticae* (Koch), is one the most polyphagous agricultural pests in the world. It feeds on over 150 crops such as cucumbers, tomatoes, corn and beans, leading to considerable yield losses. Currently, chemical acaricides are the principal means for *T. urticae* management. However, spider mites can rapidly develop acaricide-resistance, often within two to four years after an introduction of a new product. Thus, there is a need to develop new control strategies for *T. urticae* with an independent mode of action.

In this study, five spider mite gene fragments targeting *vacuolar ATPase*, *ABC-Transporter*, *Acetylcholinestrase*, *Prospero* and *Octapamine receptor* were transferred into *Arabidopsis* plants. The potential applications of RNAi-expressing plants as a method of dsRNA delivery through digestion and the effects of these *in-planta* RNAi constructs on spider mite development, fecundity and mortality were evaluated.

P16. Plant immunology and immune homeostasis. Jacqueline Monaghan*. Dept. of Biology, Queen's University.

Plants have evolved a multi-faceted immune system to fight against pathogen infection. While necessary for survival, pathogen perception and the activation of immune responses are energetically taxing for the host and have been linked to considerable fitness costs. Although defense signaling pathways must therefore be tightly regulated, very little is known about the biochemical mechanisms that tailor signaling to maintain cellular homeostasis. Our new research program at Queen's University focuses on understanding the basic mechanisms that allow plants to defend against a vast array of potential pathogens while maintaining normal growth and development. To this end, our work address the following biological questions using varied approaches: (1) What is the role of and interplay between different post-translational modifications on proteins involved in immune homeostasis? (2) What are the key regulators maintaining immune homeostasis and how do they function biochemically at the molecular level? (3) What developmental pathways are affected by immune signaling? Understanding the complexity of signaling events that underlie immune systems is integral to combating plant diseases that threaten food security world-wide.

P17. **Establishment of *in vitro* tissue culture and transformation methods in apple grown in Canada.** Yang Qiu[12*], M. Lu[2], A. Svircev[3], L. Tian[2]. [1]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, [2]Agriculture and Agri-Food Canada, London, and [3]Agriculture and Agri-Food Canada, Vineland.

Development of plant *in vitro* regeneration and efficient genetic transformation systems for apple grown in Canada is important to modify the agricultural traits by modern biotechnology such as genome editing. Apple *in vitro* regeneration and genetic transformation are cultivar dependent. Establishing "clean" apple *in vitro* culture using explants collected from fields is always challenging due to contamination of various microorganisms residing in plant issues. We tested plant tissues collected at different times of a year from field. Plant tissues collected in summer months usually resulted in heavy contamination in tissue culture. On the other hand, contamination rate decreased significantly when plant tissues were collected in spring. The browning induced by phenol substances is another serious problem. We tested different time lengths of disinfection, tested liquid medium, solid medium added with activated charcoal. Disinfection time of 30 minutes, followed by culture on liquid medium and then solid medium with charcoal appeared to be effective for preventing tissue browning. We have successfully established *in vitro* culture for two apple varieties, Fuji and Macintosh. Clean tissue culture can be well maintained and efficiently proliferated and propagated *in vitro*. Upon transfer to rooting medium, plantlets can develop. We then initiated research to develop genetic transformation system using plantlets established in tissue culture. The leaf strips were inoculated with *Agrobacterium tumefaciens* strain GV3101 carrying the binary vector pCambia3301 with *GUS* reporter gene and *bar* gene selectable marker. The infected explants were transferred to the medium containing BASTA to select transformed tissues. The regenerated plantlets will be analyzed to confirm transformation.

P18. Novel techniques to analyze protoplast calcium signalling in *Arabidopsis* and *Nicotiana* species. Van Phan[1*], M. Toyota[2,3], T.A. DeFalco[1], W. Moeder[1], K. Yoshioka[1,4]. [1]Dept. of Cell & Systems Biology, University of Toronto, [2]Dept. of Botany, University of Wisconsin, [3]PRESTO, JST, Japan, and [4]CAGEF, University of Toronto.

Cytosolic calcium (Ca^{2+}) elevation is an early signalling event in plant cells in response to environmental cues, such as abiotic and biotic stimuli. Studies using fluorescent genetically encoded Ca^{2+} indicators to visualize cellular Ca^{2+} levels have advanced our knowledge of such Ca^{2+} signalling. However, the use of protoplasts to study plant Ca^{2+} signalling remains under-developed. Here we explore the use of *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Nicotiana tabacum* leaf mesophyll protoplasts, each derived from stable transgenic lines expressing a GCaMP Ca^{2+} indicator. Using fluorescence microscopy, variations of Ca^{2+} signals in protoplasts can be imaged and assessed by both quantitative and qualitative approach to examine the effects of exposure to both abiotic and biotic stimuli on signalling in protoplasts. This use of diverse leaf mesophyll protoplasts provides a versatile method to elucidate the Ca^{2+} signalling in response to various stress elicitors.

P19. A large-scale study of the wheat transcriptome and its regulation. Elie Raherison[*], L. Lukens. Plant Agriculture, University of Guelph.

Studies of the wheat transcriptome and its regulation have contributed to identifying the genetic bases for trait diversity. Natural variation in gene expression is also central to crop diversification, and positive selection has acted upon gene regulatory loci. Here, we describe a pipeline to identify wheat transcripts, discover the regulatory loci controlling transcript abundances, and test transcripts for evidence of positive selection. We have harvested RNA from 154 genotypes drawn from a doubled haploid population derived from a cross between an early wheat Red Fife and a recent cultivar (Stettler). We are sequencing 30 million 100nt paired end reads from each genotype. Our analysis pipeline first involves TopHat and BowTie for read alignment, transcript identification, and transcript abundance estimation. Given the depth of sequence data in this study, we expect to detect low level transcripts with very high precision. We will use GATK for SNP calling, MadMapper and MSTmap to construct a genetic map, and non-parametric interval mapping to map eQTL. eQTL data will highlight the genetic basis for gene transcript regulatory control. We expect genetic variation at regulatory factors located at the same loci in different genomes to often act to regulate gene expression levels. We also expect genetically variable transcripts are those that are expressed at low levels and are environmentally responsive. We will also use a novel test for selection on gene regulation by determining if one parent's regulatory loci have consistent effects on gene expression. Finally, we will determine if eQTL overlap QTL for traits including chlorophyll content, leaf area, photosynthesis, respiration, biomass and spectral reflectance.

P20. Plant characterization using advanced sealed environment technology. M. Stasiak[1*], R. Quiring[2], P. Lyssa[3], M. Dixon[1]. [1]Controlled Environment Systems Research Facility, University of Guelph, [2]Conviron, Winnipeg, MB, and [3]Intravision Group AS, 1367 Snarøya, Norway

Building on technological advancements in growth chamber technology (Guelph BlueBox), a hardware collaboration between the University of Guelph's Controlled Environment Systems Research Facility, Conviron and Intravision Lighting has resulted in the development of a sealed environment chamber capable of high resolution measurement of whole plant photosynthesis and evapotranspiration. By precisely manipulating the environment conditions within the chamber, detailed plant/environment responses to light (intensity and quality), carbon dioxide concentration, vapour pressure deficit and temperature can be easily obtained. Plant environment response is unique between plant species and there are often notable differences between cultivars developed for improved growth characteristics such as drought, insect and disease tolerance. These differences can be demonstrated in time periods considerably shorter than a typical field season, allowing for improved analytical throughput for phenotypic characterization.

P21. Discovery and transmission of mutations in a chimeric cucumber population. Danielle Schnekenburger[1,2]*, M. Pautler[2], T. Banks[2], J. O'Neill[2], R. LeBlanc[2], P. Pauls[1], D. Somers[2]. [1]Dept. of Plant Agriculture, University of Guelph and [2]Dept. of Applied Genomics, Vineland Research and Innovation Centre.

Mutation breeding has been a popular approach to crop improvement for nearly a century. More recent approaches to mutation breeding, such as TILLING (targeted induced local lesions in genomes), aim to discover point mutations in individuals of a large population. Creating these large variant populations is a resource intensive process that requires advancement to the second generation (M2) before genetic screening can occur. This is necessary, because M1 plants possess multiple genotypes as a result of the action of the chemical mutagen ethyl methanesulfonate (EMS) on meristem tissue during the seed treatment process and it is unknown which mutations will be stabilized in M2 progeny. Using cucumber (*Cucumis sativus*) as a model, this study aims to establish a reliable method for detecting mutations in specific genes in M1 plants that might transmit into the M2. Despite a high level of chimerism, we were able to detect point mutations in target genes using Deep Variant Scanning in a population of 3072 M1 plants. The stability of these mutant lineages was tracked in several plants by repeated tissue sampling and genotyping using high-resolution melting analysis. Successful transmission of the mutant allele to the M2 generation was observed in the progeny of two plants. Techniques to encourage formation of uniform sectors will be further explored. These methods will form the basis of a reverse genetics platform used to develop traits such as disease resistance for the Canadian greenhouse industry.

P22. High throughput chemical screening using an Arabidopsis ShHTL7 system to identify strigolactone signaling agonists. Asrinus Subha*, S. Toh, S. Lumba, P. McCourt. Dept. of Cell and Systems Biology, University of Toronto.

Strigolactones (SLs) are newly discovered collection of terpenoid-derived small molecule hormones that control germination and shoot architecture in Arabidopsis. Unfortunately, SLs have been co-opted as a germination cue enabling parasitic plants of the genus *Striga* to coordinate their life cycles with their host plants. For example, *Striga hermonthica* has evolved strong seed dormancy, which is broken only when they sense SLs extruded from host roots. Upon germination, *Striga* attaches to the host root and depletes it of nutrients. In sub-Saharan Africa alone, *Striga* has infested up to two-thirds of the arable land affecting over 100 million people in 25 countries. Chemical control has mostly followed the idea of making SL mimics that germinate *Striga* in the absence of a host resulting in the death of these obligate parasites. High throughput chemical screening (HTS) could identify potent and inexpensive SL analogs that germinate *Striga* seed. However, screening directly for compounds that germinate *Striga* seeds is problematic because *Striga* seeds vary greatly in their SL responsiveness, and therefore are not amenable to consistent high throughput screening methodologies. The identification of *ShHTL7* as a picomolar sensitive SL receptor in Arabidopsis allows the design of such a system. I have developed a germination assay using Arabidopsis *ShHTL7* seeds in a 96 well format for HTS of SL agonists and have screened approximately 4000 compounds and identified 5 leads that germinate this line. More importantly, of the 5 leads found from HTS, one compound was found to initiate germination of the obligate parasitic plant *Striga.*

P23. The negative effect of high temperature on pollen development in *Camelina sativa* offers direction for identifying novel genes for thermotolerance. Cameron So*, T.L. Sage, V. Lundsgaard-Nielsen. Dept. of Ecology and Evolutionary Biology, University of Toronto.

The oilseed crop *Camelina sativa* has recently sparked interest as a sustainable biofuel. Oil production in *Camelina* is comparable to that of canola and greater than that of soybean. *Camelina* also benefits from a short growing season and resistance to many common biotic and abiotic stresses. Despite this, *Camelina* yields are reduced by even moderately elevated temperatures (>32°C). We used a population of recombinant inbred lines (RILs) to assess variation in thermotolerance and to test the hypothesis that high temperature negatively affects reproduction in *Camelina*. Surprisingly, the parental lines were found to be more temperature tolerant than any individuals in the RIL population. Our results indicate that for every degree increase in temperature, seed set decreases in the RIL population by approximately one seed. To determine the cause of reduced seed set at high temperature (HT; 35°C), we assessed both male and female reproductive traits. Reductions in seed set at HT were attributed to decreased pollen deposition, and to a lesser extent, reduced ovule initiation. This suggests that HT has an adverse impact on pollen development, a response documented in many agronomic species. The publication of the genome and genetic marker data of *Camelina* has advanced molecular genetic analyses in this oilseed crop. Molecular markers allow for the assessment of genetic variation within a species, leading to the identification of novel genes for traits of interest. Our study identifies a significant factor contributing to yield stability in *Camelina* and paves the way for identifying novel genes involved in pollen thermotolerance.

P24. Artificial selection for cold tolerance in garden roses. E. Derivry[1], P. Sandhu[2], C. Rouet[2], D.J. Somers[2]*. [1]Dept. of Agrosciences, Universite d'Avignon, [2]Dept. of Applied Genomics, Vineland Research and Innovation Centre.

Canada's national landscape rose breeding program is located at the Vineland Research and Innovation Centre in Vineland ON (USDA climate zone 5). This breeding program is unique in the world for its history of producing some of the most cold tolerant roses available on the global market. This was achieved because the rose breeding program was previously located in Morden MB (zone 3) where there was natural pressure for cold tolerance. Vineland wishes to continue the successful selection and release of cold tolerant varieties but needs a cost effective, artificial method to pre-screen progeny prior to multi-year testing in western Canada (zone 2-3). We chose to examine the use of electrolyte leakage as a measure of cellular damage due to freezing in plant tissues which are subjected to programmed freezing. A number of variables were tested including leaves vs stems, duration of pre-acclimation at 4C and amount of replication needed. Commercial roses known to be adapted to different environments (zones 3-6) showed a good correlation between the known climate zone and the degree of electrolyte leakage / cellular damage after freezing. In addition, several elite rose selections were rated for winter survival and cold tolerance from a nursery in Saskatoon SK and Olds AB between 2014-2016. Again the correlation between replicated nursery trials for cold tolerance with electrolyte leakage (r2=0.73) was strong. We are confident this artificial approach will be satisfactory to pre-screen progeny in Vineland prior to winter testing in Saskatoon SK.

P25. Assessment of *cpn60* PCR amplicon-based phylogeny for subgenus bacterial and eukaryotic community profiling. F. Kanji[1]*, B. Haug[2], M.G. Links[3], T. Dumonceaux[3], E.L. McCarthy[2], S.M. Hemmingsen[2]. [1]Dept. of Biology, McMaster University, [2]National Research Council Canada and [3]Agriculture and Agri-Food Canada.

PCR-amplifying microbial phylogenetic markers using template DNA extracted from rhizosphere, phyllosphere or plant material, sequencing the amplicons, and matching the resulting amplicon sequences to previously classified sequences has emerged as a cost-effective tool for profiling distinct plant-associated microbial communities. While published amplicon-based microbial community profiling studies overwhelmingly use a region of the gene encoding 16S ribosomal RNA (rRNA) as a bacterial phylogenetic marker, several intrinsic characteristics of this gene make it unsuitable for providing phylogenetic classification at the subgenus level. Thus, the Beneficial Biotic Interactions pillar of the Canadian Wheat Alliance between Agriculture and Agri-Food Canada, the University of Saskatchewan, the province of Saskatchewan, and the National Research Council Canada routinely employs a microbial community profiling technique based on amplicon sequencing of the Group I chaperonin-encoding gene *cpn60*. Nonetheless, here we show that a *cpn60* amplicon-based bacterial community profile created using a standard primer mix is significantly different than a similarly-prepared profile based on 16S rRNA gene sequences. As well, while the *cpn60* amplicon-based profile can target eukaryotic OTU, we have found that eukaryotic OTU are not well represented in *cpn60* amplicon libraries. Because a robust method for species-level microbial community profiling of distinct plant systems can highlight microorganisms implicated in plant physiology and disease, investigating the bias against eukaryotic OTU representation in *cpn60* amplicon libraries presents an important lead for future work.

P26. An unbe-leaf-able model: Transient expression assays in Nicotiana benthamiana. Lauren E. Grubb*, A. Thomson, K.R. Siegel*, D.R. Holmes*, J. Monaghan. Dept. of Biology, Queen's University.

The Australian native *Nicotiana benthamiana* is a model organism in plant virology, molecular biology, and biochemistry. While several factors have been attributed to its broad use, *N. benthamiana* is primarily recognized for its susceptibility to viruses and other pathogens, and its ease of genetic transformation. Here, we will discuss how we utilize *Agrobacterium tumefaciens*-mediated transient transformation of *N. benthamiana* to express *Arabidopsis thaliana* proteins in our lab at Queen's University. We use this method routinely to facilitate protein-protein interaction studies such as split-luciferase assays and co-immunoprecipitation, as well as subcellular localization using proteins tagged with fluorescent epitopes. We will also discuss utility of this system to screen mutant allele constructs based on over-expression coupled with a functional assay

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