CSPB / SCPV Eastern Regional Meeting

November 22nd & 23rd · Brock University · St. Catharines, Ontario

The Canadian Society of Plant Biologists

La Société canadienne de biologie végétale

Welcomes you to the ***Niagara Region***

Program Booklet

Message from the Organizing Committee Chair

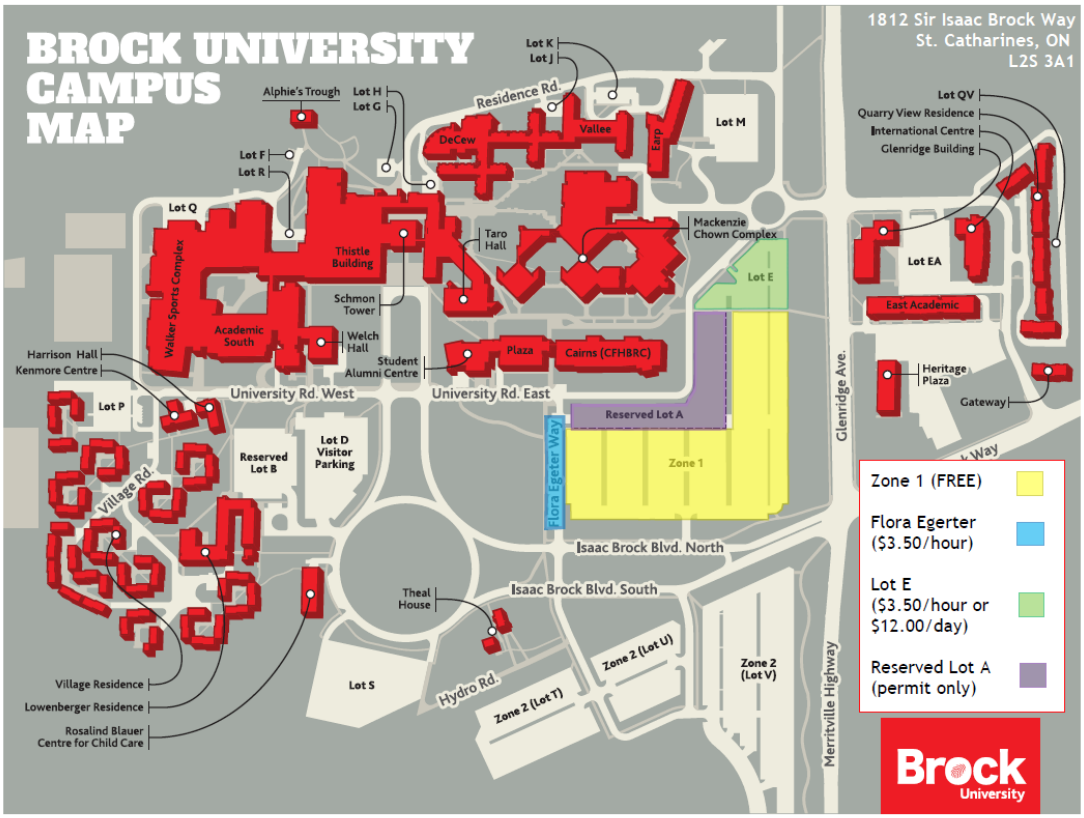
Dr. Vincenzo De Luca
Professor, Biological Sciences

Dear Meeting Participants:

Welcome to Brock University for the 2019 Eastern Regional Meeting of the Canadian Society of Plant Biologists. The last time the meeting was held at Brock was in 2010, and those of you who attended the 2010 meeting will notice how much this Niagara-based University has changed. The large glass-encased structure you will see upon your arrival on campus is the ***Cairns Family Health and Bioscience Research Complex*** **(CFH&BRC)**. Cairns complex is home to laboratories for the departments of Biology, of Chemistry and of Applied Health, in addition to hosting some McMaster University extension services and other Brock University departments. This state-of-the-art building was completed in 2012 and contains a modern greenhouse and plant tissue culture facility on the fifth floor in addition to a growth chamber farm and excellent NMR and mass spectrometry facilities. We will welcome registrants who arrive on **Friday** evening between 6:00 pm to 9:00 pm in the atrium of the Cairns complex, where you can enjoy hors d'oeuvres and complimentary drinks (wine, beer, soft drinks, and water). For those of you who are interested, we may arrange visits to some of the modern laboratories and the greenhouse during the welcome cocktail hour.

The following day, on **Saturday**, we will meet in the ***Academic South building***. You will notice that the campus is undergoing further renovations and construction, and we apologize for any inconvenience. The Academic South building is towards the back of the campus and adjacent to the Walker Sports Complex. There will be a light breakfast available from 7:30 to 9:00 am before the conference begins in AS202. We are expecting two excellent talks from our plenary speakers, Dr. David Smith and Dr. Isabel Desgagné-Penix. There will be 18 oral presentations in 2 concurrent sessions, in the morning and the afternoon. This year, we are proud to have 45 poster presentations and an hour and thirty minutes for everyone to absorb all the rich knowledge gathered within those posters. We are hoping that our students and fellow researchers will have a great opportunity to learn and to share their findings. So, from the organizing committee and our volunteers, we look forward to hosting you at Brock University on November 22nd & 23rd. Welcome to St. Catharines!

Map



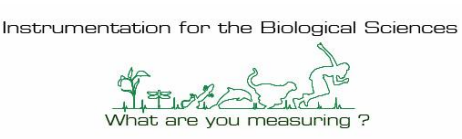
Friday & Saturday - park for *free* in <u>zone 1</u> (highlighted in yellow)

***Friday* welcome cocktail hour held in <u>Cairns complex (CFH&BRC)</u>**
***Saturday*, all events held in <u>Academic South building (AS)</u>**

For Saturday, if you park your car in Zone 1 in front of the Cairns complex, you can walk along University Road east (5 min, 400 m) past the General Brock Statue, past the Schmon Tower (tallest bulding on campus), and the David Howes theater to reach the Academic South building.

Parking is also available closer to AS in Lot D ($12.00 per day)

Sponsors



Organizing Committee

Dr. Mehran Dastmalchi, Postdoctoral Fellow, Biological Sciences, Brock University

Dr. Vincenzo De Luca, Professor, Biological Sciences, Brock University

Dr. Charles Després, Professor, Biological Sciences, Brock University

Dr. David Liscombe, Research Scientist, Vineland Research & Innovation Centre & Adjunct Professor, Brock Unversity

Schedule

Friday, November 22nd
Venue: The Atrium of the Cairns Family Health and Bioscience Research Complex

6:00-9:00 pm
- Registration
- Conference mixer with various hors d'oeuvres and refreshments

Saturday, November 23rd
Venue: Academic South block (AS)

7:30 - 9:00 Registration, coffee, light breakfast (**Atrium, AS**)
Poster and sponsor setup (**Atrium AS**)

9:00 - 9:10 Welcome remarks (Tim Kenyon, Vice President Research) (**Room AS202**)
9:10 - 9:25 CSPB Bylaw amendment issues and vote (Daphne Goring) (**Room AS202**)
9:25 - 9:30 Welcome remarks by Vincenzo De Luca (head of Organizing committee) (**Room AS202**)

9:30 - 10:20 Plenary Lecture (**AS202**)
Dr. David Smith
Stranger things: the organelle genome edition

10:20 - 10:40 *Coffee Break* **(Atrium AS)**

10:40 - 11:55 Concurrent Sessions I **(AS202 & AS203)**

AS202 **Chair: Dr. Jacqueline Monaghan**
- 10:40 - 10:55 – **Nicolas Dimopoulos** How does intraspecific variation between different white spruce (*Picea glauca* L.) genotypes affect their responses to extreme climate stress events?
- 10:55 - 11:10 – **Joshua Frank** Root-associated fungi alter tree growth and phytohormone concentrations under elevated temperature and CO_2
- 11:10 - 11:25 – **Dr. Aravind Harikumar** An approach to classification of tree genotypes in Spruce forests using UAV based hyperspectral remote sensing time-series data
- 11:25 - 11:40 – **Dr. Vera Velasco** Photosynthesis and chlorophyll fluorescence of coastal and interior Douglas-fir under extreme heatwave
- 11:40 - 11:55 – **Christopher Wong** Remotely tracking the phenology of photosynthesis using carotenoid sensitive vegetation indices in a temperate evergreen and mixed deciduous forest

AS203 **Chair: Dr. David Liscombe**
- 10:40 - 10:55 – **Christine Kempthorne** Role of pyrrolidine and phenanthroindolizine alkaloids in highly invasive *Vincetoxicum rossicum*
- 10:55 - 11:10 – **Danielle Williams** Enantiomeric-specificity of hydroxylases leads to competitive inhibition between opposing monoterpendoid indole alkaloid pathways in *Catharanthus roseus* roots

- 11:10 - 11:25 – **Matthew Bergman** Distinct metabolic pathways drive monoterpenoid biosynthesis in a natural population of *Pelargonium graveolens*
- 11:25 - 11:40 – **Natalie Hoffman** Setting the stage for lignin deposition: spatial distribution and anchoring of enzymes directing lignification in Arabidopsis
- 11:40 - 11:55 – **Radesh Nattamai Malli Pooranachandhiran** Characterization of the genome structure of lavender (*Lavandula angustifolia*), an essential oil plant

11:55 - 13:00 Lunch (**Atrium AS**)
11:55 - 13:30 Executive meeting (**AS 207**)

13:00 - 14:30 Poster Sessions (**Atrium AS**)

14:30 - 15:30 Concurrent Sessions II (**AS202 & AS203**)

***AS202* Chair: Dr. Yang (Vince) Qu**

- 14:30 - 14:45 – **Emily Clayton** The secret of AtADT5 nuclear localization
- 14:45 - 15:00 – **Jenny Crick** Role of boundary three-amino-acid-loop-extension (TALE) homeodomain transcription factors in programmed separation of plant organs
- 15:00 - 15:15 – **Nathan Doner** Investigating the role of the newly-discovered Arabidopsis lipid droplet protein SENR in plant abiotic stress response
- 15:15 - 15:30 – **Victoria Lessy** Characterizing the underlying molecular mechanism of the sugarcane anti-florigen ScFT2

***AS203* Chair: Dr. Charles Després**

- 14:30 - 14:45 – **Jordan Van Brenk** All in the Family: AROGENATE DEHYDRATASE expression
- 14:45 - 15:00 – **Mark Minnow** Small RNA changes with big consequences
- 15:00 - 15:15 – **Dr. Solmaz Irani** Different tolerance of two ecotypes of the extremophyte crucifer *Eutrema salsugineum* to low phosphate
- 15:15 - 15:30 – **Bridget Murphy** Comparing the effects of extreme warming on tamarack under differing experimental designs

15:30 - 16:00 Coffee break (**Atrium AS**)
15:30 - 16:00 Deliberations for Best Poster **(AS207)** and Best Oral presentations **(AS208)**

16:00 - 16:50 Plenary Lecture (AS202)
Dr. Isabel Desgagné-Penix
Cracking the code of Amaryllidaceae alkaloid biosynthesis

16:50 - 17:20 Closing Remarks & Awards

Plenary Speakers

Dr. David Smith

Stranger things: the chloroplast genome edition

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

I have always been intrigued by and attracted to strange genomes, genomes that break all the rules and leave researchers scratching their heads. It is no surprise, then, that I have devoted my career as a researcher to exploring organelle genomes, many of which seem to show no bounds in their propensity for peculiar and noncanonical characteristics. The past five years, in particular, have been exciting times for studying unusual organelle DNAs, especially those of chloroplasts. Recent high-profile studies have uncovered some of the most interesting chloroplast DNAs on record, including ones with massive or miniature sizes, unprecedented conformations, such as single-stranded hairpins, and other surprising features, including outright genome loss. Here, I look closely at the varying extremes in chloroplast genomic architecture, with a particular focus on those from the Chlamydomonadales, comparing them to other types of genomes, and discuss how these data are changing our understanding of organelle genome evolution.

Plenary Speakers

Dr. Isabel Desgagné-Penix

Cracking the code of Amaryllidaceae Alkaloid Biosynthesis

Université du Québec à Trois-Rivières, Department of Chemistry, Biochemistry and Physics, Trois-Rivières, QC, Canada

Amaryllidaceae alkaloids (AAs) are plant specialized metabolites comprising an estimated 600 identified structures. In the AA biosynthetic pathway, norbelladline is a key branch-point intermediate that can be converted into several AA subtypes with different structural configurations. The galanthamine-type alkaloids are one subgroup of AAs produced in low quantities in different Amaryllidaceae plant species. Galanthamine is the only AA used commercially to treat the symptoms of Alzheimer's disease. RNA-seq coupled with comparative transcriptome analysis of Narcissus plant parts led to the identification of norbelladine synthase (NBS), the key biosynthetic enzyme regulating the production of norbelladine and consequently of all AAs. Next-generation sequencing and assembly of transcriptomes for several Amaryllidaceae species highlight the potential for discovery of genes involved in AA metabolism with new technologies, particularly of the galanthamine path. To expedite the alternative biological production of these compounds, an understanding of their metabolic pathway is required. While only a few genes are known, the majority of the reactions are generally catalyzed by a collection of characterized enzyme families. The knowledge of these families can help inform efforts through homology searches to identify candidate genes to crack the code of AA biosynthesis.

Abstracts · Concurrent Sessions

Part I

Venue: AS202

Photosynthesis and climate change

10:40 - 10:55

How does intraspecific variation between different white spruce (*Picea glauca* L.) genotypes affect their responses to extreme climate stress events?

Dimopoulos N, Huang K, Maho T, Zhou Y, Ensminger I
University of Toronto Mississauga, Biology Department, Mississauga, ON, Canada

Climate change will expose Canadian forests to increasingly frequent bouts of extreme weather events, often occurring in the form of heatwaves (combination of drought with acute heat episodes), and are predicted to have negative impact on coniferous trees. White spruce (*Picea glauca* L.) is an abundant coniferous tree native to Canada, and being genetically diverse leads to intraspecific variation that can cause individuals to respond differently to stresses. Heatwaves can be simulated using temperature free-air enhancement (T-FACE) field experiments that factorially combine heat and drought stress together. In 2020, we plan on studying the effect of intraspecific variation on the stress response of different white spruce genotypes to heatwave (heat + drought) stress in contrast to drought, heat, and control field conditions over 7 weeks in the growing season. Populations of 10 different genotypes of 4-year old white spruce seedlings will be studied to see how they respond in terms of mortality, morphology, photosynthetic gas exchange, photosynthetic pigment and cuticular wax composition, and differential transcriptome regulation. In preparation, growth and phenological measurements over the 2019 growing season were conducted and have shown intraspecific differences in the growth rates between the different genotypes. We hypothesize that heatwave stress will have more severe detrimental effects on plant stress responses than drought or heat stress individually, and that slower growing genotypes will be less affected than faster growing ones when under stress.

10:55 - 11:10

Root-associated fungi alter tree growth and phytohormone concentrations under elevated temperature and CO2

Frank JJR[1], Abou-Zaid M[1,2], Ramsfield, T[1,3], Way D,[1,4,5]
[1]Department of Biology, Western University, London, ON
[2]Great Lakes Forestry Centre, Natural Resources Canada, Ottawa, ON

[3]Northern Forestry Centre, Natural Resources Canada, Edmonton, AB
[4]Nicholas School of the Environment, Duke University, Durham, NC
[5]Terrestrial Ecosystem Science & Technology Group, Environmental & Climate Sciences Department, Brookhaven National Laboratory, NY

Growth of *Populus spp*., an ecologically and economically important group of trees, has been declining due to elevated temperatures and droughts associated with climate change. Symbiotic microbes, such as root-associated fungi (RAF), may increase plant growth under climate change conditions by altering tree metabolic profiles and increasing tree access to water and nutrients. To address this hypothesis, three RAF were isolated from *Populus tremuloides* roots in the field. We then determined the effects of RAF inoculation on hybrid poplar (*Populus x canadensis*) growth under a range of future climate scenarios: ambient CO_2 (400 ppm) or elevated CO_2 (750 ppm) with either ambient temperatures, or a +4 ˚C or +8˚C warming treatment. Plant metabolites were extracted from poplar tissues (roots, stems and leaves) and analyzed using HPLC-MS. Colonization of poplar roots by RAF increased with elevated temperature and CO_2 with some RAF having up to a 336% increase. Inoculation with RAF did not increase tree height or total mass, with the exception of trees grown under moderate (+4 ˚C) warming, where total biomass increased ~15% compared to trees from current conditions. RAF inoculation increased the concentration of jasmonic acid by up to 25% and decreased salicylic acid concentrations ~10% under all climatic conditions. Abscisic acid concentrations decreased up to 40% when compared to non-inoculated controls, with the highest decrease induced under extreme warming conditions. Our results suggest that RAF increase tree growth under moderate warming and affects plant hormone regulation, which may affect resilience to future climatic stresses.

11:10 - 11:25

An approach to classification of tree genotypes in Spruce forests using UAV based hyperspectral remote sensing time-series data

Harikumar A, Ensminger I
University of Toronto Mississauga, Department of Biology, Mississauga, ON L5L 1C6, Canada

Spruce trees constitute majority of the global temperate and boreal forests. In addition to being carbon sinks that control the global climate, they are also major source for high quality wood and fibre that are widely used in the industry. However, spruce forests are greatly affected by variations in the climate, insects and pests. Thus, efforts to produce spruce trees which are resistant to both biotic and abiotic stress are essential to survive global climatic change. The biophysical parameters of tree depend on the genotype, and thus identifying the genotype of a tree is an important input to spruce breeding and reforestation programs such as the Spruce-Up. Hyperspectral remote sensing data contain a huge amount of details that can be used to accurately quantify several biophysical parameters of trees, including leaf water content, leaf chlorophyll concentration and evapotranspiration. Here, we quantify the parameters using the

Leaf Water Content Index, and the Normalized Difference Red-Edge index, and the Normalized Difference Vegetation Index feature respectively. In this study, we use high resolution hyperspectral data acquired using the Nano-Hyperspec sensor mounted on an Unmanned Aerial Vehicles (UAV) platform to perform the analysis. The temporal information required for the study is derived from data acquired in mid-spring, mid-summer and mid-autumn periods. To this end, we plan to capture the unique spectral signatures of 10 different tree genotypes for which we have the field-collected data to do validation. An automatic framework to delineate and classify individual trees would be developed using a machine learning framework.

11:25 – 11:40

Photosynthesis and chlorophyll fluorescence of coastal and interior Douglas-fir under extreme heatwave

Velasco VME, Sen T, Noordermeer D, Ensminger I
University of Toronto, Department of Biology, Mississauga, ON, Canada

Heatwaves are extreme climatic events characterized by prolonged drought followed by concurrent intense heat and drought. The occurrence of these extreme climatic events are predicted to become more frequent and more intense in the future. Here, we simulated extreme drought, heat and heatwave conditions in greenhouses and investigated their effects on photosynthesis and chlorophyll fluorescence in Douglas-fir originating from contrasting local climates. A total of four coastal and interior Douglas-fir provenances were used in the study and were exposed to four weeks of 22°C/13°C (C) or 40°C/33°C day/night (H) temperatures. One subset of the plants was watered regularly (CW, HW) and another was withheld water (CD, HD). Plants exposed to four weeks of CD, HW and HD had lower water potential (Ψw) and relative water content (RWC), however we only observed wilting on plants exposed to HD. Interestingly, coastal relative to interior provenances had less negative Ψw even with similar RWC. We also observed inhibition of maximum photosynthetic rates under CD, HW and HD, likely resulting from lower stomatal conductance. Light energy balance was also altered under CD, HW, and HD. Relative to interior, coastal provenances showed a large decrease in the proportion of light used towards photosynthesis with subsequent increase in light dissipated as heat or light. All together, our data presents varying levels of responsiveness to stress among Douglas-fir, suggesting that these plants have different capacities to tolerate extreme heat and prolonged drought events through modulation of photosynthesis and regulation of light energy balance.

11:40 – 11:55

Remotely tracking the phenology of photosynthesis using carotenoid sensitive vegetation indices in a temperate evergreen and mixed deciduous forest

Wong CYS[1,2], Ingo Ensminger[1,2,3]

[1]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada
[2]Graduate Program in Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
[3]Graduate Program in Cells and Systems Biology, University of Toronto, Toronto, ON, Canada

Photosynthetic phenology is an important controller of annual gross primary productivity (GPP). Assessing photosynthetic phenology remotely with traditional greenness-based techniques is difficult for evergreen conifers as they remain green year-round. Carotenoid-based vegetation indices such as the photochemical reflectance index (PRI) and chlorophyll/carotenoid index (CCI) are promising tools to remotely track the invisible phenology of photosynthesis by assessing carotenoid pigment dynamics. PRI and CCI may act as proxies of photosynthetic efficiency (ε) and photosynthetic activity, respectively, important parameters in light-use efficiency (LUE) models. To understand the physiological mechanisms reflected by PRI and CCI and their ability to act as proxies of photosynthetic activity, we measured leaf pigment composition, PRI, CCI, and photosynthetic activity over 2-years in an evergreen and mixed deciduous forest. PRI and CCI captured the large seasonal carotenoid/chlorophyll ratios changes and were good proxies of ε and photosynthetic activity, respectively. We propose that carotenoid-based vegetation indices may provide a useful proxy of photosynthetic activity and could improve remote sensing-based models of GPP in evergreen and deciduous forests.

Venue: AS203
Specialized Metabolism

10:40 - 10:55

Role of pyrrolidine and phenanthroindolizine alkaloids in highly invasive *Vincetoxicum rossicum*

Kempthorne C[1,2]. Kanellis V[3], McNulty J[3], Liscombe D[1,2]
[1]Brock University, Department of Biotechnology, St. Catharines, ON, Canada
[2]Vineland Research and Innovation Centre, Biochemistry, Vineland Station, ON, Canada
[3]McMaster University, Department of Chemistry, Hamilton, ON, Canada

Invasive plants have devastating effects on Canadian ecosystems and agriculture. *Vincetoxicum rossicum*, also known as dog-strangling vine, is a restricted species in Ontario exhibiting resistance to native herbivores and pathogens, outperforming other species, and difficult to control mechanically or with current herbicides. *V. rossicum* forms dense monocultures in North America and produces unique pyrrolidine and phenanthroindolizidine alkaloids (PIAs) – a class of compounds demonstrating allelopathic and cytotoxic effects in plant and mammalian model systems – that we hypothesize are contributing to its invasiveness. In this work we use high-resolution mass spectrometry to examine the chemical diversity of *V. rossicum* populations and related invasive *Vincetoxicum nigrum*, assay for phytotoxic

properties of purified compounds and extracts, and begin to elucidate PIA biosynthesis through non-targeted metabolite profiling and stable isotope labelled precursor studies. We also discuss work in progress to identify genes that may serve as targets for the metabolic engineering of these alkaloid pathways. Ultimately, understanding the chemistry of *V. rossicum* enables us to harness potentially valuable natural products for the control of other weedy species and provides unique targets for species-specific herbicides in the control invasive *Vincetoxicum* spp.

10:55 - 11:10

Enantiomeric-specificity of hydroxylases leads to competitive inhibition between opposing monoterpendoid indole alkaloid pathways in *Catharanthus roseus* roots

Williams D, Qu Y, De Luca V
Brock University, Department of Biological Sciences, St. Catharines, ON, Canada

Investigations into the biosynthesis of monoterpenoid indole alkaloids (MIAs) in the medicinal plant, *Catharanthus roseus*, have led to discoveries of multiple branch pathways leading to the unique MIA ring structures and diverse biological activities. The aspidosperma MIAs are the most prevalent root-specific alkaloids in *C. roseus*, and it was previously thought that these MIAs were derived through decorations of the tabersonine backbone. Recently, it was shown that separate hydrolases in *C. roseus* are responsible for the production of two aspidosperma MIAs with opposite optical rotations, (-)-tabersonine and (+)-vincadifformine, from a common intermediate (PNAS 2018, 115(12):3180-3185; Plant J 2019, 97(2):257-266). Following the formation of these opposing aspidosperma backbones, separate enantiomer-specific hydroxylases and O-acetytransferases catalyse parallel pathways in the formation of their respective derivatives, 19-O-hörhammericine and (+)-echitovenine (Plant J 2019, 99(4):626-636). Follow-up biochemical analysis showed that the hydroxylases have reduced activity when fed an enantiomeric mixture, and it was discovered that the enantiomer of (+)-vincadifformine is a reversible competitive inhibitor of V19H. The enantiomeric-specificity of these enzymes for their respective (-)- and (+)-substrates and their susceptibility to enantiomeric-inhibition sheds new light on the evolution of specialized metabolism and on the importance of taking stereochemistry into consideration in the discovery of new pathways.

11:10 - 11:25

Distinct metabolic pathways drive monoterpenoid biosynthesis in a natural population of *Pelargonium graveolens*

Bergman ME, Phillips MA
University of Toronto, Department of Cellular and Systems Biology, Toronto, ON, Canada

Pelargonium graveolens is a wild species of scented geranium (Geraniaceae) that serves as the predecessor to valuable rose-scented geraniums that are prized for their essential oils. Despite the industrial usefulness of geranium oil in fragrances and flavorings and its efficacy as an antimicrobial and acaricidal food preservative, little is known about essential oil biosynthesis in this genus. Here we present metabolic evidence that at least two distinct biosynthetic pathways are responsible for the suite of volatile monoterpenoids that impart value to geranium oil; namely the cyclic p-menthanes such as (-)-isomenthone and acyclic citronelloids such as geraniol and (-)-citronellol and their derivatives. We establish their common origin via the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway, but found no indication that these pathways share common intermediates beyond geranyl diphosphate (GDP). Untargeted volatile profiling of a natural population of seed grown *P. graveolens* lines yielded distinct chemotypes that preferentially accumulate (-)-isomenthone, geraniol, or (-)-citronellol along with a myriad of other volatile minor products. Whole plant 13CO2 isotopic labelling allowed the measurement of in planta rates of monoterpenoid accumulation in each of these chemotypic lines which further supported the independence of the p-menthane and citronelloid biosynthetic pathways. Whole plant isotopic labelling time course experiments paired with chiral GC-MS analysis of the products of the chemotypes revealed (+)-limonene via (+)-piperitone as the likely precursors to (-)-isomenthone rather than (+)-pulegone as seen in peppermint. Exploitation of this natural population enabled a detailed dissection of the relative rates of monoterpenoid accumulation in *P. graveolens* glandular trichomes.

11:25 – 11:40

Setting the stage for lignin deposition: spatial distribution and anchoring of enzymes directing lignification in Arabidopsis

Hoffmann N[1], Chou EY, Schuetz M, Samuels AL
University of British Columbia, Department of Botany, Vancouver, BC, Canada.
[1]Current address: Department of Cell and Systems Biology, University of Toronto

Lignin is a critical phenolic polymer that reinforces secondary cell walls of plant cells, enabling strength in fibers and water transportation in xylem vessel elements. Secreted enzymes, laccases (LACs) and peroxidases (PRXs), facilitate lignin polymerization by oxidizing lignin monomers called monolignols. In *Arabidopsis thaliana* there are 17 LACs and 73 PRXs and the isoenzymes involved in lignification are poorly characterized. Using spinning disk microscopy, we show that putative lignin-associated LACs and PRXs localize within distinctive cell wall domains of flowering stems. Whereas *LAC4-mCherry*, *LAC17-mCherry*, and *PRX72-mCherry* localized exclusively to the cellulose-rich secondary cell wall layers of both vessel elements and fibers, *PRX64-mCherry*, *PRX71-mCherry*, and *LAC10-mCherry* were restricted to the middle lamella and cell corners of interfascicular fibers. To test whether timing of expression dictates localization, we fused the coding sequence of *LAC4* and *PRX64* to the promoter of the cellulose synthase gene *CesA7*. *prCESA7-LAC4-mCherry* and *prCESA7-PRX64-mCherry* had altered but identical localization in the roots, indicating that timing of

expression plays a role in cell wall distribution. We next tested the mobility of *LAC4-mCherry* in the cell wall using fluorescence recovery after photobleaching (FRAP). Unlike a control secreted protein (*sec-mCherry*), which was mobile in the cell wall, *LAC4-mCherry* was mobile when ectopically expressed in the primary cell wall but immobile in the lignified secondary cell wall. Distinct spatio-temporal localization and anchoring of oxidative enzymes in cell wall domains could therefore act to control lignin deposition to cell types and regions of the cell wall during development.

11:40 – 11:55

Characterization of the genome structure of lavender (*Lavandula angustifolia*), an essential oil plant

Nattamai Malli Pooranachandhiran R[1], Sjaarda C[1], Baldwin R, Mahmoud S[2], P Liang[1],
[1]Brock University, Centre for Biotechnology, St. Catharines, ON, Canada
[2]Department of Biology, University of British Columbia-Okanagan, Kelowna, British Columbia, Canada

Lavender (*Lavandula angustifolia*) is a perennial plant native to the Mediterranean region, best known for its essential oils (EOs) that has numerous applications in various industries. Here, we report a detailed analysis of the recently sequenced, highly duplicated and complex genome, focusing on genome size, ploidy, and repeat content. The lavender genome was estimated to be around 870 Mbp (1C=0.96 pg) using multiple methods. The repeat element (RE) composition of the genome was estimated to be around 45% of the full genome or ~57% of the non-gap genome sequences. Further characterization of the REs revealed Long Terminal Repeat (LTRs) retrotransposons as the major repeat component (~18%) of the genome and an unusually high number of miniature inverted-repeat transposable elements (MITEs) (~88,000). Interestingly, unlike most other plant genomes, the lavender genome has much more Copia than Gypsy elements, both showing a trend of recent increasing activity and with Copia elements showing active participation in gene function including genes for EO production. Analysis also revealed the lavender genome with high proportion of regions at polyploidy levels, strongly biased towards regions containing EOs genes. Analysis of Ks substitution rates estimated the occurrences of polyploidization events in the lavender genome between 16 to 41 Mya, which may be partially responsible for the presence of high copy numbers of EO genes in the genome. In conclusion, our results reveal the lavender genome to be highly duplicated and with past and ongoing active retrotransposition, favouring EO production.

Abstracts · Concurrent Sessions

Part II

Venue: AS202
Molecular mechanisms and localization

14:30 - 14:45

The secret of AtADT5 nuclear localization

Clayton EJ, Abolhassani Rad S, Kohalmi SE
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The essential amino acid Phe is only synthesized *de novo* by plants and microorganisms. In *Arabidopsis thaliana*, the last step of Phe biosynthesis is catalyzed by a family of six enzymes called AROGENATE DEHYDRATASES (ADTs). All AtADTs localize to the chloroplast, however AtADT5 is the only AtADT to also localize to the nucleus. Intriguingly, *in silico* and wet lab analyses have not identified a nuclear localization signal (NLS) for AtADT5. However, a yeast-two-hybrid library (Y2H) screen identified a unique interactor for AtADT5, and heterodimers localize to the nucleus. As this interactor contains a predicted bipartite NLS, we believe this interactor is “piggybacking” AtADT5 into the nucleus. To investigate this, deletion constructs were made of the interactor NLS sequence, and heterodimer localization was examined using bimolecular fluorescence complementation (BiFC). We are also interested in what sequences define this interaction. Sequence comparisons of AtADT5 and AtADT4, the most closely related AtADT to AtADT5, revealed a unique motif in the C-terminus of AtADT5. To determine if this motif is responsible for the interaction, constructs containing targeted mutations of this unique motif were generated for AtADT4 and AtADT5 to be tested with Y2H and BiFC analyses. Understanding the mechanism of AtADT5 nuclear localization will help determine its role in the nucleus, possibly as a retrograde signal or transcriptional regulator. This research will provide insight on the relationship between sequence and function of closely related family members, and how small differences in sequence can have large effects on protein localization and function.

14:45 - 15:00

Role of boundary three-amino-acid-loop-extension (TALE) homeodomain transcription factors in programmed separation of plant organs

Crick J, Corrigan L, Pautot V, Hepworth SR
Carleton University, Department of Biology, Ottawa, ON, Canada; Institut Jean-Pierre Bourgin, INRA, Versailles, France

The shedding or “abscission” of plant organs occurs at predetermined positions in the plant called abscission zones (AZs). In *Arabidopsis thaliana*, the floral organs abscise soon after pollination. Abscission is induced by a signalling network that begins with IDA peptide binding to cell surface receptors. Boundary TALE homeodomain transcription factor genes were first recognized for their role in establishing the borders that define a newly forming organ. We show here that these genes also play a role in abscission, by maintaining tightly ordered cells in the AZ where separation takes place. The AZ is composed of a layer of cells on the plant body that secretes hydrolytic enzymes and an opposing layer of lignified cells on the edge of the separating organ that acts as a brace. Progressive loss of TALE activity leads to disorganization of the AZ. Loss of neatly aligned rows causes the two layers of cells which normally separate cleanly to become staggered and interlocked. Loss of TALE activity also reduces responsiveness to abscission signals. Formation of a lignin brace is progressively impaired in combination TALE mutants. Epistasis analyses support that TALE boundary genes act downstream of the initiating signalling peptide IDA and may be involved in promoting the hydrolytic enzymes that complete the separation process. We propose that the TALE genes act both upstream and downstream of IDA and contribute to abscission by maintaining integrity of cells in the AZ.

15:00 – 15:15

Investigating the role of the newly-discovered Arabidopsis lipid droplet protein SENR in plant abiotic stress response

Doner NM, Kretzschmar FK, Seay D, Ischebeck T, Chapman KD, Dyer JM, Mullen RT
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Lipid droplets (LDs) are evolutionarily-conserved organelles that function not only in neutral lipid storage, but also in several other cellular processes, such as membrane remodeling, sequestration of nuclear factors, and stress responses. The molecular mechanisms underlying LD biogenesis, maintenance, and breakdown are largely unknown, particularly in plants, where relatively few LD proteins have been studied. To address this, proteomes of isolated *Arabidopsis thaliana* LDs and protein-protein interaction assays with known Arabidopsis LD proteins as bait were screened for new protein players in plant LD biology. Here we present the results of preliminary experiments focusing on one of several novel LD proteins identified, SENR, which is a senescence-related protein with no known function(s). Confocal microscopy confirmed that SENR is localized to the LD surface, as well as to the cytoplasm, suggesting that the protein transitions between both compartments. Furthermore, senr mutant Arabidopsis seedlings accumulated nearly twice as many LDs in their leaves compared to wild-type seedlings, indicating that SENR plays a role in LD homeostasis. SENR is constitutively expressed throughout growth and development but is highly induced during abiotic stresses, particularly drought and cold. LDs are known to proliferate in leaves during these stresses. Notably, yeast two-hybrid screens with SENR identified several regulators of abiotic stress response, suggesting that SENR might play a role

in stress response by modulating the activity of other stress-related proteins at LDs. The characterization of newly-discovered LD proteins will better our understanding of this dynamic organelle, which has functions that go beyond simply cellular lipid storage.

15:15 - 15:30

Characterizing the underlying molecular mechanism of the sugarcane anti-florigen *ScFT2*

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Flowering time is a tightly regulated process that is essential for seed production and proliferation in higher plants. The vegetative to reproductive transition is controlled by diverse cues acting through distinct genetic pathways that converge at mobile floral integrators called florigens. These conserved phosphatidylethanolamine-binding proteins (PEBPs) are synthesized in leaves and migrate to the shoot apex to form a floral activation complex (FAC), which initiates reproductive growth by activating floral organ specificity. In sugarcane (*Saccharum* spp.), several florigen gene candidates have been identified, such as *ScFT2*. Interestingly, overexpression of this gene in *Arabidopsis* disrupts the floral transition and causes a dramatic change in shoot architecture. This research aims to characterize the mechanism behind this protein to determine how *ScFT2* overexpression causes an extreme vegetative phenotype. Localization experiments and interaction assays will be conducted to determine if ScFT2 interferes with FAC formation by outcompeting endogenous PEBPs, and to identify new entities involved in floral regulation. Mutants of well characterized floral genes will be complemented with *ScFT2* overexpression to prove clues as to the potential players involved in the anti-flowering phenotype. *ScFT2* will also be transformed into other species to fine-tune flowering for agricultural applications.

Venue: AS203

Expression and phenotypic plasticity

14:30 - 14:45

All in the Family: *AROGENATE DEHYDRATASE* expression

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In plants, phenylalanine (Phe) is an aromatic amino acid and precursor to numerous specialized metabolites. Phe-derived metabolites such as lignin and flavonoids are important for plant survival and protection. The final step in Phe synthesis is performed by AROGENATE DEHYDRATASES (ADTs), which are named ADT1-ADT6 in *Arabidopsis thaliana*. Promoter sequences of each ADT have unique compositions of promoter motifs that are associated with endogenous and exogenous cues. Additionally, in silico data from publicly accessible databases show that while there is ubiquitous ADT expression, the levels of tissue-specific expression vary considerably among the ADTs. To provide more resolution to the available in silico data, in vivo research to determine transcript expression patterns for ADT promoter sequences were performed. Briefly, either the intergenic region, or up to 1 kb upstream of each ADT translational start site was cloned 5'to an eGFP/GUS reporter sequence. For intron-containing ADTs, the start of the promoter region to the end of the first intron was also cloned. The resulting eight constructs were stably transformed into wildtype Arabidopsis, and expression of the reporters was visualized. Plants were grown under standard growth conditions with a 16/8 hr light/dark regime and select tissues were collected at different developmental stages. Data will be presented to show if and how ADT expression patterns differ. The goal is to create a comprehensive, high-resolution catalogue of ADT expression patterns in Arabidopsis tissues over developmental time.

14:45 - 15:00

Small RNA changes with big consequences

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Plant small RNA (sRNA) regulates gene expression by repressing mRNA translation or by triggering DNA methylation that can inhibit transcription. The phloem contains sRNA, but it is not known whether sRNA can move from the phloem to regulate gene expression in the shoot apical meristem (SAM). We designed a transgenic synthetic biology scheme to investigate sRNA trafficking from the phloem to the distal-most stem cells in the SAM. This system provides evidence of phloem-to-SAM sRNA movement. Unexpectedly, crosses involving these transgenics revealed a wide range of unexpected transgressive phenotypes. This phenotypic instability was apparent in F2 populations and affected many aspects of shoot morphology including sterility, seedling lethality, altered flowering time, aberrant siliques and meristem defects. Importantly, this instability was observed in F2 populations derived from close relatives that should be genetically identical, suggesting a possible underlying epigenetic cause. In contrast, individuals that are habitually selfed do not exhibit the same level of phenotypic variability in their progeny. These results raise some intriguing questions, although further study of this system is required to unveil what contributes to this morphological variation.

15:00 – 15:15

Different tolerance of two ecotypes of the extremophyte crucifer *Eutrema salsugineum* to low phosphate

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Eutrema salsugineum is halophytic relative of *Arabidopsis thaliana* with a close phylogenic relationship to Brassicaceae crops. In the present study we compared two *E. salsugineum* ecotypes originating from the Yukon, Canada and Shandong, China for their capacity to cope with low phosphate (Pi). High tolerance to low Pi has been reported for Yukon E. salsugineum but this trait has not been studied in other E. salsugineum ecotypes. We compared root growth and architecture for Yukon and Shandong seedlings grown on a gel medium lacking added Pi (0 mM Pi) versus supplemented with 0.5 mM Pi. Shandong seedlings were intolerant to 0 mM Pi showing overall reduced growth, total root length and root biomass relative to Yukon seedlings. The addition of 100 mM NaCl to gel media did not alter the accession-specific, Pi-responsive, root architecture. Histochemical staining for endogenous phosphatases in roots of seedlings grown on 0 mM Pi gel showed lower phosphatase activity in roots and root hairs of Yukon relative to Shandong. Total Pi content on a fresh weight basis in both roots and shoots of Shandong was approximately 1.5-fold lower relative to Yukon plants. Our results show that Shandong and Yukon *E. salsugineum* plants are differentially tolerant to low-Pi, an unexpected phenotypic distinction given that both are extremophytes. The differential response to low-Pi between the ecotypes provides an opportunity to use comparative functional genomics to identify genetic targets associated with an efficient use of Pi for improving nutrient use of crops.

15:15 - 15:30

Comparing the effects of extreme warming on tamarack under differing experimental designs

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Trees will respond in adverse ways to future shifts in temperature with climate change. Previous studies have found plants may have the ability to acclimate to increasing temperatures. However, in nature, one of the more extreme responses observed is a greater frequency of climate-induced mortality events. While interactive effects of drought and heat stress have been studied, there is little known about the impact of heat stress alone on tree mortality. We grew tamarack under two experimental designs to study heat stress-induced mortality. In the first experiment, seedlings were grown in glasshouses under ambient (400 ppm) and elevated (750 ppm) CO2 concentrations combined with ambient (average daily London, ON temperatures), ambient +4 ˚C, and ambient +8 ˚C growth temperatures. In the second experiment, seedlings were grown in growth chambers under ambient (400 ppm) and

elevated (750 ppm) CO2 concentrations combined with ambient (24/11˚C) and elevated (32/19˚C) diurnal growth temperatures. We examined seedling carbon fluxes to investigate whether high growth temperatures lead to carbon limitations and mortality. The glasshouse experiment found +8 ˚C warming led to considerable mortality when paired with ambient CO2, whereas the growth chamber experiment had no mortality. Evaluation of carbon fluxes were contradictory as well. The glasshouse experiment showed no acclimation of photosynthesis, but thermal acclimation of respiration. Comparatively, the growth chamber experiment had no acclimation of respiration, but thermal acclimation of photosynthesis. Overall, the lower light intensity and constant temperature conditions in the growth chamber were less realistic than the glasshouse conditions.

Abstracts

Posters

#1

Phytochrome regulation of nitrate accumulation is an early mechanism of resource-independent weed-crop competition

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Neighbouring weeds compromise light quality (red to far-red ratio; R/FR) and severely impact early development of crop plants in the absence of direct resource competition with a lasting effect on yield potential via unknown mechanisms. To gain insight into these mechanisms, early physiological responses of soybean, maize and Arabidopsis to neighbouring weeds were characterized using a biological weedy system that eliminates direct resource competition. An early response of soybean, maize and Arabidopsis to neighbouring weeds was nitrate accumulation in root and shoot tissues (≈28%). Similar increases in nitrate content were found in rosette leaves of Arabidopsis phytochrome B mutant and in leaf and root tissues of maize and soybean under simulated FR light. This supports the idea that nitrate accumulation in response to neighbouring weeds is mediated by reflected FR light. Increased nitrate content of soybean unifoliate leaves in response to neighbouring weeds was not due to decreases in nitrate reductase and nitrite reductase activities suggesting the involvement of nitrate transporters. Ongoing analysis of expression of Arabidopsis nitrate transporter genes in response to neighbouring weeds has revealed decreased expression of AtNRT1.13 (syn. AtNPF4.4), which has uncharacterized substrate and function. These results suggest that FR light-mediated accumulation of nitrate is an integral component of resource-independent weed-crop competition. Neighbouring weeds may generate a nitrate-limited condition via actions of nitrate transporters and compromise early development of crop plants.

#2

Boreal tree responses to combined elevated CO2 and temperature: a comparison between eight common species

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Canadian boreal forests are major above ground carbon pools in the Northern latitude. Saving this carbon sequester from the impact of global climate warming is a major issue of this century. Declining dominated boreal species is the negative feedback to global climate change that is projected with elevated CO2 and temperature. Contemporary strategic planning will be necessary to mitigate future climate change response towards this carbon sequestration and subsequently the forest economy. My research goal is to determine the response of dominated boreal species towards increased temperature and CO2. Considering the current declining rate of dominated boreal species, I will investigate the combined effect of elevated CO2 and temperature treatments on eight boreal species that represent the tree biodiversity in Canadian boreal forest: four evergreen conifers (Abies balsamea, Picea mariana, Picea glauca, Pinus banksiana), three deciduous broad-leaf species (Betula papyrifera, Populus balsamifera, Populus tremuloides) and one deciduous conifer (Larix laricina). I will conduct my experiments in glasshouses with the seedlings of these species at six different levels of combined CO2 and temperature treatments. I will compare acclimatization capacity of these species by evaluating their photosynthetic and respiratory traits. I plan to investigate their net carbon assimilation rates and leaf dark respiration rates by assessing photosynthetic capacity (Vcmax) and maximum electron transport rates (Jmax). Determining the impact of rising CO2 and temperature on plant carbon metabolism processes will direct us to address acclimatization strategies of forests under future climatic conditions.

#3

Weekly treatment with pipecolic acid induces resistance in treated and untreated neighbouring cucumber plants

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As of 2017, Canadian greenhouse vegetables represent the largest sector of Canadian horticulture. The enclosed, humid environment of a greenhouse can quickly become an ideal breeding ground for disease-causing microbes. Once infected plants are identified, it can be difficult to stop disease spread in the greenhouse. Most plants initiate innate defense in response to microbial infections and as a result are resistant to most pathogens, however some microbes possess virulence weapons that overcome defense responses of a particular species, resulting in disease. Exogenous application of small defense-related compounds can activate plant-wide resistance in numerous crop species to effectively protect them from microbial diseases. Cucumber, the second most-harvested greenhouse crop in Canada, was chosen to study the effect of treatment with pipecolic acid, a non-proteinaceous amino acid observed to contribute to long-distance signaling during Systemic Acquired Resistance, a plant-wide resistance response. Plants received three weekly soil-drench treatments of pipecolic acid or water, followed 24 hours after the last treatment by inoculation of leaves with virulent Pseudomonas syringae pv lachrymans and determination of bacterial growth 3 days later. Plants treated with pipecolic acid supported lower bacterial levels in leaves compared to water-treated plants. Our preliminary data also suggests that weekly treatment with pipecolic

acid induced resistance in untreated plants growing nearby, perhaps via inter-plant volatile communication. These findings may be useful for the greenhouse industry as pipecolic acid resistance could be induced in greenhouses by treating a small number of plants, thus reducing crop production costs.

#4

Characterizing the role of mechanosensitive ion channels in the basal compatible response pathway

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Following pollination, the dry stigmas in the Brassicaceae rapidly regulate pollen-stigma interactions, thereby leading to the acceptance of compatible pollen or the rejection of self-incompatible pollen. The basal compatible pollen response pathway confers pollen hydration, and germination, ultimately leading to the fertilization of ovules. The objective of this research is to investigate the role of mechanosensitive ion channels of small conductance-like proteins (MSLs), specifically MSL7 in the compatible pollen response pathway in Arabidopsis thaliana. The A. thaliana genome encodes for 10 MSL proteins (MSL1-10) and previous studies have shown the function of the pollen-specific MSL8 in the regulation of osmotic stress in the pollen during fertilization. Interestingly, MSL8 is tandemly linked to MSL7, and MSL7 displays stigma-specific expression. Thus, we hypothesize that MSL7 is involved in the transport of ions to facilitate turgor pressure homeostasis in the stigma during compatible pollinations. Our experimental approach utilizes the CRISPR/Cas9 system to generate single knockout lines of MSL7 and double knockout lines of MSL7 and MSL8. We have found that the deletion of MSL7 reduces compatible pollen hydration and pollen acceptance, indicating its role in the compatible pollen response pathway. We are currently in the process of generating double knockout lines, and additional msl7 mutant alleles. All of these MSL mutant combinations will be analyzed for pollen-stigma interactions to characterize the role of these mechanosensitive ion channels in the compatible pollen response pathway.

#5

Elucidating the genetic basis for accumulation of akuammicine, a by-product of vinblastine biosynthesis, in a *Catharanthus roseus* mutant

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Catharanthus roseus is an important medicinal plant and a source of a class of compounds known as monoterpenoid indole alkaloids (MIAs), which includes potent anticancer drugs vincristine and vinblastine. These MIAs are produced sparingly in plants, and their stereochemical complexity renders chemical synthesis difficult. Elucidating the biosynthetic pathways in plants allows for potential transfer into bacterial systems for increased MIA production. A cost effective and efficient method for the study of these biosynthetic pathways

is the generation of mutant plants through ethyl methanesulfonate (EMS) mutagenesis. EMS plants were screened by thin-layer chromatography (TLC), in order to select altered MIA phenotypes. M-117523 is one such mutant and displays dwarfed growth and necrotic lesions on its leaves, coupled with increased akuammicine accumulation. Here, we attempt to identify the genetic basis for this unique phenotype. MIA extracts of mutant leaves contain significantly higher levels of akuammicine, as compared to the wild type. Necrotic tissue excised from the mutant leaves yielded higher amounts of akuammicine than healthy tissues, within mutant leaves. Redox1, a key branch point enzyme, is being analyzed for mutations that might lead to the observed phenotype. Comparative transcriptional analysis of pathway genes between the wild type and mutant line is also being conducted. The scarcity of akuammicine in nature and its medicinal applications as an anti-diabetic and mild analgesic have sparked interest in understanding the biosynthesis in the plant. Elucidation of the pathway could enable future breeding of *C. roseus* and potential production of akuammicine in engineered microbes.

#6

Does the GABA shunt regulate the cytosolic concentration of the signal molecule GABA?

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Guelph University, Dept of Plant Agriculture, Guelph, Canada .

The gamma aminobutyrate (GABA) shunt refers to a metabolic pathway that is well documented. However the function (s)of the GABA shunt is not clear. The shunt catalyses a bypass around the Krebs cycle reaction catalysed by alpha ketoglutarate dehydrogenase. The shunt converts alpha ketoglutarate to the Krebs cycle intermediate succinate. It involves the conversion of alpha ketoglutarate to glutamate , glutamate to GABA in the cytosol, and GABA to succinate in the mitochondria. Recent findings demonstrate that GABA is a signal molecule stimulating the activity of anion transporters which facilitate anion efflux at the plasma membrane. Homeostatic regulation of signal molecule concentrations regulates the activity of signal receptors . Many plant stresses are known to result in calcium influx, which activates cytosolic glutamate decarboxylase and causes rapid GABA accumulation. Conversely transport of GABA from the cytosol to the mitochondria or the apoplast has been documented . Consequently the cytosolic portion of the GABA shunt has the molecular mechanisms required for the production and removal of cytosolic GABA.

#7

Diversification of Effector-Triggered Immune Responses in Brassicaceous Plants

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The phytopathogenic bacteria *Pseudomonas syringae* can inject type III secreted effector (T3E) proteins directly into plant cells through the type III secretion system. Once in host cells, T3Es can function to increase bacterial virulence by counteracting basal plant

immunity. In response, intracellular resistance proteins known as nucleotide-binding leucine rich repeat domain proteins (NLRs) can recognize specific T3Es and induce effector-triggered immunity (ETI). In the model plant *Arabidopsis thaliana* multiple families of T3Es are known to trigger an ETI response through interaction with specific NLRs. Although several NLRs are known to be conserved in close relatives of A. thaliana in the Brassicaceae family, little is known about ETI responses in non-Arabidopsis Brassicaceous species. This project aims to characterize the diversity of ETI responses in members of the Brassicaceae family by screening through a compendium of representative *P. syringae* T3Es on two species of Brassicaceae, *Camelina sativa* and *Brassica napus*. We've developed an infection protocol on *C. sativa* and *B. napus* and found that *P. syringae* pv. maculicola strain ES4326 causes distinctive and consistent symptoms on both species. We've identified one potential ETI response on *C. sativa* and six potential ETI responses on *B. napus*. Results of this study will contribute to our understanding of the diversity of ETI responses in plants.

#8

Investigation of FLOWERING LOCUS T of Coffea sp as a putative flowering inducer

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The transition from the vegetative to reproductive phase in angiosperms is a highly regulated process in the plant life cycle. Flowering in most plants can be controlled by temperature, day length, gibberellin, vernalization and autonomous cues. The Arabidopsis thaliana FLOWERING LOCUS T (FT) gene and orthologs in other species integrates all these signals to control the flowering process. FT encodes a florigen that is translated in leaf and translocated by phloem to the apical shoot meristem where it interacts with FD to activate the genetic network responsible for flower induction. Coffee is an important agricultural commodity traded worldwide and its asynchronous fruit maturation is a problem because it decreases final product quality. Floral induction begins the reproductive phase and thus can influence all subsequent development stages. The objective of this work is to investigate the involvement of FT in coffee as a flowering inducer. FT sequences were aligned against the Coffea canephora and Coffea arabica genomes to identify putative Coffea homologs. Phylogenetic analysis was performed and four FT-like genes were found in Coffee; CaFT1, CaFT2, CaTFL1 and CaMFT1. Expression analysis by RT-qPCR assay was conducted for CaFT1 on leaf samples collected at the beginning and end of a single day in 5 different points of the year (December 2016, February, April, July and October 2017). Difference of expression was observed between different development flowering stages. Current experiments aim to test CaFT as a florigen by heterologous expression Arabidopsis.

#9

Elucidating the role of poplar clade II BAHD acyltransferases in very long chain fatty acid biosynthesis

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Cuticular waxes cover the epidermis of vascular plants to protect them from environmental stresses, particularly drought and pests. Cuticular waxes are functionalized, long-chain aliphatic compounds, and are derived from very long chain fatty acids (VLCFAs). Despite the presence of C30 VLCFA-derived waxes in Populus, the biosynthesis of C30 VLCFAs is unclear. Here, we report the role of a previously uncharacterized clade of BAHD acyltransferases (termed CER2-likes) in the biosynthesis of C28 and C30 very long chain fatty acids. Transgenic poplar (P. alba x grandidentata) expressing GUS under the control of the CER2-like promoter showed tissue-specific expression in epidermal cells, consistent with its role in VLCFA and cuticular wax biosynthesis. To test the biochemical function of the five CER2-likes, each gene was heterologously expressed in S. cerevisiae alongside a condensing enzyme (CER6), then analyzed by fatty acid methyl ester (FAME) analysis. FAME analysis showed that expression of the condensing enzyme alone yielded C28 in low abundance. When expressed with CER2-likes however, C28 and C30 were up to 3-fold and 10-fold more abundant, respectively. Functional complementation of a cer2 wax-deficient Arabidopsis mutant with the poplar CER2-likes also partially restored the levels of C28 and C30 derived waxes as determined by gas chromatography. Across assays, each of the five CER2-likes exhibited different activities and substrate specificity. Thus, preferential expression of certain CER2-likes may allow plants to regulate the carbon chain lengths of cuticular waxes and adapt to different environments. Together, these results identify the role of BAHD acyltransferases (CER2-likes) in VLCFA biosynthesis.

#10

Functional complementation of *Arabidopsis thaliana* with maize starch branching enzymes represents a potential method to increase biomass as well as to shape our understanding of transient starch

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Starch is an agronomically important polyglucan synthesized by plants as a means to store photoassimilates as an osmotically inactive carbon store for subsequent use in metabolism and growth. The synthesis of starch involves several enzymes working in concert, including starch branching enzyme (SBE), responsible for cleaving alpha-(1,4)-bonds on pre-existing glucan chains and reattaching them to the same or to neighbouring chains via alpha-(1,6)-linkages. SBE activity creates branch points and contributes to starch's semi-crystalline structure. Recently, SBEI and SBEIIb cloned from maize (Zea mays L.) endosperm were constitutively expressed in a starchless, null line of Arabidopsis, lacking endogenous SBEs. While both ZmSBEI and ZmSBEIIb were able to individually restore starch synthesis in the null

line, transformants exhibited altered starch metabolism. Here, we have re-transformed the null line (sbe2.1-/sbe2.2-) as well as Columbia (Col-0) with ZmSBEI and ZmSBEIIb under the control of a CaMV 35S promotor, and are in the process of characterizing these lines. Functional complementation of Arabidopsis SBE null and Col-0 lines with maize SBEs represents a promising method to increase biomass and production in agronomically important oilseed crops such as canola, as well as to shed light on the physical characteristics of transient starch.

#11

Environmental Regulation of Plant Defence Pathways

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Environmental factors, like temperature and humidity, significantly impact the development of plant diseases. Production of the salicylic acid (SA) defence hormone has been shown to be a temperature-sensitive component of the plant immune system. Host SA globally regulates thousands of defence-related genes that are important to counteract pathogenesis. We have recently demonstrated that elevated temperature suppresses SA production in the model plant Arabidopsis thaliana by downregulating the expression of a master calmodulin-binding transcription factor that control the expression of various SA biosynthetic genes and other immune regulators in plants. Because the underlying molecular mechanisms and evolutionary significance of this environmental regulation are unclear, the research in the Castroverde Lab (https://castroverdelab.ca) at Laurier aims to investigate these important next-level questions. Our long-term vision is to define how plants defend themselves against pathogens a dynamically changing environment. We will integrate molecular, genetic and biochemical approaches to: (1) identify essential transcription factors using in silico, in vitro and in vivo methods; (2) discover new regulators by performing genetic and proteomic screens; and (3) dissect how defence pathways in other plant species are affected by elevated temperature. The knowledge gained from our research program will shed light on various plant resilience mechanisms, which should inform crop disease prevention efforts in the agricultural sector.

#12

Tracking carbon allocation into terpenoid biosynthesis and other metabolic domains in Arabidopsis rosette tissue using $^{13}CO_2$ labeling and ammonia chemical ionization mass spectrometry

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Plant terpenoids represent high value natural products with central roles in plant biology in addition to their importance in industry and medicine. Efforts to manipulate carbon flux towards these compounds have been limited by an incomplete knowledge of the regulatory program

that dictates carbon partitioning among the major metabolic domains, including cell wall biosynthesis, shikimate derived phenolics, lipid biosynthesis, and energy metabolism. Here we describe a metabolomics-based characterization of carbon use profiles in *Arabidopsis thaliana* to quantify carbon allocation to the chloroplast terpenoid precursor pathway and compare this to other major metabolic processes. This technique relies on 13CO2 time course labeling of intact plants under physiological conditions and subsequent mass spectrometric (MS) analysis of label in various metabolite pools. For gas chromatography – MS analysis, we have developed a soft ammonia chemical ionization technique which simplifies calculation of label incorporation calculations due to its preservation of the molecular ion cluster. We have coupled these targeted MS data to elemental analysis/isotopic ratio MS to normalize substrate commitment by pathway to the global carbon budget. By way of this approach, we have determined that approximately 20% of fixed carbon is dedicated to sucrose export under standard conditions, while less than 0.5% of total fixed carbon was detected in intermediates of the MEP pathway. This global carbon profiling approach provides a basis for directly assessing the impact of environment or genetic manipulation on substrate commitment to plastidic terpenoid biosynthesis relative to other metabolic processes.

#13

Discovery of medicinal pathways through accessible bioinformatics for the average researcher: de novo transcriptome assembly of *Ochrosia elliptica* using Galaxy

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Monoterpenoid indole alkaloids (MIAs) produced by members of the Apocynaceae family display valuable pharmacological properties, such as the dimeric anticancer MIA vinblastine which is produced from catharanthine and vindoline in *Catharanthus roseus*. The biosynthetic pathways leading to catharanthine and vindoline formation in C. roseus were recently completed, and this is facilitating discovery of homologous genes involved in MIA biosynthesis in other species. *Ochrosia elliptica* produces the MIAs ellipticine and apparicine, which possess anticancer and analgesic activities, respectively. Ellipticine and apparicine may be derived from stemmadenine, a common intermediate in the catharanthine and vindoline pathways. In order to identify potential gene candidates for ellipticine and apparicine biosynthesis, the leaf transcriptome of *O. elliptica* was assembled de novo from 159.2 million trimmed Illumina reads into 60 564 predicted coding sequences using the Trinity pipeline on the European Galaxy server. Further clustering of similar peptide sequences with CD-HIT-PROTEIN yielded 33 699 sequences with an average length of 435 amino acids.

Querying the assembled transcriptome with known MIA biosynthetic genes indicated that *O. elliptica* possesses orthologues with ≥70% amino acid identity to 14 out of 15 genes involved in the assembly of stemmadenine, which comprises secologanin, tryptamine, strictosidine, and stemmadenine biosynthesis. Eleven out of 14 orthologues encoded full-length open reading frames with the exception of 7-deoxyloganic acid 7-hydroxylase, strictosidine β-D-glucosidase, and Redox2. These promising results suggest that the annotated

O. elliptica transcriptome will be a vital resource for identifying gene candidates required for the assembly of ellipticine and apparicine from stemmadenine.

#14

Metabolic engineering of pyruvate production in plant chloroplasts

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Plant terpenoids represent high value primary and secondary metabolites with fundamental roles in plant and animal biology as well as many applications in industry and medicine. Metabolic engineering aimed at increasing flux towards high value terpenoid end products have focused on overexpression of rate limiting steps, but factors limiting the supply of upstream substrates have received little attention. The chloroplast 2C-methyl-D-erythritol 4-phosphate (MEP) pathway synthesizes the universal terpenoid intermediates isopentenyl and dimethylallyl diphosphate from D-glyceraldehyde 3-phosphate and pyruvate. However, chloroplast pyruvate supply is dependent on import of cytosolic phosphoenolpyruvate (PEP) due to the presumed absence of the glycolytic enzymes ENOLASE1 (ENO1) and PHOSPHOGLYCERATE MUTASE (PGM) in chloroplasts whose downregulation beyond the seedling stage is thought necessary to protect the Calvin cycle. In this study, we aimed to test this hypothesis and investigate the potential of increasing PEP/pyruvate availability for the MEP pathway via endogenous production in this compartment.

We generated double transgenic Arabidopsis lines expressing both ENO1 and PGM under control of the 35S promoter and observed no obvious developmental defects when the plastid isoforms of these genes were expressed constitutively. We have further devised a strategy to evaluate the effects of a fully functional glycolytic pathway in chloroplasts using a mutant defective in the PEP transporter and whole plant isotopic labelling strategies of MEP pathway intermediates.

#15

Characterizing the structure of a putative reverse transit peptide-like sequence at the C-Terminus of Toc159 and its role in chloroplast targeting.

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The majority of proteins targeted to the chloroplast contain a transit peptide (TP) which directs them to the plastid where they are recognized by Toc159, a member of the translocon at the outer membrane of chloroplasts (Toc complex) and imported. Outer envelope proteins (OEPs) typically reach and insert into the chloroplast outer membrane (COM) via common membrane spanning. Recently, it has been demonstrated that Toc159 targeting to the COM is achieved by a previously uncharacterized pathway involving a putative reverse TP-like

sequence (rTP) at the C-terminus of the Toc159 membrane (M-) domain. This rTP has been shown, experimentally, to target green fluorescent protein to the COM. Little research has been done to characterize the structure or exact function(s) of the Toc159 M-domain and how these structures achieve selectivity for and interface with the COM. Bioinformatic and biophysical approaches were used to determine the structure and oligomeric organization of the Toc159 rTP in order to understand how it might interact with the COM. Bioinformatic analysis suggests that the rTP contains an amphipathic helix which may interact with the membrane. Our most recent bioinformatic and biophysical results will be presented.

#16

Investigating the role of BKNs in pollen-stigma interactions

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The Brassicaceae family, are a family of plants that contain many scientifically as well as economically important plants. Brassicaceae have “dry stigma”, meaning that the papillae lack surface secretions and allows the plants to have greater control over the early post-pollination stages, with only compatible pollen grains receiving water from the stigma, this facilitates pollen germination and fertilization. The basal compatible response pathway in the stigma is initiated upon recognition of compatible pollen, followed by exocyst-mediated transduction of secretion from the stigma towards the pollen, and this pathway remains largely elusive. A previous attempt to identify candidate genes in the compatible pollen signal transduction pathway led to the discovery of pseudokinases, BRASSIKIN1 (BKN1) and BKN2. It was also discovered that BKN orthologous are found throughout the Brassicaceae family. The BKN loci between different A. thaliana ecotypes as well as different Brassicaceae species differ. A. thaliana ecotype Col-0 has a truncated BKN1 caused by a premature stop codon, but A. thaliana ecotype Hh-0 as well as A. lyrata have fully intact BKN1 and 2 genes. For this project, BKN1 and BKN2 mutants will be generated in Hh-0 and A. lyrata using the CRISPR-Cas9 system. As well, overexpression BKN1 constructs will be transformed into Col-0. Pollen response characterization assays will be performed to further understand the potential importance of the BKN genes in the compatible pollen response pathway.

#17

An amphipathic-helix-containing sequence is responsible for targeting AT3G11620 to lipid droplets,

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Lipid droplets (LDs) are the primary sites of neutral lipid storage in eukaryotic cells. They have a unique structure consisting of a hydrophobic lipid core surrounded by a phospholipid monolayer and numerous, surface-associated 'coat' proteins. While LDs are known to form at the surface of the endoplasmic reticulum (ER) and acquire both their core and monolayer lipids from the ER, how LD coat proteins target specifically to LDs is an open question. Unlike other organelles, LDs do not have dedicated protein-targeting/import machinery and no canonical targeting sequences have been identified for LD proteins. Here we have taken advantage of the general conservation of LDs across evolutionarily-diverse organisms to identify and characterize a new LD protein in Arabidopsis, AT3G11620, which is a homolog of □□□ hydrolase-fold-containing LD proteins found in yeasts, insects and mammals. We show that AT3G11620 is constitutively expressed throughout plant growth and development and that its ectopic over-expression or loss-of-expression results in an increase or decrease in LD abundance, respectively, suggesting AT3G1160 functions like its fly counterpart in regulating LD turnover. AT3G11620 also localizes to both the ER and LDs and targets to LDs via an internal sequence that includes a predicted amphipathic □ - helix. Current efforts to elucidate the nature of this LD targeting signal in AT3G11620, as well as in a similar sequence in its fly homolog, will be presented, including the possibility that large, hydrophobic residues in their amphipathic helices convey targeting specificity by recognizing phospholipid packing defects on the LD surface.

#18

Characterization of *Camelina sativa* seed germination: The effect of GA on vacuolation and embryo- endosperm interaction

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Camelina sativa is an important oilseed crop, which has recently gained attention in biofuel production as more information emerges about its genomic characteristics and crop performance. The aim of this research is to compare germination of Camelina with that of Arabidopsis, as molecular mechanisms of germination are well studied in this species. Arabidopsis protein storage vacuoles (PSVs) increase in size and decrease in number as a consequence of germination progression. Previously, through diphenyl boric acid aminoethyl ester (DPBA) staining of seed embryo and endosperm with gibberellins (GA), Camelina showed similar results to Arabidopsis, however with distinct time points of vacuolation, confirming that vacuolation is an indication of germination in Camelina as well. It was also observed that micropylar regions of the endosperm had higher ratios of vacuolated cells, suggesting that these regions may have different roles in the control of seed germination and may be more sensitive to hormonal regulation. To observe if this result is dependent on attachment of the embryo to the endosperm and to further elucidate the cellular physical and chemical interaction between these tissues, the embryo and endosperm were separated and

vacuolation staining patterns are being observed currently. This result will indicate whether, similar to Arabidopsis, vacuolation and endosperm to embryo cellular trafficking is necessary and potentially regulated by GA.

#19

Minor impacts of +9°C warming and increased CO2 on mature boreal conifers in the field

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Boreal forests cover much of the world's northern latitudes and hold one-third of the earth's forest carbon stocks. These same northern latitudes are projected to experience up to 10 °C warming and more than a doubling of CO2 concentration by the end of this century. To evaluate how these climate changes might impact common boreal tree species, we studied mature trees of two boreal conifers, Picea mariana and Larix laricina, exposed to ambient (400 ppm) or elevated (900 ppm) CO2 treatments combined with warming treatments ranging from +0 to +9°C above ambient temperatures at the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experimental site in Minnesota. Measurements of net photosynthesis (Anet) and dark respiration (Rd) were taken at common (25°C and 400 ppm) and growth conditions. Under common measurement conditions, Anet was lower in the elevated CO2 (EC) trees than the ambient CO2 (AC) trees in both species, indicating CO2 acclimation. At growth conditions, Anet increased with rising leaf temperature (Tleaf) in EC Picea, but not in AC Picea. In Larix, however, Anet was stable as Tleaf rose for both CO2 treatments, although elevated CO2 increased Anet. In EC Picea, respiration increased with temperature in common conditions, but Rd rates remained constant for all other measurements regardless of treatment. Taken all together, Picea shows sign of acclimation to CO2 but not temperature, while Larix shows sign of acclimation to both. The implications of our results for boreal forest carbon flux under future climate conditions will be discussed.

#20

Investigating the resistance mechanism of Arabidopsis thaliana ecotype Kz-9 against Pseudomonas syringae pv. tomato DC3000

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Pseudomonas syringae pv. tomato DC3000 is a phytopathogen widely used in plant immunity research. Plants possess a layer of immunity called effector-triggered immunity (ETI), wherein they employ resistance or R-proteins that detect microbial effector proteins to impede

their virulence function. Although the DC3000-Arabidosis pathosystem has been prevalently used to study disease and effector function, there has not yet been thorough investigations of ETI against DC3000's effectors in this species. We have identified an ecotype of Arabidopsis called Kz-9, that demonstrates resistance against DC3000. DC3000 grows poorly in Kz-9 relative to the susceptible Col-0 ecotype, leading us to hypothesize that Kz-9 may be mounting an ETI response. In order to determine if any DC3000 effectors were being recognized in this potential ETI response, we individually transformed 47 known DC3000 effector genes into the hypervirulent strain, Pseudomonas syringae pv. maculicola ES4326. Conducting infection assays using these ES4326 strains, we have currently identified six effectors as positive hits, which allow Kz-9 plants to remain healthy relative to the empty vector control. Using next-generation mapping we found a 1.7 Mbp region, containing 162 genes (17 being R-genes), in the genome of Kz-9 that tightly correlates with resistance to DC3000. We are creating CRISPR mutations of R-genes in this region to ultimately deduce which are required for resistance to DC3000.

#21

Characterizing the Expression of CYP707A3 in regulating ABA Levels in Response to Excess Water in *Arabidopsis thaliana*

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With fluctuating environmental conditions, plants are increasingly facing changes in water availability. In plants, water is taken up by the roots and moves through the plant through the xylem, enabling the movement of nutrients, and eventually leaves the plant through the stomata via transpiration. A plant's ability to respond to water deprivation is mediated in large part by the phytohormone abscisic acid (ABA). In excess water conditions ABA levels are in part regulated by ABA 8'-hydroxylases that catalyze the conversion of ABA to 8'-hydroxy ABA, which forms phaseic acid, a less active ABA derivative. In Arabidopsis thaliana there are four ABA 8'-hydroxylases (CYP707A1, CYP707A2, CYP707A3, CYP707A4) responsible for ABA catabolism. CYP707A1 and CYP707A3 have been shown to be involved in submergence and high humidity conditions. To visualize CYP707A3 expression in response to altered water availability, we used an upstream promoter region of CYP707A3 fused to a □-glucuronidase (GUS) reporter gene. We found that GUS expression was more pronounced in the shoot of plants subjected to submergence and high relative humidity, compared with those under control conditions. This suggests that CYP707A3 plays a role in regulating ABA levels in plants in response to changing water availability. These results will allow us to further characterize how CYP707A3 regulates ABA catabolism in the presence of differing water conditions.

#22

Towards understanding the basis of substrate specificity in medium-chain acyl-lipid thioesterase (ALT) enzymes from plants

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Acyl-ACP thioesterase enzymes, which cleave fatty acyl thioester bonds to release free fatty acids, contribute to much of the fatty acid diversity in plants. ACYL LIPID THIOESTERASES (ALTs), a novel class of plastid-localized thioesterases occurring in all plant taxa, generate medium-chain (C6-C14) fatty and β–keto fatty acids as secondary metabolites. Their volatile products likely serve to defend against predatory insects and pathogens. We investigated the catalytic diversity of ALT enzymes by expressing 15 ALTs from monocots, eudicots, a lycophyte, a green microalga, and Ginkgo biloba in Escherichia coli. Based on their substrate preferences in terms of chain length and oxidation state, the chosen ALTs can be classified into four catalytic groups comprising enzymes from diverse species and taxa. Through primary sequence alignments and structure-based phylogenetic analyses using three-dimensional models of ALTs, we identified unique features of ALTs with preference for C6-10 acyl-ACPs and C14 β-ketoacyl-ACPs respectively. We performed targeted mutagenesis experiments to determine whether these features influence ALT substrate specificity. Profiling the products of mutant enzymes in E. coli led to the identification of amino acid sequence fragments that affect acyl-ACP chain length preference, oxidation state preference, and enzyme activity, establishing the first links between ALT protein sequence and substrate specificity. Medium-chain fatty acids are used to manufacture insecticides, pharmaceuticals, and biofuels, and information from this study could be used to engineer recombinant ALTs with substrate specificities that suit particular industrial purposes. ALTs may also be promising tools for improving crop resistance to insect and pathogen attack.

#23

A role for Receptor Kinases in regulating compatible pollen responses in the Brassicaceae stigma

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Brassicaceae pistils have evolved mechanisms to recognize pollen grains as providing nutrients to the wrong mating partner would be disadvantageous. The dry stigmas lack surface secretion that would enable automatic pollen germination, and this allows the stigma to tightly regulate pollen acceptance following pollen-stigma contact. The initial cellular processes in the stigma leading up to fertilization are becoming more clearly defined, but the initial upstream signalling components are yet to be identified. Previous work in the Goring lab identified a set of receptor-like cytoplasmic kinases, BRASSIKINS (BKNs), as candidate stigmatic signalling proteins in this pathway. However, the BKNs are pseudokinases, which lack essential catalytic motifs, and are likely to function with other signalling proteins such as active kinases in the signalling complex. Thus, we hypothesize that the BKNs mediate signal transduction by forming a complex with membrane-bound receptor kinases to facilitate pollen acceptance and

hydration. To address this hypothesis, the BKNs were used to screen for putative protein interactors by testing pairwise combinations with kinase domains from stigma-expressed receptor kinases in the yeast two-hybrid system. Further characterization of the putative interactors identified two distinct clusters of receptor kinases, and so far, the loss-of-function pistils show defects in early to late stages fertility. Current work is focused on testing whether these receptor kinases can rescue the mutant pistil phenotypes. Overall, we aim to better understand how these receptor kinases facilitate the early to late stages of compatible pollen acceptance and whether they represent a conserved signalling module across the Brassicaceae.

#24

Uptake and translocation of cadmium in soybean in response to sulfite addition.

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Cadmium (Cd) is a toxic metal that is increasing in concentration in agricultural soil due to anthropogenic activity (e.g., industrial waste, impure fertilizers). Contaminated crops could pose a potential health risk for consumers. Many studies have reported the positive effects of exogenous sulfur (S) in reducing Cd uptake and translocation in rice (Oryza sativa). However, the extent to which this phenomenon applies to other crop species is unknown. Soybean (Glycine max) seedlings were grown hydroponically in a full factorial design with 0 or 20 □M CdCl2 and 0, 2.5, or 5 mM Na2SO3. The total amount of Cd taken up decreased from 73 ± 0.1 □g per plant (no S added) to 19 ± 0.04 □g per plant (5 mM S added) despite the S-induced 10-15% increase in bioavailable Cd in solution. Within S-treated root cross sections, more Cd was localized at the exodermis, less Cd was in the stele, and Casparian bands were thicker. These patterns suggest reduced opportunity for Cd to enter the xylem and to be translocated aboveground. However, proportionately more of the Cd was translocated to the shoots (31% of total Cd with no S added; 88% of total Cd with 2.5 and 5 mM S added). Soybean is therefore an unsuitable crop for growth on Cd-contaminated soils treated with exogenous sulfite.

#25

Investigating the role of secretion in the *Arabidopsis thaliana* compatible pollen response pathway

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The acceptance of compatible pollen in the Brassicaceae is tightly regulated through interactions between the pollen and the pistil. Secretion in the stigmatic papillae is proposed to be key to this interaction to provide resources to the pollen for hydration and germination. The objective of this research is to investigate components of the Arabidopsis thaliana

secretory pathway machinery for their requirement in the stigma for compatible pollen acceptance. Fluorescently-tagged markers that identify different compartments in the endomembrane system are being used to gain a fuller understanding of the secretory activity that occurs following compatible pollinations. In addition, the requirement of SNARE complex subunits, which are implicated in vesicle fusion and cargo release, is also being investigated through loss-of-function mutants. Our preliminary results have shown that combining SNARE knockout mutants leads to a reduction in compatible pollen hydration, supporting their role in the compatible pollen acceptance pathway. Higher order SNARE knockout mutants will be used in the future to further characterize the SNARE complex's role in compatible pollen acceptance. Together with fluorescently-tagged endomembrane marker lines, this research will provide a better understanding of the stigmatic papilla's secretory system, and how this system is employed in the acceptance of compatible pollen.

#26

Post-Translational Regulation of Starch Branching Enzyme 2.2 from *Arabidopsis thaliana*

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Starch is a water insoluble carbon storage polymer found in most higher plants. Transient starch is produced and degraded in chloroplasts following a diurnal cycle, to provide fixed carbon when photosynthesis ceases. Biosynthesis of transient starch occurs through the coordinated activity of multiple classes of enzymes. Starch synthases utilize ADP-glucose to polymerize growing α-glucan chains with α-1,4 linkages, while starch branching enzymes (SBE) introduce branch points to the growing glucan using α-1,6 linkages. Two functional isoforms of SBE (SBE2.1 and SBE2.2) have been identified in Arabidopsis, of which SBE2.2 accounts for most of the branching activity. Recombinant SBE2.2 was phosphorylated by soluble chloroplast extracts on residues Ser290 and Ser301. SBEs form phosphorylation-dependent multi-enzyme complexes with other starch biosynthetic enzymes. Previous studies have shown SBE2.2 to be redox regulated. A putative protein-protein interaction domain has been identified by bioinformatics. Site-directed mutagenesis is being used to alter this predicted interaction domain, phosphorylation sites and Cys residues of SBE2.2 in order to investigate their importance in catalysis and regulating the formation of protein complexes. The effect of these modifications in vitro will be assessed based on changes to catalytic activity, as well as protein-protein interactions as assessed by affinity chromatography. The in vivo relevance of these modifications will be investigated by functional complementation of a sbe2.1-/sbe2.2-knockout Arabidopsis line. Endpoints being compared are overall plant growth, sugar and starch accumulation, as well as changes to starch granule structure. This research is significant for its potential applications to plant growth and crop production.

#27

Metabolic Analysis of Bioactive Triterpene Saponins in *Quillaja brasiliensis* Cell Cultures,
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Quillaja brasiliensis Mart. (Quillajaceae) is a plant native to Brazil known as the “soap tree”. Its triterpenoid saponins exhibit pronounced adjuvant activity when used in veterinary vaccines. Saponins from Q. brasiliensis are marked by an unusually high degree of substituent complexity, which consists of multiple glycoside residues and branched lipid chains attached to the C30 triterpene quillaic acid. This study investigates carbon flux into saponins in cultured cells. Callus formation was induced from seedling-derived explants initiated on solid medium, and a fast growing lineage derived from hypocotyls was chosen to start suspension cultures. We determined that calli produce a mixture of saponins which resembled those detected in intact trees, as judged by thin layer chromatography (TLC). Saponin accumulation in cell culture may be limited by diversion of carbon to phytosterols, which are expected to diverge at squalene-2,3-epoxide, although little information is available regarding saponin biosynthesis in this species . We have devised a strategy to quantify carbon partitioning between sterols and saponins under different environmental conditions using a stable isotopic labeling approach. Further analyses and quantification of saponins and sterols by LC-MS/MS and GC-MS are ongoing. There is evidence that Q. brasiliensis cell cultures constitute an alternative source of bioactive saponins to augment vaccine efficacy.

#28

How will climate change affect floral traits and carbon dynamics in an economically important crop?

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Rising atmospheric CO2 concentrations are increasing air temperatures, with a global mean warming of 3-4 °C predicted for 2100. Increases in both temperature and CO2 concentrations are having a variety of effects on plant life, including decreasing the nutritional value of important crops, like soybeans and wheat. While some research has been conducted on the effects of climate change on human nutrition, we know much less about how pollinator food sources may be affected by these same climate drivers. Furthermore, in light of recent declines in insect pollinator populations, investigating the effects of climate change on pollinator food supplies is increasingly important because available nutrition can alter pollinator susceptibly to stressors. To investigate how flowers and nectar produced by honeybee-pollinated Cucumis sativus respond to climate change, we exposed C. sativus plants to elevated CO2 and temperature treatments using a full factorial design. We looked at the interactive and singular effects of these drivers on nectar production, flower number, and flower

size for both male and female flowers, as well as biomass partitioning and net photosynthetic rates, to better understand the whole-plant carbon balance. We found negative effects of temperature on flower size, number, and pollinator rewards. These changes in floral displays may decrease pollination efficiency and attractiveness to pollinators and decreases in nectar rewards could place pollinators in greater jeopardy than currently predicted.

#29

Plant Cell Biology & Cell Wall Signalling

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The plant cell wall surrounds and protects all plant cells. In addition to their essential role in plant growth and development, cell walls provide us with food, clothing, shelter, and energy. However, modifying plant cell walls often decreases plant yield. This is because plants sense changes to the cell wall by largely unknown mechanisms, called “cell wall signalling”, and often respond by limiting their growth. The McFarlane Lab recently joined the Department of Cell and Systems Biology at the University of Toronto, where our long term goal is to discover how plants sense and respond to cell wall changes. We study cell wall signaling using a variety of approaches, including plant genetics, molecular biology, biochemistry, quantitative live cell imaging, high-resolution electron microscopy, and computational analyses of these datasets. We tackle biological questions, such as: What molecular components are required for cell wall signaling? How is cell wall signaling turned “on” or “off”? Do all cell wall changes activate the same signals? How do plants modify their cell walls in response to these signals? How much can we modify the cell wall before the plant responds? Does cell wall signaling affect other plant signaling pathways, such as immune responses or hormone signaling? By understanding the mechanisms of cell wall signaling, it may be possible to improve plant cell walls without decreasing yield. This work is important for current and future challenges in Canada, such as advancing sustainable agriculture, enhancing food security, and developing next-generation biofuels.

#30

Plant immunology and immune homeostasis

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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 threateb food security world-wide.

#31

Investigating the role of plastid molecular chaperone HSP90C in Arabidopsis embryo development and seed maturation

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HSP90C in Arabidopsis is a chloroplast stroma-localized HSP90 family molecular chaperone. It is required for active chloroplast protein import and protein transport into the thylakoid. Sequence alignment of HSP90 family proteins reveals unique sequence motifs for each subfamily HSP90 isoforms and highlights specific feature for proteins in different subcellular compartments. Specifically, the plastidic HSP90Cs contain an extra sequence at the C-terminus that is different from that in the cytosolic HSP90 proteins. It has been previously reported that T-DNA insertion mutants of the HSP90C gene is embryonic lethal and the development of the embryo is blocked at the heart stage. We generated transgenic Arabidopsis plants that express a C-terminal truncated form of HSP90C and in planta complementation analysis indicates that the truncated form can rescue the embryonic lethality phenotype of the previously identified HSP90C T-DNA insertion mutant line. Further analysis of the HSP90C truncation mutant in understanding the specific role of HSP90C in seed development and maturation is in progress.

#32

Investigating Protein Translocation to the Chloroplast Outer Membrane,

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The majority of chloroplast-localized proteins are transcribed by the nuclear genome, translated on cytosolic ribosomes and translocated to the chloroplast. Proteins destined for the chloroplast contain addition peptide information, called a transit peptide (TP), which directs translocation. For example, transit peptides on stromal-localized proteins are typically an N-terminal extension and carry hallmark physiochemical characteristics1. Chloroplast outer membrane proteins use five strategies to facilitate translocation, one of which includes using a C-terminal peptide which shares similar features with N-terminal TPs. The N-terminal TP

prediction tool ChloroP was used to predict a C-terminal TP on the reversed Toc159 sequence in Bienertia sinuspersici. Localization assays using GFP fusions and western blots confirmed the C-terminus of BsToc159 is necessary and sufficient for outer membrane translocation3. ChloroP has since been used to predict C-terminal transit-peptides in eight additional outer membrane bound proteins, including Outer Envelope Protein 16-2 (OEP16-2)2. However, localization assays using GFP OEP16-2 fusion proteins have revealed OEP16-2 does not use a C-terminal transit peptide. Additionally, OEP16-2 does not utilize the other four currently known chloroplast outer membrane translocation strategies. Therefore, we hypothesize that OEP16-2 utilizes a novel strategy to target the chloroplast outer membrane.

#33

Characterizing 3'-fluoro- abscisic acid as a probe for studying ABA transport

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The phytohormone abscisic acid (ABA) has an essential role in plant response to various biotic and abiotic stresses. Properties of ABA transport have been documented, but the method to study ABA transport in vivo remains a challenge. The application of Positron Emission Tomography (PET) in planta allows for a real-time and non-destructive method of studying the movement of the radiotracer. PET imaging requires the modification of the target chemical to introduce a positron emitting isotope such as 18F by radiochemistry. This study aims to assess whether 3'-fluoro-ABA (3'-F-ABA) has similar functions to ABA in terms of transport and biological activity in plants. In the present study at the University of Toronto, we compared ABA-induced gene expression in Arabidopsis thaliana plants treated with 3'-F-ABA and ABA. The transport of 3'-F-ABA was assessed by a destructive quantitative phytochemical analysis using a liquid chromatography tandem-mass spectrometry (LC-MS/MS) with stably labeled ABA and F-ABA standards. The distribution of 3'-F-ABA and ABA and metabolites in treated Arabidopsis thaliana and Brassica napus plants was found to be similar. These experiments showed that 3'-F-ABA shares similar properties with ABA, indicating that 3'-F-ABA serves as a probe for studying ABA transport by PET imaging. The quantitative analysis of ABA and 19F-ABA validates results of PET imaging studies with radiolabeled 18F-ABA being carried out in parallel at the University of Saskatchewan.

#34

Autumn warming delays downregulation of photosynthesis and does not increase risk of freezing damage in interior and coastal Douglas-fir seedlings

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In the next several decades, warming in the northern hemisphere will result in asynchronous phasing between the temperature and photoperiod signals that evergreen

conifers rely upon for development of cold hardening in autumn. We aimed to characterize the intraspecific variation in photosynthetic and photoprotective mechanisms in Douglas-fir (Pseudotsuga menziesii) originating from contrasting climates during simulated summer and autumn conditions, as well as how autumn warming affects the downregulation of photosynthesis and development of cold hardening. Following growth under summer photoperiod (day/night 16 h/8 h) and temperature (22 °C/13 °C), Douglas-fir seedlings from two interior and two coastal provenances were exposed to 6 weeks of autumn photoperiod (8 h/16 h) with either cool temperature (4 °C/-4 °C) or warm temperature (19 °C/11 °C). Exposure to cool temperature induced increase in size and de-epoxidation of the xanthophyll cycle pigment pool, development of sustained nonphotochemical quenching, and downregulation of photosynthetic activity. Seedlings exposed to warm temperature exhibited no downregulation of photosynthesis, corresponding with no change in xanthophyll cycle pigment de-epoxidation and no development of sustained nonphotochemical quenching. After 9 weeks of treatment, we measured freezing tolerance to assess cold hardiness. Freezing tolerance development for all provenances was not impaired by warm temperature relative to cool temperature, and interior Douglas-fir provenances developed greater freezing tolerance under both treatments relative to coastal provenances. Our findings suggest that short photoperiod alone is insufficient to induce downregulation of photosynthesis in autumn for Douglas-fir. However, this prolonged period of photosynthetic activity does not appear to bear a trade-off of impaired freezing tolerance.

#35

Investigate the role of ER-localized HSP90 family chaperone in cellular auxin homeostasis

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Auxin is a class of plant hormones that influence nearly every aspect of plant growth and development with indole-3-acetic acid (IAA) being the naturally occurring form. In order to be an effective signaling molecule, auxin levels are tightly regulated by biosynthesis, conjugation, catabolism, compartmentation and transport. IAA is primarily synthesized in shoot apexes via tryptophan-dependent pathways and participates in meristem cell differentiations. Arabidopsis ER-localized HSP90 family chaperone is required for protein secretion and has been reported to regulate shoot apical meristem maintenance. In an RNA sequencing study, we found that reduced expression of ER-localized HSP90 results in repression of genes in auxin responsive pathway. In a proteomics study, we noticed that a nitrilase NIT1 is enriched with ER-localized HSP90.7. To better understand the role of the ER-localized HSP90 family chaperone in auxin homeostasis, we obtained an NIT1 overexpression line and investigated how altered expression of HSP90.7 affects the root phenotype induced by NIT1 overexpression. Additionally, we collected all 8 auxin efflux PIN GFP fusion lines and will analyze the abundance and function of both ER- and plasma membrane localized PIN proteins in Arabidopsis plants that have altered HSP90.7 expression. It is anticipated that this study will

shed light on the mechanism of ER HSP90 family molecular chaperone in regulating cellular auxin homeostasis.

#36

Further Characterizing the C-terminal Transit Peptide Targeting Pathway in Using OEP15,
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The chloroplast contains roughly 3000 proteins, 95% of which are encoded by the nuclear genome and must be targeted to the chloroplast post-translationally. Most stroma targeted proteins possess a cleavable N-terminal transit peptide (TP) which is recognized by the translocon at the outer envelope membrane of chloroplasts (TOC complex). Recently, a TP-like sequence has been described at the C-terminus of a subset of chloroplast outer envelope proteins (OEPs), including one of the proteins in the TOC complex, Toc 159. This C-terminal TP-like sequence is hypothesized to represent a novel mechanism of OEP chloroplast targeting. One of the proteins in the set of OEPs predicted to contain a C-terminal TP-like sequence has been named OEP15, has two isoforms and no confirmed function. Subcellular localization using transient expression assays have been performed in both onion epidermal cells and Arabidopsis thaliana protoplasts using an EGFP fusion constructs to examine the subcellular localization of OEP15. The results of these localization assays are expected to aid in understanding of the new chloroplast outer membrane targeting pathway.

#37

Fruit Tree Decline in the Niagara Region
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Tree Fruit Decline (TFD) is an emerging disease that results in rapid decline and death of stone fruit trees including apricots, peaches, plums, nectarines, and cherries. TFD has been observed across Canada, with the Niagara region being particularly affected. TFD affects young trees between 2-8 years of age where the trees look healthy then begin to rapidly decline and are dead within a few weeks. Numerous symptoms have been observed including increased suckering, leaf chlorosis and wilting, leaf cupping, increased gummosis and cankers at the branching unit, and severe necrosis that begins at the graft union and progresses upwards. Various farms have displayed similar symptoms, samples were taken from the trees displaying symptoms as well as trees that appear to be healthy. There are many viruses that display symptoms similar to TFD, however, one virus in particular is known to be present in the region and infects stone fruit trees. Tomato ringspot virus (ToRSV) is plant pathogenic that infects Prunus species and could be linked to TFD. Some of the symptoms associated with ToRSV include leaf chlorosis, leaf size deduction, leaf cupping, and graft union necrosis.

Samples that were taken this past summer from various farms, were tested for the presence of ToRSV. 23 sites were sampled from around Ontario, of which eight had samples that tested positive for ToRSV. These results suggest that ToRSV is not the sole cause of TFD. The regional distribution of ToRSV in the Niagara region will be discussed further.

#38

Leveraging phosphoproteomics to uncover mechanisms of cell wall signaling

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Our food, clothing, shelter and fuel stem indirectly or directly from plant-based products. The bulk of plant biomass is the plant cell wall, and its major components are cellulose, hemicellulose and pectins. The cell wall regulates the form, growth and development of plant cells. Because of the obvious relationship between polysaccharide production and biomass, attempts to engineer plants have focused on altering the cell wall to increase biomass. However, success has been limited, which implies that regulatory components underly cell wall composition and cell growth. To date, several cell membrane-bound kinases have been implicated in cell wall signal perception, yet the downstream signal communication mechanisms remain undefined. To address this gap, we conducted a phosphoproteomic survey to detect downstream phosphorylation events on plants under cell wall integrity stress. We employed a proteomics approach by treating plants with the cellulose synthase inhibitor isoxaben and analyzing TiO2 enriched fractions of phosphorylated peptides through high resolution mass spectrometry. Altogether, our data from 9 independent biological replicates shows 187 upregulated and 189 downregulated phosphopeptides in the isoxaben-treated vs control plants. Notably, we found 2 sites of the malectin domain receptor-like kinase FERONIA are differentially phosphorylated and further identified 9 out of the 16 FERONIA phosphosites reported in the literature. This supports our hypothesis that the detected phosphorylation changes are a response to the stress on cellulose production. In the future, this dataset will enable us to identify other proteins involved in cell wall signal transduction mechanisms for further characterization.

#39

Intelligent Agent: A webtool to aggregate and synthesize information for plant biology

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With recent advancements in data science, machine learning and natural language processing, it is possible to create a tool that can interact with diverse data repositories and analyze large volumes of data to coherently summarize information to help researchers to get to the "right information" quickly according to their interest. This is the premise behind work on a new tool called the Intelligent Agent that can aggregate and synthesize information to create

an executive summary that will include either an overview of a query or a response to a question. The tool creates new centralized and automatically updated databases that have been designed to assist in the timely processing of the data. The output is intuitive and accessible within a web browser. Using a natural language processing tool called Compromise and modern web development tools (including the React JavaScript library), we were successfully able to prototype the Intelligent Agent to aggregate data from different data repositories (such as the BAR, NCBI and Google) and synthesize the collected information to create either an overview of the query or a direct answer to a researcher's question. All data is currently from Arabidopsis thaliana, but the plan is to expand the Intelligent Agent to other plant species shortly.

#40

Identification and characterization of novel targets for a subfamily of Arabidopsis calmodulin-like (CML) proteins

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Calcium ions serve as ubiquitous second messengers in eukaryotes. Calcium sensor proteins, such as calmodulin (CaM), detect and transduce calcium signals by regulating downstream target proteins. Arabidopsis possesses 7 CaM and 50 CaM-like (CML) genes. Whereas many of the downstream targets of CaM are well characterized, very few CML targets have been identified. Here, using a range of biochemical and molecular approaches, we have identified several target proteins that interact exclusively with a small subfamily of Arabidopsis CML paralogs which display unusual calcium-binding and structural properties relative to other members of the CML family. Using the yeast two-hybrid system, we identified putative targets of these CMLs, delineated the CML-target interaction domains, and corroborated the high specificity of this interaction using in vitro assays and the in planta split-ubiquitin system with Nicotiana benthamiana. Structural and biophysical analyses indicate both CaM and CMLs interact with the target proteins that we have identified. We will discuss the significance of this interaction and the proposed roles for these CMLs and their targets based on our analyses using T-DNA insertion knockout-lines, and promoter activity assays using CML promoter:GUS reporter transgenic plants.

#41

Identifying drought tolerance traits through comparative phenotyping of two Eutrema salsugineum accessions

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Drought accounts for over 80% of global agricultural losses. Despite the importance of improving crop drought tolerance, little progress has been made towards this objective.

Our approach to identifying traits underlying drought tolerance involves a comparison between two accessions of the crucifer, Eutrema salsugineum, that display differential tolerance to water deficits. The accessions, originating from the semi-arid Yukon, Canada, and a monsoonal region of Shandong, China, were subjected to a two-step, water deficit and recovery protocol to identify physiological characteristics that discern their drought-responsive behaviour. Traits that discriminate between the ecotypes will be used to screen recombinant inbred lines (RILs) that were generated by crossing Yukon and Shandong parents. Features selected for testing include: anthocyanin accumulation, relative water content, static leaf water content, cut rosette water loss (CRWL), specific leaf area (SLA), and fluorescence emission. Of the measurements taken, CRWL and anthocyanin content distinguish the parent lines after the first drought exposure whereas SLA and fluorescence responses are better at differentiating the accessions following recovery from the first drought. Testing a randomly selected sample of RILs has identified segregation with respect to drought responsive phenotypes. Continued phenotypic screening for drought recovery and subsequent water deficit responses will help identify quantitative trait loci associated with the water-use efficient Yukon ecotype and genes meriting consideration for use in crop improvement.

#42

Biofilm formation contributes to *Pseudomonas syringae* pv. tomato success and suppression of bacterial biofilm formation contributes to PAMP-triggered immunity in *Arabidopsis*

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A biofilm is a community of bacteria in a matrix of extracellular polysaccharides and DNA that is thought to protect bacteria from plant defence. Using *Pseudomonas syringae* pv. tomato (Pst) and Arabidopsis, we investigated the role of biofilms in bacterial pathogenesis by determining if biofilm formation contributes to bacterial success in planta and if PAMP-Triggered Immunity (PTI) limits biofilm formation. PTI was induced by flg22 treatment in wild-type Col-0, fls2 (PTI mutant) and sid2-2 (SA biosynthesis mutant). In vivo Pst aggregate formation was monitored using Pst DC3000 pDSK-GFPuv. Bacterial success (growth in planta) was positively associated with Pst aggregate occurrence in fls2 and sid2-2, while fewer and smaller aggregates were observed in Col-0 during PTI indicating SA-dependent suppression of Pst aggregate formation. Alginate is a polysaccharide biofilm component of many Pseudomonas strains grown in culture. Pst ΔalgD ΔalgU ΔmucAB (alginate biosynthesis mutant with reduced virulence) growth was reduced 10-fold in Col-0 compared to wild-type Pst and formed few aggregates suggesting that Pst biofilm formation is important for Pst pathogenicity. Compared to wild-type Pst, Pst ΔalgD ΔalgU ΔmucAB grew to higher levels and formed more aggregates in sid2-2 suggesting that biofilm formation is important for coping with SA-dependent PTI. In vivo staining of aggregates for extracellular DNA (DAPI) and polysaccharides (calcofluor white), suggests that Pst aggregates are indeed biofilms. Together, these results provide compelling evidence that the ability to form biofilms contributes to Pst

pathogenicity and success in Arabidopsis and suppression of biofilm formation is an important component of PTI.

#43

Exploring patterns of gene expression in *Eutrema salsugineum* (acc. Yukon) under phosphate and sulfur deficiency

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Improving the efficiency by which crops use nutrients is critical for maintaining high crop productivity while reducing fertility management costs and eutrophication related to nutrient runoff. A Yukon ecotype of a native crucifer and crop relative, Eutrema salsugineum. grows on highly saline soil that is high in sulfur (S) but typically low in available phosphate (Pi). In this study, we manipulated Pi and S content of soil used to grow E. salsugineum in cabinets for four weeks. The phenotype of plants growing on varying levels of Pi and S were very similar. We analyzed transcriptome profiles to help identify traits associated with deficiencies in S and/or Pi. Transcriptomes comprising >28,000 expressed genes were analyzed using weighted gene co-expression network analysis and principal component analysis. Surprisingly, transcript levels between the combinations of low and high Pi and S treatments showed little evidence for clustering related to nutrient regime. Gene expression for protein-coding and long non-coding RNA (lncRNA) analyzed by DESeq2 corroborated the clustering approaches in that < 10 treatment-specific, differentially expressed genes (DEGs) were associated with the nutrient treatments with the majority (3%) related to altered S and not Pi. Interestingly, although lncRNAs are associated broadly with stress, we found no predicted treatment-specific lncRNAs and only 31 of the 618 predicted lncRNAs were DEGs, suggesting that Yukon E. salsugineum used in the study were unstressed by our treatments despite altered internal S and Pi levels.

#44

Enhancement of Plant Growth Promoting Bacteria in the Root Microbiome of Haricot Bean Following Amendment with the Fungus, *Metarhizium*,

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The microbial community in the plant rhizosphere is vital to plant productivity and disease resistance. Alterations in microbial species richness and composition of this community could be detrimental if microbes suppressing the activity of pathogens are removed. Species of the insect-pathogenic fungus, *Metarhizium,* commonly employed as biological control agents against crop pests, have recently been identified as plant root colonizers and provide a variety of benefits (e.g. growth promotion, drought resistance, nitrogen acquisition). However, the impact of *Metarhizium*amendment on the rhizospheric microbial community has yet to be elucidated. Here we examined, by Illumina sequencing, the microbiome profiles (bacteria and fungi) of common bean (*Phaseolus vulgaris*) rhizosphere (loose soil and plant root) after

amendment with *M. robertsii* conidia. Although alpha diversity was not significantly affected overall, there were numerous examples of plant growth-promoting bacteria that significantly increased with *Metarhizium*amendment. For example, *Bradyrhizobium*, a nitrogen fixing bacteria, was detected by Illumina sequencing, then isolated on agar plates from soil suspensions and confirmed using qPCR with genus-specific primers. In spite of the presence of some known fungal pathogens (i.e. *Ilyonectria, Fusarium solani*), bean plants were without signs of disease. Successful amendment of agricultural soils with biocontrol agents such as *Metarhizium*necessitates a comprehensive understanding of the effects on the diversity of the rhizosphere microbiome. Such research is fundamentally important towards sustainable agricultural practices to improve overall plant health and productivity.

#45

Plant Immune Response to Endophytic Colonization by *Metarhizium* .

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Endophytes are defined as microorganisms present in plant tissues without causing any visible symptoms of disease. As endophytic insect-pathogenic fungi, *Metarhizium* species can improve plant growth, enhance plant tolerance to abiotic stresses and antagonize to plant pathogens, such as *Fusarium solani.* However, the plant immune responses to endophytic colonization by *Metarhizium* spp. are still unknown. One strategy that may indicate the immune status of the plant during this symbiosis is to measure a change in the concentration of plant hormones. We applied UPLC ESI-MS/MS to comprehensively test the concentration of plant hormones in leaves of bean plant colonized by *M. robertsii* and/or *F. solani.* Compared to the un-inoculated control, the concentration of abscisic acid (ABA) decreased significantly in plants colonized by *M. robertsii* and increased in those colonized by *F. soloni*. To further confirm the different roles of ABA during endophytic or pathogenic colonization, colony-forming units (CFUs) of *M. robertsii* and *F. solani* in bean root homogenate were examined after exogenous application of ABA. Compared to the control without ABA application, the CFUs of *M. robertsii* decreased significantly and CFUs of *F. solani* increased in bean roots with exogenous ABA application. Both the chemical analyses and exogenous application experiment confirmed that the ABA plays a role in differential plant immune responses to *M. robertsii* and *F. solani*. This research can provide clues towards understanding the mechanism behind plant colonization of endophytes and may help to improve the application of *Metarhizium* as a biological method to maintain the plant health.