



Canadian Society of Plant Biologists -  
Société Canadienne de Biologie Végétale  
Eastern Regional Meeting

November 27, 2021

PROGRAM AND ABSTRACTS

## **Welcome!**

The Canadian Society of Plant Biologists (CSPB-SCBV) holds its Eastern Regional Meeting each fall. This annual event brings together plant biology researchers from academic, government, and industry labs in Eastern Canada for a day of talks, posters, and networking on all aspects of plant science.

Given the non-resolved coronavirus pandemic worldwide, the CSPB-SCBV and the Organizing Committee have opted for a virtual format. This format ensures the continuity of knowledge exchange and offers flexibility to participants.

Following in the tradition of the society, this year's event will include two plenary lectures by leaders in their field together with oral and e-poster presentations given mostly by post-doctoral fellows and graduate students covering all aspects of plant science and technology.

**The virtual conference program presents the schedule in your time zone.** All plenary and concurrent talks will be live or pre-recorded followed by a Q&A session with the speakers. Posters will be accompanied by short pre-recorded talks, and the poster sessions will include opportunities for live interaction with presenters. The concurrent sessions will be available for on-demand viewing shortly after the conference is complete.

## **Organizing Committee**

**Shelley Hepworth** (Carleton University)

**Owen Rowland** (Carleton University)

**Tim Xing** (Carleton University)

**Catherine Cullingham** (Carleton University)

**Rajagopal Subramaniam** (Agriculture and Agri-Food Canada)

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## Plenary Speakers



**Dr. Catherine Cullingham, Carleton University**

**Title: *Using genomics to predict forest resiliency in the mountain pine beetle system***

Dr. Catherine Cullingham is an Assistant Professor in the Department of Biology at Carleton University. She received her BSc from the University of Guelph, earned her PhD at Trent University, and completed her postdoctoral work at the University of Alberta. Her research uses landscape genetics and population genomics to fill knowledge gaps and develop tools that can be applied to issues in forestry and wildlife management. She has been working on the mountain pine beetle system for over 10 years, and has contributed to confirming host-expansion to jack pine, redefining the spatial complexity of the lodgepole x jack pine hybrid zone, and identifying genetic markers potentially associated with MPB resilience.



**Dr. Sylvie Cloutier, Agriculture and Agri-Food Canada, Ottawa**

**Title: *The challenges and promises of pre-breeding***

Sylvie received her B.Sc. in Agronomy from Université Laval (1987), M.Sc. in Plant Science from University of Guelph (1990) and Ph.D. in Biology from Université de Montréal (1994). She joined AAFC's Cereal Research Centre in Winnipeg in 1995, first as a Visiting Fellow and then as a Research Scientist prior to moving to the Ottawa Research and Development Centre as a Principal Research Scientist in 2014. Dr. Cloutier's research focuses on genetics, genomics and epigenetics of wheat and flax. She currently leads a pre-breeding program in wheat to identify new sources of resistance for FHB, leaf rust, stripe rust and powdery mildew from wild relatives using genome-wide markers, GWAS and genomic selection approaches. She coordinates a national phenotyping program in both winter and spring *Triticum* and *Aegilops* species. She co-lead the TUFGEN (flax) large scale Genome Canada project (2009-2014) and she is currently co-leading a second one called 4DWheat (2019-current). She has published more than 115 scientific publications and 12 book chapters. She is an adjunct professor at the Universities of Ottawa and Guelph where she currently supervises 4 PhD and 2 MSc students. She was awarded the Rosemary Davis award in 2013 for leadership in Agriculture and recently received the 2021 Borlaug Global Rust Initiative Gene Stewardship group award for her contribution to the sustainability of rust resistance in wheat. Throughout her career, she has mentored more than 100 individuals including Visiting Scholars, Post-docs, Graduate, undergraduate and high-school students.

## PROGRAM OVERVIEW

Time	Saturday November 27, 2021
9:00 am	Conference Opens
9:10 – 9:30 am	<b>Welcome Remarks</b>
9:30 – 10:15 am	<b>Plenary Lecture</b> <b>Dr. Catherine Cullingham</b> (Carleton University) <i>“Using genomics to predict forest resiliency in the mountain pine beetle system”</i>
10:15 – 10:30 am	Coffee Break 1
10:30 – 12:00 pm	<b>Concurrent Sessions 1 to 3</b>  C1 – Biotic interactions C2 – Metabolism and signaling C3 – Plant development
12:00 – 12:30 pm	Lunch Break
12:30 – 2:00 pm	<b>Poster Hall</b>
2:00 – 3:30 pm	<b>Concurrent Sessions 4 to 6</b>  C4 – Biochemistry and cell biology C5 – Genomics and systems biology C6 – Environment and technology
3:30 – 3:45 pm	Coffee Break 2 (Awards Deliberation)
3:45 – 4:30 pm	<b>Plenary Lecture</b> <b>Dr. Sylvie Cloutier</b> (Agriculture and Agri-Food Canada) <i>“The challenges and promises of pre-breeding”</i>
4:30 – 5:00 pm	<b>Student Awards and Closing Remarks</b>
5:00 pm	Conference Ends

## Concurrent Session 1 – Biotic interactions

C1-1	10:30	Christian Danve Castroverde	Molecular studies on the CALMODULIN-BINDING PROTEIN 60-LIKE (CBP60) protein family in tomato plants
C1-2	10:45	Eric Marchetta	Temperature regulation of plant and rhizobacterial mechanisms during induced systemic resistance (ISR) in tomato
C1-3	11:00	Márcia Gonçalves Dias	Investigating the responses of black raspberry ( <i>Rubus occidentalis</i> ) to late leaf rust ( <i>Thekopsora americana</i> )
C1-4	11:15	Charles Roussin-Leveillee	Chloroplastic ROS integrate immune signaling with phytohormone biosynthesis in pattern-triggered immunity
C1-5	11:30	Matthew Toffoli	Growing the future: Investigating the PGPR capabilities of 13 bacterial isolates from the Canadian soilborne bacteria library
C1-6	11:45	Andreea Bosorogan	Assessing the role of plant-defense compounds on insect-associated bacterial communities

## Concurrent Session 2 – Metabolism and signaling

C2-1	10:30	Josephine Payment	Investigating the waterlogging and cold stress combination in <i>Nicotiana tabacum</i>
C2-2	10:45	Frederik Nguyen	Characterizing metabolism and transport of 3'-fluoro- abscisic acid in <i>Brassica napus</i> L.
C2-3	11:00	Artyom Gritsunov	Investigating quinate and chlorogenic acid metabolism in plants
C2-4	11:15	Jessica Sinka	Metabolic flux analysis during wound-healing in potato tubers
C2-5	11:30	Paul Jerome Gamueda	Characterizing guard cell-specific drought-responsive genes in <i>Arabidopsis thaliana</i>
C2-6	11:45	Amal Jaballi	The phytohormone abscisic acid modulates protein carbonylation in <i>Arabidopsis thaliana</i>



## Concurrent Session 3 – Plant development

C3-1	10:30	Stuart Macgregor	The role of autophagy in the Arabidopsis self-incompatibility response
C3-2	10:45	Jasmin Patel	Investigating the NAC-FUS3 molecular module in seed coat development in <i>Arabidopsis thaliana</i>
C3-3	11:00	Natalie Hoffmann	Alteration of xyloglucan biosynthesis disrupts endomembrane structure and function
C3-4	11:15	Deka Mohamed	Drought-responsive RING E3 ligase XERICO maintains proper stomatal density and distribution during leaf epidermal development
C3-5	11:30	Mariann Lobbezoo	Investigating florigenic mechanisms that regulate flowering in temperate <i>Zea mays</i> and its tropical progenitor, teosinte
C3-6	11:45	Stephen Bordeleau	Elucidating the role of RKF1 interactors in post-pollination responses in <i>Arabidopsis thaliana</i>

## Concurrent Session 4 – Biochemistry and cell biology

C4-1	2:00	Bona Mu	The C-terminal extension region regulates the plastid molecular chaperone HSP90C function
C4-2	2:15	Jenan Nouredine	Investigating the mechanistic role of Arabidopsis HSP90.7 in plant development
C4-3	2:30	Thakshila Dharmasena	Conserved multitaskers: Investigating the role of a family of multi-functional enzymes in <i>Arabidopsis thaliana</i>
C4-4	2:45	Michael Kanaris	Elevated tyrosine results in the cytosolic retention of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase in <i>Arabidopsis thaliana</i>
C4-5	3:00	Adesola Tola	Ammonium sulfate prefractionation-assisted detection of the carbonylated proteins in the <i>Arabidopsis thaliana</i> leaves
C4-6	3:15	Olivia Friesen Kroeker	Organelle interactions involve endoplasmic reticulum membranes sandwiched between them

## Concurrent Session 5 – Genomics and systems biology

C5-1	2:00	Kifah Gharzeddin	Phenotyping a diverse collection of chickpea lines field-grown in Eastern Ontario for major agronomic traits
C5-2	2:15	Galyna Vakulenko	Heat stress and cold adaptation in the Antarctic alga <i>Chlamydomonas</i> sp. UWO241
C5-3	2:30	Julia Hooker	Transcriptome-wide approach to address lower seed protein content in soybean grown in Western Canada
C5-4	2:45	Aparna Haldar	Investigation into the evolution and expansion of a leucine-rich repeat receptor kinase family in land plants
C5-5	3:00	Hasna Khan	Genome-wide analysis of alternative splicing in <i>Arabidopsis thaliana</i> using a guard cell-specific transcriptome
C5-6	3:15	Nour Nissan	Revealing the truth behind a previously presumed locus, E6, in soybean variety Paranagoiana

## Concurrent Session 6 – Environment and technology

C6-1	2:00	Allison McDonald	Training graduate students and post-doctoral scholars in science communication
C6-2	2:15	Alexandra Smith	Exploring genetic and environmental effects on Canadian winter wheat yields over time
C6-3	2:30	Carly Charron	Production of plant-based vaccine candidates in <i>Nicotiana benthamiana</i> to prevent Salmonella infection in poultry
C6-4	2:45	Matei Dan-Dobre	Investigation into the evolution and expansion of a leucine-rich repeat receptor kinase family in land plants
C6-5	3:00	Pooja Kaushik	Identification of the EPF signaling peptides regulating grass stomatal development and patterning
C6-6	3:15	Ingo Ensminger	Something in the air - Drone based phenotyping of phenology and drought stress

## Plenary lecture abstracts

### Using genomics to understand risk and resiliency in the mountain pine beetle system

Catherine I. Cullingham<sup>1</sup>

<sup>1</sup>Department of Biology, Carleton University, Ottawa, ON Canada.

The most recent mountain pine beetle outbreak has affected over 18 million hectares of forest in Canada impacting industry, carbon cycling and ecosystem function. In 2006 the beetle expanded into central Alberta where it has encountered a novel host, jack pine. Many questions have arisen since then including, is jack pine an appropriate host for mountain pine beetle? What is the potential for continued spread across the Boreal forest? What genes underlie host susceptibility? Using population genetics, spatial ecology, and molecular biology we help to answer some of these important questions, and provide useful outputs for management and predictive modelling. Through this integrated approach we provide a meaningful first step towards identifying the genetic component of pine host suitability to mountain pine beetle. Given the increasing frequency and intensity of biological invasions in forest ecosystems, approaches that consider interactions from the landscape to the individual will be critical for ensuring forest resiliency in the future.

### The challenges and promises of pre-breeding

Sylvie Cloutier<sup>1,2</sup>, George Fedak<sup>1</sup>, Fizza Fatima<sup>2</sup>, Sampurna Bartaula<sup>2</sup>, Brent McCallum<sup>3</sup>, Maria Antonia Henriquez<sup>3</sup>, Barb Blackwell<sup>1</sup>, Reem Aboukhaddour<sup>4</sup>, Adam Foster<sup>5</sup>, Colin Hiebert<sup>3</sup>, Gavin Humphreys<sup>1</sup>, Curt McCartney<sup>4,6</sup>, Curtis Pozniak<sup>7</sup>, Frank You<sup>1</sup>

<sup>1</sup> Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON,<sup>2</sup> Department of Biology, University of Ottawa, Ottawa, ON,<sup>3</sup> Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB,<sup>4</sup> Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, AB,<sup>5</sup> Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, Charlottetown, PEI,<sup>6</sup> Department of Plant Science, University of Manitoba, Winnipeg, MB,<sup>7</sup> Crop Development Center, University of Saskatchewan, Saskatoon, SK.

Crop breeding programs aim to release cultivars with improved attributes that are adapted to targeted agro-ecological zones. Pre-breeding programs aim at identifying new sources of genetic diversity that could be useful in breeding programs, and to transfer the diversity into germplasm that can be used by breeders to create new cultivars. These new sources of genetic diversity could reside in already adapted germplasm but they are often found in more exotic germplasm frequently referred to as crop wild relatives (CWR). The identification of potentially useful genetic diversity is challenging because CWR lack adaptation traits and their phenology complicate their phenotyping. In addition, the genomic resources for these species are limited to non-existent. There are significant challenges to the transfer of genetic diversity from CWR to crops such as interspecific crossing barriers, infertility, chromosomal rearrangements and linkage drag. This presentation will use wheat as an example to illustrate how new strategies are currently being implemented to make pre-breeding more efficient. These strategies

capitalize on genome sequencing, high-throughput phenotyping, genome-wide association studies, genomic selection and the potential for CRISPR-based technologies. The presentation will explain the need for tapping into CWR to improve crops, the challenges associated with CWR to crop genetic transfer and the potential of integrating today's technologies to design new pre-breeding solutions to overcome these challenges.

## Concurrent session abstracts

### C1-1: Molecular studies on the CALMODULIN-BINDING PROTEIN 60-LIKE (CBP60) protein family in tomato plants

Christian Danve Castroverde<sup>1</sup>, Vanessa Shivnauth<sup>1</sup>, Sonya Pretheepkumar<sup>1</sup>, Keaun Amani<sup>1</sup>

<sup>1</sup>Wilfrid Laurier University.

Cellular signalling generates calcium (Ca<sup>2+</sup>) ions, which are ubiquitous secondary messengers decoded by calcium-dependent protein kinases, calcineurins, calreticulin, calmodulins (CAMs) and CAM-binding proteins. Previous studies in the model plant *Arabidopsis thaliana* have showed the critical roles of the CAM-BINDING PROTEIN 60-LIKE (CBP60) protein family in growth, stress responses and immunity. Certain CBP60 factors can regulate immune responses, like pattern-triggered immunity, effector-triggered immunity, and synthesis of major plant immunometabolites salicylic acid (SA) and N-hydroxypipecolic acid (NHP). Although homologous CBP60 sequences have been identified in the plant kingdom, their function and regulation in most species remain unclear. Here, we have identified 981 CBP60 homologs across various plant taxa (63 genomes). Specifically, we characterized 11 members of the CBP60 family in the agriculturally important crop tomato (*Solanum lycopersicum*). Phylogenetic analyses reveal that three homologs have the closest amino acid identity to *Arabidopsis* CBP60g and SARD1, master transcription factors involved in plant immunity. Strikingly, these three tomato *CBP60* homologs also exhibit similar gene expression profiles as their *Arabidopsis* counterparts. Conserved domain analyses revealed that they possess CAM-binding domains, reflecting their potential involvement in tomato Ca<sup>2+</sup> signalling. We are currently using deep learning-assisted approaches to predict their protein structures to infer function. In parallel, we have isolated mutants in these three genes and will be conducting various phenotyping assays. Overall, we present a kingdom-wide identification of these central Ca<sup>2+</sup>-sensing plant regulators and provide evidence on their potential function and regulation in tomato plants.

### C1-2: Temperature regulation of plant and rhizobacterial mechanisms during induced systemic resistance (ISR) in tomato

Eric Marchetta<sup>1</sup>, Keiko Yoshioka<sup>2</sup>, Wolfgang Moeder<sup>2</sup>, Eric Déziel<sup>3</sup>, Danve Castroverde<sup>1</sup>

<sup>1</sup>Wilfrid Laurier University, <sup>2</sup>University of Toronto, <sup>3</sup>Institut National de la Recherche Scientifique, Institut Armand-Frappier.

The rhizosphere is an active site of direct microbe-microbe and plant-microbe interactions. Beneficial root microbiomes can enhance nutrient uptake, improve root structure, protect against biotic stressors, and/or prime plant defenses through induced systemic resistance (ISR). While the environmental influence on plant pathogenesis and immune responses are well studied, the impacts on plant and microbial mechanisms during ISR remain largely unexplored. In this study, we aim to determine how the physiology of ISR-inducing bacterial strains isolated from Canadian soils and the resulting systemic signaling in host plants, are affected by temperature. To investigate this, we are conducting in-vitro growth rates, phosphate solubilization abilities, and direct anti-pathogenic effects of 16 ISR-inducing bacterial strains

across three temperatures (12°C, 23°C, and 30°C). Next, the in-situ effects of temperature on ISR-inducing bacteria will be determined by investigating bacterial colonization of roots, bacterial proliferation in the rhizosphere and persistence on the rhizoplane. Finally, temperature effects on the plant immune response will be determined after colonization with ISR-inducing bacterial strains through gene expression analysis. Preliminary results have identified two *Bacillus* sp. strains that maintain phosphate solubilization ability at elevated temperatures. Also, pilot growth rate monitoring of all strains has provided a baseline for future comparative temperature experiments. With climate change intensifying the severity of temperature fluctuations, our study seeks to identify temperature-resilient rhizobacteria for biofertilizer applications to enhance crop protection. Our anticipated discoveries will benefit the agricultural industry to ensure global food security and could also promote environmental and human health due to reduced agrochemical usage.

### **C1-3: Investigating the responses of black raspberry (*Rubus occidentalis*) to late leaf rust (*Thekopsora americana*)**

Márcia Gonçalves Dias<sup>1, 2</sup>, Jacqueline Monaghan<sup>2</sup>, Marcel Bellato Spósito<sup>1</sup>, Beatriz Appezzato da Glória<sup>1</sup>

<sup>1</sup>Luiz de Queiroz College of Agriculture, University of São Paulo, Brazil, <sup>2</sup>Department of Biology, Queen's University, Canada.

Late leaf rust is a disease caused by the fungus *Thekopsora americana* (Farl.) Aime & McTaggart that infects raspberries, impacting productivity and fruit quality. While red raspberries (*Rubus idaeus* L.) are highly susceptible to this pathogen, it was previously reported that black raspberries (*Rubus occidentalis* L.) might be more resistant to this disease, which could be useful in breeding programs. However, no studies have explored the ultrastructural, histochemical, or biochemical properties of the *R. occidentalis* – *T. americana* interaction. Here we present ultrastructural micrographs showing that *T. americana* is able to form appressoria that penetrate *R. occidentalis*, indicating that black raspberries can also be infected by this pathogen. In addition, our histochemical analyses indicate that *R. occidentalis* produces phenolic compounds in response to infection by *T. americana*. We are now attempting to establish a molecular toolkit that will enable the deeper characterization of the early infection stage. This work seeks to provide new information about the *R. occidentalis* defense mechanisms against *T. americana* infection that may give directions to biotechnological applications such as plant breeding targeting resistance to diseases.

### **C1-4: Chloroplastic ROS integrate immune signaling with phytohormone biosynthesis in pattern-triggered immunity**

Charles Roussin-Leveillee<sup>1</sup>, Méliane St-Amand<sup>1</sup>, Philippe Desbiens-Fortin<sup>1</sup>, Jonghum Kim<sup>2</sup>, Sheng Yang He<sup>2</sup>, Peter Moffett<sup>1</sup>

<sup>1</sup>Université de Sherbrooke, <sup>2</sup>Duke University.

Chloroplasts are emerging as essential contributors to plant immunity, mainly through their involvement in the biosynthesis of defense-related phytohormones, as well as in the generation of reactive oxygen and nitrogen species. Following the activation of pattern recognition receptors by immunogenic pathogen-derived molecules, biosynthesis of the phytohormone salicylic acid (SA) is induced in chloroplasts to amplify immune responses. Although much has been unraveled about SA biosynthesis in the chloroplast, which signal(s) trigger(s) its production during plant immunity remains unknown. We have characterized an intimate link between chloroplast-derived reactive oxygen species (cROS) and the initiation of SA biosynthesis and signaling. We have studied this phenomenon using an experimental system based on infection of *Arabidopsis thaliana* by *Pseudomonas syringae* as well as a defense priming assay wherein protection from infection is afforded by pre-treating plants with the immunogenic peptide flg22. We find that, while flg22 triggers cROS bursts, application of SA does not. Using chemical inhibitors, we show that inhibition of cROS bursts during immune activation completely abrogates the protection given by flg22 pre-treatment, as well as the induction of SA biosynthesis and signaling pathways. Interestingly, *Arabidopsis thaliana* SA biosynthesis and signaling mutants are not impaired in inducing cROS bursts during an immune response. Furthermore, we observed that the timeframe of induction of cROS bursts correlates with that of SA biosynthesis. Altogether, these observations position flg22-induced cROS upstream of SA biosynthesis in the molecular events happening in the chloroplast during immunity.

#### **C1-5: Growing the future: Investigating the PGPR capabilities of 13 bacterial isolates from the Canadian soilborne bacteria library**

Matthew Toffoli<sup>1</sup>, Winfield Yim<sup>1</sup>, Nadia Morales-Lizcano<sup>1</sup>, Wolfgang Moeder<sup>1</sup>, Eric Déziel<sup>2</sup>, Keiko Yoshioka<sup>1</sup>, Thomas Berleth<sup>1</sup>

<sup>1</sup>University of Toronto, <sup>2</sup>Institut National de la Recherche Scientifique.

The ability of soil microorganisms to protect plants has been known for a century. Our goal is to harness microbe/microbiota-associated protection (MAP) for agriculture. However, to date, the use of microorganisms in agriculture is limited, due to minimal understanding of the complex nature of rhizospheric plant-microbe interactions. MAP occurs in the rhizosphere, a narrow region of soil surrounding plant roots housing a thriving microbial community. Beneficial rhizospheric bacteria provide robust defense against pathogens via direct competition and a phenomenon known as Induced Systemic Resistance (ISR). ISR confers broad-spectrum resistance against pathogens and is induced by non-pathogenic microorganisms. Studies show that ISR-inducing bacteria often also promote host plant growth, classifying them as both ISR inducers and Plant Growth Promoting Rhizobacteria (PGPRs). Recently, our team characterized 13 bacterial isolates from a pre-established Canadian Soilborne Bacteria library. These isolates conferred resistance to a broad spectrum of pathogens including *Botrytis cinerea* and *Pseudomonas syringae*. This current study investigates these isolates from a PGPR point-of-view to observe whether these strains confer growth benefits on their hosts, specifically increasing lateral root number and shoot fresh weight. Additionally, this study aims to elucidate the involvement of hormones and hormonal signaling pathways by which this PGPR effect is

conferred to the host through examining PGPR effects in salicylic acid, jasmonic acid, and ethylene mutant plants. Ultimately, this study aims to answer whether these 13 isolates both induce ISR and promote plant growth, thereby expanding the repertoire of bacterial strains that can be used as both biopesticide and biofertilizer.

#### **C1-6: Assessing the role of plant-defense compounds on insect-associated bacterial communities**

Andreea Bosorogan<sup>1, 2</sup>, Eliana Gonzales-Vigil<sup>1, 2</sup>

<sup>1</sup>Department of Biological Sciences, University of Toronto Scarborough, Canada, <sup>2</sup>Department of Cell & Systems Biology, University of Toronto, Canada.

Plants and insects have many characterized defense strategies against each other. However, bacteria are overlooked players in this well-studied interaction. In the past decade, bacteria have been implicated in modulating defenses among insects and plants, yet there is little evidence about how plant defense compounds affect these interactions with bacteria. To investigate if bacterial communities are affected by plant-associated defense compounds and physical defenses, the interaction between the cabbage looper (*Trichoplusia ni*) and tomato (*Solanum lycopersicum*) is being studied. Cabbage loopers were fed on several defense-deficient tomato mutants, and larval weight measurements and frass (insect feces) samples were collected. Frass is a homogenous material that acts as a proxy for insect-associated bacterial diversity. Larval weight data suggest significant differences in insect performance across tomato mutant lines. Frass 16S rRNA gene sequencing results show shifts in bacteria population abundance and diversity at the genus level. A negative correlation was found between plant terpenes and *Enterobacteriaceae* sp. abundance. The bacterial  $\alpha$ - and  $\beta$ -diversities displayed significant differences relative to the plant the insects were fed on. Overall, these results suggest that herbivore insect-associated bacterial communities are affected by key plant defense compounds.

#### **C2-1: Investigating the waterlogging and cold stress combination in *Nicotiana tabacum***

Josephine Payment<sup>1</sup>, Marina Cvetkovska<sup>1</sup>

<sup>1</sup>University of Ottawa.

In their natural environments, plants are vulnerable to a wide array of stressful conditions, often occurring in combination. Recently it has become clear that plants respond to these combined stresses differently than isolated stressors, and that these responses are not easily predicted. Cold and waterlogging represents a stress combination that is prevalent in Canadian spring conditions, yet it remains understudied. Preliminary work suggests that this stress combination may be less damaging than expected, for reasons which have yet to be fully explained. To further investigate this stress combination, *Nicotiana tabacum* plants were exposed to cold and waterlogging stress both in isolation and in combination. Physiological experiments were performed to determine the plant's response to the various stressors. Additionally, effects on the photosynthetic system were examined. Our results indicate that



exposing a plant to the stress combination is no less damaging than waterlogging stress and no more damaging than cold stress; future work will aim to better understand the mechanisms behind this stress combination.

## **C2-2: Characterizing metabolism and transport of 3'-fluoro-abscisic acid in *Brassica napus* L.**

Eiji Nambara<sup>1</sup>, Frederik Nguyen<sup>1</sup>, Zhen Xu<sup>1</sup>, Christine Nguyen<sup>1</sup>, Sue Abrams<sup>2</sup>, Chris Phenix<sup>2</sup>, Devin Brown<sup>2</sup>, Morshed Chowdury<sup>2</sup>, Leon Lai<sup>2</sup>, Naveen Diddi<sup>2</sup>, Shankar Pahari<sup>3</sup>, Raju Soolanayakanahally<sup>3</sup>

<sup>1</sup>University of Toronto, Dept. of Cell and Systems Biology, <sup>2</sup>University of Saskatchewan, Dept. of Chemistry, <sup>3</sup>Agriculture and Agri-Food Canada, Saskatoon, SK Canada.

The phytohormone abscisic acid (ABA) is known for its essential role in regulating plant responses to biotic and abiotic stresses. Though properties of ABA transport have been previously documented, the ability to monitor dynamic flow of ABA *in vivo*, under different biological settings remains a challenge. Positron Emission Tomography (PET) *in planta* allows for a real-time and non-destructive method for imaging the movement of phytohormones. We synthesized [<sup>18</sup>F]-3'-fluoro-abscisic acid (3'-<sup>18</sup>F-ABA) as a radiotracer for autoradiography and PET imaging experiments. This study aims to assess whether the transport and metabolism of 3'-F-ABA is similar to ABA in plants thus validating 3'-<sup>18</sup>F-ABA as a valuable radiotracer. Firstly, 3'-<sup>18</sup>F-ABA was applied to the *Brassica napus* NAM-0 line. After 1 hour of incubation, plant extracts assessed by HPLC with a radioactive detector confirmed that ~90% of the radiolabel corresponded to 3'-<sup>18</sup>F-ABA. Using authentic fluorinated standards, we also confirmed that [<sup>18</sup>F]-3'-fluoro-phaseic acid (F-PA) is the primary catabolite formed in NAM-0. In parallel, destructive phytochemical analysis of 3'-F-ABA via liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used to compare the transport and metabolism of 3'-F-ABA to ABA. Deuterium labeled d<sub>3</sub>-3'-F-ABA and d<sub>3</sub>-F-PA were used as internal standards for quantitation. LC-MS/MS experiments revealed that 3'-F-ABA and ABA were similarly distributed in NAM-0 45 min and 90 min after administration. These experiments reveal that 3'-F-ABA has similar biological properties of ABA thus validating 3'-<sup>18</sup>F-ABA as a radiotracer for studying ABA transport by PET imaging.

## **C2-3: Investigating quinate and chlorogenic acid metabolism in plants**

Artyom Gritsunov<sup>1</sup>, Dinesh Christendat<sup>1</sup>

<sup>1</sup>University of Toronto, Dept. of Cell and Systems Biology, Toronto, ON Canada.

Quinate is abundant in plant green tissues and is used for the biosynthesis of chlorogenic acids. These compounds serve as important protectants of plants against herbivory, UV light and have other significant functions. However during ripening and maturation levels of quinic acid drop in multiple plant species. Until recently, factors responsible for the build up and disappearance of quinate were not known. In the Christendat's lab, we uncovered a set of genes responsible for the metabolism of quinic acid in plants. Specifically, we established the presence of two different, an anabolic and a catabolic, quinate dehydrogenases which are responsible for the

metabolism of quinate. We also investigated the evolutionary relationships of these enzymes in plants. Currently, we are investigating the biological role of qdhs and are trying to understand how quinic acid metabolism affects plants. We generated transgenic *Arabidopsis* lines which overexpress catabolic and anabolic qdhs. Quinate production was identified in some lines. Future work will involve more detailed metabolic analysis of transgenic lines.

#### **C2-4: Metabolic flux analysis during wound-healing in potato tubers**

Jessica Sinka<sup>1</sup>, Mark A. Bernards<sup>1</sup>

<sup>1</sup>University of Western Ontario.

Large losses of principal food crops occur annually due to threats during pre- and post-harvest such as herbivory, pathogens, abiotic stressors, and harvest-related wounding. To counter these threats, plants possess innate defense mechanisms, including the deposition of the suberin biopolymer in wound-adjacent cells. Suberin, a two-domain macromolecule, acts as a physical barrier to pathogens and water loss. The objective of this project is to better understand the regulation of suberin biosynthesis and deposition by quantifying the temporal allocation of carbon between the two spatially distinct domains of the polymer; one phenolic in nature, and the other aliphatic in nature. Through flux analysis, based on stable isotope labeling, metabolism leading to the individual suberin domains was tracked over seven days in a potato model system. To date, shikimate and L-phenylalanine were used as 'proxy' metabolites to measure phenolic metabolism, while palmitic acid and stearic acid were used to measure aliphatic metabolism. Data from untargeted metabolic analysis revealed a time-based shift in the metabolic profile. Additionally, preliminary quantification of the proxy metabolites suggests carbon is preferentially partitioned into suberin phenolics early during wound induced-metabolism, before being partitioned between both phenolic and aliphatic monomer biosynthesis. Further understanding of suberin synthesis will inform new approaches to protecting against wound related threats, improving yield, quality, and storage of food crops.

#### **C2-5: Characterizing guard cell-specific drought-responsive genes in *Arabidopsis thaliana***

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Drought, an abiotic stress factor, is projected to negatively impact crop growth in more than 50% of arable lands by 2050 consequently posing a threat to global food security. Stressors such as salt and drought stimulate guard cell (GC) ion channels to close stomata, which are pores found on the leaf epidermis surrounded by guard cells. Stomatal width changes in size throughout the day and in response to stimuli, allowing for photosynthetic gas exchange regulation and the occurrence of transpiration. Since stomata can regulate water content, many studies have investigated the potential of manipulating stomata to regulate transpiration as a possible countermeasure to the projected increase in drought incidence. To this end, Anna van Weringh, a Ph.D. candidate in the Provart lab, profiled differentially expressed GC-specific drought-responsive genes using a nuclear RNA isolation assay. I aim to characterize upregulated

GC-specific drought-responsive genes in *A. thaliana* using a reverse genetics approach. To identify phenotypically interesting candidates, initial screening was performed using a thermal imager to estimate wild-type (WT) vs. knockout (KO) temperatures. Candidates will be further characterized using assays to determine stomatal aperture width, water loss rate, and leaf anthocyanin level. The KO plants are hypothesized to have cooler temperatures, and increased stomatal aperture width, water loss rate and leaf anthocyanin levels compared to WT plants. Study of these GC-specific genes can identify possible mechanisms for plant acclimation to drought conditions and add to the current understanding of drought response mechanisms of genes that influence water use.

### **C2-6: The phytohormone abscisic acid modulates protein carbonylation in *Arabidopsis thaliana***

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Improving crop tolerance to stress conditions requires a deep understanding of plant responses to environmental changes. The phytohormone abscisic acid (ABA) is produced in response to a variety of stresses and controls the expression of stress-responsive genes in plants. ABA activates the respiratory burst oxidase-homolog NADPH oxidases (RBOH) in guard cells to generate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a second messenger to induce stomata closure. H<sub>2</sub>O<sub>2</sub> is linked to the production of reactive carbonyl species (RCS), and the treatment of *Arabidopsis thaliana* leaves was shown to trigger the production of RCS that modulate the effect of ABA on stomatal closure. However, the mechanism of action of the RCS is unclear. Here, we hypothesized that ABA-induced H<sub>2</sub>O<sub>2</sub> or RCS leads to carbonylation of certain proteins of the ABA signaling pathways. To verify this hypothesis, we profiled the carbonylated proteome extracted from *A. thaliana* leaves after ABA treatment. The carbonylated protein samples were enriched by affinity chromatography and subjected to liquid chromatography tandem mass spectrometry. We identified 180 carbonylated proteins. Of these, 26 proteins became carbonylated upon ABA treatment whereas 163 proteins found to be carbonylated in untreated samples were no longer detected in the ABA-treated samples. The identified carbonylated proteins are involved in metabolic pathway, amino-acid biosynthesis process, cellular response to oxidative stress, reactive oxygen species metabolic process and glycolic process. These results obtained from plants grown under normal conditions and in the absence of stress indicate that ABA dynamically controls protein carbonylation in *A. thaliana*.

### **C3-1: The role of autophagy in the *Arabidopsis* self-incompatibility response**

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Interactions between pollen and the pistil are critical for controlling sexual reproduction in plants, both in maintaining genetic diversity and rejecting foreign pollen. The self-

incompatibility (SI) trait is an additional layer of regulation during pollen-pistil interactions, which allows for the recognition and rejection of self-pollen. The SI pathway is triggered following pollination through interactions between the pollen S-locus protein 11/S-locus cysteine-rich peptide and the pistil S Receptor Kinase. While this initial recognition event is well characterized, downstream events in the SI pathway are not fully understood. There is a rapid increase in cytosolic calcium levels, absence of a focused actin cytoskeleton, and our previous work on the ARC1 E3 ubiquitin ligase as a downstream signalling protein. We have also previously found that autophagic bodies were present following self-pollinations in *Arabidopsis lyrata* and transgenic *Arabidopsis thaliana*, although the role that autophagy was playing in the SI pathway was unclear. To investigate the requirement of autophagy for the rejection of self-incompatible pollen, two different accessions of transgenic SI-A. *thaliana* were crossed with autophagy deficient mutants for the *AUTOPHAGY7* (ATG7) or *AUTOPHAGY5* (ATG5) genes. In both accessions, SI-A. *thaliana* carrying either of these autophagy deficient mutations resulted in an impairment of several key steps in self-pollen rejection. Additionally, the fluorescently tagged autophagosome marker, GFP:ATG8a was used to track and quantify autophagosomes in stigmatic papillae using confocal microscopy. Overall, this study has more clearly defined a role for autophagy in the SI pathway, adding to a broader understanding of the SI pathway.

### **C3-2: Investigating the NAC-FUS3 molecular module in seed coat development in *Arabidopsis thaliana***

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Seeds assure the spread and survival of angiosperms and other higher plants. The *Arabidopsis* seed comprises three major compartments: the embryo, the endosperm, and the seed coat. FUSCA3 (FUS3) is a master transcriptional regulator of seed development and its roles in promoting embryo development and seed maturation have been well researched. FUS3 also plays important functions in ovule and endosperm development, however, its role in seed coat development is unknown despite the presence of *FUS3* transcripts in this tissue. Through Y1H screening and DAP-sequencing data analysis, two seed coat-specific and uncharacterized NAC domain transcription factors were shown to bind to the *FUS3* genomic region. *fus3* and *nac* mutant seeds show less mucilage secretion compared to wild type. Mucilage, a pectin-based carbohydrate that is synthesized in the seed coat, is released during seed imbibition and enhances water uptake capacity of imbibed seeds. SEM analysis shows defects in *fus3* and *nac* seed coat morphology, while light microscopy uncovered differences in the shape of the mutants' outer integuments, which are responsible for mucilage production and secretion. Interestingly, mucilage-related genes are transcriptionally regulated by NACs. Lastly, in NAC overexpression lines, mature green seeds display a striking seed coat peeling phenotype, in which the seed coat is detached from the embryo. These same seeds are also more permeable to tetrazolium salts and more sensitive to bleach sterilization compared to wild type. Taken together, these results illustrate a role for the NAC-FUS3 module in shaping seed coat development, including mucilage deposition, seed coat permeability and adhesion.

**C3-3: Alteration of xyloglucan biosynthesis disrupts endomembrane structure and function**

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Plant cell walls are essential for plant growth and development, and are mainly comprised of the polysaccharides cellulose, hemicellulose, and pectins. Hemicellulose and pectins are produced by multiple glycosyltransferases located in the Golgi and then secreted through the endomembrane system to the cell wall. Altering biosynthesis of the hemicellulose xyloglucan (XyG) by mutating a glycosyltransferase called *MURUS3* (*MUR3*) was previously shown to cause large intracellular aggregates containing endomembrane organelles in *Arabidopsis*, although the ultrastructure and underlying mechanism for formation of these aggregations was not elucidated. In this study we use high resolution confocal microscopy and transmission electron microscopy techniques to show that the large internal aggregations in *mur3* are comprised of multiple diverse organelles, some of which are trapped within an abnormal intracellular cell wall/protein matrix. In addition, formation of these aggregates compromises endomembrane trafficking by disrupting secretion to the cell wall and plasma membrane. To test whether aggregate formation is unique to *mur3*, we screened through all known XyG biosynthetic mutants for presence of aggregations. Only XyG mutants disrupted in formation of the galactose-containing side chain showed aggregates, suggesting that specific loss of this side chain on XyG causes aggregate formation. This study provides insight into how disrupting polysaccharide biosynthesis in the endomembrane system broadly disrupts cellular trafficking.

**C3-4: Drought-responsive RING E3 ligase XERICO maintains proper stomatal density and distribution during leaf epidermal development**

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Environmental cues influence stomatal aperture, density and patterning to ensure an optimal number and distribution of stomata for response under stress. While several environmentally induced factors capable of regulating stomatal development have been identified, the genetic and molecular mechanisms by which they affect stomatal development is not well characterized. *Arabidopsis* XERICO (XER) is a stress-responsive RING E3 ubiquitin ligase that increases the levels of the phytohormone abscisic acid (ABA) and promotes drought tolerance when overexpressed. Analysis of *xer* null mutants revealed that *XER* inhibits stomatal development and ensures proper stomata spacing. *XER* maintains stomatal spacing by directing proper orientation of stomatal cell division. Repression of stomatal density and coordination of stomatal patterning by *XER* appears to be independent of its altered ABA content. Genetic analysis revealed that *XER* functions upstream of *SPEECHLESS* within the stomatal signalling module. To identify potential substrates and regulators of *XER* that function in stomatal development and stress response, a high throughput yeast two-hybrid screen was conducted

against a library of ABA-responsive genes. A glycosyltransferase (GT) implicated in cell wall biosynthesis interacted with XER *in yeast* and *in planta*. Phenotypic analysis of hypomorphic mutants in *GT* demonstrated that this interactor also functions in stomatal development. *in vivo* localization studies and proteasomal inhibition assays revealed GT is dynamically regulated during early leaf epidermal patterning and suggests that XER may regulate GT stability. Taken together, this study has uncovered two novel stomatal development regulators, which may integrate environmental signals and modulate stomatal density and patterning.

### **C3-5: Investigating florigenic mechanisms that regulate flowering in temperate *Zea mays* and its tropical progenitor, teosinte**

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Flowering in all higher plants occurs when the shoot apical meristem (SAM) transitions from vegetative to reproductive growth. Timing of the floral transition is controlled by environmental and autonomous factors that vary, depending on the species and geographic location. Under optimal conditions, reproductive growth is initiated by mobile signalling proteins known as ‘florigens’ that are synthesized in leaves and travel to the SAM. Maize (*Zea mays* ssp. *mays*) contains several florigens encoded by a family of genes known as *Zea CENTRORADIALIS* (*ZCN*). The floral transition in tropical maize and in its progenitor teosinte (*Zea mays* ssp. *parviglumis*), relies on short day photoperiods. When grown in long days or with an interrupted night cycle, tropical varieties remain vegetative. Conversely, flowering in temperate maize is determined by autonomous signals such that plants flower at the same time regardless of photoperiod. The maize *indeterminate1* (*id1*) gene controls autonomous flowering and *id1* mutants are severely delayed in flowering. Expression of maize florigens such as *ZCN8* are significantly reduced in *id1* plants. To investigate how *id1* controls flowering in relation to photoperiod, near isogenic lines of *id1* teosinte were created. Similar to *id1* maize mutants, homozygous *id1* teosinte mutants do not flower in short days, suggesting that *id1* controls photoperiod flowering as well. This study elucidates the florigenic mechanism in *Zea mays* by further exploring florigen function in temperate and tropical maize, and the role of the *id1* gene in relation to the autonomous and photoperiod floral induction pathways.

### **C3-6: Elucidating the role of RKF1 interactors in post-pollination responses in *Arabidopsis thaliana***

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Plant reproduction is a complex, multistep process following pollination and ultimately leads to pollen tubes growing into ovules for fertilization and the initiation of seed development. The dialogue between pollen and pistil is essential for successfully mediating this process. Understanding this process is crucial for potentially improving yields in crops such as canola where seeds are harvested for seed oil. Recently, a new subgroup of Receptor-Like Kinases

(RLKs), the Leucine-Rich Repeat (LRR) VIII-2 RLKs, have been identified as key players in the early stages of this dialogue. These RLKs are transmembrane proteins which possess a characteristic domain architecture of an extracellular domain, transmembrane domain, and cytosolic kinase domain. Specifically, the *Receptor-Kinase in Flowers 1 (RKF1)* gene cluster was found to be primarily involved in the stigma to promote compatible pollen hydration, as knocking out this gene cluster resulted in decreased wild-type pollen hydration. Using the cytosolic kinase domain of RKF1 as bait, potential binding partners were identified through a yeast-2-hybrid screen of an *Arabidopsis* flower cDNA library (Hybrigenics Services). Here, we work to confirm these interactions and identify their roles in post-pollination processes. Top candidates identified from this screen were the Related to Apetala (RAP) 2.12 and 2.3 transcription factors which belong to the Ethylene Response Factor VII (ERF-VII) group. Various interaction combinations were tested between RKF1 and these ERF-VII factors to determine the ERF-VII interaction domains. In addition, phenotypic analyses of ERF-VII T-DNA knockout lines are currently underway. These novel interactions potentially illuminate undescribed ERF-VII pathways regulating pollen-pistil dialogue.

#### **C4-1: The C-terminal extension region regulates the plastid molecular chaperone HSP90C function**

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Chloroplasts carry out photosynthesis in plants and provide most food sources for living organisms on earth. Due to their symbiotic origins, plastid proteins are either nuclear-encoded and imported, or expressed from the plastid genome. Chloroplasts develop from proplastids or etioplasts upon light perception, which triggers thylakoid formation, a process in which the plastid chaperone HSP90C is essential by assisting chloroplast protein import and thylakoid protein transport. The HSP90C C-terminal extension has a unique feature compared to its homologs, wherein the D-P-W amino acid triplet is conserved across most species in planta. This suggests a functional constraint. Both HSP90C and C-terminal extension truncated proteins could complement the embryonic lethality of the knockout in *Arabidopsis*. Yeast-two-hybrid has shown that HSP90C binding capacity in-vivo to client proteins is greatly reduced or absent if having the C-terminal extension truncated. I set out to investigate whether the molecular mechanism of the C-terminal extension function in client binding is either through direct interaction with client proteins or indirectly via modulating HSP90C conformational change. The role of the C-terminal extension in affecting HSP90C dimerization, ATP hydrolysis and general chaperone activity by prevention of heat-induced model substrate aggregation will be investigated. It has been shown that the C-terminal extension may not be involved in the dimerization of the apoprotein, as revealed by size-exclusion chromatography. Finally, the role of the C-terminal extension of HSP90C in the maturation of client proteins in photosynthesis machinery and those in the thylakoid protein transport machinery will also be explored.

**C4-2: Investigating the mechanistic role of Arabidopsis HSP90.7 in plant development**

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HSP90 family chaperones are highly conserved for their critical role in maintaining cellular protein homeostasis. The *Arabidopsis* HSP90.7, an ortholog of GRP94, reportedly modulates pollen tube germination and ER stress tolerance. The HSP90.7 knock-down mutant *shepherd* (*shd*) displays defects in pollen tube germination and compromised root and shoot development, demonstrating the critical role of HSP90.7 in meristem regulation and male fertility. Previously, we demonstrated the importance of a highly charged region in the middle domain of HSP90.7 in ER stress tolerance and its impact on the chaperone's ATP hydrolysis activity. In this study, we identified and characterized a new HSP90.7 knock out mutant (*hsp90.7-1*) to further investigate its role in plant development. Unlike the *shd* mutant, *hsp90.7-1* exhibits seedling lethality, accentuating the importance of HSP90.7 in plant development, particularly during vegetative growth. To investigate the underlying mechanism behind the arrest growth of *hsp90.7-1* mutants, we conducted a transcriptome comparative analysis via RNA sequencing to gain insight on the global gene expression in the *hsp90.7-1* mutant. Interestingly, pathways such as response to auxin and regulation of auxin metabolic processes were significantly repressed, suggesting a potential role for HSP90.7 in regulation of cellular auxin homeostasis. With auxin being a key regulator of cell division and root growth, we hypothesize that altered expression of HSP90.7 induces alterations in the local auxin signaling pathways resulting in abnormal root growth. It is anticipated that this study will shed light on the mechanistic role of HSP90.7 in plant development and its importance in cellular auxin homeostasis.

**C4-3: Conserved multitaskers: Investigating the role of a family of multi-functional enzymes in *Arabidopsis thaliana***

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Phosphorylation and ubiquitination are two major post-translational modifications that co-exist in nature, respectively catalyzed by protein kinases and ubiquitin ligases. The interplay between these modifications and the enzymes that catalyze them has been well documented in many signal transduction pathways, however, the biochemical mechanisms underlying these interactions are only starting to be revealed. While protein kinases and E3 ligases are usually encoded by distinct genes, we have come across a conserved family of proteins that contain both domains. Although these proteins are found across the plant lineage and have a long evolutionary history, their molecular and biological functions are completely unknown. Here, I will present ongoing work aimed at understanding this intriguing protein family in the model plant *Arabidopsis thaliana*.



**C4-4: Elevated tyrosine results in the cytosolic retention of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase in *Arabidopsis thaliana***

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The shikimate pathway plays a central role in the biosynthesis of aromatic amino acids and specialized metabolites in plants. The first enzyme, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS) serves as a key regulatory point for the pathway in microbes and plants. DAHPS is well-characterized in microbial systems, whereby allosteric feedback inhibition of enzyme activity by the aromatic amino acids has been shown to be the most dominant form of regulation. The mechanism of regulation for DAHPS is poorly understood in plants, and the role of tyrosine (Tyr) with respect to the three DAHPS isozymes from *Arabidopsis thaliana* was investigated. *In vitro* enzymatic analyses established that Tyr does not function as an allosteric regulator for the *A. thaliana* DAHPS isozymes. In contrast, *Arabidopsis* T-DNA insertional mutants for the *DAHPS1* locus, *dahps1*, are hypersensitive to elevated Tyr. Tyr hypersensitivity can be reversed with tryptophan and phenylalanine supplementation, indicating that Tyr is affecting the shikimate pathway flux in the *dahps1* mutant. Interestingly, Tyr treatment of *Arabidopsis* seedlings showed reduced accumulation of overexpressed DAHPS2 in the chloroplast, and not DAHPS1 nor DAHPS3. Further, BiFC studies indicated that DAHPS2 interacts with a 14-3-3 protein in the cytosol and this interaction is enhanced with Tyr treatment. We hypothesize that this interaction with 14-3-3 retains nascent DAHPS2 in the cytosol which prevents its ability to function in the chloroplast, and is facilitated by elevated Tyr in the plant.

**C4-5: Ammonium sulfate prefractionation-assisted detection of the carbonylated proteins in the *Arabidopsis thaliana* leaves**

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Protein carbonylation is a posttranslational modification related to reactive oxygen species, but its physiological importance is less understood in plants than in animal cells. Carbonylated proteins represent a small subset of the total proteome under physiological conditions, and accordingly, the current methods of detection of carbonylated proteins only favor the most abundant proteins. In this study, we hypothesized that ammonium sulfate-based protein precipitation can help prefractionate the sample and facilitate the detection of more proteins sensitive to carbonylation. To verify this, the proteins were extracted from the *Arabidopsis thaliana* leaves and subjected to stepwise precipitation with ammonium sulfate to 40%, 60%, and 80% saturation prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for protein identification. We found that this prefractionation method not only reduced the complexity of the total proteome but also facilitated the identification of additional 749 proteins (45% gain), compared to the number of proteins identified from the non-fractionated

sample. All the 1672 proteins identified in the non-fractionated sample were also found in the pre-fractionated samples. Distinct metabolic and biological processes were enriched in the different fractions compared to the non-fractionated extract. The prefractionation with ammonium sulfate was combined with the labeling of the carbonylated proteins by a fluorescent hydrazide probe. Several carbonylated proteins only became visible in the pre-fractionated samples after SDS-PAGE. Our results indicated that the ammonium sulfate-based prefractionation is efficient in detecting more proteins sensitive to carbonylation and revealed a potential link between protein carbonylation and the gene ontology groups overrepresented in the fractions.

#### **C4-6: Organelle interactions involve endoplasmic reticulum membranes sandwiched between them**

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Our understanding of the eukaryotic plant cell rests on two key concepts; one, the recognition of discrete and functionally different subcellular domains, named as organelles and, two, continuous interactivity between the different organelles to sustain optimal functionality of the cell as a unit. Strong biochemical evidence supports both tenets. Moreover, based on ultrastructural transmission electron microscopy images a general notion is that increased organelle proximity facilitates interactions and exchanges between them. Notably, the ultrastructural images have been obtained from fixed and dead plant tissue and do not portray the dynamic nature of the living plant cell. Our investigations using Arabidopsis plants transgenic for fluorescent fusion protein probes targeted to chloroplasts, mitochondria and peroxisomes show that under normal conditions these three organelles and their tubular extensions, called stromules, matrixules and peroxules, respectively, do not come in physical contact with each other. However, each organelle is surrounded by a loose cage comprising of endoplasmic reticulum (ER) membranes. Our observations suggest that even when organelles appear to be near each other an ER membrane is always sandwiched between them. Live imaging-based evidence on the role of the ER will be presented to support our evolving views on organelle behaviour and interactions.

#### **C5-1: Phenotyping a diverse collection of chickpea lines field-grown in Eastern Ontario for major agronomic traits**

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Chickpea (*Cicer arietinum* L.) is a self-pollinated pulse crop, widely grown worldwide, due to its high nutritional value and dietary uses (seeds contain greater than 20% protein). There are two major chickpea types; Desi and Kabuli, which differ in several morphological and phenological characteristics. The current study incorporates phenotyping of 200 Desi and Kabuli chickpea

lines under field conditions in Eastern Ontario to assess various agronomic traits. The phenotyping was designed for future genome-wide association mapping and Marker Assisted Selection. Our preliminary results from the field trial demonstrate significant differences among the genotypes for several traits such as Days to Flowering (DTF), Number of Pods per Plant (NPP), Plant Height (PH), and Ascochyta Blight resistance (ASC), represented by Ascochyta Blight Score. The genetic diversity among the current set of genotypes were highly significant for NPP and DTF. Principal Component Analysis was performed to analyses the variability among the genotypes. PCA components exhibited that PC1 captured 49.2 % of the total variance, while 35% proportion of variance was explained by PC2 and PC3. PPT and PH had to the maximum amount of variance interpreted by PC1. The diversity map constructed using the Single Nucleotide Polymorphism (SNP) markers also supported significant genetic variation among the genotypes. Our initial results have identified distinct genotypes that have significant diversity and high values for the yield components, which can be used as parents to genetically improve agronomic traits of chickpea including for growth in Eastern Canada.

### **C5-2: Heat stress and cold adaptation in the Antarctic alga *Chlamydomonas* sp. UWO241**

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*Chlamydomonas* sp. UWO241 is a psychrophilic alga found 17 m below the permanently ice-covered surface of the Antarctic Lake Bonney, where it experiences a myriad of harsh environmental conditions such as low temperature, low light, and high salinity. While this habitat is extreme, it is also very stable, and this alga rarely experiences changes in its environment. Heat shock proteins (HSPs) are a ubiquitous family of chaperone proteins that perform important housekeeping roles. In general, HSP expression is induced during abiotic stress to regain protein homeostasis – a process regulated by heat shock transcription factors (HSFs). However, our work has shown that UWO241 constitutively accumulates high protein levels of HSPs in steady-state conditions but fails to induce additional HSP accumulation during heat stress. This is the first known green alga without a classical heat stress response. It is hypothesized that UWO241 has lost the ability to regulate HSPs in its extreme but unchanging environment. In this study, a single HSF was identified in UWO241 genome. Comparative sequence analysis revealed that all characteristic domains are conserved. Next, we performed targeted analysis of the UWO241 transcriptome in heat-stressed cultures. We show that 26% of UWO241 HSPs were differentially expressed during heat stress; however, the HSF1 transcript was not. More work needs to be done to characterize the mechanism behind HSP regulation in UWO241.

### **C5-3: Transcriptome-wide approach to address lower seed protein content in soybean grown in Western Canada**

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Soybean is an important agronomic crop in Canada with widespread uses in human consumption, animal feed, and biotechnology. The capacity to fix nitrogen gives soybean an important role in sustainable agricultural practices by reducing the need for nitrogen fertilizers. Adapting Canadian soybean agriculture to changing climate conditions is necessary to produce an adequate crop yield with acceptable levels of seed protein. The Canadian Grain Commission has reported lower seed protein from soybeans grown in western Canada compared to eastern Canada, regardless of genotype. This project will uncover key genes underlying differences in seed protein content across Canada. Using a transcriptome-wide approach, we identify differences in expression of genes contributing to seed protein content, and to study the effect of environmental variation on geographically-dependent gene expression (West vs East). Three groups of genes are being investigated; seed protein and oil biosynthesis genes, stress response genes, and genes that code for regulatory proteins capable of influencing gene expression. Ten soybean lines ranging low to high in seed protein are growing in four locations across western and eastern Canada from 2018-2021. RNA sequencing is used to establish a large, high-quality expression library for all samples across all years. Differential expression used together with gene ontology and novel transcript discovery are key bioinformatic tools for determining candidate genes. Results to date have identified potential candidate genes underlying the differences in seed protein and oil content. This research offers novel information for geographically-tailoring Canadian soybean and developing allele-specific markers to breed high protein soybean cultivars.

#### **C5-4: Investigation into the evolution and expansion of a malectin-containing leucine-rich repeat receptor kinase family in land plants**

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Plants cannot see danger – instead, they use a large network of cell surface receptors to recognize external signals. The leucine-rich repeat receptor kinases (LRR-RKs) are the largest family of plant cell-surface receptors. These LRR-RKs are further divided into 15 subfamilies in *Arabidopsis thaliana* and play a role in a variety of functions from growth to immunity. The LRR-RKs in each subfamily are thought to share common functions, such as the biotic stress-related LRR-XII subfamily in *Arabidopsis thaliana*. In this work, we investigated LRR-RK subfamilies across 112 plant species to identify the fingerprints of stress-related families. In doing so, we observed increased expansion and tandem duplication in a subfamily of malectin-like domain containing LRR-RKs (MLD-LRR-RKs). Further investigation and comparison to the stress-related LRR-RK-XII subfamily revealed contrasting gene expansion patterns between LRR-I and LRR-XII in Brassicales and Poales, with Brassicales demonstrating LRR-I preferential expansion and Poales showing LRR-XII preferential expansion. These findings demonstrate lineage-biased expansion of one LRR-RK subfamily over another with a similar predicted function, a pattern which could be widespread given the overlapping roles of many other subfamilies.

**C5-5: Genome-wide analysis of alternative splicing in *Arabidopsis thaliana* using a guard cell-specific transcriptome**

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Alternative splicing is a powerful method of increasing proteome diversity and regulating gene expression in eukaryotes. In *Arabidopsis thaliana*, 60-70% of all multiexonic genes undergo alternative splicing in at least one tissue type or environmental condition. Drought stress is a condition of particular interest, as drought frequency and severity continue to increase under climate change. Guard cells (GCs) are critical for the drought response since they make up stomata, the pores on the leaf surface that control water loss through transpiration. Our lab has successfully generated a GC-specific transcriptome in *Arabidopsis* plants subjected to a physiologically-relevant progressive drought regimen. In addition to identifying thousands of differentially expressed genes, analysis of this transcriptome has revealed genome-wide changes in alternative splicing in GCs subjected to drought stress. We found that GCs under severe drought stress have the greatest number of differentially spliced genes compared to their age-matched well-watered controls. For example, we found that the gene *XTH31* (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 31) undergoes alternative splicing during severe drought stress. Specifically, the first intron of the gene is retained, leading to the introduction of a premature stop codon which would disrupt gene function. This could occur through degradation of the alternatively spliced transcript, or translation into a truncated protein. If translation occurs, the resulting protein will lack its catalytic domain, including both of its active sites. Our study reveals genome-wide changes in GC splicing patterns under severe drought stress and opens the door to functional analyses of alternative splicing consequences in the drought response.

**C5-6: Revealing the truth behind a previously presumed locus, E6, in soybean variety Paranagoiana**

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Soybean grown in latitudes of ~20° or lower, produce reduced grain yield and quality due to the shorter days within those latitudes. One way to overcome this limitation is by growing varieties carrying the long juvenile (LJ) trait, identified as the J gene, Glyma.04g050200. The LJ trait allows the plant to remain in the vegetative state for a much longer period, delays flowering and produces higher yields. E6 is a locus mapped to the same region as J on chromosome 04 which up until this point, has also been hypothesized to be involved in the LJ trait. Here we aimed to determine if E6 and J are the same locus or linked loci and to answer the puzzling and ongoing debate in the soybean research community. PCR amplification and DNA sequencing analysis of contrasting soybean lines (E6, e6, J, j-1) identified the presence of a *Ty1-copia* retrotransposon within the 4th exon of the J gene in soybean line “Paranagoiana”, where the E6

locus was first mapped. This was confirmed to impact the mRNA of the gene through further cDNA sequencing of Glyma.04g050200. Our results show that the previously identified soybean maturity locus, E6, does not exist, and that the LJ trait phenotyped in Paranagoiana is due to a new variation in J, now called j-x.

### **C6-1: Training graduate students and post-doctoral scholars in science communication**

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There are few formalized science communication opportunities built into the training curriculum for highly qualified personnel in Canada. Tri-Agency funded scientists have a responsibility to make the results of their work accessible to the public, and a lack of science communication skills are a barrier to engagement with non-expert audiences. During the summer of 2021, we organized and delivered a Science Writing Internship Program funded by an NSERC Science Communication Skills grant. We recruited graduate students and post-doctoral scholars based in Canada and elsewhere, and provided opportunities to both receive training in science communication and to put these skills into practice. Our goals were to: i) educate science, technology, engineering, and mathematics (STEM) trainees in how to effectively communicate with non-expert audiences, ii) create a free online resource of good science communication practices based upon expert workshops, and iii) build a supportive network of STEM trainees and science communication leaders in Canada. We'll describe our program, explain how we met our goals, and offer advice to others who aim to offer science communication training to members of their own lab groups, departments, or institutions in the future.

### **C6-2: Exploring genetic and environmental effects on Canadian winter wheat yields over time**

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Crop performance is dependent on the genetics of the cultivar, and the environment in which it is grown. As climate change causes environments to become more extreme and unpredictable, the extent to which yields are influenced by genetics versus the environment is of increasing importance. We investigated the role of genetic and environmental factors on the performance of winter wheat in Ontario, using historical data from variety trials conducted between 1988 and 2018. Over this period, wheat yields steadily increased by 38 kg ha<sup>-1</sup> yr<sup>-1</sup>, or 0.82% yr<sup>-1</sup>, relative to 1988. While fungicide treatment of trials contributed a one-time, large, 670 kg ha<sup>-1</sup> yield increase, yields were otherwise unaffected by long term changes in agronomic practice, climate, or other non-genetic factors. Genetic improvement entirely accounted for yield improvement. Winter wheat cultivars may be classified into distinct germplasms. Pedigree and molecular analyses revealed that genetic increases in yield were similar across all germplasm groups. Entry yield estimates calculated from genomic prediction models strongly correlated with entry field estimated yields with a mean  $r=0.68$ . Genomic prediction accuracies arose

because yields differed across genetically distinct subpopulations. Because environmental changes over the past three decades in these trials have had no notable negative effects on winter wheat productivity, we are cautiously optimistic about environmental effects in the future. Genetic improvement coupled with genomic prediction will likely lead to higher winter wheat yields.

### **C6-3: Production of plant-based vaccine candidates in *Nicotiana benthamiana* to prevent *Salmonella* infection in poultry**

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*Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) is one of the most prevalent causes of gastrointestinal disease in humans, and infection commonly results from the consumption of poultry products. Vaccination is one of the most effective strategies to prevent *S. Enteritidis* infection in poultry flocks, however the vaccines that are currently available have limitations such as safety concerns and poor immunogenicity. Because of this, innovative approaches are being pursued to develop novel *S. Enteritidis* vaccine candidates. One approach is the use of self-assembling protein nanoparticles to present antigenic epitopes to the immune system to stimulate long-lasting immunity. The goal of this project is to create safer, and more effective *S. Enteritidis* vaccine candidates. To address this, antigenic epitopes from a surface-exposed protein from *S. Enteritidis* were genetically fused to a protein nanoparticle. The fusion constructs were expressed in the leaves of *Nicotiana benthamiana*, where the proteins accumulated to high levels. Structural analysis of the fusion proteins indicated that the fusion of the epitopes did not interfere with nanoparticle assembly. Further analysis will be performed to verify that the epitopes are surface exposed on the assembled nanoparticle where they will be visible to the immune system, and animal trials will be performed to assess whether the fusion proteins elicit an immune response. Overall, the success of this project could lead to the development of promising *S. Enteritidis* vaccine candidates, which could reduce the prevalence of *S. Enteritidis* in poultry flocks, and subsequently lower the rate of human infection.

### **C6-4: Characterization of the role of SPL4 in the regulation of drought stress and trichome development in alfalfa**

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The impacts of climate change are expected to increase the demand for crops resistant to drought stress. Understanding how molecular mechanisms control plant response to stress is crucial to prevent losses in crop yield. Studies conducted in *Medicago sativa* (alfalfa) have shown that genetic modifications can induce plant mechanisms to increase stress tolerance. miR156 is a long non-coding RNA which negatively regulates gene expression at the post-

transcriptional level. Trichomes are physical structures present on plant tissues that can aid plants in transpiration reduction. We studied the role of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 4 (SPL4), a target of miR156, in alfalfa's response to drought and trichome development. We found that SPL4 regulates trichome development and drought stress response. Transgenic alfalfa plants with RNAi-silenced *SPL4* (SPL4-RNAi) have an increased trichome density under both control and drought conditions. In response to 14 days of withholding water, SPL4-RNAi plants had an increased root length, an increased water content in roots, shoots and leaves, and an increased water potential in the leaves when compared to wild-type plants. This study shows SPL4 has a role in trichome development and drought stress response, making it a potential target for modification to improve plant tolerance to drought stress.

#### **C6-5: Identification of the EPF signaling peptides regulating grass stomatal development and patterning**

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Proper density and distribution of stomata are critical for efficient gas exchange between a plant and the atmosphere. Several EPIDERMAL PATTERNING FACTOR (EPF) peptides have been discovered as important signalling molecules, controlling stomatal patterning and differentiation in Arabidopsis. However, although exciting progress has been made in understanding EPF signalling in Arabidopsis, how EPF peptides control different stomatal patterns and morphologies in agriculturally important monocots, such as wheat, is poorly understood. By examining expression patterns, overexpression transgenics, and cross-species complementation, we identified the antagonistic stomatal ligands orthologous to Arabidopsis AtEPF2 and AtSTOMAGEN/AtEPFL9 peptides in wheat and the grass model organism *Brachypodium distachyon*. Application of bioactive BdEPF2 peptides inhibited stomatal initiation, but not the progression or differentiation of stomatal precursors in the *Brachypodium*, and the inhibitory roles of these EPF peptides during grass stomatal development were suppressed by the contrasting positive peptide BdSTOMAGEN in a dose-dependent manner. We are exploring the biological functions of the remaining *EPF* genes in *Brachypodium* and have identified new EPF peptides specifying grass stomatal patterning. These results not only demonstrate how conserved EPF peptides that control different stomatal patterns exist in nature but also suggest new strategies to improve crop yield through the utilization of plant-derived peptides that optimize stomatal density and patterning on the plant epidermis.

#### **C6-6: Something in the air - Drone based phenotyping of phenology and drought stress**

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The field of forest genomics has seen unprecedented advances during the past decade. A suite of genomic resources is now available for enhanced genomic selection and can be used to accelerate breeding cycles and to identify genotypes that are better adapted and more resilient to future climate change and diseases. The large-scale phenotyping of populations has become the bottleneck for identifying and connecting the different genomic resources with adaptive traits in populations with thousands of trees. Measuring leaf optical properties using spectral reflectance sensors carried by drones is an innovative approach for large-scale phenotyping of tree responses to drought, monitor phenology, and assess differences between tree genotypes in large-scale field experiments. In this presentation I will give an overview of leaf-level, canopy-level and drone based observations of leaf spectral reflectance. I will demonstrate that some of the widely used vegetation indices such as the normalized difference vegetation index (NDVI) and photochemical reflectance index (PRI) vary in their ability to adequately track important traits such as phenology or photosynthetic efficiency. I will conclude with a brief discussion of technical challenges of using optical sensors when monitoring complex canopies and why using carotenoid based vegetation indices is particularly useful in order to monitor evergreen conifer canopies.

## Poster abstracts

### Investigating the role of LRR-VIII-2 receptor-like kinases in intra- and inter-species pollinations in *Arabidopsis thaliana*

Laura Canales Sanchez<sup>1</sup>, Francis Lee<sup>2</sup>, Daphne Goring<sup>1</sup>

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Successful fertilization of a flowering plant requires a tightly controlled interaction between the pollen and the pistil. In *Arabidopsis*, most research thus far has focused on the later stages of compatible pollen tube growth and ovular reception in the pistil, identifying many key players belonging to the large family of predicted Receptor Like Kinases (RLKs) which serve as primary sensors that perceive and translate extracellular signals. Recent studies have also shown that some of the key players involved in pollen pistil-interactions also promote self-pollen over interspecies pollen. Previous work in the Goring lab identified Leucine-Rich Repeat (LRR) VIII-2 subgroup RLKs which were characterized to function in the upper part of the female reproductive tract in the pistil to support compatible pollen. Here, I further explore the function of these LRR-VIII-2 RLKs in compatible pollen responses and their role in establishing interspecies barriers for pollen specificity. Different pollen donors were used to test for interspecific specificity and to test for a breakdown of an interspecies barrier in the mutant LRR-VIII-2 RLK pistils. In addition, a rescue experiment was performed to assess whether the expression of one member, RKF1, in the stigma could rescue the altered compatible pollen phenotypes as the pollen tubes grew through the mutant LRR-VIII-2 RLK pistils. From these studies, we are further defining the functions of a new group of receptor kinases and their roles in the pistil to support the earlier stages of compatible pollen-pistil interactions and block interspecies pollen.

### Identification of novel maturity-related QTLs in a *G. max*/*G. soja* RIL population

Simon Lackey<sup>1, 2</sup>, Elroy Cober<sup>2</sup>, Andrew Bird<sup>2</sup>, Ashkan Golshani<sup>1</sup>, Bahram Samanfar<sup>1, 2</sup>

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The soybean, *Glycine max* (L.) Merr., is one of the most economically important crops in Canadian agriculture. Canadian soybean breeders will need new sources of genes controlling early flowering and maturity to further contribute to the expansion of growing regions, particularly for Western provinces and Northern regions. The wild relative of the domesticated soybean, *Glycine soja* Sieb. & Zucc., has significantly more genetic diversity than *G. max*, and has the potential to provide novel alleles for short growing seasons that have not been explored or exploited. Studies investigating *G.max/G.soja* populations have previously identified QTLs related to seed weight, but the polygenic nature of seed weight requires further study. Identification and selection against seed dormancy related genes is essential before *G. soja* derived germplasm can be released. This study aims to identify novel quantitative trait loci related to time of flowering and maturity, seed weight, and seed dormancy in a *G.max/G.soja* recombinant inbred line population. Preliminary results suggest that lines from the population reach R7, beginning of maturity, around eleven days earlier than the domesticated parent (71

days instead of 82 days) and R8, full maturity, around ten days earlier than the domesticated parent (82 days instead of 92 days). Identification of the underlying genes and development of allele-specific markers for marker-assisted selection (MAS) will allow for an accelerated pipeline of cultivar development in short season soybean breeding programs and allow breeders to easily introgress these new sources of early maturity from *G. soja*.

### **Natural variation in *Arabidopsis thaliana* immunity at elevated temperatures**

Christina Rossi<sup>1</sup>, Christian Danve M. Castroverde<sup>1</sup>

<sup>1</sup>Wilfrid Laurier University.

Climate change threatens food security worldwide as rising global temperatures worsen plant disease prevalence, resulting in increased crop losses. Previous studies on the model plant-pathosystem *Arabidopsis thaliana*-*Pseudomonas syringae* pv. *tomato* (*P. syringae*) showed that host salicylic acid (SA) production is lost at elevated temperatures (ET). These studies investigating *Arabidopsis* immunity at ET have only focused on the reference *Arabidopsis* accession Columbia-0 (Col-0). However, it has been well-established that there is a profound intraspecific and pangenomic variation within the >1000 accessions of *A. thaliana*. As the natural variation of *Arabidopsis* immune responses at ET has not been fully investigated, I will test various *Arabidopsis* accessions from diverse regions/climates and identify those with greater resistance to *P. syringae* infection when grown at ET. Specifically, I will measure basal and systemic immunity, as well as defence gene and SA levels, under ambient and warm temperatures. Currently, I am optimizing the *Acinetobacter* sp. *ADPWH lux* SA quantification assay, in addition to growing various *Arabidopsis* accessions. Determining *Arabidopsis* accessions with temperature-resilient defence responses will help uncover the fundamental molecular and genetic components underpinning temperature-sensitive plant immunity, and this could aid in predicting how plants may respond to climate change. Overall, this research will potentially lay the mechanistic groundwork on engineering disease-resistant and climate-resilient crops, which are anticipated to have broad impacts on the agricultural industry.

### **Investigating the role of PUBs in the self-incompatibility pathway of transgenic SI *A. thaliana* of the C24 accession**

Paula Beronilla<sup>1</sup>, Daphne Goring<sup>1</sup>

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In order to promote outcrossing and to increase genetic diversity, members of the Brassicaceae family possess an intraspecific mating barrier, known as the self-incompatibility system, that allow the pistil to recognize and reject self-pollen. The SI system is initiated by the *S*--haplotype specific interactions between the highly polymorphic *S* locus genes: *SCR/SP11*, the male determinant gene, and *SRK*, the female determinant gene. This interaction is followed by the activation of *ARC1*, a member of the PLANT-U-BOX family of E3 ubiquitin ligases, which targets its substrates for degradation by ubiquitination. One of the identified downstream targets of *ARC1* is the exocyst complex and this event disrupts the delivery of water and resources

required by the pollen to hydrate and germinate. Species have evolved to be self-compatible due to the pseudogenization of *SCR* and *SRK*, and the deletion of *ARC1*. Interestingly, when the self-compatible *Arabidopsis thaliana* of the C24 accession was transformed with *SCRb* and *SRKb* from the self-incompatible *Arabidopsis lyrata*, the transgenic lines exhibited strong SI phenotypes despite lacking *ARC1*. We hypothesize that there is an unidentified factor in the transgenic SI *A. thaliana* C24 that performs a similar biological function as *ARC1*. The two candidates for this study are *PUB16* and *PUB17* as they are the two most closely related PUBs to *ARC1*. Our experimental approach uses the CRISPR/Cas9 system to generate single knockout mutants and the double knockout mutants in the SI *A. thaliana* C24 background, which will then be assessed for pollen-pistil interactions.

### **Effects of sulfur on cadmium uptake and translocation in soybean (*Glycine max*).**

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Soybean (*Glycine max*) is an important crop species for global agriculture and production. With soybean being one of the top five produced crop species in Canada, it is important to maintain healthy and safe to eat beans. Cadmium (Cd) is a non-essential element that can lead to plant growth inhibition. If it accumulates in edible crops, Cd poses a health risk to consumers. Cadmium concentrations are increasing globally in agricultural soils due to anthropogenic contamination. Sulfur (S) addition to Cd-contaminated soils has been shown to reduce Cd toxicity in rice and wheat. It might do this by either forming an insoluble plaque on the root surface, which prevents Cd uptake into the roots, or by increasing the production of chelators that bind to and sequester Cd within plant cells. The aim of this study is to determine the underlying mechanisms behind reduction of Cd toxicity due to S addition in soybean. The objectives are to determine if S addition will cause decreased Cd uptake, increased chelator production, and increased sequestration of Cd in vacuoles, resulting in reduced Cd toxicity. The results can be applied to agronomy if they confirm that S addition will help reduce Cd uptake and toxicity in soybean plants resulting in higher crop yield and safer beans.

### **Investigating the role of a *Pseudomonas putida* KT2440 IclR-type regulator in the *A. thaliana* rhizosphere**

Elina Kadriu<sup>1</sup>, Sophie Qin<sup>1</sup>, Stephanie Prezioso<sup>1</sup>, Dr. Dinesh Christendat<sup>1</sup>

<sup>1</sup>University of Toronto.

p-hydroxybenzoic acid (PHB) is a phenolic acid exuded into the plant rhizosphere, where it acts as one of many signaling molecules responsible for the recruitment and establishment of the rhizosphere microbiome, including the soil bacterium *Pseudomonas putida* KT2440 (*P. putida* KT2440). Although PHB improves abiotic stress tolerance, high concentrations of this metabolite in the rhizosphere induces autotoxicity, affecting many agriculturally important crops. The utilization of rhizosphere microbes is critical for the detoxification of this compound to ensure plant survival and propagation. Here, we have identified a novel isocitrate lyase type

transcriptional (lclR) regulator PP\_2609, in *P. putida* KT2440. We show that PHB interacts with the effector binding domain of PP\_2609 and induces gene expression of its associated operon. Deletion of *pp\_2609* improves *P. putida* KT2440 growth and promotes lateral root counts of *A. thaliana*, likely due to a stress-induced systemic response to avoid toxicity. Studying this pathway may provide crucial information for how microorganisms maintain metabolite homeostasis in the rhizosphere as these compounds not only change the soil chemistry, but the overall microbiome diversity and plant development. Furthermore, this work may facilitate improved bioremediation techniques to prevent the phytotoxic accumulation of PHB which inhibits crop growth in agriculturally used soils.

### **Root-associated fungi alter tree growth and phytohormone concentrations under elevated temperature and CO<sub>2</sub>**

Danielle Way<sup>1, 2, 3</sup>, Joshua Frank<sup>4</sup>, Mamdouh Abou-Zaid<sup>1, 5</sup>, Tod Ramsfield<sup>1, 6</sup>

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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<sub>2</sub> (400 ppm) or elevated CO<sub>2</sub> (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<sub>2</sub> 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. Absciscic 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 increases tree growth under moderate warming and affects plant hormone regulation, which may affect resilience to future climatic stresses.

## **Improving plant growth and increasing biomass of greenhouse-grown tomatoes by engineering the soil microbiome**

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Global food production is facing a number of challenges such as freshwater scarcity, shrinking arable land, and more frequent extreme weather events due to global warming, all of which lead to reduced plant growth and yields. A newly emerging eco-friendly approach for improving plant growth harnesses naturally occurring plant growth-promoting microbes. Akin to probiotic supplement for the human gut microbiome, introducing specific microbes into the plant microbiome has been shown in a number of studies to improve plant growth and increase plant biomass under highly controlled laboratory conditions. The objective of this research project is to determine if these types of improvements to plant growth and biomass can be reproduced in commercial tomato varieties grown under commercial greenhouse conditions. Initially, a library of over 500 pure microbial strains isolated from a field-site by the Yergeau laboratory at INRS-CAFSB were screened for their ability to promote plant growth. Eight candidate microbes were identified that significantly increased biomass in 5-week-old tomatoes grown in growth chambers. The efficacy of these candidate microbes at improving plant growth under commercial greenhouse conditions has been tested in a number of recently completed as well as ongoing greenhouse trials. The mechanism(s) of action of these candidate microbes is being investigated through a combination of lab-based characterization and whole genome sequencing. In the future we intend to develop the most promising microbes into commercial products with our industrial partner, Axter Agrosience Inc., a leading supplier of liquid fertilizers and biostimulants, followed by distribution nationwide to Canadian farmers.

## **Temperature regulation of pipecolic acid-mediated plant systemic immunity**

Alyssa Shields<sup>1</sup>, Eric Marchetta<sup>1</sup>, Danve Castroverde<sup>1</sup>

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Crop losses are exacerbated by increasing global temperatures due to climate change. Plant disease susceptibility is influenced by elevated temperatures that inhibit components of the plant immune system, including pattern-triggered immunity (PTI), effector-triggered immunity (ETI), and basal resistance. Salicylic acid (SA) is associated with these local immunity mechanisms, and also with systemic defences through interactions with the mobile immune signal pipecolic acid (Pip) and its metabolically active derivative *N*-hydroxypipecolic acid (NHP). Previous studies have shown that increased temperatures negatively affect PTI, ETI and SA production in the local/primary sites of infection; however, it is currently unclear if and how elevated temperature affects plant systemic immunity. Here we show that elevated temperatures effectively eliminate systemic acquired resistance (SAR) in *Arabidopsis thaliana*. We first primed plants with the model bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 at both normal (23°) and elevated (28°) temperatures. At 23°C, plants were more resistant to future infection, but this systemic protection was absent at 28°C. This was

associated with decreased expression of the Pip-NHP biosynthetic genes *ALD1* and *FMO1* at both primary (local) and secondary (systemic) leaf tissues after pathogen infection. Furthermore, ongoing experiments show that exogenous Pip application is unable to restore systemic immunity to *Pst* DC3000 at elevated temperature. These results demonstrate that elevated temperature extensively impacts the plant immune system, providing a more comprehensive understanding of systemic and local immune responses in our warming climate.

### **The effect of elevated temperature on floral traits in cucumber**

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Pollination is an essential ecosystem service that can be affected by anthropogenic climate warming. These warmer temperatures have the potential to change plant-pollinator interactions by affecting floral traits that are essential for pollination services. Current studies have focused on how elevated temperature affects flower development (including onset of flowering, flower size, and number of flowers produced), however, we lack consistent data on how visual traits that are important for pollinator visitation change in flowers grown at elevated temperatures. To determine how warming affects key floral traits that are important to pollinators, I will grow *Cucumis sativus* under ambient and +4°C temperatures. I will evaluate changes in human-visible flower pigmentation using high performance liquid chromatography (HPLC) analysis. Additionally, I will quantify the size and shape of UV floral guides (signals to pollinators that are only visible under UV wavelengths of light) using UV-visible photography. I will correlate changes in visual floral traits with flower development and plant performance traits (i.e. plant height, shoot biomass, root biomass) to determine the effects of warming. From this research I will be able to determine how warming will affect key floral traits that are important to pollinators.

### **Defense of the plants - leveraging natural variation of *Arabidopsis thaliana* to identify stress-adapted receptor kinases**

Zachary Kileeg<sup>1</sup>

<sup>1</sup>University of Toronto Scarborough.

Plant disease is a major contributor to crop loss around the world. Although many effective methods are in use to reclaim these losses, tuning basal immunity through receptor kinases remains an underutilized solution. Receptor kinases play an important role in plant immune responses, yet our understanding of them is limited. In this research, I sought to leverage the natural variation that exists within *Arabidopsis thaliana* to fingerprint stress-related adaptation in the receptor kinases. To do so, I identified all receptor kinases across 26 ecotypes of *Arabidopsis thaliana*, clustered them into orthogroups, and examined their presence/absence variation. With this, I found a subset of receptor kinase families containing rare genes with

evidence of tandem duplication - a pattern indicative of stress adaptation. I then used this natural variant collection to infer selection and identify potential stress-related genes in the leucine-rich repeat receptor-kinase (LRR-RK) family. Identification of stress-related genes will then be confirmed using high-throughput immune assays.

### **Determinants of substrate specificity in a catalytically diverse family of medium-chain acyl-acyl carrier protein thioesterases from plants**

Becky Kalinger<sup>1</sup>, Owen Rowland<sup>1</sup>

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The catalytic diversity of the ACYL-LIPID THIOESTERASE (ALT) family of medium-chain acyl-acyl carrier protein (ACP) thioesterases sets them apart from other, similar enzymes. ALT enzymes can act on substrates of varied oxidation states, producing medium-chain (6-14 carbon) fatty acids, 3-hydroxy fatty acids, and methylketone precursors. While ALTs have biotechnological potential as sources of these industrially valuable compounds, little understanding of what dictates their widespread substrate preferences currently limits their biotechnological use. Through targeted mutagenesis guided by computational modelling, we identified the first known determinants of ALT substrate specificity, including a six-amino-acid motif that dictates oxidation state preference and influences chain-length selectivity, and other regions of sequence that influence enzyme productivity or give rise to unnatural chain-length selectivity profiles when modified. *In silico* docking of homology models of ALTs with ACP bearing 6-, 10-, and 14-carbon acyl chains revealed that the identified structural regions may influence interaction with ACP or alter acyl binding pocket structure. The findings from this work represent an advancement towards being able to engineer mutant ALTs with product profiles suited to specific biotechnological purposes. The homology models of ALTs can be used to predict the effects of further mutagenesis on ALT activity, and mutant ALT enzymes generated in this study with increased productivity or unique activity profiles are promising starting points for additional rational design or directed evolution experiments.

### **Clade III TGACG-motif binding basic leucine zipper transcription factors as mediators of BLADE-ON-PETIOLE regulated plant development**

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TGACG-motif binding (TGA) transcription factors are a class of basic leucine zipper (bZIP) proteins in plants. In *Arabidopsis thaliana*, there are ten TGA factors grouped into five clades. Widely involved in ROS-mediated stress responses, the role of TGA factors in development is poorly characterized. BLADE-ON-PETIOLE 1 and 2 (BOP1/2) are BTB-ankyrin proteins that regulate plant architecture. As co-activators without a DNA-binding domain, these proteins use TGA bZIP transcription factors for recruitment to DNA. Thus far, we found specific roles for clade I and clade V TGAs in this capacity. We show here that clade III TGA transcription factors, TGA3 and TGA7, previously associated with plant defense, also interact with BOP1/2 in



regulating development. Our data show that *TGA3* and *TGA7* are co-expressed with *BOP1* and *BOP2* at organ boundaries and function in a boundary module that regulates meristem integrity, flowering, and inflorescence architecture. The dwarf stature and vascular patterning defects in *BOP1* and *BOP2* overexpressing plants were substantially corrected by *tga3* and *tga3 tga7* mutations. Lignin deposition and related transcripts, elevated in *BOP1* and *BOP2* overexpressing plants, were normalized in *tga3* and *tga3 tga7* mutants. Two-hybrid assays confirmed an interaction between TGA3/TG7 and BOP1/BOP2 proteins in yeast. These data suggest that clade III TGAs are key mediators of BOP1/2 co-activator function. The next steps will explore a link between TGA factors and ROS-regulated aspects of development.

### **Investigating interactions between plastid stromules and lipid droplets**

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Oils are long-term energy resources stored in lipid droplets (LDs) in plants. While extensive research exists for LDs in seeds, there is little information on the function of LDs that are found in the vegetative tissues. The role of LDs in the survival of vegetative cells is also unknown. In plants, plastids are the site of de novo fatty acid (FA) biosynthesis while LDs are the final storage sites for neutral lipids. The common link to the acyl-lipid biosynthesis pathway led to our hypothesis that vegetative LDs and plastids interact. Further, since plastids produce thin stroma-filled tubules named as stromules, we speculated that plastid-LD interactions may involve these stromules. This study utilized different fluorescent proteins targeted to plastids and LDs in various Arabidopsis mutants to uncover a possible relationship. Preliminary results suggested that the LDs drifted past stromules and the two organelles did not dwell together for a prolonged period. Thus, direct interaction between these organelles seems unlikely. However, both plastids and LDs exhibited a diurnal rhythm in their behaviour which suggested that there might be conditional interactions between the two. Various aspects of our approach and the impact of these observations in understanding plastid-LD interactions will be presented.

### **Regulation of the shikimate pathway entry point in the earliest land plant and its evolutionary implications**

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In plants and microorganisms, the shikimate pathway leads to the biosynthesis of chorismate, a precursor to specialized metabolites, including the aromatic amino acids (AAAs) tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe). The first enzyme of the pathway, 3-deoxy-D-arabino-heptulosonate synthase (DAHPS), well-characterized in bacteria, is known to be feedback-inhibited by the AAAs, and has been classified into subtypes based upon structural and regulatory differences. However, plant DAHPS enzymes have yet to be as robustly characterized, representing a gap in the understanding of how the shikimate pathway is

regulated in plants. Unlike higher land plants, the earliest land plant (liverworts) only possesses a single DAHPS isozyme. This expansion in DAHPS copy number over evolutionary time raises the possibility of a similar expansion in regulatory modes, where liverworts possess the most ancestral mode. We conducted enzyme kinetics assays on the DAHPS enzyme from the model liverwort *Marchantia polymorpha* (MpDAHPS), which revealed this mode to be inhibition by the AAAs, similar to bacterial DAHPS enzymes. Our lab has previously shown that DAHPS isozymes in *Arabidopsis thaliana* are regulated not by the aromatic amino acids, but by a 14-3-3 protein. Phylogenetic studies of DAHPS evolution from a bacteria-like ancestral mode of regulation to a plant-specific mode in later land plants may uncover the evolutionary trajectory of DAHPS regulation in plants as a whole, as well as delineate the plant family of DAHPS enzymes.

### **Leveraging phosphoproteomics to uncover mechanisms of cell wall integrity signaling**

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Plant cell walls are highly dynamic structures that surround and protect plant cells. These polysaccharide-based matrixes are finely tuned to balance rigidity for protection and structure, and flexibility to grow and respond to environmental cues. As a result, the cell wall has become a primary target for bioengineers interested in increasing the production of its components. However, increasing the expression of polysaccharide biosynthetic enzymes has had limited success. These results hint at an underlying regulatory mechanism, called 'cell wall integrity' (CWI) signaling that perceives changes to the cell wall, and in turn, remodels the cell wall and/or regulates plant growth. Several cell membrane-bound kinases have been implicated in the signal perception component of this mechanism, but the downstream components remain uncharacterized. We employed a proteomics approach by treating plants to induce cell wall stress and analyzing phosphorylated peptides through high resolution mass spectrometry. Altogether, our data from 3 independent experiments (n=9) shows 242 differentially phosphorylated phosphopeptides, corresponding to 241 proteins. We conducted a bioinformatic analysis for all 241 candidates integrating gene ontology terms, protein-protein interaction networks, phosphomotif analysis, and comparative analysis with other stress-induced phosphoproteomes. These results guided selection of 30 candidates for a reverse genetics screen under cell wall stress conditions. Results from this screen implicate a set of three intracellular kinase candidates, now referred as ISOXABEN PHOSPHO-RESPONSIVE or IPR, in CWI.

### **Effect of oxidative treatments on the activity of *Arabidopsis thaliana* cytosolic NAD-dependent glyceraldehyde 3-phosphate dehydrogenases.**

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Environmental stresses can lead to an increased production of reactive oxygen species in plant cells. Plants being sessile organisms, they use metabolic strategies to deal with the effects of

oxidative stress. Growing evidence point to the involvement of post-translational modifications and redox-dependent regulation of key metabolic proteins. With increasing stress level, protein cysteine thiols susceptible to deprotonation are sequentially oxidized to their sulfenic, sulfinic and sulfonic acid forms, the later being considered irreversible. Cytosolic NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPC) is a key glycolytic enzyme sensitive to oxidative conditions owing to its deprotonated catalytic cysteine. Redox-dependent modifications of this residue has a strong impact on GAPC enzymatic activity and could play a role in the regulation of the enzyme in response to stress. The physiological conditions that can trigger such modifications as well as the impact on primary metabolism are still poorly understood. In order to better understand the conditions under which oxidation of this residue can occur, we cloned and heterologously expressed GAPC1 and GAPC2, the two GAPC isoforms in *Arabidopsis thaliana*. The recombinant proteins were subjected to oxidative treatments to characterize the oxidation of the enzymes and determine if the process was reversible. We investigated the occurrence of reversible oxidation of GAPC activity *in vivo* using *A. thaliana* cell cultures subjected to stresses and Arabidopsis mutants with impaired enzymatic machinery for detoxification of reactive oxygen species. These studies will help us better understand the conditions under which oxidative stress can irreversibly damage an important enzyme in plant primary metabolism.

### **The effect of after-ripening on nitrate promoted seed germination in *Arabidopsis thaliana***

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After-ripening (AR) is a period of dry storage that allows seeds to increase their germination potential. It is known that extended periods of AR increase the seed's sensitivity to environmental regulators such as nitrate. Absciscic acid (ABA) is a phytohormone that promotes seed dormancy and other plant developmental processes. *Arabidopsis* NIN-like protein (NLP8) is a crucial regulator for nitrate signaling as well as nitrate-promoted seed germination. Upon the induction of nitrate, NLP8 binds to nitrate responsive elements (NRE) in the promoters of nitrate-regulated genes to induce gene expression. NLP8 is known to bind directly to the NRE of ABA catabolic gene *CYP707A2* to effectively decrease ABA levels during seed germination. AR has been known to increase the production of reactive oxygen species (ROS) in imbibed seeds. In this study, we found that H<sub>2</sub>O<sub>2</sub> increases NO release in imbibed Columbia seeds, however nitrate inhibits it. Moreover, we found that AR effectively increases the transcriptional activity of NLP8, while it is inhibited by NO donor sodium nitroprusside (SNP) when nitrate is co-applied during seed imbibition. Taken together, we conclude that although NO and nitrate are both positive regulators of seed germination, these two pathways act competitively during the progression of AR.

### **Arabidopsis LIPID DROPLET PROTEIN OF SEEDS controls lipid droplet size and number in seeds and seedlings**

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Lipid droplets (LDs) are organelles found in cells across all kingdoms of life. They consist of a hydrophobic core of neutral lipids surrounded by a monolayer of phospholipids and are coated with numerous proteins that carry out a wide range of functions. In plant oilseeds, for example, OLEOSINs are the predominant LD proteins and are thought to stabilize LDs during seed development. Following germination, OLEOSINs are selectively degraded, allowing for LD fusion and catabolism and providing the energy required for seedling establishment. However, despite ongoing advances in understanding LD biology in plants, the molecular mechanisms underlying their formation, maintenance, and turnover, as well as their molecular roles, remain largely unknown, in part because relatively few LD proteins have been studied. Recently, we discovered several new families of plant-specific LD proteins and here we describe experiments aimed at characterizing one of these, termed LIPID DROPLET PROTEIN OF SEEDS (LDPS). LDPS in *Arabidopsis* is annotated to be of unknown function and, akin to the OLEOSINs, is expressed exclusively in developing and mature seeds, as well as in young seedlings. Notably, mature *ldps* mutant seeds have smaller LDs and less storage oil relative to wild-type seeds. Furthermore, LDs in *ldps* mutant seedlings do not appear to fuse following germination, as they do in wild-type seedlings. Taken together, these findings and those from proteomics and lipidomics analyses of *ldps* mutant seeds indicate that LDPS plays a key role, perhaps in concert with OLEOSINs, in controlling LD homeostasis in seeds and young seedlings.

### **Characterizing very-long-chain fatty acids in *Chlamydomonas reinhardtii***

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Very-long-chain fatty acids (VLCFAs) are fatty acids that contain greater than 18 carbons. They are precursors to diverse storage and signalling lipids found ubiquitously in nature. The model organism, *Chlamydomonas reinhardtii*, is a green algal species that has been shown to accumulate triacylglycerols under stress conditions, such as during periods of nitrogen deprivation and high salinity. This has led to the efforts of engineering *C. reinhardtii* for lipid accumulation as a vehicle for the production of biofuels. Despite this fact, the VLCFA profile of *C. reinhardtii* remains largely uncharacterized. *C. reinhardtii*'s genome contains homologs for all

the components of the fatty acid elongation machinery required for the synthesis of VLCFAs, yet the function of these genes in green algae remains unknown. This study aims to characterize the VLCFA profile of *C. reinhardtii* and explore how its composition is altered under various conditions, such as in limiting nitrogen and high salinity. Furthermore, our goal is to determine if *C. reinhardtii*, being evolutionary closer to higher plants, is a more suitable model to study VLCFA biosynthesis when compared to yeast which has traditionally been used. These are the initial steps to develop *C. reinhardtii* as a prospecting model for the reconstitution of higher-plant lipid biosynthetic pathways.

### Effects of warming on crop yield: A meta-analysis

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Under current trends of population growth, food production must increase up to 70% by 2050 to feed a population exceeding 9 billion people. Any efforts to increase food production will be done in a warming climate with elevated levels of atmospheric CO<sub>2</sub>, conditions that will alter crop yield and quality. Current research has shown that higher levels of CO<sub>2</sub> will decrease protein, zinc, and iron concentrations, exacerbating nutrient deficiencies globally, especially in disadvantaged and marginalized populations. While we know that crop yield declines with warming, we lack understanding in how nutritional quality is also affected. To address this issue, I used the Web of Science database to compile articles for a meta-analysis on the effects of warming on crop yield, harvest index, and nutritional quality. In total, 50 of the original 4,873 articles returned from the search query were assessed using a random-effects meta-analysis. Compared to control conditions, elevated temperatures saw significant decreases in yield (Kg/Ha, g/m<sup>2</sup>), and thousand grain weight, while no significant change was detected in harvest index. The articles compiled in the database did not return enough data to perform analysis on nutritional quality, emphasizing that our understanding of how the nutritional quality of major crops changes with warming is not well enough investigated. A better understanding of how nutritional quality is impacted by warming will be crucial to provide an adequate and nutritious food supply for the future population.

### Characterization of flower cutin mutants to reveal potential roles in *Arabidopsis thaliana* floral organ abscission

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Plants undergo a process called abscission to shed their organs. To better understand how and why this process occurs, the model organism, *Arabidopsis thaliana* (*Arabidopsis*) has been studied in depth. *Arabidopsis* flowers shed several of their organs (petals, sepals, and stamens) in order to make way for the silique (seed pod). An abscission zone (AZ) forms at the point of

organ detachment. This zone consists of specialized cells, some of which function to create a protective layer that helps to seal the plant from pathogen entry and water loss. Preliminary chemical analysis has shown that this protective layer is a form of flower cutin. In addition to providing protection for exposed surfaces during abscission, this cutin layer might provide a smooth surface that facilitates organ detachment. To test this hypothesis, flower cutin mutants will be characterized. Changes in the timing of abscission will be assayed. The structure of the break plane and exposed surface layers will be observed using scanning electron microscopy. Sealing of the protective layer will be monitored using a dye permeability assay. These studies will help us gain a better understanding of the different functions of the protective layer in the AZ.

### **Photosynthetic capacity in Jack pine does not acclimate to growth under elevated CO<sub>2</sub> and warming**

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Boreal forests are an important reservoir of terrestrial carbon (C) and a key component of the global C cycle. Rising atmospheric carbon dioxide levels (CO<sub>2</sub>) are expected to warm boreal regions by up to 8°C by 2100, yet we have relatively little data on the physiological responses of boreal trees to climate change. To resolve this, we grew Jack pine [*Pinus banksiana* (Lamb.)] from seed at either ambient (410 ppm, AC) or elevated CO<sub>2</sub> (750 ppm, EC) and at either ambient temperature (T0) or +4°C (T4) or +8°C (T8) warming. After four months, we measured the maximum rate of Rubisco carboxylation ( $V_{\text{cmax}}$ ), the maximum rate of electron transport ( $J_{\text{max}}$ ), and total biomass. Although neither CO<sub>2</sub> nor growth temperature had a significant effect on  $V_{\text{cmax}}$  or  $J_{\text{max}}$ , T4EC and T8EC seedlings had lower  $V_{\text{cmax}}$  and  $J_{\text{max}}$  than T0EC seedlings when measured at high leaf temperatures. Total biomass trended downward with warming but was unaffected by CO<sub>2</sub>. These results suggest that photosynthetic capacity in Jack pine does not acclimate to high CO<sub>2</sub> or warming, and that growth in this species is unlikely to benefit from CO<sub>2</sub> enrichment. Future work will characterise these responses in other boreal tree species with the goal of improving our models of plant-atmosphere C exchange and providing insight on the productivity of Canada's boreal forest in the future.

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