

CSPB-ERM 2025 Program

Wilfrid Laurier University

**2025 Canadian
Society of Plant
Biologists / La société
canadienne de
biologie végétale
Eastern Regional
Meeting**

*Wilfrid Laurier University,
Waterloo, Ontario*

November 28th-29th, 2025

<https://event.fourwaves.com/erm2025>

#CSPB_ERM



LAURIER 
WILFRID LAURIER UNIVERSITY

WELCOME FROM THE CSPB-ERM ORGANIZERS!

It is our great pleasure to welcome all of you to Wilfrid Laurier University for the **2025 Canadian Society of Plant Biologists • la Société Canadienne de Biologie Végétale (CSPB • SCBV) Eastern General Meeting**. This year's theme is

"Canadian plant sciences 2025: From fundamental discoveries to future-defining innovations," as we mark the quarter-centenary of the first plant genome, which has led to major conceptual advances and transformative technologies. This year's scientific program features Plenary Talks by Prof. Eliana Gonzales-Vigil (University of Toronto Scarborough) and Prof. Edel Pérez-López (Université Laval), who are leaders in their respective fields. Our

conference centrepiece is highlighting the work of graduate students, post-doctoral fellows and early career PIs in six Concurrent Sessions and a lunch Poster Session. We invite you to share your ideas, establish new connections and enjoy the enriching conversations!



In partnership with the *CSPB-SCBV Education Committee* (chaired by Dr. Miranda J. Meents, Simon Fraser University), we want to take this opportunity to encourage nominations for the newly launched *Society Award for Teaching Excellence*. This is the first year that CSPB is accepting nominations for this new award, which recognizes excellence in teaching over the previous five years. You can find more information on the [CSPB-SCBV website](#)."

Finally, we extend our sincerest gratitude to the volunteers for their tremendous commitment to organize a vibrant and intellectually stimulating conference.

2025 CSPB-ERM Organizing Committee

Christian Danve M. Castroverde, Allison McDonald, Zhongwei Zou and Matt Smith
Wilfrid Laurier University

Land Acknowledgement

Laurier's Waterloo campus is located on the shared traditional territory of the Neutral, Anishnaabe (Anish-nah-bay) and Haudenosaunee (Hoe-den-no-show-nee) peoples. This land is part of the Dish with One Spoon Treaty between the Haudenosaunee and Anishnaabe peoples and symbolizes the agreement to share, protect our resources and not to engage in conflict.

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ACKNOWLEDGEMENTS

Local Organizing Committee



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Student/Post-Doc Volunteers

- **Anissa Francis** (MSc student, McDonald lab)
- **Ayomi Thilakarathne** (PhD student, Zou lab)
- **Christina Rossi** (PhD student, Castroverde Lab)
- **Danya Maclean** (BSc student, Zou lab)
- **Fatima Warraich** (PhD student, McDonald Lab)
- **Holly Ferguson** (MSc student, Smith Lab)
- **Janet Velázquez Pérez** (Post-doctoral Fellow, Castroverde, Poynter and De-Witte-Orr Labs)
- **Mohsen Shojaei-Barjoui** (BSc student, Smith Lab)
- **Payten Saunders** (BSc student, Zou lab)
- **Sashini Samarakone** (MSc student, Zou Lab)
- **Tetiana Borysenko** (MSc student, Zou lab)
- **Vanessa Shivnauth** (MSc student, Castroverde Lab)
- **Vidushika Yapa Mudiyansele** (MSc student, Zou Lab)
- **Yaruby Gerardo** (BSc student, Smith Lab)

A sincere THANK YOU to all our volunteer Student Presentation Judges and Concurrent Session Chairs! The time and effort you invested into assessing the student oral/poster presentations and chairing the concurrent sessions are much deeply appreciated.

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MEETING SCHEDULE AT A GLANCE

Friday, 28th November 2025	
6:15 - 8:00 PM	Friday Bowling Social/Mixer (RSVP Required) <i>Splitsville Waterloo, 341 Marsland Dr Unit 1, Waterloo, ON N2J 3Z2</i>

Saturday, 29th November 2025			
08:00 - 08:50 AM	Poster Set-up <i>Science Atrium, Science Building</i>		
08:00 - 09:10 AM	Registration and Light Breakfast <i>Lobby, Bricker Academic Building</i>		
08:50 - 09:00 AM	Conference Welcome <i>BA-201, Bricker Academic Building (2nd floor)</i>		
09:00 - 09:45 AM	Plenary Lecture I (Prof. Eliana Gonzales-Vigil, University of Toronto Scarborough) <i>BA-201, Bricker Academic Building (2nd floor)</i>		
09:45 - 10:15 AM	Morning Coffee/Refreshment Break <i>Lobby, Bricker Academic Building</i>		
10:15 AM - 12:00 PM	Concurrent Session 1 <i>BA-110, Bricker Academic Building (1st floor)</i>	Concurrent Session 2 <i>BA-111, Bricker Academic Building (1st floor)</i>	Concurrent Session 3 <i>BA-112, Bricker Academic Building (1st floor)</i>
12:00 - 02:15 PM	Lunch and Poster Sessions <i>Science Atrium, Science Building</i> <i>Presenters of odd # posters should be by their poster by 12:35 PM. Presenters of even # posters should be by their poster by 01:25 PM.</i>		
02:15 - 04:00 PM	Concurrent Session 4 <i>BA-110, Bricker Academic Building (1st floor)</i>	Concurrent Session 5 <i>BA-111 Bricker Academic Building (1st floor)</i>	Concurrent Session 6 <i>BA-112, Bricker Academic Building (1st floor)</i>
04:00 - 04:45 PM	Afternoon Coffee/Refreshment Break <i>Lobby, Bricker Academic Building</i>		
04:00 - 04:45 PM	Deliberations for Best Poster <i>BA-110, Bricker Academic Building (1st floor)</i>		
04:00 - 04:45 PM	Deliberations for Oral presentations <i>BA-111, Bricker Academic Building (1st floor)</i>		
04:45 - 05:30 PM	Plenary Lecture II (Prof. Edel Pérez-López, Université Laval) <i>BA-201, Bricker Academic Building (2nd floor)</i>		
05:30 - 06:00 PM	Award Announcements and Closing Remarks <i>BA-201, Bricker Academic Building (2nd floor)</i>		

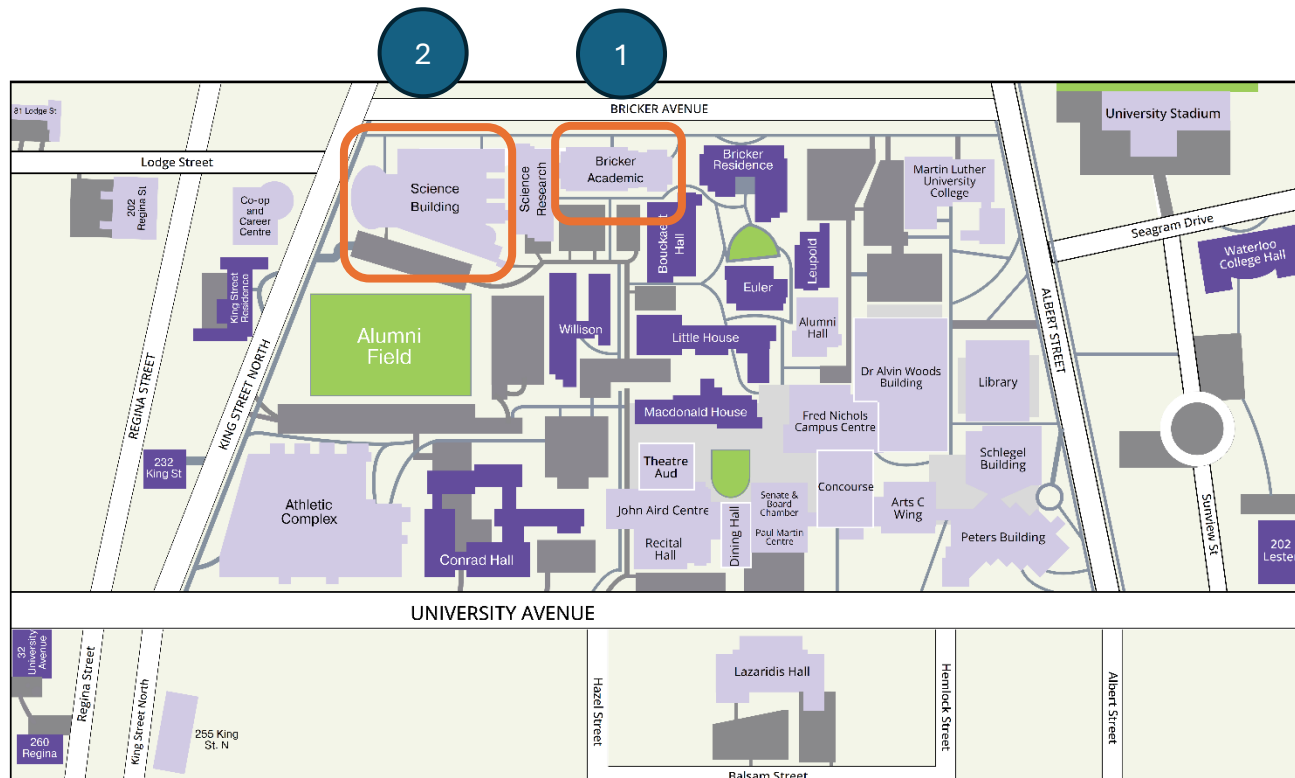
GENERAL INFORMATION

Meeting Venues

The 2025 Canadian Society of Plant Biologists • la Société Canadienne de la Biologie Végétale (CSPB • SCBV) Eastern Regional Meeting is being held at Wilfrid Laurier University (Waterloo, Ontario; see map below) on November 29th, 2025 (Saturday).

Conference registration, Plenary Sessions, Concurrent Sessions and coffee breaks take place in the **Bricker Academic Building** (see 1 on map; [Google Maps link](#)). Plenary and award presentations are held in BA-201 on the second floor of Bricker Bldg. Concurrent Sessions take place in rooms BA-110, BA-111 and BA-112 on the first floor of Bricker Bldg.

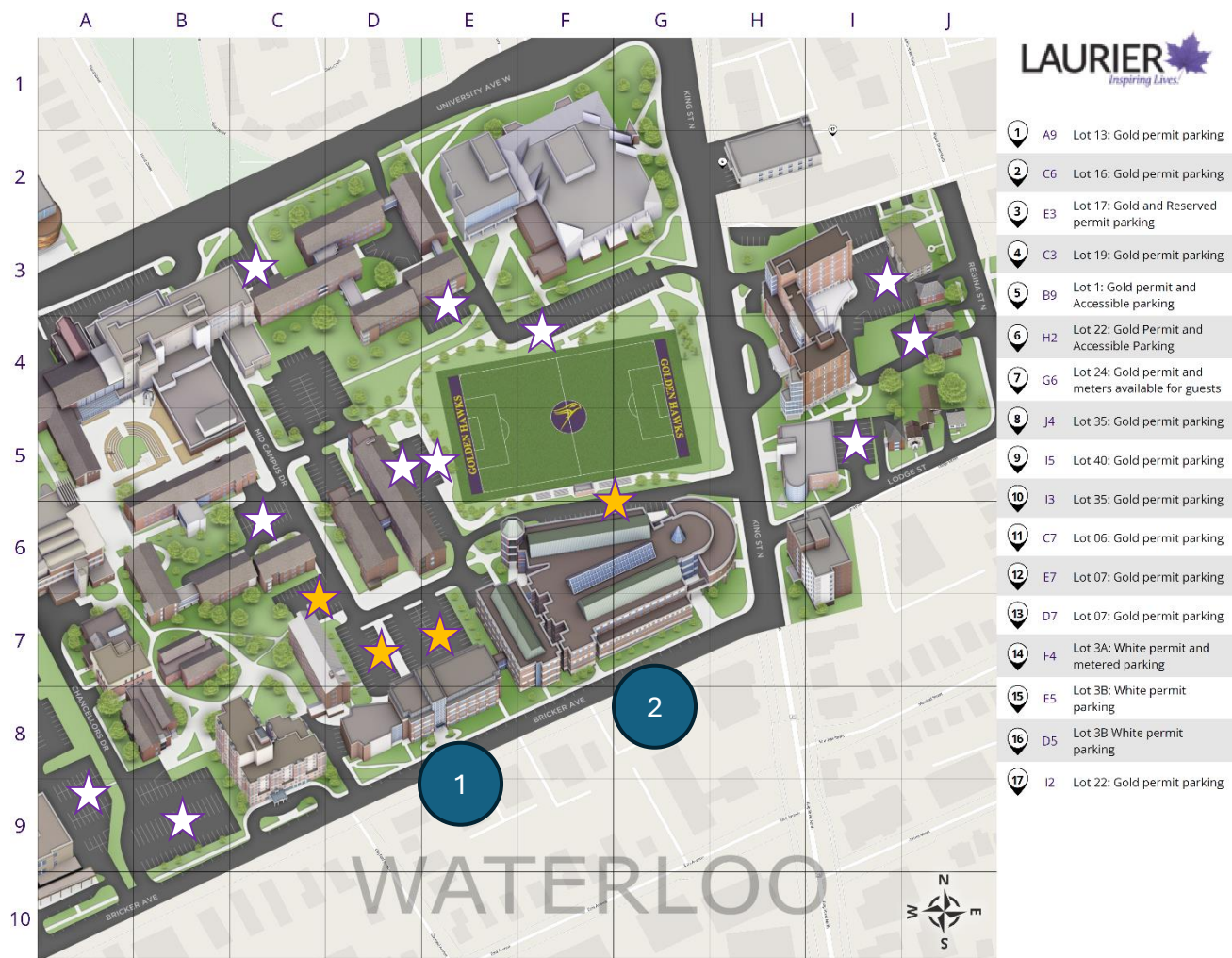
Poster Sessions and lunch are held in the Science Atrium on the first floor of the **Science Building** (see 2 on map; [Google Maps link](#)).



Parking

Parking is available at **Gold** and **White** lots (indicated with golden stars) close to the Bricker Academic Bldg. (#1 on map) and Science Bldg. (#2 on map). Other Gold or White parking lots are indicated as white stars. There is no charge for parking on campus on Saturday if

you park in Gold or White parking lots. Please see the [Laurier parking website](#) for more information.



Internet Access

Eduroam is a secure network accessible to educational and research communities. You can access network services while at Laurier using your own institutional login credentials, without making special arrangements. All faculty, students, staff and alumni from Canadian universities can access Eduroam in Laurier buildings using their institutional credentials during regular building hours. [Set up eduroam](#) at your home institution first to confirm that it works.

Guest Wi-fi Through Eduroam: Please connect with the Registration Table to receive Eduroam Visitor Access (eVA). This enables secure, temporary Wi-Fi access to visitors that are not from an institution that participates in Eduroam.

Please note that you will be responsible for complying with the terms of use outlined in [Policy 9.1 – Use of Information Technology](#) while using Laurier ICT services.

Food

Light breakfast (muffins and fresh fruit) will be served during Registration in the lobby of the Bricker Academic Bldg. (#1 on map). The two Coffee Breaks (Morning and Afternoon) will also serve light snacks (e.g. cookies). Coffee, tea and other beverages will be available during Registration and the two Coffee Breaks.

Boxed lunches, including a sandwich, cookie, fruit, chips and a drink, will be provided during the Lunch and Poster Session in the Atrium of the Science Bldg. (#2 on map). Food served at Registration, Coffee Breaks and Lunch are included in the registration fee.

Conference Social/Mixer

The Conference Social/Mixer (RSVP needed during registration) will be held at Splitsville Bowling Waterloo ([Google Maps link](#)) on November 28th, 2025 (Friday) from 6:15 PM to 8:00 PM. The bowling lanes are reserved for 6:30 PM, but we request everyone to arrive 15 minutes early for registration. Snacks and non-alcoholic refreshments will be provided. Alcoholic beverages can be purchased separately. For more information, please visit the Splitsville website: <https://www.splitsvillebowl.ca/>

Code of Conduct

CSPB/SCBV is committed to ensuring that the meeting is a welcoming and inclusive space for sharing ideas and knowledge. To this end, we will provide a safe and productive environment that promotes equal opportunity and treatment for all participants and that is free of harassment and discrimination. This code of conduct applies to all CSPB/SCBV meeting attendees, speakers, exhibitors, staff, contractors, volunteers, and guests. Please make sure that you have read the [Code of Conduct](#).

Reporting: Any harassment, whether experienced directly or witnessed, should be reported promptly to **a member of the CSPB/SCBV local organizing committee**. All complaints will be taken seriously and responded to promptly. Confidentiality will be maintained to the extent that it does not compromise the rights of others.

Social Media Policy: The CSPB/SCBV encourages the use of social media, such as Facebook, Instagram, X (formerly Twitter), LinkedIn, and blogging, as ways to engage the

community in events. Please make sure that you have reviewed the [Social Media Policy](#) in detail.

For any questions about the policy, please contact:

CSPB/SCBV President (president@cspb-scbv.ca)

This Code of Conduct was adapted from the Code of Conduct developed for the Plant Biology 2018 Meeting organized by ASPB, CSPB/SCBV, and ISPR (2018).

This Social Media Policy was adapted from the Code of Conduct developed for the Plant Biology 2021 Meeting organized by ASPB (2022).

CSPB/SCBV requires all participants to read and agree to this Code of Conduct and Social Media Policy as part of the meeting registration process.

PLENARY AND CONCURRENT SESSION TITLES

Plenary I (Room BA-201)			
	<i>First name</i>	<i>Last name</i>	<i>Plenary talk title</i>
09:00-09:45 a.m.	Eliana	Gonzales-Vigil	The reactive cuticle: Alkene oxidation and aldehyde release in poplar leaves.

Plenary II (Room BA-201)			
	<i>First name</i>	<i>Last name</i>	<i>Plenary talk title</i>
04:45-05:30 p.m.	Edel	Pérez-López	Linking ANK effectors to virulence evolution of the clubroot pathogen

Concurrent Session 1: Biochemistry and Metabolism (Room BA-110) <i>Chair: Fathy El-Gebaly</i>			
	First name	Last name	Submission title
10:15-10:28 a.m.	Michele	Antonacci	Multiple Detoxification Mechanisms in <i>Tetranychus urticae</i> Enable Metabolic Resistance to the <i>Arabidopsis</i> -Derived Indole-3-Acetonitrile
10:28-10:41 a.m.	Rachel	Hindle	Managing starch to survive stress: An investigation of plastidial starch phosphorylase (Pho1) in <i>Arabidopsis thaliana</i>
10:41-10:54 a.m.	Fathy	Elgebaly	Development of bioproducts and circular bioeconomy through the valorization of agri-food waste
10:54-11:07 a.m.	Andreea	Bosorogan	When seeing is believing: Using the visual reporter RUBY for teaching and engineering medicinal metabolites in <i>Atropa belladonna</i>
11:07-11:20 a.m.	Christine	Nguyen	Expression and functional analyses of ABCG25 efflux transporter in ABA transport in <i>Arabidopsis thaliana</i>
11:20-11:33 a.m.	Ivan	Monsalvo	ANAC042 Regulates the Biosynthesis of Conserved and Lineage-Specific Phytoalexins in <i>Arabidopsis</i>
11:33-11:46 a.m.	Lorena	Yeung	Heterologous expression of <i>CrMATE1</i> increases flux of intermediates in monoterpene indole alkaloid pathway
11:46-11:59 a.m.	no talk		

Concurrent Session 2: Bioinformatics, Computational Biology, and Omics (Room BA-111)

Chair: Sylvain Colomer

	First name	Last name	Submission title
10:15-10:28 a.m.	Bridget	Murphy	From summer stress to winter rest: transcriptomic insights into autumn phenology under drought and warming in white spruce
10:28-10:41 a.m.	Mingze	Xu	Gene gains and losses in the evolution of a conserved KCS gene cluster
10:41-10:54 a.m.	Yifan	Wu	Benchmarking AlphaFold 3, AlphaFold-Multimer, and ColabFold for Heterodimeric Complex Prediction in <i>Arabidopsis thaliana</i>
10:54-11:07 a.m.	Sylvain	Colomer	Large-scale individual tree segmentation across entire white spruce forests using UAV hyperspectral imagery and deep learning
11:07-11:20 a.m.	Colette	Picard	An antagonistic epigenetic mechanism regulating gene expression in pollen revealed through single-nucleus multiomics
11:20-11:33 a.m.	Racquel	Singh	An evolutionary and functional analysis of virulence gene promoters in <i>Pseudomonas syringae</i> .
11:33-11:46 a.m.	Jie	Lin	Identifying Missing Glyceollin Transcription Factors in Soybean
11:46-11:59 a.m.	Anissa	Francis	Analysis of alternative oxidase in non-angiosperm plants

Concurrent Session 3: Plant Immunity (BA-112)

Chair: Yan Lai

	First name	Last name	Submission title
10:15-10:28 a.m.	Ujomonigho	Omoregie	Pinpointing candidate genes in a quantitative trait locus associated with resistance to bacterial brown spot in adzuki bean
10:28-10:41 a.m.	Mylene	Corzo-Lopez	Proteomic Analysis of <i>Phaseolus vulgaris</i> L Responses to <i>Xanthomonas phaseoli</i> pv. <i>phaseoli</i> Infection.
10:41-10:54 a.m.	Yan	Lai	The <i>Pseudomonas syringae</i> conserved effector locus is a prominent target of soybean effector-triggered immunity
10:54-11:07 a.m.	Mack	Loranger	Rhizobacteria-induced systemic priming against fungal pathogens involves hydroxycinnamic acid amides
11:07-11:20 a.m.	Tamar	Av-Shalom	Effector-triggered stomatal immunity prevents leaf invasion by bacterial pathogens

11:20-11:33 a.m.	Jacob	Lund	Long-distance movement of the lipid transfer protein DIR1 during plant immunity requires N-hydroxyphenylacetic acid signaling
11:33-11:46 a.m.	Evan	Krysmanski	Investigating the Activation of DEFECTIVE IN INDUCED RESISTANCE 1 Movement during Systemic Acquired Resistance in <i>Arabidopsis</i>
11:46-11:59 a.m.	Christina	Rossi	A Multi-Omics Approach to Uncover Novel Regulators of Temperature-Sensitive Plant Immunity

Concurrent Session 4: Biotic Interactions (BA-110)

Chair: Julia Pastor-Fernandez

	First name	Last name	Submission title
2:15-2:28 p.m.	Vidushika	Madduma Bandara	Understanding the interaction between <i>Verticillium longisporum</i> and <i>Leptosphaeria maculans</i> in <i>Brassica napus</i>
2:28-2:41 p.m.	Sashini	Samarakone	Exploring genetic resistance to sclerotinia stem rot in canola through VQ gene expression profiling
2:41-2:54 p.m.	Ayomi	Thilakarathne	Integrated Transcriptomic, Enzymatic and Phenotypic Analysis of <i>Brassica napus</i> Responses to <i>Verticillium longisporum</i> Infection
2:54-3:07 p.m.	Uliana	Galaktionova	Tomato responses to different tomato-adapted spider mite populations
3:07-3:20 p.m.	Julia	Pastor Fernandez	Glutathione S-Transferases Mediate Two-Spotted Spider Mite Adaptation to <i>Arabidopsis</i>
3:20-3:33 p.m.	Jorden	Maglov	Uncovering the complexity of <i>Arabidopsis thaliana</i> defenses against <i>Tetranychus urticae</i> herbivory
3:33-3:46 p.m.	Annabelle	Audet	Determinants of Pokeweed Mosaic Virus' Host Range
3:46-3:59 p.m.	Tevon	Pitambar	Overcoming the biological and technical barriers of <i>Agrobacterium tumefaciens</i> -mediated transformation in the forage legume sainfoin (<i>Onobrychis viciifolia</i> Scop.)

Concurrent Session 5: Abiotic Stress (BA-111)

Chair: Emilie Isasa

	First name	Last name	Submission title
2:15-2:28 p.m.	Olivia	Di Biase	Changes in photosynthetic and anatomical traits in response to incremental drought and recovery in white pine and white spruce
2:28-2:41 p.m.	Vincent	Riedel	Differences in Chlorophyll Fluorescence and Vegetation Indices for White Spruce (<i>Picea glauca</i>) Grown under Contrasting Climates at Two Eastern Canadian Sites

2:41-2:54 p.m.	Haoran	Jia	Salt tolerant ecotypes of <i>Eutrema salsugineum</i> respond differently to salinity
2:54-3:07 p.m.	Grace	Cheng	Environmentally driven variation in drought-relevant traits among 16 white spruce genotypes from two Eastern Canadian sites
3:07-3:20 p.m.	Emilie	Isasa	Microtopography and soil moisture gradients drive variation in turgor loss point and growth in white spruce seedlings
3:20-3:33 p.m.	Anchalya	Balasubramaniam	Divergent Photoprotective Strategies in Eastern White Pine and White Spruce Under Moderate Drought
3:33-3:46 p.m.	Allison	Szenasi	Environmental stress in extremophilic <i>Chlamydomonas</i> : Comparative analysis of closely related Arctic and Antarctic species
3:46-3:59 p.m.	Danielle	Bowman	Understanding the Genetic Underpinnings of Climate Change Acclimation and Temperature Stress Resilience in an Antarctic Green Algal Psychrophile

Concurrent Session 6: Cell biology, signalling, and transcriptomics (BA-112)

Chair: Hermann Prodjino

	First name	Last name	Submission title
2:15-2:28 p.m.	Anka	Colo	Identification of Novel RLCK Substrates in Receptor Kinase Pathways Governing Plant Growth and Development
2:28-2:41 p.m.	Carly	Charron	Green vaccines: Plant-produced nanoparticles to prevent non-typhoidal <i>Salmonella enterica</i> colonization in poultry
2:41-2:54 p.m.	Sophia	Mastrella	Exploring the function of protein palmitoylation in auxin-induced ROP6 signaling
2:54-3:07 p.m.	Angela	Sidsworth	Identifying New Components in the <i>Arabidopsis</i> Self-Incompatibility Pathway
3:07-3:20 p.m.	Noelle	Perkins	Winter is Coming: Uncovering a Dehydrin Protein's Role in Conifer Cold Acclimation
3:20-3:33 p.m.	Holly	Ferguson	Unraveling the targeting pathway of OEP6: Insights into chloroplast and mitochondrial outer membrane trafficking
3:33-3:46 p.m.	Max	Carito	Uncovering the mechanistic determinants of pathway specificity in the RLCK-VII family
3:46-3:59 p.m.	Hermann	Prodjino	Dissecting Ionic and Osmotic Components of Salt Stress in African Rice (<i>Oryza glaberrima</i>): Physiological and Gene Expression Insights

POSTER SESSION TITLES

12:35 - 1:25 PM: Odd-numbered poster session

(Presenters are requested to be stationed by their posters during this time period)

1:25 - 2:15 PM: Even-numbered poster session

(Presenters are requested to be stationed by their posters during this time period)

Plant-Pathogen Interactions			
1	Nick	Archibald	Investigating the Role of <i>BnNAC19</i> Overexpression in <i>Brassica napus</i> Responses to <i>Verticillium longisporum</i>
2	Annabelle	Audet	Determinants of Pokeweed Mosaic Virus' Host Range
3	Tetiana	Borysenko	Role of <i>BnWRKY40</i> Overexpression in Enhancing Resistance of <i>Brassica napus</i> to <i>Verticillium longisporum</i> and <i>Sclerotinia sclerotiorum</i>
4	Janis Hiu Tung	Cheng	Mapping the physical and genetic interactome of <i>Pseudomonas syringae</i> effectors
5	Samantha	Reis	Canola <i>Verticillium</i> stripe disease: pathogenicity variations, co-existence with <i>Leptosphaeria maculans</i> , and transcriptome profile at adult plant stage.

Plant Immunity			
6	Nicole	Iori	Characterizing the Role of LRK10L Proteins in Plant Immune and Stress Signalling
7	Jeongrae	Kim	Optimization of a novel <i>Pseudomonas syringae</i> - <i>Nicotiana benthamiana</i> pathosystem for the study of effector-triggered immunity
8	Ritchi	Sarin	Investigating the Roles of WAK1 and SVB in Temperature-Dependent Regulation of Salicylic Acid-Mediated Defence in <i>Arabidopsis thaliana</i>
9	Rylan	Vincent	Shooting the Messenger: The Importance of Small RNAs in Plant Immunity
10	Jaya	Vos	Genome-Wide Association Study to Identify Causative SNPs Involved in Temperature-Regulated Immunity in <i>Arabidopsis thaliana</i>

Molecular Signaling and Regulation			
11	Fayeza Tabassum	Azad	Tracking cell wall signaling via transcriptional and biochemical outputs
12	Colleen	Barghout	Identification of novel components in <i>Arabidopsis</i> CEP peptide signalling

13	George	Del Grosso	Expression pattern alone does not encode receptor-like cytoplasmic kinase signaling specificity
14	Punita	Lalchand	Function and Regulation of Protein Palmitoylation in Auxin Signaling
15	Leo	Tullo	Investigate structural motifs of the HSP90C C-terminal extension region in mediating substrate binding
16	Hannah	Waterson	Illuminating Phosphorylation: Visualizing ADT1 and ADT4 Interactions with 14-3-3 <i>In Planta</i>
17	Come	Luciat-Labry	Aggregation of HTL/KAI2 elucidates a novel mechanism of signalling in <i>Arabidopsis</i>
18	Yifan	Wu	Benchmarking AlphaFold 3, AlphaFold-Multimer, and ColabFold for Heterodimeric Complex Prediction in <i>Arabidopsis thaliana</i>

Abiotic Stress and Environmental Responses

19	Sean	Balogh	Adaptive Drought-Induced Lipid Modulations in Soybean (<i>Glycine max</i> L.)
20	Maria	Barsamyan	Investigating the roles of NAC transcription factors in <i>Arabidopsis thaliana</i> seed coat development and salt stress response
21	Kristen	Huang	Evaluating SIF response to optimization of UAV flight parameters in coniferous and mixed deciduous forests
22	Theo	Kristanto	Understanding cold-induced sweetening in potato tubers
23	Laura	Li	Identifying differential low phosphate responsive traits in <i>Eutrema salsugineum</i> and their recombinant inbred lines.
24	Sofia	Mahmood	Altered expression of NAC transcription factors impairs seed coat development and germination under salinity and cold stress.
25	Nur	Hussain	Assessment of Temporal Dynamics of Photosynthetic Activity in Deciduous and Mixed Forests Using Solar-Induced Chlorophyll Fluorescence

Biochemistry and Metabolism

26	Elizabeth	Copley	Breaking it down: investigating the biological function of cuticular waxes in the poplar cuticle
27	Hannah	Lye	Identification and functional analysis of lipases associated with oil metabolism in the microalga <i>Nannochloropsis oceanica</i>
28	Nick	Sheppard	Investigating Mechanisms Underlying Increased Biomass and Seed Yield in Canola Expressing Maize Starch Branching Enzymes
29	Fatima	Warraich Kahloon	Characterizing the Structure and Post-Translational Regulation of the Plant Alternative Oxidase (AOX) Proteins Using a Yeast Expression System

30	Kajsa	Yates	Linum usitatissimum (flax) diacylglycerol acyltransferases interact with lysophosphatidylcholine acyltransferase
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Genomics and Epigenetics			
31	Pradiksha	Aravindpathy	Characterizing the Relatedness of Moringa Trees
32	Colette	Picard	An antagonistic epigenetic mechanism regulating gene expression in pollen revealed through single-nucleus multiomics
33	Tanya	Sarin	Optimization of sequence-specific dsRNA transient expression in <i>Nicotiana benthamiana</i>
34	Mohammad	Erfatpour	Toward a Navy Bean Pangenome for Dissecting Common Bacterial Blight Resistance and Tepary Bean Introgressions

ABSTRACTS

PLENARY SESSIONS

PLENARY SESSION I

The reactive cuticle: Alkene oxidation and aldehyde release in poplar leaves



Prof. Eliana Gonzales-Vigil¹

¹Department of Biological Sciences at the University of Toronto in Scarborough

Plant cuticles serve as frontline defences, shielding internal tissues from abiotic and biotic stresses. Although their protective role is well-established, cuticles are traditionally viewed as non-reactive physical barriers, primarily composed of a mixture of very-long chain lipids. Our lab has been investigating the accumulation of monounsaturated hydrocarbons (cis-9 alkenes) on the cuticular wax of *Populus* (poplar), with the ultimate goal of understanding their functional role for the plant. Unlike other cuticular waxes, alkenes exhibit a distinctive pattern of accumulation; new leaves contain

trace levels that increase as the leaves expand. Notably, the trend reverses in more mature leaves, where a decline in very-long chain alkenes coincides with an increase in shorter-chain aldehydes. The initial rise in alkenes is strongly correlated with the expression of a 3-ketoacyl CoA synthase, *PtKCS1*, a gene showing hallmarks of recent specialization. In contrast, the subsequent decline in alkenes is attributed to spontaneous oxidation into aldehydes upon exposure to air and light. Interestingly, nonanal is one of the volatile aldehydes released and is a well-studied semiochemical with key roles in plant and insect communication. Moreover, the production of shorter aliphatics from long-chain cuticular waxes can be extended to other components, such as the generation of caprolactones from diketones in bread wheat (*Triticum aestivum*). These observations uncover a new mechanism for the generation of plant volatiles that are structurally encoded within cuticular waxes.

PLENARY SESSION II

Linking ANK effectors to virulence evolution of the clubroot pathogen



Prof. Edel Pérez-López¹

¹Department of Phytopathology,
Université Laval

Plasmodiophora brassicae, the causal agent of clubroot disease, is a soil-borne obligate biotroph that threatens canola production worldwide, with resting spores capable of surviving in soil for decades. Deployment of clubroot-resistant (CR) canola cultivars has been the most effective management strategy, but it has also imposed strong selective pressure, driving the emergence of new virulent isolates in Canada. Effector proteins are central to these evolutionary arms races yet remain poorly characterized in this pathosystem. To address this, we assembled the first telomere-to-telomere reference genome of *P.*

brassicae (20 chromosomes, 10,521 genes, 806 predicted effectors) and performed population genomics across 142 isolates from 20 countries. We observed three major genetic clusters, with Canadian-dominated clusters exhibiting higher virulence. Pangenome analysis of 45 isolates revealed a dynamic ~250 kb hotspot containing five ankyrin-repeat (ANK) effectors, including ANK2, which carried multiple intragenic deletions and flanking structural variants in highly virulent isolates. Using AlphaFold2 structural predictions, we discovered that ANK effectors represent a significantly expanded family in gall-forming plasmodiophorids and are highly expressed during infection. Interaction screens identified ANK1 and ANK2 targets in *Arabidopsis*, all host proteins associated with stress sensing and cell expansion. BiFC assays confirmed their localization at both the plasma membrane and the nucleus, suggesting that ANK effectors interfere with host stress-signaling networks in multiple cellular compartments. Altogether, our findings link population-genomic signatures, structural variation, and functional evidence, revealing ANK effectors as key players in virulence evolution and disease establishment.

ABSTRACTS

CONCURRENT SESSIONS

Concurrent Session 1 - Biochemistry and Metabolism

Multiple Detoxification Mechanisms in *Tetranychus urticae* Enable Metabolic Resistance to the Arabidopsis-Derived Indole-3-Acetonitrile

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The interaction between herbivorous pests and their plant hosts reflects a co-evolutionary “arms race,” where plants produce toxic metabolites to deter herbivory and herbivores evolve countermeasures to survive. Generalist herbivores, unlike specialists restricted to few hosts, cope with diverse defenses through metabolic detoxification. The two-spotted spider mite (*Tetranychus urticae*, TSSM) exemplifies this adaptability, feeding on more than 1,100 plant species yet thriving only on specific hosts. Remarkably, TSSM can adapt to novel plants within about 25 generations.

Arabidopsis thaliana represents a challenging host due to its indole glucosinolates, defensive metabolites typical of Brassicaceae. Upon tissue damage, these compounds yield indole-3-isothiocyanate and indole-3-acetonitrile (IAN), both implicated in plant defense. IAN, a nitrile capable of releasing cyanide through enzymatic oxidation, inhibits mitochondrial respiration and is toxic to TSSM when supplemented artificially, confirming its defensive role.

Here, we investigated TSSM’s metabolic strategies for detoxifying Arabidopsis-derived IAN through untargeted metabolomics of mites after IAN ingestion. Several metabolites detected in mites overlapped with plant products, allowing reconstruction of potential detoxification routes. Detection of γ -glutamyl- β -cyanoalanine suggests oxidation of IAN, cyanide release, and detoxification via β -cyanoalanine synthase. Glutathione and glucose conjugates of IAN were also found, indicating conjugation mechanisms that reduce free IAN levels. Additionally, we identified indole-3-oxoacetamide—a metabolite not reported in *Arabidopsis*—which may arise from nitrile hydratase activity, providing an alternative, cyanide-free detoxification route.

Overall, TSSM exhibits remarkable metabolic flexibility, employing multiple enzymatic pathways to neutralize IAN toxicity and adapt to Brassicaceae-specific chemical defenses.

Managing starch to survive stress: An investigation of plastidial starch phosphorylase (Pho1) in *Arabidopsis thaliana*

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Starch is the primary carbohydrate reserve and energy source for plants. The metabolism of starch allows for sustained growth during the night, and under stress conditions. Plastidial starch phosphorylase (Pho1) provides an alternative, non-energy-consuming pathway for starch biosynthesis and degradation, with activity largely favoring biosynthesis. Recent work has shown that oxidative stress conditions catalytically activate Pho1, suggesting an additional link between stress signalling and starch metabolism.

Reactive oxygen species generated under abiotic stress disrupt normal cellular processes, reducing growth and yield. Plants lacking *Pho1* display heightened sensitivity to stress, yet a comprehensive understanding of the physiological consequences of its loss remains unclear. I hypothesize that under abiotic stress, *Pho1* expression level will positively correlate with leaf starch accumulation and therefore, plant resilience. An comprehensive analysis of a titration of *Pho1* expression levels under drought and salinity stress will be conducted including the quantification of carbohydrates, gene expression and enzyme activity. By integrating metabolic, genetic and phenotypic data, this work seeks to clarify how Pho1 contributes to maintaining energy balance and fitness during stress.

The findings enhance understanding of starch-associated stress responses and identify potential regulatory connections between carbohydrate metabolism and oxidative signalling. Pho1 is conserved across the plant kingdom, insights gained from *Arabidopsis thaliana* will have broad relevance to major crop species. Ultimately, this research aims to advance our knowledge of how plants adjust primary metabolism to endure fluctuating environmental conditions, offering new perspectives for improving stress tolerance and productivity in a changing climate.

Development of bioproducts and circular bioeconomy through the valorization of agri-food waste

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Agricultural food waste (AFW) constitutes approximately one-third of Ontario's overall waste stream, including ~ 6.2 million tons of crop residues like corn stover and 12 to 15 million pounds of cucumber and tomato grade-outs from commercial greenhouses, annually. These figures highlight the huge amount of AFW in Ontario and resulting disposal issues. Therefore, an opportunity exists to convert AFW using hydrothermal carbonization (HTC) to create high-value bioproducts, such as biocarbon and plant growth & immunity biostimulants. HTC involves heating wet biomass to 180 to 280°C under pressure to produce biocarbon solids (hydrochar) and process water (PW). Hydrochar can be used as a solid fuel, while PW contains plant metabolites that may enhance plant growth and resistance to pathogenic disease. *Nicotiana benthamiana* and cucumber plants were treated by foliar spray or soil drench with PW produced at 180°C or 260°C. No harmful effects were observed on plants treated with PW (180, 260 °C) as determined by measuring shoot length, leaf number, and plant biomass. Next, the ability of PW treatment to enhance plant immunity was investigated in cucumber to the bacterial pathogen *Pseudomonas syringae* pv *lachrymans* (*PsI*) and in *N. benthamiana* to tobacco rattle virus (TRV). PW treatments (180 to 280°C) reduced *PsI* multiplication in PW-treated compared to mock-treated cucumber, while TRV levels were reduced only systemic leaves of PW-treated compared to mock-treated *N. benthamiana* plants. Future work will focus on understanding how PW treatments result in enhanced resistance to bacterial and viral pathogens.

When seeing is believing: Using the visual reporter RUBY for teaching and engineering medicinal metabolites in *Atropa belladonna*

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Atropa belladonna is a medicinally important plant that synthesizes atropine, an anticholinergic tropane alkaloid (TA) widely used to treat asthma, pain, and bradycardia. However, *A. belladonna* accumulates low levels of TA compounds, which can be improved by genetic engineering. To achieve this goal, we optimized both transient and stable transformation protocols with *A. belladonna*, using the visual reporter plasmid pCambia2300-RUBYGREEN (RUBY) that includes three enzymes for the production of the red pigment betalain. Transiently infiltrated leaves developed a strong red colour within 4-5 days, which remained visible for over 5 weeks. The visual reporter allowed adapting this technique as a teaching tool in undergraduate laboratories to demonstrate the principles of plant transient transformation and metabolic engineering. In contrast, stable transformation of *A. belladonna* with the RUBY construct led to patchy accumulation of betalains, ranging in colour from red to green and purple. Common phenotypes included betalain accumulation in the roots, floral reproductive organs, and immature fruits. Moreover, we achieved a 35% transformation efficiency (9/26 T₀ lines), confirmed by reporter expression. In T₁ lines, betalain accumulation was retained but varied widely among seedlings, likely due to segregation. The betalain persistence further supports the robustness of these protocols for future TA-targeted engineering in *A. belladonna*. Together, these results establish a foundation for metabolic engineering in *A. belladonna*, with applications in both research and education aimed at improving the biosynthesis of valuable medicinal compounds.

Expression and functional analyses of ABCG25 efflux transporter in ABA transport in *Arabidopsis thaliana*

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The plant hormone abscisic acid (ABA) plays a pivotal role in plant adaptation to environmental stress. ABA regulates growth and coordinates physiological processes throughout the plant via a network of ABA transporters that facilitate its cellular and long-distance transport. The identification of several ABA transporters has enhanced our understanding of ABA transport in leaves and seeds. Although long-distance ABA transport from shoots to roots has been described, the mechanisms governing ABA movement and distribution within specific root tissues remain poorly understood. The ATP-binding cassette (ABC) transporter AtABCG25 functions as an ABA exporter and is primarily expressed in hypocotyls, roots, and vascular tissues of leaves. Long-distance transport assays in *abcg25* mutants revealed increased accumulation of deuterium-labelled ABA in the roots compared to the wild type, indicating that ABCG25 could be responsible for fine-tuning whole-plant ABA levels in the plant. To further elucidate the role of ABCG25, we developed ABCG25 reporter lines to analyze its tissue-specific expression in roots. We aim to gain deeper insights into the mechanisms underlying whole-plant ABA movement and to investigate

how ABCG25 regulates ABA levels in roots, which is a crucial site for maintaining ABA homeostasis in plants.

ANAC042 Regulates the Biosynthesis of Conserved and Lineage-Specific Phytoalexins in *Arabidopsis*

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Throughout evolution, plants have developed diverse defence mechanisms in response to pathogen attack. These include the production of specialized metabolites called phytoalexins. A prevailing paradigm in transcription factor (TF) biology is that conserved TFs regulate specific branches of specialized metabolism across plant lineages. In *Arabidopsis thaliana*, the NAC family TF ANAC042 (also known as JUNGBRUNNEN1 or JUB1) regulates the biosynthesis of the lineage-specific phytoalexin camalexin (Tryptophan-derived). Interestingly, its homologs in soybean (*Glycine max*) and Chinese wild grape (*Vitis quinquangularis*) regulate the biosynthesis of glyceollin and resveratrol, which are phytoalexins that are biosynthesized from phenylalanine. In *Arabidopsis*, there are two main Phe-derived phytoalexins, scopoletin and pathogen-inducible monolignols, which are phytoalexins broadly conserved in vascular plants including dicots, monocots, and magnoliids. This study investigates whether ANAC042 broadly regulates conserved (Phe-derived) and lineage-specific (Trp-derived) phytoalexins in *Arabidopsis*. Using a novel matrix-assisted laser desorption ionization high resolution mass spectrometry (MALDI-HRMS) method, we show that the *anac042-1* loss-of-function mutant is deficient in camalexin, 4-hydroxyindole-3-carbonyl nitrile (4OH-ICN), and pathogen-inducible monolignols and scopoletin following Flg22 elicitation. ANAC042 overexpression restored or enhanced phytoalexin levels, correlating with transcript levels of the corresponding biosynthetic genes. Yeast one-hybrid and promoter-reporter assays in *Nicotiana benthamiana* demonstrated that ANAC042 directly activates Trp- and Phe-derived phytoalexin biosynthetic gene promoters. The role of ANAC042 aligns with the current paradigm as a broad regulator of phytoalexin synthesis. Our results suggest that ANAC042 is a cooperative and opportunistic TF that has coopted conserved and lineage-specific metabolic pathways into phytoalexin biosynthesis.

Heterologous expression of CrMATE1 increases flux of intermediates in monoterpenoid indole alkaloid pathway

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The medicinal plant *Catharanthus roseus* is the source of many therapeutically potent monoterpenoid indole alkaloids (MIAs). However, their low abundance can often restrict investigations into new bioactivities. This hurdle can be circumvented by heterologous reconstitution of MIA pathways in microbial hosts. Critically, reassembly is often compromised by

low product titers, by-product formation, and a lack of pathway orthogonality. Transporters can provide strategic means to engineer metabolic organization and guide flux. We previously characterized CrMATE1 as a vacuolar importer of secologanin. Once in the vacuole, secologanin is condensed with tryptamine to form strictosidine, the central MIA scaffold, which is exported by CrNPF2.9. Here, we demonstrate the utility of these two transporters in engineering MIA biosynthesis and the production of the antihypertensive drug, ajmalicine. We recapitulate CrMATE1 substrate specificity for secologanin and show that the co-expression of CrMATE1 and CrNPF2.9 in a *de novo* ajmalicine-producing yeast strain significantly enhances product titers. Notably, CrMATE1 expression alone conferred the most substantial improvement, with our assays suggesting that vacuolar secologanin import is the critical bottleneck for ajmalicine biosynthesis. Relocating strictosidine synthesis to the cytosol has a negative impact on titers, highlighting the importance of compartmentalization and transporter co-expression. In parallel, *CrMATE1* overexpression in *C. roseus* petals redirected metabolic flux, elevating accumulation of branch-pathway MIAs, including akuammicine and serpentine. Our work establishes transporters as critical regulators of MIA pathway flux and reiterates their significance in bioengineering efforts for complex plant metabolic pathways.

Concurrent Session 2 - Bioinformatics, Computational Biology, and Omics

From summer stress to winter rest: transcriptomic insights into autumn phenology under drought and warming in white spruce

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The future productivity and carbon sequestration potential of boreal forests is uncertain under climate change. Projected increases in drought and warming will likely alter the seasonal regulation of photosynthesis in evergreen conifers, especially during autumn. Local adaptation within conifer species will also affect how different populations respond to these changes. To investigate the genetic regulation of autumn phenology under climate change, we examined transcriptomic responses in two latitudinally-distinct white spruce (*Picea glauca*) families exposed to summer drought, autumn warming, and their combination in a field experiment spanning summer to winter. To expand transcriptome coverage beyond the white spruce reference genome, we applied a hybrid transcriptomics approach to generate *de novo* and reference genome-guided transcriptome assemblies. The resulting *de novo* long-read (LR) transcriptome was 92.7% complete (BUSCO), compared to the 47.9% completeness in the reference genome, and included 30,286 high-quality unique transcripts with an average length of 1081 bp. We mapped short-read libraries from needle samples collected during the field experiment to the *de*

novo LR transcriptome to assess gene regulation across treatments and families. Preliminary findings show that 900 to 1400 genes were differentially regulated between families at each treatment and time point. Across treatments, warming and warming combined with drought induced the strongest upregulation of transcripts during October and December, including those related to photosynthesis, growth, and pigment biosynthesis. These transcriptomic patterns align with the physiological data and suggest family-specific responses to climatic stress during autumn.

Gene gains and losses in the evolution of a conserved KCS gene cluster

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Very-long-chain fatty acids (VLCFAs) are important precursors of membrane, surface, and storage lipids in plants. The 3-ketoacyl-CoA synthase (KCS) family plays a crucial role in the biosynthesis of VLCFAs, through controlling the substrate-specific and rate-limiting condensation step during their elongation. A tandem KCS gene cluster in *Populus trichocarpa*, herein referred to as the KCS-zeta cluster, was previously investigated, and two distinct subclades (Clade A & B) were identified based on divergent activity and phylogenetic relationships. To evaluate sub- or neo-functionalization after KCS tandem duplication, their evolutionary history in angiosperms was investigated, using a combination of techniques including comparative genomics, codon-based selection analysis, and functional characterization. Synteny analysis showed that the KCS-zeta gene cluster is widely conserved in angiosperms, with Clade A and B arising by tandem duplication in the common ancestor of Eudicots, followed by further divergence into smaller subclades in the Rosids. Notably, divergent selection pressures were detected between the two clades, with Clade B experiencing stronger purifying selection. Next, we expressed representatives from both clades in *Saccharomyces cerevisiae* to determine if the clades differ in activity. Our results indicate stronger conservation of function for clade B, in terms of total VLCFAs being produced and the ability to use positional isomers of monounsaturated VLCFAs. Altogether, these observations suggest partial subfunctionalization following gene duplication in the common ancestor of Rosids, while the majority of duplicates undergo pseudogenization.

Benchmarking AlphaFold 3, AlphaFold-Multimer, and ColabFold for Heterodimeric Complex Prediction in *Arabidopsis thaliana*

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The Nobel Prize-winning program AlphaFold has revolutionized biological research and demonstrated its capabilities in modeling both protein monomers and protein-protein interactions (PPIs). Despite its widespread application, AlphaFold's performance in plant PPI modeling remains underexplored. With the recent release of AlphaFold 3 and its source code, we conducted a benchmark study comparing three AlphaFold variants, AlphaFold 3 (AF3), AlphaFold-Multimer (AFM), and ColabFold (CF), focusing on the model plant species *Arabidopsis thaliana*. Due to the lack of a perfectly matched benchmark dataset, we constructed a dataset of 201 heterodimeric interfaces by querying the Protein Data Bank (PDB) and improving its quality

through filtering and redundancy reduction. Our analysis revealed limited variation in overall performance among the three programs, based on success rate and mean accuracy. There are 165 jobs where at least one program produced successful predictions. Notably, AF3 yielded a greater proportion of both high-quality and incorrect models compared to AFM and CF. Although overall performance was similar, individual job results varied, with 24 cases where only one program produced a successful prediction. We further evaluated the impact of template access and intrinsic interface properties on modeling success. Specifically, we showed that binary interactions originating from large assemblies (megacomplexes) possess distinct properties from other interfaces, and enabling template search significantly improves modeling accuracy for these cases. Additionally, we assessed the effectiveness of model confidence metrics and explored model selection strategies. Our findings highlight AlphaFold's potential in modeling Arabidopsis PPIs and provide practical insights into its application in plant biology.

Large-scale individual tree segmentation across entire white spruce forests using UAV hyperspectral imagery and deep learning

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The development of high-performance, affordable UAVs has transformed vegetation monitoring, enabling detailed observation of forest canopies at unprecedented spatial and temporal resolutions. UAV-derived datasets now provide high-fidelity structural and physiological information at the individual tree level across entire forest stands, offering novel insights into canopy function and forest dynamics. In the context of accelerating climate change and increasing tree mortality, such data are becoming essential for understanding forest resilience and adaptation. However, exploiting this data requires effective individual tree segmentation algorithms at the forest scale, capable of tackling large scale data, multi-sensor complexity, and variability introduced by illumination.

To address these challenges, we developed a comprehensive deep learning workflow designed to process UAV hyperspectral imagery at forest scale, enabling automated individual tree segmentation and analysis. Our pipeline integrates advanced preprocessing, hyperspectral-to-RGB conversion, instance segmentation using deep learning and centroid-based mask fusion, allowing robust detection of individual trees across entire forest canopies and multiple seasons.

To assess the performance of our pipeline, we evaluated the model on two twin white pine forests in Canada, each comprising approximately 6,000 trees of similar age and structure. Flights were conducted across multiple months to capture seasonal variability, including differences in illumination and weathers. Our experiments showed that the proposed pipeline is very effective, correctly detecting an average of 87% of individual trees over the course of a year. The model consistently processed full-forest and maintained good performance across different conditions.

An antagonistic epigenetic mechanism regulating gene expression in pollen revealed through single-nucleus multiomics

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Arabidopsis MBD5, MBD6, and MBD7 are CG-specific methyl-readers with opposite functions: MBD5 and MBD6 (MBD5/6) repress methylated loci in pollen vegetative nuclei (VN), while MBD7 prevents transgene silencing, possibly by promoting DNA demethylation. Here we show that loss of *MBD7* rescues transcriptional defects at a large subset of MBD5/6-bound loci. Using simultaneous profiling of DNA methylation and transcription in single pollen nuclei, we found that MBD5/6-bound loci that are actively demethylated in immature VN lose additional methylation in *mbd5/6*, prior to transcriptional derepression. A subset of these loci is also bound by MBD7, correlating with demethylation and transcriptional derepression in *mbd5/6* that are both reversed by loss of *MBD7*. Conversely, ectopically recruiting the MBD7 complex to MBD5/6 targets causes partial demethylation and upregulation. We propose that MBD5/6 maintain silencing in VN in part by preventing the MBD7 complex from enhancing the active demethylation that occurs during VN maturation.

An evolutionary and functional analysis of virulence gene promoters in *Pseudomonas syringae*.

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Plants are locked in an evolutionary arms race for survival with the pathogens that infect them. To guard against pathogen attack, plants have evolved robust innate immune systems to detect microbes and initiate immune signalling. Successful pathogens, however, have evolved mechanisms to suppress these defenses and promote infection. The bacterial phytopathogen *Pseudomonas syringae* infects hundreds of plant species worldwide, causing annual agricultural losses. To establish infection, *P. syringae* injects virulence proteins (effectors) directly into plant cells to disrupt immunity. However, immune receptors in resistant plants can detect effectors and activate effector-triggered immunity (ETI). While effector diversification at the protein-coding level is well characterized, the role of effector regulatory variation in immune evasion remains poorly understood. We hypothesize that *P. syringae* modulates effector expression via sequence variation in effector promoters ("hrp box promoters") to minimize immune activation. To test this, we identified and analyzed 9,126 promoters upstream of effector genes across 494 diverse *P. syringae* genomes. Phylogenetic and motif analyses revealed 574 unique sequences with conserved core motifs and variable sites. To quantify functional variation, we constructed a library of over 220 promoter variants fused to GFP and optimized a high-throughput microplate assay to measure promoter activity. Our results suggest that specific polymorphisms in hrp box promoters significantly influence effector expression. Ongoing work aims to assess how promoter variation impacts host-microbe interactions *in planta*. These findings will expand our understanding of how changes in pathogen regulatory sequences influence disease outcomes and may reveal molecular targets for improved disease management in crops.

Identifying Missing Glyceollin Transcription Factors in Soybean

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Plants have evolved sophisticated defense mechanisms to combat pathogen attacks, including the production of antimicrobial metabolites known as phytoalexins. In soybean (*Glycine max*), glyceollins are the primary phytoalexins and play a critical role in defense against pathogens such as *Phytophthora sojae*. Previous studies identified *GmMYB29A2* and *GmNAC42-1* as positive regulators of glyceollin biosynthesis in response to the wall glucan elicitor (WGE) from *P. sojae*. However, overexpressing each of these TFs alone was insufficient to fully activate glyceollin biosynthesis, suggesting the involvement of additional regulatory components. This study aimed to deepen our understanding of the complex regulatory networks governing phytoalexin synthesis and to explore strategies for enhancing their production. Specifically, we (1) investigated whether overexpressing *GmMYB29A2* and *GmNAC42-1* together could activate glyceollin biosynthesis in the absence of an elicitor; (2) established an optimized soybean hairy root transformation protocol for functional gene analysis; (3) identified negative regulators of the glyceollin biosynthetic pathway; and (4) identified a network of transcription factor proteins that is held together by the SG2 and KEEP motifs of *GmMYB29A2* and *GmWRKY33* proteins, which is required to activate phytoalexin biosynthesis. By characterizing negative regulators, such as *GmJAZ1-9* and *GmWRKY72*, we have elucidated why the overexpression of positive regulators alone cannot activate glyceollin biosynthesis without an elicitor. My research has revealed that the regulation of phytoalexin biosynthesis is more complex than previously understood, involving interplay between negative and positive regulators. My results provide clues to the possible existence of a conserved network of core regulators of phytoalexin biosynthesis in plants.

Analysis of Alternative Oxidase in Non-Angiosperm Plants

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As environmental conditions become increasingly volatile, the resiliency and adaptability of plant systems are tested. Investigating plant systematics, particularly through studying the alternative oxidase (AOX) pathway, offers a promising avenue to understand how plants respond to biotic and abiotic stressors.

AOX is an enzyme found within the electron transport system (ETS) of many organisms across almost all domains of life. When activated, AOX offers mitochondrial flexibility during times of stress. While its presence has been confirmed in protists, fungi, and animals, most AOX research focuses primarily on flowering plants (i.e. angiosperms). AOX research on non-angiosperms, such as mosses, ferns, etc., is limited.

Based on the research gap in the scientific literature, it is worth asking: to what extent is AOX distributed across plant lineages? I hypothesize that AOX exists within all non-angiosperm plants. My research will determine the taxonomic distribution of AOX in non-flowering plants using two approaches. (i) First, I will conduct a bioinformatic survey using publicly available databases to search for AOX sequences in non-flowering plants using Basic Local Alignment Search Tool (BLAST) searches and multiple sequence alignments. (ii) Second, I will conduct cDNA analysis using reverse-transcriptase polymerase chain reaction (RT-PCR) technology and gel

electrophoresis. Additionally, protein analysis will be conducted using specifically designed antibodies to analyze proteins through SDS-PAGE and Western Blots.

These findings may offer new insights into the evolutionary history of AOX and the physiological function of the protein. Discoveries such as these could help mitigate the impacts of climate change on our ecosystems.

Concurrent Session 3 - Plant Immunity

Pinpointing candidate genes in a quantitative trait locus associated with resistance to bacterial brown spot in adzuki bean

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Bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* van Hall can cause up to 40 % yield loss in the adzuki bean crop. Genetic resistance might be the most effective strategy for managing this disease; however, currently, there are no resistant commercial adzuki varieties available. In this study, a QTL spanning approximately 6 Mbp was identified for bacterial brown spot resistance on a linkage map constructed from 2,911 filtered SNPs segregating in 105 RILs from a cross between Erimo and a Chinese variety (CV). One hundred and eighty candidate genes were identified within the QTL. Comparative genomics and pathogenicity screening on Erimo and CV were carried out using *P. syringae* pv. *syringae* (Pss27, obtained from AAFC Harrow), *P. syringae* pv. *aceris* (Me2 and Me3, isolated in this study) and *P. syringae* pv. *dysoxyl* (Li6, isolated in this study). Erimo showed more resistance than the CV ($p < 0.0001$). Unique virulence factors (VF) were identified in the bacterial strains. Pss27 was the most aggressive strain in adzuki and possessed 10 unique VFs. Two of the unique VFs (hopAB1 and hopL1) had a total of seven cognate target genes within the QTL. Of all the target genes, only ethylene-responsive transcription factor (ERF024) was polymorphic between Erimo and CV. Copy number variation was observed between Erimo and CV, with two extra copies of ERF024 present in tandem in Erimo. ERF024 was located 20 kbp from the QTL peak marker, S02_48822524, and was also the closest candidate gene to the peak marker.

Proteomic Analysis of *Phaseolus vulgaris* L Responses to *Xanthomonas phaseoli* pv. *phaseoli* Infection.

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Xanthomonas phaseoli pv. *phaseoli* (*Xpp*) is a causal agent of common bacterial blight (CBB) in *Phaseolus vulgaris* L. To infect the host, *Xpp* injects effector proteins into plant cells, enabling survival, replication, and colonization. Plant responses depend on the immune pathway triggered, either Pathogen-Associated Molecular Pattern-Triggered Immunity (PAMP-PTI) or Effector-Triggered Immunity (ETI). PAMP-PTI activates defense gene expression via MAPK cascades and reactive oxygen species, while ETI involves resistant proteins recognizing pathogen effectors. To

investigate early molecular mechanisms of host-pathogen interactions, we conducted a comparative bottom-up proteomics analysis using two recombinant inbred lines (RILs), one CBB-resistant and one CBB-susceptible, challenged with pathogenic and non-pathogenic *Xpp* strains. The resistant RIL showed a robust and coordinated defense against the pathogenic strain, with proteins involved in secondary metabolite biosynthesis, structural reinforcement, and signalling pathways such as ubiquitin-mediated proteolysis. Its response to the non-pathogenic strain resembled a primed, energy-efficient state associated with induced systemic resistance (ISR). In contrast, the susceptible RIL showed limited immune activation upon challenge with the pathogenic strain, suggesting delayed or insufficient mobilization of defense mechanisms. Its response to the non-pathogenic strain was minimal and predominantly metabolic, indicating poor microbial recognition and possible pathogen manipulation of host metabolism. Overall, this study reveals distinct proteomic responses in resistant and susceptible bean genotypes to *Xpp* challenge. These findings provide valuable insights into the molecular mechanisms underlying compatible and incompatible interactions that may be used to develop effective strategies to manage CBB in common bean

The *Pseudomonas syringae* conserved effector locus is a prominent target of soybean effector-triggered immunity

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Soybean (*Glycine max* (L.) Merr.) is one of the most economically important crops in the world, but every year pathogens cause billions of dollars' worth of losses worldwide. The domestication of this critical crop was associated with several severe genetic bottlenecks that resulted in a dramatic loss of genetic diversity (~50%) relative to its wild progenitor. Although domestication selected for favorable agricultural traits, it is unclear how the reduced genetic diversity affected the immunity landscape of cultivated soybean relative to its wild ancestors. Here, we performed a systematic screen for effector-triggered immune (ETI) responses in both cultivated soybean (*Glycine max*) and wild soybean (*Glycine soja*) against the bacterial pathogen *P. syringae*. This screen uncovered an extensive ETI landscape with 121/529 alleles (22.9%) from 32/65 effector families (49.2%) and 125/529 alleles (23.6%) from 23/65 effector families (35.4%) eliciting ETI in *G. max* and *G. soja* respectively. Interestingly, the conserved effector locus (CEL) effectors of *P. syringae* (AvrE, HopM and HopAA) are the most prevalent ETI- eliciting effector families in soybean. Finally, we demonstrate that pretreatment of soybean with the CEL effectors can immunize soybean against subsequent infection. Overall, we are providing a comprehensive ETI interaction landscape between soybean and *P. syringe*, laying the foundation for the discovery of novel plant immune receptors serving the agronomic improvement of soybean.

Rhizobacteria-induced systemic priming against fungal pathogens involves hydroxycinnamic acid amides

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The rhizosphere, a narrow region of soil surrounding roots, contains a large diversity of microorganisms with a composition that is distinct from the surrounding soil. Some rhizosphere bacteria can trigger a heightened state of immunity in the whole plant, termed Induced Systemic Resistance (ISR). Which has a large potential agriculture application, acting as a natural method to reduce crop loss related to pest and pathogens. To understand the mechanisms behind this enhanced resistance we investigated the metabolic profile of tomato plants treated with *Pseudomonas defensor* WCS374r and *Bacillus velezensis* VFB49, bacterial strains known to elicit ISR in tomato, using an untargeted approach, from which we identified a set of differentially accumulated metabolites (DAMs). We show that some of these metabolites - the hydroxycinnamic acid amides (HCAAs) feruloyl putrescine and caffeoyl putrescine - exhibit direct antimicrobial properties against two fungal pathogens, *Botrytis cinerea* and *Fusarium virguliforme*. Further, we show that the *Arabidopsis* agmatine coumaroyl transferase (*AtACT*), which catalyses the final step in HCAA biosynthesis, is induced by both the ISR inducing strains and is required for resistance to *B. cinerea*. The apoplastic location of these compounds is also crucial for their function as a knockout line of the multidrug and toxin extrusion (MATE) transporter, DTX18, lost the resistance phenotype. Overall, this work offers novel insights into the mechanism of ISR, suggesting that the accumulation of HCAAs is one of the factors conferring enhanced resistance to pathogen infection.

Effector-triggered stomatal immunity prevents leaf invasion by bacterial pathogens

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The bacterial phytopathogen, *Pseudomonas syringae*, begins its disease cycle when it invades the apoplastic space in the leaf by entering through natural openings such as stomata. Plants restrict pathogen access to the leaf apoplast by closing stomata upon recognition of microbe associated molecular patterns (MAMPs). Successful *P. syringae* strains reopen stomata using phytotoxins and effectors facilitating colonization of the apoplastic space. In this study we show that stomatal reopening is inhibited by the recognition of the conserved *P. syringae* type III effector AvrE1 by the *Arabidopsis* resistance protein CAR1; a nucleotide-binding leucine-rich repeat (NLR) protein that is expressed 10-fold higher in guard cells compared to mesophyll tissues. We show that CAR1 triggers a stomatal immune response that reinforces and extends MAMP induced stomatal closure further preventing pathogen invasion. We also show that the CAR1 immune response suppresses virulence when the pathogen must invade the leaf tissue after surface inoculation but does not provide any protection when strains are directly injected into the tissue, which bypasses stomatal immunity. As such, the pre-invasive immune response provided by CAR1 against this conserved effector represents a significant barrier to early colonization of this microbe in the leaf tissue. Our work describes the first tissue specific effector-triggered immune response in plants.

Long-distance movement of the lipid transfer protein DIR1 during plant immunity requires N-hydroxypipicolinic acid signaling

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Localized attack by microbial pathogens induces Systemic Acquired Resistance (SAR) in distant leaves, resulting in resistance to subsequent infections. The lipid transfer protein, Defective in Induced Resistance 1 (DIR1), is required for SAR and upon infection it accumulates in the phloem of induced leaves and travels to distant leaves to establish SAR. However, it is unknown how DIR1 becomes activated to move into the phloem at the site of infection to travel to distant leaves. The plant immunometabolite N-hydroxypipicolinic acid (NHP) is essential for resistance at the local and distant sites of pathogen attack. To determine whether NHP contributes to activation of DIR1 movement during SAR, we collected phloem exudates from *Arabidopsis* plants treated with NHP, and NHP biosynthesis mutants induced for SAR with avirulent *Pseudomonas syringae*. In NHP-treated plants, DIR1 signal was observed in local and distal phloem exudates, suggesting that NHP, or downstream signalling, is involved in activation of DIR1 movement. Interestingly, in exudates collected from the NHP biosynthesis mutant, *fmo1-1*, DIR1 signal was detected in exudates collected from induced, but not distant leaves, suggesting NHP is necessary for the DIR1 long distance movement. Together, these results demonstrate that FMO1-generated N-hydroxypipicolinic acid (NHP) is required for long-distance movement of DIR1 and propagation of systemic immunity to *Pseudomonas syringae*. This suggests a role for NHP upstream of signal propagation in the SAR mechanism, potentially in activating or facilitating the movement of signalling components into the phloem.

Investigating the Activation of DEFECTIVE IN INDUCED RESISTANCE 1 Movement during Systemic Acquired Resistance in Arabidopsis

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Following perception of pathogens, plants initiate overlapping signaling pathways to suppress the multiplication of pathogens at infection sites and activate a systemic defense response called Systemic Acquired Resistance (SAR). Induction of SAR includes activation of mobile signals at local infection sites, including the protein DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1), which accumulates in the phloem and travels to distant leaves, where signals are perceived, initiating a primed state resulting in resistance upon subsequent infection. To identify signaling components that contribute to DIR1 activation for movement, the contributions of PAMP- or Effector-triggered immunity (PTI or ETI) were examined by collecting phloem exudates from plants independently induced by PTI or ETI and assessing DIR1 presence using DIR1 immunoblot analysis. Following SAR induction by PTI or ETI in a local leaf, DIR1 was detected in exudates collected from induced and distant uninduced leaves, suggesting a common downstream component of PTI and ETI signaling pathways contributes to activation of DIR1 movement. The effect of metabolites common to PTI and ETI signaling was then investigated, beginning with the plant defense hormone salicylic acid (SA), by treating leaves with exogenous SA and examining an SA

biosynthesis mutant, *sid2-2*. SA treatment did not induce DIR1 accumulation in phloem exudates of induced or distant leaves, suggesting that SA or its downstream effects do not contribute to DIR1 movement. Moreover, the DIR1 signal was observed in induced and distant leaves of SAR-induced *sid2-2* mutants, providing further evidence that SA-mediated signaling does not contribute to the activation of DIR1 movement.

A Multi-Omics Approach to Uncover Novel Regulators of Temperature-Sensitive Plant Immunity

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Rising global temperatures result in decreased crop yields by negatively intercepting the plant immune system. In *Arabidopsis thaliana*, elevated temperature negatively impacts plant immunity by downregulating *CALMODULIN BINDING PROTEIN 60G* (*CBP60g*) and *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1* (*SARD1*), key genes encoding master transcription factors of plant immunity. Downregulation of *CBP60g/SARD1* at warm temperatures is controlled by dissociation of GBPL3 defence-activated condensates (GDACs), leading to the suppression of the central plant defence hormone salicylic acid (SA). Although we have begun to build our understanding of the plant immune system under changing temperatures, little is known about the upstream molecular players that regulate this temperature-sensitive plant immunity. Our research aims to identify and characterize these molecular players using a multi-omics approach integrating genomics (Genome Wide Association Study), transcriptomics (RNA-Seq), and (phospho)proteomics (tandem mass spectrometry). Leveraging both natural and engineered immune variation in *A. thaliana*, our analyses are expected to reveal candidate genes, proteins, and/or phosphosites that act as potential regulators of thermosensitive plant immunity. I have identified several candidates with various roles to date, but further functional analyses are still being undertaken to establish if these candidate molecular players mediate plant immunity under changing temperatures. Overall, this study will offer detailed insights into key *Arabidopsis* genes, proteins, and phosphosites that are influenced by elevated temperatures and how they are linked to plant immune function under changing environmental conditions. These important factors will offer potential targets for genetically engineering climate-resilient, disease-resistant plants.

Concurrent Session 4 - Biotic Interactions

Understanding the interaction between *Verticillium longisporum* and *Leptosphaeria maculans* in *Brassica napus*

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Brassica napus (canola) is one of the most commercially important oilseed crops in the world. Canada accounts for the world's largest canola production. Increasing stresses from fungal diseases are threatening the yield and quality of canola in Canada. Blackleg, majorly caused by

the fungal pathogen *Leptosphaeria maculans* results in significant yield losses in Canadian canola. Verticillium stripe caused by the soil-borne fungus *Verticillium longisporum* has recently emerged as a potential threat to domestic canola production. In the field, Verticillium stripe disease symptoms can be misdiagnosed with those of blackleg, and the two diseases may co-occur in canola. Although the resistance of canola to blackleg is well studied, how this resistance might be affected by Verticillium stripe remains unclear. This project aims to understand the interaction between *L. maculans* and *V. longisporum* in canola through transcriptomic and genetic analysis. Canola genotypes carrying *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*, *LepR1* and *LepR2* genes were inoculated with both *L. maculans* and *V. longisporum* under greenhouse conditions to screen a genotype showing a significant change in resistance against both diseases. Canola genotype 01-23-2-1, which exhibited a significant breakdown of the *Rlm7* gene resistance, was selected for genome-wide transcriptome analysis. Additionally, the disease severities of both blackleg and Verticillium stripe of these canola genotypes will be evaluated using phenotypic data. The overall outcome of this study will help to identify genes with strong breeding potential, which can be utilized to enhance canola resistance against *V. longisporum* and *L. maculans*.

Exploring genetic resistance to sclerotinia stem rot in canola through VQ gene expression profiling

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Sclerotinia Stem Rot (SSR), caused by *Sclerotinia sclerotiorum*, poses a significant threat to canola (*Brassica napus*), which is crucial for Canada's agricultural economy. This disease can lead to drastic yield losses and adversely affect seed quality, impacting export values. Current management strategies, including the use of fungicides and crop rotation, are inconsistent, costly, and can harm the environment. Since there is currently no evidence of Resistance (R) genes conferring complete qualitative resistance against *S. sclerotiorum* in canola, other genes involved in building quantitative resistance must be studied. Valine-glutamine (VQ) genes are involved in plant immune responses to biotic and abiotic stress. In this study, various canola lines were screened for enhanced SSR resistance. Quinta and 1065 were found to exhibit the greatest susceptibility, while Surpass 400 and Jet Neuf demonstrated the strongest resistance. RNA sequencing was done to obtain gene expression data from Jet Neuf and a more susceptible line, Westar. Inoculation was performed in planta using mycelial agar plugs, and samples were collected at 24- and 48-hours post-inoculation (hpi). Focusing on VQ genes, we identified differentially expressed genes (DEGs) between the two genotypes. DEG analysis was complemented by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses to pinpoint genes potentially involved in SSR resistance. These candidate genes can be used to develop transgenic canola lines with enhanced SSR resistance. Improving SSR resistance will help reduce disease burden for farmers and improve the profitability and sustainability of canola production.

Integrated Transcriptomic, Enzymatic and Phenotypic Analysis of *Brassica napus* Responses to *Verticillium longisporum* Infection

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Canola (*Brassica napus*) is a valuable crop with high global demand, and as its cultivation expands, pathogen invasions have concurrently increased, threatening yield stability. Verticillium stripe, caused by *Verticillium longisporum*, is a recently emerged and significant disease that causes significant damage to canola. This study focuses on the impact of *V. longisporum* infection on the expression of genes involved in plant resistance, plant growth hormone biosynthesis, and antioxidant enzyme activity, which are the areas that remain poorly studied and understood in canola. Phenotypic assessments indicated that Westar seedlings exhibited partial cotyledon necrosis, while Quinta seedlings showed complete cotyledon death. However, at the adult stage, most Westar plants were infected and displayed severe symptoms. Transcriptome analysis revealed genes associated with antioxidant enzyme activities and plant growth hormone regulation during disease progression. By narrowing down the differentially expressed genes identified in roots and leaves, we selected several candidates for CRISPR/Cas9-mediated genome editing to enhance resistance at the seedling stage of *B. napus*. Spectrometric analysis showed that most antioxidant enzymes were more highly expressed in leaves than in roots in both Westar and Quinta varieties, which are susceptible and partially resistant to *V. longisporum*, respectively. These findings enhance our understanding of the role of antioxidant enzymes and other important functional genes in canola defense mechanisms against *V. longisporum* infection.

Tomato responses to different tomato-adapted spider mite populations

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The two-spotted spider mite (*Tetranychus urticae*) is an extreme generalist herbivore capable of feeding on over 1100 plant hosts across more than 100 families. Yet, individual populations do not perform equally well when transferred to novel hosts due to xenobiotic stress that reduces their survivorship and fecundity. However, mites can adapt to and restore their fitness on previously unsuitable hosts within ~25 generations. In this study, we investigated how mites adapt to tomato (*Solanum lycopersicum*) by analyzing transcriptomic responses in tomato plants challenged with non-adapted and six independently tomato-adapted mite populations.

Herbivores counter plant defenses through different adaptation strategies, such as metabolic detoxification or by attenuation of plant defense responses. Our transcriptomic analysis revealed that adapted mite populations developed different strategies to cope with tomato defenses. A subset of tomato-adapted mite populations induced tomato transcriptomic responses similar to those induced by the non-adapted mite populations, suggesting that these mites have evolved mechanisms to attenuate tomato's induced defenses. In contrast, other tomato-adapted mite populations triggered robust tomato responses, indicating that these mites developed an ability to tolerate tomato defenses. Analysis of differentially expressed genes provides insights into specific pathways involved in tomato defense and their modulation, depending on mite adaptation strategy.

Glutathione S-Transferases Mediate Two-Spotted Spider Mite Adaptation to *Arabidopsis*

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Plants produce defensive metabolites to deter pests, yet herbivores have evolved countermeasures to overcome these defenses. The two-spotted spider mite (*Tetranychus urticae*) is a highly polyphagous pest feeding on over 1100 plant species. Despite their broad host range, they are considered composite generalists as individual populations perform better on specific groups of plant species. While initial performance declines on new hosts, *T.urticae* adapt within ~25 generations.

To study how mites overcome Arabidopsis defenses, we adapted bean-reared mites to Arabidopsis wildtype Col-0 (Col-a) and the cyp79B2/B3 mutant (cyp-a), which lacks tryptophan (Trp)-derived compounds. Arabidopsis is a challenging host for ancestral mites, but mite performance increases on the mutant, implicating Trp-derived compounds as key defense components.

Transcriptomic analyses identified 293 differentially expressed genes in Col-a and cyp-a mites versus bean-reared mites. Among these, 37 were upregulated detoxification genes. Some genes were specifically upregulated in Col-a mites, suggesting roles in overcoming Trp-derived defenses. Others were shared between Col-a and cyp-a mites, indicating broader roles in detoxifying non-Trp defenses. RNA interference-based gene silencing revealed that silencing most of these genes reduced mite fecundity, underscoring their essential, non-redundant roles in adaptation. Recombinant proteins of selected genes were used in *in vitro* reactions to identify their substrates and to illustrate their mode of action.

Using this approach, we showed that glutathione-S-transferase of the *mu* family is required for isothiocyanate detoxification and mite adaptability to Arabidopsis. Together, our findings highlight the complexity of mite adaptation to *Arabidopsis*, revealing the diverse detoxification strategies that underlie host adaptability.

Uncovering the complexity of *Arabidopsis thaliana* defenses against *Tetranychus urticae* herbivory

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Tetranychus urticae (two-spotted spider mite) is an extreme generalist herbivore capable of feeding on over 1100 plant species. Despite this broad host range, individual mite populations perform well on only a subset of host plants but can rapidly adapt to new environments, reflecting a balance of specialist and generalist traits. This rapid adaptation underlies their ability to overcome diverse plant defenses. To understand mite counteradaptations to host defenses, it is first necessary to identify host defenses that initially limit mite fitness. Our lab addressed this by experimentally adapting a bean-reared ancestral mite population to Arabidopsis, a challenging and non-preferred host. Arabidopsis defenses against mites are mediated by the jasmonic acid (JA)-responsive transcription factors MYC2,3,4. We previously found that MYC2,3,4-dependent synthesis of glucosinolates restricts mite performance. While glucosinolates are a hallmark of Arabidopsis antiherbivore defenses, mites perform significantly better on a *myc2,3,4* mutant defective in JA-induced responses relative to a *cyp79b2/b3* mutant defective in tryptophan-derived glucosinolates. This indicates the involvement of additional MYC2,3,4-dependent

defenses in the Arabidopsis response against mites. To identify these defenses, we conducted RNA-seq and UPLC-MS/MS on mite-infested Col-0 and *myc2,3,4*. This comparative analysis implicated phenylpropanoid biosynthesis in the plant defense response. Using pathway-specific mutants, we identified sinapate esters, hydroxycinnamic acids, and flavonoids as candidate anti-mite compounds. Feeding assays confirmed that these phytochemicals cause mortality and reduce fecundity. In summary, we identified novel Arabidopsis phytoalexins and phytoanticipins effective against spider mites within the phenylpropanoid pathway and uncovered new nodes of *myc2,3,4* regulation.

Determinants of Pokeweed Mosaic Virus' Host Range

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Plant viruses, particularly the potyviruses that form the largest group of agriculturally important pathogens, threaten agriculture by causing yield losses with substantial economic consequences. Pokeweed mosaic virus (PkMV) is a single-stranded RNA potyvirus that commonly infects American pokeweed (*Phytolacca americana*), yet its extended host range remains undefined. Host range influences a virus's ability to infect new crops, shapes disease emergence, and reveals molecular "compatibility rules" for prediction and prevention. To assess host specificity, we used tobacco (*Nicotiana benthamiana*), a standard potyvirus model. Pokeweed and tobacco were mechanically inoculated with PkMV; inoculated and upper, non-inoculated leaves were sampled from 0-7 days post-inoculation (dpi). Viral genomic RNA was quantified by RT-qPCR, and coat protein (CP) accumulation was assessed by immunoblot. In pokeweed, PkMV replicated robustly: CP was detectable by 5 dpi and systemic viral RNA by 4 dpi. In tobacco, genomic RNA and replication intermediates did not increase over time, CP was undetectable, and no systemic movement occurred, indicating a block at the replication or movement stages. A genome-scale phylogeny tree built from 150 reference potyvirus genomes placed PkMV near three *Nicotiana*-infecting potyviruses, focusing the research question: what early compatibility determinant(s) prevent PkMV from establishing infection in tobacco despite close relatives succeeding? Ongoing experiments prioritize defining the restricted step and testing candidate viral-host interactions, with the broader aim of linking phylogenetic relatedness to host outcomes to improve host-range prediction and resistance strategies. Together, these findings will improve how we predict host shifts and design durable crop resistance.

Overcoming the biological and technical barriers of *Agrobacterium tumefaciens*-mediated transformation in the forage legume sainfoin (*Onobrychis viciifolia* Scop.)

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Onobrychis viciifolia Scop. (sainfoin) is a perennial forage legume with resurgent popularity over the last decade due to its non-bloating and anthelmintic properties, attributed to its relatively high proanthocyanidin content compared to other forage legumes. However, as a standalone crop sainfoin has lower biomass yield, waterlogging tolerance, and stand persistence when compared

to already established forage legumes such as alfalfa. Conventional breeding strategies have had little success with this crop, therefore molecular-based breeding strategies are needed to accelerate the development of improved cultivars, but this has lagged behind due to the lack of a reliable genetic transformation method. Sainfoin is recalcitrant to genetic transformation, thus the goal of this research was to establish an effective *Agrobacterium tumefaciens*-mediated transformation protocol for sainfoin by addressing factors known to contribute to this phenomenon. Transformation success in recalcitrant species is typically genotype and explant-type specific, so trials were conducted using multiple explant types from five distinct cultivars. To minimize stress in explants during regeneration and transformation, various supplements such as pH stabilizers, antioxidants, and synthetic polymers were added to tissue culture media to provide an optimal environment for successful T-DNA integration while reducing consequent necrosis in explants. Using this protocol, we have successfully generated transgenic plants that have been validated by PCR and Sanger sequencing.

Concurrent Session 5 - Abiotic Stress

Changes in photosynthetic and anatomical traits in response to incremental drought and recovery in white pine and white spruce

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Climate change is increasing the frequency and length of drought, reshaping ecosystems worldwide. In Canada's boreal forest, which contains 30% of the global total, declining water availability threatens native species like *Picea glauca* (White spruce) creating opportunities for species isolated to southern areas of the boreal such as *Pinus strobus* (White pine) to expand northward and compete with northern species. Understanding species-specific drought responses is crucial to understand which species will respond better to future climate. In this study, we examined trait relationships in white spruce and white pine to determine whether drought resilience emerges through integrated physiological, anatomical, and structural adjustments, or through independent, species-specific mechanisms. Drought was implemented in phases through decreasing field capacity by 60%, 30%, 0% FC and then rewatering. We found that white pine exhibited more pronounced changes than white spruce to drought. While both white pine and white spruce decreased stomatal conductance and CO₂ assimilation, and increased water-use efficiency the degree of change differed. White pine showed greater increases in the de-epoxidation state of xanthophyll pigments, showing a greater shift towards photoprotection. Changes in photosynthesis also reflected in growth where white pine maintained diametric growth rates during drought, altering latewood xylem anatomy through increased mechanical support and hydraulic safety, while white spruce slowed growth, limiting ability to adjust the xylem. While both species recovered growth during the rewatering period, the ability of white pine to grow and make strong adjustments to drought reflects its capacity for dynamic trait integration across physiological and anatomical domains.

Differences in Chlorophyll Fluorescence and Vegetation Indices for White Spruce (*Picea glauca*) Grown under Contrasting Climates at Two Eastern Canadian Sites

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Rapid climate change is leading to more frequent wildfires and drought-induced tree mortality in Canadian forests. For white spruce (*Picea glauca* (Moench) Voss), one of the most abundant tree species in Canada, these changes are expected to affect productivity and health. Non-destructive measurement techniques, such as leaf spectral reflectance and chlorophyll fluorescence (ChlF), have emerged as valuable tools for assessing tree stress levels and monitoring annual phenological changes. The aim of this study is to compare monthly measured vegetation indices (VI), obtained from leaf spectral reflectance and ChlF of two climatically contrasting experimental field sites. ChlF and VI data of 180 trees were included in the present analysis. Our analysis reveals that ChlF and the normalized difference vegetation indices (NDVI) do not show any significant difference within a same measurement period. By contrast, the photochemical reflectance index (PRI) and the chlorophyll/carotenoid index (CCI), which both detect variations in carotenoids, show a significantly higher CCI and slightly lower PRI at the colder site. Overall, the carotenoid related PRI and CCI demonstrate strong potential to capture acclimation of photosynthesis to environmental stress, including low temperatures.

Salt tolerant ecotypes of *Eutrema salsugineum* respond differently to salinity

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Eutrema salsugineum is a highly stress-tolerant crucifer closely related to *Arabidopsis thaliana* and canola. Two accessions have been compared, one from Shandong, China, that grows in a temperate and monsoonal area, and a second ecotype from a sub-Arctic, semi-arid area in Yukon, Canada. The shared condition is tolerance to highly saline soil. We hypothesized that both ecotypes would display similar salt-responsive traits, including differentially expressed genes (DEGs), given their overlapping tolerance to salt. We produced a *de novo* genome assembly for Yukon plants and compared it to one available for Shandong plants. Overall genome structure was 99% similar for these ecotypes but we found some distinct polymorphisms. For phenotyping we grew seedlings up to 17 days on agar with 0 or 150 mM NaCl. Relative to Shandong plants, Yukon seedlings developed significantly larger shoots and longer lateral roots indicating that salt tolerance between the ecotypes was different. Comparisons between transcriptomes showed Yukon plants had 1,825 DEGs in response to salinity, whereas Shandong had more than 3,846 DEGs; evidence of different transcriptional reprogramming between the ecotypes in response to NaCl. Additionally, between the ecotypes, 1,006 genes were constitutively and more highly expressed in Yukon compared to Shandong plants. We hypothesize that Yukon plants grew better on 150 mM NaCl because the constant expression of salt-responsive genes “primes” this ecotype

for improved osmotic stress tolerance, providing evidence these ecotypes have locally adapted differently to meet a similar osmotic challenge.

Environmentally driven variation in drought-relevant traits among 16 white spruce genotypes from two Eastern Canadian sites

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Climate change is expected to increase the frequency and severity of drought events, impacting the growth and productivity of Canadian forests. White spruce (*Picea glauca*), a dominant conifer species, is sensitive to water limitation and may experience higher mortality under future drought conditions. In this study, we examined white spruce seedlings of different genotypes to identify trees most resilient to water stress for reforestation efforts. We selected 16 white spruce genotypes from clone-replicated genetic trials in Pickering, ON and Pintendre, QC, to characterize drought-relevant hydraulic and anatomical traits: turgor loss point (π_{tlp}), osmotic potential at full turgor (π_o), hydraulic diameter, cell wall reinforcement, and theoretical xylem-specific hydraulic conductivity. We sampled branches from three clones per genotype, per site, and constructed pressure-volume curves using Scholander's pressure chambers to relate leaf water potential to water content and determine hydraulic traits. We imaged cross-sections of preserved branches and used ROXAS, a software for automated cell detection, to quantify xylem anatomical traits. Trees from the drier Pickering site exhibited significantly lower mean π_{tlp} and π_o values than trees from the wetter Pintendre site, indicating greater osmotic adjustment capacity to buffer against leaf wilting during drought. In contrast, trees from Pintendre showed traits associated with increased xylem safety (e.g. reduced hydraulic diameter, increased cell wall reinforcement), possibly reflecting acclimation to waterlogging. Our results suggest that environmental conditions exert a stronger influence than genetics on drought resistance in white spruce, a factor that should be considered when selecting seedlings for reforestation in future warmer and drier climates.

Microtopography and soil moisture gradients drive variation in turgor loss point and growth in white spruce seedlings

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In contrast to the response of trees to drought and water limitation, the effects of excess soil moisture and waterlogging are understudied, especially in conifers. Prolonged saturation of the soil with water can induce root hypoxia, promote root infection and disease, limiting water

transport, reducing growth, and compromising drought tolerance. Yet, the extent to which microtopography-driven variation in soil moisture influences intraspecific hydraulic traits remains poorly characterized. We assessed how microsite conditions variation in soil moisture influences seedling growth and water relations of white spruce (*Picea glauca*) to better understand how fine-scale environmental heterogeneity shapes physiological performance. In a field experiment from clone-replicated genetic trials in Pickering, ON, 42 seedlings from 14 genotypes were planted across a natural topographic gradient. Daily volumetric soil moisture was recorded using 13 sensors throughout the 2023 growing season. For each seedling, we measured final height and turgor loss point (Ψ_{Tlp}). We hypothesized that seedlings growing in chronically wet, low-elevation microsites would exhibit reduced height and less negative Ψ_{Tlp} compared to those in drier, higher elevation microsites. Our findings show that seedlings in wetter low-elevation microsites were 33% shorter and had less negative Ψ_{Tlp} (-1.93 ± 0.29 MPa) compared to seedlings in drier high-elevation ($\Psi_{Tlp} -2.30 \pm 0.23$ MPa), reflecting reduced growth and impaired turgor maintenance under waterlogged conditions. These results underscore the role of microtopographic soil moisture in shaping intraspecific hydraulic traits, with implications for reforestation and climate-resilient forest management.

Divergent Photoprotective Strategies in Eastern White Pine and White Spruce Under Moderate Drought

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Northern hemisphere forests are warming, with some regions projected to rise 11 °C by century's end. Moderate droughts are more frequent, and conifers increasingly face high-light and water stress. Unlike extreme drought that triggers hydraulic failure, moderate drought imposes recurring sublethal stress expected to dominate future growing seasons. We hypothesized that dynamic photoprotection and stability of photosystem I (PSI) are essential components of the drought response of white pine (*Pinus strobus*) and white spruce (*Picea glauca*). Photoprotection in this context includes non-photochemical quenching, NPQ, which safely dissipates excess absorbed light as heat, and cyclic electron transport, CET, which routes electrons around PSI to make ATP and balance energy without generating extra NADPH. Zeaxanthin, a xanthophyll-cycle carotenoid that drives this heat dissipation, remains under drought because de-epoxidation is sustained, buffering excess excitation and supporting NPQ capacity. Seedlings grew under greenhouse conditions; drought was applied at ~60%, ~30%, and 0% field capacity, then rewatered. The maximum quantum efficiency of PSII (F_v/F_m) remained stable under mild drought and decreased at peak stress. The effective quantum yield of PSII (Φ_{PSII}) declined, especially in spruce. NPQ increased, shifting toward sustained NPQ. Pine maintained CET, PSI stability, and higher levels of PSI subunit (PsaD); spruce lost more of the PSII core protein D1 (PsbA), indicating greater PSII damage. Pine's larger xanthophyll-cycle pigment pool and higher zeaxanthin suggest greater NPQ than spruce. Post-rewatering, all parameters recovered. These results indicate pine withstands drought through dynamic photoprotection and PSI stability, whereas spruce relies on sustained NPQ and pigment restructuring.

Environmental stress in extremophilic *Chlamydomonas*: Comparative analysis of closely related Arctic and Antarctic species

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Psychrophilic algae are obligate cold extremophiles that thrive under multiple extremes (low temperatures, high salinities, extreme photoperiods and shading) but are sensitive to environmental change and are increasingly threatened with the rapid warming of the Polar Regions. The Arctic and Antarctic are both cold, but otherwise different environments: the Arctic is more dynamic and sensitive to warming and is increasingly less saline due to ice loss, while the Antarctic is more stable. Here, we compare closely related but geographically distant Polar green algae to identify if habitat and geographical distances affect algal physiology and resilience in the face of climate change. The order *Chlamydomonadales* is the ideal group for this work, as it contains a model species and over one-third of described algal psychrophiles.

Chlamydomonas sp. ICE-MDV is a recently described Antarctic alga, and we identified several close relatives from both poles (the ICE clade). We examine two Arctic (RCC2041, NA36) and two Antarctic species (RCC4618, ICE-MDV). All algae are grown at a range of salinities (500-10 mM NaCl) and temperatures (4-16°C) to examine growth, survival, photosynthetic efficiency, and lipid accumulation under native and climate-relevant scenarios. We hypothesized that, despite close evolutionary relationships, algae from the more dynamic Arctic will be more resilient to environmental change. Psychrophilic algae support all polar food webs and are crucial for ecosystem health, and our work will inform on how these fragile ecosystems will adapt to our changing climate.

Understanding the Genetic Underpinnings of Climate Change Acclimation and Temperature Stress Resilience in an Antarctic Green Algal Psychrophile

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Climate change is one of the most pressing problems our world faces today. The Polar Regions are particularly affected, as minor fluctuations in air temperature can lead to ice melt, causing significant changes in salinity and light availability.

The perennially ice-covered Antarctic Lake Bonney is an excellent example of how global warming affects polar aquatic habitats. This lake is home to one of the best studied psychrophilic (cold-loving) algae *Chlamydomonas priscui*. This alga resides 17 meters below the ice, and is accustomed to very low light (10 $\mu\text{mol m}^{-2} \text{s}^{-1}$), high salinity (700 mM), and low temperatures (2-5°C), but experiences severe heat shock at 24°C.

My goal is to understand adaptation to conditions that accompany climate change and examine whether acclimation to such conditions impacts the ability of *C. priscui* to withstand acute heat stress. To determine this, cultures were grown until mid-exponential growth phase then subjected to 24°C for a period of 12 hours, followed by a recovery at 4°C for 24 hours. RNASeq analysis

revealed that native conditions (high salinity, low light) prime the algae to withstand heat stress through upregulation of storage lipid biosynthesis and catabolism, production of Reactive Oxygen Species (ROS) and robust antioxidant defenses. In contrast, cells acclimated to climate change scenarios (lower salinity, higher light) suffered significant damage and were unable to recover. This study is the first of its kind to examine the ability of this vital primary producer to withstand climate change conditions and recover from acute heat stress.

Concurrent Session 6 - Cell Biology, Signalling, and Transcriptomics

Identification of Novel RLCK Substrates in Receptor Kinase Pathways Governing Plant Growth and Development

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In plants, receptor kinases (RKs) are the predominant cell surface receptors that perceive self- and environment-derived signals to initiate critical signalling pathways involved in growth, development, and immunity. Functioning downstream of RKs are receptor-like cytoplasmic kinases (RLCKs), which are activated through RK-mediated phosphorylation, and in turn, phosphorylate their respective substrate proteins. Previous research has predominately focused on the discovery of signals and RKs however, less is known about downstream components of signalling cascades such as RLCKs and their substrate proteins. Our research focuses two RLCKs: PBL34, which function in CLE peptide signalling to control plant meristem maintenance and development, and SGN1 which functions in CIF peptide signalling to regulate Casparian strip formation, an integral transcellular barrier in the root endodermis. Using substrate motifs targeted by PBL34 and SGN1 identified through a positional screening peptide array (PSPA) with collaborators, all proteins in model *Arabidopsis thaliana* were scored as potential substrates and filtered to create a list of candidate substrate proteins. To date, three proteins have been validated as PBL34 substrates via *in vitro* kinase assays. *In planta* assays will characterize these candidate substrates and elucidate their roles, further validating them as PBL34 or SGN1 substrates. Together, this research will uncover new components of RK-mediated signalling and advance our understanding of developmental and immune pathways in plants.

Green vaccines: Plant-produced nanoparticles to prevent non-typhoidal *Salmonella enterica* colonization in poultry

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Non-typhoidal *Salmonella enterica* serovars (NTS) are common causes of foodborne illness (salmonellosis) in humans, primarily through contaminated poultry products. Vaccination is the most effective strategy to prevent NTS colonization in poultry, but current vaccines have limitations, including safety concerns and poor immunogenicity. This project aims to develop

novel, plant-based nanoparticle vaccines that are safer, more effective, and inexpensive alternatives.

Antigenic epitopes from three surface-exposed NTS proteins were genetically fused to two self-assembling nanoparticles: encapsulin (ENC) and *Brucella* lumazine synthase (BLS). The fusion proteins were transiently expressed in *Nicotiana benthamiana*, achieving high accumulation levels (0.8-1.0 mg/g leaf fresh weight). Nanoparticle assembly was confirmed by size-exclusion chromatography and transmission electron microscopy, and surface display of NTS epitopes was verified with immunogold labelling.

In mice, both ENC and BLS vaccine candidates induced strong immune responses upon subcutaneous administration, and antibodies from BLS-immunized mice bound to NTS cells. A chicken immunization and challenge study was subsequently conducted. Sera, feces, and organs collected during the study are being analyzed to assess antibody responses and protection against NTS colonization. Preliminary results will be discussed.

To develop a cost-effective oral vaccine, ongoing work focuses on formulating the vaccine candidates to enhance stability in the digestive tract prior to an oral immunization trial in chickens. Overall, this research demonstrates the feasibility of producing plant-based nanoparticle vaccines and presents a promising approach to reduce NTS colonization in poultry and salmonellosis in humans.

Exploring the function of protein palmitoylation in auxin-induced ROP6 signaling

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The membrane-bound Rho-like GTPases from plants (ROPs) are master regulators of diverse cell signaling events, controlling various agriculturally important traits such as leaf morphogenesis, lateral root and root hair development, and responses to biotic and abiotic stresses. Despite decades of research, critical knowledge gaps remain in understanding how ROPs function at the membrane to achieve signaling specificity. Recent studies have shown that ROP6, a member of the ROP family, clusters into plasma membrane nanodomains in response to the phytohormone auxin. However, the molecular mechanisms underlying this lateral segregation remain poorly understood. To identify potential regulators of this process, we mapped the ROP6 proxitome using TurboID-based proximity labeling combined with mass spectrometry (PL-MS). Among the candidates, we focused on a palmitoyl acyltransferase (PAT), an enzyme known to catalyze the addition of saturated palmitic acid to target proteins, thereby influencing their membrane association and domain partitioning. Using biochemical and cell imaging approaches, we showed that this PAT directly interacts with ROP6, and that the interaction is enhanced by auxin. We are currently investigating whether PAT can palmitoylate ROP6 and regulate its dynamics and nano-organization at the plasma membrane. This work has the potential to reveal a novel class of regulatory enzymes within the auxin-triggered ROP6 signaling pathway, offering new strategies to modulate auxin responses and optimize plant developmental traits.

Identifying New Components in the *Arabidopsis* Self-Incompatibility Pathway

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Self-incompatibility (SI) is a genetic mechanism in flowering plants that prevents self-fertilization and promotes outcrossing. In the Brassicaceae family, SI is initiated by allele-specific interactions between the SP11/SCR ligand on the pollen and the S-Receptor-Kinase (SRK) on the stigma surface. This interaction activates a signaling cascade that rejects self-pollen. Several SI signaling components in *Brassica* SI species have been characterized: ARC1E3 ubiquitin ligase, which targets compatible pollen factors, FERONIA (FER) receptor kinase, which activates RBOHD to generate reactive oxygen species (ROS) to inhibitory levels, and M-Locus-Protein-Kinase (MLPK), also connected to RBOHD activation. However, some elements of the related SRK signaling cascade in *Arabidopsis* species remain unknown. This research aims to identify and characterize new regulators of the *Arabidopsis* SI response. Using yeast two-hybrid screening, novel *Arabidopsis* SRK-interacting proteins from the Receptor-Like-Cytoplasmic-Kinase (RLCK) family were identified. To investigate their function, T-DNA insertion mutants for these RLCKs are being crossed into the transgenic *Arabidopsis thaliana* SI-Col-0 line, which expresses functional SCR, SRK and ARC1 alleles. Additionally, the roles of FER and RBOHD in *Arabidopsis* SI signaling will be assessed. Loss-of-function *fer* and *rbohD* mutants are being crossed into the SI-Col-0 line to test whether disrupting ROS signaling compromises SI. Pollen hydration assays and aniline blue staining will be used to evaluate SI defects. This research will uncover new components of the SRK-mediated-SI-pathway in *Arabidopsis* and provide mechanistic insights into ROS signaling in reproductive self-recognition. Findings may inform strategies to manipulate SI systems for hybrid seed production and crop improvement in Brassicaceae.

Winter is Coming: Uncovering a Dehydrin Protein's Role in Conifer Cold Acclimation

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Conifers are evergreen trees that thrive in environments where they are subjected to prolonged periods of low temperature and short photoperiod. To cope with these conditions, they utilize a suite of biochemical and physiological adaptations. This includes the upregulation of dehydrin proteins, a subclass of the late embryogenesis abundant (LEA) protein family. Dehydrins are intrinsically disordered proteins that are upregulated during dehydration stress, which includes freezing. We identified a 16kD dehydrin protein in white spruce (*Picea glauca*) that is only upregulated during low temperature and short photoperiod. We hypothesized that this protein is involved in the cold acclimation of conifers and so we aimed to determine its protein sequence and to better characterize its structure and potential function. To achieve this, we used mass spectrometry to obtain the protein's amino acid sequence. Gene expression patterns were then analyzed using *in silico* transcriptomic data and validated through RT-qPCR. Our results identified nine transcripts likely involved in the upregulation of the 16kD dehydrin protein. Structural features, including intrinsic disorder and potential interaction motifs were assessed using computational tools. We also performed comparative sequence analysis to identify homologous

proteins in other species, providing insight into their evolutionary conservation. Our findings contribute to a deeper understanding of the molecular mechanisms underlying cold acclimation in conifers.

Unraveling the targeting pathway of OEP6: Insights into chloroplast and mitochondrial outer membrane trafficking

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The chloroplast is arguably the most important organelle in plants, playing a key role in photosynthesis and other metabolic pathways, which relies on the import and post-translational trafficking of nuclear-encoded proteins. These proteins interact with protein complexes TOC and TIC at the outer and inner membranes, respectively, where they are subsequently guided to different compartments within the chloroplast. The targeting and insertion of nuclear-encoded proteins to the chloroplast outer membrane (COM) is less well understood and my research aims to explore it further by focusing on a novel outer envelope protein, OEP6, which is thought to be a tail-anchored protein but with a poorly understood targeting mechanism. I aimed to characterize OEP6 features previously thought to be important for its targeting, as well as unexplored features such as the α -helical transmembrane domain and positively charged flanking regions. To do so, I generated a series of eGFP-OEP6 fusion constructs with truncations and/or mutations and used them to transform onion epidermal cells and *A. thaliana* mesophyll protoplasts. Expression and sub-cellular location of fusion proteins were determined using confocal microscopy and cellular fractionation assessed using Western blots probed with anti-GFP, and antibodies against chloroplast and mitochondrial markers. It was found that positively charged residues flanking the transmembrane α -helix are key for OEP6 chloroplast targeting, and more specifically, a set of positive charges are responsible for distinguishing targeting between the chloroplast and mitochondria. These findings aid in overall understanding of protein trafficking, specifically for proteins targeting to the outer membrane of chloroplasts.

Uncovering the mechanistic determinants of pathway specificity in the RLCK-VII family

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To ensure their fitness, plants are compelled to respond to a variety of environmental and endogenous signals. Perception of these extracellular signals is coupled with intracellular responses by members of the receptor-like kinase (RLK) superfamily. At the plasma membrane, the presence of a ligand induces oligomerization of receptors with their respective co-receptors, leading to activation of their cytoplasmic kinase domains, which then activate downstream substrates via transphosphorylation. The immediate targets of these activated receptor complexes are receptor like cytoplasmic kinases (RLCKs)—a subset of this same superfamily—who act to relay this extracellular signal to intracellular components. The RLCK-VII family has proven to be of central importance in the context of both immunity and development. However, the mechanistic basis for activated RLCK-VIIs acting within certain pathways but not others remains unclear. Employing an innovative semi-degenerate peptide library approach has enabled the

characterization of the intrinsic substrate preferences across the PBL family. Previously, attempts to characterize substrate preferences have relied on kinase-substrate pairs reported in literature which are relatively scarce. Such an abundance of experimental data will allow the high-resolution mapping of kinase features that underlie distinct substrate preferences. This work will lay the groundwork for future re-engineering of extant pathways in both developmental and immune signalling as well as the design of novel signalling circuits.

Dissecting Ionic and Osmotic Components of Salt Stress in African Rice (*Oryza glaberrima*): Physiological and Gene Expression Insights

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Salinity is a major environmental constraint that significantly reduces crop yields worldwide. Rice (*Oryza sativa*) is known to be salt-sensitive, and improving its salt tolerance remains a key objective for plant breeders. Wild and cultivated relatives within the *Oryza* genus may serve as valuable sources of tolerance genes. Compared to the well-studied Asian rice (*O. sativa*), the African rice (*Oryza glaberrima*) remains poorly characterized regarding its physiological and molecular responses to salt stress. This study aimed (1) to identify salt-tolerant *O. glaberrima* varieties, (2) to distinguish between the osmotic and ionic components of salt stress, and (3) to assess the specific effects of individual ions on plant performance. Plants were exposed to salinity both in nutrient solution and soil culture, and several parameters related to photosynthesis, mineral nutrition, water status, yield, and antioxidative properties were evaluated. Among the tested accessions, the cultivar TOG5307 maintained higher net photosynthesis and demonstrated superior tolerance to Na⁺ accumulation and better osmotic adjustment than the salt-sensitive TOG5949. Yield-related traits were also less affected in TOG5307 under salinity. Na⁺ ions were more toxic than Cl⁻, though an additive effect occurred when both ions were present as NaCl. Using PEG as a non-penetrating osmotic agent revealed additive effects of osmotic and ionic stress in the sensitive cultivar, whereas antagonistic interactions were observed in the tolerant one for several parameters. Data were further analyzed in relation to the expression of genes encoding ion transporters, providing insights into the mechanisms underlying salt tolerance in *O. glaberrima*.

ABSTRACTS

POSTER SESSIONS

Poster Session - Plant-Pathogen Interactions

01 - Investigating the Role of *BnNAC19* Overexpression in *Brassica napus* Responses to *Verticillium longisporum*

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Canola (*Brassica napus*) is one of the most important cash crops in Canada. *Verticillium longisporum* is an emerging soilborne fungal pathogen in Canada that causes Verticillium stripe disease in canola, resulting in significant yield losses. It produces microsclerotia in the stalks, causing epidermal peeling and host plant decomposition, allowing microsclerotia to re-enter the soil, where they lie dormant until the next growth season. Previously, we have identified that the overexpression of a transcription factor *BnNAC19* (NAM, ATAF, and CUC) can enhance canola resistance against another important fungal disease, blackleg. This study aimed to investigate whether the overexpressed *BnNAC19* transgenic canola line also exhibits enhanced resistance against *V. longisporum*. Two-week-old seedlings of Westar and *OEBnNAC19* transgenic lines were inoculated with *V. longisporum* isolate and monitored. Seedling phenotypic evaluations were conducted at various post-inoculation dates (dpi). Plant roots and leaves were sampled at 7 and 14 dpi, respectively, for gene expression analysis. Our initial results indicated that overexpression of *BnNAC19* in canola resulted in greater stunting, necrosis, and yellowing of the cotyledons and leaves in seedlings. Gene expression analysis showed an upregulation of pathogenesis-related gene 1 (*PR1*) and a downregulation of *PR2* in overexpression lines. This suggested that *BnNAC19* may act as a negative regulator for Verticillium stripe disease resistance. To validate these findings, we will further assess the expression of several pathogenesis-related (*PR*) genes and measure antioxidant activity in the overexpression of *BnNAC19* transgenic plants, aiming to characterize the role of *BnNAC19* in response to *V. longisporum*.

02 - Determinants of Pokeweed Mosaic Virus' Host Range

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Plant viruses, particularly the potyviruses that form the largest group of agriculturally important pathogens, threaten agriculture by causing yield losses with substantial economic consequences. Pokeweed mosaic virus (PkMV) is a single-stranded RNA potyvirus that commonly infects American

pokeweed (*Phytolacca americana*), yet its extended host range remains undefined. Host range influences a virus's ability to infect new crops, shapes disease emergence, and reveals molecular "compatibility rules" for prediction and prevention. To assess host specificity, we used tobacco (*Nicotiana benthamiana*), a standard potyvirus model. Pokeweed and tobacco were mechanically inoculated with PkMV; inoculated and upper, non-inoculated leaves were sampled from 0-7 days post-inoculation (dpi). Viral genomic RNA was quantified by RT-qPCR, and coat protein (CP) accumulation was assessed by immunoblot. In pokeweed, PkMV replicated robustly: CP was detectable by 5 dpi and systemic viral RNA by 4 dpi. In tobacco, genomic RNA and replication intermediates did not increase over time, CP was undetectable, and no systemic movement occurred, indicating a block at the replication or movement stages. A genome-scale phylogeny tree built from 150 reference potyvirus genomes placed PkMV near three *Nicotiana*-infecting potyviruses, focusing the research question: what early compatibility determinant(s) prevent PkMV from establishing infection in tobacco despite close relatives succeeding? Ongoing experiments prioritize defining the restricted step and testing candidate viral-host interactions, with the broader aim of linking phylogenetic relatedness to host outcomes to improve host-range prediction and resistance strategies. Together, these findings will improve how we predict host shifts and design durable crop resistance.

03 - Role of *BnWRKY40* Overexpression in Enhancing Resistance of *Brassica napus* to *Verticillium longisporum* and *Sclerotinia sclerotiorum*

Tetiana Borysenko¹, Zhongwei Zou²

¹she/her, ²he/him

Canola (*Brassica napus*) is a globally important oilseed crop and a cornerstone of Canadian agriculture, contributing nearly one-quarter of national farm crop receipts. However, fungal diseases pose a major threat to canola production, causing severe yield losses. Among these, *Verticillium longisporum* (the causal agent of Verticillium stripe) and *Sclerotinia sclerotiorum* (the causal agent of Sclerotinia stem rot) are particularly damaging, both leading to significant yield loss in canola. In this study, we demonstrate that *BnWRKY40* may function as a positive regulator of defense responses against both pathogens. Quantification of *V. longisporum* gDNA in infected tissues revealed that overexpression of *BnWRKY40* significantly reduced fungal growth and enhanced resistance to Verticillium stripe at early infection stages, improving seedling survival. Following *S. sclerotiorum* inoculation, lesion areas on leaves of *OE-BnWRKY40* canola lines were significantly smaller at 24 hpi compared with the wild type, though no differences were observed at 48 hpi, suggesting an early-stage defense role. Gene expression analysis indicated that defense-related genes exhibited different expression patterns regulated by *BnWRKY40* in response to fungal pathogens. These findings highlight the strong genetic potential of *BnWRKY40* as a key player in plant immunity, particularly at early infection stages, emphasizing its value for the development of canola lines with improved resistance to fungal pathogens.

04 - Mapping the physical and genetic interactome of *Pseudomonas syringae* effectors

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Many bacterial pathogens employ a type III secretion system to deliver effector proteins into host cells to promote virulence. One of the best-studied collections of type III secreted effectors (T3Es) belongs to the phytopathogen *Pseudomonas syringae*, which can infect a broad range of agriculturally significant crop species. The dynamic relationships between pathogen effectors have been shown to dramatically influence the outcome of plant-microbe interactions. Despite the importance of effector coordination in modulating plant immune responses and pathogen fitness, few have systematically studied this phenomenon. Here, I describe a study to comprehensively and systematically map all genetic and physical interactions between a library of ~500 effectors representing the pangenome *P. syringae* effector repertoire. Using high-throughput yeast genetic screens, our work has interrogated >270,000 pairwise effector combinations, covering the entire effector-effector interaction space across the *P. syringae* species complex. We constructed a network representing approximately 2,100 binary physical interactions, revealing patterns of specificity in effector-pairing and complexity in effector repertoire composition. This data provides testable hypotheses regarding mechanisms underlying infection strategies and effector regulatory relationships that play central roles in virulence.

05 - Canola *Verticillium* stripe disease: pathogenicity variations, co-existence with *Leptosphaeria maculans*, and transcriptome profile at adult plant stage.

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Canola (*Brassica napus*) is an essential and economically important crop to the Canadian economy. Canola is affected by many fungal diseases, including those caused by *Verticillium longisporum* and *Leptosphaeria maculans*, which allows for severe yield loss. *Leptosphaeria maculans*, the causal agent of blackleg disease, contains avirulence genes (Avr) that have a gene-for-gene interaction with resistance (*Rlm*) genes in canola. *V. longisporum* is the pathogenic agent of *Verticillium* stripe disease in canola. Together, *L. maculans* and *V. longisporum* have a positive synergistic effect when a plant is co-infected. The relationship between *V. longisporum* and *L. maculans* is not well understood and will be addressed in this study. The variability of pathogenicity of *V. longisporum* populations in western Canada will also be tested. In this study,

we will demonstrate that there is heterogeneity among *V. longisporum* genotypes and virulence across western Canada and identify *L. maculans* Avr gene profiles that can live synchronously with *V. longisporum*. This will be accomplished by using molecular identification and cotyledon inoculation tests. Dual-RNA sequencing analysis will be conducted to underline critical defence genes during the infection of canola by *V. longisporum* at the adult plant stage. This study provides insight into the broadness of *V. longisporum* pathogen variation and its interaction with blackleg pathogen. The identified differentially expressed genes will serve as valuable genetic resources that can improve canola resistance against *V. longisporum*, which can be leveraged in future breeding programs.

Poster Session - Plant Immunity

06 - Characterizing the Role of LRK10L Proteins in Plant Immune and Stress Signalling

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Plants rely on a multilayered immune system to defend against pathogens. The first layer, pattern-triggered immunity (PTI), is initiated when pattern recognition receptors (PRRs) on the cell surface detect conserved microbial molecules called microbe-associated molecular patterns (MAMPs). This triggers an early defence response involving reactive oxygen species (ROS) bursts and transcriptional reprogramming. Among the key mediators of PTI are receptor-like kinases (RLKs), a large family of transmembrane receptors that perceive external signals and activate downstream immune responses. A subfamily of RLKs, the LRK10-like (LRK10L) proteins, remains poorly characterized; however, phylogenetic and transcriptomic analyses suggest these receptors may act as PRRs in immune signalling. My project utilizes the model plant *Arabidopsis thaliana* to investigate whether LRK10L proteins function as PRRs, contributing to early immune signalling. I also aim to determine whether LRK10L proteins interact with abscisic acid (ABA)-mediated pathways, given the crosstalk between immune and drought stress signalling. To characterize the potential roles of LRK10L receptors in immune and stress signalling, I will employ a combination of physiological and molecular assays. Early immune activity is assessed through ROS burst, seedling growth, and bacterial growth assays following bacterial lysate exposure, identifying PTI-related phenotypes in LRK10L mutants relative to wild type. Abiotic stress responses are evaluated on media supplemented with ABA, NaCl, or mannitol to assess growth and stress sensitivity under hormonal, salt, and osmotic stress conditions. These approaches aim to elucidate how LRK10L receptors act in immune and drought-response pathways, improving understanding of plant stress signalling and guiding crop resilience strategies.

07 - Optimization of a novel *Pseudomonas syringae* - *Nicotiana benthamiana* pathosystem for the study of effector-triggered immunity

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Many phytopathogens rely on secreted virulence proteins, termed effectors, to modulate host immunity and cause disease. While effectors function to promote disease, individual effectors can be recognized by plant intracellular receptors called nucleotide binding leucine-rich repeat proteins (NLRs), leading to effector-triggered immunity (ETI). The bacterial species *Pseudomonas syringae* uses type III-secreted effectors (T3Es) to facilitate infection in a wide range of plant species, including the model plant *Nicotiana benthamiana*. Despite its versatility and broad usage in understanding the genetic and biochemical basis of immunity, only few NLRs have been well characterized in *N. benthamiana*. Here, we present a new infection protocol to rapidly screen for ETI responses in *N. benthamiana* using the pathogenic *P. syringae* strain DC30000ΔHopQ1. Using this optimized pathology assay, we assayed 50 diverse *P. syringae* effectors for ETI-elicitation to study the conservation of documented immune responses in *N. benthamiana*. This work aims to uncover novel ETI responses and identify cognate NLRs responsible for *P. syringae* effector recognition, providing the foundation to map the ETI-landscape in this model Solanaceous plants.

08 - Investigating the Roles of WAK1 and SVB in Temperature-Dependent Regulation of Salicylic Acid-Mediated Defence in *Arabidopsis thaliana*

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ABSTRACT

Climate warming poses a major threat to plant immunity by disrupting temperature-sensitive defence signalling networks. In *Arabidopsis thaliana*, elevated temperatures (28 °C) suppress the salicylic acid (SA) pathway, a central component of basal immunity and systemic acquired resistance against various pathogens. This suppression occurs through downregulation of the transcription factor-encoding genes *CBP60g* and *SARD1*, which regulate ICS1-dependent SA biosynthesis, leading to increased susceptibility to certain pathogens such as *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000. However, the upstream molecular mechanisms that connect extracellular damage sensing and intracellular proteostasis to this temperature-dependent loss of immunity remain unknown. Based on a previous *Arabidopsis* proteomics screen for temperature-regulated proteins, we propose that the cell surface damage receptor WALL-ASSOCIATED KINASE 1 (WAK1) and endoplasmic reticulum (ER) stress regulator SMALLER TRICHOMES WITH

VARIABLE BRANCHES (SVB) act as putative regulators of SA-mediated immunity under elevated temperatures. To test our hypothesis, successfully genotyped *Arabidopsis* Col-0, *wak1*, and *svb* mutants will be inoculated with *Pst* DC3000 and then incubated at normal (23°C) and elevated temperatures (28°C). Initial PCR-based screening for the mutant will be conducted, and pathogen growth will be measured as colony-forming units (CFU/mL) at 0 and 3 days post-infection. If the disease resistance phenotypes of the mutants differ significantly from Col-0 plants, then further immune mechanisms will be assessed by measuring SA accumulation and defence gene expression under different temperatures. Our results may reveal how cell wall-damage signals and ER proteostasis interact with SA-based defences during heat stress, enhancing our understanding of temperature-sensitive immune regulation.

09 - Shooting the Messenger: The Importance of Small RNAs in Plant Immunity

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Understanding the workings of plant immunity is essential for improving agricultural efficiency and sustainability. Plants employ a two-tiered immune response. Pattern-triggered immunity (PTI) is the first line of defense in plant immunity, and leads to a relatively mild immune response. Effector-triggered immunity (ETI) is a second and more severe immune response and results in localized cell death. Previously, these pathways were thought to be independent from one another, but recent research has revealed signaling crosstalk between the two pathways. However, the mechanisms behind this mutual reinforcement remain unclear. Previous research has shown the importance of miRNAs in plant immunity, which modulate degradation and translation of mRNAs based on sequence complementarity. We investigated if post-transcriptional gene silencing by miRNAs could serve as an avenue of crosstalk between PTI and ETI. miR825-5p is known to suppress intracellular receptors involved in ETI, helping to prevent accidental activation of immunity. We demonstrate using RNAseq analyses that loss of PTI sensitivity results in altered miRNA expression, including substantial downregulation of miR825-5p, suggesting that disruption of PTI signaling leads to upregulation of ETI receptors. These results further implicate miRNAs as an avenue of crosstalk between the two layers of plant immunity. Further research will investigate the role of immune-correlated miRNAs by assessing how efficiently they reduce accumulation of their targets. Understanding the role miRNAs play in plant immunity will allow us to further elucidate how plants respond to pathogens and to understand how the two layers of plant immunity interact to mount an effective anti-pathogen response.

10 - Genome-Wide Association Study to Identify Causative SNPs Involved in Temperature-Regulated Immunity in *Arabidopsis thaliana*

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Anthropogenic climate change threatens global food security by elevating temperatures and altering water availability, which can fundamentally alter plant-pathogen interactions. Elevated temperature weakens immunity in *Arabidopsis thaliana* (*A. thaliana*) plants by downregulating the master defense regulators, *CALMODULIN-BINDING PROTEIN 60g* and *SAR DEFICIENT 1* (*CBP60g* and *SARD1*). This negative impact suppresses the biosynthesis of the central defence hormone salicylic acid (SA), increasing susceptibility to certain pathogens, such as *Pseudomonas syringae* pv. *tomato* DC3000. Although temperature-sensitive and resilient *A. thaliana* accessions have been observed previously, the genetic determinants of naturally occurring temperature-regulated immunity remain unknown. This study aims to identify novel genetic regulators associated with heat-resilient immunity using a Genome-Wide Association Study (GWAS). Eighty natural *A. thaliana* accessions will be infected with *Pst* DC3000 and then grown under normal (23 °C) and elevated (28 °C) conditions. Bacterial proliferation will be quantified through serial dilution plating to evaluate immune performance. Based on bacterial counts, accessions will be categorized as temperature-resilient or sensitive. GWAS will then associate immune phenotypes with single-nucleotide polymorphisms (SNPs), followed by post-GWAS validation of candidate genes potentially underlying temperature resilience. The findings of this project will provide deeper insights into the mechanisms involved in temperature-resilient immunity in *A. thaliana*, which could serve as the groundwork for climate-resilient crop engineering and sustainable agriculture in a changing environment.

Poster Session - Molecular Signaling and Regulation

11 - Tracking cell wall signaling via transcriptional and biochemical outputs

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Plant cells continuously monitor the integrity of their cell walls through mechanisms known as cell wall signalling (CWS). Changes that negatively affect the homeostasis and/or integrity of the cell wall will trigger transcriptional reprogramming and subsequent cell wall remodelling. However, it remains unclear how plant cells distinguish between different cell wall-related stressors and fine-tune their responses accordingly. Particularly, the extent of transcriptional similarity between CWS and other cell wall-related pathways (e.g., cell wall damage, pattern-triggered immunity) is poorly understood. Here, using RT-qPCR, we investigated the transcriptional response of six genes,

previously implicated in cell wall stress-related pathways, across 14 treatments selected to best represent these pathways. Treatments included the CWS induction via the cellulose synthesis inhibitor, isoxaben; wounding; osmotic stress; and flg22 to induce pattern-triggered immunity. Further, we applied a phloroglucinol-HCl stain to investigate patterns of ectopic lignification as a proxy for changes in cell wall composition. Our results indicate that chronic stress (7-day) treatments drastically alter seedling growth but induce minimal transcriptional changes compared to long-term (24-hour) treatments. Furthermore, 24-hour cell wall stress treatment is sufficient to induce treatment-specific ectopic lignification patterns, whereas 4-hour treatments did not induce ectopic lignification. In contrast to 24-hour treatment with 200 nM isoxaben, 600 nM isoxaben strongly upregulates genes associated with multiple stress-related pathways, suggesting activation of pathways beyond CWS at higher isoxaben concentrations. Together, these findings reveal treatment-specific patterns in both gene expression and lignin deposition, indicating specificity in recognizing distinct stressors and tailoring both transcriptional and structural changes during CWS responses.

12 - Identification of novel components in *Arabidopsis* CEP peptide signalling

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C-TERMINALLY ENCODED PEPTIDES (CEPs) are small peptide hormones that regulate a plethora of physiological processes in plants including root growth and development, root system architecture, responses to nitrogen stress, and immunity. *Arabidopsis* CEPs are recognized by receptor kinases CEPR1 and CEPR2, while CEP4 signals through the related receptor-like kinase 7 (RLK7) as well, however, the downstream components involved in CEP signaling remain undefined. Receptor-like cytoplasmic kinases (RLCKs), are known to be involved in signal transduction during root development and involved in pattern-triggered immunity signaling, often acting downstream of other receptor kinases. This overlap in signaling outputs suggests a potential role for RLCK-VIIs in CEP signaling.

Previous studies have shown that when CEPs (CEP1/4) are exogenously applied to *Arabidopsis*, plants exhibit reduced root and seedling growth, increased resistance to infection, and strong MAPK activation. However, little is known about the signaling pathways that lie between perception of CEPs by receptor-like kinases and the response. To investigate the potential roles of RLCK-VIIs, I will test *Arabidopsis* CEP-induced responses in 9 *rlck-vii* polymutant lines, each containing multiple loss-of-function alleles within a given RLCK-VII subfamily. If RLCK-VII members are involved in CEP signaling, I anticipate RLCK-VII knockouts disrupting the CEP pathway resulting in immune signaling disruption, less severe root/seedling growth inhibition, and reduced MAPK activation compared to CEP treated wild type plants. I anticipate 1 or 2 of the 9 lines will demonstrate involvement in the pathways. I will present the results of screening the lines for involvement in CEP immunity and growth signaling.

13 - Expression pattern alone does not encode receptor-like cytoplasmic kinase signaling specificity

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Plant signaling pathways involve complex networks of proteins that enable plants to perceive and respond to both environmental and endogenous signals. Crucial to these processes are receptor kinases (RKs) and receptor-like cytoplasmic kinases (RLCKs). Together, they regulate essentially all aspects of plant life including growth, development, reproduction and immunity. RKs are transmembrane-bound proteins that perceive external stimuli leading to the initiation of signaling cascades. RLCKs function downstream of associated RK complexes to facilitate intracellular signal transduction via phosphorylation and regulation of substrate proteins. Interestingly, individual RLCKs can exhibit pathway-specific function despite sharing similarities in coding sequence, activation mechanisms and substrate interaction. For instance, BIK1 and SGN1 belong to the RLCK-VII/PBL subfamily, yet they function in distinct signaling pathways. BIK1 functions as a key positive regulator of immunity while SGN1 mediates proper Casparian strip formation. However, the mechanisms governing this signaling specificity are unknown. Thus, our research aims to determine the molecular basis of RLCK signaling specificity. By generating promoter-swapped BIK1 and SGN1 constructs for expression *in plants*, we demonstrated that expression pattern alone does not encode signaling specificity. Notably, SGN1 cannot rescue an immune phenotype even when driven by the BIK1 promoter. These findings contribute to our understanding of these molecular signaling networks that regulate diverse physiological and developmental processes in plants.

14 - Function and Regulation of Protein Palmitoylation in Auxin Signaling

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Protein S-palmitoylation is a posttranslational modification in which a fatty acid, typically palmitic acid, covalently attaches to specific proteins. Although this modification is widespread in plants, its role in cell signaling and regulation remains largely unexplored. My research aims to use *Arabidopsis thaliana* as a model system to investigate whether and how protein palmitoylation influences auxin signaling, a pathway that governs nearly every aspect of plant growth and development. The enzymes responsible for S-acylation, known as palmitoyl acyltransferases (PATs), are encoded by a large gene family in *Arabidopsis*. Using biochemical, genetic, and cell biological approaches, we have gathered evidence suggesting that a specific PAT may play a role in auxin signaling. We are currently investigating the mechanisms by which this PAT is regulated by auxin. This work has the potential to reveal a novel class of regulatory enzymes within the auxin signaling

pathway, offering new strategies for manipulating auxin responses to optimize plant developmental traits.

15 - Investigate structural motifs of the HSP90C C-terminal extension region in mediating substrate binding

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Chloroplasts perform various biochemical processes such as photosynthesis, and amino acids and lipid biosynthesis. Although chloroplasts generally contain thousands of proteins, the majority of them are nuclear encoded, and must be translated in and imported post-translationally from within the cytosol. The Arabidopsis stromal-localized heat shock protein 90 (AtHSP90C) has been shown to function as a molecular chaperone involved in cytosol-to-stroma and stroma-to-thylakoid protein translocation, and stromal protein folding and refolding. The interaction between HSP90C and the photosystem II subunit protein PsbO1 has been well characterized by multiple biochemical approaches, and truncation of the HSP90C C-terminal extension (CTE) region has recently been shown to reduce the chaperone binding activity to this client. This study aims to investigate structural motifs within the CTE & C-terminal domain that may mediate the chaperone-client interaction. We performed site-directed mutagenesis to introduce substitutions or deletions in some conserved motifs within the CTE as well as those within the C-terminal dimerization domain, and then tested the mutant protein interaction with model substrate by yeast two-hybrid assays. Our results showed that these mutations significantly reduced the interaction between HSP90C and PsbO1. This differential binding affinity suggests HSP90C likely contains more than one substrate binding site. We propose to conduct in-depth biochemical and enzymatic analyses of the different HSP90C mutant proteins with an attempt to better understand its molecular mechanism and particularly the ATP-dependent functional cycle.

16 - Illuminating Phosphorylation: Visualizing ADT1 and ADT4 Interactions with 14-3-3 *In Planta*

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Phenylalanine (Phe) is an essential aromatic amino acid that is a precursor for many specialized metabolites. In *Arabidopsis thaliana*, six AROGENATE DEHYDRATASES (ADT1-ADT6) localize to the chloroplast and catalyze the final step of Phe biosynthesis through a dehydration/decarboxylation reaction, converting arogenate to Phe. While ADTs generally favour the use of arogenate as their substrate, some isoforms (ADT1, ADT2, ADT6) can also utilize prephenate, suggesting differences in substrate specificity. Additionally, ADTs are known to form all combinations of homo- and heterodimers, potentially modulating their enzymatic activity and

the channeling of Phe for different end uses. Recent *in silico* analyses predict that all ADTs undergo phosphorylation- a post-translational modification (PTM) in which phosphate groups are added to select amino acids. Phosphorylation is the most common PTM observed in plants, and we aim to determine if it alters the subcellular localization, substrate specificity, and protein-protein interactions of ADTs. As such, the goal of this study is to determine if ADT1 and ADT4 are phosphorylated *in planta*, and I hypothesize that both ADT1 and ADT4 proteins undergo phosphorylation and function as phospho-proteins in *Arabidopsis*. To test this, we use 14-3-3 proteins as molecular sensors as 14-3-3s are known to only interact with phosphorylated proteins, as well as Bimolecular Fluorescent Complementation (BiFC) assays and confocal microscopy to visualize interactions between 14-3-3 SGF14I and ADT1 or ADT4 *in planta*. This work is the first demonstration that ADT1 and ADT4 are phosphorylated *in planta* and is the first step in understanding how phosphorylation may impact ADT function.

17 - Aggregation of HTL/KAI2 elucidates a novel mechanism of signalling in *Arabidopsis*

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Plants survey their environment and integrate cues like light and temperature to determine the best time to germinate. The α/β hydrolase receptor HYPOSENSITIVE TO LIGHT/KARRIKIN INSENSITIVE 2 (HTL/KAI2) allows pyrogenic and root parasitic species like *Striga hermonthica* to bypass the requirement for light and germinate in response to a ligand. This same receptor and signalling cascade are present in all land plants, however the mechanism by which signalling partners are recruited and germination is initiated remains unclear. By expressing KAI2 in baker's yeast, I show that the receptor forms protein aggregates in response to its ligand. I also show that higher temperature alone can induce aggregate formation and that sensitivity to ligands and temperature vary by ortholog. These results were validated in native and over-expression lines in *Arabidopsis thaliana*. The elucidation of these germination mechanisms allows better management of *Striga hermonthica* and its impact on crop species worldwide.

18 - Benchmarking AlphaFold 3, AlphaFold-Multimer, and ColabFold for Heterodimeric Complex Prediction in *Arabidopsis thaliana*

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The Nobel Prize-winning program AlphaFold has revolutionized biological research and demonstrated its capabilities in modeling both protein monomers and protein-protein interactions (PPIs). Despite its widespread application, AlphaFold's performance in plant PPI

modeling remains underexplored. With the recent release of AlphaFold 3 and its source code, we conducted a benchmark study comparing three AlphaFold variants, AlphaFold 3 (AF3), AlphaFold-Multimer (AFM), and ColabFold (CF), focusing on the model plant species *Arabidopsis thaliana*. Due to the lack of a perfectly matched benchmark dataset, we constructed a dataset of 201 heterodimeric interfaces by querying the Protein Data Bank (PDB) and improving its quality through filtering and redundancy reduction. Our analysis revealed limited variation in overall performance among the three programs, based on success rate and mean accuracy. There are 165 jobs where at least one program produced successful predictions. Notably, AF3 yielded a greater proportion of both high-quality and incorrect models compared to AFM and CF. Although overall performance was similar, individual job results varied, with 24 cases where only one program produced a successful prediction. We further evaluated the impact of template access and intrinsic interface properties on modeling success. Specifically, we showed that binary interactions originating from large assemblies (megacomplexes) possess distinct properties from other interfaces, and enabling template search significantly improves modeling accuracy for these cases. Additionally, we assessed the effectiveness of model confidence metrics and explored model selection strategies. Our findings highlight AlphaFold's potential in modeling *Arabidopsis* PPIs and provide practical insights into its application in plant biology.

Poster Session - Abiotic Stress and Environmental Responses

19 - Adaptive Drought-Induced Lipid Modulations in Soybean (*Glycine max* L.)

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Drought represents a critical environmental constraint for soybean production in North America, reducing yield up to 50% and significantly impacting seed quality. Lipids play essential roles in plant development and environmental stress adaptation. However, the specific stress-adaptive functions of lipids in mediating soybean drought responses remain under examined. This study investigated lipid-based drought responses in Ontario-adapted short-season (maturity groups 0.8-1.6) soybean cultivars. Leaf, root, and seed tissues were sampled at several vegetative and reproductive stages to characterize any changes in lipid abundance and fatty acid composition under drought relative to optimal watering conditions. Drought stress resulted in elevated total leaf lipid content and substantial variation in α -linolenic acid ($18:3\Delta^{cis9,12,15}$) abundance across genotypes. The relative proportions of key lipid classes including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylcholine, and phosphatidylglycerol (PG) were differentially affected among the cultivars, with some exhibiting increased MGDG:DGDG ratios under drought. Drought-responsive remodeling of photosynthetic membrane lipids

(MGDG, DGDG, and PG) occurred to varying degrees across different cultivars. Dynamic changes in fatty acid saturation and acyl chain length for these key lipids suggest structural adjustments that may support membrane stability under drought stress. Ongoing investigations are focused on assessing the impacts of drought on cuticular waxes, root and seed lipid composition, as well as associated gene expression networks. Together, these findings advance the understanding of lipid-mediated mechanisms underlying drought adaptation and provide insight into the metabolic basis of soybean stress tolerance.

20 - Investigating the roles of NAC transcription factors in *Arabidopsis thaliana* seed coat development and salt stress response

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The seed coat is a multi-layered structure that regulates seed germination and dormancy while protecting against environmental stress and pathogens. As climate change intensifies biotic and abiotic stressors, understanding seed coat development is increasingly important for improving seed viability and resilience. NAC transcription factors, members of the NAM, ATAF, and CUC family, are key regulators of secondary cell wall formation and other developmental processes. However, the roles of several seed coat-expressed NACs remain poorly understood.

This study investigates three NAC transcription factors—NAC-A, NAC-D, and NAC-E—expressed in the distal seed coat of *Arabidopsis thaliana*. The objective was to determine their roles in seed coat differentiation and assess potential redundancy or synergistic interactions through analysis of single, double, and triple *nac* mutants. Seed coat characteristics, including pectin and cellulose deposition, mucilage secretion, epidermal surface structure, and starch accumulation, were examined using histochemical assays. Seed permeability was evaluated via tetrazolium staining, and germination under salt stress was assessed to examine NAC involvement in abiotic stress response.

Preliminary results indicate that co-knockout mutants of NAC-D and NAC-E exhibit aberrations in columella structure, irregular mucilage production and extrusion, increased seed permeability, and loss of seed coat epidermal cells. These phenotypes are further intensified in the NAC-B/D/E triple knockout, which also displays delayed germination and impaired early development.

This research uncovered a critical role for these NAC transcription factors in the regulation of seed coat development and highlights their potential cooperative functions in supporting seed viability and stress resilience.

21 - Evaluating SIF response to optimization of UAV flight parameters in coniferous and mixed deciduous forests

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The occurrence of climate change-induced droughts has increased over the past decades, with detrimental effects on photosynthetic carbon uptake and forest productivity. Solar-induced fluorescence (SIF) is closely connected to carbon uptake through photosynthetic light reactions. SIF can therefore be used as a highly sensitive, rapid, and non-invasive indicator of plant health and carbon uptake. We aim to develop a protocol for reliable and precise retrieval of SIF from highly complex tree canopies using UAVs. For this purpose, we performed a series of test flights over forests of Southern Ontario. A drone-based SIF system was used, where height, speed, and distance parameters were modified using a flight control and mission planning software. SIF data was evaluated to observe the effects of varied flight parameters and identify the combination that would yield the highest fluorescent signal. Our study established a first SIF data collection protocol that will be further tested and validated in upcoming field data collections, aiming to quantify photosynthetic activity from highly complex tree canopies. Findings from this study will highlight the applications of UAV-based SIF sensing for forest health monitoring across wider spatial ranges.

22 - Understanding cold-induced sweetening in potato tubers

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Cold-induced sweetening (CIS) is a phenomenon affecting potato tubers stored at temperatures around 4°C that results in accelerated starch degradation and soluble sugar accumulation. CIS has significant economic impact, particularly to the potato processing industry, due to the undesirable traits associated with sugar accumulation, among which is the production of the toxic and carcinogenic acrylamide induced by the high temperatures of deep-frying. Among enzymes of starch-sugar interconversion, plastidial α -glucan phosphorylase (Pho1) and vacuolar invertase (VINV) are understood to be highly active in tubers subjected to CIS-inducing temperatures. However, the physiological role and complex mechanisms of regulation of CIS remain poorly understood. By assessing changes in starch and sugar levels, measuring activity of Pho1 and VINV, as well as conducting transcriptome analyses, this project aims to evaluate the metabolic

responses of tubers from known CIS-resistant and -susceptible cultivars subjected to long-term storage at CIS-inducing temperatures.

23 - Identifying differential low phosphate responsive traits in *Eutrema salsugineum* and their recombinant inbred lines.

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In plants, phosphorus plays a key role in many cellular processes and components. To maximize crop yields and combat food insecurity, agriculture relies on applications of phosphorus-containing fertilizers. However, we must reduce our reliance on fertilizers because they pollute the environment and accessible global rock phosphate (Pi) stores for their production are diminishing. We compared two ecotypes of *Eutrema salsugineum*, an extremophyte closely related to *Arabidopsis thaliana*. An ecotype from Yukon, Canada, tolerates low-Pi conditions while an ecotype from Shandong, China, does not. Using both ecotypes and their recombinant inbred lines, we tested seedlings on agar (prepared with 0, 0.05 or 0.5 mM Pi) and using plants on soil (grown with 0 or 2.5 mM Pi) to identify screening conditions for low-Pi tolerance. Root architecture of seedlings grown on media lacking added Pi (0.0 mM Pi) proved best in revealing low-Pi responsive traits and for some RILs this condition was necessary to identify low-Pi sensitive root-based phenotypes. Rosette area and non-photochemical quenching (NPQ) of soil-grown plants treated with 0 mM Pi fertilizer did not always agree with predictions for low-Pi tolerance based on seedling tests. Of note, NPQ data suggested Yukon plants grown with low-Pi have a greater capacity to safely dissipate excess energy and this response was not seen for Shandong plants. Identifying phenotypic traits associated with low-Pi tolerance, along with transcriptome sequencing, offers a way to identify genes responsible for low-Pi tolerance and can be applied to generate Pi use efficient crops.

24 - Altered expression of NAC transcription factors impairs seed coat development and germination under salinity and cold stress.

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Seed crops provide most of the world's calories, but climate change and environmental stress threaten their yield and global food security. To sustainably boost production, it is crucial to understand how seeds develop under stress. NAC transcription factors are key regulators of plant growth and stress responses, and transcriptomic analyses in *Arabidopsis thaliana* show that NAC-D and NAC-E are highly expressed in the seed coat, which protects the developing embryo.

Previously, knockout mutants *nac-d nac-e* were shown to have increased seed coat permeability and salt sensitivity, indicating impaired barrier function. Calcofluor staining analyses revealed disrupted cell wall organization. RNA-seq showed downregulation of genes in phenylpropanoid, suberin, and cell wall pathways. These findings suggested that *NAC-D* and *NAC-E* may preserve seed coat impermeability by regulating both biochemical barriers and structural components of the outer integuments. Here we show that 35S:*NAC-D* and 35S:*NAC-E* transgenic lines exhibited a broad spectrum of phenotypes. RT-qPCR confirmed variable upregulation of *NAC-D* and *NAC-E* transcripts among lines. Interestingly, some 35S:*NAC* lines showed markedly increased seed coat permeability resembling the knockout mutants, while others displayed wild-type-like traits. Correspondingly, calcofluor white staining of epidermal cellulosic structures revealed patterns consistent with both knockout mutants and wild-type phenotypes. We hypothesize that precise regulation of these NAC transcription factors is essential for maintaining seed coat impermeability and stress resilience. We propose that *NAC-D* and *NAC-E* are key regulators of signaling networks governing outer seed coat development, offering potential insights to enhance seed resilience and improving agricultural sustainability.

25 - Assessment of Temporal Dynamics of Photosynthetic Activity in Deciduous and Mixed Forests Using Solar-Induced Chlorophyll Fluorescence

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Forests are significant components of the global carbon cycle, contributing as major carbon sinks and regulators of climate change. Understanding their photosynthetic activity is essential for assessing ecosystem levels of carbon exchange. This study quantifies seasonal variations in photosynthetic activity between deciduous and mixed forests in the Great Lakes region of southern Ontario, Canada. Continuous measurements of Solar-Induced Chlorophyll Fluorescence (SIF) and photosynthetically active radiation (PAR) were collected from April to September 2025 at the Turkey Point Deciduous Forest (TPD) and Turkey Point Mixed Forest (TP39) sites. SIF was obtained from a Fluorescence Box (FloX) system installed on eddy-covariance towers, which included upwelling and downwelling spectrometers (400-950 nm) and fluorometers (650-800 nm). SIF was calculated using the improved Fraunhofer Line Discriminator (iFLD) method, enabling high-temporal-resolution assessment of canopy photosynthesis and forest photosynthetic. Both sites showed strong seasonal variation, with SIF and PAR peaking in summer. Deciduous forest exhibited higher average PAR ($1396.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) and SIF ($0.24 \text{ mW m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$), while mixed forest recorded slightly lower PAR ($1373.7 \mu\text{mol m}^{-2} \text{s}^{-1}$) but higher mean SIF ($0.29 \text{ mW m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$). Maximum SIF was observed in July at Deciduous site ($0.44 \text{ mW m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$) and in June at mixed forests ($0.41 \text{ mW m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$). These findings emphasize that SIF exhibits distinct seasonal dynamics between deciduous and mixed forests, reflecting differences in canopy structure, leaf phenology, and photosynthetic capacity. The study confirms that FloX-based SIF measurements can reliably track photosynthetic activity in different forest ecosystems.

Poster Session - Biochemistry and Metabolism

26 - Breaking it down: investigating the biological function of cuticular waxes in the poplar cuticle

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Land plants have developed a waxy, hydrophobic protective layer called the cuticle to defend against various stressors, including invading insects and pathogens. Various poplar (*Populus trichocarpa*) accessions accumulate unsaturated hydrocarbons called alkenes in the cuticle of leaves throughout development. As the leaves age, alkene accumulation declines as they spontaneously oxidize into aldehydes: one of which is nonanal. Nonanal has been previously reported to be a semiochemical that mediates communication, interaction and mating in insects. Moreover, it has been implicated in priming defences in neighbouring plants against microbial pathogens.

Since nonanal is released from alkene breakdown in the cuticle of *P. trichocarpa* leaves, we hypothesize that nonanal could play a role in plant-insect interactions and/or defence from foliar pathogens. This research aims to understand the biological relevance of alkenes (and consequently, nonanal) in the cuticle of poplar leaves. To achieve this, plants were exposed to either insect herbivory or fungal pathogen infection to quantify changes in cuticular waxes using gas chromatography-mass spectrometry (GC-MS). Herbivory of white-marked tussock moth larvae and poplar leaf rust infection revealed contrasting changes in alkene accumulation and other cuticular waxes. The plant responses appeared to be genotype-dependent, highlighting the diversity within accessions of a species. Complementary assays, such as applying stress hormones, may give further evidence for the role of cuticular waxes in stress responses.

27 - Identification and functional analysis of lipases associated with oil metabolism in the microalga *Nannochloropsis oceanica*

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Plant-based oils are widely used by humans, yet their production relies on unsustainable amounts of arable land to harvest oil crops. As the global population continues to rise, the amount of arable land available will limit us from being able to grow these crops to meet the increased demand.

Photosynthetic algae offer a sustainable alternative, as they do not depend on arable land for growth. *Nannochloropsis oceanica* stands out as it amasses high amounts of oils, which also contain health-beneficial fatty acids under stress conditions. However, oil metabolism in this species remains largely unexplored. Lipases, a class of hydrolases involved in oil metabolism, are severely understudied despite evidence their targeted manipulation can enhance oil production. Here, 45 putative lipases were identified in *N. oceanica* through expression profiling under stress conditions and conservation and phylogenetic analysis to previously characterized lipases. The strongest candidate lipases were selected for further investigation. Their functions have been explored through a yeast chassis. Lipases have been recombinantly expressed and have shown to significantly impact the lipid profile. Additionally, fatty acid feeding experiments using eicosapentanoic acid, one of the valuable fatty acids in the species, are currently being performed with the yeast. Future work will entail creating gene knock-out and overexpression lines of these lipases in *N. oceanica*, with the goal of clarifying their specific roles in oil metabolism. Ultimately, the objective of this research is to identify critical lipases involved in oil metabolism in *N. oceanica*, offering valuable genetic targets for the biotechnological improvement of oil biosynthesis.

28 - Investigating Mechanisms Underlying Increased Biomass and Seed Yield in Canola Expressing Maize Starch Branching Enzymes

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Starch is a complex carbohydrate that plants use as a transient energy reserve for night-time metabolism or for long term storage. It consists of two glucose polymers, linear amylose and branched amylopectin. The synthesis of starch granules is controlled by four major types of enzymes: Starch Synthase, Starch Branching Enzyme (SBE), Debranching Enzyme, and Starch Phosphorylase. SBEs catalyze the cleavage of linear α -glucan chains and the introduction of branching points, which largely determine the structure and physiochemical properties of starch granules; accordingly, their activity is essential for proper starch architecture. Building on our previous finding that replacing endogenous *Arabidopsis* SBEs with a maize SBE isoform that is exclusively expressed in monocot endosperm increased plant seed yield, we applied this strategy to the oil crop canola using a combination of CRISPR-Cas9 gene editing and conventional transgenic approaches. Under controlled environmental conditions, the transgenic canola plants produced more stems and pods, resulting in a 30-40% increase in total seed yield. The thicker stems observed in these lines also conferred enhanced tolerance to heat and drought. To uncover the underlying mechanisms, we are quantifying the spatial and temporal fluxes of starch and soluble sugars, as well as analyzing cell wall composition. A small-scale field trial in Rockwood, Ontario is currently underway to validate whether the traits observed in greenhouse are reproducible under field conditions.

29 - Characterizing the Structure and Post-Translational Regulation of the Plant Alternative Oxidase (AOX) Proteins Using a Yeast Expression System

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Alternative oxidase (AOX) is a mitochondrial inner membrane protein that transfers electrons directly from ubiquinol to oxygen, bypassing complexes III and IV of the electron transport chain. In plants, AOX is critical for maintaining metabolic flexibility, reducing reactive oxygen species (ROS), and sustaining redox balance, particularly under environmental stress. AOX has been implicated in plant responses to drought, salinity, temperature extremes, and pathogen attack, highlighting its role in stress tolerance and resilience. However, questions remain regarding AOX protein structure, conserved motifs, and post-translational regulation. This project focuses on plant AOX by analyzing sequence and structural features across representative species using bioinformatics. Initial analysis using predictive modeling software, such as AlphaFold and SwissModel, found that all AOX proteins examined are predicted to exist as monomers. Additionally, early sequence analysis revealed conserved domains, motifs, and potential post-translational modification sites, contributing to a better understanding of evolutionary and functional differences among AOX proteins. In parallel to bioinformatics, AOX cDNA sequences have been synthesized and inserted into the pYES2.1 protein expression vector designed for *Saccharomyces cerevisiae*. Future work will involve expressing these AOX proteins in yeast to study their biochemical properties and functional differences. Additionally, respirometry experiments will be conducted to assess AOX activity and its impact on cellular respiration under varying conditions. This research will advance our understanding of plant AOX regulation and its role in stress adaptation, with potential applications for improving crop resilience. Findings from plants will also provide a framework for broader comparisons with AOX from fungi, protists, and animals.

30 - *Linum usitatissimum* (flax) diacylglycerol acyltransferases interact with lysophosphatidylcholine acyltransferase

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Flax (*Linum usitatissimum*) seed oils are rich in α -linolenic acid (ALA; 18:3 Δ^9 *cis*, 12*cis*, 15*cis*), an important polyunsaturated fatty acid (PUFA) in food and oleochemical industries. PUFAs are modified on phosphatidylcholine (PC) before they are eventually accumulated into triacylglycerols (TAGs), the major component in seed oils. Various enzymes participate in the channelling of PUFA from PC to TAG, including diacylglycerol acyltransferase (DGAT) and lysophosphatidylcholine acyltransferase (LPCAT). DGAT catalyzes the final addition of an acyl-CoA (a fatty acid derivative) to

form TAG, while LPCAT catalyzes the addition and removal of acyl-CoAs from PC. Recently, select flax DGAT and LPCAT isoforms have been found to physically interact with each other, possibly facilitating the channelling of ALA from PC into TAG, although how these interactions happen remains largely unknown. This study aims to identify and characterize the putative regions and sites responsible for DGAT-LPCAT interaction by using AlphaFold2 AI based protein structure prediction, site-directed mutagenesis, and membrane yeast two-hybrid (MYTH) assays. The results show the N-termini and the in silico predicted interactive domain of DGAT and LPCAT are potentially important for their interactions. These findings provide further insights into the mechanisms of lipid biosynthetic interactomes, which may aid future efforts to engineer the production of modified fatty acids such as ALA.

Poster Session - Genomics and Epigenetics

31 - Characterizing the Relatedness of Moringa Trees

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The Moringa or Drumstick Tree, *Moringa oleifera*, is one of 13 species in the genus *Moringa* that belongs to the *Moringaceae* family. *M. oleifera* is traditionally known for providing valuable medicinal, nutritional, and therapeutic effects in humans due to its numerous secondary metabolites and antioxidants. Currently, it is found and commercially grown in various countries with semi-arid climates that suit its growth criteria. Despite its many beneficial properties and wide cultivation, there has been little genomic study on the species. The most complete available genome assembles approximately 90% of the genome, with the complete genome size reported as 315 Mb. The introduction of Moringa trees to Mogadishu, the capital of Somalia, may have resulted from a single introduction event within living memory. These seeds are thought to have been distributed among people in local communities and spread to the surrounding regions, which would indicate that the trees are all closely related. However, it remains unclear whether there have been additional independent introductions, or whether the initial seeds brought to Somalia were from a single tree or a collection of seeds from various Moringa trees. Using restriction site-associated DNA sequencing methods for analysis of high-quality DNA isolated from dry leaf samples, we aim to characterize the relatedness of various *M. oleifera* trees from different regions of Somalia to uncover whether they are all genetically related.

32 - An antagonistic epigenetic mechanism regulating gene expression in pollen revealed through single-nucleus multiomics

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Arabidopsis MBD5, MBD6, and MBD7 are CG-specific methyl-readers with opposite functions: MBD5 and MBD6 (MBD5/6) repress methylated loci in pollen vegetative nuclei (VN), while MBD7 prevents transgene silencing, possibly by promoting DNA demethylation. Here we show that loss of *MBD7* rescues transcriptional defects at a large subset of MBD5/6-bound loci. Using simultaneous profiling of DNA methylation and transcription in single pollen nuclei, we found that MBD5/6-bound loci that are actively demethylated in immature VN lose additional methylation in *mbd5/6*, prior to transcriptional derepression. A subset of these loci is also bound by MBD7, correlating with demethylation and transcriptional derepression in *mbd5/6* that are both reversed by loss of *MBD7*. Conversely, ectopically recruiting the MBD7 complex to MBD5/6 targets causes partial demethylation and upregulation. We propose that MBD5/6 maintain silencing in VN in part by preventing the MBD7 complex from enhancing the active demethylation that occurs during VN maturation.

33 - Optimization of sequence-specific dsRNA transient expression in *Nicotiana benthamiana*

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Nicotiana benthamiana (bushy tobacco) is a well-characterized model for transient and stable transgene expression and a promising platform for double-stranded (ds) RNA-based therapeutics. However, optimizing growth conditions to maximize dsRNA yield is essential for advancing this system for commercial applications. RNA interference (RNAi) defends plants against viruses by processing viral dsRNA into small interfering RNAs (siRNAs) that are loaded into the RNA-induced silencing complex (RISC), which targets and silences viral genes to limit infection. Previous studies have shown that different temperatures modulate RNAi efficiency by altering small RNA composition and reducing siRNA accumulation. Therefore, suppression of RNAi might enhance transgene-derived dsRNA expression and stability. This research investigates how elevated temperature affects sequence-specific dsRNA accumulation in *N. benthamiana*. Tobacco plants will be infiltrated with an *Agrobacterium* strain carrying a dsRNA-expression construct and then grown under control (22 °C) and elevated (28 °C) temperatures. The dsRNA accumulation will be quantified by western blotting with dsRNA-specific J2 monoclonal antibody, RT-PCR, and qPCR. Expression of key RNAi components, including genes encoding DICER-LIKE and ARGONAUTE proteins, will also be assessed to test whether elevated temperature suppresses RNAi-mediated silencing machinery in tobacco. Based on prior evidence linking heat stress to altered small RNA dynamics and dsRNA stability, we hypothesize that elevated temperature reduces RNAi activity,

increasing dsRNA yield. These results will identify optimal thermal conditions for transient dsRNA production in tobacco, improving plant-biofactory systems for dsRNA therapeutics and revealing how thermal regulation influences RNA silencing pathways during abiotic stress.

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34 - Toward a Navy Bean Pangenome for Dissecting Common Bacterial Blight Resistance and Tepary Bean Introgressions

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas fuscans* subsp. *fuscans* (Xff), is a major constraint on dry bean production worldwide. The genetic control of CBB resistance in common bean (*Phaseolus vulgaris* L.) is complex. Although the major QTL on Pv06 (BC420), Pv08 (SU91), and Pv10 (SAP6) have been characterized, none confer complete resistance to aggressive Xap or Xff strains. To address this limitation, some of the first navy bean cultivars with interspecific introgressions from tepary bean (*P. acutifolius* A. Gray, PI 440795) were developed (e.g., OAC Rex, Apex, Mist, and Rexeter) at the University of Guelph, providing enhanced resistance to CBB. However, the extent of *P. acutifolius* genome integration in these cultivars and the potential presence of additional resistance- or agronomically relevant alleles remain unclear. To address this, we are constructing a navy bean pangenome using long-read sequencing of 11 cultivars with contrasting CBB responses. Resistant cultivars carrying *P. acutifolius* introgressions and susceptible cultivars (e.g., Ex Rico 23, Gryphon, Laser, Cruiser, AC Compass, Envoy, and T9905) are being sequenced on the Oxford Nanopore PromethION 24 platform at 60× coverage. These assemblies will be integrated into a graph-based pangenome to capture the full spectrum of genomic diversity, including structural variants and presence-absence variation. Our objectives are to (1) validate the previously reported Niemann-Pick-associated locus (now annotated as a patched family protein) for CBB resistance across multiple navy bean backgrounds, (2) quantify the genomic contribution of *P. acutifolius* to resistant cultivars, and (3) identify novel candidate genes and allelic variation that may contribute not only to disease resistance but also to enhanced resilience to biotic and abiotic stress. This work will establish the first navy bean pangenome, providing a foundation for genome-wide association, comparative genomics, and marker discovery. Ultimately, this resource will accelerate the development of improved cultivars with durable CBB resistance and inform breeding strategies to balance resistance gains with potential introgression-associated trade-offs.

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