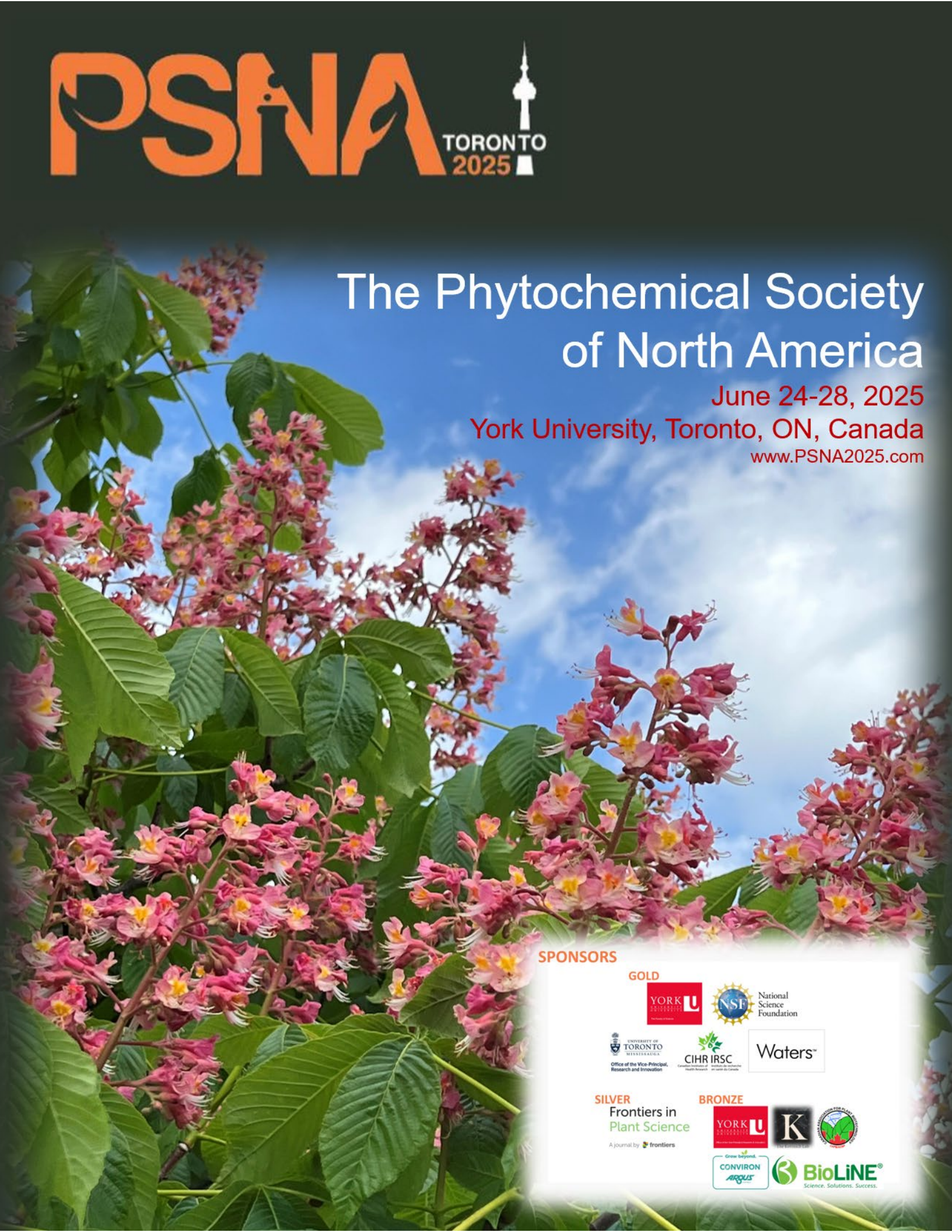
PSNA TORONTO 2025

The Phytochemical Society of North America

June 24-28, 2025

York University, Toronto, ON, Canada

www.PSNA2025.com



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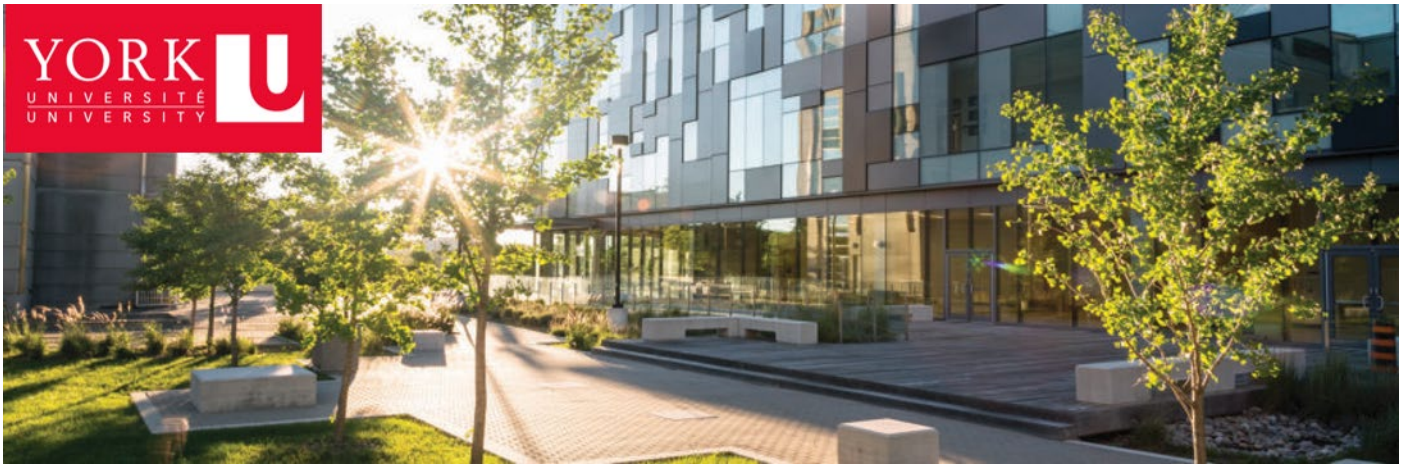


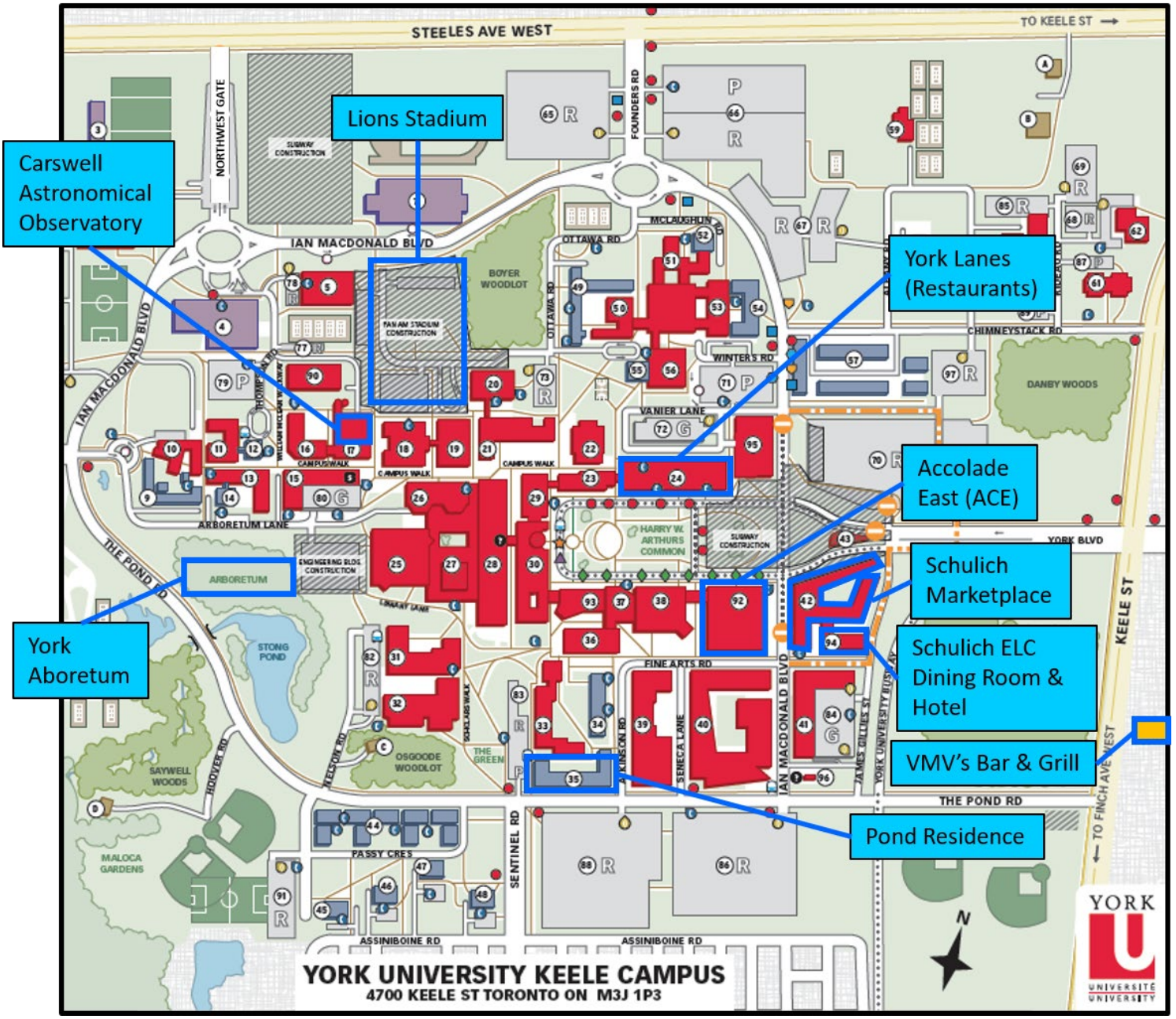
Welcome to York University!

York University (*a.k.a.* YorkU or YU), Canada's third-largest university, is located in northern Toronto bordering York Region, the traditional territory of the Anishinaabeg, Haudenosaunee, Huron-Wendat, and Métis. YorkU has approximately 53,500 students, 7,000 faculty and staff, and over 375,000 alumni worldwide. It has 11 faculties, including Science, Schulich School of Business, Osgoode Hall Law School, Lassonde School of Engineering, and various research centers. The mission of YU is the pursuit, preservation, and dissemination of knowledge. In pursuit of its motto *Tentanda Via* (The way must be tried), YorkU makes innovation its tradition. For more information about York University and its research visit the Faculty of Science and Research & Innovation webpages.

We wish you a good time in Toronto!

Nik Kovinich, Sangeeta Dhaubhadel, Michael Phillips, and Mark Lange

York University Campus Map



For an interactive campus map visit
https://map.concept3d.com/?id=1200#!ce/34557?s/

YORK UNIVERSITY, TORONTO, ON, CANADA

SYMPOSIA

Gene Discovery and Functional Plant Genomics
Plant Metabolomics and Pathway Discovery
Metabolic engineering and Plant Synthetic Biology
Cannabis and Plant-derived Pharmaceuticals
Indigenous Connections and Traditional Botanical Medicines
Phytochemistry of Functional Foods and Human Nutrition
Chemical Ecology and Plant-Organismal Interactions
Plant Immunity and Microbiome Interactions

PLENARY AND KEYNOTE SPEAKERS

Vincenzo De Luca
Brock University

Elizabeth Sattely
Stanford University & HHMI

Mark Lange
Washington State University

Nicholas Provart
University of Toronto

Yang Qu
University of New Brunswick

Jonathan Ferrier
Dalhousie University

Diana Roopchand
Rutgers University

Cristiana Argueso
Colorado State University

Bao-Hua Song
University of North Carolina at Charlotte

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The Kovinich Lab

Canadian Association for Plant Biotechnology
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PSNA 2025

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Sangeeta Dhaubhadel, *PSNA President, Agriculture and Agri-Food Canada*
Michael Phillips, University of Toronto **Mark Lange**, Washington State University

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Philipp Zerbe, *PSNA Treasurer*
Agriculture and Agri-Food Canada, Canada

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University of Oregon, USA

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Aida Shahabi
York University, Canada

Melissa Ly
York University, Canada

Sarah Pullano
York University, Canada

Hyejung Kwon
York University, Canada

Program Overview

Tuesday, June 24th

- OPENING SESSION, PLENARY TALK: Vincenzo De Luca, Brock University
- RECEPTION

Wednesday, June 25th

- SYMPOSIUM I: Gene Discovery and Functional Plant Genomics
- LUNCH – CIBC Marketplace
- SYMPOSIUM II: Plant Metabolomics and Pathway Discovery
- POSTER SESSION I (odd-numbered posters) and RECEPTION

Thursday, June 26th

- SYMPOSIUM III: Metabolic engineering and Plant Synthetic Biology
- LUNCH
- SYMPOSIUM IV: Cannabis and Plant-derived Pharmaceuticals
- POSTER SESSION II (even-numbered posters) and RECEPTION
- TRIVIA NIGHT at VMV's Bar & Grill

Friday, June 27th

- YMC BREAKFAST Career Round Table
- SYMPOSIUM V: Indigenous Connections and Traditional Botanical Medicines
- SYMPOSIUM VI: Phytochemistry of Functional Foods and Human Nutrition
- MEMBERS MEETING
- LUNCH
- SYMPOSIUM VI (continued)
- GROUP PHOTO
- Free afternoon with opportunities for local excursions

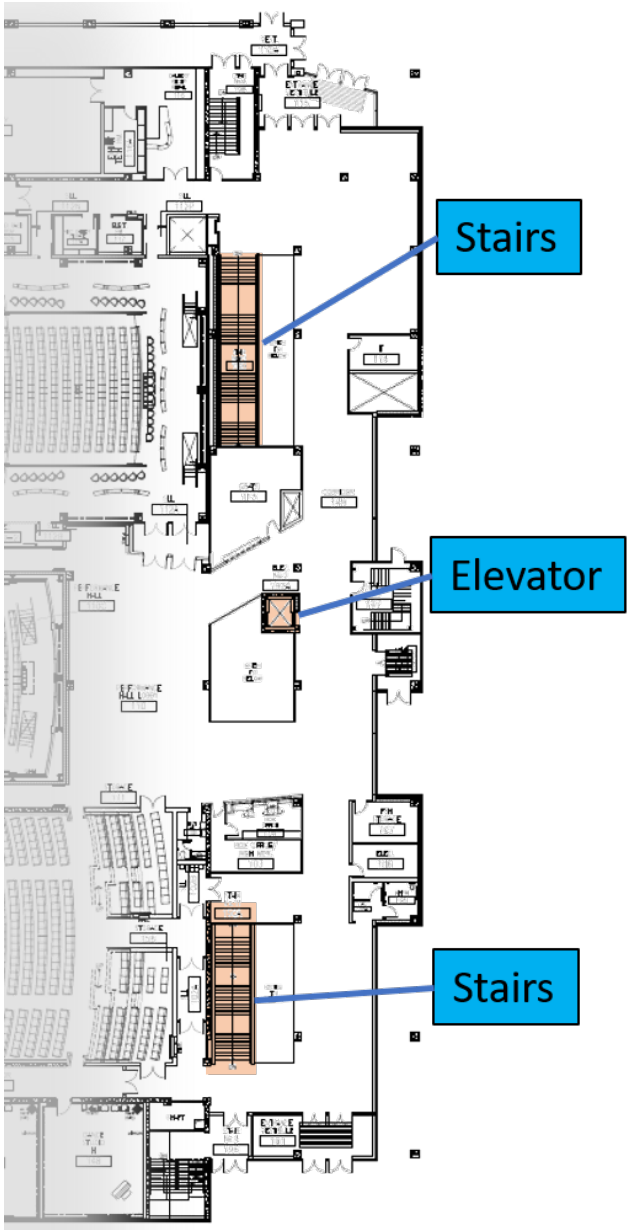
Saturday, June 28th

- SYMPOSIUM VIII: Chemical Ecology and Plant-Organismal Interactions
- PMN WORKSHOP
- LUNCH
- SYMPOSIUM VII: Plant Immunity and Microbiome Interactions
- CONFERENCE BANQUET and PRESENTATION of PIONEER, LIFETIME ACHIEVEMENT, NEISH and The PLANT JOURNAL-PSNA EARLY-CAREER AWARDS as well as TRAVEL and POSTER PRESENTATION

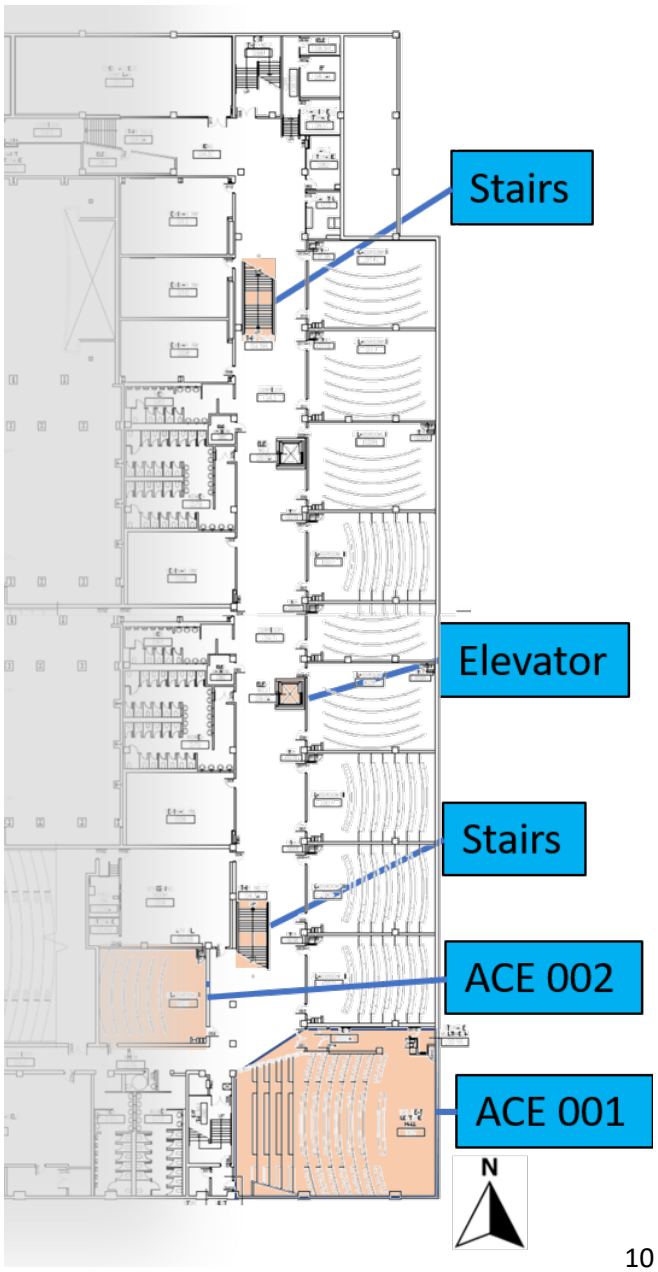
AWARDS

- ANNOUNCEMENTS of ORAL PRESENTATION AWARDS
- FINAL REMARKS and ADJOURNMENT

First floor



Basement



2025 PSNA Pioneer Award

VINCENZO DE LUCA
Professor Emeritus
Department of Biological Sciences
1812 Sir Isaac Brock Way
St. Catharines, Ontario
Canada, L2S 3A1
vdeluca@brocku.ca

Upon completion of a B.Sc. (Honors Biology, 1975) at Loyola College in Montreal, Quebec, Dr. De Luca did his M.Sc. in Plant Biochemistry (1978) in the department of Biology at Queens University in Kingston, Ontario. This was followed by a one-year research stage at McGill University (1979) and a PhD in Chemistry (1984) at Concordia University in Montreal, Quebec. In the summer of 1984, he joined the National Research Council of Canada (Plant Biotechnology Institute) as a Research Associate in Saskatoon, Saskatchewan and was appointed to a permanent position as a Research Officer in 1986. In 1989 he was appointed Associate Professor in the Biology Department of the University of Montreal with his research laboratory located in the "Institut de Recherche en Biologie Végétale" within the Montreal Botanical Gardens and he was promoted to Full Professor in 1995. In 1998 he took a three-year leave of absence to join the Novartis Agricultural Biotechnology Institute in Research Triangle Park in North Carolina as Principal Scientist II where he led a group in vegetable biotechnology. In 2001 he returned to Canada where he joined the Department of Biological Sciences at Brock University as a Tier 1 Canada Research Chair in Plant Biotechnology (CRC). This Tier 1 CRC was renewed three times and this supported part of his salary and research for the rest of his career that ended in 2022. During his career he received the C.D. Nelson Award (1991) of the Canadian Society of Plant Biologists (CSPB) that is awarded for 'outstanding research contributions to plant biology within 10 years of initiating an independent research position'. In 2010 he received the Brock University distinguished research and creativity award and in 2021 he received the Society Medal for 'outstanding career research and leadership contributions in Plant Biology primarily in Canada' from the CSPB. Most recently (2025) he was awarded the Phytochemical Pioneer Award for 'pioneering and lasting contributions shaping and influencing research in the field of phytochemistry' by the Phytochemical Society of North America (PSNA). During his career he served as President of the PSNA (1997-98) and of the CSPB (2013-15). He also served on the editorial boards of Phytochemistry (1993-2025), Molecular Plant (2015-22), The Plant Cell (2010-15), Plant Cell Reports (1995-98), Biotechnology and Bioengineering (1996-97) and Canadian Journal of Botany (1991-94). In addition to serving on several grant panels in Canada, he also served on biotechnology grant panels in the European Union and has held long-term memberships in the PSNA, CSPB and the American Society of Plant Biologists. His publication record includes >130 peer reviewed papers in international journals over a career that spanned more than 40 years.

Award Presentation: **A Trip Through the Scientific and Personal Life of Vincenzo De Luca**

The Phytochemical Pioneer Award from the PSNA reflects a recognition by my scientific peers of a lifetime of achievements in plant biology, particularly focussed on phytochemical research and dedication to our scientific society since I became a graduate student member in the summer of 1983, over 40 years ago! It is a great honor to be considered in the same category as former award winners like Norman Lewis, Richard Dixon, Eric Conn, Richard Hemmingway, Meinhart Zenk, Helen Stafford, Ulrich Matern, Frank Loewus, Neil Towers, Stewart Brown and Nikolas Fischer. The success of the PSNA since its founding 64 years ago is however rooted in the value of our wonderful annual meetings and in the friendly and cordial atmosphere that encourages and nurtures our students in scientific presentations and discussions. As I reflect on this award I have become aware that my life journey and career path could have been different if early life events would have followed a different course. I hope to highlight some of these events during our conference banquet to reflect how a positive, collaborative and altruistic motivation combined with some luck provided my career path and a life's passion for scientific research.

Friday Afternoon Excursions

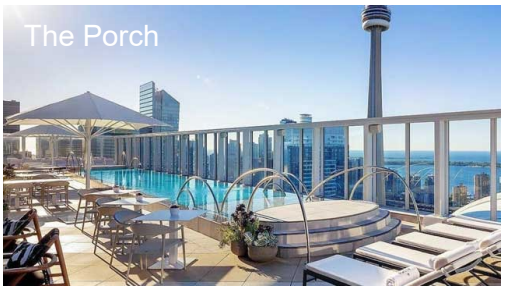
Toronto is a city full of excitement, culture, and natural beauty—all easily accessible by public transit. With York University's TTC Subway Station adjacent the PSNA 2025 conference venue, make the most of your free afternoon by visiting these local attractions:

Toronto Botanical Garden & Edwards Gardens
A serene oasis with 17 themed gardens, perfect for inspiration.
Public Transit: Line 1 to Eglinton Station, then Bus 51, 54, or 162 to Leslie & Lawrence (~45 min).

CN Tower & Ripley's Aquarium
Iconic views and a stunning aquatic tunnel with sharks and vibrant marine life.
Public Transit: Line 1 to Union Station (~45 min).

Canada's Wonderland
An amazing adventure with Rollercoasters, waterpark, and live entertainment.
Public Transit: GO Bus from York University to Vaughan Mills, then Shuttle.

Distillery District & Breweries
Bask in waterfront views at Amsterdam Brewhouse and Bellwoods Brewery, or enjoy the rooftop atmosphere at The Porch. For a complete summer experience, grab the Toronto Brew Pass.
Public Transit: Line 1 to Union Station, then walk east.



Royal Ontario Museum & Art Gallery of Ontario
World-class exhibitions of art, culture, and natural history.
Public Transit: Line 1 to Museum or St. Patrick (~40 min).

High Park
Toronto's largest park with trails and gardens.
Public Transit: Line 2 to High Park or St. Clair West, plus streetcar.

Casa Loma
A fairytale castle in midtown and a bustling historic food market downtown.
Public Transit: Subway to Dupont (Casa Loma) or Union (Market).

Harbourfront & Toronto Islands
Relax by the lake or take a ferry to the Toronto Islands for a tranquil escape.
Public Transit: Line 1 to Union Station.

Open Air Astro Fair
Discover the wonders of space at York University's first-ever outdoor astronomy festival, featuring telescopes, planetarium shows, and hands-on science booths. This one-day event will run on June 27 only if the weather permits. If interested, check out the website in the morning of June 27 for confirmation and ticket details.

Toronto is ready to welcome you with open arms. Whether you're exploring gardens, sipping craft beer, or enjoying skyline views, you're never far from an adventure. All attractions listed are accessible via TTC or GO Transit.

Enjoy your time at PSNA 2025!

Detailed Program

All events are in the Accolade East Basement Room 001, except as noted (see map on page 5 for locations of Schulich Marketplace and Schulich ELC Dining Room)

All times are in North America Eastern Standard Time (Toronto / New York / Cancún)

June 24 (Tuesday)	**CONFERENCE OPENING**
2:00 - 5:00 pm	**Conference registration** – Accolade East Basement Corridor (look for signage)
3:00 – 8:00 pm	**Poster hanging and preview** – Accolade East Basement Corridor
5:00 - 5:20 pm	**Land acknowledgements and Opening remarks** – Accolade East Room 001 **1.** Nik Kovinich *(Chair, PSNA 2025 Conference, PSNA Secretary)* ***2.*** Sangeeta Dhaubhadel *(PSNA President)* **3.** Michael Phillips *(University of Toronto - Mississauga)* **4.** Mark Lange *(Washington State University)*
5:30 - 6:30 pm	***Plenary talk:*** **Vincenzo De Luca** (*Brock University, Canada*) Forty Years of Discoveries into the assembly of Monoterpenoid indole alkaloids within Plant species
6:30 - 8:00 pm	**Welcome reception** – Accolade East Basement Corridor
June 25 (Wednesday)	**SYMPOSIA I & II, POSTER SESSION I**
7:45 - 8:45 am	**Breakfast** – Accolade East Basement Corridor
8:45 - 9:00 am	**Opening remarks/announcements** – Accolade East Room 001
	Symposium I: Gene Discovery and Functional Plant Genomics
9:00 - 9:40 am	***Keynote talk & Symposium Chair:*** **Nicholas Provart** *(University of Toronto, Canada)* Raising the BAR for Hypothesis Generation in Plant Biology: Guard Cell Drought Transcriptomes Use Case
9:40 - 9:55 am	***Short presentation 1:*** **Jie Lin** *(York University, Canada)* Identifying Missing Glyceollin Transcription factors in Soybean
9:55 - 10:10 am	***Short presentation 2:*** **Soheil Mahmoud** *(University of British Columbia, Canada)* Promoters for the Metabolic Engineering of Lavender Glandular Trichomes
10:10 - 10:25 am	***Short presentation 3:*** **Gabrielle Wyatt** *(University of California, Davis, USA)* Cytochrome P450 gene family expansion and diterpenoid biosynthesis in the bioenergy crop switchgrass (*Panicum virgatum*)
10:25 - 10:40 am	***Short presentation 4:*** **Brenda Winkel** *(Virginia Tech, USA)* Flavonoids may influence nuclear clock gene expression via interactions with peroxiredoxins in the chloroplast
10:40 - 10:55 am	***Short presentation 5:*** **Joseph Lynch** *(University of Missouri, USA)* Dual Pathways: Catalysis and Control in Cytosolic Phenylalanine Biosynthesis
10:55 - 11:10 am	***Short presentation 6:*** **Basanta Lamichhane** *(Université du Québec à Trois-Rivières, Canada)* N-methyltransferase from *leucojum aestivum* facilitates galanthamine biosynthesis via two routes
11:10 - 11:30 am	**Coffee break** – Accolade East Basement Corridor
11:30 - 11:55 am	***Arthur Neish Award presentation:*** **Satya Swathi Nadakuduti** *(University of Florida, USA)* Integrating Mitragyna omics data sets to study diversification of monoterpene indole alkaloid metabolism
11:55 - 12:10 pm	***Short presentation 7:*** **Jia Wang** *(University of Calgary, Canada)* Myc2 transcription factor identified as a central regulator of laticifer-associated metabolism in lettuce (*Lactuca sativa*)

12:10 - 12:25 pm	***Short presentation 8:*** **Josh Vermaas** *(Michigan State University, USA)* Sorgoleone Transport from Root Hairs Explored by Molecular Simulation
12:25 - 12:40 pm	***Short presentation 9:*** **Arnold William Tazon** *(Université du Québec à Trois-Rivières, Canada)* Characterization of intergenic regions for bidirectional expression of recombinant proteins in the *Chlamydomonas reinhardtii* chloroplast
12:40 - 12:55 pm	***Short presentation 10:*** **Fanfan Li** *(McGill University, Canada)* Describing the orchestrated gene shift in a mutant of Madagascar periwinkle accumulating akuammicine
1:00 - 2:00 pm	**Lunch** – CIBC Marketplace
	Symposium II: Plant Metabolomics and Pathway Discovery
2:20 - 3:00 pm	***Keynote talk & Symposium Chair:*** **Yang Qu** *(University of New Brunswick, Canada)* Expanding the Genomic and Biochemical Landscapes of Monoterpenoid Indole Alkaloid Biosynthesis
3:00 - 3:30 pm	***Invited speaker:*** **Eiji Nambara** *(University of Toronto, Canada)* Abscisic acid metabolism and environmental signals
3:30 - 3:50 pm	**Coffee break**– Accolade East Basement Corridor
3:50 - 4:20 pm	***TPJ-PSNA Award presentation*****: Ryo Yokoyama** *(University of Missouri, USA)* Metabolism of Aromatic Amino Acids and Aromatic Natural Products in Plants
4:20 - 4:35 pm	***Short presentation 11:*** **Van-Hung Bui** *(University of British Columbia, Canada)* Single-cell multi-omics in *Camptotheca acuminata*
4:35 - 4:50 pm	***Short presentation 12:*** **Sajjad Sobhanverdi** *(Université du Québec à Trois-Rivières, Canada)* Deciphering the role of CYP96T enzymes in the biosynthesis of galanthamine-type Amaryllidaceae Alkaloids from *Hippeastrum papilio*
4:50 - 5:05 pm	***Short presentation 13:*** **Jannelle Andrews** *(Washington State University, USA)* Investigating the Substrate Specificity of a Mint Monoterpenoid Double Bond Reductase
5:05 - 5:12 pm	***Flash talk 1:*** **Mwafaq Ibdah** *(Newe Yaar Research Center, Israel)* Biosynthesis of elemicin and isoelemicin in *Daucus carota* leaves
5:14 - 5:21 pm	***Flash talk 2:*** **Scott Mann** *(University of New Brunswick, Canada)* Regiospecific hydroxylase and O-methyltransferase for the biosynthesis of anticancer alkaloids in toad tree
5:23 - 5:30 pm	***Flash talk 3:*** **Lucas Busta** *(University of Minnesota Duluth)* Systematizing Decades of Phytochemical Research with Language Models
5:32 - 5:41 pm	***Flash talk 4:*** **Jue Wang** *(University of British Columbia)* Site-Selective Hydroxylation and derivatization of evodiamine via cytochrome P450s
	Poster Session I
6:00 - 7:30 pm	**Poster presentation (odd numbers)** – Accolade East Basement Corridor
7:30 - 8:30 pm	**Advisory Board Meeting** –Accolade East Room 002
June 26 (Thursday)	**SYMPOSIA III & IV, POSTER SESSION II**
7:45 - 8:45 am	**Breakfast** – Accolade East Basement Corridor
8:45 - 9:00 am	**Opening remarks/announcements**
	Symposium III: Metabolic Engineering and Plant Synthetic Biology
9:00 - 9:40 am	***Keynote talk:*** **Elizabeth Sattely & Symposium Chair** *(Stanford University, USA & HHMI)* No title provided.

9:40 – 10:10 am	***Invited speaker:*** **Yang Xu** *(University of Guelph, Canada)* Protein interactomes for lipid biosynthesis in plants
10:10 - 10:25 am	***Short presentation 1:*** **Jorge El-Azaz** *(University of Wisconsin-Madison, USA)* Atypical Phenylalanine Ammonia Lyases that Enhance Phenylpropanoid Production in Planta
10:25 - 10:40 am	***Short presentation 2:*** **Michael A. Phillips** *(University of Toronto, Canada)* A Synthetic Carbon Assimilation Shunt Boosts Terpenoid Biosynthesis in Plants
10:40 - 10:55 am	***Short presentation 3:*** **Quentin Dudley** *(University of Wisconsin-Madison, USA)* Reconstitution of monoterpene indole alkaloid biosynthesis in genome engineered *Nicotiana benthamiana*
10:55 - 11:10 am	***Short presentation 4:*** **Codruta Ignea** *(McGill University, Canada)* Unlocking the catalytic potential of yeast geranylgeranyl diphosphate synthase through metabolic-driven epistatic interactions
11:10 - 11:30 am	**Coffee break** – Accolade East Basement Corridor
11:30 - 11:55 am	***Arthur Neish Award presentation:*** **Thuy Dang** *(University of British Columbia, Canada)* Cytochrome P450s in Alkaloid Biosynthesis: Catalysts of Chemical and Structural Diversity
11:55 - 12:10 pm	***Short presentation 5:*** **Solihu Kayode Sakariyahu** *(Western University, Canada)* Deciphering the genetic regulation of proanthocyanidin biosynthesis in *Lotus corniculatus* forage
12:10 - 12:25 pm	***Short presentation 6:*** **Lee-Marie Raytek** *(McGill University, Canada)* A Bridge and an Anchor: Optimizing the isoflavonoid metabolon in a yeast chassis
12:25 - 12:40 pm	***Short presentation 7:*** **Reza Sajaditabar** *(University of British Columbia, Canada)* CRISPR/Cas9-Mediated Knockout of Borneol Diphosphate Synthase to Reduce Production of Borneol and Camphor in Lavender
12:40 - 12:55 pm	***Short presentation 8:*** **Ngoc-Mai Huynh** *(University of British Columbia, Canada)* Yeast-based Engineered biosynthesis for De novo production of Spirooxindole
1:00 - 2:00 pm	**Lunch** – CIBC Marketplace
	Symposium IV: Cannabis and Plant-derived Pharmaceuticals
2:20 - 3:00 pm	***Keynote talk:*** **Mark Lange & Symposium Chair** *(Washington State University, USA)* Follow Your Nose – The Chemistry, Biochemistry, and Genetics of Cannabis Aroma
3:00 - 3:30 pm	***Invited speaker:*** **Igor Kovalchuk** *(University of Lethbridge, Canada)* Cannabis sativa – breeding new varieties and analyzing their medicinal properties
3:30 - 3:50 pm	**Coffee break** – Accolade East Basement Corridor
3:50 - 4:20 pm	***Invited speaker*****: Zamir Punja** *(Simon Fraser University, Canada)* Challenges to Plant Health From Emerging Pathogens – A Case Study of Cannabis sativa L. (Cannabis)
4:20 - 4:35 pm	***Short presentation 1:*** **Adam Sumner** *(Virginia Tech, USA)* Cannabis Flavonoid Biosynthesis: CsAP2L1
4:35 - 4:50 pm	***Short presentation 2:*** **Amit Jaisi** *(Walailak University, Thailand)* In vitro culture of *Phlegmariurus carinatus* and production of neuroactive lycopodium alkaloid
4:50 - 5:05 pm	***Short presentation 3:*** **Tayah Bolt** *(University of California, Davis, USA)* From farm to (robot) stomach: testing beans with diverse seed coat patterns and phenolic profiles in California growing environments
5:05 - 5:40 pm	**Discussion panel: Phytochemical Products for the Treatment of Chronic Pain**
	Poster Session II

6:00 - 7:30 pm	**Poster presentation (even numbers)**
	Trivia Night
7:30 - 11:00 pm	**VMV's Bar & Grill** – 4207 Keele St Unit 1-2, North York, ON M3J 3T8

June 27 (Friday)	**SYMPOSIA V & VI**
7:45 - 8:45 am	**Breakfast** – Accolade East Basement Corridor
	YMC Breakfast Career Round Table – Accolade East Room 002
8:45 - 9:00 am	**Opening remarks/announcements**
	Symposium V: Indigenous Connections and Traditional Botanical Medicines
9:00 - 9:40 am	***Keynote talk:*** **Jonathan Ferrier & Symposium Chair** *(Dalhousie University, Canada)* Missinihe Michi Saagiig Mashkiki miinwa Midaaswi ashi-niswi Keezis (Credit River Mississauga Phytochemistry and the Thirteenth Moon)
9:40 - 9:55 am	***Short presentation 1:*** **Basil Anukam** *(Federal University of Technology, Owerri, Nigeria)* Phytochemical content and antimicrobial Properties of *Piper guineenses* leaf extract
9:55 - 10:10 am	***Short presentation 2:*** **Chieme Chukwudoruo** *(Federal University of Technology, Owerri, Nigeria)* Biochemical Effects And Anticholinesterase Activities Of Selected Leaf Extracts On Aluminium Lactate-Induced Alzheimer Disease In Albino Rats
10:10 - 10:40 am	***TPJ-PSNA Award presentation:*** **Boaz Negin** *(Boyce Thompson Institute, USA)* Catechol acetylglucose: A newly identified benzoxazinoid-regulated defensive metabolite in maize
10:40 – 11:10 am	**Members Meeting**
11:10 - 11:30 am	**Coffee break** – Accolade East Basement Corridor
	Symposium VI: Phytochemistry of Functional Foods and Human Nutrition
11:30 - 12:10 pm	***Keynote talk & Symposium Chair:*** **Diana Roopchand** *(Rutgers University, USA)* Dietary polyphenols and metabolic resilience: insights from meta-omics
12:10 - 12:25 pm	***Short presentation 1:*** **Jeongim Kim** *(University of Florida, USA)* Enhancement of crop yield and nutritional value through metabolic engineering
12:25 - 12:40 pm	***Short presentation 2:*** **Itzel Aviña** *(Tecnológico de Monterrey, Mexico)* Transcriptional Coordination Enhances Folate Biosynthesis in Common Bean Seeds During Postharvest Storage
12:40 - 12:55 pm	***Short presentation 3:*** **Andrew Rabas** *(Western University, Canada)* Ginseng Replant Disease and the Ginsenoside Connection
1:00 - 2:00 pm	**Lunch** – Schulich ELC Dining Room
2:00 – 2:15 pm	***Short presentation 4:*** **Karla Lizbeth Chávez-Rivera** *(Tecnológico de Monterrey, Mexico)* Evaluation of a Temporary Immersion System as a platform for the obtention of secondary metabolites from *Agave salmiana*.
2:15 - 2:30 pm	***Short presentation 5:*** **Frédéric Marsolais** *(Agriculture and Agri-Food Canada)* Identification and characterization of enzymes in the asparagine transamination pathway in higher plants
2:30 – 3:00 pm	**Group photo**
	Free Afternoon
3:00 - Evening	**Free Afternoon** – Enjoy exploring Toronto!

June 28 (Saturday)	SYMPOSIA VII & VIII
7:45 - 8:45 am	**Breakfast** – Accolade East Basement Corridor
8:45 - 9:00 am	**Opening remarks/announcements**
	Symposium VII: Chemical Ecology and Plant-Organismal Interactions
9:00 - 9:40 am	***Keynote talk & Symposium Chair:*** **Bao-Hua Song** *(University of North Carolina at Charlotte, USA)* Wild Soybean Meets Omics – Insights into Plant Chemical Defense
9:40 - 9:55 am	***Short presentation 1:*** **Dorothea Tholl** *(Virginia Tech, USA)* Metabolic modifications in differential host resistance of wild and cultivated carrots to above and belowground parasitic plants
9:55 - 10:10 am	***Short presentation 2:*** **Amanda Agosto Ramos** *(University of California Davis, USA)* Convergence and constraint in glucosinolate evolution across the Brassicaceae
10:10 - 10:25 am	***Short presentation 3:*** **Julia Pastor-Fernandez** *(Western University, Canada)* Detoxification of plant secondary metabolites by Two-Spotted Spider Mites as a mechanism of host adaptability
10:25 - 10:40 am	***Short presentation 4:*** **Jorden Maglov** *(Western University, Canada)* Uncovering the complexity of *Arabidopsis thaliana* defenses against *Tetranychus urticae* herbivory
10:40 - 10:55 am	***Short presentation 5:*** **John Jelesko** *(Virginia Tech, USA)* Is Poison Ivy Urushiol an Ecological Non-Functional (aka Anachronistic) Chemical Defense?
10:55 - 11:10 am	***Short presentation 6:*** **Marjori Thays da Silva** *(East Tennessee State University)* Heterospecific Pollen Tolerance in Natural Communities: The Role of Flowering Synchrony
11:10 - 11:30 am	**Coffee break**– Accolade East Basement Corridor
11:30 - 11:55 am	***Invited speaker:*** **Gustavo MacIntosh** *(Iowa State University, USA)* Manipulation of soybean defenses and induced susceptibility effected by aphids
11:55 - 12:10 pm	***Short presentation 7:*** **Elizabeth Copley** *(University of Toronto, Canada)* Harnessing cuticular waxes from poplar leaves to produce insect pheromones
12:10 - 12:25 pm	***Short presentation 8:*** **Andreea Bosorogan** *(University of Toronto, Canada)* Specialized metabolite diversity in *S. habrochaites*: Unlocking new sources of resistance against two insect herbivores
12:25 - 1:00 pm	**PMN Workshop: Gabrielle Wyatt** *(University of California, Davis, USA),* **Matt Stata** *(Michigan State University)* **and Charles Hawkins** *(Michigan State University)* The Plant Metabolic Network: A Unified Resource for the Plant Metabolism Research Community
1:00 - 2:00 pm	**Box Lunch** – Schulich ELC Dining Room
	Symposium VIII: Plant Immunity and Microbiome Interactions
2:20 - 3:00 pm	***Keynote talk & Symposium Chair:*** **Cristiana Argueso** *(Colorado State University, USA)* Phytohormonal Control of Plant Immunity and Susceptibility in Changing Climates
3:00 - 3:15 pm	***Short presentation 1:*** **Annabelle Audet** *(York University, Canada)* A Cysteine Protease Cleaves Pokeweed Antiviral Protein to Enhance Apoplastic Immune Signaling
3:15 - 3:30 pm	***Short presentation 2:*** **Ivan Monsalvo-Montiel** *(York University, Canada)* ANAC042 Regulates the Biosynthesis of Conserved and Lineage-Specific Phytoalexins in Arabidopsis
3:30 - 3:50 pm	**Coffee break** – Accolade East Basement Corridor
3:50 - 4:20 pm	***Arthur Neish Award presentation:*** **Cynthia Holland** *(Williams College, USA)* Molecular fates of anthranilate in plants
4:20 - 4:50 pm	***Invited speaker:*** **Katalin Hudak** *(York University)* Plant RNA Toxins and their Role in Signaling During Stress

4:50 - 5:05 pm	***Short presentation 3:*** **Deepak Duhan** *(Western University, Canada)* Soybean - *Sclerotinia sclerotiorum* interaction: identification of factors involved in partial resistance against Sclerotinia stem rot disease

Banquet

6:30 – 10:30 pm	**Schulich ELC Dining Room** **2025 PSNA Phytochemical Pioneer Award Presentation: Vincenzo De Luca** *(Brock U)* **Award Ceremony**

ORAL PRESENTATION BIOGRAPHIES AND ABSTRACTS

June 24 (Tuesday) **OPENING SESSION**

VINCENZO DE LUCA
Brock University
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Plenary talk: **Forty Years of Discoveries into the assembly of Monoterpenoid indole alkaloids within Plant species**

Vincenzo De Luca

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

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Monoterpenoid indole alkaloids (MIA) are among the largest, most complex and biologically active group of nitrogen containing specialized metabolites that have evolved within the Apocynaceae, Loganiaceae, and Rubiaceae families. The discovery of the pathways involved has taken place over the period of >60 years beginning with the use of radiotracer feeding experiments in the 1960's that led to the discovery that tryptophan/tryptamine and secologanin were the primary precursors for their biochemical formation. During this period extensive phytochemical studies led to the structural identification of many MIAs with biological activities in a range of medicinal plants, with hopes to produce them in vitro in cell suspension cultures derived from those plants. The difficulties associated with MIA production in cell culture systems triggered biochemical studies to identify enzymes and the pathways involved leading to some partial pathway characterizations. The molecular cloning and functional characterization of tryptophan decarboxylase and strictosidine synthase in 1989 triggered the next more rapid phase of pathway elucidation. Since then, our group has used Catharanthus roseus, the only commercial source of anti-cancer vinblastine and vincristine dimeric MIAs, as a model system for identification of the dozens of genes responsible for their formation. The development of novel methods greatly increased the speed of gene and function identification to yield the entire pathway involved, its expression in recombinant yeast and limited production of target MIAs. In addition, our group has provided information on the cell-specific organization of MIA biosynthesis, where the pathway is divided between 3 or more cell types within leaves and its expression appears to be controlled by developmental and environmental cues that may explain why such low levels of dimers accumulate in C. roseus leaves. We will present selected examples of problems encountered over a 40-year period in these studies and provide information on the solutions that led to successful pathway elucidation. The knowledge generated from these studies is now leading to rapid identification of candidate genes involved in the assembly of MIAs in diverse members of these plant families and have great implications understanding their biological roles and for commercial applications in biotechnological processes.

Nicholas Provart, Ph.D.
University of Toronto
Toronto, Ontario,
Canada

Dr. Nicholas Provart is a professor of plant cyberinfrastructure and systems biology and is chair of the Department of Cell & Systems Biology at the University of Toronto. Currently his Bio-Analytic Resource (BAR) at bar.utoronto.ca, comprising tools for coexpression analysis of publicly-available gene expression data, cis-element prediction, identifying molecular markers, generating "electronic fluorescent pictographic" (eFP) representations of gene expression patterns, and exploring protein-protein interactions in Arabidopsis and other plants, receives ~4M page views a month by researchers worldwide. He is one of the founding members of the International Arabidopsis Informatics Consortium, is past President of the Multinational Arabidopsis Steering Committee, and is teaching five MOOCs on bioinformatic methods, plant bioinformatics, and data visualization for genome biology on Coursera.org.

Keynote talk: **Raising the BAR for Hypothesis Generation in Plant Biology: Guard Cell Drought Transcriptomes Use Case**

Nicholas J. Provart

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We have developed tools, available as part of the Bio-Analytic Resource (BAR) at http://bar.utoronto.ca, for exploring large data sets from plants, to allow deeper insights into biological questions. My lab's three visual analytic tools for transcriptomic data (eFP Browser, ePlant, and eFP-Seq Browser) allow for rapid access to comprehensive gene expression compendia we have curated for identifying tissues, cell-types, or perturbations in which a gene is active or alternatively spliced. Interactions, be they protein-protein or regulatory, create networks. We have developed new tools for exploring such data, either from large collections of experimentally-supported protein-protein or protein-DNA interactions or from predicted interactions, including protein-protein interactions inferred from molecular docking studies. Our lab is also interested in generating data to help understand drought responses and we have modified the INTACT system to isolate transcriptomes from guard cells and whole leaves of plants subject to slowly developing drought. Using some of the above-mentioned tools and other bioinformatic analyses, we have been able to show that guard cells exhibit unique patterns of response both at early and later stages of drought. Thermal imaging of reverse genetic lines, prioritized in part using BAR tools, suggests the physiological importance of several differentially expressed genes in guard cells from droughted plants.

Short presentation 1: **Identifying Missing Glyceollin Transcription factors in Soybean**

Jie Lin, Ivan Monsalvo, Melissa Ly, Hyejung Kwon, Dasol Wi, Nik Kovinich*

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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. Despite their promising pharmaceutical properties, including anticancer and neuroprotective activities, glyceollins are synthesized transiently and in low quantities. Transcription factors (TFs) from the MYB (Myeloblastosis), NAC (NAM, ATAF, and CUC), and WRKY (WRKYGQK) families regulate diverse biological processes, including phytoalexin biosynthesis. 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 in the absence of 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.

Short presentation 2: Promoters for the Metabolic Engineering of Lavender Glandular Trichomes

Reza Sajaditabar and Soheil Mahmoud

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The arial parts of lavender plants are covered with glandular trichomes (GT), which produce and accumulate large quantities of mono- and sesquiterpenes as the main constituents of an economically important essential oil. These trichomes represent excellent bio-factories for the mass production of valuable terpenes through metabolic engineering. This requires the expression of the required biosynthetic genes in transformed plants. However, the ectopic expression of such transgenes often adversely affects the host plant, presumably due to the cytotoxic effects of metabolites produced in non-specialized plant cells. To address this issue, we have cloned the regulatory regions (promoters) for two strongly expressed monoterpene biosynthetic genes including linalool synthase (LinS) and cineole synthase (CinS). In this study various truncations of these promoters were used to drive the expression of the Green Fluorescent Protein (GFP) in *Lavandula latifolia*. Preliminary results indicate that some of these promoter fragments are non-specific. However, two of the larger fragments can drive the expression of GFP in GT secretory cells, and can potentially be used to produce valuable phytochemicals in lavender glandular trichomes.

Short presentation 3: Cytochrome P450 gene family expansion and diterpenoid biosynthesis in the bioenergy crop switchgrass (*Panicum virgatum*)

Gabrielle Wyatt[1], Andrew Muchlinski[1], Megumi Gutleben[1], Lucas Crispi Cuhna[1], Elissa Nakano[1], Ayna Muftic[1], Alicia Ross[2], Dean Tantillo[2], Philipp Zerbe[1]

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Given the finite availability of arable land, the continued advancement of plant-derived biofuels is vital for developing sustainable and regenerative fuel alternatives. Switchgrass (Panicum virgatum) is a native North American prairie grass valued for its high biomass production and potential as a model bioenergy crop. Its broad native range, ability to thrive on marginal soils, and high energy efficiency make it a compelling candidate for biofuel production that does not compete with food crops. As the pressures of climate change intensify, it is essential to understand the mechanisms of crop stress resilience to develop and improve crops for an increasingly uncertain future. Diterpenoids are important metabolites that plants use to mediate interactions with their environment. Some diterpenoids, such as the gibberellin phytohormones, are highly conserved across plant lineages, while others are restricted to few or even single species. These species-specific diterpenoids often serve as key defensive metabolites against biotic and abiotic stressors and are of increasing interest as crop improvement targets to minimize agricultural inputs such as pest control applications. The first committed biosynthetic step of diterpenoid biosynthesis involves modular combinations of diterpene synthases (diTPS) which convert the common substrate, geranylgeranyl pyrophosphate (GGPP), into a diverse range of scaffolds. Next, cytochrome P450 monooxygenases (P450s) decorate the hydrocarbon scaffolds through regio- and position- specific oxygenations rendering highly bioactive metabolites. Switchgrass contains large and diverse gene families of diTPS and P450s that lead to an expansive arsenal of diterpenoid metabolites. This work highlights the discovery and functional characterization of several members of the large switchgrass P450 gene family involved in diterpenoid metabolism. We investigated gene family size across four switchgrass accessions and observed the rapid expansion of the CYP99A subfamily. Several of the P450 genes show stress elicited tissue-specific expression. We functionally characterized enzymatic activity of encoded proteins by heterologously expressing CYP99A and CYP701A genes in Nicotiana benthamiana and elucidated the structures of the resulting metabolite products using nuclear magnetic resonance (NMR) spectroscopy. The switchgrass P450s tested displayed high substrate and product promiscuity resulting in several new diterpenoid structures and prompted the examination of structure–function relationships driving the diversification of these switchgrass-specific diterpenoid metabolites. Together, this work advances our understanding of diterpenoid metabolism and its potential physiological roles in switchgrass.

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Short presentation 4: **Flavonoids may influence nuclear clock gene expression via interactions with peroxiredoxins in the chloroplast**

W. Keith Ray[1,4], Evan S. Littleton[2,4], William Hanrahan[3], Anne Brown[3], Richard F. Helm[1,3,4], Shihoko Kojima[2,4], and Brenda S.J. Winkel[2,4]

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Flavonoids are plant specialized metabolites with a wide array of biological functions including in defense, cell signaling, and protection from reactive oxygen species. We have recently reported that flavonoids may modulate the expression of genes of the core circadian clock, with Arabidopsis flavonoid-deficient mutants (tt4 and tt7) exhibiting altered clock gene expression as well as elevated circadian amplitude of the clock gene reporter, TOC1:LUC. More recent efforts to uncover the underlying mechanism indicate that dihydroxy B-ring flavonoids, in particular, are responsible for the effects on clock gene expression. We also found that the elevated amplitude of TOC1:LUC expressioin correlates with the elevated cellular H2O2 content of flavonoid-deficient seedlings, and that this can be rescued by reducing the production or accumulation of reactive oxygen species. This suggests that flavonoids affect clock gene expression by influencing H2O2 levels. To explore possibilities beyond simply serving as antioxidant scavengers, an affinity pulldown assay was performed using immobilized rutin, a quercetin glycoside that is highly abundant in Arabidopsis, against protein extracts from young seedlings. Among the 152 high-confidence binding targets identified in this experiment were peroxiredoxin A and B (AtPrxA/B), plastid-localized antioxidant enzymes essential for maintaining redox homeostasis, the homologs of which are known targets of flavonoid binding in mammals. Interaction of quercetin with AtPrxA was confirmed by isothermal titration calorimetry, while molecular docking analysis indicated that quercetin and rutin both bind to the AtPrxA active site, with binding energies of -21.6 and -28.5 kcal/mol, respectively. Compartmentspecific [Ca++] biosensors showed that flavonoid-deficient lines have lower levels of rhythmic Ca++ in chloroplasts, as well as a smaller spike in chloroplast [Ca++] at the lightdark transition. These findings suggest that flavonoids may influence core clock gene expression by modulating retrograde signaling triggered by rhythmic H2O2 and/or Ca++ levels, potentially through interactions with chloroplast antioxidant regulators such as the peroxiredoxins.

Short presentation 5: **Dual Pathways: Catalysis and Control in Cytosolic Phenylalanine Biosynthesis**

Joseph H Lynch[1]

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In plants, the aromatic amino acids phenylalanine, tyrosine, and tryptophan serve not only as essential building blocks of proteins, but also as precursors to thousands of primary and specialized metabolites with diverse roles in plant survival and fitness. It is well established that a complete set of all enzymes necessary to synthesize these amino acids from phosphoenolpyruvate and erythrose-4-phosphate are present in the plastids. However, classic biochemical evidence was used to justify the original formulation of the “Dual Pathway Hypothesis,” which posited that an extraplastidial pathway operates in parallel to contribute to total cellular aromatic amino acid production. While this hypothesis failed to gain widespread support as the dominance—and perceived exclusivity—of the plastidial pathway became evident, recent evidence has led us to revisit the Dual Pathway Hypothesis. Here I describe efforts to discover and characterize extraplastidial enzymes involved in aromatic amino acid biosynthesis, as well as factors involved in differential regulation of plastidial and cytosolic pathways. The findings of this research have implications not just for our understanding of fundamental plant function, but also for metabolic engineering of plant-based platforms for sustainable phytochemical production.

Short presentation 6: **N-Methyltransferase From Leucojum Aestivum Facilitates Galanthamine Biosynthesis Via Two Routes**

Basanta Lamichhane[1], Sarah-Eve Gélinas[1], Natacha Merindol[1], Ricard Simon[1], Hugo Germain[1,2], Isabel Desgagné-Penix[1,2*]

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Galanthamine, a pharmaceutically valuable Amaryllidaceae alkaloid (AA), is a key treatment for early-stage Alzheimer’s disease. Its biosynthesis originates from 4´-*O*-methylnorbelladine (4´OM), a key precursor for numerous AAs. Cytochrome P450-mediated C–C coupling converts 4’OM into *N*-demethylnarwedine, which can follow two possible distinct routes: (i) reduction to norgalanthamine, followed by *N*-methylation to form galanthamine, or (ii) *N*-methylation to narwedine, which is subsequently reduced to galanthamine. To elucidate the key *N*-methylation steps in this pathway, we identified three candidate *N*-methyltransferases (NMTs) from the transcriptome of *Leucojum aestivum*: two belonging to the tocopherol methyltransferases (TMTs) family and one to the coclaurine NMT family. Subcellular localization studies revealed that TMT1 is chloroplastic, TMT2 is localized to the Golgi complex, and NMT is dual localized in the ER and cytosol. "Functional characterization in *Nicotiana benthamiana* via agroinfiltration, using TMT1, TMT2, and NMT in the presence of N-demethylnarwedine or norgalanthamine, revealed that TMT1 exhibits the highest catalytic efficiency. TMT1 could catalyze the N-methylation of N-demethylnarwedine to narwedine and norgalanthamine to galanthamine and NMT displayed similar activity in in-vitro assays.

These findings identify TMT1 and NMT as promiscuous NMT involved in multiple steps of galanthamine biosynthesis, offering new insights into AA metabolic pathways and potential strategies for enhancing galanthamine production through metabolic engineering.

Satya Swathi Nadakuduti, Ph.D.
University of Florida
Gainesville, Florida,
USA

Dr. Satya Swathi Nadakuduti is an Assistant Professor in the Department of Environmental Horticulture at the University of Florida, where she leads an interdisciplinary research program at the intersection of genetics, biochemistry, and molecular biology. Her work focuses on understanding plant specialized metabolism, exploring the chemical diversity of plant-derived compounds, especially those with pharmaceutical value and agricultural potential. Dr. Nadakuduti earned her Bachelors in Horticulture in India, followed by a Masters in plant genetics from Leibniz Universität Hannover, Germany. She completed her Ph.D. in Plant Breeding, Genetics, and Biotechnology from Michigan State University under the mentorship of Dr. Cornelius Barry. She then pursued postdoctoral research with Drs. Robin Buell and David Douches before establishing her lab at the University of Florida in 2020. In addition to research, Dr. Nadakuduti is actively involved in teaching and mentoring, training the next generation of plant scientists in the field of plant natural products.

Arthur Neish Award presentation: **Integrating Mitragyna omics data sets to study diversification of monoterpene indole alkaloid metabolism**

Satya Swathi Nadakuduti[1,2*], Larissa C. Laforest[1], Tuan-Anh M. Nguyen[3], Abhisheak Sharma[4], Christopher R. McCurdy[5], Thu-Thuy Dang[3]

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Monoterpenoid indole alkaloids (MIAs) represent a diverse family of specialized plant metabolites, including FDA-approved chemotherapeutics like vinblastine, and vincristine. The Mitragyna genus within the Rubiaceae (coffee family) produces pharmaceutically significant MIAs and spirooxindole alkaloids. We sequenced and assembled high-quality chromosome scale genomes of four Mitragyna species/chemotypes. We conducted comparative genomics analyses including comprehensive synteny and phylogeny across the Gentianales to investigate the role of genome structure in the diversification of the post-strictosidine pathways in MIA biosynthesis. We generated RNA-seq and corresponding targeted metabolite datasets from various MIA accumulating tissues and developmental stages to facilitate genome mining and gene discovery, particularly for MIAs and oxindole alkaloids, laying the groundwork for pathway discovery and synthetic biology applications. Future work will leverage comparative genomics to characterize genome-wide variations responsible for the evolution of the specialized MIAs.

Short presentation 7: **Myc2 transcription factor identified as a central regulator of laticifer-associated metabolism in lettuce (*Lactuca sativa*)**

Jia Wang[1], Eman M. Salama[1], Connor L. Hodgins[1], Justin B. Nichol[1], Marcus A. Samuel[1] and Dae-Kyun Ro[1,*]

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Lettuce (Lactuca sativa, Asteraceae) is a major vegetable crop in North America and an ideal candidate for indoor cultivation, contributing to its prominence in urban agriculture. Beyond its agronomic importance, lettuce serves as a model system for the Asteraceae, the largest plant family encompassing over 32,000 species. Its annual, self-pollinating nature, short life-cycle (~4 months), and robust transformation protocols make it highly amenable to both forward and reverse genetic studies. Modern genome editing tools, such as CRISPR/Cas9, further enable efficient functional genomics and trait improvement. A unique feature of lettuce is the differentiation of laticifers from cambium. The laticifers produce latex, which contains two major metabolites: natural rubber (cis1,4-polyisoprene) and sesquiterpene lactones. Despite their importance, the molecular mechanisms underlying laticifer development and metabolism remain poorly understood.

To address this, transcriptomic analysis was conducted comparing laticifers and whole stem tissue, identifying Myc2 as one of the most highly expressed transcription factors in laticifers. To investigate its role, a CRISPR/Cas9-mediated knockout of Myc2 was generated through tissue culture. The resulting myc2 mutant line exhibits slower growth compared to wild-type but shows no major developmental abnormalities. Notably, stem incisions in myc2 mutants fail to exude latex, in contrast to the characteristic milky outflow in wild-type plants. Histological analysis revealed that laticifers are present in the mutant but display reduced cell-wall degradation between laticifer cells. These findings indicate that Myc2 is not required for laticifer cell identity but plays a critical role in establishing laticifer-specific metabolic function. Differential expression analysis revealed that several hundred genes are downregulated in the myc2 mutant, and metabolite profiling showed a complete absence of natural rubber. Disrupted pathways include isoprenoid and fatty acid metabolism. Together, our results suggest that Myc2 is a key transcriptional regulator of laticifer metabolism, likely acting downstream of a yet unidentified master regulator of laticifer cell fate.

Short presentation 8: **Sorgoleone Transport from Root Hairs Explored by Molecular Simulation**

Saad Raza[1], Sakiko Okumoto[2], and Josh V. Vermaas[1]

[1]*Plant Research Laboratory and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA*

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Competition for soil nutrients and water with other plants fosters competition within the biosphere for access to these limited resources. The roots for the common grain sorghum produce multiple small molecules that are released via root exudates into the soil to compete with other plants. Sorgoleone is a compound that suppresses weed growth near sorghum by acting as a quinone analog and interferes with multiple metabolic processes, including photosynthesis. Since sorghum also grows photosynthetically and may be susceptible to sorgoleone action if present in tissues above ground, sorgoleone needs to be excreted efficiently. However, since the P450 enzymes that synthesize sorgoleone are intracellular, the release mechanism for sorgoleone remains unclear. In this study, we conducted an *in silico* assessment for sorgoleone and its precursors to passively permeate biological membranes. When an organic sorgoleone phase is already present, passive permeation of extraction of sorgoleone into the membrane is relatively favorable, but there was no clear mechanism to create an organic phase in the first place. Our collaborators have identified a putative ABC transporter for sorgoleone in sorghum. Through molecular simulation of this transport protein in the context of a membrane bilayer, we find that sorgoleone can accumulate both on the ATP domain, as well as the exit site on the transport protein. Thus, the action of this transporter may yield an initial sorgoleone droplet that can grow and excrete sorgoleone into the soil that grows through passive diffusion.

Short presentation 9: **Characterization of intergenic regions for bidirectional expression of recombinant proteins in the *Chlamydomonas reinhardtii* chloroplast**

Arnold William Tazon[1], Natacha Mérindol[1], Elisa Ines Fantino[1], Fatma Meddeb-Mouelhi[1 2], and Isabel Desgagné-Penix[1 2]

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Chloroplasts have emerged as promising intracellular factories for engineering complex metabolic pathways in microalgae. However, the efficiency of multigene engineering is limited by the lack of characterized regulatory elements. In this study, we used a comprehensive semi-rational analysis of intergenic regions (IRs) between divergent genes in the chloroplast genome of *Chlamydomonas reinhardtii* to develop a bidirectional promoter (BDP)-based engineering strategy. Three selected BDP were isolated and inserted by gene gun bombardment into the *psbN-trnE2* locus of the chloroplast genome to express *mVenus* and *tdTomato* in opposite orientations. Using RT-qPCR, flow cytometry, confocal microscopy, and immunoblotting, we demonstrate that *BDP1* exhibits the highest bidirectional activity for both transgenes at the protein level, whereas *BDP2* shows more balanced activity at the transcriptional level, and *BDP3* displays more limited activity. Additionally, we investigated the effect of methyl jasmonate (MeJA), a plant hormone known to activate specialized metabolite pathways, on *BDP1*-mediated gene expression. MeJA treatment significantly increased tdTomato fluorescence, while mVenus fluorescence remained unchanged. These findings highlight the potential of natural BDPs, particularly *BDP1*, as versatile tools for multigene engineering in *C. reinhardtii* and other microalgae, while providing a baseline for understanding the fundamental transcriptional mechanisms in the chloroplast. This study also opens new avenues for optimizing chloroplast gene expression using environmental or chemical stimuli like MeJA.

Short presentation 10: **Describing the orchestrated gene shift in a mutant of Madagascar periwinkle accumulating akuammicine**

Fanfan Li[1], Vincenzo De Luca[2], Mehran Dastmalchi[1]

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Catharanthus roseus is a medicinal plant that produces an abundance of monoterpenoid indole alkaloids (MIAs), notably the anticancer compounds vinblastine and vincristine. While the canonical pathway is resolved at the enzymatic level, the mechanisms controlling many lateral branches of MIA biosynthesis remain largely unknown. Here, we describe an EMS-generated mutant of *C. roseus*, which accumulates high levels of MIAs, including akuammicine, a promising analgesic compound. In addition to its unique chemical profile, we observed a striking lesion-mimic mutant (LMM) phenotype. Spontaneous and sporadic lesions appeared on the leaves and were the primary site of MIA enrichment. To pinpoint the causal mutation, phenotype segregation via reciprocal crosses was performed. The LMM and high-MIA phenotypes co-segregated in the F_2 generation in a 3:1 ratio. We followed a bulk-segregant analysis sequencing (BSA-seq) approach, using the F_2 WT-like and Mu-like populations and the parental lines. Thereby, the locus of interest was pinpointed to chromosome 5. In parallel, we conducted a transcriptomics analysis, comparing WT to mutant healthy (hlth) and lesion (les) sectors to reveal the gene network underlying the chemical and physical phenotypes. Over 8,000 differentially expressed genes (DEGs) were identified, and a subset of 700 DEGs was unique to hlth and les samples. Many defence-related genes were upregulated in the mutant libraries, indicating an overall upheaval of the associated pathways. From these DEGs, we have identified known players in MIA metabolism, such as CrMATE1 (a vacuolar secologanin uptake transporter). This multi-omics study can help unravel the link between core metabolism, especially its plastidic components, and specialized defences, including MIA biosynthesis.

June 25 (Wednesday) **SYMPOSIUM II: Plant Metabolomics and Pathway Discovery**

Yang Qu, Ph.D.
University of New Brunswick
Fredericton, New Brunswick, Canada

Dr. Yang Qu completed his Ph.D. in Biochemistry under the supervision of Dr. Dae-Kyun Ro at the University of Calgary, following a B.Sc. in Life Sciences from Peking University, China. His doctoral research focused on the characterization of a heterodimeric prenyltransferase for the biosynthesis of natural rubber, a long-chain cis-polyisoprenoid exceeding 1 million g/mol. He then joined Dr. Vincenzo De Luca's group at Brock University, where he contributed to elucidating the biosynthetic pathway of the anticancer drug vinblastine, an iconic monoterpenoid indole alkaloid (MIA) that has been the subject of several decades of intensive research. Dr. Qu's identification of 11 enzymes completing the 30-step biosynthesis of anhydrovinblastine represents a landmark achievement in the study of MIA and plant specialized metabolism.

Now leading his research group at the University of New Brunswick, Dr. Qu continues to explore the biochemistry, genomics, and synthetic biology of MIAs. His recent work includes the full elucidation and yeast-based reconstitution of the biosynthetic pathway for the antiarrhythmic MIA ajmaline, the biosynthesis of mitragynine and its heterologous production in yeast, the biochemical and genomic basis of MIA C3-epimerization, and the functional characterization of numerous MIA-diversifying enzymes. These include

many transferases, oxidoreductases and a unique dioxygenase that catalyzes an unusual redox-neutral reaction.

Keynote talk: **Expanding the Genomic and Biochemical Landscapes of Monoterpenoid Indole Alkaloid Biosynthesis**

Jaewook Hwang, Scott Galeng Alexander Mann, Hannah Tran, Mathew Bailey Richardson, Jorge Jonathan Oswaldo Garza-Garcia, Mahamadamin Shasavarani, Jacob Owen Perley, Yang Qu

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Monoterpenoid indole alkaloids (MIAs) rank among the most structurally intricate and pharmacologically powerful plant-derived natural products. Recent milestones in the field include the full biosynthetic reconstruction of cornerstone MIAs such as the 30-step anhydrovinblastine and the 20-step ajmaline pathways. In parallel, advances in single-cell sequencing have offered a modern lens on the spatial compartmentalization of MIA biosynthesis, expanding upon decades of classical cellular studies.

Building on this foundation, our research delves into the multi-omics landscape of MIA-producing species across four plant families. By integrating pangenomic analyses with tissue-specific transcriptomes and metabolomes, we uncover previously cryptic biosynthetic gene clusters, novel enzymes, and lineage-specific variations. This talk will highlight recent discoveries from our group that expand the genomic and biochemical map of MIA biosynthesis, laying the groundwork for future metabolic engineering strategies aimed at sustainable and scalable production of high-value alkaloids.

Invited speaker: **Abscisic acid metabolism and environmental signals**

Saad Hussain[1], Mohammad Ismu Daud[1], Christine Nguyen[1], Hiraku Suda[2], Masatsugu Toyota[2], Keiko Yoshioka[1], and Eiji Nambara[1]

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Abscisic acid (ABA) is a plant hormone that regulates plant growth and physiology to adjust to environmental changes, especially water status. ABA levels tend to be high under water deficit conditions, while they are low under water-sufficient conditions. CYP707A is an ABA 8'-hydroxylase that catalyzes the committed step in ABA catabolism, converting ABA to 8'-hydroxyABA, which non-enzymatically isomerizes to phaseic acid, a less bioactive metabolite. CYP707A is critical for decreasing ABA levels under various water-sufficient conditions. When Arabidopsis plants are exposed to high humidity (HH) conditions, the expression of CYP707A3 is induced within 10 minutes, followed by a decrease in ABA levels. We found that HH induces an increase in cytosolic calcium concentrations within a few minutes, activating calcium signaling to induce the transcription of CYP707A3. Calcium signaling also impacts the metabolism of ABA and other hormones in response to environmental changes, triggering various downstream physiological responses. We will present our recent results depicting spatial and temporal regulation of plant hormone metabolism under changing environments.

Ryo Yokoyama, Ph.D.
University of Missouri
Columbia, Missouri, USA

TPJ-PSNA Award presentation: **Metabolism of Aromatic Amino Acids and Aromatic Natural Products in Plants**

Ryo Yokoyama[1]

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Plants produce a diverse array of aromatic natural products using CO2 as a solo carbon source through photosynthesis. These aromatic phytochemicals play crucial roles in plant development and adaptation (e.g., lignin, phytohormones, pigments, and defense compounds) and are also utilized in our society as nutraceuticals, pharmaceuticals, and biomaterials. In plants, a significant portion of photosynthetically fixed carbon is allocated to the biosynthesis of aromatic amino acids - tyrosine, phenylalanine, and tryptophan - to synthesize massive amounts of aromatic amino acid-derived natural products. However, we have limited knowledge about how plants convert photosynthates into stable forms of aromatic phytochemicals and utilize them for plant growth and fitness. This knowledge gap has been a bottleneck in enhancing the production of high-value compounds in plants and designing new engineered plants with resilience to harsh environmental stresses. This talk aims to highlight my recent efforts to understand and engineer the metabolism of aromatic amino acids and their derivatives with the focus on phenylalanine and phenylalanine-derived specialized metabolites including anthocyanin. I will provide a potential framework for developing strategies to enhance plant chemical production and resilience.

Short presentation 1: **Single-cell multi-omics in Camptotheca acuminata**

Van-Hung Bui[1], Chenxin Li[2,3], Hai-Anh Vu[5], Lorenzo Caputi[5], Robin Buell[2,3,4,] Thu-Thuy Dang[1]

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Monoterpene indole alkaloids (MIAs) are a diverse class of plant natural products highly valued for their pharmaceutical properties. Camptothecin, an important MIA from Camptotheca acuminata, serves as a precursor to the anticancer drugs topotecan (Hycamtin®) and irinotecan (Camptosar®). In this study, we integrated single-cell RNA sequencing with single-cell metabolomics to investigate how camptothecin metabolism is spatially organized. Our results demonstrated that the camptothecin biosynthetic pathway is partitioned across at least two distinct cell types in the stem, supported by complementary single-cell metabolomic data. Additionally, we quantified six key intermediates of camptothecin biosynthesis in the individual meristem, stem, and leaf cells, revealing significant variability in metabolite concentrations, and special compartmentalization of camptothecin biosynthesis. Single-cell RNA sequencing also identified novel gene candidates potentially involved in camptothecin biosynthesis, underscoring the pathway's cell-type-specific organization. This single-cell multi-omics approach provides critical resources and establishes a robust methodological framework for exploring the spatial complexity of camptothecin biosynthesis at single-cell resolution.

Short presentation 2: **Deciphering the role of CYP96T enzymes in the biosynthesis of galanthamine-type Amaryllidaceae Alkaloids from Hippeastrum papilio**

Sajjad Sobhanverdi[1], Karen Cristine Gonçalves dos Santos[1], Natacha Merindol[1], Hugo Germain[1,2], Isabel Desgagné-Penix[1,2]

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Amaryllidaceae alkaloids (AA) are well-known specialized metabolites with versatile antitumor, antiviral, and anti-acetylcholinesterase activities. Among the more than 650 reported AAs, galanthamine has gained particular interest due to its widespread use in treating mild symptoms of Alzheimer's disease. While the biosynthetic pathway of galanthamine has been partially elucidated, key steps remaine unresolved—particularly the cyclization of the central intermediate, 4'-O-methylnorbelladine (4'OMN), which leads to the formation of distinct AA scaffolds, including galanthamine-, lycorine-, and crinine-type of alkaloids. These cyclization reactions are catalyzed by cytochrome p450 enzymes (CYP) with distinct regioselectivities, including para-ortho', parapara', and ortho-para'. In this study, we identified specific CYP96T enzymes capable of converting the 4OMN into nornarwedine, the precursor scaffold of galanthamine-type AA, via a para-ortho' C-C phenolic coupling. To determine whether additional CYP96T isoforms could catalyze such steps, we performed co-expression network analysis and correlated metabolite abundance with gene expression patterns in Hippeastrum papillio, a species known for its high galanthamine production. The results suggest the involvement of several CYP96T candidate enzymes in the cyclization of 4OMN. Phylogenetic analysis revealed that these CYP96T candidates belong to the oxidoreductase family and possess a different oxygen-binding motif, A(Q/G)X(NTQ), compared to the superfamily CYP86. Structural modeling and docking simulations were conducted to identify key residues within the substrates-binding sites, providing insights for engineering enzymes increased specificity and catalytic efficiency. The functional activities of the identified CYP96T candidates will be verified through heterologous expression in yeast. This study provides new insights into the enzymatic mechanism underlying galanthamine biosynthesis and lays the foundation for targeted metabolic engineering strategies to enhance its production through synthetic biology approaches.

Short presentation 3: **Investigating the Substrate Specificity of a Mint Monoterpenoid Double Bond Reductase**

ANDREWS, Jannelle A., NARAYANAN, Srividya., LANGE, Iris., and LANGE, B. Markus

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The monoterpenoid pathway of mint produces metabolites that constitute the bulk of extracted essential oil, widely used in consumer products. Menthone and menthol are key monoterpenoids of peppermint (*Mentha × piperita* L.), where a double bond reductase, initially termed pulegone reductase (PulR), catalyzes a critical step in menthone formation. PulR belongs to the medium-chain dehydrogenase/reductase (MDR) superfamily, which has broad phylogenetic distribution. Other mint species and accessions accumulate different oil constituents, such as piperitenone and piperitenone oxide, the biosynthesis of which likely also involves a double bond reduction, and we thus investigated if PulR can accept monoterpenoids other than pulegone. First, PulR was assayed with a diverse panel of monoterpenoid substrates varying in three-dimensional structure and double bond placement. Second, kinetic assays were employed to compare the catalytic efficiency and selectivity with all substrates that gave measurable activities in the first set of assays. Third, molecular docking was used to further explore the binding of different substrates in the active site pocket of PulR. Fourth, residues with potential roles in substrate recognition were subjected to alanine scanning mutagenesis, which involves systematically replacing each non-alanine amino acid residue of interest, one at a time, with alanine, an amino acid lacking a side chain to interact with potential ligands. The impacts of this substitution were then evaluated in the context of their contribution to substrate binding. Rules for the double bond positioning in a potential monoterpenoid substrate and the determinants of specificity for catalysis by PulR will be discussed.

Flash Talk 1: **Biosynthesis of elemicin and isoelemicin in Daucus carota leaves**

Xing-Qi Huang[1,2], Mosaab Yahyaa[3], Prasada Rao Kongala[3], Itay Maoz[1,2], Natalia Dudareva[1,2,4], Mwafaq Ibdah[3]

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Volatile phenylpropenes comprise one of the largest groups of plant phenylalaninederived volatiles that not only possess ecological roles but also exhibit numerous pharmacological activities. Despite their wide distribution in the plant kingdom, biosynthesis of only a small subset of these compounds has been discovered. Here, we elucidated yet unknown steps in the biosynthesis of isoelemicin and elemicin using carrot (Daucus carota subsp. sativus), which produces a wide spectrum of volatile phenylpropenes, as a model system. Comparative transcriptomic analysis combined with metabolic profiling of two carrot cultivars producing different spectrums and levels of phenylpropene compounds revealed that biosynthesis of isoelemicin and elemicin could proceed via the (iso)eugenolindependent pathway, which diverges from the lignin biosynthetic pathway after sinapyl alcohol. Moreover, in planta results showed that two different NADPHdependent reductases, a newly identified 5-methoxy isoeugenol synthase (DcMIS) and previously characterized (iso)eugenol synthase (DcE(I)GS1), both of which use sinapyl acetate as a substrate, are responsible for the biosynthesis of immediate precursors of isoelemicin and elemicin, respectively. In contrast to penultimate reactions, the final steps in the formation of these phenylpropenes are catalyzed by the same newly characterized methyltransferase, S-adenosyl-Lmethionine:5-methoxy(iso)eugenol O-methyltransferase, that methylates the parahydroxyl group of their respective precursors, thus completing the (iso)eugenolindependent route for the biosynthesis of isoelemicin and elemicin.

Flash Talk 2: **Regiospecific hydroxylase and O-methyltransferase for the biosynthesis of anticancer alkaloids in toad tree**

Scott Galeung Alexander Mann*, Matthew Bailey Richardson*, Rochelle Nicola Young, Ghislain Deslongchamps, and Yang Qu

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**Equal contributions.*

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Monoterpenoid indole alkaloids (MIAs) are a structurally diverse class natural products with significant medicinal properties. Bis-MIAs, such as chemotherapeutic vinblastine and anti-autophagic conodurine, are synthesized through enzymatic coupling of monomeric MIAs, often requiring specific modifications to activate reactive centers. In this study, we report the identification and characterization of a regiospecific enzyme pair, coronaridine 11-hydroxylase (TeC11H) and 11-hydroxycoronaridine *O*-methyltransferase (TeHCOMT), from *Tabernaemontana elegans* (toad tree). The C11-methoxylation of coronaridine activates C10 and C12 on the indole for subsequent coupling with a vobasinyl monomer, representing a critical transformation in the biosynthesis of a series of 11-methoxycoronaridine derived bis-iboga-vobasinyl MIAs in *T. elegans* root with potent anticancer and anti-autophagy activities. Biochemical analysis and homology modeling of TeC11H and TeHCOMT, along with their *Tabernanthe iboga* (iboga) homologs for coronaridine C10-methoxylation, reveal key residues at their highly similar active sites responsible for distinct regioselectivity, demonstrating how coronaridine is oriented to favour C11 or C10 methoxylation. Our discovery sheds light on the coordinated specification of active sites in metabolically linked enzymes during evolutionary adaptation in different lineages. TeC11H and TeHCOMT represent a valuable tool for metabolic engineering, offering new opportunities to biosynthesize anticancer alkaloids and explore the therapeutic potential of novel bis-MIAs.

Flash Talk 3: **Systematizing Decades of Phytochemical Research with Language Models**

Alan R. Oyler and Lucas Busta

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Language models are emerging as powerful tools for a wide array of tasks, with a particularly promising role in processing scientific literature. Scientific articles compile results from decades, if not centuries, of effort by researchers worldwide. In phytochemistry, one form in which these efforts manifest is as data describing the occurrence of specific phytochemicals across the plant tree of life. Studying the phylogenetic distribution of these occurrences is clearly important, as such studies have helped uncover, for example, (i) mechanisms by which lineage-specific metabolic pathways evolve and (ii) variant metabolisms with agricultural or biotechnological importance. Despite the value of understanding plant chemical distributions and the tools that have been developed for searching, archiving, and systematizing plant chemical distribution data, streamlined processing of such scientific literature remains a challenge. Recently, we have been exploring the potential for new, transformer-based language models to systematize plant chemical occurrence data on large scales. This presentation will first briefly touch on our published work in which we demonstrate that large language models can accurately evaluate compound occurrence information in unstructured text (scientific abstracts). Next, I will detail our recent, unpublished experiments with small language models – tools that can be run with personal computing resources. These studies show that such models can accurately extract phytochemical occurrence data from unstructured text. As a proof-of-concept for larger database assembly, I will also report the results from our application of the small language model pipeline to a dataset of 42,000 abstracts. Finally, I will also cover important findings from the benchmarking of our small language model pipeline that other researchers will need to be aware of if they wish to use language models to create their own large, structured datasets of phytochemical information.

Flash Talk 4: **Site-Selective Hydroxylation and derivatization of evodiamine via cytochrome P450s**

Jue Wang[1], Brooke D. Kwan[1], Anh Nguyen[1], Hoa HT Nguyen[1], Thu-Thuy T. Dang[1]

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Evodiamine is an indole-quinazoline alkaloid derived from the Evodiae fructus (Wuzhuyu in Chinese), a traditional medicine with a long history of being used. Evodiamine shows numerous bioactivities, including anti-inflammation, antibacterial, myocardial protection, anti-obesity and analgesic. Besides, evodiamine has anticancer activity by blocking cell cycle, promoting cell apoptosis, promoting autophagy, and inhibiting tumor invasion and metastasis via the various signaling pathways: phosphatidylinositol 3-kinase (PI3K)-serine/threonine kinase (AKT), NF-κB signaling pathway because its specific L-shaped conformation similar to the structure of topoisomerase (Topo) inhibitors such as Camptothecin. However, the low solubility, poor bioavailability, and potential toxicity limit its clinical application. Engineering structural diversity can increase the hydrophilicity and drug-like properties. However, it is challenging to achieve high regio- and stereoselectivity via chemical synthesis due to its inert C-H bonds. Alternatively, hydroxylation of the inert C-H bonds on the scaffold via enzymes such as cytochrome P450s could provide a solution for late-stage C-H functionalization of evodiamine. To achieve this, we mined the database for new cytochrome P450s that can accept evodiamine, and discovered three new enzymes capable of site-selective hydroxylation at the C-3 and C-10 positions of evodiamine. Hydroxylated evodiamine analogues can then be used to produce more diverse structure via organic synthesis. This chemo-enzymatic approach allows us to generate a small library of structurally novel analogs with potentially improved pharmacological properties.

Elizabeth Sattely
Stanford University & Howard Hughes Medical Institute
Stanford, California,
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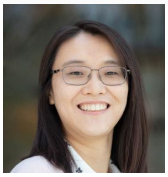

Yang Xu
University of Guelph
Guelph, Ontario, Canada

Dr. Yang Xu is an Assistant Professor in the Department of Molecular and Cellular Biology at the University of Guelph, Canada. She received her Ph.D. in Plant Science from the University of Alberta, where she also worked as a postdoctoral fellow. She later continued her research as a Postdoctoral Research Associate at Michigan State University before joining the University of Guelph. Dr. Xu's research focuses on lipid metabolism in plants and microalgae, with the goal of developing biotechnological strategies to engineer and produce high-value designer oils.

Invited speaker: **Protein interactomes for lipid biosynthesis in plants**

Yang Xu*
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Acyl lipids play a wide range of vital roles in plants, functioning both as energy-rich storage compounds (oils) and as essential components of cellular membranes involved in key biological processes, including photosynthesis. Plant lipids, particularly oils, are valuable agricultural commodities and renewable sources of high-energy products for food, biofuel, and industrial applications. Understanding the metabolic pathways of lipid production in plants offers a promising avenue to optimize photosynthesis, enhance oil yields, and improve stress tolerance, ultimately boosting agricultural productivity. While numerous proteins involved in plant lipid metabolism have been identified over the past few decades, our knowledge of the dynamic protein interactomes that govern lipid biosynthesis remains limited, though it is advancing rapidly. In this talk, I will present our recent findings on protein interactomes in lipid biosynthesis, focusing on enzymes and proteins involved in oil production and photosynthetic membrane lipid synthesis. I will also discuss ongoing work investigating the composition of these interactomes and the specific regions mediating protein–protein interactions

Short presentation 1: Atypical Phenylalanine Ammonia Lyases that Enhance Phenylpropanoid Production in Planta

Jorge El-Azaz, Yuri Takeda-Kimura, and Hiroshi A. Maeda

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Plants synthesize a diverse array of phenylpropanoids, which serve as essential nutrients, medicines, and valuable biomaterials while also acting as a major global carbon sink. These compounds primarily originate from the aromatic amino acid (AAA) phenylalanine (Phe) through the action of Phe ammonia lyase (PAL), an enzyme that is tightly regulated by various transcriptional and posttranscriptional mechanisms. Despite being one of the most studied enzymes in the phenylpropanoid pathway, the diversity of PAL regulatory mechanisms across plant species remains underexplored, as does the extent to which PAL regulation determines metabolic flux into phenylpropanoids metabolism. Using a phylogeny-guided biochemical screening, we found that PAL enzymes undergo complex and varied feedback regulation across different plant lineages. This screening identified atypical PAL enzymes uniquely feedback-activated by phenylpropanoids. Overexpression of feedback-activated PALs in *Nicotiana benthamiana* combined with enhanced Phe supply effectively redirected accumulated AAAs toward phenylpropanoid production. Similarly, the expression of the atypical PAL enzyme from *Elaeis guineensis* (oil palm) in *Arabidopsis thaliana*, unlike overexpression of endogenous Arabidopsis *AtPAL1*, boosted the accumulation of varied phenylpropanoids. Our findings uncovered the evolutionary diversity in PAL regulation across plants species, and discovered atypical PALs as valuable tools to release a key bottleneck and enhance phenylpropanoid production *in planta*.

Short presentation 2: A Synthetic Carbon Assimilation Shunt Boosts Terpenoid Biosynthesis in Plants

Anya Franks[1], Matthew Bergman[2], Sonia Evans[1], Mateja Perc[2], and Michael Phillips[1,3]

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Improving photosynthesis is a central objective in plant biotechnology. Although ribulose1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the path for most CO2 assimilation in plants, the introduction of novel carbon fixation pathways presents opportunities to fine tune downstream carbon utilization. Here we describe genetically modified plants that fix CO2 through reversible steps of the pentose phosphate pathway and channel this carbon preferentially towards terpenoids. Tobacco plants transiently expressing plastidial 6- phosphogluconate dehydrogenase (PGD) during the day reductively carboxylate ribulose-5-phosphate (Ru5P) to 6-phosphogluconate (6PG) using NADPH reducing power provided by photosynthesis. CO2 assimilated through this route was coupled to a synthetic Entner-Doudoroff (ED) pathway, a 2-step prokaryotic shunt consisting of a dehydratase (EDD) that converts 6PG to 2-keto-3-deoxy-6-phosphogluconate (KDPG) and an aldolase (EDA) that cleaves KDPG into GAP and pyruvate, the substrates for the first enzyme of the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Metabolite profiling of plants transiently expressing chloroplast targeted isoforms of PGD, EDD, and EDA demonstrated that Ru5P-derived GAP and pyruvate were readily incorporated into the MEP pathway, which supplies the precursors to terpenoids in the chloroplast. Increased MEP pathway flux was also observed by co-expressing PGD and EDD in the absence of EDA. Biochemical characterization indicated that endogenous fructose 1,6- bisphosphate aldolase (FBA) can replace KDPG aldolase (EDA) activity as a result of the substrate promiscuity afforded by aldolase enzymes. When plants were incubated in the dark or the same enzymes were targeted to the cytosol, the impact on the MEP pathway was absent, verifying that this synthetic route was dependent on the light reactions of photosynthesis. The photosynthetically driven coupling of this reversible pentose phosphate pathway to terpenoid biosynthesis through this ED/MEP shunt demonstrates the potential of alternative carbon assimilation routes to channel carbon directly into valuable natural products. In addition, this synthetic route to augmenting plastid terpenoid biosynthesis has significant implications for engineering plants for climate change tolerance and biofuel engineering.

Short presentation 3: **Reconstitution of monoterpene indole alkaloid biosynthesis in genome engineered *Nicotiana benthamiana***

Quentin Dudley

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Monoterpene indole alkaloids (MIAs) are a diverse class of plant natural products that include several medicinally important compounds such as the cancer drug vinblastine. We set out to reconstitute the pathway for strictosidine, a key intermediate of all MIAs, from central metabolism in Nicotiana benthamiana. A disadvantage of this host is that its rich background metabolism results in the derivatization of some heterologously produced molecules. Here we use transcriptomic analysis to identify glycosyltransferases that are upregulated in response to biosynthetic intermediates and produce plant lines with targeted mutations in the genes encoding them. Expression of the early MIA pathway in these lines produces a more favorable product profile. Strictosidine biosynthesis was successfully reconstituted, with the best yields obtained by the co-expression of 14 enzymes, of which a major latex protein-like enzyme (MLPL) from Nepeta (catmint) is critical for improving flux through the iridoid pathway. The removal of endogenous glycosyltransferases does not impact the yields of strictosidine, highlighting that the metabolic flux of the pathway enzymes to a stable biosynthetic intermediate minimizes the need to engineer the endogenous metabolism of the host. The production of strictosidine in planta expands the range of MIA products amenable to biological synthesis. The recently established Dudley lab is continuing this effort using cell-free protein synthesis as a tool to screen enzyme variants and next-generation sequencing as a high throughput method for selecting promoter, terminator, and transfer-DNA architectures to optimize enzyme expression levels.

Short presentation 4: **Unlocking the catalytic potential of yeast geranylgeranyl diphosphate synthase through metabolic-driven epistatic interactions**

Jean-Alexandre Bureau[1], Yueming Dong[1], Damien Bretagne[1], Attia Iram[1], Mohsin MD Patwary[1], Asia Vighi[1], Liv Toft[1], Dan Voicu[1], Olga Sofianovich[1], Yu Xia[1,2], Codruta Ignea[1,2]

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Geranylgeranyl diphosphate (GGPP), a crucial precursor for protein geranylgeranylation, is essential for cellular metabolism and function. In plants and microorganisms, GGPP also serves as a substrate for specialized metabolites like diterpenoids and carotenoids. GGPP synthases thus exhibit diverse catalytic activities across organisms, adapting to specific metabolic needs. Here, we evolved yeast geranylgeranyl diphosphate synthase (GGPPS) to enhance its catalytic potential for efficient GGPP-derived compound production in a pathway-regulated manner. Our results reveal that mutation epistasis in GGPPS synergistically responds to cellular metabolic requirements by modulations of enzyme conformational dynamics. Pathway-driven most-fit GGPPS variants resulted from positive sign epistasis of substitutions that are absent or nearly absent in nature complementing mutations at permissive surface residues. Using a highly efficient variant, we achieved 31.5 mg/L β-carotene production in an industrial Saccharomyces cerevisiae strain. This research highlights fitness dynamics in tailored environments, providing a framework for leveraging enzyme function in fundamental and applied research.

Thuy Dang
University of British Columbia
Kelowna, BC Canada

Dr. Thu Thuy Dang is currently an assistant professor and a UBCO Principal Research Chair in Natural Product Biosynthesis and Biotechnology at the Department of Chemistry, University of British Columbia, Okanagan. She's also a Michael Smith Health Research Foundation Scholar in Biochemistry. Thuy has been awarded the Arthur Neish Award (2025) and TPJ-Early Career Award from PSNA. Before joining UBC, she was a postdoctoral fellow (EMBO) at the John Innes Centre in the laboratory of Dr. Sarah O'Connor (Norwich, UK). Thuy obtained her PhD in Biochemistry from the University of Calgary (Canada) in 2014. Her research program integrates biochemistry, chemistry, bioinformatics, and molecular genetics to understand and engineer the biosynthesis of valuable alkaloids from medicinal plants.

Arthur Neish Award presentation: **Cytochrome P450s in Alkaloid Biosynthesis: Catalysts of Chemical and Structural Diversity**

Thu-Thuy T. Dang[1], Tuan-Anh M. Nguyen[1], Larissa C. Laforest[2], Satya Swathi Nadakuduti[2]*

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Plants produce a vast diversity of nitrogen-containing heterocyclic metabolites known as alkaloids, which serve critical ecological functions and have been used as medicines for centuries. Among them, monoterpene indole alkaloids (MIAs) are a structurally and functionally diverse class of natural products with potent pharmacological activities. Clinically relevant MIAs include the anticancer agent camptothecin, the antiarrhythmic drug ajmaline, and the immunomodulatory alkaloid mitraphylline. This presentation will explore recent discoveries of key biosynthetic enzymes involved in both the scaffolding and diversification of MIA structures. The roles of cytochrome P450 monooxygenases enzymes in generating chemical diversity from shared precursors will be highlighted. In addition, how combinatorial chemoenzymatic C–H functionalization strategies can be applied to expand the chemical space of MIAs and facilitate the sustainable production of high-value alkaloid derivatives will be discussed. Our work contributes to a deeper understanding of MIA metabolism and enables greener biocatalytic approaches to medicinal alkaloid synthesis.

Short presentation 5: **Deciphering the genetic regulation of proanthocyanidin biosynthesis in Lotus corniculatus forage**

Solihu Kayode Sakariyahu[1,2], Tim McDowell[1], Ling Chen[1], Justin Renaud[1], Yousef Papadopoulos[3], Kathleen Glover[3], Susanne Kohalmi[2], and Abdelali Hannoufa[1,2]

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Proanthocyanidins (PAs) are diverse specialized metabolites derived from the phenylpropanoid pathway. PAs are widely known for their beneficial properties, including the ability to confer disease resistance and reduce the incidence of pasture bloat and methane emissions in ruminant animals. Whereas studies have been reported on PA biosynthesis in seeds of model plants, such as Arabidopsis thaliana and Medicago truncatula, the regulatory mechanisms governing PA accumulation in shoots of forage legumes remain elusive. Here, we investigated the genetic and environmental regulation of PA accumulation in Lotus corniculatus, a temperate forage legume highly valued for its substantial herbage PA content. PA levels and compositions were quantified using the DMACA and butanol-HCl assay combined with high-resolution liquid chromatographymass spectrometry (LC-MS) to understand the environmental and genetic factors impacting PA quantity and metabolite profiles. We observed pronounced fluctuations in PA levels among eight genotypes and across multiple geographic growth locations. Further, transcriptomic analyses using RNA sequencing from three PA-contrasting L. corniculatus genotypes revealed 42 differentially expressed genes involved in the phenylpropanoid pathways. Clustering analysis grouped these genes into four primary expression modules, with genes associated with PA biosynthesis showing elevated expression in high-PA accumulating genotypes. Promoter region analyses of PA biosynthetic genes indicated enrichment for MYB transcription factor binding sites, suggesting their potential regulatory roles in PA biosynthesis. To elucidate the regulatory relationships between the PA biosynthetic genes and transcription factors, we employed the GENIE3 algorithm to construct predictive gene regulatory networks. This analysis identified multiple MYB and bHLH transcription factors linked to the biosynthesis and accumulation of PAs in forage tissues. Our findings identified potential key regulatory factors essential for the genetic engineering of PA biosynthesis in the leaves of temperate forage species. Further studies of loss-of-function mutants in these regulatory genes will further advance our understanding of PA biosynthesis and regulation, presenting opportunities for targeted breeding strategies to optimize PA levels, improve forage quality, and enhance stress resilience in L. corniculatus.

Short presentation 6: A Bridge and an Anchor: Optimizing the isoflavonoid metabolon in a yeast chassis

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The plant kingdom is abundantly rich with beneficial and medicinal compounds, providing us with a biochemical playground for engineering microbes. Among these compounds, phenylpropanoid-derived isoflavonoids are characteristic legume metabolites that function as antimicrobial phytoalexins, symbiosis signals, and phytoestrogens in the human diet. While translating isoflavonoid biosynthesis into a yeast chassis has been realized, it is inherently linked to the competing pathway for flavonoid biosynthesis due to promiscuous enzymes with broad substrate or product ranges. Currently, the resulting isoflavonoid yields are often too low to consider this a scalable, sustainable source of these bioactive compounds. However, reconstituting such biosynthetic "assembly lines" can lead to pitfalls associated with stripping away the context of the native cellular environment. Considering the broader isoflavonoid metabolon, we have identified key points for engineering a more efficient complex to improve isoflavonoid rather than flavonoid production. Our strategies focus on realizing the potential of a non-catalytic auxiliary protein, chalcone isomerase-like (CHIL), and engineering a non-catalytic domain in the rate-limiting cytochrome P450, isoflavone synthase (IFS). Notably, engineered yeast co-expressing CHIL with the biosynthetic components of the isoflavonoid pathway exhibited a 67% increase in flux through the target pathway and a 33% increase in the major isoflavone daidzein titers, without diverting additional flux to the competing flavonoid pathway. By extending CHIL characterization to the isoflavonoid pathway, we have revealed an expanded role for this auxiliary protein and underscored its utility in engineered metabolic contexts. Both CHIL and IFS represent critical infrastructure in the isoflavonoid metabolon that can be leveraged to enhance pathway flux and improve yields. This systems-level strategy highlights the often-overlooked impact of noncatalytic proteins and the broader metabolon architecture in shaping plant specialized metabolism, offering complementary approaches to pathway reconstitution that can further enhance the potential of microbial biosynthesis platforms.

Short presentation 7: CRISPR/Cas9-Mediated Knockout of Borneol Diphosphate Synthase to Reduce Production of Borneol and Camphor in Lavender

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Lavender is a perennial aromatic shrub cultivated worldwide for its valuable essential oil (EO). The EO is constituted of over 50 monoterpenes, including borneol and camphor, which contribute off odor reducing quality. The metabolic pathway leading to the biosynthesis of camphor initially involves conversion of the monoterpene linear precursor geranyl diphosphate (GPP) to bornyl diphosphate (BPP), by the enzyme borneol diphosphate synthase (BPPS). BPP is then enzymatically transformed into borneol, which is subsequently dehydrogenated to produce camphor. In this study, we used the CRISPR/Cas9 technology to knock out the BPPS gene in order to reduce production of borneol and camphor in spike lavender (Lavandula latifolia). The lavender genome and the genomic sequence of BPPS were scanned to identify candidate guide RNA molecules (gRNAs). Among the 151 gRNAs identified, the best candidates with minimal or no offtargets were selected. Plasmids carrying these gRNAs along with Cas9 were constructed and transformed into Agrobacterium tumefaciens cells, which were used to transform lavender plants. Transformed plants are currently being analyzed by sequencing to detect potential mutations in the BPPS gene, and by GC-MS analysis to evaluate changes in the EO borneol and camphor content. This study aims to establish a genome editing approach to enhance EO profile in lavender, which could contribute to developing highquality cultivars with improved commercial value.

Short presentation 8: Yeast-based Engineered biosynthesis for De novo production of Spirooxindole

Ngoc-Mai T. Huynh[1], Hong-Hoa T. Nguyen[1], Thu-Thuy T. Dang[1]

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Monoterpene indole alkaloids (MIAs) constitute a structurally diverse class of plantderived natural products with significant pharmaceutical potential. Spirooxindole derivatives, particularly mitraphylline, a subclass of MIAs, have reported beneficial medicinal properties; however, their natural abundance in Mitragyna speciosa (kratom) plants is extremely limited. Furthermore, the biosynthetic pathway of spirooxindoles has recently been elucidated, paving the way for synthetic biology approaches to produce these molecules in heterologous hosts. Yeast engineering has emerged as a promising solution, enabling the stable integration of multiple genes into the host genome and facilitating systematic evaluation of gene functions and pathway reconstruction. Here, we aim to achieve the production of mitraphylline, a promising anticancer candidate from kratom via a multiple-step enzymatic pathway, reconstituted in a yeast-based platform. This system is expected to serve as a powerful tool for in the production of mytragynine and other spiroxindole alkaloids and sustainable biomanufacturing of this pharmaceutically valuable alkaloid.

Mark Lange
Washington State University & Dewey Scientific
Pullman, Washington,
USA

Mark was trained as a chemist and received an M.Sc. degree from the University of Bonn and a PhD in phytochemistry from the University of Munich (both in Germany). Following postdoctoral studies, he spent four years in group leader positions at biotechnology companies (Novartis, Syngenta, and Diversa). In 2004, Mark joined Washington State University, where he rose through the ranks and is currently a professor and director at the Institute of Biological Chemistry. His research program is focused on unraveling the biochemical pathways leading to bioactive natural products. Mark is also a serial entrepreneur, currently serving as Co-Founder and Chief Scientific Officer at Dewey Scientific LLC, a cannabis science and breeding company in Washington State, USA.

Keynote talk: **Follow Your Nose – The Chemistry, Biochemistry, and Genetics of Cannabis Aroma**

Mark Lange

Institute of Biological Chemistry, Washington State University

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Cannabis (Cannabis sativa L.) is renowned not only for its psychoactive and therapeutic properties but also for its distinctive and complex aroma. This aroma is produced by a diverse array of volatiles, including terpenoids, esters, aldehydes, alcohols, and sulfur containing compounds (VSCs). For example, the "gassy" or "fuel-like" aroma characteristic of certain cannabis chemotypes is a highly sought-after trait of specific genetic lineages such as OG Kush and Sour Diesel, and this main body note is imparted to a large extent by terpenoids. "Skunkiness" is a distinctive, sulfurous, and intensely pungent smell found prevalent across cannabis chemotypes. While revered by many consumers, it is often considered a nuisance odor of cannabis cultivation. Recent research has shown that VSCs such as 3-methyl-2-butene-1-thiol are key contributors to this characteristic odor. Other VSCs underlie exotic and fruity notes, with important contributions to widen range of aroma characteristics. These sulfur compounds are present in very low concentrations but have extremely low odor thresholds, meaning that even trace amounts can significantly influence a chemotype's aroma. This presentation will discuss the progress that has been made in recent years toward unraveling the biosynthetic origins of various types of cannabis volatiles. An overview will be given on how this information is integrated into aroma-forward breeding programs

Igor Kovalchuk
University of Lethbridge
Lethbridge, AB
Canada

Invited speaker: **Cannabis sativa – breeding new varieties and analyzing their medicinal properties**

Igor Kovalchuk, Andriy Bilichak, Yaroslav Ilnytskyy, Darryl Hudson, Salma Shujat, Santosh Suryavanshi, Bo Wang, DongPing Li, Gregory Robinson, Viktoriia Cherkasova, Marta Gerasymchuk, Esmaeel Ghasemi Gojani, Olga Kovalchuk

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In the past, we have generated over 1,000 Cannabis sativa hybrids by crossing various hemp and marijuana varieties. We have partially sequence 63 varieties and identified various cannabidiolic acid (CBDA) synthases. Recently, we cloned several of the genes encoding CDDAS, made transgenic tobacco plants carrying these genes and analyzed the activity of CBDAS in *in vitro* enzymatic essays, testing conversion efficiency of cannabigerolic acid (CBGA) to CBDA. In a separate work, we have isolated several enthomopathogenic fungi and demonstrated their efficiency in controlling cannabis aphids (*Phorodon cannabis*); we showed that this natural approach is superior to a tested commercial insecticide for preserving the flower weight and cannabinoids/terpenes concentration in aphid-infected plants. Also, in a series of experiments, we screened over 500 different extracts and demonstrated that certain cannabis extracts have anti-cancer and anti-inflammatory properties, as well as are able to preserve viability of skin fibroblasts and beta-cells exposed to high glucose/high lipid stress. In reconstitution experiments, we showed that certain individual cannabinoids and terpenes posses anti-inflammatory properties resembling whole extracts. Also, we demonstrate the effect of single cannabinoids on reducing inflammation and aiding chemo drugs reducing cancer cell growth. Finally, we have developed an algorithm allowing to evaluate the anti-inflammatory potential of various extracts using mRNA and methylome profiling.

Zamir Punja
Simon Fraser University
Burnaby, BC
Canada

Zamir completed a BSc degree in Plant Sciences at the University of British Columbia in Vancouver, and MSc and PhD degrees in plant pathology from the University of California, Davis. He joined Campbell Soup Company and worked jointly with North Carolina State University in Raleigh on management of carrot diseases. He was appointed Manager of Plant Biotechnology research for Campbell's in Davis, California to develop innovative methods for crop improvement. Zamir joined Simon Fraser University in 1989 as Associate Professor and was promoted to Professor in 1996. His research interests include the etiology and management of plant diseases on vegetable and horticultural crops, and the applications of plant biotechnology for disease management. More recently, his work has shifted to cannabis, where his group has described a range of previously unreported pathogens affecting the crop and has evaluated various methods for disease management. He is a Fellow of the Canadian Phytopathological Society. Zamir has received numerous research and teaching awards, including the Sterling Prize for Controversy for his work on GMO foods. He was Editor-in-Chief of the *Canadian Journal of Plant Pathology* for 18 years. His current research is focused on cannabis pathogens.

Invited speaker: **Challenges to Plant Health From Emerging Pathogens – A Case Study of Cannabis sativa L. (Cannabis)**

Zamir K. Punja

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

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Cannabis sativa L. (cannabis, marijuana) is cultivated widely in Canada following its legalization in 2018 for the medicinal and recreational markets. The plants are grown for their complex inflorescences (racemes) which develop on female plants and produce an abundance of glandular trichomes. The trichomes manufacture and store a range of cannabinoid and terpene compounds, which are the a priori reason for commercial cultivation of these plants. The quality of the harvested plants can be severely impacted by plant pathogens that infect the roots, stems and inflorescences. Reports of pathogens infecting these plants have increased significantly since 2018, reflecting the susceptibility of a range of cultivated cannabis genotypes to previously undescribed and emerging pathogens. Molecular characterization of pathogen strains infecting cannabis has shown that many are closely related to strains found previously infecting other hosts, including hops, barley, tomato, blueberry, and others, which are grown in proximity to cannabis greenhouses. Spread of inoculum from these hosts is facilitated by wind, rain and movement of plant materials. Other pathogen strains are carry-overs from the previous illicit cultivation of cannabis prior to 2018 and have spread through dissemination of infected plant materials. The most devastating pathogens are those found infecting the inflorescences directly, causing a rot, and include species of Botrytis, Fusarium and Alternaria. Other recently described pathogens, such as Hop latent viroid and Fusarium stem rot, are shown to spread via cuttings taken from infected asymptomatic mother plants. The challenges faced by cannabis cultivators in managing these pathogens will be described, and the potential methods for disease management will be reviewed. These include identification of potential genetic resistance among the wide range of genotypes under cultivation, sanitary practices, and the use of tissue culture to obtain pathogen-free planting stock.

Short presentation 1: **Cannabis Flavonoid Biosynthesis: CsAP2L1**

Adam Sumner and Bastiaan Bargmann

School of Plant and Environmental Science, Virginia Tech University, Blacksburg, Virginia, United States

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Demand for medicinal cannabis metabolites has rapidly increased in recent years, leading to research into the understanding of the pathways in which hemp produces these metabolites. The end goal is to enable farmers to have true control over the modulation of the levels of valuable secondary metabolites in the hemp that they cultivate. Cannflavin A and B are cannabis-specifc prenylated flavonoids that have been found to elicit non-psychoactive and anti-inflammatory effects. Little research has so far been conducted into the mechanisms of their synthesis. The transcription factor CsAP2L1 has been found to be connected to cannabis secondary metabolism, and this study works to demonstrate it as a future modulator of cannabis flavonoid biosynthesis. Utilizing transgenic leaf tissue cell suspensions, an over-expression study of CsAP2L1 was completed, starting with an RNA-Seq analysis and validated with promoter analysis, RT-qPCR, and metabolite analysis. Genes in the cannflavin pathway and flavonoid related genes were elucidated in the study and demonstrate steady modulation of this secondary metabolism in cannabis. The information gained from these findings serves as a crucial stepping stone in the continued attempt to produce the valuable, pharmaceutical cannflavins in higher amounts.

Short presentation 2: **In vitro culture of Phlegmariurus carinatus and production of neuroactive lycopodium alkaloid**

Amit Jaisi[1, 2*], Mubeen Fatima[1]

[1]*School of Pharmacy, Walailak University, Nakhon Si Thammarat, Thailand*

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Phlegmariurus carinatus (Desv. ex Poir.) Ching, a species in the Lycopodiaceae family, serves as a notable source of the neuroactive compound huperzine A and various lycopodium alkaloids (LAs). Despite their promising applications, these compounds face significant limitations due to their low natural abundance, slow plant growth rates, and challenges associated with cultivation, in vitro propagation, and chemical synthesis (Xu et al., 2018). Recent advancements in biosynthetic research have identified critical genes involved in LA production (Nett et al., 2021, 2023). In our ongoing investigation of bioactive compounds and alkaloid biosynthesis in plants (Xu et al., 2018; Zen et al., 2021), we adopted three primary methodologies: establishing in vitro sporophyte cultures, employing elicitation to replicate biotic and abiotic stress conditions, and using deuterium oxide (D_2O) feeding experiments. Additionally, we utilized MALDI imaging to map the tissue-specific spatial distribution of LAs. High-resolution mass spectrometry (HRMS) analysis revealed that LAs were most abundant in leaf tissues. The application of coronatine to gemmae cultures was particularly effective in enhancing LA metabolism. D_2O labeling experiments indicated that LA biosynthesis, particularly for huperzine A and fawcettimine, is concentrated in the rapidly growing tips of sporophytes, where these alkaloids accumulate in significant quantities. Furthermore, spatio-chemical analysis revealed an uneven distribution of LAs within the plant, with notable accumulation in leaves, vascular tissues of the stem, and root cortex. This study is the first to examine the effects of elicitation on LA metabolism and to characterize the cell-type-specific accumulation of LAs in sporophytes. The findings provide valuable insights into lycopodium alkaloid biosynthesis and pave the way for future research in this field.

Short presentation 3: **From farm to (robot) stomach: testing beans with diverse seed coat patterns and phenolic profiles in California growing environments**

Tayah M. Bolt[1], Margaret Riggs[1], Weiyi Sun[2], Shu Yu[1], Larry Lerno[3], Li Tian[1], Paul Gepts[1], Antonia Palkovic[1], Travis A. Parker[1], Gail M. Bornhorst[2,4], Christine H. Diepenbrock[1]

[1] *Department of Plant Sciences, University of California, Davis, Davis, CA, USA* [2] *Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA, USA* [3] *Food Safety and Measurement Facility, University of California, Davis, Davis, CA, USA* [4] *Riddet Institute, Palmerston North, New Zealand*

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Common bean (*Phaseolus vulgaris L.*) is a critical crop for direct human consumption, grown in diverse environments worldwide. It serves as a primary protein source in many countries, offering high protein and fiber content, gluten-free status, and antioxidant properties. These characteristics also make it a valuable ingredient for improving the nutritional profile of food products. Common bean includes several commercially relevant market classes, such as kidney, pinto, black, and navy beans, with considerable diversity in seed coat colors and patterns (underlain by phenolic compounds) helping consumers identify their preferred varieties. While macronutrients (e.g., fat, protein, starch) are typically prioritized in crop evaluations, there has been less focus on micronutrients and anti-nutrients, including certain phenolic compounds and phytate, which can act as inhibitors of mineral nutrient bioavailability. This study first aimed to assess the at-harvest nutrient levels in common bean samples with differing seed coat coloration and patterning, considering genotype by growing environment (GxE) interactions. In addition to differing phenolic compound profiles, the results showed distinct groupings based on growing year and location for macronutrients. Although these values are useful for plant breeding programs and are historically the metric for such endeavors, these values do not necessarily reflect the bioaccessibility of these nutrients (i.e. the nutrients which are released from the food matrix during digestion). Measuring bioaccessible nutrients during digestion in a physiologically relevant yet high-throughput manner is a significant challenge in plant breeding and food product development. The second aim of this study was to develop and compare simulated digestion models for small sample masses, to examine bioaccessible nutrient levels in a subset of these common bean samples selected to represent a range of protein and starch values while also representing overall macronutrient profiles (e.g., in principal component space). In a comparison of digestion methods on one sample type, while the highest trait values (starch and protein hydrolysis, total phenolics, and antioxidant power) were observed from more dynamic digestion models, even simple dynamic models showed higher values than a static digestion model. The use of these in vitro models varying in complexity provided insight into nutrient bioaccessibility; e.g., in a comparison of two genotypes grown in each of two locations (with differential extent of seed coat patterning), differences were observed during digestion between genotypes for protein hydrolysis and between environments for both total phenolics and protein hydrolysis, even in a higher-throughput digestion model. These results inform the application of simulated digestion models to assay bioaccessible nutrient levels in plant-based foods.

Discussion panel: Phytochemical Products for the Treatment of Chronic Pain

Gregg Robideau
Supreme Optimization
Ottawa, ON
Canada

Gregg Robideau obtained his PhD and Postdoctoral Degrees from Agriculture and Agri-Food Canada (AAFC) where he developed molecular diagnostic methods to detect and identify plant pathogens and pests from complex environmental samples. Gregg has 10 years' experience in industry, serving R&D Scientist at Genotek, Head of R&D at TerraCycle, and is currently Senior Digital Marketing Stragist at Supreme Optimization, helping companies grow their business online with smarter and more effective digital marketing strategies.

Mary-Ann Fitzcharles
Alan Edwards Pain Management Centre and McGill University
Montreal, QC
Canada

Mary-Ann Fitzcharles is a clinician, teacher and clinical researcher at McGill University, Montreal, Canada since 1982. She is a founder member of the Royal College of Physicians and Surgeons of Canada specialty committee for Pain Medicine, is a past chair of the Canadian Rheumatology Association (CRA) Therapeutics committee and has led the Canadian Fibromyalgia Guidelines and the CRA 2019 position statement on Medical Cannabis.

Mark Lange
Washington State University & Dewey Scientific
Pullman, Washington,
USA

Mark was trained as a chemist and received an M.Sc. degree from the University of Bonn and a PhD in phytochemistry from the University of Munich (both in Germany). Following postdoctoral studies, he spent four years in group leader positions at biotechnology companies (Novartis, Syngenta, and Diversa). In 2004, Mark joined Washington State University, where he rose through the ranks and is currently a professor and director at the Institute of Biological Chemistry. His research program is focused on unraveling the biochemical pathways leading to bioactive natural products. Mark is also a serial entrepreneur, currently serving as Co-Founder and Chief Scientific Officer at Dewey Scientific LLC, a cannabis science and breeding company in Washington State, USA.

Jonathan Ferrier
Dalhousie University
Halifax, Nova Scotia,
Canada

Keynote talk: **Missinihe Michi Saagiig Mashkiki miinwa Midaaswi ashi-niswi Keezis (Credit River Mississauga Phytochemistry and the Thirteenth Moon)**

Jonathan Ferrier

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Anishinaabe (Ojibwe) Missinihe Michi Saagiig phytochemical knowledge, carried by societies like the Midewiwin and Waabunowin, encodes complex ecological relationships across the territory now known as the Greater Toronto Area. This knowledge system has been carried for millennia in oral history, regalia, pictographs, petroglyphs, and birchbark scrolls. The Ojibwe lunar calendar—found on the back of Mikinaak (Turtle)—represents Midaaswi ashi-niswi Keezis (13 Moons), aligning with seasonal peaks in biosynthesis of secondary metabolites across Mississauga territory. Alkaloids, phenylpropanoids, and terpenes arise from core biosynthetic pathways including methylerythritol phosphate (MEP), shikimic acid, and phenylpropanoid routes. These compounds express adaptive, ecological roles within the biodiversity of Mikinaak Minising (Turtle Island), especially on the Missinihe (Credit River), where Eastern salt waters meet the largest freshwater system on earth, and Northern Mixed Woodlands meet Carolinian forest diversity. Language, land, and medicine lines intersect here, forming a uniquely rich biocultural landscape. Colonization disrupted these systems. The 13th Moon was removed from the calendar, and by 1872–73, the Credit River Mississaugas were reduced to a few hundred. Community members were displaced, assimilated, or killed. Traditional medicine families were named "Illegitimate" for maintaining spiritual and traditional phytochemical practices. Ryerson's 'Credit Experiment' and the Industrial Residential Schools, enforced by Indian Commissioner Lt. Col. Jasper Tough Gilkison, criminalized regalia and ceremonies under the Indian Act. Yet seeds of knowledge survived. A 1920 photo of an Ojibwe Strap Dress worn despite bans on traditional regalia marks that resistance. This work seeks to restore Mississauga mashkiki phytochemistry as cultural heritage and scientific knowledge. With clear use of Etuaptmumk (Two-Eyed Seeing), this review draws on decades of research, with ethnobotanical, metabolomic, and historical evidence to revitalize the phytochemical relationships with the Mississauga Thirteen Moon calendar in Missinihe Michi Saagiig territory. Nii'kinaaganaa.

Short presentation 1: **Phytochemical content and antimicrobial Properties of Piper guineenses leaf extract**

Anukam Basil Ngozichukwu* and Chieme Sunday Chukwudoruo[1]
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Phytochemical and antimicrobial analysis of *Piper guineenses* was carried out on the dry and ground seed of the plant using standard laboratory procedures. The sample was screened for its phytochemical content and quantified. Results revealed that the seed sample contained 3.66% Alkaloid, 3.40% Flavonoid, 3.86% Tannin. The extracted phytochemical compounds were subjected to antimicrobial screening with certain selected human pathogens and the diameter of inhibition was obtained;For Alkaloids; Escherichia coli 8mm, Pseudomonas aureginosa 6mm, Klebsiella 3mm, Staphylococcus aureus. 4mm and Streptococcus aureus 5mm. For Flavonoids; Escherichia coli 2mm, Pseudomonas aureginosa 3mm, Klebsiella 6mm, Staphylococcus aureus 2mm and Streptococcus aureus: 2mm. For Tannins; Escherichia coli 2mm, Pseudomonas aureginosa 6mm, Klebsiella 3mm, Staphylococcus aureus 2mm and Streptococcus aureus, 6mm. These values were compared with those from 1mg standard antibiotic; Oxacillin which gave values of 12mm, 21mm, 14mm, 13mm, 20mm respectively for Escherichia coli, Fseudomonas aureginosa, Klebsiella and Staphylococcus aureus respectively with minimum inhibitory concentration (MIC) of 6.5mg/cm', 12.5mg/cm', 12.5mg/cm, 6.5mg/cm' and 12.5mg/cm' respectively. From the findings it revealed that the bioactive compounds present in the leaf extract of the plant possesses antimicrobial property which could be a cheaper and alternative source of antibiotics for the locals.

Short presentation 2: **Biochemical Effects And Anticholinesterase Activities Of Selected Leaf Extracts On Aluminium Lactate-Induced Alzheimer Disease In Albino Rats**

Chieme Sunday Chukwudoruo and Anokam Basil, Tasi'U Uba Mohammad
Federal University of Technology, Owerri, Imo State, Nigeria.

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The biochemical effects and anticholinesterase activities of *Annona muricata* (soursop) and *Solanum macrocarpon* (African garden egg) leaf extracts on aluminium lactate-induced Alzheimer's-like disease in albino rats were studied. Thirty-two (32) albino rats (120g-140g) which were grouped into 8 groups (I-VIII) of 4 each were used. With the exception of group I, all the groups were treated for 28days followed by induction of 7.5 mg/Kg aluminium lactate (the toxicant) via intra-peritoneal injection from the 21st day to the 28th day (1 week). They were labelled: group I (normal control which was administered only distilled water), group II (negative control which was not treated after the toxicant induction), group III (standard control-it was treated with 2mg/Kg Niostigmine- standard drug), groups IV and V (were treated with 250mg/Kg and 500mg/Kg of *A. muricata*), groups VI and VI (were treated with 250mg/Kg and 500mg/Kg of *S. macrocarpon*) and group VIII: (was treated with 500mg/Kg of *S. macrocarpon* and *A. muricata*). The biochemical analysis was carried out and revealed significant ($p<0.05$) alteration induced by the toxicant in all the biomarkers in consideration (RBC, PVC, WBC, Hb, PLT, MCV, MCH, MCHC, ESR, MDA, TNF-α, Vitamin C, Vitamin E, AChE and BuChE) but treatments with standard drug, *S. macrocarpon* and *A. muricata* significantly ($p<0.05$) countered the effects of the toxicant at varying capacity and according to concentrations; with *S. macrocarpon* (at 500mg/Kg bw) having more potency than the standard drug and *A. muricata* in parameters like RBC, PVC, WBC (only *S. macrocarpon* at the low and high doses was able to counter the effect of the toxicant), PLT, MDA, TNF-α and Vitamin E; *A. muricata* more potency than the standard drug and *S. macrocarpon* in parameters like MCH, ESR and BuChE; the combination of the 2 plants' extracts were found more potent in Vitamin C (which was followed by 250mg/Kg *A. muricata*) and AChE (which was followed by 250mg/Kg *S. macrocarpon*) and followed the standard drugs in MCHC and Vitamin C were *S. macrocarpon* at 250mg/Kg and *A. muricata* at 500mg/Kg respectively. From the results of histopathology, the negative control group showed moderate, multifocal, widespread cerebral neuronal degeneration indicated the extensive damage to neurons which is a hallmark feature of advanced Alzheimer's disease pathology, the effect of the toxicant was slightly ameliorated by the standard drug in group III and 500mg/Kg *A. muricata* leaf extract in group V as the groups showed mild to moderate, widespread neuronal degeneration in the cerebrum with a few normal cells, while groups IV, VI, VII and VIII showed total mitigation of the effect just as it was observed in the normal control group, indicating their potential in the management of the disease.

TPJ-PSNA Award presentation: **Catechol acetylglucose: A newly identified benzoxazinoid-regulated defensive metabolite in maize**

Annett Richter[1], Allen F. Schroeder[1], Caroline Marcon[2], Frank Hochholdinger[2], Georg Jander[1], and Boaz Negin[1, *]

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An enormous diversity of specialized metabolites is produced in the plant kingdom, with each individual plant synthesizing thousands of these compounds. In addition to the established functions of many specialized metabolites in protection against biotic interactors, some also regulate the production of additional defensive compounds. Benzoxazinoids, a class of abundant Zea mays (maize) defensive metabolites, were shown to have such a role, regulating callose deposition in response to insect feeding and fungal infection. In this study, we searched for additional maize metabolites that are regulated by benzoxazinoids. This identified two previously uncharacterized compounds, catechol glucoside, and catechol acetylglucose, which are produced from salicylic acid but whose accumulation is regulated by benzoxazinoids. Using genome-wide association studies, we identified a gene encoding a predicted acetyltransferase whose expression level was negatively correlated with catechol glucoside abundance. Maize knockout mutants of this gene displayed low catechol acetylglucose and elevated catechol glucoside abundance. Following tissue disruption, maize plants accumulated catechol, which inhibited Spodoptera frugiperda (fall armyworm) growth when fed in an artificial diet. Analysis of the caterpillar frass showed that these caterpillars detoxify catechol by glycosylation, and that the reduction in caterpillar growth while feeding on artificial diet containing catechol was correlated with their ability to glycosylate this compound. These findings also suggest that the success of S. frugiperda as an agricultural pest may depend partially on its ability to detoxify catechol, which is produced by maize as a defensive compound.

Diana Roopchand
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Diana Roopchand, PhD is an Associate Professor in the Department of Food Science at Rutgers University and her research laboratory is located in the New Jersey Institute for Food, Nutrition, and Health (IFNH). Her team investigates how food components and natural products compounds may interact with the gut microbiota to promote health and resilience to chronic disease. Her lab uses preclinical murine models, cell culture, microbiology, and *in silico* tools with the goal of translating bench research into human intervention studies. Dr. Roopchand received her doctorate from the Biochemistry Department of McGill University (Montreal, Canada) and completed a NIH-funded Postdoctoral Fellowship at Rutgers University. In addition to her academic training, she has worked in both the pharmaceutical and dietary supplement industry, gaining perspective for the diverse approaches available for health maintenance and disease prevention. She is also a founding partner of Nutrasorb, LLC which develops functional botanical ingredients, foods, and cosmetics.

Keynote talk: **Dietary polyphenols and metabolic resilience: insights from meta-omics**

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Dietary polyphenols are associated with protection from chronic metabolic disease despite low bioavailability. Based on preclinical data, grape polyphenol (GP) extracts rich in B-type proanthocyanidin (PAC) compounds likely mediate their health benefits via the gut microbiota and microbial metabolites. To investigate the links between PACs, the gut microbiota, and metabolic health, a human intervention study was performed (NCT04018066). Longitudinal metabolomic, metagenomic, and metaproteomic changes were measured in healthy participants (n= 27) before and after 5 days of soy protein isolate (SPI) supplementation alone followed by 10 days of supplementation with GPs complexed to SPI (GP-SPI). Serum, fecal, and urine samples were collected before and during the 17 day study and prepared for shotgun metagenomic sequencing, mass spectrometry-based metaproteomics, and targeted metabolomics (i.e., bile acids and polyphenols metabolites). Most multi-omic changes observed after 2 and/or 4 days of GP-SPI intake were temporary, returning to pre-supplementation profiles by day 10, suggesting microbial adaptation to PAC-rich GPs. Shotgun metagenomics sequencing provided insights that could not be captured with 16S rRNA amplicon sequencing. Notably, 10 days of GP-SPI supplementation decreased fasting blood glucose in association with increased serum hyocholic acid (HCA), a bile acid associated with improved glucose tolerance, and decreased abundance of a gut bacterial guild. While causal relationships remain to be investigated, this is the first study suggesting a link between PAC-rich GPs and serum HCA, a bile acid known for its inverse relationship with fasting blood glucose and increased metabolic resilience.

Short presentation 1: Enhancement of crop yield and nutritional value through metabolic engineering

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Plants produce diverse specialized metabolites essential for stress adaptation, some of which also benefit human health. Engineering these metabolites offers a promising strategy for developing crops with enhanced stress resilience and improved nutritional value. However, modifying metabolite composition and content is challenging due to the complexity of biosynthetic pathways and their multilayered regulatory mechanisms. Our recent research reveals the critical role of the 5′ untranslated region (5′ UTR) in the translational regulation of specialized metabolite production in plants. Although the 5′ UTR is not translated into protein, it plays a key role in controlling translation. While investigating metabolic networks in Arabidopsis thaliana, we identified two dominant mutant alleles exhibiting distinct morphological and biochemical traits. Genetic and transcriptomic analyses revealed that gain-of-function mutations in the 5′ UTR of a transcription factor drive these phenotypic changes. The mutated 5'UTR increases protein levels without altering its transcripts. Since this transcription factor serves as a master regulator of glucosinolate biosynthesis, plants carrying the mutated 5′ UTR show increased expression of its target genes leading to enhanced glucosinolate production. This discovery suggests that 5′ UTRs can be strategically leveraged for crop trait improvement.

Short presentation 2: Transcriptional Coordination Enhances Folate Biosynthesis in Common Bean Seeds During Postharvest Storage

Itzel Astrid Aviña-Ávalos[1,2], Sara Margarita Garza-Aguilar[2], Arturo Tlelo Reyes[2], Liliana Elizabeth García-Valencia[2], Perla Azucena Ramos-Parra[2], Rocío Isabel Díaz de la Garza[1],*

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Micronutrient preservation in stored legume seeds represents a critical yet understudied aspect of global food and nutrition security, particularly for vulnerable populations that rely on beans as a dietary staple. Folates, essential cofactors in one-carbon metabolism (1CMet), are highly susceptible to degradation during postharvest storage, yet the molecular mechanisms governing their stability remain poorly understood. In this study, we investigated folate accumulation and its molecular regulation during extended storage in three commercial common bean varieties: Mayocoba, Negro Jamapa, and Pinto Saltillo. Seeds were stored under controlled temperature and humidity conditions, and were sampled at defined intervals over a six-month period. We combined targeted metabolic profiling with comprehensive transcriptomic analysis to uncover previously unrecognized regulatory networks.

Specific storage conditions triggered a remarkable 36-48% increase in total folate content over six months, which contrasted sharply with the 30-60% degradation observed under alternative conditions. Polyglutamylation patterns shifted significantly, with Glu6-7 forms accumulating during folate preservation, while longerchain Glu8 species degraded in suboptimal storage environments. These changes occurred despite metabolic quiescence, challenging traditional assumptions about

postharvest biochemical inactivity.

Metabolic profiling of biosynthetic pterins, key folate precursors, revealed an early decline followed by partial recovery by day 180, suggesting a transient pause and later reactivation of folate synthesis. In contrast, oxidized pterins remained stable across time and varieties, supporting the notion that late-stage folate accumulation reflects active biosynthesis rather than passive preservation.

Concurrently, all varieties showed significant protein loss (17.7% by day 180, $p<0.05$) and declines in 1CMet-related free amino acids (serine, glycine and methionine decreased significantly within 30 days), possibly reflecting seed adjustments to storage-induced stress.

Transcriptomic analysis revealed coordinated folate regulation through: (1) Sustained upregulation of core folate biosynthesis genes (GCHI, 1.4-1.8-fold; and ADCS, 1.3-1.6-fold); (2) Induction of folate polyglutamylases (2.5-5.3-fold); (3) Activation of compartment-specific transporters (plastidial FOLT1, 1.7-2.4-fold; and vacuolar MRP1/2, 1.4-2.5-fold); and (4) Coordinated downregulation of folate salvage pathways (PTAR, 0.35-0.69-fold) and mitochondrial interconversion enzymes.

One-carbon metabolism pathways (including those for methionine, serine, and glycine biosynthesis) are broadly downregulated during storage, likely reflecting seed dormancy and metabolic slowdown. The temporal pattern of transcriptional activation preceding folate accumulation suggests a pre-activation mechanism that is conserved across varieties, yet sensitive to environmental conditions.

These findings suggest that seeds may possess evolved mechanisms to prime micronutrient availability for post-storage metabolic requirements. Understanding these metabolic mechanisms will allow for the design of postharvest strategies focused on maximizing the nutritional value of foods. This approach could positively impact food safety and public health in the medium term while also providing valuable knowledge for future research on grain nutritional quality.

Short presentation 3: **Ginseng Replant Disease and the Ginsenoside Connection**

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American Ginseng (*Panax quinquefolius*) is a perennial herbaceous plant, primarily cultivated for its high value in Traditional Chinese medicine, that matures over several years. However, ginseng growers face a significant challenge known as ginseng replant disease (GRD), which refers to the enduring negative effects of ginseng cropping on subsequent plantings, regardless of intervals, extending beyond normal pathogen carryover. Alongside pathogens, it is speculated that ginsenosides, triterpenoid saponins produced by ginseng, might contribute to GRD. Ginsenosides can be further categorized as protopanaxadiols (PPD) and Protopanaxatriols (PPT). To better understand the role of ginsenosides in GRD, we conducted experiments aimed to explore the behaviour of ginsenosides in ginseng garden soil, focusing on binding capacity, movement and the accumulation of ginsenoside breakdown products in field soils. Five different concentrations of ginsenosides were applied to columns packed with autoclaved soil from a GRD garden, and eluted slowly with water over ten weeks. The ginsenosides in the flowthrough were extracted and processed using LC-MS. After ten weeks, each soil column was segmented and the soil analyzed for ginsenoside content. In a separate experiment, soil collected from a commercial ginseng garden over the course of four years of ginseng cultivation was analyzed for putative ginsenoside breakdown products identified through in vitro experiments. From the soil column experiment we demonstrate that PPDs bound more tightly to the soil column, while PPTs were more mobile and nearly completely eluted from the columns within the first four weeks. Moreover, a ginsenoside breakdown product accumulated in ginseng garden soil in a seasonal manner over the course of a four year ginseng cultivation cycle. These findings provide insight into ginsenoside dynamics in soil and allow a better understanding of ginsenoside breakdown products in ginseng garden soils during cultivation.

Short presentation 4: **Evaluation of a Temporary Immersion System as a platform for the obtention of secondary metabolites from Agave salmiana.**

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Mexican *Agave salmiana* holds great economic, cultural, and gastronomic value with its derived food products offering notable health benefits. Phytochemicals associated with the medicinal properties of *Agave salmiana* include saponins and flavonols, which are valuable to the food and pharmaceutical industries. However, the prolonged maturation periods of *Agave salmiana*. and fluctuating phytochemical content associated with maturity stage and environmental factors, necessitate the exploration of tissue culture techniques. This study aims to assess the effects of the in vitro culture system on plant development and saponin and flavonols content in *Agave salmiana*, using temporary immersion systems (TIS) as a novel approach to induce saponin and flavonols accumulation. Three-week-old *Agave salmiana* vitroplants, initiated from aseptic seeds, were transferred to either semi-solid medium or the TIS (Biocoupler™), at two-time intervals with varying parameters for the TIS (immersion times and frequencies, volume of culture medium, and inoculant density). Saponin and flavonol content were quantified using HPLC-DAD-ELSD, while plant growth parameters were evaluated to determine overall plant quality. Findings revealed superior growth parameters such as plant height, leaf and root number, and biomass —including a 2.5 increase in dry weight- and variations in total saponin and total flavonol content among plants cultured in the TIS compared to those in the semi-solid system. This ongoing investigation offers insights into optimizing in vitro culture conditions to enhance plant development and modulate saponin content in *Agave salmiana*, thereby contributing to its broader agricultural, pharmaceutical, and industrial applications.

Short presentation 5: **Identification and characterization of enzymes in the asparagine transamination pathway in higher plants**

Frédéric Marsolais[1,2], Dristy Zaman[1,2] and Aga Pajak[1]

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Improving the quality of economically and nutritionally valuable legume crops, such as soybean, requires a comprehensive understanding of nitrogen metabolism. L-asparagine (Asn) is an important source of nitrogen stored and transported in higher plants, favoured in legumes due to its advantageous 2N:4C ratio. The catabolism of Asn in tissues occurs through two major pathways: deamidation and transamination. The transamination pathway employs an unidentified α-ketosuccinamate (α-KSM) reducing dehydrogenase (α-KSDH), as well as an ω-amidase, which has not been fully characterized. The goal of this research is to identify α-KSDH in soybean and characterize the role of ω-amidase in the transamination pathway in *Arabidopsis*. Fractionation, ammonium sulfate precipitation, size exclusion chromatography, and LC-MS/MS were employed to sequentially purify and identify an α-KSDH that showed activity with α-KSM. Altogether, these techniques revealed that soybean hydroxyphenylpyruvate reductase (GmHPPR) and *Arabidopsis* hydroxypyruvate reductase 2 (AtHPR2) catalyze the reduction of α-KSM into α-HSM. Based on this activity, these are likely the enzymes responsible for α-KSDH function *in vivo* of their respective species, however *in vivo* studies are required to confirm this function. The broad substrate specificity of HPPR and HPR2 and its activity with 2-hydroxyacids indicate that its main role *in vivo* is to convert intermediary metabolites into metabolites that can be directly used for other important pathways within the plant. An ω-amidase T-DNA insertion loss of function mutant was found to be embryo lethal in *Arabidopsis*, cause shortened siliques in heterozygotes, and overall have a detrimental affect on reproduction.

Like ω-amidase, GmHPPR is known as a “clean-up” enzyme, and it can be assumed that it also plays a role in mitigating the levels of toxic intermediary metabolites as a “repair” enzyme. This research has advanced our understanding of Asn metabolism in higher plants and may ultimately contribute to advancements in crop nitrogen use efficiency.

June 28 (Saturday) SYMPOSIUM VII: Chemical Ecology and Plant-Organismal Interactions

Bao-Hua Song
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Dr. Bao-Hua Song is a Professor of Biological Sciences and joint faculty member in the School of Data Science at the University of North Carolina at Charlotte. Her research lies at the intersection of Ecological, Phytochemical, and Agricultural Genomics, with a focus on understanding the molecular basis and evolution of complex traits significant to agriculture, human health, and climate adaptation. Dr. Song’s lab integrates multidisciplinary approaches, including omics, molecular genetics, ecology, and evolutionary biology, to investigate plant multiple stress resistance and phytochemical diversity using crop wild relatives as study systems. She currently leads several major research grants, including an NSF-funded interdisciplinary collaborative project, an NIH-supported phytochemistry project, and a grant from the North Carolina Biotechnology Center.

Bao-Hua got her Ph.D. from Beijing Institute of Botany, Chinese Academy of Sciences. She completed her postdoctoral training with Dr. Thomas Mitchell-Olds at the Max Planck Institute for Chemical Ecology and later at Duke University. She joined the faculty at UNC Charlotte in 2012. Throughout her career, Dr. Song has received multiple honors, including the Knobel Prize for Research and Scholarship, the DBS Outstanding Research Award, and the Outstanding Data Science Faculty Research Award. Several of her publications have been recognized for their scientific impact.

Keynote talk: **Wild Soybean Meets Omics – Insights into Plant Chemical Defense**

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Climate change poses growing threats to agricultural sustainability and global food security, demanding innovative strategies to develop crops resilient to increasingly variable environmental conditions. Crop wild relatives (CWRs), which harbor untapped genetic diversity, represent a promising resource for addressing these challenges. Wild soybean (Glycine soja), native to East Asia, serves as an ideal model for studying plant resistance to pests and environmental stresses. The soybean cyst nematode (Heterodera glycines, SCN) is the most devastating pest of cultivated soybean, causing over $1.5 billion in annual yield losses in the U.S. alone. Rapid SCN evolution and lack of novel resistant resources in cultivated soybean represent the two biggest challenges for SCN management. To address this, we employed an integrative approach combining genome-wide association studies (GWAS), RNA-sequencing, metabolomics, and molecular genetics to uncover new mechanisms of SCN resistance in wild soybean. This work has led to the identification of key candidate genes and phytochemicals associated with broad-spectrum resistance across different SCN types. Ongoing single-cell omics studies are poised to further elucidate the dynamic cellular interactions between wild soybean and SCN. These insights not only deepen our understanding of plant chemical defense mechanisms but also provide valuable resources for accelerating the development of new SCN-resistant soybean cultivars.

Short presentation 1: **Metabolic modifications in differential host resistance of wild and cultivated carrots to above and belowground parasitic plants**

Sukhmanpreet Kaur[1,6], Mwafaq Ibdah[2], Riko Sakioka[3], Kyogo Nagano[4], Kaori Yoneyama[4], Philipp Simon[5], James Westwood[6], and Dorothea Tholl[1]

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The cultivation of carrots (Daucus carota) faces global threats from infestation with parasitic plants, particularly the root parasite Phelipanche aegyptiaca (Egyptian broomrape) and shoot parasite Cuscuta gronovii (swamp dodder). These parasites extract water and nutrients from their host through specialized structures called haustoria, which cause severe damage and yield losses. We explored the resistance mechanisms of carrots against parasitic plants by examining different cultivated carrots and wild carrot relatives. Our study found wild carrot species (Daucus glaber and Daucus littoralis) but not cultivated carrots to be resistant to P. aegyptiaca. This resistance was based on the reduced release and modified profile of strigolactones that stimulate germination of the parasite seeds. In addition to the pre-attachment resistance, we observed limited development of the parasite after attachment to D. glaber roots. In contrast to their response to P. aegyptiaca, wild carrots were found to be susceptible to the shoot parasite C. gronovii, while a domesticated carrot cultivar showed high levels of resistance. A so far uncharacterized host response was identified, where the parasite penetrates the host epidermis and cortex but its haustorium is unable to establish a vascular connection in conjunction with the accumulation of phenolics and/or lignin around the haustorium. Bulksegregant and transcriptome analysis will help identify gene loci associated with this host response. Taken together, this work introduces distinct resistance mechanisms against two separate parasitic plants, highlighting carrots as a system for exploring resistance to a range of plant parasites.

Short presentation 2: **Convergence and constraint in glucosinolate evolution across the Brassicaceae**

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Diversity in plant specialized metabolites plays critical roles in plant-environment interactions and plant physiology. In long-term evolutionary times, e.g. between families or orders, there is evidence that this diversity arises by whole-genome and tandem duplication events. However, less is known about the evolutionary patterns that shape the chemical diversity observed at shorter time scales, e.g. within a family. Utilizing the aliphatic glucosinolate pathway we seek to explore how the terminal structural modification enzyme GSL-OH has evolved across the Brassicaceae and the genomic processes that controls presence-absence variation of its products (R)-2-hydroxy-but-3-enyl and (S)-2-hydroxy-but-3-enyl. To explore this, we implemented a phylo-functional approach where we functionally validated GSL-OH orthologs across the Brassicaceae and used that information to map the genomic origin and trajectory of the loci. We uncovered a complex mechanism with at least three ancestral loci that were unequally retained across groups independent of their phylogenetic relationship and showed extensive gene loss across all species. In addition to these processes, some tandem duplicates often diverged in function with preference towards the R or S enantiomer, with this convergently occurring across the phylogeny. To explore potential biological differences between the enantiomers we performed *Trichoplusia ni* herbivory assays and *Botrytis cinerea* detached leaf assays. We found that the S enantiomer was more susceptible to *B. cinerea* infection while the R enantiomer seemed more susceptible to *T. ni* herbivory. This variation in enantiomeric activity and function may shape the recurrent evolution of the gene.

Short presentation 3: **Detoxification of plant secondary metabolites by Two-Spotted Spider Mites as a mechanism of host adaptability**

Julia Pastor-Fernandez[1], Vinayak Singh[1], Sameer Dixit[1], Luis C. Romero[2], Vladimir Zhurov[1], Mark A. Bernards[1], Ricardo Hernandez[3], Brendan Abiskaroon[3], Maksymilian Chruszcz[3], Miodrag Grbic[1] and Vojislava Grbic[1]

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Plants exhibit a complex array of defensive secondary metabolites to fight pest attacks. However, herbivorous arthropods developed strategies to counteract these plant defensive compounds in order to feed on plants. The herbivore we focus on is the twospotted spider mite (TSSM; Tetranychus urticae), a highly polyphagous pest that feeds on over 1100 plant species belonging to more than 100 different families. Despite their broad host range, they are considered composite generalists, as individual populations tend to perform better on smaller groups of plant species. When introduced to a new host, their performance initially declines significantly; however, after approximately 25 generations, they adapt and perform well, demonstrating a rapid capacity for host adaptation.

To investigate mite strategies to cope with Arabidopsis defensive compounds, we adapted bean-reared mite populations to Arabidopsis, using wildtype Col-0 (Col-a mites) and cyp79B2/B3 (cyp-a mites) plants. Arabidopsis represents a challenging host to beanreared ancestral population, but mite performance increases on cyp79B2/B3 mutant that lacks tryptophan-derived compounds. Among these compounds, we showed that indole glucosinolates play a significant role in Arabidopsis defense against TSSM and are key targets for mite adaptation.

Transcriptomic analysis of mite responses when fed on bean and the two Arabidopsis genotypes identified 293 DEGs in Col-a and cyp-a mites vs bean-reared mites. Among them, 37 upregulated genes encode detoxification enzymes and are associated with mite adaptation to Arabidopsis. Some of these genes were specifically upregulated only in Cola mites after transfer to both genotypes, indicating a role in adaptation to tryptophanderived defenses. Others were upregulated in both Col-a and cyp-a mites upon transfer to either Arabidopsis genotype, suggesting a general role in adaptation to Arabidopsis defenses that are synthesized outside of the Trp-dependent biosynthetic pathway. Both sets of genes were selected for further functional analysis.
To test their requirement for mite adaptability to Arabidopsis we performed RNA interference-based gene silencing. Most of the tested genes led to reduced mite fecundity when silenced, indicating their essential role in mite adaptation to Arabidopsis. These enzymes despite belonging to the same protein families have specific, non-redundant functions, as silencing individual genes negatively impacted mite fitness. 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 demonstrated that Arabidopsisadapted mites gained the ability to detoxify Trp-derived defenses by at least two different mechanisms. We demonstrated that the activity of β -cyanoalanine synthase (TuCAS) is necessary for the detoxification of hydrogen cyanide released in Arabidopsis leaves upon indolic glucosinolates' breakdown during mite feeding. Additionally, we showed that glutathione-S-transferase of the mu family is required for the detoxification of the isothiocyanate. Together, our findings highlight the complexity of mite adaptation to Arabidopsis, revealing the diverse detoxification strategies that underlie mite host adaptability.

Short presentation 4: **Uncovering the complexity of Arabidopsis thaliana defenses against Tetranychus urticae herbivory**

Jorden Maglov[1], Julia Pastor-Fernandez[1], Alexander Harrison[1], Michele Antonacci[1], Brendan Abiskaroon[2], Tony Schilmiller[2], Vladimir Zhurov[1], Maksymilian Chruszcz [2], and Vojislava Grbic[1]

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Tetranychus urticae (two-spotted spider mite) is an extreme generalist herbivore with the ability to feed on over 1100 plant species. Its wide host range suggests that mites can disarm a wide array of host defenses. However, as a composite generalist herbivore, individual mite populations perform well on only a subset of host species within their host range. These populations can also rapidly adapt to new host environments, showcasing a balance between specialist and generalist traits. Thus, rapid adaptation is one of the key features underlying mites' wide host range, allowing mites to render initially effective plant defenses, ineffective. Understanding mite counteradaptations to host defenses requires an understanding of plant defenses that were initially effective in limiting mite fitness. To study the mite-host interaction, our lab experimentally adapted an ancestral bean-reared mite population to Arabidopsis. Arabidopsis is a challenging and non-preferred host for bean-reared mites, making it an excellent model to identify Arabidopsis defenses that restrict mite herbivory. Arabidopsis defenses against mites are primarily mediated by jasmonic acid-induced transcription factors MYC2,3,4. We have previously established that MYC2,3,4-dependent synthesis of tryptophan-derived indole glucosinolates contributes to the Arabidopsis defense response against mites. While glucosinolates are considered a hallmark of Arabidopsis antiherbivore defenses, we showed that 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 secondary metabolites. This indicates the existence of other classes of MYC2,3,4-dependent phytochemicals that contribute to Arabidopsis defenses against mite herbivory. To unbiasedly identify additional Arabidopsis compounds that restrict mite herbivory, we combined untargeted metabolomics and transcriptomics approaches. This comparative analysis revealed several pathways enriched in differentially expressed genes and differentially abundant metabolites in response to mite herbivory. One of the identified pathways is phenylpropanoid biosynthesis. Using mutants that disrupt the phenylpropanoid pathway, we identified sinapate esters, hydroxycinnamic acids, and flavanol glycosides as compounds that may contribute to the Arabidopsis defense response against mites. To directly test the toxicity of these compounds, we next fed mites with individual phytochemicals and measured resulting mite mortality and fecundity levels. To determine how Arabidopsis defensive phenylpropanoids are regulated within the plant, we measured metabolite abundance in a mutant myc2,3,4 background. We have discovered that the phenylpropanoid pathway consists of both constitutive and inducible defenses, where inducible defenses accumulate in a manner both dependently and independently of canonical regulatory systems like MYC2,3,4. Thus, we identified novel Arabidopsis phytoalexins and phytoanticipins against spider mites and have begun to uncover the complexity of defense responses within a single plant species.

Short presentation 5: **Is Poison Ivy Urushiol an Ecological Non-Functional (aka Anachronistic) Chemical Defense?**

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In North America, poison ivy (Toxicodendron radicans) produces a potent allergen called urushiol that can initiate intense allergenic contact dermatitis (aka “poison ivy rash”) in humans. While urushiol is a potent defense against humans, no contemporary North American fauna displays urushiol-induced contact dermatitis comparable to humans. Humans cannot be a primary co-evolved target animal because humans immigrated to North America only ~14,000 ago. Therefore, we investigated whether arthropods and microbes may be coevolved targets of urushiol chemical defense.

Many specialized phytochemicals against arthropod herbivores and microbial pathogens are inducible by the plant stress hormones jasmonic acid or salicylic acid, respectively. Axenic poison ivy seedlings treated with methyljasmonic acid or salicylic acid showed comparable urushiol levels relative to buffer treated controls. We utilized trait-trait correlations to evaluate whether urushiol levels in naturally recruited poison ivy plants were associated with either insect herbivory or surface microbe levels. Neither insect foliar herbivory area, drupe-associated bacterial colony forming units (CFU), nor drupeassociated fungal CFUs were correlated with urushiol levels. Field exclosure experiments demonstrated that only large herbivores significantly reduced poison ivy biomass and height. Urushiol levels showed a negative allometric tradeoff with foliar biomass, like other functional plant chemical defenses. Urushiol levels accumulate as a constitutive chemical defense within resin canals, suggesting urushiol targets a chewing herbivore. In the absence of a convincing contemporary native North American target organism, we propose there were prehistoric coevolved North American urushiol-sensitive megaherbivores. However, they are now extinct – leaving contemporary urushiol as an anachronistic ecological trait.

Megaherbivores (>2,000 kg body mass) are typically top-down ecosystem architects. At the end of Pleistocene, the extinction of megaherbivore species resulted in dramatically altered North American landscapes and their ecological functioning (e.g. decreased nutrient cycling, altered dominant plant species composition, increased forest size, and greatly increased fire impacts). Poison ivy prefers forest edge habitats, where it grows as both ground-creeping and tree-climbing lianas. During the Pleistocene, browsing megaherbivores (e.g. mastodons and giant ground sloths) would have browsed on vulnerable young trees at forest edges, as well as tree-climbing poison ivy lianas. We posit that poison ivy lianas provided a potent allergenic chemical defense against megaherbivores, discouraging their browsing both on poison ivy and young trees hosting climbing poison ivy lianas. Poison ivy urushiol and megaherbivore browsers would have exerted mutual co-evolutionary processes such as: 1) purifying natural selection on urushiol levels and composition; and 2) megaherbivore (in)sensitivity to urushiol allergens. However, since the end-of-Pleistocene megaherbivore extinctions, the poison ivy urushiol trait might now be shaped by mostly neutral evolutionary processes. These evolutionary hypotheses are amendable to future investigations using poison ivy evolutionary genomics and Paleoimmunogenetics.

This posited urushiol chemical anachronism may be the “tip of an iceberg” of other plant specialized metabolites that are perhaps likewise contemporary anachronisms - disconnected from their extinct coevolved (mega)herbivores or pollinators. The breadth of contemporary phytochemical diversity is well measured and estimated. Whether this observed phytochemical diversity also corresponds to contemporary functional ecological traits is generally neither critically considered nor adequately validated.

Short presentation 6: **Heterospecific Pollen Tolerance in Natural Communities: The Role of Flowering Synchrony**

Marjori Thays da Silva[1] and Gerardo Arceo-Gómez[1]

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Sexual reproduction in flowering plants is vulnerable to heterospecific pollen (HP) transfer, which reduces fitness through physical (e.g., stigma clogging) or chemical interference. While HP typically decreases seed production by ~20%, effects vary widely across species suggesting divergence in HP tolerance. Recent studies suggest tolerance correlates with history of HP exposure across populations, but differences in HP exposure also exist across temporal scales (mediated by variation in flowering overlap), and this remains untested. Here, we use controlled hand-pollination experiments across species pairs with varying co-flowering overlap to quantify HP effects on pollen tube growth, and ovule fertilization. Specifically, we test the hypothesis that species with high flowering synchrony may have evolved greater HP tolerance. Our preliminary results showed that the species not only tolerate HP from strongly overlapping co-flowering species but also benefit from them, as their reproductive fitness increases even further. Our work establishes the first experimental link between phenological overlap and HP tolerance, advancing understanding of plant-plant interference in natural communities. These results provide a foundation for future studies on phytochemical mechanisms underlying HP tolerance, including potential selective pressures on pollen-level traits (e.g., adhesion proteins or inhibitory compounds). This work is a critical step toward unraveling reproductive adaptation in biodiverse systems.

Gustavo MacIntosh
Iowa State University,
Ames, Iowa,
USA

Invited speaker: **Manipulation of soybean defenses and induced susceptibility effected by aphids**

Jessica Hohenstein, Charles Kanobe, Martha Ibore Natukunda, Kia Barry, Gustavo MacIntosh

Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, USA

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The soybean aphid (Aphis glycines), an economically important insect pest of soybean (Glycine max), can induce susceptibility on its host during colonization. The mechanism for this process is not known. Based on previous transcriptome analyses, we hypothesized that aphids block effective jasmonate (JA) defenses by induction of an antagonistic abscisic acid (ABA) signal. Analysis of soybean plants exposed to seven days of previous aphid feeding showed that these plants had attenuated JA-, wound-, and herbivore-induced JA responses compared to control plants. Growth of a chewing caterpillar Helicoverpa zea was facilitated by the aphid-regulated suppression of JA responses. Aphid-treated plants had increased levels of cis-JA but not biologically active JA-isoleucine, and aphid feeding differentially induced the expression of genes associated with JA-Ile catabolism. In parallel, aphid-treated plants had higher ABA content compared with control plants. ABA treatment and knockdown lines impaired in ABA biosynthesis (aba2-RNAi) or signaling (scof-1-RNAi), showed that both endogenous and exogenous ABA suppressed wound-induced JA responses. Furthermore, aphid populations were significantly reduced on plants defective in ABA signaling and aphidregulated attenuation of JA signaling was abolished in these lines. Remarkably, plants defective in ABA signaling had increased JA signaling in the absence of stressors, suggesting that, in soybean, ABA is necessary to control basal levels of JA.

Long-term (21 days) exposure to aphids induced isoflavone biosynthesis, and several isoflavones accumulated in soybean plants. Daidzein, one of the most highly elevated isoflavones, acts as deterrent of aphid feeding in choice experiments, suggesting that these phytoalexins could protect plants from aphid infestations. However, ABA seems to block isoflavone production during the colonization process. Our results indicate that soybean aphids exploit a preexisting ABA-JA antagonism to suppress effective JAmediated plant defenses and to block phytoalexin production.

Short presentation 7: **Harnessing cuticular waxes from poplar leaves to produce insect pheromones**

Elizabeth Copley[1,2] & Eliana Gonzales-Vigil[1,2]

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Damage to crops by insect pests is a serious concern for farmers worldwide due to immense economic losses, compromised food product quality, and reduced yield. To combat environmental stressors, including insect damage, land plants have developed a waxy, hydrophobic protective layer on the surface of the aerial organs called the cuticle.

Various poplar (Populus trichocarpa) accessions accumulate unsaturated hydrocarbons called alkenes in the cuticle of leaves throughout development. Alkene accumulation starts low, then eventually becomes the dominant compound class in fully expanded leaves. As the leaves age, alkene accumulation declines as they spontaneously oxidize into aldehydes: one of which is a known volatile insect pheromone called nonanal.

The use of insect pheromones in traps may be a more sustainable way to control pests as opposed to insecticide application. However, their use has been limited by the high cost of chemically synthesizing them. Alternative methods of producing insect pheromones, such as nonanal from poplar alkenes, are needed.

The goal of this project is to develop a novel, environmentally sustainable pest management tool using the insect pheromone, nonanal, produced from poplar alkenes in insect traps to reduce the application of pesticides. To determine the applicability of alkenes as pheromone baits for traps, a preliminary field trial using alkene- and nonanalbaited traps at the UTSC Campus Farm was completed last summer. Sticky traps baited with either a 23-carbon cis-9 alkene, tricosene, or with nonanal in glass vials were deployed and the number of various insect species on the traps were counted to determine if any local insects were responding to the treatments.

In addition, this research will elucidate the biological function of cuticular waxes, including alkene accumulation, in the poplar leaf cuticle by discerning their roles in plant-insect interactions via insect bioassays. Preliminary results from insect herbivory assays have shown changes in leaf cuticular wax profiles due to mechanical wounding and in response to insect herbivory. Interestingly, the responses are unique for each compound class and are largely genotype-dependent. The results of this project will lead to developing insect traps that can specifically target insect pests using plant-produced alkenes.

Short presentation 8: **Specialized metabolite diversity in S. habrochaites: Unlocking new sources of resistance against two insect herbivores**

Andreea Bosorogan[1,2], Sundas Rehman[2], Osmond Hui [2], Eliana Gonzales-Vigil [1,2]

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Conventional breeding has improved several agronomic traits in cultivated tomato (Solanum lycopersicum), yet herbivore resistance remains a critical challenge in tomato production. Plants use a plethora of strategies, including specialized metabolites (e.g., terpenes, cuticular waxes), to reduce insects' feeding and development. While the abundance of specialized metabolites varies across Solanum spp., this diversity has been lost in the cultivated tomato. S. habrochaites, a wild relative of tomato, harbours striking chemical diversity within its trichomes, yet the underlying selective pressure behind this chemical diversity remains elusive. In this study, we screened 17 accessions of S. habrochaites against two insect pests: Trichoplusia ni (Lepidoptera) and Leptinotarsa decemlineata (Coleoptera). Significant differences in insect mortality and weight gain were observed across the accessions, with twelve S. habrochaites accessions displaying high resistance – causing over 50% mortality and reduced weight gain in either T. ni or L. decemlineata. Of these, six accessions showed resistance to both insect species, five were resistant only to T. ni, and one only to L. decemlineata. These observations suggest that S. habrochaites accessions use common and distinct mechanisms to defend against different insect species. Moreover, choice assays implicate olfactory cues driving insect deterrence. To determine if chemical diversity is responsible for the differences in insect performance among cultivated and wild tomatoes, terpenes and cuticular waxes from leaves were profiled. Terpene diversity and abundance varied significantly across the accessions, yet the cuticular wax profiles of S. habrochaites were similar to those of cultivated tomato. Accessions with elevated levels of several terpenes, including bergamotene, santalene, and germacrene, showed high resistance to insect damage, suggesting potential repellent or toxic effects on T. ni. Negative correlations between several other terpenes, particularly germacrene B, and insect performance also suggest that terpene diversity contributes to the resistance. Altogether, this study enhances our understanding of plant-insect interactions in S. habrochaites and identifies key terpenes for breeding insect-resistant tomato cultivars.

Workshop: **The Plant Metabolic Network: A Unified Resource for Plant Metabolism Research Community**

Charles Hawkins[1], Bo Xue[1], Gaëlle Cassin-Ross[1], Gabrielle Wyatt[2], Matt Stata[1], Marcos de Oliveira[3], Farida Yasmin[2], Johanna Murray[1], Hiroshi Maeda[3], Philipp Zerbe[2], and Seung Y. Rhee[1]

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The Plant Metabolic Network (PMN) is a free, online resource for plant metabolism available at plantcyc.org. The current version (PMN 16) features 155 single-species databases for plants and green algae as well as a pan-plant reference database called PlantCyc. PlantCyc version 16.0.2 contains 1,162 pathways, 3,770 enzymes, 4,960 metabolites, and 7,696 cited papers detailing experimentally supported plant metabolism data from 607 species. The resource was created using PMN's bespoke pipeline and integrates computationally predicted information with experimental data from the literature to create a comprehensive picture of the metabolism of each species. PMN serves as a bridge between genome biology and plant metabolism, allowing researchers to create, query, visualize, and analyze metabolism data. Users can also interpret omics data in the metabolic context using Pathway Tools' Omics Viewer. This workshop demonstrates various ways to use PMN's databases for plant research. Participants will learn how PMN can bridge the gap between genomics and metabolism, transforming datasets and generating insights into a plant's metabolism. Having seen what PMN databases can be used for, participants will then learn how to create their own databases for any plant or green algal species with a sequenced and annotated genome, using PMN's newly-released pipeline. The workshop will also highlight PMN's recent efforts to increase experimentally validated data on enzyme functions from the Zerbe and Maeda labs within the Plant Enzyme Consortium. Finally, we will show how the plant metabolism research community can contribute experimental enzyme function data or species-specific databases to PMN using our new curation form to increase the reach, visibility, and utility of their work.

Cristiana Argueso
Colorado State University
Fort Collins, Colorado, USA

Keynote talk: ***Phytohormonal Control of Plant Immunity and Susceptibility in Changing Climates***

Cris Argueso[1]

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Phytohormones are small molecules that regulate plant development and responses to the environment, including responses to pathogens. The importance of phytohormones in plant-pathogens interactions is underscored by the variety of these molecules that are essential for immunity activation, as well as the number of pathogen effectors that manipulate phytohormonal signaling and metabolism to aid in plant cell colonization. Due to changes in the climate leading to higher temperatures and more frequent heat waves, several crop species have become more susceptible to plant pathogens, a process known as Heat-Induced Disease Susceptibility (HIS). Furthermore, as global human populations continue to grow and temperatures are expected to rise, the pressure to increase food productivity and develop more HIS-resistant crop varieties and agricultural strategies intensifies. Given the importance of phytohormones to plant responses to both biotic and abiotic stresses, we set out to determine their contribution to HIS in Arabidopsis. We show that the lack of functional cytokinin signaling makes plants less susceptible to bacterial pathogens at elevated temperatures, and that this decreased HIS is not attributed to stronger defense responses, but rather to changes in plant metabolism associated with pathogen feeding. We also present possible genetic and chemical strategies to translate the findings discovered in Arabidopsis to combat HIS in crop species.

Short presentation 1: **A Cysteine Protease Cleaves Pokeweed Antiviral Protein to Enhance Apoplastic Immune Signaling**

Annabelle Audet[1], Jennifer Chivers[1], and Katalin A. Hudak[1]

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Plant defenses rely on protein-protein interactions within the apoplast, the first point of contact between plant cells and invading pathogens. The apoplast serves as a critical site for immune signaling, facilitating recognition of external threats and activation of defense responses. Pokeweed antiviral protein (PAP), a ribosome-inactivating protein (RIP) from Phytolacca americana (pokeweed), is localized to the apoplast where it contributes to plant defense. PAP functions by inactivating ribosomes through the removal of a specific adenine residue in the sarcin-ricin loop of the 25S ribosomal RNA, thereby inhibiting translation. However, how PAP may interact with other proteins in the apoplast remains unclear. To investigate potential extracellular interactions, we identified Phytolacca americana cysteine protease 1 (PaCP1), an extracellular papain-like cysteine protease, as a novel PAP interactor. Using pull-down assays, mass spectrometry, and yeast twohybrid analysis, we confirmed that PAP specifically binds the mature, active form of PaCP1. Sequence and structural analyses classified PaCP1 as a member of the C1A subfamily of papain-like cysteine proteases, sharing high similarity with Arabidopsis thaliana xylem cysteine protease 1, a protease involved in Arabidopsis immunity. Both PAP and PaCP1 were localized to the apoplast, where PaCP1 cleaves PAP at its N- and C-termini, generating fragments that lack ribosome-inactivating activity. The small peptides released from this cleavage were found to enhance MAPK phosphorylation in pokeweed leaves, indicating a potential role in immune or stress signaling. The interaction between PAP and PaCP1 suggests a mechanism by which protease-mediated cleavage diversifies the functions of ribosome-inactivating proteins beyond their canonical role of inhibiting translation. Our findings present a novel extracellular function for PAP and advance our understanding of how dynamic protein interactions in the apoplast contribute to the modulation of plant immune responses.

Short presentation 2: **ANAC042 Regulates the Biosynthesis of Conserved and Lineage-Specific Phytoalexins in Arabidopsis**

Ivan Monsalvo[1], Leonardo Parasecolo[2], Sarah Pullano[1], Jie Lin[1], Aida Shahabi[1], Melissa Ly[1], Hyejung Kwon[1], Khushi Mathur[1], Demian R. Ifa[2], and Nik Kovinich[1]

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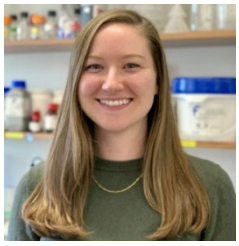
Throughout evolution, plants have developed diverse defence mechanisms in response to pathogen attack. These include the production of specialized metabolites called phytoalexins. A prevailing paradigm in transcription factor (TF) biology is that conserved TFs regulate specific branches of specialized 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.



Cynthia Holland
Williams College
Williamstown, Massachusetts, USA

Cynthia Holland is an Assistant Professor at Williams College, a small liberal arts college in Williamstown, Massachusetts. Before starting at Williams, Cynthia completed her Ph.D. research on aromatic amino acid and hormone biosynthesis and regulation in Joe Jez's lab at Washington University in St. Louis, and conducted postdoctoral research on cardenolide biosynthesis in wallflowers in Georg Jander's lab at the Boyce Thompson Institute. At Williams, her lab of undergraduate researchers investigates the metabolic pathways branching off from anthranilate, an intermediate in tryptophan biosynthesis. This NSF-funded research has uncovered how plants regulate a key enzyme in the tryptophan pathway and the molecular basis of substrate recognition in anthranilate O-methyltransferases, which produce a grape-scented defense volatile. Current work in the Holland lab focuses on how plants perceive anthranilates, including both N- and O-methyl anthranilate derivatives.

Arthur Neish Award presentation: **Molecular fates of anthranilate in plants**

Cynthia K. Holland, Aracely Watson, Michael Fallon, Ellia Chiang, and Chris Flores

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Plant chemical defenses are often secondary or specialized metabolites derived from primary metabolites such as amino acids. While all plants synthesize anthranilate as an intermediate in tryptophan biosynthesis, several species have co-opted anthranilate for defensive functions. The O-methyl ester of anthranilate is a volatile anti-herbivory compound responsible for the characteristic grape aroma and is emitted by grapes, citrus, maize, and other angiosperms. Citrus also produces N-methyl anthranilate esters with analgesic activity in mammals. Our recent work has identified and biochemically characterized the enzymes responsible for synthesizing these bioactive esters in grape and citrus. We have also begun exploring how plants perceive and respond to anthranilates. This has revealed a role for anthranilates in seed germination and early development in Arabidopsis thaliana. In addition, in vitro assays have identified methylesterases capable of demethylating anthranilate esters, and we are investigating the physiological relevance of these interactions in planta and the implications of these findings on inter-plant communication and plant physiology. Together, this research expands our understanding of the biosynthesis and biological functions of anthranilates in agriculturally important plants.

Katalin Hudak
York University
Toronto, ON, Canada

Dr. Kathi Hudak is a Professor in the Department of Biology at York University in Toronto, Canada. She received her PhD from the University of Waterloo and continued as a Postdoctoral Research Associate at the Biotechnology Center of Rutgers University. She then returned to Canada to accept a faculty position at York University. Dr. Hudak's research focuses on RNA glycosylases and how their activity alters gene expression during plant stress and virus infection.

Invited speaker: **Plant RNA Toxins and their Role in Signaling During Stress**

Katalin A. Hudak

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Cells rely on various RNAs, in the form of mRNAs, tRNAs and rRNAs, to transmit information stored in DNA for the production of functional proteins. These nucleic acids may be modified by alkylation, oxidation and base loss during their lifetime, which alters their function. Though substantial research has been devoted to the detection and repair of damaged DNA, much less attention has been paid to RNA as it is viewed as short-lived and quickly degraded. However, recent results from our lab and others show that damaged RNAs are important signaling molecules during stress. Plant enzymes called ribosome inactivating proteins (RIPs) remove a purine base from the sarcin/ricin loop of rRNA, thereby inhibiting protein synthesis. Current work from our group illustrates how this damage slows the rate of elongation, which we think is the key element recognized by translational surveillance factors. Moreover, we have shown how mRNAs with abasic sites stall ribosomes, resulting in the degradation of these messages. Expression of many RIPs is also upregulated by biotic and abiotic stresses, suggesting that they function in defense, either by depurinating the RNA of the invading pathogen or the plant itself, eliciting death that would limit infection spread. Most of our knowledge of RIPs stems from biochemical analyses of individuals; therefore, we performed an unbiased search among all annotated plant genomes for proteins with RIP domains and identified more than 800 from 120 species, many with novel associated domains and physicochemical characteristics. Surprisingly, most of these RIPs lacked a signal peptide, indicating they may be localized to the cytoplasm of cells, raising questions about their toxicity against conspecific ribosomes. The distribution of RIPs throughout 21 plant orders, with many species expressing more than one type of RIP, indicates a more diverse group of proteins than previously known. This diversity, together with their ability to remove bases from RNA, suggests that RIPs function to control gene expression during stress and defense against pathogens.

Short presentation 3: **Soybean - *Sclerotinia sclerotiorum* interaction: identification of factors involved in partial resistance against Sclerotinia stem rot disease**

Deepak Duhan[1,2], Justin Renaud[1] and Sangeeta Dhaubhadel[1,2]

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Glyceollins are soybean-specific phytoalexins that play a crucial role in the plant's defense. Sclerotinia stem rot disease caused by the fungal pathogen *Sclerotinia sclerotiorum* poses a significant threat to soybean production. In this study, we aimed to explore if glyceollins and other isoflavonoids contribute to resistance in soybean against *S. sclerotiorum*. Targeted metabolomics on four soybean cultivars that are either partially resistant (OAC Drayton and OAC 13- 500C) or susceptible (Nattosan and OAC Kent) was conducted using liquid chromatography-mass spectrometry (LC-MS). Our results revealed distinct differences in the metabolite profiles between the partially resistant and susceptible soybean cultivars. Constitutive metabolites, such as naringenin, isoliquiritigenin, and daidzein, were present in all cultivars; however, their levels increased significantly higher level in the partially resistant lines following infection. Among the induced metabolites, glyceollin I, II, and III showed pronounced accumulation in response to infection, particularly in the partially resistant cultivars. Conversely, glyceocarpin levels remained relatively stable across conditions and cultivars. Our findings suggest that the early and robust induction of glyceollin is contributes to the partial resistance in soybean. The elevated baseline levels and infection-responsive increases of key isoflavonoid metabolites, such as daidzein, may also contribute to the development of a resistant phenotype. Together, these compounds could serve as valuable biomarkers in resistance screening.

IN-PERSON POSTER PRESENTATION ABSTRACTS

POSTER SESSION I (odd-numbered posters) – Wednesday June 25, 2025
POSTER SESSION II (even-numbered posters) – Thursday June 26, 2025

Poster 1: Mapping multi-substrate specificity of Arabidopsis aminotransferases

Kaan Koper[1], Marcos V.V. de Oliveira[1], Sebastian Huß[2], Shogo Hataya[3], Fayaz Soleymani-Babadi[2], Charles Hawkins[4], Seung Y. Rhee[4], Taichi E. Takasuka[3], Zora Nikoloski[2], Hiroshi A. Maeda[1]

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Nitrogen is an essential element in all organisms, and its availability and use efficiency directly impacts organismal growth and performance, especially in plants. Aminotransferases are core enzymes of the nitrogen metabolic network for synthesizing various organonitrogen compounds. Although each aminotransferase can potentially catalyze hundreds of transamination reactions with different combinations of amino and keto acid substrates, the full functionality of many aminotransferases remains elusive. Here we employed high-throughput gene synthesis and enzyme assay platforms to determine substrate specificities of 38 aminotransferases of Arabidopsis thaliana and unveiled many previously unrecognized activities among a total of 4,104 reactions tested. Integration of the obtained biochemical data in an enzyme-constrained metabolic model of Arabidopsis and in silico simulation further revealed that the promiscuity of aminotransferases alters nitrogen distribution profiles and contributes to the robustness of the nitrogen metabolic network. The study provides foundational knowledge towards understanding the functionality of nitrogen metabolism and improving nitrogen use efficiency in crops while limiting fertilizer usage.

Poster 2: Metal-Induced Activation of Isoflavonoids and Phytoalexin Pathways in Legumes: Comparative Analysis of Soybean, Pea, and Lentil

Allison Bergen[1,2], Tim McDowell[1], Justin Renaud[1], Hodan Halane[1,3], Sangeeta Dhaubhadel[1,3]
[1]London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada
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Legume crops such as soybean (*Glycine max*) and pea (*Pisum sativum*) deploy specialized metabolites known as phytoalexins—predominantly isoflavonoid-derived compounds—as part of their defense response against biotic and abiotic stresses. Among these, glyceollins in soybean and pisatin in pea are reported as stress-induced compounds. In contrast, the phytoalexin of lentil (*Lens culinaris*), an economically important yet less studied legume, remains largely unknown. This study aims to investigate the potential of metal stress to induce phytoalexins and isoflavonoid biosynthetic pathways across these three legumes, with a specific interest in identifying novel defense-related metabolites in lentil. To induce the stress response, seeds of each species were treated with $AgNO_3$, $CuCl_2$, $CuSO_4$, and a water control- and metabolites were extracted post-treatment. Extracts were analyzed using liquid chromatography–mass spectrometry (LC-MS), with preliminary metabolite profiling completed using authentic standards and untargeted feature detection methods. As expected, soybean treated with $AgNO_3$ exhibited elevated levels of glyceollins, aligning with previous literature. Pea samples treated with $CuCl_2$, particularly at 5 mM, showed significant upregulation of pisatin, confirming the efficacy of metal ions as elicitors. In lentil, several features were upregulated across treatments, including some compounds matching known isoflavonoid standards; however, many of the most prominent peaks could not be matched to available standards, suggesting the presence of novel or poorly characterized metabolites. Ongoing work focuses on deeper annotation of these lentil-

specific features for potential phytoalexins unique to lentil. This could provide valuable insight into the innate defense mechanism of lentil and contribute to broader efforts to enhance disease resistance in pulse crops. Our results demonstrate that targeted metal stress is an effective tool to activate defense pathways in legumes. More importantly, this study provides a foundational step toward identifying and characterizing lentil-specific phytoalexins, an area currently underexplored.

Poster 3: Investigating Diterpenoid Biosynthesis and Diversity in Maize (*Zea mays*) Through Pathogen-Induced Metabolomics and Enzyme Discovery

Siena Schumaker[1], Farida Yasmin[1], and Phillip Zerbe[1]
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Plants produce a diverse array of specialized metabolites to adapt to their environment. Among these, terpenoids represent the largest class, playing key roles in growth, defenses against pathogens, and responses to abiotic stress. Previous studies in maize have demonstrated that plants deficient in diterpenoid compounds are more susceptible to attack by fungal pathogens such as *Fusarium graminearum* and insect pests like the European corn borer. To further investigate the role of diterpenoids in maize defense, I am conducting a metabolomic study across a panel of genetically diverse maize lines. Plants will be inoculated with *Fusarium verticillioides*, a major fungal pathogen, to evaluate induced diterpenoid responses. Untargeted metabolite profiling will be used to characterize the diversity and abundance of diterpenoids across genotypes in response to infection. In parallel, candidate gene mining of the B73 maize genome has identified one diterpene synthase and seventeen cytochrome P450 monooxygenase enzymes potentially involved in diterpenoid biosynthesis. These genes will be functionally characterized using *in vivo* combinatorial enzyme assays, employing *Agrobacterium*-mediated expression in *Nicotiana benthamiana* and *E. coli*, followed by GC-MS analysis. Together, these untargeted and targeted approaches will advance our understanding of diterpenoid-mediated defense and may lead to the discovery of novel inducible metabolites.

Poster 4: Phylotranscriptomics Analysis of the Betalain Biosynthesis in Caryophyllales

Alma Y. Gutierrez-Vences[1] and Carlos E. Rodriguez-Lopez[1]
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Betalains are tyrosine-derived pigments found exclusively in the Caryophyllales order, where they coexist with the more ubiquitous anthocyanins. These two pigment classes are mutually exclusive and exhibit a complex homoplastic distribution pattern. While the biosynthetic pathway has been characterized, the molecular mechanisms that resulted in their mutual exclusion and dominance in the major lineages of the order remain understudied. To address this, we conducted orthology-guided comparative coexpression and phylogenetic analyses to identify modules associated with betalain biosynthesis and its mutual exclusion with anthocyanins. Our findings suggest that alongside transcriptional regulation, pigment transport plays a role in both the emergence and exclusion of betalains. This is the first report implicating transport in betalain emergence and anthocyanin exclusion. Furthermore, our results support the hypothesis of multiple independent origins of betalains, with key mechanisms occurring in a family-specific manner, providing new insights into the molecular basis of pigment specialization in the Caryophyllales.

Poster 5: Understanding the role of SABP2-Interacting Proteins SIP432: a Premnaspirodiene Oxygenase Enzyme in Stress Signaling

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Climate change has had a significant impact on crop yield in recent years. The increase in temperature and drought has caused a decrease in plant survival and an increase in plant pathogens that also affect plant survival. Plants have evolved mechanisms to counter the adverse effects of biotic and abiotic stresses. Several proteins that contribute to the mechanisms that allow for resistance against pathogens and environmental stressors have been identified, but the overall metabolic pathway remains unknown. To better understand the defense mechanisms, it is important that we piece together these important genes/proteins for their role in biotic and abiotic stress responses. This study focuses on the characterization of a SABP2-interacting protein (SIP)-432. SIP432 is a putative premnaspirodiene oxygenase-like enzyme. Premnaspirodiene is a 15-carbon compound that can be converted into antimicrobial compounds by a hydroxylation reaction. The interaction of SIP432 with SABP2, a critical component of salicylic acid-mediated plant immunity, implies a role for SIP432 in plant defense signaling. This study uses the *Arabidopsis thaliana* SIP432 knockout mutants can help understand its role in abiotic and biotic stress responses. Wild-type (Col-0) and SIP432 mutant plants were subjected to abiotic stress conditions to understand their role in stress response pathways. All this will allow for the discovery of signaling in plant-pathogen interaction. Discovering the function of this protein could help uncover the environmental stress pathways in plants.

Poster 6: Metabolic Profiling of Early Land Plants using High Performance Liquid Chromatography – Mass Spectrometry

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The earliest land plants, which emerged around 470 million years ago during the Ordovician period, were non-vascular bryophytes like mosses, liverworts, and hornworts, all reproducing by means of sporic meiosis. High performance liquid chromatography paired with quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) provides a means to analyze a wide range of polar and semi-polar metabolites. Representative bryophytes were extracted with methanol and the resulting extracts containing semi-polar metabolites were subjected to HPLC-QTOF-MS on a reversed phase (RP) column and subsequent ionization in both negative and positive polarity mode. Furthermore, tandem mass spectrometry (MS/MS) was performed to assess fragmentation patterns of metabolites at several collision energies (10, 20, 30, and 50 eV). Following extraction with 80 % methanol and removal of non-polar constituents by liquid-liquid extraction or solid phase microextraction (SPME), polar metabolites were separated by hydrophilic interaction (HILIC) chromatography, followed by HPLC-QTOF-MS under the same conditions as those listed above. Strategies for the comprehensive characterization of different metabolite classes using streamlined workflows will be presented and the implications on the evolution of chemical diversity in bryophytes will be discussed.

Poster 7: Exploring carotenoid metabolism for increased β-carotene levels in wheat grains

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Vitamin A deficiency poses a serious threat to food security and can be addressed by enriching staple food crops, such as wheat, with β-carotene, the most effective provitamin A carotenoid. However, tetraploid (pasta) wheat grains mainly accumulate lutein, a non-provitamin A carotenoid that competes with β-carotene for biosynthetic precursors due to the activity of lycopene ε-cyclase (LCYe). Additionally, β-carotene levels can be reduced by the action of β-hydroxylases (HYDs) and carotenoid cleavage dioxygenases (CCDs). To better understand the impact of carotenoid metabolic genes on β-carotene accumulation in tetraploid wheat, we identified loss-of-function mutants of *LCYe*, *HYD,* and *CCD* homoeologs, generated mutant combinations, and compared them to control plants. Our biochemical and physiological analyses indicated that mutant combinations of *HYD2* and *LCYe* homoeologs led to significant increases in β-carotene in the grain endosperm, while maintaining comparable physiological traits to controls. In addition, we generated and analyzed combinatorial mutants of *HYD1* and *HYD2* homoeologs and observed the subfunctionalization of *HYD* genes and homoeologs across different tissues. Furthermore, our characterization of *ccd4* mutants revealed the functions of CCD4 in carotenoid cleavage in leaves, and uncovered several growth traits regulated by CCD4, Poltergeist-like (PLL), or interactions between CCD4 and PLL. Our research not only demonstrates strategies for enhancing β-carotene levels in tetraploid wheat grains, but also provides insights into the roles and interactions of carotenoid metabolic genes. These findings in tetraploid wheat set the stage for developing hexaploid (bread) wheat varieties with increased β-carotene levels and offer a sustainable solution to address vitamin A deficiency and improve food security.

Poster 8: The Elucidation of the Gene Regulatory Network Underlying Cannabinoid Biosynthesis in Cannabis sativa

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Cannabis sativa is a plant species valued for its ability to produce over 180 cannabinoids, specialized metabolites with diverse therapeutic and agricultural applications. However, the transcriptional regulation of cannabinoid biosynthesis remains poorly understood. To address this, we employed yeast one-, two-hybrid, promoter-luciferase, and bimolecular fluorescence complementation assays to investigate for protein-DNA and protein-protein interactions amongst putative metabolism-regulating transcription factors and the promoters of cannabinoid biosynthetic genes. Yeast one-hybrid assays revealed that putative metabolism-regulating transcription factors bound the promoters of numerous cannabinoid biosynthetic genes. Promoter-luciferase assays suggested that the candidate cannabinoid regulators, CsE23 and CsAL1, transactivated the promoter of the *OLS* gene for cannabinoid biosynthesis. Yeast two-hybrid and bimolecular fluorescence complementation assays demonstrated that putative metabolism-regulating transcription factors interact in numerous combinations. These findings point towards a network of transcription factors that can guide future breeding and bioengineering efforts.

Poster 9: Unlocking Phytoalexin Biosynthesis – Overcoming JAZ1-mediated Suppression and Biosynthetic Gene Transactivation through Multigene Engineering)

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Phytoalexins are plant defense metabolites whose biosynthesis remains suppressed until elicited by a pathogen or stress. We recently found that*JAZ1*, a negative regulator of the canonical jasmonic acid (JA) signaling pathway, suppresses phytoalexin biosynthesis by physically interacting with the transcription factor (TF) ANAC042 in Arabidopsis (*Arabidopsis thaliana*). ANAC042 directly activates the expression of phytoalexin biosynthetic genes and the MYB family TF *MYB15*, which is another activator of

phytoalexin biosynthesis. This research investigates whether simultaneously knocking-out *JAZ1* and overexpressing *ANAC042* or *MYB15* can derepress and/or enhance phytoalexin production. Using a novel matrix-assisted laser desorption ionization high-resolution mass spectrometry (MALDI-HRMS) method, we compared the metabolite profiles of eight Arabidopsis phytoalexins. We demonstrated that the Arabidopsis loss-of-function *jaz1-1* mutant elicited by Flg22 alone was insufficient in phytoalexin production. However, stacking the overexpression of *ANAC042* and *MYB15* in the *jaz1-1* mutant either exceeds or restores wildtype (Col-0) and jaz1-1 mutant metabolite amounts under Flg22 elicitation. When ANAC042 was overexpressed in the jaz1-1 knock-out mutant, a 42-fold change amount of 4OH-ICN was observed when compared to Col-0 under Flg22 elicitation. Similarly, MYB15 overexpression in jaz1-1 mutant background led to a 10-fold change in 4OH-ICN under Flg22 treatment. In contrast, under non-elicited conditions, overexpression of ANAC042 or MYB15 in jaz1-1 mutants resulted in a 20- and 8-fold increase, respectively, relative to the elicited Col-0. In addition, the expression of these biosynthetic genes in the overexpression lines corresponds to the accumulation of metabolites. Our findings demonstrate that JAZ1 suppression coupled with the overexpression of individual positive regulators can significantly boost phytoalexin production. Future work will focus on unlocking the phytoalexin synthesis by overexpressing multiple TFs in the jaz1-1 deficient plants.

Poster 10: Transcription factor regulation of a cannabinoid gene promoter from C*annabis sativa*

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Background: *Cannabis sativa* (*C. sativa*) produces over 120 cannabinoids, primarily within its trichomes which are hair-like structures on the surface of the plant. OLIVETOLIC ACID SYNTHASE (OLS) is of particular importance as a rate-limiting enzyme in the biosynthesis of all cannabinoids. The amount of OLS activity *in planta* correlates with the level of *OLS* gene transcription, yet the transcription factors (TFs) that regulate *OLS* expression remain unknown. By RNA-sequencing, gene promoter sequence analysis, and yeast one-hybrid assays, our team has identified 14 TFs that bind 13 cannabinoid gene promoters, including MYB-family TFs that bind the *OLS* gene promoter. Objective: To test whether four MYB-type TFs (namely CsMYB1, CsMYB2, CsMYB3 and CsMYB4) directly transactivate or repress the *OLS* gene promoter using dual-luciferase assays in the *Nicotiana benthemiana* system. Methodology: The coding sequences of the four TFs will be cloned into the *p62SK-GW* vector and the promoter into the *pGreenII-0800-LUC* vector, which contains a firefly luciferase (LUC) reporter and a 35S-driven Renilla luciferase (REN) gene for normalization. By measuring the amount of light emitted by LUC, we can assess the activity of each TF on the *OLS* promoter. Conclusion: We have identified three MYB-type TFs that activate and one that represses the OLS promoter, suggesting a complex network of TFs regulate *OLS* gene expression. These results help improve our understanding of the genetic mechanisms that regulate cannabinoid biosynthesis, thereby bringing us closer to targeted metabolic engineering of *C. sativa* to manipulate cannabionoid production and profiles.

Poster 11: Engineering aromatics in C_3 and C_4 Crops: From Atmospheric Trash to Biochemical Treasure

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The shikimate pathway's initial enzymatic step, catalysed by 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DHS), represents a critical control point in aromatic amino acid biosynthesis. A previous study using *Arabidopsis thaliana* has demonstrated that *suppressor of tyra2* (*sota*) mutations in DHS circumvent endogenous feedback regulation, leading to aromatic amino acid hyperaccumulation and an up to 30% enhancement in net CO_2 assimilation1. Building on this discovery, we propose to engineer commercially relevant C_4 monocot sorghum (*Sorghum bicolor*) and C_3 dicot *Nicotiana tabacum* with the feedback-insensitive *sota*-DHS variant, coupled with aromatic-metabolising enzymes (aroEz), to redirect carbon flux towards high-value compounds e.g. vanillin and related aromatic chemicals. We anticipate this approach will yield three key outcomes: firstly, measurable increases in target compound production (target 3.5% d.w.) quantified by LC-MS metabolomics; secondly, characterisation of any associated growth trade-offs under controlled and field-realistic conditions; and thirdly, identification and implementation of appropriate glycosyltransferases to facilitate vacuolar storage of target compounds, thereby improving both yield and stability. This multidisciplinary strategy combines advanced metabolic engineering with sustainable crop improvement, offering a novel pathway for renewable aromatic chemical production with applications spanning pharmaceuticals, nutraceuticals, fragrance and industrial feedstocks. The findings will provide both fundamental insights into aromatic metabolic engineering and practical solutions to convert CO_2 into high-value compounds.

Poster 12: Dynamic Regulation of Pisatin Biosynthesis Underlies Cultivar-Specific Resistance to *Aphanomyces euteiches* in Pea

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Pisatin, a legume-specific isoflavonoid phytoalexin, is a key component of pea (*Pisum sativum*) defense against the soilborne pathogen *Aphanomyces euteiches*. While previous studies have established its antifungal activity, the regulation and spatial dynamics of pisatin biosynthesis and secretion across pea cultivars remain poorly understood. In this study, we investigate cultivar-specific differences in pisatin pathway activation in relation to resistance or susceptibility to A. euteiches infection. Using a time-course infection assay, we performed targeted metabolomics on both root tissues and root exudates of two pea cultivars: AAC Chrome (susceptible) and PI557501 (partially resistant). We quantified pisatin and its precursor metabolites, integrating high-resolution LC-MS data analysis. Root border cell morphology and disease phenotyping further contextualized metabolite responses. Our data reveal that PI557501 exhibited consistently higher levels of pisatin precursor metabolites in uninfected root tissues compared to AAC Chrome, indicating a cultivar-specific elevation in basal flux through the phenylpropanoid pathway. In response to infection, AAC Chrome accumulated pisatin precursors at higher level compared to PI557501. However, the increased accumulation of precursor molecules in AAC Chrome did not result into higher level of pisatin suggesting a bottleneck or suboptimal conversion in the final biosynthetic step. Moreover, a lower levels of intermediates corresponded with higher pisatin accumulation in PI557501 suggesting an efficient precursor utilization. Pisatin was detected not only in root tissues but also in root exudates, marking the first report of pisatin release into the rhizosphere during A. euteiches infection. Microscopic analysis of root tips showed that PI557501 maintained a more abundant border cell layer, which may aid in the secretion of defense compounds and in establishing an effective barrier against pathogen entry. Together, these findings highlight how the timing, metabolic flux, and secretion of defense metabolites shape cultivar-specific responses to root rot and suggest a possibility of exudate-based defenses as a viable strategy for breeding disease-resistant pea cultivars.

Poster 13: The Metabolic Profile of the Rhizosphere of Phytoremediation Willow is Altered by Salt Stress

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Soil salinization is a growing environmental issue resulting from human activities such as intensive agriculture and industrialization, threatening soil functionality and ecosystem services. Salt stresses often relate to sodium chloride (NaCl) due to its prevalence, but sodium sulfate (Na_2SO_4) derived from natural and anthropogenic sources represent another widespread salt notably in Canada. The ecological restoration of saline soils can benefit from vegetation establishment, where salt-tolerant plants like willows may not directly remove salts but rather help maintain vegetation cover, preserve ecosystem functions, and potentially facilitate long-term soil recovery. Willows (*Salix spp.)* are particularly well-suited for this approach due to their fast growth, high biomass production, and tolerance to environmental stresses. Despite this potential, further understanding of molecular mechanisms involved in salt stress tolerance is required to optimize their use in saline soil management. Plants respond to salt stress through a complex network of physiological, molecular, and biochemical changes. Salt-induced changes in the metabolic profile are still largely unknown, especially in roots exudates. These exudates comprise metabolites, enzymes and mucilage, that are released into the rhizosphere where they play essential roles in detoxification, microbial interactions, nutrient uptake and stress signaling. The objective of this study was to investigate the impact of salt stress on root exudation in willows and to identify metabolites involved in stress tolerance. Willows were cultivated in soil treated with either sodium chloride (NaCl) or sodium sulfate (Na_2SO_4). Rhizosphere samples were collected, and an untargeted metabolomic analysis was conducted using high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (HPLC-HRMS/MS). Approximately 2,500 features were detected per treatment, with 939 successfully matched to database entries for putative annotation. Metabolites from numerous biosynthetic pathways were identified, including amino acids, alkaloids, lipids, phenolics, organic acids, terpenoids, carbohydrates and carbonyl compounds. Salts impacted around 15% of the metabolome, with anion-specific alterations. Under NaCl treatment, a total of 141 detected metabolites were were altered to the control condition. Among them, 98 metabolites increased, while 43 decreased. These changes affected all biosynthetic pathways; however, the enriched compounds were predominantly phenolics (29), including flavonoids known for their antioxidant properties, such as eriodictyol and tangeritin identified through putative annotation. Under Na_2SO_4 treatment, 126 metabolites showed changes following salt exposure: 46 were enriched, while 80 were depleted. The most affected pathways were amino acids, phenolics and organic acids. Noteworthy, lipids (10), including persin, were increased in Na_2SO_4-treated soils. This work highlights the reconfiguration of the rhizosphere's metabolic profile in response to salt stress, an adaptation specific to the anion applied. These results will contribute to a deeper understanding of the plant strategies to tolerate salt stress including plant-rhizosphere interactions. Ultimately, these findings may support the development of more effective strategies for managing salt-affected soils and potentially supporting the growth of less salt-tolerant plant species in these challenging environments.

Poster 14: Do Cyanobacteria use the Entner-Doudoroff Pathway?

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The Entner-Doudoroff Pathway (EDP) offers an alternative metabolic shortcut to glycolysis, which is used by many proteobacteria organisms such as in *Escherichia coli*. The EDP generates glyceraldehyde-3-phosphate (GAP) and pyruvate from 6-phosphogluconate in two steps, which could potentially augment flux into the methylerythritol 4-phosphate (MEP) pathway in synthetic biology context for the synthesis of terpenoids and other biofuel precursors. The model cyanobacterium *Synechocystis sp.* PCC 6803 has long been used to investigate the EDP in photosynthetic microbes, but recent findings by our group has led us to reconsider whether cyanobacteria actually use the EDP. To assess the presence or absence of the EDP in *Synechocystis sp.*

PCC 6803, we quantified the signature intermediate of the EDP, 2-keto-3-deoxy-6-phosphogluconate (KDPG), in both *Synechocystis* and *E. coli* using liquid chromatography - mass spectrometry. Extraction of phosphorylated metabolites from *Synechocystis* and *E. coli* was performed using the standard addition method and external calibration curves. The results revealed that while KDPG was readily detectable in *E. coli* cells at a concentration of 10.92 pmol/mg cells, KDPG could not be detected in *Synechocystis*, evidencing the absence of a functional EDP. In contrast, most metabolites from the MEP pathway could be easily quantified in *Synechocystis*, validating the extraction procedure and analytical method. This result is consistent with the absence of the key dehydratase gene from the genome of *Synechocystis sp.* PCC 6803 (EDD) required for a functional EDP and highlights the potential of metabolic engineering to introduce the EDP into *Synechocystis sp.* PCC 6803 for the first time. This novel approach to engineering cyanobacteria would enhance the production of GAP and pyruvate to feed into the MEP pathway and create new possibilities to engineer the biosynthesis of biofuel precursors in a photosynthetic microbe.

Poster 15: **A double bond matters: Investigating 3-ketoacyl-CoA Synthase in *Populus trichocarpa***

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Very-long-chain fatty acids (VLCFAs), defined by carbon chain lengths exceeding 20 atoms, are essential components in a wide range of developmental and physiological processes in eukaryotes, particularly in higher plants. Their biosynthesis is mediated by the fatty acid elongation (FAE) complex, in which 3-ketoacyl-CoA synthase (KCS) catalyzes the initial and rate-limiting step, thereby governing substrate specificity and the determining the length, position of the double bond and stereochemistry of the products. In *Populus trichocarpa*, the gene *PtKCS1* has been identified as a key regulator within the alkene-forming pathway, where it elongates unsaturated VLCFAs that are subsequently modified into alkenes that are incorporated into the epicuticular wax of leaves. To further investigate the residues that confer PtKCS1 the ability to utilize monounsaturated fatty acids as substrates, two rationally engineered chimeric proteins were constructed by combining key regions of PtKCS1 into the structural framework of a closely related PtKCS2, resulting in promiscuous enzymatic activity whose mechanistic basis remains to be fully elucidated. Here, I present recent findings focused on exploring the underlying cause of this high promiscuity by detailed investigation of enzyme activity using heterologous expression in yeast. Upon supplementing the media with a range of fatty acid substrates, the chimeric enzymes consistently maintained elevated activity levels, higher than the original PtKCS1 and PtKCS2 backbone. This peculiar activity profile provides an opportunity to expand the repertoire of substrates that are incorporated into epicuticular waxes. To accomplish this, we used two *Arabidopsis* KCS promoters to drive *in planta* expression of the chimeric constructs. These transgenic lines provide a platform for dissecting the alkene-forming pathway and identifying downstream components involved in cuticular wax.

Poster 16 ***Rethinking Downregulation of Glycolysis in the Chloroplast***

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Phosphoenolpyruvate (PEP) and pyruvate constitute key precursors for the synthesis of terpenoids, phenolics, and fatty acids in plants. Chloroplasts of photosynthetic cells cannot synthesize PEP due to the downregulation of two key enzymes of glycolysis, enolase (ENO) and phosphoglycerate mutase (PGM). Instead, PEP is synthesized in the cytosol and reimported into chloroplasts through the PPT1 translocator, where it is thought to supply the biosynthesis of aromatic amino acids through the shikimate pathway as well as undergo conversion to pyruvate, a precursor to terpenoids and lipids. This arrangement is assumed to be necessary to protect the Calvin-Benson-Bassham (CBB) cycle from depletion. Here we provide an alternative explanation: Downregulation of ENO and PGM is necessary to prevent PEP re-entry into the CBB cycle as 3-phosphoglycerate (3PGA), which is thermodynamically favoured over PEP by ~12:1. This conclusion is based on upregulation of ENO and PGM in the Arabidopsis cue1 mutant, whose defective PPT1 transporter results in a distinctive reticulated phenotype due to a deficiency of PEP in the plastid. This makes it ideal for visually tracking the effects of restoring daytime glycolysis in chloroplasts. Stably transformed cue1 plants that overexpress plastidial ENO and PGM displayed partial complementation of the reticulated phenotype, whereas wild-type plants overexpressing these genes suffered growth impairments. We argue that restoration of lower glycolysis benefits the near absolute PEP deficit in cue1 chloroplasts (3PGA→2PGA→PEP), while the same thermodynamic equilibrium disadvantages wild-type plants with normal plastidic PEP levels (PEP→2PGA→3PGA). We conclude that when the CBB is active, the lower steps of glycolysis must be blocked to avoid depletion of PEP needed to sustain the shikimate pathway. Therefore, the downregulation of glycolysis in the chloroplast is an adaptation that permits otherwise incompatible biosynthetic processes to function together seamlessly in the same compartment.

Poster 17: **Tracking the changes of cutin in Populus species**

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Cutin and suberin are cell-wall associated polymers that serve distinct, yet complementary protective functions in plants. Cutin is found in the cuticle of aerial plant tissues, creating a hydrophobic barrier composed mainly of hydroxy fatty acids and glycerol. This external matrix, together with cuticular waxes, protects plants from water loss, radiation damage, and pathogen intrusion. On the other hand, suberin is a structural component found in the periderm of bark, wound sites and roots. Suberin consists of, fatty acids, dicarboxylic acids and phenolic compounds, forming a waterproof barrier that prevents water and solute movement, while shielding plants from soil-borne pathogens. These polymers enable plants to thrive the diverse environmental challenges. In this study, we

investigated the chemical diversity in cutin monomers found in the stems and leaves of *Populus trichocarpa* and *P. balsamifera*, two closely related species that have colonized different environments in the Northern Hemisphere. The chemical analysis **revealed** variation in monomer accumulation depending on the species tissue, and developmental stage. While cutin monomer quantity changed **with** the developmental stage, the chemical components remained largely unchanged. The quantity of the monomers increased as stems aged in *P. trichocarpa*, however, it remained consistent as the stem aged in *P. balsamifera*. The predominant monomers were hydroxy fatty acids, with the exception of the leaf tissue in *P. trichocarpa*, where fatty acids constituted the major compounds Furthermore, phenolic components showed larger variation throughout the development in *P. balsamifera*. Next, we examined the impact of drought stress on the cuticle of *P. trichocarpa* by analyzing the polymer composition across various tissues, including leaves, stems, and roots. The results show these tissues respond differently to the applied stress. For instance, total monomer content increased in stem and root tissues, but showed reduction in mature leaves. Accumulation of individual compounds also varied among tissues with mature leaves accumulating lower fatty acids under the stress. These observations highlight the adaptive complexity of the lipid polymers, emphasizing their role in protecting plants from environmental challenges like drought. Future studies will delve deeper into the mechanisms underlying the variation, offering valuable insights to enhance tree resilience.

Poster 18: Evolutionary Divergence and Functional Specialization of a Conserved KCS Gene Cluster in Angiosperms

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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*) gene family plays a crucial role in the biosynthesis of VLCFAs, as this enzyme catalyzes the substrate-specific and rate-limiting condensation step in their elongation processes. While the functional roles and substrate specificity of individual *KCS* genes have been extensively studied in various species, the evolutionary history and conservation of *KCS* gene clusters across angiosperms remain unclear. Previously, a *KCS* gene cluster named *PtKCS1* was identified on chromosome 10 of *Populus trichocarpa*. Two distinct subclades of *KCS* were identified based on their divergent activity and phylogenetic relationships (clade A and B). With the increasing number and quality of sequenced plant genomes, comparative synteny analysis allows us to understand the evolution and conservation of gene families across a range of angiosperms. In this study, we used a combination of comparative genomics, synteny analysis, and functional characterization to reveal that this *KCS* gene cluster is widely conserved in basal angiosperms, monocots, and eudicots. The expanded phylogenetic analysis supported the split into two clades, with one being retained throughout angiosperms (clade B), while the other is unique to the Malvids (clade A). To investigate the diversification in enzyme function, representative enzymes from each clade were heterologously expressed in *Saccharomyces cerevisiae*, followed by product characterization via VLCFA profiling using GC-MS. Due to the limited amount of free monounsaturated substrates found endogenously in yeast, cells were supplemented with exogenous palmitoleic (C16:1 *cis*-ω7) and oleic acid (C18:1 *cis*-ω9) to further investigate enzyme substrate specificity towards monounsaturated VLCFAs. Altogether, our results show distinct activity profiles between the two clades in terms of total VLCFAs being produced, the amount of monounsaturated VLCFAs, and the positional isomers of the monounsaturated VLCFAs, indicating possible sub-functionalization between clades A and B following gene duplication in the common ancestor of Malvids. Overall, these findings shed light on the evolution of *KCS* genes and their unique role in promoting the fatty acid diversification in plants.

Poster 19: Elucidating Cafestol and Kahweol Biosynthesis in *Coffea arabica*

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The large class of plant terpenoids provides a rich source of bioproducts. However, terpenoids are often difficult to source from nature and few complete biosynthetic pathways have been characterized. Elucidating complete biosynthetic pathways enables the use of synthetic biology approaches for engineering natural and “new-to-nature” terpenoids in microbial or plant host systems. Here, we present research on the biosynthesis of Cafestol and Kahweol, two diterpenoids naturally produced in *Coffea arabica.* These two compounds have important health benefits with demonstrated anti-cancer and anti-inflammatory activity, making them promising drug targets in the pharmaceutical industry. To functionally characterize candidate enzymes involved in Cafestol and Kahweol biosynthesis, we used a *Nicotiana benthamiana* expression system and tested combinations of terpene synthase and cytochrome P450 enzymes. The resulting metabolic product profiles were analyzed via GCMS. To date, we have identified the class I and II terpene synthase enzymes that catalyze the production of *ent*-kaurene, a predicted intermediate in the biosynthesis of Cafestol and Kahweol. We further characterized four P450 enzymes capable of downstream modifications on *ent*-kaurene via various hydroxylations. Continuing to unravel the biosynthetic pathways of Cafestol and Kahweol will be critical to provide the resources necessary to establish these medicinal compounds as therapeutics and pave the way for improved production at an industrial-scale.

Poster 20: Turning red: using the RUBY reporter to visualize metabolite accumulation in *Atropa belladonna*

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Atropa belladonna, also known as Deadly Nightshade, is a highly poisonous but medicinally relevant plant. Its important medicinal metabolites make *A. belladonna* an excellent subject for transgenic studies to increase the production of these compounds. To perform these transgenic studies, reliable protocols for *A. belladonna* transformation are required. To develop and optimize transformation protocols with *A. belladonna*, we used the visual reporter plasmid RUBY. This plasmid encodes three enzymes that synthesize betalain from L-tyrosine: cytochrome P450 76AD1 (CYP76AD1), L-3,4-dihydroxyphenylalanine 4,5-dioxygenase (DODA), and glucosyltransferase. Betalain is the bright red pigment that gives beets their characteristic colour. Plants successfully transformed with the RUBY operon thus accumulate betalain and turn red. *A. belladonna* was transformed with RUBY using two different techniques: infiltration of leaves (transient expression), and *in vitro* regeneration (callus transformation). The transiently infiltrated leaves of *A. belladonna* accumulated a strong red colour in 4-5 days, which was still visible after 5 weeks. These results provide insight into determining the best timeline for tissue collection with future infiltration studies. In contrast, callus transformation with RUBY was more challenging. There was some betalain accumulation in the callus tissue, but the colour and coverage of this accumulation varied strongly. The callus tissue had varied phenotypes ranging from fully red to green and purple, which resembled the purple pigments that naturally accumulate in the shoots, flowers, and berries of *A. belladonna*. Out of 26 screened regenerated plants, 35% were confirmed to be transgenic by amplification of one of the RUBY-encoded genes. These results indicate the suitability of these transformation protocols for future transgenic studies on *A. belladonna* targeting its medicinal components. Beyond informing future protocol development with *A. belladonna* for research purposes, the visual RUBY reporter will also be applied as a teaching tool in undergraduate laboratories to help students understand how transgenic plants are generated and improved for higher accumulation of medicinal compounds.

Poster 21: Scaling Titers: Leveraging Plant Transporters in Microbial Systems

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The medicinal plant *Catharanthus roseus* produces a suite of pharmaceutically important monoterpenoid indole alkaloids (MIAs), including the chemotherapy agents—vinblastine and vincristine. Despite their medicinal value, these target compounds are produced in trace quantities in plants. Therefore, the integration of plant pathways into microbial systems provides a tangible alternative. However, current efforts are plagued by vanishingly small yields, often due to aberrant by-products or lack of pathway orthogonality. Indeed, the transition from a multicellular plant to a single-cell yeast metabolic chassis has proven challenging. Transporters can help simulate a degree of pathway organization and compartmentalization that could benefit target compound titers. Recently, our lab characterized CrMATE1 as a vacuolar importer of secologanin, a key precursor in MIA biosynthesis. We hypothesized that optimizing its expression in yeast could reduce intermediate loss and improve MIA yield. Here, we present data from biotransformation assays evaluating the function of CrMATE1 in a heterologous system and various factors underlying its performance. The present study can aid future efforts for the scalable biosynthesis of pharmaceutically relevant MIAs and strategies for domesticating foreign pathways in yeast.

Poster 22: Comparative Profiles of Volatile Organic Compounds in Red Pepper (*Capsicum annuum* L.) Powders as Affected by Drying Methods

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To understand drying-induced alterations in flavoring properties of red pepper (*Capsicum annuum* L.) powders, volatile organic compounds in fresh and dried pepper powders produced under heated air (HA), far-infrared (IR), and sun drying (SD) conditions were semi-quantified by HS-SPME/GC-MS. A total of 113 volatiles, of which 78 were odor-active, were identified in fresh and dried red peppers. Drying significantly reduced the total amount of volatiles by 55%, 45%, and 23% for HA, IR, and SD, respectively, compared to fresh peppers. Major decrements were observed in hydrocarbons, ethers, and esters. According to their changing patterns, identified volatiles (113) could be categorized into five groups: decreased (39), disappeared (4), increased (13), newly formed (51), and variable depending on drying methods (6). Among the drying methods, SD resulted in a higher amount of total volatiles, mainly due to elevated levels of furans, bases, and pyrans; e.g., 1-(furan-2-yl)hexan-1-one in SD exhibited 123.25 and 79.71 times higher amounts compared to IR and HA, respectively. In contrast, alcohols and ketones were more abundant in IR. As unique volatiles, 2,3,5,6-tetramethylpyrazine could be detected only in IR, while 1-methylpyrrole-2-carbaldehyde could be observed exclusively in SD red peppers. Partial least squares discriminant analysis (PLS-DA) of volatile profiles revealed distinctive differentiation among drying methods, with hexanoic acid, 2-methylbutanoic acid, 2-ethenyl-6-methylpyrazine, and furan-2-ylmethanol as volatiles of high Variable Importance in Projection (VIP) scores. These results suggest the importance of drying methods on the qualitative and quantitative properties of volatiles in red pepper powders, which in turn may affect their flavor and market quality.

Poster 23: The Metabolite Maysin and its Regulation and Defense-Related Biological Activity in the Vegetation of Maize

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Vascular plants produce a variety of specialized metabolites, including defensive compounds, which help compensate for their sessile nature by enhancing survival and adaptation to environmental stresses. Investigating how these metabolites are regulated, and

mediate biological effects provides insights into the selective pressures that led to their development. This study focused on the flavone maysin in maize (Zea mays). Maysin is a C-glycosyl flavone with well-documented regulation, synthesis, and growthinhibitory effects on the lepidopteran pest, corn earworm, in maize silks. However, its accumulation and biological activity in vegetative tissues, such as stems and leaves, are less understood. The presence of maysin in both reproductive (silk) and vegetative tissues emphasizes its potential role in defense across different tissues. Using whole seedlings and detached leaf assays, we examined maysin's impact on the growth of the fall armyworm (Spodoptera frugiperda), a major lepidopteran pest that causes severe damage to the foliage. Our results show that larvae-fed maize leaves with higher maysin content exhibit reduced growth, similar to the effect observed in silk tissue. To explore the genetic regulation of maysin in maize seedlings, we performed differential gene expression analysis using above-ground vegetative tissue from two near-isogenic lines differing in maysin levels. We identified several differentially expressed genes within a 32 Mbp introgression region between the two lines. Among the known structural genes in themaysin pathway, only the salmon silk2 (sm2) gene, encoding a rhamnosyl transferase, was differentially expressed. These findings underscore the need to further characterize maysin regulation in vegetative tissues and its role in pest defense.

Poster 24: **Biochemical and Functional Analysis of Carotenoid Biosynthetic Enzyme Complexes in Tomato Fruits**

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Carotenoids are essential pigments in plants, playing fundamental roles in photosynthesis, photoprotection, and human nutrition. While the enzymatic steps in the carotenoid biosynthetic pathway are well characterized, the spatial organization and dynamic assembly of biosynthetic enzyme complexes within chromoplasts remain largely unexplored. This study investigates the structural and functional organization of carotenoid biosynthetic enzyme complexes in tomato (*Solanum lycopersicum*) fruits, focusing on the interplay between enzyme composition, metabolic flux, and carotenoid accumulation. Using CRISPR-Cas9 genome editing, we generated a *Zeta-carotene isomerase (Z-ISO)* knockout mutant, which is analyzed alongside well-characterized tomato mutants' *yellow flesh (r), tangerine (t), Beta, and Delta,* each exhibiting distinct carotenoid profiles compared to the wild-type *Ailsa Craig* (AC) background. To examine the native enzyme complexes responsible for carotenoid biosynthesis, chromoplast isolation followed by ultracentrifugation and size-exclusion chromatography (SEC) was performed to fractionate protein complexes, while mass spectrometry (MS) is being employed to identify and characterize their composition. Additionally, protein-protein interaction assays, such as Bimolecular Fluorescence Complementation (BiFC), are being used to study enzyme interactions and localization within chromoplast compartments, offering insights into potential metabolic channeling mechanisms. Furthermore, high-performance liquid chromatography (HPLC) is being used to analyze carotenoid accumulation and metabolic flux across different tomato mutants, helping to determine how enzyme organization influences the efficiency and direction of carotenoid biosynthesis. Understanding these mechanisms has far-reaching implications for crop biofortification, metabolic engineering, and food security, particularly in addressing vitamin A deficiency—a major global health issue. Additionally, this research provides fundamental insights into how plastidial enzyme complexes regulate metabolic fluxes, offering strategies to optimize carotenoid content in horticultural crops. These findings could be leveraged for synthetic biology applications, precision breeding, and genetic engineering, ultimately contributing to the development of nutritionally enhanced crops with higher carotenoid content. Our findings advance knowledge on chromoplast biology and the functional role of metabolic enzyme complexes, paving the way for novel approaches in plant metabolic engineering. By integrating genetics, proteomics, and biochemical characterization, this study provides a comprehensive understanding of the organization and dynamics of carotenoid biosynthetic enzyme complexes, which will be essential for future efforts to optimize carotenoid production for both plant physiology and human health benefits..

Poster 25: **Directed Evolution to Enhance Phytoene Synthase Enzyme Activity for Metabolic Engineering**

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Imagine a tomato that not only looks vibrant but also packs a powerful nutritional punch. Tomatoes contain carotenoids that are crucial for plant growth and human nutrition. They play critical roles as light-harvesting pigments and structural components of photosystems. In humans, Provitamin A carotenoids, such as β-carotene and α-carotene, are the dietary precursors of vitamin A, which is an essential dietary supplement for eyes and immune system. Vitamin A deficiency leads to diseases such as blindness especially in developing countries. Metabolic engineering efforts have focused on increasing carotenoid production in crops, particularly targeting provitamin A biosynthesis. Phytoene synthase (PSY), the first committed enzyme in the carotenoid pathway, is a major rate-limiting step. Therefore, it is the main target for provitamin A biofortification in many crops. Tomato has multiple copies of PSY, while PSY1 is dominant in fruits, it is less efficient in promoting carotenoid biosynthesis compared to PSY2. This provides us a target for further improvement not only in tomato but also in other crops since PSY sequences have high homology in plants. In this study, we took advantage of a bacteria system, which can accumulate different level of colored carotenoid products based on the activity of PSY introduced. Structure-guided site-directed and random mutagenesis are introduced in *PSY2* gene. Therefore, the mutants of the highest enzymatic activity can be easily screened out by the color accumulated in bacteria culture, which will provide novel targets for carotenoid metabolic engineering. This will be performed using a high throughput screening robotic method (Opentrons OT-2). This platform enables the parallel screening of up to 96 mutants with minimal waste and short time. By applying random mutation and directed evolution**,** we aim to identify key amino acid residues which can increase PSY activity significantly**.** As a next step, a trained machine learning model could learn the relationship between the amino acid sequence of PSY2 variants and their enzymatic activity. Once trained, this model can be used to predict the activity of unseen PSY2 variants. These predictions could be further used to guide subsequent rounds of directed evolution by prioritizing the creation and testing of PSY2 variants that the model predicts to have improved activity. Ultimately, those sites can be used for prime editing to improve provitamin A carotenoid production in crops.

Poster 26: A Patchwork Model for Morphinan Biosynthesis in *Papaver*

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The evolution of morphinan alkaloid biosynthesis in *Papaver* species has involved gene duplication, fusion, neofunctionalization, and deletion, shaping the present-day chemotaxonomy. A key fusion event led to the bifunctional enzyme reticuline epimerase (REPI), which catalyzes the stereochemical inversion of (S)-reticuline and likely preceded neofunctionalization of downstream enzymes in the morphine biosynthesis pathway of *Papaver somniferum*. The aldo-keto reductases 1,2-dehydroreticuline reductase (DRR) and codeinone reductase (COR) play crucial roles in this pathway, catalyzing the second and penultimate steps, respectively. DRR occurs in some species as part of the REPI fusion protein. We examined orthologs of DRR and COR from transcriptomes of 12 *Papaver* species, including those representing evolutionary intermediates prior to the emergence of REPI. Functional assays confirmed that these orthologs retain enzymatic activity in species with partial or complete morphinan pathways, supporting a patchwork model of metabolic evolution. Structural and substrate specificity analyses of DRR and COR orthologs provided insights into their functional divergence and latent enzymatic properties, revealing molecular mechanisms that contributed to pathway evolution. These findings enhance our understanding of the assembly of specialized metabolism in *Papaver* species.

Poster 27: Cytochrome P450s in Alkaloid Biosynthesis: Catalysts of Chemical and Structural Diversity

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Plants produce a vast diversity of nitrogen-containing heterocyclic metabolites known as alkaloids, which serve critical ecological functions and have been used as medicines for centuries. Among them, monoterpene indole alkaloids (MIAs) are a structurally and functionally diverse class of natural products with potent pharmacological activities. Clinically relevant MIAs include the anticancer agent camptothecin, the antiarrhythmic drug ajmaline, and the immunomodulatory alkaloid mitraphylline. This presentation will explore recent discoveries of key biosynthetic enzymes involved in both the scaffolding and diversification of MIA structures. The roles of cytochrome P450 monooxygenases enzymes in generating chemical diversity from shared precursors will be highlighted. In addition, how combinatorial chemoenzymatic C–H functionalization strategies can be applied to expand the chemical space of MIAs and facilitate the sustainable production of high-value alkaloid derivatives will be discussed. Our work contributes to a deeper understanding of MIA metabolism and enables greener biocatalytic approaches to medicinal alkaloid synthesis.

Poster 28: Integrating *Mitragyna* omics data sets to study diversification of monoterpene indole alkaloid metabolism

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Monoterpenoid indole alkaloids (MIAs) represent a diverse family of specialized plant metabolites, including FDA-approved chemotherapeutics like vinblastine, and vincristine. The *Mitragyna* genus within the Rubiaceae (coffee family) produces pharmaceutically significant MIAs and spirooxindole alkaloids. We sequenced and assembled high-quality chromosome scale genomes of four *Mitragyna* species/chemotypes. We conducted comparative genomics analyses including comprehensive synteny and phylogeny across the Gentianales to investigate the role of genome structure in the diversification of the post-strictosidine pathways in MIA biosynthesis. We generated RNA-seq and corresponding targeted metabolite datasets from various MIA accumulating tissues and developmental stages to facilitate genome mining and gene discovery, particularly for MIAs and oxindole alkaloids, laying the groundwork for pathway discovery and synthetic biology applications. Future work will leverage comparative genomics to characterize genome-wide variations responsible for the evolution of the specialized MIAs.

Poster 29: A Synthetic Carbon Assimilation Shunt Boosts Terpenoid Biosynthesis in Plants

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Improving photosynthesis is a central objective in plant biotechnology. Although ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the path for most CO2 assimilation in plants, the introduction of novel carbon fixation pathways presents opportunities to fine tune downstream carbon utilization. Here we describe genetically modified plants that fix CO2 through reversible steps of the pentose phosphate pathway and channel this carbon preferentially towards terpenoids. Tobacco plants transiently expressing plastidial 6-phosphogluconate dehydrogenase (PGD) during the day reductively carboxylate ribulose-5-phosphate (Ru5P) to 6-phosphogluconate (6PG) using NADPH reducing power provided by photosynthesis. CO2 assimilated through this route was coupled to a synthetic Entner-Doudoroff (ED) pathway, a 2-step prokaryotic shunt consisting of a dehydratase (EDD) that converts

6PG to 2-keto-3-deoxy-6-phosphogluconate (KDPG) and an aldolase (EDA) that cleaves KDPG into GAP and pyruvate, the substrates for the first enzyme of the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway. Metabolite profiling of plants transiently expressing chloroplast targeted isoforms of PGD, EDD, and EDA demonstrated that Ru5P-derived GAP and pyruvate were readily incorporated into the MEP pathway, which supplies the precursors to terpenoids in the chloroplast. Increased MEP pathway flux was also observed by co-expressing PGD and EDD in the absence of EDA. Biochemical characterization indicated that endogenous fructose 1,6-bisphosphate aldolase (FBA) can replace KDPG aldolase (EDA) activity as a result of the substrate promiscuity afforded by aldolase enzymes. When plants were incubated in the dark or the same enzymes were targeted to the cytosol, the impact on the MEP pathway was absent, verifying that this synthetic route was dependent on the light reactions of photosynthesis. The photosynthetically driven coupling of this reversible pentose phosphate pathway to terpenoid biosynthesis through this ED/MEP shunt demonstrates the potential of alternative carbon assimilation routes to channel carbon directly into valuable natural products. In addition, this synthetic route to augmenting plastid terpenoid biosynthesis has significant implications for engineering plants for climate change tolerance and biofuel engineering.

Poster 30: The role of *Peroxidase* genes in suberin assembly in potato

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Background: Potatoes (*Solanum tuberosum L.*) are a vital crop for sustenance and economic development in many developing countries due to their high productivity and nutritional value. Their adaptability to diverse climates and soils makes them a key component of global food security, especially in regions vulnerable to climate change. Potatoes are rich in carbohydrates, vitamin C, potassium, and B vitamins, and they provide dietary fiber and antioxidants, supporting health in low-resource settings. In the United States, however, the potato industry faces significant post-harvest challenges. Up to 33% of the national yield is lost due to post-harvest deterioration, resulting in an estimated $1.2 billion in annual revenue loss. Improving post-harvest resilience requires a deeper understanding of how potatoes defend against environmental and biological stresses. One critical defense mechanism is the development of a suberin-rich skin, which protects tubers from pathogens and controls water and ion loss. Suberin is composed of poly(phenolic) (SPPD) and poly(aliphatic) (SPAD) domains, deposited in a sequential process. However, the role of the SPPD in facilitating the deposition of the SPAD remains unclear. Suberin assembly is facilitated by various factors, including *Suberization-Associated Anionic Peroxidase*. This work seeks to inform strategies to enhance post-harvest quality, reduce losses, and support food security globally. Objective: This study aims to elucidate the role of the SPPD in the deposition of the SPAD in a wound-inducible potato suberin model system. My approach involves disrupting SPPD formation using *RNAi*-mediated knockdown of targeted *peroxidase* genes to prevent phenolic polymerization, and to monitor the impact on SPAD deposition. Results: Three peroxidase genes with potential involvement in the polymerization of wound-induced SPPD, namely *Peroxidase 19 (PRX19)*, *Peroxidase 55 (PRX55)*, and *Peroxidase 105 (PRX105)* were amplified via PCR, cloned into the Gateway® RNAi expression vector, and introduced into *Agrobacterium tumefaciens* for plant transformation. Successful transformants were selected (three lines per gene) used to generate microtubers for wound-induced suberin formation. Quantitative RT-PCR (qRT-PCR) analyses confirmed that all knockdown lines exhibited over 80% reduction in target gene transcript levels, relative to an empty vector control line. Total peroxidase enzyme activity assays corroborated these findings, showing significantly reduced peroxidase activity in wounded *PRX19*-RNAi tissue; however, no statistically significant changes in total peroxidase activity were observed for wound-induced *PRX55*-RNAi and *PRX105*-RNAi tissues. Chemical analysis revealed a marked reduction in total poly(phenolic) and poly(aliphatic) content in *PRX19* -RNAi lines. This suggests a pivotal role for *PRX19* in SPPD polymerization, and the need for the SPPD for SPAD deposition. In contrast, *PRX55*-RNAi and *PRX105*-RNAi lines did not differ from EV control lines in wound-induced SPPD or SPAD content. Finally, permeability assays across all lines revealed that the reduced suberin phenotype of wound-healed *PRX19*-RNAi lines rapidly lost water when exposed to a desiccating environment. Significance: Understanding the suberization process is crucial for enhancing potato resistance to post-harvest stresses. My study provides insights into the critical role of the SPPD in the overall deposition of a functional suberized wound periderm, and the central role of *PRX19* in the process. These findings contribute basic knowledge that can be used to improve potato storage and reduce yield losses.

Poster 31: Altered Shikimate Kinase-Like 1 gene expression results in elevated MecDP, a MEP intermediate involved in oxidative stress responses

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In microorganisms and plants, the shikimate pathway is a fundamental component of aromatic amino acid biosynthesis, redirecting the flow of organic carbon from primary metabolism towards the production of chorismate. The functional diversification of shikimate kinase (SK), to shikimate kinase-like 1 (SKL1) represents an expansion of molecular complexity unique to terrestrial plants. However, little is known about the phytochemical role of SKL1. Previous studies show that SKL1 no longer supports shikimate pathway flux but is required for chloroplast biogenesis. Here, we report a detailed phytochemical characterization of the variegated *Arabidopsis thaliana* SKL1 T-DNA insertional line, *skl1-3*. The attenuation of SKL1 gene expression in these mutants causes a significant reduction in the overall chlorophyll and carotenoid accumulation, resulting in a heteroplastidic photomorphogenic phenotype. Furthermore, the *skl1-3* mutant showed an accumulation of 2-C-Methyl-D-erythritol-2,4-cyclodiphosphate (MEcDP), an intermediate of the methylerythritol phosphate (MEP) pathway, which generates precursors for isoprenoid biosynthesis. These results have important implications for the mechanistic features of chloroplast biogenesis that are unique to land plants.

Poster 32: MS/MS Spectra Comparison of Cannabinoids Using Collision-Induced Dissociation (CID) and Electron-Activated Dissociation (EAD)

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Minor cannabinoids show promising bioactive properties for managing chronic pain. Their isolation and characterization are essential for identifying therapeutic compounds. Collision-induced dissociation (CID) MS/MS is commonly used for structural identification of cannabinoids by fragmenting molecules through controlled collisions with N2. However, CID provides fragmentation spectra with limited information, particularly in its inability to differentiate closely related cannabinoid isomers. Therefore, additional structural studies are needed. Electron-activated dissociation (EAD) MS/MS produces unique fragments compared to CID MS/MS. Here, we compare positive ion mode EAD and CID MS/MS spectra for cannabinoids to evaluate whether EAD MS/MS is a more efficient and diagnostic technology for identifying cannabinoids in cannabis. A SCIEX ZenoTOF 7600 System collected CID and EAD MS/MS data from thirty-five cannabinoid standards (Caymen Chemical). EAD MS/MS displays unique fragment ions for cannabinoids that are not observed in CID MS/MS spectra, giving us the ability to differentiate bicyclic, tricyclic, and tetracyclic cannabinoids without additional confirmation studies, such as NMR. For example, EAD MS/MS spectrum of CBD revealed multiple fragment ions, including m/z 91, 107, 207, 247, and 273, that were not observed (or in low abundance) in the CID MS/MS spectrum. Additionally, we have developed fragmentation pathways of minor cannabinoids using EAD MS/MS data. EAD MS/MS assists us in identifying novel minor cannabinoids that have not yet been evaluated for their pain therapeutic potential.

Poster 33: Plant RNA Toxins and their Role in Signaling During Stress

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Cells rely on various RNAs, in the form of mRNAs, tRNAs and rRNAs, to transmit information stored in DNA for the production of functional proteins. These nucleic acids may be modified by alkylation, oxidation and base loss during their lifetime, which alters their function. Though substantial research has been devoted to the detection and repair of damaged DNA, much less attention has been paid to RNA as it is viewed as short-lived and quickly degraded. However, recent results from our lab and others show that damaged RNAs are important signaling molecules during stress. Plant enzymes called ribosome inactivating proteins (RIPs) remove a purine base from the sarcin/ricin loop of rRNA, thereby inhibiting protein synthesis. Current work from our group illustrates how this damage slows the rate of elongation, which we think is the key element recognized by translational surveillance factors. Moreover, we have shown how mRNAs with abasic sites stall ribosomes, resulting in the degradation of these messages. Expression of many RIPs is also upregulated by biotic and abiotic stresses, suggesting that they function in defense, either by depurinating the RNA of the invading pathogen or the plant itself, eliciting death that would limit infection spread. Most of our knowledge of RIPs stems from biochemical analyses of individuals; therefore, we performed an unbiased search among all annotated plant genomes for proteins with RIP domains and identified more than 800 from 120 species, many with novel associated domains and physicochemical characteristics. Surprisingly, most of these RIPs lacked a signal peptide, indicating they may be localized to the cytoplasm of cells, raising questions about their toxicity against conspecific ribosomes. The distribution of RIPs throughout 21 plant orders, with many species expressing more than one type of RIP, indicates a more diverse group of proteins than previously known. This diversity, together with their ability to remove bases from RNA, suggests that RIPs function to control gene expression during stress and defense against pathogens.

Poster 34: Exploring the biosynthesis of chlorogenic acid in plants

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Chlorogenic acid has emerged as an important compound in crop protection and an attractive antioxidant with widespread health benefits. CGA is produced by the condensation of shikimate pathway derived compounds caffeoyl-CoA and quinic acid. Although quinic acid is a highly abundant compound produced by plants, the metabolic route for its production was only recently discovered. Protein biochemistry and structural biology played fundamental roles in mapping the metabolic route for biosynthesis of quinic acid and its regulated accumulation in plants. In this presentation, I will map the processes involved in establishing the biosynthesis of chlorogenic acid and how the accumulation of this compound is regulated and its distribution amongst plants species. Further, I will review the antioxidant properties of CGA and how it has emerged as an important nutritional compound in society.

Poster 35: Cytochrome P450 enzyme CYP716A is a gatekeeper of bitter and hemolytic oleanolic acid biosynthesis in *Chenopodium quinoa*

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Chenopodium quinoa is a nutritionally rich and climate-resilient pseudocereal gaining attention globally. Quinoa seeds are gluten-free and rich in protein and micronutrients. However, they taste bitter due to the presence of antinutritional oleanane-type triterpenoid

saponins *viz.* oleanolic acid, hederagenin and ursolic acid. Oleanolic acid sapogenin is the major saponin found in *C. quinoa* seeds. Oleanolic acid containing saponins are bitter and hemolytic. Oleanolic acid is synthesized by the cyclization of 2,3-oxidosqualene by beta-amyrin synthase followed by the oxidation of beta-amyrin. Oxidation of beta-amyrin is catalyzed by the action of cytochrome 450 enzymes. Plant genomes contain cytochrome P450 (CYP) supergene family involved in the biosynthesis of sapogenin aglycones. Here, by performing homology-based sequence analysis we identified a *CYP716A* in *C. quinoa*, which converts beta-amyrin into oleanolic acid. The functional validation was carried out by homologous transient overexpression and virus-induced gene silencing (VIGS) of *CYP716A* through agro-infiltration in the leaves of *C. quinoa*, followed by UPLC-MS quantification of the metabolites. Furthermore, heterologous expression in tobacco and Arabidopsis demonstrated the biological functionality of *CYP716A* in growth and stress responses. In summary, we discovered a novel beta-amyrin 28-oxidase enzyme (CYP716A) that catalyzes the biosynthesis of oleanolic acid in *C. quinoa* and explored its role in growth and defense. These results provide a strong foundation for understanding triterpenoid saponin biosynthesis in *C. quinoa* and designing saponin-free varieties.

Poster 36: ***Tetranychus urticae*** **metabolic responses to** ***Arabidopsis thaliana*** **defensive phenylpropanoid sinapoyl malate**

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Tetranychus urticae – the two-spotted spider mite (TSSM) – is an extreme generalist herbivore that feeds on more than 1400 plant hosts across 140 plant families. TSSMs are composite generalists consisting of locally adapted populations that perform well on a subset of hosts but can quickly establish high performance on new, initially unfavorable, plants. This suggests that rapid adaptation is a driving factor that allows the TSSM to overcome a wide range of plant defenses. The TSSM does so partly through xenobiotic detoxification of plant chemical defenses that involves the conversion of a toxic compound into non-toxic product. To date, indole glucosinolates constitute a largely characterized group of Arabidopsis defenses against TSSM herbivory. However, it remains unclear what other compounds may contribute to Arabidopsis defense against TSSM herbivory. Our lab recently found that TSSM performance on Arabidopsis mutants with disrupted biosynthesis for sinapoyl malate, a highly abundant hydroxycinnamate ester present in Brassica leaves, are significantly higher. This implicates sinapoyl malate as a novel Arabidopsis defense against TSSM herbivory. To determine if sinapoyl malate is contributing to Arabidopsis defenses, we tested its direct toxicity towards an ancestral TSSM population adapted to bean (bean-adapted) and its derived population that is adapted to Arabidopsis (Arabidopsis-adapted). We found that sinapoyl malate is acaricidal to both bean- and Arabidopsis-adapted TSSMs when they are exposed to high concentrations of this compound. Further, bean-adapted TSSMs are more susceptible to sinapoyl malate toxicity, demonstrated by a reduction of fecundity. To determine the pattern of sinapoyl malate modification in Arabidopsis- and bean-adapted TSSMs, we performed an untargeted metabolomic analysis of bean- and Arabidopsis-adapted TSSMs exposed to sinapoyl malate. This analysis revealed a conserved accumulation of multiple sinapoyl malate derivatives, such as sinapoyl glucose, within bean- and Arabidopsis-adapted TSSMs. This suggests that both populations respond similarly to sinapoyl malate. Of these identified derivatives most are also found in plants. Assuming that the enzymatic modifications of sinapoyl malate and its derivatives are conserved between plants and TSSMs, and using knowledge of the metabolism of structurally similar hydroxycinnamates to sinapoyl malate in other models, like humans, we reconstituted a TSSM detoxification pathway of sinapoyl malate. We propose a model of sinapoyl malate metabolism that implicates TSSM detoxification enzymes, such as esterases and glycosyltransferases, being involved in the initial steps of sinapoyl malate metabolism. Further work is needed to validate this model by demonstrating the ability of TSSM enzymes to metabolize sinapoyl malate and its derivatives.

Poster 37: **Multiple Detoxification Mechanisms in** ***Tetranychus urticae*** **Enable Metabolic Resistance to the Arabidopsis-Derived Indole-3-Acetonitrile**

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The co-evolutionary dynamics between herbivorous pests and their plant hosts are shaped by an ongoing 'arms race,' where plants deploy a complex array of defensive metabolites to deter feeding, while herbivores evolve counter-defensive strategies to overcome them. The chemical diversity of defenses among plant families has driven herbivores to evolve different coping mechanisms. Specialist herbivores have specific adaptations to circumvent the defenses of a limited number of host plants, typically confined to a single family. In contrast, generalist herbivores are capable of feeding on a wider range of plant species by deploying a combination of counter-defensive strategies, including metabolic resistance through detoxification of host defenses. *Tetranychus urticae*, the two-spotted spider mite (TSSM), is a cosmopolitan agricultural pest that can feed on over 1,100 plant species across over 100 families. Despite the wide host range, individual TSSM populations thrive only on a subset of plants. However, TSSM shows an extraordinary ability to rapidly adapt to new hosts within ~25 generations. *Arabidopsis thaliana* is a challenging host for TSSM, partly due to its production of indole glucosinolates—secondary metabolites unique to Brassicaceae family. Upon tissue damage, indole glucosinolates are enzymatically hydrolyzed into indole-3-isothiocyante and indole-3-acetonitrile (IAN), both of which contribute to Arabidopsis defense. IAN accumulates in leaves upon TSSM feeding and is thus considered a candidate defense metabolite. IAN belongs to the nitrile class of compounds and release cyanide through enzymatic oxidation. Cyanide inhibits cellular respiration by targeting the mitochondrial electron transport chain. In line with its mode of action, artificial supplementation of IAN resulted in high

toxicity to TSSM, supporting its role as a chemical barrier in Arabidopsis. In this study we explore the metabolic strategies employed by TSSM toward the detoxification of Arabidopsis-derived IAN. We performed untargeted metabolomic analysis of mite extracts after IAN ingestion to determine the pattern of IAN modification and to infer potential detoxification pathways. Many of the metabolites detected in mites were also present in plants, allowing us to propose a detoxification pathway based on known plant biochemical routes. We detected γ-glutamyl-β-cyanoalanine that may have been derived from β-cyanoalanine, suggesting the oxidation of IAN, the release of cyanide and its detoxification by the β-cyanoalanine synthase. In addition, we found glutathione and glucose conjugates of IAN. These modifications may act to reduce the IAN pool available for the oxidation and generation of cyanide. Finally, we identified indole-3-oxoacetamide, a compound not reported in Arabidopsis. While Arabidopsis converts IAN into indole-3-acetamide in auxin biosynthesis, the oxoacetamide form in mites may reflect a novel detoxification route, possibly via nitrile hydratase activity that circumvents cyanide release. These findings reveal that TSSM employs multiple metabolic pathways to neutralize IAN toxicity, highlighting the metabolic flexibility of generalist herbivores in coping with host-specific chemical stress.

Poster 38: **Targeted knockout of anthocyanidin reductase in fig (*Ficus carica*) hairy roots via CRISPR/Cas9 - Laying the groundwork for enhanced fruit flavor**

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The common fig (Ficus carica), a member of the Moraceae family, is native to the Eastern Mediterranean and has long been a staple food crop in the region, with annual production exceeding one million tonnes. In addition to its nutritional value, fig has also been appreciated for its medicinal potential due to a wide range of secondary metabolites particularly phenolics and isoprenoids found in both fruits and latex. These compounds have shown antioxidant, anti-parasitic, and antimicrobial activities. Hairy roots induced by Agrobacterium rhizogenes have been widely used for various applications, such as metabolic engineering, recombinant protein production, and phytoremediation. More recently, the hairy root system has become a useful platform to rapidly evaluate the efficiency of CRISPR/Cas9 constructs, especially before committing to time-intensive stable transformations. In this work, we aimed to explore gene function in fig through genome editing, which to our knowledge has not been reported previously in Ficus carica. We focused on the anthocyanidin reductase (ANR) gene, a key enzyme in the biosynthesis of tannins (proanthocyanidins) and knocking out ANR in fig can improve its flavors in fruits. Because generating stable transgenic fig plants can take several years, we established a hairy root system using A. rhizogenes, which allows us to evaluate CRISPR/Cas9 constructs in a few weeks. To identify the most effective strain for transformation, we tested five A. rhizogenes strains: K599, AR10, AR1193, R1000, and R1200. Among them, R1000 showed the highest efficiency. In total, we generated 630 hairy roots, and 116 of them displayed a distinct reddish color. Sequencing confirmed that CRISPR-induced mutations were successfully introduced into the ANR gene. To understand the basis of the color change, we analyzed methanol extracts from the red hairy roots using LC-MS/MS and compared them with extracts from white (control) roots. We detected cyanidin, a red pigment and a direct precursor of ANR, consistent with our expectation that knocking out ANR leads to its accumulation. This system provides a rapid and practical platform for functional genomics in fig and opens the door for future advances in F. carica biotechnology. It represents an important step toward establishing fig as a genetically tractable species, with broader applications in fruit crop improvement and secondary metabolite engineering.

Poster 39: **Ancient gene clusters initiate monoterpene indole alkaloid biosynthesis and C-3 stereochemistry inversion**

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The inversion of C3 stereochemistry in monoterpenoid indole alkaloids (MIAs), derived from the central precursor strictosidine (3S), is essential for synthesizing numerous 3R MIAs and oxindoles, including the antihypertensive drug reserpine found in Rauvolfia serpentina (Indian snakeroot) and Rauvolfia tetraphylla (devil pepper) of the plant family Apocynaceae. MIA biosynthesis begins with the reduction of strictosidine aglycone by various reductases, preserving the initial 3S stereochemistry. In this study, we identify and biochemically characterize a conserved oxidase/reductase pair from the pocynaceae, Rubiaceae, and Gelsemiaceae families of the order Gentianales: the heteroyohimbine/yohimbine/corynanthe C3-oxidase (HYC3O) and C3-reductase (HYC3R). These enzymes collaboratively invert the 3S stereochemistry to 3R across a range of substrates, resolving the long-standing question about the origin of 3R MIAs and oxindole derivatives, and facilitation of reserpine biosynthesis. Notably, HYC3O and HYC3R are located within gene clusters in both the R. tetraphylla and Catharanthus roseus (Madagascar periwinkle) genomes, which are

partially homologous to an elusive geissoschizine synthase (GS) gene cluster we also identified in these species. In R. tetraphylla, these clusters occur closely in tandem on a single chromosome, likely stemming from a single segmental duplication event, while in C. roseus, a closely related member of rauvolfioid Apocynaceae, they were later separated by a chromosomal translocation. Collectively, our work uncovers the genomic and biochemical basis for key events in MIA evolution and diversification, providing insights beyond the well-characterized vinblastine and ajmaline biosynthetic pathways.

Poster 40: Engineering *Chlamydomonas reinhardtii* for cannabidiolic acid biosynthesis: Challenges and prospects

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Cannabinoids constitute a structurally diverse family of specialized metabolites synthesized naturally in *Cannabis sativa*, extensively studied for their therapeutic potential. Among these, cannabigerolic acid (CBGA) serves as substrate of the cannabidiol acid synthase (CBDAS) to generate CBDA, a molecule of increasing interest for pharmaceutical and wellness applications. This study evaluates the feasibility of employing microalgae *Chlamydomonas reinhardtii* as a photosynthetic host for CBDA biosynthesis, with a focus on the cytosolic expression of the CBDAS enzyme. Two expression cassettes carrying *CBDAS*, driven by either the *pHSP70A-RBCS2* or *pSAD* promoters, were constructed and transformed into the nuclear genomes of three *C. reinhardtii* strains (CC-1690, CC-125, and CC-5415). Integration of the transgenes was confirmed by genomic PCR, while transcript accumulation was verified by qRT-PCR across three independent clones per construct. Despite successful gene integration and transcription, CBDAS protein remained undetectable by Western blot, and enzymatic assays using crude microalgae extracts failed to reveal functional activity in any of the transformants. These findings underscore the intrinsic challenges associated with heterologous protein expression in the green microalgal cytosol. In future work, we will adopt a dual optimization approach, combining alternative subcellular targeting strategies, notably to the endoplasmic reticulum or chloroplast, to promote proper protein folding and catalytic activity with refined cultivation conditions aimed at enhancing metabolic flux and overall expression efficiency. Collectively, these strategies are expected to advance the development of *C. reinhardtii* as a sustainable platform for cannabinoid production.

Poster 41:

Poster 42: Itching to Identify Gene Candidates Involved in Late Urushiol Biosynthetic Steps

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Urushiol is the natural product allergen responsible for the dreaded skin rash symptoms caused by poison ivy (*Toxicodendron radicans*). Beyond its well-documented allergenicity in humans, urushiol also has a long history of material science applications. It is the principal component in the sap of *T. vernicifluum,* which has been used as a natural lacquer to coat wooden objects in Japan for >9000 years. Additionally, in this polymerized state, urushiol reacts with cellulose or other biomolecules to function as an adhesive. Urushiol is not a single compound but rather a group of catechol congeners differing in either a C17- or a C15-alk(en)yl side chain. Although none of the urushiol biosynthetic enzymes or genes have been characterized, there are two alkylphenol metabolites present in poison ivy - anacardic acid and cardanol – predicted as penultimate metabolic intermediates leading to urushiol. Urushiol is hypothesized to be synthesized from either of these metabolites through a hydroxylation at the 2-position of the aromatic ring. Thus, the objective of this work is to identify poison ivy transcripts that encode for oxidative enzymes capable of converting anacardic acid or cardanol into urushiol. To identify potential candidate genes responsible for catalyzing these hydroxylations, we first identified differentially expressed transcripts that are positively correlated with differential urushiol accumulation levels. The gene expression of poison ivy tissues with high urushiol levels (drupes) and low urushiol levels (leaves) was quantified via Illumina short-read RNA sequencing for all 163,846 transcripts in the poison ivy transcriptome. I initially identified 13,087 differentially expressed poison ivy transcripts (≥ 3-fold difference with a P-value ≤ 0.05) that were also significantly correlated with differential urushiol accumulation levels (Pearson correlation P-value ≤ 0.05). These differentially expressed transcripts were subsequently filtered for enzyme families known to catalyze hydroxylation reactions on an aromatic ring. This resulted in eighteen transcripts, representing three different enzyme families, that are high priority candidates for encoding predicted enzymes responsible for catalyzing hydroxylation reactions of either anacardic acid or cardanol to produce urushiol. Currently, we are testing each candidate open reading frame using Agrobacterium-mediated transient expression in *Nicotiana benthamiana* leaves to produce recombinant poison ivy enzymes leading to the biochemical conversion of anacardic acid or cardanol into urushiol. Following the approach of Lau & Sattely (2015), each recombinant candidate gene will be infiltrated into *N. benthamiana* leaves and subsequently infiltrated with anacardic acid or cardanol. Recombinant enzymes will also be expressed in yeast or *E.coli*, purified, and assayed for *in vitro* hydroxylation of either anacardic acid or cardanol, resulting in urushiol production. Most products used for surface coatings or adhesives are derived from petrochemicals, which are a finite and environmentally harmful resource. Urushiol is a natural “green chemistry” thermoset polymer with historical utility as an adhesive and surface coating, making it a sustainable alternative to fossil petrochemical feedstocks. Thus, identifying the genes responsible for urushiol biosynthesis is the first step towards developing a sustainable biomaterial economy in which urushiol can be used for a variety of high-performance material science applications.

Poster 43: Drought-Induced Alterations in Lipid Metabolism of Soybean (*Glycine max L.*): Dynamic Changes in Photosynthetic Membrane Lipids

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Soybean (*Glycine max L.*) is an economically important oilseed crop that supplies oils and proteins for human consumption and livestock feed. However, its production is severely threatened by drought, which can lead to up to 50% yield losses. Lipids, a diverse group of plant metabolites, play vital roles in plant development and in responses to environmental stressors such as drought. Despite this, the molecular mechanisms linking lipid metabolism to drought tolerance in soybean remain poorly understood. This study aims to investigate how lipid metabolism contributes to the drought stress response in soybean plants. Select soybean cultivars were grown under drought and optimal watering conditions to assess their agronomic performance and the lipids profiles of different tissues across growth stages. Our results showed that soybean cultivars vary in their tolerance to drought, and their leaf lipid abundance and composition respond dynamically to drought stress. Specifically, total lipid abundance increased in response to drought, with changes in α-linolenic acid (C18:3) content. Photosynthetic membrane lipids – including monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol, and phosphatidylglycerol – exhibited some degree of drought-responsive remodelling of constituent fatty acid saturation and chain length. Interestingly, drought tolerant cultivars exhibited stress-induced changes in the abundance of phosphatidylcholine and MGDG, along with a shift in the saturation of their constituent fatty acids. Research on the impact of drought stress on cuticular wax components, root lipids, and seed lipids is ongoing, along with complimentary transcriptomic analysis. These observations provide insight into the drought-responsive lipid alterations in soybean, suggesting a potential role for lipid remodelling in supporting drought resistance in soybean.

Poster 44: ***Physalis*** **acylsugar diversity: chemical profiles and biosynthetic insights across tissues and species**

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Plants have evolved a structurally diverse chemical repertoire to mediate various environmental interactions. Yet, little is known about the chemical complexity and metabolic pathways across many plant genera, which limits the ability to use biologically active metabolites to enhance plant resilience. Acylsugars are an important class of specialized metabolites widely distributed across the Solanaceae that play a role in plant defense. Acylsugars have been extensively characterized from glandular trichomes, however these metabolites have also been detected in other tissues, such as roots and fruit. Here, we performed tissue specific metabolomics using LC-MS across 30 *Physalis* species, an emerging model genus that is closely related to more economically important *Solanum* crops. Automated mass feature identification and library searches from surface extracts of leaf, calyx, and fruit tissues revealed a large amount of metabolite diversity including putative metabolite classes of flavonoids, phenolics, and terpenoids. Given the lack of acylsugars in public repositories, mass spectrometry features were manually annotated and revealed close to 300 unique acylsugar types, most of which were found only in a single species. Principal coordinate analysis (PCoA) of acylsugar profiles indicated that species with similar metabolite profiles also cluster taxonomically. Acylsugars had variable structures, with chain lengths ranging from 2 to 12 carbons, and number of acylations from 2 to 4. Species varied in abundance and number of acylsugars, with some species producing as few as 5 acylsugars in a single tissue, while others produced more than 30. Acylsugar accumulation patterns across different tissues also varied within *Physalis*. Some, but not all, *Physalis* species accumulated acylsugars on the fruit surface. To determine the biochemical mechanism underlying species-specific acylsugar variation, we biochemically characterized the first step of acylsugar biosynthesis, catalyzed by an acylsugar acyltransferase (ASAT), from *Physalis* species with different acylsugar profiles. Recombinantly purified ASAT1s from three species were tested *in vitro* with acyl-CoA substrates of various lengths, and displayed slightly different substrate preferences, which may explain the difference in acylsugar profiles. The extensive chemical diversity and fruit-localized acylsugars within *Physalis* can inform engineering strategies for increased crop resilience.

Poster 45: **Agricultural Management Influences the Ecometabolomic Profile of *Zea mays***

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Research in plant ecometabolomics may allow us to establish linkages between soil and plant function, crop quality, and the overall health of agricultural systems. However, the way plant metabolomics are affected by and respond to agricultural management is not well understood. Understanding how agricultural management influences the ecometabolomic response of plants may lead to agricultural practices that require less inputs, reducing harmful environmental impacts, and creating agricultural systems that are more resilient to various biotic and abiotic stressors. As part of the Long-Term Agroecosystem Research (LTAR) Network, we assessed ecometabolomic profiles of corn (*Zea mays* L.) leaves and roots between contrasting prevailing (prevailing practice, PP) and alternative (alternative practice, AP) cropping practices which utilized cover crops and cover crop interseeding. Corn plants were collected during the reproductive (R3) growth stage of corn. Our untargeted metabolomic analyses resulted in 124 annotated features, with 68 features significantly different between AP and PP treatments. We detected 43 features annotated as PSMs, 39 of which were greater ($p \leq 0.10$) in the AP than PP treatments. For instance, corn leaf tissue in AP treatments was greater in shikimates and alkaloids, while corn root tissue in AP treatments was greater in shikimates, alkaloids, benzenoids and terpenoids. This study indicates the way we manage our agricultural systems influences the composition of PSMs produced by corn. Increased production of PSMs in the AP treatment may allow corn to better respond to various abiotic and biotic stresses, enhancing the resilience of plants within their ecosystem.

Poster 46: **Characterising a Second Cytochrome P450 Reductase in the Artemisinin Biosynthetic Pathway**

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Malaria places an epidemiological burden on more than 87 countries. Although it is fully treatable, it contributes to more than half a million annual deaths. Artemisinin, which is extracted from the plant *Artemisia annua,* is a critical medicine for the treatment of severe malaria. Unfortunately, the artemisinin content varies from plant to plant, which disrupts its ability to meet the demand for treatment. Thus, enhancing metabolic engineering strategies *in-planta* is critical for increasing drug availability. Cytochrome P450 71AV1 (CYP71AV1), a membrane-bound monooxygenase, oxidises several steps in the artemisinin pathway. Its catalysis requires partner membrane enzymes like cytochrome P450 reductase (CPR1) to pass electrons to its functional heme group. Our lab has identified a gene with 80% homology to the CPR1 amino acid sequence that shares key motifs such as flavin mononucleotide (FMN) and flavin adenine mononucleotide (FAD) binding groups, which are essential for electron shuttling among reducing enzymes. I am characterising this enzyme, hereby CPR2, to understand its possible interaction with CYP71AV1 and consequent involvement in artemisinin biosynthesis. Here, I present the expression and purification of a truncated version of CPR2 from *Escherichia coli* for the first time and its resulting kinetic analysis. The data from both qualitative (colour changes) and quantitative kinetic results show that CPR2 has reducing capabilities *in-vitro* and is a highly active and thermostable enzyme. Enzyme saturation was reached at 20uM of substrate and was subsequently inhibited at higher concentrations, as reflected in Michaelis-Menten curves. Its kinetic activity closely mimics that of CPR1 and more experimentation is necessary to determine if a true functional difference exists for these two enzymes. Follow-up experiments will determine whether this enzyme partners with CYP71AV1 and contributes to the production of artemisinin and/or its derivatives.

Poster 47: Understanding salinity tolerance mechanisms in finger millet through metabolomics

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Soil salinization is one of the major abiotic stresses that leads to a reduction in growth and yield by affecting various physiological and metabolic processes of plants. Finger millet (*Eleusine coracana* Gaertn L.) is an underutilized but nutritionally rich food crop that is generally cultivated in the marginal lands. Apart from its nutritional quality, finger millet has substantial resilience against several abiotic stresses. Previous reports on finger millet suggests its relatively higher salinity tolerance than many other leading crop species. The existence of genotypic variation for salt tolerance in finger millet suggests the genetic control of the character and possible crop improvement via plant breeding. Identification of genotypes with greater stress tolerance is essential for understanding tolerance mechanisms and the development of elite cultivars. Based on the consensus of several phenotypic data of a prior experiment at the germination and seedling stages, we selected two accessions (IE 518 and IE 405) for further evaluation with the morphophysiological parameters and metabolomics. Significant phenotypic separation of IE 518 and IE 405 for salt tolerance was reflected through differences in plant height (PH), maximum quantum yield of photosystem II (F_V/F_M), electrolyte leakage (EL), net photosynthesis rate (P_n), shoot Na^+ ion accumulation, and malondialdehyde (MDA) content. However, both accessions showed retention of K^+ ions even at a higher concentration than Na^+, which underscores the possibility of root mediated Na^+ exclusion or compartmentalization in finger millet. Pathway enrichment analysis with the uniquely regulated metabolites due to salt identified key metabolic pathways having potential association with salt tolerance mechanisms in IE 518. Important pathways that were found to be influenced in IE 518 due to salinity include stress signaling (butanoate metabolism), biotin metabolism, energy metabolism (Tricarboxylic acid cycle, pyruvate metabolism, and photosynthesis), amino acid biosynthesis, sugar metabolism, etc.

Poster 48: Deciphering spirooxindole biosynthesis in *Mitragyna parvifolia* using multi-omics approach

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Monoterpenoid indole alkaloids (MIAs) found in Rubiaceae are associated with varied pharmaceutical uses. Spirooxindole alkaloids constitute a structural subtype of MIAs with the unique spiro[pyrrolidine-3,3′-oxindole] ring system. Despite their intriguing structures and potent bioactivities, the evolution and diversification of spirooxindole alkaloids remain poorly understood. *Mitragyna parvifolia,* a tree species of the Rubiaceae family that predominantly produces anti-proliferative spirooxindole alkaloid mitraphylline. Transcriptomic analyses of juvenile, mature leaves, stems, stipules, and roots integrated with MIA profiling and genomic analyses revealed several candidates in the MIA biosynthetic pathway. Functional characterization of selected candidates led to the elucidation of the biosynthesis of the anti-proliferative spirooxindole mitraphylline in *M. parvifolia*. The study discovered several biosynthetic enzymes, a pair of FAD-dependent oxidase (*Mp*AO), and a reductase (*Mp*SOS) which is capable of converting the 3*S*-ajmalicine substrate to the epimer 3*R*-ajmalicine *in planta*. Then, a new cytochrome P450 enzyme (*Mp*SOS) is found converting the 3*R*-ajmalicine to the corresponding spirooxindoles, mitraphylline and isomitraphylline. The combination of the FAD-dependent oxidase and reductase favor the heteroyohimbane scaffold, in particular, the epimerization of 3*S*-tetrahydroalstonine (ajmalicine-stereoisomer) to its 3*R*-epimer has conversion rate higher than 90%. Intriguingly, all of the biosynthetic enzymes toward mitraphylline and isomitrayphylline are tightly coexpressed with the upper pathway gene strictosidine synthase, dictating the specific specialized metabolism to this *M. parvifolia* species. There is no evidence of spirooxindole presence in *Ophiorrhiza pumila*, and *Cinchona pubescens* other member of the Rubiaceae family. Such distinct presence strongly suggests that the biosynthetic step catalyzed by SOS enzymes plays a key role in spirooxindole specialization from *Mitragyna* species.

Poster 49: Investigation of the initial steps in cherylline-type alkaloids biosynthesis in *Crinum x. powellii*

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Cherylline, a unique type of Amaryllidaceae alkaloid (AA), has demonstrated antiviral activity against Zika and dengue viruses. While its biosynthetic pathway is presumed to follow the general AA framework, it remains largely uncharacterized. This study investigates the initial steps of cherylline biosynthesis, focusing on the formation of its first intermediates. We analyzed homologs of Norcraugsodine reductase (NR) and Norbelladine synthase (NBS) in the cherylline-producing species *Crinum x. powellii* and compared their activity to homologs in non-cherylline-producing species *Narcissus papyraceus*. *In vitro* and *in vivo* assays revealed that the *C. x powellii* NBS homolog exhibits distinct catalytic properties compared to its *N. papyraceus* counterpart, suggesting functional divergence in AA biosynthesis. This comparative analysis provides insights into key enzymatic differences underlying cherylline production. Additionally, we explore putative biosynthetic pathways, emphasizing norbelladine and its methylated derivatives as critical intermediates. These findings may support the development of sustainable production strategies for cherylline and related AAs, advancing their potential pharmaceutical applications.

Poster 50: **Investigating SIP68, a UDP-Glucosyltransferase, and Its Role in Plant Stress Tolerance Through Cytokinin Mediated Pathway**

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UDP-glucosyltransferases (GTs) play a pivotal role in plant metabolism by catalyzing the transfer of glucosyl groups from UDP-glucose to a wide range of acceptor molecules, thereby modifying their stability, activity, and transport properties. SIP68, a UDPglucosyltransferase, was identified through a yeast two-hybrid screen as an interactor of Salicylic Acid-Binding Protein 2 (SABP2), implicating it in salicylic acid (SA)-mediated defense signaling. Prior studies in tobacco plants have revealed its involvement in stress responses; however, its broader role in plant development and immunity remains largely unexplored. This study aims to characterize SIP68's function by integrating bioinformatics analyses, transgenic plant studies, and biochemical assays. Through comprehensive in silico analysis, we examined the SIP68 gene family, gene structure, chromosomal localization, and exon-intron organization. Additionally, homology modeling, domain architecture, motif analysis, and phylogenetic comparisons were conducted to infer evolutionary relationships and potential functional attributes. In silico predictions suggest that SIP68 likely participates in cytokinin-mediated metabolic pathways, potentially regulating plant growth and development by modifying key signaling molecules through glucosylation. To experimentally validate SIP68's developmental role, SIP68-silenced transgenic tobacco plants were generated and analyzed. Phenotypic assessments revealed significant changes in root length, shoot height, and leaf width compared to wildtype plants, demonstrating its critical function in growth regulation. Additionally, SIP68 appears to be a key player in plant immune responses. To evaluate its role in biotic stress resistance, SIP68-silenced and wild-type plants were challenged with the Tobacco Mosaic Virus. The SIP68-silenced plants displayed increased susceptibility to both pathogens, further reinforcing its role in SA-mediated defense. To understand SIP68's biochemical function, in vitro substrate-binding assays were performed, revealing its strong affinity for flavonols. This suggests a potential role in modifying secondary metabolites that influence plant physiology and stress responses. However, identifying SIP68's specific in planta substrates remains a challenge, necessitating further studies to elucidate its precise metabolic role. In conclusion, our study provides significant insights into SIP68's dual role in plant development and immunity. The observed phenotypic alterations in SIP68-silenced plants underscore its regulatory role in plant growth, potentially via cytokinin metabolism. Furthermore, its contribution to plant defense, particularly in SA-mediated pathogen resistance, highlights its importance in biotic stress responses. Future studies should focus on identifying SIP68's direct molecular targets, elucidating its regulatory networks, and determining its impact on cytokinin and flavonoid metabolism. Understanding SIP68's function will advance our knowledge of UDP-glucosyltransferases in plant biology and could provide novel strategies for crop improvement.

Poster 51: **Non Targeted Metabolomic Analysis in *Sechium edule* (Jacq)SW and *Sechium edule* var Albus minor: A descriptive study among the domesticated and wild form**

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This study investigated the impact of domestication on the metabolic profile of two genotypes of *Sechium edule* (Jacq.) Sw.: a wild accession (653 d) and a cultivated edible variety (*S. edule* var. *albus minor*, 261-05). Using an untargeted and targeted metabolomics approach (UHPLC-ESI-Q-TOF-MS and HPLC), differences in the chemical composition of both genotypes were characterized, with a particular focus on secondary metabolites such as flavonoids, phenols, and alkaloids, as well as primary compounds (sugars, amino acids, and organic acids). Multivariate analyses (PCA, heatmaps, and volcano plots) revealed that the wild form exhibits a greater diversity of defense-related pathways (flavonoid, isoflavonoid, and certain cucurbitacin synthesis), while the domesticated variety showed enrichment in primary metabolism pathways (e.g., steroid and fatty acid biosynthesis). Additionally, accession 653 d has high concentrations of phenolic compounds (chlorogenic acid, quercetin, catechin, and protocatechuic acid), whereas accession 261-05 retains only a subset of these metabolites but still maintains the ability to produce some of nutraceutical interest (including rosmarinic acid). These results suggest that artificial selection favored the channeling of metabolic resources toward desirable agronomic traits, such as palatability and yield, at the expense of reducing metabolites involved in biotic and abiotic stress tolerance. Nevertheless, the persistence of conserved pathways in both genotypes suggests the possibility of manipulating or reintroducing defense-related genes. Overall, this study confirms the value of wild S. edule germplasm as a source of chemical diversity and points the way for new genetic improvement strategies and biotechnological applications aimed at obtaining varieties with fruits with a greater number of applications.

Poster 52: Elucidation of Biosynthetic Pathway of potential nutraceutical compound (monotropein) using bioinformatics and molecular biology approaches

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Blueberry is the second most important crop in the US, owing to increased awareness of its health benefits due to the presence of several bioactive compounds. One such plant bioactive compound is monotropein. It has been reported that monotropein is found in only three plant species, namely *Monotropa Uniflora*, *Morinda officinalis* (*Vaccinium* spp). Most work on monotropein has been done in *Morinda officinalis* where it has been shown to impart several human health benefits. Recent work has also identified monotropein in both wild and cultivated blueberries. However, how monotropein is produced and the type of candidate genes involved in the biosynthesis of monotropein in blueberries has yet to be elucidated. Our research group used bioinformatics, comparative genomics and protein engineering techniques to functionally characterize the ISY (Iridoid Synthase) gene. Transcriptome-based identification and functional characterization of iridoid synthase revealed that it is co-expressed with UDP-glucuronosyltransferase, which is a likely downstream step in the formation of monotropein. Currently, we are focusing on functionally characterizing Geraniol Synthase (GES) gene, as recent work has found the expression of GES is significantly associated with the production of iridoids in other plant families. Our overarching goal is to elucidate the biosynthetic pathway of production of monotropein in blueberry to breed blueberries with higher production of monotropein.

Poster 53: A Dichotomy of Trichome Functions in *Cannabis sativa* – Glandular Versus Non-Glandular – as Revealed by Scanning Electron Microscopy

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Cannabis sativa L. (cannabis, marijuana) is cultivated widely in Canada after it was legalized in 2018 for the medicinal and recreational markets. The plants are harvested for their complex inflorescences (racemes) which develop on genetically-female plants and produce an abundance of glandular trichomes. These trichomes are formed on the bract tissues that are found within the inflorescences. During maturation of the inflorescences, the glandular trichome heads enlarge in size and the trichome stalks elongate as they progress developmentally from sessile trichomes. The trichome heads manufacture and store a range of cannabinoid and terpene compounds, which are the *a priori* reason for commercial cultivation of these plants. The most well-known cannabinoids include the psychoactive THC and the non-psychoactive CBD, as well as a wide range of other compounds which accumulate to high levels within the cuticle-covered heads. Breeding efforts have targeted enhanced production of these compounds to where they can reach levels of up to 30% by dry weight. Bulbous trichomes also seen on bract tissues have no know function assigned to them. On vegetative tissues which include young stems, leaves and petioles, non-glandular trichomes (leaf hairs) are produced in abundance, especially during the early stage of plant growth. Their functions are presumed to be for protection against predation and UV irradiation, and in reducing moisture loss and surface temperatures. During an investigation of symptoms of nutrient toxicity caused by high fertilizer regimes on young cannabis plants, a previously undescribed function of the non-glandular trichomes was discovered. Under these high fertilizer conditions, a white precipitate that was found to consist of salt crystals developed along leaf veins, especially on the underside of the leaves. The white deposits were also found on the stems of young plants. Necrotic tissues developed in association with the crystals. Under the scanning electron microscope, the crystals were formed in association with the non-glandular trichomes, which were observed to secrete a crystalline-like substance from their tips. The secretions also originated along the entire trichome surface in what appeared to be exudations forced through the cuticular surface, possibly from underlying osmotic pressure. The crystals were analyzed by SEM-EDAX and shown to contain calcium, magnesium, potassium, silicon, chloride and other ions. These chemicals are all present in the fertilizer regime applied to cannabis plants. The excess fertilizer applied to the plants appears to have been taken up through the roots and forced out of the trichome glands, perhaps as a mechanism to reduce the toxicity to cells. The secretory capacity of non-glandular trichomes to exude inorganic salts is reported for the first time. Whether this represents an adaptive strategy to deal with high salt stress conditions remains to be determined. The response was genotype-specific and only observed on two genotypes. The plants all eventually recovered from the toxicity and grew normally.

Poster 54: Mapping the Subcellular Compartmentalization of Enzymes in Monoterpene Indole Alkaloid Biosynthesis

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Monoterpene Indole Alkaloids (MIAs) constitute a large and diverse group of specialized plant metabolites known for their potent biotherapeutic properties and high drug candidacy rates. This class includes clinically significant compounds such as vinblastine and camptothecin, used in chemotherapy, and reserpine, a treatment for hypertension. MIA biosynthesis is characterized by intricate enzyme compartmentalization across various cell types and subcellular organelles, including the vacuole, nucleus, endoplasmic reticulum, and peroxisome. Recent discoveries have expanded our understanding of MIA biosynthetic enzymes, including cytochrome P450 monooxygenases, berberine bridge enzyme-like oxidases, reductases, and methyltransferases. To further investigate the subcellular localization of these enzymes, we employed fluorescent tagging combined with transient expression in Nicotiana benthamiana. This approach aims to provide deeper insights into MIA biosynthesis and trafficking, clarifying how compartmentalization influences metabolic flux. A better understanding of these spatial dynamics will facilitate targeted engineering strategies to enhance the production of therapeutic MIAs in heterologous systems.

Poster 55: Biosynthesis of omega-3 fatty acids and derived jasmonates in *Marchantia polymorpha*

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In the model bryophyte *Marchantia polymorpha*, omega-3 polyunsaturated fatty acids (n3PUFAs) with 16, 18, or 20 carbon atoms (C16, C18, or C20) are precursors for bioactive jasmonates. Nevertheless, the role of omega-3 fatty acid desaturases (n3FADs) in the production of n3PUFAs and jasmonates has not yet been fully explored. In this study, we characterized two n3FAD enzymes in *Marchantia polymorpha*. We found that MpFAD3 (localized in the endoplasmic reticulum) prefers arachidonic acid (ARA, 20:4n6) as its substrate, thereby regulating the biosynthesis of C20-derived jasmonates. In contrast, MpFAD7 (localized in the chloroplast) efficiently uses hexadecadienoic acid (HDA, 16:2n6) and linoleic acid (LA, 18:2n6) as substrates, impacting the production of C16/18-derived jasmonates. Both enzymes showed a certain degree of promiscuity, which is reflected by increased n6PUFA levels in the double *Mpfad3fad7* mutant compared to single mutant lines individually. All three mutant lines, comprising both single and the double mutant, displayed enhanced resistance to *Fusarium oxysporum* infection relative to the wild type. This observation is consistent with earlier findings obtained from jasmonate-biosynthesis or -signaling mutants. Interestingly, *Spodoptera exigua* caterpillars exhibited similar performance when feeding on the wild type or on the significantly reduced jasmonate-dependent defenses, *Mpfad3fad7* mutant plants. This was likely due to a shortage of essential n3PUFAs. Our findings suggest that two conserved yet specialized n3FADs control the biosynthesis of n3PUFAs and jasmonates in *Marchantia*. Furthermore, the quality of food, specifically the type and quantity of n3PUFAs, contributes to insect performance in addition to the plant's jasmonate-dependent defenses.

Poster 56: **Examining Wax Layers for Water Retention in Plants**

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Chemical layers found just outside a plant's epidermal cells, often called wax layers, are instrumental in a plant's defense against external stressors. Such stressors can include retaining water in a dry environment. However, the ideal chemical composition of these wax layers, leading to effective water retention, has not yet been identified. Therefore, the goal of this project was to measure the permeation characteristics of various wax mixtures using synthetic wax discs. Mixtures of varying concentrations of aliphatic (docosane, docosanol) and triterpenoid (betulin) compounds were dissolved in chloroform, then deposited dropwise on to discs made of stainless-steel wire mesh (~178 um). More than 500 discs were created and tested over the course of this project so far. These synthetic discs were mounted individually in 3D-printed retention chambers, and placed inside an incubator where the temperature and relative humidity were monitored and controlled. The rate of water loss was then observed over two days. Two controls were also tested: parafilm discs for the minimal rate of water loss and waxless mesh scaffolds for the maximum rate. It was observed that chemical mixtures can have greater water retention capabilities than individual chemical compounds. At all temperatures tested (ranging from 23°C to 37°C), a binary 50-50 mixture of docosane to docosanol had the lowest permeability rate. Elevated temperatures resulted in higher permeability rates, regardless of chemical composition. The effect on permeability of added betulin into aliphatic mixtures depended on that specific base aliphatic chemistry. Future research will compare these synthetic layers to leaf samples to determine whether synthetic compositions are accurate representations of real waxes found in plants. If the two are indeed comparable, it is likely that we can use synthetic discs to discover the chemical components of effective plant wax layers - and apply such knowledge to crops to aid their performance in adverse conditions.

Poster 57: **Investigating genetic and environmental effects on photosynthetic pigment concentrations in *Lactuca sativa* and *Spinacia oleracea***

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Vitamin A deficiency affects approximately 30% of children under five globally, leading to impaired vision, increased risk of infection, and in severe cases, mortality. Green leafy vegetables such as lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) are widely consumed for their phytochemicals and nutritional composition. Leaves of these crops tend to be dense in chlorophylls and provitamin A and other carotenoids; the latter are pigments that serve as a photoprotective mechanism in the photosynthetic apparatus. In human and animal systems, certain carotenoids including **β**-carotene act as dietary sources of provitamin A; lutein and zeaxanthin have been associated with reduced risk of age-related macular degeneration; and lactucaxanthin has been linked to the mitigation of hyperglycemia-related pathogenesis. However, water limitation can disrupt photosynthesis, increase oxidative stress, and reduce crop quality. Breeding for enhanced pigment concentrations may improve both nutritional quality and photosynthetic performance under well-watered and/or water-limited conditions. This study aims to characterize the genetic basis

of carotenoid and chlorophyll concentrations in diverse and elite cultivars of spinach and lettuce grown under water-limited and well-watered conditions and apply genomics- and sensing-enabled prediction tools to accelerate breeding for these traits and environment types. Chlorophyll a, b, their sum, and total carotenoids were quantified across multiple field trials via colorimetric assays, and individual compound concentrations are being determined by high-performance liquid chromatography. In spinach, most pigment combinations showed positive correlations across genotypes, except for chlorophyll b and total carotenoids. Similarly, in lettuce, all traits were found to be highly correlated across and within field trials and treatments. These wet-chemistry data are being cross-analyzed with two sensing modalities - backpack-based hyperspectral sensing and a Resonon pushbroom hyperspectral camera - to assess consistency and complementarity across platforms. Overall, the identification of genomic regions associated with these compounds and/or the development of predictive models for them could be used to select parents in breeding programs aimed at enhancing the concentrations of photosynthetically relevant pigments alongside improved agronomic performance.

Poster 58: **Leveraging AlphaFold and Molecular Operating Environment to Investigate Enzyme Catalysis in Monoterpenoid Indole Alkaloid Biosynthesis**

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The revolutionary advancement of protein structure modeling, driven by AlphaFold, has transformed protein homology modeling, making accurate structure prediction widely accessible to the scientific community. Understanding enzyme catalysis through homology modeling and substrate docking provides invaluable insights into enzyme function, evolution, and engineering for improved performance. In this study, I leveraged AlphaFold and Molecular Operating Environment (MOE) to investigate key enzymes involved in the biosynthesis of monoterpenoid indole alkaloids (MIAs), one of the largest and most structurally diverse classes of alkaloids in nature. My targets include cytochrome P450 monooxygenases, cinnamyl alcohol dehydrogenase-like reductases, and *S*-adenosylmethionine-dependent methyltransferases. This work establishes a workflow for homology modeling and substrate docking, offering mechanistic explanations for enzyme specificity, kinetic differences, and key residues that may dictate substrate binding poses within highly similar active sites. Notable examples include pseudoakuammigine 10-hydroxylase and vincaminoreine 10-hydroxylase from *Vinca minor*; coronaridine 11-hydroxylase, 11-*O*-methyltransferase, and perivine *N*-methyltransferase from *Tabernaemontana elegans*; and coronaridine 10-hydroxylase, tabersonine β-epoxidase, and pseudovincadifformine 18-hydroxylase from *Tabernaemontana litoralis*.

Poster 59: **Supply or Demand? The Relationship between Straight-Chain Ester Production and Free Fatty Acid Availability in *Malus Domestica***

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Apple fruit flavor and, consequentially, value are intrinsically tied to the specialized volatile organic compound pathways in the fruits' skin. These, in combination with more primary pathways like sugar and acid metabolism, make up the many unique flavor profiles found in apple cultivars. Amongst these volatiles, the short straight-chain ester, hexyl acetate is especially important as it conveys a desirable sweet, fresh, fruity flavor to the apple. Recent studies into the synthesis pathways of apple esters have identified the important role of alcohol acyltransferase, the final step in ester formation. There remains, however, a lack of clarity in the upstream steps and influences that determine how much hexyl acetate or other straight-chain esters are produced compared to branched-chain esters which utilize the same ripening-dependent acyltransferases. Our research investigates the relationship between hexyl acetate production and free fatty acid precursors in apple peels across a panel of apple cultivars. We also examined the expression patterns of specialized genes in the lipoxygenase straight-chain ester synthesis pathway between relatively high and low hexyl acetate-producing apple cultivars. The findings of this research represent progress towards a clearer understanding of aroma synthesis in apple fruit. This knowledge not only will help guide future research in tree fruit metabolism but also will help guide apple breeders and farmers in the development of more desirable apple cultivars which may lead to both greater consumption and nutrition in consumers and greater value for the producers of these fruits.

Poster 60: **Analysis of Environmental and Seasonal Influences on Condensed Tannins and Other Metabolites in Birdsfoot Trefoil (*Lotus corniculatus* L.) Cultivars**

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***Lotus corniculatus* L.**, commonly known as birdsfoot trefoil (BFT), is a perennial, non-bloating forage plant grown in temperate regions, renowned for its ability to accumulate high levels of condensed tannins (CT) in its foliage. BFT has been shown to reduce parasitic nematode burdens in ruminants. Although health benefits for ruminants have been linked to BFT consumption, the variability in condensed tannin concentrations presents a significant challenge, as high CT levels can lead to antinutritional effects

in cattle. While many studies have examined the impact of environmental factors on CT accumulation in BFT under controlled conditions, the effects of environmental and seasonal variations on CT levels and other plant metabolites in BFT cultivars grown in the field remain relatively unexplored. In this study, we combine traditional CT quantification and metabolomic profiling with high-resolution liquid chromatography-mass spectrometry (LC-MS) to investigate the genetic and environmental factors influencing both CT content and other metabolite profiles. We analyzed eight BFT cultivars from locations in Kentville (Canada), Rhode Island, and Utah (USA), and found significant variation in soluble CT content and overall metabolite composition. Notably, CT levels fluctuated considerably across cultivars and geographic regions, with plants from Kentville exhibiting the highest levels. Geographic location emerged as the most influential factor on CT levels. Our LC-MS analyses identified various metabolites in BFT samples, including flavonol, flavonol glycosides, lysophospholipids and saponins. A targeted metabolomic approach revealed the presence of key proanthocyanidin compounds such as (epi)catechin monomers, procyanidin B1, and trimeric procyanidin compounds in the samples. Like CT levels, metabolite profiles were largely determined by geographic location, with metabolites such as catechin, procyanidin B1, and lysophospholipids, including PCs (Phosphatidylcholines), lysoPEs (Lysophosphatidylethanolamines), and lysoPCs (Lysophosphatidylcholines), mostly responsible for location-specific sample clustering. These findings, along with further genetic analysis, will provide deeper insights into the adaptability of CT production in response to environmental factors, offering potential pathways for breeding and management strategies to improve the nutritional quality, resilience, and health benefits of birdsfoot trefoil, while also reducing methane emissions in livestock.

Poster 61: **The Role of Bacterial VOCs and Plant Endogenous Auxin in PGPR-Induced Root Morphogenesis**

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The rhizosphere is the narrow region of soil immediately surrounding plant roots, characterized by its rich microbial diversity. Among these root-colonizing microbes, a group of bacteria known as Plant Growth Promoting Rhizobacteria (PGPR) can enhance plant growth. They can confer various benefits to their plant hosts, such as making soil nutrients phytoavailable and enhancing abiotic and biotic stress tolerance. One possible mechanism by which PGPR promotes growth is through root morphological changes, primarily seen as an increase in lateral root number (LRN). This change is thought to be driven by the manipulation of the plant growth hormone auxin. While past research has focused on the ability of some PGPR to produce auxin, comparatively little work has been done on how diverse PGPR strains affect plant growth by manipulation of endogenous plant auxin levels.

Here, we investigated how different PGPR alter root architecture using four distinct strains, representing three species and two genera of PGPR. When treated directly with any of these strains, plants displayed increased LRN and reduced primary root length (PRL), which is a typical response to increased auxin. Interestingly, when plants were exposed solely to bacterial Volatile Organic Compounds (VOCs), the strains maintained their ability to increase LRN without reducing PRL, while also increasing fresh weight, indicating the crucial role of VOCs in the growth-promoting effect of these PGPR. To determine the involvement of auxin, we analyzed in planta auxin levels following bacterial treatment using Arabidopsis thaliana DR5 promoter reporter systems in wild type plants and auxin synthesis mutant wei2-1. We also observed the changes in root architecture in both auxin synthesis and response mutants. Through this study, we begin to reveal a more detailed picture of the role of auxin and bacterial VOCs in PGPR-mediated root architecture changes.

Poster 62: **Chemical and Physical Analysis of *Sorghum bicolor* Varieties: Understanding Wax's Role in the Defense Against Fungal Pathogens**

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Cultivating *Sorghum bicolor*, an important cereal crop used worldwide for its drought tolerance and high yields, comes with the challenges of pests and disease. Plants, including sorghum, coat themselves in complex mixtures of hydrophobic molecules to waterproof their surfaces. These molecules are commonly referred to as waxes and provide the plant with a "fingerprint", a unique identifier for the surface of a given plant species and variety. Previous research has shown that the waxes on plant surfaces play a key role in fungal infection, mediating recognition, differentiation, and defense signaling. However, the leaf wax composition and antifungal activity of *S. bicolor* varieties that are resistant to fungal pathogens have yet to be described. Currently, I am analyzing the surface wax of seven varieties of sorghum, four of which have been described as pathogen resistant (Della, Dorado, Rio, and Gaolian), one as susceptible (BTx623), and two that were obtained from a collection of sorghum EMS mutants (SNP-1390 and SNP-0928). My gas chromatography-mass spectrometry (GC-MS) analysis has revealed significant differences in wax compound abundance between the varieties. Very-long-chain aldehydes (VLCAs) were found to be a major wax constituent in three of the four previously described pathogen-resistant varieties. VLCAs were not present in BTx623, Rio, SNP-0928, or SNP-1390. In addition, triterpenoid abundance varied greatly across varieties, with SNP-0928 seemingly possessing a mutation that leads to the overproduction of the triterpenoids (71.8% of the total wax), which are major leaf wax constituents of Sorghum, while Della produces the lowest proportion of triterpenoids (6.6%). Thus, my preliminary results indicate that triterpenoids and VLCAs may play a key role in mediating pathogen-host interactions in sorghum. To follow up on these preliminary results, scanning electron microscopy will be leveraged to characterize any potential topographical differences caused by these chemical variations. As an additional follow-up, I am using *in vitro* growth assays with betulin, a triterpenoid found in Birch bark, and *Fusarium nygamai*, a sorghum pathogen. My results so far indicate that betulin inhibits the growth of fungi; further in vitro growth assays with pathogenic fungi and various

triterpenoids are currently underway to support these findings. Eventually, these assays will be repeated with the leaf waxes extracted from the seven sorghum varieties selected in this study to assess their antifungal activity. My presentation will outline results collected to date and describe future plans in more detail.

Poster 63: Cell line engineering for secondary metabolite production in plant cell culture

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Plant secondary metabolites show significant therapeutic properties, such as anti-inflammatory, antitumor, antimicrobial, and analgesic effects. Most pharmaceutically relevant secondary metabolites have no production alternative besides industrial-scale cultivation of the species producing the target compound. Plant cells cultivated in cell-suspension culture offer a promising alternative for consistent secondary metabolite production in a controlled environment.
The development of suitable cell lines is a complex and time-intense procedure. Economic viability of a plant cell-based process hinges on three main attributes: 1) shear resistance, 2) doubling time and 3) aggregate formation. Shear sensitivity and doubling time are key determinants of the economic viability of a bioprocess, while aggregate formation limits nutrient uptake and pose technical difficulties like blocking of bioreactor tubing. Therefore, we are establishing a standardized cell adaptation strategy to address these challenges. We base our strategy on an extensive media optimization screen to meet the specialized nutrient need of individual cell lines and on continuous cultivation of plant cells in a cost-effective bioreactor system. Cultivating plant cells at constant cell density in a bioreactor (turbidostat) from the early stages of cell line development offers the following advantages: 1) shorter effective generation time due to the eliminated growth lag associated with passaging, 2) continuous cultivation inherently supports the enrichment of single cells and small aggregates 3) the bioreactor environment supports the selection shear-resistant cell lineages. Consequently, fast-dividing cells with low aggregation tendency will be enriched.

Poster 64: Towards a complete annotation of plant enzyme families using machine learning approaches

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In the genomics era, characterized enzymes remain vastly outnumbered by millions of unannotated protein sequences, creating a critical bottleneck in functional annotation that hinders progress in plant biochemistry. This bottleneck largely stems from the slow and labor-intensive process of manual biocuration, delaying the inclusion of experimentally validated enzyme functions in public databases. To bridge this gap, we developed FuncFetch, a high-throughput pipeline integrating NCBI E-Utilities, OpenAI's GPT-4, and Zotero to automate the extraction of enzyme activities from the scientific literature. Evaluation of FuncFetch on enzyme-substrate data extraction from journal PDFs demonstrated a precision of 0.86 and recall of 0.64. When applied to nine large plant enzyme families across 5,459 publications, FuncFetch compiled a comprehensive dataset of 32,605 enzyme activities at a total cost of under 1,500 USD. Notably, analysis of the BAHD acyltransferase family revealed that approximately 70% of experimentally characterized enzymes in literature remain uncurated in public databases. To address this curation gap, our BAHD activity dataset has been submitted to UniProt and will soon be publicly accessible. Moreover, the extensive data compiled facilitates enzyme function predictions through machine learning and orthology-based methods. Leveraging embeddings from the state-of-the-art protein transformer model ESM-3 and a set representation of known substrate molecules, we are training a neural network to predict enzyme specificity toward individual substrates and broader compound classes. Overall, this integrated workflow of automated data retrieval, manual curation, and predictive modeling promises to accelerate the functional annotation of plant genes on a large scale.

Poster 65: Towards Cytochrome P450 gene network analysis reveals key enzymes in soybean glyceollin pathway

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Phytoalexin glyceollin plays a crucial role in plant defense against pathogens in soybean. Our study aims to elucidate glyceollin biosynthetic pathway, focusing on the role of cytochrome P450 monooxygenases. Through a genome-wide search, we identified 346 P450s in soybean, analyzed their conserved motifs and gene expression patterns upon *Phytophthora sojae* infection. This research led to the establishment of a soybean P450 database, providing a valuable resource for future studies. Utilizing soybean-*P. sojae* interaction transcriptome data, we constructed a co-expression network to identify P450s involved in the glyceollin pathway, leading to the identification of participating P450s such as cinnamate 4-hydroxylases (C4Hs), isoflavone hydroxylases (IFHs) and glyceollin synthases. Furthermore, we characterized three C4H and nine IFH multigene family members in soybean and identified catalytically efficient candidates through the enzyme kinetic studies. Furthermore, a comparative transcriptome analysis of soybean cultivars differing in the level of partial resistance against *P. sojae* revealed a rapid change in the transcriptome of susceptible cultivar during early infection period, while the strong partial resistant cultivar that contained higher basal expression levels of glyceollin biosynthetic genes, did not show such change. These findings offer significant insights into the regulatory mechanisms underlying glyceollin biosynthesis and highlight the potential of leveraging P450s for improving disease resistance in soybean. Additionally, identifying catalytically efficient P450s involved in glyceollin production also addresses bottlenecks in glyceollin biosynthesis, with potential applications in metabolic engineering.

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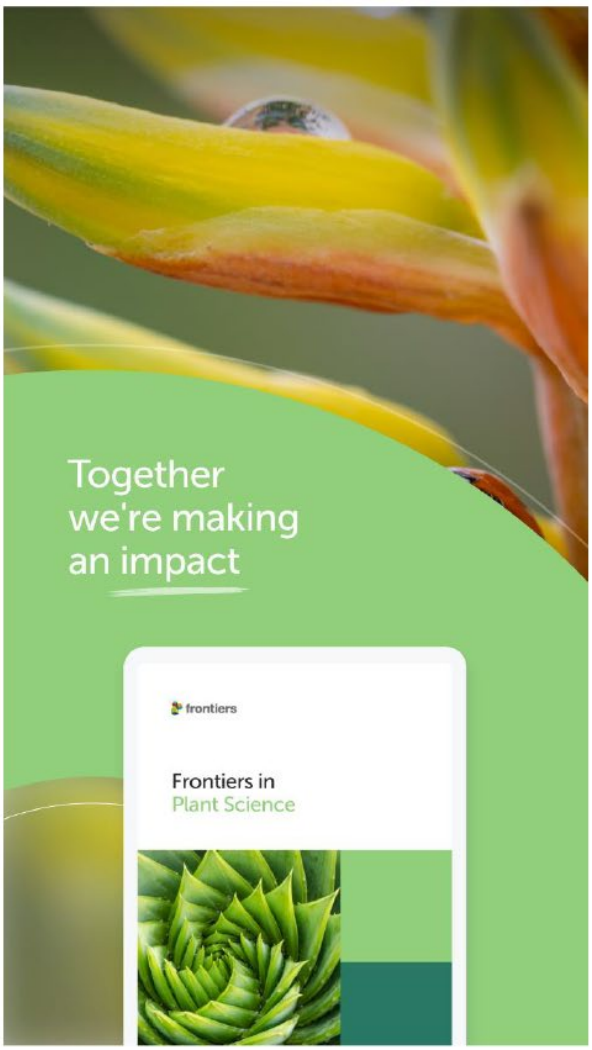

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