

60TH ANNIVERSARY MEETING

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
SOCIÉTÉ PHYTOCHIMIQUE DE L'AMÉRIQUE DU NORD
SOCIEDAD FITOQUÍMICA DE AMÉRICA DEL NORTE



July 25th–30th, 2021 | Kelowna, BC, Canada

TOPICS

Plant volatile emission: Beyond the traditional view
New advances in integrated *omics* and phytochemical research
Chemistry and biochemistry of therapeutic plants
Chemical ecology
Natural products discovery, synthesis and biosynthesis in the *omics* era
Breakthrough approaches in elucidating phytochemical biosynthesis
Evolution of plant specialized metabolism
Enzymology and organization of plant metabolism
Phytochemistry of the forest, the far north, and underutilized plants
Biochemistry and production of high-value phytochemicals
Synthetic biology and metabolic engineering of plant metabolism

PLENARY AND KEYNOTE SPEAKERS



Dr. Natalia Dudareva
Purdue University



Dr. Lloyd Sumner
University of Missouri



Dr. Paula Brown
British Columbia Institute
of Technology



Dr. Reuben Peters
Iowa State University



Dr. Sarah O'Connor
Max-Planck Institute
of Chemical Ecology



Dr. Jing-Ke Weng
Massachusetts Institute
of Technology



Dr. Jörg Bohlmann
University of British Columbia



Dr. Anastasios Melis
University of California, Berkeley



Dr. Dae-Kyun Ro
University of Calgary



Dr. Vincent Martin
Concordia University



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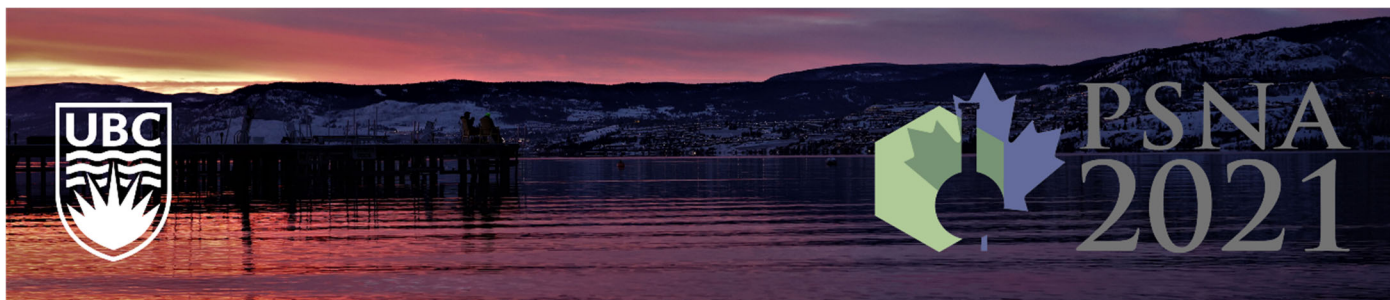
University of California, Davis

Dr. Dharendra Kumar

East Tennessee State University

Dr. De-Yu Xie

North Carolina State University



Meeting Program

All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)

AGENDA SUMMARY

- July 25 (Sunday) Opening/plenary session
- July 26 (Monday) Symposium 1: New advances in integrated *omics* and phytochemistry research
PSNA Member's Meeting
Symposium 2: Chemistry and biochemistry of therapeutic plants
- July 27 (Tuesday) Symposium 3: Chemical ecology
Early Career Researcher Workshop
Symposium 4: Natural products discovery, synthesis, and biosynthesis in the *omics* era
Poster session 1 (P1–P30)
- July 28 (Wednesday) Symposium 5: Breakthrough approaches in elucidating phytochemical biosynthesis
PSNA's Young Members Trivia Game
Symposium 6: Evolution of plant specialized metabolism
- July 29 (Thursday) Symposium 7: Enzymology and organization of plant metabolism
Symposium 8: Phytochemistry of the forest, the far north, and underutilized plants
Poster session 2 (P31–P60)
- July 30 (Friday) Symposium 9: Biochemistry and production of high-value phytochemicals
Symposium 10: Synthetic biology and metabolic engineering of plant metabolism
Closing

JULY 25 (SUNDAY)

July 25 (Sunday) Afternoon	Opening/plenary session Chair: Soheil Mahmoud (<i>University of British Columbia, Canada</i>)
15:00–15:30	1. Soheil Mahmoud – Chair, PSNA 2021 Local Organization Committee 2. Philip Barker – Associate Vice-President & Vice-Principal, Research, UBC 3. Argelia Lorence – President, Phytochemical Society of North America
15:30–16:45	Plenary speaker: Natalia Dudareva (<i>Purdue University, USA</i>) Plant volatile emission: Beyond the traditional view

JULY 26 (MONDAY)

July 26 (Monday) Morning	Symposium 1: New advances in integrated omics and phytochemistry research Chair: Dorothea Tholl (<i>Virginia Polytechnic Institute and State University, USA</i>)
9:00–9:50	Keynote speaker: Lloyd Sumner (<i>University of Missouri, Columbia, USA</i>) Advances in phytochemistry achieved through integrated metabolomics
9:50–10:00	[short break]
10:00–10:25	Benjamin Lichman (<i>University of York, UK</i>) Omics approaches to unravel complex alkaloid biosynthesis in <i>Daphniphyllum</i>
10:25–10:50	Gerald F. Schneider (<i>Utah State University, USA</i>) Comparative metabolomics of fruits and leaves in a hyper diverse lineage suggests fruits are a key incubator of phytochemical diversification
10:50–11:00	[short break]
11:00–11:25	Evangelos Tatsis (<i>Center for Excellence in Molecular Plant Sciences, China</i>) The genome of medicinal plant <i>Scutellaria barbata</i> and the evolution of clerodane pathway
11:25–11:30	Lovely Mae F. Lawas (<i>Auburn University, USA</i>) <u>Flash talk</u> : Unravelling iridoid production in blueberry: using omics to investigate plant natural products for human health
11:30–11:35	Radin Sadre (<i>Michigan State University, USA</i>) <u>Flash talk</u> : The beautiful lady's secret: Untargeted metabolomics uncovers the roles of cytochromes P450 in a pseudotropine-dependent branch of modified tropane alkaloid biosynthesis
11:35–11:40	Jeffrey Simpson (<i>Purdue University, USA</i>) <u>Flash talk</u> : Application of genome wide association in <i>Arabidopsis</i> leads to the functional classification of a new BAHD acyltransferase

July 26 (Monday) Noon	Phytochemical Society of North America's Member's Meeting
12:00–13:00	PSNA Member's Meeting (open to all)

July 26 (Monday) Afternoon	Symposium 2: Chemistry and biochemistry of therapeutic plants Chair: Susan Murch (<i>University of British Columbia, Canada</i>)
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- 13:30–14:20 **Keynote speaker: Paula N. Brown** (*British Columbia Institute of Technology, Canada*)
Green machine or tropical breeze: What's in a name when talking cannabis chemistry & research
- 14:20–14:30 **[short break]**
- 14:30–14:55 **Peyman Azhdary** (*University of British Columbia, Canada*)
Application of molecularly imprinted polymers as recognition elements in a liquid-phase electrochemical sensor for selective detection of a psychoactive substance, Δ^9 -tetrahydrocannabinol (THC)
- 14:55–15:20 **Sandra Irmisch** (*University of British Columbia, Canada*)
A novel anti-diabetic compound from plants: Biosynthesis, gene discovery, and metabolic engineering of montbretin A
- 15:20–15:30 **[short break]**
- 15:30–15:55 **Adam Clapp** (*California State University, Sacramento, USA*)
Elucidating the Taxol biosynthetic pathway and engineering taxadiene synthase for improved product specificity
- 15:55–16:00 **Eszter Sas** (*University of Montreal, Canada*)
Flash talk: Untargeted metabolite assessment reveals biorefinery opportunities from wastewater treatment using willow phytoremediation
- 16:00–16:05 **Rocio Esmeralda Hernández-Rubio** (*Universidad Autónoma de San Luis Potosí, Mexico*)
Flash talk: *Alternanthera flava* prevents acute liver damage induced by CCl₄ in rat
- 16:05–16:10 **Alison Edge** (*Brock University, Canada*)
Flash talk: Completion of the vindoline and catharanthine pathways in *Catharanthus roseus* facilitates characterization of MIA pathways in *Ochrosia elliptica*

JULY 27 (TUESDAY)

July 27 (Tuesday) Morning	Symposium 3: Chemical ecology Chair: Dharendra Kumar (<i>East Tennessee State University, USA</i>)
9:00–9:50	Keynote speaker: Reuben J. Peters (<i>Iowa State University, USA</i>) Digging into rice diterpenoid biosynthesis
9:50–10:00	[short break]
10:00–10:25	Arthur Neish Award recipient: Heejin Yoo (<i>Oklahoma State University, USA</i>) Uncovering regulatory mechanisms of salicylic acid biosynthesis for plant immunity in <i>Arabidopsis</i> and Brassicaceae oilseed crops
10:25–10:50	Dorothea Tholl (<i>Virginia Polytechnic Institute and State University, USA</i>) Above and belowground dynamics of terpene volatiles in switchgrass
10:50–11:00	[short break]
11:00–11:25	John Jelesko (<i>Virginia Polytechnic Institute and State University, USA</i>) The enigmatic nature of poison ivy urushiol chemical ecology
11:25–11:30	Abraham J. Koo (<i>University of Missouri, Columbia, USA</i>) <u>Flash talk</u> : Differential regulation of the ribosomal association of mRNA transcripts in an <i>Arabidopsis</i> mutant defective in jasmonate-dependent wound response
11:30–11:35	Jacob Walsh (<i>University of Western Ontario, Canada</i>) <u>Flash talk</u> : Synthesis of deoxynivalenol metabolites for use as analytical standards

July 27 (Tuesday) Noon	Early Career Researcher Workshop with Monica Borghi, Ellaine De Guzman, and Haejin Kim
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11:45–12:45 **Monica Borghi** (*Assistant Professor – Utah State University, USA*)
Ellaine De Guzman (*Journal Specialist – Frontiers in Plant Science*)
Haejin Kim (*Genome Editing Scientist – Pairwise Plants*)

July 27 (Tuesday) Afternoon	Symposium 4: Natural products discovery, synthesis, and biosynthesis in the omics era Chair: Peter Constabel (<i>University of Victoria, Canada</i>)
13:30–13:55	Arthur Neish Award recipient: Soon Goo Lee (<i>University of North Carolina, Wilmington, USA</i>) Bitter and sweet: Molecular basis for branched steviol glucoside biosynthesis
13:55–14:20	Craig A. Schenck (<i>Michigan State University, USA</i>) Production of structurally diverse acylsugars through rapid screening of a promiscuous, yet specific, <i>Nicotiana acuminata</i> acylsugar pathway
14:20–14:30	[short break]
14:30–14:55	Isabel Desgagné-Penix (<i>University of Quebec, Trois-Rivières, Canada</i>) Cloning and characterization of norbelladine synthase and noroxomaritidine reductase, catalyzing the first key steps in Amaryllidaceae alkaloid metabolism
14:55–15:20	Elizabeth Mahood (<i>Cornell University, USA</i>) Leveraging integrative omics analyses for stress-responsive metabolic pathway elucidation in <i>Brachypodium</i>

July 27 (Tuesday) Evening	Poster session 1
15:30–17:00	Concurrent poster presentations (abstracts P1 to P29)

JULY 28 (WEDNESDAY)

July 28 (Wednesday) Morning	Symposium 5: Breakthrough approaches in elucidating phytochemical biosynthesis Chair: Thu-Thuy Dang (<i>University of British Columbia, Canada</i>)
9:00–9:50	Keynote speaker: Sarah E. O'Connor (<i>Max-Planck Institute of Chemical Ecology, Germany</i>) Harnessing the chemistry of plant natural product biosynthesis
9:50–10:00	[short break]
10:00–10:25	PSNA TPJ Young Investigator Award recipient: Lucas Busta (<i>University of Minnesota, USA</i>) A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry
10:25–10:50	Nishat S. Islam (<i>University of Western Ontario, Canada</i>) Postharvest darkening of seed coat and proanthocyanidin biosynthesis in pinto bean
10:50–11:00	[short break]
11:00–11:25	B. Mark Lange (<i>Washington State University, USA</i>) Mathematical models of plant metabolism: Awesome research tools for everything from gene discovery to complex network analysis
11:25–11:30	Lira Palmer (<i>Max-Planck Institute of Chemical Ecology, Germany</i>) <u>Flash talk</u> : Development of an <i>in vivo</i> method in <i>Nepeta cataria</i> (catnip) to characterize the biosynthetic pathway behind nepetalactone stereoisomer production
11:30–11:35	Hannah Parks (<i>Michigan State University, USA</i>)

11:35–11:40 Flash talk: Expanding the tropane alkaloid metabolic network: Metabolite discovery in *Atropa belladonna*
Radesh P. Nattamai Malli (*Brock University, Canada*)
Flash talk: *De novo* genome assembly, annotation and characterization of the essential oil plant *Lavandula angustifolia* (lavender)

July 28 (Wednesday) Noon **PSNA's Young Members Trivia Game**

12:00–13:00 PSNA's Young Members Trivia Game

July 28 (Wednesday) Afternoon **Symposium 6: Evolution of plant specialized metabolism**
Chair: Reinhard Jetter (*University of British Columbia, Canada*)

13:30–14:20 **Keynote speaker: Jing-Ke Weng** (*Massachusetts Institute of Technology, USA*)
 Mechanistic basis for metabolic evolution in plants

14:20–14:30 [short break]

14:30–14:55 **Yulin Sun** (*University of British Columbia, Canada*)
 Unravelling the biosynthesis of the very-long-chain β -diketones sealing barley (*Hordeum vulgare*) surfaces: A polyketide synthase condensing non-canonical substrates

14:55–15:20 **Jeff Y. Chen** (*University of Toronto, Canada*)
 Divergence of very-long-chain fatty acid substrate specificity in a recently duplicated gene cluster of poplar β -ketoacyl-CoA synthases

15:20–15:30 [short break]

15:30–15:55 **Narayanan Srividya** (*Washington State University, USA*)
 What determines if monoterpene synthases produce monocyclic or bicyclic products? An excursion into structure-function relationships

15:55–16:00 **Praveen Khatri** (*University of Western Ontario, Canada*)
Flash talk: Genome-wide identification of cytochrome P450 monooxygenases provide insight into their role in partial resistance against *Phytophthora sojae* in soybean (*Glycine max*)

16:00–16:05 **Lars Kruse** (*University of British Columbia, Canada*)
Flash talk: Large-scale comparative genomics and biochemical analyses reveal ancestral promiscuity as a driver of functional diversification of BAHD acyltransferases in plants

16:05–16:10 **Sonia Ehi-Eromosele** (*University of Toronto, Canada*)
Flash talk: Is the Entner-Doudoroff pathway functional in higher plants?

JULY 29 (THURSDAY)

July 29 (Thursday) Morning **Symposium 7: Enzymology and organization of plant metabolism**
Chair: Björn Hamberger (*Michigan State University, USA*)

9:00–9:25 **Arthur Neish Award recipient: Nitzan Shabek** (*University of California, Davis, USA*)
 Structural insights into strigolactone signaling regulation by the ubiquitin system

9:25–9:50 **Kristin Roach** (*Iowa State University, USA*)
 Structure-function investigation of class II diterpene cyclases

9:50–10:00 [short break]

10:00–10:25 **Jessica Sinka** (*University of Western Ontario, Canada*)
 Metabolic flux analysis during wound-healing in potato tubers

10:25–10:50	Paul D. Fiesel (<i>Michigan State University, USA</i>) Swapping sugars: The biochemical evolution of eggplant trichome defensive metabolites
10:50–11:00	[short break]
11:00–11:25	Shu Yu (<i>University of California, Davis, USA</i>) Mutant combinations of lycopene ϵ -cyclase and β -carotene hydroxylase 2 homoeologs increased β -carotene accumulation in endosperm of tetraploid wheat (<i>Triticum turgidum</i> L.) grains
11:25–11:30	Ayelign M. Adal (<i>University of British Columbia, Canada</i>) <u>Flash talk</u> : A monoterpene synthase controls bornyl diphosphate biosynthesis from <i>Lavandula x intermedia</i>
11:30–11:35	Jedrzejj Gozdziak (<i>University of British Columbia, Canada</i>) <u>Flash talk</u> : In-depth analysis of unusual very-long-chain ketones found in cuticular waxes of wild-type and mutant Welsh onion (<i>Allium fistulosum</i> L.)

July 29 (Thursday) Afternoon **Symposium 8: Phytochemistry of the forest, the far north, and underutilized plants**
Chair: **Dae-Kyun Ro** (*University of Calgary, Canada*)

13:30–14:20	Keynote speaker: Jörg Bohlmann (<i>University of British Columbia, Canada</i>) Oleoresin defenses in conifers: Chemical diversity and limitations under climate change
14:20–14:30	[short break]
14:30–14:55	Arthur Neish Award recipient: Rebecca Roston (<i>University of Nebraska, Lincoln, USA</i>) Cold tolerance of membranes is a matter of timing and metabolic state – not just a saturation story
14:55–15:20	Matthew E. Bergman (<i>University of Toronto, Canada</i>) Cytosolic geraniol and citronellol biosynthesis mediated by a Nudix hydrolase in <i>Pelargonium graveolens</i>

July 29 (Thursday) Evening **Poster session 2**

15:30–17:00	Concurrent poster presentations (abstracts P30 to P60)
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JULY 30 (FRIDAY)

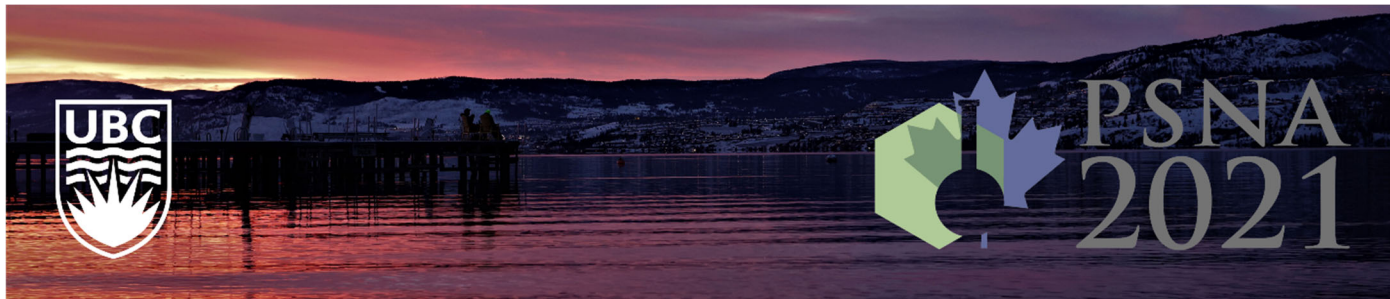
July 30 (Friday) Morning **Symposium 9: Biochemistry and production of high-value phytochemicals**
Chair: **Yang Qu** (*University of New Brunswick, Canada*)

9:00–9:50	Keynote speaker: Anastasios Melis (<i>University of California, Berkeley, USA</i>) Plant essential oils production in cyanobacteria
9:50–10:00	[short break]
10:00–10:25	Courtney P. Leisner (<i>Auburn University, USA</i>) Medicinal genomics: Exploring the diversity of iridoid compounds in blueberry for human health benefits
10:25–10:50	De-Yu Xie (<i>North Carolina State University, USA</i>) Anti-COVID-19 activities of flavan-3-ols in crops
10:50–11:00	[short break]
11:00–11:25	M. Soledade C. Pedras (<i>University of Saskatchewan, Canada</i>) Tropalexins A and B suggest evolutionary conservation of phytoalexin biosynthetic enzymes in Brassicales

- 11:25–11:30 **Sergio M. Espinoza** (*San Simón University, Bolivia*)
Flash talk: Proximate analysis and antioxidant capacity of the edible mushroom *Ustilago maydis* (Musuru) grown in creole maize varieties
- 11:30–11:35 **Yue Zhu** (*North Carolina State University, USA*)
Flash talk: Flavonols and dihydroflavonols inhibit the main protease activity of SARS-CoV-2

July 30 (Friday) Afternoon	Symposium 10: Synthetic biology and metabolic engineering of plant metabolism Chair: De-Yu Xie (<i>North Carolina State University, USA</i>)
13:15–13:30	Poster/Flash Talk Awards: Björn Hamberger (<i>Michigan State University, USA</i>)
13:30–14:20	Keynote speaker: Dae-Kyun Ro (<i>University of Calgary, Canada</i>) CRISPR/Cas9 in yeast engineering for producing plant specialized metabolites
14:20–15:10	Keynote speaker: Vincent Martin (<i>Concordia University, Canada</i>) Breakthrough technologies in metabolic engineering: a yeast tetrahydroisoquinoline platform
15:10–15:20	[short break]
15:20–15:45	Nathan D. Tivendale (<i>University of Western Australia, Australia</i>) Analysis of plant enzymes as consumable parts for synthetic biology
15:45–16:10	Nikola Micic (<i>University of Copenhagen, Denmark</i>) Glutathione conjugates: A tool to decipher glutathione transferase functions
16:10–16:20	Closing Remarks Dorothea Tholl – (<i>Virginia Polytechnic Institute and State University, USA</i>) – <i>Incoming President, Phytochemical Society of North America</i>

All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)



ORAL PRESENTATION ABSTRACTS

July 25 (Sunday)
Afternoon

Plenary session

15:30–16:45

Plant volatile emission: Beyond the traditional view

Natalia Dudareva

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Plants emit an amazing diversity of volatile organic compounds (VOCs) that play numerous roles in plant growth and development. Due to the plethora of biological processes dependent on VOCs, significant progress has been made towards understanding the biosynthesis of plant VOCs and their regulation. Much less is known about how VOCs move within and between cells and are released into environment. Until recently, it was presumed that VOCs simply diffuse out of cells. However, to achieve observed emission rates by diffusion alone VOCs have to accumulate to toxic levels in membranes, in which they preferentially partition due to their lipophilic nature. The presentation will cover different aspects of VOC emission: from involvement of transporters in VOC trafficking across the plasma membrane to the role of cuticle as an integral member of the overall VOC biosynthetic network.

JULY 26 (MONDAY)

July 26 (Monday)
Morning

Symposium 1: New advances in integrated *omics* and phytochemistry research

9:00–9:50

Keynote:

Advances in phytochemistry achieved through integrated metabolomics

Vered Tzin, Bonnie Watson, Dong Sik Yang, John H. Snyder, Zhentian Lei, David V. Huhman, Stacy Allen, Yuhong Tang, Derek Nedveck, John Stanton-Geddes, Peter Tiffin, Nevin Young, Rajarshi Ghosh, Feng Qiu, Anil Bhatia, Dennis Fine, Daniel Wherritt, Lloyd W. Sumner

Metabolomics Center, University of Missouri, Columbia, USA

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Metabolomics is significantly advancing our understanding of biological systems. This presentation will illustrate how we are using integrated metabolomics for the discovery and characterization of specialized metabolic pathways in the model plant legume, *Medicago truncatula*. The presentation will introduce our basic metabolomics technologies and the biosynthesis pathway of triterpene saponins. The presentation will further describe how genetic and metabolic diversity are being exploited for novel gene discovery. Multiple gene discoveries obtained through correlated gene expression analysis with metabolomics data as well as genome wide association studies will be presented. The presentation will then describe the molecular and biochemical evidence used for the functional characterization of the novel genes. Although metabolomics is currently advancing our understanding of metabolism, its full scientific promise has not been realized due to multiple grand challenges. These grand challenges include the confident identification of all observed metabolites as well as greater depth-of-coverage. This presentation will also introduce an ensemble of advanced instrumentation; i.e. UHPLC-MS-SPE-NMR, that has been developed to address these grand challenges and applied to elucidating saponin biosynthesis.

10:00–10:25

Omic approaches to unravel complex alkaloid biosynthesis in *Daphniphyllum*

PSNA 2021

All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)

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Benjamin Lichman

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Plants from the genus *Daphniphyllum* produce a variety of unique alkaloids of remarkable complexity. Over 320 *Daphniphyllum* alkaloids (DA) have been identified, with over 30 different carbon skeletal forms all featuring multiple fused rings and stereogenic quaternary carbon centres. Applications of these compounds has been not been widely investigated, though the use of the plant in traditional medicine and reports of bioactivities in isolated compounds indicate DAs may have pharmaceutical potential. In addition, enzymes and pathways capable of forming complex structures may have wider applications in biochemical synthesis. To understand the biosynthetic origins of DAs, we have performed metabolomics analysis on multiple tissues from different plants, 177 samples in total. Through a combination of clustering, annotation and feature based molecular networking, we have identified subtypes of alkaloids that appear to be differentially abundant across the dataset. Furthermore, mass spectrometry imaging indicates that these structural subtypes are differentially localised within tissues. Together this indicates that DA metabolism is subdivided into pathways that are under different regulatory and/or spatial controls. Next, to discover genes related to DA biosynthesis, we sequenced a selection of samples using a combination of Nanopore and Illumina technologies, and performed a *de novo* transcriptome assembly. Gene expression data analysis using a network approach was integrated with metabolomics data to reveal gene modules and specific transcripts that correlate closely with DA accumulation. This work represents the first multi-omics investigation into a neglected alkaloid class and sets the stage for the discovery of new enzymes involved in the biosynthesis of *Daphniphyllum* alkaloids.

10:25–10:50

Comparative metabolomics of fruits and leaves in a hyper diverse lineage suggests fruits are a key incubator of phytochemical diversification

Gerald F. Schneider^{1,2}, Diego Salazar³, Sherry B. Hildreth^{1,4}, Richard F. Helm⁴, Susan R. Whitehead¹

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²Department of Biology, Utah State University, Logan, USA

³International Center for Tropical Botany, Florida International University, Miami, USA

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Interactions between plants and leaf herbivores have long been implicated as the major driver of plant secondary metabolite diversity. However, other plant-animal interactions, such as those between fruits and frugivores, may also be involved in phytochemical diversification. Using 12 species of *Piper*, we conducted untargeted metabolomics and molecular networking with extracts of fruits and leaves. We evaluated organ-specific secondary metabolite composition and compared multiple dimensions of phytochemical diversity across organs, including richness, structural complexity, and variability across samples at multiple scales within and across species. Plant organ identity significantly influenced secondary metabolite composition, both independent of and in interaction with species identity. Leaves and fruit shared a majority of compounds, but fruits contained more unique compounds and had higher total estimated chemical richness. While organ-level chemical richness and structural complexity varied substantially across species, fruit diversity exceeded leaf diversity in more species than the reverse. Furthermore, the variance in chemical composition across samples was higher for fruits than leaves. By documenting a broad pattern of high phytochemical diversity in fruits relative to leaves, this study lays groundwork for incorporating fruit into a comprehensive and integrative understanding of the ecological and evolutionary factors shaping secondary metabolite composition at the whole-plant level.

11:00–11:25

The genome of medicinal plant *Scutellaria barbata* and the evolution of clerodane pathway

Haixiu Li¹, Song Wu^{1,2}, Ruoxi Lin¹, Ana Rita Almeida Pinheiro^{1,2}, Cathie Martin³, Evangelos Tatsis^{1,4}

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²University of the Chinese Academy of Sciences, Shanghai, China

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Scutellaria L., also known as the skullcaps, is the second largest genus in Lamiaceae family with more than 300 species. Skullcaps have been used extensively for centuries in applications in traditional Chinese medicine (TCM). *Scutellaria baicalensis* Georgi is a famous medicinal plant widely used in TCM, mainly due its bioactive 4' deoxyflavones which are produced at high levels, specifically from its roots (*huang qin*). *Scutellaria barbata* D. Don, is used extensively in TCM treatments of cancers; particularly in the advanced metastatic cancers, where its efficacy at reducing cancer progression as

well as an absence of harmful side effects, has resulted in renewed interest in using this traditional prescription (*ban-zhi-lian*) as therapy complimentary to Western chemotherapies.

The aerial parts of *S. barbata*, mainly used for TCM decoctions, are rich in clerodane diterpenoids. Recently, scutebarbatine A, the major clerodane diterpenoid identified from *S. barbata* extracts, was found to induce tumor-selective cytotoxicity by taking the brakes off apoptosis; suggesting that the chemotherapeutic bioactivities of *S. barbata* extracts could be enhanced. Despite the numerous phytochemical works on diterpenoid content of *S. barbata* and other *Scutellaria* species, there is a large knowledge gap regarding the medicinal properties of those compounds and genetic information on how, and why they are synthesized by the plant.

In order to elucidate the biosynthesis of clerodane diterpenoids we sequence the genome of *S. barbata*. Mapping the diterpene metabolism by functional characterizing the diterpene synthases from *S. barbata*, *S. baicalensis* and *Salvia splendens* we identified three basic diterpene metabolic pathways leading to giberrelins, abietane and clerodane diterpenoids. Biochemical activity paired with extensive phylogeny and comparative genomics revealed the origin and evolution of class II and class I clerodane synthases in species of Lamiaceae family.

11:25–11:30

Flash talk: Unravelling iridoid production in blueberry: using omics to investigate plant natural products for human health

Lovely Mae F. Lawas, Courtney P. Leisner

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Fruit crops provide humans with essential nutrients and are sources of natural products that have diverse biological activities. Among these are blueberries, which produce the specialized metabolite iridoids that have health-promoting benefits. Monotropein, an iridoid glycoside known to have anti-inflammatory and antinociceptive properties in other plants, has been detected in several wild *Vaccinium* species but in only a small number of cultivated highbush blueberries. It is not known however, how iridoids are produced in blueberry, nor the molecular mechanism behind differential iridoid production among accessions. To address these knowledge gaps, RNA-Seq was performed on a tissue diversity panel of monotropein-positive (Ornablu) and monotropein-negative (Concord) accessions. The derived transcriptome assemblies allowed the identification of candidate genes using orthology analysis with genes known to be involved in iridoid biosynthesis in *Catharanthus roseus*, a medicinal plant species wherein the iridoid pathway has been well studied. Orthologs for iridoid synthase (ISY), an enzyme in a key step in the iridoid biosynthetic pathway, have been identified in both Ornablu and Concord transcriptomes. Bioinformatically-derived sequences of the two blueberry ISY were used to obtain synthetic ISY genes and expressed in *E. coli*. The ISY proteins were purified and used for in vitro enzyme assay with 8-oxogeraniol as substrate. Analysis of the organic phase of the resulting reactions by gas chromatography-mass spectrometry showed the detection of nepetalactol, which is the expected product in the enzymatic reaction. This implies that the putative blueberry ISY sequences from Ornablu and Concord produce a functional protein that catalyzes the formation of iridoids. Other key enzymes in the biosynthetic pathway will also be functionally characterized with the aim of determining the differentiating step between monotropein-producing and non-producing accessions. This new finding paves the way to understanding iridoid biosynthesis in blueberry that could open up opportunities for increasing its health benefits to humans.

11:30–11:35

Flash talk: The beautiful lady's secret: Untargeted metabolomics uncovers the roles of cytochromes P450 in a pseudotropine-dependent branch of modified tropane alkaloid biosynthesis

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The Solanaceae family represents an invaluable source of bioactive phytoalkaloids including hyoscyamine and scopolamine, anticholinergic and antispasmodic tropane alkaloids. However, the diversity and biosynthesis of modified tropane alkaloids remains underexplored in Solanaceae preventing systematic advances for biotechnological production of novel medicinal alkaloids. In plants, the cytochrome P450 (CYP) superfamily catalyzes a wide range of chemical modifications and is a major driving force for the structural diversification of alkaloids. The functional characterization of plant CYPs is challenging as most substrates are unknown and the production of these membrane-bound enzymes is often incompatible with microbial hosts. The *Atropa belladonna* (Solanaceae family) transcriptome encodes approximately 200 unique transcripts for CYPs of which many are produced in root, the site of tropane-dependent scopolamine biosynthesis. Using *A. belladonna* as a model system

for Solanaceae, we combined virus-induced gene silencing with UHPLC/MS-based untargeted metabolomics and multivariate statistical analysis to investigate the roles of candidate enzymes. This metabolomics-based approach identified two root-produced CYPs that are essential for the production of highly diverse hydroxylated and/or esterified *N*-demethylated nortropane alkaloids in a largely unknown, pseudotropine-dependent branch of alkaloid biosynthesis. The comparative analyses of the metabolic changes in the roots of gene-silenced vs. control *A. belladonna* lines discovered more than 40 modified tropane alkaloids and allowed predicting the distinct roles of the CYPs. To produce and assay the CYPs, we established an *in planta* transient biochemical assay system using *Nicotiana benthamiana* as host and screened numerous candidate substrates. *N. benthamiana* is a member of the Solanaceae family that, in contrast to *A. belladonna*, does not produce modified tropane alkaloids. These assays confirmed the CYPs catalyze early sequential reaction steps in the biosynthesis of modified tropane alkaloids. Our study demonstrates the power of this metabolomics-guided approach for enzyme discovery when knowledge of the biosynthetic pathways and intermediate identities is extremely limited.

11:35–11:40

Flash talk: Application of genome wide association in *Arabidopsis* leads to the functional classification of a new BAHD acyltransferase

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The identification of plant natural products and the genes required for their biosynthesis can be achieved by exploring natural metabolic and genetic diversity within a plant species. In this study, variation in leaf and stem metabolites from over 400 *Arabidopsis thaliana* natural accessions were associated to 1.5 million single nucleotide polymorphisms among the population. Nucleotide diversity within an uncharacterized BAHD-family acyltransferase gene that has the highest sequence similarity to hydroxycinnamoyl-CoA shikimate hydroxycinnamoyltransferase (HCT), a core enzyme in phenylpropanoid and lignin biosynthesis, exhibited strong associations to three previously undescribed leaf and stem metabolites. MS/MS and isotopic labeling identified these metabolites as glucuronosyl glycerol esters of various acids, including phenylalanine-derived phenylacetic acid. Analysis of T-DNA knockout lines and overexpression of this BAHD acyltransferase in three different *Arabidopsis* accessions and *Nicotiana benthamiana* verified that it is necessary and sufficient for the production of the metabolites. In addition, overexpression of two different gene products identified from *Arabidopsis* accessions that make varying amounts of the metabolites revealed different capacities of the two genes to ectopically produce the metabolites. This BAHD gene is a Brassicaceae-specific homolog to HCT; however, the metabolites it influences in *Arabidopsis* are detectable in multiple plants species, including sorghum and maize. Together this study demonstrates how genome wide association can be leveraged to identify novel gene functions and metabolites in plants.

July 26 (Monday)
Afternoon

Symposium 2: Chemistry and biochemistry of therapeutic plants

13:30–14:20

Keynote:

Green machine or tropical breeze: What's in a name when talking cannabis chemistry & research

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Along with the incredibly diverse array of cannabis-based products available to Canadians for medical and recreational use, there is a plethora of anecdotal information suggesting different "types" or "strains" produce different effects. However, after a long history of informal breeding programs the term "strain" does not reflect distinct cultivated varieties and many products are chemotaxonomically indistinguishable. Although a pathway for access to cannabis for medical purposes has existed in Canada since 1999, products were not subjected to Health Canada's traditional drug approval process. Under the *Cannabis Act*, which came into force on October 17, 2018, the consumption and sale of cannabis for non-medical use was legalized. These regulations also established a framework for drugs containing cannabis; exempt from certain provisions of the *Cannabis Act* but compliant with the rules for drugs. While legalization has provided a much-needed opportunity to better explore relationships between cannabis phytochemistry and potential therapeutic benefits, there remains a disconnect between requirements to legally produce medical cannabis and requirements to conduct clinical research. The result is medical cannabis products already being bought and consumed by Canadians are not deemed suitable for clinical study. Suggested solutions in an open letter to the Government of

Canada by Canada's cannabis health research community include provision of an omnibus product approval for existing medical cannabis products or creation of a single-source contract to produce products. From a phytochemical perspective both suggestions are problematic given that products are classified based on delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) content, yet products with similar THC/CBD profiles can exhibit significantly different pharmacological effects. It is well-established that phytochemical characterization and quality control are key factors for translating clinical trials outcomes. While targeting specific compounds or chemical classes can yield invaluable information, only a fraction of the chemical information is captured. The combination of targeted and untargeted chemometric approaches can provide more comprehensive phytochemical characterizations, elucidating relationships across plant metabolites, and potentially improving clinical research outcomes.

14:30–14:55

Application of molecularly imprinted polymers (MIPs) as recognition elements in a liquid-phase electrochemical sensor for selective detection of a psychoactive substance, Δ^9 -tetrahydrocannabinol (THC)

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Cannabis is widely used in North America as a recreational and medicinal drug. The primary substance responsible for the psychoactive effects of this plant is Δ^9 -tetrahydrocannabinol (THC). Selective detection of THC in the liquid phase is important because of its presence in body fluids after use and the complex nature of the liquid samples containing THC and other different analytes. Molecularly imprinted polymers (MIPs) can improve the selectivity of sensors by using them as recognition elements. This ability of MIPs originates from the presence of recognition cavities which are complementary in size, shape, and functional groups to the template which is the same as the target analyte for detection. These cavities are created after the extraction of the target template from the polymer synthesized in the presence of both a monomer and the template. In the present study, we developed an MIP-based electrochemical sensor for the detection of THC in methanol. The polymer was synthesized as a thin film on a gold interdigitated electrode using pyrrole and THC as monomer and template, respectively. This synthesis was done using the electropolymerization technique with the cyclic voltammetry (CV) method (-0.2 to 0.8 V, 6 cycles at a scan rate of 50 mV/s). The THC template was extracted by keeping the electrode in a solution of acetic acid and methanol for approximately 2 hours while shaking slowly. The resulting MIP-based electrochemical sensor was tested using the electrochemical impedance spectroscopy (EIS) technique. It showed remarkable responses when it was exposed to a solution of THC in methanol, with no considerable response towards pure methanol, thus, demonstrating the efficacy of the sensor for the selective detection of THC in solution. The developed sensor can be applied for the selective detection of different cannabinoids present in cannabis extracts for purity and quality control measurements.

14:55–15:20

A novel anti-diabetic compound from plants: Biosynthesis, gene discovery, and metabolic engineering of montbretin A

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Plant metabolites have been employed by humans for millennia in traditional and modern medicine, and they remain an important source for the discovery of novel pharmaceuticals. The plant metabolite montbretin A (MbA) is being developed as a novel therapeutic for type 2 diabetes and obesity, two diseases that are affecting human health worldwide at epidemic levels. MbA is a complex acylated flavonol glycoside and its only known source are the belowground storage organs (corms) of the ornamental plant montbretia (*Crocsmia x crocosmiiflora*). Due to its low abundance *in planta* and its complex chemical structure, field cultivation and chemical synthesis, respectively, are not feasible to provide sufficient amounts for drug development and application. Our goal is to develop a scalable and sustainable production system for MbA through metabolic engineering of a microbial or plant production system. However, this first requires fundamental knowledge of the MbA biosynthetic system. We elucidated the complete MbA biosynthetic pathway using an approach that combined montbretia biology, metabolite profiling, transcriptomics, molecular biology and biochemistry. MbA biosynthesis occurs in corms during a narrow window of time during seasonal young corm development. Metabolite

profiling and whole protein activity data allowed us to reconstruct the linear, step-wise assembly sequence of MbA. Using differential transcriptome analysis and heterologous enzyme expression in *E. coli* and *N. benthamiana* we were able to identify and characterize five UDP-sugar dependent glycosyltransferases and a BAHD-acyltransferase which together catalyze the complete MbA biosynthesis. This fundamental knowledge serves as the foundation for engineering approaches. We successfully reconstructed MbA biosynthesis in *N. benthamiana* by transiently expressing MbA biosynthetic enzymes and modifying flavonoid metabolism. In addition, work on reconstructing MbA biosynthesis in the yeast *Saccharomyces cerevisiae* is in progress. This demonstrates the potential of MbA metabolic engineering and production in a heterologous system.

15:30–15:55

Elucidating the Taxol biosynthetic pathway and engineering taxadiene synthase for improved product specificity

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Plant terpenoids are a diverse group of natural products with important uses in the pharmaceutical and bioproduct industries. Yet, these metabolites are difficult to obtain through isolation from the natural source or through complex chemical synthesis. Among these compounds, Taxol is a leading chemotherapy drug with a multi-billion-dollar industry. Current production of Taxol utilizes complex and expensive semi-synthesis and cell culture techniques, resulting in the drug being price-prohibitive for many people around the globe, particularly in impoverished countries. This project aims to develop microbial pathway engineering approaches for improving the scalable and sustainable production of Taxol.

Key challenges in microbial Taxol production include, firstly, that the complete biosynthetic pathway has not yet been fully elucidated; to this date, only 11 of the 17 genes required for full biosynthesis have been discovered and characterized. Secondly, the enzyme catalyzing the first committed step in the pathway, taxadiene synthase (TXS) has relatively low catalytic efficiency and yields multiple side products. To address these limitations, we used transcriptome mining to identify additional biosynthetic genes and enzymes as well as variants of known pathway enzymes. Using this approach, several cytochrome P450 enzyme candidates have been identified that are predicted to facilitate positionspecific oxygenations on the taxadiene scaffold. In addition, structure-guided site-directed as well as directed evolution approaches are being applied to generate TXS variants with improved catalytic properties. Here, site-directed mutagenesis of active residues revealed key active site residues (Y688, Y684, Q609) effect TXS product specificity.

15:55–16:00

Flash talk: Untargeted metabolite assessment reveals biorefinery opportunities from wastewater treatment using willow phytoremediation

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Conventional municipal wastewater treatment represents a worldwide economic and environmental burden which is driving exploration of alternative biotechnologies, such as the use of fast-growing willow phytoremediation plantations. These plantations can simultaneously generate sustainable biomass and phytochemicals for green biorefinery; however, the impact of wastewater irrigation on the willow phytochemicals is yet to be explored. A one-hectare field trial was established in Quebec (Canada) where replicated blocks of *Salix miyabeana* 'SX67' compared trees left unirrigated with those irrigated with primary effluent wastewater at around 29,500,000 L ha⁻¹ yr⁻¹ over three years. In addition to sustainable biomass yields, biomass composition and bioenergy conversion yields, persistent methanol-extracted phytochemicals were compared using untargeted LC-MS/MS with an annotation pipeline including MS/MS fragment clustering, *in silico* formula, structure and chemical class prediction, as well as comparison to Salicaceae and public metabolite libraries. Biomass production was substantially improved by wastewater treatment, increasing by 200%, while the influence on structural carbohydrates, lignin content, as well as on ionic liquid pretreatment, enzymatic saccharification and lignin recovery yields were minor. From a total of 213 consistently detected phytochemical features, 83

were significantly depleted and 14 were significantly enriched due to wastewater irrigation, six lignans and flavonolignans, including salcolin, quiquelin and ramontoside, and three flavonoids, including glabraoside. Although untargeted metabolite assessment in non-model crops is challenging due to the high number of yet-to-be-characterised compounds, it is powerful in revealing the underlying biological mechanisms of abiotic stress tolerance. These findings are both biologically intriguing, representing the phytochemical toolkit of wastewater tolerance and a high yield phenotype in a non-model species, as well as important from a biorefinery perspective, by revealing added-value phytochemicals induced in a bioenergy crop. Integrating biorefinery opportunities with environmental wastewater treatment could potentially help improve the economic feasibility of this clean biotechnology.

16:00–16:05

Flash talk: *Alternanthera flava* prevents acute liver damage induced by CCl₄ in rat

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Liver failure reflects a disruption of many normal processes, such as protein synthesis, glucose formation, glycogen storage and ammonium detoxification. This research utilizes a Mexican medicinal plant and provides an opportunity to develop new drugs to help control liver diseases. *Alternanthera flava* (*A. flava*) is a species belonging to the *Amaranthaceae* family whose species have shown antioxidant and hepatoprotective properties. The study is mainly focused on the histopathological evaluation and hepatoprotective activity of the ethanolic extract of *A. flava* in acute liver injury induced by CCl₄ in Wistar rats. For this purpose, the division of 4 groups was performed: Control group (rats administrated with mineral oil, which is vehicle of CCl₄), CCl₄ group (a single dose of 1.6 g/kg i.p.), CCl₄ + *A. flava* group (50 mg/kg, p.o. of *A. flava* was daily administered for 3 days prior CCl₄ intoxication and *A. flava* Group (rats administered with mineral oil and ethanolic extract of *A. flava*). Macroscopically the livers of CCl₄ + *A. flava* showed morphological characteristics like healthy groups (Control and *A. flava* groups), unlike the CCl₄ group. Microscopic analysis by Hematoxylin and eosin and periodic acid-Schiff stains showed less necrosis and inflammatory infiltrate in comparison with livers of CCl₄ group. Macroscopic and microscopic observation were consistent with serum markers of liver damage (alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase). Alanine aminotransferase activity was significantly decreased, and alkaline phosphatase and gamma-glutamyl transpeptidase increases were completely prevented. Therefore, *A. flava* is a potential treatment for prevention of acute liver damage.

16:05–16:10

Flash talk: Completion of the vindoline and catharanthine pathways in *Catharanthus roseus* facilitates characterization of MIA pathways in *Ochrosia elliptica*

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Monoterpenoid indole alkaloids (MIAs) produced by members of the Apocynaceae family display valuable pharmacological properties, such as the dimeric anticancer MIA vinblastine, which is produced from catharanthine and vindoline in *Catharanthus roseus*, and the antiarrhythmic MIA ajmaline, which is produced in *Rauwolfia serpentina*. Catharanthine, vindoline, and ajmaline are derived from strictosidine, which is the central intermediate in MIA biosynthesis. The biosynthetic pathways leading to catharanthine and vindoline formation in *C. roseus* were recently completed, and most genes involved in ajmaline biosynthesis in *R. serpentina* have been characterized. These pathways now provide a foundation to facilitate discovery of homologous genes involved in MIA biosynthesis in other species. *Ochrosia elliptica* produces a wide array of MIAs including ellipticine and apparicine, which possess anticancer and analgesic activities, respectively, and which may be derived from stemmadenine, a common intermediate in catharanthine and vindoline biosynthesis. To identify potential gene candidates for MIA biosynthesis, the leaf transcriptome of *O. elliptica* was assembled *de novo* from 184 million trimmed Illumina reads into 365 339 predicted coding sequences using the Trinity pipeline on the European Galaxy server. Further clustering of similar peptide sequences with CD-HIT-PROTEIN yielded 48 935 sequences with an average length of 292 amino acids, and removal of peptides shorter than 200 amino acids yielded 25 102 sequences with an average length of 439 amino acids. Querying the assembled transcriptome with known MIA biosynthetic genes indicated that *O. elliptica* possesses orthologues with ≥70% amino acid identity to 13 out of 15 genes involved in the assembly of stemmadenine, which comprises secologanin, tryptamine, strictosidine, and stemmadenine biosynthesis. All thirteen putative orthologues encoded full-length open reading frames. These

promising results suggest that the annotated *O. elliptica* transcriptome will be a vital resource for identifying gene candidates required for the assembly of MIAs in *O. elliptica*.

JULY 27 (TUESDAY)

July 27 (Tuesday)
Morning

Symposium 3: Chemical ecology

9:00–9:50

Keynote:

Digging into rice diterpenoid biosynthesis

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Rice (*Oryza sativa*) is an important crop plant that produces numerous diterpenoids. My group has taken a systematic approach towards elucidating both the underlying metabolic network and biological activity of these natural products. In particular, our elucidation of the early steps in rice diterpenoid metabolism has enabled a reverse genetic approach towards investigation of physiological relevance, which has begun to reveal surprising complexity in the impact of these natural products in not only microbial disease resistance but also regulation of more basic aspects of plant physiology such as stomatal opening as well as genome organization. Our most recent results will be discussed.

10:00–10:25

Arthur Neish Award presentation: Uncovering regulatory mechanisms of salicylic acid biosynthesis for plant immunity in *Arabidopsis* and Brassicaceae oilseed crops

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Brassicaceae oilseed plants such as *Brassica napus* and *Camelina sativa* are outstanding sources for food, feedstock, and biofuels. Especially, canola oil derived from *B. napus* cultivars with reduced levels of erucic acid and glucosinolate is popular for both food and biodiesel. However, *B. napus* is highly susceptible to various pathogens including fungal pathogen *Leptosphaeria maculans*. While *C. sativa* is generally more resistant to various pathogens than *B. napus*, underlying mechanism of contrasting disease responses is largely unknown. Here, we discovered that *B. napus* has distinct transcriptional regulatory mechanisms of salicylic acid (SA) biosynthesis from those in *C. sativa* and *Arabidopsis thaliana* (*Arabidopsis*). SA is a plant defense hormone controlling immune responses in both local infected tissue and uninfected systemic tissue. SA can be synthesized by two pathways, the isochorismate synthase 1 (ICS1) pathway and the phenylalanine ammonia lyase (PAL) pathway. In the well-studied Brassicaceae family model species *Arabidopsis*, SA is mainly synthesized via ICS1 pathway with transcriptional regulation of *ICS1* as a key regulatory mechanism. Preliminary results show that *C. sativa* *ICS1* expression is induced to control immune mechanism for SA biosynthesis in systemic tissue similar to *Arabidopsis*, while *B. napus* *ICS1* expression is not induced in response to pathogen infection in systemic tissue. This contrasting regulation of SA biosynthesis is suggested to explain more disease susceptibility in *B. napus*. Our research aims to elucidate molecular mechanisms for distinct transcriptional regulation of SA biosynthesis in *B. napus*, *C. sativa*, and *Arabidopsis* in response to various pathogens to uncover the links between SA biosynthesis and pathogen resistance in oilseed crops. Understanding conserved or specific regulatory mechanisms in diverse plant species will provide novel and effective genetic engineering strategies to improve disease resistance while minimizing fitness costs.

10:25–10:50

Above and belowground dynamics of terpene volatiles in switchgrass

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Many grasses are known to synthesize terpene specialized metabolites; but the formation of these compounds has been largely studied in domesticated species. Switchgrass (*Panicum virgatum*), a perennial warm season prairie grass, represents an important species in natural and anthropogenic grasslands of North America. Despite its resilience to abiotic and biotic stresses, still little is known about the role of terpenes in switchgrass resistance to pathogens and herbivores. Many genes of the switchgrass terpene synthase (TPS) family are expressed constitutively in above- and belowground organs. However, volatile terpenes are largely emitted only upon herbivory and stress hormone treatment suggesting the existence of downstream pathways to non-volatile derivatives involved in defense. This excludes the two monoterpenes borneol and camphor, which accumulate at high levels under non-stress conditions in roots of many switchgrass accessions. We found that exposure of switchgrass roots to nitrogen fixing isolates and rhizobacterial communities stimulates and stabilizes borneol accumulation. We further investigated possible effects of borneol on root colonizing bacteria in the switchgrass related grass *Setaria viridis*. TPS04 silenced lines did not show significant differences in root bacterial colonization in comparison to wild type. It is possible that borneol functions mostly as an antifungal defense as indicated by *in vitro* assays. These findings support a model of microbe-stimulated volatile accumulation for belowground defense.

11:00–11:25

The enigmatic nature of poison ivy urushiol chemical ecology

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Based on human allergic dermatitis symptoms resulting from contact with poison ivy urushiol, it is a foregone conclusion that urushiol is a plant chemical defense. However, humans are an invasive species within the native range of poison ivy, and thus not a co-evolved target of a urushiol chemical defense. In the scientific literature, there are no reports of native (or domesticated) fauna that show comparable urushiol-induced allergic dermatitis symptoms. Attempts to develop clinical animal models do not adequately recapitulate human allergic dermatitis symptoms/pathology. We performed a variety of observational and manipulated field and laboratory studies to gain insights about the ecological role of urushiol and poison ivy. Using a trail-transect sampling approach in SW Virginia, poison ivy is mostly present under forest canopy as ground crawling lianas with ramets. However, the probability of observing poison ivy lianas greatly increases in both agricultural and low density human land use categories, indicating poison ivy prefers disturbed landscapes typical of forest edges. In establishment experiments, 91% of germinated poison ivy seedlings quickly disappeared due to presumed browsing. Exclosure experiments demonstrate that large fauna (i.e. white tailed deer) had a significantly greater impact on poison ivy plant height and biomass compared to either small fauna exclosure or insecticide treatment. Regression analysis on naturally-recruited climbing liana leaves demonstrated there was no significant correlation between leaf insect herbivory and foliar urushiol levels. Along the same lines, axenic grown poison ivy seedlings treated with methyl jasmonate had comparable urushiol levels to ethanol-treated plants. Poison ivy is a woody liana, and urushiol accumulates in specialized resin canals/ducts as an apparent preformed chemical defense against browsing fauna. Constitutive plant chemical defenses are typically an eco-evolutionary strategy against either frequent herbivory or herbivory from large fauna. However, the identity of such posited extant urushiol-susceptible native North American fauna remains enigmatic.

11:25–11:30

Flash talk: Differential regulation of the ribosomal association of mRNA transcripts in an *Arabidopsis* mutant defective in jasmonate-dependent wound response

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(+)-7-iso-Jasmonoyl-L-isoleucine (JA-Ile) is a powerful oxylipin responsible for the genome-wide transcriptional re-programming in plants that results in major physiological shifts from growth to defense. The double T-DNA insertion *Arabidopsis* mutant, *cyp94b1cyp94b3* (*b1b3*), defective in cytochrome p450s, CYP94B1 and CYP94B3, which are responsible for oxidizing JA-Ile, accumulates several fold higher levels of JA-Ile yet displays dampened JA-Ile-dependent wound responses – the opposite of what is expected. Transcriptomic and proteomic analyses showed that while the transcriptional response to wounding was largely unchanged in *b1b3* compared to wild-type (WT), many proteins were found to be significantly reduced in the mutant. To understand this protein phenotype and their hypothesized contribution to the *b1b3* phenotypes, wounded rosette leaf samples from both WT and *b1b3* were subject to a translating ribosome affinity purification RNA sequencing (TRAP-Seq) analysis. Over 1,600 genes whose transcripts do not change in abundance by wounding changed their

association with the ribosomes after wounding in WT leaves. The total pool of mRNA transcripts was similar between WT and *b1b3*, however, the ribosome-associated pool of transcripts was changed significantly. Most notably, fewer transcripts were associated with the ribosome pool in *b1b3* than in WT potentially explaining the reduction of many proteins in the mutant. Among those genes with fewer ribosome-associated transcripts in *b1b3* were genes relating to stress response, specialized metabolism, protein metabolism, ribosomal subunits, and transcription factors, consistent with the biochemical phenotypes of the mutant. These results show previously unrecognized regulations at the translational level that are affected by mis-regulation of JA homeostasis during the wound response in plants.

11:30–11:35

Flash talk: Synthesis of deoxynivalenol metabolites for use as analytical standards

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Fusarium graminearum is fungal species capable of infecting maize and wheat throughout the world; it is responsible for producing a few known mycotoxins, including a particular toxin class of concern is deoxynivalenol (DON). DON is commonly detected as 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) in grain and maize, and it has been linked to health concerns in mammalian systems such as diarrhea, vomiting, gastro-intestinal inflation, and intestinal necrosis. As such its detection and consequent monitoring is of growing importance. DON can also exist in other conjugated forms, which are produced as part of the host plants defense mechanism, namely 15-ADON-3-glucoside, and DON-3-glucoside. These conjugated products are difficult to monitor for and can lead to an underreporting of the mycotoxin exposure in maize and wheat products. After consumption of DON, mammalian metabolism converts it to the less toxic DON-3-glucuronide, which is the key biomarker of DON exposure. Mass spectrometry is an effective tool to quantify these DON derivatives; however, chemical standards of these compounds are not commercially available, especially those labelled with stable isotopes. Our group therefore pursued the synthesis of both labelled and unlabelled versions of 15-ADON3G and DON3-glucuronide to facilitate the mass spectral analysis of DON in crops and for human exposure studies. We used recombinant enzyme catalysed semi-synthesis and a modified könings-knorr reaction to produce the glucoside and glucuronide standards respectively. DON-glucuronide standards were produced and validated for the liquid chromatography high-resolution mass spectrometry monitoring of DON exposure in a cohort of Rwandan women. The recombinant enzyme Os79 derived from *oryza sativa* was used to conjugate DON and 15ADON to produce DON-3-glucoside and 15ADON-3-glucoside. These conjugated standards will allow for the accurate quantification of total DON concentrations in crops and exposures in human populations.

July 27 (Tuesday)
Afternoon

Symposium 4: Natural products discovery, synthesis, and biosynthesis in the omics era

13:30–13:55

Arthur Neish Award presentation: Bitter and sweet: Molecular basis for branched steviol glucoside biosynthesis

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Steviol glucosides, such as stevioside and rebaudioside A, are natural products roughly 200-fold sweeter than sugar and are used as natural, noncaloric sweeteners. Biosynthesis of rebaudioside A, and other related stevia glucosides, involves formation of the steviol diterpenoid followed by a series of glycosylations catalyzed by uridine diphosphate (UDP)-dependent glucosyltransferases. UGT76G1 from *Stevia rebaudiana* catalyzes the formation of the branched-chain glucoside that defines the stevia molecule and is critical for its high-intensity sweetness. Here, we report the 3D structure of the UDP-glucosyltransferase UGT76G1, including a complex of the protein with UDP and rebaudioside A bound in the active site. The X-ray crystal structure and biochemical analysis of site-directed mutants identifies a catalytic histidine and how the acceptor site of UGT76G1 achieves regioselectivity for branched-glucoside synthesis. The active site accommodates a two-glucosyl side chain and provides a site for addition of a third sugar molecule to the C3' position of the first C13 sugar group of stevioside. This

structure provides insight on the glycosylation of other naturally occurring sweeteners, such as the mogrosides from monk fruit, and a possible template for engineering of steviol biosynthesis.

13:55–14:20

Production of structurally diverse acylsugars through rapid screening of a promiscuous, yet specific, *Nicotiana acuminata* acylsugar pathway

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Acylsugars are defensive, trichome-synthesized sugar esters produced across the Solanaceae family. Although built from simple core metabolites and synthesized by a relatively short biosynthetic pathway, tremendous acylsugar structural variation exists. To advance our understanding of the synthesis of acylsugar diversity, acylsugars were profiled across the *Nicotiana* genus coupled to transcriptomics-guided pathway discovery and in vitro acylsugar network reconstruction. Differences in the types of sugar cores, numbers of acylations, and acyl chain structural diversity contributed to over 200 different acylsugar structures annotated throughout *Nicotiana*. Acyl chain length varied from 2-8 carbons and placement into a phylogenetic context revealed that while most acyl chains are ubiquitous, others like tiglyl acyl chains were phylogenetically restricted. A comparative transcriptomics approach was used to identify trichome-enriched *Nicotiana acuminata* acylsugar biosynthesis candidate enzymes. Enzyme assays with four acyltransferase enzymes (NaASAT1-4) together with acyl-CoAs and sucrose produced acylated sucroses, demonstrating their in vitro metabolic capacity. Rapid LC/MS screening of the acylsugar pathway with diverse substrates enabled synthesis of >20 acylsugar types in one assay with acyl chain compositions not found in the *Nicotiana* genus, while also providing insight into acylsugar pathway promiscuity and specificity. For example, NaASAT1 used diverse CoA substrates ranging from 5-12 carbons in length and even aromatic CoAs, however NaASAT1 only used sucrose as the acyl acceptor and not other mono or disaccharides. NaASAT4 added acetyl-CoA to structurally diverse mono, di, tri and tetra-acylated acyl acceptors, but was unable to use other CoA acyl donors. NaASATs were combined with other Solanaceae ASATs enhancing the in vitro metabolic output and revealing the strict regiospecificity of ASATs. A rapid in vitro biochemical screen identified the metabolic potential and constraints of the *N. acuminata* acylsugar pathway providing a first step towards a metabolic engineering approach to optimize production of structurally diverse and biologically active acylsugars.

14:30–14:55

Cloning and characterization of norbelladine synthase and noroxomaritidine reductase, catalyzing the first key steps in Amaryllidaceae alkaloid metabolism

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Amaryllidaceae alkaloids (AAs) are a large group of plant specialized metabolites with diverse biological and pharmacological properties. Norbelladine is the entry compound in AAs biosynthesis and is produced from the condensation of tyramine and 3,4-dihydroxybenzaldehyde (3,4-DHBA). There are two reported enzymes capable of catalyzing this reaction both with low yield. The first one is norbelladine synthase (NBS), which was shown to condense tyramine and 3,4-DHBA. The second one, noroxomaritidine reductase (NR), catalyzes a reduction reaction to produce norbelladine. To confirm and establish the enzyme(s) catalyzing the first committed step of AAs biosynthesis, both NBS and NR coding sequences (CDS) were identified from the transcriptome databases of two Amaryllidaceae plant species *Narcissus papyraceus* and *Leucojum aestivum*. Both the genes were cloned, expressed in *Escherichia coli* and enzymatic assays were performed with tyramine and 3,4-DHBA to test the production of norbelladine. The assays included each enzyme separately and combined in one reaction. The production of norbelladine was detected using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Our results suggest that both NBS and NR function together in a sequential manner for the condensation of tyramine and 3,4-DHBA into norcraugsodine followed by a reduction into norbelladine. Moreover, using transient expression of yellow fluorescent protein (YFP) fusions in *Nicotiana benthamiana* leaves, NBS and NR, which lack predicted signal peptides, were both localized to the cell cytoplasm confirming their colocalization to work together in the same cellular compartment. In addition, the protein homology modeling and molecular docking studies predicts the binding of tyramine and 3,4-DHBA to NBS and norcraugsodine to NR. Together, our study establishes that both NBS and NR participates in biosynthesis of norbelladine and will further strengthen the establishment of synthetic biology platforms to produce AAs.

Leveraging integrative omics analyses for stress-responsive metabolic pathway elucidation in *Brachypodium*

Elizabeth Mahood¹, Lars Kruse², Alexandra Bennett³, Armando Bravo⁴, Maryam Rahmati Ishka⁴, Chinmaey Kelkar⁵, Yulin Jiang⁶, Maria Harrison⁴, Olena Vatamaniuk⁷, Gaurav Moghe¹

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The biosynthesis of specialized metabolic genes has historically been difficult to elucidate, as they often belong to large gene families that undergo frequent gene duplication. In my talk, I will describe the results of an integrated transcriptomics and metabolomics experiment in the model C3 species *Brachypodium distachyon*, designed to elucidate metabolic functions of genes. *B. distachyon* plants were grown under different conditions (heat, copper [Cu] deficiency, combined heat-Cu deficiency stress, low phosphate [P], and arbuscular mycorrhizal symbiosis [AMS]). Transcripts and metabolites were simultaneously extracted from leaves and roots, and RNA-seq and untargeted metabolomics was performed. After an exploratory analysis of the data, metabolic genes contributing to specialized metabolite production were identified through a combination of correlation and phylogenetic approaches.

Principal Components Analysis of the metabolomic data generated an expected clustering of metabolomic datasets by organs and growing conditions. Information theoretic analysis further revealed that while leaves had the most complex metabolite profiles, root metabolomes were more inducible and produced more condition-specific metabolites, especially under heat stress and AMS. Differential analysis of metabolite levels coupled with deep learning techniques helped identify broadly stress-responsive metabolite types such as sphingolipids, glycerolipids and phospholipids. Many condition-specific metabolites – such as blumenols (C13 apocarotenoids) – that can potentially serve as stress biomarkers were also found.

After metabolite structural annotation and gene expression quantification, we identified genes with conserved expression under AMS and with structurally similar, highly correlated metabolites as candidates of biosynthetic pathways. This workflow successfully identified multiple metabolic genes of known importance in AMS as well as identified new candidate genes regulating plant metabolism under AMS in *B. distachyon*.

Associating stress-induced genes with metabolic pathways may provide an efficient method of gene function prediction in understudied species, as well as yield breeding targets for the creation of more stress-tolerant plants.

JULY 28 (WEDNESDAY)

July 28 (Wednesday) Morning **Symposium 5: Breakthrough approaches in elucidating phytochemical biosynthesis**

9:00–9:50

Keynote:

Harnessing the chemistry of plant natural product biosynthesis

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Plants, which make thousands of complex natural products, are outstanding chemists. Through the concerted action of enzymes that are assembled into metabolic pathways, nature creates enormous chemical complexity from simple starting materials. This talk will highlight the discovery process for enzymes that catalyze unusual or unprecedented enzymatic transformations, mechanistic and structural characterization of these enzymes, and methods by which these enzymes can be harnessed for metabolic engineering to generate pharmacologically important compounds. A variety of different plants and molecules are used for these studies, most notably the monoterpene indole alkaloids and the monoterpenes known as iridoids.

10:00–10:25

PSNA TPJ Young Investigator Award presentation: A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry

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Virtually all land plants are coated in a cuticle, a waxy polyester that prevents non-stomatal water loss and is important for heat and drought tolerance. Here, we describe a likely genetic basis for a divergence in cuticular wax chemistry between *Sorghum bicolor*, a drought tolerant crop widely cultivated in hot climates, and its close relative *Zea mays* (maize). Combining chemical analyses, heterologous expression, and comparative genomics, we reveal that: (i) sorghum and maize leaf waxes are similar at the juvenile stage but, after the juvenile-to-adult transition, sorghum leaf waxes are rich in triterpenoids that are absent from maize, (ii) biosynthesis of the majority of sorghum leaf triterpenoids is mediated by a gene that maize and sorghum both inherited from a common ancestor but that is only functionally maintained in sorghum, and (iii) sorghum leaf triterpenoids accumulate in a spatial pattern that was previously shown to strengthen the cuticle and decrease water loss at high temperatures. These findings uncover the possibility for resurrection of a cuticular triterpenoid-synthesizing gene in maize, which could create a more heat-tolerant water barrier on the plant's leaf surfaces. They also provide a fundamental understanding of sorghum leaf waxes that will inform efforts to divert surface carbon to intracellular storage for bioenergy and bioproduct innovations.

10:25–10:50

Postharvest darkening of seed coat and proanthocyanidin biosynthesis in pinto bean

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Postharvest seed coat darkening affects the value of pinto beans (*Phaseolus vulgaris*), one of the leading market classes of dry beans worldwide. Pinto bean producers and vendors encounter significant crop value loss due to the poor canning quality, longer cooking time, higher storage costs and the decreased consumer preference for the darker pintos. Regular darkening (RD) pintos darken faster after harvest by accumulating a higher level of proanthocyanidins (PAs) compared to the slow darkening (SD) cultivars. However, the SD cultivars show poor agronomic performances in different environmental conditions. Thus, the identification of the gene/s responsible for slow darkening in pinto beans was demanded to develop improved varieties. Using multiple Omics approaches and inheritance study, we identified a unique allele of the *P* (Pigment) gene, responsible for the slow darkening phenotype. *P* encodes for a bHLH transcription factor protein, regulates late proanthocyanidin biosynthetic genes, and is an *Arabidopsis* TT8 orthologue. Alteration in *P* sequence determines the seed coat color in common beans including pintos. Gene-specific molecular markers, specific for the SD trait are developed, to develop improved SD cultivars faster and resolve this economically important issue for bean producers. Mechanism of regulation of PA biosynthetic genes in pintos is under investigation.

11:00–11:25

Mathematical models of plant metabolism: Awesome research tools for everything from gene discovery to complex network analysis

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Understanding the regulation of biochemical pathways is among of the oldest applications for mathematical modeling. For more than a century, the options for formulating the characteristics of reactions or structuring a model have been growing, and the choices can sometimes be difficult to maneuver for novices and experts alike. In this presentation, we will outline the design processes for different modeling frameworks. We will use a diverse set of experimental systems to illustrate the power of modeling for supporting scientific discovery and informing breeding and engineering efforts. Experimental topics will include the organ-specificity of terpenoid formation in *Arabidopsis*, feedback regulation of oil production in mint, resin biosynthesis in pine, and an across-species analysis of flux

distribution through primary and specialized metabolism in glandular trichomes. The goal is to make modeling more accessible to experimentalists, in particular those with an interest in capturing the genetic basis of plant chemical diversity.

11:25–11:30

Flash talk: Development of an *in vivo* method in *Nepeta cataria* (catnip) to characterize the biosynthetic pathway behind nepetalactone stereoisomer production

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The Lamiaceae plant family, colloquially known as the mint family, is well known for its chemical diversity and economical importance, especially amongst members of the sub-family the Nepetoideae. Most members of this sub-family are well known for their diverse terpene-based natural products; however, one genus, the *Nepeta*, stands out amongst the Nepetoideae for its unique ability to produce nepetalactone, an iridoid-scaffold compound known for its psychoactive effect on cats and potential use as a bio-based pest control in agriculture due to its influence on various insect species. Chemical profiling on *Nepeta* spp. and within varieties of a single species have revealed the production of different nepetalactone stereoisomers varies widely across plants. Previous work has identified *Nepeta* spp. biosynthetic enzymes that can synthesize different stereoisomers of nepetalactones *in vitro*. The role these biosynthetic genes play in planta to synthesize different stereochemical ratios of nepetalactone has not been addressed. I developed a virus-induced gene silencing (VIGS) tool for *N. cataria* to explore the *in vivo* function of the putative biosynthetic genes. By simultaneously targeting a visual marker gene, magnesium chelatase subunit H (CHLH), and each member of the genes involved in the production of the various nepetalactone stereoisomers, I am able to precisely select the tissue under the knockdown phenotype of VIGS and characterize this pathway *in vivo*. Furthermore, using this tool, along with expression analysis tools such as qPCR and RNAseq, I aim to untangle the mechanisms behind isomer regulation and gene expression in nepetalactone production, as well as to understand the effect of this pathway on other physiological processes.

11:30–11:35

Flash talk: Expanding the tropane alkaloid metabolic network: Metabolite discovery in *Atropa belladonna*

Hannah M. Parks¹, Maris A. Cinelli¹, Josh M. Grabar², A. Daniel Jones¹, Cornelius S. Barry²

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Understanding how metabolic pathways emerge and networks evolve is a key challenge in biology. Advances in high resolution mass spectrometry, coupled with multivariate statistical analyses, provides a powerful platform for metabolite discovery and we are combining these approaches with gene-silencing to reveal novel insight into plant specialized metabolism. Tropane alkaloids are a pharmacologically important class of plant specialized metabolites synthesized by just a few plant families. *Atropa belladonna* (Solanaceae) synthesizes the anticholinergics hyoscyamine and scopolamine through a multistep pathway that was recently elucidated. However, little is known about the alternative metabolic fates of tropane pathway intermediates or their role in generating novel metabolic diversity. Virus-induced gene silencing (VIGS) was utilized to silence key tropane pathway genes in *A. belladonna* roots and the metabolite profiles of these plants were captured using liquid chromatography coupled with mass spectrometry (LC-MS). Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was used to identify metabolites that differ between control and silenced lines, revealing the existence of many novel metabolites that arise from the repurposing of tropane alkaloid pathway intermediates. These metabolites were targeted for downstream analyses, including liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) experiments, compound purification, structural characterization by nuclear magnetic resonance (NMR) spectroscopy, and pathway reconstruction in *Nicotiana benthamiana*. These data highlight the potential of utilizing non-targeted metabolite profiling of silenced lines for advancing the discovery of novel compounds and improving understanding of metabolic networks.

11:35–11:40

Flash talk: De novo genome assembly, annotation and characterization of the essential oil plant *Lavandula angustifolia* (lavender)

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Lavender (*Lavandula angustifolia*) is a perennial plant native to the Mediterranean region and known for its essential oil (EOs) that has numerous applications in various industries. We have recently sequenced the *L. angustifolia* (Maillette) genome (Malli et al., 2019; DOI: 10.1007/s00425-018-3012-9), and here report a detailed analysis of this highly duplicated and complex genome, focusing on genome size, ploidy, and repeat content. The lavender genome was estimated to be around 870 Mbp (1C=0.96 pg) using a quantitative PCR method. Genome size was also validated through analysis of raw genome sequence data using KmerGenie software. The repeat element composition was estimated to be around 45% of the full genome or ~57% of the non-gap genome sequences. Further characterization revealed Long Terminal Repeat (LTRs) retrotransposons as the major repeat component, comprising ~18%, followed by DNA transposons at ~8.5% of the genome. Interestingly, unlike most other plant genomes, the lavender genome has more *Copia* than *Gypsy* elements, both showing a trend of recent increasing activity. Furthermore, both types of the LTRs, but more so for *Copia*, have shown active participation in gene function including genes for EO production, with *Copia* elements impacting ~30 % of the CDS regions, in addition to promoter, intron and UTR regions. The lavender genome has an unusually high number (~88,000) of miniature inverted-repeat transposable elements (MITEs) compared to other model plant genomes. Analysis also revealed the lavender genome contains a high proportion at polyploidy level, with bias towards regions containing essential oil genes. Analysis of Ks substitution rates estimated that polyploidization events in the lavender genome occurred between 16 to 41 million years ago, and are partly responsible for the presence of high copy numbers of EO genes. In conclusion, our results reveal the lavender genome to be highly duplicated and with past and ongoing active retrotransposition, all favouring EO production.

July 28 (Wednesday) Afternoon **Symposium 6: Evolution of plant specialized metabolism**

13:30–14:20

Mechanistic basis for metabolic evolution in plants

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Metabolic pathways are often considered “perfected” or at least predictable as substrates efficiently rearrange into products through the intervention of an optimized enzyme. Moreover, single catalytic steps link up, forming a myriad of metabolic circuits that are often modeled with a high degree of certainty. However, on closer examination, most enzymes are not precise with respect to their activity, using not just one substrate but often a variety and producing not just one product but a diversity. Hence, the metabolic systems assembled from enzymes possessing varying degrees of what can be termed catalytic promiscuity are not clear-cut and restrictive; rather, they may at times operate stochastically in the intracellular milieu. This “messiness” complicates our understanding of normal and aberrant cellular behavior, while paradoxically sowing the seeds for future advantageous metabolic adaptations for host organisms. In this talk, I will discuss the evolutionary implication of catalytic promiscuity widely observed in plant specialized metabolic systems, and how we can now exploit plant metabolism and chemodiversity for new catalysts, materials, and drugs.

14:30–14:55

Unravelling the biosynthesis of the very-long-chain β -diketones sealing barley (*Hordeum vulgare*) surfaces: A polyketide synthase condensing non-canonical substrates

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The cuticle, a thin lipophilic film lining above-ground plant surfaces, plays an essential role in plant adaptation for terrestrial environments. The cuticular waxes of Poaceae, including major staple crops, are dominated by very-long-chain β -diketones crucial for drought resistance in adult/reproductive stages and, therefore, crop yield. Previous studies had identified a gene cluster encoding the enzymes responsible for β -diketone formation in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), but left their biochemical functions unknown. Here, in-depth GC-MS analysis first showed that the barley wax β -diketones had isomer and homolog structures that could not be explained by the hitherto assumed iterative decarboxylative condensation pathway. The enzyme thought to catalyze the first reaction en route to β -diketones, upon expression in *E. coli*, was found to be a hydrolase likely intercepting thioester intermediates of fatty acid synthesis to produce β -ketoacids. However, the enzyme

previously implicated in the following reaction, annotated as a type III polyketide synthase (PKS), was not able to utilize these β -ketoacids as starters for decarboxylative condensation with malonate extenders analogous to other plant PKSs. Instead, yeast (*Saccharomyces cerevisiae*) heterologous expression and *in vitro* assays showed that this barley PKS used the β -ketoacids as extenders, in a non-canonical reaction with long-chain acyl-CoAs as starters. Finally, we explored the chain-length specificities of the PKS for both its substrates, revealing that the characteristic homolog/isomer composition of the barley wax β -diketones is determined by the combined specificities of the PKS and the hydrolase feeding substrates to it. Our findings thus define wax β -diketone formation as a unique two-step pathway involving an unprecedented PKS reaction.

14:55–15:20

Divergence of very-long-chain fatty acid substrate specificity in a recently duplicated gene cluster of poplar β -ketoacyl-CoA synthases

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Cuticular waxes are composed of a diverse group of lipids which protect plants against biotic and abiotic stresses. Although cuticular waxes typically consist of saturated hydrocarbons, the waxes of *Populus trichocarpa* contain an unusual series of long chain alkenes that are associated with increased biomass and pathogen resistance. The occurrence of alkenes can be largely predicted by the expression of PotriKCS1, a β -ketoacyl-CoA synthase (KCS) involved in the elongation of very-long-chain fatty acids, precursors to plant cuticular waxes. Interestingly, PotriKCS1 is part of an eight-gene tandem-duplicated cluster on Chromosome 10, and while PotriKCS1 preferentially elongates unsaturated fatty acids, a duplicate, PotriKCS2, prefers saturated substrates. Our study aimed to (1) understand how substrate specificity evolved in this KCS gene cluster, and (2) identify the key amino acid substitutions responsible for driving changes to substrate specificity. To do this, we characterized the gene cluster using heterologous expression in yeast and GC-MS fatty acid profiling. Functional characterization of the gene cluster showed that the paralogs had distinct preferences towards fatty acid substrates of varying lengths and degrees of unsaturation. Treatment with chloroacetamide covalent inhibitors also showed a differential pattern of inhibition between paralogs, suggesting the presence of structural changes near the active site. Finally, we used natural polymorphisms, structure-homology modelling, and chimeric proteins to identify two N-terminal regions as major determinants of substrate specificity. Together, our results illustrate the evolution of a recently duplicated gene cluster of KCSs and the amino acid substitutions that drive changes to substrate specificity in these enzymes.

15:30–15:55

What determines if monoterpene synthases produce monocyclic or bicyclic products? An excursion into structure-function relationships

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Monoterpene synthases (MTSs) catalyze the conversion of a C10 prenyl diphosphate into acyclic, monocyclic or bicyclic monoterpenes. While some MTSs generate multiple products, others are highly specific and produce only one major monoterpene. A shared characteristic of catalysis by MTSs is the occurrence of highly reactive carbocation intermediates. The nature of the interaction of residues in the active site of these enzymes with these carbocations determines the course of the reaction. In the present study, we used two exceptionally well characterized MTSs, (-)-limonene synthase from spearmint (LMNS) and (+)-bornyl diphosphate synthase (BPPS) from common sage, to better understand why the critical carbocation intermediate is deprotonated in the former, while it proceeds through further cyclizations in the latter. To address this fundamental issue, we employed state-of-the-art computational approaches, including molecular dynamics, quantum mechanics/molecular mechanics, umbrella sampling and statistical mechanics. These simulations were complemented by experimental site saturation mutagenesis to evaluate the effects of altered active site residues. Through integration of these information-rich data sets, we were able to deduce a model that allows us to explain the product profiles formed by LMNS and BPPS. More importantly, our study lays the foundation for predicting the functions of MTSs in newly sequenced plant genomes.

15:55–16:00

Flash talk: Genome-wide identification of cytochrome P450 monooxygenases provide insight into their role in partial resistance against *Phytophthora sojae* in soybean (*Glycine max*)

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The cytochrome P450 monooxygenases (P450s) are the heme-thiolate superfamily of enzymes that function as oxidoreductases with the heme group as a catalytic centre. Plant P450s have proven their potentials in the synthesis of many specialized metabolites including alkaloids, terpenoids, phenylpropanoids and saponins. Plants utilize these metabolites to combat biotic as well as abiotic stress. To identify the P450s involved in the partial resistance against stem and root rot disease caused by *Phytophthora sojae*, we performed a genome-wide analysis of soybean genome and found 359 *GmP450* encoded by 342 genes. The analysis of protein motifs showed conserved EXXR, PXRX and CXG motifs among all *GmP450s*. A search for datasets containing differential gene expression in soybean in response to *P. sojae* resulted into 5 publically available transcriptomic datasets, where 21 *GmP450s* were differentially expressed in all datasets. Overall analysis of *GmP450s* in soybean showed that members of CYP71, CYP82 and CYP93 actively participate in partial resistance mechanism in soybean to provide inherent immunity.

16:00–16:05

Flash talk: Large-scale comparative genomics and biochemical analyses reveal ancestral promiscuity as a driver of functional diversification of BAHD acyltransferases in plants

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Large enzyme families play an important role in metabolic diversification in plants by virtue of their ability to use diverse chemical scaffolds. How frequently this functional diversity arises through emergence of entirely new activities vs. specialization of ancestrally possible activities is not clear. In this study, we addressed this question using the functionally diverse BAHD acyltransferase family (BAHDs) as a model for metabolic enzyme families. This enzyme family has been shown to be involved in many different plant specialized metabolite pathways, using multiple structurally distinct acceptor substrates. Previous studies have also shown that some BAHDs have a broad substrate range, however the extent of substrate promiscuity within this family and the consequential evolutionary implications have not been addressed adequately. Our study found that this fast-evolving family expanded drastically during land plant evolution from 1-5 copies in algae to ~100 copies in diploid angiosperm genomes. *In vitro* characterization of various BAHDs against a structurally diverse substrate panel, in combination with phylogenetic analyses revealed that the ancestral enzymes prior to origin of land plants were likely already capable of using most of the substrate classes used by characterized BAHD enzymes today. For example, the ability to acylate anthocyanins was inferred to have existed millions of years prior to its fixation in specialized anthocyanin acyltransferases in angiosperms. Motif enrichment analysis in anthocyanin-acylating BAHDs identified two motifs that are fixed in the largest anthocyanin acylating clade. Molecular dynamic simulations, ancestral sequence reconstruction, and enzyme kinetics further resolved the potential roles of these motifs in evolution of anthocyanin acylation. Our results illuminate how promiscuity in robust and evolvable enzymes contributes to functional diversity in enzyme families and the metabolic diversification in plants.

16:05–16:10

Flash talk: Is the Entner-Doudoroff pathway functional in higher plants?

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The 2C-methyl-D-erythritol-4-phosphate (MEP) pathway converts pyruvate and D-glyceraldehyde-3-phosphate (GAP) into isopentenyl (IDP) and dimethylallyl diphosphate (DMADP) for isoprenoid biosynthesis in the plastid. Pyruvate and GAP are supplied in turn by the catabolic Embden-Meyerhof-Parnas (EMP) pathway (i.e., classical glycolysis) and the pentose phosphate pathway (PPP) pathway. In some prokaryotes, the Entner-Doudoroff (ED) pathway constitutes a third catabolic route which produces pyruvate and GAP from the PPP intermediate 6-phosphogluconate (6-PG) in a 2-step process consisting of 6-PG dehydratase (EDD) and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase

(EDA). It has been suggested the ED pathway may also operate in some plants. Here, we aimed to determine whether a fully functional ED pathway could increase substrate availability for the MEP pathway in the model plant *Arabidopsis*. The unique ED pathway intermediate KDPG was detected in leaf tissue of *Arabidopsis*, soybean, and tomato by LCMS/MS. However, BLAST searches revealed that *Arabidopsis* lacks an *EDA* gene for conversion of KDPG into pyruvate and GAP. To supplement this step in *Arabidopsis*, the *EDA* gene from soybean was first cloned and expressed in *E. coli*. The recombinant protein converted KDPG to pyruvate and GAP. Stable *Arabidopsis* transformants expressing soy *EDA* were then generated to probe the potential for providing additional substrate for plastid terpenoid biosynthesis through a functional ED pathway. The ED pathway may offer new alternatives for optimization of the MEP pathway for plant terpene production. Hence, elucidation of a potential role of the ED pathway in plants to augment MEP pathway flux is currently in progress.

JULY 29 (THURSDAY)

July 29 (Thursday)

Symposium 7: Enzymology and organization of plant metabolism

Morning

9:00–9:25

Arthur Neish Award presentation: Structural insights into strigolactone signaling regulation by the ubiquitin system

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The newly discovered phytohormones, strigolactones (SLs), regulate numerous essential growth and developmental processes including branching, leaf senescence, root development, and act as rhizospheric signals for communication with symbiotic fungi and parasitic plants. It has been shown that SL signaling requires three distinct enzymes: D14 α/β hydrolase, F-box MAX2/D3 ubiquitin (Ub) ligase, and SMXLs transcription repressors. SMXLs levels are controlled by MAX2 and the phytohormone receptor D14. Despite these findings, key questions concerning the phytohormone perception and regulation by the conformational dynamics remain unanswered. Here we show that MAX2/D3 has multiple functional states that are determined by distinct structural conformations. We demonstrate biochemically and *in planta* the key role of MAX2/D3 plasticity in the recruitment of D14, SMXLs, and SL perception. Altogether, this work provides new insights into the regulation phytohormones signaling cascades by the ubiquitin proteasome system in plants.

9:25–9:50

Structure-function investigation of class II diterpene cyclases

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Diterpenoids are a diverse class of natural products commonly produced by plants, bacteria, and fungi. Many of these specialized metabolites are known to have industrial applications as flavorings, fragrances, biofuels, cosmetics, and because of anti-inflammatory, antimicrobial, and even antitumor properties they are of great interest in pharmaceutical and agricultural sectors as well. The complexity of these metabolites makes them difficult to produce with synthetic chemistry alone and biosynthesis using metabolic engineering is often alternatively applied. For this reason, it is important to understand the structure and mechanisms of the enzymes that perform the committed step in diterpenoid biosynthesis, class II diterpene cyclases (DTCs). While available structures have enabled certain aspects of DTC catalysis to be uncovered such as identification of the catalytic base, there are no precatalytic structures. Such structures would help overcome current limitations for computational molecular modeling with these enzymes. Additionally, there are subtle but important details underlying substrate bound enzyme structure and how agriculturally relevant inhibition occurs. Recent advancements in Cryo-EM technology suggest such precatalytic structures could be solved using this technique. A concurrent understanding of these enzymes can be established using mutational analysis. DTCs show functional plasticity where minor alterations such as a single residue switch in the active site can dramatically alter product outcome. Utilizing the highly conserved nature of these enzymes, mutations can be introduced to mimic a DTC of alternative function altering catalysis to produce diterpenes of different carbon scaffolds or varying stereochemistries. Results in this work provide insights characterizing key active site residues determining product outcome and highlight the opportunity to use Cryo-EM to solve a substrate-bound structure.

10:00–10:25

Metabolic flux analysis during wound-healing in potato tubers

PSNA 2021

All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)

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Many principal food crops face threats such as herbivory, pathogens, abiotic stressors, and harvest-related wounding (allowing for pathogens to infect the exposed tissues); these threats result in large losses of crops annually, both pre- and post-harvest. In response to wounding, the biopolymer suberin is deposited between the cell wall and plasma membrane, to act as a physical barrier to pathogens and water loss. Suberin is characterized by two spatially distinct domains. One is phenolic in nature while the other is an aliphatic-based polyester. The objective of this project is to quantify the temporal allocation of carbon between the domain specific pathways leading to the (poly)phenolic and (poly)aliphatic domains during wound-induced suberization. Through flux analysis, based on stable isotope labeling, the metabolism leading to suberin deposition in a *Solanum tuberosum* (potato) tuber model system was tracked over seven days. Tubers were treated with uniformly labeled D-Glucose-¹³C₆ and downstream metabolites possessing labelled carbon analysed by gas chromatography-mass spectrometry (GC-MS) and identified based on fragmentation patterns. Erythrose, shikimate, glutamine, and L-phenylalanine were used as “proxy” metabolites indicative of phenolic metabolism, while citric acid, malic acid, and stearic acid were used as “proxy” metabolites indicative of aliphatic metabolism; glycerol was also tracked as it is hypothesized to play a key role in linking of the domains. Quantification of these proxies revealed a time-based differential partitioning of carbon between phenolic and aliphatic metabolic fates. Preliminary data suggests carbon is preferentially partitioned into suberin phenolics early on during wound induced-metabolism, before being partitioned between both phenolic and aliphatic monomer biosynthesis. Advancement in the understanding of regulation and synthesis of wound-associated suberin will inform new approaches to bolstering plant defense against wound related threats, thereby improving yield, quality, and storage of food crops.

10:25–10:50

Swapping sugars: The biochemical evolution of eggplant trichome defensive metabolites

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Solanaceae species synthesize a remarkable array of specialized metabolites in clade- and tissue-specific patterns. Acylsugars, one class of structurally diverse metabolites, defend against herbivores and pathogens, and defense is impacted by acylsugar chemistry differences. Glandular trichomes manufacture acylsugars from the simple building blocks of sugars and acyl coA esters. Sucrose is the sugar core in most species, while the acyl chains come from a variety of primary metabolic networks and differ greatly in number, length, and branching patterns. Investigating inter-species acylsugar differences has uncovered metabolic evolutionary mechanisms. Considerable attention was paid to acylsugar diversity and biosynthesis within the tomato clade of *Solanum* but not within the large, phylogenetically distinct clade of ‘spiny’ *Solanum*, *Leptostemonum*. We describe the results of an integrated approach to document the diversity, biosynthesis, and evolution of acylsugars within the *Leptostemonum* clade of *Solanum*. Mass spectrometry and nuclear magnetic resonance spectroscopy analyses identified unique lineage-specific acylsugar characteristics restricted to the clade. The presence of *myo*-inositol sugar cores, glycosylated acylsugars, and hydroxylated medium-length acyl chains in early-diverging clades of *Leptostemonum* indicates that lineage-specific evolutionary events led to the current acylsugar phenotype. The evolutionary origin of *myo*-inositol sugar cores was investigated in *S. melongena* (brinjal eggplant), and *S. quitoense* (lulo or naranjilla), through tissue-specific RNAseq, *in vitro* enzyme assays, and *in vivo* gene knockout/knockdown experiments. As acylsugar acyltransferases exhibit high acyl acceptor specificity, we hypothesized that enzymes not orthologous to the tomato pathway evolved in *Leptostemonum*. In support of the hypothesis, *S. melongena* does not contain a functional ortholog of tomato’s first acylating enzyme, SIASAT1. Instead, we identified a homolog of SIASAT1 expressed in glandular trichomes and capable of acylating *myo*-inositol. Identification of the full acylinositol pathway will allow functional testing of acylinositol defense capabilities. Application of acylinositols in crop defense may be a valuable agricultural tool.

11:00–11:25

Mutant combinations of lycopene ϵ -cyclase and β -carotene hydroxylase 2 homoeologs increased β -carotene accumulation in endosperm of tetraploid wheat (*Triticum turgidum* L.) grains

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Vitamin A deficiency (VAD) has been recognized as a public-health issue in developing countries due to the insufficient intake of vitamin A and/or provitamin A from diet. Generating staple crops with high accumulation of β -carotene (the most efficient provitamin A carotenoid) provides long term alleviation to VAD. Grains of tetraploid wheat (*Triticum turgidum* L.) accumulate mainly lutein, a non-provitamin A carotenoid competing with β -carotene for the common biosynthetic precursor lycopene. Lycopene ϵ -cyclase (LCYe) directs the lycopene to Lutein. β -carotene can be further catabolized by multiple enzymes including β -carotene hydroxylase (HYD). The increase of β -carotene accumulation in storage tissue has been achieved in many species by blocking LCYe and HYD to divert carbons from lutein to β -carotene biosynthesis and reduce the turnover of β -carotene. To understand the individual and combined effects of LCYe and HYD2 mutations on β -carotene accumulation in tetraploid wheat grain endosperm (flour), loss-of-function Targeting Induced Local Lesions in Genomes (TILLING) mutants of LCYe and HYD-2 homoeologs were identified and crossed to generate higher order mutant combinations of *lcye-A*, *lcye-B*, *hyd-A2*, and *hyd-B2*. Significant increase of β -carotene in endosperm were achieved in multiple mutant combinations. Our results on molecular, biochemical, and physiological characterization of the mutant combinations will be presented.

11:25–11:30

Flash talk: A monoterpene synthase controls bornyl diphosphate biosynthesis from *Lavandula x intermedia*

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In lavender, geranyl diphosphate (GPP), the linear precursor to regular monoterpenes, can be transformed to bornyl diphosphate (BPP), an intermediate that is rapidly converted to borneol, which is subsequently oxidized to camphor. The latter two monoterpenes contribute off odor, and are considered undesired constituents of lavender essential oil. In this study, we isolated and functionally characterized a bornyl diphosphate synthase gene (*LiBPPS*) from *Lavandula x intermedia*. The ORF excluding transit peptide of *LiBPPS* was expressed in *E. coli*, purified with Ni-NTA agarose affinity chromatography, and assayed *in vitro*. The recombinant LiBPPS protein converted GPP into BPP as the major product, and a few other monoterpenes as minor products. We further studied the *in planta* role of *LiBPPS* in essential oil metabolism through its overexpression in sense and antisense orientations in separately transformed *Lavandula latifolia* plants. As anticipated, overexpression of *LiBPPS* in sense lead to increased borneol and camphor production, and overexpression in antisense resulted in decrease borneol and camphor synthesis. Our results demonstrate that the cloned *LiBPPS* could be used in metabolic engineering studies aimed at improving essential oil compositions and scent in lavender and other plants.

11:30–11:35

Flash talk: In-depth analysis of unusual very-long-chain ketones found in cuticular waxes of wild-type and mutant Welsh onion (*Allium fistulosum* L.)

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The waxy cuticle that coats the aerial organs of most terrestrial plants consists typically of very-long chain compounds with terminal functional groups, which play important roles in plant water retention as well as protecting the plant from UV light, pathogens and insects. In cases where mid-chain functionalities are present in plant wax, they usually make up the majority of wax constituents. The work here aims to investigate the wax biosynthesis in Welsh onion (*Allium fistulosum* L.) as a model for formation of very-long-chain ketones. To this end, we performed an in-depth analysis of the wax constituents that coat the leaves of wild-type onion as well as a previously uncharacterized wax mutant. Gas Chromatography-Mass Spectrometry (GC/MS) and Flame Ionization Detection (FID) analysis revealed that the wax coverage in wild type consisted mainly of C31 ketone (70% of total wax) with carbonyl group mainly on C-14 and C-16 (55% and 35% of C31 homolog). Among other wax constituents, trace amounts of ketols (oxo-alcohols) were also found, with isomer compositions similar to those of the ketones. This suggests that both compound classes are biosynthesized on a common pathway, introducing the mid-chain functionality during chain elongation and not by oxidation of fully elongated alkanes. Only traces of ketones were found in the mutant leaf wax, along with alcohols and esters (52% and 28% of total wax, respectively) similar to other species. Our research on the wax composition of *A. fistulosum* as well as the wax mutant provides a basis for exploration of the

biosynthetic machinery forming the very-long-chain ketones that are crucial for stress tolerance of Welsh onion and other crops.

July 29 (Thursday)
Afternoon

Symposium 8: Phytochemistry of the forest, the far north, and underutilized plants

13:30–14:20

Keynote:

Oleoresin defenses in conifers: Chemical diversity and limitations under climate change

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Conifers have evolved complex oleoresin-based chemical defenses against herbivores and pathogens. In co-evolved bark beetles, the terpene components of the conifer oleoresin also serve various chemo-ecological functions as pheromone precursors, chemical barcodes for host identification, or nutrients for insect-associated microbiomes. This presentation highlights the genomic, molecular and biochemical underpinnings of the large chemical space of oleoresin terpenes produced by trees. While oleoresin terpenoid defenses have contributed much to the evolutionary success of conifers, under new conditions of climate change, these chemical defenses may become inconsequential against range-expanding forest pests. Another line of conifer defense against insects involves the deposition of stone cells as a physical barrier against stem feeding insect larvae. Physical stone cell defenses can provide a durable resistance, that may be difficult to overcome by insects.

14:30–14:55

Arthur Neish Award presentation: Cold tolerance of membranes is a matter of timing and metabolic state – not just a saturation story

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Cold limits agricultural production, particularly of crops that were domesticated in warm regions, such as maize, sorghum, and many panicoid grasses. An important portion of a plant's response to cold is to remodel their membranes to improve membrane fluidity and prevent breakage and catastrophic leakage. Because any single membrane composition can only remain fluid at a small range of temperatures, plant membranes are rapidly remodeled in response to temperature fluctuations. We tested the relationship between glycerolipid membrane changes, evolutionary distance and cold tolerance using a trio of panicoid grasses. Two of the grasses are closely related but differ in cold tolerance – foxtail millet and urochloa. Two of the grasses are more distantly related and similar in cold tolerance – urochloa and sorghum. We took densely timed lipid measurements after gentle cold onset, pairing them with transcript measurements to identify causal genes. We found many lipid responses to cold were similar in all species, and that differences occurred at discrete time points. To our surprise, the data showed strong daily rhythms existed in both lipids and lipid-controlling genes. We re-measured model species *Arabidopsis thaliana* lipids responding to cold and found many of the patterns we observed in panicoid grasses were generalizable. This is in contrast to published studies that frequently differ in the direction and amplitude of specific lipid changes. We conclude that membrane cold tolerance is not the result of a single change, but a series of changes. We hypothesize that lipid changes in response to cold must be understood within the rhythmic cycles of metabolism.

14:55–15:20

Cytosolic geraniol and citronellol biosynthesis mediated by a Nudix hydrolase in *Pelargonium graveolens*

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Geraniol, citronellol, and related esters are high-value acyclic monoterpenes used in food technology, perfumery, and cosmetics. Essential oil from rose-scented geranium (*Pelargonium graveolens*) is a major source of these 'citronelloids'. Although many species produce geraniol from geranyl diphosphate (GDP) in the plastid via a monoterpene synthase, a few make it in the cytosol through an alternative route involving a nudix hydrolase. We provide evidence that *Pelargonium* biosynthesizes citronelloids

in the cytosol via geranyl monophosphate (GP) through the action of a Nudix hydrolase and phosphatase. A Nudix hydrolase cDNA from *Pelargonium* glandular trichomes dubbed PgNdx1 encoded a cytosolic protein capable of hydrolyzing GDP to GP with a K_M of ~750 nM but is only weakly active towards farnesyl diphosphate. Leaf protein preparations converted GDP to geraniol in *in vitro* assays, a process which could be blocked by phosphatase inhibitors, suggesting a two-step conversion of GDP to geraniol. *P. graveolens* chemotypes enriched in either geraniol or (-)-citronellol accumulate GP or citronellyl monophosphate (CP), respectively, the presumed precursors of their monoterpenoid end products. In contrast, (-)-isomenthone rich lines lack these prenyl monophosphates and monoterpene alcohols and instead feature high levels of cyclic *p*-menthane monoterpenes derived exclusively from the plastid. In citronellol rich lines, GDP, GP, and CP were readily detected, while citronellyl diphosphate was absent, suggesting that citronellol biosynthesis proceeds by reduction of GP to CP in this species. These findings highlight the cytosol as a compartment that supports monoterpene biosynthesis in *Pelargonium* and expands the role of Nudix hydrolases in plant volatile biosynthesis.

JULY 30 (FRIDAY)

July 30 (Friday) Morning	Symposium 9: Biochemistry and production of high-value phytochemicals
9:00–9:50	<p>Keynote: Plant essential oils production in cyanobacteria <u>Anastasios Melis</u> <i>Department of Plant & Microbial Biology, University of California, Berkeley, USA</i> E-mail: melis@berkeley.edu</p> <p>The work aims to convert the secondary slow metabolism of the isoprenoid biosynthetic pathway into a primary activity in cyanobacteria and to generate heterologous products using these photosynthetic microorganisms as cell factories. Case study is the production of the 10-carbon monoterpene β-phellandrene (PHL) in <i>Synechocystis</i> sp. PCC 6803. Barriers to this objective and solutions will be discussed.</p>
10:00–10:25	<p>Medicinal genomics: Exploring the diversity of iridoid compounds in blueberry for human health benefits <u>Courtney P. Leisner</u>¹, Lovely Mae F. Lawas¹, Mohamed O. Kamileen², Sarah E. O'Connor², C. Robin Buell³ ¹<i>Department of Biological Sciences, Auburn University, Auburn, USA</i> ²<i>Department of Natural Product Biosynthesis, Max Planck Institute of Chemical Ecology, Jena, Germany</i> ³<i>Department of Plant Biology, Michigan State University, East Lansing, USA</i> E-mail: cpl0013@auburn.edu</p> <p>Blueberry (<i>Vaccinium corymbosum</i>) is an economically important fruit crop that is native to North America. Fresh market production of blueberries in the United States was valued at \$5.68 billion in 2015 and was planted over 36,349 hectares. In addition to its commercial value, blueberries are prized for their positive health benefits, containing high levels of antioxidants, which has been linked to a decreased risk of cancer and heart disease. Another class of known pharmacologically important, yet under studied compounds in blueberries are iridoids. Iridoids are present in over 15 plant families and are potent natural products with a wide range of biological activities in humans including, anticancer, antibacterial and anti-inflammatory. Previous work has identified monotropein, an iridoid glycoside compound, in several wild <i>Vaccinium</i> species, as well as American cranberry (<i>V. macrocarpon</i>), but limited work has been done to identify this compound in cultivated North American blueberry species (<i>V. corybosum</i>, <i>V. angustifolium</i>, <i>V. virgatum</i>). To address this research limitation I have collected over 80 berry and leaf samples from multiple species and commercial varieties of blueberry to survey for monotropein production. The glycoside iridoid monotropein was successfully identified in only 5 of the 71 cultivars included in the panel, indicating that the presence of iridoid compounds is determined by the specific ecotype and pedigree of a given cultivar. Additionally, all wild blueberry species analyzed contained monotropein. This indicates that one way to increase the presence of iridoid compounds in a broader range of cultivars and ecotypes of cultivated blueberry would be through targeted breeding efforts that incorporate wild germplasm. Currently, we have generated both metabolite and transcriptomic data to identify key iridoid biosynthetic pathway genes in blueberry and cloned a key enzyme in the pathway. This is a key step in understanding iridoid biosynthesis in blueberry for future research.</p>

10:25–10:50

Anti-COVID-19 activities of flavan-3-ols in crops

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Since SARS-COV-2 was reported to cause COVID-19 in December, 2019, this virus has infected more than 161 million people and caused about 3,383,265 deaths worldwide by May 15, 2021. Fortunately, mRNA vaccines were rapidly developed for emergent uses to vaccinate the public in about one year. However, this great success does not exclude the necessity to development medicines to treat COVID-19. Especially, medicines are important to prevent people from potential death caused by new variants. In this presentation, we report to use docking simulation to screen flavan-3-ols and test potential candidates in vitro. This screening identified flavan-3-ol gallates and dimeric procyanidins that showed promising activity to inhibit the main protease activity of SARS-Cov-2. In addition, extracts of green tea, muscadine grapes, cacao, and dark chocolate that are rich in flavan-3-ols and procyanidins showed inhibitory activity against the main protease activity. Furthermore, numerous COVID-19 volunteers shared with us that drinking green tea helped their recovery from infection. Moreover, a correlation analysis revealed a negative correlation between per capita tea consumption and death rates in 115 and 121 countries worldwide. These data indicate the therapeutic promising of flavan-3-ol gallates and their rich plant products in help the recovery from COVID-19.

11:00–11:25

Tropalexins A and B suggest evolutionary conservation of phytoalexin biosynthetic enzymes in Brassicales

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Crucifers (order Brassicales, family Brassicaceae), as for example the widely cultivated *Brassica* spp. canola, rapeseed, cauliflower, cabbage, rutabaga and turnip, respond to microbial pathogens and abiotic stress employing de novo biosynthesis of chemical defenses known as phytoalexins. Although the order Brassicales encompasses 17 families, to date only species of the family Brassicaceae (commonly known as crucifers and comprising the most economically valuable crops) has been investigated for phytoalexin production. Hence, it is of great interest to expand searches to other families within the Brassicales, as such work could uncover unique structures critical to understand the evolution of defense pathways.

Toward this goal, nasturtium (*Tropaeolum majus* L.), a member of the family Tropaeolaceae (order Brassicales), was investigated for phytoalexin production. Details of this investigation, including isolation and elucidation of new chemical structures and biosynthetic intermediates will be reported. These plant defenses contain a 1,3- benzothiazine ring and are shown to derive from (S)-Phe via the phenyl glucosinolate glucotropaeolin. That is, more than three decades after the first report on Brassicales-Brassicaceae (cruciferous) phytoalexins, the first Brassicales-Tropaeolaceae phytoalexins will be disclosed. The discovery of the first phytoalexins from Tropaeolaceae, tropalexins A and B, suggests that *T. majus* uses a pathway similar to that of *Brassica* spp. that synthesize the phytoalexins cyclobassinone and rutalexin. This work indicates that Tropaeolaceae species are of enormous importance as genetic sources of novel phytoalexin pathways and suggests an evolutionary conservation of key biosynthetic enzymes within the order Brassicales. Furthermore, we suggest that phylogenetically closely related tribes in Brassicales synthesize structurally similar and/or identical phytoalexins.

11:25–11:30

Flash talk: Proximate analysis and antioxidant capacity of the edible mushroom *Ustilago maydis* (Musuru) grown in creole maize varieties

Sergio M. Espinoza, Sthefany A. Villanueva-Machaca, Sonia Torrico-Vallejos

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Ustilago maydis is a phytoparasitic fungus that grows in maize ears producing a disease commonly known as corn smut. This disease is spread in several areas of the world and is capable of producing important economic losses on corn production. Nonetheless, the fruiting bodies that are produced during the development of this fungus are edible and have been traditionally consumed since ancient times across different cultures and places. This mushroom is known as “Musuru” in the rural areas of Bolivia and is employed as an alternative food due to its wide availability and unique taste; although widespread commercialization is highly hampered since there is no local knowledge about its properties and benefits. In this regard, the present research focused on assessing the nutritional properties of this

mushroom along with its phenolic content and antioxidant activity in order to revalorize and support its consumption. It is known that the properties of *Ustilago maydis* depend on the host on which it grows, so the unique maize varieties locally available could lead to unprecedented functional properties. Thus, the proximate composition of mushrooms growing in four local maize varieties (“Hualtaco”, “Pairumani”, “Kulli” and “Huillcaparu”) was determined following the AOAC (2019) guidelines for total ash content, crude fat, protein content, crude fiber, and total carbohydrates. In addition, the total phenolic content was estimated for methanolic and aqueous extracts that were obtained by maceration and decoction in boiling water, respectively. The antioxidant capacity was determined for the same extracts by using the CUPRAC method. Important differences were detected between aqueous and methanolic extracts regarding their phenolic content and antioxidant activity. Similarly, different nutritional properties were evident for mushrooms growing in different maize varieties. The antioxidant potential of this species could support its use as a functional food and increase its value.

11:30–11:35

Flash talk: Flavonols and dihydroflavonols inhibit the main protease activity of SARS-CoV-2

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SARS-COV-2 is the abbreviation of the novel severe acute respiratory syndrome coronavirus 2. Since December 2019, this deadly virus has caused the current pandemic. Although since January 2021, vaccines have been available to prevent the infection of this virus, medicines are not excluded to help control the pandemic. The main protease (M^{pro}) of SARS-CoV-2 is an essential enzyme required for the virus multiplication in the host cells, thus is an appropriate target to screen potential medicinal compounds. Flavonols and dihydroflavonols, two groups of plant flavonoids, have antiviral activities. In this study, we hypothesized that these two groups could inhibit the M^{pro} activity. To test this hypothesis, we performed docking simulation and *in vitro* assays. Docking results predicted that (+)-dihydrokaempferol, (+)-dihydroquercetin, (+)-dihydromyricetin, kaempferol, quercetin, myricetin, isoquercetin, and rutin could bind to at least two subsites (S1, S1', S2, and S4) in the binding pocket of M^{pro}. Their affinity scores ranged from -8.8 to -7.4, which showed a potential inhibitive activity. Seven available compounds were further used to test their inhibitive activity *in vitro*. The resulting data demonstrated that seven compounds effectively inhibited the M^{pro} activity and their IC50 values ranged from 0.125 to 12.9 μ M. Of them, rutin showed the most inhibitive activity. Moreover, we used human coronavirus 229E (HuCov 229E) to test the inhibitory effects on virus replication in Huh-7 cells. The resulting data demonstrated that quercetin effectively inhibited HuCov 229E replication and its EC50 value was 4.88 μ M. These findings indicate that these nutraceutical flavonols and dihydroflavonols are appropriate candidates for screening anti-COVID-19 drugs.

July 30 (Friday)
Afternoon

Symposium 10: Synthetic biology and metabolic engineering of plant metabolism

13:30–14:20

Keynote:

CRISPR/Cas9 in yeast engineering for producing plant specialized metabolites

Sihyun Baek, Joseph C. Utomo, Dae-Kyun Ro

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Saccharomyces cerevisiae is a versatile microbial platform to build synthetic metabolic pathways to produce diverse chemicals. To expedite the construction of complex metabolic pathways by multiplex CRISPR-Cas9 genome-edit, eight desirable intergenic loci, located adjacent to highly expressed genes selected from top 100 expressers, were identified and fully characterized for three criteria after integrating green fluorescent protein (GFP) gene. i) CRISPR-mediated GFP integration efficiency, ii) expression competency assessed by levels of GFP fluorescence, and iii) assessing growth rates of GFP integrated strains. Five best performing intergenic loci were selected to build a multiplex CRISPR platform, and a synthetic 23-bp DNA comprised of 20-bp synthetic DNA with a protospacer adjacent motif (PAM) was integrated into the five loci using CRISPR-Cas9 in a sequential manner. This process resulted in five different yeast strains harboring 1–5 synthetic gRNA-binding sites in their genomes. Using these pre-engineered yeast strains, simultaneous integrations of 2-, 3-, 4-, or 5-genes to the targeted loci were demonstrated with efficiencies from 85% to 98% using beet pigment betalain (3-gene pathway), hygromycin and geneticin resistance markers. Integrations of the multiple, foreign genes in the targeted loci with 100% precision were validated by genotyping. Finally, we further developed the strain to have 6th synthetic gRNA-binding site, and the resulting yeast strain was used to generate a

yeast strain producing a sesquiterpene lactone, kauniolide by simultaneous 6-gene integrations. We also tested the temporary disabling of NHEJ (non-homologous end-joining) mechanism in yeast in the hope of increasing multiplex CRISPR/Cas9 efficiency. This study demonstrates the effectiveness of a single gRNA-mediated CRISPR platform to build complex metabolic pathways in yeast.

14:20–15:10

Keynote:

Breakthrough technologies in metabolic engineering: a yeast tetrahydroisoquinoline platform

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The tetrahydroisoquinoline (THIQ) moiety is a privileged substructure of many bioactive natural products and semi-synthetic analogues. Plants manufacture more than 3,000 THIQ alkaloids, including the opioids morphine and codeine. While microbial species have been engineered to synthesize a few compounds from the benzyloisoquinoline alkaloid (BIA) family of THIQs, low product titers impede industrial viability and limit access to the full chemical space. Here we report on the engineering of a yeast THIQ platform by increasing production of the central BIA intermediate (S)-reticuline to $>4.6 \text{ g L}^{-1}$. Using this platform, we demonstrate high titer *de novo* biosynthesis of dihydrosanguinarine. In addition, we engineered yeast to produce cholesterol and introduced the human mu opioid GPCR receptor, creating an opioid biosensor capable of detecting the opiate morphine at an EC_{50} of 882 nM. Our sterol-optimized platform will be a valuable tool in generating human GPCR-based biosensors, aiding in ongoing receptor deorphanization efforts, and providing a framework for high-throughput screening of receptors and effectors, such as plant natural products.

15:20–15:45

Analysis of plant enzymes as consumable parts for synthetic biology

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In all cellular organisms, enzyme pools are continually renewed through a cyclic process of synthesis, degradation and re-synthesis (enzyme turnover). However, the factors and mechanisms that control this process are poorly understood. It is unclear why some enzymes turn over faster than others and how 'old' enzymes differ from 'new' enzymes structurally and functionally.

To unlock the mysteries of this process, we considered enzyme turnover rates (measured by stable isotope labelling) and metabolic flux rates (calculated using flux balance analysis) in plants. Considering these rates together as a unitless ratio of catalytic-cycles-till-replacement (CCR) provides a new quantitative tool to assess the replacement schedule of and energy investment into enzymes as they relate to function. We assessed CCRs of selected enzymes in plants (and bacteria for comparison) to reveal a range of seven orders of magnitude for this ratio. These values will allow genetic engineers and synthetic biologists to seek CCR-based improvements in crop productivity.

To better understand the structural and functional differences between newly-synthesised enzymes and enzymes that have existed in cells for some time, we investigated tagging and enriching nascent enzymes using azidohomoalanine (AHA) and homopropargylglycine (HPG). These methionine surrogates have previously been used for enzyme tagging in mammalian systems and we assessed their suitability for this purpose in plants (proteins tagged with AHA or HPG can be separated from the rest of the protein pool using commercially-available kits). Unlike animal cells, plant cells synthesise methionine, which potentially complicates the use of such surrogates in plants. We show that AHA and HPG influence the cellular levels of methionine and related metabolites in Arabidopsis. We also show the effects of AHA and HPG on cell growth, the enzymes in plants that can be shown to incorporate AHA and HPG and discuss the efficiency of this approach in plants vs. mammals.

15:45–16:10

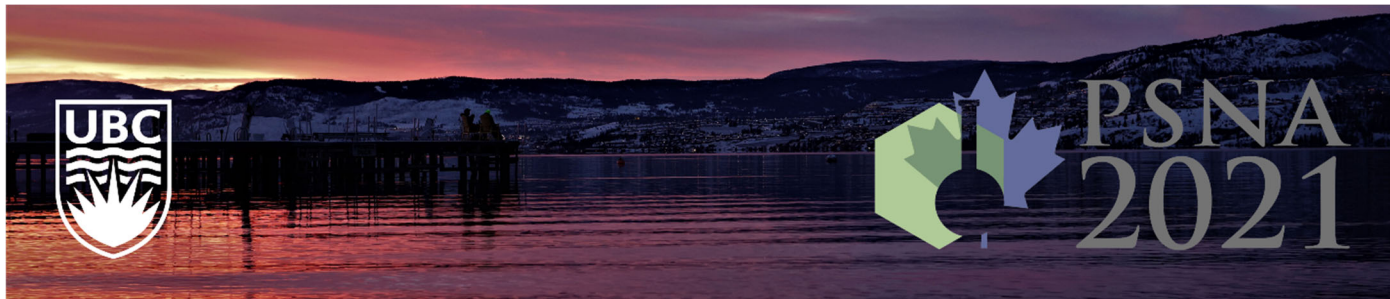
Glutathione conjugates: A tool to decipher glutathione transferase functions

Nikola Micic, Huijun Liu, Mette Sørensen, Nanna Bjarnholt

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Glutathione conjugates are compounds generated in a reaction between the tripeptide glutathione (GSH) and reactive organic species. The reaction can be catalyzed by glutathione-transferases (GSTs), a group of enzymes that are highly abundant throughout the plant kingdom. Traditionally related to xenobiotic detoxification and antioxidant response, the numbers of GSTs in plants are too high to be explained by these functions alone. Indeed, a few GSTs have been identified as having functions in specialized metabolism where GSH conjugates are biosynthetic pathway intermediates. However, the lack of identification of multiple endogenous GSH conjugates is holding back progress in elucidation of additional GST functions. To solve this issue, we employed liquid chromatography-mass spectrometry (LC-MS) to perform an untargeted search for GSH conjugates, detected by their specific fragmentation pattern, used as a valuable diagnostic tool. Field samples of four barley cultivars (*Hordeum vulgare*) and a greenhouse-grown fern (*Phlebodium aureum*) were used in the initial screening. Although evolutionarily distant, both plants contain species-specific GSH conjugates. The detected conjugates in barley were identified as aroma compound precursors (3-GS-pentanal, 3-GS-pentanol and 3-GS-hexanal or 3-GS-2-methyl-pentanal) as well as a conjugate of 12-oxo-phytodienoic acid (GS-OPDA), the precursor of jasmonic acid. Also, five unknown conjugates were detected in field samples of infected barley. In the case of fern, 3-GS-octanal and one unknown conjugate was detected. To induce GSH conjugate biosynthesis for identification of GSTs, leaves were subjected to mechanical damage. However, it is unclear whether the resulting increase were caused by enzymatic or chemical reactions. In both plant species, precursors of identified conjugates can be linked to oxylipins, a group of oxidized lipids prevalent during stress. Considering the regulatory and signalling potential of conjugate precursors and the detection of GSH conjugates in infected samples, we can speculate a potential regulatory role of GSTs in plant development, defense and signalling.



POSTER PRESENTATION ABSTRACTS

POSTER SESSION 1

15:30–17:00, JULY 27 (TUESDAY)

P1. Aldoximes: A metabolic hub for stress-triggered growth regulation

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Plant growth and stress response are tightly linked. Enhancement of plant stress tolerance often comes at the cost of growth, and vice versa. However, how stress response and growth are coordinated simultaneously remains poorly understood. Our recent studies revealed that a class of compounds called aldoximes play a role in stress-triggered growth regulation. Aldoximes are amino-acid derivatives and are best known as precursors of defense compounds such as glucosinolates in Brassicales. We found that aldoximes affect plant growth by modulating the production of growth hormone auxins and phenylpropanoids such as lignin. The accumulation of aldoximes represses phenylpropanoid production by activating the expression of the F-box genes that function in the degradation of phenylalanine ammonia-lyase (PAL). Since PAL functions at the entry point of the phenylpropanoid pathway, aldoxime accumulation affects the phenylpropanoid pathway significantly. Tryptophan- or phenylalanine-derived aldoximes are precursors of two major auxins, indole-3-acetic acid (IAA) and phenylacetic acid (PAA), respectively. Since well-characterized aldoxime-derived defense compound glucosinolates are found exclusively in Brassicales, aldoxime-derived auxin biosynthesis is thought to be family-specific. However, aldoxime production enzymes are found widely in the plant kingdom. Consistently, our study showed that various species, including maize produce auxins from aldoximes, suggesting the significance of this metabolic network in a multitude of different plants. Given that stress or stress hormones activate aldoxime production, the aldoxime-mediated metabolic network may play a crucial role in stress-triggered growth regulation.

P2. *Alternanthera flava* extract prevents bile duct ligation-induced cholestatic liver damage *in vivo*

Jesus Ramos-Herbert¹, Jazmín Guadalupe Hernández-Martínez¹, Liseth Rubí Aldaba-Muruato¹, Alejandro Hernández-Morales², José Roberto Macías-Pérez¹

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Liver inflammation plays an important role in the pathophysiology of cholestatic liver diseases. *Alternanthera flava* (*A. flava*) is a plant used in Mexican traditional medicine as muscle anti-inflammatory. Therefore, the present study aimed to evaluate the ability of *A. flava* to prevent acute liver injury in a murine model. For the *in vivo* study, bile duct ligation (BDL) for 48 h was used as a model of hepatobiliary damage. Wistar rats were divided into four groups: Sham group, BDL group, BDL + *A. flava* group (50 mg/kg, p.o., daily dose, 3 days before surgery and until sacrifice) and *A. flava* group (Sham + *A. flava* treatment). Macroscopically, the livers of the BDL + *A. flava* group showed less liver damage than the BDL group. Meanwhile, the liver weight/body weight ratio was significantly higher in the BDL + *A. flava* group. In addition, hematoxylin and eosin tissue stain showed that the BDL group presented marked liver necrosis, inflammatory cell infiltration, and bile ducts proliferation while *A. flava* treatment prevented this damage. Likewise, periodic acid-Schiff stain exposed that the BDL + *A. flava* group showed a higher amount of liver glycogen than the BDL group. On the other hand, *A. flava* prevented the significant serum increase in the alanine aminotransferase enzyme activity while the gamma-glutamyl transpeptidase enzyme activity have increased significantly in the BDL group but remained normal in the BDL + *A. flava* group. Finally,

the total and direct bilirubin showed slight decreasing tendencies in the BDL + *A. flava* group. These results show for the first time that *A. flava* protects against the liver damage caused by acute obstructive cholestasis, highlighting its anti-necrotic and anti-cholestatic properties.

P3. Analysis of table grapes for its bioactive composition and fungal endophytes

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Grape (*Vitis vinifera*) is a rich source of bioactive molecules including flavonoids, anthocyanins and stilbene compounds. In addition to their nutritive values, these compounds possess antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic activities and have wide applications in food and nutraceutical industries. Endophytes are systematically distributed asymptotically in plant tissues such as roots, stems, leaves, seeds and fruits. In the last decade, endophytes have sparked a great deal of interest with their immense diversity and unique features that show potential in being useful tools in agricultural, industrial, and pharmaceutical applications. Interestingly, recent evidence showed few strains of fungal endophytes producing polyphenol compounds in grapes as well. Grape phytochemical and fungal endophyte community studies are more focused on wine grapes whereas documented endophyte research on table grapes are limited. The objective of this study is to show the composition of bioactive phytochemicals and fungal endophytes of table grapes in Winnipeg market to identify their potential interactions. Grape polyphenols were extracted and analyzed using high performance liquid chromatography (HPLC) in cultivars such as Flame, Autumn Royal, Sweet Scarlet and Red Globe. Amplicon ITS (internal transcribed spacer) metagenomics approach was used to profile the fungal communities of the table grape endophyte microbiome. This study showed containing bioactive compounds and fungal endophyte diversity in commercial table grapes found in market. However, further research is needed to develop a deeper understanding of bioactive-endophyte-host relationships and the metabolic contributions given through this exchange.

P4. Analysis of the macroscopic signs of the skin and in an *in vivo* model using a flavanone of natural origin as a possible anti-psoriasis compound

Karla Fernanda González-Pedroza^{1,2}, Valeri Dominguez-Villegas³, Berenice Andrade-Carrera³, Isaura Quintana-Padilla⁴, Ana Calpena Campmany⁵, María Luisa Garduño-Ramírez²

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Psoriasis is a skin condition considered an autoimmune disease that causes: Deterioration of the epidermal layer, severe itching, plaque formation, red peeling and papules; It may be due to a genetic predisposition, since the affected genes are involved in the control of the immune system. Affecting a third of patients with psoriatic arthritis due to the resistance of TNF-alpha blockers, for which the research for new molecules for its treatment. It is known that the interleukins IL-23 and IL-12 are key in psoriasis, stimulating skin lesions that occur due to chronic inflammation, triggered by proliferation of T lymphocytes, for which the search for inhibitors represents an important and new therapeutic target. In this contribution, the photographic analysis showed macroscopic signs of the skin to demonstrate the *in vivo* anti-psoriatic efficacy of flavanone (2S)-5,7- dihydroxy-6-methyl-8-prenyl-flavanone

P5. Antiproliferative activity and antimicrobial susceptibility of *Hippocratea Excelsa* Kunth. and *Annona muricata* L., in MDA-MB-231, MCF-7 and HeLa cancer cells

Alejandra Ortiz-González¹, María Guadalupe Hernández-Linares², Maura Cárdenas-García¹, Ana Marta De Los Ángeles Lobo-Sánchez³

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The population uses products of natural origin indiscriminately to alleviate various ailments among which are mentioned infectious processes and cancer, which has caused that in recent years the consumption of medicinal plants is increasing, being used as

substitutes or in combination with therapies prescribed by doctors, without considering that there are no systematic studies to evaluate their effect and safety. In Mexico, traditional medicine continues to be a reference for the use of these remedies, including two native species, *Hippocratea Excelsa* "cancerina" and *Annona Muricata* "guanábana". The objective of this work was to evaluate and assess the antiproliferative activity of these two species in breast and cervical cancer cells, as well as their effect on antimicrobial susceptibility in multiresistant strains of intrahospital origin such as *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The extracts were prepared from the dosage suggested by the people who practice Traditional Medicine in Puebla, by the Soxhlet extraction method, with 2 different solvents; ethanol and water. Showing that the solvent used and the concentration of the extract influence the activity presented. For the antiproliferative effect on cancer cells, a significant decrease in proliferation was found with the aqueous extracts especially for breast cancer cells, as well as an effect on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* strains by the ethanolic extract of cancerin, with results even better than those obtained with the control antibiotics. These results make it clear that more tests should be carried out on the action of natural products to suggest their use as adjuvants, in a standardized and safe way by the population.

P6. Assessing the effects of volatile repellents on the invasive spotted lanternfly, *Lycorma delicatula* (White)

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Spotted lanternfly [*Lycorma delicatula* (White)] (Hemiptera: Fulgoridae) (SLF) is a large piercing-sucking insect, native to Asia and newly invasive to the United States. Since its arrival in Pennsylvania in 2014, SLF has spread to an additional 10 states and has caused damage to a variety of ornamental plants, trees, and fruit crops, most notably, grapes. The insect is poised to have devastating consequences to agroecosystems and forests, demanding a better understanding of its ecology. While classical insecticides kill nymphs and adults, the ability to repel SLF from crops is of greater importance to prevent infestations in the first place. Volatile secondary plant metabolites are used to repel arthropods, managing infestations from planting seed to post-harvest storage. Recent studies in Asia suggest that the essential oil of lavender [*Lavandula* spp.] and its predominant monoterpene constituent, linalool, repel SLF nymphs and adults in field and laboratory settings. To corroborate these findings under field conditions in the United States, we tested the efficacy of lavender oil in a field site in Virginia. Retention of adult SLF was monitored on its preferred host plant, tree-of-heaven [*Ailanthus altissima* (Mill.) Swingle] in comparison to cultivated grape [*Vitis rotundifolia* 'Carlos']. Preliminary analysis showed significant repellent effects on SLF adults positioned on tree-of-heaven, while effects on grapes were less pronounced. Overall retention rates were higher on tree-of-heaven compared to grape, which may be due to prior experience of individuals feeding on tree-of-heaven. Additional assays in the laboratory and field are required to determine host plant effects on the efficacy of repellents. Moreover, we will test other plant-derived repellents in olfactometer and choice assays for subsequent field studies and applications in vineyards.

P7. Assessing the role of the ascorbate content of rice accessions of the RDP1 on high night temperature stress tolerance

Cherry Quiñones¹, Kharla Mendez¹, Shannon Cunningham¹, Wenceslao Larazo¹, R. Shea Harris¹, Zachary C. Campbell¹, Karina Medina-Jimenez¹, Hannah Seats¹, Abigail Wilkie¹, Mathew Luster¹, Arlene Adviento-Borbe³, Harkamal Walia⁴, Argelia Lorence^{1,2}

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Greater than 3.5 billion people globally depend on rice for more than 20% of their daily calories. Rice is Arkansas' second-highest-value commodity and the top agricultural export. However, rice production is continuously challenged by abiotic stresses, including high night temperature stress (HNT). HNT affects both rice yield and grain quality, but the underlying physiological and metabolic processes are not yet fully understood. Ascorbate (AsA) in plants is a key component in the heat, cold, drought, and salinity stress response. Previous studies have concluded elevated ascorbate content contributes to a plant's abiotic stress tolerance; however, the potential role of AsA in HNT stress tolerance is unknown in rice. In this study, six high-tunnel greenhouses (3 ambient; 3 HNT) were successfully built in a state-of-the-art field experimental station in Harrisburg, AR. These movable infrastructures, equipped with heating and cyber-physical systems, housed 310 rice accessions from Rice Diversity Panel 1 (RDP1) arranged in a randomized block design with three replications. The rice accessions were exposed to HNT stress of +4°C relative to ambient air temperature for two weeks during the reproductive stage. Rice accessions Baldo and NSF-TV 27 showed high AsA content and no difference in AsA content, respectively, when exposed to HNT compared to ambient treatment. Other accessions, such as Binulawan and Kasalath, showed low ascorbate content after exposure to HNT compared to ambient. Quantification of reduced, oxidized, and total AsA in the

rest of the RDP1 is ongoing to fully assess the role of AsA in HNT tolerance. This study will lead to the identification of novel genes and pathways which can be used by rice breeders and molecular biologists to develop rice varieties resilient to HNT stress.

P8. Developing omics for investigating poison ivy (*Toxicodendron radicans* (L) Kuntze) urushiol metabolism

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Urushiols are alkylcatechol natural products responsible for causing the clinical symptoms of poison ivy rash. While urushiol chemical structures are well documented, none of the genes and enzymes responsible for urushiol biosynthesis are empirically characterized. The Jelesko laboratory has developed a suite of poison ivy “omic” resources to pursue a molecular and biochemical characterization of urushiol biosynthesis. A general framework for alkylphenol biosynthesis was proposed by Giessman in 1966 and serves as framework for investigating urushiol biosynthesis. GC-MS is routinely used to identify urushiol congeners, as well as intermediary metabolites proposed for urushiol biosynthesis. Posited alkylresorcylic and alkylresorcinol metabolites have not been identified in our poison ivy extracts. In contrast, both anacardic acid and cardanol metabolites accumulate in poison ivy tissues. Nascent germinating seedlings produce unusual cardanol isomers and corresponding urushiol isomers, suggesting the conversion of cardanol to urushiol may be the final step in urushiol biosynthesis. We have developed a leaf and root de novo transcriptome assembly, as well as an unpublished draft poison ivy whole genome sequence assembled from PacBio and Minlon reads. The poison ivy WGS is approximately 404 MB, assembled into 3,743 scaffolds, with 22,843 gene. Physiological validation of putative urushiol biosynthetic genes will require gene editing and/or gene suppression in differentiated poison ivy tissues. To these ends, we have developed both Agro-infiltration transient expression in poison ivy leaves, as well as stable genetic transformation and regeneration of poison ivy hairy root culture cultures. Transgenic poison ivy hairy roots produce significantly less urushiol than wild type control roots, but greater than the undetectable urushiol levels in undifferentiated poison ivy callus culture. In summary, we have developed a suite of omics resources suitable for urushiol metabolic gene discovery and characterization in poison ivy.

P9. Effect of different Cu(II)-to-Mo(VI) ratio in nutrient solution on the concentrations of some mineral nutrients in *Helianthus annuus* seedlings

Alan Alexander Gonzalez Ibarra, Eunice Yanez Barrientos, Kazimierz Wrobel, Alma Rosa Corrales Escobosa, Katarzyna Wrobel

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Molybdenosis is a disease affecting ruminants exposed to excessive dietary intake of molybdenum. Mo overload is a principal cause of the secondary deficiency of copper, leading to the oxidative damage and the disturbed carbohydrate metabolism in these animals. To prevent adverse effects of Mo-Cu antagonism, it is recommended that animal feeds contain at least two times more Cu than Mo. Since ruminant diet includes the elemental composition of the plant material consumed, this work has been focused on the selection of the concentrations and of the molar ratio between of Cu and Mo in the nutrient solution to achieve favorable profile of principal mineral micronutrients in hydroponically grown *Helianthus annuus*. Twenty-seven seedling cultures were obtained using Hoagland solution containing different concentrations of Cu (0-10 μ M), Mo (0-50 μ M) and their ratios (nine different conditions, three replicates per each). The determination of Cu, Mo, Na, K, Ca, Mg, Fe and Zn was performed in roots and in aerial parts by atomic emission spectrometry with excitation in microwave plasma (MP-AES). To observe data structure in the reduced dimensionality, the obtained results were submitted to principal component analysis (PCA). In the PCA model based of two PCs, the samples exposed to the increased Cu concentrations (with respect to typical Hoagland composition) were clearly separated from those exposed to the increased Mo concentrations whereas the cultures obtained under typical or “intermediate” conditions, formed a third group. The variables associated with Cu- and Mo- exposed seedlings were Cu, K and Mo, Fe, respectively; it is of note that Fe was also inversely dependent on Cu level in medium. The obtained results were further analyzed by computing surface responses. It was concluded that the levels of Cu, Mo and other mineral nutrients in hydroponically grown sunflower might be at least partly regulated by selecting appropriate concentrations of the two elements in medium.

P10. Effects of auxins on anthocyanin biosynthesis in suspension culture of engineered *Artemisia annua* red PAP1 cells

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All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)

PAP1 is a R2R3-MYB transcription factor that regulates the biosynthesis of anthocyanin. *Artemisia annua* is the only natural source that provide artemisinin for malarial treatment. To develop novel cells from *A. annua*, we used the *PAP1* gene to engineer red cells and further developed suspension culture of *PAP1* red cells. Naphthaleneacetic acid (NAA), indole 3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D) are three auxins used in our study. In this report, we report effects of the three auxins on the growth suspension cells and anthocyanin biosynthesis. Ten concentrations, 0, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 15, and 20 μM , were tested in suspension media for each auxin. Suspension cells were cultured for 20 days to collect cells to measure fresh weight and analyze anthocyanins. The resulting data revealed different effects of the three auxins and their concentrations on cell growth and anthocyanin biosynthesis. These data are valuable to understand anthocyanin biosynthesis in suspension cells. Detailed mechanisms will be discussed in our presentation.

P11. Establishing a characterization strategy for *Withania somnifera* extracts using LC-HRMS/MS in combination with GNPS networking analysis

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The dynamic metabolome of plants combined with unstandardized extraction procedures creates several challenges in achieving botanical integrity, batch-to-batch reproducibility and product stability of botanical supplements. The phytochemical composition of the plant extract will govern not only the bioactivity and potential health benefits but also the reproducibility of clinical trials. Knowledge of chemical profiles and quantification of marker compounds for standardization purposes is necessary to minimize variability in biological response. Here, in addition to the quantification of well-established *Withania somnifera* marker compounds, we have developed a method for the characterization of *Withania somnifera* in which GNPS networking analysis assists in dereplication. *Withania somnifera* (WS) is a medicinal plant used to support resilience to neurological changes associated with aging. WS is getting attention due to its cognitive, antidepressant and anxiolytic effects found in preclinical models. WS produces a complex group of steroidal lactones known as withanolides that are considered as the main active compounds. In this study, WS water and ethanolic extracts were analyzed by combining liquid chromatography with high-resolution mass spectral data acquisition. More than 10,000 *m/z* molecular features (deconvoluted detected ions) containing MS/MS data were recorded using positive electrospray ion mode acquisition. Using the same raw data, we quantified seven phytochemicals by external calibration with authentic standards, including six withanolides. We uploaded the WS spectral data onto the Global Natural Products Social Molecular Networking (GNPS) algorithm. GNPS is a web-server tool for MS/MS data classification, matching with available online spectral data to identify known compounds, and for sharing of results with the research community. The GNPS algorithm creates a network of structurally related compounds whose nodes are connected by similarity in their mass fragmentation patterns. This procedure adds in dereplication and may guide prioritization of further structure elucidation efforts by orthogonal techniques such as NMR spectroscopy and testing of bioactivity.

P12. Evolution of diterpenoid gene clusters in Lamiaceae

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Evolution is the driving force behind the multitudes of biological diversity seen today. However, the full scope of evolutionary mechanisms are still not well understood because of their complexity and the long timeframe over which the process takes place. Advancements in sequencing technologies over the last few decades have seen a tremendous spike in accessibility and understanding evolution. With the wide availability of genomes today, it is important to study evolution so that we can have a better understanding of how various traits become favorable at a fundamental level. Lamiaceae, or the mint family, contains a variety of aromatic species including culinary and medicinal herbs. In this family, terpene metabolism has expanded to produce an expansive and diverse list of natural products, with over 3,000 distinct diterpenoids reported. Many terpenoids are high-value compounds used in pharmaceuticals, agriculture, cosmetics, and flavors. Genes involved in the same specialized metabolism pathways have commonly been found in biosynthetic gene clusters, where genes are physically clustered within the genome. Beautyberry shrub, or *Callicarpa americana*, is one of several species known to host a biosynthetic gene cluster containing the pathway for producing the diterpene miltiradiene, a precursor to many compounds providing a variety of health-promoting benefits. Several other species are known to host a similar cluster, and these species have highly diverged from each other within the Lamiaceae. Terpenoid biosynthetic gene clusters represent an evolutionarily flexible system, allowing us to use genomic evidence to expand our knowledge of specialized metabolism evolution.

P13. Exploring the *Cannabis* microbiome to develop safe and sustainable bio-fungicides

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The importance of the plant-microbiome with regard to overall plant-health has been highlighted by research in the 21st century. Plants adapt to their environment by hosting beneficial microorganisms that confer selective advantages in stressed conditions. These microorganisms can contribute to plant-health by assisting in nutrient acquisition, secreting plant-growth promoting phytohormones, or antagonizing pathogens to prevent disease. Pathogenic microorganisms are responsible for around \$220 billion in crop loss annually, and in general, growers are heavily reliant on the use of chemicals to control plant-pathogens. The widespread use of pesticides is often costly to growers, the environment, and human health. Pathogen control in *Cannabis* has seen little research. Both industrial hemp and drug-type *Cannabis* are limited in the tools available to control pathogens, and currently no conventional fungicides are approved under current regulation. The use of "biologicals" has become a widespread practice in modern agriculture, and the use of biocontrol agents such as *Bacillus* and *Trichoderma* have become practice in the *Cannabis* industry. However, there has been limited bioprospecting for beneficial microorganisms well adapted to *Cannabis* and its major pathogens. My research investigates the seed-vectored and inherited microorganisms that act as the founding microbiome inside root and shoot tissue of new *Cannabis* seedlings. These endophytes possess a variety of beneficial characteristics, are vertically transmitted across plant generations, and are being screened for their ability to control the *Cannabis* pathogens: fusarium, botrytis, and pythium. My thesis investigates whether these microorganisms can be used as safe and sustainable biofungicides in the emerging *Cannabis* market.

P14. Flavonol specific 3-O glucosyltransferase (Cp3GT) mutant S20G+T21S: Enzyme structure and function

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Flavonols are a major subclass of flavonoids and are considered the most abundant subclass of flavonoids and are widely spread in nature. Flavonols are classified as having a hydroxyl group in the 3rd position of the C ring. The most prevalent modification to flavonols is glucosylation which adds glucose to an acceptor molecule. The flavonol specific 3-O glucosyltransferase (Cp3GT) enzyme from grapefruit (*Citrus paradisi*) is the topic of this research and specifically adds glucose to flavonols at the 3-OH position. The level of activity varies depending on the flavonol structure. This makes Cp3GT an ideal model system for studying the structure/function relationship of Cp3GT site-directed mutants. S20G+T21S is a mutant form of Cp3GT that was created by site directed mutagenesis previously. As compared to the wild type Cp3GT, S20G+T21S has significantly higher activity with kaempferol as well as altered levels of activity with other flavonols. One of the more striking differences of S20G+T21S is its ability to add glucose to the 7-OH position of the flavanone, naringenin thus showing a change in flavonoid class specificity as well as regiospecificity for position of glucose attachment. The S20G+T21S mutant was transformed into *Pichia pastoris* using electroporation. Transformation was verified by colony PCR and DNA sequencing and a time course analysis of expression conducted. Optimal expression previously occurred at 24-48 hours and will be verified by SDS page and western blot. Progress on optimizing purification in preparation for crystallization will be reported.

P15. Foxglove, *Digitalis lanata*, transcriptome and genome leads to identity of digoxin biosynthesis enzymes including a putative cholesterol monooxygenase P450scc

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Digoxin is an important heart medication that is produced from the leaves of *Digitalis lanata*. It is part of a larger group of molecules known as cardenolides that have a wide variety of therapeutic effects. Efforts to improve the production of these compounds have been hindered due to the lack of knowledge of the enzymes that produce these molecules. The currently proposed digoxin pathway begins with cholesterol and consists of nine steps that are catalyzed by cytochrome P450s (CYPs), malonyltransferases (MTs), and glucuronosyltransferases (UGTs). Previous research has identified two enzymes of the pathway; however, lack of available genomic data has hindered efforts to identify more candidates. Here we report our draft assembly of the *D. lanata* genome with a size of 779 Mb, a contig N50 of 281Mb, and BUSCO score of 92.5%. In addition, we present transcriptomic data which was used to identify possible digoxin biosynthesis genes. Utilizing differential expression analysis of the leaf and root transcriptome, we identified 256 digoxin biosynthesis enzymes, consisting of CYPs, MTs, and UGTs whose expression was increased in the leaves, correlating to cardenolide production. These data combined with protein homology and phylogenetic analysis, allowed us to identify 23 candidate enzymes that are likely involved in cardenolide production. Protein modeling and docking have revealed one candidate enzyme to be a putative cholesterol side-chain cleaving enzyme. Preliminary biochemical analysis shows its ability to modify cholesterol. Our data

reveals potential cardenolide-producing enzymes which, when fully characterized, can be introduced into microbes for the quick and efficient production of digoxin and other cardenolides.

P16. Hepatoprotective effect of *Mansoa hymenaea* during acute cholestasis in rat

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Cholestasis refers to bile ducts obstruction and the subsequent accumulation of toxins in the liver, which can induce inflammation, necrosis, fibrosis, cirrhosis, and finally end-stage liver disease. Therefore, this study aimed to evaluate the hepatoprotective effect of the ethanolic extract of *Mansoa hymenaea* (*M. hymenaea*), a plant used in traditional Mexican medicine to treat colds, fever, and rheumatic pain. Male Wistar rats were divided in four groups: Sham group, BDL group (bile duct ligation, 48 h), BDL+ *M. hymenaea* (*M. hymenaea* extract, 200 mg/kg, p.o. daily doses, 3 days before surgery and until sacrifice) and *M. hymenaea* group. Our results showed that macroscopic architectures of livers were preserved in the BDL+ *M. hymenaea* group concerning the BDL group. These observations were consistent with microscopic analysis by Hematoxylin and eosin and periodic acid-Schiff stains, which showed less necrosis, inflammatory infiltrate and ductular proliferation in the BDL+ *M. hymenaea* compared to BDL group. The serum markers of liver damage (alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, and total and direct bilirubin) were significantly reduced in the BDL+ *M. hymenaea*. Our results show for the first time that ethanolic extract of *M. hymenaea* can prevent cholestatic liver damage in rat.

P17. *In silico* and *in vitro* prediction of the antiproliferative activity of *Opuntia joconostle* in breast cancer cell lines

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Cancer is considered one of the first causes of death in the Mexican population and breast cancer is especially the first cause in women due to the presence of malignant tumors. In spite of the existence of treatments, society looks for complementary treatments in Traditional Medicine, in species whose effects have not been studied. This is one of the reasons why patients abandon therapies or prolong the time of diagnosis, without scientific support, attending in advanced stages of diagnosis and with poor prognosis. Therefore, the objective of this work was to clarify the biological activity of *Opuntia joconostle*, a native species of Mexico used against cancer, in breast cancer cells, through a multidisciplinary work in order to determine its antiproliferative effect and the possible mechanisms of action in cancer cells. This was done *in silico* using the Big Data Cellulat program where the possible inhibitory concentrations at which the extracts could be used *in vitro* were determined and the simulation of hypotheses of the mechanism of action of the extract on different signaling pathways was also carried out. The extracts were performed by conventional and Soxhlet method in different solvents and then characterized by spectroscopic methods and titrated by XTT to determine the antiproliferative effect. The results showed a relationship between the concentration of the extract and its inhibitory effect, being the aqueous extract the one that presented a better effect on the cell lines. In conclusion, the aqueous extract of *Opuntia joconostle* shows a promising outlook for the treatment of breast cancer, while promoting the use of national products.

P18. Knockout of OsWRKY36 and OsWRKY102 boosts lignification with altering culm morphology of rice

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Breeding approaches to enrich lignins in biomass could be beneficial to solid fuel use and improving the biorefinery process, because lignins have much larger heating values than polysaccharides and represent a potent source of valuable aromatic chemicals [Umezawa, *Phytochem. Rev.*, 17, 1305–1327 (2018), Umezawa et al., *Lignin*, 1, 30–41 (2020)]. Our recent work has revealed that heterologous expression of *Arabidopsis thaliana* transcriptional activator, AtMYB61, in rice (*Oryza sativa*), a model grass species, enriched grass-specific lignin components, such as p-coumaroylated and triclin lignin units in cell walls, both of which are typical components in grass lignins [Koshiba et al., *Plant Biotechnol.*, 34, 7–15 (2017)]. In addition, the grass characteristic units were also augmented in rice mutants deficient in the transcriptional repressor gene, OsMYB108 [Miyamoto et al., *Plant J.*, 98, 975–987 (2019), Miyamoto et al., *Curr. Plant Biol.*, 24, 100174 (2020)]. In this study, we generated other rice transgenic lines deficient in OsWRKY36 and OsWRKY102, which encode putative transcriptional repressors for secondary cell wall formation, using CRISPR/Cas9-mediated targeted mutagenesis. Both OsWRKY36 and OsWRKY102 mutations significantly increased lignin content by up to 28 % and 32 %, respectively. Additionally, OsWRKY36/OsWRKY102-double-mutant lines displayed lignin enrichment of cell walls (by up to 41 %) with substantially altered culm morphology over the single-mutant lines as well as the wild-type controls. Chemical and NMR analyses showed that relative abundances of guaiacyl and p-coumarate units were slightly higher and lower, respectively, in the WRKY mutant lignins compared with those in the wild-type lignins. The results provide evidence that both OsWRKY36 and OsWRKY102 are associated with repression of rice lignification, strongly suggesting the WRKYs and their close grass homologs are promising breeding targets for improving the utilization properties of grass biomass [Miyamoto et al., *Plant Sci.*, 296, 110466 (2020)].

P19. LC-MS metabolomic analysis of Se-exposed onion roots performed after derivatization with ethyl chloroformate

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Mechanisms underlying plants tolerance and Se accumulation/volatilization have been extensively studied within the context of plant science but also in relevance to human health and nutrition. Different species of *Allium* genus can transform inorganic selenium into organic species with demonstrated biological activity. To get further insight on Se biotransformation process, onion roots were hydroponically growth in the presence of sodium selenite (5mgSe L⁻¹, 25 mgSe L⁻¹) and non-exposed controls were obtained for comparative purposes. Ethanolic root extracts were submitted to derivatization with ethyl chloroformate, in order to focus the analysis on amino acids and other compounds susceptible to this derivatization; since non-polar derivatives were extracted to chloroform, sample clean-up was achieved at this stage. Analysis was performed by capillary liquid chromatography - electrospray ionization quadrupole-time of flight mass spectrometry (maXis impact ESI-QTOF-MS, Bruker Daltonics). Raw data were pre-processed using Data Analysis 4.1. software (Bruker) and analyzed on XCMS platform (<https://xcmsonline.scripps.edu/>). Under criterion of fold change >8 at p < 0.05, several features presenting higher abundance in the exposed plants were detected. For identification, mass difference corresponding to derivatization, cationization and adduct formation were defined. Formation of two selenium metabolites (Se-methylselenocysteine and 2-selanylideneacetic acid) was observed together with up-regulation of some amino acids; alteration in formation of sulfur compounds has also been detected. The obtained results contribute to better understanding of mechanism underlying biosynthesis of methyl-Se-cysteine in onion roots.

P20. Mammalian melatonin agonist pharmaceuticals stimulate rhomboid receptors in plants

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Melatonin is a human neurotransmitter and plant signaling metabolite that perceives and directs plant metabolism but the mechanisms of melatonin action in plants are undefined. We hypothesized that roots have a melatonin-specific receptor and/or transporter. To test this hypothesis *Arabidopsis* seedlings were grown with melatonin pharmaceutical receptor agonists: Ramelteon and Tasimelteon, and/or antagonists: luzindole and 4-P-PDOT. Ramelteon was found both to mimic and competitively inhibit melatonin in plants. Due to the higher selectivity of Ramelteon for the MT1 receptor type, a sequence homology search for MT1 in *Arabidopsis* identified the rhomboid-like protein 7 (RBL7). In physiological studies, *Arabidopsis* RBL7 mutants were less responsive to both Ramelteon and melatonin. Quantum dot imaging studies of Ramelteon effects on melatonin binding to root cell membranes revealed a binding mechanism. We propose that RBL7 is a receptor for melatonin that directs root architecture and growth in a mechanism that is responsive to environmental factors.

P21. Metabolic organization and enzymology of the flavonoid biosynthetic pathway in *Citrus sinensis*

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The metabolic organization and enzymology of biosynthetic pathways is critical for plant survival and has direct impacts on the production of agriculturally and medicinally significant metabolites. It has long been considered that metabolic channeling impacts cellular metabolism. Enzymes of a biosynthetic pathway that form protein-to-protein interactions which generate a supramolecular complex that can channel products among the components are known as metabolons. In this work, the formation of flavonoid biosynthesis as a metabolon is being analyzed in blonde and blood orange (*Citrus sinensis*) varieties. Blood orange uniquely accumulates anthocyanins, a late pathway flavonoid subclass, producing a darkly pigmented fruit. By analyzing flavonoid metabolon organization in both varieties, we are investigating the hypothesis that the production of unique metabolic end products is impacted by metabolon organization. Furthermore, current metabolon models are being expanded to include flavonoid derivatizing enzymes, such as glycosyltransferases. Metabolomic analysis in all plant species to date has shown very little, if any, accumulation of aglycone flavonoid structures. Therefore, the contribution of derivatizing enzymes to metabolon formation as well as impacts on accumulation of flavonoid end products is an overlooked and critical component of the system. A long-term goal of this research is to develop a better understanding of enzyme pathways and their organization that will contribute to improved strategies for increasing the production and biosynthesis of specific metabolites of interest.

P22. On the extractability, stability, and accumulation of nepetoidins in *Ocimum basilicum* L. leaves and cell cultures

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Antioxidant polyphenols nepetoidin A and B are widely spread in Lamiaceae family yet often remain undetected due to their purported instability and unusual exclusive extractability with nonpolar solvents. Here we analyzed the extractability and occurrence of nepetoidins in tissues of sweet basil (*Ocimum basilicum* L.). Extractability tests showed co-extraction of nepetoidins with the major polyphenol rosmarinic acid was possible with solvent mixtures, yet substantially less efficient than that with the respective preferred solvents. Nepetoidin, but not rosmarinic acid levels in extracts from dried leaves were dramatically reduced. However, this was not due to degradation suggested by earlier reports, but to reduced extractability, which could be restored by wetting the tissue with water prior to addition of nonpolar solvent. Nepetoidins were found to accumulate at high levels in basil callus and suspension cultures. Correlation analysis revealed several novel compounds that are coextracted with nepetoidins. Candidate signals for nepetoidin and rosmarinic acid glycosides were also detected in dedifferentiated basil cells. Metabolomics analysis of callus extracts produced with different solvents suggested the use of mixed solvent for the most comprehensive coverage of the metabolomic diversity. By contrast, nepetoidins were not enriched in basil peltate trichomes. This study establishes optimized recovery procedure for nepetoidins, reports their production by dedifferentiated basil cells, and illustrates how sample preparation methodology affects the phytochemical findings.

P23. Preparation of a unique flavonol glycosyltransferase Cp3GT for crystallization

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Glycosyltransferases (GTs) are enzymes found throughout the plant and animal kingdoms that catalyze the transfer of a sugar moiety to secondary metabolites such as flavonoids. In plants, glycosylated flavonoids have been shown to protect plants from UV light, attract pollinators, and facilitate the colonization of nitrogen-fixating *Rhizobia* species. In humans, glycosylated flavonoids have been shown to lower risk of cardiovascular disease, reduce inflammation, and inhibit tumor growth in some cancers. While ubiquitous in nature and structurally similar, GTs vary greatly with regards to substrate/regio-specificity. Utilization of GTs for the engineered production of physiologically active flavonoid glucosides relies on high resolution structural data generated using X-ray crystallography. A GT found in grapefruit (Cp3GT) exclusively glycosylates flavonols at the 3-OH position and is structurally similar to 2 previously crystallized plant GT's with complimentary but distinct specificities for anthocyanidins and flavonols (VvGT1 and UGT78K6). Structural mechanisms have been proposed using solved crystal structures that explain anthocyanidin and flavonol/anthocyanidin specificity, however, no structural model exists that explains exclusive flavonol specificity exhibited by Cp3GT. Wild type Cp3GT was recombinantly expressed in *Pichia pastoris*, purified using cobalt affinity chromatography, and concentrated. A 24-well matrix was designed to screen conditions necessary to induce crystallization. This was conducted using the hanging drop method of vapor diffusing with varying concentrations of a PEG 10000 precipitant. Concentrations of 18-22% PEG 10000 were previously shown to induce precipitation. Additional screens will be performed to optimize other conditions. Progress toward obtaining a Cp3GT crystal of suitable size and quality for X-ray diffraction and analysis.

P24. Quality and consistency of ma'afala as a source of flour

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Breadfruit (*Artocarpus altilis*) is an underutilized staple crop originating from the Pacific Islands and recently introduced into worldwide distribution for food security and economic benefits. Ma'afala is a commercial breadfruit cultivar that contains essential amino acids at levels higher than many other staples including wheat, corn, rice, soybean, and yellow pea. Flour produced from the cultivar Ma'afala is more digestible than wheat flour, has high-energy value, is gluten-free, and has no toxicity in chemical and histological analysis. To determine the potential impacts of differing growth practices and environments we investigated the starch, and protein content of Ma'afala fruit and flour grown under different agroforestry regimes and environmental conditions. We hypothesize that all Ma'afala trees have similar nutritional content irrespective of their growing conditions. We analyzed fruit, starch, and flour characteristics from 86 clones originating from a single Ma'afala tree (maternal) that were planted at 6 locations in Hawaii. The locations were selected to represent the wet and dry sides of the islands, irrigated and non-irrigated trees, and different agricultural management practices. We found significant differences in starch yield, starch-bound protein content, and flour protein content obtained from the different locations. Precipitation was positively correlated to breadfruit nutritional content. The maternal tree had the highest starch and flour protein content which was similar to its clone grown under the same model and in the same location, but clones grown in other locations were variable. These results showed that the location where Ma'afala is grown influences its nutritional quality and future studies should investigate other agronomic conditions that might influence these differences.

P25. Redundant roles of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase isoforms of the shikimate pathway during *Sclerotinia sclerotiorum* infection in *Arabidopsis thaliana*

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The shikimate pathway directs carbon flow from central carbon metabolism towards the biosynthesis of aromatic amino acids (AAAs) in plants and microbes. AAAs are not only essential for protein synthesis in all organisms, but are also used to produce countless plant specialized metabolites important in the nutrition, pharmaceutical, and biomaterial industries. 3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase (DHS) enzymes catalyze the first committed reaction in the shikimate pathway and are a potential bottleneck for the production of AAAs and their derived compounds. Of the three *Arabidopsis thaliana* DHS isoforms (AthDHSs), only AthDHS2 is feedback inhibited by tyrosine and tryptophan. The expression of *AthDHS1* and *AthDHS3*, but not *AthDHS2*, is strongly induced under various abiotic and biotic stresses, such as pathogen infection, suggesting that AthDHS isoforms have distinct roles in plants. In this study, to determine the *in vivo* roles of the three AthDHSs, the *dhs1*, *dhs2*, and *dhs3* single, and the *dhs2 dhs3* double knockout mutants were isolated and challenged to *Sclerotinia sclerotiorum*. Compared to wild type, these *dhs* mutants did not show any abnormal phenotypes under normal growth conditions nor significant changes in disease progression after *S. sclerotiorum* infection. Soluble metabolite analyses via liquid chromatography–mass spectrometry (LC-MS) showed that there were significant changes in levels of AAAs and tryptophan-derived defense compounds, such as indol-3-ylmethyl (I3M) and camalexin, between mock and fungal treatments. However, levels of these compounds were not significantly different between wild type and *dhs* mutants, including the *dhs2 dhs3* mutant that only has the AthDHS1 isoform. These findings revealed that *AthDHS1* alone was sufficient to complete the *A. thaliana* life cycle and maintain shikimate pathway activity, even during *S. sclerotiorum* infection. Thus, the lack of each AthDHS isoform could be compensated by the presence of other isoforms in *A. thaliana*, at least under the conditions tested in this study.

P26. Regulation of anthocyanin biosynthesis in engineered red *PAP1* calli of *Artemisia annua*

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Artemisia annua is an effective antimalarial medicinal plant. We overexpressed *Arabidopsis Production of Anthocyanin Pigmentation 1* in *A. annua* cells and engineered novel red cells. In this presentation, we report effects of auxins on cell growth and anthocyanin biosynthesis in different calli types. We tested three auxins, indole 3-acetic acid (IAA), naphthaleneacetic acid (NAA) and 2, 4-dichlorophenoxyacetic acid (2, 4-D). Ten concentrations (0, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 15, 20 μ M) were tested for the three auxins. Based on these concentrations, different media were prepared to grow cells for 20 days and then collected to measure fresh weight and anthocyanins. The resulting data showed that three auxin and their different concentrations differentially affected cell growth and anthocyanin production. Details of impacts will be discussed in our presentation.

P27. Synthesis of a new amidesteroid from diosgenin and evaluation of its antiproliferative effect in breast and cervical cancer cell lines

PSNA 2021

All times are in North America Pacific Time (Vancouver / San Francisco / Tijuana)

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Cancer is the second leading cause of death worldwide and in Mexico it is the third leading cause of death after heart and metabolic diseases. Especially in Mexican women, breast cancer is the leading cause of death due to malignant tumors, followed by cervical cancer, with figures that are increasing year after year in terms of incidence and prevalence. Although there are already known treatments, resistance to these and the presence of side effects makes it necessary to synthesize new compounds from active ingredients obtained from natural products, such as Diosgenin, which is obtained from *Dioscorea composita*, a tuberacle commonly known as "cabeza de negro". The production of these new compounds is intended to increase the options for new antineoplastic therapies with active principles that present greater biosafety and fewer negative effects. The aim of this work was to synthesize a new amidesteroid from Diosgenin and evaluate its antiproliferative effect in Hela and MDA-MB-231 cell lines. Performing modifications selectively on the B-ring of Diosgenin, through optimized methodologies, following a retrosynthetic analysis of 4 reactions in total; acetylation, oxidation, condensation and Beckmann rearrangement, thus obtaining 3 by-products and the final compound Amide (25R)-3 β -acetoxy-7-aza-B-homo-5-spirosten-8-one, which showed a good antiproliferative action on triple negative breast cancer cells. Leaving for the future the option of making modifications in this structure in order to achieve a more effective therapy against this type of cancer, which currently has the worst prognosis and the lowest survival rate.

P28. Terpenoid bioproduction in plants using product compartmentalization via lipid droplet scaffolds and engineered plastid pathways

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Leveraging synthetic biology approaches, engineered plants offer a sustainable production platform for high-value chemicals and other bioproducts. Squalene, a C30 hydrocarbon, is a biofuel candidate and the precursor to high-value triterpenoids, a diverse class of natural products with applications in the health, cosmetic, and other biotechnological industries. In this work, two strategies have been developed to increase plant production yields of squalene and triterpenoids by hijacking existing cell compartments or building novel compartments to sequester products within cells. Natively, squalene biosynthesis occurs in the cytosol through farnesyl diphosphate synthase (FDPS) and squalene synthase (SQS). The first strategy re-localizes this pathway to plastids, natural intracellular compartments, where biosynthesis can occur separate from native competing enzymes. In plastids, squalene yields were optimized through screening of diverse orthologs and engineered variants of key steps in the pathway. The second strategy re-engineers cytosolic lipid droplets as synthetic storage organelles with biosynthetic enzymes anchored to the surface, synthesizing and storing products in the same location. Fusing FDPS and SQS to the algal Lipid Droplet Surface Protein (LDSP) enables enzyme scaffolding on the surface of lipid droplets, which sequester the hydrophobic products. This SQS-LDSP-FDPS fusion protein can also be targeted to plastids, where it associates with plastid envelopes, thylakoid membranes, and plastoglobules. Furthermore, targeting the SQS-LDSP-FDPS fusion protein to plastids mediates negative effects on photosynthesis, possibly through further squalene sequestration within the associated membranes. These strategies effectively increased squalene yields using transient expression in *N. benthamiana* and are being implemented in stable poplar transformants, a target production crop. Effective compartment engineering improves plant production of squalene, while also providing platforms to expand towards higher-value triterpenoids and other bioproducts.

P29. The unique tyrosine-derived lignin pathway of grasses coordinately evolved with an enhanced tyrosine supply

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Grasses (the Poaceae family) undoubtedly represent one of the most agriculturally relevant crops and a dominant plant group in multiple biomes around the planet. The remarkable set of metabolic and physiologic innovations exhibited by this plant family include the enzyme phenylalanine/tyrosine ammonia lyase (PTAL), which confers the unique ability to synthesize lignin and other phenylpropanoids from the aromatic amino acid tyrosine, instead of relying exclusively on phenylalanine like other plants do. As vascular plants divert massive amounts of carbon into the biosynthesis of lignin, grasses may have undergone significant changes in the biosynthesis and homeostasis of tyrosine, the preferred substrate of PTAL. In this work, we have characterized arogonate dehydrogenase (TyrA) family enzymes, which catalyze the final step of tyrosine biosynthesis, in various grasses and other closely

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related monocot species. In most plants, TyrA activity is subjected to a tight tyrosine-mediated feedback inhibition, which is essential for preventing the over production of tyrosine and maintaining a fine balance with the other products of the shikimate pathway. By combining *in vitro* kinetic characterization and heterologous expression *in planta*, we have found that the TyrA3 isoform of grasses has a low sensitivity to feedback inhibition by tyrosine. This de-regulated isoform is co-expressed with many lignin biosynthetic enzymes, including PTAL, in the vascular tissues of grasses. Moreover, the characterization of TyrA3 orthologs in various species across the Poales and grass lineage revealed that TyrA3s have been subjected to positive selection, becoming increasingly more active and less sensitive to feedback inhibition during their evolution. Our findings uncover that the unique tyrosine-derived lignin biosynthesis of grasses is accompanied by a major change in the regulation of aromatic amino acid biosynthesis leading to increased allocation of carbon into tyrosine.

POSTER SESSION 2

15:30–17:00, JULY 29 (THURSDAY)

P30. Airborne fungus-induced biosynthesis of anthocyanins in *Arabidopsis thaliana* via jasmonic acid and salicylic acid signaling

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Anthocyanins are plant-specific pigments, the biosynthesis of which is stimulated by pathogen infection in several plant species. *A. thaliana* seedlings injected with airborne fungi can accumulate a high content of anthocyanins. The mechanism involved in fungus-induced anthocyanin accumulation in plants has not been fully described. In this study, the fungus *Penicillium corylophilum* (*P. corylophilum*), isolated from an *Arabidopsis* culture chamber, triggered jasmonic acid (JA), salicylic acid (SA), and anthocyanin accumulation in *A. thaliana*. Inhibitors of JA and SA biosynthesis suppressed the anthocyanin accumulation induced by *P. corylophilum*. The anthocyanin content was minimal in both the null mutant of JA-receptor *coi1* and the null mutant of SA-receptor *npr1* under *P. corylophilum* stimulation. The results indicate that JA and SA signaling mediated fungus-induced anthocyanin biosynthesis in *A. thaliana*. *P. corylophilum* led to different levels of anthocyanin generation in null mutants for MYB75, bHLH, EGL3, and GL3 transcription factors and WD40 protein, demonstrating that multiple MYB–bHLH–WD40 transcription factor complexes participated in fungus-induced anthocyanin accumulation in *A. thaliana*. The present study will help further elucidate the mechanism of plant resistance to pathogen infection.

P31. Are all kratom products created equal? Metabolomics of *Mitragyna speciosa* and commercial kratom products

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Mitragyna speciosa (Korth.) Havil. or kratom is a plant native to south east Asia traditionally consumed for its psychoactive properties. Commercial kratom products are sold through online vendors in Canada and in person shops in USA. In spite of widespread use, the regulatory status of kratom products is being debated and the FDA recently issued an import alert to detain products crossing the border. Better understandings of the phytochemistry of *M. speciosa* and kratom, including how the active alkaloids are biosynthesized, and how the chemical profiles of different cultivars vary will help to clarify issues of product quality, safety and regulation. We hypothesized that the chemical profiles of commercially produced kratom products are inconsistent. We used an untargeted UPLC-MS/MS metabolomics approach for experiments to: (1) establish a collection of plant material for studies, (2) determine the effects that different growth conditions have on the chemical profile of three *M. speciosa* varieties, and (3) compare six commercially available kratom products to authentic plant materials. We generated two untargeted metabolomics datasets. Putative identification annotated predicted intermediates in the alkaloid biosynthetic pathway leading to the main bioactive mitragynine. Chemometric analysis found little variance between varieties of *M. speciosa*, however, there was significant variance across the commercial samples. Further work is needed to understand the source of this variability that may be due to adulterants, fillers, or different post harvest treatments and to establish a standard chemical profile that represents high quality kratom products.

P32. Biocatalytic conversion of synthetic geranylgeranyl diphosphate derivatives by terpene synthases

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Terpenoids are the largest class of specialized metabolites in plants, with a range of practical applications such as antifedants, pharmaceuticals, flavors, and fragrances. This range of uses is reflected in their structural diversity, which arises from sequential modifications of a small subset of starting substrates leading to thousands of different compounds. Diterpenoids (C₂₀) are nearly all derived from a single molecule—geranylgeranyl diphosphate (GGPP)—with the first step in their biosynthesis carried out by either a single terpene synthase (TPS) or pair of TPSs to make terpene backbones. Given the use of a single starting substrate and the known promiscuity of many TPSs, we sought to evaluate the capability of a library of characterized TPSs to convert a range of synthetic GGPP derivatives. Here we have synthesized twenty GGPP derivatives and tested them with twenty-seven TPSs, leading more than a hundred successful combinations out of 516 unique combinations tested. Out of the TPSs tested, a range of promiscuity can be seen with some that have an exceptional ability to convert a range of synthetic substrates in high quantities, while others are highly specific for their native substrate. Current work is focused on solving the structures of a handful of products to gain mechanistic insight into the conversion of these substrates, and future work will involve the addition of downstream pathway enzymes such as cytochrome P450s and acetyltransferases to further modified these non-natural terpene backbones.

P33. Carbohydrates reserve: A potential critical phenotypic marker for high night temperature stress tolerance in rice?

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The relationship between high night air temperature (HNT) stress and carbohydrate partitioning is not yet fully elucidated, specifically in rice. Plant tissue carbohydrate status drives night-time respiration in rice, and an increase in HNT stress can elevate respiration rate. Plants with a higher capacity to store non-structural carbohydrates (NSC) in the stem can better tolerate changes in climatic conditions, including elevated night air temperature. However, this claim was proven only in two rice varieties grown under greenhouse conditions. In this study, a subset population was selected from 310 rice accessions cultivated out in the field, including 10 HNT-tolerant, 10 HNT-sensitive, and 10 hybrid varieties. The field experiment was laid out in randomized complete block design with three replications. Using high-tunnel greenhouses equipped with heating infrastructure, HNT stress was imposed when approximately 50% of the panel reached the flowering stage. A +4°C temperature difference was maintained between the ambient and heated greenhouses. Two main tillers were collected during flowering and dough growth stages and were separated into leaves, stem, and panicles. The anthrone colorimetric method was used to quantify soluble sugar and starch contents. NSC was computed by summing all soluble sugar and starch. In stems, during the flowering stage, preliminary results showed that non-structural carbohydrates were higher in a tolerant variety (NSF-TV 27), compared to a sensitive one (Tog 7178) and a hybrid (XP754). Additionally, the proportion of starch and soluble sugars was different among rice accessions/varieties. The tolerant variety had higher starch content, while soluble sugar was higher in the sensitive variety. NSC analysis is on-going for the remaining plant samples. The findings of this study will provide a vital phenotypic marker through quantification of carbohydrate reserves and will be useful in the development of high-yielding, HNT-tolerant rice varieties.

P34. Cardenolides in plant-insect interactions: *Syntomeida epilais* and its host plants

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Interspecific relationships between plants and insects are of particular interest, because of the different adaptations generated to evade predators. Plants belonging to the Apocynaceae family are known producers of cardenolides as part of their defense mechanisms; however, these metabolites can be sequestered by insects to use them as protection against the attack of predators. *Nerium oleander* (Apocynaceae) has been reported as the main host plant of the *Syntomeida epilais*, a moth whose larvae feed on the leaves of the plant and sequester oleandrin, a cardenolide which they use for their defense. Recent field observations made it possible to identify *Pentalinon andrieuxii* (Apocynaceae) as a new host plant of *S. epilais*; to date, however, the production of

cardenolides has not been reported for *P. andrieuxii*. In view of this, the objective of this work is to investigate the presence of cardenolides in *P. andrieuxii* and to establish their role in the interaction between the plant and *S. epilais*.

P35. Characterization of P297F glucosyltransferase in *Citrus paradisi*

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Grapefruit flavonol-specific 3-O-glycosyltransferase, Cp3GT, exhibits a high level of substrate and regioselectivity in its glycosylation of plant secondary metabolites. This reaction promotes stability, solubility, and detoxification allowing the plant to maintain homeostasis of secondary metabolites, more specifically flavonoids. Flavonoids are prominent plant antioxidants, provide protection against herbivores, provide attraction for pollinators, and many other functions. Despite glucosyltransferase's prominence throughout all eukaryotes, much about the structure and specificity of this enzyme has yet to be discovered. Of the hundreds of putative glucosyltransferases found in plant genetic databases, very few have been characterized and even fewer have been crystallized. This leaves much of the protein's precise structure unknown. Previously, a flavonol specific 3-O-glycosyltransferase found in *Citrus paradisi* was isolated from a cDNA library of young leaf tissue. Site directed mutagenesis was conducted on wild type Cp3GT to recombinantly produce a substitution at position 297 from proline to phenylalanine. This single mutation caused a total loss of GT activity. A freezer failure resulted in loss of this mutant which was subsequently reconstructed. Sequencing data showed the appropriate mutation with all tags in frame downstream from a thrombin cleavage site. P297F will be transformed into *Pichia pastoris*. The P297F mutant will then be expressed and purified via affinity chromatography with the intention of crystallizing the P297F protein and of determining three-dimensional structure via X-ray crystallography. Progress will be reported.

P36. Characterization of SIP-428: A NAD⁺-dependent deacetylase enzyme, in plant stress signaling

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SABP2-interacting protein 428 (SIP428) is a SIR2-type deacetylase, also called sirtuins. The SIP428 proteins belong to a family of NAD⁺-dependent deacetylase enzyme that was identified in a yeast-two hybrid screen using SABP2 as a bait. SABP2 is an important methyl salicylate esterase enzyme that catalyzes the conversion of methyl salicylic acid (MeSA) into salicylic acid (SA) during the pathogenic infection. Accumulation of SA induces systemic acquired resistance (SAR), a broad-spectrum defense mechanism in other uninfected distal parts of the plant. Sirtuins are known to play diverse roles in DNA repair, apoptosis, and stress responses. Cellular proteins are known to undergo posttranslational modifications such as protein acetylation which is a reversible modification that plays role in regulating transcription, activation, and deactivation of certain pathways by transferring acetyl group to lysine residues. This change neutralizes the positive charge of the amino group thereby affecting the biological function of the affected proteins. The main goal of this research is to understand the role of SIP-428 in abiotic stress, and to determine if its expression is altered upon pathogen infection. To understand better about the role of SIP-428 in plant physiology and how it plays a vital role in SABP2 signaling pathway we will be using transgenic tobacco plant in which the expression of SIP 428 has been silenced/knocked down. The SIP428 RNAi transgenic lines will be subjected to various abiotic stresses and to test the changes in gene expression upon pathogen infection, the bacterial pathogen, *Pseudomonas syringae* will be used. Subsequently, biochemical activity will be tested, and reverse transcriptase polymerase chain reaction (RT-PCR) will be used to analyze the SIP428 expression.

P37. Characterizing *Cannabis* metabolite profile and production at multiple developmental stages

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Cannabis sativa has a long history of medical, cosmetic, and textile application alongside the human civilization. Since the 1930s, restrictions on Cannabis production around the world has left a gap in the scientific literature reporting intensive research of *C. sativa*. However, recent legislative changes in the United States has led to a renewed investment from industry and an increase in academic research. To date, numerous studies that have been undertaken have sought to optimize growth conditions to maximize plant yield. This includes optimization of various environmental parameters such as soil, moisture, humidity, altitude, and light quantity and quality. On the one hand, progress has been made in the improvement of plant yields, on the other hand, whether the yield of desired metabolites have been increased remains open for investigation. Here, based on the fact that metabolite profiles and production vary across multiple developmental stages, we propose a different approach to understand the extent of those variations and the effects of variation on yields. To overcome the effects of segregation occurred in the progeny on metabolites, we use micropropagation to

generate genetically uniform populations and the grow plants in different growth conditions for metabolic comparison and measurement of desired metabolites at different development stages. Our approaches and findings are anticipated to enhance evaluation of desirable metabolites in quality and quantity.

P38. Composition and formation of epicuticular wax lining the abaxial and adaxial leaf surfaces of *Drimys winteri*

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The plant cuticle covering aerial organ surfaces of land plants is a crucial adaptation for preventing non-stomatal water loss and, thus, stress tolerance. Some cuticle surfaces are glossy, while some appear glaucous because of wax crystals protruding from them. These crystals are important for plant protection against insect herbivores/pathogens/high-light stress/dust accumulation. Here, we aimed to study the composition of surface wax crystals, to lay the groundwork for biosynthesis studies. For this, we investigated winter's bark (*Drimys winteri*), comparing its dark-glossy upper leaf surface with the pale-glaucous lower side. Nanotubule-shaped crystals were observed by SEM on the lower surface, in contrast to a smooth upper surface. Detailed chemical analyses (by GC-MS and GC-FID) showed that the wax mixture on the abaxial surface comprised more than 75% secondary alcohols (mainly C29 alkane-10-ol) and diols (mainly C29 alkane-10,X-diols), while the adaxial wax mixture contained only 25% of these compounds along with primary alcohols, alkyl esters and triterpenoids. Therefore, the amount of secondary alcohols and diols is ten times higher on the glaucous than on the glossy leaf surface, in accordance with previous reports on other species implying that the surface crystals are formed by the secondary alcohols. More detailed analysis (involving TLC fractionation) demonstrated that the functional groups of all secondary alcohol isomers and homologs detected were exclusively located on even-numbered carbon atoms along the hydrocarbon chain, suggesting that the secondary hydroxyls are introduced via Claisen condensation of biosynthetic precursors for the secondary alcohols in *D. winteri*. This is in stark contrast to *Arabidopsis*, where wax secondary alcohols are known to be formed via P450-dependent hydroxylation. Several C29 triols were also identified in the *D. winteri* wax mixtures, which underline the hydroxylation pattern found in diols. Those findings are essential for understanding hydrophobic wax formation, and for future studies into the biosynthesis of crystal-forming wax alcohols and bioengineering of artificial plant-like coating materials.

P39. Creating an *E. coli* platform strain to produce plant terpenes

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Terpenes are a large and diverse class of secondary metabolites produced by plants primarily for defense. This class of compounds includes myriads of medicinally and commercially important chemicals such as taxol, digoxin and geraniol. Currently, terpenes are produced industrially from plants or by chemical synthesis, however, these production methods are largely resource-/cost-inefficient. An economical alternative to large-scale production of terpenes is through the metabolic engineering of microbes. *E. coli*, a commonly used model organism, natively expresses the methyl-erythritol phosphate (MEP) pathway to provide the terpene building blocks for cell wall biosynthesis. This native pathway can be exploited to produce diverse terpenes by introducing heterologous genes. However, the productivity is often low due to the limited metabolic flux through the native MEP pathway. Though this challenge can be overcome by overexpressing the pathway in *E. coli*, an optimal level of overexpression is essential to avoid imposing a metabolic burden on the cells and maximize the productivity. To create an *E. coli* strain that can be effectively used as a platform to produce plant terpenes, we inserted an additional copy of its native MEP pathway into the genome using CRISPR/Cas9 technology coupled with λ -Red recombineering, as genomic integration imposes a low metabolic burden. We then used the monoterpene geraniol as a reporter of the pathway flux. We also modulated the expression level of the genomically integrated additional MEP pathway by using different promoters to maximize productivity.

P40. Distinct localisation patterns of alkaloids in *Daphniphyllum macropodum* revealed by Imaging MS

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Daphniphyllum macropodum (Daphniphyllaceae) plants are dioecious evergreens known for their ability to produce structurally diverse and complex alkaloids with unique polycyclic skeletons. Several of these molecules have shown valuable bioactive properties such as cytotoxicity against tumor cell lines, as well as antioxidant activity and vasorelaxant effects. Elucidating the localisation of these metabolites within the plant tissues has the potential to provide insights into their possible biological role, as well as, clues about

their biosynthesis. We investigated the localisation pattern of major alkaloids in *D. macropodum* using Matrix-assisted laser desorption/ionisation (MALDI)-MS imaging. Stems and leaves sections of the plant were coated with 2,5-dihydroxybenzoic acid (DHB) matrix and then analysed by MALDI-MS. The identified alkaloids in the stem and leaves, showed two distinct distributions. The first localisation pattern coincided with what we identified as the phloem from histochemical staining studies. The second group of compounds were localised to the epidermis. These contrasting localisation patterns seem to correlate with the differences in the chemical structure, with alkaloids putatively assigned to the same skeleton subtype having similar distributions. Alkaloid localisation within the epidermal cells may suggest a role in defence, as the epidermis is the most exposed tissue to environmental stressors. The localisation around the vascular tissues could be the result of presence of specialised cells associated with the phloem. Our observations indicate that *Daphniphyllum* alkaloids sub-types have distinct spatial distributions in the plant. Combining these observations with the localisation of the enzymes involved in the biosynthesis, for instance through *in situ* PCR, will improve our understanding of metabolic pathways leading to these compounds and the possible transport elements involved.

P41. Dynamics of anthocyanin biosynthesis during the growth of engineered red callus *Artemisia annua*

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Artemisia annua is the only anti-malarial medicinal crop to produce artemisinin, an effective medicine for the treatment of severe and malignant malaria caused by *Plasmodium falciparum*, which is resistant to other antimalarial drugs. In this presentation, we report anthocyanin biosynthesis in engineered red calli of *A. annua*. Red cells are engineered via the overexpression of PAP1, a master regulator of the anthocyanin biosynthesis in *Arabidopsis thaliana*. To characterize anthocyanin biosynthesis, we design experiments to collect calli at different culture days, including 0, 5, 10, 15, 20, 25, and 30 days. Biomass is weighed at each time point to measure the growth curve of calli. Anthocyanins are measured at each time point to characterize the dynamics of productions. The expression of pathway genes is measured with qRT-PCR to understand their profiles. These findings reveal that the anthocyanin biosynthesis in engineered red cells is associated with callus growth. The mechanism behind this phenomenon will be discussed in our presentation.

P42. Effect of light and auxin on the *in vitro* culture of *Crinum powellii*.

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Crinum powellii, also known as Powell's swamp lily, is an Amaryllidaceae plant species. It produces different types of Amaryllidaceae alkaloids (AAs) with therapeutical values including lycorine and cherylline, which are known for their antiviral and anti-acetylcholinesterase properties, respectively. Hence this specific species might be a valuable source of medical compounds. Light and hormone treatments used during *in vitro* culture have an important role in tissue differentiation, organogenesis and eventually in the synthesis and accumulation of different phytochemicals like AAs. To date, there is no report on *in vitro* culture of *C. powellii* and the study of the effect of different variables on their growth. Hence, our aim was to optimize callus culture conditions for *C. powellii*. In our study, we cultured twin scale size of *C. powellii* explants in dark and light conditions (14/8 light/dark, 185 $\mu\text{mol}/\text{m}^2$) and with treatment with various auxins (NAA: α -naphthaleneacetic acid and 2,4-D: 2,4-Dichlorophenoxyacetic acid) at different concentrations (0, 2 and 4 mg/L). Results show that light intensity has a negative impact on *C. powellii in vitro* cultures growth and that tissue differentiation and organogenesis vary with types and concentration of auxin in dark condition. In our study, 2 mg/l of 2,4-D was significant to induce the callus from bulbs explant of *C. powellii* at dark condition. Our work can be beneficial for the conservation purpose, as well as to develop biotechnological approaches to produce valuable AAs from *in vitro* culture of *C. powellii*.

P43. Functional characterization of CYP76M17 and CYP76M14 via combinatorial biosynthesis

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Plants produce secondary metabolites such as diterpenoids in response to biotic and abiotic stresses. Diterpenoids are a group of chemically heterogenous compounds with a 20 carbon skeleton based on four isoprene units. Two diterpenoid biosynthetic gene clusters (BGC) have been discovered in rice, one located on chromosome 4 and the other on chromosome 2. These contain not only the diterpene synthases but also CYP P450s. The gene cluster located on chromosome 2 contains four diterpene synthase genes (OsCPS2, OsKSL5, 6-7) and six P450 genes, CYP76M5-8 and CYP71Z6-7. The chromosome 2 gene cluster has been found to be involved in phytocassane, oryzalides and ent-pimara-8(14),15-diene pathways. An interesting feature of the chromosome 2 cluster is the presence of the closely related paralogs CYP76M5-8. Previous study of the CYP76M5-8 cluster has shown that, they are involved

in diterpenoid biosynthesis. The mechanism of formation of the CYP76M subfamily within the BGC2 cluster is unknown. It is hypothesized that a common CYP76M ancestor was inserted earlier into the BGC2 cluster and underwent duplication to give rise to CYP76M5-8. In addition to these, there are other similar CYP76M paralogs elsewhere in the genome. These are CYP76M14 and CYP76M17, which are on chromosome 1 and chromosome 6 respectively (Miyamoto et al, 2016). In this study, we utilize an optimized metabolic engineering system for CYP expression to functionally characterize CYP76M14 and CYP76M17.

P44. Functional evolution of the SABATH family and characterization of phenylacetic acid methyltransferase (PAMT) in potato

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Plant SABATH methyltransferases catalyze the methylation of various important hormones and other small molecule metabolites. Phenylacetic acid (PA), as an important phytohormone, not only plays a role in plant growth but is also important for plant systemic resistance. Headspace analysis of potato (var. Desiree) plants showed the presence of methyl phenylacetate in flowers. Thus we rationally assumed that a SABATH member may be responsible for the methylation of PA. In this study, homologous gene search of the potato genome revealed 28 SABATH methyltransferases genes. Transcriptome analysis showed that 19 of them expressed in flowers and 20 of them expressed in tubers. Phylogenetic analysis indicated that methyltransferase groups were relatively conserved. Since indole-3-acetic acid (IAA) and phenylacetic acid (PAA) are structurally similar, two indole-3-acetic acid methyltransferase (IAMT) candidates (P22754 and P38439) were subject to cloning and activity assay. We found that P22754 showed main activity towards IAA and lower relative activity towards PAA. Therefore, we hypothesize that P22754 is bifunctional enzyme and is responsible for methyl phenylacetate in flowers. The functions of selected additional SABATH genes in potato were also characterized, enabling a better understanding of functional evolution of the SABATH family in this plant.

P45. Identification and characterization of candidate genes in the cardenolide biosynthetic pathway in wormseed wallflower

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Cardiac glycosides, or cardenolides, have evolved independently in at least 12 plant families as an herbivore defense mechanism. Historically, humans have used cardenolide-producing plants, including foxglove and milkweed, for treating heart arrhythmias, and emerging research suggests that these glycosylated steroids may have a future role in cancer treatment. To our knowledge, only one crucifer genus, *Erysimum*, produces cardenolides, and the complete cardenolide biosynthetic pathway remains elusive. Using the rapidly growing, self-pollinating annual wormseed wallflower (*Erysimum cheiranthoides*) as a model, we have identified candidate genes in cardenolide metabolism using a functional genomics pipeline. Using comparative genomics, correlation between metabolites and transcripts among tissues with and without defense elicitation, and co-expression network analyses, we have narrowed down our list of candidate genes involved in forming and decorating the steroid core. We've developed mass spectrometry-based enzyme assays using purified proteins as an untargeted and unbiased approach to identifying substrates for these enzymes. Initial data suggests that we have identified *UDP-dependent glycosyltransferases* involved in cardenolide biosynthesis. Our next steps involve confirming our initial findings using targeted LC-MS and *in vitro* enzyme assays and generating CRISPR knockouts in the plant. Ultimately, these findings will allow us to identify additional pathway genes with the ultimate goal of elucidating the entire cardenolide biosynthetic pathway.

P46. Identification of intracellular targets for tobacco SIP68: A UDP-glucosyltransferase and its role in growth regulation

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All living organisms must be capable of eliciting appropriate responses to various stresses in order ensure survival. These stresses include viral, bacterial, and fungal infections (biotic) as well as temperature changes, UV exposure, and other environmental factors (abiotic). To combat biotrophic infections, many species of plants use a secondary metabolite known as Salicylic Acid (SA). Intracellular concentrations of active SA are largely mediated by Salicylic Acid Binding Protein 2 (SABP2), which has also been shown to interact with SIP68, a highly conserved UDP-glucosyltransferase (GT) enzyme. While this protein is still a novel one, previous studies have shown that SIP68 is a negative regulator of SA mediated defense signaling in tobacco. This was confirmed by observing increased resistance in SIP68 silenced tobacco when exposed to viral and bacterial infection. These plants also showed a significant

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decrease in the rates of germination and development compared to wildtype tobacco. In silico analysis revealed that SIP68 may play a role in the zeatin metabolic pathway which promotes plant growth and cell proliferation. On average, SIP68 silenced lines took an additional week to reach cotyledon formation when compared to WT. Nine-week-old SIP68 silenced lines also develop about half the rate as WT in respects to root length, shoot length, leaf width, and wet mass. While there is evidence to suggest that SIP68 utilizes UDP-glucose as a donor substrate, the specific substrate(s) of SIP68 have not yet been identified but interaction with various classes of flavonols has been observed. Therefore, this research is looking to investigate intracellular targets for SIP68. To investigate this, GT activity screening assays are being conducted which will allow for spectrophotometric detection of GT activity with various zeatin substrates. With this information, we suggest that SIP68 is a UDP-glucosyltransferase that has a direct role in plant development and immune response.

P47. Investigating the gene regulation of 1,8-cineole synthase by transcription factor LiNAC in *Lavandula latifolia*

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Lavandula latifolia (spike lavender) produces essential oil comprised mainly of monoterpenes, including 1,8-cineole. The enzyme 1,8-cineole synthase (CinS) catalyzes the conversion of the linear precursor geranyl diphosphate (GPP) to 1,8-Cineole. Regulation of expression of the *CinS* gene is currently poorly understood. A recent study identified 8 potential transcription factors (TFs), one of which showed a positive regulation of the 1,8-cineole synthase (CinS) promoter in *N. benthamiana*. However, the exact function of this TF in the regulation of *CinS* expression in lavender remains unknown. This study aims to stably express the NAC TF from *L. x intermedia* (LiNAC) in sense and antisense versions in *L. latifolia* to examine the expression of *CinS* and accumulation of 1,8-cineole in transgenic plants. The full-length cDNA of LiNAC in sense or antisense versions were placed under the control of CaMV 35S promoter and used to stably transform *L. latifolia* via *Agrobacterium*-mediated transformation. Transgenic plants expressing 35S::LiNAC-sense or 35S::LiNAC-antisense, containing a hygromycin resistance gene, are being selected for and are elongating on Murashige and Skoog media containing hygromycin. After shoot elongation, the plants will be rooted, acclimated to soil, and analyzed for the expression of the transgenes by PCR. As well, the transformed plants will be examined for the expression of the 1,8-cineole synthase gene by PCR, and for 1,8-cineole content by gas chromatography/mass spectroscopy (GC-MS). The results from this study will give us a better understanding of the fine-tune regulation of monoterpene metabolism by transcription factors which will further our ability to use biotechnology to produce transgenic plants yielding higher concentrations of medicinally important compounds such as 1,8-cineole.

P48. LC-MS and software tools for profiling flavonoids in tea

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Tea is the second most consumed beverage worldwide after water. Systems biology studies have applied metabolomics in tea to show the benefits of phytochemicals such as flavonoids, xanthines, and theanine. Geographical origin in combination with manufacturing and packaging processes can all impact levels of these phytochemicals. Here we describe an untargeted metabolomics approach utilizing liquid chromatography-mass spectrometry for the detection and annotation of phytochemicals in tea. Twenty-four different types of tea including green, black, and herbal varieties were brewed in triplicate for the analysis. Pools of each variety of tea were also created for MSn data acquisition using the AcquireX workflow. Samples were injected and separated using a Thermo Scientific™ Vanquish™ UHPLC system and data was acquired with a Thermo Scientific™ Orbitrap ID-X mass spectrometer. Data analysis was performed using Thermo Scientific™ Compound Discoverer™ for unknown identification and differential analysis. The data displayed robust mass accuracy and allowed for elemental composition prediction and putative annotation of thousands of phytochemicals. The AcquireX intelligent data acquisition strategy maximized the number of phytochemicals interrogated by MS/MS through inclusion of only sample relevant features across the tea variety pools and enabled spectral matching against the online Thermo Scientific™ mzCloud library using both identity and similarity algorithms. The use of a pooled quality control sample enabled normalization of compound responses over time across the entire batch and allowed for reproducible detection of over two thousand compounds across the tea varieties. Compounds were further classified using compound class scoring and neutral loss searching to annotate flavonoids. Differential analysis tools were used to detect differences in the phytochemical profiles of the various tea samples to allow for stratification and fingerprinting.

P49. Magnesium-isotope fractionation in chlorophyll-a extracted from two plants with different pathways of carbon fixation (C3, C4)

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Relatively few studies have been focused so far on magnesium-isotope fractionation during plant growth, element uptake from soil, root-to-leaves transport and during chlorophylls biosynthesis. In this work, maize and garden cress were hydroponically grown in identical conditions in order to examine if the carbon fixation pathway (C4, C3, respectively) might have impact on Mg-isotope fractionation in chlorophyll-a. The pigment was purified from plants extracts by preparative reversed phase chromatography and its identity was confirmed by high resolution mass spectrometry. The green parts of plants and chlorophyll-a fractions were acid-digested and submitted to ion chromatography coupled through desolvation system to multiple collector inductively coupled plasma-mass spectrometry. Clear preference for heavy Mg-isotopes was found in maize green parts ($\Delta^{26}\text{Mg}_{\text{plant-nutrient}}$ 0.65-0.74) and in chlorophyll-a ($\Delta^{26}\text{Mg}_{\text{chlorophyll-plant}}$ 1.51-2.19). In garden cress, heavy isotopes were depleted in green parts ($\Delta^{26}\text{Mg}_{\text{plant-nutrient}}$ (-0.87)-(-0.92)) and the preference for heavy isotopes in chlorophyll-a was less marked relative to maize ($\Delta^{26}\text{Mg}_{\text{chlorophyll-plant}}$ 0.55-0.52). The observed effect might be ascribed to overall higher production of energy in form of ATP, required for carbon fixation in C4 compared to C3, which could reduce kinetic barrier and make equilibrium fractionation prevailing during magnesium incorporation to protoporphyrin ring.

P50. Market analysis of phytochemical biopesticides applied to California crops

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Government policy and consumer demand are driving the increased use of biopesticides to protect crops from microbial and insect pests. The global market for biopesticides, crop protection products based on living microbes, microbial products or plant derived chemicals, is predicted to represent 20% of total pesticide use in 2025. In provision of our goal to develop new biopesticides with secondary phytochemicals as the main active ingredients, we analyzed the 2010 to 2018 pesticide use data from the California Department of Pesticide Regulation's databases to discern trends in the agricultural application of phytochemical biopesticides. Total pounds of active ingredients applied to crops increased from 94,000 lbs in 2010 to 151,000 lbs in 2018, a 61 % increase. The five crops that received the highest amount of biopesticides, ranked from highest to lowest, were berries, greens, crucifers, grapes and onions, with grapes receiving the highest diversity of biopesticide products. Generally, extracts of the giant knotweed (*Reynoutria sachalinensis*) or the neem tree (*Azadirachta indica*) had the highest adoption across all crops. In addition to these, grape also received numerous biopesticides based on terpenoids and saponins. Many of the pest control phytochemicals highlighted in this survey can be extracted from agri-food waste or purpose grown biomass. As an effort to determine the economic feasibility of obtaining new phytochemical biopesticides from agri-food waste, our cost analysis of phytochemicals extracted from hop (*Humulus lupulus*) crop residue using a 600 L supercritical CO₂ batch reaction, revealed a break even cost of \$9.76 USD per kilogram of feedstock in a commercial toll extraction scenario. As jurisdictions around the world are searching for solutions to agri-food waste, this low-cost feedstock can offer economic opportunity as well as environmental benefits within the context of the circular bioeconomy.

P51. Poison ivy urushiol accumulation: a currency for contemporary chemical defense, or money for nothing?

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A foundational principle of evolutionary ecology is that adaptive traits are eventually constrained by natural selection and/or resource allocation. To assess the impact of local environmental conditions on urushiol accumulation, a single branch from each of 10 naturally recruited climbing poison ivy lianas at two locations in Blacksburg VA were harvested, total leaf area was measured, and urushiol congener composition and levels were measured. ANOVA analysis indicated that location had no significant impact on urushiol congener composition or levels. Instead, urushiol levels and composition were significantly different based on the particular plant sampled, suggesting that plant genotype had a greater impact than shared environment. Deployment of specialized plant metabolites that reduce herbivory need to be balanced with the metabolic demands of plant vigor and growth. So, we performed pilot experiments that evaluated the allometric relationships between biomass vs. urushiol levels & composition. Poison ivy drupes from five states were germinated in vitro and tissues from different developmental stages were evaluated. Across accessions from five states, nascent germinated intact poison ivy seedlings showed a statistically significant negative relationship between biomass and predominantly 15-urushiols. In contrast, when six accessions from different states were evaluated separately, the allometric relationships between 1st true leaf biomass vs. either C15 or C17 urushiols showed remarkably different (statistically significant) patterns between the six

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accessions. When joint regression analyses were performed across all six poison ivy accessions, there was no significant allometric relationship between 1st true leaf biomass vs. either C15- or C17-urushiol levels. The hypervariability of foliar urushiol levels between individual plants in similar environments, combined with the variable allometric relationships of biomass vs. urushiol levels across poison ivy's native geographical range are unexpected findings for an adaptive trait under a consistent pattern of natural selection.

P52. Quantifying chalkiness in rice kernels using PlantCV2

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Chalkiness is the opaque area in the translucent white endosperm of rice caused by a lower density of starch granules compared to translucent rice seeds. Chalkiness reflects an undesirable appearance, reduces the value of the rice crop in the markets and also contributes to decrease rice yield because chalky kernels are more susceptible to breakage during milling. The ability to detect chalkiness in individual rice seeds is important in rice breeding programs. Since chalkiness is a visible feature, inspection under a microscope has been the traditional method used for many years to quantify it. However, this method is a slow and subjective process. Recent developments in high-throughput plant phenotyping methods and imaging processing techniques have enabled a detailed characterization of seed physical characteristics in an economical, fast, and accurate way without risking breakage. Here, we employed a high throughput platform and Plant Computer Vision (PlantCV2) software to phenotype 320 rice accessions to quickly and objectively quantify the percentage of chalkiness in rice. The digital images of the rice grains were captured with a visible (RGB) sensor using a Scanalyzer HTS, and the images were analyzed using a PlantCV2 workflow. During the analysis process, the proportion of chalkiness was defined by calculating the proportion between the chalk area and the total area of the rice seed. In addition, with this pipeline we are able to extract other important seed parameters including area, perimeter, width, and length.

P53. Seasonal variation in the content of alkaloids, polyphenols and saccharides contained in *Ephedra sinica* under Japanese cultivation condition

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Genus *Ephedra* is an important medicinal plant in Asia. In Japan, dried stems of *Ephedra sinica* L. is used as an important ingredient for traditional prescription with the entire supply of *Ephedra* being imported from China. This is because *Ephedra* plants do not grow naturally in Japan and are not cultivated commercially either. We started cultivation studies to produce *Ephedra* herb (called “*mao*” in Japan) domestically but these studies have not revealed seasonal variations of internal compounds of *Ephedra* plants under Japanese cultivation condition. Therefore, we investigated seasonal variation in alkaloids and polyphenols as they have been reported as a bioactive compound of *Ephedra* herb. Saccharides were quantified to indicate growth responses. The alkaloids norpseudoephedrine, norephedrine, pseudoephedrine, ephedrine and methylephedrine were quantified by HPLC. The total amount of polyphenols (TP) was quantified by Folin-Ciocalteu method. Saccharides were quantified by LCMS. In the first trial between May and December 2018, we investigated the content of alkaloids in the stem. The results show that norpseudoephedrine (0.056%) and norephedrine (0.062%) were the highest in June, pseudoephedrine (0.241%) was the highest in September, ephedrine (0.399%) was the highest in July, and methylephedrine (0.018%) was higher in August and December. In the second trial between June and November 2020, we investigated the content of alkaloids again along with TP and saccharides. Alkaloid content was higher in the summer and the changes in the content of TP were the same as alkaloids. Sucrose was relatively abundant from July to October, very little was present in November. In contrast, glucose and fructose were more abundant in November. Mannitol was the highest in July. In conclusion, we revealed seasonal variation in the content of alkaloids, polyphenols and saccharides which is useful information for the production of *mao* in Japan.

P54. Semisynthetic studies of diterpenoid rollozatrione and biological evaluation of its derivatives

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Riolozatrione is a diterpenoid isolated from *Jatropha dioica* roots. This plant grows in Northeast Mexico and is commonly called "sangre de drago". The chemical structure and absolute configuration of riolozatrione, were determined previously by X-ray crystallography, VCD, and NMR spectroscopy. There are only two known diterpenoids possessing the riolozane skeleton, riolozatrione and 6-epi-riolozatrione. Recently, it was demonstrated that riolozatrione exhibits moderate *in vitro* activity against herpes simplex virus (HSV), while 6-epi-riolozatrione is inactive against this virus. Due to the uniqueness of the riolozane skeleton, and to the biological activity, the aim of this work was to study the reactivity of riolozatrione and evaluate the antiherpetic activity of the obtained derivatives. Rioloatrione was subjected to different reaction conditions, using two types of bases (MeOK and *t*-BuOK), one Lewis acid (Yb(OTf)₃), and two reducing agents (NaBH₄ and H₂/Pd/C).

P55. The absence of a C2-C3 double bond on a novel flavonoid influence its cytotoxic activity on HER2 positive breast cancer cells

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Breast cancer is the most frequently diagnosed cancer in women across the world, with an estimated 2.3 million new cases occurring in 2020. Additionally, over 684,000 deaths annually are attributed to breast cancer across the globe, making it the most common cause of cancer-related death in women. Further, treatment of breast cancer relies heavily on whether the cancer cells express estrogen, progesterone, and HER-2 receptors and this expression profile is often related to how quickly the cells grow and spread. In the United States, hormone receptor positive and HER-2 negative cancers make up about 68% of cases, 14% are HER-2 positive, and 10% do not express any receptor, known as triple negative. Further, triple negative and HER-2 positive breast cancers are associated with poor prognosis. Two novel flavonoids, CT1 and CT3 which differ only by the presence of the C2 C3 double bond have been identified as potential chemotherapeutic agents by showing cytotoxic effects on cell lines that represent about 82% of all breast cancer subtypes in the United States. The objective of this study was to investigate the anti-proliferative effects of these compounds on MCF7, SKBr3, and MDA-MB-231 breast cancer cell lines. The leaves of *Chromolaena tacotana* that contain CT1 and CT3 were dried and placed in a Soxhlet extractor followed by column chromatography, isolation and purification of the compounds. Cells were treated with CT1 and CT3 at concentrations of 5, 10, 20, 40 and 80 µM and MTT assays were conducted to assess cell viability. CT1 showed the most cytotoxic effects on MCF7 and MDA-MB-231 as compared to CT3. The opposite effect was observed for SKBr3, with CT3 demonstrating greater toxicity. The results in MDA-MB-231 cells suggest that the observed cytotoxic effect is not dependent on the presence of hormonal or HER-2 receptors. Further studies are necessary to elucidate the molecular targets of CT1 and CT3.

P56. The pharmacokinetics of digesting Cannabis derived compounds

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Cannabinoids are natural occurring plant compounds from *Cannabis sativa*. The most abundant cannabinoids are cannabidiol (CBD) and tetrahydrocannabinol (THC), however CBD is not psychoactive like THC. Previous studies have shown various health beneficial effects of CBD such as reducing pain, anxiety, tumor, inflammation, and seizure. Since human studies are arduous, expensive, and limited by the ethical issues, *in vitro* models have been developed as valid methods to estimate the bioaccessibility of compounds using simulated gastrointestinal conditions. Many *in vitro* studies have shown low bioavailability of CBD when administered orally as an isolate or powder. However, CBD, a lipophilic compound, is easily dissolved in fat containing food matrices. In this study, the bioavailability of CBD was tested in two *in vitro* digestion experiments: 1) a fed state with baby food and olive oil, and 2) a starved state with no food. In addition, our work aimed at understanding the pharmacokinetics of digestion enzymes and food matrices on bioavailability of CBD. In an assay, lipase, an enzyme found in the gut, was shown to break down more lipids in the presence of bile salts, and in a digestion, this can result in higher micellarization efficiency of lipid soluble compounds. When digested with food, CBD's bioavailability was 22.7%, while its bioavailability without food was only 0.1%. In conclusion, digestion and bioavailability of these *Cannabis* derived compounds are greatly influenced by the food they are consumed with, thus adding to the pharmacological research of cannabinoids.

P57. Uncovering the genetic and biochemical impacts of modern breeding on aroma metabolism in strawberry (*Fragaria x ananassa*)

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The US strawberry industry earned \$3.2 billion in 2017; 90% of that fruit was grown in California. Strawberry (*Fragaria x ananassa*) is an allo-octoploid species that arose from the crossing of two wild octoploid species, *F. virginiana* x *F. chiloensis*, in 18th century Europe. Continued strawberry breeding has prioritized the optimization of major commercial traits including yield, pest and pathogen resistance, as well as fruit size, firmness, and shelf life. However, breeding for the latter traits has affected ripening mechanisms and flavor complexity possibly due to pleiotropic and biochemical effects on aroma metabolism. We hypothesize that these inadvertent off-target effects on aroma metabolism are caused by a combination of directed selection and breeding bottlenecks in the pursuit of larger, firmer, longer lasting strawberries. Although over 300 aroma-defining specialized metabolites have been described in strawberry, as few as 20 represent key determinants of strawberry flavor and overall consumer liking including several fruity esters, floral terpenes, peachy lactones, and caramel furanones. Furthermore, inheritance of and the underlying biosynthetic and regulatory genes and pathways determining aroma diversity across a wide range of wild and domesticated strawberries remains largely unknown. Using both a set of segregating breeding populations and a diversity panel of wild and cultivated species we are performing SPME-GC-MS volatile metabolite analysis coupled with fruit quality analysis, differential gene expression analysis, and genome-wide association studies. We are identifying drivers of aroma metabolite diversity and gaining further insight into the biosynthesis, regulation, and evolution of strawberry aroma metabolism. We hope to generate a metabolic and genetic atlas that can be leveraged for the advancement of aroma-enhanced breeding varieties.

P58. Using ¹³C-NMR dereplication to aid in the identification of xanthenes present in the stem bark extract of *Calophyllum brasiliense*

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Xanthenes are metabolites with a variety of biological properties. They are present in only a few families of higher plants, lichens, and fungi; in plants these metabolites are found mainly in the Clusiaceae family. The genus *Calophyllum*, which until recently was included in the Clusiaceae family, is a rich source of xanthenes, with mono, di, and poly-oxygenated patterns; even though this genus includes 190 species, *C. brasiliense* is the only *Calophyllum* spp. known to occur in the Yucatan peninsula. Recently, ¹³C-NMR dereplication analysis has been used to identify secondary metabolites in a fast and efficient way. In this case, the spectroscopic data obtained from the ¹³C-NMR analyses of a crude extract or semipurified fraction is compared with predicted/theoretical or experimental data contained in one or more databases; the results are used to predict the composition of the analyzed sample. In this investigation a combination of traditional phytochemical methods and ¹³C-NMR dereplication analysis were used to identify xanthenes in the stem bark extract of *C. brasiliense*.

P59. Value-added biopesticidal nanoemulsions from hemp

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When cannabinoids are extracted from hemp (*Cannabis sativa*), terpene-rich by-products (TP) are produced and often discarded. The goal of this research was to develop effective nanoemulsion-containing biopesticides both from TP and enriched distillation products (DTP) of TP. Nanoemulsions of TP and DTP (TPne and DTPne) were fabricated by sonication and scaled-up by microfluidization (four passes at 30,000 psi). Activity against the bean beetle, *Callosobruchus maculatus* was determined in an experiment with five treatments at the same terpene or neem concentrations, four of which were formulated at experimentally determined optimal conditions: 1) 5% TPne, 2) 5% DTPne, 3) 5% neem oil (neem), and 4) emulsifier alone (control). Fifth treatment was 5% DTPne with 1:3 oil-to-surfactant mixing ratio (vs 1:2 for other treatments). Each treatment was replicated five times; experiment was repeated three times. Mung bean (*Vigna radiata*) seed soaked in treatments for 5 min were available to two adult pairs of *C. maculatus* for two days. Viable eggs and adults were counted. Both TP and DTP contained monoterpenes, sesquiterpenoids, and terpenoids. Cannibidiol (CBD) was the primary component of TP but absent in DTP. (-)-*trans*-caryophyllene

was the primary component in DTP. Optimal conditions for nanoemulsion formulation were: 13.5 lipophilic balance (HLB); 5% (wt) oil concentration; 1:2 oil-to-surfactant mixing ratio; and 1.5 min sonication. DTPne was more stable than TPne. Numbers of mung bean seed with viable eggs and total viable eggs were greater in control than in 5% DTPne treatments (1:2 and 1:3). Numbers of emerged adults were lower in the 5% DTPne (1:3) and the 5% TPne than in control. This study has demonstrated that an agricultural waste product can be reformulated and used as an effective biopesticides. When used to treat mung beans, nanoemulsion from DTP enriched in monoterpenes, reduced losses due to infestation of mung beans by *C. maculatus*.