58th Annual Meeting

Phytochemical Society of North America
20-24th July, 2019, Johnson City, TN 37614

SYMPOSIUMS:

- Natural Product Metabolism: Pathway Discovery and Regulation:
  - *Keynote Speaker: Dr. Michael Phillips, Univ. of Toronto.*
- Secondary Metabolism of Bryophytes, Ferns, and Lycophytes: Enormous Diversity from Primitive Plants:
  - *Keynote Speaker: Dr. Yoshinori Asakawa, Tokushima Bunri Univ. Japan*
- Natural Product Enzymology: Bridging the Gap between Bioinformatics and Biochemical Function
  - *Keynote Speaker: Dr. IsabelDesgagne-Penix, Uni. of Quebec at Trois Rivier, Canada*
- Advances in Phytochemical Tools and Applications:
  - *Keynote Speaker: Dr Marianela Rodriguez, BASF, USA*
- Chemical Ecology: Interactions of Plants with Other Organisms:
  - *Keynote Speaker: Sybille Unsicker, Max Planck Institute, Germany*
- Signaling in Development, Stress, and Defense:
  - *Keynote Speaker: Dr. Jean Greenberg, Univ. of Chicago, USA*
- Translational Phytochemistry: Commercialization of Discoveries:
  - *Keynote Speaker: Dr. Ryan Philippe, Manus Bio, USA*
- Arthur Neish New Investigator Symposium
  - *Speakers Dr. Ruthie Angelovici, USA; Dr. Dylan Kosma, USA; Dr. Patrick Horn, USA*
- Natural Products in Agriculture: Harnessing the Potential of Secondary Metabolites to Improve Crop Function:
  - *Keynote Speaker: Dr. Martha Vaughan, USDA ARS, USA*
- Natural Products in Medicine: Drug Development and Discovery:
  - *Keynote Speaker: Dr. Victoria Palau, ETSU, USA*

58th Annual Meeting of the Phytochemical Society of North America

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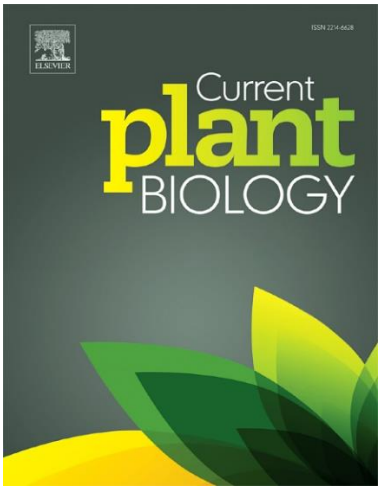

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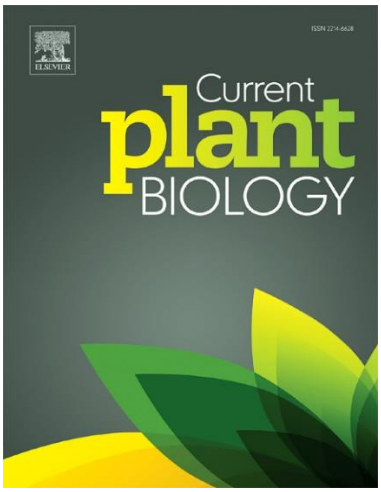

We would also like to express our sincere appreciation to the ETSU Office of Research and Sponsored Programs for the loan of the poster boards

2019 Scientific Organizing Committee Members:

Dr. Dhirendra Kumar, Co-Chair	East Tennessee State University
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Dr. Mark Berhow	USDA-ARS, Peoria, IL
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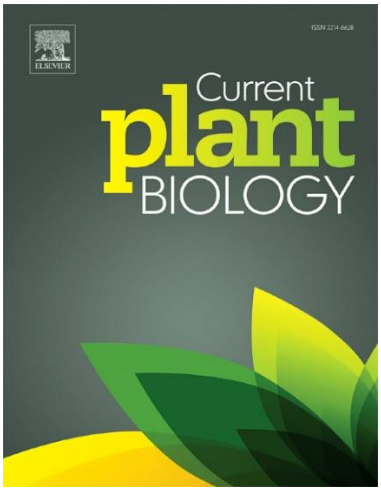

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AGENDA SUMMARY

Venue: Carnegie Hotel

Saturday, July 20, 2019

2 – 9:00 pm **Registration** at entry to Old Soldier's Ballroom, Carnegie Hotel

4pm Executive Committee Meeting (Reeves Room)

6pm Awards Committee Pre-meeting (Reeves Room)

6pm-9pm **Welcome Reception, Wilder Room**, Opportunity to set up posters in the Wilder Room

Sunday, July 21, 2019

7:30-5pm **Registration** desk open

7:30-8:45am **Continental Breakfast, Old Soldier's Ballroom entry**

8:45 am **Conference Introduction**: Organizing Committee Co-chairs, Dhirendra Kumar and Cecilia McIntosh

8:50 am **Welcome**, PSNA President Deyu Xie

8:55 am **Symposium I Natural Product Metabolism: Pathway Discovery and Regulation, chair Laurence Davin**

8:55am **S-1 Dr. Michael Phillips**, University of Toronto, Mississauga, Canada: **UNDERSTANDING THE CONTROL OF PLANT METABOLISM USING STABLE ISOTOPES**

9:40 am **OP – 1 PSNA/Elsevier New Investigator Awardee Presentation**: Phillip Zerbe, University of California Davis, USA: **DISCOVERY, BIOSYNTHESIS, AND BIOLOGICAL FUNCTION OF DITERPENOID DEFENSES IN MAIZE**

10:10 am **OP - 2** L.H. Xie, College of Landscape Architecture and Arts, China: **TREE PEONY SPECIES AS AN EFFICIENT SOURCE FOR a-LINOLENIC ACID PRODUCTION**

10:30 am **Coffee Break – Old Soldiers Ballroom**

11:00 am **OP – 3** R. Judd, North Carolina State University, USA: **ARTEMISININ IS SYNTHESIZED IN HIDDEN PLACES: A NEW HOPE FOR THE ANTI-MALARIAL MEDICINE**

11:20 am **OP – 4** B.J. Leong, Michigan State University, USA: **EVOLUTION OF METABOLIC NOVELTY: A TRICHOME-EXPRESSED INVERTASE CREATES SPECIALIZED METABOLIC DIVERSITY IN WILD TOMATO**

11:40am **OP – 5** Reinhard Jetter, University of British Columbia, Canada: **BIOSYNTHESIS, LOCALIZATION, AND POTENTIAL FUNCTION OF AROMATIC COMPOUNDS IN CUTICULAR WAXES OF CEREALS**

12:00 pm **OP – 6** Soheil Mahmoud, Canada: **REGULATION OF ISOPRENOID PRECURSOR PARTITIONING IN LAVENDER**

12:20pm **OP – 7** Monica Borghi, Max Plank Institue of Molecular Plant Physiology, Germany: **METABOLOMICS OF FLOWER ANTHESIS**

12:40-2:00 pm **Lunch – Old Soldiers Ballroom**

2:00 pm **Announcements**

2:05 pm **Symposium 2 Natural Product Enzymology: Bridging the Gap between Bioinformatics and Biochemical Function, chair Bjoern Hamberger**

2:05 pm **S - 2 Dr. Isabel Desgagne-Penix**, University of Quebec at Trois Rivier, **Canada: CRACKING THE AMARYLLIDACEAE ALKALOID BIOSYNTHETIC CODE**

2:50 pm **OP – 8** Lucas Busta, University of Nebraska – Lincoln, USA: **THE DIVERSITY, ACTIVITY, BIOSYNTHESIS, AND EVOLUTION OF BIOACTIVE POLYACETYLENES IN DAUCUS CAROTA**

3:10 pm **OP – 9** Qingyan Meng, Washington State University, USA: **PROBING DIRIGENT PROTEIN BIOCHEMICAL MECHANISMS IN DIVERSE METABOLIC PATHWAYS**

3:30 pm **Coffee Break Old Soldiers Ballroom**

4:00 pm **OP – 10** Daniel Owens, University of Hawaii, USA: **DETERMINING THE METABOLIC ORGANIZATION AND ENZYMOLOGY OF THE CITRUS SINENSIS FLAVONOID BIOSYNTHETIC PATHWAY**

4:20 pm **OP – 11** Kruse LH, Cornell University, USA: **THE DIVERSITY AND EVOLUTION OF RESIN GLYCOSIDE BIOSYNTHESIS IN THE MORNING GLORY (CONVOLVULACEAE) FAMILY**

4:40 pm **OP – 12** Lewis, N.G., Washington State University, USA: **PLANT TERRESTRIAL COLONIZATION AND DIRIGENT PROTEINS: METABOLIC DIVERSITY KNOWN THUS FAR AND INSIGHTS GAINED**

5:00 pm **OP – 13** Garcia DC, Oak Ridge National Laboratory, USA: **COMPUTATIONALLY-GUIDED DISCOVERY AND EXPERIMENTAL VALIDATION OF INDOLE-3-ACETIC ACID SYNTHESIS PATHWAYS**

5:20 pm **BE SURE ALL POSTERS ARE SET UP**

6-8 pm **Poster Session I, WILDER ROOM, refreshments. Poster presenters with odd numbered posters are asked to be by your poster during this session**

8 pm Dinner on your own

Monday, July 22, 2019

7:30am-5pm **Registration** desk open

7:30-8:45am **Continental Breakfast** – Old Soldiers Ballroom

8:45 am **Announcements**

8:50 am **Symposium 3 Secondary Metabolites of Bryophytes, Ferns, and Lycophytes: Enormous Diversity from Primitive Plants, chair Mark Berhow**

8:50 am **S – 3 Dr. Yoshinori Asakawa**, Tokushima Bunri University, Japan: **LIVERWORTS: A GOOD SOURCE OF BIOLOGICALLY ACTIVE ENT-SESQUI- AND DITERPENOIDS**

9:35 am **OP – 14** Dr. Feng Chen, University of Tennessee, USA: **BIOSYNTHESIS OF TERPENOIDS IN NONSEED LAND PLANTS**

9:55 am **Symposium 4 Advances in Phytochemical Tools and Applications, chair Phillip Zerbe**

9:55 am **S – 4 Dr. Marianela Rodriguez**, BASF, USA: **PHYTOCHEMICALS AND NATURAL PRODUCTS: INNOVATIONS, CHALLENGES AND APPLICATIONS**

10:40 am **Coffee Break** – Old Soldiers Ballroom

11:10 am **Symposium 4 (Continued)**

11:10 am **OP – 15** Fred Stevens, Oregon State University, USA: **COMPUTATION-ASSISTED ANNOTATION OF BIOLOGICAL ACTIVITY TO NATURAL PRODUCTS IN HOPS**

11:30 am **OP – 16** Mark Berhow, USDA, USA: **PURIFICATION OF QUERCETIN SOPHOROSIDE AND EVALUATION OF ITS METABOLISM IN A RAT GUT MODEL**

11:50 am **OP – 17** Bob Standaert, East Tennessee State University, USA: **PRODUCTION OF INDOLE-3-ACETIC ACID AND RELATED COMPOUNDS BY PANTOEA SP. YR343 AND ITS ROLE IN PLANT COLONIZATION**

12:10 pm **OP – 18** Armando Alcazar-Magana, Oregon State University, USA**: CHARACTERIZATION OF BOTANICAL EXTRACTS BY INTEGRATION OF MASS SPECTRAL FINGERPRINTING WITH PRECURSOR ION QUANTIFICATION: APPLICATION TO EXTRACTS OF CENTELLA ASIATICA**

12:30-2:00pm **Box Lunch**; Separate lunch for **early career members in Library** with a panel discussion (F. Stevens, T. Kutchan, N. Lewis) on "*Improving Writing Skills – How to Get a Scientific Paper Accepted for Publication*"; **other participants in Ballroom or on hotel grounds.**

2:00 pm **Symposium 5 Chemical Ecology: Interactions of Plants with Other Organisms, chair Dorothea Tholl**

2:00 pm **S – 5 Dr. Sybille Unsicker**, Max Planck Institute for Chemical Ecology, Germany: **PATHOGEN EFFECTS ON TREE-INSECT INTERACTIONS**

2:45 pm **OP – 19** Mahdieh Mirzaei, Boyce Thompson Institute, USA: **CHEMICAL CHARACTERIZATION OF A NOVEL EMS INDUCED MUTANT WITH ALTERED CARDIAC GLYCOSIDE PROFILE IN ERYSIMUM CHEIRANTHOIDES**

3:05 pm **OP - 20** D.A. Barker, East Tennessee State University, USA: **CONGRUENCE AND WITHIN-SEASON VARIATION IN FLORAL VISITATION AND POLLEN TRANSPORT NETWORKS IN SOUTHERN APPALACHIAN PLANT-POLLINATOR COMMUNITIES**

3:25 pm **OP - 21** Demissie, ZA, National Research Council Canada, Canada: **EXOMETABOLOMIC PROFILING OF CLONOSTACHYS ROSEA CO-CULTURED WITH FUSARIUM GRAMINEARUM: OLD AND NEW SUSPECTS MEDIATE THEIR ANTAGONISM AND MYCOTOXIN TOLERANCE**

3:45 pm **coffee break** Old Soldiers Ballroom

4:15 pm **OP – 22** A. Stanley, East Tennessee State University, USA: **THE EFFECTS OF URBANIZATION ON AVIAN SEED DISPERSAL SUCCESS OF EASTERN POISON IVY (ANACARDIACEAE)**

4:35 pm **OP - 23** Kimberly Gwinn, University of Tennessee at Knoxville, USA: **SWITCHGRASS EXTRACTIVES, A NEW SOURCE FOR BIOPESTICIDES AND BIODISINFECTANTS**

4:55 pm **announcements**

4:55 pm **PSNA Members Meeting – All Conference Participants Should Attend** – Old Soldiers Ballroom

6-8 pm **Poster Session 2, WILDER ROOM, refreshments. Poster presenters with even numbered posters are asked to be by your poster during this session**

8pm Dinner on your own

Tuesday, July 23, 2019

7:30-5pm **Registration** desk open

7:30am **Continental Breakfast** – Old Soldiers Ballroom

8:45am **Announcements**

8:50 am **Dr. David Stern,** Boyce Thompson Institute: *Report on Outcomes of the Plant Summit: implications for PSNA*

9:15am **Question and Answer/Discussion; chair/facilitator Dhirendra Kumar**

9:45am **Symposium 6 Signaling in Development, Stress, and Defense, chair Feng Chen**

9:45 am **S – 6 Dr. Jean Greenberg and Dr. Zeeshan Z. Banday**, University of Chicago, Chicago, USA: **ROLE OF NOVEL PLASTID ENVELOPE PROTEINS IN SIGNAL MOBILIZATION AND DEFENSE RESPONSE**

10:30 am **OP - 24** N. Kovinich, West Virginia University, USA: **GLYCEOLLIN TRANSCRIPTION FACTOR GmMYB2 IS A REGULATOR OF SOYBEAN RESISTANCE TO PHYTOPHTHORA SOJAE**

10:50 am **Coffee Break** – Old Soldiers Ballroom

11:20 am **OP - 25** Dhirendra Kumar, East Tennessee State University, USA: **TOBACCO SIR2 DEACETYLASE SIP-428 IS A NEGATIVE REGULATOR OF PLANT IMMUNITY**

11:40 am **OP - 26** Winkel BSJ, Virginia Tech, USA: **EVIDENCE FOR A CONNECTION BETWEEN FLAVONOID METABOLISM AND THE PLANT CIRCADIAN CLOCK**

NOON-1:30 pm **Lunch – Old Soldiers Ballroom**

1:30 pm **Symposium 7 Translational Phytochemistry: Commercialization of Discoveries, chair Argelia Lorence**

1:30 pm **S-7 Dr. Ryan Philippe**, Manus Bio, Cambridge, MA 02138, USA: **COMMERCIAL BIOPRODUCTION OF PLANT NATURAL PRODUCTS: THE MANUS BIO APPROACH**

2:15 pm **OP - 27** J.L. Dahmen, Conagen Inc., USA: **FROM DISCOVERY TO MARKET: FERMENTATION FOR INGREDIENTS AND NATURAL PRODUCTS**

2:35 pm **ARTHUR C. NEISH NEW INVESTIGATOR SYMPOSIUM, chair Daniel Owens**

2:40 pm **N - 1 Dr. Ruthie Angelovici**, University of Missouri, USA: **UNCOVERING THE METABOLIC AND GENETIC REGULATION OF FREE AND BOUND AMINO ACIDS IN SEEDS**

3:10 pm **Coffee Break** – Old Soldiers Ballroom

3:40 pm **ARTHUR C. NEISH NEW INVESTIGATOR SYMPOSIUM, (Continued)**

3:40 pm **N – 2 Dr. Dylan Kosma**, University of Nevada Reno, USA: **PLANT BANDAGES: IDENTIFICATION OF TRANSCRIPTIONAL REGULATORS OF THE PLANT WOUND HEALING PROCESS**

4:10 pm **N – 3 Dr. Patrick Horn**, East Carolina University, USA: **PEROXOREDOXIN Q ACTIVATES AN UNUSUAL FATTY ACID DESATURASE THROUGH REDOX REGULATION IN ARABIDOPSIS THALIANA**

4:40 pm **Announcements**

6:00-9:00 pm **Banquet and Award Ceremony – Old Soldiers Ballroom**

Wednesday, July 24, 2019

7:30-11:30am **Registration** desk open

7:30am **Breakfast – Old Soldiers Ballroom**

8:45am **Announcements**

8:50am **Symposium 8 Natural Products in Agriculture: Harnessing the Potential of Secondary Metabolites to Improve Crop Function, chair Victoria Palau**

8:50am **S – 8 Dr. Martha Vaughan**, USDA ARS NCAUR, Peoria IL 61604, USA: **METABOLOMICS OF CROP RESILIENCE**

9:35 am **OP - 28** Yao Shengbo, Anhui Agricultural University, China: **DISCOVERY AND CHARACTERIZATION OF TANNASE GENES IN PLANTS**

9:55 am **OP - 29** De-Yu Xie, Noth Carolina State University, USA: **TRANSGENIC CAPTURE OF ISOPRENE LEADS TO BIOAMSS INCREASE OF PLANTS**

10:15 am **OP - 30** Toshiaki Umezawa, Kyoto University, Japan: **MYB108 LOSS OF FUNCTION ENRICHES P-COUMAROYLATED AND TRICIN LIGNIN UNITS IN RICE CELL WALLS**

10:55 am **Coffee Break – Old Soldiers Ballroom**

11:20 am **Symposium 9 Natural Products in Medicine: Drug Development and Discovery, chair Sangeeta Dhaubhadel**

11:20 am **S – 9 Dr. Victoria Palau,** East Tennessee State University, USA**: DIFFERENTIAL ANTI-NEOPLASTIC ACTIVITY OF FLAVONOIDS DERIVED FROM ANDEAN PLANTS WITH ETHNOBOTANICAL IMPORTANCE**

12:05 am O**P - 33** Mohamed Ali M. Ibrahim, The University of Mississippi, USA: **NATURE PRODUCT LEADS FOR DRUG RESISTANT HUMAN AND PLANT PATHOGENS**

12:25 am **OP - 34** A.E. Ubhenin, Federal University Lafia, Nigeria: **CARIOPROTECTIVE AND HYPERCHOLESTEROLEMIC EFFECT OF ETHANOLIC EXTRACT OF MORMODICALCHARANTIAL IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN ADULT WISTAR RATS**

1:05 pm **conference ends**

Explore Northeast Tennessee (Roan Mountain Natural Park, Jonesborough (oldest town in Tennessee), NASCAR, Birthplace of Country Music Museum and more!) ON YOUR OWN

Symposium I Natural Product Metabolism: Pathway Discovery and Regulation

Michael Phillips, Ph.D.
University of Toronto
Mississauga, Canada

The Phillips' laboratory was recently established at the University of Toronto. My research relies on isotopic labeling, metabolomics, and flux analysis to understand the control of plant metabolism. Part of the research in our lab deals with the interface of primary and secondary metabolism as it relates to chloroplast isoprenoid precursor biosynthesis via the 2C-methyl-D-erythritol-4-phosphate pathway. Using Arabidopsis, we develop new mass spectrometry based methods to quantify the allocation of carbon to isoprenoids relative to other major metabolic domains. The remainder of our research deals with isoprenoid secondary metabolite biosynthesis, in particular terpenoid volatiles. We have recently developed isotopic labeling methods to measure the kinetic rates of monoterpenoid biosynthesis in the glandular trichomes of intact rose-scented geraniums.

[S – 1] UNDERSTANDING MONOTERPENE BIOSYNTHESIS IN ROSE SCENTED GERANIUM USING STABLE ISOTOPES

Phillips, M.
Department of Cell & Systems Biology, University of Toronto, Mississauga, ON L5L 1C6, CANADA

Pelargonium graveolens is a wild predecessor to rose-scented geranium hybrids prized for their essential oils used as fragrances and flavorings in the cosmetics, food, and perfumery industries. It is characterized by an especially complex mixture of volatiles that accumulate in captitate glandular trichomes. However, little is known about their biosynthesis. Here we describe an isotopic labeling technique aimed at understanding the biosynthetic network of monoterpenoid volatiles in rose-scented geraniums as well as quantifying their rates of formation in intact plants. At least two distinct monoterpene biosynthetic pathways contribute to their volatile profiles; namely, cyclic *p*-menthanes such as (-)-isomenthone and acyclic monoterpene alcohols such as geraniol and (-)-citronellol and their derivatives (referred to here as citronelloid monoterpenes). We established their common origin via the 2*C*-methyl-D-erythritol-4-phosphate pathway but found no indication these pathways share common intermediates beyond geranyl diphosphate.

We exploited the natural variation in a population of *P. graveolens* to dissect the metabolic details of this monoterpenoid biosynthetic network. Untargeted volatile profiling of 22 seed-grown *P. graveolens* lines demonstrated distinct chemotypes that preferentially accumulate either (-)-isomenthone, geraniol, or (-)-citronellol along with approximately 80 minor volatile products. Whole plant $^{13}CO_2$ isotopic labeling performed under physiological conditions permitted us to measure the *in vivo* rates of monoterpenoid biosynthesis in these lines and quantify differences in metabolic modes between chemotypes. We observed distinct carbon allocation patterns among *p*-menthane or citronelloid monoterpenoid. In (-)-isomenthone accumulating chemotypes, we observed appearance of ^{13}C label in the (-)-isomenthone pool of illuminated whole plants in ~3 h, increasing at a rate of 1,719 pmol $^{13}C \cdot mg^{-1}$ $FW \cdot h^{-1}$. In contrast, (-)-citronellol and geraniol specialists dedicated only 10-20% as much carbon flux towards (-)-isomenthone and instead preferentially allocated freshly fixed carbon to (-)-citronellol and geraniol at rates of 465 and 380 pmol $^{13}C \cdot mg^{-1}$ $FW \cdot h^{-1}$, respectively. By following ^{13}C incorporation into half a dozen likely upstream intermediates, we further determined that *p*-menthane monoterpenoids in *Pelargonium* are likely synthesized from (+)-limonene via (+)-piperitone rather than (+)-pulegone, the comparable intermediate of the related (-)-menthol pathway in peppermint.

The metabolic analysis of this natural population provides the first real-time rates of monoterpenoid biosynthesis in intact glandular trichomes. It further establishes a physiological framework from which to evaluate the roles of individual biosynthetic genes involved in the structural steps of this network, whose characterization is currently underway.

[OP – 1] DISCOVERY, BIOSYNTHESIS, AND BIOLOGICAL FUNCTION OF DITERPENOID DEFENSES IN MAIZE

Mafu Sibongile[1], Ding Yezhang[2], Murphy Katherine M[1], Huffaker Alisa[2], Schmelz Eric A[2], Zerbe Philipp[1]

[1]Department of Plant Biology, University of California Davis, One Shields Avenue, Davis, CA, USA [2]Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA, USA

Diterpenoids constitute a diverse class of metabolites with critical functions in plant development, defense, and ecological adaptation. Major monocot crops, such as maize (Zea mays) and rice (Oryza sativa), deploy diverse blends of specialized diterpenoids as core components of biotic and abiotic stress resilience. Here we describe the biosynthesis and biological function of a novel class of maize diterpenoids, named dolabralexins. Integrating genome-wide gene discovery and rapid biochemical characterization using multi-enzyme co-expression assays identified the dolabralexin pathway. Sequential activity of two diterpene synthases, the ent-copalyl diphosphate synthase (ZmAN2) and kaurene synthase-like 4 (ZmKSL4), and a cytochrome P450 monooxygenase (CYP71Z16) produce the diterpene epoxides 15,16-epoxy-dolabradiene and 3β-hydroxy-15,16-epoxy-dolabradiene. Absence of dolabralexins in Zman2 and Zmksl4 mutants under elicited conditions confirms the in planta metabolic dependency on these enzymes. Combined MS and NMR studies demonstrated that much of the 3β-hydroxy-15,16-epoxy-dolabradiene is further converted into 3β,15,16-trihydroxydolabradiene (THD). Metabolite profiling of field-grown maize cultivars supports the widespread biosynthesis of dolabralexins in roots with THD as the predominant constituent. Pathogen and oxidative stress induce dolabralexin accumulation and transcript expression of ZmAN2 and ZmKSL4 in root tissues, suggesting roles in both biotic and abiotic stress responses. Indeed, dolabralexins significantly inhibit growth of major Fusarium pathogens in vitro at <10 µg/ml. Fungal bioassays with structurally distinct diterpenoids illustrate how individual functional groups contribute to antimicrobial activity. In addition, the diterpenoid-deficient Zman2 mutant shows significant different in the root microbiome composition. These findings support a role for dolabralexins in cooperative and defensive plant-microbe interactions and expand the known chemical space of diterpenoid defenses as genetic targets for understanding and ultimately improving maize resilience.

[OP – 2] TREE PEONY SPECIES AS AN EFFICIENT SOURCE FOR a-LINOLENIC ACID PRODUCTION

Xie LH[1], Zhang QY[1], Kilaru A[2], Zhang YL[1]

[1] College of Landscape Architecture and Arts, Northwest A&F University, Xianyang, China, [2] Department of Biological Sciences, East Tennessee State University, Johnson City, TN, USA

The increasing need for healthy edible oil has driven us to identify α-linolenic acid (ALA)-rich species and identify key biochemical steps in ALA synthesis. Seeds of tree peony species are rich in unsaturated fatty acid content with > 40% ALA in the total fatty acid. However, fatty acid content and composition is variable among the tree peony germplasm. To this extent, a comparative study was carried out to identify the key genes responsible for differential oil accumulation among nine wild tree peony species. Subsequent to analyzing fatty acid content and composition of the seeds from nine tree peony species, a high- (*P. rockii*) and low-oil (*P. lutea*) accumulating species were selected for transcriptome analysis. Gene expression analysis revealed upregulation of select genes involved in plastidial fatty acid synthesis, and acyl editing, desaturation and triacylglycerol assembly in the endoplasmic reticulum in seeds of *P. rockii* relative to *P. lutea*. Also, in association with ALA content in seeds, transcript levels for fatty acid desaturases (SAD, FAD2 and FAD3), which encode for enzymes necessary for polyunsaturated fatty acid synthesis were higher in *P. rockii* compared to *P. lutea*. Additionally, we showed that the overexpression of PrFAD2 and PrFAD3 in Arabidopsis increased linoleic and α-linolenic acid content, respectively and modulated their final ratio in

the seed oil. In conclusion, we identified the key steps that contribute to efficient ALA synthesis and validated the necessary desaturases in *P. rockii* that are responsible for not only increasing oil content but also modulating 18:2/18:3 ratio in seeds. Together, these results will aid to improve essential fatty acid content in seeds of tree peonies and other crops of agronomic interest.

[OP – 3] ARTEMISININ IS SYNTHESIZED IN HIDDEN PLACES: A NEW HOPE FOR THE ANTI-MALARIAL MEDICINE

Judd, R., Bagley, M., Zhu, Y., Mingzhou, L., Pu, G., Zhao, X., Li, C., Ekelof, M., Muddiman, D., Xie, D.
NC State University, Raleigh, NC, USA

Artemisinin-based combination therapy (ACT) forms the first line of malaria treatment. However, the yield fluctuation of artemisinin has been an unsolved problem for the global ACT demand. This problem is mainly caused by the sole glandular trichome (GT) specificity of artemisinin biosynthesis in all current *Artemisia annua* crops. Herein, we report new discoveries that non-GT cells in a novel self-pollinated (SP) *A. annua* cultivar and a natural GT-free mutant express the artemisinin biosynthetic pathway. Transcriptional analysis using qRT-PCR demonstrates the transcripts of genes of the artemisinin biosynthetic pathway in leaves of the mutant, and nearly GT-free leaves and calli of the SP inbred cultivar. LC-qTOF-MS/MS analysis shows that the three types of GT-free tissue samples produce artemisinin, artemisinic acid, and arteannuin B. Moreover, detailed IR-MALDESI imaging profiling demonstrate that these three metabolites and dihydroartemisinin are localized in non-GT cells of leaves. In conclusion, non-GT cells of the two types of genotypic *A. annua* biosynthesize artemisinin and its derivatives. This fundamental discovery not only adds new knowledge to revise the current dogma of artemisinin biosynthesis, but also expedites innovation of novel metabolic engineering technologies for high and stable production of artemisinin in the future.

[OP – 4] EVOLUTION OF METABOLIC NOVELTY: A TRICHOME-EXPRESSED INVERTASE CREATES SPECIALIZED METABOLIC DIVERSITY IN WILD TOMATO

Leong BJ., Lybrand D., Lou Y-R., Fan P., Schilmiller AL., Last RL.

Department of Plant Biology, Michigan State, East Lansing, MI, USA, 48824

Plants produce myriad taxonomically restricted specialized metabolites. This diversity – and our ability to correlate genotype with phenotype – makes the evolution of these ecologically and medicinally important compounds interesting and experimentally tractable. Trichomes of tomato and other nightshade family plants produce structurally diverse protective compounds termed acylsugars. While cultivated tomato (*Solanum lycopersicum*) accumulates strictly acylsucroses, the South American wild relative *Solanum pennellii* produces copious amounts of acylglucoses. Genetic, transgenic and biochemical dissection of the *S. pennellii* acylglucose biosynthetic pathway identified a trichome gland cell expressed invertase-like enzyme that hydrolyzes acylsucroses. This enzyme acts on the pyranose ring-acylated acylsucroses found in the wild tomato but not the furanose ring-decorated acylsucroses of cultivated tomato. These results show that modification of the core acylsucrose biosynthetic pathway leading to loss of furanose ring acylation set the stage for co-option of a general metabolic enzyme to produce a new class of protective compounds.

[OP – 5] BIOSYNTHESIS, LOCALIZATION, AND POTENTIAL FUNCTION OF AROMATIC COMPOUNDS IN CUTICULAR WAXES OF CEREALS

Sun Y, Luna-Cortes A, Jetter R

Department of Botany, University of British Columbia, Vancouver, Canada

To protect plants against biotic and abiotic stress, the waxy cuticle must coat all epidermis cells. While the bulk of cuticular waxes consists of very-long-chain acyl compounds, significant amounts of alicyclic and/or aromatic compounds are also present in the wax mixtures of many species. In particular, cereal species are known to accumulate alkyl-substituted resorcinols in surface tissues. We have isolated and characterized alkylresorcinol synthases, enzymes capable of synthesizing these aromatic compounds, from rye and Brachypodium. Their expression patterns correlated with the spatial and temporal distribution of cuticular alkylresorcinols, showing that the enzymes are dedicated to cuticle biosynthesis. However, the alkylresorcinol products were found exclusively in the intracuticular wax, buried deep inside the cuticle and not in direct contact with the biotic or abiotic environment, suggesting structural functions in the cuticular layer instead.

[OP – 6] REGULATION OF ISOPRENOID PRECURSOR PARTITIONING IN LAVENDER

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Lavender essential oil is composed of both regular and irregular monoterpenes, which are derived from linear precursors geranyl diphosphate (GPP) and lavandulyl diphosphate (LPP), respectively. Although this plant strongly expresses genes responsible for the biosynthesis of both monoterpene classes, regular monoterpenes dominate the essential oil. Given that the small subunit of GPP synthase (GPPS.SSU) modifies the activity of geranylgeranyl diphosphate synthase GGPPS to produce GPP, we hypothesized that GPPS.SSU can similarly affect the activity of LPP synthase. To test the hypothesis, we cloned and investigated GPPS, LPPS, and GGPPS cDNAs from Lavandula x intermedia, and investigated potential interaction between the recombinant forms of these proteins encoded by these genes. LiLPPS and LiGGPPS were each encoded by a single cDNA. However, LiGPPS was a heteromeric protein, consisting of a large subunit (LiGPPS.LSU) and a small subunit for which two different isoforms (LiGPPS.SSU1 and LiGPPS.SSU2) were detected. Expression of LiLPPS or LiGGPPS cDNA in E. coli produced an active protein. On the other hand, neither recombinant LiGPPS subunits was active alone. However, when co-expressed in E. coli, LiGPPS.LSU and LiGPPS.SSU1 formed an active heteromeric GPPS, while co-expression of LiGPPS.LSU with LiGPPS.SSU2 did not yield an active protein. When co-expressed, LiGPPS.SSU1 modified the activity of LiGGPPS (to produce GPP) in bacterial cells. LiGPPS.SSU1 also modified the activity of the endogenous GGPPS (to GPPS) in transformed N. benthamiana plants. However, LiGPPS.SSU1 did not affect the activity of LiLPPS. Given that LiLPPS and LiGPPS subunits are strongly expressed (at the level of transcription) in L. x intermedia flowers, our results suggest that regulatory mechanisms other than transcriptional control of these genes control the IPP/DMAPP partitioning in lavender flowers.

[OP – 7] METABOLOMICS OF FLOWER ANTHESIS

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How metabolism changes during development and what drive those changes are open questions in flower biology. During flower development, the onset of anthesis is a time of remarkable transformations: petals

unroll and stretch out, style and filaments elongate, scent emission and nectar secretion commence and pollination occur. As such, a complete reprogramming of flower metabolism is expected to occur in pre-anthesis when flowers prepare for pollination as well as in post-anthesis when functional flowers transition to fruits to sustain development and maturation of the embryo and seed set. To shed light onto the physiological process occurring at time of flower anthesis, we combined metabolomics and transcriptomics approaches and measured the whole set of primary (sugars, amino acids, and organic acids), specialized (flavonoids, carotenoids, and volatile organic compounds) metabolites and transcripts in a developmental series of flowers of *Arabidopsis thaliana*. We observed a dramatic metabolic shift characterized by two opposite trends of primary metabolite accumulation. One first group of primary metabolites showed low-abundance in pre-anthesis and high abundance in post-anthesis and a second group of primary metabolites showed the opposite behavior. Conversely, secondary metabolites of the class of flavonoids showed progressive accumulation all thought flower development. Feeding experiments with 13C and 14C labeled glucose were performed to assess the flux through the pathways of central metabolism, which reveal utilization of sugar resources for the production of the cell wall and partitioning into nectar. These metabolic changes and the transcripts that support these changes will be discussed.

Symposium 2 Natural Product Enzymology: Bridging the Gap between Bioinformatics and Biochemical Function

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When she was a child, **Isabel Desgagné-Penix**, professor in the Department of Chemistry, Biochemistry and Physics at the University of Québec at Trois-Rivières (UQTR) in Canada, saw women in her community pick up small fruits and plants to concoct herbal remedies. Since, she has always been interested in the way plants make medicines. She holds a Ph.D. in Cell and Molecular Biology from the University of Texas - San Antonio and has become a world pioneer in the use of advanced technologies for specialized plant metabolism research. Today, she directs the plant specialized metabolism research laboratory at the head of a research team made up of more than twenty people (students, postdocs, professionals). Her expertise on plant biochemistry specifically on medicinal plants is recognized worldwide. Strongly involved in the promotion of women and indigenous in science, Isabel Desgagné-Penix contributes, through her work in plant biochemistry, to design and build new biological systems for purposes useful to humanity.

[S – 2] CRACKING THE AMARYLLIDACEAE ALKALOID BIOSYNTHETIC CODE.

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Amaryllidaceae alkaloids (AAs) are a group of nitrogen-containing plant specialized metabolites comprising an estimated of 600 identified structures. In AA metabolism, norbelladline is a key branch-point intermediate that can be directed into several AA subtypes with different structural skeleton configurations. The galanthamine-type alkaloids are one subclass of AAs produced in low quantities in different Amaryllidaceae plant species. Galanthamine is the only AA used commercially for treatment of the symptoms of Alzheimer's disease. Next-generation sequencing and comparative transcriptome analysis of *Narcissus* plant parts led to the recent identification of norbelladine synthase (NBS), an Amaryllidaceae alkaloid biosynthetic enzyme regulating the production of AAs. The *de novo* assembly of transcriptomes for several other Amaryllidaceae species highlight the potential for discovery of AA biosynthetic genes with new technologies, particularly of the galanthamine branch pathway. Recent technical advances of interest include those in enzymology, next-generation sequencing, genetic modification, metabolic engineering, and synthetic biology. To further the biological production of these compounds, an understanding of their biosynthetic genes is requisite and, although only few genes are known, the majority of the reactions are reaction types that are typically

catalyzed by a collection of characterized enzyme families. The knowledge of these families can help inform efforts through homology searches to identify candidate genes to crack the AA biosynthetic code.

[OP – 8] THE DIVERSITY, ACTIVITY, BIOSYNTHESIS, AND EVOLUTION OF BIOACTIVE POLYACETYLENES IN DAUCUS CAROTA

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Polyacetylenic lipids are produced in various Apiaceae and Asteraceae species in response to pathogen attack. It has long been suspected that these compounds are natural pesticides; potentially valuable resources for creating crops with enhanced pathogen resistance. The release of a high-quality carrot genome has enabled functional genomics approaches to exploring polyacetylene structure, function, and biosynthesis. We began with a detailed analysis of carrot polyacetylene chemical structures and distribution among carrot tissues in the cultivar Danvers. We identified five major (two novel) and seven trace polyacetylenes, with falcarindiol and falcarinol predominating. In this cultivar, total polyacetylene concentrations were around 2 µg/mg. At this concentration, we found that purified falcarinol inhibited the growth rate of mycelia of the necrotrophic fungus *Sclerotinia sclerotiorum* by 25%. Next, an analysis of five carrot cultivars revealed falcarinol levels ranging from ca. 1 to 5 ug/cm2 that were positively correlated with resistance to S. sclerotiorum. These data provided a rationale and framework for searching for underlying biosynthetic genes. Previous work had identified that polyacetylene biosynthesis begins with the conversion of the monounsaturated fatty acid oleate into the polyunsaturated, acetylenic fatty acid dehydrocrepenynate. In other plant species, these steps are catalyzed by the fatty acid desaturase (FAD2) family. We found that the carrot FAD2 family is massive, with 24 members. To identify carrot FAD2s associated with polyacetylene production, we correlated polyacetylene abundance with both public RNAseq data from diverse carrot tissues and RNAseq data from carrot cell cultures before and after elicitation with an extract of fungal mycelia. By testing top candidate genes in yeast and/or Arabidopsis seeds, we identified carrot genes capable of generating dehydrocrepenynate. We are now (i) creating knockout and overexpression lines with altered polyacetylene content to test their pathogen resistance and (ii) examining the evolution of polyacetylene biosynthesis and structure in the euasterid clade.

[OP – 9] PROBING DIRIGENT PROTEIN BIOCHEMICAL MECHANISMS IN DIVERSE METABOLIC PATHWAYS

Meng, Q.[1], Moinuddin, S. G. A.[1], Kim, S-J.[1], Bedgar, D. L.[1], Smith, C. A.[2], Cort, J. R.[3], Davin, L. B.[1], and Lewis, N. G.[1]

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Dirigent proteins (DPs) are encoded by large multi-gene families throughout the plant kingdom. Pterocarpans are well known phytoalexins involved in plant defense mechanisms in plants, such as pea (*Pisum sativum*) and licorice (*Glycyrrhiza echinata*). Here we describe the first 3D DP structures of stereoselective pterocarpan-forming dirigent proteins (DPs) from pea and licorice, affording the phytoalexin medicarpin from isoflavonoid precursors. Determination of their crystal structures, and observation of their differential abilities to process 4 distinct diastereomeric isoflavonoids [(3R,4R)-7,2'-dihydroxy-4'-methoxyisoflavanol (DMI), (3S,4R)-DMI, (3R,4S)-DMI and (3S,4S)-DMI] into (-)- and (+)-medicarpin at vastly different rates, also allowed for comparison to other stereo-selective lignan and aromatic terpenoid forming homologues. Studies of these DPs suggest a common biochemical mechanism in binding and stabilizing distinct plant phenol-derived mono- and bis-quinone methide intermediates.

[OP – 10] DETERMINING THE METABOLIC ORGANIZATION AND ENZYMOLOGY OF THE *CITRUS SINENSIS* FLAVONOID BIOSYNTHETIC PATHWAY

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The focus of our research program is the analysis of secondary metabolites important for the development and survival of plants that can also be utilized for advantage to humanity. A metabolon is a group of enzymes in a biosynthetic pathway that organize spatially by forming protein-protein interactions generating a supramolecular complex with the ability to channel metabolites among the component enzymes. This advanced level of organization influences competition for shared pathway intermediates, affects flux throughout the metabolic system, helps to increase local substrate concentrations, maintains stability of labile metabolites, as well as acts to isolate potentially damaging or toxic metabolic intermediates and transition states. Although metabolon formation of the flavonoid biosynthetic pathway has been an active area of study, there is little known about formation in species that accumulate early pathway flavonoid subclasses or the role played by core structure derivatization enzymes, such as glycosyltransferases. *Citrus sinesis* is a particularly suitable and agriculturally significant system in which to perform flavonoid metabolon studies as it produces a popularly consumed food product, flavonoid compounds directly affect its taste characteristics influencing marketability, it uniquely accumulates early flavonoids such as flavones and flavanones, and its genome sequence has recently become available. Furthermore, the existence of "blood" varieties, which exclusively accumulate anthocyanins among citrus species, represents a unique opportunity to investigate the specific impact metabolon formation may have upon anthocyanin biosynthesis. We are currently determining the enzymology and metabolic organization of the *C. sinensis* flavonoid biosynthetic pathway to identify targets for improving the content and quality of flavonoid metabolites for agricultural, nutraceutical, and medicinal applications.

[OP – 11] THE DIVERSITY AND EVOLUTION OF RESIN GLYCOSIDE BIOSYNTHESIS IN THE MORNING GLORY (CONVOLVULACEAE) FAMILY

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Over a million metabolites are estimated to be produced within the plant kingdom across all taxa. One such class of compounds is referred to by multiple names such as acylsugars (Solanaceae), resin glycosides (RGs; Convolvulaceae), fatty acid glycosides (Lamiaceae), caroliniasides (Geraniaceae) and gallicosides (Caryophyllaceae). Whether these structurally analogous classes represent an ancestral state of angiosperms or scenarios of independent/convergent evolution is not clear. To obtain further insights, we examined the diversity and evolution of RGs as defense metabolites unique to the morning glory family. RG is a structurally diverse compound class, characterized by a variable length oligosaccharide core esterified with diverse short-chain fatty acids and a singular long chain fatty acid uniquely hydroxylated at the 11th position, forming a macrolactone ring. Root and leaf metabolites from ~30 different species were screened via UHPLC-MS/MS, revealing hundreds of RGs per species. Very few RGs were shared between species, suggesting rapid evolution of this phenotype. In most species, RGs were found in both organs, with variations in the number of unique compounds and relative concentration. However, some species such as *Dichondra argentea* showed differential accumulation patterns, with RGs present predominantly in *D. argentea* roots but not in leaves. Under the assumption that this pattern is caused by differential expression of underlying biosynthetic genes, RNA-seq was performed from two distantly related species with different RG accumulation patterns. This analysis identified one acyltransferase candidate capable of adding small acyl chains to the tricoloric acid substrate in vitro. Evolutionary analysis revealed gene homologs across Convolvulaceae and Solanaceae species, but best hits in Solanaceae were only 40-60% identical, suggesting rapid evolution of the enzyme. In vivo confirmation of this candidate is underway. Elucidating the RG pathway will provide a better understanding of how novel metabolic pathways emerge and why some compound classes show such a remarkable diversity.

[OP – 12] PLANT TERRESTRIAL COLONIZATION AND DIRIGENT PROTEINS: METABOLIC DIVERSITY KNOWN THUS FAR AND INSIGHTS GAINED

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Evolutionary transition of aquatic plants to a dry land habitat resulted in much of the basis of life as we know it, whether as regards: our aesthetic pleasure of land plants in their diverse native habitats; our critical dependence on land plants for food, medicines, fuels, commodity chemicals/polymers and structural materials; and our seasonal reliance on land plants from which birds, animals, insects and humanity, etc., all critically depend. We describe herein our discovery of the large dirigent protein multi-gene families that enabled land plant adaptation, and the growing discovery of their physiological functions known thus far which encompass diverse biochemical metabolic pathways and physiological functions. More specifically, a pivotal step in land plant adaptation was the emergence of dirigent proteins (including their massive multigene families), the various manifestations of which enabled emergence of both plant structural and plant chemical defenses. Yet, while more than 90% of dirigent protein functions still remain unknown, our mechanistic studies now begin to provide evidence for a common biochemical mechanism in diverse metabolic pathways including lignan, lignin, aromatic terpenoid, and pterocarpan biosynthetic pathways. These, in turn, now provide valuable insights as to how investigate - and identify - the remaining unknown biochemical pathways in plant phenol biochemical pathways that remain to be discovered, and which are envisaged to provide a more complete insight into the key processes that evolved during the various phases of land plant evolution to date.

[OP – 13] COMPUTATIONALLY-GUIDED DISCOVERY AND EXPERIMENTAL VALIDATION OF INDOLE-3-ACETIC ACID SYNTHESIS PATHWAYS

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Elucidating the interaction networks associated with secondary metabolite production in microorganisms is an ongoing challenge made all the more daunting by the rate at which DNA sequencing technology reveals new genes and potential pathways. Developing the culturing methods, expression conditions, and genetic systems needed for validating pathways in newly discovered microorganisms is often not possible. Therefore, new tools and techniques are needed for defining complex metabolic pathways. Here we describe an in vitro computationally-assisted pathway description approach that employs bioinformatic searches of genome databases, protein structural modeling, and protein-ligand docking simulations to predict the gene products most likely to be involved in a particular secondary metabolite production pathway. This information is then used to direct in vitro reconstructions of the pathway and subsequent confirmation of pathway activity. As a test system, we elucidated the pathway for biosynthesis of indole-3-acetic acid (IAA) in the plant-associated microbe *Pantoea sp*. YR343. This organism is capable of metabolizing tryptophan into the plant phytohormone IAA. BLAST analyses identified a likely three-step pathway involving an amino transferase, an indole pyruvate decarboxylase, and a dehydrogenase. However, multiple candidate enzymes were identified at each step, resulting in a large number of potential pathway reconstructions (32 different enzyme combinations). Our approach shows the effectiveness of crude extracts to rapidly elucidate enzymes leading to functional pathways. Further, in vitro testing of the pathway reconstructions revealed the underground nature of IAA metabolism in Pantoea sp. YR343 and the various mechanisms used to produce IAA. Importantly, our experiments illustrate the scalable integration of computational tools and cell-free enzymatic reactions to identify and validate metabolic pathways in a broadly applicable manner.

Symposium 3 Secondary Metabolites of Bryophytes, Ferns, and Lycophytes: Enormous Diversity from Primitive Plants

Prof. **Dr. DHC Yoshinori Asakawa**
Tokushima Bunri University
Japan

Since a half century, Asakawa and his group investigate phytochemicals of bryophytes, ferns and inedible mushrooms, their biological activity and chemosystematics as well as microbial biotransformation of secondary metabolites to produce functional products such as nootkatone, the grape fruit aroma. They found that the terpenoids (mono-, sesqui- and diterpenes) in liverworts are enantiomers of those found in higher plants.

[S – 3] LIVERWORTS: A GOOD SOURCE OF BIOLOGICALLY ACTIVE ENT-SESQUI- AND DITERPENOIDS

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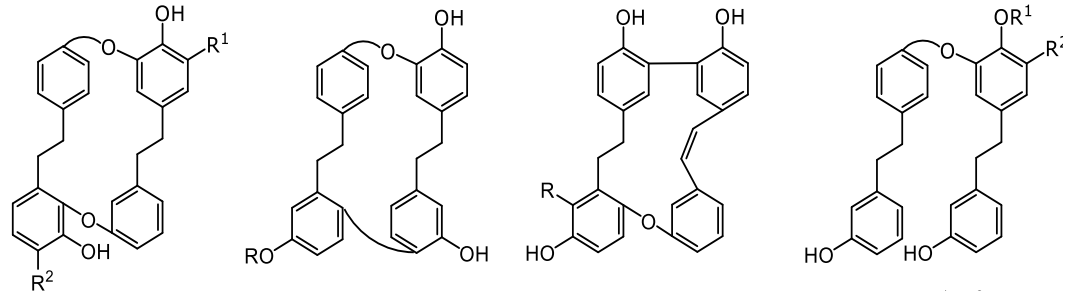
Although more than 23000 species of bryophytes have been recorded, their phytochemical studies have been neglected almost for one century, because they are useless for human diet, difficulty of their identification, collection of a large amount and some bad information that nothing biologically active compounds are included in bryophytes. Since 50 years, we focus on the isolation, structural elucidation of secondary metabolites of bryophytes, especially liverworts, since they contain beautiful oil bodies and many of them show incredibly pungent and bitter taste, and characteristic scent. At present, more than 1500 terpenoids, aromatics and polyketides were found in liverworts. The most characteristic chemical phenomenon in liverworts is that most mono-, sesqui- and diterpenoids isolated are enantiomers of those found in higher plants and discovery of bis-bibenzyls (**1-8**) in three orders, the Jungermanniales, Metzgeriales, and Marchantiales. Another chemical interest is that the isolation and detection of nitrogen and/or sulfur-containing compounds in liverworts are very rare. A few liverworts produce the exactly same sex pheromones, as those found in some brown algae. It is also chemically interesting that the secondary metabolites are not similar to those of green algae at all, but to brown algae. In this paper, characteristic chemical compounds found in liverworts, their biological activity (antimicrobial antifungal, antiviral, muscle and application of these secondary metabolites to the taxonomy of liverworts will be summarized [1a-e].



1: Marchantin A: R^1=OH; R^2=H
2: Marchantin C: R^1=R^2=H
3: Riccardin A: R=Me
4: Riccardin C: R=H
5: Isoplagochin A: R=H
6: Isoplagiochin B: R=OH
7: Perrottetin E: R^1=R^2=H
8: Perrottettin F: R^1=H; R^2=OH

Ref. [1] a) Asakawa, Y. (**1982**) in: *Progress in the Chemistry of Organic Natural Products*. ***42,*** 1-285. Springer, Vienna. b) Asakawa, Y. (**1995**) *ibid*. ***65***, 1-618. c) Asakawa, Y. Ludwiczuk, A., Nagashima, F. (**2013**) *ibid*. ***95***, 1-796. d) Asakawa, Y., Ludwiczuk, A., Toyota, M. (**2014**) in: *Handbook of Chemical and Biological Analytical Methods (II).* p. 1-53. John Wiley & Sons. e) Asakawa, Y. (**2016**) in: *Recent Advances in Polyphenols Research*, ***5***, 36-66. Jon Wiley & Sons.

[OP – 14] BIOSYNTHESIS OF TERPENOIDS IN NONSEED LAND PLANTS

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Terpenoids constitute the largest class of plant secondary metabolites. For their biosynthesis, terpene synthases are pivotal enzymes. While there is rich knowledge about terpenoid biosynthesis in seed plants, our understanding of terpenoid biosynthesis in nonseed land plants has been limited. Our recent studies showed that nonseed plants possesses two types of terpene synthase genes: typical plant terpene synthase genes (TPS) and microbial terpene synthase-like genes (MTPSL), unlike seed plants which contain only TPS genes. In this talk, I will focus on MTPSL genes. In the first half, the discovery and occurrence of MTPSL gene in nonseed plants will be introduced. In the second half, the liverwort Marchantia polymorpha will be used as a main model species for the description of the catalytic functions of MTPSLs and their contribution to the in-planta biosynthesis of terpenoids.

Symposium 4 Advances in Phytochemical Tools and Applications

Dr. Marianela Rodriguez,
BASF, USA

Marianela (Nela) Rodriguez-Carres is an Innovation Scout and Collaboration Manager at BASF. She identifies opportunities for external collaboration and evaluates radical technologies for innovative solutions at Bioscience Research. Her current portfolio includes topics across different business: Crop Protection, Personal Care, and Nutrition. Nela has over 20 years of experience in molecular and computational approaches for discovery and engineering of plants & microbes. She is a native of Panama and moved to the US for her Ph.D. in Plant Pathology/Molecular Biology at the University of Arizona. After finishing her graduate work, she obtained at fellowship at Duke University Medical Center, and has been living in Durham, North Carolina ever since. In her free time, she can often be spotted outdoors, playing disc golf, collecting fungal specimens, and chasing after her two sons, Greg and Alex.

[S – 4] PHYTOCHEMICALS AND NATURAL PRODUCTS: INNOVATIONS, CHALLENGES, AND APPLICATIONS

Chemistry as a cross-sectional technology plays a key role in addressing future challenges, because innovations in the field of chemistry provide answers to exactly those questions that will concern humanity in the future: raw materials, environment, climate, nutrition, and quality of life. Two examples of natural chemistry derived innovations with an impact in different segments are discussed: 1) Crop Protection – Inscalis®, a novel insecticide with a unique mode of action which key ingredient is produced by the fungus *Penicillium coprobium*. By optimizing the growth conditions and the chemical synthesis, BASF increased yield and created a precise solution for controlling piercing-sucking pests (aphids, whiteflies, psyllids, scales, and leafhoppers); 2) Personal Care – Dermagenist ®, an extract of Origanum majorana leaves, the first active ingredient that re-activates fibroblast by protecting them from epigenetic modification associated with aging. These examples demonstrate how BASF meets future challenges by driving innovation and collaborations across different segments and technologies, delivering solutions that combine economic success with environmental protection and social responsibility.

[OP – 15] COMPUTATION-ASSISTED ANNOTATION OF BIOLOGICAL ACTIVITY TO NATURAL PRODUCTS IN HOPS

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The conventional method to identify bioactive natural products in plant extracts is bioassay-guided fractionation. This approach requires multiple rounds of chromatographic separation and bioactivity testing of the chromatographic fractions until one obtains a fraction that contains a single active principle. It is therefore time-consuming, labor-intensive, and it has the risk of isolating known compounds. We explored ways to annotate bioactivity to natural products in crude chromatographic fractions by using computational models that detect predictors of bioactivity based on fluctuations in bioactivity with relative concentrations of compounds across crude chromatographic fractions. In a proof-of-concept study, we prepared a crude acetone extract of hops (Humulus lupulus) and obtained 40 fractions by chromatography of the extract on a Sephadex LH-20 column. We chemically characterized these fractions by loop-injection mass spectrometry. To detect anti-inflammatory compounds in the fractions, we quantified the iNOS inhibitory activity of the crude extract and the fractions (20 μg/mL) in lipopolysaccharide (LPS)-treated RAW264.7 cells. By ElasticNet computational analysis of the mass spectral and bioactivity data, we found xanthohumol in the top 10 list of predictors of bioactivity. In this list, xanthohumol ranked 4th ([2M-H]– ion) and 6th ([M-H]– ion). We identified xanthohumol by LC-MS/MS comparison with our in-house library of natural products as wells as by querying the Global Natural Products Social Molecular Networking (GNPS) tool. The integration of the ElasticNet approach with GNPS allowed us to narrow down the number of hops natural products associated with iNOS inhibitory activity and to focus on prenylflavonoids as possible bioactive candidates. We validated the findings by testing the iNOS inhibitory activity of 99.9% pure xanthohumol in this assay; it inhibited iNOS by >90% at 3.54 μg/mL (10 μM). This example demonstrates that this computational approach adequately performs to identify active principles in crude natural product extracts with reduced reliance on bioactivity-guided fractionation.

[OP – 16] PURIFICATION OF QUERCETIN SOPHOROSIDE AND EVALUATION OF ITS METABOLISM IN A RAT GUT MODEL

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Broccoli has a number of constituents that may have an effect on the prevention of the development of chronic diseases in mammals. However, the exact mechanism of how they are absorbed by the gut from food and in what form they are absorbed has been difficult to ascertain. Broccoli has a number of bioactive phytochemicals, including the flavonoid quercetin sophoroside, whose absorption by the body has not been examined. Quercetin sophoroside is difficult to purify in quantity from broccoli so it was instead purified from *Apocynum venetum* (sword-leaf dogbane or Luo Bu Ma) and characterized by MS2, 1H and 13C NMR. An in situ rat gut model was used to compare absorption and metabolism of quercetin sophoroside with that of the well-studied quercetin aglycone. In agreement with the literature, the aglycone was absorbed in the jejunum, where it underwent glucuronidation, sulfation and methylation. The quercetin sophoroside was shown to be absorbed intact and underwent subsequent methylation and sulfation in the jejunum. Whereas the aglycone also underwent a little phase II metabolism and absorption in the cecum, only products of catabolism were seen for quercetin sophoroside following cecal introduction. Cecal catabolism by the microbiota was similar among the two substrates, forming derivatives of benzoic acid, phenylacetic acid, and phenyl propionic acid. Although sophorosides are common glycosides in brassica vegetables, red raspberries and other plants, this is the first study of sophoroside absorption and metabolism. This shows that there are many intact and altered forms of this flavonoid glycoside which is a key piece of knowledge to lead to the assessment of the actual biological activity of these compounds in mammals that digest foods such as broccoli.

[OP – 17] PRODUCTION OF INDOLE-3-ACETIC ACID AND RELATED COMPOUNDS BY PANTOEA SP. YR343 AND ITS ROLE IN PLANT COLONIZATION

Kasey Estenson[1], Gregory B. Hurst[2], Robert F. Standaert[3], Amber N. Bible[1], David Garcia[1], Karuna Chourey[2], Mitchel J. Doktycz[1], and Jennifer L. Morrell-Falvey[1]

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Indole-3-acetic acid (IAA) is a critical growth regulator in plants. It is also produced by many microbes, generally using tryptophan as a precursor, and its potential role in the plant–microbe interaction is a subject of active investigation. In this work, we studied the production of IAA and related indoles by a plant-associated bacterium, *Pantoea* sp. YR343, and its role in establishment of the symbiotic relationship.
Pantoea sp. YR343 was isolated from the roots of *Populus deltoides*, and its genome was sequenced by the DOE Joint Genome Institute (JGI). Analysis of the genome led to the prediction that the bacterium can synthesize IAA via the indole-3-pyruvate (IPA) pathway. In particular, there was a predicted homolog of IPA decarboxylase (IpdC), which catalyzes the committed step in the pathway. We performed proteomic and metabolic analyses of wild-type cells as well as a *ΔipdC* mutant. Addition of tryptophan to the culture medium led to the production of IPA and IAA, consistent with an active IPA pathway; however, the primary metabolite was tryptophol. The *ΔipdC* mutant was unable to produce tryptophol, consistent with a loss of IpdC activity, but was still able to produce IAA at about 20% of the wild-type levels, either from spontaneous decomposition of IPA or through an unknown enzymatic pathway. This observation may account for the ability of the mutant to colonize poplar roots despite lacking *ipdC*. In the wild-type strain, IAA itself was found to cause upregulation of genes for transport of amino acids and carbohydrates, as well as production of extracellular polymeric substance (EPS), suggesting that IAA serves as a signal for the bacterium and potentially plays a role in establishing the plant–microbe interaction.

[OP – 18] CHARACTERIZATION OF BOTANICAL EXTRACTS BY INTEGRATION OF MASS SPECTRAL FINGERPRINTING WITH PRECURSOR ION QUANTIFICATION: APPLICATION TO EXTRACTS OF CENTELLA ASIATICA

Alcazar-Magana Armando[1,2]; Wright, Kirsten[3]; Vaswani, Ashish[1]; Caruso, Maya[3]; Gray, Nora[3]; Quinn, Joseph[3,5]; Soumyanath, Amala[3]; Stevens, Jan F[2,4]; Maier, Claudia S.[1]

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Botanical products are popular for maintaining health and preventing or treating disease. Plants are extraordinary factories of secondary metabolites, and the phytochemical composition of plant material governs the bioactivity and potential health benefits. Humans are exposed to a vast diversity of such phytochemicals through our diet. Despite botanical products are recognized for promoting health and resilience, the inconsistent phytochemical composition modulates the potential benefits or toxicity as well as the results of pre-clinical or clinical trials. Consequently, challenges in establishing the effects of botanical products reside in obtaining batch-to-batch reproducibility regarding their phytochemical composition. In this work, we developed an efficient method for the in-depth characterization of plant extracts and quantification of marker compounds in the same chromatographic run using a quadrupole time-of-flight analyzer in the data-dependent acquisition (DDA) mode. In addition to the spectral fingerprint acquisition of the extracts, this procedure also combines a post-acquisition precursor ion quantification procedure for determining levels of distinct phytochemicals in various *Centella asiatica* (*C. asiatica*) extracts, a member of the Apiaceae family, which has been used to improve memory and mental health. This integrated workflow allowed the tentative identification of 117 compounds, chemically interconnected based on Tanimoto 2D structure similarity. In

addition to this, the data acquired, can be mined in the future in case of new compounds are identified in the plant. We validate the accurate quantification of twenty-four phytochemicals commonly found in plant extracts, and this methodological approach is generally applicable to other botanical products. The differences in the composition of phytochemicals across different *C. asiatica* accessions was significant, proving that detailed characterization of plant extracts is crucial for reproducible application in laboratory studies, clinical trials, and safe ingestion. The authors acknowledge NIH grants R01AT008099, R61AT009629, T32 AT002688 and S10RR027878

Symposium 5 Chemical Ecology: Interactions of Plants with Other Organisms

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Sybille B. Unsicker is a Group Leader at the Max Planck Institute for Chemical Ecology (MPI-CE) in Jena and an associated member of the Chair for Terrestrial Ecology at the Technical University Munich (Germany) where she teaches courses in Chemical Ecology. Sybille obtained her Master Degree in Tropical Ecology at the University of Würzburg in 2002 and earned her PhD in Ecology at the University of Jena in 2007. After a one-year postdoc at the Max Planck Institute for Chemical Ecology Sybille established her own project group in 2008 to study the chemical ecology of poplar trees. She is particularly interested in the role of volatiles and phenolics in plant defense against insects and pathogens.

[S – 5] PATHOGEN EFFECTS ON TREE-INSECT INTERACTIONS

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The interaction and co-evolution of plants and insects were intensively studied in the last decades. However, plants are usually also colonized by microbial species such as bacteria and fungi which have the ability to modify the phytochemical properties of plants and thus indirectly affect plant-insect interactions. Herbivorous insects not only ingest plant material, but also compounds or tissues of microbial origin at the same time. Despite this complexity, our knowledge of tripartite interactions is rather poor, especially in trees, which are rarely the focus of plant-insect studies. My group investigates the influence of a widespread pathogen (poplar leaf rust fungus, *Melampsora larici-populina*) on the phytochemistry of black poplar (*Populus nigra*) trees and on the interaction of the tree with a generalist insect herbivore (gypsy moth, *Lymantria dispar*). In my talk, I will highlight some of our recent findings, including the consequences of the poplar leaf rust fungus on poplar phytochemistry and the beneficial effects of the pathogen on insect herbivore performance.

[OP – 19] CHEMICAL CHARACTERIZATION OF A NOVEL EMS INDUCED MUTANT WITH ALTERED CARDIAC GLYCOSIDE PROFILE IN ERYSIMUM CHEIRANTHOIDES

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Cardiac glycosides (CGs) are a specialized class of defensive secondary metabolites that evolved independently in at least a dozen plant families. Despite the medically relevant properties of these compounds, the complete CG biosynthesis pathway remains unknown. To open new avenues in characterizing the CG biosynthetic pathway, we developed an ethylmethanesulfonate (EMS)-mutagenized population of *Erysimum cheiranthoides* (wormseed wallflower), a member of the only known CG-producing crucifer genus. This species contains three main aglycones digitoxigenin, cannogenol, and strophanthidin, and

at least nine mono- and diglycoside CGs that are derived from these aglycones. We hypothesize that sequential hydroxylation converts digitoxigenin to cannogenol and then strophanthidin. In the present study, a set of 700 M2 plants was screened for altered CG phenotypes using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Here, we report the characterization of one mutant line, M454, which accumulates very low concentrations of cannogenol- (erycordin) and strophanthidin-derived (erysimoside, erychroside, strophalloside, and helveticoside) CGs. In contrast, digitoxigenin abundance is unchanged and the amount of digitoxigenin-derived CGs (glucodigitoxigenin, glucodigifucoside, dig-10, and dig-12) is 10-85 times higher in M454 than in wildtype. This suggests that a cytochrome P450 or other hydroxylase involved in the conversion of digitoxigenin to cannogenol is mutated. To identify the molecular basis of this altered phenotype, the M454 mutant was crossed to wild-type E. cheiranthoides and a segregating F2 population was generated. Analysis of more than 200 F2 progeny showed a 3:1 wildtype:mutant segregation of the CG phenotype. Bulked segregant analysis will reveal the causal mutation disrupting normal CG accumulation and will lead us to characterizing this CG biosynthetic enzyme.

[OP – 20] CONGRUENCE AND WITHIN-SEASON VARIATION IN FLORAL VISITATION AND POLLEN TRANSPORT NETWORKS IN SOUTHERN APPALACHIAN PLANT-POLLINATOR COMMUNITIES

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Our understanding of the ecological and evolutionary consequences of plant-pollinator interactions has relied on the study of interactions between species pairs despite these taking place within multi-species communities. The use of network theory to study complex interactions has allowed novel insights into the structure of plant-pollinator communities. However, network studies have relied on floral visitation data, although, this may be insufficient to fully characterize the diversity and strength of interactions. By using pollen transport data (i.e. pollen on pollinators), new insights can be gained on the structure and function of plant-pollinator communities. Yet studies that compare pollen-transport with floral-visitation networks are scarce. Furthermore, the strength and frequency of plant-pollinator interactions can vary across temporal scales. However, within-season (monthly variation) and within-day (morning vs. evening) variation in plant-pollinator networks has been little studied. This fine-scale temporal variation can be important in structuring plant-pollinator interactions. By evaluating variation in network structure across biologically relevant time scales, a better understanding of the factors that shape plant-pollinator communities can be achieved. Here, we build plant-pollinator interactions networks on floral visitation and pollen transport data by observing, collecting and sampling pollen from floral visitors across replicated transects in a Southern Appalachian floral community within a flowering season. We aim to 1) compare the congruence of plant-pollinator networks built on floral visitation and pollen transport data and 2) evaluate within season and within-day variation in plant-pollinator network structure. Preliminary results show that the structure of floral visitation and pollen transport networks are significantly different from each other. For instance, pollen-transport network size is four times larger than the floral-visitation network. Species in the pollen transport network tend to be more connected with five times more links per species on average than floral visitation networks. Within-season and within-day differences in network structure are currently being evaluated.

[OP – 21] EXOMETABOLOMIC PROFILING OF CLONOSTACHYS ROSEA CO-CULTURED WITH FUSARIUM GRAMINEARUM: OLD AND NEW SUSPECTS MEDIATE THEIR ANTAGONISM AND MYCOTOXIN TOLERANCE

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Clonostachys rosea strain ACM941 is a bio-control agent with activity against Fusarium graminearum, the causative agent of Fusarium Head Blight. Indirect evidence from comparative genomic and transcriptomic profiling studies have shown that *C. rosea* largely relies on its extensive secondary metabolism and ATP-binding cassette transporter genes to antagonize Fusarium and tolerate their mycotoxins. To establish the biochemical and molecular basis of this relationship, we co-cultured *C. rosea* strain ACM941 and F. graminearum strain GZ3639, and profiled their secreted metabolites. Concurrent with exometabolomic profiling, the transcriptional response of ACM941 co-cultured with GZ3639 were compared to monoculture grown ACM941 and GZ3639, respectively. A total of 70 differentially secreted metabolites were identified by pairwise comparisons of which 31 were secreted by ACM941 and 39 by GZ3639. Differentially secreted ACM941 exometabolites include the polyketides trichodimerol and associated bisorbicillinoid analogs, and other metabolites of interest. In contrast, GZ3639 exometabolites were dominated by trichothecenes including 15-acetyl deoxynivalenol and its analogs, while metabolites sharing common precursors with trichothecenes, like fusarielins and fusarins, were reduced. Differential regulation of putative and known secondary metabolite gene clusters were confirmed through RNAseq profiling and qPCR validation. We have conclusively established that *C. rosea* secretes antimicrobial metabolites including polyketides against GZ3639. Efforts are underway to functionally characterize candidates by gene deletion and heterologous expression. The molecular and biochemical basis of *C. rosea* antagonistic and mycotoxin tolerance mechanisms identified in these study would be helpful to improve its biocontrol and mycotoxin detoxification.

[OP – 22] THE EFFECTS OF URBANIZATION ON AVIAN SEED DISPERSAL SUCCESS OF EASTERN POISON IVY (ANACARDIACEAE)

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The rate of global urbanization is increasing rapidly as the human population expands, leading to species loss and biotic homogenization. Less studied, however, is the effect of urbanization on the frequency and efficiency of species interactions. Animal-based seed dispersal interactions may be especially sensitive to urbanization because they depend on several factors: 1) rate of feeding interactions, 2) diversity of dispersers, 3) probability of seed dispersal and 4) probability of germination after dispersal. How urbanization disrupts species interactions, including seed-dispersal, is still poorly known. In this study, we evaluate differences in the frequency and efficiency of seed dispersal between urban and natural sites using Eastern Poison Ivy (*Toxicodendron radicans*) as the focal species. Individual plants within natural and urban sites were observed in twelve-minute intervals (total 185.8 hours) during which the number and identity of feeding avian species was recorded. A total of 9500 fruits between natural and urban sites were marked with a UV fluorescent dye. Undispersed marked fruits were recovered via seed traps to estimate probability of dispersal. Defecated fruits were collected from natural and urban sites to evaluate germination efficiency after dispersal. Feeding rate was 2.3x higher in urban compared to natural sites (P=0.007). Seed disperser diversity was 3x higher in urban sites and species composition was significantly different between natural and urban sites (P=0.04). However, probability of seed dispersal was not significantly different between habitat type (P=0.4). Germination rate was 20% higher in defecated seeds from natural sites compared to defecated seeds from urban sites (P=0.005). Species composition differences between sites may play an important role in germinability of seeds; due to differences feeding strategies and their ability to scarify seeds in their digestive systems, a

necessary step for seeds that rely on animal seed dispersers. Urbanization thus negatively affects seed dispersal interactions for *T. radicans*.

[OP – 23] SWITCHGRASS EXTRACTIVES, A NEW SOURCE FOR BIOPESTICIDES AND BIODISINFECTANTS

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The value of switchgrass (*Panicum virgatum*) as a crop will be increased if commercially viable uses for all plant components are identified. The overall objective of this research project was to evaluate the potential of switchgrass extractives (nonstructural components) as antimicrobials. Content of free sugars, total phenolic (TPC), hydroxycinnamic acids (HCAs) derivatives, total flavonols, and other important phytochemicals content were determined for three switchgrass cultivars, harvested from four commercial farms in the second and third growing seasons. There was an effect of season on the amounts of TPC, HCAs, and total flavonols. The increased concentration of phytochemicals in the third season may have been due to environmental conditions or cultural practices. When ethanol-soluble extractives were concentrated and assessed for activity against pathogens, activity was correlated with chemical composition. In disk diffusion assays against eight plant pathogenic bacteria, Xanthomonas species were the most sensitive to extractives, whereas *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *tomato* were the least sensitive. Diseases caused by *X. perforans*, *Clavibacter michiganensis* subsp. *michiganensis, and P. syringae pv. tomato* were controlled by extractives treatment applied to leaves of Mountain Spring (MS) tomato. Activities of extractives were also evaluated against foodborne bacteria. In disk diffusion assays, extractives were active against all tested isolates of *Escherichia coli*, *Salmonella enterica serovars*, and *Staphylococcus aureus* with the exception of a methicillin-resistant S. aureus strain. Switchgrass extractives reduced E. coli population counts on Formica coupons to nondetectable levels after 30 min. Population counts of two *S. enterica* serovars were lower on Roma tomatoes for fruit washed with extractives. Extractives were also active against several plant pathogenic fungi in laboratory assays and provided effective control against disease caused by *Alternaria alternata* on MS tomato leaves. Results from this research provided evidence that switchgrass extractives have potential as value-added commercial biopesticides and disinfectants.

Symposium 6 Signaling in Development, Stress, and Defense

Dr. Jean Greenberg,
University of Chicago,
Chicago, USA

Jean T. Greenberg is a professor at the Department of Molecular Genetics and Cell Biology, The University of Chicago. She is also in the committee on Genetics, Genomics and Systems Biology and committee on Microbiology. Jean did her B.A Biochemistry from Barnard College, Columbia University, NY and earned her Ph.D. in Biophysics from Harvard University, MA. Her lab at the University of Chicago uses a combination of genetics, cell biology and biochemistry approaches to study how plants respond to pathogens and other environmental stresses. Jean works on multiple projects encompassing pathogenesis of Pseudomonas syringae and discovering immune components and their modifications in different hosts. Her recent focus is on the use of advanced proteomics to study chloroplast reprogramming during immune response.

[S – 6] ROLE OF NOVEL PLASTID ENVELOPE PROTEINS IN SIGNAL MOBILIZATION AND DEFENSE RESPONSE

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Plastid membranes are the key sites of biosynthesis of important signal molecules for plant immunity. Plastid inner and outer envelope membranes are embedded with channels and transporters that enable transport of nutrients, ions, lipids and metabolites. We are currently studying the reprogramming of plastid envelope constituents that helps plants adapt to diverse environments. Our lab has discovered two new classes of proteins that also localize to the chloroplast envelopes: prohibitins (PHBs) and a novel bipartite-signal containing LTP-like proteins. We found that PHB isoforms also localize to the chloroplast envelopes during stress and form complexes with ICS1 to regulate SA accumulation. AZI1 is the best-studied LTP-like bipartite-signal protein that employs a special signal-anchor mechanism for plastid envelope targeting. AZI1and its closest paralog EARLI1 is specifically necessary for systemic immunity. Its pool at the chloroplast envelope is enriched during infection, which is consistent with its role in mobilizing the plastid-derived priming signal azelaic acid (AZA). The distinct roles as well as the precise sub-cellular localizations of most other LTP-like proteins that use a similar noncanonical bipartite-signal to target plastids remain largely unknown. Using informatics and biochemical methods we confirm the plastids envelope localizations of many such LTP-like bipartite-signal proteins. We further provide evidence that these proteins have a role in systemic acquired resistance, root-associated bacterial growth promotion and may also help in signal mobilization at the chloroplast envelope. Together, our data supports our hypothesis that plastid envelopes are reprogrammed during stress to provide stress adaptation/plasticity to the host cells

[OP – 24] GLYCEOLLIN TRANSCRIPTION FACTOR GmMYB2 IS A REGULATOR OF SOYBEAN RESISTANCE TO PHYTOPHTHORA SOJAE

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Glyceollin isomers I, II, and III are the major pathogen-elicited secondary metabolites (i.e. phytoalexins) of soybean that, collectively with other 5-deoxyisoflavonoids, have been implicated in providing race-specific resistance against the devastating oomycete pathogen Phytophthora sojae. We recently identified the NAC-

family transcription factor (TF) GmNAC42-1 to be an essential regulator of some but not all glyceollin biosynthesis genes, indicating that other essential TF(s) of the glyceollin gene regulatory network (GRN) remain to be identified. Here, we conducted comparative transcriptomics on soybean hairy roots of the variety Williams 82 (W82) and imbibing seeds of Harosoy 63 (H63) upon treatment with wall glucan elicitor (WGE) from P. sojae and identified two homologous R2R3-type MYB TF genes GmMYB1 and GmMYB2 that were upregulated during the times of peak glyceollin biosynthesis. Overexpressing and RNAi silencing GmMYB2 increased and decreased, respectively, the levels of GmNAC42-1, GmMYB1, and glyceollin biosynthesis gene transcripts and metabolites in response to WGE. However, overexpressing GmMYB1 suppressed GmMYB2 and GmNAC42-1 and glyceollin I accumulations, identifying a negative feedback loop. The GmMYB2 protein bound the promoter of the glyceollin I-specific gene GLYCINOL 4-DIMETHYLALLYLTRANSFERASE (G4DT) in yeast one-hybrid (Y1H) and electrophoretic mobility shift assay (EMSA) experiments. Silencing GmMYB2/1 in W82 encoding the R gene Rps1k rendered it compatible with race 1 P. sojae, whereas overexpressing GmMYB2 rendered the universally susceptible variety Williams incompatible. Compatibility and incompatibility coincided with reduced and enhanced accumulations of glyceollin I and not the other 5-deoxyisoflavonoids. Thus, GmMYB29A2 is essential for regulating glyceollin I biosynthesis and race-specific resistance to P. sojae. Since glyceollins exhibit broad-spectrum anticancer and potent neuroprotective activities in humans, engineering the glyceollin GRN for increased biosynthesis will improve soybean composition not only for agriculture but also for human health.

[OP – 25] TOBACCO SIR2 DEACETYLASE SIP-428 IS A NEGATIVE REGULATOR OF PLANT IMMUNITY

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SIP-428, a SIR2 (Silent Information Regulator 2) enzyme is a SABP2-interacting protein (SABP2) that was identified in a yeast two-hybrid screen. Salicylic acid (SA) plays an important role in inducing defenses in plants resisting pathogenic infections. SABP2, a 29 kDA SA-binding protein catalyzes the conversion of methyl salicylate to SA. SIR2 enzymes exhibit NAD+ dependent deacetylase activity and catalyze the deacetylation of acetylated lysine residues in cellular proteins. Recombinant SIP-428, expressed in E. coli, exhibited NAD+ dependent deacetylase activity. The deacetylase activity of SIP-428 was positively modulated by the recombinant SABP2. Interestingly, the recombinant SIP-428 expressed in *E. coli* was found to be acetylated using anti-acetyl lysine antibodies. To better understand the role of SIP-428 in plant physiology, stable transgenic tobacco plants silenced in SIP-428 expression (via RNAi) and overexpressor transgenic lines (estradiol inducible) were generated. The SIP-428-silenced lines (T3 generation) showed enhanced basal resistance to virulent pathogens and enhanced activation of systemic acquired resistance (SAR) upon infection with avirulent pathogens. While the SIP-428 oversexpressor lines showed compromized basal resistance and SAR. These results suggest that SIP-428 is a negative regulator of both basal resistance and systemic aquired resistance. To understand the subcellular localization, eGFP-tagged SIP-428 was transiently expressed in *Nicotiana benthamiana* and visualized by confocal microscopy. Results suggest that SIP428 is likely a cytosolic enzyme.

[OP – 26] EVIDENCE FOR A CONNECTION BETWEEN FLAVONOID METABOLISM AND THE PLANT CIRCADIAN CLOCK

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Flavonoid metabolism is arguably one of the best-characterized biochemical systems in plants. The component enzymes are organized as a complex associated with one or more cytochrome P450s at the cytoplasmic face of the endoplasmic reticulum; a number of the core enzymes have also been found in the nucleus, where chalcone synthase (CHS) may interact with the histone remodeling machinery. To explore the

potential impact of CHS on gene expression, we generated an RNA-seq dataset for the Arabidopsis tt4-11 line, which lacks CHS enzyme and is devoid of flavonoids. The analysis identified 42 transcripts whose levels were at least two-fold higher and 121 that were at least two-fold lower in tt4-11 seedlings than in wild type. Surprisingly, a substantial number of these genes are associated with control of the circadian clock. qPCR was used to validate this finding for several genes, including the key circadian regulator CCA1. Analysis of CCA1p::luciferase and TOC1p::luciferase reporter constructs indicates that the amplitude, but not the phase, of the circadian cycle is affected in tt4-11 seedlings. This work forms the basis for future experiments aimed at understanding the mechanisms underlying this intriguing newly-discovered connection between flavonoid metabolism and the circadian clock in plants, which has recently also been reported in animal systems.

Symposium 7 Translational Phytochemistry: Commercialization of Discoveries

Dr. Ryan Philippe,
Manus Bio, USA

Ryan Philippe is the Director of Innovation and Partnerships at Manus Bio, where he oversees business development, technical partnership development and external research and commercial collaborations. Ryan joined Manus Bio in 2012 and has held various positions with increasing responsibilities, starting at the bench and transitioning to management prior to making the move to the business side of science. He is inventor or co-inventor on more than 20 issued patents and applications in synthetic biology and metabolic engineering. He received his B.Sc. in Genetics and Ph.D. in Botany from the University of British Columbia in Vancouver, BC, Canada, and pursued his post-doctoral work at the Salk Institute for Biological Studies in La Jolla, California, USA.

[S – 7] COMMERCIAL BIOPRODUCTION OF PLANT NATURAL PRODUCTS: THE MANUS BIO APPROACH

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Chemical manufacturing is undergoing a period of transition, from an era focused on petrochemical-derived chemical synthesis and plant extraction to one which leverages the power of biotechnology and biomanufacturing. Manus Bio was founded in 2011 with the mission of developing an economical and sustainable biomanufacturing platform, particularly for accessing rare and complex natural ingredients. We recreate plant processes in microorganisms to produce natural ingredients through fermentation. Our microbial platform has been optimized to convert inexpensive carbon and plant-derived substrates into rare and expensive ingredients with applications as flavors, fragrances, food ingredients, cosmetics, vitamins, pharmaceuticals and agricultural chemicals. Ultimately, our technology provides a low-cost, sustainable, and environmentally-friendly source for many ingredients used in our daily lives.

Manus Bio emerged when a group of biochemical engineers at the Massachusetts Institute of Technology (MIT) recognized a glaring gap which exists in the sustainable sourcing of many chemicals and ingredients used in our daily lives, from clothing and cosmetics to our medicines and food. Despite hundreds of years of innovation and progress in product development, ingredients continue to be sourced primarily from three main activities – mining, plant cultivation, and animal husbandry – none of which offer sustainable or environmentally-responsible solutions to the issue of chemical procurement. Fortunately, this realization came at a time of unprecedented scientific advances in biology, with many of the breakthroughs occurring in

Cambridge, MA itself. Together, the rapid commoditization of genome sequencing and gene synthesis and the growing understanding of cellular function offered by systems biology made the otherwise daunting task of engineering biology much more tractable.

By taking a multi-disciplinary approach that combines metabolic engineering, protein engineering, and traditional chemistry principles, Manus Bio has deployed a mature end-to-end commercial platform for the discovery and economical biomanufacturing of a variety of complex natural products, thus truly making nature more accessible, affordable and sustainable.

[OP – 27] FROM DISCOVERY TO MARKET: FERMENTATION FOR INGREDIENTS AND NATURAL PRODUCTS

Dahmen, JL
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Plants and their secondary metabolites have served as fragrances, flavors, and pharmaceuticals for millennia. While the discovery of new secondary metabolites is extremely important, cost barriers such as limited availability and high molecule complexity can prevent their adoption by consumers. The emerging field of synthetic biology is beginning to democratize this process. By determining the metabolic pathways by which plants make small molecules and re-constructing them an organism amenable to fermentation, we can greatly increase the availability of natural products. Here we present case studies in the natural flavorings (peach lactones) and sweeteners (steviol glycosides) space and how Conagen has produced natural product molecules heterologously via fermentation. Current and future applications of bioproduction will be discussed, and several case studies will be presented that illustrate the impact already being felt in the food and beverage space. We are interested in working with academics to sponsor projects for natural product and metabolic pathway discovery.

ARTHUR NEISH NEW INVESTIGATOR SYMPOSIUM

Ruthie Angelovici
Division of Biological Sciences
University of Missouri
Columbia, MO, USA

Ruthie Angelovici received her B.S. in Plant Science from the Tel Aviv University at Israel in 2001. She than joined Gad Galili's lab at the Weizmann institute at Israel where she received her PhD in plant science in 2009 researching the transcriptomic and metabolic networks, regulating Arabidopsis seed maturation and germination. From their she worked as a postdoc in the lab of Dean DellaPenna at Michigan State University researching the genetic architecture of seeds' free amino acids natural variation. In 2015 she has received a Postdoctoral Independent Career Potential Award from Michigan State University. Later that year she joined the faculty in University of Missouri, Columbia as an assistant professor in the Division of Biological science establishing her lab investigating the environmental and genetic regulation of amino acids in plant seeds.

[N – 1] UNCOVERING THE METABOLIC AND GENETIC REGULATION OF FREE AND BOUND AMINO ACIDS IN SEEDS

Amino acids play an important role in seed development maturation and desiccation. There are two functional pools of amino acids in seeds: the free, and the protein-bound amino acids. The free amino acids (FAA) comprise 5% of the total seed amino acids in seeds and provide building blocks for proteins, as well as precursors for many biological processes that are essential for seed development, maturation, desiccation and germination. The bound amino acids (PBAA) comprise 95% of the the total amino acids (TAA), from which ~60% is deposited in seed storage proteins (SSPs). Despite our vast understanding of the metabolic pathways of amino acids, we have very little understanding of the regulation of these traits, or the interplay between them - especially in seeds. Our research aims to enhance our fundamental understanding of the FAAs and PBAAs regulation and control under standard and stress conditions, as well as uncovering their genetic basis using quantitative and reverse genetic approaches. Our studies reveal that FAAs are plastic, and potentially adaptable and their levels are, at least in part, controlled by secondary metabolisms. In contrast, PBAAs are very robust traits, and their relative composition is rigorously maintained by balanced proteomic re-programing when stress is imposed. Our data strongly suggests a distinct metabolic and genetic regulation of FAA and PBAA. Comprehensive understanding of the regulation and mode of action of this metabolic system is crucial for efficient amino acids' biofortification in seeds.

Dylan Kosma
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Growing up surrounded by the Shawnee National Forest and the fertile soils of the Mississippi River Delta, Dylan Kosma developed a passion for plant biology at an early age. Dylan received a B.A. and M.S. in Plant Biology from Southern Illinois University Carbondale in 2002 and 2005, respectively. During that time his research encompassed bryophyte desiccation tolerance and different aspects of phytoremediation including plant cyanide metabolism. In 2009, Dylan received a Ph.D. in Horticulture from Purdue University where, under the supervision of Matthew Jenks, his research was centered on the functional role of plant cuticles in drought stress tolerance, fruit maturation, and insect resistance. Dylan conducted his post-doctoral research in the lab of John Ohlrogge and Mike Pollard at Michigan State University where he began to study the biochemistry and transcriptional regulation of suberin

biosynthesis. In 2015, Dylan joined the Department of Biochemistry and Molecular Biology faculty at the University of Nevada Reno where he continues to study the transcriptional regulation of suberin in plant defense responses.

[N – 2] PLANT BANDAGES: IDENTIFICATION of TRANSCRIPTIONAL REGULATORS OF THE PLANT WOUND HEALING PROCESS

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Suberin is a heteropolymer of aliphatics and phenylpropanoids whose presence is nearly universal in the plant kingdom. It is found in specific cell types including endodermis, periderm, and seed coats. It is a major component of the "skin" or periderm of potato tubers. It is also produced in response to environmental stresses like wounding. In the US, up to 33% of the potato crop is lost during storage equating to more than $1 billion in crop losses each year. A large proportion of those losses can be attributed to improper wound suberin formation. Until now, little has been known about the transcriptional regulation of suberin deposition. We recently discovered and described the first transcription factor known to regulate suberin deposition, AtMYB41. Here we describe aspects of the transcriptional regulation of wound suberin deposition including the discovery of transcription factors that regulate wound suberin deposition in potato and Arabidopsis as well as their potential connection to abscisic acid (ABA) and jasmonic acid (JA) signaling pathways. This research is funded by NSF.

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Patrick Horn received his B.S. in Biochemistry from The University of Texas at Austin in 2008. He then joined Kent Chapman's lab at the University of North Texas where he received a Ph.D. in Biochemistry in 2013 researching enabling technologies to analyze plant lipids. From there he worked as a postdoc in the labs of John Ohlrogge and Christoph Benning at Michigan State University researching areas of lipid droplet biosynthesis and unusual fatty acid production. In 2018, the International Plant Lipid Symposium presented him with the Paul K. Stumpf award recognizing an outstanding early-career plant lipid researcher. Later that year, he joined the faculty at East Carolina University as an assistant professor in the Department of Biology establishing his own research lab investigating plant lipids.

[N – 3] PEROXIREDOXIN Q ACTIVATES AN UNUSUAL FATTY ACID DESATURASE THROUGH REDOX REGULATION IN *ARABIDOPSIS THALIANA*

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Thylakoid membrane lipids comprised of glycolipids and the phospholipid phosphatidylglycerol (PG) are essential for proper plant growth and development, functioning of the photosynthetic apparatus, and responding to dynamic environments. Unlike other lipid classes, chloroplast PG contains a substantial

proportion of the unusual *trans* fatty acid 16:1$^{\Delta 3trans}$ (16:1t). Given the near universal presence of 16:1t in chloroplasts across the plant kingdom, we pursued a deeper investigation into the biosynthesis, turnover, and biochemical functions of chloroplast PG and 16:1t. We determined that in *Arabidopsis thaliana*, 16:1t biosynthesis requires both FATTY ACID DESATURASE4 (FAD4) and a thylakoid-associated redox protein, PEROXIREDOXIN Q (PRXQ), to produce normal levels of 16:1t. Co-expression of FAD4 and PRXQ in yeast resulted in the production of new, unusual Δ3 fatty acids suggesting a stimulation of FAD4 activity. Given that other chloroplast-located peroxiredoxins were not associated with unusual fatty acid production in *Arabidopsis* or yeast this FAD4-PRXQ relationship appears very specific. Assaying FAD4 and PRXQ transgenics in yeast and Arabidopsis revealed (1) a potential redox mechanism for FAD4 stimulation, (2) essential acidic residues for FAD4's catalytic activity, (3) an essential C-terminal domain required for FAD4's activity and stability, and (4) an association of FAD4 activity with copper-mediated biochemical pathways.

Symposium 8 Natural Products in Agriculture: Harnessing the Potential of Secondary Metabolites to Improve Crop Function

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Dr. Vaughan is a molecular biologist with interdisciplinary training in plant stress physiology, defense signaling, and secondary metabolism. Her research focuses on how weather affects crop-fungal pathogen interactions in a manner that influences mycotoxin production and contamination of grain. She seeks to identify sustainable and climate resilient strategies to eliminate mycotoxins from grain and enhance food safety. Martha received both her BSc (2004) and PhD (2010) from Virginia Tech. She then conducted postdoctoral research with the USDA-ARS Chemistry Unit at the Center for Medical, Agricultural and Veterinary Entomology in Gainesville FL. In 2013, she joined the Mycotoxin Prevention and Applied Microbiology Research Team at the National Center for Agricultural Utilization Research in Peoria, IL to research the effects of climate change on mycotoxin contamination of wheat and corn. In 2015, Dr. Vaughan received the Arthur C. Neish Young Investigator Award from the Phytochemical Society of North America. Dr. Vaughan was also named ARS Midwest Area Early Career Research Scientist of 2017 for her outstanding research contributions toward understanding plant defense responses against combined biotic and abiotic stress.

[S – 8] METABOLOMICS OF CROP RESILIENCE

Martha M. Vaughan[1], Miroslava Cuperlovic-Culf[2], Karl Vermillion[1], and Susan P. McCormick[1]

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Rising atmospheric CO_2 concentration and associated climate changes are increasing food safety concerns because the epidemiology and toxigenicity of mycotoxigenic fungal pathogens are closely linked to projected weather conditions. Crop resistance and resilience are dependent on precisely coordinated and ordered defense responses, but our understanding of how environmental conditions can regulate the production of defense metabolites is limited. We evaluated the metabolic profiles of wheat in response to *Fusarium graminearum (Fg)* at ambient (400ppm) and elevated (800ppm) CO_2 concentrations. While the effects of elevated CO_2 were dependent on both the *Fg* strain and the wheat variety, overall elevated CO_2 increased the amount of mycotoxin per unit pathogen biomass. An analysis of the phytohormone, transcriptomic and

metabolic responses of the host revealed some of the underlying causes of increased susceptibility. To harness the true potential of secondary metabolites involved in crop resistance, further research is needed to understand the impact of changing environmental conditions on regulation of defense response.

[OP – 28] DISCOVERY AND CHARACTERIZATION OF TANNASE GENES IN PLANTS

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Tannins play vital roles in resistance to biotic and abiotic stresses. Herein, we discover a gene in the tea genome, that we name CsTA, encoding the first known plant tannin hydrolase. Its recombinant protein expressed in Escherichia coli and Arabidopsis thaliana displayed tannase activities for galloylated catechins and pentagalloyglucose substrates. The CsTA tannase gene, and close homologs in other species, cluster as a unique tannase clade among the class I carboxylesterases, indicating a monophyletic origin of plant tannase genes >115 million years ago. The expression levels of CsTA and FvTA were found to be consistent with the timing of degradation of galloylated catechins in tea leaves and the accumulation of ellagitannins in strawberry fruit, respectively. Because these and other plants show great intraspecies variation in the levels of tannins, the discovery of the tannase genes now means that efforts to improve these traits for human consumption and crop durability can be better targeted.

[OP – 29] TRANSGENIC CAPTURE OF ISOPRENE LEADS TO BIOMASS INCREASE OF PLANTS

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Isoprene is the simplest terpene produced from plants. It is estimated that plants emit more than 600 million tons of isoprene per year. Isoprene is synthesized from dimethylallyl pyrophosphate (DMAPP) in chloroplasts. In this presentation, we report effects of isoprene capture and storage on plant growth and biomass. Two novel GPPS cDNAs were designed from sequences of aphid *Myzus persicae* (Mp), Arabidopsis, and a human influenza hemagglutinin. One, namely, Cy-MzGPPS-HA, was designed to localize protein in the cytosol and the other, namely PTP-MzGPPS-HA, was designed for localization in chloroplasts. Two versions of cDNAs were introduced into tobacco and *Camelina sativa*. The novel cDNAs encoded enzymes in the plant cells and reduced the emission of isoprene. More importantly, transgenic plants grew faster, developed bigger leaves, increased internodes, and promoted early flowering. The dry mass of transgenic plants was doubled compared to wide type plants. The significance of this technology will be discussed.

[OP – 30] MYB108 LOSS OF FUNCTION ENRICHES p-COUMAROYLATED AND TRICIN LIGNIN UNITS IN RICE CELL WALLS

Takuji Miyamoto[1], Rie Takada[1], Yuki Tobimatsu[1], Yuri Takeda[1], Shiro Suzuki[1], Masaomi Yamamura[1], Keishi Osakabe[2], Yuriko Osakabe[2], Masahiro Sakamoto[3], Toshiaki Umezawa[1,4]

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Breeding approaches to enrich lignins in biomass could be beneficial to 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)]. However, despite the fact that grasses are promising lignocellulose feedstocks, limited information is yet available for molecular-

breeding approaches to upregulate lignin biosynthesis in grass species. 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 tricin lignin units in cell walls, both of which are typical components in grass lignins [Koshiba et al., Plant Biotechnol., 34, 7–15 (2017)]. On the other hand, several members from subgroup 4 of the R2R3-MYB family have been shown to act as transcriptional repressors of phenylpropanoid biosynthesis, representing another potential target to manipulate lignin content in biomass. In this study, we generated lignin-enriched transgenic rice via targeted mutagenesis of the transcriptional repressor OsMYB108 using CRISPR/Cas9-mediated genome editing. The OsMYB108-knockout rice mutants displayed increased expressions of lignin biosynthetic genes and enhanced lignin deposition in culm cell walls. Chemical and two-dimensional nuclear magnetic resonance (NMR) analyses revealed that the mutant cell walls were preferentially enriched in p-coumaroylated and tricin lignin units. NMR analysis also showed that the relative abundances of major lignin linkage types were altered in the OsMYB108 mutants. This research is funded by JST/JICA SATREPS program.

Symposium 9 Natural Products in Medicine: Drug Development and Discovery

Dr. Victoria Palau
East Tennessee State University, USA

Dr. Palau is a tumor cell biologist, her field of work includes cell signaling in oncogenesis and mechanism of action of potential antineoplastic agents. In recent years she has studied novel compounds derived from species traditionally used by native people in the Andean regions of South America. She received her PhD (1999) from Florida International University on Biochemistry of DNA, and conducted postdoctoral research at the University of Miami School of Medicine. Her work has received editorial recognition by Science Signaling (Science STKE), and has been selected twice for symposia presentations at the annual meeting of one of the most prestigious cancer meetings: the American Association for Cancer Research. This award is given to only 50 out of approximately 5000 accepted abstracts submitted by research labs from all over the world. Her aim is to continue to promote the study of plant species with potential medicinal value, that may be endangered by deforestation and loss of habitat.

[S – 9] NEOPLASTIC ACTIVITY OF FLAVONOIDS DERIVED FROM ANDEAN PLANTS WITH ETHNOBOTANICAL IMPORTANCE

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About 6000 flavonoids have been identified to date, and many of these have been reported to have anti-proliferative and apoptotic effects on cancer cells by targeting several cellular signaling pathways. However, the basis of the relationships between chemical structures and the differential effects observed on certain cancer cells are not completely elucidated. Ethnobotanical studies in the Andean regions of South America have identified several plant species that are used traditionally for the treatment and prevention of various cancers. Our studies seek to confirm the identity of these species, and extract and isolate their bioactive flavonoids. Subsequent analysis of the effect and mechanism of action by which structurally similar compounds are able to inhibit cell viability on cancer cells may allow us to establish important relationships between structure and function.

[OP – 33] NATURE PRODUCT LEADS FOR DRUG RESISTANT HUMAN AND PLANT PATHOGENS

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Our ongoing drug discovery research has revealed many fascinating stories that resulted in the development of numerous natural product drug leads against several challenging human and plant diseases and highlights the importance of the conservation of terrestrial natural resources (Ibrahim et al., PNAS 2013, 110, 16832-16837). Methicillin-resistant Staphylococcus aureus (MRSA) is a destructive pathogen with a high mortality rate. More than 50% of S. aureus infections around the world are caused by MRSA. A group of active metabolites named "platanosides", (Ibrahim et al., 2015 patent US8633166B2), were isolated from *Platanus occidentalis*, commonly called American sycamore. The isolated metabolites were shown to prevent the growth of MRSA on surfaces and systemically. The in vitro anti-MRSA activity indicated that changing the olefinic geometry of the p-coumaroyl units greatly affects the MRSA activity. American sycamore is significant to the forest products industry and holds good potential as a dedicated biofuels crop grown on short rotations in plantations. However, the growth and productivity of sycamore plantations is hampered by bacterial leaf scorch disease (BLS) caused by *Xylella fastidiosa*. A potential ecological link has been suggested between the isolated platanosides and these serious diseases that harm many crucial American crops. Genomic DNA was isolated from sycamore leaves and subjected to PCR for DNA barcoding. Using the PCR method, the presence of *Xylella* was confirmed in all BLS-symptomatic sycamore samples. Validating this ecological link and developing these detection tools will ultimately facilitate selection of elite BLS-resistant families of sycamore as well as remedies to control *X. fastidiosa*-caused diseases.

[OP – 34] CARDIOPROTECTIVE AND HYPERCHOLSTEROLEMIC EFFECT OF ETHANOLIC EXTRACT OF MORMODICALCHARANTIAL IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN ADULT WISTAR RATS

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The ethanolic extract of the plant of Mormodicalcharantial and standard drug, metoprolol were prepared in normal saline and then administered orally to rats at the doses of 250 and 100mg/kg body weight (b.wt) respectively for a period of thirty days .ISO was freshly prepared in normal saline and was then used to induce MI by intraperitoneal injection at the dose of 100mg/kg to Wistar rats on the 30th day. Serum lipid profile and cardiac marker enzymes such as creatine phosphokinase (CK-MB) Isoenzyme , lactate dehydrogenase (LDH), Alanine transaminase (ALT)and Aspartate (AST) were obtained in the serum and in the heart homogenate of the experimental rats and then measured calorimetrically.The results show that isoproterenol-induced myocardiac infarction were associated with significant ($p<0.05$) increase in the activities of cardiac marker enzymes such as AST, ALT, CK-MB and LDH in the serum with concomitant decrease in the activities of these enzymes in the myocardial tissue as compared to control group. There were also significant ($p<0.05$) increase in serum level of total cholesterol (TC) ,trigyceride(TG), low density

lipoprotein(LDL) and very low density lipoprotein(VLDL) in the group injected with isoproterenol (group ii)as compared with control group . Pretreatment with leaf extract of Mormodicalcharantial at a dose of 250mg\kg b.wt and also by Metoprolol at dose 100 mg/kg body weight significantly ($p<0.05$) prevented this alteration of the lipid profile and also of the activities of these cardiac marker enzymes both in the serum and myocardial tissue as compared to isopreterenol-induced control group. The histological examinations of the heart further confirmed the cardioprotective effect Mormodicalcharantial as there were absent of swollen myocardium whereas ISO induced groups were characterized with these toxicological features . Mormodicalcharantialpossesses cardioprotective and hypocholesterolemic effects.

POSTER ABSTRACTS

[P – 1] PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF SOME NIGERIAN PLANTS

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Utilization of plants for medicinal purposes has been known for thousands of years as teas, powders, tinctures, poultices, and herbal formulations. Phytochemical investigation on three Nigerian plants *Piliostigma thonningii* (Leguminosae/Fabaceae), *Bridelia ferruginea* (Euphorbiaceae) and *Sphenocentrum jollyanum* (Menispermaceae) yielded thirty-six compounds. All the extracts, fractions and compounds were tested for antimicrobial, antiprotozoal and cannabinoid activities. The crude methanol extract of *S. jollyanum* root exhibited 98% and 80% antimicrobial activity against Aspergillus fumigatus Pinh and Vancomycin resistant enterococcus (VRE) at a concentration of 200 µg/mL, with IC50 11.45 and 12.95 µg/mL, respectively. The ethyl acetate fraction of methanol extract showed in-vitro activity against *A. fumigatus* Pinh at 83% with IC50 of <8 µg/mL. The total extract of B. ferruginea exhibited moderate activity towards CB2 receptor and 90% antiprotozoal activity against *Trypanosoma brucei brucei*. Phytochemical investigation on *S. jollyanum* yielded two new steroidal glycosides Sphenocentrocide A, and Sphenocentrocide B, and four known ecdysteroids. Phytochemical investigation on *P. thonningii* yielded two compounds newly isolated from natural sources, 2β-methoxyclovan-9α-ol, and methyl-ent-3β-hydroxylabd-8(17)-en-15-oate, along with 14 known compounds. Phytochemical investigation on *B. ferruginea* yielded 14 known compounds. Compounds isolated from P. thonningii, 2β-methoxyclovan-9α-ol and alepterolic acid, showed potential selectivity towards T. brucei with IC50 7.89 and 3.42 µM, respectively, while methyl-ent-3β-hydroxylabd-8(17)-en-15-oate showed activity towards *T. brucei* and *Leishmania donovani* Amastigote with IC50 3.84 and 7.82 µM, respectively. The structure activity relationship (SAR) of the isolated metabolites suggested that hydroxylation at C-2 enhances the antiprotozoal activity towards *T. brucei* in sesquiterpenes 2β-methoxyclovan-9α-ol and clovane-2β,9α-diol. Similarly, hydroxylation at C-3 in labdane diterpenes elevates the antiprotozoal activity towards *T. brucei*. Compounds isolated from *B. ferruginea*, Lutein exhibited 73% displacement in CB2 receptor with IC50 56.47 µM, and 93% inhibition towards T. brucei with IC50 4.16 µM, while Myricitrin showed 99% inhibition towards *Escherichia coli* with IC50 1.12 µM.

[P – 2] USING NANOMATERIALS AND ADVANCED IMAGING TO DISCERN PLANT PEPTIDE SIGNALING AND TRAFFICKING MECHANISMS

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Plants use intracellular signaling and cell-cell communication to coordinate their growth, development, and to maintain homeostasis. Small secreted peptides, like phytsulfokine (PSK), regulate cellular differentiation, growth, and response to stress. These peptides act via cell surface receptors to trigger short and long range signaling effects. PSK is a sulfated pentapeptide active at nanomolar concentrations through its Â receptors PSKR1 and PSKR2. Conserved among higher plants, PSK signaling regulates cell growth, cell longevity, differentiation, and stress/infection responses. A key goal in the field is to interrogate signaling mechanisms and outputs in whole plants under physiologically relevant conditions. However there are major experimental limitations to achieving this goal. In particular, sensitive signaling probes are often membrane impermeable and difficult to introduce into intact plant cells. Furthermore, genetically encoded probes require either the time-consuming production of stable transgenic lines or the use of conditions that can be non-physiological. I

propose to create nanofiber arrays for the in planta delivery of small molecules. Nanofibers are pointed structures that do not elicit wound responses and can stay embedded in plasma membranes that seal around them. I will use nanofibers together with molecular genetic manipulations in Arabidopsis to study PSK trafficking and signaling.

[P - 3] STRUCTURE ELUCIDATION OF A GRAPEFRUIT GLUCOSYLTRANSFERASE ENZYME MUTANT P297F

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Citrus fruits are widely consumed and can offer various health benefits. One enzyme found in grapefruits, CP3GT, catalyzes the addition of glucose to one specific flavonoid at only one site. These flavonoids are plant secondary metabolites that can be used in a variety of functions including signaling and protection. The only class of flavonoids that CP3GT glucosylates is flavonols, and this specificity is of interest to study. In order to study this enzyme and its structure a variety of mutants were created using site directed mutagenesis. One mutant, P297F, exhibited a loss of function. This mutant has been studied further by inserting a thrombin cleavage site, extracting the plasmid expressing the mutation and sequencing it. After sequencing the gene sequence was verified to be in frame and contain the needed thrombin cleavage site to remove tags used for protein purification. The plasmid was then digested, and transformed into yeast. After this, conditions for protein expression were tested over a 48 hour period. Conditions for protein purification are being optimized. After the protein is purified, conditions for crystallizations will be tested using a matrix of conditions based on previously crystallized plant GTs. Information obtained while testing conditions for protein expression, protein purification, and crystallization will also be presented. Successful crystallization would allow the enzyme mutant P297F structure to be solved through x-ray crystallography and compared with computer models.

[P – 4] PURIFICATION AND BIOLOGICAL ACTIVITY OF A DIARYLHEPTANOID COMPOUND FOUND IN LEAVES AND BARK OF ALNUS RUBRA (RED ALDER)

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Plants synthesize a wide range of secondary metabolites to adapt to the environment. Many of these metabolites are bioactive, and help the plant defend against pests and pathogens. Red alder (Alnus rubra) bark, root and leaf extract has a long history of use in traditional medicine and hygiene, and diarylheptanoids, especially oregonin, have been identified as major bioactive constituents. Diarylheptanoids have become of recent interest due to the discovery of their wide range of biological properties, including antioxidant, antifungal, and anti-cancer effects. We recently discovered that high oregonin concentration in alder leaves is associated with enhanced resistance to Western tent caterpillar. In order to test the biological activity of oregonin directly, a novel method was developed for the preparative extraction of oregonin from red alder leaf and bark material by spray drying aqueous extract into a concentrated powder. Oregonin was purified from the powder concentrate using flash chromatography. Both spray-dried raw red alder extract, and purified oregonin have shown promising insect repellence activity against Trichoplusia ni, a generalist Lepidopteran pest, at similar concentrations shown to reduce western tent caterpillar herbivory in alder leaf bioassays. Our purification method is rapid, inexpensive, and easily scaled up to quantities needed for further insect tests, as well as potential pesticide formulation development.

[P – 6] CHARACTERIZATION OF A MAMMALIAN ENDOCANNABINOID HYDROLYZING ENZYME IN PHYSCOMITRELLA PATENS

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The discovery of a mammalian endocannabinoid, anandamide (N-arachidonylethanolamide; AEA or NAE 20:4) in *Physcomitrella patens* but not in higher plants prompted our interest in characterizing its metabolism and physiological role in the early land plant. Anandamide acts as an endocannabinoid ligand in the mammalian central and peripheral systems and mediates various physiological responses. Endocannabinoid signaling is terminated by a membrane-bound fatty acid amide hydrolase (FAAH). Based on sequence identity and in silico analyses, we identified nine orthologs of human and Arabidopsis FAAH in P. patens (PpFAAH1 to PpFAAH9). Predicted structural analysis revealed that all the nine PpFAAH contain characteristic amidase signature sequence with a highly conserved catalytic triad and share a number of key features of both plant and animal FAAH. These include a membrane binding cap, membrane access channel, substrate binding pocket and as well as potential for dimerization. Among the nine, gene expression levels for PpFAAH1 and PpFAAH9 were enhanced with exogenous anandamide treatment. Further cloning and heterologous expression, followed by radiolabeled in vitro enzyme assays revealed that PpFAAH1 activity was optimal at 37 °C and pH 8.0. Furthermore, PpFAAH1 showed higher specificity to NAE 20:4 than to other N-acylethanolamines such as NAE 16:0. Highest in planta amide hydrolase activity was noted in microsomes of gametophyte tissues, suggesting the possibility for membrane localization of active FAAH. Interestingly, when FAAH1 was overexpressed, the moss cultures not only showed reduced growth but their transition from protonemal stage to gametophyte was inhibited, which was rescued in part by exogenous AEA. Unlike overexpressors of AtFAAH1, which showed enhanced growth and hypersensitivity to abscisic acid, PpFAAH1 overexpressors showed tolerance to abscisic acid. Together, these data suggest that the occurrence of anandamide and distinct properties of PpFAAH1 in early land plants have physiological implications that are different from that of higher plants.

[P – 7] A NOVEL FIELD INFRASTRUCTURE FOR PHENOMICS OF HIGH NIGHT AIR TEMPERATURE STRESS TOLERANCE IN THE RICE GERMPLASM

Quiñones, C. O.[1], Mendez, K. V.[1], Larazo, W.[1], Harris, R. S., Campbell, Z. C.[1], Aniemena, C.[1], Jimenez. K. M.[1], Adviento-Borbe, A.[3], Walia, H.[4] and Lorence, A.[1,2]

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Rice (*Oryza sativa* L.) is one of the major sources of calories for people worldwide. The USA is among the top global exporters and Arkansas produces ~50% of all USA rice. The production of rice is highly affected by increasing global air temperatures. High nighttime air temperature stress affects both rice yield and grain quality. The responses of rice to high nighttime air temperature stresses have been tested in few genotypes mostly under greenhouse conditions. One of the limitations in implementing these studies is the lack of high throughput phenotyping methods for field conditions. The physiological responses of rice to high nighttime air temperature stress are not yet fully elucidated, thus, further studies need to be conducted. In this study new infrastructure consisting of six heat tents fitted with phenomics sensors are being established in a state-of-the-art field experimental station in Arkansas. These tents are equipped with a retractable roof, solar-powered and fitted with light deprivation features; 320 rice accessions from the Rice Diversity Panel 1 will be grown in

each tent in a randomized block design. At maturity three of the tents will be heated at night to assess tolerance to high nigh air temperature stress in the panel. Integrated temperature control, humidity control, and light sensors will be deployed to record data throughout the growing period. These studies will lead to the identification of novel markers that can be used by rice breeders and molecular biologists to develop rice varieties that are more resilient to high nigh air temperature stress.

[P – 8] PHENOTYPIC CHARACTERIZATION OF CROSSES BETWEEN ARABIDOPSIS VACOULAR PROTON PYROPHOSPHATE 1 AND MYO-INOSITOL OXYGENASE OVER-EXPRESSERS IN RESPONSE TO ABIOTIC STRESSES

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Over-expression of Arabidopsis AVP1-1 a H+ pyro-phosphatase and of MIOX4, the first enzyme in the myo-inositol pathway for L-ascorbate (AsA) biosynthesis led to plants with increased biomass and tolerance to multiple abiotic stresses. In this work, we developed homozygous reciprocal crosses to investigate the possible synergy between these genes under salt stress. Our results indicate that the crosses contain significantly higher AsA and increased constitutive expression of AVP1-1 and MIOX4 genes compared to wild type (WT) and parent lines. We found that under normal growth conditions, the projected leaf area of the crosses was significantly higher compared to the one of controls. When the crosses were subjected to salt stress (150 mM NaCl), the projected leaf area of the crosses was significantly higher than the one of WT and MIOX4 over-expressers and comparable to the one of the AVP1-1 OE line. Interestingly, the photosystem II (PS II) efficiency, relative chlorophyll concentration, and linear electron flow of the crosses were significantly higher s compared to WT and MIOX4 OE when subjected to salt stress. Additionally, under this stress AsA content was also higher in the crosses compared to the WT and AVP1-1 OE line. These results indicate that the combination of the genes of interest can be advantageous for crop improvement as it provides additional stress tolerance than the use of the individual traits. Ongoing experiments are being carried out to assess the water limitation stress tolerance of the crosses using similar approaches.

[P – 9] QUANTIFICATION OF PHYTOCHEMICALS IN HAWAIIAN MEDICINAL PLANTS

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Globally, over 60% of deaths each year can be attributed to chronic diseases. Chronic diseases are long-lasting and debilitating, including conditions such as heart disease, cancer, diabetes, Alzheimer’s, and dementia; however, chronic diseases are among the most preventable of health concerns. Specific life-style and dietary changes have been shown to reduce the probability of developing chronic diseases by as much as 80%. Phytochemicals are of particular interest to human health because they can act as antioxidants, preventing cellular damage and the development of chronic diseases. The subtropical environment of the Hawaiian archipelago affords plants year-round development without seasonal threats, but this milder environment has led to increased competition for limited resources and driven species to develop a unique array of phytochemicals, as they strive to increase their overall fitness in a continual “chemical warfare” with

neighboring organisms. Members of the genus Sida (Malvaceae) are employed extensively in traditional medicine around the world due to their observed and reported antibacterial, antifungal, and antioxidative capacities. In Hawai‘i, the culturally-important native species, Sida fallax, was frequently administered in lā‘au lapa‘au (Hawaiian herbal medicine) to treat a range of medical conditions. The continued use of S. fallax and other native plant species in traditional medicine suggests a phytochemical-richness that has never been fully explored. Studies on native Hawaiian phytochemicals are limited and most species have never been phytochemically characterized. In this study, the total phenolic and total flavonoid contents of two co-occurring Sida species were analyzed in reference to commercially available Sida supplements. Whole plants were collected from multiple sites on the island of O‘ahu and each organ type (stems, leaves, flowers, and roots) was processed and analyzed separately. The findings of this study further our understanding of the density of phytochemicals produced by native Hawaiian flora and their ability to combat chronic diseases.

[P – 10] CREATION OF A PIPELINE TO ANALYZE INTRASPECIFIC VARIATION IN COMMON MILKWEED LATEX CHEMISTRY

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Milkweeds (Asclepias spp.) are the only food source for monarch butterfly (Danaus plexippus) larvae. These plants contain latex that consists primarily of sticky cis-polyisoprene compounds that inhibit insect feeding. Milkweed latex also contains multiple chemical defenses, including cardenolides, steroidal toxins that inhibit sodium/potassium ATPases in animal cells. Although milkweed cardenolides and latex have been studied previously, little is known about intraspecific variation in cardenolide content, cis-polyisoprene chain length, and the effects of these defenses on herbivory by monarch larvae and other insects. In particular, due to the incompatibility of cis-polyisoprene with mass spectrometry techniques, previous studies have not identified small-scale quantitative variation in latex rubber content. In order to generate information about natural variation in cardenolides, cis-polyisoprene quantity and chain length, and other milkweed defenses, we have created a high-throughput pipeline combining NMR, LC-MS, and SEC techniques, and are applying this to study a population of 150 diverse common milkweed (Asclepias syriaca) isolates collected from throughout the eastern United States.

[P – 11] AUTOMATED KERNEL PHENOTYPING OF CORN HYBRIDS GROWN IN ARKANSAS

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Maize (Zea mays L.) is one of the most economically important cereals crops on earth. Kernel traits are important in maize breeding programs and research. Measurement of the geometrical parameters of kernels such as area, length, contour, and width have been used to differentiate morphological variation in shape and in the estimation of yield and maize production. Traditionally, kernels have been measured by manual

methods, using calipers, which is a time consuming and error-prone process that limits the number and the quality of measurements that can be feasibly taken. With the development of high-throughput plant phenotyping (HTPP) methods, the generation of high-quality genotype data for maize is both attainable and advantageous. In this study using CyVerse together with an algorithm developed by ND Miller as part of the Phytomorph pipeline, we were able to extract the area, perimeter, length, and width profiles of kernels, as well as color hues from 250 hybrid corn lines grown in Arkansas as part of the Genomes to fields (G2F) project. This process uses digital images acquired with RGB, near infrared (NIR), and fluorescence (FLUO) sensors that are part of a Scanalyzer HTS instrument. Hundreds of thousands of images are being analyzed through the segmentation algorithm that information is increasing our knowledge and abilities to evaluate kernel traits in detail and with greater objectivity.

[P – 12] PHENOLIC COMPOUNDS AND WATER-SOLUBLE CARBOHYDRATES OF COOL-SEASON GRASSES OF CENTRAL KENTUCKY: POSSIBLE IMPLICATIONS FOR EQUINE HEALTH

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Pasture-associated laminitis, an inflammatory disease of grazing horses and ponies, is triggered by proliferation of certain bacteria in the equine hindgut. An excess of water-soluble carbohydrates from grasses is implicated in the bacterial proliferation. Because some natural products can inhibit some hindgut bacteria in vitro, phenolic compounds in cool-season grasses may modulate the effects of water-soluble carbohydrates on equine hindgut bacteria. Total water-soluble carbohydrates and phenolic compounds were quantified in 5 cultivars of cool-season grasses collected in the morning and afternoon of three springtime harvests, and the phenolic extracts were profiled by HPLC and UPLC-MS. Total water-soluble carbohydrates increased in the afternoon, whereas total phenolic compounds decreased or did not vary from morning to afternoon. Some variation due to harvest date or growth stage was observed among cultivars for both classes of compounds. In perennial ryegrass (Lolium perenne) “Calibra” and “Linn”, and in tall fescue (Lolium arundinaceum) “Cajun”, 3-caffeoylquinic acid was the dominant phenolic compound. The 4- and 5-caffeoylquinic acid isomers were present as well. In orchardgrass (Dactylis glomerata) “Persist”, caffeoylisocitrate dominated. Kentucky bluegrass (Poa pratensis) “Ginger” contained low concentrations of caffeoylisocitrate and some unidentified compounds. The crude phenolic extracts or individual phenolic compounds should be assayed for potential effects on bacteria implicated in laminitis.

[P – 13] DEVELOPMENT OF A HIGH THROUGHPUT METHOD TO QUANTIFY CHALKINESS IN MILLED RICE

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Chalkiness, the presence of white spots in rice, is a major problem for producers because it reduces the appearance, milling quality, and overall price of grains. Chalkiness is caused by low starch levels in rice kernels. Along with starch, the amount of ascorbate a plant biosynthesizes plays a major role in chalkiness.

Lowering ascorbate content in rice leads to plants with increased chalkiness. We hypothesize that increasing ascorbate in rice will reduce chalk levels. A pre-requisite for testing this hypothesis was to develop a method to accurately quantify chalkiness in rice. High throughput digital phenotyping technologies permit detailed characterization of seed physical characteristics without risking breakage. Using a high throughput platform, in this work we were able to phenotype multiple rice accessions with known chalkiness using visible, fluorescence, and near infrared cameras. Algorithms were developed to analyze the acquired images. Results support our hypothesis that high-throughput, digital phenotyping methods exhibit greater efficacy and accuracy in the positive detection and quantification of chalkiness in rice seeds as a percentage of total seed area compared to manual phenotyping methods. Next we are testing if high ascorbate lines we developed indeed have lower grain chalkiness.

[P – 14] MOLECULAR CLONING AND FUNCTIONAL CHARACTERIZATION OF A DIHYDROFLAVONOL 4-REDUCTASE FROM VITIS BELLULA

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Vitis bellulais a new grape crop in southern China. Berries of this species are rich in antioxidative anthocyanins and proanthocyanidins. This study reports cloning and functional characterization of a cDNA encoding a *V. bellulais* dihydroflavonol reductase (VbDFR) involved in the biosynthesis of anthocyanins and proanthocyanidins. A cDNA including 1014 bp was cloned from young leaves and its open reading frame (ORF) was deduced encoding 337 amino acids, highly similar to *V. vinifera* DFR(VvDFR). Green florescence protein fusion and confocal microscopy analysis determined the cytosolic localization of VbDFR in plant cells. A soluble recombinant VbDFR was induced and purified from *E. coli* for enzyme assay. In the presence of NADPH, the recombinant enzyme catalyzed dihydrokaempferol (DHK) and dihydroquercetin (DHQ) to their corresponding leucoanthocyanidins. The VbDFR cDNA was introduced into tobacco plants viaAgrobacterium-mediated transformation. The overexpression of VbDFRincreased anthocyanin production in flowers. Anthocyanin hydrolysis and chromatographic analysis revealed that transgenic flowers produced pelargonidin and delphinidin, which were not detected in control flowers. These data demonstrated that the overexpression of VbDFR produced new tobacco anthocyanidins. In summary, all data demonstrate that VbDFR is a useful gene to provide three types of substrates for metabolic engineering of anthocyanins and proanthocyanidins in grape crops and other crops.

[P – 15] COMPOSITION AND GENOMICS OF SURFACE CHEMICALS ON GRAIN OF SORGHUM BICOLOR AND RELATED GRASSES

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Sorghum bicolor is a multi-use crop with exceptional water- and nitrogen-use efficiency whose expanded use will likely decrease water and fertilizer consumption and create a more sustainable agricultural system. However, sorghum is generally not economically competitive with maize when irrigation is available, so sorghum harvest value needs to be increased. Natural waxes cover aerial plant surfaces where they protect against dry climates and increase water-use efficiency. On sorghum, waxes accumulate to levels higher than nearly all other plant species and sorghum kernel waxes are an emerging industrial bioproduct that could replace ubiquitous carnauba wax as sorghum waxes can be extracted during grain processing. The long-term

goal of this project is to combine wax biochemistry with genomics and bioinformatics to identify genes controlling sorghum wax deposition. The goal of the present work is to perform a detailed chemical characterization of waxes on sorghum grain and to uncover genes controlling the chemical profile of the kernel surface. We first extracted and fractionated sorghum grain surface chemicals into compound classes (primary alcohols, secondary alcohols, and aldehydes) and compared their melting properties against those of carnauba. The primary alcohol fraction from sorghum waxes had melting properties similar to those of carnauba wax. We next used the chemical composition information to guide transcriptome mining in public sorghum RNA-Seq datasets, resulting in the identification of 14 genes potentially involved in sorghum wax biosynthesis. We are currently working on functionally testing these genes using heterologous expression. The characteristics (expression, presence/absence, primary sequence) of sorghum wax biosynthesis genes are also being compared with related genes from other grass species, all in the context each grasses' grain wax profile.

[P – 17] DEVELOPING A SPECIALIZED TRAP CROP FOR THE CONTROL OF STRIPED FLEA BEETLE

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The striped flea beetle, *Phyllotretta striolata*, is an invasive pest to plants in the mustard family. It is particularly devastating to the oil seed industry where it causes an estimated US $300 million in losses a year (Knodel and Olson, 2002). Pheromone lures and trap crops are an appealing pest management approach where insect or plant volatiles are used to lure pests off crops of interest. Previously, the sesquiterpene, himachaladiene, has been identified as a key component of the *P. striolata* aggregation pheromone. Himachaladiene is synthesized by terpene synthase-1 (TPS1) from the less common farnesyl diphosphate (FDP) isomer, (Z,E)-FDP, which in turn is made from isopentenyl diphosphate (IDP) and geranyl diphosphate (GDP) by the isoprenyl diphosphate synthase IDS3 (Beran et al., 2016a, b). Here we seek to produce trap plants that emit P. striolata aggregation pheromone in addition to host plant volatiles for enhanced insect attraction. To achieve this goal, a stepwise approach to genetic engineering is being used: P. striolata IDS3 and TPS1 genes will first be transiently expressed in *Nicotiana benthamiana* to test the production of the pheromone in a plant system. Following this step, stable transformation will be tested in the model plant Arabidopsis thaliana prior to transformation in Brassica. Successful transformants will be used in behavioral assays. This research is funded by USDA NIFA AFRI.

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Beran, F., et al. (2016b) "Novel Family of Terpene Synthases Evolved from Trans-Isoprenyl Diphosphate Synthases in a Flea Beetle." Proc Natl Acad Sci U S A 113: 2922-7.

Knodel, J.J. and D.L. Olson (2002) "Biology and Integrated Pest Management of the Crucifer Flea Beetle in Canola." Fargo, North Dakota: North Dakota State University Cooperative Extension Service.

[P – 18] PLANT BIDIRECTIONAL PROMOTERS ENABLE COMPLEX ENGINEERING OF MULTIGENE METABOLIC PATHWAYS

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Genetically engineered plants offer a promising platform for sustainable bioproduction of compounds for biofuels, cosmetics, health, and other industries. Squalene, a biofuel target, is the C30 precursor to triterpenes, a diverse class of natural products with wide applications. Engineering plants for robust squalene production requires installation of upstream pathways and compartmentalization of the squalene product to increase production and reduce native metabolism interference, requiring transformation of several genes. Plant transformations, however, can take months to complete and quickly lose efficiency when using large, multigene constructs or co-transforming multiple genes on separate constructs. To maximize the number of genes in a construct for transformation, bidirectional promoters (BDPs) can enable more compact, synthetic gene clusters. When combined with "self-cleaving" peptide linkers, one BDP can divergently express four or more genes. Additionally, BDPs with differential expression on either side can be used to fine tune co-expression of several genes, providing greater regulation than common methods. In this work, a library is being built of differentially expressing BDPs in leaf tissue of Populus trichocarpa (poplar). These BDPs are being characterized in Nicotiana benthamiana and poplar through transient expression. Select BDPs will be used to engineer plants with synthetic gene clusters of up to eight genes for biosynthesis of squalene and downstream triterpenes. This library would enable complex metabolic engineering in plants, maximizing the number of genes in constructs and improving expression regulation.

[P – 19] THE SEARCH FOR NOVEL HERBICIDAL NATURAL PRODUCTS IN STRAWBERRY GUAVA

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There is a growing interest in alternatives to commercial herbicides and increased enthusiasm for organic farming practices. This is particularly true in Hawaii where the protection of land, water, and its residents are of the highest concern. The search for new herbicidal compounds is continuing utilizing strawberry guava (Psidium cattleianum). Strawberry guava is one of the most widespread invasive species to Hawaii due in part to its lack of native predators and proposed allelopathic properties. A plant having allelopathic compounds, or having the property of allelopathy, is defined as one which can produce its own natural herbicides to compete against other plants for desired resources such as nutrients or sunlight. The common or American guava, Psidium guajava, is a close strawberry guava relative that has previously been shown to have these allelopathic properties. As such, Strawberry guava represents an as of yet uninvestigated source of potential new herbicidal compounds. We intend to establish the allelopathic profile of *P. cattleianum*, and investigate any identified compounds with weed killing activity as potential new herbicide leads. Furthermore, the molecular target sites of any newly discovered compounds will be investigated, which has the potential to contribute additional herbicidal modes of action to aid in combatting evolved herbicide resistance.

[P – 20] TERPENE BIOSYNTHESIS IN RED ALGAE IS CATALYZED BY MICROBIAL TYPE BUT NOT TYPICAL PLANT TERPENE SYNTHASES

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Red algae (Rhodophyta) and land plants belong to the monophyletic clade Archaeplastida, and taxa of both groups are rich producers of terpene secondary metabolites. The terpene carbon skeletons of land plants are made by two types of terpene synthases: typical plant terpene synthases and microbial-type terpene synthases (MTPSLs); however, terpene biosynthesis in red algae is poorly understood. By systematic sequence analysis of seven genomes and 34 transcriptomes of red algae, MTPSL homologs were identified within one genome and two transcriptomes, whereas no homolog of typical plant terpene synthase genes was found. Phylogenetic analysis showed that red algae MTPSLs group with bacterial terpene synthases. Analysis of the genome assembly and characterization of neighboring genes demonstrated red algal MTPSLs to be bona fide red algal genes and not microbial contaminants. MTPSL genes from *Porphyridium purpureum* and *Erythrolobus australicus* were characterized via heterologous expression in Escherichia coli and demonstrated to have sesquiterpene synthase activities. We detected a number of volatile sesquiterpenes in the headspace of *P. purpureum* and E. australicus cultures, most identical to the in vitro products of the respective MTPSLs. Expression of the MTPSL gene in *P. purpureum* was found to be induced by methyl jasmonate, suggesting a role for this gene in host defense. In summary, this study indicates that the formation of terpene carbon skeletons in red algae is carried out by MTPSLs that are phylogenetically unrelated to typical plant terpene synthases and most likely originated in Rhodophyta via horizontal gene transfer from bacteria.

[P – 21] DISTINCT METABOLIC MODES DRIVE VARIATION IN CYCLIC AND ACYCLIC MONOTERPENOID BIOSYNTHESIS IN PELARGONIUM GRAVEOLENS CHEMOTYPES

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Pelargonium (scented geraniums) is a genus of flowering plant in the Geraniaceae known for its pleasing aromas. Its essential oils are used for fragrance and flavoring but also possess arachnicidal and antimicrobial properties. Despite its widespread use in the cosmetics and cleaning industries, little is known about Pelargonium essential oil biosynthesis. Here we demonstrate the contribution of at least two distinct metabolic pathways responsible for the characteristic monoterpenoid volatile blend in Pelargonium. The first group consists of the cyclic p-menthane monoterpenes (-)-isomenthone and (+)-limonene which resemble high value monoterpenes found in peppermint but with inverted stereochemistry. The second group, referred to here as citronelloid monoterpenes, include acyclic monoterpene alcohols such as geraniol and (-)-citronellol, and their ester and aldehyde derivatives. Using untargeted volatile profiling of 22 seed-grown lines of wild-type *P. graveolens* we identified 3 distinct chemotypes which predominantly accumulate either (-)-isomenthone, geraniol, or (-)-citronellol with minor contributions from approximately 80 other volatile compounds. We exploited the metabolic differences of these chemotypes in whole plant 13CO2 isotopic labelling assays to determine that (1) the p-menthane monoterpenoids are likely synthesized from (+)-limonene via (+)-piperitone , (2) these two groups of monoterpenes utilize a common pool of geranyl

diphosphate (GDP) precursor supplied by the 2C-methyl-D-erythritol-4-phosphate pathway and (3) downstream of GDP, these two pathways are functionally independent and do not appear to share common intermediates.

[P – 22] EXPLORING PHYTOCHEMICAL DIVERSITY IN INGA (FABACEAE) USING MSMS-BASED MOLECULAR NETWORKING

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Plants have evolved the ability to produce a wide array of small molecules, many of which are thought to be involved in defense against biotic and abiotic stressors. Investigating the structural and functional diversity of phytochemicals presents a major challenge, as most of these compounds have not been characterized. One promising approach lies in MSMS-based molecular networking, which uses characteristic fragmentation patterns of known and unknown compounds to generate a compound similarity network. Here we present methods to use this network approach to characterize both intraspecific and interspecific chemical diversity. We collected MSMS spectra of compounds from an intermediate polarity extraction of young leaf tissue from 120 Inga species and built a molecular network (gnps.ucsd.edu). Using this network, we generated two different measures of chemical diversity that are useful in studying evolutionary patterns of secondary metabolism. Intraspecific chemical diversity adapts measures of species functional diversity to estimate compound diversity within the chemical profile of a single species. Interspecific chemical similarity measures how similar the chemical profiles of any pair of species are to each other. These metrics are useful in asking a number of ecological questions: Do Inga species primarily invest in one class of secondary metabolites, or do produce many compound classes to build a wide defensive profile? Do closely related species have similar chemical profiles, or are they highly divergent? Do herbivores evolve along with Inga in a chemical arms race, or do they jump to hosts with defenses similar to those they are already adapted to?

[P – 23] RELATIONSHIP OF THE PHYTOCHEMICAL COMPOSITION OF EXTRACTS FROM COFFEE AND COCOA BY-PRODUCTS AND THEIR IN VITRO POTENTIAL AGAINST INFLAMMATION, OXIDATIVE STRESS, ADIPOGENESIS, AND DIABETES

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We aimed to compare the composition-bioactivity profile of coffee and cocoa by-products by comprehensively characterizing the composition of extracts from coffee husk, coffee silverskin, and cocoa shell and evaluating their in vitro biological activity in RAW264.7 macrophages and 3T3-L1 adipocytes. Coffee husk and coffee silverskin extracts were mainly composed of caffeine (9.8 and 19.2 mg/g, respectively) and chlorogenic acid (3.5 and 2.8 mg/g, respectively) whereas cocoa shell contained a high amount of theobromine (10.0 mg/g) and protocatechuic acid (0.8 mg/g). Likewise, other phenolics were found in significant concentrations (kaempferol-3-O-galactoside in coffee husk; caffeic acid in coffee silverskin; and the flavanols (+)-catechin, (–)-epicatechin, and procyanidin B2 in cocoa shell). Extracts effectively reduced inflammatory markers in macrophages and adipocytes (NO, PGE2, TNF-α, MCP-1, IL-6) and reduced the production of radical species (21.5-66.4%). Extracts were able to diminish lipid accumulation (4.1-49.1%) in

adipocytes by regulating lipolysis and inducing adipocyte browning. Insulin resistance was counteracted in adipocytes via of GLUT-4 translocation elicitation (52.4-72.9%) and insulin receptor signaling pathway phosphorylation modulation. Correlations were carried out to find the underlying associations among phytochemicals compounds from coffee and cocoa by-products and their in vitro biological activity, and asses their contribution to each of the observed properties. Protocatechuic, salicylic, and p-coumaric acids together with flavanols were suggested as main actors in the protection against inflammation. Oxidative stress reduction properties of the extracts correlated with the presence of gallic, protocatechuic, and p-coumaric acids. Gallic and chlorogenic acids and flavonols were associated with reduced adipogenesis. The anti-diabetic potential of the extracts from coffee and cocoa by-products was mainly associated with protocatechuic and chlorogenic acid concentrations. We established, for the first time, the relationship of the composition of different phytochemicals among coffee and cocoa by-products and their in vitro potential to reduce markers of inflammation, oxidative stress, adipogenesis, and insulin resistance.

[P – 24] DIFFERENT INDUCED 4-COUMARATE: COA LIGASES REGULATE PHENYPROPANOIDS METABOLISM BRANCH INTO LIGNIN AND FLAVONOIDS PATHWAYS IN TEA PLANT

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In tea plants, several structure genes involved in phenylpropanoid compounds biosynthesis have been identified such as CsDFR, CsF3’5’H, CsF3H, CsLAR and CsGST. However, all these proteins catalyze the down-stream bio-reactions in phenylpropaoids metabolism flux especially in flavonoids pathway, until now, if there existed different cluster 4CL genes that regulate phenyproppanids metabolism flux into lignin and flavonoids metabolism flux, is still unclear. Here, we reported two different 4CL genes in tea plant, named as Cs4CL1 and Cs4CL2. Cs4CL1 mainly expressing in root and stem, Cs4CL2 mainly expressing in leaves. Promoter and expression data suggested that Cs4CL1 transcription is more sensitive to mechanical injury and Cs4CL2 could be induced under UV-B light stress. Cs4CL1 and Cs4CL2 recombinant proteins could catalyze Coa adding reaction when using p-coumaric acid, ferulic acid and caffeic acid as substrates, caffeic acid is the optimal substrate for Cs4CL1, p-coumaric acid is the optimal substrate for Cs4CL2. Overexpressing Cs4CL1 and Cs4CL2 in model plant tobacco could increase lignin accumulation in transgenic plants, additional, in Cs4CL2 transgenic tobacco plants accumulated more flavonoids compounds. All above data suggested that the different induced Cs4CL regulated phenylpropanoids metabolism flux into lignin and flavonoids pathway.

[P – 25] BIOCHEMICAL EVALUATION OF A UNIQUE PAIR OF FERREDOXIN AND FERREDOXIN NADP+ REDUCTASE ISOFORMS IN NON-PHOTOSYNTHETIC GLANDULAR TRICHOMES

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Peppermint and its relatives in the mint family (Lamiaceae) are commercially valuable culinary herbs that accumulate high monoterpenoid essential oil concentrations within glandular trichomes (GTs). The precursors for these molecules are synthesized by the methylerythritol phosphate (MEP) pathway. Based on data from genome-scale metabolic modeling and cell type-specific transcriptome analyses, we hypothesized that a unique pair of ferredoxin (Fd) and ferredoxin NADP+ reductase (FNR) are present in non-photosynthetic GTs

to facilitate high flux through the reductive steps of the MEP pathway. In peppermint GTs, two Fd isoforms were identified: a 'minor' isoform identical to that found in roots with very low expression, and a 'major' isoform distinct from root or leaf isoforms with high expression. The FNR isoform expressed in peppermint GTs was found to be identical to the root isoform. Vectors containing the various isoforms of Fd and FNR were transformed into E. coli and the corresponding recombinant proteins purified. Electrochemical assays with these proteins indicated a wide range of redox potentials, with those representing GT isoforms enabling an electron flow particularly suitable for non-photosynthetic cell types.

[P – 26] NOVEL BIOACTIVE PEPTIDES IN *VIOLA INCONSPICUA*

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Rapidly emerging multidrug resistant pathogens and a deficit of new antimicrobial compounds entering the clinical pipeline necessitate the exploration of alternative sources of therapeutics. Botanical remedies derived from the genus Viola have been a staple of traditional medicines for centuries. Among the abundant secondary metabolites present in Viola spp., cyclotides are a rapidly growing class of highly stable, cyclic, and disulfide bound peptides with diverse intrinsic bioactivities. Herein we explore the cyclotide constituents of the botanical species *Viola inconspicua* via a reduction/alkylation mass shift analysis. Cyclotide-containing fractions of a *V. inconspicua* peptide are assayed for clinically-relevant Gram-negative antibacterial activity. Tandem mass spectrometry reveals novel bioactive cyclotide sequences and adds to the expansive suite of bioactive cyclotides found in Viola.

[P – 27] CHEMOPHENETICS OF MEZILAURUS CLADE (LAURACEAE)

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The Mezilaurus clade is an important group in Lauraceae molecular phylogeny. The clade comprises the genera *Anaueria*, *Chlorocardium*, *Clinostemon*, *Mezilaurus*, *Sextonia* and *Williamodendron*, respectively. In our study of this clade, multivariate analysis (MVA) was used to evaluate their phytochemical profiles, in order to both gain understanding about their phytochemical constituents, as well as to reveal their unexplored taxonomic potential. In the present work of the *Mezilaurus* clade members, MVA statistical treatment of HPLC-MS/MS data obtained from micro-extracts of leaves (25 mg in 1 mL methanol) was carried out. The chemical compositions of *Williamodendron*, *Anaueria brasiliensis* and 3 species of Mezilaurus are described herein for the first time. Principal component analysis (PCA) established the major constituents in these groups to be Î³-lactones, alkaloids and lignoids. Hierarchical Cluster Analysis (HCA) plots were also generated showing good correlations with the taxonomic data, especially within the Mezilaurus genus.

[P – 28] THE BZIP TRANSCRIPTION FACTOR MXBZIP10 FROM MALUS XIAOJINENSIS REGULATED IN RESPONSE TO IRON DEFICIENT STRESS

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The bZIP family transcription factors are widely involved in plant growth, development and biological and abiotic stress signaling. However, few reports have thus far been characterized in the regulation of nutrition deficiency stress. In the early study, the EST sequence of bZIP10 transcription factor was screened from the iron deficiency suppression subtractive hybridization library of apple, which implies that the transcription factors of the bZIP family were involved in the response to the iron deficiency stress in *Malus xiaojinensis*. According to the EST sequence information, MxbZIP10 was isolated from *Malus xiaojinensis*, and which includes 897 bp ORF, encodes 298 amino acids , contains a conservative bZIP domain. The subcellular localization of MxbZIP10-GFP in tobacco seedlings leaves revealed that MxbZIP10 protein localizes in nuclear. Northern blot detection using digoxigenin (DIG)-labeled oligonucleotide probesÂ indicated that expression of MxbZIP10 gene was up-regulated in roots of *Malus xiaojinensis* seedlings under iron deficiency conditions. Arabidopsis overexpression MxbZIP10 showed significantly increased root length and improved Fe, Mn, Cu and Zn content under iron deficient condition. Rhizosphere stained with bromo cresol violet indicated acidified. Perl-stained maturation zone of 2-week-old plants showed MxbZIP10 overexpressed Arabidopsis contain obviously amounts of ferric iron in their roots compared with wild type plants germinated and grown on iron-adequated or iron-inadequated media. To make clear if and how MxbZIP10 directly regulates iron homeostasis genes, we initiated find MxbZIP10 direct targets by performing Co-IP (coprecipitation) with the MxbZIP10 by the pSuper1300-MxbZIP10::GFP Arabidopsis. According to the protein prediction results, MxbZIP10 has an influence on the kinase and leaf stomata. 202 different proteins were found between the pSuper1300-MxbZIP10::GFP arabidopsis and pSuper1300-GFP *Arabidopsis thaliana* according to Co-IP, the 85 proteins are more reliable than 95%, and these proteins exist in the nucleus, chloroplasts and mitochondria.

[P – 29] TRANSPOSON-MEDIATED EXPANSION OF A BACTERIAL DEFENSE REGULON PROMOTES ARABIDOPSIS CHEMICAL DIVERSITY

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Plants synthesize hundreds of thousands of ecologically specialized, lineage-specific metabolites through biosynthetic gene duplication and functional specialization. In the latter process, it is unclear how duplicated genes become wired into existing regulatory networks. Here, we show that the duplicated gene CYP82C2 was recruited into the WRKY33 regulon and indole-3-carbonylnitrile (ICN) biosynthetic pathway through exaptation of a retroduplicated LINE retrotransposon (EPCOT3) into a novel enhancer. The stepwise development of a chromatin-accessible WRKY33-binding site on EPCOT3 potentiated the regulatory neofunctionalization of CYP82C2 and evolution of the inducible defense metabolite 4-hydroxy-ICN in Arabidopsis thaliana. Transposable elements have long been recognized to have the potential to rewire regulatory networks; these results establish a more complete understanding of how duplicated genes and transposable elements contribute in concert to chemical diversity and pathogen defense.

[P – 30] GRAPEFRUIT FLAVONOID SPECIFIC 3-O GLUCOSYLTRANSFERASE: EFFECT OF TAGS ON ACTIVITY AND PREPARATION FOR CRYSTALLIZATION

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Citrus produce secondary metabolites that are modified by glucosyltransferases. Glucosyltransferases are ubiquitous in plants but vary greatly in their specificity. One glucosyltransferase (CP3GT), found in grapefruit (Citrus paradisi), was shown to exclusively glucosylate flavonols at the 3-OH position. While many GT's have been identified in "-omic" databases, they vary greatly in their structural identity, and only a small percentage of putative GT's have been functionally characterized. Research on GT structure function relationships strengthens the reliability of genomic database annotations and makes significant contributions to the field of enzyme biotechnology. Bioenergy research and custom enzyme synthesis rely on GT structural data. X-ray crystallography is a proven method for determining structure and discerning structure/function relationships; to date 6 plant GT's have solved crystal structures. The specificity of CP3GT is different from those and thus this research was designed to prepare CP3GT for crystallization by determining the conditions necessary to collect native, active protein that is 95-99% pure. This requires the removal of a c-myc and a 6x-His tag by inserting a thrombin cleavage site upstream of the tags and digesting pure rCP3GT protein with thrombin. Wild type CP3GT and one mutant underwent site-directed mutagenesis to insert a thrombin cleavage site. rCP3GT was expressed in yeast using methanol induction and was purified using IMAC. Data showed that 3.3U of thrombin digested 1µg of protein when carried out at 4o C for 2 hours. Storage at 4o C for 2 hours resulted in retention of 70% of CP3GT activity. The effect of thrombin treatment on activity was tested by assaying pure CP3GT with and without tags using the flavonol quercetin. Data showed no significant difference in activity between tagged and native enzyme. Future experiments will test the effect of tags on enzyme kinetic characters and conditions for crystallization will be explored.

[P – 31] MAIZE BIOCHEMICAL DEFENSE AGAINST FUSARIUM GRAMINEARUM

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Natural variation in plant resistance against pests and pathogens can be explained to a large extent by variation in specialized metabolism. B73 and Mo17, two maize (Zea mays) inbred lines with contrasting resistance against the fungal pathogen *Fusarium graminearum*, have extensive differences in their constitutive and fungus-induced metabolite profiles. Genetic mapping of this metabolic diversity using recombinant inbred lines led to the discovery of a vesicular transport protein that influences the abundance of more than fifty maize root metabolites. Acetylated diferuloylsucrose was isolated and characterized as a previously unknown maize antifungal compound. To investigate maize metabolic diversity beyond that which is found in B73 and Mo17, a panel of 280 inbred lines was used for genome-wide association studies. This genetic mapping approach has resulted in the discovery of numerous quantitative trait loci that influence the production of defense-related maize metabolites.

[P – 32] PROJECT #CHEMICALBLOOM: A CITIZEN SCIENCE VENTURE TO SURVEY EPICUTICULAR WAX DEPOSITS

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To protect themselves from their environment, some plants naturally coat themselves in a thick, white layer of chemicals called a chemical bloom. The cells that create plant chemical blooms are nature's version of a chemical factory – except that nature's factories are biodegradable, self-healing, fueled by sunlight, and integrate seamlessly into their local ecosystems. Our long-term goal is to learn about these environmentally-friendly chemical factories so that someday we can use them to synthesize plant chemicals that are medicinal and/or important to the human diet. To begin working toward this goal, we aim to answer these two questions: What plant species have chemical blooms? What molecules make up chemical blooms? To answer these questions, we have started a citizen science project in which anyone can (i) report a chemical bloom using photographs and social media networks and/or (ii) collect a chemical bloom and send it to the project headquarters for mass spectrometric analysis by requesting a bioprospecting kit from the project. This poster will describe in detail how the project is organized, feature results from several citizen participants, and summarize all the results obtained so far. Bioprospecting kits will also be available to the audience should they wish to participate in the project.

[P – 33] C-TERMINAL REGION OF WRI1 PARALOGS IN AVOCADO IS A POTENTIAL TARGET FOR OIL ENHANCEMENT IN NONSEED TISSUES

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WRINKLED1 (WRI1), a member of AP2/EREBP class of transcription factors regulates carbon allocation between glycolytic and fatty acid biosynthetic pathway in plants. Additionally, among the four WRI1 paralogs in arabidopsis, WRI3 and 4 but not WRI2, are also able to increase fatty acid content in seed tissue. While the role of WRI1 is well established in seeds, the potential role of WRI1 or its paralogs as master regulators in oil-rich nonseed tissues is poorly understood. One of the basal angiosperm avocado (Persea americana) accumulates high oil content in its mesocarp throughout its fruit development. Recent transcriptome studies of avocado mesocarp revealed that the ortholog of WRI2, along with WRI1 and WRI3 were highly expressed throughout the developmental stages. Through transient expression assays, we further demonstrated that both PaWRI1 and PaWRI2 can accumulate oil in tobacco leaves. We conducted a comprehensive and comparative in silico analysis of WRI1, 2 & 3 orthologs from *A. thaliana* (dicot), *Z. mays* (monocot) and *P. americana* (basal angiosperm) to identify distinct features associated with function. Our data shows a difference in C-terminal intrinsically disordered region (IDR) and potential phosphorylation sites in PamWRI1 & 2, which might suggest their possible role in high oil biosynthesis in mesocarp tissue. Also, enrichment with hydrophobic amino acid and depletion of hydrophilic amino acid leads to high random coil secondary structure with PamWRI1 &2 showing the highest percentage among all. Absence of C-terminal PEST motif in PamWRI1 & 2 might result in their stability in nonseed tissue and thus leads to high oil biosynthesis and accumulation. These data suggest that variable C-terminal region among the WRI1 orthologs is a potential target to enhance oil biosynthesis in nonseed tissues.

[P – 34] AROGENATE DEHYDRATASES AND ISOFLAVONOID METABOLON IN SOYBEAN (GLYCINE MAX): IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION

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Specialized metabolites in plants are imperative for a variety of stress response mechanisms. Understanding the processes by which these metabolites are synthesized may help to improve the genetic manipulation of crops. Previously we demonstrated the existence of an isoflavonoid metabolon in soybean. Among many phenylpropanoid enzymes, two arogenate dehydratases (ADTs), necessary for the synthesis of phenylalanine in plants, were shown to be part of the isoflavanoid metabolon. This was surprising as the isoflavonoid metabolon is anchored to the endoplasmic reticulum, but GmADTs mostly localize to the chloroplast. Phenylalanine is a precursor to many specialized metabolites, including isoflavanoids. In other plants, some ADTs have been shown to possess prephenate dehydratase (PDT) activity, which catalyzes an alternate route of phenylalanine synthesis. Soybean contains 9 putative GmADTs, some of which may possess PDT activity. Here, we aim to functionally characterize all GmADTs for their ADT/PDT activity. Six GmADTs were cloned into a yeast expression vector and transformed into pha2, a knockout yeast strain that lacks PDT activity rendering the strain unable to synthesize phenylalanine. PDT activity of GmADTs were determined by testing transformants for their ability to complement pha2 on media without phenylalanine. Among the six GmADT isoforms, GmADTU4 and GmADT12B were able to grow on media lacking phenylalanine, demonstrating their ability to convert prephenate to phenylalanine. Characterization of other GmADTs for their PDT/ADT activities is ongoing.

[P – 35] PARTIAL STRUCTURE ASSIGNMENT OF A POLYKETIDE ISOLATED FROM *RHODOCOCCUS* BACTERIUM USING 2D-NMR TECHNIQUES

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Rhodococcus, a soil bacterium, is known for its ability to catabolize a wide range of compounds. It is under study to explore its ability to produce antibacterial compounds [Ward, A. L.; Manikindi, P.; Borisova, R.; Shilabin A. G.; Lampson, B. C. PLOS ONE. 2018, 13(12)]. The Rhodococcus strain MTM3W5.2 produces a novel antibacterial molecule in broth cultures and the compound was fractionated using a Sephedex LH-20 column. Further purification via reverse-phase HPLC yielded a pure sample at 48.90 minutes, RT48, and selected for complete structural elucidation. The scope of this project is to fully determine the structure of RT48 via spectroscopic techniques including High-Resolution Mass Spectroscopy (HRMS) as well as full 2D-NMR spectroscopic data set. HRMS data deduced an exact mass of 911.5490 Da, equivalent to a molecular formula of C52H78O13. A Bruker 600 MHz NMR was utilized to collect a complete spectral data set including: 1H; 13C; DEPT; H, H-COSY; HSQC; HMBC. Initial screening of deuterated solvents, ranging in polarity, led to the determination that CDCl3 displayed the greatest solubilizing effect of RT48 as evident by multiple sharp peaks in the 7.0-8.2 ppm region, thus indicating the presence of several methylene groups commonly associated with polyketide species. In addition, several major spin systems have been deduced from the spectra. However, due to limited sample quantity in compound with a large molecular weight and product instability, the long range HMBC signals needed to connect these fragments have not yet been

obtained. Ongoing work is being completed to purify more compound to be tested on higher resolution instrumentation at external facilities.

[P – 36] AN ADDITIONAL HYDROXYL GROUP ON 'C3 IN 3',4'-DIHYDROXY-5,7-DIMETHOXYFLAVONE (CT6) FUNCTIONS TO INHIBIT PROTEIN SYNTHESIS AND CELL PROLIFERATION IN CANCER CELLS

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Chromolaena tacotana is a member of the Asteraceae family and it is used as a medicinal plant by native people in the lower Andean regions of South America to treat various illnesses including cancer. The flavones 3',4'-dihydroxy-5,7-dimethoxyflavone (CT6) and 4-hydroxy-5,7-dimethoxy flavone (CT7) were obtained from *C. tacotana* using a Soxhlet extractor with CH2Cl2 to acquire a crude extract from the leaves. The extract was flocculated with 1:1 ethanol:water to remove fats and chlorophylls, and CH2Cl2 was used to extract the desired aqueous portion. The flavonoids were then extracted by column chromatography. The molecular structures of the CT6 and CT7 flavones differ only by an additional hydroxyl group on C3â€™. As indicated by MTT assays, this substituent confers CT6 with high cytotoxic activity against cancer cell lines, specifically pancreatic cancer Panc28 and MIA PaCa-2 cells. Its ability to inhibit cell viability is distinctly less on colon cancer HCT116 and CaCo-2 cells. Conversely, CT7 has a slight effect on Panc28 at 80Î¼M and no effect on MIA PaCa-2, HCT-116, or CaCo-2 cells at the range of concentrations tested. Immunochemistry analysis suggest that CT6 exerts its cytotoxic activity on these cells by inhibiting protein synthesis and apoptosis was detected by TUNEL assay.

[P – 37] DEVELOPING BIOPESTICIDES BASED ON SAPONINS EXTRACTED FROM AGRI-FOOD WASTE

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Globally, billions of tonnes of agri-food waste, either as crop or food processing residue, are generated annually. This represents an untapped source of phytochemicals useful in a variety of applications. One sizable and timely application, especially within the context of sustainable agriculture, is the use of phytochemicals as biopesticides. Based on 2017 data on botanical extracts or purified phytochemicals used by California farmers as biopesticides and extrapolated to Canada, we estimate the Canadian retail market for agricultural use biopesticides based on plant secondary metabolites to be worth $670 million annually. With increased scrutiny of synthetic pest control products, it is expected that this value will grow. Our research project objectives are to optimize the extraction of saponins from tomato waste, pea waste and other agri-food sources, to screen the activity against crop pests of greenhouse vegetable production and to improve pest control efficacy via innovative formulations. Preliminary findings using solvent extraction demonstrated that over 30 tonnes of saponins annually could be derived from tomato skins generated by an Ontario tomato processor. Saponins from a grain processing by-product, currently in commercial development, caused mortality in spider mite larvae and significantly inhibited the in vitro growth of *Didymella bryoniae*, causative

agent of gummy stem blight in cucumber, and *Phytophthora capsici*, causative agent of root rot of tomato. Moreover, saponins were found to protect tomato plants from *P. capsici* infection. Disease symptoms and mortality in tomato plants that received saponins were greatly reduced as compared to control tomato plants that received only water. The next steps are to: 1) compare saponin yields using more sustainable techniques such as supercritical CO_2 extraction and subcritical water extraction; 2) measure efficacy in protecting whole plants from other pests; and 3) determine the saponin mode(s) of action in each case.

[P – 38] THE MILKWEED GENOME PROVIDES INSIGHT INTO THE PATHWAY OF CARDENOLIDE BIOSYNTHESIS

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is an important host plant for the monarch butterfly (*Danaus plexippus*) and several other specialist insect herbivores that are tolerant of its cardenolide toxins. Although milkweed has important ecological value and potential medicinal and industrial uses, the genetic, genomic, and metabolomic resources for milkweed have limited the further advances in the investigation of these plants. In this study we created a genetic mapping population that includes 293 *A. syriaca* lines collected from 57 places around North America and Europe. A low-heterozygosity *A. syriaca* line from Hungary was sequenced using Illumina, Pacific Biosystems (PacBio), and Hi-C technology. The draft genome assembly is 655 Mbp, with N50 = 185,222 bp and a total of 16,583 genes that were identified using the Maker annotation pipeline and the ab initio gene predictors. All *A. syriaca* lines were subjected to genotyping by sequencing, gene expression profiling (with and without prior caterpillar feeding) by 3'RNA-seq, and HPLC-MS metabolite profiling to measure the abundance of 16 known cardenolides. To identify genes involved in the cardenolide biosynthesis pathway, we conducted a correlation analysis of gene expression and cardenolide content data. This identified 1061 candidate genes at a 0.4 confidence threshold, including five cytochrome p450 genes. Preliminary results show that levels of digitoxingenin, ascleposide, desglucosyriode, and labriformin are significantly increased when expression of a CYP714 gene is silenced in *A. syriaca*.

[P – 39] THE EFFECTS OF CT1 AND CT3 IN PANCREATIC AND COLON CELL LINES

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These experiments sought to investigate the effects of two structurally similar flavonoids that differ in the presence of a single double bond between C2 and C3 on ring C. 3,5,4'-trihydroxy-7-methoxyflavone (CT1) and 5,4'-dihydroxy-7-methoxy-flavanonol (CT3) were extracted from *Chromolaena tacotana*, a plant species traditionally regarded as medicinal by the native people of the Andean regions between 1000-1500 m above sea level in Colombia, South America. The effect of CT1 and CT3 was tested on human pancreatic and colon cancer cell lines of various differentiation status. Better-differentiated colon cancer Caco-2 and pancreatic

cancer Panc28 cells, and poorly differentiated colon cancer HCT116 and pancreatic cancer MIA PaCa-2 cells were dosed with CT1 or CT3 and cell viability was calculated from the results obtained by MTT assays. ANOVA and Tukey's multiple comparisons were used to analyze the two compounds, dosages, and cell lines. It was found that less differentiated cell lines responded better to treatment by CT1 and CT3 as compared to better-differentiated cells. Exceptions were found at high dosages for CT1, where better-differentiated cell lines also responded favorably. CT1 presented the lowest EC50 values in HCT116 and MIA PaCa-2 cells, whereas no cytotoxic effect was observed in Panc28 cells after dosing with either compound. The results found in this study suggest that the presence of the double bond on CT1 highly enhances its cytotoxic activity, and there is a preferential targeting of poorly differentiated cancer cells of the colon and the pancreas by CT1 and CT3.

[P – 40] OPTIMIZATION OF THE PURITY OF γ -TOCOTRIENOL VITAMER IN PALM OIL: A PROMISING RADIOPROTECTIVE AGENT FOR TREATMENT OF ACUTE RADIATION SYNDROME

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The health implications associated with exposure to ionizing radiation remain as one of the principal causes of death. Several research has been conducted to discover a likely counteracting agent for acute radiation syndrome (ARS) without success. The United States Food and Drug Administration (FDA) has not recognized any potent and safe countermeasure (radioprotectors) for preventing ARS. However, recent studies have indicated that γ-tocotrienol (GT-3), a vitamin E isomer essentially present in palm oil, has radioprotective capacities in mice and non-human primate (NHP) models. Although GT-3 happens to be one of the remarkable promising countermeasures discovered; nevertheless, the isolation and purification from other E vitamers or its matrix are problematic. This has limited its characterization, derivatization, biomedical application and inclusion into novel products such as pharmaceuticals, supplements, and functional foods. We have thus designed new chromatographic and fractional distillation procedures to enhance separation, improve the purity and stability of GT-3. Thin layer chromatography (TLC) was used to ascertain the best solvent system for the large column chromatography (CC). Exactly 8% ethyl acetate (EA) in petroleum ether (PE) used in TLC resulted in good spots separation (Rf = 0.3). Furthermore, a gradient elution with EA in PE led to the maximum purity based on the 1H NMR and GC-MS outcomes. Results obtained so far have revealed the precise structure and a purity of 95% of the compound. Hence, some traces of impurities, in particular, γ-tocotrienol (BT-3), in the GT-3 distillate tend to compromise its stability and solubility to some extent. The current research will be helpful in uncovering the biochemical structure of numerous complex bioactive components from plants that are difficult to separate and characterize. It is also anticipated that this work will support the development of new medications for ARS.

[P – 41] HERBIVORE DEFENSE METABOLITE EVOLUTION IN THE NON-MODEL CRUCIFER WALLFLOWER (ERYSIMUM CHEIRANTHOIDES)

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Plants have spent the past 425 million years evolving and reconfiguring enzymes from primary metabolism to generate specialized metabolites with new functions, and many of these specialized metabolites provide

protection against herbivory. Cardiac glycosides, or cardenolides, evolved independently in 12 plant families to deter herbivores by inhibiting Na+, K+-ATPases in animal cell membranes. 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. Furthermore, the mechanisms governing cardenolide biosynthesis have escaped investigation. Using the rapidly-growing, self-pollinating annual, wormseed wallflower (*Erysimum cheiranthoides*), my goal is to identify genes in cardenolide metabolism using a functional genomics pipeline. A genome and transcriptome for *E. cheiranthoides* has been generated, and candidate genes have been identified by comparative genomics. We predict that gene expansion and duplication events have led to the neofunctionalization of enzymes that can then act on cardenolides. Candidate genes were further narrowed by correlating cardenolides in various plant tissues to gene expression data. To functionally characterize candidate genes, a number of approaches including transient overexpression, RNA interference, and heterologous expression in *Nicotiana benthamiana* have been developed for testing the role of the gene in cardenolide biosynthesis and regulation. Additionally, efforts are focused on identifying hormonal regulators of cardenolide biosynthesis.

[P – 42] STABILITY AND COLORANT PROPERTIES OF BETALAIN PIGMENTS FROM VEGETABLE AMARANTH (AMARANTHUS SPP.) IN A MODEL BEVERAGE SYSTEM

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Betalains are tyrosine-derived plant pigments appearing magenta to yellow that are used in the food industry to replace synthetic colorants such as Red 40, Blue 1, and Yellow 5/6. They are a pH-stable alternative to more common anthocyanin-based natural colorants such as grape pomace extract. The only source of betalains currently used commercially is red beetroot. Vegetable amaranth is a prolific and low-maintenance alternative to beets with promising pigment yields and novel color characteristics, but little is known about the performance of amaranth color extracts in food and beverage products. In this study, a model beverage system was utilized to analyze the thermal and pH stability of 5 different amaranth cultivars in comparison to both crude and purified beet-sourced colorants. Significant differences in pigment degradation rates and color parameters were observed between amaranth cultivars, with Ames 28293 (*A. hybridus*) showing the greatest stability and highest chroma (C*) values. Ames 28293 showed similar stability to beet-colored beverages at pH 3 and 4.5 and produced significantly bluer colors. Overall, beverages showed the best stability at the lowest temperature tested (5 Â°C) and generally had the slowest pigment degradation at a pH value of 4.5. HPLC-MS is currently being used to identify extract components that significantly influence extract color and stability. Results indicate that certain Amaranthus cultivars can match the stability of beet-derived colorants and provide color hues unobtainable with beets alone.

[P – 44] TOBACCO UDP-GLUCOSYLTRANSFERASE SIP68 HAS A ROLE IN PLANT DEFENSE

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Tobacco glucosyltransferase SIP68 is a family 1 glycosyltransferase. It was identified in a yeast two-hybrid screen using tobacco SABP2 (SA-binding protein 2) as a bait. SABP2 catalyzes the conversion of methyl salicylate (MeSA) into salicylic acid and is an important component of the SA-mediated defense pathway in

plants. Glucosides, the products of glucosyltransferase catalyzed reactions are the ubiquitous class of secondary metabolites. Glucosides are involved in various roles ranging from the protection of plants against pathogens, herbivory, the physical appearance of plants, transportation of metals, and symbiotic agents between plants and microorganisms. The recombinant SIP68 expressed in *Pichia pastoris* glucosylated several flavonoids invitro including kaempferol and quercetin, while it failed to glucosylate SA or MeSA. To study the role of SIP68 in plant stress signaling, transgenic lines with altered SIP68 expression were generated using RNAi. Transgenic tobacco plants silenced in SIP68 expression showed enhanced basal resistance to virulent pathogens. These transgenic lines also showed a strong onset of systemic acquired resistance (SAR) induced by infection with tobacco mosaic virus (TMV). Both these results suggest that SIP68 is likely a negative regulator of plant defenses. These transgenic lines did not show any altered response to abiotic (salt) stress. The eGFP tagged SIP68 localized in the cytoplasm when transiently expressed in *Nicotiana benthamiana* leaves. These results were further confirmed via subcellular fractionation using differential centrifugation of the tobacco leaves transiently expressing eGFP tagged SIP68. Characterizing the role of SIP68 in plant metabolism and discovering its putative substrate/s will help to improve our general understanding of how plants respond to stresses.

[P – 45] THE HIDDEN IMPACTS OF CLIMATE CHANGE: EFFECTS OF ALTERED THERMAL PATTERNS ON PHYTOCHEMICAL PROFILES

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Phytochemicals are a key contributor to the world nutraceutical supply; plant derivatives are in a variety of consumer products from food and health supplements to prescription drugs. The production and accumulation of many phytochemicals are tightly controlled by the plants signaling and regulatory systems based on environmental conditions. Therefore, the yield and quality of these chemicals fluctuate in response to a dynamic environment. As demand for plant derivatives continue to rise, growers face challenges of inconsistent products due to shifting environmental inputs. We are investigating the impact of changing weather patterns and growth conditions on the composition of specialized metabolites, particularly those with a potential for improving human health. We are studying the effects of both high daytime and high nighttime temperatures on the profile of specialized metabolites and quality of commodity crops. This data could reveal plant varieties and environmental conditions that maximize the desired phytochemical profiles to benefit human health. Additionally, we will gain insights into the impact of changing environments on the regulation of phytochemicals, which could be applied to other crop systems targeted for the production of pharmaceuticals and other consumer products.

[P – 46] THE TASTE OF CARROTS: EXPLORING THE GENETIC DETERMINANTS OF CARROT VOLATILE TERPENE AROMA AND FLAVOR

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Carrots (*Daucus carota* L.) are among the most nutritionally and economically valuable root crops worldwide. Despite the distinctive flavor of carrot, the genetic determinants of its taste have not been well characterized. Carrot palatability is genotype specific and mainly attributed to differences in blends of volatile

terpenes and total sugar content among other compounds. Using genome and transcriptome resources, we have performed a comprehensive functional characterization of the carrot terpene synthase (TPS) gene family and identified several TPS genes responsible for the biosynthesis of terpene compounds extracted from carrot roots and aboveground tissues. Employing random forest analysis, we further determined distinct terpene representatives from carrot varieties of different color and predicted their corresponding TPS genes based on gene expression correlations. Results are discussed in comparison to TPS loci previously predicted by genome wide association mapping. Outcomes of this study may be applied to enhance carrot palatability and quality. This research was funded by the US-Israel Binational Agricultural Research and Development Fund (BARD).