56th Annual Meeting of the Phytochemistry Society of North America

August 5-9, 2017, University of Missouri, Bond Life Sciences Center, 1201 Rollins, Columbia, MO

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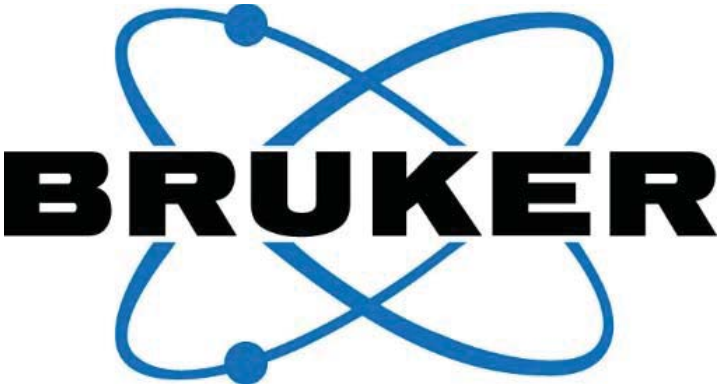

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50% female; 50% male

Graduate Students: **485**

Scholarships Granted: **$1.3 million per year; 87% of eligible applicants receive CAFNR scholarships**

Freshman Retention Rate: **86.5%**

Clubs and Organizations: **50+**

Faculty: **206**

Academic Associates: **125**

Staff: **338**

Ag Research Centers: **17 centers totalling 14,500 acres**

Facilities: **1.5 million square feet, campus, centers & farms**

Income: **$109 million**
36.5% general revenue; 30% grants & contracts; 13.6% sales; 5.9% federal appropriations; 4.3% gifts; 3.2% tuition & fees; 2.8% endowment & investment income; 2.6% other income; 1.1% research incentive funds

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UNDERGRADUATE PROGRAMS

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- Agricultural Systems Management
- Agriculture
- Animal Sciences
- Biochemistry
- Environmental Sciences
- Food Science & Nutrition
- Hospitality Management
- Natural Resource Science & Management
- Parks, Recreation & Sport
- Plant Sciences

COST TO ATTEND MIZZOU 2016-17

	In-State	Out-of-State
Undergraduate (tuition, 14 hrs/semester, room/board)		
Cost per year	$21,274	$36,556
Graduate (tuition, 9 hrs/semester)		
Cost per year	$7,382	$18,414

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- Animal Sciences
- Biochemistry
- Bioengineering
- Food Science
- Hospitality Management
- Natural Resources
- Plant Sciences
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PLANT PHENOMICS FACILITY

The Scanalyzer HTS is a powerful 2D high-throughput phenotyping tool manufactured by LemnaTec (Germany). At Arkansas State University, the facility is equipped with four cameras that collect high resolution images that allow extracting multiple plant features:

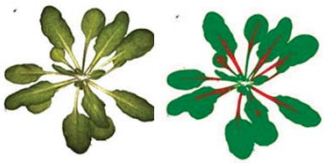
VISIBLE (VIS, RGB) – Size, architecture, chlorosis, necrosis

FLUORESCENCE (FLUO) – *In planta* chlorophyll fluorescence

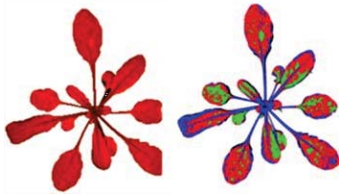
NEAR INFRARED (NIR) – *In planta* water content

INFRARED (IR) – Leaf temperature

We are equipped to extract readouts of interest from the images using commercial (e.g. LemnaGrid) as well as open source software (e.g. PlantCV 2.0).



Images taken with VIS camera. Captured image extracted from background (left); color classified (analyzed) image (right).



Images taken with FLUO camera. Captured image extracted from background (left); color classified (analyzed) image (right).

Images taken with NIR camera. Captured image extracted from background (left); color classified (analyzed) image (right).

The capabilities of the Scanalyzer HTS have been successfully tested using a variety of assays and plant species. Experimental design is not limited to those listed here.

Plant species:

- Arabidopsis
- Maize
- Rice
- Tobacco
- Tomato

Assays performed:

- Salinity stress
- Cold stress
- Heat stress/Heat shock
- Water limitation stress
- Nutrient stress

DO YOU HAVE AN **EXPERIMENT** IN MIND?

FOR PRICING AND BOOKING INFORMATION

Contact Dr. Argelia Lorence
Director of the A-State Plant Phenomics Facility
alorence@astate.edu
870-680-4322

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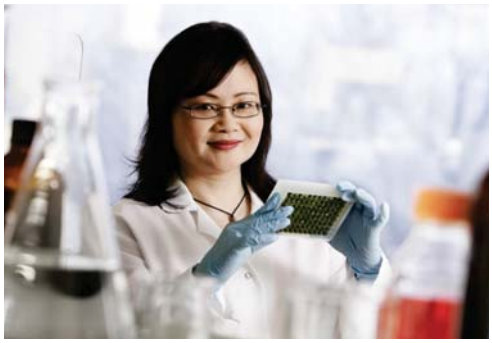

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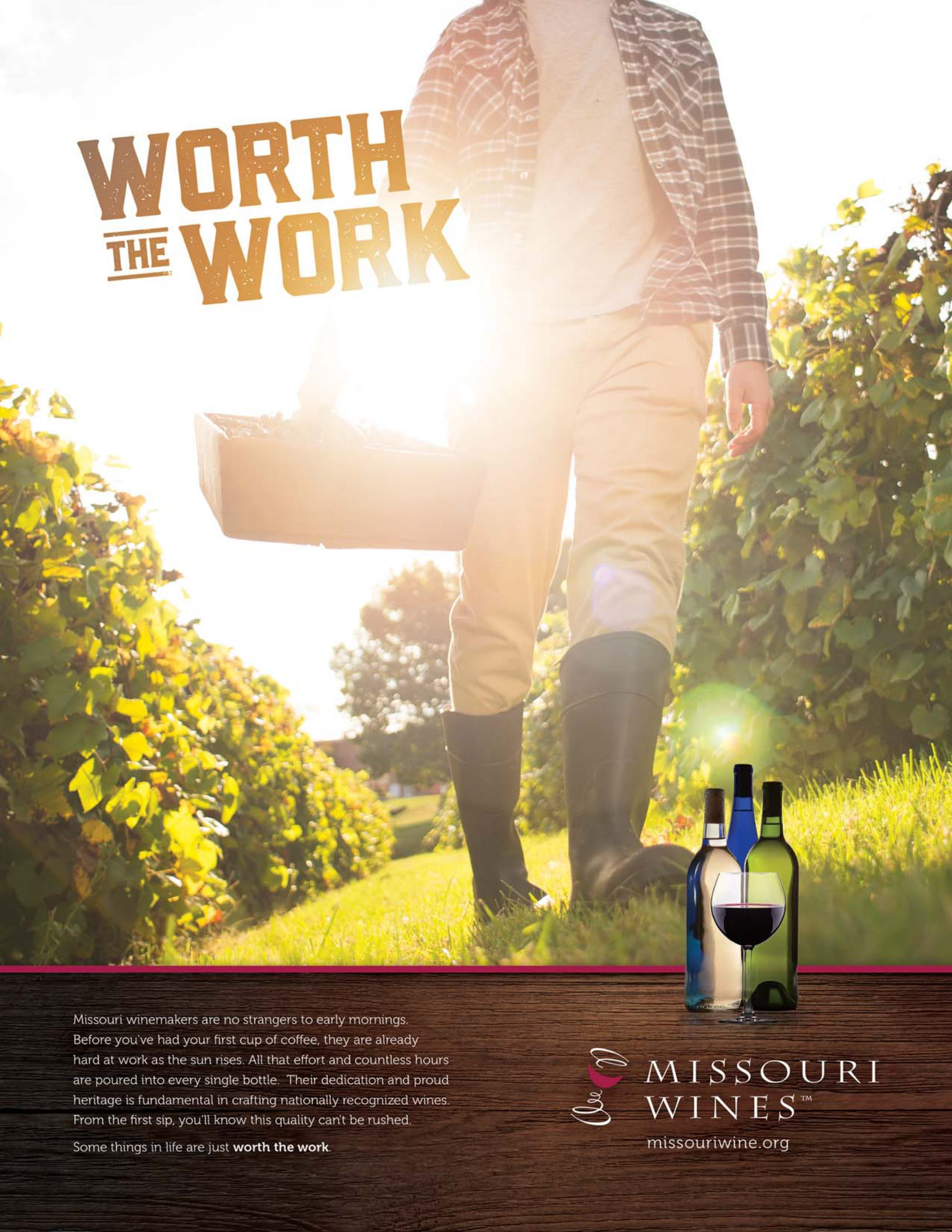

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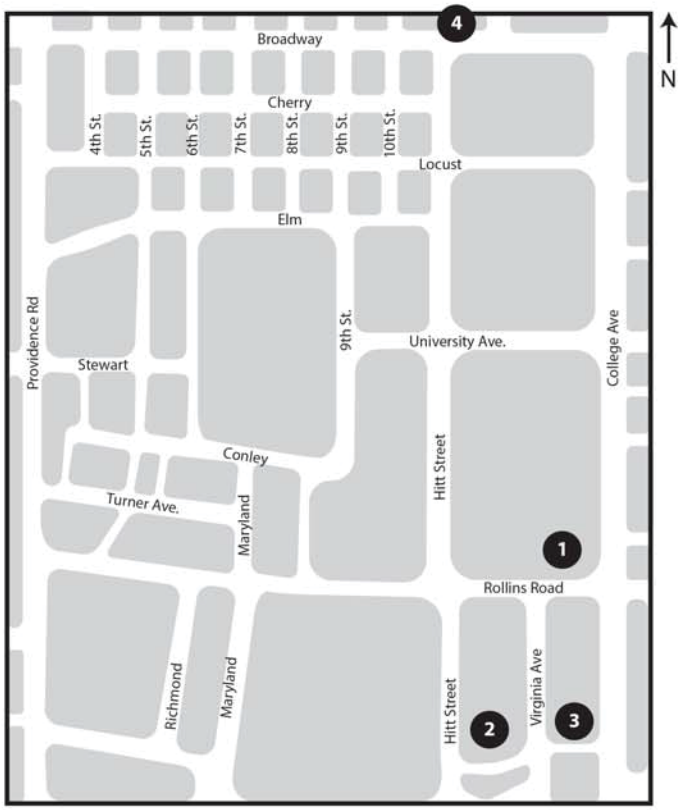

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PSNA 2017 Conference Program August 5-9, 2017

All oral presentations will be held in the Monsanto Auditorium within the Christopher S. Bond Life Sciences Center, 1201 Rollins Street, Columbia, MO

Saturday, August 5, 2017

Christopher S. Bond Life Sciences Center, University of Missouri

4:00 pm – 8:00 pm Conference Registration, Monsanto Atrium
6:00 pm – 8:00 pm Welcome Reception, Bond Life Sciences Center, McQuinn Atrium

Sunday, August 6, 2017

7:30 am – 5:00 pm Conference Registration, Monsanto Atrium and Poster Setup, McQuinn Atrium

8:45 am – 9:00 am Opening Remarks

9:00 am – 9:45 am **Plenary Symposium: Toni Kutchan,** *Donald Danforth Plant Science Center, "Plant terpenes as biomaterials and biofuels"*

9:45 am – 10:00 am Break, McQuinn Atrium

Symposium 1: Imaging, Phenotyping & Metabolomics, Monsanto Auditorium,

Symposium Sponsored by the NSF Plant Imaging Consortium

10:00 am – 10:45 am **Keynote: Richard Ferrieri**, University of Missouri, "*Isotopically Labelled Probes Provide Temporal and Spatial Information on Plant Metabolism*"

10:45 am – 11:05 am Argelia Lorence, Arkansas State University; "*Harnessing the Power of High Throughput Plant Phenotyping and Other Omics at the Plant Imaging Consortium*"

11:05 am – 11:25 am Feng Qiu, University of Missouri at Columbia, "*Development of an Expert System to Enhance Gas Chromatography-Mass Spectrometry-Based Metabolite Identification*"

11:25 am – 11:45 pm Toshihiro Obata, University of Nebraska Lincoln *"Characterization of C4 Photosynthesis in Maize by Dynamic Metabolic Flux Analysis and Cell Type Fractionation"*

11:45 am – 12:05 pm Beverly J. Agtuca, University of Missouri, *"Rapid in situ metabolic screening of soybean root nodules by laser ablation - electrospray ionization mass spectrometry (LAESI-MS)"*

12:05 pm – 12:25 pm Yuan-Chuan Tai, Washington University School of Medicine, *"In vivo Molecular Imaging Technologies for Accelerating Phytochemical Innovation and Translation"*

12:25 – 1:30 pm Lunch, McQuinn Atrium

Symposium 2: Terpenoids, Monsanto Auditorium

1:30 pm – 2:15 pm **Keynote: Reuben Peters**, Iowa State University, "*Enzymatic studies of (di)terpenoid biosynthesis*"

2:15 pm – 2:45 pm Bernd Markus Lange, Washington State University, "*Bioenergetics of mint glandular trichomes - how to power metabolism in a non-photosynthetic cell type*"

2:45 pm – 3:15 pm De-Yu Xie, North Carolina State University, "*Metabolic Network-based Strategy for Improvement of Artemisinin Production in Self-pollinated Artemisia annua*"

3:15 pm – 3:45 pm Break, McQuinn Atrium

Symposium 3: Alkaloids and More Terpenoids, Monsanto Auditorium

3:45 pm – 4:30 pm **Keynote: Vincenzo De Luca**, Brock University, Canada, "*Alkaloids Session- Molecular and biochemical characterization of monoterpenoid indole alkaloid biosynthesis pathways and their use in metabolic engineering"*

4:30 pm – 5:00 pm Dorothea Tholl, Virginia Polytechnic Institute and State University, "*Changing homoterpene synthase specificity by a single amino acid switch*"

5:00 pm – 5:30 pm Soheil Mahmoud, University of British Columbia, *"Monoterpene metabolism in Lavandula"*

5:30 pm – 7:00 pm Poster Session I, McQuinn Atrium,
Sponsored by Monsanto

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Monday, August 7, 2017

7:30 am – 5:00 pm Conference Registration and Poster Setup

9:00 am – 9:45 am **Plenary Symposium:**
Sponsored by the NSF Plant, Algae, and Microbial Metabolomics Research Coordination Network (PAMM-NET)

PAMM-NET

Georg Jander, Boyce Thompson Institute, Cornell University, *"Genetic and biochemical diversity in maize defense responses"*

9:45 am – 10:15 am Break, McQuinn Atrium

Symposium 4: Phytochemistry & Ecology, Monsanto Auditorium

10:15 am – 11:00 am **Elsevier Awardee Presentation: Hiroshi Maeda**, University of Wisconsin, "*De-regulation of tyrosine biosynthesis underlies evolutionary expansion of diverse plant natural products"*

11:00 am – 11:30 am Abbas Abdoli, University of Saskatchewan, "*Detoxification pathways of rutabaga phytoalexins by the phytopathogen Alternaria brassicicola*"

11:30 am – 12:00 pm Lloyd W. Sumner, University of Missouri, *"Integrated metabolomics for the discovery and characterization of saponin biosynthetic genes in Medicago truncatula"*

12:00 pm – 1:00 pm Lunch – Career Panel/Workshop: Hosts Drs. De-Yu Xie and Mark Berhow, Bond Life Sciences Center, Rm 572. (workshop attendees please pick up your lunch in the McQuinn Atrium and take upstairs, 5th floor)

Symposium 5: Lipids, Monsanto Auditorium

1:00 pm – 1:45 pm **Keynote: Ruth Welti**, Kansas State University, *"Using lipid analysis by mass spectrometry to understand plant response to the environment"*

1:45 pm – 2:15 pm Philip Bates, University of Southern Mississippi, "*Deciphering the Eukaryotic Pathway of Leaf Glycerolipid Assembly through Lipid Flux Analysis in Arabidopsis Mutants and Oil Accumulating Tobacco"*

2:15 pm – 2:45 pm Xiangjun Li, University of Nebraska-Lincoln, *"A Novel Hydroxy Fatty Acid Biosynthetic Pathway Revealed by Discovery of Abundant C24 Di-Hydroxy Fatty Acids in Orychophragmus Seed Oil"*

2:45 pm – 3:15 pm Lucas Busta, University of Nebraska – Lincoln, *"Digging for buried treasure in a chemical diversity database"*

3:15 pm – 3:45 pm Break, McQuinn Atrium

3:45 – 4:45 pm PSNA Business Meeting, Monsanto Auditorium

5:00 pm – 6:30 pm Poster Session II, McQuinn Atrium,
Sponsored by Monsanto

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Tuesday, August 8, 2017

8:00 am – 9:00 am Conference Registration

9:00 am – 9:45 am **Plenary Symposium: Jay Thelen**, University of Missouri, *"A new model for the regulation of de novo fatty acid biosynthesis in plants"*

9:45 am – 10:15 am Break, McQuinn Atrium

Symposium 6: Synthetic Biology and Metabolic Engineering, Monsanto Auditorium

10:15 am – 11:00 am **Keynote: Edgar Cahoon**, *University of Nebraska, "Application of Synthetic Biology to Enhance Genetic Variation for Improved Plant Quality and Performance"*

11:00 am – 11:30 am Bjoern Hamberger, Michigan State University, Department of Biochemistry and Molecular Biology, "*From plant pathway discovery to synthetic biology: engineering of diterpene production*"

11:30 am – 11:50 pm Peiqiang Wang, Anhui Agricultural University, "*Evolutionary and Functional Characterization of Leucoanthocyanidin Reductases from Camellia sinensis*"

11:50 am – 12:10 pm Mingzhuo Li, North Carolina State University, "*Transcriptional Profiles Associated with Sugar Metabolism in Tobacco Altered by Overexpression of a Tea (Camellia sinensis, Cs) R2R3-MYB4*"

12:10 pm – 12:30 pm Radin Sadre, Michigan State University, *"Boosting the production of terpenoids in lipid droplet-accumulating photosynthetic tissues"*

12:30 pm – 1:30 pm Lunch, McQuinn Atrium

Symposium 7: Industrial Phytochemistry, Monsanto Auditorium

1:30 pm – 2:15 pm **Keynote: Martin Ruebelt**, Monsanto Company, *"Monsanto's Approach to Sustainable Agriculture "*

2:15 pm – 2:45 pm Toshiaki Umezawa, Research Institute for Sustainable Humanosphere, Kyoto University, "*A new O-methyltransferase gene involved in antitumor lignan biosynthesis in Anthriscus sylvestris*"

2:45 pm – 3:15 pm Break, McQuinn Atrium

Symposium 8: Food and Nutraceuticals, Monsanto Auditorium

3:15 pm – 4:00 pm **Keynote: William Folk**, University of Missouri, *"Valuing the Safety and Efficacy of Dietary Supplements, Nutraceuticals and Traditional Medicines"*

4:00 pm – 4:30 pm Zhentian Lei, University of Missouri Columbia, *"Metabolomics of Scab Susceptible and Resistant Pecan Varieties"*

4:30 pm – 5:00 pm Jan Frederik Stevens, Oregon State University, *"Biomarkers of Glucosinolate Intake Using Labeled Broccoli Sprouts"*

5:00 pm – 5:45 pm **Neish Awardee Presentation: Daniel K. Owens**, University of Hawaii at Manoa, "*Identification and Mode of Action of Herbicidal Natural Products"*

6:00 pm – 9:00 pm Award Banquet, McQuinn Atrium, Bond Life Sciences Center

Wednesday, August 9, 2017

8:00 am – 9:00 am Conference Registration, Monsanto Atrium

Symposium 9: Phytochemical Signaling, Monsanto Auditorium

9:00 am – 9:45 am **Keynote: Abraham Koo**, University of Missouri Columbia, *"Are Metabolites of Jasmonate Cohorts For Stress Signaling? Definitely Maybe"*

9:45 am – 10:15 am Hagai Yasuor, Department of Vegetable and Field Crops, Gilat Research Center, Agricultural Research Organization, Israel, *"Role of plant hormones in flower and fruit development in tomato"*

10:15 am – 10:45 am Break, McQuinn Atrium

10:45 am – 11:15 am **Plenary Symposium: Maria Luisa Villarreal**, Universidad Autónoma del Estado de Morelos Centre of Biotechnological Research (CEIB), Cuernavaca, Morelos, Mexico, *"Advances and Opportunities in the Investigation of Selected Mexican Medicinal Plants"*

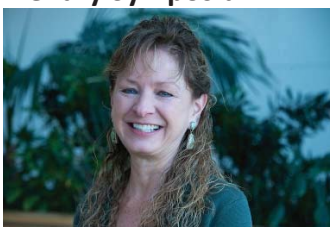
11:15 pm –11:35 pm Maria Guadalupe Beatriz Zapata Zapata and Dennis Atenea de Loera Carrera, University of San Luis Potosi, *"PSNA 2018 in San Luis Potosi, Mexico"*

12:00 pm Close of PSNA 2017

Speaker Abstracts

Sunday, August 6, Morning

Plenary Symposium



Toni M. Kutchan is the Oliver M. Langenberg Distinguished Investigator and Vice President for Research at the Donald Danforth Plant Science Center and Adjunct Professor of Biology at Washington University in St. Louis and at the University of Missouri– St. Louis. Previously, she spent twenty years leading research in Germany, most recently as Professor and Department Head at the Leibniz Institute for Plant Biochemistry in Halle, Germany, as well as Managing Director of that institute. Her primary research interests are the biosynthesis of plant medicinal compounds such as alkaloids and the development of plant synthetic biology systems. She has been a member of the Board of Scientific Advisors of the Schering Research Foundation and a member of the Central Selection Committee of the Alexander von Humboldt Foundation in Germany. She is currently a member of the Scientific Advisory Committee of the William L. Brown Center for Plant Genetic Resources of the Missouri Botanical Garden in St. Louis, of the Forshungszentrum Jülich in Germany, of the BioDiscovery Institute of the University of North Texas and of the Research Committee of the Boyce Thompson Arboretum in Arizona. She is a member of the Board of Trustees of the Academy of Science– St. Louis and a member of the STEM Advisory Committee of the Girl Scouts of Eastern Missouri. Dr. Kutchan is a member of the Berlin-Brandenberg Academy of Sciences, a Fellow of the Academy of Science– St. Louis, and a member of the German National Academy of Science Leopoldina. She holds a B.S. in Chemistry from the Illinois Institute of Technology, a Ph.D. in Biochemistry from St. Louis University, the Dr. Habil. and venia legendi in biochemistry from the University of Munich.

Plant terpenes as biomaterials and biofuels

Jörg M. Augustin[1,2], Yasuhiro Higashi[1,3], and Toni M. Kutchan[1]

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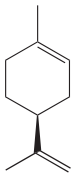
[2]Current address: Elemental Enzymes, 1685 Galt Industrial Blvd, St. Louis, MO 63132 USA
[3]Current address: RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

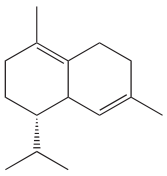
Plants provide mankind with a vast array of phytochemicals that have a wide-ranging industrial and pharmacological application. Large-scale availability of phytochemicals can limit their use. Microbial production systems such as *Escherichia coli* and *Saccharomyces cerevisiae* are well established biotechnological platforms that can often be successfully bioengineered to serve as alternative sources for natural compounds. In addition to microbes, plant cell cultures have been exploited as potential biotechnological production platforms for phytochemicals. Despite the advantages of such cell-based production systems over the native producer, a common drawback is the requirement for specialized fermentation facilities, energy input and a continuous supply of macro- and micronutrients. Bioengineering of low-input crop plants to synthesize high value compounds would allow production of phytochemicals on farmland. However, whereas production of pharmacological proteins in plants has recently significantly advanced, suitable plant feedstocks for production of small molecules remain under-explored. Many plant-derived compounds of high value for industrial or pharmaceutical applications originate from plant species that are not amenable to cultivation. Biotechnological production in low-input organisms is, therefore, an attractive alternative. Here we explore whether *Camelina sativa*, an emerging low-input non-foodstuff Brassicaceae oilseed crop grown on marginal lands or as a rotation crop on fallow land, can successfully be refactored to produce and store novel compounds in seed. As proof-of-concept, we use the cyclic monoterpene hydrocarbon (4*S*)-limonene and the bicyclic sesquiterpene hydrocarbon (+)-δ-cadinene, which have potential biofuel and industrial solvent applications. Posttranslational translocation of the recombinant enzymes to the plastid with concurrent overexpression of genes of the MEP pathway resulted in the

accumulation of (4*S*)-limonene **1** and (+)-δ-cadinene **2** up to 14 mg g^{-1} seed. This study presents the framework for rapid engineering of camelina oilseed production platforms for terpene-based high value compounds.



(4*S*)-Limonene

1

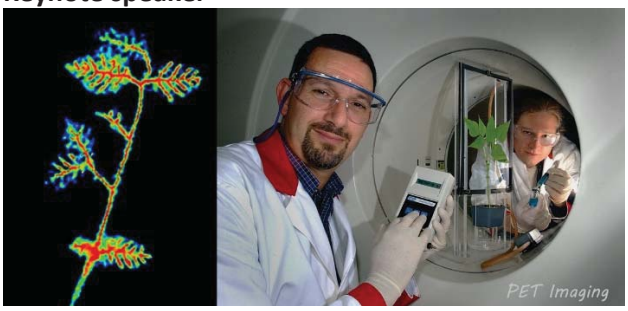


(+)-δ-Cadenine

2

Symposium 1: Imaging, Phenotyping & Metabolomics

Keynote speaker



Rich Ferrieri earned his Ph.D. degree in Radiochemistry from Texas A&M University in 1979, and then became a postdoctoral fellow under Alfred Wolf at Brookhaven National Laboratory (BNL). He was later hired onto the scientific staff at BNL working on the medical applications of Positron Emission Tomography (PET). In 2002, Rich shifted his interest away from medical research, and into plant biology leveraging many of the same imaging and radiochemistry tools to study basic plant functions. He is credited with developing the first radiolabeled PET plant hormone (jasmonate) to study its transport in living plants, as well as with developing a unique set of dynamic diagnostic tools for unraveling the physiological and metabolic mechanisms of plant stress. After 38 years of service as a tenured senior scientist at BNL, Rich moved his program to U. Missouri (Columbia) where he is building a new integrative program at the Missouri Research Reactor Center in plant imaging and metabolic flux analysis. As a Research Professor, he is faculty with the Department of Chemistry and the Interdisciplinary Plant Group at MU and is co-Director of the MU Radiochemistry Sciences Institute.

Isotopically Labelled Probes Provide Temporal and Spatial Information on Plant Metabolism.

Richard Ferrieri

Missouri Research Reactor Center, University of Missouri, Columbia, MO, USA

Agriculture in the 21st century faces many formidable challenges. On one hand, it must produce more food and fiber to feed a growing population; while on the other, it has to increase biomass feedstocks for an increasing bioenergy market. Projections indicate that feeding a world population of 9.1 billion people in 2050 will require raising overall food production by more than 50 percent while production in developing countries will need to almost double. World hunger, however, is not simply a future problem, but one that is very real today. Approximately 21,000 people die of starvation, or die from some disease linked to malnutrition every day. The scientific roadmap to ensuring global food security requires connecting the dots between the genes that regulate biological function and the plant phenotypes. Navigating this course requires drilling deeply at transcriptional, proteomic and metabolomic levels. Aided by recent advances in chemistry and imaging instrumentation, researchers now have the tools to examine a plant's changing metabolism in a whole new light of regulation by being able to coordinate temporally and spatially changes in pathway fluxes. This talk will highlight some of the recent developments in my group using short-lived radioactive isotopes coupled with dynamic whole-plant Positron

Emission Tomography (PET), Root Radiography, and Radiometabolite Flux Analysis, as well as using stable isotopes coupled with MS-based imaging technologies including nanoSIMS and MALDI-MS imaging. Taken together, these technologies have enabled us to explore the dynamic relationships that exist at the whole plant level and with the surrounding rhizosphere.

Harnessing the Power of High Throughput Plant Phenotyping and Other Omics at the Plant Imaging Consortium

A. Lorence[1,2], Z. Campbell[1], L.M. Acosta-Gamboa[1], N. Nepal[1], and S. Liu[1]

[1] Arkansas Biosciences Institute, Arkansas State University
[2] Department of Chemistry and Physics, Arkansas State University, Jonesboro, AR, USA. Email: alorence@astate.edu

Manual plant phenotyping requires a great deal of resources and is typically not feasible for detection of subtle phenotypes. Therefore, there is a growing need to develop quantitative and automated phenotyping systems to analyze large numbers of plants. Recognizing this need Arkansas State University acquired a Scanalyzer HTS. This instrument is equipped with visible, fluorescence, near infrared, and infrared sensors allowing unbiased, non-invasive, and automated screening of plant phenotypes. Additional funding allowed the establishment of the Plant Imaging Consortium (PIC, http://plantimaging.cast.uark.edu/). Two of the missions of PIC are to 1) expand access to this powerful phenotyping tool for other plant scientists in the USA and to 2) empower novel discoveries that can be applied to developing plants able to thrive under adverse environmental conditions. In order to develop and validate phenotyping protocols we have used known lines with enhanced growth and tolerance to abiotic stress. After extracting information contained in the high resolution images we have acquired, a high correlation between the digital readouts measured with the LemnaGrid software, and the manual readouts measured for these lines has been found. The power of the fluorescence and near infrared cameras has become evident after analyzing the response of these lines to stresses such as water deficit and salinity, where novel digital readouts of stress have been identified. Meaningful improvements have been made to our phenotyping protocols in three key areas: 1) use of materials to reduce water losses and improve image analysis, 2) careful monitoring of light intensity and water status in the soil and the plants, and 3) development of novel algorithms to improve image segmentation and image and data analysis. Results illustrating these improvements and the potential of these approaches to advance the study of plants in combination with radiochemistry and other omics (eg. ionomics) will be presented.

Development of an Expert System to Enhance Gas Chromatography-Mass Spectrometry-Based Metabolite Identification

Feng Qiu, Zhentian Lei, Lloyd W. Sumner

Bond S. Life Sciences Center, University of Missouri at Columbia, MO 65202, USA

The number one, grand challenge of metabolomics is the large-scale, confident identification of metabolites. GCMS is an important technique in metabolomics and particularly useful for the analysis of volatile components. While spectral libraries are commonly used in GCMS-based chemical identification, many metabolites observed in GCMS remain unknown. In addition, the development and application of computational methods for GCMS is still limited. This presentation introduces an intelligent system which combines library search, virtual derivatization, retention prediction, and structural prediction for metabolite identification. Artificial Neural Networks (ANN) were used to develop a retention index (RI) prediction model for metabolites using their molecular descriptors calculated using the Chemistry Development Kit. Partial Least Square Discriminant Analyses (PLSDA) were used to develop predictive models for the structural fingerprints generated using Molecular Access System (MACCS) and 10-fold cross-validation was performed for the determination of the number of PLSDA components in order that both the highest sensitivity and specificity were achieved. An algorithm was developed for the generation of hypothetical trimethylsilyl derivatives of polar metabolites. The identification workflow includes library searches using molecular formula followed by the prediction of RIs and

structural fingerprints. The workflow was automated with a few, simple clicks in Excel/VBA for batch identifications. The performance was evaluated by the percentage of correct identifications in top K ranks. The results showed that 32%, 43%, and 52% of test metabolites were ranked in top 5, 10, and 20, respectively, all of which outperformed MetFrag. Further evaluation using an independent dataset including 250 EI-MS spectra from MassBank showed that 31% of metabolites were correctly identified and 76% were ranked in top 20. This expert system, which is complementary to our LCMS-based PlantMAT pipeline (Qiu et al., Anal. Chem. 2016, 88), further increases the chemical space of traditional library searches and facilitates the identification of unknown metabolites.

Characterization of C_4 Photosynthesis in Maize by Dynamic Metabolic Flux Analysis and Cell Type Fractionation

Toshihiro Obata[1,2], Stéphanie Arrivault[2], Manuela Guenther[2], Melanie Hoehne[2], Alisdair R. Fernie[2] and Mark Stitt[2]

[1]Department of Biochemistry, University of Nebraska Lincoln, 1901 Vine Street, Lincoln, NE, 68588, USA
[2]Max-Planck-Insititute of Molecular Plant Physiology, Am Mühlenberg 1, Potsdam, Brandenburg, 14476, Germany

C_4 photosynthesis is based on the CO_2 concentrating shuttle (CCS) which efficiently concentrates CO_2 in proximity of RuBisCO to achieve higher carbon fixation and lower oxidation rates. This process includes primary CO_2 fixation into a C_4 molecule in the mesophyll cells (MCs), transport of fixed carbon into the bundle sheath cells (BSCs), carbon release in the BSCs and transport of carbon backbone back to MCs. Although the pathways of CCS have been fully elucidated, there are open questions about their operation. We performed dynamic metabolic flux analysis in combination with cell type fractionation to address them. We fed $^{13}CO_2$ to a fully extended maize leaf for 0 to 60 min and the metabolism is quickly quenched using custom made labeling chamber. The amounts and ^{13}C accumulation of metabolites were analyzed by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-tandem MS analyses. The overall labeling kinetics reflected the topology of C_4 photosynthesis. Our results clearly showed that photorespication takes place in a C_4 plant. Simultaneous operation of both NADP-malic enzyme (ME) and phospho*enol*pyruvate carboxykinase subtypes of CCS was also indicated. In the tested condition, around 90% of carbon was estimated to be transported by NADP-ME type CCS even though only 40% of foliar malate pool is involved in the CCS. There was evidence of rapid carbon exchange between intermediates of Calvin-Benson cycle and CCS. In contrast, very little C leaks from the large pools of CCS metabolites into respiratory metabolism. Additionally the analysis of cell type specific labeling patterns by the fractionation using differential grinding revealed concentration gradients to drive intercellular diffusion of malate but not pyruvate. These results indicate the operation of multiple pathways for carbon transport from MCs to BSCs and carbon exchange among them, which probably confer flexibility in the C_4 photosynthesis.

In vivo Molecular Imaging Technologies for Accelerating Phytochemical Innovation and Translation

Author(s) & Affiliation(s)

Yuan-Chuan Tai[1], Sergey Komarov[1], Ke Li[2], Nilantha Bandara[3], Dong Zhou[1], Wenhu Chu[1], Susan Lever[4], Buck Rogers[3], Silvia S. Jurisson[4]

[1] Department of Radiology, Washington University in St. Louis, 510 S. Kingshighway Blvd. Campus Box 8225, St. Louis, MO 63110, USA
[2] Department of Electrical and Systems Engineering, Washington University in St. Louis
[3] Department of Radiation Oncology, Washington University in St. Louis
[4] Department of Chemistry, University of Missouri in Columbia

Radiotracer techniques have been used to study biological sciences for over half a century. With the advances in multi-modality imaging instrument, there is increasing interest in radiotracer imaging technologies such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) for studying biomolecules *in vivo*. With molecular imaging, one can measure the spatiotemporal distribution of biomolecules quantitatively and nondestructively. This enables longitudinal studies of the same subjects, eliminating inter-subject variability when

establishing biological models for normal developments or disease progressions. Since the radiotracer technologies are extremely sensitive, one can image trace amount of biomolecules (<picomole) without disturbing the biological systems. This unique advantage allows pharmaceutical companies to accelerate the development and validation of new drugs by measuring pharmacokinetics and pharmacodynamics in animals and human directly.
While these *in vivo* molecular imaging technologies have been widely adopted for clinical and pre-clinical research, their applications to plant sciences remain limited, partly due to the limited availability of resources and partly due to the lack of awareness.
Leveraging NSF awards (MRI and EPSCoR Track II), the Plant Imaging Consortium (PIC) has established a Radiotracer Imaging and Radiochemistry Core to offer unique resources and imaging capabilities to plant scientists who are interested in studying the dynamics of molecular interactions in plants in real-time. We have established tools and radiolabeling techniques to enable molecular imaging of various types of plants (e.g., maize, soybean, common bean, tomato, Arabidopsis thaliana, Venus flytrap, poplar, etc.) using a wide range of radiotracers (e.g. $^{11}CO_2$, $^{13}NH_3$, $^{13}NO_3$, ^{107}Cd, ^{64}Cu, $^{13}N_2$, ^{18}FDG, ^{18}F-amino acid, etc.). This unique resource for molecular plant imaging research, combined with our vast experience in translating novel biomolecules from preclinical validation to human clinical trials, will offer an exciting opportunity to accelerate the translation of phytochemical research innovations to medical and pharmaceutical applications.

Rapid in situ metabolic screening of soybean root nodules by laser ablation - electrospray ionization mass spectrometry (LAESI-MS)

Beverly J. Agtuca[1*], Sylwia A. Stopka[2], Laith Samarah[2], Sterling Evans[1], Christopher R. Anderton[3], Minviluz Stacey[1], David W. Koppenaal[3], Ljiljana Paša-Tolić3, Akos Vertes[2], and Gary Stacey[1]

[1]Divisions of Plant Sciences and Biochemistry, C. S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211
[2]Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, The George Washington University, Washington, DC 20052
[3]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354

Soybean is an economically important legume that produces edible oil and protein. Soybean production is aided by the plant's ability to fix atmospheric nitrogen through a symbiotic relationship with rhizobia leading to the formation of root nodules. We are using a systems biology approach, specifically metabolomics, to study this symbiosis with an ultimate goal to enhance soybean productivity and sustainability. Among the approaches used, laser ablation electrospray ionization mass spectrometry (LAESI-MS) yields spatial, metabolite distributions with minimum sample preparation. We used LAESI-MS to investigate the metabolites in soybean nodules and root vascular tissue resulting from infection by B. japonicum wild type or a fix-mutant (nifH) strain. In addition, we sampled the tissues of a soybean stearoyl-acyl carrier protein desaturase (SACPD) mutant that showed increases in seed, stearic acid levels, but also showed clear changes in nodule morphology. Finally, we analyzed the soybean vascular tissue of plants grown on a variety of nitrogen sources, including under nitrogen-fixing conditions. For LAESI-MS, all tissues were subjected to nanosecond mid-IR laser pulses that coupled directly into a water adsorption band, and resulted in an ablation plume that was intercepted by electrospray for ionization and measurement with a mass spectrometer. Multivariate statistical and hierarchical clustering analyses were performed to determine sample specific metabolites between the transgenic and wild-type tissues. Preliminary results showed specific metabolites that were involved in nitrogen fixation, such as [heme B]+ at m/z 616.179, [riboflavin+H]+ at m/z 377.153, and [adenine+H]+ at m/z 136.062. Interestingly, distinctive metabolites were localized at the lesions of the infection zone in the SACPD mutant nodule. Our study demonstrates that LAESI-MS, when coupled with the 21 Telsa Fourier Transform Ion Cyclotron Resonance, an ultra-high mass resolution and mass accuracy MS, holds tremendous potential for use in further studies of plant-microbe interactions, as well as other plant processes.

Sunday, August 6, Afternoon

Symposium 2: Terpenoids

Keynote speaker

Reuben Peters is a professor in the Department of Biochemistry, Biophysics, and Molecular Biology at Iowa State University, where he is studying diterpenoid biosynthesis and physiological function. These complex natural products are important in host-microbe interactions (particularly for plants), and some have proven to be effective pharmaceutical drugs. Thus, this research has potential implications for both plant and human health. Prior to joining the faculty at Iowa State University, Peters was a Postdoctoral Fellow of the Jane Coffin Childs Memorial Fund for Medical Research at the Institute of Biological Chemistry at Washington State University with Dr. Rodney Croteau. He is a beneficiary of the University of California education system, having received his B.S. in molecular biology at U.C. San Diego and his Ph.D. in biochemistry and biophysics from U.C. San Francisco with Dr. David Agard.

Enzymatic Studies of (Di)terpenoid Biosynthesis

Reuben J. Peters[1]

[1]Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA 50011
email: rjpeters@iastate.edu

My group has taken a systematic approach towards investigating diterpenoid biosynthesis, with a strong focus on enzymatic studies. We are particularly interested in discovering the enzymatic structure-function relationships specifying variation in the catalytic activity of the terpene synthases and cytochrome P450 (CYP) mono-oxygenases that play central roles in production of these natural products. Our work has been enabled by development of a synthetic biology approach in which we reconstitute metabolic pathways in *E. coli*, which allows facile investigation of otherwise difficult to access substrates and elucidation of novel enzymatic activities. Based on such functional characterization we have significantly expanded the range of known catalytic activity for diterpene synthases and CYPs involved in diterpenoid biosynthesis. In turn, we have used the resulting phylogenetic information to identify key residues that exert significant control over product outcome in both class II diterpene cyclases and class I diterpene synthases. Notably, these residues can be used to both predict and engineer the activity of these enzymatic families. Similar studies are underway with CYPs as well. Our most current results will be presented.

Bioenergetics of mint glandular trichomes - how to power metabolism in a non-photosynthetic cell type

B. Markus Lange, Sean R. Johnson, Iris Lange, Narayanan Srividya,

Institute of Biological Chemistry and M.J. Murdock Metabolomics Laboratory,
Washington State University, Pullman, WA 99164-6340, USA

The commercially important essential oils of peppermint (*Mentha* x *piperita* L.) and its relatives in the mint family (Lamiaceae) are accumulated in specialized anatomical structures called glandular trichomes (GTs). A genome-scale

stoichiometric model of secretory phase metabolism in peppermint GTs was constructed based on current biochemical and physiological knowledge. Fluxes through the network were predicted based on metabolomic and transcriptomic data. Using simulated reaction deletions, this model predicted that two processes, the regeneration of ATP and ferredoxin (in its reduced form), exert substantial control over flux toward monoterpenes. Follow-up biochemical assays with isolated GTs indicated that oxidative phosphorylation and ethanolic fermentation were active, and that cooperation to provide ATP depended on the concentration of the carbon source. We also report that GTs with high flux toward monoterpenes express, at very high levels, genes coding for a unique pair of ferredoxin and ferredoxin-NADP+ reductase isoforms. This study provides the first evidence how bioenergetic processes determine flux through monoterpene biosynthesis in GTs.

Metabolic Network-based Strategy for Improvement of Artemisinin Production in Self-pollinated *Artemisia annua*

De-Yu Xie, Dong-Ming Ma

Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA,
email: De-Yu Xie, dxie@ncsu.edu

Artemisia annua is an effective anti-malarial medicinal plant saving more than a billion of people every year. This medicinal plant produces artemisinin, the most effective medicine for treatment of cerebral and malignant malaria infected by *Plasmodium falciparum*. We recently characterized a partial genes-terpenoids network (Ma et al., 2015, Molecular Plant, 8: 1580-1598). Based on this, we hypothesize that any a manipulation of gene expression in the network can alter metabolic network performance and enhance or reduce biosynthesis of artemisinin. In our study, an *Artemisia annua HDR* cDNA (namely *AaHDR1*) was cloned from leaves. Green florescence protein fusion and confocal microscope analyses showed that AaHDR1 was localized in chloroplasts. The overexpression of *AaHDR1* increased contents of artemisinin, arteannuin B and other sesquiterpenes, and multiple monoterpenes. By contrast, the suppression of *AaHDR1* by anti-sense of led to opposite results. In addition, an untargeted metabolic profiling showed that the overexpression and suppression altered non-polar metabolite profiles. In conclusion, the overexpression and suppression of AaHDR1 protein level in plastids differentially affect artemisinin and other terpenoid biosynthesis, and alter non-polar metabolite profiles of *A. annua*. Taken together, artemisinin biosynthesis is controlled by not only the activity of its pathway but also its network in which it is localized. This study shows a novel opportunity to improve artemisinin production for fighting against malaria.

Symposium 3: Alkaloids

Keynote speaker

Vincenzo De Luca, Professor, Department of Biological Science, Brock University, St. Catharines, ON, Canada, L2S 3A1

BIOGRAPHY:

- Professor & Tier 1 Canada Research Chair in Plant Biotechnology, Brock University (2001-08; 09-15; 16-22)
- Principal Senior Scientist, 3 years, Novartis/Syngenta Biotechnology (1998-2001)
- Professor Plant Biology, 9 years, University of Montreal (1989-1998)
- Staff scientist, 5 years, Plant Biotechnology Institute, National Research Council of Canada (1984-1989)

KEY ACHIEVEMENTS:

- More than $30 million in individual & collaborative funding since 1989.
- President, Canadian Society of Plant Biologists (2013-15).
- Member of the Board, Federation of Canadian Plant Societies (2011-15).
- Brock University Award for Distinguished Research or Creativity (2011-12).
- Vice President Canadian Society of Plant Physiologists (2011-13)
- Renewal of Tier 1 Canada Research Chair in Plant Biotechnology (2008-15 and in 2016-22)
- Tier 1 Canada Research Chair in Plant Biotechnology (2001-08)
- Member of the Science advisory board of the Vineland Research & Innovation Centre, Vineland, Ontario. (2007-11).
- Member International Scientific Advisory Board, Max-Planck Institute for Chemical Ecology, Germany (2008-11).
- Member International Scientific Advisory Board, Max-Planck Institute for Chemical Ecology, Germany (2003-08).
- President of the Phytochemical Society of North America (1998-99)
- CD Nelson Award winner (1993) from the Canadian Society of Plant Physiologists
- Editor
    - Molecular Plant (2014 to present)
    - Phytochemistry (1993 to present)
    - The Plant Cell (2010 to 2014)
    - Biotechnology and Bioengineering (1996 to 1999)
    - Plant Cell Reports (1995 to 1999)
    - Canadian Journal of Botany (1993-1997)
- Author of over 127 scientific papers published in international journals
- Member of Grant Selection panels
    - European Research Council Grants (L9 panel; 2012-13; 2014-15; 2016-17)
    - 2010-2012; NSERC Discovery: Genes Cells and Molecules
    - Other grant panels during career: NSERC Strategic; NSERC Discovery, FCAR, Ontario Graduate Scholarships, FQRNT Research Grant Panel.
- Invited speaker to national and international symposia, seminars, workshops and courses; contributor of reviews and book chapters in top journals and books in his field.

RESEARCH INVOLVES: Biochemistry, and molecular biology/genomics for gene discovery of secondary metabolism pathways. Production of: plant and/or microbial cell factories for rational metabolic pathway engineering.

IMPACT OF THE RESEARCH:

- Metabolic pathway discovery and evolution
- Value added product discovery and eco-friendly commercialization
- Adaptation of plant cell factories for inexpensive manufacturing
- Pathway transfer to plants and microorganisms.

Molecular and biochemical characterization of monoterpenoid indole alkaloid biosynthesis pathways and their use in metabolic engineering.

V. De Luca, Y. Qu, T. Kidd, A. Thamm, A. Edge, G. Jones, K.H. Kim

Brock University, Biological Sciences, St. Catharines, ON Canada L2S 3A1

The monoterpenoid indole alkaloids (MIAs) make up a large diverse class of specialized small molecules characteristically found within many thousands of species in the Apocynaceae, the Loganiaceae, & the Rubiaceae plant families. Their complexity is matched by their remarkably diverse biological activities and use in various human therapies that have triggered extensive studies to understand their mode of biosynthesis. For example extensive efforts have been made to characterize the assembly of chemotherapeutic dimeric MIAs, vinblastine and vincristine that are derived from the coupling of catharanthine and vindoline monomers. Recent advances have led to the characterization of the complement of genes required for the multistep assembly of the iridoid, secologanin that is required for the

biosynthesis of the MIA strictosidine, the central precursor to the majority of MIAs found in Nature. This led to the engineering of secologanin biosynthesis and in the assembly of strictosidine in plants (transient expression in *Nicotiana plumbaginifolia*) and in yeast. Similarly, the characterization of 2 remaining genes in the vindoline pathway permitted the reconstitution of the tabersonine to vindoline pathway in yeast. While the successful assembly of the strictosidine and tabersonine to vindoline pathways are promising, many genes remain to be discovered before it will be possible to produce MIA anticancer drugs like vinblastine, since the multistep pathways from strictosidine to catharanthine and tabersonine remain to be elucidated. Present efforts include screening efforts to identify MIA mutants and to characterize the genetic basis for these mutations. The significant progress and discoveries that have been made will be presented.

Changing homoterpene synthase specificity by a single amino acid switch

Dorothea Tholl[1], Sungbeom Lee[2], Somayesadat Badieyan[3], and Qiang Wang[4]

[1]Department of Biological Sciences, Virginia Tech, 409 Latham Hall, Blacksburg, VA 24060, USA
[2]Research Division for Biotechnology, Advanced Radiation Technology Institute, Jeongeup-Si, Jeollabuk-Do 580-185, South Korea
[3]Department of Chemistry, University of Michigan, 930 North University Ave, Ann Arbor, USA
[4]Institute of Ecological Agriculture, Sichuan Agricultural University, Chengdu, China

Volatile homoterpenes such as (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E*,*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) are emitted from the foliage of a large number of flowering plants upon pathogen and insect attack. These volatiles have been shown to aid in indirect defense and elicit defensive responses in neighboring plants. We previously identified a cytochrome P450 monooxygenase of the CYP82 family (CYP82G1) in Arabidopsis, which catalyzes an oxidative C-C cleavage reaction of (*E*)-nerolidol and (*E*,*E*)-geranyllinalool leading to the formation of DMNT or TMTT, respectively (Lee et al. 2010). In an attempt to identify homoterpene synthases of the CYP82 family in other dicots, we mined the genome of poplar, which has been shown to release both DMNT and TMTT from leaves upon herbivory. Out of several poplar genes in the CYP82 family, PtCYP82L1 and PtCYP82L2 showed highest sequence similarity to CYP82G1 and the expression of both genes was induced in leaves upon treatment with the fungal elicitor alamethicin. Enzyme assays with recombinant CYP82L1 and CYP82L2 proteins demonstrated different product profiles with DMNT being the main product of CYP82L1 and CYP82L2 producing primarily TMTT and some DMNT. Homology modeling and substrate docking of (*E*)-nerolidol and (*E*,*E*)-geranyllinalool suggested differences in substrate binding based on the presence of a Phe109 residue in the CYP82L1 enzyme narrowing the binding pocket and possibly compromising the cleavage of geranyllinalool. Reciprocal changes of Phe109 and Gly109 between CYP82L1 and CYP82L2 indeed resulted in distinct changes of the DMNT/TMTT product profiles. The results demonstrate that minimal modifications in enzyme structure drastically affect volatile homoterpene formation.

Lee S. et al. (2010) Herbivore-induced and floral homoterpene volatiles are biosynthesized by a single P450 enzyme (CYP82G1) in Arabidopsis. Proc. Natl. Acad. Sci. USA, 107:21205-10.

Monoterpene metabolism in Lavandula

Soheil Mahmoud

Department of Biology, University of British Columbia, Kelowna, BC, Canada

Lavandins (*Lavandula x intermedia*) are widely cultivated for their essential oil (EO), which is constituted of both regular and irregular monoterpenes with the former class dominating the oil. We have developed genomics resources to facilitate the discovery of structural and regulatory genes that control EO formation, secretion and storage in Lavandula. This presentation summarizes recent findings concerning the molecular aspects of EO metabolism in these plants.

Monday, August 7- Morning

Plenary Symposium

Georg Jander completed a PhD in Microbiology and Molecular Genetics at Harvard Medical School in 1995. As a postdoc at Massachusetts General Hospital he began studying plant specialized metabolism, investigating the role of glucosinolates in Arabidopsis-insect interactions. From 1998 to 2002 Jander worked as a scientist at Cereon Genomics, a Monsanto Company research site in Cambridge, Massachusetts. In 2002 he moved to his current position at the Boyce Thompson Institute, where he is continuing to study the function of plant specialized metabolism in defense against pests and pathogens. Current research projects in the Jander lab involve maize, potato, milkweed, and Arabidopsis.

Genetic and biochemical diversity in maize defense responses

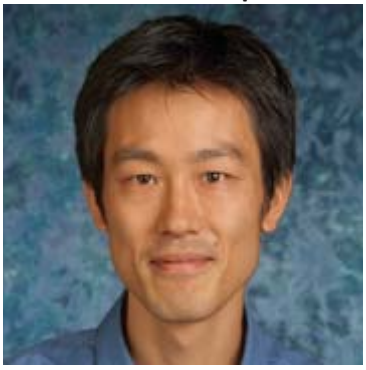
Georg Jander

Boyce Thompson Institute, Cornell University, Ithaca, New York, USA

Natural variation in plant resistance against biotic stresses can be explained to a large extent by diversity in the biosynthetic genes of plant specialized metabolism. We discovered extensive differences in both constitutive and pathogen-induced metabolite profiles of two maize (Zea mays) inbred lines with contrasting resistance to seedling infection by the fungal pathogen Fusarium graminearum. This natural variation allowed us to perform metabolite quantitative trait locus (mQTL) mapping at both the single-metabolite and metabolome scales. To pinpoint the causative gene(s) underlying these mQTL, we performed comparative and correlative network analysis based on transcriptomic and metabolomic data obtained from the same mapping population. These experiments identified a likely vesicular transport protein, an ethylene receptor, and a biosynthetic enzyme that influence the constitutive abundance of metabolites related to Fusarium resistance. Our findings underline the metabolic diversity and essential defensive functions of maize specialized metabolism

Symposium 4: Phytochemistry & Ecology

Elsevier Award Recipient and Keynote speaker



Hiroshi Maeda is assistant professor in the Department of Botany at University of Wisconsin-Madison. He recived BS and MS degree in Biotechnology at Osaka University. He then moved to the US and obtained PhD at Michigan State

University in 2006, working with Dr. Dean DellaPenna on tocopherol (vitamin E) functions in photosynthetic organisms. After working as postdoc with Dr. Natalia Dudareva at Purdue University on phenylalanine and benzenoid volatile biosynthesis in petunia flowers, he started his current position at UW-Madison from the fall 2011. Dr. Maeda's laboratory has been investigating evolutionary diversification of the tyrosine biosynthetic pathway in various plant species. Dr. Maeda is the recipient of the 2006 Anton Lang Memorial Graduate Student Award from MSU DOE-Plant Research Laboratory, the 2011 Eric Conn Young Investigator Award from the American Society of Plant Biologists, and the 2016 Arthur Neish Young Investigator Award from PSNA.

De-regulation of tyrosine biosynthesis underlies evolutionary expansion of diverse plant natural products

Samuel Lopez-Nieves[1], Ya Yang[2,4], Alfonso Timoneda[3], Minmin Wang[1], Tao Feng[3], Stephen A. Smith[2], Samuel F. Brockington[3], and Hiroshi A. Maeda[1]

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[2]Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, USA.
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Plants synthesize numerous natural products, which play crucial roles in plant adaptation and human health. In contrast to well-documented diversification of specialized metabolic enzymes, little is known about the evolution of primary metabolic enzymes that provide precursors to the production of various specialized metabolites. Here we uncovered that relaxed regulation of tyrosine (Tyr) biosynthesis in the plant order Caryophyllales underlies the evolution of diverse Tyr-derived specialized metabolites, such as betalain pigments and neurotransmitters, dopamine and epinephrine. Betalains are Tyr-derived pigments uniquely found in the plant order Caryophyllales and replaced the otherwise ubiquitous phenylalanine (Phe)-derived anthocyanins. We found that table beets (*Beta vulgaris*), which produce high levels of betalains, synthesizes Tyr via plastidic arogenate dehydrogenases (TyrAa/ADH) encoded by two *ADH* genes (*BvADHα* and *BvADHβ*). Unlike BvADHβ and other plant ADHs that are strongly inhibited by Tyr, BvADHα exhibited relaxed sensitivity to Tyr. Phylogeny-guided enzyme characterization revealed that BvADHα orthologs arose before the evolution of betalain pigmentation in the core Caryophyllales. Metabolite profiling further discovered that various Tyr-derived compounds, besides betalains, accumulate in BvADHα-containing Caryophyllales, including tyramine accumulation in *Simmondsia chinensis* that has little betalains. Finally, heterologous expression of Tyr-insensitive BvADHα, but not BvADHβ, in *Nicotiana benthamiana* increased Tyr and Phe levels. These results together suggest that de-regulation of Tyr biosynthesis redirected carbon flow from Phe to Tyr biosynthesis and supported the evolutionary expansion of diverse specialized metabolites derived from Tyr in a lineage-specific manner. The finding can now be used to systematically identify and enhance production of Tyr-derived plant natural products.

Detoxification pathways of rutabaga phytoalexins by the phytopathogen *Alternaria brassicicola*

Abbas Abdoli and M. S. C. Pedras*

Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, SK, Canada

Phytoalexins are antimicrobial plant metabolites produced in response to different kinds of stress like pathogen attack, while not present in healthy plants. Rutalexin, brassicanate A, isalexin and rapalexin A are phytoalexins of rutabaga (*Brassica napus* L. ssp*. rapifera*) that inhibit the growth of several cruciferous fungal pathogens, including *Alternaria brassicicola* (Schwein.) Wiltshire, an economically important plant pathogen. In this work, the biotransformation of rutalexin, brassicanate A, isalexin and rapalexin A by *A. brassicicola* was investigated, the detoxification pathways were established and the antifungal activity of metabolites against *A. brassicicola* was evaluated. Results of this work showed that brassicanate A and rapalexin A inhibited strongly the mycelial growth of *A. brassicicola* but rutalexin and isalexin showed weaker inhibitory activity against *A. brassicicola*. While the biotransformation of rutalexin, brassicanate A, and

isalexin were fast, rapalexin A was resistant to fungal transformation. In fungal cultures rutalexin, brassicanate A and isalexin were enzymatically transformed to less toxic metabolites. Details of this work will be presented and discussed.

Integrated metabolomics for the discovery and characterization of saponin biosynthetic genes in *Medicago truncatula*

Lloyd W. Sumner[1,2], Bonnie S. Watson[2], David V, Huhman[2], Daniel Wherritt[3], Zhentian Lei[1,2], Yuhong Tang[2], Derek Nedveck[3], Peter Tiffin[3], and Nevin Young[3]

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[2]Plant Biology Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK, USA
[3]Department of Chemistry, University of Texas at San Antonio, San Antonio, TX, USA
[4]Plant and Microbial Biology, University of Minnesota, St. Paul, MN, USA

Triterpene saponins are structurally diverse specialized metabolites found in many plant families, including the Leguminosae. They possess a broad spectrum of bioactivities including anticancer, antifungal, antibacterial, anti-insect, alleopathic and anti-nutritive. Although the functional importance of saponins are known, the biosynthetic pathways for saponins remain largely uncharacterized. Here we report the use of integrated metabolomics, correlated gene expression profiling and genome wide association studies (GWAS) for the discovery and characterization of novel saponin biosynthetic genes in the model legume *Medicago truncatula*, which accumulate a large variety of glycosylated saponins. In this project, saponins from aerial and root tissues of approximately 200 accessions, most of which were also associated with the Medicago Hapmap project, were profiled using UHPLC-QTofMS. High variations in the saponin content were found in different tissues as well as in different accessions. Multiple lines with differential saponin accumulation were chosen for further characterization, including correlated gene expression analyses using microarrays and RNAseq relative to the differential saponin accumulation. Genome wide association studies (GWAS) were performed to identify candidate loci responsible for variation in the production of specific saponin compounds in *M. truncatula.* The correlated gene expression and GWAS results guided the selection and prioritization of gene candidates for subsequent molecular and biochemical characterization. In vitro biochemical assays revealed the specific activity of saponin biosynthetic enzymes. Additional molecular genetic confirmations were performed through the analysis of Tnt1 insertional mutations within the targeted saponin genes and through the analysis of plants stably transformed with known and putative saponin genes. This presentation will describe the integrated technologies and approaches used and provide examples of novel gene discoveries.

Acknowledgements: This work was supported by the University of Missouri, Noble Research Institute, Bruker Daltonics Gmbh, USA National Science Foundation MCB#1024976, NSF MRI#1126719, NSF #1139489, and NSF IOS#1340058

Monday, August 7- Afternoon

Symposium 5: Lipids

Keynote speaker

Ruth Welti obtained her BS from the University of Connecticut and her PhD from Washington University in St. Louis. She is a long-time lipid biochemist who became a plant biologist around the turn of the century. She currently serves as director of the Kansas Lipidomics Research Center and is a university distinguished professor and Lillian J. Brychta professor of biology at Kansas State University. She has been instrumental in developing mass spectrometry-based lipid analysis for the plant community.

Using lipid analysis by mass spectrometry to understand plant response to the environment

Ruth Welti

Department of Biology, Kansas State University, Manhattan, KS, USA

A goal of lipid analysis by mass spectrometry is to link variations in plant lipid levels with plant response to the environment and with genes encoding catalytic or regulatory proteins underlying the lipid variation. Welti will describe lipidomic analyses and their applications to identify the genetic and biochemical basis for alterations in lipids involved in plant responses to abiotic stresses.

Deciphering the Eukaryotic Pathway of Leaf Glycerolipid Assembly through Lipid Flux Analysis in Arabidopsis Mutants and Oil Accumulating Tobacco

Philip D. Bates[1], Nischal Karki[2], Xue-Rong Zhou[3]

[1,2]Department of Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, MS, USA.
[3]CSIRO, Canberra, ACT, Australia.

The parallel “Eukaryotic” and “Prokaryotic” pathways of glycerolipid assembly in plant leaves have been well established for over 35 years. Recent work has indicated that TGD1,2,3 proteins are involved in the transfer of eukaryotic assembled lipid substrates from the endoplasmic reticulum (ER) to the plastid for eukaryotic galactolipid synthesis (predominantly monogalactosyldiacylglcerol (MGDG)). However, the class of lipid transferred from the ER to the plastid is still unclear. Diacylglycerol (DAG), phosphatidic acid (PA), and lysophosphatidylcholine (LPC) have all been indicated as possible ER to plastid transport molecules. Lysophosphatidylcholine acyltranserase (LPCAT) activity is abundant in chloroplast envelopes and could be involved a pathway where LPC is transferred from the ER to the plastid, converted to phosphatidylcholine (PC) by LPCAT, and then subsequently turned over to DAG for MGDG synthesis. To investigate the role of LPCATs within leaf lipid fluxes the Arabidopsis *lpcat1/lpcat2* double mutant was crossed with the *act1* mutant which eliminates the

prokaryotic pathway and requiring MGDG synthesis through the eukaryotic pathway. *In vivo* [^{14}C]acetate acyl flux analysis indicated that nascent acyl groups still followed a PC-MGDG precursor-product relationship indicative of the eukaryotic pathway, suggesting that LPCAT activity (and thus LPC) is not required for the import of lipids into the plastid. Transgenic tobacco leaves expressing an acyl-CoA:diacylglycerol acyltransferase (DGAT) that produce oil up to 15% of leaf dry weight were analyzed for eukaryotic pathway fluxes with [^{14}C]glycerol. The results indicated that DGAT uses a DAG pool derived from PC for oil synthesis, and that the PC-MGDG precursor-product relationship was almost completely eliminated in the oil accumulating leaves. Therefore, oil synthesis out competes MGDG synthesis for a PC-derived DAG pool. These combined results suggest that DAG (not LPC or PA) is the class of lipid transferred from the ER to the plastid within the eukaryotic pathway of galactolipid synthesis.

A Novel Hydroxy Fatty Acid Biosynthetic Pathway Revealed by Discovery of Abundant C24 Di-Hydroxy Fatty Acids in *Orychophragmus* Seed Oil

Xiangjun Li[1], Alicen M. Teitgen[2], Wei Zhang[3], Chunyu Zhang[3], Robert E. Minto[2], and Edgar B. Cahoon[1]

[1]Center for Plant Science Innovation & Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA,
[2]Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA,
[3]National Key Lab of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China

The Brassicaceae *Orychophragmus violaceus* (Chinese violet cress, February orchid) is native to China and has been used in intergenic crosses with *Brassica napus* to study genome stability. Previously published analyses have indicated that the seed oil of this plant is enriched in linoleic acid (18:2) and has low very long-chain fatty acid content. Re-evaluation of *O. violaceus* oil by thin layer chromatographic analysis serendipitously revealed the presence of a highly polar oil composition. Consistent with this, two fatty acids with extended retention times accounting for 40% to 50% of the seed oil were identified in gas chromatographic analysis of silylated fatty acid methyl esters from *O. vi*olaceus seed oil. Data from GC-MS and NMR analyses were consistent with the identity of these fatty acids as the C24 dihydroxy fatty acids 7,18-OH-24:1Δ15 and 7,18-OH-24:2Δ15,21. In addition to these fatty acids, small amounts of C18-C24 monohydroxy fatty acids were detected in O. violaceous seed oil. Co-expression of cDNAs for an oleic acid 12-hydroxylase and a variant 3-keto-acyl-CoA synthase found in the *O. violaceus* seed transcriptome was sufficient to confer nebraskanic acid synthesis in Arabidopsis seeds, without the need for a second fatty acid hydroxylase. These data together with the detection of C_{20} 3-OH and C_{22} 5-OH dihydroxy intermediates in fatty acid and acyl-CoA pools of *O. violaceus* seed indicated a novel route for hydroxy fatty acid synthesis involving premature or “discontinuous” elongation of the 3-OH intermediate during a carbon chain extension cycle.

Digging for buried treasure in a chemical diversity database

Lucas Busta[1] and Reinhard Jetter[2,3]

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[3]Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada

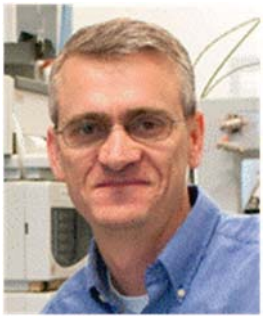
The structures of phytochemicals encrypt biochemical information. This is especially true for cuticular waxes, lipids that coat and provide crucial protection for plants, because they occur in multidimensional mixtures that contain the signatures of their underlying modular biosynthetic pathways. Although the biosynthesis of ubiquitous C26 – C32 wax alcohols, aldehydes, alkanes, fatty acids, and esters has been characterized, the most abundant wax compounds on many species, including several major crops, contain secondary functional groups and thus pathways to these compounds are, at least in part, different from the ubiquitous wax compounds with which they co-occur. So far, details of these pathways are largely unknown.

We have used CAS SciFinder to perform an optimized, methodical literature search leading to a comprehensive database of specialty wax compound structures. With this, we have developed and refined hypotheses for specialty wax compound biosynthesis through systematic analysis of their structural diversity.

Across the plant kingdom, specialty wax compounds with one, two, and three secondary functional groups have been identified. Where multiple specialty compounds were reported, they frequently occurred as homologous series and/or mixtures of isomers. Among these, it is possible to recognize series of homologs with predominantly odd or even total carbon numbers, and mixtures of isomers with functional groups on adjacent or alternating carbons. Based on these structural patters, specialty compounds can be categorized and their biosynthetic mechanisms may be predicted: mixtures of isomers with secondary functions on adjacent carbons almost certainly arise from P450 oxidation, while mixtures of isomers with alternating group positions are probably formed by malonate condensation reactions mediated by polyketide or ketoacyl-CoA synthase enzymes, or else by head-to-head condensation of long-chain acyls. Though it is possible that some enzymes generating ubiquitous compounds also participate in specialty wax compound biosynthesis, our analyses strongly suggest that some dedicated specialty wax compound machinery exists.

Tuesday, August 8- Morning

Plenary Symposium



Jay Thelen earned a Bachelors of Science in Biological Sciences from the University of Nebraska-Lincoln in 1993. His graduate research training was on the regulation of the maize mitochondrial pyruvate dehydrogenase complex in Doug Randall's lab at the University of Missouri-Columbia, earning his Ph.D. in 1998. In 1999 he began a postdoctoral position in John Ohlrogge's lab at Michigan State University investigating the regulation of plastid heteromeric acetyl-CoA carboxylase. In 2004 he accepted a tenure-track faculty position in the Biochemistry Department at the University of Missouri and was promoted to full professor in 2016. His research interests include metabolic regulation, mutlienzyme complexes, de novo fatty acid synthesis, protein phosphorylation, and quantitative proteomics approaches to study biological systems. He has authored or co-authored 110 original research publications and was honored by the University of Missouri System with both a Presidential Early Career Award for Research Excellence as well as the Chancellor's Award for Outstanding Research and Creative Activity.

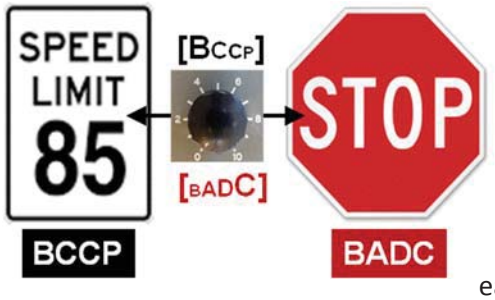
A new model for the regulation of *de novo* fatty acid biosynthesis in plants

Jay Thelen

Department of Biochemistry, University of Missouri, Columbia, MO, USA

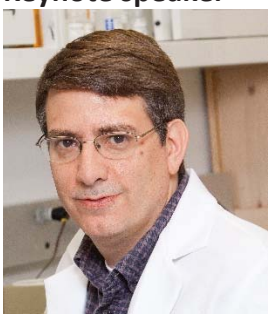
Acetyl-coenzyme A carboxylase (ACCase) catalyzes the committed step of *de novo* fatty acid biosynthesis. In prokaryotes, green algae, and most plants, this enzyme is a heteromeric complex requiring four different subunits for catalytic activity. Using proteomics we recently discovered a novel interacting protein to the plant ACCase, tentatively annotated as a 'biotin/lipoyl attachment domain containing' (BADC) protein. This small family of proteins resembles the biotin carboxyl carrier protein (BCCP) catalytic subunit but is not biotinylated due to a mutated biotinylation motif. Although lacking a biotinyl active site, BADCs are capable of assembling into holo-ACCase inhibiting activity of both *E. coli* and *A. thaliana* ACCase. Down-regulation of this gene family by stacked, hairpin RNAi substantially increases seed

oil content in Arabidopsis. We conclude the BADC proteins are ancestral BCCPs that lost their biotinylation motif and adopted a neomorphic role as attenuators of ACCase activity. Consistent with this interpretation, quantitative expression of all known ACCase genes revealed BADC genes are regulated in an inverse pattern to genes for the catalytic subunits in response to light and exogenous fatty acids. A "rheostat" model for regulation of plant heteromeric ACCase, and thus *de novo* fatty acid biosynthesis, is proposed whereby the *in vivo* ratio of sibling proteins BCCP and BADC dictates pathway flux. Given the growing list of subunits to the heteromeric ACCase, we recently developed and validated a multiplexed, absolute quantitative assay to monitor *in vivo*, steady-state subunit levels in Arabidopsis. Absolute quantitation of each ACCase subunit (and isoforms therein) using heavy- labeled peptides coupled to multiple reaction monitoring-tandem mass spectrometry revealed limiting levels of the alpha-carboxyltransferase catalytic subunit in developing siliques. Transgenic overexpression of this limiting subunit increased both ACCase specific activity and seed oil content in Arabidopsis. We are now leveraging these two new findings to optimize ACCase function in the cover crop camelina and soybean.



Symposium 6: Synthetic Biology and Metabolic Engineering

Keynote speaker



Edgar Cahoon is the George Holmes University Professor of Biochemistry and Director of the Center for Plant Science Innovation at the University of Nebraska-Lincoln. Dr. Cahoon's lab conducts basic and applied research on plant lipid metabolism to enhance the nutritional and industrial value of crop plants and to probe the synthesis and function of bioactive lipids, including sphingolipids, for improved crop performance and quality. He is the author of more than 100 publications and inventor or co-inventor on 30 issued U.S. patents in plant biotechnology. Dr. Cahoon received his B.S. in biochemistry from Virginia Tech, an M.S. in plant physiology from Cornell University, and Ph.D. in plant biochemistry and molecular biology from Michigan State University.

Application of Synthetic Biology to Enhance Genetic Variation for Improved Plant Quality and Performance

Edgar B. Cahoon, Hae Jin Kim, and Tom E. Clemente

Center for Plant Science Innovation, E318 Beadle Center, University of Nebraska-Lincoln, Lincoln, NE, USA

Genes underlying extremes in chemical diversity found in plants and other organisms provide a deep reservoir of tools for improving plant quality and agronomic performance through metabolic engineering. The speed and complexity associated with the introduction of genetic variation in plants through metabolic engineering can be enhanced by the use of these genes in combination with emerging tools of synthetic biology. In this presentation, the use of large-scale gene synthesis and modular transgene assembly will be described for metabolic engineering of seed composition and agronomic traits in soybean and camelina. As one example, the metabolic engineering of soybean and camelina with nine transgenes for aquaculture feed traits that target omega-3 fatty acid composition, high value carotenoids, and vitamin E antioxidants will be described. As a second example, efforts to improve camelina as a platform for production of biofuel and industrial oils will be described. In these studies, modular gene assembly methods have been used to

introduce transgenes that target the expression or suppression of up to 12 genes for oil quality and composition, seed protein composition, and agronomic traits. These examples highlight the ability of synthetic biology to revolutionize plant genetic improvement beyond the capabilities of breeding and traditional metabolic engineering approaches that have typically involved altering expression of only small numbers of genes.

From plant pathway discovery to synthetic biology: engineering of diterpene production

Nikolaj L. Hansen[c], Jakob Nissen[c], Victor Forman[c], Britta Hamberger[a,b,c] Aparajita Banerjee[a,b], Wajid Waheed Bhat[a,b], Sean Johnson[a,b], Radin Sadre[a,b] and Bjoern Hamberger[a,b,c]

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[b]Great Lakes Bioenergy Research Center
[c]Department of Plant and Environmental Sciences, University of Copenhagen, DK.

Plants synthesize an impressive diversity of specialized (i.e. secondary) metabolites with roles in adaptation and interaction with the environment. Traditionally used virtually exclusive as source of therapeutic preparations, natural small molecule specialized metabolites still represent, or inspired more than a third of the approved pharmaceuticals over the last three decades. Terpenoids, with over 50,000 known structures, constitute the oldest and largest class of plant specialized metabolites, and a vast repository of bioactive natural products. Formal chemical synthesis of high-profile plant derived terpenoid pharmaceuticals remains challenging, despite recent strategies mimicking natural routes. Extraction from plant biomass, and semi-synthesis from biotechnogically-produced intermediates have been approached as alternative strategies. With the recent development of multi-species transcriptome repositories the discovery of typical terpenoid pathways is no longer a limitation and new approaches are emerging. I will discuss three facets illustrating our approaches in the Chinese medicinal herb *Tripterygium wilfordii* (Celastraceae), accumulating structurally unique diterpenoids. Specifically, characterization of the terpene synthase gene family revealed emergence of a new function in a terpene synthase subfamily. Two closely related diterpene synthases with distinct functions were found to highlight the evolutionary potential of this family driving rapid diversification of diterpene specialized metabolites. Lastly, limitations in the biotechnological production of potential drug intermediates motivated exploration of strategies to engineer improved platforms.

The Evolution and Function characterization Analysis of Leucoanthocyanidin Reductase from *Camellia sinensis*

Wang Peiqiang[a]， Gao Liping[b]

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[b] School of Life Science, Anhui Agricultural University, Hefei, Anhui 230036, China

Tea, is rich in polyphenolic compounds, in which the catechins are the major polyphenols. Their biosynthesis, are closely corresponded with the expression of *Leucoanthocyanidin Reductase* (*LAR*) and *Anthocyanidin Reductase* (*ANR*) genes. In this paper, the evolution analysis and function characterization of three CsLARs is elaborated. The phylogenetic tree showed plant LARs could be grouped into three groups including gymnosperms, monocotyledons and dicotyledons (cluster Ⅰ and Ⅱ). Interestingly, the eighth amino acid residue in a conserved LAR-specific motif is changeable due to a transversion (G→T) and transition (G→C) happened in the codon of this residue. Thereby, plant LARs could be classified as G-type, A-type and S-type LARs due to the changeable amino acid residue. Although (2R, 3S) -*trans*-flavan-3-ols were the products of recombinant CsLARs proteins expressed in *E. coli*, both (2R, 3S) -*trans* and (2R, 3R) -*cis*-flavan-3-ols were detected in over-expressing CsLARs tobaccos. However, butanol / HCl hydrolysis indicated that the overexpression of CsLARs caused the decrease of polymerized catectins. The hybridization experiment of CsLARc + AtPAP1 also showed no polymer was detected in addition to epicatechin, catechin and glucoside, though the accumulation of anthocyanin was decreased a lot. Interestingly, CsMYB5b promoted the biosynthesis of both flavan-3-

ols and proanthocyanidins (PAs) *via* up-regulating the expression of *NtANR* and *NtLAR* genes. Therefore, LARs alone can only participate in the biosynthesis of catechins. Co-expression of LAR and ANR was involved in the synthesis of both catechins monomer and PAs.

Key words : Phylogenetic analysis, LARs, (2R, 3R) -*cis*-flavan-3-ols, *Camellia sinensis*

Acknowledgement:

This work was supported by the Natural Science Foundation of China (31470689, 31270730, 31200229 and 31570694), the Natural Science Foundation of Anhui Province, China, and the Biology Key Subject Construction of Anhui. We thank Prof. Huarong Tan for assistance with LC/MS analysis.

Transcriptional Profiles Associated with Sugar Metabolism in Tobacco Altered by Overexpression of a Tea (*Camellia sinensis, Cs*) R2R3-MYB4

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[2]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, 27695, USA

A R2R3 MYB4 transcription factor isolated from green tea, namely (CsMYB4a), was shown to suppress the activity of plant shikimate and phenylpropanoid pathways in transgenic tobacco plants. Here, we report that the heterogeneous expression of *CsMYB4a* leads to dramatic alteration of transcriptional profiles associated with sugar metabolism in transgenic tobacco plants. A genome-wide transcriptomics is completed with leaf tissues. Data mining identifies that 12 genes involved in the tricarboxylic acid (TCA) cycle, glycolysis, and sugar metabolisms are downregulated in transgenic plants. In addition, 8 genes associated with starch and sucrose metabolism are up-regulated. Furthermore, GC-MS based metabolic profiling shows significantly increased accumulation of ribose, fructose, glucose, galactose, and sucrose in transgenic plants, supporting the transcriptional alterations by CsMYB4a. This regulation is likely associated with an interaction between CsMYB4a and DNAs of those tobacco genes, which is discussed in our presentation.

Boosting the production of terpenoids in lipid droplet-accumulating photosynthetic tissues

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Terpenoids are specialized metabolites with broad applications such as specialty fuels, biomaterials, bioherbicides, drugs, flavors and fragrances. We demonstrated previously that in *Coleus forskohlii*, specialized root cells sequester high levels of terpenoids in lipid droplets.(1) Using a transient expression system in *Nicotiana benthamiana*, we investigated novel engineering strategies to increase the synthesis and accumulation of high-value terpenoids in combination with triacylglycerols (TAGs) in plants. In *Nicotiana* leaves, transient overexpression of different terpenoid precursor pathway genes from various bacteria and plants resulted in up to 6-fold higher levels of engineered terpenoids compared to controls. Lipid droplets were produced in *Nicotiana* leaves through ectopic transient expression of the transcription factor WRINKLED1 which induces fatty acid biosynthesis and indirectly, TAG accumulation.(2) Our data provide evidence that the mere presence of lipid droplets is sufficient to increase terpenoid product levels by several folds when a terpene synthase is co-expressed.

(1)Pateraki et al. (2014) Plant Physiol. 164:1222-36, PMID: 24481136
(2)Ma et al. (2015) Plant J. 83:864-874, PMID: 26305482

Symposium 7: Industrial Phytochemistry

Keynote speaker

Martin Ruebelt leads the R&D Analytics Platform at Monsanto in St. Louis, MO. His team provides analytical leadership, expertise, and resources to enable product discovery, development and stewardship across Monsanto's Technology functions. The team is using state of the art analytical tools to analyze and characterize small and macro molecules. Martin joined Monsanto in 2001 and has held various positions with increasing responsibilities. He received a MS degree in Food Chemistry from the Technical University of Kaiserslautern, Germany and received his PhD in Food Chemistry/Biochemistry from the Technical University of Munich, Germany. His academic research was on proteomics for evaluating genetically modified plants in the context of natural variability. Prior to his PhD, he managed an analytical contract laboratory in Germany focusing on food, water and soil analyses.

Monsanto's Approach to Sustainable Agriculture

Martin Ruebelt

Agriculture Productivity Innovations, Monsanto, St. Louis, MO, USA

Climate change has created a more volatile environment for farming - threats like drought, insect populations, and new/renewed disease continue to increase. By delivering environmentally compatible agricultural products that overcome these challenges, we are moving to stem the tide. Our pipeline of seed production, genetic trait integration, pest control and yield protection/enhancement through chemical and biologically derived products, and data science enables the delivery of integrated solutions that can help produce more food using fewer natural and renewable resources on an ever-shrinking footprint. This talk focuses on our integrated strategies for chemical pest control, biological products, and data science. Offering industry leading crop protection tools afford pest management through multiple modes of action, which is essential to a farmer's ability to have a good harvest. Our crop protection offerings provide solutions by coupling pesticide(s) with resistant germplasm(s) and allow farmers to apply the right protection, in the right amount, at the right time. Our biological portfolio focuses on two core technologies: Microbials and BioDirect® technology. Microbials are products derived from microbes that can complement or replace agricultural chemical products. Microbes can colonize in soil and improve the nutrient concentrations, as well as help control fungi, bacteria, nematodes, insects and weeds. Monsanto collaborates with world leaders in microbe research and production to create value-add solutions for the farmer. BioDirect® technology is a topically applied RNA-based technology that leverages our genomics expertise. BioDirect® technology is in the early phases of research and development for weed management, insect management, virus control, and bee health. Overarching all of the aforementioned strategies is the integration of our field data science capabilities. We offer technology where farmers can customize agronomic practices to get the most from their fields. Offering the tools to measure nitrogen availability, soil types, or customizable management zones allows each farmer the ability to turn their fields into their own laboratory. We believe through full integration as described above we can harness science solutions that can sustain biodiversity and help nourish the world.

A new *O*-methyltransferase gene involved in antitumor lignan biosynthesis in *Anthriscus sylvestris*

Masato Kumatani[1], Masaomi Yamamura[1], Eiichiro Ono[2], Satoshi Shiraishi[3], Honoo Satake[3], Toshiaki Umezawa[1,4]

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[2]Suntory Global Innovation Center Limited, 8-1-1 Seikadai, Seika-cho, Soraku-gun, Kyoto 619-0284, Japan
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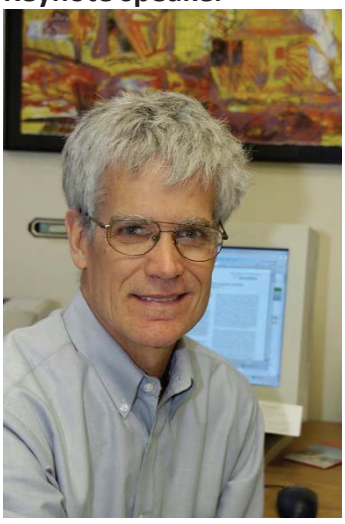
[4]Research Unit for Development of Global Sustainability, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

Lignans are phenylpropanoid dimers that are linked at the C-8 position of their propyl side chains, and are known to have antitumor, antiviral, and antioxidative physiological activities (Umezawa 2003). Podophyllotoxin is a well-known antitumor lignan used as a precursor for the chemical synthesis of the anticancer drugs etoposide, teniposide, and etopophos (Farkya et al. 2004). However, the availability of this lignan is limited because of the overharvesting of podophyllotoxin-forming plants. Hence, research has focused on the stable and large-scale production of podophyllotoxin (Farkya et al. 2004). Previously, Sakakibara et al. (2003) proposed the use of the pathway from matairesinol to the podophyllotoxin precursor yatein in *Anthriscus sylvestris,* which is known to produce podophyllotoxin and related lignans. This pathway has two methylation steps: methylation of the hydroxy group at position C-5 in thujaplicatin, and methylation of the C-4 hydroxy group in 5-*O*-methylthujaplicatin. Recently, we identified a cDNA encoding *A. sylvestris* thujapilcatin *O*-methyltransferase (TJOMT), which converts thujaplicatin to 5-*O*-methylthujaplicatin (Ragamustari et al. 2013). However, 5-*O*-methylthujaplicatin *O*-methyltransferase (5MTJOMT) that methylates 5-*O*-methythujaplicatin to 4,5-*O*,*O*-dimethylthujaplicatin has not been identified. In this study, we used correlation analysis between patterns of 5MTJOMT activity and gene expression among several samples of *A. sylvestris* to select putative OMT sequences. Then, based on the biochemical characterization of recombinant putative OMTs, we identified a cDNA encoding As5MTJOMT. AsTJOMT and As5MTJOMT are located in different clades of the OMT amino acid sequence phylogenetic tree. Moreover, *Anthriscus* lignan OMTs are located in clades that are distant from those of *Podophyllum hexandrum* OMTs involved in podophyllotoxin biosynthesis (Lau and Sattely 2015), strongly suggesting that podophyllotoxin biosynthetic pathways in *A. sylvestris* and *P. hexandrum* are examples of convergent evolution.

Symposium 8: Food and Nutraceuticals

Keynote speaker



William Folk is currently Professor of Biochemistry and Adjunct Professor of Public Health, University of Missouri. He trained with Nobel Laureate Dr. Paul Berg at Stanford University and as a Helen Hay Whitney Fellow at the Imperial Cancer Research Fund, U.K., and has served on the faculties of the University of Michigan-Ann Arbor and the University of Texas-Austin. At the University of Missouri, he served as Chair of Biochemistry and Sr. Associate Dean of Research in the School of Medicine, and Director of the International Center for Indigenous Phytotherapy Studies. He has worked

with traditional medical practitioners, physicians and natural product chemists in basic and clinical studies of South African traditional medicines. For a partial list of publications go to:
http://www.ncbi.nlm.nih.gov/sites/myncbi/william.folk.1/bibliograpahy/42226497/public/?sort=date&direction=ascending

Valuing the Safety and Efficacy of Dietary Supplements, Nutraceuticals and Traditional Medicines – a Case Study of a South African 'Adaptogen": *Sutherlandia frutescens*

William R. Folk

Department of Biochemistry, University of Missouri, Columbia, MO, USA

Most people use dietary supplements, nutraceuticals and traditional medicines to improve health and treat disease. There is need for special attention to potential antagonisms and risks caused by concurrent use of these products and 'conventional' medicines – and for ensuring good communication about these risks occurs between healthcare providers and the public. I will illustrate these points with a case study of *Sutherlandia frutescens*, traditionally used in South Africa for treatment of symptoms of HIV infection and Tuberculosis, and for chronic diseases such as diabetes and cancer. Recent clinical and laboratory experiments indicate that metabolites in Sutherlandia alter uptake and metabolism of first line antiretrovirals and TB medicines used worldwide. Such interactions will increase development of pathogen drug resistance, which is a growing global concern, and underscore the need for better understanding of these interactions and predicting/searching for analogous interactions in the many other phyto-health products used worldwide.

Metabolomics of Scab Susceptible and Resistant Pecan Varieties

Zhentian Lei[1], David Huhman[2], Daniel Wherritt[3], Barbara Sumner[1], Santosh Kumar and[1] Lloyd W. Sumner[1]*

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Pecan scab, caused by the fungal plant pathogen *Fusicladium effusum*, is the single most destructive disease of pecans. It can infect pecan leaves, twigs, petioles, shucks, and nuts. Infection of the nuts can cause them to either fall prematurely or fail to reach full size, thus reducing yields and quality. Scab is active throughout the growing season and is spread to susceptible tress by wind and rain. Scab control is typically achieved by fungicide spray. It is costly and environmentally unfriendly. Moreover, repeated and widespread use of fungicides has resulted in emergence of fungicide resistant scab. In nature, there exit some naturally scab-resistant pecan varieties. These varieties normally require little or no fungicide spray. However, the mechanism of scab resistance in these resistant varieties is not known. In addition, our knowledge of pecan secondary metabolism is still very limited. Secondary metabolism is important to plant defense and fitness. To understand the biochemical basis of scab resistance, we developed the ultrahigh performance liquid chromatography (UPLC)-mass spectrometry (MS)-solid phase extraction (SPE) nuclear magnetic resonance (NMR) based metabolomics platform, and applied it in metabolic profiling of two specific varieties, Kanza (scab resistant) and Pawnee (scab susceptible). Comparative metabolome study has revealed secondary metabolites that are potentially important in pecan resistance.

Neish Awardee speaker

Daniel K. Owens earned a BSc degree with a concentration in biochemistry from East Tennessee State University where he first began research into natural products and flavonoid metabolism by developing a novel assay system for flavanone-3-hydroxylase. He continued working with flavonoids in the lab of Brenda Winkel at Virginia Tech and was awarded his PhD for examining the labile dioxygenase enzymes involved in flavonol biosynthesis in *Arabidopsis thaliana* with a particular focus on the flavonol synthase isozyme family. He then began a postdoctoral position in the lab of Cecilia McIntosh where glucosyltransferase enzymes with the potential to influence flavor chemistry and other aspects of metabolism in Citrus species were identified and thoroughly characterized. Subsequently, he moved to a plant physiologist postdoctoral position with the USDA-ARS Natural Product Utilization Research Unit in Oxford, MS where natural products were investigated as herbicide leads and herbicide resistant crop plants were characterized in the labs of Franck Dayan and Stephen Duke. Daniel is currently an assistant professor in Molecular Biosciences and Bioengineering at the University of Hawaii - Manoa in Honolulu, HI where his lab is investigating the herbicidal potential of natural products from allelopathic tropical and subtropical plants as well as beginning to study the potential of glucosyltransferase enzymes to interact within the flavonoid metabolon.

Identification and Mode of Action of Herbicidal Natural Products

Joey Ooka and Daniel K. Owens

University of Hawaii at Manoa, Department of Molecular Biosciences and Bioengineering, 1955 East West Rd., Agricultural Sciences 218, Honolulu ,HI 96822, USA

Among agronomic pests worldwide, weeds are considered to be of the greatest concern to farmers. Weeds are conservatively estimated to cause greater than $40 billion in annual agricultural losses. This is particularly problematic issue in the Hawaiian islands where no winter kill offs and a temperate climate allow for essentially year round growth of pest and invasive species. Developing herbicidal products with novel modes of action as part of an overall integrated pest management strategy to combat weed and invasive plant infestations as well as continued problems with evolving herbicide resistance is a critical challenge. There is also a need for cheaper, environmentally safe, organically-approved herbicides to aid producers in meeting the increasing demand for organically grown products. Plant natural products are a valuable source for the discovery of new herbicidal compounds. Having naturally evolved herbicidal activity instead of being designed against a previously identified molecular target site, as with the majority of synthetic compounds, improves the likelihood of identifying natural compounds with novel target sites and modes of action. We propose to characterize herbicidal compounds from allelopathic plants, such as the Hawaiian invasive species strawberry guava (*Psidium cattleianum*), for activity against broadleaf and grassy weeds. Identified herbicidal compounds are to be tested for their specific molecular target sites of action and further developed for usage as novel modes of action are discovered.

Biomarkers of Glucosinolate Intake Using Labeled Broccoli Sprouts

Jan F. Stevens[1,2], Jaewoo Choi[1], Bob Durst[1], Laura M. Beaver[1,3], Emily Ho[1,3]

[1]Linus Pauling Institute, [2]College of Pharmacy, [3]College of Public Health and Human Sciences, Oregon State University, Corvallis, Oregon 97330, USA

Cruciferous vegetables and their bioactive components in food, including indoles and isothiocyanates, appear to modulate cancer risk but observational data to date in humans are inconsistent. Such discrepancies arise in part owing to methodological limitations of accurately assessing dietary exposures on breast and prostate cancer risk. Despite widespread use, classical dietary-intake instruments including food frequency questionnaires and other dietary recall methods are subject to well-known limitations. To circumvent and address the unmet current need for reproducible measures of dietary intake and metabolic impacts of crucifers, we used deuterium-labeled broccoli sprouts in combination with mass spectrometry based metabolomics approaches to quantify and differentiate between broccoli-specific metabolites and their interactions with their molecular targets of action.
Broccoli seeds were germinated for 5 days on H_2O or 25% deuterium (D_2O), refreshed twice per day, and the harvested sprouts were extracted and analyzed on a Sciex 5600 TripleToF instrument. The raw ToF-MS data were processed with XCMS software for peak detection and retention-time alignment, which yielded 1,428 spectral features. The XCMS output was subsequently processed with X^{13}CMS software to identify deuterium labeled compounds. To detect deuterium-enriched metabolites, we set the input parameter at 1.00628 Da as the mass difference between ^{1}H and ^{2}H (deuterium). This step yielded 152 isotopologue groups representing 152 deuterium-enriched metabolites covering a wide range of phytochemicals, including, but not limited to, metabolic precursors of sulforaphane, glucobrassicin, phenolic acids, caffeoylquinic acids, catechins, and other polyphenols. Consumption of labeled broccoli sprouts in combination with deuterium-assisted metabolomics provides a strategy to distinguish between dietary metabolites and endogenously produced metabolites.

Wednesday, August 9- Morning

Symposium 9: Phytochemical Signaling

Keynote speaker

Abe Koo did his B.S. and M.S. degree in South Korea at Korea University. He moved to Michigan State University (MSU) in 1999 to do his Ph.D. under Dr. John Ohlrogge where he studied lipid metabolism in higher plants and wrote a dissertation on the topic of fatty acid trafficking. He joined Dr. Gregg Howe's group in Department of Energy-Plant Research Laboratory (DOE-PRL) at MSU where he was first introduced to the field of jasmonate. During this time he identified enzymes involved in JA biosynthesis and published papers on long-distance JA signaling. In 2012, he moved to University of Missouri as assistant professor in the Division of Biochemistry. The research in the Koo lab centers on metabolism and signaling of jasmonate. Dr. Koo was the recipient of the Anton Lang Memorial Research Excellence Award from MSU DOE-Plant Research Laboratory in 2010 and the Arthur C. Neish Young Investigator Award from the

Phytochemical Society of North America in 2015. His 2009 paper was selected as Top Five Most-Cited Papers Award by the journal Phytochemistry and this year he contributed a comprehensive review on jasmonate metabolism to the journal Phytochemistry Review.

Are Metabolites of Jasmonate Cohorts For Stress Signaling? Definitely Maybe

Arati N. Poudel[b,c], Rebekah E. Holtsclaw[a,c], Athen Kimberlin[a], Sidharth Sen[d], Shuai Zeng[d], Trupti Joshi[d,e], Zhentian Lei[a,c,f], Lloyd W. Sumner[a,c,f] and Abraham J. Koo[a,c,*]

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[c]Interdisciplinary Plant Group, University of Missouri, Columbia, MO, 65211, USA
[d]Department of Computer Science, Christopher S. Bond Life Science Center, University of Missouri, Columbia, MO, 65211, USA
[e]Department of Molecular Microbiology and Immunology, Medical Research Office School of Medicine, Informatics Institute, University of Missouri, Columbia, MO, 65211, USA
[f]Metabolomics center, University of Missouri, Columbia, MO, 65211, USA

Plants very rapidly perceive tissue wounding, such as that inflicted by insect feeding, and activate several key defense responses. The critical function of the 3-oxo-2-(2-pentenyl)cyclopentaneacetic acid known as jasmonic acid and its derivatives (JA) in controlling bulk of these defense responses, including chemical and morphological defense responses, has been recognized for many years, and there has been a considerable progress in elucidating the molecular mechanisms of JA perception and signaling. These discoveries highlighted the need to understand biochemical processes underlying JA homeostasis. Upon synthesis, JA is metabolized into a variety of derivatives including jasmonoyl-L-isoleucine (JA-Ile) which is the known endogenous bioactive form of JA responsible for JA mediated responses. In contrast to the well-studied functions of JA-Ile, biological functions of other JA metabolites are less clear. Actions of metabolic enzymes that convert JA-Ile to these other JA metabolites are expected to have impacts on plant's response either by attenuation of the JA-Ile signal or by creation of new signals derived from JA-Ile. Transcriptional and translational dynamics of the positive and negative regulators of the JA signaling components which themselves are feedback regulated by fluctuating JA-Ile levels contribute to the fine tuning of plant growth under stress. The interplay among the metabolites, genes, and proteins of JA pathway will be discussed.

Role of plant hormones in flower and fruit development in tomato

Andrei Vainer[1,2], Irina Panizel[1], Sayantan Panda[1], Adi Faigenboim[2] Asaph Aharoni[1], Hagai Yasuor[2*]

[1]Department of Plant Sciences, The Weizmann Institute of Science, Rehovot, Israel
[2]Department of Vegetable and Field Crops, Gilat Research Center, Agricultural Research Organization, Israel

Hormones play a pivotal role in most physiological processes in plants. The aim of this research was to elucidate the role of plant hormone from different chemical families during flower and fruit development. In order to elucidate which components participate in the hormone homeostasis and possible interaction of phytohormones from different chemical families. We used transcriptome analysis to identify hormone-related genes using Illumina digital gene expression technology and by comparing the hormone metabolome using analytical hormone profiling method, in which the analytes are concentrated from plant extracts and separated by chemical properties using consecutive SPE followed by analysis on UPLC-ESI-MS/MS. The gene expression and metabolome were determined in different flower organs (sepals, petals, stamen, pollen, stigma and ovary) and fruit (embryo, seeds, placenta, jelly, pericarp+peel) during different developmental stages under control conditions. The hormonal related gene expression and metabolome data shows differential hormone expression between flower organ, fruit organs, tomato genotypes during tomato flower and fruit development. The RNASeq data and hormone content analysis shows a complicated spatial and temporal expression pattern of the hormone-related gene expression and metabolome during flower organ and fruit development. These dynamic expression patterns might point on the important role of hormone homeostasis and interaction during developmental events. We are in the process of identifying transcripts for the different hormones

biosynthesis, metabolism, transport, responsive genes, and response factors. We currently trying to modify auxin homeostasis by silencing the GH3 gene (hormone conjugate-related gene family) and using transgenic approaches in order to improve the ability of coping with abiotic stress. More basic research is needed in order to clarify the hormone interactions during these developmental events.

Wednesday, August 9- Afternoon

Plenary Symposium

Professor **María Luisa Villarreal** was born in México city where she got her B.Sc. (biology) at the Universidad Nacional Autónoma de México, and her PhD (biotechonology) in 1997. She was exchange visitor at the Faculty of Pharmacy of the University of Illinois in Chicago and at the National Cancer Institute in Bethesda Maryland as well as visiting professor at the University of Picardie Jules Verne in Amiens, France (2008,2009. 2010), and at the Department of Pharmacognosy of Leiden University in the Netherlands (2012). She is currently Senior Professor in Plant Biotechnology at the Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, and Head of the Natural Products Department. She has a primary research interest in the systematic scientific study of endemic Mexican medicinal plants using system biology and biotechnological models. She has published over 90 research peer reviewed indexed articles in scientific journals including co-autorships with scientists from France, United States, Brazil and the Netherlands. She has served as the Mexican Representative at Expert Meetings in Medicinals Plants (ONUDI) in Trieste Italy (2001, and 2012) and as the President of the Mexican Society of Biotechnology and Bioengineering (2008-2010). She has received different national and international recognitions, among others, the MEXWII gold award given by the Global Women Inventors and Innovators from United Kingdom (2006), the Special Recognition for Academic Excellence given by the Congress of the State of Morelos, México (2000), and the National Award "Martín de la Cruz" given by the Mexican Ministry of Health (2013) for her outstanding work in biology and chemistry of natural products.

Advances and Opportunities in the Investigation of Selected Mexican Medicinal Plants

María Luisa Villarreal

Centro de Investigación en Biotecnología (CEIB) Universidad Autónoma del Estado de Morelos, México

Mexico is one of the 17 megadiverse countries in the world harboring 24,000 botanical species, from which 13,000 are endemic. The use of medicinal plants in the country is widespread, and 6,000 different species are utilized in rural communities with 80% of the total population relying in Mexican Traditional Medicine as the primary source of health care. The rich indigenous knowledge system offers unique opportunities for bioprospecting and for developing basic and applied research in pharmacognosy. However, there is a need to use robust methodologies to study the medicinal autochthonous flora and to offer new possibilities for the transformation of herbal products into standardized medicines.

In this presentation strategies of selection for pharmacognosy studies as well as planning procedures in natural products chemistry and biotechnology of selected Mexican species, will be presented. Examples on the study of different populations of *Galphimia glauca* that synthesize sedative and anxiolytic nor-secofriedelanes will illustrate the benefits of a combined multidisciplinary approach using tissue culture techniques, metabolic profiling systems and molecular markers, for the correct characterization and identification of species of the genus *Galphimia*.

Poster Abstracts

All posters should be put up before Poster Session I at 5:30 pm on Sunday, August 6th and should remain up until after the banquet dinner Tuesday evening, August 8th

Symposium 1: Imaging, Phenotyping & Metabolomics

P1

Molecular Mechanisms Mediating the Enhanced Growth and Abiotic Stress Tolerance Phenotype of Arabidopsis MIOX Over-expressers

N. Nepal[1], J.P. Yactayo-Chang[1], L.M. Acosta-Gamboa[1], K. Medina-Jiménez[2], M.A. Arteaga[2], and A. Lorence[1,3,]

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Email: alorence@astate.edu

Myo-Inositol oxygenase (MIOX) is first enzyme in the inositol route to vitamin-C (L-ascorbate, AsA). MIOX Arabidopsis over-expressers have elevated AsA and exhibited enhanced biomass and increased tolerance to abiotic stresses. The molecular mechanisms underlying this phenotype are not well understood. In this work RNA-Seq analysis was used to study the global gene expression profile of a high AsA line. In addition, RT-qPCR was used to validate the expression of hallmark genes and a hand-held fluorometer was used to measure photosynthetic efficiency of the MIOX line compared to wild type controls. Our *in silico* and RT-qPCR analysis indicate increased expression of transcripts involved in auxin biosynthesis, hydrolysis, transport, and metabolism possibly leading to elevated active auxin content. Additionally we detected up-regulation of transcripts involved in photosynthesis. In support of this finding we measured increased efficiency of the photosystem II and lower non-photochemical quenching in the high AsA line compared to controls. These changes in auxin metabolism and efficient photosynthesis are likely explanations for the enhanced biomass accumulation and growth rate of the MIOX line. Multiple gene families conferring plants tolerance to cold, drought, and heat stresses were found to be elevated in the MIOX over-expressers. Elevated expression of amylases and increased level of reducing sugars in the high AsA line possibly confer tolerance to cold stress and act as signal molecules to initiate biotic defense responses. Interestingly, we detected upregulation of transcripts involved in defense hormones biosynthesis (eg. jasmonates), defense proteins (plant defensin), secondary metabolites (eg. glucosinolates) and transcription factors that are known to be important in biotic stress tolerance in the high AsA line. Further quantification of auxin and glucosinolates via GC/MS and HPLC, respectively, and bioassays challenging the MIOX line with bacterial pathogens and nematodes will be carried out in follow up studies.

P2

Structure Determination and Enzyme Activity of a Flavonol-Specific-3-O Glucosyltransferase Found in Grapefruit

Aaron Birchfield[1], Cecilia McIntosh[1] Ranjan Chakraborty[2]

East Tennessee State University, [1]Department of Biological Sciences, [2]Department of Health Sciences, Johnson City, TN 37601, USA

Citrus and other fruits produce secondary metabolites that are synthesized, regulated, and modified by a class of enzymes called glycosyltransferases. This class of enzymes is of interest to this lab due to their unique structural and functional properties. Glycosides of flavonoids produced by glycosyltransferases are a critical part of plant metabolism

and many have health benefits when consumed. One such glycosyltransferase, found in Duncan Grapefruits (*Citrus paradisi*), was identified, recombinantly expressed, and shown through biochemical characterization to exclusively glycosylate the flavonol class of flavonoids. The structural basis that accounts for a glycosyltransferase's selectivity has been determined by protein crystallization in other labs, yet no structural basis currently exists for the specificity exhibited by this flavonol-specific glycosyltransferase, CP3GT. The WT enzyme and two mutants were expressed in E. coli and underwent site-directed mutagenesis to insert thrombin cleavage tags for removal of protein purification vectors. The plasmid was transformed into yeast and protein was expressed through methanol induction. After purification, crystallization screens will begin for formation and acquisition of glycosyltransferase crystals. Analysis of diffraction patterns of CP3GT crystals will reveal the enzyme's complete structure. It is hypothesized that obtaining a crystal structure for this enzyme will illuminate the structural basis of its specificity. Additionally, it is hypothesized that a thrombin- cleavage gene vector inserted for removal of purification tags will have no impact on enzyme activity or specificity.

P3

Effects of ploidy level on chemotype and antimicrobial activity in the *Achillea millefolium* complex

K. Sammons[1,2], A. Hegeman[1,2,3]

University of Minnesota, [1]Department of Plant and Microbial Biology, [2]Department of Horticultural Science, [3]Microbial and Plant Genomics Institute, 290 Alderman Hall, 1970 Folwell Ave, St. Paul, MN, 55108, USA

Achillea millefolium, commonly known as yarrow, is a multiploid species complex, which occurs globally and has a long history of medicinal use as a vulnerary, anxiolytic, digestive aid, and antimicrobial. Ploidy differences in yarrow have been associated with highly varied essential oil composition, particularly that of chamazulene. Variable quality is available in commerce, due in part to indiscriminate use of plants of various ploidy levels. Genome sizing by flow cytometry is underway on nearly 100 populations of *A. millefolium* and related *Achillea* species. Crude extracts of flowers will be analyzed with ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) to correlate chemical profile with ploidy level using partial least squares-discriminatory analysis (PLS-DA). Antimicrobial activity is assessed in a 96-well plate assay with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* and visualized by the resazurin viability test.

P4

Imbalance of L-tyrosine by modulating arogenate dehydrogenase activity alters plant development

de Oliveira M.V.V.[1], Wang M.[1], Griffith D.[1], Batchu S.[1 2], Jin X.[1], Pan E.[1], Chen X.[1] and Maeda H.[1]
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Amino acids are central to plant metabolism but little is known about how amino acid homeostasis is linked to overall plant physiology. Tyrosine (Tyr) is an aromatic amino acid synthesized mainly in the plastids by arogenate dehydrogenase, encoded by *TyrA1* and *TyrA2* genes in *Arabidopsis*. Here we found that *TyrA1* suppression reduces seeds yield due to a delayed anther dehiscence, whereas *tyra2* knockout leads to a dwarf plant with reticulate leaves. These *tyra2* phenotypes can be rescued by Tyr feeding, *TyrA2*, or even *TyrA1* expression driven by *TyrA2* promoter. Notably, *tyra2* had lower levels of tocopherols than wild-type but had similar levels of Tyr when normalized by weight, suggesting that *tyra2* is adjusting its growth not to deplete Tyr. Indeed, low light conditions synchronized *tyra2* and wild-type growth rate and ameliorated the *tyra2* reticulate phenotype. After shifting to normal light, *tyra2* transiently decreased Tyr levels during the first two days and subsequently increased aspartate levels before the reticulate phenotype appeared. Metabolite analysis of other reticulate mutants also showed high aspartate contents. Furthermore, overexpression of a de-regulated TyrA resulted in an accumulation of Tyr over 50-fold compared to control, which also led to dwarf and reticulate leaf phenotypes that can be rescued by feeding L-phenylalanine. Based on these results, we proposed that imbalance of Tyr, rather than a simple lack of Tyr, impacts plant growth and development, which are tightly associated with increased aspartate.

P5

"Cre-ative imaging": a method of imaging stress responses by enhancing the output and suppressing the background.

Vibha Srivastava[1,2], Eliott Preutt[1,3], Huazhong Guan[1,4], and Jamie Underwood[1]

University of Arkansas, [1]Department of Crop, Soil, and Environmental Sciences, [2]Department of Horticulture, [3]Cell and Molecular Biology Program, Plant Science Building, Fayetteville, AR 72701, USA. [4]Present address: Fujian Agriculture & Forestry University, Fuzhou, Fujian, China.

The classical approach of studying gene expression patterns is by expressing a fluorescent protein under the control of the promoter of the gene-of-interest. The detection of fluorescence in this approach is, however, dependent on the promoter-strength, limiting its application on imaging transiently induced, tissue-specific or weakly induced genes. Here, a novel approach is described that allows clear imaging of gene expression by irreversibly amplifying the fluorescence in response to gene activity. The approach consists of the Cre-lox recombination, hence termed "Cre-ative" to suppress the background at the uninduced states, and activate fluorescence upon treatment. The efficacy of the approach was verified by imaging heat-induced gene of heat-shock protein. The intensity of induced fluorescence in Creative imaging, as expected, was several fold higher than in classical imaging. Most interestingly, the expression patterns in Creative imaging was more precise indicating response to gene activation, whereas, classical imaging produced minor enhancement of leaky (uninduced) expression, blurring the gene response to the external stimuli. The Creative approach will be useful for imaging tissue-specific, conditional responses, in addition to capturing very early transient or weak responses that may have eluded plant biologists due to the lack of robust bio-imaging strategies.

P6

MetaboDEX: An R Shiny Web Application for Metabolomic Data Exploration, Analysis, and Visualization

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R has become a popular software for statistical analysis and visualization of biological data. As a powerful and highly customizable language, R can perform user defined functions over large data sets quickly and efficiently. This is particularly helpful with metabolomic and sequence data which may exist as a matrix of tens of thousands of variables across many samples. These large matrices of numbers can present researchers with an intimidating data processing pipeline and is often outsourced to bioinformatics specialists. Therefore, it is advantageous to have the tools required for data interpretation within the labs conducting the research.

Though R is undeniably a valuable asset in data analysis, it can be challenging to new users with no prior experience in computer programming. MetaboDEX combines the power and efficiency of the R environment with the dynamic user interface of R Shiny to create a web application that is user friendly to those with little or no experience with the R language. Users can upload their data in the specified format and easily view statistical information with a few simple clicks. Presentation quality graphs are automatically generated with the uploaded data but can be customized using simple text boxes and slider bars.

While the software presented is still in the initial stages of development, long term objectives include the addition of LC-MS data processing as described in [Grace and Hudson 2016] as well as GC-MS data analysis using AMDIS batch results. This application will provide a streamlined, easy-to-use data processing interface for large data sets. All scripts will be open source allowing users with a foundation in R to create custom modules with additional functionality.

1. Stephen C. Grace and Dane A. Hudson (2016). Processing and Visualization of Metabolomics Data Using R, Metabolomics - Fundamentals and Applications, Dr Jeevan Prasain (Ed.), InTech, DOI: 10.5772/65405. Available from: https://www.intechopen.com/books/metabolomics-fundamentals-and-applications/processing-and-visualization-of-metabolomics-data-using-r

Symposium 2: Terpenoids

P7
DEAD: leveraging databases to drive discovery of new diterpene synthases

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Diterpenoids are a diverse and widespread class of plant natural products. The first committed step in diterpenoid biosynthesis is the cyclization of geranylgeranyl diphosphate by one or two diterpene synthases. After initial cyclization the diterpene skeleton can be decorated or modified by downstream enzymes. While some of the observed diversity in diterpene skeletons has been found to result from later modifications of a diterpene synthase product, our analyses suggest that many novel diterpene synthase activities remain to be discovered. Previously, the Hamberger laboratory has developed methods for rapid activity screening of diterpene synthase candidate genes. To aid in the search for new candidate genes, we are developing a set of three cross-referenced databases. The Diterpene Enzyme Assay Database: a comprehensive list of characterized enzymes involved in diterpenoid biosynthesis, including the gene sequences and the structures of substrates and products. The Diterpene Chemotaxonomy Database: a collection of structures and the plants from which they were isolated. Finally, the Plant Transcriptome Database: a list of plants with publicly available transcriptome data and hyperlinks to access that data. We leverage the information in these three databases to predict the functional space of diterpene synthases, and to guide the discovery of diterpene synthase genes with new activities.

P8
Unraveling the mechanism of Soyasapogenol E biosynthesis in *Medicago truncatula*

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Medicago truncatula is a model legume and close relative of the world's most important forage legume, alfalfa (*Medicago sativa*). It is a rich source of natural products including flavonoids, isoflavonoids and triterpene saponins, which impact the forage properties of legumes. The saponins are amphipathic molecules composed of a lipophilic aglycone (also known as sapogenin) which can be triterpenoid, steroidal, or steroidal alkaloid, covalently linked via a glycosidic bond to one or more hydrophilic glycosides (sugars). Saponins have immense biological activity including but not limited to antimicrobial, anticarcinogenic, allelopathic, hepatoprotective and antiretroviral. These molecules can be classified based on their aglycone skeleton into 11 main groups which arise from cyclization of oxidosqualene to either chair-chair-chair or chair-boat-chair confirmation. *Medicago* and other legumes are rich sources of triterpenoid saponins. Although many of the reactions in the pathway following oxidosqualene cyclization are performed by CYP450s, it is our speculation that formation of Soyasapogenol E from Soyasapogenol B is governed by a dehydrogenase enzyme(s). Genome wide association studies (GWAS) with metabolic profiling of *Medicago truncatula* (www.medicagohap- map.org/) has led to the discovery of two genes likely related to the formation of Soyasapogenol E from Soyasapogenol B. These genes were also found to be co-expressed with a beta-amyrin synthase gene, a key gene in saponins biosynthetic pathway. Current efforts are in progress for functional characterization of these genes.

P9

A (-)-kolavenyl diphosphate synthase catalyzes the first step of salvinorin A biosynthesis in *Salvia divinorum*

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Salvia divinorum (Lamiaceae) is a powerful hallucinogenic annual herb used by indigenous cultures of Mexico for medicinal and ritual purposes. It produces an array of bioactive neo-clerodane diterpenoids, with salvinorins A as the major accumulated products of the biosynthetic network. Salvinorin A is a highly selective kappa-opioid receptor agonist. This investigation aimed to identify the enzyme that catalyzes the first reaction of salvinorin A biosynthesis, the formation of (-)-kolavenyl diphosphate ((-)-KPP), which is subsequently dephosphorylated to afford (-)-kolavenol. Peltate glandular trichomes were identified as the major and perhaps exclusive site of salvinorin accumulation in *S. divinorum,* using detection approaches including MALDI-based imaging mass spectrometry (MALDI-IMS). The trichome-specific transcriptome was used to identify candidate diterpene synthases (diTPSs). *In vitro* and *in planta* characterization of a class II diTPS designated as SdKPS confirmed its activity as (-)-KPP synthase and its involvement in salvinorin A biosynthesis. Mutation of a phenylalanine into histidine in the active site of SdKPS completely converts the product from (-)-KPP into *ent*-copalyl diphosphate. Structural elements were identified that mediate the natural formation of the neo-clerodane backbone by this enzyme and suggest how SdKPS and other diTPSs may have evolved from *ent*-copalyl diphosphate synthase.

P10

Transcriptome guided discovery of diterpene synthases in Lamiaceae

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Plants of the mint family (Lamiaceae) produce an immense diversity of diterpenoids. More than one-fourth of all the known diterpenes are produced by plants belonging to Lamiaceae[1]. These diterpenes are a rich source of known and potentially new pharmaceuticals, fragrances and industrial feedstocks. Some examples of high-value diterpenes include forskolin, marrubiin, tanshinones, carnosic acid, and sclareol. Despite the substantial body of extant literature on diterpenoid pathways in mints, there remain many diterpene skeletons for which the corresponding diterpene synthase genes are not known. Out of more than 7000 species in Lamiaceae, diterpene synthases have been characterized from just 11. We mined the leaf transcriptomes of 48 distinct species from mint family (The Mint Genome Project[†]) in search of novel diterpene synthases. Three distinct strategies were used to prioritize candidate plants and genes: 1) plants with unusual diterpene backbones, 2) plants where no diterpenes have been reported, but where diterpene synthase genes were found in the transcriptome, and 3) novel diterpene synthase sequences from plants with previously characterized diterpene synthases. We have so far identified 30 new candidate genes from 10 selected species and functional testing is currently underway. Based on the mass spectrometric data, predicted diterpene biosynthetic pathway of few selected species will also be presented.

[1]3,320 of all the known diterpenes (13,775) are produced by plants belonging to Lamiaceae (Dictionary of Natural Products ver. 25.2)
[†]http://mints.plantbiology.msu.edu/

Symposium 3: Alkaloids

P11
Erythrina Alkaloids as source of valuable chemicals

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The Erythrina genus of the Leguminosae family contains 115 species with ample morphological variation and great ecological diversity. Around the world, the major distribution of the *Erythrina* species it is found in southern Mexico and Central America. The genus has been studied since different points of view, e.g. ornamental or culinary until studies of their chemical composition or its ethnomedical applications. This is because the plants contain in different parts compounds as flavonoids, isoflavonoids, alkaloids, tripsin inhibitors, hemaglutinins or saponins. The stem bark, flowers and seeds of them have been studied intensely with this series of compounds but are the alkaloids and isoflavonoids who have received special attention by their vary structures or their biological applications. In this work we tested the insecticidal activity of the crude extracts of seeds of *Erythrina americana* Miller against *Trialeurodes vaporariorum* and found that the crude extract and also the purified fractions contain alkaloids with repellence activity, and inhibition of oviposition, being the purified fractions those of higher activity than the crude extracts.

Symposium 4: Phytochemistry & Ecology

P12
Synthesis of Stable Isotope-labeled Precursors for Probing Biosynthetic Pathways of the Cruciferous Phytoalexins Nasturlexins

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In response to biotic and abiotic stress, plants produce phytoalexins, a group of secondary metabolites that are biosynthesized *de novo* from primary precursors. Possessing antifungal activity against a variety of plant pathogens, phytoalexins play an important role in the chemical defence of plants.
Crucifers (family Brassicaceae) are known to produce tryptophan-derived phytoalexins, with more than 45 metabolites discovered to date. Recently, nasturlexins, a group of phenylalanine-derived phytoalexins have been isolated from watercress (*Nasturtium officinale*), upland cress (*Barbarea verna*), and winter cress (*B. vulgaris*). The occurrence of another biosynthetic pathway in addition to the previously known tryptophan-derived pathway poses an opportunity to explore this intriguing discovery.
In this work, the biosynthesis of nasturlexins is proposed, starting from homophenylalanine via the glucosinolate gluconasturtiin. To probe this pathway, a variety of precursors labelled with stable isotopes have been synthesized from simple and commercially available starting materials. Synthesized labeled precursors include gluconasturtiins, isothiocyanates, dithiocarbamates, and their structurally related derivatives. Current work is in progress to administer these labelled precursors into watercress and upland cress to determine biosynthetic intermediates, necessary to discover the corresponding biosynthetic enzymes and genes present in crucifer species.

P13

Changes in the metabolite profile of field-grown *Scrophularia lanceolata* Pursh and *Scrophularia marilandica* L.

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Scrophularia lanceolata Pursh and *Scrophularia marilandica* L. are two of the most common species within the Scrophulariaceae endemic to North America. Historically, they were used by Native Americans and colonialists to treat sunburn, sunstroke, frostbite, edema, as well as for blood purification and in women's health. A number of phenylethanoid /phenylpropanoid and iridoid glycosides detected in these species possess anti-inflammatory properties, including the iridoid glycoside, harpagoside. Due to the presence of these anti-inflammatory metabolites and the historical uses of these species, we performed a field study at the University of Missouri-Southwest Research Center to determine the optimal production of these important compounds. We subjected the plants to shade treatment and analyzed metabolite composition differences between the two species and each of their tissues. We determined that *S. lanceolata* leaves grown in full sun produced more harpagoside per dried weight (0.63%) compared to shade grown plants (0.43%). *S. lanceolata* also produced more harpagoside compared to *S. marilandica* (0.25%). We also analyzed how seasonal variation affected metabolite content. We found that after early summer (May and June), there was a decline in the production of harpagoside in the leaves of *S. lanceolata* and *S. marilandica*. We also observed changes in the abundances of other potentially bioactive phenylethanoid / phenylpropanoid and iridoid glycosides.

P14

Building Phytochemical Identification Protocols and Databases

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Fully elucidating the phytochemical composition of plant tissues, powders, and extracts is a fundamental need for research, as well as for all aspects of agricultural product evaluation and processing. An optimized single LC-MS evaluation that would accurately determine the elemental composition of as many compounds present in an extract would greatly aid in the evaluation of plant tissues. For phytochemicals, we have used accurate mass analysis to quickly characterize the potential chemical formulas and structures for both known and unknown compounds in plant extracts. Several laboratories and instrument companies are working on phytochemical evaluations and have similar protocols, but what is lacking is 1) a curated set of a large library of pure phytochemical standards that have been evaluated by MS, 2) a centralized location for accessing phytochemical spectral libraries and databases, and 3) accurate software programs for predicting unknown chemical structures from MS and other data. The PSNA is considering initiating a big data project to address these needs. Developing a practical protocol for the evaluation of plant materials for their complete phytochemical composition would aid in the authentication, detection of contamination, detection of adulteration; and aid in the assessment of the effect of cultivar, growing conditions, and stress on phytochemical composition.

P15

Synthesis and evaluation of inhibitors of brassinin oxidase, a phytoalexin-detoxifying enzyme from the plant pathogen *Leptosphaeria maculans*

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Brassinin oxidase (BOLm) is an inducible phytoalexin-detoxifying enzyme produced by a fungal pathogen of crucifer crops (*Leptosphaeria maculans* (Desm.) Ces. et de Not. [asexual stage *Phoma lingam* (Tode ex Fr.) Desm).]) that catalyzes the detoxification of brassinin to indole-3-carboxaldehyde. Brassinin is one of the most important phytoalexins produced in plants of the family Brassicaceae (common name crucifer) due to its role as a biosynthetic precursor of other cruciferous phytoalexins and also antimicrobial activity against several pathogenic fungi of crucifers. An investigation on the inhibition of detoxifying enzymes of cruciferous pathogenic fungi revealed that the phytoalexins camalexin and 6-methoxycamalexin could inhibit BOLm degradation activity. In this work mono- and di-methoxycamalexins were synthesized and evaluated for inhibition of BOLm. In general, dimethoxycamalexins showed weaker mycelial growth inhibitory activity than mono-methoxycamalexins against *L. maculans*. Amongst all tested dimethoxycamalexins, 5,6- and 6,7-dimethoxycamalexins showed significantly higher BOLm inhibitory activity than 4,5- and 4,7-dimethoxycamalexins. Similarly, 5- and 6-methoxycamalexins showed higher BOLm inhibitory activity than 4- and 7-methoxycamalexins. Details of this work will be presented and discussed.

P16

Use of untargeted metabolomics analyses to explore the effect of cultivation strategy on phytochemical composition of native plant extracts

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Native plants are important components of landscapes, but their use on agricultural landscapes has been limited because of the low economic return potential. Recent research has shown that crude extracts from wild-collected native and naturalized Midwestern plants contain high-value antimicrobial and antioxidant compounds. Consumer demand for natural ingredients in personal care products has been rapidly increasing over the past decade and these naturally-occurring chemical compounds are potentially useful for inclusion in “natural” pharmacological and cosmetic products. Cultivation of these plants is desirable as it alleviates many of the problems associated with wild collection (e.g. the destruction of native habitats and the loss of biodiversity) and cultivated plant materials have more consistent phytochemical concentrations due to the uniformity of cultivation. However, there is little information on the capacity of these species to produce biologically-active compounds in agricultural settings, or on specific agronomic practices that may optimize the production of these natural compounds. Three of these perennial plant species – purple coneflower (*Echinacea purpurea*), Canada milk vetch (*Astragalus canadensis*), and showy tick trefoil (*Desmodium canadense*) – were established in six different types of plant communities at two distinct environments in Minnesota. Five tissue types – leaf, stem, root, flower, and seed – were harvested from each plant species under each treatment and crude extracts were analyzed using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) to examine 1) the effects of experimental treatments on phytochemical composition of the three plant species and 2) the production of phytochemicals throughout individual plants. Analyses will be focused on identifying phytochemical patterns that are influenced by specific agronomic designs, which will inform cultivation strategies that optimize phytochemicals of interest. Data processing and untargeted statistical analysis will be accomplished using the Galaxy M metabolomics platform.

Symposium 5: Lipids

P17

Cardioprotective and Hypocholesterolemic Effect of Ethanolic Extract of *Mormodical charantial* in Isoproterenol-Induced Myocardial Infarction in adult Wistar Rats

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The present study was carried out to evaluate cardioprotective effect of ethanolic extract of *Mormodical charantial* in isoproterenol (ISO) induced myocardial infarction(MI) in Wistar rats. The ethanolic extract of the plant of *Mormodical charantial 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 *Mormodical charantial* 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. *Mormodical charantial* possesses cardioprotective and hypocholesterolemic effects.

Keywords: cardioprotective, isoproterenol, interaperitoneally, metoprolol
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P18

A TDNA insertion in *At5g41900* affects Arabidopsis thaliana growth and lipid composition

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The *Arabidopsis thaliana* gene *At5g41900* encodes a protein annotated as a member of the alpha/beta hydrolase protein superfamily. *At5g41900* is one of five genes called *BODYGUARD* (*BDG*). One family member, *At1g64670* (*BDG*) is expressed in the epidermis and has a role in cuticle development, but the other four genes, *At5g41900* (*BDG2*), *At4g24140* (*BDG3*), *At5g17780* (*BDG4*), and *At5g17720* (*BDG5*), have not been characterized in detail. Genome-wide studies indicate that *At5g41900* is expressed at moderate levels throughout the plant. Compared to wild-type plants, a T-DNA insertion line of *At5g41900* has larger leaves, longer petioles, and more branched stems. It also flowers earlier and grows faster overall. There are multiple differences in the leaf lipid composition of the *At5g41900* T-DNA insertion mutant, compared to wild type. These include increases in lysophospholipids and steryl glucosides and increased levels

of the fatty acid 18:3 in several phospholipids. Additional analyses of *At5g41900* gene function are planned. This work was funded by National Science Foundation (MCB 1413036) and awards from the Johnson Center for Cancer Research at Kansas State University.

P19

Identification of Arabidopsis Mutant Lacking Glycerolipid Assembly in the Chloroplast

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Lipid analysis of leaves from the Arabidopsis T-DNA insertion line Salk_109175C revealed a significantly decreased ratio between MGDG(34:6) and MGDG(36:6), and other lipid alterations, consistent with the mutant lacking the ability to assemble glycerolipids in the chloroplast. Salk_109175C has a confirmed mutation at the At5g64790 locus, a gene which is mostly expressed in pollen. To confirm that the lipid phenotype observed was due to the known insertion, the Salk line was crossed to WT and the heterozygous progeny of this cross were selfed to obtain an F2 generation. Analyzing the F2 generation for lipid levels and genotyping using PCR demonstrated that the mutation and the phenotype were not linked. These results indicate that the mutation causing the observed phenotype is actually located in another gene. We are using map-based cloning to identify the locus associated with the observed lipid compositional alterations and study its effect on plant physiology.
This work was funded by National Science Foundation (MCB 1413036). Hannah Luskis a McNair Scholar and a Kansas INBRE awardee. She also received funding from the KSU College of Arts and Sciences.

P20

Anandamide and Eicosapentaenoyl Ethanolamide in the Moss *Physcomitrella patens*

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N-acylethanolamines (NAEs) are nitrogen-containing lipid-derived signaling molecules, which include an endocannabinoid anandamide (*N*-arachidonoylethanolamide, AEA, NAE20:4). NAEs regulate diverse biological and physiological functions in eukaryotes. NAEs with 20C polyunsaturated fatty acids (PUFA) are mostly limited to early land plants. Bryophyte *Physcomitrella patens* possesses higher levels of PUFAs that includes arachidonic acid (AA, 20:4) and eicosapentaenoic acid (EPA, 20:5), which account for ~ 24 and 7 % of the total fatty acid content, respectively. Selective lipidomic analyses revealed the occurrence of distinct levels NAEs and their corresponding *N*-acylphosphatidylethanolamine (NAPE) precursors in various developmental stages of moss. Moreover, previously unidentified 20C NAEs in angiosperms, AEA and also EPEA (eicosapentaenoyl ethanolamide, NAE20:5) were detected in all haploid developmental stages of moss, accounting for ~ 23 and 5 % of total NAEs, respectively. Corresponding NAPE precursors of AEA and EPEA were ~ 49 and 30 % of the total NAPEs in protonemata and ~ 55 and 6 % in mature gametophytes, respectively. Mature gametophytes showed about 12 % increase in *N*-20:4-PE and ~ 20 % decline in *N*-20:5-PE relative to protonemal NAPE content. Contrary to AEA, EPAE levels declined with maturation and were lowest in mature gametophytes, relative to protonemata. Exogenous application of AEA and EPEA and their corresponding fatty acids showed inhibitory effects in a dose dependent manner on moss protonemal growth. Additionally, confocal imaging of protonemal tips showed depolymerized F-actin and tip growth inhibition, exclusively in response to short term AEA treatment. Together, these data indicate that while occurrence of NAEs is evolutionarily conserved, composition of PU-NAEs in early land plants is unique and their biological implications and metabolic pathway are yet to be determined.

P21

The chemical diversity, activity, and biosynthesis of bioactive carrot polyacetylenes

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As our climate becomes more variable and unpredictable, phytochemicals that contribute to plant disease resistance become ever more important research targets. A class of lipid compounds called polyacetylenes are produced in various Apiaceae (e.g. carrot, coriander) and Asteraceae (e.g. sunflower, artichoke) species in response to pathogenesis. Accordingly, it has long been suspected that these compounds contribute to pathogen resistance. If this is indeed the case, knowledge of the genes involved in polyacetylene biosynthesis and accumulation could be a valuable resource for creating crops lines with improved pathogen resistance.

The recent publication of a high quality carrot genome and transcriptomes has enabled functional genomics approaches to exploring polyacetylene structure, function, and biosynthesis in this species. We began with a detailed analysis of carrot polyacetylene chemical structures and their distribution in diverse carrot tissues. After TLC purification, we identified five major (two novel) and seven trace polyacetylenes, with falcarindiol and falcarinol being the major constituents of the whole polyacetylene pool. These compounds accumulate primarily in the peel of the carrot root. Next, we purified falcarinol and falcarindiol found that mycelia of the necrotrophic fungus *Sclerotinia sclerotiorum* exhibited a 25% reduction in growth rate on substrate containing just 20μg/ml polyacetylenes. We then prepared carrot cell cultures and elicited then with mycelial protein extracts from the mold *Phytophthora megasperma*. This treatment caused the accumulation of several different polyacetylene species and, based on RNA-seq, the upregulation of several fatty acid acetylenase genes putatively involved in the initial steps of polyacetylene biosynthesis. We are currently in the process of evaluating the activity of these genes in heterologous systems.

P22

Cuticular wax compositional changes during leaf develop in *Camellia sinensis* cultivar 'Fuyun 6'

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Cuticular waxes is the second most important barrier to restrict water loss after stomata, and protect plants against drought stress. In tea tree cuticular waxes also affect leaf processing property and aroma production, thus affect the quality of made tea. Long-chain aliphatic primary alcohols (C20-C36) possess health promoting activities, was reported to be abundant in green tea, may mainly present in cuticular waxes. The leaf position in the newly-developed shoot serves as reliable indicator of different developmental stages, with the second leaf in the shoot representing the early immature stage of leaf development, and the leaf reach full maturity after the forth leaf. To understand if and how cuticular waxes changes with leaf development, we isolated the epi-, intra- as well as total cuticular waxes from the second and the fifth leaf of *Camellia sinensis* cv 'Fuyun 6', then analyzed their coverage and composition by Gas Chromatography Mass Spectrometry and Flame Ionization Detection. We found that the wax coverage was increased with leaf maturation, the fifth leaf (28.41μg.cm^{-1}) contains 3 folds more cuticular wax compared with the second leaf (8.45μg.cm^{-1}). The second leaf wax rich in very-long-chain fatty acid (VLCFA) derivatives such as alcohols, alkyl esters, acids, and alkanes in their waxes; in contrast, the fifth leaf wax was dominated by alicyclic compounds especially terpenoids. Although the epicuticular waxes showed difference between the young and mature leaves, the intracuticular waxes showed more dramatic changes, with 1-alkanols and terpenoids are highly enriched in the young and mature leaf, respectively. Our data suggested that intracuticular waxes are extensively remodeled in response to leaf development

or environmental conditions, this could result in different water sealing properties between young and mature leaves, thus affect their response to drought tolerance.

P23

Understanding the role of Fatty Acid Amide Hydrolase in NAE metabolic pathway in *Physcometrella patens*

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In plants, saturated and unsaturated *N*-Acylethanolamines (NAEs) with acyl chains 12C to 20C are reported for their differential levels in various tissues and species. While NAEs were shown to play a vital role in mammalian neurological and physiological functions, their metabolism and functional implications in plants however, remains incomplete. We studied the fate of NAEs in vitro and in vivo in *Physcomitrella patens*. Fatty acid amide hydrolase (FAAH) is one of the metabolic enzymes that break the amide bond in NAEs, and release the free fatty acid and ethanolamine. We identified *Physcometrella patens* fatty acid amide hydrolase (*Pp*FAAH) and heterologously expressed in *E. coli* for *in vitro* characterization. Both *in vivo* and *in vitro* studied showed that unsaturated NAEs substrates are hydrolyzed faster than saturated NAEs; more than 50 times higher in *in vitro* and 10 times higher in *in vivo*. *In vitro* studies indicated that *Pp*FAAH showed the highest hydrolysis activity in a reaction with pH 8.0 at 30°C. Time-based studies disclosed that the hydrolysis rate was faster in first 30 minutes and then declined gradually. In *in vivo*, amidohydrolase activity was mostly associated with microsome compared with cytoplasmic fractions. Additionally, microsome fraction of mature gametophytes showed higher amide hydrolase activity than protonemal or early gametophyte microsome fractions; however, *PpFAAH* expression did not show any significant difference between the developmental stages. Functional characterization of NAE metabolic pathway in the moss is underway. We are generating moss mutants, mainly PpFAAH knock out (KO) and overexpressor (OE) to investigate biological implications of FAAH in moss growth and development.

Symposium 6: Synthetic Biology and Metabolic Engineering

P24

Biochemical Characterization of Substrate Specificity of *Citrus paradisi* Flavonol Specific 3-*O*-Glucosyltransferase Mutant D344P

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Plants produce a vast array of secondary metabolites. Flavonoids are phenolic metabolites ubiquitous among plants and are known to aid in processes such as plant reproduction, UV defense, pigmentation and development. In relation to human health, flavonoids have been found to possess anti-inflammatory, anti-cancer, and antioxidant properties. Flavonoid's ability to participate in so many interactions is due in part to their subclass variation and further chemical modification, such as glucosylation, where a glucose molecule is added to the flavonoid substrate. The enzymes that catalyze these reactions are known as glucosyltransferases (GT). *Citrus paradisi* contains a glucosyltransferase that is specific for adding glucose to the 3-O position of flavonols (Cp-F3-*O*-GT). To further understand the reactions it catalyzes, Cp-F3-*O*-GT structure was modeled against an anthocyanidin/flavonol 3-*O*-GT found in *Vitis vinifera* to identify candidate amino acids for mutations. Mutants were then generated using site-directed mutagenesis, and one mutant, D344P, was constructed by an aspartate being replaced with a proline. Biochemical characterization of the mutant D344P protein was performed in order to determine whether the mutation has an effect on the substrate specificity of Cp3-O-GT. An initial quick-screening assay using radioactive UDP-glucose as a sugar donor suggested there may have

been an expansion of substrate acceptance. The time course assays did not support this observation. Results show that D344P protein has decreased activity with flavonols as compared to the wild-type Cp3-O-GT with no expansion of substrate specificity. Homology models were analyzed, some of which supported experimental results.

P25

Arabidopsis Gluconolactonase, the First Enzyme Involved in Ascorbate Biosynthesis Localized in the Chloroplast Protects Plants From Light Stress

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L-Ascorbic acid (AsA, vitamin C) is the most abundant water-soluble antioxidant found in plants. Ascorbate has a wide variety of physiological roles including being an enzyme cofactor, a scavenger of free radicals, and donor/acceptor of electron in the chloroplast. Ascorbate protects tissues against damage caused by reactive oxygen species produced through normal oxygenic metabolism or generated from biotic and abiotic stresses. The *myo*-inositol route to ascorbate involves four enzymes: *myo*-inositol oxygenase, glucuronate reductase, gulonolactonase (GNL), and L-gulono-1,4-lactone oxidase. The first two enzymes have been already characterized by the Lorence Laboratory. The third enzyme, GNL, has been characterized in rats and multiple bacteria but not in plants. To identify candidates, we aligned GNL sequences from rat and bacteria to the *Arabidopsis thaliana* genome, and this led us to identify 18 genes. One of them (*At*GNL) possesses a chloroplastic signal peptide. Expression data available at Genevestigator, shows that there is suppression of the expression of this gene when plants are exposed to dark conditions. We have found that homozygous *gnl* knockouts have lower foliar AsA content compared to wild type controls, and display stunted growth, and chlorotic lesions, indicating the involvement of this enzyme in AsA synthesis and in maintaining a healthy redox balance in the leaves. On the other hand, *At*GNL over-expressers and restored lines (knockouts that have been complemented with the functional enzyme) have elevated AsA content, grow faster, accumulate more biomass, and produce higher seed yields than the corresponding controls. In this work, we present the detailed characterization of the phenotype of lines with normal (wild type), lower (knockout) and higher (over-expressers and complemented lines) GNL expression growing under low, normal, and high light conditions.

P26

Engineering of *Physcomitrella patens* for diterpenoid production

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Terpenoids are the largest group of specialized metabolites with diverse roles in various biological processes in plants including growth, development, defense, and adaptation. In addition, different terpenoids have commercial applications as nutraceuticals, pigments, flavors and fragrance molecules, polymers, and drugs. Many diterpenoids with backbones consisting of 20 carbon atoms have therapeutic activities and are valuable industrial targets. Sustainable bio-production of these compounds is an alternative to isolation from the natural sources or chemical synthesis. Yet this requires the knowledge of their biosynthetic pathways. Over the last two decades, a rich repository of the diterpene synthases has been established. Often a pair of diterpene synthase is required for the formation of their scaffolds. Novel combinations of these enzymes lead to the biosynthesis of different diterpenes that increase the chemical diversity. *Physcomitrella patens* (moss) is a promising host for stable production of diterpenoids as it (i) is a photosynthetic organism requiring only sunlight, CO_2, and water, (ii) allows convenient genome editing, and (iii) has diterpeneoid biosynthetic pathway that can be easily manipulated. This work describes the engineering of *P. patens* with various combinations of diterpene synthase modules. Pieces of DNAs with overlapping ends are assembled *in vivo* and integrated into the genome of *P. patens*. Production of diterpenoids is demonstrated by GC-MS analysis.

Symposium 7: Industrial Phytochemistry

P27
Chemistry at Monsanto – Great Career Opportunities

Martin Ruebelt

Agriculture Productivity Innovations, Monsanto, USA

Monsanto is one of the world's leading agricultural technology companies developing seeds & traits through agrochemical productivity products, traditional breeding and world-class biotechnologies. Monsanto offers farmers a wide range of solutions from row crop seeds like corn, soybean, cotton, wheat, canola, and sorghum, to vegetable seeds, crop protection products, and data solutions. Investing over $3 million in research daily, Monsanto helps feed the world by helping farmers produce more with less resources. Our products are designed to meet modern agriculture's ever-evolving needs. Monsanto offers solutions to customers through brands aimed at providing solutions that will help them have a successful harvest. Come and explore the various career opportunities at Monsanto.

P28
Identifying Health Promoting Phytochemicals in Black Walnuts (*Juglans nigra L.*)

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People have relentlessly been searching for natural treatments from plants as alternatives to synthetic medicines in fighting against the diseases. Consumption of black walnuts has been scientifically proven to have many health benefits including decreased risk of cardiovascular disease, and coronary heart disease. The consumption of black walnuts has also contributed to reducing the risks of type II diabetes, and prevention and treatment of certain cancers, and the lessening of symptoms attributed to age-related and other neurological disorders. However, information about the levels of the health promoting phytochemicals and the chemical profiles among different cultivars cross the geographic gradients are lacking. The objectives of this study are to 1) identify and characterize the health-promoting phytosterols, polyphenols, and other health-promoting secondary metabolites in black walnuts using targeted as well as non-targeted global metabolomics approach (XCMS), 2) identify the metabolic pathways of these compounds and elucidate their roles in promoting health and 3) compare the levels of these compounds between different walnut varieties cross the geographic and environmental gradient. The findings of this project will help identify the immediate applications of these compounds, and therefore foster the rural economic development for state of Missouri.

P29

Identifying Bioactive Compounds from Switchgrass (*Panicum virgatum*) through Global Metabolite Profiling and High-Throughput Bioassay Screening

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Integrated biorefining incorporates biomass conversion processes and equipment to produce multiple products including fuels, power, heat, and value-added chemicals from biomass. By identifying multiple high-value products, a biorefinery supply chain can maximize the value derived from the biomass feedstock. The objectives of this study are to 1) identify and characterize the valued bioactive phytosterols and phytophenolics in switchgrass (SW; *Panicum virgatum*) using global metabolomics approach (XCMS), 2) compare the chemicals profiles between four varieties of switchgrass (Liberty, Alamo, Kanlow, and Showme), 3) perform an array of high-throughput screening bioassays to identify the commercial applications, and 4) explore potential new uses and applications in cosmetic, personal care products, and pharmaceutical industries. The compounds extracted from SW will be analyzed by ultra-high pressure liquid chromatography coupled with mass spectrometry (UPLC-MS). The ion chromatograms will be submitted to XCMS platform operated by Center for Metabolomics at the Scripps Research Institute. The spectra will be annotated and the compounds will be identified and categorized by the integration of the METLIN, the world's largest metabolite database. Multivariate analysis and principle component analysis (PCA) will be performed by XCMS to compare the chemical profiles between the four varieties. An array of bioassays (e.g., anti-inflammatory and antioxidant) will be performed at High-Throughput Screening Laboratory at the University of Kansas (Directed by Dr. Anuradha Roy) using biochemical and cell-based high throughput screens (HTS). The niche market of the bioactive compounds will be identified. We anticipate that our findings will increase the overall revenues of the chain production and benefit all the participants involved in the supply chain of cellulosic biorefinery industry.

P30

Identifying Bioactive Phytochemicals in Spent Coffee Grounds for Cosmetics Application Through Global Metabolite Analysis

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Annually, more than 6 million tons of spent coffee grounds (SCG) are generated worldwide. The present study explores the possible use of spent coffee grounds as the raw materials for cosmetics industry. The objective of this project is to investigate the chemical profiles and identify the bioactive compounds for cosmetics application through global metabolite analysis. The compounds extracted from SCG of Ethiopia coffee (Yirgacheffe), Costa Rican coffee (Tarrazu) and Hawaiian coffee (Kona Blend) were analyzed by ultra-high pressure liquid chromatography coupled with mass spectrometry (UPLC-MS). The ion chromatograms were submitted to XCMS platform operated by Center for Metabolomics at the Scripps Research Institute. The peak detection, peak grouping, spectra extraction, retention alignment, were processed by XCMS. The spectra were annotated and the compounds were identified and categorized by the integration of the METLIN, the world's largest metabolite database. Multivariate analysis and principle component analysis (PCA) were performed by XCMS to compare the chemical profiles between the three coffee

cultivars. Each identified compound was assigned and related its biological pathway through XCMS biological pathway/network analysis. The findings of this study will help identify the bioactive compounds in the SCG and their immediate applications for skin care application (e.g., anti-oxidant, anti-inflammatory, skin-whiting, and anti-aging).

P31

Exploring New Commercial Opportunities for Osage Orange

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Muclura pomifera (raf.) Schneid., belong to the Moraceae, the mulberry family, most commonly called Osage orange. It is a native species that was widely distributed in the United States and parts of Southeastern Canada. Osage orange is extremely durable and is considered to be one of the most decay resistant woods. Osage orange provides an abundant resource of the bioactive lipids and polyphenolics (e.g., isoflavonoid and xanthones) that have not been systematically studied. Although Osage orange is not a human food source, it is considered to be safe and, therefore, a possibly good source of antioxidant nutraceuticals and functional food ingredients, and active ingredients for personal care products. The objectives of this project are to 1) identify and characterize the valued bioactive phytosterols and phytophenolics in fruits and seeds using global metabolomics approach (XCMS), 2) compare the chemicals profiles between different tissues, 3) perform an array of high-throughput screening bioassays to identify the commercial applications, and 4) explore potential new uses and applications in cosmetic, personal care products and pharmaceutical industries. We anticipate that our findings will help turning the low value renewable materials into a lucrative industry in the region.

P32

Determine the Role of Bacteriophage for Diterpenoid-A Resistance in *Staphylococcus aureus*

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Staphylococcus aureus is a bacterium commonly found on human skin and is a frequent cause of skin and respiratory infections. Some S. aureus strains have developed resistance to multiple antibiotics, including beta-lactam antibiotics, and they are known as methicillin resistant *Staphylococcus aureus* or MRSA. Every year, an average of 19,000 deaths and 94,000 infections resulted from MRSA infection according to CDC. These microbes limit the treatment method; thus, the development of the new antibiotics is urgent. Our lab had isolated a meroterphene from Eastern Redcedar named diterpenoid A. Our studies showed that it exhibits strong antimicrobial activity against many Gram positive bacteria pathogens. Moreover, it exhibits strong inhibitory effect against MRSA. To further investigate the actual mode of action of diterpenoid A, two diterpenoid-A resistant S. aureus strains were created by ethyl methane sulfonate mutagenesis followed by diterpenoid A selection. A bioinformatics analysis on genomic sequences of the mutant strains and the parent strain was performed using BWA alignment and SAMtools following by snpEff to determine the SNPs candidate for diterpenoid A-resistance. Approximately 40 allele-candidates were identified through the bioinformatics approach, and most of the SNPs are located within Staphylococcal temperate phage genes. This suggests the mutated bacteriophage-encoded proteins might be responsible for the resistance to diterpenoid A.

Symposium 8: Food and Nutraceuticals

P33
Deciphering the theanine biosynthesis mechanism: bifunctional enzymes of glutamine synthetase for accumulation of theanine and glutamine in tea plant

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As the major non-protein amino acid in tea, the world largest non-alcohol beverage, theanine has unique health-promoting functions and contribute to tea drinks with the umami and sweet tastes. It is long been proposed that theanine is derived from glutamate and ethylamine catalyzed by theanine synthetase (TS), similar to glutamine derivation from glutamate and ammonium by the action of glutamine synthetase (GS) in tea plant. However, the molecular identity of theanine synthetase (TS) so far is not clearly defined, nor its acting mechanism. We investigated the functions of putative *CsTS/GS* genes for theanine biosynthesis in tea plant. Six GS genes, one type I GS, CsGSI-1, four cytosolic type II GS (CsGSII-1a, b, c, and d), and one chloroplastic type II GS, GSII-2a, were identified from tea genome. They displayed distinct tissues specific expression patterns, and only parts of them in consistent with theanine accumulation in roots and young buds and leaves. We studied the CsGSI-1, CsGSII-1a, CsII-1b for their functions as TS or GS. When expressed in Arabidopsis plants or soybean hairy roots, the glutamine contents were significantly higher than the control under normal growth conditions, although no theanine was detectable. However, when fed with ethylamine, significant levels of theanine were produced, although glutamine content decreased. Therefore, both GS and TS activities were displayed with these CsGS proteins when overexpression plants were fed with ethylamine. The theanine biosynthesis is primarily dependent of ethylamine availability, as GS showed higher affinity to ammonium over ethylamine. However, tea plant CsGS/TSs are predicted to have higher affinity to ethylamine, but lower preference to ammonium. We also tried to dissect the evolutionary cues for CsTS and CsGS from their genomic gene structures in tea plants, as compared with GSs from other species.

P34
Exploring health benefits of phytochemicals in black walnuts (*Juglans nigra* L.)

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Black walnut (*Juglans nigra* L.) is widely distributed throughout the U.S. eastern forest, with the highest concentrations occurring in Missouri. Consumption of walnuts has been linked to many health benefits including reduced risk of cardiovascular disease, reduced levels of cholesterol, stimulated cognitive functions and prevented certain cancers (e.g., prostate and breast cancers). Black walnut contains more than 50 health-promoting bioactive phytosterols and phytophenolics, such as ß-sitosterol, campesterol, stanol esters, tocopherols, ergosterol, pedunculagin, and stigmasterol. However, systematic characterization of the bioactive compounds in walnuts using modern bioinformatics and metabolomics approach has never been explored. Additionally, the links between the specific compounds and the

health benefits have not been well established. Fifteen cultivars of black walnut grown in Missouri are collected and the potent chemicals are identified and isolated. We conducted bioassay-guided purification to identify the bioactive compounds (e.g., antibacterial, anti-inflammatory and antioxidant) and perform mice studies to evaluate the pharmacokinetic of these compounds in the serum/tissue. The metabolic pathways and biological functions of the identified health promoting phytochemicals will be elucidated by using XCMS metabolomics platform, transcriptomic analysis and gene expression in the mice studies. The final goal of this study is to identify the mode of the actions of the bioactive compounds in black walnut.

P35

3,3'-Diindolylmethane inhibits Advanced Prostate Cancer in TRAMP Mice

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3,3'-Diindolylmethane (DIM) is an acid-derived dimer of indole-3-carbinol, found in many cruciferous vegetables, such as broccoli, and has been shown to inhibit prostate cancer (PCa) in several *in vitro* and *in vivo* models at high concentrations. We **hypothesize** that DIM will inhibit advanced PCa at lower concentrations and that DIM inhibits advanced PCa via stimulation of ERβ. To further study the effects of DIM on inhibiting advanced PCa development, we used FVB/C57 TRAMP (**TR**ansgenic **A**denocarcinoma of the **M**ouse **P**rostate) mice as our model. The control group of mice were fed with a western diet to mimic the common high-fat diet eaten by U.S. men. Three additional groups of mice were fed the western diet supplemented with 0.04%, 0.2% and 1% DIM in diet. Incidence of advanced PCa [poorly differentiated carcinoma (PDC)] in the control group was 60%. 1% DIM dramatically reduced PDC incidence to 24% (p=0.001), while 0.2% and 0.04% DIM reduced PDC incidence to 38% (p=0.056) and 45% (p=0.18) respectively. However, DIM did affect mice weight, so to eliminate any possible confounding effect of body weight on PDC incidence, we did logistic regression with our data. This showed a clear negative association between DIM concentration and PDC incidence with p=0.004, while the association between body weight and PDC incidence is not significant (p=0.998). Offsetting this confirmation of our hypothesis, there was also a trend of increasing well differentiated carcinoma (WDC) along with the increase of DIM concentration in the diet. 1% DIM increased WDC incidence from 40% to 66% (p=0.02) when compared to control, while other diets had no statistically significant effects. ER activity data showed DIM stimulated both receptors, but surprisingly it did not compete with E2 binding. In conclusion, our results show that dietary DIM can inhibit the most aggressive stage of prostate cancer.

P36

Commiphora wightii: Metabolic Profiling of a desert plant for health care

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Commiphora wightii (Arnott) Bhandari, commonly known as guggul, produces a medicinally important gum resin which is used extensively by Ayurvedic physicians to treat various ailments. Metabolic profiling provides a plethora of information about metabolites, and thus, it is an efficient tool to screen plants for novel bioactive compounds from phytomaterials. Metabolic profiling was performed on aqueous and nonaqueous extracts of leaves, stem and latex samples of chemotyopes of *C. wightii* using GC-MS, HPLC and NMR spectroscopy. Univariate and multivariate analysis were used to classify metabolites in plant parts as well as differentiation of various chemotypes of *C. wightii*. One hundred and thirty two metabolites were identified and quantified from aqueous and nonaqueous extracts of leaves, stem and latex samples from *C. wightii*. High concentrations of free quinic acid were found in fruits (553.5 ± 39.38 mg g−1 dry wt.) and leaves of *C. wightii*. The concentration of quinic acid in leaves may also be used as a chemo marker for identifying the high guggulsterone yielding elite variety of *C. wightii* because of its negative correlation with the concentration of guggulsterone. Decisions based the quinic acid levels can avoid unnecessary tapping of gum resin for

collection of guggulsterones from stem of *C. wightii* which is fatal to the plant. We also report the isolation of a fungal endophyte, (Nigrospora sps.) from this plant. The fungal endophyte produced a substantial quantity of bostrycin and deoxybostrycin known for their antitumor properties. A vast array of primary and secondary metabolites were also identified and quantified from *C. wightii*. Non-targeted metabolite profiling of *C. wightii* results illustrated remarkable differences in the levels of various biologically active metabolites among chemotypes suggesting that additional tissues beyond latex of particular chemotype can be used as potential nutraceuticals and healthcare supplements.

P37

Characterization and quantification of phytochemical constituents in *Centella asiatica* extracts by High Resolution Mass Spectrometry

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Centella asiatica (CA) has been used as a brain tonic in Ayurvedic medicine. The results of several studies in humans and rodent models have caused excitement in using CA preparations as a potential alternative and complementary medicine to improve memory in Alzheimer's disease. CA has been reported to have several other biological activities desirable for human health such as antibacterial, antioxidant, anti-inflammatory, wound healing, and immunostimulant properties. Genetic, geographical and post-harvest processing all influence the secondary metabolite composition in CA products, potentially affecting biological effects and study reproducibility. Modern mass spectrometry (MS) platforms offer accurate mass measurements in combination with collision-induced dissociation techniques for structural analysis and quantification, allowing detailed targeted and untargeted characterization of plant extracts.
Several aqueous and ethanolic CA extracts were characterized using an Acquity UPLC connected to Synapt G2 HDMS system. For quantification of phytochemicals known to occur in CA, an AB Sciex Triple TOF 5600 mass spectrometer equipped with a TurboSpray electrospray ionization source operated in the negative ionization mode was used. The method developed allows quantification of a) seven flavonoids, b) three structural isomers of caffeoylquinic acids, c) five di-caffeoylquinic acids, d) six caffeic acids derivatives and e) the major saponins and related sapogenins. Recovery experiments were carried out for CA extracts. CA Samples were spiked with 24 available standards at two different concentration levels (0.25 ng and 5 ng on column for each compound) recoveries for individual compounds were in the range 91–132 %. For accuracy testing, three standard mixtures of known concentration (low, medium and high) were analyzed in the range of 87-125 %, confirming the feasibility of the proposed procedure for quantitative analysis of CA samples.

Differences were observed in the chemical profiles of the CA extracts, illustrating that standardization and detailed characterization of CA extracts are pre-requisites to reliably and reproducibly study the biological activity of CA preparations.

P38

Identification of Natural Products in Scab Resistant Pecan Trees as Candidates for Natural Fungicides

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Pecan scab is a fungal plant pathogen infecting Pecan tree (*Crya illinoinensis*) leaves, twigs, petioles, shucks, and nuts. Infection presents as mottled, dark colored lesions that ultimately decrease pecan yield and quality and can cause

enough damage to provide a route for secondary infection. Currently scab is controlled with multiple applications of expensive and harmful fungicide spray throughout the growing season. As such, there remains a need for improved methods to control scab that have both lower environmental and economic costs. Within the multitude of pecan tree cultivars there are both scab resistant and scab susceptible strains. As there are pecan tree varieties that are inherently resistant, there must be a natural product produced by these cultivars that could serve as a natural fungicide in susceptible varieties. To probe what this possible natural product could be we evaluated the metabolomes of three resistant (Kanza, Major, and Peruque) and two susceptible (Pawnee and Shoshone) cultivars using ultrahigh performance liquid chromatography (UHPLC)-quadrupole time of flight (QTOF) mass spectrometry (MS) along with gas chromatography (GC)-QTOF-MS. Analysis revealed secondary metabolites that are potentially important in scab resistance and are candidates for future fungal assay studies.

Symposium 9: Phytochemical Signaling

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Investigating the role of oxidized JA-Ile in plant growth and defense response

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Plant hormone jasmonic acid (JA) controls immune response against insects and regulates plant development. Among the JA derivatives, jasmonoyl-isoleucine (JA-Ile) is known to be the endogenous bioactive form of JA responsible for JA mediated responses. JA-Ile is metabolized into a variety of JA derivatives including 12-hydroxy-JA-Ile (12OH-JA-Ile), upon rapid synthesis following elicitation by tissue injury. 12OH-JA-Ile has been reported to be the first metabolite in the catabolic pathway to deactivate JA-Ile signal and is synthesized by CYP94 members of the Arabidopsis cytochrome P450s. Contrary to the widely held belief that 12OH-JA-Ile is largely an inactive signal, our genetic analyses has provided indirect evidence that 12OH-JA-Ile may function as an active signal. Consistently, Arabidopsis seedlings treated with 12OH-JA-Ile strongly accumulated anthocyanin and were also increased in leaf trichome cell numbers to levels comparable to that induced by JA-Ile. Both are anti-herbivory features known to be regulated by JA-Ile. In addition, expression of several JA-Ile responsive marker genes was upregulated by 12OH-JA-Ile. Genome-wide transcript analyses and untargeted metabolomics experiments showed that 12OH-JA-Ile could mimic a significant part of JA-Ile effect both at the transcriptional and metabolic level. Mutation in CORONATINE INSENSITIVE 1 (COI1) blocked 12OH-JA-Ile effect on anthocyanin and trichome induction, indicating that 12OH-JA-Ile signals through the common receptor and signaling mechanism as JA-Ile. 12OH-JA-Ile was able to trigger anthocyanin accumulation in tomato seedlings in COI1-dependent manner indicating that 12OH-JA-Ile signaling system is likely to be conserved in the eudicots. These results show that 12OHJA-Ile likely plays a role in the JA-regulated wound response.

P40

SIP355, a BURP-domain containing protein mediates both biotic and abiotic stress

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The salicylic acid pathway in plants is crucial to defense against microbial pathogens and also known to modulate environmental stress. This pathway enables the plant to induce systemic acquired resistance, whereby regions of the plant not initially exposed to a pathogen become resistant. One of the key enzymes in this pathway is known as Salicylic Acid Binding Protein 2 (SABP2). It is likely that SABP2 may have many important interacting proteins which assist the plant in resisting both biotic and abiotic stressors. Several SABP2-inetracting proteins were identified in a yeast-two-hybrid screen using SABP2 as bait. One of these interacting proteins, SIP355, a putative BURP-domain containing protein is expressed at higher levels during osmotic stress. It is our goal to determine the role that SIP355 plays in abiotic and biotic stress. We have generated stable transgenic lines of *Nicotiana tabacum* silenced for SIP355 using RNAi. Preliminary experiments were performed using leaf disk assays as well as tissue culture experiments relating to the plant's ability to cope with salt and osmotic stress. These initial experiments indicate that the SIP355 transgenic lines exhibit an altered response to salt stress and osmotic stress as compared to wild-type plants. Some of these silenced lines also showed a significant effect on plants ability to induce systemic acquired resistance. We have generated an eGFP fusion of SIP355 to study its subcellular localization using confocal microscopy.

P41

Characterization of SIP68 for its Role in SA Mediated Stress Signaling in Plant

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SIP68 is an SABP2-interacting protein identified in a yeast two-hybrid screen. SABP2 is an important plant protein which catalyzes the conversion of methyl salicylate to salicylic acid. Salicylic acid is one of the important plant hormones that provides defense at both local as well as distal uninfected plant organs known as systemic acquired resistance. SIP68 was characterized as UDP-glucosyltransferase (UGT). Since SABP2 has a role in plant defense and UGT's are involved in many important plant processes, there is the possibility of a role for SIP68 in plant biotic and abiotic stress signaling. Full length SIP68 was cloned and expressed in *Pichia pastoris*. The recombinant affinity purified SIP68 glucosylates flavonols (kaempferol, quercetin, gossypetin, fisetin), flavanones (hesperetin, naringenin), flavones (apigenin, luteolin), and isoflavones (4-acetone-7 Hydroxy-6-methoxy-isoflavone) with varying degree. The highest activity was detected with kaempferol followed by quercetin. However, SA was not a substrate for glucosyltransferase activity of SIP68. Our aim is to assess the role of SIP68 in abiotic and biotic stress signaling in the plant. One of the approaches is to alter the expression of SIP68 in the plant using CRISPR-Cas9 gene editing system. Transgenic plants with altered SIP68 expression will be analyzed for their response to pathogen infection (biotic) and environmental stresses (abiotic). We also aim to localize SIP68 inside tobacco cells using the enhanced Green Fluorescent Protein (eGFP) fusion. This research will help us to add another clue in understanding the plant defense as well as localization of our protein of interest inside the plant cell.

P42

Role of SIR2 like deacetylase enzyme in plant stress signaling mechanism

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Salicylic acid (SA) is an important hormone responsible for inducing various plant defense genes in response to pathogenic infection. Synthesis of SA takes place in effector-triggered immune (ETI) response which is essential for activating the systemic acquired resistance (SAR). SABP2, a 29 kDA protein catalyzes the conversion of methyl salicylate to SA. SABP2 is critical for mounting an effective SAR response. SIP-428 is one of the SABP2-interacting tobacco protein that shows high similarity with SIR2 (Silent Information Regulator 2) like proteins. SIR2 enzymes exhibit NAD+ dependent deacetylase activity and catalyzes the post-translational deacetylation of acetylated lysine residues in cellular proteins. The presence of acetylated lysine residues in some cellular and organellar proteins implicated in physiological and metabolic pathways open up the possible role of SIP-428 in plant physiology including plant immunity. Recombinant SIP-428 from tobacco, when expressed heterologously in *E. coli*, exhibited NAD+ dependent deacetylase activity. To better understand the role of SIP-428 in plant physiology, we are taking two approaches. First one is *in vivo* analysis of transgenic tobacco plant with altered SIP-428 expression (transgenic silenced lines via RNAi and Inducible overexpressor lines of SIP-428 is being generated), and another is *in vitro* analysis of recombinant SIP-428. We have already generated hairpin RNAi-silenced stable transgenic tobacco lines as well as inducible SIP-428 overexpression transgenic tobacco lines. T2 generation line of both SIP-428 silenced, and overexpression will be used to understand the role of SIR2 deacetylase enzyme in SABP2 mediated plant stress signaling mechanism.

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SIP-470, a plant lipid transfer protein and its role in biotic stress

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SIP-470 is a SABP2-interacting protein that was identified in a yeast two-hybrid screen. Bioinformatics analysis shows that SIP-470 is a putative plant lipid transfer protein (PLTP). SABP2 catalyzes the conversion of inactive methyl salicylic acid into an active salicylic acid for defense responses. An accumulation of SA is essential to induce systemic acquired resistance. In plants, LTPs have roles in pathogen defense and environmental stress responses. With both SA and SABP2 having some form of direct role in the plant's defense mechanisms, it is likely that SIP-470 also has a role in modulating plant defenses. To test this hypothesis, *Arabidopsis* T-DNA insertion knockout mutants lacking SIP-470 homolog were analyzed for their response to pathogens. The mutant plants did not show any significant effect in inducing SAR but showed an effect in basal resistance responses. Since SIP-470 was originally identified in tobacco plants, it is prudent to study its role directly in tobacco plants. Towards this, transgenic tobacco lines that are silenced in SIP-470 via RNAi have been generated and are being analyzed for their response to pathogen infection. The overexpressor transgenic lines of SIP-470 have been generated under the control of an estradiol-inducible promoter. These plants are being tested for their response to basal resistance and systemic acquired resistance. Because PLTPs are known to have a role in environmental stress in plants, abiotic stress tests are also being conducted using these transgenic lines. To learn more about this protein, the biochemical analysis is also being conducted. Recombinant SIP-470 was expressed in *E. coli* and purified using metal affinity chromatography. In vitro analysis has confirmed that SIP-470 is a lipid binding protein. An eGFP fusion with SIP470 protein has been created to study the subcellular localization of SIP470 in tobacco cells using the transient expression.

P44

Biochemical Study on Initial Steps of Jasmonate Biosynthesis

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Jasmonic acid (JA) is a key plant hormone that regulates resistance to insects, and is also involved in various aspects of plant development. While the core JA biosynthetic pathway has been elucidated, how the pathway is regulated is still mostly unknown. This project involves the study of the initial steps of JA biosynthesis, which is induced rapidly by physical tissue damage. JA biosynthesis begins in the chloroplast, where its precursor, α-linolenic acid (ALA), is cleaved from membrane lipids through lipase activity and is then converted to 12-oxophytodienoic acid (OPDA), an intermediate of JA. The substrate (ALA) availability has been generally believed to be the rate-limiting step for JA biosynthesis in intact leaves. To test this, we conducted in vitro and in vivo experiments using both isolated pea chloroplasts and transgenic Arabidopsis lines expressing a lipase (DAD1) driven by a dexamethasone (DEX) inducible promoter. Exogenous addition of ALA supported a strong burst of OPDA production that quickly saturated (<5 min) in isolated chloroplasts, indicating that the isolated chloroplasts has ability to synthesize OPDA independently, that ALA is a limiting factor, and that there are additional mechanisms to restrain OPDA production. In Arabidopsis, transcriptional activation of DAD1 by exogenous application of DEX alone could trigger JA synthesis. However, wounding and DEX co-treatment amplified JA production well beyond the level induced by either of the two treatments separately. Taken together these data suggest that the lipases responsible for cleaving ALA from chloroplast lipids are a key regulatory point for JA production that is under both transcriptional and post-transcriptional regulation.

P45

SRFR1, a negative regulator in plant immunity, regulates ethylene-mediated phosphate starvation response and secondary metabolism

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The role of phosphate (Pi) in enhancing disease resistance has been demonstrated for several decades. However, plants are always faced with low Pi stress in nature. The molecular mechanism that coordinating Pi uptake and disease resistance is still poorly understood. Previously, we showed that mutation of SRFR1 elevated immune response, including activation of SNC1- and EDS1-dependent effector-triggered immunity and enhanced resistance to herbivory in the shoot, and enhanced resistance to cyst nematode in the root. In this study, we further characterized root phenotypes of three *srfr1* alleles. Surprisingly, even under high Pi (1 mM) conditions, all of the three *srfr1* mutants displayed short primary roots with more lateral roots and root hairs typical of phosphate starvation response (PSR). We demonstrated that the PSR mimic phenotype of *srfr1* mutants under high phosphate conditions was independent of *SNC1* or *EDS1*, but was related to ethylene biosynthesis. Accordingly, *ACS2*, *ACS6*, *ACS7*, *ACS8* and *ACS9* were significantly up-regulated in *srfr1* mutants. RNA-seq analysis showed a most significant up-regulation of pentacyclic triterpenoid biosynthesis-related genes in the root of *srfr1*. A possible link among phosphate uptake, secondary metabolism and plant immunity will be discussed.

P46

Investigating the role of protein dynamics in JA production and JA mediated wound responses

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The plant hormone jasmonic acid (JA) is involved in defense against insects and the regulation of plant development. In plants, wounding quickly triggers the formation of JA metabolites that results in a typical JA defense response. Jasmonoyl-isoleucine (JA-Ile) is known to be the bioactive form of JA responsible for downstream JA mediated responses. Both the synthesis and degradation of JA-Ile involves numerous enzymes and intermediary substrates that may have regulatory importance. One set of enzymes, the CYP94s, are members of the Arabidopsis cytochrome P450 family and are involved in oxidizing JA-Ile to 12OH-JA-Ile in a degradation step to attenuate the JA-Ile signal. Mutation of these CYP94s (*b1b3*) results in increased JA-Ile, loss of 12OH-JA-Ile, and a reduction in overall JA defense responses – the opposite of what is expected. This would indicate that loss of CYP94s confers resistance to JA-Ile dependent wound responses. In addition the *b1b3* mutant has normal JA responses to exogenously applied JA, indicating that the mutant phenotypes are distinctly wound related. Transcriptomic data of wounded *b1b3* showed either no change or hyper-induction patterns in JA-responsive gene expression. Interestingly JA related protein accumulation/degradation seems to be affected in *b1b3* pointing to potential translational or post-translational regulation. The results indicate that the *b1b3* mutant may be used to unravel a previously unknown regulatory system involving JA synthesis and degradation.

35th Annual Symposium

IPG 2018

Advances in Plant Metabolism

May 30 - June 1, 2018

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- Waters Xevo triple quadrupole MS coupled to UPLC (UPLC-QQQ MS).
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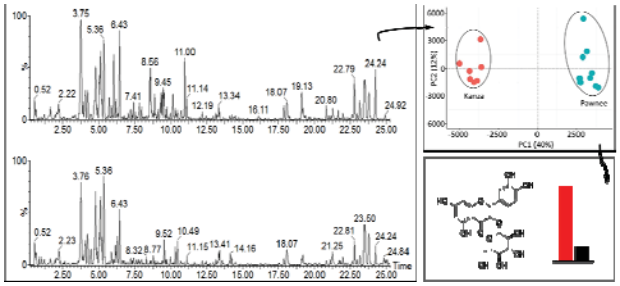

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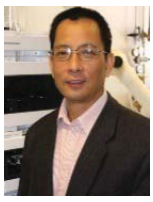

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57th Annual Meeting of the Phytochemical Society of North America (PSNA), August 4-8, 2018

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