The Phytochemical Society of North America

August 8–12, 2015
University of Illinois at Urbana-Champaign

conferences.illinois.edu/psna
psna-online.org

Meeting Venues and Hotels

Contents

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Greetings!

Welcome to the twin cities of Urbana and Champaign, in the heart of east central Illinois. In case you're wondering, they're not identical twins; their births were separated by about 30 years. Urbana dates back to 1822, when William Tompkins built the first homestead on the site that 11 years later was officially platted as a new city. Champaign didn't come into existence until 1852, when the Illinois Central Railroad laid tracks two miles outside of Urbana and residents who settled close by rejected annexation efforts and instead incorporated their own city a few years later. Despite the fact that the two cities now share many services (as well as Wright Street, the official dividing line) and are cooperative and cordial with each other, referenda to merge the two into a single metropolis have been, since 1855, consistently and resoundingly defeated. The courthouse in Urbana, the permanent county seat for Champaign County, was frequented in the 1840s and 1850s by a young lawyer who practiced law in the 8th Judicial Circuit. In 1854, this lawyer—named Abraham Lincoln—delivered one of his first public speeches against slavery in the county courthouse in downtown Urbana.

About the University of Illinois at Urbana-Champaign

The lives of the residents of Urbana and Champaign were changed forever when, in 1867, the Illinois Industrial University, later to become the University of Illinois at Urbana-Champaign, was chartered. The University of Illinois at Urbana-Champaign is a comprehensive, major public university that is ranked among the best in the country. Founded in 1867 as a state-supported, land-grant institution under the terms of the Morrill Land Grant Act of 1862 with a threefold mission of teaching, research, and public service, the UIUC opened its doors to students in 1868 and rapidly earned a reputation as an educational institution of international stature. Today, scholars and educators rank it among a select group of the world's great universities. For more information, see the university website (**www.illinois.edu**).

UIUC is a residential campus of classrooms, laboratories, libraries, residence halls, and recreational and cultural facilities with 200 major buildings on the central campus of 1,783 acres. Nearby are the University's 1,650-acre Willard Airport; Robert Allerton Park, the campus's 1,768-acre nature and conference center; and 3,600 acres of agricultural land. An additional 3,700 acres of farmland elsewhere in Illinois are used by the College of Agricultural, Consumer, and Environmental Sciences as experimental fields. The University also owns close to 1,000 acres of natural areas within a 50-mile radius of the campus, held for the express purpose of conducting ecological research.

The University of Illinois and its various campuses have had a long history of scientific exploration into the world of plants, both in production and in biological and chemical characterization. The Morrow Plots are the oldest agronomic experiment fields in the United States which include the longest-term continuous corn plot in the world. In 1968, the Morrow Plots became a National Historic Landmark. Some of the seminal work

understanding on photosynthesis and carbon dioxide fixation was done in part here including the work of the labs of Govindjee and W.L. Ogren. The University continues to feature a diverse array of agricultural-based research in the College of Agricultural, Consumer and Environmental Sciences, one the hosts of this meeting.

Today, UIUC is home to life scientists across the campus using new genomic tools, computational methods and chemical analytical techniques to explore mechanisms of interest to phytochemists. In addition to departmental programs, the UIUC Institute for Genomic Biology, is an international center for genomics research. Other unique campus research resources include SoyFACE, a 60-acre facility instrumental in documenting the chemical ecology of global climate change in agriculture, the world's largest public university library (with 24 million-plus items in its catalogue), and Blue Waters, the petaflop supercomputer that is the fastest anywhere in the world on a university campus), capable of over 13 quadrillion calculations per second. With 22 Nobel Prize winners and 20 Pulitzer Prize winners among faculty and alumni, the University of Illinois is the number one return on investment in the Midwest.

"The best way to predict the future is to create it."

—Abraham Lincoln

Welcome to Illinois!

May Berenbaum
Mark Berhow
Elvira de Mejia

Registration Desk Hours

The registration desk is located outside Illini Rooms ABC on the main level of the Illini Union, except as noted.

Saturday, August 8	4:30–7:30 pm
I Hotel and Conference Center—Lincoln Room	
Sunday, August 9	7:30 am–5:30 pm
Monday, August 10	7:30 am–5:30 pm
Tuesday, August 11	7:30 am–5:30 pm
Wednesday, August 12	7:30–11:00 am

Your registration includes the conference program, the welcome reception on Saturday, refreshment breaks Sunday-Wednesday, lunch Sunday-Tuesday, poster sessions on Sunday and Monday, and the banquet on Tuesday. A limited number of guest banquet tickets may be available for purchase at the registration desk during the conference.

Meeting Venues

Illini Union (Sunday–Wednesday)
1401 W. Green St.
Urbana, IL 61801
Phone: 217-333-4666
union.illinois.edu

I Hotel and Conference Center
(Saturday's welcome reception)
1900 S. 1st St.
Champaign, IL 61820
Phone: 217-819-5000
stayatthei.com

Alice Campbell Alumni Center
(Tuesday's banquet)
601 S. Lincoln Ave.
Urbana, IL 61801
Phone: 217-333-1471
uiaa.org/alumnicenter

Meeting Hotels

Illini Union Hotel
1401 W. Green St.
Urbana, IL 61801
Phone: 217-333-4666
union.illinois.edu

I Hotel
1900 S. 1st St.
Champaign, IL 61820
Phone: 217-819-5000
stayatthei.com

Comfort Suites
2001 N. Lincoln Ave.
Urbana, IL 61801
Phone: 217-328-3500
comfortsuites.com/hotel-urbana-illinois-IL366

Transportation

Some area hotels have a complimentary shuttle that makes trips between the airport, the hotel, and campus. Please call your hotel or check at their front desk to arrange for a ride.

Champaign-Urbana MTD Bus System
Champaign-Urbana has a great public transportation system, with several stops on campus and near the conference hotels. The 27 Air Bus runs between Willard Airport in Savoy and the Illini Union several times a day. A standard ride costs $1, one-way, with free transfers from route to route. Only coins and $1 bills accepted. Drivers can make change for up to $5 prior to 7:00 pm. Exact cash is required after that time. Visit cumtd.com for maps and schedules, or call 217-384-8188.

Other Bus and Train Services
Peoria Charter coach buses depart from campus and the Illinois Terminal in Champaign daily (800-448-0572 or peoriacharter.com). Greyhound buses also depart from the Illinois Terminal daily (217-352-4150 or greyhound.com). A few Amtrak trains serve Champaign-Urbana via the Illinois Terminal in Champaign (amtrak.com).

Taxi Service
Yellow Checker Cab, Phone: 217-355-3553
Orange Taxi, Phone: 217-363-1500
Green Taxi, Phone: 217-721-5533
Quality Limo & Taxi, Phone: 217-552-7400

Willard Airport
11 Airport Rd., Savoy, IL 61822
Phone: 217-244-8618
flycmi.com

Parking

Parking is free and plentiful at the I Hotel and Conference Center and Comfort Suites. If you are staying at the Illini Union Hotel, you will be given a complimentary parking pass to park in their adjacent lot. For those staying elsewhere, there is limited metered parking near the Illini Union and Alice Campbell Alumni Center. Be sure to bring quarters to feed the meters or plan to pay by phone. If you are willing to walk a few blocks, you may be able to find free parking in the residential area east of the Illini Union, just past Lincoln Avenue.

Wi-Fi Access

Wireless Internet access is free at the meeting venues. Please select the network **UIpublicWiFi**, open your browser, and enter your contact information when prompted.

Emergency Numbers and Procedures

Emergency (Police, Fire, or Ambulance): 911

Non-Emergency
University Police: 217-333-1216
Urbana Police: 217-384-2320
Champaign Police: 217-351-4545

Medical Assistance
Carle Foundation Hospital: 217-383-3311
Christie Clinic: 217-366-1200
Provena Covenant Medical Center: 217-337-2000

Tornado Preparedness
East-central Illinois is prone to summer thunderstorms; often conditions are conducive to tornadoes. If you hear the sirens go off, a tornado warning is in effect. Take cover immediately in the lowest floor of a building, and stay away from windows.

Fire Procedure
If a fire alarm goes off, exit the building in a calm and orderly manner. If you are on an upper level floor, exit by the nearest stairwell. Do not use elevators.

Moderators, Speakers, Poster Presenters, and Exhibitors

Moderators
If you are moderating a session, please arrive a few minutes early to ensure that audio-visual equipment is in place and functional. At each session, a student volunteer should be on hand to assist you. The speaker's allotted time includes time for questions. The moderator should alert speakers when 3 minutes remain and again indicate when one minute remains. After their allotted time, all speakers should be asked to leave the podium. If the speaker has used his/her entire allotted time, then the speaker cannot take questions. Moderators, please announce this format at the beginning of the session, and *stay on schedule*.

Speakers
Please consult the program ahead of time to confirm the time and location of your talk. Arrive early at your session, and if you are planning to use slides, email them in advance and also bring them on a USB stick. Find the session moderator and identify yourself so that he or she is aware that you are present. Please try to stay within your allotted time—it's a courtesy to your audience and fellow speakers in your session.

Poster Presenters
The maximum size is the standard for scientific posters, 4-feet by 4-feet. Landscape is the typical orientation, but portrait is also acceptable as long as it isn't wider or taller than 4-feet. The display boards will be numbered to indicate where to place your poster. Push pins will be provided to affix your poster to the board. Please put up your poster by 12:00 pm on Sunday, and stand by your poster during your designated poster session. Any posters remaining after Tuesday will be discarded.

Poster Session I
Odd-numbered posters (e.g. P1, P3, P5...)
Sunday, August 9, 5:30–7:30 pm
Illini Room AB

Poster Session II
Even-numbered posters (e.g. P2, P4, P6...)
Monday, August 10, 5:30–7:30 pm
Illini Room AB

Exhibitors
Exhibitors will be set up in Illini Room AB from Sunday–Tuesday.

Refreshments

Refreshments will be available in Illini Room AB at the start of each day and during the break times. There are also a few eateries in the Illini Union such as Jamba Juice and Espresso Royale (main level) and Einstein Bros. Bagels (lower level).

Meeting Agenda

All events take place in Illini Rooms ABC on the main level of the Illini Union, except as noted.

Saturday, August 8

2:00–5:00 pm | Executive Committee Meeting, I Hotel and Conference Center—Lincoln Room

5:30–7:30 pm | Welcome Reception, I Hotel and Conference Center—Lincoln Room

Sunday, August 9

Symposium 1: Phytochemical and Plant "omics"
Chairs: Lloyd Sumner and Cecilia McIntosh

- 8:00 am | Welcome by Robert Hauser, Dean, College of ACES, University of Illinois at Urbana-Champaign
- 8:15 am | Miroslava Cuperlovic-Culf, National Research Council. Cheminformatics and metabolomics converge on wheat—fungal pathogen interactions.
- 9:00 am | Dean DellaPenna, Department of Biochemistry and Molecular Biology, Michigan State University. Transorganellar complementation functionally demonstrates a new interface for the synthesis of non-polar metabolites by membrane spanning pathways
- 9:45 am | Elsevier Award Speaker Nicole Clay, Department of Molecular, Cellular & Developmental Biology, Yale University. Evolutionary toolkits for chemical innovation in land plants.
- 10:30 am | Break
- 10:40 am | Lloyd Sumner, Noble Foundation. Large-scale, computational and empirical UHPLC-MS-SPE-NMR annotation of plant metabolomes.
- 11:00 am | Fred Stevens, Oregon State University. Shotgun metabolomics and lipidomics for mechanistic elucidation of the anti-obesity effects of xanthohumol from hops *(Humulus lupulus).*
- 11:20 am | Parnian Lak, Oregon State University. LC-MS/MS investigation of the active phytochemicals in *Centella asiatica*.
- 11:40 am | Marcos Soto Hernadez, Colegio de Postgraduados. NMR-1H metabolomic analysis of Mexican races of avocado.

12:00–1:30 pm | Lunch

Symposium 2: Phytochemical Lipids & Metabolism
Chairs: Aruna Kilaru and Argelia Lorence

- 1:30 pm | Kent Chapman, Department of Biological Sciences, University of North Texas. Metabolism and functions of N-acylethanolamines in seedling development.
- 2:15 pm | Pradeep Kachroo, Department of Plant Pathology, University of Kentucky. Chemical- and lipid-mediated systemic acquired resistance in plants.
- 3:00 pm | Neish Award Speaker: Abe Koo, Department Biochemistry, University of Missouri. Metabolism of plant hormone jasmonate: A sentinel for tissue damage and master regulator of stress response.
- 3:35 pm | Break
- 3:50 pm | Aruna Kilaru, East Tennessee State University. Transcriptome analysis of avocado mesocarp reveals key genes necessary to improve oil yield.
- 4:10 pm | Md Mahbubur Rahman, East Tennessee State University. Identification, and heterologous expression analysis of Avocado DGAT1 and DGAT2

- 4:30 pm | Reinhard Jetter, University of British Columbia. Wheat waxes revisited: composition and biosynthesis.
- 4:50 pm | Samiddhi Senaratne, University of Melbourne. Monoterpene acid glucose esters in *Eucalyptus* foliar oil glands.
- 5:10 pm | Ebenezer Ajewole, University of Western Ontario/Agriculture and Agri-Food Canada. K+ Dependent and K+ independent Asparaginase from common Bean (*Phaseolus vulgaris):* mechanism of activation by K+.

5:30–7:30 pm | Poster Session I

Monday, August 10

Symposium 3: Phytochemical Biosynthesis
Chairs: Li Tian and De-Yu Xie

- 8:00 am | Greetings
- 8:15 am | Xiao-Ya Chen, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Science. Regulation of sesquiterpene biosynthesis in plants.
- 9:00 am | Joe Chappell, Department of Plant & Soil Science, University of Kentucky. *Valeriana officinalis* as a novel platform for plant natural product drug discovery.
- 9:45 am | Cecilia McIntosh, mcintosc@etsu.edu, Department of Biological Sciences, East Tennessee State University. Structure and function of flavonoid glucosyltransferases: using a specific grapefruit enzyme as a model.
- 10:30 am | Break
- 10:40 am | Hiroshi Maeda, University of Wisconsin-Madison. Diversification of tyrosine biosynthetic pathways in plants: Non-plastidic, tyrosine-insensitive prephenate dehydrogenases in legumes.
- 11:00 am | Mark Lange, Washington State University. Evolution of chemical diversity in plants
- 11:20 am | Li Tian, University of California, Davis. Investigations on early steps of hydrolyzable tannin biosynthesis.
- 11:40 am | Toshiaki Umezawa, Kyoto University. Structural modification and increase of lignin in *Oryza sativa* for biomass refinery.

12:00–1:30 pm | Lunch

Symposium 4: Chemical Ecology I
Chairs: Dorothea Tholl and Massuo Kato

- 1:30 pm | May Berenbaum, Department of Entomology, University of Illinois. Honey bees as phytochemists: how pollen and nectar are converted into beebread and honey.
- 2:15 pm | Walter S. Leal, Department of Molecular and Cellular Biology, University of California at Davis. Mosquito odorant receptor for the insect repellent DEET and plant-derived semiochemicals.
- 3:00 pm | Neish Award Speaker: Martha Vaughan, USDA, ARS, Peoria, IL. Climate change and crop phytochemical defenses: Potential implications for food security and food safety.
- 3:35 pm | Break
- 3:50 pm | Katharina Schramm, University of Utah. Metabolomics of juniper detoxification in a generalist and specialist mammalian herbivore.
- 4:10 pm | Felipe Christoff Wouters, Max Planck Institute for Chemical Ecology. Detoxification of maize chemical defenses by Lepidopteran herbivores.
- 4:30 pm | Verena Jeschke, Max Planck Institute for Chemical Ecology. Toxicity of glucosinolate-derived isothiocyanates to generalist-feeding caterpillars.
- 4:50 pm | De-Yu Xie, North Carolina State University. Gene-to-terpene landscapes in self-pollinating *Artemisia annua*.

- 5:10 pm | Michael Sullivan, US Dairy Forage Research Center, ARS-USDA. Engineering alfalfa to accumulate useful caffeic acid derivatives and characterization of hydroxycinnamoyl-CoA transferases from legumes.

5:30–7:30 pm | Poster Session II

Tuesday, August 11

Symposium 5: Chemical Ecology II
Chairs: Dhirendra Kumar and De-Yu Xie

- 8:00 am | Greetings
- 8:15 am | Jonathan Gershenzon, Department of Biochemistry, Max Plank Institute for Chemical Ecology. Metabolic tag in the cornfield: Biosynthesis and activation of benzoxazinoids by corn plants and deactivation by herbivores.
- 9:00 am | Eric Schmelz, Department of Biological Sciences, University of California at San Diego. Inducible small molecule defenses in maize: more than a few surprises.
- 9:45 am | Jyoti Shah, Department of Biological Sciences, University of North Texas. Signaling function for an abietane diterpenoid in plant defense and development.
- 10:30 am | Break
- 10:40 am | Franck Dayan, USDA/ARS, Oxford, MS. Sarmentine, a natural piper amide herbicide with multiple mechanisms of action.
- 11:00 am | Dimitre Ivanov, University of Western Ontario. The chemoattractant potential of ginsenosides in the ginseng - *P. irregulare* pathosystem,
- 11:20 am | Chintamani Thapa, University of Saskatchewan. Chemical interaction between the cruciferous phytoalexin camalexin and *Colletotrichum* species.
- 11:40 am | Mitchell Wise, Research Chemist USDA, Cereal Crops Research Unit. Field application of benzothiadiazole on oat: Effect on avenanthramide production and crown rust resistance.

12:00–1:30 pm | Lunch

Symposium 6: Botanical Medicines
Chairs: Dejan Nikoli and Fred Stevens

- 1:30 pm | Richard van Breemen, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago. Development of safe and effective botanical dietary supplements for women's health.
- 2:15 pm | Ehab Abourashed, Department of Pharmaceutical Sciences, Chicago State University. The non-volatile constituents of nutmeg—a key to solving an old riddle?
- 3:00 pm | Neish Award Speaker: Philipp Zerbe, Dept of Plant Biology, University of California, Davis. Elucidating modular diterpenoid metabolism in non-model plant systems: From chemical diversity to biotechnology applications.
- 3:35 pm | Break
- 3:50 pm | Foluso Oluwagbemiga Osunsanmi, University of Zululand. Antiplatelet aggregation and cytotoxicity activity of betulinic acid and its acetyl derivative from *Melaleuca bracteates* var. revolution gold.
- 4:10 pm | Tristesse Burton, University of Illinois at Chicago. American Indian botanicals as possible alternatives to hormone therapy.
- 4:30 pm | Massuo Kato, University of Sao Paulo. Molecular diversity and evolution of *Peperomia* species.
- 4:50 pm | Break

5:00–6:00 pm | Annual Meeting of the PSNA

6:30–9:00 pm | Award Banquet, Alice Campbell Alumni Center Ballroom

Welcome by Neal Merchen, Associate Dean for Research, College of ACES, University of Illinois at Urbana-Champaign

Wednesday, August 12

Symposium 7: Nutritional & Medicinal Phytochemistry
Chairs: Elvira de Mejia and Shelly Nickols-Richardson

- 8:00 am | Greetings
- 8:15 am | Mary Ann Lila, Plants for Human Health Institute, North Carolina State University. Phytoactive chemicals: discovery is just at the "starting line" of the research.
- 9:00 am | John (Jack) Juvik, Department of Crop Sciences, University of Illinois at Urbana-Champaign. Manipulation of glucosinolate biosynthesis, hydrolysis, and hydrolysis product anticancer bioactivity in broccoli (*Brassica oleracea* L. ssp.).
- 9:45 am | Okiemute Rosa Johnson-Ajinwo, Keele University. Anti-malarial activities of *Margaritaria discoidea* and other Nigerian medicinal plants.
- 10:15 am | Syeda Hussain, University of Sao Paulo. Phytochemical analysis for phenol and saponin in *Brachiaria* sp.
- 10:45 am | Pavel Somavat, University of Illinois at Urbana-Champaign. Coproduct yield comparisons between purple corn, blue corn, and yellow dent corn for different milling processes.
- 11:15 am | Richard Licayan, Rutgers University. Investigation of α-glucosidase inhibition properties, antioxidant activities, and cytotoxicity of Philippine herbal vines containing polyphenols.

Speaker Abstracts

Sunday, August 9, Morning

Symposium 1: Phytochemical and Plant "omics"

Chairs: Lloyd Sumner and Cecilia McIntosh

Miroslava Cuperlovic-Culf obtained Ph.D. in Biophysical Chemistry at University of California, Santa Barbara followed by post-doctoral training in Biophysics with Prof. Myer Bloom at University of British Columbia. Currently she is a Senior Research Officer at National Research Council of Canada and Adjunct Professor of Chemistry in the department of Chemistry & Biochemistry at Universite de Moncton. Miroslava is particularly interested in metabolomics and computational analysis of biological system under different conditions and treatments.

[S1-1] Cheminformatics and metabolomics converge on wheat–fungal pathogen interactions

Presenter and Author: Miroslava Cuperlovic-Culf, National Research Council, Canada

Pathogenic fungi are increasingly recognized as a foremost treat to plant, animal and human health. Fungal pathogens are leading to some of the most destructive diseases in plants including major damage to agricultural crops such as rice, wheat and corn. In addition to devastating plant yield, fungal toxins can present a danger to animal and human health with many historical examples of fungal toxins causing catastrophic losses of life. The development of fungal infection resistant plants, novel, environmentally friendly, anti-fungal treatments as well as more informative methods for treatment follow-up is sorely needed. Unbiased analysis of metabolites provided by different metabolomics approaches delivers fast, inexpensive way for molecular follow-up in treatment development and assessment as well as determination of markers for targeted sensors development. Although current metabolomics approaches can only view several hundreds of the estimated 200,000 compounds, information about the concentration changes in this subset of metabolites in different conditions provides valuable overview of distinct compound classes and regions in the metabolic network. Metabolic changes are a significant part of plants' response to pathogens. Recently published studies have determined number of resistance related metabolites as well as important metabolic pathways involved in the response of plants to fusarium head blight and rust. Function of the majority of these metabolites in the resistance remains, however, unknown. At the same time, a number of plant metabolites have been extensively studied for their health effect in mammalian cells. In this work we are combining results of metabolomics measurements in plant-pathogen interactions with cheminformatics analysis of properties of molecules to describe major metabolic changes inducing resistance in plants to fusarium head blight and rust fungi. For known resistance related metabolites we are subsequently working on determining molecular functions and targets in either fungus or plant using methods adopted from drug discovery practices.

Although function of the majority of resistance related metabolites remains unknown, several fungal proteins have been determined as possible targets. Out of these in this work we have explored histone deacetylases, carbonic anhydrases, cytochrome P450 and lipoxygenases. All four groups of proteins have experimentally proven roles in fungal pathogenesis with number of plant and fungal metabolites acting as their potent inhibitors.

Specific focus in the talk will be on the interaction of resistance related metabolites with these protein targets. Structures of these four groups of proteins are conserved across species and therefore can be inhibited by the same molecules. Roles of these groups of proteins in plant—fungus interaction were explored using computational modelling and metabolomics analysis. Computational analysis was also performed to determine inhibitory power of significantly over-concentrated metabolites against plant and fungal proteins. Experimental testing has also been initiated exploring the effect of inhibition of these proteins on fungal metabolism and toxin production using NMR and FT-IR spectroscopy. Results of these studies will be presented.

Dean DellaPenna is a MSU Foundation Professor and University Distinguished Professor in the Department of Biochemistry and Molecular Biology at Michigan State University. He earned a B.S. in Cellular Biology at Ohio University in 1984 and a Ph.D. in Plant Physiology at the University of California at Davis in 1987. He was on the faculty in the Plant Sciences Department at the University of Arizona from 1990 to 1996 and then in the Biochemistry Department at the University of Nevada, Reno before moving to Michigan State University in 2000. Dr. DellaPenna received the Distinguished Faculty Award from MSU in 2008 and was elected a Fellow of the American Association for Advancement of Science (AAAS) in 2009. His scientific interests lie at the interface of plant biochemistry and human health. He has pioneered genomics-enabled approaches to understand the synthesis and accumulation of essential dietary nutrients in plant tissues and he currently leads a consortium employing genome wide association studies and deep sequencing to understand and compare the control of essential nutrient content and composition in model plants and crops. He is a vocal proponent of using such knowledge to breed and engineer crops to provide balanced nutrition on a global scale. He was also a member of the Medicinal Plant Consortium; a multi institutional effort generating transcriptome and metabolome data from medicinal plants for use in dissecting the biosynthesis of plant-derived compounds of medical significance.

[S1-2] Transorganellar complementation functionally demonstrates a new interface for the synthesis of non-polar metabolites by membrane spanning pathways

Presenter and Author: Dean DellaPenna, Department of Biochemistry and Molecular Biology, Michigan State University

Plastids are subcellular factories that participate in the synthesis of a bewildering array of compounds, often by initiating biosynthetic pathways that are subsequently completed in other organelles. Such organelle-spanning pathways require extensive exchange of metabolites with the extraplastidic environment, which for polar metabolites, is handled by dozens of well-characterized envelope membrane transporters. However, for the many thousands of plastid-synthesized nonpolar compounds synthesized by membrane-spanning pathways, such transporters have remained elusive. This talk will highlight recent data from an approach we have termed transorganellar complementation that functionally demonstrates enzymes in one organelle can directly access nonpolar metabolites from a companion organelle. We propose a mechanism, based on hemifused-membranes at plastid:ER contact sites, that allows enzymes in one organelle direct, transporter-independent access to a range of nonpolar compounds in both organelle membranes. Such an interface would facilitate inter-organellar metabolism, allosteric regulation between organelles and allow the observed rapid evolution of chemical diversity by membrane-spanning pathways to be

uncoupled from coevolution with nonpolar metabolite transporters.

[S1-3: Elsevier Award Speaker] Evolutionary toolkits for chemical innovation in land plants

Presenter and Author: Nicole Clay, Department of Molecular, Cellular and Developmental Biology, Yale University.

Plant secondary metabolites, both constitutive and pathogen-inducible, have recently been shown to be involved in controlling several evolutionary conserved plant innate immune responses, such as callose deposition and programmed cell death. Despite advances in our understanding of the contribution of plant secondary metabolites to plant immunity, many pathogen-inducible genes, pathways and metabolites remain uncharacterized in even the most-characterized model plant species. Using expression profiling, co-expression analysis and untargeted metabolic profiling, we have uncovered the complete biosynthetic pathway to a previously unknown class of tryptophan-derived cyanogenic metabolites in *Arabidopsis*. The enzymes catalyzing the first committed steps of the novel cyanogenic indole biosynthetic pathway and the ancestral camalexin biosynthetic pathway are encoded by paralogous cytochrome P450 genes, a finding that suggests that gene duplication and neofunctionalization of core pathway enzymes can serve as drivers of chemical diversity in plant secondary metabolism. In addition, we have identified two multiprotein regulatory complexes involved in the production of clade-specific phenylpropanoids and aromatic alkaloids in *Arabidopsis*. The stable subnetworks of transcription factors at the core of these complexes may be highly conserved in distantly related plant species, a finding that suggests that the recruitment and remodeling of ancient gene regulatory networks during land plant evolution can also serve as drivers of chemical diversity in plant secondary metabolism.

Nicole K. Clay received her B.S. in Biology (1996) from Massachusetts Institute of Technology, and her Ph.D. in Biology (2005) from Yale University. She received her postdoctoral training in the field of plant-microbe interactions (2005-2010) at Massachusetts General Hospital, an affiliate of the Harvard Medical School. In 2011, she joined the faculty at Yale University as an Assistant Professor of Molecular, Cellular & Developmental Biology. Her research program is focused on understanding how the function and cell-surface abundance of immune receptors are regulated by the secretory and endocytic pathways, and how the plant defense network of specialized metabolic pathways is evolutionarily driven to diversification and functionalization.

[S1-4] Large-scale, Computational and Empirical UHPLC-MS-SPE-NMR Annotation of Plant Metabolomes

Presenter(s): Lloyd W. Sumner, Plant Biology Division, the Samuel Roberts Noble Foundation

Author(s): Lloyd W. Sumner, Feng Qiu, Dennis Fine, Daniel Wherritt, Zhentian Lei, Plant Biology Division, the Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

Integrated metabolomics is a revolutionary systems biology tool for understanding plant metabolism and elucidating gene function. Although the vast utility of metabolomics is well documented in the literature, its full scientific promise has not yet been realized due to multiple technical challenges. The number one, grand challenge of metabolomics is the large-scale confident chemical identification of metabolites. To address this challenge, we have developed powerful software entitled Plant Metabolite Annotation Toolbox' (PlantMAT) and are coupling it to a sophisticated ensemble composed of Ultrahigh pressure liquid chromatography coupled to mass spectrometry coupled to automated solid-phase extraction and NMR (UHPLC-MS-SPE-NMR) for the

large-scale systematic and biological directed annotation of plant metabolomes.

UHPLC-QTofMS/MS metabolite profiling was performed on 80% aqueous methanol extracts of Medicago truncatula, and the data processed using Bruker's Compass DataAnalysis 4.1 for peak deconvolution and formula prediction. Metabolite identifications were first attempted through spectral matching with custom libraries generated with authentic compounds. However, authentic compounds are not available for all metabolites; especially plant natural products, and a large number of the detected peaks could not be identified using spectral matching. Thus, the orthogonal data was imported into PlantMAT and structures for approximately 100 saponins and polyphenolic glycosides were efficiently predicted. Ten of these were chosen for further structural validation. These ten compounds were isolated, purified and concentrated by mass directed UHPLC-MS-SPE. The SPE isolated compounds were eluted with approximately 250 ul of deuterated methanol and 1D and 2D NMR spectra acquired. The NMR spectral data confirmed a 100% accuracy in the PlantMAT predicted structures. The results demonstrated that the cumulative platforms allows for higher-throughput and high confidence metabolite identifications necessary for metabolome 'sequencing'.

[S1-5] Shotgun Metabolomics and Lipidomics for Mechanistic Elucidation of the Anti-obesity Effects of Xanthohumol from Hops (*Humulus lupulus*)

Presenter(s): Jan Frederik Stevens, Linus Pauling Institute & College of Pharmacy

Author(s): J. Choi, C.L. Miranda, J.F. Stevens, Linus Pauling Institute & College of Pharmacy, Oregon State University, OR 97331, USA

Xanthohumol (XN) is a prenylated flavonoid found in hops, beer, and in dietary supplements. There is substantial evidence in the literature that XN improves glucose and lipid metabolism of mammalian cells in vitro, but relatively little is currently known about the potential benefits/risks of chronic exposure to XN in vivo. Even less is known about the underlying mechanisms of action. The objective of this study is to identify the molecular targets of XN in obesity rodent models that mimic metabolic syndrome in humans. Zucker fa/fa rats and diet-induced obese C57BL/6J mice, both accepted models of obesity and metabolic syndrome, were treated for 6-12 weeks with XN at three dose levels (n=12-16/dose group). The XN-treated animals had significantly lower plasma glucose levels and smaller body weight gain compared to the control groups, while food intake was not affected by treatment. Metabolome and lipidome profiles of plasma and liver tissue were recorded in addition to single endpoints of glucose and lipid homeostasis. Metabolites were identified using an in-house library of >600 metabolites (IROA Technologies) and by online database searching. We detected >5,000 metabolites by LC-QToF mass spectrometry, of which >200 metabolites were identified by mass, isotope distribution, MS/MS fragmentation pattern, and when standards were available, retention time. The shotgun metabolomics and lipidomics analyses showed a dose-dependent decrease of hepatic triglyceride content and metabolic products of dysfunctional lipid metabolism (medium-chain acylcarnitines, dicarboxy fatty acids, hydroperoxy and hydroxy fatty acids). Taken together, the results indicate that xanthohumol improves beta-oxidation of fatty acids. These anti-obesity effects are also consistent with our preliminary results showing that XN inhibits de novo lipogenesis in differentiating 3T3-L1 adipocytes, determined by deuterium labeling and subsequent mass isotopomer distribution analysis (MIDA) of triglycerides.

[S1-6] LC-MS/MS investigation of the active phytochemicals in *Centella asiatica*

Presenter(s): Parnian Lak, Department of Chemistry, Oregon State University, Corvallis, OR; Linus Pauling Institute, Oregon State University, Corvallis, OR;

Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR.

Author(s): Parnian Lak[1,2,5]; Nora Gray[3]; Christopher Harris[3]; Jeff Morré[1]; Margeux Hunter[3]; Trevor Shear[1]; William Bisson[2]; Joseph Quinn[3,4]; Amala Soumyanath[3]; Jan F. Stevens[2,5]; Claudia Maier[1,2]

[1]Department of Chemistry, Oregon State University, Corvallis, OR; [2]Linus Pauling Institute, Oregon State University, Corvallis, OR; [3]Department of Neurology, Oregon Health and Science University, Portland, OR; [4]Department of Neurology and Parkinson's Disease Research Education and Clinical Care Center (PADRECC), Portland Veterans Affairs Medical Center, Portland; [5]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR

Centella asiatica (CA) has been traditionally used for centuries to improve memory and cognition. We have previously shown that the water extract of CA leaves (CAW) can enhance cognitive function in the Tg2576 mouse model of Aβ toxicity and in wild type old mice. Major known constituents are triterpenoids and caffeoylquinic acids. We have investigated the underlying mechanisms of the effects of CAW on healthy aged mice brain, and the active compounds in CAW that contribute to this effect, by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). So far we have discovered that CAW increases brain levels of fatty acid amides, and inhibits fatty acid amide hydrolase (FAAH).

To isolate and characterize the active compounds, CAW was first subject to liquid-liquid extraction. The fractions were tested for their bioactivity, and the butanol fraction was shown to contain all active phytochemicals. This fraction was further separated by silica gel column chromatography and gel filtration chromatography. In each step the fractions and partitions were analyzed by TLC/MS or LC/MS for structural identification. In house library, commercially available programs and on-line sources were used to comprehensively analyze the plant metabolites. By taking a multistep separation and analysis approach, a few structural candidates have been identified and characterized for their inhibitory effects on FAAH. Computational methods have also been used to investigate the molecular characteristics of CAW phytochemicals inhibiting FAAH.

[S1-7] NMR-1H metabolomic analysis of mexican races of avocado

Presenter(s): Marcos Soto-Hernandez, Colegio de Postgraduados México

Author(s): Emmanuel Ibarra-Estrada, Colegio de Postgraduados, postgrado en Fisiología Vegetal.

Alejandro Barrientos-Pliego, Universidad Autonoma Chapingo

The avocado is one of the fruits more important in México, and its consumption has increased considerably. The variety 'Hass' is the most traded and consumed around the world, and it is necessary to find news genotypes that compete in quality with Hass variety. In México coexist several races and diverse species of Persea, which shows the diversity and importance of this crop. The aim of this study was to analyze the close relations and differences in chemical composition in the pulp and skin of three races of avocado and two species of Persea. A metabolomic analysis based on NMR-1H was done It was studied three genotypes of the West Indians race, three of the guatemalan race, seven of the Mexican race, *P. nubigena*, *P. schiedeana* and 'Hass'. With the data it was done a component principal analysis and one discriminant analysis to determine the variability and the correlations of the variables with the differences and close relations between the genotypes. In pulp, the results showed that the mexican race, the guatemalan, *P. nubigena* and 'Hass' are very similar, whereas the West Indians race and *P. shiedeana* were very different to those. In skin, the grouping and separations between genotypes were very similar. 'Hass', a breeding between the guatemalan and mexican race, in both pulp and skin was close to the mexican race. The West Indians race in both structures was characterized by its content of aminoacids and sugars, whereas the mexican genotypes were distinguished by aromatic compounds.

Sunday, August 9, Afternoon

Symposium 2: Phytochemical Lipids and Metabolism

Chairs: Aruna Kilaru and Argelia Lorence

[S2-1] Metabolism and functions of N-acylethanolamines in seedling development.

Presenter: Kent Chapman, Department of Biological Sciences, University of North Texas

Author(s): Kent D. Chapman[1], Jantana Keereetaweep[1], Elison B. Blancaflor[1,2]

[1]University of North Texas, Center for Plant Lipid Research, Department of Biological Sciences, Denton, TX 76203

[2]Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, OK

N-Acylethanolamines (NAEs) are fatty acid derivatives conjugated to ethanolamine via an amide bond. In seeds of most plants including Arabidopsis thaliana, NAEs with linolenic (18:3) and linoleic (18:2) acids together make up more than 70% of the total NAE pool, and they both decline markedly with the progression of seed germination and seedling development. Evidence indicates that both NAE types are metabolized by specific lipoxygenase (LOX) isoforms during the course of seedling establishment, leading to an array of NAE oxylipin metabolites. Specific ethanolamide oxylipins of the NAEs are capable of inhibiting seedling development in a tissue-specific manner. Seedling sensitivity to growth inhibition by ethanolamide oxylipins overlaps a secondary dormancy stage induced by abscisic acid (ABA) that is believed to support survival from stresses during seedling establishment. Collectively, our data suggest that the formation of ethanolamide oxylipins may participate in the coordinate control of seedling establishment under unfavorable environmental conditions in cooperation with phytohormone and light signaling pathways.

Kent Chapman leads the Center for Plant Lipid Research at the University of North Texas which focuses on basic and applied aspects of plant lipid metabolism. Center scientists use contemporary cellular, biochemical, and molecular genetic approaches to understand how lipids influence the growth and development of plants. Efforts also contribute to the discovery of new products and uses for plant derived lipids and their potential public benefit. Kent Chapman earned a B.A. degree in biology from Lycoming College in Williamsport, PA, and then traveled Tempe, AZ, where he earned a Ph.D. degree in botany (plant cell biology) from Arizona State University. After completing his doctoral degree, Chapman was awarded a 2-year NSF postdoctoral fellowship to study plant biochemistry at Louisiana State University in Baton Rouge, LA. In 1993, he accepted a position as a tenure-track, Assistant Professor of Biochemistry at the University of North Texas (UNT) in Denton, TX. During the last 20+ years, Chapman has developed an internationally-recognized research program in plant biochemistry and cell biology at UNT. Research in the Chapman laboratory is focused mostly in the areas of cellular signaling pathways and lipid storage. The Chapman lab has contributed more than 100 publications to the primary plant biology and biochemistry literature, and new ideas about the evolutionary conservation of lipid metabolism and function in eukaryotes have emerged from these efforts. Chapman is co-inventor on six patents (issued or pending). In January, 2014, he took leave from UNT for one year to serve as Program Director at the National Science Foundation's Division of Integrative Organismal Systems. Chapman currently holds the title of Regents Professor at the University of North Texas and serves as Executive Editor for the journal, Progress in Lipid Research.

[S2-2] Chemical- and lipid-mediated systemic acquired resistance in plants.

Presenter and Author: Pradeep Kachroo, Department of Plant Pathology, University of Kentucky

Systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of pathogens. SAR involves the generation of a mobile signal at the site of primary infection, which arms distal portions of a plant against subsequent secondary infections. The last decade has witnessed considerable progress and a number of diverse chemical signals contributing to SAR have been isolated and characterized. Among these, salicylic acid (SA) functions in parallel to nitric oxide (NO)- and reactive oxygen species (ROS)-derived signaling leading to SAR. Other chemical signals involved in SAR azelaic acid (AzA) and glycerol-3-phosphate (G3P) function in the NO-ROS branch of the SAR pathway. The plant galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are also required for SAR and of these DGDG contributes to plant NO as well as SA biosynthesis. In contrast, MGDG regulates the biosynthesis of the SAR signals AzA and G3P. Interestingly, replacement of galactose sugar with glucose in DGDG is unable to restore SAR in *dgd1* plants even though it does rescue their morphological and fatty acid phenotypes. These results suggest that MGDG and DGDG are required at distinct steps and function exclusively in their individual roles during the induction of SAR. Relationship among recently identified mobile inducers of SAR will be discussed.

Pradeep Kachroo conducted his Ph.D. research on transposable elements in the rice blast pathogen Magnaporthe grisea. He received his degree from Maharaja Sayajirao University of Baroda, India and from the University of Wisconsin-Madison. He was a Research Associate with Professor Dan Klessig at the Boyce Thompson Institute, NY until his appointment as Assistant Professor at the Department of Plant Pathology in September 2002.

[S2-3: Neish Award Speaker] Abe Koo, Department Biochemistry, University of Missouri. Metabolism of plant hormone jasmonate: A sentinel for tissue damage and master regulator of stress response.

Abraham (Abe) Jeong-Kyu Koo did his undergraduate and Master of Science studies at Korea University in South Korea. He moved to Michigan State University (MSU) in 1999 to do his PhD under Dr. John Ohlrogge where he studied lipid metabolism in higher plants and wrote a dissertation on topics of fatty acid trafficking across the chloroplast membranes. Upon receiving his PhD degree in 2004, 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. A Phytochemistry review article that he had co-authored with his postdoctoral mentor was later awarded the Top Five Most-Cited Papers Award (from 2009 through 2013) by the journal publisher. He was also awarded the Anton Lang Memorial Award for Research Excellence by the DOE-PRL (2010) and in the same year was promoted to research assistant professor in the DOE-PRL. In 2012, he moved to University of Missouri to start his independent group as tenure-track assistant professor in the Division of Biochemistry. Since then he has been engaging in mentoring and leading his own research group as well as participating in departmental teachings. He has served in various committees and also served as ad-hoc reviewer for multiple journals including Nature Chemical Biology, Plant Cell,

Plant Journal, Plant Physiology, and Phytochemistry.

[S2-4] Transcriptome Analysis of Avocado Mesocarp Reveals Key Genes Necessary to Improve Oil Yield

Presenter(s): Aruna Kilaru, East Tennessee State University

Author(s): Xia Cao, Bayer CropSciences, Parker B. Dabbs, East Tennessee State University, Ha-Jung Sung, East Tennessee State University, Mahbubur Md. Rahman, East Tennessee State University, Keithanne Mockaitis, Indiana University, John B. Ohlrogge, Michigan State Univer

Avocado is an economically important crop with ~70% oil in its fruit tissue, which is an essential component of human diet. The steady increase in global demand for avocado production (9%/year) has drawn attention to the importance of understanding the genetic regulation of triacylglycerol (TAG) accumulation. Using RNA-seq approach, mesocarp-specific regulation and biosynthesis of TAG in developing avocado fruit was analyzed. During the period of TAG accumulation in the mesocarp, an increased expression was noted for genes mostly associated with hexose metabolism in plastids, including pyruvate kinase, relative to cytosol, which is likely associated with the need for higher pyruvate flux directed toward plastid fatty acid synthesis. A corresponding increase in expression for plastidial fatty acid synthesis genes was also noted but not for TAG assembly genes. Additionally, WRINKLED1 (WRI1), a regulatory element typically associated with seed oil biosynthesis, was also highly expressed in oil-rich mesocarp of avocado, along with two other isoforms of WRI. Transcriptomics also revealed that multiple acyltransferases that participate in rate-limiting step in TAG synthesis might be active concomitantly in mesocarp to achieve higher levels of TAG accumulation. Similar observations were previously made with transcriptome analysis of oil-rich seed and non-seed tissues. Together these data suggest a ubiquitous role for WRI1 and that a major point of regulation of oil biosynthesis in oil-rich mesocarp tissue most likely occurs at the level of source and not sink. Overall, this study provides a foundation for functional genomics required to direct metabolic engineering efforts to enhance avocado oil yield.

[S2-5] Identification, and Heterologous Expression Analysis of Avocado DGAT1 and DGAT2

Presenter(s): Md Mahbubur Rahman, East Tennessee State University at Johnson City

Author(s): M. M. Rahman[1], J. Shockey[3], A. Kilaru[1,2]

[1]Department of Biological Sciences, [2]Department of Biomedical Sciences, East Tennessee State University, Johnson City, TN 37614, USA, [3]USDA-ARS, New Orleans, LA 70124, USA

The neutral lipid triacylglycerol (TAG) is the main storage lipid in plants. When stored in seeds, TAG provides the carbon and energy source during germination. There is significant human nutritional demand for vegetable oil, but its use in production of renewable biomaterials and fuels has intensified the need to increase oil production. In plants, the final and committed step in TAG biosynthesis is catalyzed by diacylglycerol acyltransferases (DGAT) and/or a phospholipid: diacylglycerol acyltransferases (PDAT). Both DGAT and PDAT contribute to seed TAG biosynthesis in an independent or overlapping manner, depending on the species. However, in nonseed tissues such as mesocarp of avocado, the regulation of TAG biosynthesis is not well-studied. Based on the transcriptome data of Persea americana it is hypothesized that both DGAT and PDAT are likely to catalyze the conversion of diacylglycerol to TAG. In this study, putative DGAT1 and DGAT2 were identified and comprehensive in silico analyses were conducted to determine the respective start codons, full-length coding sequences, transmembrane domains, predicted protein structures and phylogenetic relationships with other known DGATs. These data reveal that the putative DGATs of a basal angiosperm species retain features that are conserved not only

among angiosperms but also other eukaryotes. For further biochemical characterization, the avocado DGATs were expressed in a TAG-deficient yeast strain and lipotoxicity rescue assays were conducted. The complementation of this yeast strain confirmed enzyme activity and supported the possible role of both avocado DGATs in TAG biosynthesis. Future studies will be focused on determining the substrate specificity of DGAT and its role, relative to PDATs in TAG biosynthesis in avocado mesocarp.

[S2-6] Wheat waxes revisited: composition and biosynthesis

Presenter(s): Reinhard Jetter, University of British Columbia

Author(s): R. Jetter, University of British Columbia, R. Racovita, University of British Columbia, A. Aharoni, Weizmann Institute of Science, Z. Wang, Northwest A&F University

Plant leaves, stems, fruits and flowers are covered by a skin consisting of the polyester cutin and waxes. The latter are mixtures of homologous aliphatics that perform a number of crucial functions in protecting the plant against biotic and abiotic stress. Although the waxes of various wheat cultivars had been analyzed before, many compounds in the mixtures remained unidentified. Here, comprehensive analysis of leaf and stem waxes of wheat cv. Bethlehem revealed more than 200 compounds, many of them with novel structures. Based on the characteristic isomer and homolog patterns, biosynthetic pathways leading to the formation of various compound classes could be hypothesized. To test these models, diverse candidate genes were characterized through expression studies, subcellular localization, mutant chemotyping and heterologous expression in various hosts. Several enzymes involved in different wax-forming pathways were identified, and their (partially redundant) roles will be discussed.

[S2-7] Monoterpene acid glucose esters in *Eucalyptus* foliar oil glands

Presenter(s): Samiddhi Lankani Senaratne, The University of Melbourne, Australia

Author(s): Samiddhi L. Senaratne, Jason Q. D. Goodger, Ian E. Woodrow, School of BioSciences, The University of Melbourne, Victoria 3010, Australia

Monoterpene acid glucose esters (MAGEs) are a group of non-volatile plant secondary metabolites, composed of a monoterpene acid esterified to glucopyranose. A growing number of MAGEs have been isolated from different plant species, but they appear to be particularly prevalent in trees of the genus *Eucalyptus*. *Eucalyptus* is rich in species diversity and eucalypt leaves are well known for the presence of a vast range of secondary metabolites including their characteristic terpene essential oils. Non-volatile resinous materials including MAGEs co-occur with the volatile essential oil components of eucalypts in sub-dermal embedded foliar secretory cavities, commonly known as oil glands. Despite the high species diversity and the predominance of MAGEs, relatively few species have been studied in great detail, particularly with respect to the chemistry of the non-volatile compounds in their foliar oil glands. Our group conducted a survey of 30 *Eucalyptus* species from subgenus *Symphyomyrtus* and subgenus *Eucalyptus* to determine the presence and ubiquity of MAGEs in their leaf glands. A number of known MAGEs were identified based on their characteristic mass fragmentation patterns using ESI-LCMS. In addition, several novel MAGEs were also present in some species. The results show that MAGEs are the most abundant non-volatile compounds in eucalypts in the subgenus *Symphyomyrtus*, but this appears not to be the case in subgenus *Eucalyptus*. The distribution pattern of MAGEs in the two subgenera is likely to assist in the identification of taxonomical relationships in the genus *Eucalyptus*. Moreover, the discovery of new non-volatiles in the genus may help inform biosynthetic studies and possibly open

up avenues for the development of novel natural products.

[S2-8] K+ Dependent and K+ Independent Asparaginase from Common Bean (*Phaseolus vulgaris*): Mechanism of Activation by K+

Presenter(s): Ebenezer Ajewole, University of Western Ontario

Author(s): Ebenezer Ajewole[1,2], Agnieszka Pajak[2], Mariusz Jaskolski[3,4] and Frédéric Marsolais[1,2]

[1]Department of Biology, University of Western Ontario, London, ON, N6A 5B7, Canada [2]Genomics and Biotechnology, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, ON, N5V 4T3, Canada

[3]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland [4]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland

Asparagine is broadly known to be the major nitrogen (N) transport and storage form in most plants, especially legumes. Catabolism of L-asparagine provides N for protein biosynthesis in developing plant tissues. The enzyme, L-asparaginase, is responsible for the catalytic deamidation of L-asparagine into L-aspartate and ammonia, which is necessary for further transformation into other amino acids. Previous studies showed that there are two isoforms of the hydrolytic enzyme (potassium dependent - PvASPG1 and potassium independent asparaginase - PvASPG-T2) as a result of their K+ requirements for activation. The crystal structure of the potassium dependent asparaginase (PvASPG1) suggested that Ser118 in the activation loop of PvASPG1 plays a critical role in coordinating potassium for activation; however, this amino acid residue is replaced by an isoleucine in PvASPG-T2. Reciprocal mutants of the enzymes were created by site directed mutagenesis and the effect of the amino acid residue substitution on the kinetic parameters, optimum pH and K+ dependence of the wild type and mutant enzymes were studied, both in the presence and absence of potassium. There appears to be a catalytic switch on enzyme's dependence on K+ for activation, while activity was optimum at pH 7.5 for both enzymes. The extent of conformational changes, stability and binding affinities of the enzyme as a result of the point mutation is also being investigated. This study seeks to elucidate the mechanism of catalytic activation of the plant-type asparaginase by K+.

Monday, August 10, Morning

Symposium 3: Phytochemical Biosynthesis

Chairs: Li Tian and De-Yu Xie

[S3-1] Regulation of sesquiterpene biosynthesis in plants.

Presenter(s): Xiao-Ya Chen, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Science

Author(s): Xiao-Ya Chen[1,2], Chang-Qing Yang[1]

[1] National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

[2] Plant Science Research Center, Shanghai Chenshan Botanical Garden, Shanghai 201602, China

Plant synthesizes a wealth of secondary metabolites, among which terpenoids form the largest group. Sesquiterpenes, derived from the 15-carbon farnesyl diphosphate, play important roles in plant adaptability to environments, particularly in protecting plants from herbivores and pathogens. We are interested in sesquiterpene biosynthesis in cotton (*Gossypium* spp.), *Arabidopsis* and some medicinal plants, and the role of phytochemicals in plant-insect interactions and co-evolution.

The phytohormone jasmonate (JA) plays an important role in regulating secondary metabolism. In *Arabidopsis* the bHLH transcription factor MYC2 is a key component of JA signaling pathway. Interestingly, we

found that, in *Arabidopsis* inflorescence, MYC2 integrates both gibberellin (GA) and JA signals in regulation of expression of terpene synthase genes, leading to inducible biosynthesis and release of volatile sesquiterpenes and monoterpenes. Another group of JA-responsive transcription factors, AP2/ERF, is also involved in regulating sesquiterpene biosynthesis in *Artemisia annua*.

In addition to response to environmental factors, secondary metabolism also changes during plant development and growth. Our recent study of *Pogostemon cablin* demonstrated that the miR156-targeted SPLs directly regulate sesquiterpene synthase genes, linking the plant age cue to the elevated accumulation of patchouli oil in mature plant. Over-expression of *MIR156* gene nearly abolished and, on the contrary, expression of a miR156-resistant SPL gene promoted the biosynthesis of sesquiterpenes. This provides a strategy to engineer plant for accelerated growth and enhanced production of secondary metabolites simultaneously.

*We thank Zong-Xia Yu, Gao-Jie Hong and Ying-Bo Mao for their contributions.

Xiao-Ya Chen is Professor at the Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences. He currently serves as President of Chinese Society of Plant Biology and Director of Shanghai Chenshan Plant Science Research Center. He obtained B.Sc. (1982) from Nanjing University, China, and Ph.D. (1985) from Reading University, UK. Dr. Xiao-Ya Chen is interested in plant secondary metabolism, cotton biology and biotechnology. He has made important contributions in the investigation of biosynthesis of plant terpenoids (cotton, *Arabidopsis* and selected medicinal plants), transcription factors and hormone signaling pathways regulating secondary metabolism, the role of plant secondary metabolites in plant-insect interactions, plant-mediated RNAi technology for insect pest control, and cotton fiber development with emphasis on key transcription factors. Chen was elected member of Chinese Academy of Sciences in 2005, member of The World the Academy of Science (TWAS) in 2008, and received HLHL Foundation Prize for Scientific and Technological Progress in 2008. He currently serves as Editor-in-Chief for *Science Bulletin* and Associate Editor for *Plant Biotechnology Journal*.

[S3-2] *Valeriana officinalis* as a novel platform for plant natural product drug discovery.

Presenter: Joe Chappell, Department of Plant & Soil Science, University of Kentucky

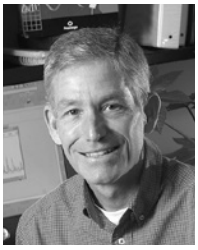
Authors: Santosh Kumar[1], Scott Kinison[1], Eric Nybo[1], Vincent Ricigliano[2], Christopher Brooks[2], Dianella G. Howarth[2], Joe Chappell[1]

[1]Dept. of Pharmaceutical Sciences, University of Kentucky, Lexington, KY

[2]Dept of Biological Sciences, St. John's University, Jamaica, NY,

Valerian is a nutraceutical preparation from the roots of *Valeriana officinalis* that is commonly recommended for relief of tension, anxiety and insomnia. The greatest biological efficacy of valerian has been correlated with freshly harvested and carefully dried root preparations, and with the iridoid alkaloid and sesquiterpene content of these preparations. The valepotriates are epoxyiridoid esters with the dominant species being valtrate. Because of putative instability and water insolubility of the valepotriates, some investigations have suggested that the sesquiterpene compounds are more important for the biological activity of valerian. To determine which chemical components of *V. officinalis* are important for its biological activities, we have developed several important capabilities and tools to support this effort. First, we have developed and relied upon transcriptomic and metabolomic resources to identify the genes encoding for

these unique biosynthetic capabilities. Second, the methodology for genetic engineering hairy root cultures of *V. officinalis* having diverse chemical profiles has been developed. Third, we have been working on the development of a novel test platform for assessing the anxiolytic activity of the various engineered hairy root culture lines. Progress in all of these areas will be presented.



Joe Chappell's laboratory is dedicated to understanding the mechanisms that plants use to make the dizzying array of terpene/isoprenoid compounds. For many years, and like many laboratories, we focused our attention on how plants regulate the biosynthesis of antimicrobial terpene-based phytoalexins. Our interests have expanded from there. Our work utilizes a wide range of experimental strategies including genetic engineering, structure-function comparisons of genes and proteins, and cross-comparisons between many different plants and other organisms.

[S3-3] Structure and function of flavonoid glucosyltransferases: using a specific grapefruit enzyme as a model.

Presenter and Author: Cecilia A. McIntosh, Department of Biological Sciences, School of Graduate Studies, and Institute for Quantitative Biology, East Tennessee State University, Johnson City, TN 37614, USA

Flavonoids are a major group of ubiquitous plant natural products and the majority are found natively in glycosylated forms. They have significant roles in the plant life cycle and also have impact on consumer acceptance and health properties of food crops and processed products. Secondary product glycosyltransferases are key enzymes in the biosynthesis of the compounds that accumulate in plant tissues. Results from our studies to elucidate the dynamics of flavonoid metabolism and regulation in grapefruit revealed that, in addition to other factors, properties of the GT enzymes themselves have significant impact on the compounds found. We have characterized several enzymes isolated from grapefruit leaf tissue and expressed from grapefruit putative GT clones. One of the clones codes for a flavonol-specific 3-*O*-GT, CpF3*O*GT. Unlike many flavonoid 3-O-GTs, this enzyme does not glucosylate anthocyanidins. There are some reports of more promiscuous flavonoid GTs from other plants, including those that add sugars to multiple positions. In order to study CpF3*O*GT structure and function, we have used bioinformatics and modeling approaches to identify residues for site-directed mutagenesis to test hypotheses related to acceptor substrate specificity and regiospecificity. Over 30 single, double, triple, and more extensive mutants have been synthesized. Characterization of the mutant proteins is ongoing, however the majority have been tested and experimental results used to refine structural models.

Cecilia McIntosh earned her Ph.D. from the University of South Florida in 1990 with Richard Mansell and was a postdoctoral research fellow with Dr. David Oliver at the University of Idaho (1990-93). She joined the faculty of the East Tennessee State University Department of Biological Sciences in 1993 where she is now a full professor. She served as a program director at the National Science Foundation from 2003-04, and was named Dean of ETSU's School of Graduate Studies in 2006. With a lifelong interest in phytochemistry and plant natural products, her research program at ETSU has focused on different aspects of flavonoid biosynthesis and metabolic regulation using *Citrus paradisi* (grapefruit) as the model system. This research has been funded from the U.S. Department of Agriculture and the U.S. National Science Foundation. She has served on multiple advisory boards including the Arkansas ASSET external advisory board and on the technical advisory board for the

Arkansas EPSCoR "P3" project from 2008-13. She is a founding fellow of ETSU's Institute for Quantitative Biology. In addition to her research, she is extensively involved in K-12 student outreach and professional development for graduate students and faculty. She has been active in the PSNA, serving on several annual meeting committees, the awards committee, multiple terms on the advisory board, and as treasurer. She was PSNA president during its 50th anniversary year.

[S3-4] Diversification of Tyrosine Biosynthetic Pathways in Plants: Non-Plastidic, Tyrosine-Insensitive Prephenate Dehydrogenases in Legumes

Presenter(s): Hiroshi A. Maeda, Department of Botany, University of Wisconsin-Madison

Author(s): Hiroshi A. Maeda[1], Craig Schenck[1], Siyu Chen[1]

[1]Department of Botany, University of Wisconsin-Madison, WI 53706 USA.

Tyrosine (Tyr) is an essential aromatic amino acid synthesized de novo by plants and microbes. Besides being a protein building block, in plants Tyr and a Tyr-pathway intermediate 4-hydroxyphenylpyruvate (HPP) serve as key precursors of a diverse array of natural products, such as cyanogenic glycosides, betalains, tocopherols (vitamin E), plastoquinone, and isoquinoline alkaloids. Tyr biosynthesis can occur via two intermediates, arogenate and HPP. Arogenate dehydrogenase (ADH) and prephenate dehydrogenase (PDH) are key enzymes for the arogenate and HPP pathways, respectively, which are generally feedback regulated by Tyr. Most plants only have plastidic ADH activity and likely synthesize Tyr using the arogenate route within the plastids, whereas both ADH and PDH activity was detected in some legume species. However, the genes encoding enzymes responsible for the plant PDH activity are unknown. Here we report identification and biochemical characterization of PDH enzymes from soybean and Medicago. Comparative genomics and phylogenetic analyses together with recombinant enzyme characterization identified a legume-specific monophyletic clade that contains PDH enzymes. Kinetic analyses revealed that soybean and Medicago PDHs specifically use prephenate but not arogenate substrate. Unlike previously-characterized plant ADHs, both legume PDHs were insensitive to Tyr regulation and localized to the cytosol. Our study provides molecular evidence for the diversification of the primary metabolic Tyr pathway, likely to support the chemodiversity that exists downstream of HPP and Tyr in different plant species.

[S3-5] Evolution of chemical diversity in plants

Presenter and Author: Bernd Markus Lange, Washington State University

The ability to sequester secondary (or specialized) metabolites and defense proteins in secretory structures was a critical adaptation that has shaped plant-herbivore and plant-pathogen interactions for hundreds of millions of years. Secretory structures in terrestrial plants appear to have first emerged as intracellular oil bodies in liverworts. In vascular plants, internal secretory structures, such as resin ducts and laticifers, are usually found in conjunction with vascular bundles, while subepidermal secretory cavities and epidermal glandular trichomes generally have more complex tissue distribution patterns. An overview of the evolution of these secretory structures, and the chemical diversity found within them, will be presented. Although particular emphasis is placed on describing the evolution of pathways leading to terpenoids, the emergence of other metabolites is assessed as well to outline metabolic capabilities of different plant lineages. Online resources to capture and interrogate plant chemical diversity and biosynthetic pathways will be assessed as well.

Recent publications on the topic:

• Turner G.W., Lange B.M. (2015) Ultrastructure of grapefruit secretory cavities and immunocytochemical localization of

(+)-limonene synthase. Int. J. Plant Sci., in press.

• Lange B.M. (2015) The evolution of plant secretory structures and emergence of terpenoid chemical diversity. Annu. Rev. Plant Biol. 66, 139-159.

• Fischedick J.T., Johnson S.R., Ketchum R.E.B., Croteau R.B., Lange B.M. (2015) NMR spectral search module for Spektraris, an online resource for plant natural product identification—taxane diterpenoids from Taxus x media cell suspension cultures as a case study. Phytochemistry 113, 87-95.

• Johnson S.R., Lange B.M. (2015) Open-access metabolomics databases for natural product research: present capabilities and future potential. Frontiers Bioeng. Biotechnol. 3, 22.

[S3-6] Investigations on early steps of hydrolyzable tannin biosynthesis

Presenter(s): Li Tian, University of California, Davis

Author(s): Li Tian, Nadia N. Ono, Xiaoqiong Qin and Alexander E. Wilson, University of California, Davis

Hydrolyzable tannins (HTs) are structurally diverse polyphenolic compounds derived from a shikimate pathway intermediate. This group of metabolites has been studied for their function in plant-plant interactions, defensive role against insects and herbivores, and impact on soil nutrient profiles. More recently, much attention has been given to these compounds due to their health promoting activities in humans. Although previous biochemical studies have outlined the biosynthetic steps leading to the production of HTs, the biosynthetic genes and enzymes of the pathway have yet to be identified. We chose pomegranate as our model system for interrogation of the HT biosynthetic pathway. Our transcriptomic sequencing of pomegranate fruit peel, a tissue enriched in HTs, revealed multiple candidate genes that are potentially involved in HT formation. We have also established a pomegranate hairy root culture system in our lab that allows genetic characterization of candidate HT biosynthetic genes. Our results from transcriptome sequencing, pomegranate hairy root culture, and the biochemical properties of candidate HT biosynthetic enzymes will be presented.

[S3-7] Structural modification and increase of lignin in Oryza sativa for biomass refinery

Presenter(s): Toshiaki Umezawa, Kyoto University

Author(s): Yuri Takeda, Kyoto University, Taichi Koshiba, Kyoto University, Yuki Tobimatsu, Kyoto University, Masaomi Yamamura, Kyoto University, Masahiro Sakamoto, Kyoto University, Toshiyuki Takano, Kyoto University, Shiro Suzuki, Kyoto University, Takefumi Hattor

Lignin has long been considered recalcitrant in the polysaccharide utilization processes such as pulping and bioethanol fermentation. Lignin is, however, a potential feedstock for aromatic products. Hence, structural modification to produce lignins that are suitable for lignin utilization as well as higher lignin production in biomass plant species are important breeding objectives. Herein, we aimed to develop transgenic rice plants which have lignin with modified structures by the use of metabolic engineering of lignin biosynthesis. We produced a transgenic rice plant in which *Oryza sativa* ferulate 5-hydroxylase 1 (OsF5H1 or OsCAld5H1) was downregulated by RNAi technique. The OsCAld5H1-knock down did not significantly alter the total lignin depositions but severely decreased syringyl (S) lignin content in rice cell walls; reduced S lignin levels in the transgenic lines were compensated with increased guaiacyl (G) and p-hydroxyphenyl (H) lignin levels. Lower S/G ratio contributes an increase of heating values of biomass, because G lignin has higher carbon content than S lignin. In addition, we produced transgenic rice plants with increased lignin content. We selected several genes which were predicted to be involved in lignin biosynthesis. These genes were overexpressed under the control of CaMV35S promoter in rice, and we obtained transgenic rice T1 lines with about

1.5-fold lignin contents in culms compared to control wild-type plants, which was estimated to contribute about 7% increase of heating value. This strategy may be applicable to lignin upregulation in large-sized grass biomass plants, such as Sorghum, Miscanthus and Erianthus, which are suitable for direct fuel utilization of biomass.

Monday, August 10, Afternoon

Symposium 4: Chemical Ecology I

Chairs: Dorothea Tholl and Massuo Kato

[S4-1] Honey bees as phytochemists: how pollen and nectar are converted into beebread and honey

Presenter and Author: May R. Berenbaum, Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-3975

The western honey bee (*Apis mellifera*) and its congeners are unusual among pollinators in that they rarely consume raw pollen and nectar; rather, they process nectar into honey and pollen into beebread, which are stored in the hive and shared among nestmates. This processing allows honey bees to convert raw materials into forms that can be stored in large quantities for long periods of time without spoilage. The perennial nature of honey bee colonies necessitates collecting pollen and nectar over many months from a broad diversity of successively blooming flowers, exposing them to hundreds of different phytochemicals. Moreover, by concentrating nectar to make honey, reducing its water content from about 90 percent to 18-20 percent, honey bees also increase the relative concentrations of phytochemicals in their food. Despite the diversity of floral sources utilized to support the eusocial life style and the concomitant exposure to a tremendous diversity of phytochemicals, the honey bee genome is markedly reduced in terms of its inventory of detoxification genes. The uniquely complex behavioral repertoire of *A. mellifera* may compensate for this deficit via a "social detoxification system," whereby collective behaviors may reduce the phytochemical complexity of the diet. Behavioral mechanisms that may reduce the diversity and quantity of toxins requiring enzymatic transformation include selective foraging, toxin dilution by mixing nectars and pollens from multiple sources, maintenance of high hive temperatures to promote toxin breakdown, enzymatic processing of honey, and culturing fermentative microbes to convert pollen into beebread. Remarkably, many of these mechanisms resemble processing techniques used by humans to reduce the toxic phytochemical content of plant-based foods.

May Berenbaum, Ph.D. has been on the faculty of the Department of Entomology at the University of Illinois at Urbana-Champaign since 1980, serving as head since 1992 and as Swanlund Chair of Entomology since 1996. She is known for elucidating chemical mechanisms underlying interactions between insects and their hostplants, including detoxification of natural and synthetic chemicals, and for applying ecological principles in developing sustainable management practices for natural and agricultural communities. Her research, supported primarily by NSF and USDA, has produced over 230 refereed scientific publications and 35 book chapters. A member of the National Academy of Sciences, she has chaired two National Research Council committees, the Committee on the Future of Pesticides in U.S. Agriculture (2000) and the Committee on the Status of Pollinators in North America (2007). Devoted to teaching and fostering scientific literacy through formal and informal education, she has authored numerous magazine articles and six books about insects for the general public. She graduated summa cum laude, with a B.S. degree and honors in biology, from Yale University in 1975 and

received a Ph.D. in ecology and evolutionary biology from Cornell University in 1980.

[S4-2] Mosquito odorant receptor for the insect repellent DEET and plant-derived semiochemicals.

Presenter and Author: Walter S. Leal, Department of Molecular and Cellular Biology, University of California, Davis, CA 95616 USA

Insect repellents have been used since antiquity as prophylactic agents against diseases transmitted by mosquitoes and other arthropods, including malaria, dengue fever, and encephalitis. They evolved from smoke generated by burning plants (e.g.: lemon gum) and topical applications of essential oils (e.g: lemon eucalyptus extract) into repellent substances, including those isolated from plants (e.g.: p-menthane-3,8-diol, PMD) and a broad-spectrum synthetic repellent *N,N*-dimethyl-3-methylbenzamide (DEET), which was discovered in the early 1940s from a screening of thousands of compounds. Thereafter, other synthetic repellents have been developed (e.g: IR3535, 3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester), but DEET remains the most widely used repellent substance world-wide. Molecular modeling led to the development of picaridin ((*RS*)-*sec*-butyl 2-(2-hydroxyethylpiperidine), but progress in drug design has been slow, in part because of the unknown mode of action. Although it is well established that DEET acts on the insect's gustatory and olfactory systems, its mode of action as an odorant was a matter of considerable debate, with two dichotomous hypotheses. One school suggests that DEET modulates the activity of many odorant receptors (ORs), whereas there is growing evidence suggesting that repulsion is triggered by excitatory responses of odorant receptor neurons and that OR are involved. As adult mosquito ORs remained elusive, it has been postulated that the ionotropic receptor IR40a could account for the widespread effect of DEET olfactory repellency. To thoroughly test these hypotheses, we carried out molecular, behavioral, and electrophysiological studies mostly with the vector of West Nile virus in California, the southern house mosquito, *Culex quinquefasciatus*. Here, we report on a DEET receptor, which is also sensitive to plant-derived semiochemicals.

Walter Leal's laboratory research is aimed at unraveling the molecular mechanisms that make the insect's olfactory system so sensitive and selective. Insect prominence among other animals is due in large part to a key physiological element for their survival and reproduction—a refined olfactory system. Olfaction is orchestrated at various levels starting with reception of odorants at the periphery, processing and integration of olfactory and other sensory modalities in the brain, and ultimately translation of olfactory signal into behavior. Thus, the cornerstone of a sophisticated olfactory system is the ability of the insect's peripheral system to selectively detect and rapidly inactivate minute amounts of odorants. Reception of odorants is mainly mediated by three olfactory proteins, namely, odorant-binding proteins (OBPs), odorant receptors (ORs), and odorant-degrading enzymes (ODEs). OBPs are involved in the uptake, transport and delivery of odorants to ORs. By using biochemical, electrophysiological, RNAi, and kinetic studies we have demonstrated that OBPs are essential for the sensitivity of moth's olfactory system. In collaborations with structural biologists, including UC Davis colleagues, we study the molecular mechanisms of odorant binding, release, and transport, including novel, pH-dependent conformational changes in moths and mosquitoes. By comparative kinetic studies of odorant degradation by recombinant and native enzymes we have demonstrated for the first time that ODEs are involved in the fast inactivation of odorants. Using bioinformatics and molecular approaches coupled with the Xenopus oocyte recording system we

investigate how ORs contribute to the selectivity of the insect's olfactory system.

[S4-3: Neish Award Speaker] Climate change and crop phytochemical defenses: Potential implications for food security and food safety.

Presenter: Martha Vaughan, USDA, ARS, Peoria, IL

Authors: Martha M. Vaughan, Alisa Huffaker, Eric A. Schmelz, Shawn Christensen, Leon Hartwell Allen, Peter E. A. Teal, USDA, ARS, Peoria, IL

Elevated atmospheric carbon dioxide concentration ([CO_2]) increased maize susceptibility to *Fusarium verticillioides* stalk rot. Even though the pathogen biomass accumulated to significantly higher levels at double ambient [CO_2] (2x[CO_2]), the projected [CO_2] concentration to occur at the end of this century, the quantity of fumonisin mycotoxin contaminants were unaltered. Combined effects of [CO_2] and drought on pathogen infection were contradictory; drought reduced *F. verticillioides* biomass in maize stalks at ambient [CO_2] (1x[CO_2]), but increased pathogen biomass in stems at 2x[CO_2]. In the absence of the host plant, 2x[CO_2] hindered *F. verticillioides* growth but enhanced fuminison production on media. The soluble carbohydrate, starch, protein and fatty acid content of the maize stem tissues were not significantly altered by 2x[CO_2]. However, in response to *F. verticillioides* infection, the accumulation of soluble carbohydrates, total proteins, fatty acids, MBOA and phytoalexins was absent or reduced in maize plants at 2x[CO_2]. Examination of early JA levels in response to *F. verticillioides* infection, indicated that the phytohormone defense response maybe dampened at 2x[CO_2]. There was a direct correlation between JA levels and the levels of defense metabolites, and an inverse correlation with pathogen proliferations. The attenuation of JA signaling and the reduction of fumonisin produced per unit *F. verticillioides* biomass are potentially a result of limited free fatty acid substrate for oxylipin biosynthesis. By stimulating the defense response prior to *F. verticillioides* infection, maize plants grown at 2x[CO_2] were capable of restricting pathogen proliferation to comparable levels as maize at 1x[CO_2]. The adaptation of new disease management strategies, such as priming, may be necessary to improve the production and safety of the future maize crop at higher [CO_2].

Martha Vaughan is a molecular biologist with interdisciplinary training in plant stress physiology, defense signaling, and secondary metabolism. Her research focuses on how climate affects crop-fungal pathogen interactions in a manner that influences downstream mycotoxin production and grain contamination. She has expertise in evaluating the response of plants to combined abiotic and biotic stress factors both above and belowground. Her work has led to the identification, molecular characterization and functional elucidation of novel defense metabolites. She has contributed to the understanding of how climate changes will effect crop susceptibility to Fusarium pathogens and mycotoxins. She has also been involved in the development of methods necessary to conduct climate change research including a plant culturing method that enables long term plant growth at a consistent level of drought. Through the evaluation of phenomena that naturally regulate mycotoxin production, such as phytochemicals, she seeks to identify sustainable and climate resilient strategies to eliminate mycotoxin contamination in grain and enhance food safety.

As a born and bred Hokie, Martha received both her BSc (2004) and PhD (2010) from Virginia Tech. She then conducted postdoctoral research with the USDA-ARS Chemistry Unit at the Center for Medical, Agricultural and Veterinary Entomology in Gainesville FL. In 2013, she joined the Bacterial Foodborne Pathogens & Mycology Research Team at the National Center for Agricultural Utilization Research in Peoria, IL to pursue research on

the effects of climate change on mycotoxin contamination of grain crops.

[S4-4] Metabolomics of juniper detoxification in a generalist and specialist mammalian herbivore.

Presenter(s): Katharina Schramm, University of Utah

Author(s): Michele Skopec, Weber State University, James Cox, University of Utah, James Halpert, University of Connecticut, Denise Dearing, University of Utah

Herbivory is common in mammals, yet our understanding of the detoxification processes used by mammals to biotransform plant secondary compounds (PSC) is limited. Specialist herbivores are thought to have evolved unique detoxification mechanisms to process high levels of PSC in their diets. We explored this hypothesis by comparing the detoxification metabolite patterns of a specialist and a generalist herbivore from the genus Neotoma. Stephen's woodrat, *N. stephensi*, is a specialist on one-seeded juniper (*Juniperus monosperma*). The desert woodrat, *N. lepida*, is a generalist across its range; yet populations in the Great Basin often feed on a diet predominantly made up of juniper (*J. osteosperma*). While both woodrat species are naturally exposed to high levels of terpenes (the predominant class of PSC in juniper) the terpene profiles and quantities differ between the two juniper species.

Individuals from both woodrat species were fed diets of each juniper in a cross-over design. Urine, collected over a 24h period, was extracted and analysed in an untargeted metabolomics approach by both GC-MS and HPLC-MS/MS. When feeding on *J. osteosperma*, the native diet of *N. lepida*, both woodrat species exhibited very similar patterns of metabolites. On *J. monosperma*, native diet of *N. stephensi*, the profiles were distinctly different between species. These results suggest that the species used different metabolic pathways to biotransform *J. monosperma*-PSC. Species-specific differences also existed with respect to glucuronic acid conjugation. Our results suggest that the specialist *N. stephensi* uses different detoxification pathways when feeding on its native juniper compared to *N. lepida*, whereas both woodrat species use the same pathways when feeding on the native host of *N. lepida*, *J. osteosperma*.

[S4-5] Detoxification of Maize Chemical Defenses by Lepidopteran Herbivores

Presenter(s): Felipe Christoff Wouters, Max Plank Institute for Chemical Ecology

Author(s): Daniel Giddings Vassão, Michael Reichelt, Katrin Luck and Jonathan Gershenzon, Max Plank Institute for Chemical Ecology, and Matthias Erb, Universtity of Bern

In order to avoid or overcome damage by herbivores, grasses (Poaceae) such as rye, wheat, and maize rely on a family of indole-derived chemical defenses called benzoxazinoids (BXDs). These compounds are stored in plant cells as stable glucosides and, upon herbivory, are converted into toxic aglucones by the action of plant glucosidases. However, some herbivores evolved adaptations that enable them to feed on well-defended plants, leading to the emergence of some species as serious agricultural pests. Although BXDs biosynthesis in plants is well studied, little is known about their metabolism in insects. In this context, we aim to better understand the strategies used by lepidopteran herbivores to avoid toxicity from BXDs.

Comparing lepidopteran herbivores feeding on maize, we observed that *Spodoptera frugiperda, S. littoralis* and *S. exigua* excrete the non-toxic DIMBOA-Glc (the most abundant BXD in maize) in the frass. We performed in vitro assays with gut tissue preparations and confirmed that this compound is result of enzymatic reglucosylation of DIMBOA by the insect. Surprisingly, the compound produced by the insect is (2S)-DIMBOA-Glc, an epimer of the plant-derived (2R)-DIMBOA-Glc. We demonstrate that plant glucosidases are not able to hydrolyze the insect metabolite, thus preventing it from exerting its toxicity after it

is transformed by the insect. Therefore, we identified the reglucosylation of DIMBOA with inversion of stereochemical configuration as a detoxification mechanism employed by *Spodoptera* species. Moreover, we present our ongoing work on identifying the UGT enzymes relevant for BXD detoxification in *S. frugiperda*, based on a transcriptomic approach, and how we plan to further explore BXD metabolism through feeding assays using radiolabeled BXDs. Collectively, the elucidation of BXD metabolism in insects clarifies their coevolution with plants and provides targets for innovative approaches in ecological and agricultural research.

[S4-6] Toxicity of glucosinolate-derived isothiocyanates to generalist-feeding caterpillars

Presenter(s): Verena Jeschke, MPI for Chemical Ecology

Author(s): Verena Jeschke, Emily E. Kearney, Jonathan Gershenzon and Daniel G. Vassão, MPI for Chemical Ecology

Glucosinolates are major plant defense compounds in the order Brassicales (e.g. cabbage, broccoli, mustard). Upon tissue damage caused e.g. by chewing herbivores, the non-toxic parent glycosides are transformed into toxic isothiocyanates (ITCs) and other metabolites. Still, generalist-feeding insect herbivores sometimes feed successfully on glucosinolate-containing plants. Previous studies in our group revealed that a proportion of ITCs is metabolized to glutathione conjugates; yet, a large amount is excreted in unmodified form [1]. These unmodified ITCs cause decreased rates of growth and delayed development. However, the mode of action of these nucleophilic and lipophilic toxins in caterpillars is poorly understood.

We compared the effects of Met- and Trp-derived glucosinolates, which lead principally to ITC and non-ITC hydrolysis products, respectively, on two generalist-feeding caterpillars in the laboratory. The development of larvae of *Spodoptera littoralis* (African cotton leafworm) and *Mamestra brassicae* (cabbage moth) was investigated from hatching until adult emergence while the larvae were reared on *Arabidopsis thaliana*, accession Col-0, and three different glucosinolate-deficient mutants. We found that both glucosinolate types negatively affected larval development when fed separately, but in combination their effect was significantly stronger. To our surprise, larvae fed on glucosinolates gave rise to heavier pupae and adults. Using artificial diets, we conducted detailed investigations on the effect of the aliphatic 4-methylsulfinylbutyl-ITC (sulforaphane) on the the biochemistry and metabolism of *S. littoralis* larvae. The most typical effect was the decrease of glutathione in midgut tissue and hemolymph, likely due to losses by conjugation to ITC during detoxification. As a consequence, the levels of free amino acids were altered, with a sharp decrease in cysteine leading to reductions in protein content, but an increase in lipids. To learn more about the mechanism of glucosinolate toxicity, we are now performing a comprehensive study of the conjugation of ITC to the proteome in vivo.

[1] Schramm K, Vassão DG, Reichelt M, Gershenzon J, Wittstock U (2012) Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. Insect Biochemistry and Molecular Biology 42, 174-182.

[S4-7] Gene-to-Terpene Landscapes in Self-pollinating *Artemisia annua*

Presenter(s): De-Yu Xie, Department of Plant and Microbial Biology, North Carolina State University

Author(s): De-Yu Xie[1], Dong-Ming Ma[1], Fatima Alejos[1], Zhilong Wang[1], Liangjiang Wang[2] and Ming-An Sun[3]

[1]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA, [2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA, [3]Virginia Bioinformatics Institute, Virginia Tech, VI, USA

Artemisia annua is an antimalarial medicinal plant that saves more than 100 million of people annually. Here, we report to use an integrated approach of metabolomics,

transcriptomics and gene function analysis to characterize gene-to-terpene scenarios in different tissues of a self-pollinating variety. Fifty-one identified terpenes were differentially produced by leaves and flowers from lower to higher positions. 18,871 contigs were assembled from sequences of six cDNA libraries. Sequence mining identified forty-seven genes that were mapped and integrated to networks composed of the artemisinin, non-amorphadiene sesquiterpene, monoterpene, triterpene, 2-C-methyl-D-erythritol 4-phosphate (MEP) and mevalonate pathways. Manipulation of genes significantly altered both individual pathway activities and genome-wide terpene profiles. This study provides useful gene-to-terpene landscape data for rational metabolic engineering of artemisinin.

[S4-8] Engineering alfalfa to accumulate useful caffeic acid derivatives and characterization of hydroxycinnamoyl-CoA transferases from legumes

Presenter(s): Michael L. Sullivan, U.S. Dairy Forage Research Center

Author(s): Michael L. Sullivan, U.S. Dairy Forage Research Center and Heather A. Green, U.S. Dairy Forage Research Center

Some forages crops, such as red clover, accumulate high levels of caffeic acid derivatives. Oxidation of these o-diphenols to o-quinones by endogenous polyphenol oxidases (PPOs) and the subsequent reactions of these quinones (probably with endogenous plant proteases) result in a significant reduction in post-harvest protein degradation. Reduced protein loss in forage crops should lead to more efficient nitrogen utilization in ruminant animal production systems, providing both economic and environmental benefits. One of the most commonly grown legume forage crops, alfalfa, does not accumulate PPO-utilizable phenolics. For this reason, we have been working to engineer biosynthetic pathways for accumulation of caffeic acid derivatives in this crop. Having identified a hydroxycinnamoyl-CoA:malate transferase (HMT) in red clover responsible for phaselic acid (caffeoyl-malate) accumulation, we transferred this gene to alfalfa. Leaves of the resulting transgenic alfalfa accumulated mostly p-coumaroyl and feruloyl-malate rather than the desired caffeoyl derivative. Simultaneous down-regulation of endogenous caffeoyl-CoA O-methyl transferase (CCOMT) resulted in increased accumulation of phaselic acid in alfalfa leaves and even higher levels in stems, possibly due to the relatively higher levels of phenylpropanoid pathway enzymes, particularly HCT and C3'H, in this tissue that would normally be involved in monolignol biosynthesis. The levels of phaselic acid produced in these plants should be sufficient to significantly impact post-harvest proteolysis. We are currently testing this via in vitro assays and small-scale ensiling experiments. We have also identified a gene from red clover encoding a hydroxycinnamoyl-CoA transferase responsible for clovamide (caffeoyl-L-DOPA amide) production; a gene from bean encoding a hydroxycinnamoyl-CoA transferase capable of transferring hydroxycinnamoyl moieties to tetrahydroxyadipic acid isomers; and hydroxycinnamoyl-CoA transferase activities from bean and perennial peanut capable of transferring hydroxycinnamoyl moieties to malic and tartaric acid, respectively. Characterization of these enzymes should provide insights into the structure/function relationships of this class of BAHD transferases.

Tuesday, August 11, Morning

Symposium 5: Chemical Ecology II

Chairs: Dhirendra Kumar and De-Yu Xie

[S5-1] Metabolic tag in the cornfield: Biosynthesis and activation of benzoxazinoids by corn plants and deactivation by herbivores.

Presenter: Jonathan Gershenzon, Department of Biochemistry, Max Planck Institute for Chemical Ecology

Authors: Vinzenz Handrick, Felipe C. Wouters, Christelle A.M. Robert, Tobias G. Köllner, Daniel Giddings Vassão, Georg Jander, Matthias Erb and Jonathan Gershenzon

Max Planck Institute for Chemical Ecology, Jena, Germany

Boyce Thompson Institute for Plant Research, Ithaca, New York

A major group of phytochemicals in corn and other grasses are the benzoxazinoids (BXDs), a group of indole-derived, hydroxamic acid derivatives that are produced in plants as glucosides. The best known of these substances, DIMBOA, has a wide range of biological properties, including anti-insect, antibiotic and allelopathic activities. While the core pathway of BXD biosynthesis is well-studied, little is known about the steps leading to a number of derived structures whose occurrence is induced by herbivore damage. We have discovered a number of genes involved in these later steps by quantitative trait locus mapping and transcriptomic methods, and characterized the corresponding enzymes after heterologous expression. Among these are several O-methyltransferases and a 2-oxoglutarate-dependent dioxygenase. Maize inbred lines that are naturally mutated in these genes generally show increased herbivore damage suggesting that the inducible, late pathway BXD metabolites have roles in herbivore defense. Curiously, an inactive copy of one of these genes leads to plants that are more susceptible to caterpillar damage, but more resistant to aphids due to increased callose deposition. This trade-off indicates a role for BXDs not only as direct toxins but also as signals for other defensive responses.

Herbivores that feed readily on maize foliage must possess some type of metabolic or behavioral adaptation to avoid BXD toxicity, which is based on hydrolysis of the glucose residue by a plant β-glucosidase and reaction of the resulting aglycone with nucleophilic targets in the herbivore. We investigated the metabolism of BXDs by the fall armyworm (*Spodoptera frugiperda*), a major pest of corn and other small grains in North and South America. After BXDs are activated by hydrolysis mediated by plant β-glucosidases, the armyworm larvae were found to re-glucosylate them in their guts using a glucosyltransferase. Although the plant β-glucosidase also present in the gut could in theory rehydrolyze and reactivate the new glucoside, the herbivore actually creates a glucoside of opposite configuration to the plant compound which can no longer be activated by the plant enzyme. This metabolic trick appears to be the critical adaptation allowing the fall armyworm to feed on maize without negative consequences.

After studying biology as an undergraduate at the University of California in Santa Cruz, **Jonathan Gershenzon** received his PhD in botany from the University of Texas in 1984. From 1985 until 1997 he worked as a scientist at the Institute for Biological Chemistry, Washington State University in Pullman. Since 1997 he is a Director and Scientific Member at the Max Planck Institute for Chemical Ecology in Jena, Germany, where he heads the Department of Biochemistry. He was appointed Honorary Professor at Friedrich Schiller University Jena in 1999.

Gershenzon studies the biochemistry of secondary plant metabolites, their mode of action on herbivores, the regulation of secondary metabolisms in plants and the

evolution of pathways. Most of the work in his department focuses on two major groups of plant defenses: glucosinolates and terpenoids.

[S5-2] Inducible small molecule defenses in maize: more than a few surprises.

Presenter and Author: Eric Schmelz, Department of Biological Sciences, University of California at San Diego

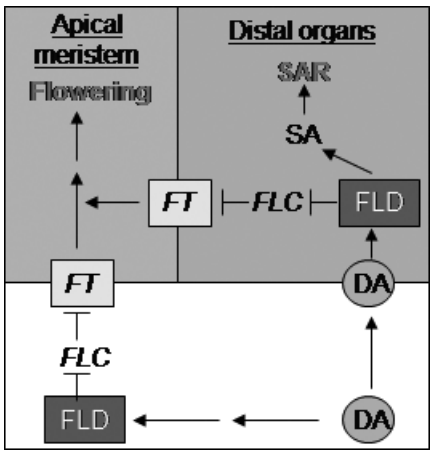
In response to pathogen and insect attack, plants produce an array of small molecule biochemicals that either directly or indirectly function in plant protection. Pathogen inducible transcript profiling in maize *(Zea mays)* reveals a large number of terpene synthases, cytochrome P450s, O-methyl transferases, proteases and numerous proteins of unknown function. Long considered absent in maize, high concentrations of antimicrobial phytoalexins are now known to predominate at the site of infection. As key biosynthetic precursors of zealexin class sesquiterpenoids, ZmTps6 and ZmTps11 encode b-macrocarpene synthases that are broadly elicited by fungi at both the transcript and protein level. With similar regulation, ZmAn2 encodes an ent-copalyl diphosphate synthase required for kauralexins, acidic diterpenoid phytoalexins. Outside of classical phytoalexin activities, zealexins and kauralexins display inducible accumulation in drying roots and are surprisingly associated with drought protection. Pathogen-induced cyclopenten(an)one oxylipins with structural parallels to phytohormones are an additional class of maize phytoalexins. In addition to fungal growth suppression, 9-lipoxygenase derived cyclopentenones are phytotoxic and display partially overlapping transcriptional activities with jasmonates suggesting additional roles as endogenous signals. Despite being among the worlds largest annually harvested crops, recent findings indicate that many key regulatory and multifunctional secondary metabolite pathways in poaceous species remain largely unknown.

Eric Schmelz completed his PhD within the Center for Insect Science/Plant-Insect Interactions Group at the University of Arizona, Tucson. He then carried out his postdoctoral and research scientist studies at the USDA-Agricultural Research Service located at the University of Florida, Gainesville. He joined the Division of Biological Sciences faculty at U.C. San Diego in July 2014.

[S5-3] Signaling function for an abietane diterpenoid in plant defense and development.

Presenter and Author: Jyoti Shah, Department of Biological Sciences, University of North Texas, Denton, Texas

Abietane diterpenoids, which are major constituents of conifer resins, have industrial and medicinal applications. They are also found in flowering plants. However, their function in Angiosperms is poorly understood. We recently co-purified dehydroabietinal (DA) with a systemic acquire resistance (SAR) promoting activity that accumulates in the vascular sap of *Arabidopsis thaliana* leaves [1]. Further experiments demonstrated that DA itself was a very potent inducer of plant immunity against pathogens. Local application of picomolar solutions of DA resulted in the systemic induction of salicylic acid (SA) signaling, thereby conferring enhanced disease



resistance. In our efforts to further understand DA signaling, we identified *Arabidopsis thaliana* mutants (*ida*) that are unresponsive to DA. These *ida* mutants are deficient in SAR. In addition, several of these *ida* mutants exhibit a delayed flowering phenotype, thus suggesting that in addition to its role in promoting systemic signaling associated with plant defense, DA may have a role in promoting flowering. The effect of DA on flowering is mediated through promoting expression of *FLOWERING LOCUS D* (*FLD*) and *FT*, both positive regulators of flowering, and the simultaneous repression of *FLC*, an inhibitor of *FLC* expression. As opposed to flowering, the effect of DA on SAR did not involve *FLC* and *FT*, but required FLD [2]. These results suggest that DA, a newly identified signaling molecule, has functions in plant defense as well as development. We propose that DA promoted flowering and defense utilize common components leading to *FLD* expression, beyond which the signaling pathway utilized for promoting SAR and flowering bifurcates [3].

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Jyoti Shah was born in India, where he studied Microbiology at University of Bombay (Mumbai) (BSc 1983; MSc 1985). He started his own research group in the Division of Biology, Kansas State University after his PhD from University of Notre Dame (1991) and postdoctoral work at the Waksman Institute, Rutgers University. He is currently Professor in Biological Sciences at the University of North Texas. His research, which has been funded by the National Science Foundation and the US Department of Agriculture, has centered on signaling in plant defense against pests, and the engineering of defense signaling mechanisms for developing plants that are inherently resistant to pests.

[S5-4] Sarmentine, a natural Piper amide herbicide with multiple mechanisms of action

Presenter(s): Franck E. Dayan, USDA-ARS Natural Products Utilization Research Unit

Author(s): Franck E. Dayan and Daniel K. Owens, USDA-ARS Natural Products Utilization Research Unit, and Ratnakar Asolkar and Louis Boddy, Marrone Bio Innovations

Sarmentine, 1-(1-pyrrolidinyl)-(2E,4E)-2,4-decadien-1-one, is a natural product isolated from the fruits of Piper species. The compound has a number of interesting biological properties, including its broad-spectrum activity on weeds as a contact herbicide. Initial studies highlighted a similarity in response between plants treated with sarmentine and herbicidal soaps such as nonanoic acid (pelargonic acid). However, little was known about the mechanism of action leading to the rapid desiccation of foliage treated by sarmentine. In cucumber cotyledon discs assays, sarmentine induced rapid light-independent loss of membrane integrity at 100 µM or higher concentration, whereas 3 mM pelargonic acid was required for similar effect. Sarmentine was between 10 and 30 times more active than pelargonic acid on wild mustard, velvetleaf, redroot pigweed and crabgrass. Additionally, the potency of 30 µM sarmentine was greatly stimulated by light, suggesting that this natural product may also interfere with photosynthetic processes. This was confirmed by observing a complete inhibition of photosynthetic electron transport at that concentration. Additional studies on isolated thylakoid membranes confirmed that sarmentine acted as a PSII inhibitor by competing for the binding site of plastoquinone. This can be attributed in part to structural similarities between herbicides like diuron and sarmentine. While this mechanism

of action accounts for the light stimulation of the activity of sarmentine, it does not account for its ability to destabilize membrane in darkness. In this respect, sarmentine has some structural similarity to crotonoyl-CoA, the substrate of enoyl-CoA reductase, a key enzyme in the early steps of fatty acid synthesis. Inhibitors of this enzyme, such as triclosan, cause rapid loss of membrane integrity in the dark. Sarmentine inhibited the activity of enoyl-CoA reductase, with an I50app of 18.3 μM. Therefore, the herbicidal activity of sarmentine appears to be a complex process associated with multiple mechanisms of action.

[S5-5] The Chemoattractant Potential of Ginsenosides in the Ginseng - P. *irregulare* Pathosystem

Presenter(s): Dimitre A. Ivanov, Department of Biology and the Biotron, The University of Western Ontario

Author(s): Ivanov, D.A., Georgakopolous, J.R.C., Bernards, M.A.

Department of Biology and the Biotron, The University of Western Ontario, 1151 Richmond St. N., London, ON, Canada, N6A 5B7

Ginsenosides, the triterpenoid saponins produced by American ginseng (*Panax quinquefolius* L.), have been extensively studied for their medicinal value, however their function in the rhizosphere remains unknown. Similar to other saponins, ginsenosides, possess antifungal properties against root and non-root pathogenic fungal species. However, growth of the ginseng root pathogen *Pythium irregulare*, is stimulated when exposed to ginsenosides. Presently, the chemoattractant potential of ginsenosides for *P. irregulare* was evaluated through (1) an in vivo pot experiment that monitored the pathogenicity of *P. irregulare* toward ginsenoside-treated and -untreated one- and two- year old ginseng plants and (2) by monitoring the affects of a purified total ginsenoside extract (GSF) and pure ginsenosides (Rb1, Re and F2) on the growth of the pathogen, in vitro. Disease severity and Time to Infection (TTI) was evaluated in vivo, by monitoring the chlorophyll fluorescence parameter ΦNO through non-invasive Chl fluorescence imaging in whole leaves of infected plants. Treatment of ginseng roots with a relatively high dose of ginsenosides prior to planting resulted in delayed infection by *P. irregulare*, of both one- and two-year old ginseng plants. Meanwhile, in vitro exposure of *P. irregulare* to pure ginsenoside extracts and GSF enhanced and altered mycelial growth. While, these results do not definitively show that ginsenosides act as chemoattractants for *P. irregulare*, they do demonstrate that rhizosphere ginsenosides affect the growth pattern of *P. irregulare* in vivo, which can affect the severity of its pathogenicity.

[S5-6] Chemical Interaction between the cruciferous phytoalexin camalexin and *Colletotrichum* species

Presenter(s): Chintamani Thapa, Department of chemistry, University of Saskatchewan, SK S7N 5C9, Canada

Author(s): Chintamani Thapa, M. Soledade C. Pedras, Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, SK, S7N 5C9, Canada

To defend against pathogens, crucifers (e.g. canola, mustard, cabbage, rutabaga) possess numerous pathways, some of which include de novo biosynthesis of defense metabolites called phytoalexins. Phytoalexins are antimicrobial compounds produced by plants under biotic or abiotic stress, but not present under non-stressed conditions. Colletotrichum species have been known to cause significant yield losses in various crops due to anthracnose, a fungal disease that infects a wide range of plant families, including crucifers.

The objective of this project is to investigate the transformation of the cruciferous phytoalexin camalexin by *Colletotrichum* species. Firstly it was established that some cruciferous phytoalexins are highly antifungal against various *Colletotrichum* species. Next,

investigation of the metabolism of camalexin using fungal cultures of *C. higginsianum*, *C. dematium* and *C. lentis* (previously *C. truncatum*) and chemical analyses (HPLC and LC-ESI-MS) has demonstrated that camalexin was slowly transformed by all species. The chemical structures of the biotransformation products were established after isolation and spectroscopic analyses (NMR, HRMS, UV, IR) of each compound. The structures of new metabolites were confirmed by chemical syntheses. The antifungal activity of the metabolic products of camalexin established that they are less antifungal than camalexin. Furthermore, camalexin was able to induce the production of the fungal metabolite chrysogine in *C. higginsianum*. Altogether, relative to cruciferous pathogens, the transformations of camalexin by *Colletotrichum* species are substantially slower (e.g. 12 hours vs. 8 days). Hence, based on these results, it is suggested that *Colletotrichum* species, unlike cruciferous pathogens (e.g. *Alternaria brassicicola*), use non-specific enzymes ("house-keeping" enzymes) to metabolize camalexin. These results will be presented and compared with reported work on the metabolism of camalexin by other fungal pathogens.

[S5-7] Field Application of Benzothiadiazole on Oat: Effect on Avenanthramide Production and Crown Rust Resistance

Presenter(s): Mitchell L. Wise, USDA, ARS, Cereal Crops Research Unit

Author(s): Mitchell L. Wise[1], Marcus Vinje[1], Shawn Conley[2]

[1]USDA, ARS, Cereal Crops Research Unit, 502 Walnut St., Madison, WI. 53726, USA

[2]Department of Agronomy, University of Wisconsin, 1575 Linden Dr, Madison, WI. 53706, USA

Plant defense activators such as benzothiadiazole (BTH) are known to elicit the biosynthesis of plant phytoalexins. In oat, BTH treatment has been shown to up-regulate avenanthramide production in both the vegetative tissue and filling grain in greenhouse studies. Avenanthramides are phenolic antioxidants demonstrating several nutraceutical effects in laboratory experiments including: inhibition of nuclear factor kappa beta activation, suppression of the aberrant Wnt/b-catenin signaling pathway in HeLa cells and activation of anti-oxidant response element (ARE) associated genes in vitro. Avenanthramides are also reported to function as phytoalexins in response to Crown Rust infection. Presented here are the results of application of BTH under field conditions. Field trials were conducted in 2013 using four application rates on two cultivars of oat and in 2014 using three application rates on four oat cultivars. The results showed a subtle but demonstrable effect of increased avenanthramide production in vegetative tissue, mature grain and enhanced resistance to Crown Rust infection. Gene expression of pathogenesis related protein-1 (PR-1), phenylalanine ammonia lyase (PAL) and hydroxycinnamoyl-CoA:hydroxyanthranilate hydroxycinnamoyl-CoA transferase (HHT) was also monitored by qRT-PCR. Both PR-1 and PAL expression were demonstrably up-regulated in the field trials, HHT was not. However, greenhouse experiments showed all three genes to be elicited with 68, 9, and 3 fold increases, respectively.

Tuesday, August 11, Afternoon

Symposium 6: Botanical Medicines

Chairs: Fred Stevens and Dejan Nikoli

[S6-1] Development of safe and effective botanical dietary supplements for women's health.

Presenter and Author: Richard B. van Breemen, UIC/NIH Center for Botanical Dietary Supplements Research, University of Illinois College of Pharmacy, 833 S. Wood St., Chicago, IL 60612

To ensure a safe and effective product, botanical dietary supplements can be

developed in a manner analogous to pharmaceuticals that involves identification of mechanisms of action and active constituents, chemical standardization based on the active compounds, biological standardization based on activity, preclinical evaluation of toxicity and potential for drug-botanical interactions, metabolism of active compounds, and finally, clinical studies of safety and efficacy. Unlike pharmaceuticals, botanical also require authentication, which will be discussed in view of the current controversy regarding DNA barcoding vs. chemical fingerprinting for finished products. Completing these steps enable the translation of botanicals from the field to safe human use as dietary supplements. Focused since 1999 on botanicals used by menopausal women as alternatives to hormone therapy, the UIC Botanical Center has carried out this systematic approach for a series of botanicals including red clover (*Trifolium pratense*), black cohosh (*Actea racemosa*), and hops (*Humulus lupulus*) culminating in Phase I and II clinical trials. Our current investigation of licorice species will be highlighted with a focus on safety studies such as metabolism of active compounds and potential for drug-botanical interactions. Supported by NIH grant P50 AT000155 from the NIH Office of Dietary Supplements and the National Center for Complementary and Integrative Health

Richard B. van Breemen is the Matthias C. Lu Collegiate Professor of Pharmacy and Professor of Medicinal Chemistry and Pharmacognosy at the University of Illinois College of Pharmacy at Chicago. He serves as Director of the UIC/NIH Center for Botanical Dietary Supplements Research and leads the Mass Spectrometry, Metabolomics and Proteomics Facility for the University of Illinois Cancer Center. Prof. van Breemen received his B.A. in chemistry from Oberlin College in 1980 and Ph.D. in Pharmacology and Experimental Therapeutics from the Johns Hopkins University (with Catherine Fenselau) in 1985. He carried out post-doctoral research in laser desorption mass spectrometry at Johns Hopkins with Robert Cotter before joining North Carolina State University as assistant professor of Chemistry and Biotechnology. Until 2010, he served as the first Editor-in-Chief of *Combinatorial Chemistry & High Throughput Screening* and is currently on the editorial board of *Assay and Drug Development Technologies* and a Regional Editor of *Biomedical Chromatography*. Prof. van Breemen received a University Scholar faculty award from the University of Illinois, a 2010 Expert Methods Panel award from the AOAC International for his work on analytical methods for dietary supplements, and the 2008 Harvey W. Wiley Award from the AOAC International. His research concerns the discovery and development of natural products as chemoprevention agents and the investigation of botanical dietary supplements as alternatives to hormone therapy for menopausal women. His translational research has involved phase I and phase II clinical trials of lycopene for the prevention of prostate cancer in men and studies of the safety and efficacy of botanical dietary supplements for the management of menopausal symptoms in women. Prof. van Breemen has published over 300 papers and book chapters concerning natural products, botanical dietary supplements and the use of mass spectrometry for drug discovery and development from natural product sources.

[S6-2] The non-volatile constituents of nutmeg—a key to solving an old riddle?

Presenter: Ehab A. Abourashed[1]
Authors: Ehab A. Abourashed[1] *and Abir T. El-Alfy*[1]
[1]*Department of Pharmaceutical Sciences, College of Pharmacy, Chicago State University, Chicago, IL*

Nutmeg, the kernel of *Myristica fragrans* (Myristicaceae), is a kitchen spice and a common ingredient of many food products. The characteristic taste and aroma of nutmeg is mainly attributed to its essential oil rich in terpenes and phenylpropenoids. Nutmeg has also been reputed for its CNS

activity and is often classified as a natural hallucinogenic agent. Due to its ease of accessibility, nutmeg is often used within confined communities as a cheap substitute for marijuana and narcotic agents. Moreover, sporadic reports have been published on the anxiolytic/anxiogenic, antidepressant and anti-inflammatory effects of nutmeg preparations. Based on earlier speculations, the CNS effects of nutmeg were attributed to biotransformation of myristicin, the major constituent of its volatile oil, to amphetamine-like metabolites after consumption by abusers. Recent studies, however, failed to identify any amphetamine-like metabolites in test animals or human volunteers. On the other hand, recent evaluation in our laboratory of HPLC-fingerprinted extracts further established the psychoactivity of nutmeg in the mouse tetrad assay. Subsequent *in vitro* evaluation of the same extracts against an extended panel of CNS receptors, including cannabinoid type 1 & 2 receptors, did not identify any specific target.\. Due to the apparently contradictory reports, i.e. lack of coherent chemical and molecular evidence alongside of significant *in vivo* activity, the phytopharmacology of nutmeg had to be revisited in an attempt to resolve this issue. Following a systematic and reproducible phytochemical procedure, the major non-volatile constituents of nutmeg were isolated, characterized and evaluated *in vitro* against novel targets that significantly modulate neurotransmission. Twelve compounds were identified and the majority had a lignan skeleton with one new compound reported for the first time. A number of the isolated compounds showed significant activity against the newly identified targets and may thus contribute towards the reputed psychoactivity of nutmeg. These compounds may have therapeutic potential in various psychological conditions including substance abuse withdrawal. Phytochemical and pharmacological details of the 'road map' leading to our proposed solution to the riddle of nutmeg will be the focus of this presentation.

Ehab A. Abourashed, M.S., Ph.D. is a graduate of the Faculty of Pharmacy, Cairo University. He received an M.S. in Organic Chemistry from the University of Tennessee and a Ph.D. in Pharmacognosy from the University of Mississippi. He is currently Associate Professor at the Chicago State University College of Pharmacy, which he joined in 2009 during its second year of inception, and Adjunct Research Associate Professor at the National Center for Natural Products Research, University of Mississippi.

Prior to Chicago State University, Dr. Abourashed held other positions in academia, as Research Scientist at the University of Mississippi National Center for Natural Products Research and Associate Professor at King Saud University College of Pharmacy. In the private sector, he was Senior Scientist at GlaxoSmithKline, New Jersey and Director of Quality Assurance at ElSohly Labs, Mississippi.

Dr. Abourashed has more than 40 peer-reviewed publications, is the co-author of one textbook and is an editorial board member of the journal 'Antioxidants'. His research interests and publications are focused on isolation, structure elucidation and microbial transformation of biologically active natural products; application of HPLC and LC-MS in phytochemical, environmental and biochemical analysis; and phytopharmacology of herbal medicines. Exposing professional pharmacy students to natural products drug discovery through investigating traditional medicinal plants with psychoactive properties and antioxidant activities is the main focus of Dr. Abourashed's current research activities.

[S6-3: Neish Award Speaker] Elucidating modular diterpenoid metabolism in non-model plant systems: From chemical diversity to biotechnology applications.

Presenter and Author: Philipp Zerbe, Department of Plant Biology, University of California, Davis, CA 95616 USA

Plants are nature's master chemists; they produce a myriad of natural products that are essential for life on the planet. Mankind has utilized this chemical diversity for millennia for ceremonial purposes, as flavors, and to cure various diseases. Among these bioactive metabolites, terpenoids form the largest and most diverse class with a wide range of ecological functions and industrial uses. We established deep transcriptome resources for more than a dozen plant species that produce terpenoid metabolites of economic importance, such as clary sage, balsam fir, horehound, and maize. To decipher the corresponding biosynthetic genes, enzymes and pathways across various species, we developed a gene discovery strategy that integrates tissue-specific metabolite and transcriptome analysis with phylogeny-guided gene identification and efficient *in vitro* and *in vivo* enzyme characterization. This approach resulted in the discovery of more than 60 diterpene synthases and several hundred cytochrome P450-dependent monooxygenases, as key enzymes in generating terpenoid metabolic diversity. Functional characterization revealed numerous novel diterpene synthase functions as part of dynamic modular pathways, where catalytically distinct enzymes may function in different combinations to enhance chemical diversity. Following nature's lead, we developed proof-of-concept yeast expression platforms for several diterpenoids through combinatorial expression of functionally distinct diterpenoid pathway genes.

Philipp Zerbe is an Assistant Professor at the Department of Plant Biology, University of California at Davis. His research interests focus on the evolutionary diversification of terpenoid metabolism in various medicinal and other economically relevant plants, and the development of tools for the production of terpenoid bioproducts with human benefit. Dr. Zerbe received his PhD from the Ruhr-University Bochum, Germany (2007) under mentorship of Professor Elmar Weiler, studying the structural and functional interrelations of key enzymes in jasmonate biosynthesis. Prior to his position at the University of California, Davis, Dr. Zerbe worked as a Postdoctoral Fellow and later Research Associate with Professor Jörg Bohlmann at the Michael Smith Laboratories, University of British Columbia (Vancouver, Canada). Here, his research focused on the discovery of specialized diterpene pathways in coniferous trees and medicinal plants as part of two Genome Canada funded research consortia (SMarTForests, PhytoMetaSyn).

[S6-4] Antiplatelet aggregation and cytotoxicity activity of betulinic acid and its acetyl derivative from *Melaleuca bracteates* var. revolution gold.

Presenter(s): Foluso Oluwagbemiga Osunsanmi, University of Zululand at South Africa

Author(s): Oluwagbemiga S. Soyinbe, University of Zululand and Idiat. B. Ogunyinka , University of Zululand and Rebamang A. Mosa and Andy R.Opoku, University of Zululand at South Africa.

Platelet dysfunctions are implicated in cardiovascular diseases. Management of abnormal Platelet aggregations with natural products is a promising approach to the treatment of cardiovascular diseases. In this study, betulinic acid (BA) and its acetyl derivative (3-β acetylbetulinic acid) (BAA) from *Melaleuca bracteata* var. revolution gold were investigated for their antiplatelet aggregation and cytotoxicity. BA was

isolated from Melaleuca bracetata by column chromatography and some portion of BA were used to synthesize BAA. The antiplatelet aggregation activity of the compounds were separately evaluated on collagen, ADP, thrombin and epinephrine to induce rat platelet aggregations. The MTT cytotoxicity assay was used to determine the cytotoxic effect of the compounds against human embryonic kidney (HEK293) and hepatocellular carcinoma (HEPG2) cell lines. BA and BAA exhibited significant ($p<0.05$) dose dependent antiplatelet aggregation activity. BA and BAA showed the highest platelet aggregation inhibition on epinephrine induced platelet aggregation with IC50 values 0.78 mg/ml and 0.85 mg/ml respectively. BA and BAA showed less cytotoxicity effect on both HEK293 cell (IC50 1027 µg/ml and 1051 µg/ml respectively) and HEPG2 cells (IC50 448 mg/ml and 672 mg/ml respectively). The results suggest that the compounds could serve as template for synthesis of new antiplatelet drugs.

[S6-5] American Indian botanicals as possible alternatives to hormone therapy.

Presenter: Tristesse Burton, University of Illinois at Chicago

Authors: Tristesse Burton[1], Tareisha Dunlap[1], Huali Dong[1], Guannan Li[1], Judy Bolton[1], Djaja Soejarto[1], and Richard B. van Breemen[1]

[1]UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612, USA

Although hormone therapy (HT) remains the standard treatment for managing menopausal symptoms, many women seek alternatives such as botanical dietary supplements since HT has been associated with increased risks of breast cancer, coronary heart disease and stroke. The leading botanicals that women take for menopause are black cohosh and red clover, which were historically used by American Indians. Although these two botanicals have been investigated extensively, there are still numerous American Indian plants that lack scientific studies on their safety and efficacy. This talk will discuss the pharmacognostic process to identify if a subset of American Indian botanicals can serve as alternatives to HT while also protecting women from hormone-dependent cancers. This project is expected to find novel and effective drug leads for menopausal symptoms, cancer, and inflammation while also supporting American Indian traditional knowledge.

Fifteen native plants were selected to examine estrogenic, chemopreventive, and anti-inflammatory potential through bioassay-guided fractionation. *Amorpha canescens* Pursh. (Fabaceae)—leadplant, *Echinocystis lobata* (Michx.) Torr. & A. Gray (Cucurbitaceae)—wild cucumber, and *Silphium perfoliatum* L. (Asteraceae)—cup plant inflorescent tissue was selected for initial screenings based on American Indian ethnobotany and published biological data. Leadplant MeOH extract was selected as the best candidate for bioassay-guided fractionation due to high anti-estrogenic activity in the Ishikawa cell-based assay and anti-inflammatory activity in the Griess assay. Cup plant and *Lespedeza capitata* Mixch. (Fabaceae) roundhead lespedeza (another species within the 15 species) are being pursued as alternatives due to significant anti-estrogenic and estrogenic activity respectively.

[S6-6] Molecular diversity and Evolution of *Peperomia* species

Presenter(s): Massuo J. Kato, University of São Paulo

Author(s): M.J. Kato[1], L.Y.Valero-Gutiérrez[1], M.M. Martins, M.C.R. Oliveira-Junior[1], V. Bueno[1], L.F. Yamaguchi[1], A.P.M. Egydio[2], A. Salatino[2], M.L. Salatino[2]

[1]Department of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, 05508-000, São Paulo, SP Brazil

[2]Department of Botany, Institute of Biosciences, Universidade de São Paulo, Rua do Matão, 277, 05429-970, São Paulo, SP, Brazil

One remarkable aspect among basal Angiosperm is the diversification observed in some families. While some families such as Myristicaceae (e.g. nutmeg—*Myristica fragrans*) contain nearly 440 species (20 genera), Piperaceae (e.g. black pepper—*Piper nigrum*) has about 3600 species (5 genera). The family Piperaceae contains the two major genera *Piper* and *Peperomia* with approximately 2000 and 1600 species, respectively. Although both genera are mostly Neotropical, phytochemical studies on *Peperomia* are scarce as compared to *Piper* species. Thus, the major aim of this work was to determine the phylogenetic relationship among Brazilian *Peperomia* species (matK, ITS, and CHS as markers) as well as their chemical profiles obtained by means of HPLC-DAD, LC-ESI-MS, NMR, and GC-MS data. The phylogenetic trees provided by ITS and matK sequences were congruent with that from CHS. The Hierarchical Cluster Analysis (HCA) and Discriminant Analysis of Principal Components (DAPC) of crude extracts obtained from leaves indicated some trends with the largest group encompassing species containing lignoids such as *P. blanda* (tetrahydrofurans) and *P. glabella* (peperomins), while the second group contains the species *P. tetraphylla* (amides) and *P. trineura* (polyketides). Due to the occurrence of orsellinic acid derivatives in several species, the corresponding OAS (orsellinic acid synthase) was investigated in the plants and in the 27 endophytic fungi isolated from them. Each fungi amplified for at least one fungal PKS excepting the strain POS1 (an endophyte from stems of *P. oreophila*) while the OAS was confirmed in several isolates. Although the orsellinic acid was not detected in the extracts of these endophytes growing in PDB culture media, sequencing of the amplified fragment for the PUS3 fungi with 60% of homology with the OAS from Streptomyces viridochromogenes do not preclude the hypothesis of horizontal transfer from fungi to the plants.

Wednesday, August 12, Morning

Symposium 7: Nutritional & Medicinal Phytochemistry

Chairs Elvira de Mejia and Shelly Nickols-Richardson

[S7-1] Phytoactive chemicals: discovery is just at the "starting line" of the research.

Presenter and Author: Mary Ann Lila, Plants for Human Health Institute, North Carolina State University

Research on the bioactive properties of phytochemicals, whether from edible food crops or in herbals used in supplements or medicines, should generally include a three-pronged approach: 1) Biodiscovery, 2) Bioefficacy, and 3) Effective Delivery to Consumers. Biodiscovery includes rigorous examination of the health-protective constituents in well-recognized fruits and vegetables, as well as exploration of novel resources which may have historic incidence of use by some localized cultures, but, may not have been examined in a scientific context. Bioefficacy involves comprehensive testing of natural products/phytoactive chemicals to decipher their unique biological activities against chronic human diseases, to discover mechanisms of action, and/or to determine effective dosages relevant to human interventions. Bioefficacy can be investigated at the lab bench in chemical bioassays, in vitro, in animal studies, or (as the gold standard) in human clinical trials. But unless we as researchers can follow through with that last prong of the research agenda—i.e. *effective delivery to consumers*—everything else may be end up as just an academic exercise. We need to make sure that efficacious natural product discoveries can be deliverable to consumers in a format that they will willingly embrace, and chose to adopt for their own health benefit. Despite rigorous informational and educational campaigns, consumers have largely shown reluctance and/or chronic inability *even* to incorporate adequate phytoactive-rich fruit and

vegetable produce into routine diets. In order to address this disconnect, a novel strategy was developed to concentrate immunoprotective fruit and vegetable phytochemicals for delivery in convenient, and highly bioavailable functional food formats. Phytoactives from candidate fruits and vegetables were captured and concentrated into lightweight, portable protein-dense ingredients. Sensory panels confirmed the favorable organoleptic properties of the ingredient, and recommended wider applications to counteract the negative trends of Western diets. Most recently, the phytoactive-protein chimeric ingredients have been incorporated into snack food products with direct utility for meals in transit, and even for humanitarian aid efforts in undernourished populations. Simultaneously, the complexing of phytoactive-protein particles addresses structural and formulation challenges (e.g. bar hardening, thermal degradation, or ingredient separations) that are current challenges in the industries.

Mary Ann Lila is Director of the *Plants for Human Health Institute*, North Carolina State University, North Carolina Research Campus. She holds the endowed David H. Murdock Chair, and is a Professor in the Department of Food, Bioprocessing, and Nutrition Sciences. Through ground-breaking, transdisciplinary discovery and outreach, the team of faculty at the Plants for Human Health Institute (PHHI) pioneers a dramatic shift in the way the American public views and uses plant food crops—not merely as a source of nutrients and flavorful calories, but as a powerful resource for components that protect and enhance human health. Integrated research in metabolomics, biochemistry, pharmacogenomics, breeding and postharvest attributes are aimed at development and promotion of mainstream fruit and vegetable produce with enhanced health benefits, *and* introduction of new or underappreciated crops and products from various sites throughout the globe, allowing consumers to make proactive, responsible dietary choices that benefit their own, and their families' health. Dr. Lila is currently a co-Director of an ambitious Plant Pathway Elucidation Project (P2EP) which synergizes the talents from 7 UNC system universities, campus industry partners, and partnering local 2 and 4-year colleges.

Recent projects include a Bill&Melinda Gates Foundation Grand Exploration Challenges project in Zambia, an NIH NIDDKD project on functional food innovations, the Medicines for Malaria Venture which examines promising plant-derived chemicals with potent anti-malarial efficacy; a major blueberry genome sequencing initiative using state-of-the-art NextGen sequencing capacity, which focuses on the genes relevant to health-protective properties in the fruits; and a USDA program on tribal resources and STEM education in American Indian/Alaska Native communities, the health protective properties of traditionally-used medicinal planta, and the threats imposed by climate change.

Lila was formerly Director (2006-2008) of ACES Global Connect (the international arm of the College of ACES, University of Illinois) and Associate Director of the nationally acclaimed Functional Foods for Health Program (1997-2000) at the University of Illinois. Dr. Lila has been honored with the Paul A. Funk Scholarship Recognition Award (the premier research award in the College of ACES, University of Illinois), the Spitze Professorial Career Excellence Award, the Faculty Award for Excellence in Research, the University Scholar Award, the Amoco Award for Excellence in Undergraduate Instruction, and the Lilly Endowment Teaching Fellowship. Dr. Lila has ongoing research projects in Egypt, Central Asia, Oceania, Mexico, Ecuador, Chile, and Africa, and is Vice President of the Global Institute for BioExploration (GIBEX). In 1999, Dr. Lila won a Fulbright Senior Scholarship to conduct research and outreach in New Zealand, and returns to Australasia at least once/year.

[S7-2] Manipulation of glucosinolate biosynthesis, hydrolysis, and hydrolysis product anticancer bioactivity in broccoli (*Brassica oleracea* L. ssp.).

Presenter and Author: John A. Juvik, Department of Crop Sciences, University of Illinois at Urbana-Champaign

Epidemiological studies have long suggested that an inverse relationship exists between the consumption of *Brassica* vegetables and the induction of cancer. This response is associated with the hydrolysis of endogenous glucosinolates generating indolyl and isothiocyanate products that display anti-carcinogenic bioactivities. Using a population of broccoli F2:3 lines varying in glucosinolate composition and concentration in floret tissue our lab has identified hydrolysis products and key genes associated with quinone reductase (QR) activity, an in vitro biomarker for anti-cancer activity. In another study, preharvest spray treatment of the plant signal transduction compound, methyl jasmonate (MeJA) and postharvest treatment of 1-methylcyclopropene (1-MCP) was found to increase floret QR activity and prolong broccoli postharvest quality.

John (Jack) A. Juvik, is the Director of Graduate Studies for the Department of Crop Sciences and the Director of the Illinois Plant Breeding Center at the University of Illinois. Dr. Juvik's research program has focused on breeding vegetables for improved flavor, insect and disease resistance and for enhanced levels of phytochemicals associated with health promotion. He has investigated transformation strategies and tissue culture ploidy level manipulation in *Brassica* vegetables and *Miscanthus*. His program has pioneered the use of DNA markers for the creation of linkage maps of tomatoes, sweet corn, broccoli and more recently for the bioenergy crop, *Miscanthus*. These maps have been used for the identification of favorable QTLs and for marker-assisted introgression of beneficial alleles for improved quality, yield, and other traits into elite germplasm for commercial release. Most recently he is working in collaboration with Kraft Foods on a project investigating the feasibility of using pigments from corn as natural colorants in processed foods and beverages. Professor Juvik has published over 100 peer-reviewed journal articles and has advised 36 M.S. and Ph.D. students during his tenure at the University of Illinois.

[S7-3] Anti-Malarial Activities of *Margaritaria discoidea* and Other Nigerian Medicinal Plants

Presenter(s): Okiemute Rosa Johnson-Ajinwo, Institute for Science and Technology in Medicine, Keele University, UK.

Author(s): Okiemute Rosa Johnson-Ajinwo[1, 2], Imran Ullah, Christopher Lee [1], Haima Raman[1], Nicolas Ellerby,[1] Paul Horrocks[1] and Wen-Wu Li[1]

[1]Institute for Science and Technology in Medicine, Keele University, UK

[2]Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria

We previously evaluated in vitro anti-cancer activity of a Nigeria medicinal plant—Margaritaria discoidea (Baill.) G.L.Webster [1]. The aim of current study is to investigate crude extracts of ten medicinal plants including M. discoidea for their anti-malarial activities and identification of potential bioactive compounds.

Methods: Ten selected medicinal plants (*Anchomanes difformis* (Blume) Engl., *Mucuna pruriens* var. pruriens, *Bombax buonopozense* P. Beauv, *Millettia thonningii* (Schum & Thonn.) Baker, *Corchorus olitorus* L., *Margaritaria discoidea* (Baill.) G.L. Webster, *Moringa oleifera* Lam., *Newbouldia laevis* (P. Beauv.) Seem., *Fleurya* sp and *Platycerium bifurcatum* (Cav.) C. Chr.) were sourced from a bio reserve in Nigeria. Extracts of the plants were screened for anti-malarial activities on plasmodium falciparum. Following bioassay-guided fractionation, potential bioactive compounds from *M. discoidea* were identified by gas

chromatography mass spectrometry following trimethylsilylation derivation. Their anti-malarial activity was confirmed and a preliminary structural modification was undertaken.

Results: Preliminary screening of extracts from the ten plants disclosed that the organic extracts of *M. discoidea* had the most promising anti-malarial activities. The potential bioactive compounds in *M. discoidea* are securinine (IC50 of 11.3 μM) and gallic acid (IC50 of 26.8 μM) against *P. falciparum*

Conclusions: *M. discoidea* possesses anti-malarial activity against the intraerythrocytic stage of development. This is the first report of the anti-malarial activity of this plant. Securinine and gallic acid appear the main anti-malarial compounds in this plant.

Reference: [1] Okiemute Rosa Johnson-Ajinwo, Alan Richardson, Wen-Wu Li. Cytotoxic effects of stem bark extracts and pure compounds from Margaritaria discoidea on human ovarian cancer cell lines. Phytomedicine 2015, 22, 1-4.

[S7-4] Phytochemical analysis for phenol and saponin in *Brachiaria* sp.

Presenter(s): Syeda Maryam Hussain (university of sao paulo)

Author(s): Syeda Maryam Hussain, University of Sao Paulo at Pirassununga, SP, Brazil.

Valdo Rodrigues Herling, University of Sao Paulo at Pirassununga, SP, Brazil.

Lilian Elgalise Techio Pereira, University of Sao Paulo at Pirassununga, SP, Brazil.

Plants secondary metabolites (PSM) can act as a toxin or detoxifying agents, depending on their origin and nature. Tannins and saponins are PSM found in *Brachiaria* sp., although the former in negligible quantity, while the later in higher concentration. In order to comprehend how sward management affects PSM, the concentration of tannins and saponins were determined in *Brachiaria decumbens* cv. Basilisk (BD) and *Brachiaria brizantha* cv. Xaraés (BB) in summer, autumn, winter and spring. Sward managements comprised four levels of nitrogen fertilization (0, 150, 300 and 450 kg N/ ha) combined with two cutting heights (10 and 20 cm (BD) and 15 and 30 (BB) and were applied according to complete randomized design in a 2x4x4 factorial arrangement, and season was considered a repeated measure. Sampling was done after every 28 days for phytochemical tests (saponin (foam test) and phenols (ferric chloride). Foaming index varied only with season (P= 0.001) in BB, and lowest values were reported in spring (58.9±2.06%). However, in BD there was a significant interaction between all factors (P=0.0006) on foam stability. There were no differences between seasons in non-fertilized swards and those receiving 450 kg N/ha, while lowest values were reported on treatments 300/20, 300/10 and 150/20, respectively in autumn, winter and spring. There was a significant interaction between all factors for phenols in BD and BB (P<0.0001 for both species). In BD, regardless cutting heights,highest values were observed during summer and spring for all nitrogen levels. While, in BB the highest phenol concentration occurred in summer and autumn or during summer and spring for all nitrogen levels on plant cutting heights of 15 cm and 30 cm, respectively. Overall, for both species, increasing on nitrogen fertilization tends to decrease phenols concentration regardless the cutting height. However, variations on saponin seems more related to season of the year.

[S7-5] Coproduct yield comparisons between purple corn, blue corn and yellow dent corn for different milling processes

Presenter(s): Pavel Somavat, University of Illinois at Urbana-Champaign

Author(s): Pavel Somavat, Qian Li, Elvira Gonzalez de Mejia, Vijay Singh, University of Illinois at Urbana-Champaign

Purple corn (*Zea mays* L.) and blue corn (*Zea mays* var. saccharata) are varieties of colored corn rich in anthocyanins. Studies have shown that anthocyanins possess anti-oxidant/anti-carcinogenic properties and help fight obesity, tissue injury and inflammation. Various products

made from anthocyanin rich colored corn are already available in market. In this study, we fractionated purple and blue corn using wet-milling, dry-milling and dry-grind processes at 1 kg scale with three replications and compared various coproduct yields against traditional yellow dent corn (*Zea mays* var. indentata). In wet-milling process, starch yield was largest for yellow dent corn 70.1% (d.b.) while it was 63.3 and 61.5% (d.b.) for purple and blue corn. Gluten solids yield was largest in blue corn 14.2% (d.b.) while it was 13.8 and 10.5% (d.b.) for purple and yellow dent corn. Fiber yields were 10.9, 10.6 and 8.1% (d.b.) for purple, blue and yellow dent corn, respectively. In dry-milling process, yellow dent corn yielded greatest amount of total endosperm fraction 84.9% (d.b.), total endosperm yield for purple and blue corn were 80.1 and 77.0% (d.b.), respectively. Pericarp yields for blue, purple and yellow dent corn were 12.5, 9.9 and 6.9% (d.b.), respectively. In dry-grind process, final ethanol concentration was 17.1% (v/v) for yellow dent corn while ethanol concentration for purple corn and blue corn were 14.5 and 14.4% (v/v), respectively. DDGS yield was greatest for purple corn 41.6% (d.b.) and yields were 38.0 and 32.9% (d.b.) for blue and yellow dent corn, respectively. Purple corn and blue corn can potentially be used in all the three milling processes, yield differences being offset by health promoting properties of various coproducts recovered. However, further techno-economical analysis of the three processes is required.

[S7-6] Investigation of α-Glucosidase Inhibition Properties, Antioxidant Activities, and Cytotoxicity of Philippine Herbal Vines Containing Polyphenols

Presenter(s): Richard I. Licayan, Rutgers the State University of New Jersey

Author(s): Thomas Villani, Rutgers the State University of New Jersey, Romeo M. Del Rosario, Mindanao University of Science and Technology, Philippines, James E. Simon, Rutgers the State University of New Jersey

The prevalence of diabetes mellitus is rising globally. The World Health Organization (WHO), reports that this disease is one of the leading causes of deaths in the Philippines and is responsible for about five percent of global deaths. Diabetes can be divided into two types. In Type I, the pancreas does not produce sufficient insulin and in Type II, the insulin that is produced, does not enter the cells properly. Treatment of diabetes is done by injecting insulin and/or changing the diet. Providing insulin can generate an elevation of postprandial hyperglycemia (PPHG) that causes a spike in the production of glucose. Decreasing the level of PPHG may be accomplished by inhibiting a carbohydrate-hydrolyzing enzyme like α-glucosidase. This study, with funding by the USAID, investigated leaf polyphenols content, alpha glucosidase inhibition, antioxidant, and cytotoxic activities of four Philippine herbal vines including *Merremia peltata, Ipomoea cairica, Ipomoea lacunosa,* and *Ipomoea pes-caprae*. The antioxidant activity was determined by ABTS, FRAP, DPPH, Hydroxyl radical, and Superoxide radical inhibition assays. Cytotoxicity of the leaf extract was evaluated in terms of LC_{50} using brine shrimp lethality assay. Results revealed that *I. lacunosa* had the highest polyphenolic content (71.54 mg GAE per 100 gram dry weight). HPLC/UV/MS analysis indicated that the four vines contained a large number of polyphenols and the majority of the peaks were identified as flavonoids. *Ipomoea cairica* showed the highest antioxidant capacity in five methods used for radical scavenging assays. The *M. peltata, I. cairica, I. lacunosa,* and *I. pes-caprae* extracts demonstrated good α-glucosidase inhibition with IC_{50} values of 500, 250, 234, and 62.5 µg/mL, respectively. Results from the cytotoxicity screens showed that the extracts of *M. peltata, I. cairica, I. lacunosa,* and *I. pes-caprae* were potent against the brine shrimp with LC_{50} values of 29.66, 61.54, 96.08, and 30.70 µg/mL, respectively. These plants appear to be rich sources of natural antioxidants and may be useful in treatment of diabetes mellitus.

Poster Abstracts

Poster Session I

Odd-numbered posters (e.g. P1, P3, P5...)

Sunday, August 9, 5:30–7:30 pm
Illini Room AB

Poster Session II

Even-numbered posters (e.g. P2, P4, P6...)

Monday, August 10, 5:30–7:30 pm
Illini Room AB

[P1] Phenomics Approaches to Elucidate the Role of the Various Ascorbate Pathways to Abiotic Stress Tolerance in Arabidopsis

Presenter(s): Lucia Acosta-Gamboa, Arkansas Biosciences Institute and Department of Molecular Biosciences

Author(s): Zachary Campbell, Arkansas Biosciences Institute, Arkansas State University

Raquel Torres, Arkansas Biosciences Institute, Arkansas State University

Lindsay Mull, Arkansas Biosciences Institute, Arkansas State University

Argelia Lorence, Arkansas Biosciences Institute and Department of Chemistry and Physics, Arkansas State University

L-Ascorbic acid (AsA, vitamin C) is a key antioxidant and enzyme cofactor in plants. Ascorbate controls cell division, affects cell expansion, and plays an important role in modulating plant senescence. It also protects plants against reactive oxygen species that are produced in response to abiotic and biotic stresses. Biosynthesis of AsA in plants is carried out by a complex metabolic network involving D-mannose/L-galactose, D-galacturonate, L-gulose, and myo-inositol as main precursors. We have previously shown by manual phenotyping that Arabidopsis lines over-expressing enzymes in the myo-inositol pathway have elevated AsA, accumulate more biomass of both aerial and root tissues and are also tolerant to abiotic stresses including salt, cold, and heat. The downside of manual phenotyping is that it is time consuming, low throughput, subjective, and limited to the resolution of the human eye. On the other hand, high throughput phenotyping technologies are accurate, non-destructive, and more sensitive, allowing the detection of subtle phenotypes. We have used a Scanalyzer HTS system to phenotype our high AsA Arabidopsis lines with visible, fluorescence, and near infrared cameras. We have shown that by using this approach, high AsA lines grow faster, accumulate more biomass, and display healthier chlorophyll fluorescence and water content profiles than controls. By studying abiotic stress in a high throughout fashion using optimized protocols, we have also shown that these high AsA lines are tolerant to salt and drought stresses. Our ongoing experiments aim to dissect the contribution of the various AsA pathways to abiotic stress tolerance.

[P2] Isolation of flavonoids from *Delonix elata* and determination of its rutin content using capillary electrophoresis

Presenter(s): Areej Altaweel, Pharmacognosy Department, College of Pharmacy, King Saud University, P.O. Box 22452 Riyadh 11495, Saudi Arabia

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[3]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, El-Messalah, Alexandria 21521, Egypt

Delonix elata (L.) Gamble is an important, traditionally used plant in Saudi Arabia. It is

used to relieve rheumatic pain, flatulence and the seeds are employed as purgatives. The aim of the present study was to isolate chemical constituents of the n-butanol fraction (BF) of *D. elata* and to find out, by capillary electrophoresis, percentage of rutin present in this BF. Three quercetin glycosides and one kaempferol rutinoside were isolated from the BF of aerial parts of *D. elata;* namely, Quercetin 3-O-rutinoside-7-O-glucoside (1), Quercetin 3,7-diglucoside (2), Rutin(3) and Kaempferol 3-O-rutinoside (4). Rutin, an active constituent has been reported to possess good pharmacological as well as therapeutic potentials. A sensitive and rapid procedure for quantitative determination of rutin by capillary electrophoresis was developed and the content of RUT was found to be 7.349 mg/gm, relative to n-butanol fraction and 18.373 mg%, relative to the dry powder of *D. elata*. The method could be recommended for approval and use in the pharmaceutical and food industries.

[P3] Characterization of SBIP-470, a Family 1 Lipid Transfer Protein and its Role in Plant Stress Signaling

Presenter(s): Timothy Audam, East Tennessee State University, Johnson City, TN-37604

Author(s): Timothy Audam, East Tennessee State University, Johnson City, TN-37604; Dr. Dhirendra Kumar, East Tennessee State University, Johnson City, TN-37604, Danda Chapagai, Department of Biological Sciences, University of South Carolina, Columbia, SC-29208

SBIP-470 is a tobacco SABP2-interacting protein. Nucleotide sequence analysis of SBIP470 clone obtained from the yeast two-hybrid screening shows that it is a putative lipid transfer protein that belongs to nsLTP1 subfamily. SABP2 is a methyl esterase like tobacco enzyme that catalyzes the conversion of methyl salicylate; an important lipid mobile signal to Salicylic Acid (SA). This is a critical step in the development of a systemic acquired resistance (SAR) against pathogens. Plant lipid transfer proteins (PLTPs) are a group of highly conserved proteins found in tissues of higher plants primarily responsible for the shuttling of phospholipids and other fatty acid groups between cell membranes. Studies also reveal that PLTPs play a very important role in the defense against phytopathogens. Previous studies using the *Arabidopsis* T-DNA insertion knockout plants were shown to be defective in the growth phenotype as well as its basal resistance to pathogens. Based on this interesting finding and in order to fully delineate the role of SBIP-470 in plants, we are generating transgenic tobacco RNAi lines that are silenced in SBIP-470 expression. SBIP-470 overexpressing transgenic tobacco lines using inducible system is also being generated. The role of SBIP-470 in inducing SAR is currently being investigated using Arabidopsis T-DNA knockout lines and in the SBIP-470 RNAi tobacco transgenic lines when available. Defense gene expression studies under biotic and abiotic stresses (drought and salinity) in the RNAi-silenced plants compromised in SBIP-470 expression will also be carried out. In order to biochemically characterize SBIP-470 in terms of its ability to transfer and bind to plant lipids, lipid binding and transfer assays will also be done. Fully understanding the role of SBIP-470 in stress response of plants can lead to the development of disease resistant plants which can have a direct impact on food production globally.

[P4] Exploring the Transcriptome of the Glucosinolate/Myrosinase System in Broccoli (*Brassica oleracea* var. italica)

Presenter(s): Talon M. Becker, University of Illinois at Urbana-Champaign

Author(s): Kang Mo Ku, University of Illinois at Urbana-Champaign

Elizabeth H. Jeffery, University of Illinois at Urbana-Champaign

John A. Juvik, University of Illinois at Urbana-Champaign

The glucosinolate/myrosinase system is a plant defense system found in the Brassicaceae

family, which includes the genera of *Brassica* and *Arabidopsis*. This system has been linked primarily to plant defense against insect herbivory and pathogen infection, but also has been shown to affect human health. Several glucosinolate hydrolysis products, the active compounds created from the reaction of glucosinolates and the enzyme, myrosinase, have been shown to be bioactive in humans. Specifically, the ingestion of these compounds can increase the transcription rates, and consequently the enzymatic activity, of certain detoxification enzymes in the human body. This has led to these compounds being described as having cancer-preventive/ chemopreventive activity, making the ability to accurately manipulate this system an important breeding objective in the *Brassica* vegetable market. In this research, we show the effect of spraying the stress elicitor compound, methyl jasmonate (MeJA), on the transcriptome of the glucosinolate/ myrosinase system as well as final glucosinolate content and measurable cancer-preventive bioactivity. In addition, we have explored the feasibility of using transcriptomic data of genes from this system to build predictive models for final phenotypes, such as glucosinolate levels or measureable cancer-preventive bioactivity. If viable, this type of transcriptomic selection could be used in breeding programs focused on manipulating these traits.

[P5] Synergistic hepatoprotective effect of Phyllanthus niruri and Andrographis paniculata extracts in the presence of Piper longum in wistar rats.

Presenter(s): Onkar Bedi,Department of Pharmacology, ISF College of Pharmacy, Moga (Punjab, INDIA)

Author(s): Puneet Kumar,Department of Pharmacology, ISF College of Pharmacy, Moga (Punjab, INDIA) and Vinod Gauttam,Department of Pharmacognosy, ISF College of Pharmacy, Moga (Punjab, INDIA)

Objective

The scientific and traditional data revealed that *Phyllanthus niruri* (Euphorbiaceae) (PN), Andrographis paniculata (Acanthaceae) (AP) and Piper longum (Piperaceae) (PL) have their antioxidant and hepatoprotective role. In the present study, we have investigated the synergistic effect of these herbs against alcohol and HFD (High Fat Diet) induce hepatotoxicity in rats. Further mechanistic study was conducted to establish the mechanism of the developed formulation with respect to Hemeoxygenase-1 (HO-1) which involve in the hepatoprotective effect.

Material and method

The hepatoprotective activity of three herbal extracts combinations *Phyllanthus niruri* (PN), *Andrographis paniculata* (AP) and *Piper longum* (PL) were first studied in in-vitro on HepG-2 cell lines by using ethanol (100mM) and then in-vivo activity was explored again with 40% Alcohol 2ml/100g and HFD induce hepatotoxicity in Wistar rats. Oxidative stress parameters, liver enzymes, Lipid profile and histopathological and mechanistic study were assessed in liver homogenate. The body weight and urine analysis was done on 7th, 14th, 21st day.

Results

The herbal combinations with different ratios (AP: PN: PL, 1:1:1, 2:2:1, 3:1:1) showed significant hepatoprotective effect using in vitro (HepG-2) and in vivo models. Further herbal combination with equal ratio group showed significant hepatoprotective effect as compare to other groups. The herbal combination group showed significant reduction in oxidative stress parameters and improved antioxidant and liver enzymes level.

Conclusion

The present studies suggest that the combination of herbal drugs showed synergistic hepatoprotective effect by antioxidant and induction of HO-1 mechanism. The combination of these three herbal drugs provide base for their clinical implications.

[P6] Reduction of Total Glucosinolates in Canola Meal via Thermal Treatment and Fungal Bioprocessing

Presenter(s): Mark Berhow, USDA ARS NCAUR

Author(s): Jason R. Croat, Biology & Microbiology Department, South Dakota State University, Brookings, SD 57007, USA, Mark Berhow, USDA, Agricultural Research Service, National Center for Agricultural Utilization Research; Peoria, IL 61604, USA, Bishnu Karki, Agricultural & Biosystems Engineering Department, South Dakota State University, Brookings, SD 57007, USA, Kasiviswanathan Muthukumarappan, Agricultural & Biosystems Engineering Department, South Dakota State University, Brookings, SD 57007, USA, and William R. Gibbons, Biology & Microbiology Department, South Dakota State University, Brookings, SD 57007, USA

On a worldwide basis, canola (*Brassica napus*) meal is second only to soybean meal as a protein source for livestock. A general limitation of *Brassica* spp. meals is the presence of glucosinolates (GLS). GLS and the enzyme myrosinase are compartmentally stored separately in the plant. Upon disruption of plant tissues, myrosinase cleaves glucose from GLS and releases toxic compounds such as nitriles, thiocyanates, and isothiocyanates to defend the plant. For this reason canola was bred to contain lower levels of GLS and erucic acid. However feed inclusion rates are still limited to 25-30% of livestock diets. The objective of this research was to develop a microbial process to metabolize GLS and the resulting breakdown products into non-toxic components, to enable higher inclusion levels in livestock rations. An additional objective was to boost the protein levels and digestibility. Cold pressed and hexane extracted canola meals were autoclaved (thermal treatment) then processed using several metabolically diverse fungal cultures. *Aurobasidium pullulans*, *Trichoderma reesei*, *Fusarium venenatum*, *Pichia kudriavzevii*, and *Mucor circinelloides* are grown in solid-state and submerged culture at 50 and 10% solid loading rate, respectively. Total GLS levels were reduced up to 65.5 and 79.8% by thermal treatments while microbial conversion further reduced GLS up to 97.8 and 97.5% in solid-state and submerged fungal incubation, respectively. Optimized trials will be analyzed for toxic GLS breakdown compounds.

[P7] GC-MS and NMR Metabolomic Profiling with Solid-state NMR Analysis of FHT-RNAi Silenced Potato Periderm Tissues

Presenter(s): Qing Cai, City College of New York, CUNY Graduate Center and Institute for Macromolecular Assemblies,

Author(s): Qing Cai, City College of New York; Wenlin Huang, City College of New York; Olga Serra, University of Girona, Campus Montilivi Mercè Figueras, University of Girona, Campus Montilivi Marisa Molinas, University of Girona, Campus Montilivi; Ruth E. Stark, City College of New York

Suberin is a biopolyester constituent of specialized plant periderm tissues formed within the phellem cell walls. Suberin and waxes of the periderm layer act to prevent water diffusion, mechanical breakdown and pathogenic invasion in plants. Ferulic esters are considered to be the most important linkage between aliphatic and aromatic suberin domains and also linked with cell-wall polysaccharides. The potato gene FHT (fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase) esterifies ferulic acid to suberin. Diminished levels of feruloyl transferase activity have been associated with lowered amounts of feruloyl esters of fatty acids in both suberin-associated waxes and suberin breakdown products derived from transesterification. FHT knockdown by RNAi silencing is accompanied by increases in periderm water permeability but unaltered periderm ultrastructure. To investigate how this potato suberin biosynthesis pathway is affected in FHT-RNAi silenced potato cultivars, metabolite profiles for non-polar periderm tissue extracts were examined by complementary GC-MS and solution NMR measurements, and solid suspensions after polar and nonpolar extractions were assessed by solid-state 13C NMR. Metabolomic analyses (PCA and OPLS-DA) showed consistency among biological

replicates and discrimination between FHT-RNAi and wild type samples, allowing identification of 25 out of 31 biomarkers including alkanes, α,ω-fatty diacids, fatty acids/alcohols, methyl esters and glyceryl esters. The relative numbers of each major carbon type in the solid residue were estimated from quantitatively reliable DPMAS 13C NMR spectra. The FHT-RNAi cultivar has oxygenated aromatic-to-(CH2)n and oxygenated-aliphatic-to-(CH2)n ratios that exceed the values for wild type cultivars by ~60 and 80%, respectively. These compositional trends indicate an enhanced hydrophilic-hydrophobic balance in FHT-RNAi suberin samples, consistent with augmented water permeability and reduced relative amounts of fatty acid and fatty alcohol-containing polymeric products deduced from suberin breakdown. The solid-state results complement our GC-MS data showing large amounts of fatty acid and fatty alcohol metabolites accumulated in non-polar extracts from FHT-RNAi native periderms.

[P8] Effects of Anandamide on Development, Growth and Cellular Organization of *Physcomitrella patens*

Presenter(s): Jedaidah Chilufya, East Tennessee State University

Author(s): Shiva Devaiah and Aruna Kilaru, East Tennessee State University.

Mosses are bryophytes with a simple cellular organization and distinctive growth stages. With their unique lipid profile, most mosses are also tolerant to various stressors. A ubiquitous class of bioactive fatty acid ethanolamides in eukaryotes called N-acylethanolamines (NAEs) also occurs in the moss *Physcomitrella patens*. Unlike in higher plants, where saturated and unsaturated NAE types are limited to those with acyl chains 12C to 18C, *P. patens* also contains anandamide, NAE 20:4. In higher plants, NAEs are most abundant in desiccated seeds and mediate plant growth, development, cellular organization and response to stress, in an abscisic acid (ABA)-dependent or independent manner. In mammals, NAE 20:4 acts as an endocannabinoid ligand and mediates a multitude of physiological responses. This unique NAE type, NAE 20:4 is hypothesized to effect development, growth and cellular organization of *P. patens*. To

determine the role of NAEs in moss development, NAE content and composition in protonema, early and late gametophyte stages, and sporophytes, will be quantified from their total lipid extracts, using selective lipidomics. The effects of anandamide on growth will be studied by culturing moss in the presence of exogenous NAE 20:4 in a dose-dependent manner. Temporal changes in growth patterns will be determined by the evaluation of digital images using ImageJ tool. The effect of anandamide on cytoskeletal organization will be visualized by immunostaining the phyllodes exposed to NAE 20:4 and observing them under confocal microscope. Preliminary results indicate that the endogenous NAE content and composition is variable, depending on the developmental stage and that NAE 20:4 is a potent negative regulator of moss growth. More detailed studies are expected to provide novel insights into the role NAEs, specifically NAE 20:4 might play in mediating growth and development of seedless plants.

[P9] Isolation of new phenolics with anticancer and antioxidant potential from roots of *Rhodiola imbricata* and their quantification by validated HPLC method

Presenter(s): Alka Choudhary, aDepartment of Natural Products, National Institute of Pharmaceutical Education and Research, Sector-67, S.A.S. Nagar, 160062, Punjab, India

Author(s): Alka Choudhary, Sunil Kumar Surapaneni, Kulbhushan Tikoo, and Inder Pal Singh, National Institute of Pharmaceutical Education and Research, Sector-67, S.A.S. Nagar, 160062, Punjab, India.

Raj Kumar, Division of Phytochemistry, Defence Institute of High Altitude research (DRDO), C/o 56 APO, Leh-Ladakh, 901205, J&K, India

Several plants of genus *Rhodiola* (family Crassulaceae) have been widely used in

traditional/modern medicine for their ability to enhance physical endurance and to treat impotence, fatigue, gastrointestinal, cardiac and central nervous system disorders. One such species with cytoprotective/radioprotective properties is *Rhodiola* imbricata. Aqueous extract of *R. imbricata* rhizome has shown anti-cancer activities, which might be useful in leukemia cancer treatment. The proliferation of K-562 cell line was significantly decreased after incubation with aqueous extract of *R. imbricata* due to induction of apoptosis and cell cycle arrest at G2/M phase. Inspite of several biological studies, no phytochemical studies have been reported for this plant. As a part of our ongoing programme to isolate and characterize natural products, we planned phytochemical investigations on *R. imbricata* in search of new molecules from nature. In the present study, phytochemical investigations led to isolation of several phenolic glycosides (including three new compounds) along with simple phenolics from the roots of *R. imbricata*. The structures of isolated molecules were established by NMR spectroscopy (1D and 2D NMR) and Mass spectrometry (ESI HRMS). The compounds were evaluated for antioxidant activity in various antioxidant assays and anticancer activity against A549 and MCF7 cell lines. the percentage of isolated molecules was determined in the extract by a validated HPLC method.

[P10] Application of annatto hairy roots for the production of bioactive compounds for human health

Presenter(s): Jarrod Creameans, Arkansas Biosciences Institute

Author(s): Jarrod Creameans, Lingling Fang, Denzel McGregory, and Luis Nopo-Olazabal, Arkansas Biosciences Institute, Fabricio Medina-Bolivar Arkansas Biosciences Institute and Dept. of Biological Sciences, Arkansas State University

Annatto (*Bixa orellana*) is a tropical plant native to South America which is currently used as a traditional medicine to treat multiple diseases including skin infections, respiratory problems and malaria. Extracts from leaves, seeds, fruits and roots of this plant have shown to possess biological activities in vitro, however the bioactive constituents of these extracts have not been elucidated. Furthermore, the seeds of this plant are the source for the natural pigment "annatto dye" which is commonly used in the food industry. Previously, we established hairy root cultures of annatto to produce and identify potential bioactive compounds from this species. This work led to the characterization of candidate chemicals with anti-malarial activity in vitro, including ishwarane, tocotrienol and stigmasterol (Molecules 2014, 19:756-766). To increase the levels of these bioactive compounds, the hairy root cultures of annatto were treated with different combinations of elicitors, including methyl jasmonate and cyclodextrin, for different time periods. HPLC analyses showed that several compounds were induced and excreted into the culture medium. Further characterization of these compounds is underway. We propose that hairy root cultures of annatto can be developed as a sustainable source for the production and discovery of bioactive compounds with distinct bioactivities.

[P11] Black bean (*Phaseolus vulgaris* L.) anthocyanins: extraction, thermal stability and shelf-life at different pHs and temperatures

Presenter(s): Luis Mojica, University of Illinois

Author(s): Luis Mojica, University of Illinois, Andy Tan, University of Illinois, Mark Berhow, United States Department of Agriculture, Agricultural Research Service, and Elvira Gonzalez de Mejia, University of Illinois

The aim of this research was to optimize the anthocyanins extraction from black bean coats and evaluate their thermal and shelf-life stability at different pHs and temperatures. Anthocyanins were extracted from black bean coats using different ethanol concentrations, temperatures and solid-to-liquid ratios. Anthocyanin solutions were adjusted to different pHs according to commercially available beverages (pH 2.5, 3.0, 3.5, 4.3).

From the black bean coats, it was possible to obtain a 7.1% dry powder yield of anthocyanins. Optimal extraction conditions were ethanol 24%, solid-to-liquid ratio 1:40 and 29 ˚C ($P < 0.0001$). Bean anthocyanins were more stable at pH 2.5 and 4 ˚C (89.6%) in comparison to 22 ˚C (19.3%), with a half-life of 273 and 56 days, respectively. Anthocyanins presented the lowest degradation rate value at 70 ˚C ($k = 0.061/h$) and pH 3.0, and the highest half-life values (11 h). Energy of activation ranged from 55.9 to 84.8 kJ/mol, having higher values the solutions with lower pHs. The highest Q10 change was observed at pH 2.5 with a value of 2.99 for 70 to 80 °C, while the lowest Q10 was found at pH 4.3 (1.18) for 80 to 90 ˚C change. Three anthocyanins were identified by their mass ions as delphinidin 3-O-glucoside (M+ = 465.1), petunidin 3- O- glucoside (M+ = 479.1) and malvidin 3-O-glucoside (M+ = 493.1). Black beans are a good source of anthocyanins with potential to be used as food colorants.

[P12] Development of a Combined Electrospray Ionization/Atmospheric Pressure Chemical Ionization, Polarity Switching High Resolution-Accurate Mass Liquid Chromatography-Mass Spectrometry Method and Comparative Metabolomics of Five Grass Species

Presenter(s): Bradley S. Evans, Donald Danforth Plant Science Center

Author(s): Elizabeth A. Kellogg, Donald Danforth Plant Science Center

The two most widely utilized ionization source types in liquid chromatography-mass spectrometry (LC-MS) are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Each can be used in either positive or negative ionization modes. The ESI+, ESI-, APCI+ and APCI- modes of operation provide complementary information about the molecular composition of a sample, yet if all these modes are used for analysis it is in series rather than parallel. High resolution accurate mass (HRAM) mass spectrometry enables the confident assignment of elemental compositions to ions and facilitates identification of known compounds and begins structure elucidation of unknowns. In order to collect more comprehensive metabolomic profiles in a single analysis we have developed a combined ESI-APCI ion source coupled to a HRAM LC-MS system capable of polarity switching. We applied this technique for the metabolomic profiling of five species of grasses: *Arundinella hirta, Capillipedium spicigerum, Coelorachis tuberculosa, Ischaemum hubbard* and *Miscanthus junceus*.

[P13] Biomimetic Preparation of Halogenated Norcoclaurine Analogs via Tyrosinase for Directed Biosynthesis In Benzylisoquinoline Alkaloid Producing Cells

Presenter(s): Jordan Fauser, DePaul University

Author(s): Jordan E Fauser[1], Sean O Crowe[1], Realino Gurdiel[1], Greg Gillespie[1], Michael J Sislow[1], Justin J Maresh[1]

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We present a low-cost, one-pot, aqueous synthesis of halogenated norcoclaurine analogs, as potential substrates for precursor directed biosynthesis. Using a cross linking technique we form an insoluble aggregate of tyrosinase from plant and fungal sources such as sweet potato, avocado, and white button mushroom. This aggregate catalyzes oxidation of halogenated tyramine to halogenated dopamine, a reaction with an inherently unfavorable equilibrium. High-yield turnover is achieved by coupling dopamine production to Pictet-Spengler condensation with 4-hydroxy-phenylacetaldehyde to generate halogenated analogs of the tetrahydroisoquinoline norcoclaurine. The resulting norcoclaurine analogs may be isolated by extraction or used directly from the reaction mixture for precursor-directed biosynthesis in plant cell cultures to produce halogenated benzylisoquinoline alkaloids. To the best of our knowledge, this work is the first to successfully utilize tyrosinase

for high-yield production of unnatural catechol products.

[P14] Gas chromatography-based myrosinase activity and QTL mapping in broccoli (Brassica oleracea L. var. italica)

Presenter(s): Alicia M. Gardner, University of Illinois at Urbana-Champain

Author(s): Alicia M. Gardner, University of Illinois at Urbana-Champaign, Kang Mo Ku, University of Illinois at Urbana-Champaign, Allan F. Brown, North Carolina State University, and John A. Juvik, University of Illinois at Urbana-Champaign

Vegetables in the *Brassica* genus have been shown to contain a number of compounds that exhibit pharmacological activity. This research elucidates genetic loci involved in the production of glucosinolate hydrolysis products, which are associated with anti-cancer bioactivity in broccoli (*Brassica oleracea* L. var. italica). The myrosinase (thioglucosidase EC 3.2.1.147) activities of 104 F2:3 families from the mapping population BNC x VI158, grown in 2009 and 2010, were determined by the quantification of sinigrin and benzyl glucosinolate (exogenous glucosinolate) hydrolysis products on a gas chromatography-flame ionization detector system. QTL analysis of the data was conducted in the software package MapQTL 5 (Van Ooijen, 2004) using a dense linkage map with 590 mapped SNP marker loci, covering an estimated 96% of the B. oleracea genome. This map was developed by Brown et al. (2014) using a 60K Illumina SNP array of Brassica napus, and is anchored to the genomic sequence of the rapid cycling Brassica oleracea TO1000. The results of this study will provide useful information for the identification of genes involved in the chemopreventative activity of broccoli and support the breeding of Brassica cultivars that can serve as functional foods.

[P15] Identification and quantification of bioactive compounds from Panax ginseng using HPLC-CAD

Presenter(s): Rajarshi Ghosh, Middle Tennessee State University

Author(s): Rajarshi Ghosh, Anthony Farone, Elliot Altman and Paul Kline, Middle Tennessee State University

Panax ginseng is one of the most popular and widely used medicinal plants worldwide. Ginseng roots have been shown to have broad spectrum pharmacological effects on the immune system, cardiovascular system, and central nervous system. The ginsenosides and polysaccharides have been credited to be the active ingredients responsible for the various pharmacological effects of ginseng. With the increasing amount of ginseng products in the market, it is essential to have proper quality control procedures to standardize the presence of various ginsenosides and polysaccharides in adequate quantities. There is a need for new and simple methodologies for detection and quantification of these compounds. In the present study, an attempt has been made to identify and quantify the various bioactive compounds in ginseng using High performance liquid chromatography (HPLC) coupled with charged aerosol detector (CAD). CAD offers an attractive alternative detection technique for various compounds as it does not depend on the spectral properties of the analyte. This feature is especially helpful in detecting compounds such as carbohydrates which have poor UV absorptivity. Quantification of compounds can be performed based on calibration curve of a single standard. A Waters XBridge amide column with gradient elution was used to separate the compounds. The detected compounds were identified based on retention times of respective standards.

[P16] Synthesis of 1,3,4-oxadiazole derivatives as potential and selective COX-2 inhibitors

Presenter(s): Jagdeep Grover, Department of Natural Products, National Institute of

Pharmaceutical Education and Research, Sector-67, S.A.S. Nagar-160062, Punjab

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Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to inflammatory mediators such as prostaglandins (PGs), prostacyclins and thromboxanes. Ongoing safety concerns pertaining to the use of non-selective NSAIDs have spurred development of coxibs with improved safety profile.1 As a part of our continued efforts to discover new COX inhibitors2,3, a novel series of 2,5-biaryl-1,3,4-oxadiazoles have been synthesized and evaluated for in vitro COX-1 and COX-2 inhibition, in vivo anti-inflammatory potential and cytotoxicity evaluation in J774A.1 and RAW 264.7 cell lines. Almost all the compounds displayed selective COX-2 inhibition with SI range from 30-100. Compounds with nitro and tert-butyl substituents emerged as most potent with IC50 value in the range of 0.81-0.89 μM against COX-2. The same compounds also exhibited superior anti-inflammatory activity (50-59% inhibition of edema at 150 μmol/Kg) in comparison to celecoxib. Molecular docking studies further predicted the binding orientation of ligands at the active site of enzyme. Cytotoxicity study of potent compounds revealed the cell viability of more than 90% in the said cell lines. The present study suggests the significance of bulky substituent like methylsulfonyl and tert-butyl as a pharmacophoric feature for selective inhibition of COX-2 enzyme.

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[P17] Metabolomic Profiling and Thioacidolysis Analysis of FHT-RNAi Silenced Potato Periderm Tissues

Presenter(s): Liqing Jin, City College of New York, CUNY Graduate Center and Institute for Macromolecular Assemblies

Author(s): Liqing Jin, City College of New York, Wenlin Huang, City College of New York, Olga Serra, University of Girona, Campus Montilivi, Joan Rigau, Centre for Research in Agricultural Genomics (CRAG), Consorci CSIC-IRTA-UAB-UB, Campus de Bellaterra UAB, Mercè Figueras, University of Girona, Campus Montilivi, Marisa Molinas, University of Girona, Campus Montilivi, Ruth E. Stark, City College of New York

The potato (*Solanum tuberosum*) is an important staple crop. Potato tubers are protected from dehydration and pathogens by a covering peel (periderm) impregnated with suberin, a complex cross-linked heteropolymer that contains both polyaliphatic and lignin-like aromatic domains. Current models describing the macromolecular structure of suberin assume that ferulic acid cross-links both domains as it may form carboxyl esters bonds with aliphatic monomers and non-ester radical coupled bonds with phenolics. Ferulic acid also links by ester bonds to glycans and acts in cross-linking polysaccharides and lignins. Fatty alcohol/ω-hydroxyacid hydroxycinnamoyl transferase (FHT) is a BADH acyltransferase responsible of the synthesis of akyl-ferulates necessary for suberin biosynthesis. Periderm from tubers deficient in FHT showed a significant decrease in suberin ferulate esters and an increase in soluble phenolics. This periderm also showed changes in the texture and water permeability but, surprisingly, the ultrastructure of the suberin is not altered. To further investigate the consequences of FHT silencing in potato periderm, polar extracts of native tuber periderm have been analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS), revealing significantly enhanced amounts of six identified phenolic amines including feruloyl tyramine, feruloyl putrescine and caffeoyl putrescine, which are derived from feruloyl-CoA. The insoluble part of suberin has also

been subjected to thioacidolysis and analyzed by Gas Chromatography-Flame Ionization Detector (GC-FID), showing that FHT-RNAi aromatic suberin contains larger amounts of guaiacyl units, which are also derived from feruloyl-CoA. These metabolite and polymer changes for FHT-RNAi periderms and the associated fate of feruloyl-CoA in the suberin biosynthetic pathway suggest that FHT-RNAi silencing disfavors alkyl ferulate biosynthesis and favors an alternative disposition of the feruloyl-CoA substrate.

[P18] Responses of two parasitoids of the emerald ash borer, *Agrilus planipennis* Fairmaire, the introduced *Spathius agrili* Yang, and native *Spathius floridanus* Ashmead, to volatile host-associated cues

Presenter(s): Todd David Johnson, University of Wisconsin-Madison (where the work was conducted);

University of Illinois Urbana-Champaign (current affiliation)

Author(s): Todd David Johnson (as above), Jonathan P. Lelito, USDA APHIS PPQ, EAB Unit, and Kenneth F. Raffa, University of Wisconsin-Madison

The introduced specialist parasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae) has been released as a biological control agent of the emerald ash borer, *Agrilus planipennis* Fairmaire. *Spathius floridanus* Ashmead (Hymenoptera: Braconidae) a native generalist parasitoid, opportunistically attacks *A. planipennis* and may contribute to control of the insect. We currently know little about the host location behaviors of these wasps. We evaluated responses of female wasps to components of this host complex, *Fraxinus pennslyvanica* (green ash) stem tissue, *F. pennslyvanica* foliage, and an *A. planipennis* larva within a stem. Experiments were conducted in Y-tube olfactometer bioassays, using wasps reared on *A. planipennis* larvae feeding in *F. pennslyvanica* twigs at USDA APHIS in Brighton, MI. Naïve *S. agrili* were attracted to the entire complex, and leaf tissue alone, relative to blank controls. *S. agrili* were also significantly attracted to a stem with a larva and leaf tissue together, relative to leaf tissue alone. In contrast, naïve *S. floridanus* were attracted to a larva within a stem, but not to any other component. Thus, naïve *S. agrili* and *S. floridanus* appear to employ different host-location strategies. Because *S. floridanus* attack multiple species of *Agrilus*, host-associated cues perceived during emergence from their natal hosts may improve attraction to emerald ash borer larvae. Subsequent experience may elicit attraction to plant foliage, and thereby facilitate location of host habitat. In contrast naïve *S. agrili* may learn additional host-associated cues during oviposition. Further understanding of host-location behavior may improve the utility of these parasitoids for biological control, both by suggesting strategies for pre-release conditioning, and by providing tools for assessing post-release establishment.

[P19] Design, Synthesis, Drug-Likeness and Anti-ovarian Cancer Activity of Thymoquinone Analogues

Presenter(s): Okiemute Rosa Johnson-Ajinwo, Institute for Science and Technology in Medicine, Keele University, UK.

Author(s): Okiemute Rosa Johnson-Ajinwo[1,2], Alan Richardson[1], and Wen-Wu Li[1]

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Cancer-related deaths claim about 20,000 lives daily. Thymoquinone (TQ), a bioactive compound isolated from *Nigella sativa* has demonstrated in vitro moderate activities against breast, colon, ovarian, pancreatic and prostate cancers, including leukaemia, but the compound is poorly soluble.1 Recently, thymquinone has been shown to block the pSer/pThr recognition by Polo-like-kinase 1 Polo-Box-Domain at the atomic level. In this work, the synthesis of TQ analogues via structure-based design, cytotoxicity, solubility,

drug-likeness and structure-activity relationship (SAR) investigation were carried out.

Methods: Eleven TQ analogues were synthesised via reduction and amine addition to quinone, while nine analogues were procured from Sigma Aldrich. The in vitro Sulforhodamine B assay and Trypan blue method were used for the evaluation of the cytotoxic activities of TQ and its analogues on three ovarian cancer cell lines; A2780, OVCAR8 and A2780CIS. The in vitro Selective index (SI) was determined using the human ovarian epithelial cells (HOE). Phosphate buffer was employed in the aqueous solubility study. ChemBioDraw software was used to estimate the log P values of the compounds.

Results/Conclusion: TQ analogues disclosed a structure-activity relationship. Three of the analogues showed significant cytotoxic activity and were twice-more potent than TQ. The solubility data validated the Log P values estimated by the software. An evaluation of the drug-likeness of the analogues showed that 'Lipinski rule of 5' was obeyed; justifying their potentials for further research as possible clinical agents.

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[P20] In-vivo Hepatoprotective and Antioxidative Activity of *Glycosmis Pentaphylla* Leaves Against CCl4-Induced Oxidative Hepatocellular Damage

Presenter(s): Pradeep Kamboj, JCDM College of Pharmacy

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Ajudhia N. Kalia, Department of Pharmacognosy, Sri Sai College of Pharmacy, Pathankot, Punjab, India-145001

The present study was conducted to evaluate the hepatoprotective and antioxidative potential of hydroalcoholic extract of *Glycosmis pentaphylla* leaves (GpE) and chloroform, ethyl acetate & n-butanol fractions of GpE. The toxicant CCl4 (1 ml/kg in olive oil; sc) was administered on 4th and 5th day of study to induce oxidative hepatotoxicity in rats. The hepatoprotection of the test drugs where compared with the reference drug silymarin (50 mg/kg). The pre-treatment of rats with higher dose of GpE (400 mg/kg) and EAf (150 mg/kg) for 7 days produced a significant ($p<0.05$) dose dependent hepatoprotective and antioxidative affect evident by decreased levels of serum (AST, ALT, ALP & TB) and tissue TBARS in addition to increased levels of serum (TP & ALB) and reduced tissue GSH. The histological examination provided the supportive evidences that strongly demonstrate antioxidative affect on hepatocytes and restoring their normal functional ability. The present study scientifically validates the free radical-scavenging property to be one of the mechanisms of hepatoprotection and justify the traditional medicinal claims attributed to *Glycosmis pentaphylla* leaves. Further detailed studies are in progress to elucidate bioactive constituent/s and their concentration in the plant.

[P21] In-vitro Cytotoxicity Activity of *Eclipta alba* Linn Against A-549, HEp-2 and HeLa Cell Lines

Presenter(s): Sonia Kamboj, Dept. of Biology, Satluj Public School, Sirsa

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The present study was aimed to evaluate the in-vitro cytotoxicity activity of ethanolic extract of *Eclipta alba* aerial parts (EaE) against three human cancer cell lines namely lung (A- 549), liver (Hep-2) and Cervix (HeLa) cell lines. The cytotoxicity potential of EaE was evaluated

by SRB and MTT assays and the percentage viability of the cell lines were carried out using Trypan blue dye exclusion method. The EaE extract showed maximum percentage of growth inhibition against A-549 cell line; where as in case of Hep-2 cell lines no cytotoxicity activity was observed. In concentration range of 1000 to 31.25 (µg/ml), EaE significantly produced cytotoxicity effect on A-549 and HeLa cell lines with CTC50 values 44.60 and 51.30 in SRB and MTT assays, respectively. Therefore, the results of present study scientifically validate the use of *Eclipta alba* as an anticancer agent in traditional system of medicine, and provide potential information for its further in-vivo and phytochemical studies.

[P22] Structural and functional analysis of Grapefruit Cp-3-O-GT mutant P145T.

Presenter(s): Sangam Kandel, East Tennessee State University, Johnson City, TN

Author(s): Sangam Kandel, Sarah Khaja, Shivakumar Devaiah, McIntosh A. Cecilia, East Tennessee State University, Johnson City

Flavonoids are a class of secondary metabolites, the majority of which are present in glucosylated form. Glucosyltransferases are the enzymes that mediate glucosylation by transferring glucose from a high energy sugar donor to the acceptor substrates. Our study focuses on the structural and functional analysis of a flavonol-specific 3-O-glucosyltransferase (Cp-3-O-GT) clone from *Citrus paradisi* that has been characterized previously in our lab. Multiple sequence alignment and homology modeling was done to identify candidate residues for mutation. Cp-3-O-GT was modeled with a flavonoid 3-O-GT from Vitis vinifera (VvGT) that can glucosylate both flavonols and anthocyanidins. We identified a proline residue at position 145 of Cp-3-O-GT that corresponded to a threonine residue in VvGT and designed a Cp-3-O-GT- P145T mutant to test the hypothesis that that mutation of proline by threonine in Cp-3-O-GT could alter substrate or regiospecificity of Cp-3-O-GT. While the mutant P145T enzyme did not glucosylate anthocyanidins, it did glucosylate flavanones and flavones in addition to flavonols. This is significant because flavanones and flavonols do not contain a 3-OH group. HPLC was performed to identify the reaction products. Early results indicated that the mutant protein glucosylates naringenin at 7-OH position forming prunin. Product identification with other substrates is in progress. Results are being used to revisit and refine the structure model. Structural and functional analysis of flavonoid GTs may contribute to custom design of GTs for the synthesis of novel glucosides by changing glucosylation patterns

[P23] The identification of biochemical pathway genes from non-model plant species

Presenter(s): Matthew B. Kilgore, Donald Danforth Plant Science Center

Author(s): Megan M. Augustin, Donald Danforth Plant Science Center

Gregory D. May, National Center for Genome Resources

John A. Crow, National Center for Genome Resources

Toni M. Kutchan, Donald Danforth Plant Science Center

Secondary metabolites are often restricted in their distribution to different groups of organisms. For this reason, attempts to study these often useful and interesting forms of metabolism require an ability to work in a diversity of non-model species. Methods for gene discovery with low investment and high efficiency are needed to effectively identify the biosynthetic genes in these diverse pathways. During this work, a workflow for efficiently identifying biosynthetic genes is developed and applied to Amaryllidaceae alkaloid biosynthesis. Genes discovered during this work include a cytochrome P450 capable of phenol-phenol coupling 4′-O-methylnorbelladine to noroxomaritidine, a norbelladine 4′-O-methyltransferase (NpN4OMT) and a short chain dehydrogenase/reductase capable of forming norbelladine

from tyramine and 3,4-dihydroxybenzaldehyde. These enzymatic discoveries support the future application of this workflow to other biosynthetic pathways and organisms.

[P24] The Effect of R382w Mutation on *C. Paradisi* Flavonol Specific 3-O-Glucosyltransferase

Presenter(s): Kathleen King, East Tennessee State University

Author(s): Shivakumar Devaiah, East Tennessee State University and Cecilia McIntosh, East Tennessee State University

Flavonoids are a class of plant metabolites with C6-C3-C6 structure responsible for many biological functions, including coloration and defense. *Citrus paradisi*, grapefruit, contains a wide variety of flavonoids which are grouped by the extent of modification, examples of which are flavonols, flavones, and flavanones. A major modification is the addition of glucose by glucosyltransferases (GTs) to stabilize the structure and provide ease of transport. This process can be highly substrate and regiospecific. With Cp3OGT, glucose is added at the 3-hydroxy position. This 3GT only accepts flavonols as its substrate; however, a *Vitis vinifera* (grape) 3-GT can accept both flavonols and anthocyanidins. Homology modeling using the crystallized structure of the *V. vinifera* GT predicted sites of amino acids that could influence substrate binding site. The 382 position was of particular interest with arginine in *C. paradisi* and tryptophan in *V. vinifera*. This change is hypothesized to cause a shift in substrate specificity of the Cp3OGT to accept anthocyanidins as well as flavonols. Site-directed mutagenesis was performed to form the R382W mutant Cp3OGT and transformed into yeast for expression. Western blot determined the optimal protein induction period for the cells, after which the cells were broken to extract the recombinant mutant protein. Purification of the R382W 3GT allowed for enzyme analysis to be performed by measuring the incorporation of radioactive glucose into the reaction product. HPLC will be used to identify reaction products. An enzyme kinetics study will show the extent of any biochemical change in function as a result of this mutation; results will then be incorporated into a refined protein model.

[P25] SABP2, A Tobacco Methyl Salicylate Esterase has Role in Modulating Nonhost Resistance

Presenter(s): Dhirendra Kumar, East Tennessee State University

Author(s): MD Imdadul Haq, East Tennessee State University

Pavan Chigurupati, East Tennessee State University

Nonhost resistance is a type of broad-spectrum resistance exhibited by a given plant species to most strains of a pathogen which are generally pathogenic to other plant species. Studies show that gene-for-gene resistance activates SA-signaling but if it also activated during nonhost resistance is not clear. SABP2, a methyl salicylate esterase is a critical component in SA-signaling pathway in tobacco plants, it also has a role in nonhost resistance. To study this, SABP2-silenced tobacco plants were infected with tobacco nonhost pathogen, *Pseudomonas syringae* (P.s) pv. phaseolicola (Psp) and its growth was monitored. Data analysis revealed slightly enhanced growth of Psp suggesting SABP2 plays a minor role in inducing nonhost resistance against Psp in tobacco plants. RT-PCR analysis of SABP2 transcripts upon Psp infection showed that its transcript level increased with time in both control plants as well as in SABP2-silenced tobacco plants suggesting the involvement of SABP2 in nonhost resistance. To fully suppress the SABP2 activity, SABP2-silenced plants were further treated with 2,2,2,2′-tetra-fluoroacetophenone (tetra-FA), a known inhibitor of SABP2 prior to the inoculations with Psp. The tetra-FA treated SABP2-silenced plants showed compromised resistance to Psp suggesting a role for SABP2 in nonhost resistance. This observation was supported by gene expression analysis of tetra-FA treated plants. The expression of other nonhost marker genes such as CDM1 and HIN1

was also monitored in P.s. tabaci (Pst, virulent/host pathogen on tobacco), P.s. phaseolicola and P.s. tomato (Psp and Psto, avirulent/nonhost pathogens on tobacco), and TMV infected control and SABP2-silenced tobacco plants. This revealed no significant difference which suggest that these two genes are activated in SABP2-SA independent manner. Overall results suggest that SABP2-mediated SA signaling has a role in nonhost resistance in tobacco plants. Understanding the mechanism of nonhost resistance is likely to help in developing broad-spectrum disease-resistant crop plants.

[P26] Characterization of Volatile Compounds in Grains of Newly Developed Aroma Rice Varieties by using HS-SPME-GCMS

Presenter(s): Young-Sang Lee, Soonchunhyang University

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To characterize flavor property of newly developed aroma rice varieties (cv. Gahyangchal-1-ho and cv. GoldenQueen-3-ho), brown rice powders were transferred into a headspace vial and solid phase microextraction (SPME) combined with a gas chromatography mass spectrometry (GCMS) were used for the analysis of volatile compounds and comparison to non-aroma variety (cv. Chucheong). Based upon NIST mass spectral library total 64 volatile compounds consisting of 28 hydrocarbons, 10 alcohols, 7 ketones, 4 aldehydes, 3 esters, and some bases etc. could be identified in all 3 tested varieties. Among those 64 identified, 17 volatiles such as hexanal and 2-pentyl-furan were found commonly in both aroma and non-aroma rice varieties suggesting these compounds as general volatiles in rice grain. However, 23 volatiles such as 2-acetyl-1-pyrroline (responsible for popcorn flavor), heptanal, 2-octanone could be observed only in aroma rice varieties, while 14 volatiles such as 3,7,11-trimethyl-1-dodecanol and 1,3-dimethyl-Benzene could be observed only in non-aroma rice variety. Two tested aroma varieties even showed different volatile profiles probably due to the difference in flavor-inducing genes introduced during the breeding program.

[P27] Sulfur-containing amino acids enhanced the antioxidant activity of peptides present in common bean (*Phaseolus vulgaris* L.) non-digestible fraction

Presenter(s): Diego Luna-Vital, University of Illinois at Urbana-Champaign

Author(s): Diego Luna-Vital, University of Illinois at Urbana-Champaign; Michael Kolman, University of Illinois at Urbana-Champaign; Guadalupe Loarca-Pina, Universidad Autonoma de Queretaro and Elvira de Mejia, University of Illinois at Urbana-Champaign

The objective of this research was to assess the antioxidant potential of five pure synthesized peptides (GLTSK, LSGNK, GEGSGA, MTEEY and MPACGSS) originally identified from common bean non-digestible fraction (NDF). The in vitro evaluations were performed using CCD-33Co normal colon cells treated simultaneously with hydrogen peroxide (H_2O_2) and the bean peptides for 4 h. Intracellular reactive oxygen species (ROS) were detected with the cell permeant reagent 2',7' –dichlorofluorescein diacetate (DCFDA), a fluorogenic dye that measures several ROS activities. The cytoprotective effect of the peptides on CCD-33Co cells exposed to H_2O_2 was determined treating the cells simultaneously with H_2O_2 and the peptides for 24 h and evaluating cell viability. Also, superoxide (SO), hydroxyl (HO) and nitric oxide (NO) radicals scavenging activity and the effect on linoleic acid oxidation of the peptides were assessed. The peptides reduced the intracellular concentration of ROS ranging from 18.2% to 72.1% in CCD-33Co cells.

Besides, the peptides increased the viability (68.2% to 124.8%) of CCD-33Co cells in comparison when they were treated with H_2O_2 only (21.3%). Furthermore, it was observed that peptides had scavenging properties with radical inhibition over NO (16.1% to 73.8%), SO (9.5% to 64.6%) and HO (4.9% to 56.0%) radicals. Finally, the peptides promoted a delay in linoleic acid oxidation ranging from 0.0066 to 0.426 AU/h (absorbance units are proportional to oxidation level), compared to the control (0.516 AU/h). The number of sulfur-containing amino acids significantly correlated with all the antioxidant activities tested; NO (r=0.95), SO (r=0.95) and HO (r=0.69), the reduction on linoleic acid oxidation (r=-0.86), and the in vitro determinations of ROS decreased (r=0.66) and cytoprotective effect (r=0.70). In general, MPACGSS was the most potent peptide followed by MTEEY in all the evaluations, correlating with the presence of sulfur-containing amino acids methionine and cysteine, known to provide antioxidant properties to peptides.

[P28] Biochemical and genetic characterization of tyrosine aminotransferases in *Arabidopsis thaliana*

Presenter(s): Hiroshi A Maeda, University of Wisconsin-Madison

Author(s): Minmin Wang, Kyoko Today, Hiroshi A. Maeda, University of Wisconsin-Madison

In plants, tyrosine (Tyr) serves as a key precursor of various plant natural products (e.g., tocochromanols, plastoquinone, ubiquinone, rosmarinic acid, morphine alkaloids), which are crucial for plant environmental responses and of pharmaceutical or nutritional importance to human. Tyrosine aminotransferases (TAT) catalyze the reversible transamination between Tyr and 4-hydroxyphenylpyruvate (HPP), the key entry reaction to many of the Tyr-derived compounds. Here we report phylogenetic, biochemical, genetic, and subcellular localization analyses of TAT enzymes from *Arabidopsis thalian*. TAT1 and TAT2 formed a monophyletic clade that is sister to a cystine lyase clade. Detailed biochemical characterization showed that the recombinant enzymes of both TAT1 and TAT2 converted Tyr to HPP, as previously reported, but also used various keto acceptors and other amino acids such as phenylalanine (Phe) and methionine (Met). The tat1 and tat2 single and double mutants of *A. thaliana* exhibited only partial reduction in total TAT activity, increased Tyr levels, and partially reduced levels of tocopherol and plastoquinone. GFP localization and subcellular fractionation studies further showed that TAT1 and TAT2 as well as subsequent HPP dioxygenase (HPPD) are located outside of the plastids. These results indicate that TAT1 and TAT2 partially contribute to the conversion of Tyr to HPP outside the plastids, leading to tocopherol and plastoquinone biosynthesis. However, TAT1 and TAT2 likely have additional functions and also yet to be identified aminotransferases having TAT activity exist in both cytosol and plastids in *A. thaliana*. The study highlights biochemical and genetic complexity of Tyr catabolism leading to the formation of natural products in plants.

[P29] Solgen 40, the high Genistin/ Genistein soy concentrate prevents bone mineral loss during menopause in a dose concentrate manner preventing the oxidized bone microenvironment.

Presenter(s): Jose Angel Marañon, Tradichem Innovation Center

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Estrogen deficiency accelerates the effects of aging on bone by decreasing defense against oxidative stress (OS). Genistin/genistein

and daidzin/daidzein, the most common phytoestrogens from soy isoflavones seems to protect the adult skeleton against bone loss by slowing the rate of bone remodeling and by maintaining a focal balance between bone formation and resorption. Recent studies report that reactive oxygen species (ROS) may play a role in postmenopausal bone loss by creating a more oxidized bone microenvironment and increase the intracellular concentration of the antioxidant glutathione in bone prevents bone loss during estrogen deficiency in mice. For understanding how soy isoflavones can prevent the bone damage by ROS and help in the maintenance of glutathione levels we have studied the capacity of genistin/genistein, daizin/daidzein for blocking a common ROS as DPPH radical, by a novel HPLC-DPPH-DAD in vitro test. Our results has revealed that the high genistin/genistein soy concentrate SOLGEN 40 blocks ROS species in a dose dependent manner. In the other hand soy concentrates containing high daidzin/daidzein just partially blocks ROS and the increase of dadizin/ daidzein concentration do not increase the block of ROS. Therefore, the administration of high dosages of SOLGEN 40 and other high genistin/genistein soy concentrates seems to be a safe an efficient strategy during menopause for preventing bone mineral loss by diminishing the intracellular concentration of reactive oxygen species

[P30] DNA Intercalating Alkaloids Isolated From *Chelidonium Majus* (Papaveraceae)

Presenter(s): Tamer Mohamed, Institute of Pharmacy and Molecular Biotechnology, Department of Biology, University of Heidelberg, Germany

Author(s): Wink Michael, Institute of Pharmacy and Molecular Biotechnology, Department of Biology, University of Heidelberg, Germany

DNA intercalating agents increase the stability of DNA which can be demonstrated by measuring the melting temperature (Tm). Tm can be determined in a spectrophotometer in which the cell temperature is increased gradually. The resulting absorption data comes as a sigmoidal curve from which melting temperature can be determined when half of the DNA has denatured. The current study aims to assess DNA intercalating activities of four pure bioactive isoquinoline alkaloids: sanguinarine, berberine, allocryptopine and chelerythrine which were isolated from *Chelidonium majus* (Papaveraceae) by repeated silica gel column chromatography, recrystallization and preparative thin layer chromatography (TLC). The isolated compounds were identified by comparing their physical properties and mass spectra with those of the published data. The results showed that sanguiarine is the most active intercalating agent with Tm value of 83.55 ± 0.49 followed by berberine, chelerythrine and allocryptopine with Tm values 62.58 ± 0.47, 51.38 ± 0.37 and 50.94 ± 0.65, respectively, relative to 49.78 ± 1.05 of bacteriophage DNA alone and 86.09 ± 0.5 for ethidium bromide as a positive control.

[P31] Improving stability of bean anthocyanins by β-cyclodextrin in a sport beverage

Presenter(s): Luis Mojica, University of Illinois at Urbana-Champaign

Author(s): Luis Mojica, University of Illinois at Urbana-Champaign; Y., Aguilera, Universidad Autónoma de Madrid; M.A. Berhow, United States Department of Agriculture; María A Martín-Cabrejas, Universidad Autonoma de Madrid; and E. González de Mejia, University of Illinois at Urbana-Champaign

The objective was to investigate the color stability of common bean anthocyanins from two black bean cultivars (Otomi and Idaho) co-pigmented with β-cyclodextrin in a sport beverage, as potential replacements for synthetic colorants. The model beverage system was exposed to white fluorescent light and stored at 32 °C for 10 days. Antioxidant capacity, total anthocyanins, and individual anthocyanin characterization (LC-ESI-MS) were performed. Cultivar Idaho bean exhibited the highest antioxidant capacity (325.1 μM Trolox/g dry coat) and presented the highest

concentration of anthocyanins (183.0 mg/100 g dry coat). For both cultivars, petunidin 3-O-glucoside (1.2-37.8 mg/100 g dry coat), delphinidin 3-O-glucoside (55.8-60.0 mg/100 g dry coat), petunidin (48.5-85.3 mg/100 g dry coat) and malvidin 3-O-glucoside (5.1-14.5 mg/100 g dry coat) were found. The degradation reaction of anthocyanins in the presence of β-cyclodextrin followed the first-order kinetics (6.2 and 5.1 d-1 in Otomi and Idaho cultivars, respectively). A similar behavior occurred in the absence of β-cyclodextrin (4.5 and 3.9 d-1 in Otomi and Idaho cultivars, respectively). The half-life ($t_{1/2}$) of anthocyanins was increased significantly ($p < 0.05$) with the addition of 2% β-cyclodextrin (8 vs. 13 days). Moreover, in the co-pigmented sport beverage the color difference values (ΔE*) were higher (43.7 and 47.1 in Otomi and Idaho cultivars, respectively) than the no co-pigmented (34.4 and 30.2 in Otomi and Idaho cultivars, respectively) under the same experimental conditions. Thus, data demonstrated that bean anthocyanins have the potential to be used as natural pigments in beverages stabilized by β-cyclodextrin.

[P32] CASMI 2014: Challenges, solutions and results

Presenter(s): Dejan Nikolic, University of Illinois at Chicago

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Steffen Neuman, Leibnitz Institute of Plant Biochemistry, Halle, Germany

The Critical Assessment of Small Molecule Identification (CASMI) contest, inaugurated in 2012, was designed as the medium for researchers to exchange ideas as to how to best identify and annotate small molecules based on mass spectrometric data. In this contest participants are asked to solve the structures of unknown compounds based on a set of high resolution MS and MS/MS data provided by the organizers. The third round of CASMI was organized by an international team of researchers from the United Kingdom and the United States. The challenges were divided into four groups: endogenous metabolites, natural products, synthetic chemicals and unknown compounds. In addition to high resolution MS and MS/MS data, additional information such as chromatographic data and sample origin was also provided. Mass spectrometric data were acquired on different types of instruments including qTOF, Orbitrap, IT-TOF and FTMS. The contest consisted of two categories: best molecular formula (Category 1) and best structure (Category 2). Seven participants took part in the contest. The winner in the category 1 correctly solved 34 out of 42 challenges; the winner in the Category 2 correctly solved 23 out of 42 challenges. Natural products proved to be the most challenging group of compounds with the smallest number of correct solutions submitted.

[P33] Molecular Identification of Tuliposide B-Converting Enzyme: a Lactone-Forming Carboxylesterase from the Pollen of Tulip

Presenter(s): Taiji Nomura, Toyama Prefectural University

Author(s): Taiji Nomura, Toyama Prefectural University;

Shinjiro Ogita, Toyama Prefectural University;

Yasuo Kato, Toyama Prefectural University

6-Tuliposides A (PosA) and B (PosB), which are the major secondary metabolites in tulip (*Tulipa gesneriana*), are enzymatically converted to the antimicrobial lactonized aglycons, tulipalins A (PaA) and B (PaB), respectively. We recently identified a PosA-converting enzyme (TCEA) as the first reported member of the lactone-forming carboxylesterases, Herein, we report the identification of another lactone-forming

carboxylesterase, PosB-converting enzyme (TCEB), which preferentially reacts with PosB to give PaB. This enzyme was isolated from tulip pollen, which showed high PosB-converting activity. Purified TCEB exhibited greater activity towards PosB than PosA, which was contrary to that of the TCEA. Novel cDNA (TgTCEB1) encoding the TCEB was isolated from tulip pollen following peptide sequencing of the purified TCEB. TgTCEB1 belonged to the carboxylesterase family of alpha/beta-hydrolase fold and was approximately 50% identical to the TgTCEA polypeptides. Functional characterization of the recombinant enzyme verified that TgTCEB1 catalyzed the conversion of PosB to PaB with an activity comparable with the native TCEB. RT-qPCR analysis of each part of plant revealed that TgTCEB1 transcripts were limited almost exclusively to the pollen, despite the presence of significant levels of PosB-converting activity in several other tissues (e.g., roots), which suggested the presence of a TgTCEB1 isozyme gene being expressed in such tissues. Immunostaining of the anther cross-section using anti-TgTCEB1 polyclonal antibody verified that TgTCEB1 was specifically expressed in the pollen grains, but not in the anther cells. N-Terminal transit peptide of TgTCEB1 was shown to function as plastid-targeted signal. Taken together, these results indicate that mature TgTCEB1 is specifically localized in plastids of pollen grains. Interestingly, PosB, the substrate of TgTCEB1, accumulated on the pollen surface, but not in the intracellular spaces of pollen grains. This sequestration system of the enzyme (TgTCEB1) from its substrate (PosB) represents a unique chemical defense mechanism of the pollen of tulip.

[P34] Characterization of SBIP68 a Putative Tobacco Glucosyltransferase and Its Role in Plant Defense

Presenter(s): Abdulkareem Odesina, East Tennessee State University

Author(s): Abdulkareem Odesina and Dhirendra Kumar, East Tennessee State University

SBIP68, a tobacco protein was found was found to interact with SABP2 in a two-hybrid screening. SABP2 is an important component of the salicylic acid pathogen response pathway in tobacco and other plants. Based on bioinformatics, SBIP68 was predicted to be a putative UDP-glucose: flavonoid glucosyltransferase, having a GT1_Gtf_like conserved domain as well as the 44-amino acid PSPG Box characteristic of Family 1 glycosyltransferases (UGTs). Glucosides are a ubiquitous class of secondary metabolites involved in roles ranging from the protection of plants against pathogens and herbivory to the physical appearance of plants, transportation of metals, symbiotic agents between plants and microorganisms, and acting as sexual hormones. Glucosyltransferases transfer glucose molecules from activated glucose donors like UDP-glucose to potential aglycones, producing the corresponding glucosides. The full length SBIP68 gene was cloned and expressed heterologously in E. coli as well as in Pichia pastoris. Purified recombinant protein from Pichia was further used for biochemical analyses. A total of fourteen different substrates have been tested so far, including flavonoids and simple phenolics. It showed relatively high activity with several flavonoids known to have roles in stress signaling. Biochemical analyses using simple phenolics as aglycones is in progress. Research is also ongoing to determine the effect of SBIP68's interaction with SABP2 on its biochemical activity. Earlier studies on the gene expression analyses of SBIP68 showed that it is likely modulated upon viral pathogen infection. Studying SBIP68 will help to improve our general understanding of how plants respond to pathogen attacks.

[P35] Peptides from beans increased insulin secretion, reduced oxidative stress and inhibited lipid accumulation in *in vitro* models

Presenter(s): Miguel Eduardo Oseguera Toledo, University of Illinois at Urbana-Champaign/ Universidad Autonoma de Queretaro

Author(s): Miguel E. Oseguera-Toledo, Universtity of Illinois at Urbana-Champaign/Universidad Autónoma de Querétaro: Silvia Amaya-Llano,Universidad Autónoma de Querétaro; Elvira González de Mejía, Universtity of Illinois at Urbana-Champaign

The objective was to evaluate the effect of de-hulled hard-to-cook (HTC) bean bioactive peptide fractions on important proteins for type-2 diabetes, oxidative stress, lipid accumulation and glucose uptake in in vitro models. Protein isolates from two common bean varieties, pinto Durango and Negro 8025 beans, were hydrolyzed (120 min) with either alcalase® or bromelain and then peptides < 1 kDa were fractionated using an ultrafiltration membrane system. The hydrolysate and peptide fraction < 1 kDa were further hydrolyzed with pepsin-pancreatin simulating a gastrointestinal digestion. To evaluate the role of the peptides as insulin secretagogues and antioxidants, iNS-1E pancreatic beta-cells were used. Pinto-bromelain peptide fractions < 1 kDa were able to increase glucose-stimulated insulin secretion by 20-150 µIU insulin/mL in a dose-dependent manner (1 µg/mL and 100 µg/mL, respectively) in iNS-1E cells; Pinto-alcalase peptide fractions < 1 kDa increased the relative protein expression of IGF-II, IGFBP-2 and 3 and reduced VEGF. Oxidative stress and reactive oxygen species (ROS) in basal conditions did not affect cell viability however decreased ROS production (70%). Pre-treatment of iNS-1E cells with hydrolysates or their fractions (1–100 µg/ml) for 20 h significantly reduced cell damage induced by 100 µM tert-butylhydroperoxide (t-BOOH) for 2 h, up to 70% for complete hydrolysate of pinto Durango-alcalase (100 µg/ml). The effect of peptides reduced intracellular fat accumulation (28%) when applied during 3T3-L1 adipocyte differentiation and (25%) applied directly to mature 3T3-L1 cells. In addition, treatments improved insulin-induced glucose uptake (64%) in insulin resistant adipocytes. This study demonstrates that protein hydrolysates and their peptide fractions, even after gastrointestinal digestion, improved insulin secretion and protected pancreatic cells against oxidative stress, inhibited lipid accumulation and improved glucose uptake in adipocytes.

[P36] A Survey of Anthocyanin Concentration and Composition in Diverse Maize Germplasm

Presenter(s): Michael Paulsmeyer, University of Illinois at Urbana-Champaign

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Increasing consumer demand for more natural ingredients has spurred interest in the replacement of artificial dyes in foods and beverages with naturally occurring phytopigments. In this study, our lab used HPLC to screen the anthocyanin content of over 375 accessions of maize from diverse backgrounds. Anthocyanin concentrations in our collection ranged from less than 1 to 2547 mg anthocyanins per kg whole corn. After HPLC analysis, each sample was categorized based on the location and abundance of various anthocyanin structural types. Our findings showed that pericarp tissue produces more pigment than aleurone tissue on average and pericarp tissue has the unique ability to produce anthocyanins condensed with flavonols. Aleurone tissue was found to be important as a source of high ratios of malonylglucoside anthocyanins. In general, cyanidin derivatives were the most

common anthocyanin types, but we have found 43 accessions segregating for the purple aleurone1 (pr1) gene that produce primarily pelargonidin types of anthocyanins. Finally, we are now characterizing a new phenotype that, to our knowledge, has never been documented before in maize. Several accessions in our collection are unable to produce anthocyanins containing acylated glycosides. The information collected in this study represents the most comprehensive screen of colored maize lines to date, providing a valuable tool for the production of high anthocyanin yielding corn lines.

[P37] Phytochemical diversity drives tropical plant-insect community diversity

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What are the ecological causes and consequences of variation in phytochemical diversity within and between plant taxa? Despite decades of natural products discovery by organic chemists and research by chemical ecologists, our understanding of phytochemically-mediated ecological processes in natural communities has been restricted to either broad classes of compounds or a small number of well-characterized molecules. Until now, very few studies have assessed the ecological causes or consequences of quantified phytochemical diversity across taxa in natural systems. Consequently, hypotheses that attempt to explain variation in phytochemical diversity among plant taxa remain largely untested. We take advantage of recent technological advances for the generation of spectral data from crude plant extracts to characterize phytochemical diversity in a suite of co-occurring plants in the tropical genus, *Piper* (Piperaceae). In combination with 20 years of data on Piper associated insects, we found that phytochemical diversity has a direct and positive effect on the diversity of herbivores, but also reduces overall herbivore damage. Elevated chemical diversity is associated with more specialized assemblages of herbivores, and the cascading positive effect of phytochemistry on herbivore enemies is stronger as herbivore diet breadth decreases. It is clear from these results that high phytochemical diversity not only enhances the diversity of plant-associated insects, but also contributes to the ecological predominance of specialized insect herbivores.

[P38] P450 from woad is involved in formation of indigo and indirubin in recombinant *Escherichia coli*

Presenter(s): Laxmi Sagwan-Barkdoll, Southern Illinois University Carbondale

Author(s): Laxmi Sagwan-Barkdoll, Parminder Multani and Aldwin Anterola, Southern Illinois University Carbondale

The plant *Isatis tinctoria*, commonly known as woad, is a natural source of the blue dye indigo and its structural isomer indirubin, which is considered to be the active ingredient in antileukemic Chinese herbal preparations. Oxidation of indole can produce indigo and indirubin, but the enzymes that catalyze this reaction in plants are unknown. We cloned a P450 gene from *I. tinctoria* and expressed it in *E. coli*, which then produced a water-insoluble blue precipitate. Extraction of these bacterial cultures with chloroform and dimethyl formamide, followed by HPLC and UV-Vis spectrophotometry, showed the presence of both indigo and indirubin. Indigo production was increased approximately 3-fold when the P450 is coexpressed with isatin hydrolase (from *Pseudomonas*), an enzyme that degrades isatin

to isatic acid. Meanwhile, indirubin production was improved by supplementation with 500 mg/l of 2-oxindole and 100 mg/l of isatin. We now propose that P450 genes are involved in the biosynthesis of indigo precursors and indirubin in plants.

[P39] Structure-function investigations of site directed mutants of Citrus paradisi flavonol specific 3 O glucosyltransferase—Impact of mutations of serine, histidine and glutamine residues

Presenter(s): Preethi Sathanantham, East Tennessee State University at Johnson City

Author(s): ShivaKumar Devaiah, East Tennessee State University at Johnson City and Cecilia McIntosh, East Tennessee State University at Johnson City

Glucosyltransferases (GTs) are enzymes that enable transfer of glucose from an activated donor (UDP-glucose) to the acceptor substrates. A flavonol specific glucosyltransferase cloned from *Citrus paradisi* has strict substrate and regiospecificity (Cp3OGT). The amino acid sequence of Cp3OGT was aligned with a purported anthocyanin GT from *Clitorea ternatea* and a GT from *Vitis vinifera* that can glucosylate both flavonols and anthocyanidins. Using homology modeling to identify candidate regions followed by site directed mutagenesis, three double mutations of Cp3OGT were made. Biochemical analysis of the three mutant proteins was performed. S20G+T21S protein retained activity similar to the wildtype (WT- Kmapp-80 µM; Vmax = 16.5 pkat/µg, Mutant- Kmapp-83 µM; Vmax -11 pkat/µg) but the mutant was more thermostable compared to the WT and this mutation broadened its substrate acceptance to include the flavanone, naringenin. S290C+S319A mutant protein retained 40% activity relative to wildtype, had an optimum pH shift, but had no change in substrate specificity (Kmapp-18 µM; Vmax-0.5 pkat/µg). H154Y+Q87I protein was inactive with every class of flavonoid tested. Product identification revealed that the S20G+T21S mutant protein widened the substrate and regio-specificity of CP3OGT. Docking analysis revealed that H154 and Q87 could be involved in orienting the ligand molecules within the acceptor binding site. H363, S20, and S150 were also found to make close contact with the 7-OH, 4-OH and 3'-OH groups, respectively.

[P40] The Effects of Coconut Water as an Undefined Media Amendment on the Growth of *Panax Quinquefolius* Calli.

Presenter(s): Shannon Smith, Middle Tennessee State University

Author(s): Shannon Smith, Middle Tennessee State University

Dr. John Dubois, Middle Tennessee State University

Dr. Elliot Altman, Middle Tennessee State University

Panax quinquefolius, or American ginseng, has been harvested and cultivated for generations. Research is ongoing to illuminate the relationship between secondary metabolites present and physical responses to the metabolites in humans. Due to the recalcitrance of the plant to tissue culture, research into the optimization of quality tissue production for scientific analysis has been undertaken. Calli were initiated for experimentation, and initial results pointed to the beneficial affects certain media amendments had on tissue growth. A study was performed to determine if the promising media mixture has a long term statistically significant effect on the growth of calli. Samples of calli were weighed and plated on media containing the undefined nutrient supplement (+S media), and media without the undefined nutrient supplement (-S media). Differences in growth rate, overall mass, phenolic production, and culture count were tracked over a six month period, with cultures passaged once every four weeks. Data were statistically analyzed via Sigma Stat. Results suggest the media supplement does affect the calli, giving an initial boost in growth with effects tapering off over time. The initial differences were sufficient to result in the variance in mass increase between

the two media types. This information has been used to better optimize tissue growth protocols, supplying tissue for chemical analysis and experiments to optimize metabolite production.

[P41] Characterization of a Tobacco SIR2 like Enzyme for its Role in Plant Stress Signaling

Presenter(s): Bal Krishna Chand Thakuri, East Tennessee State University

Author(s): Mackenzie Davenport1, East Tennessee State University

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SBIP-428 is a SABP2-interacting tobacco protein identified in yeast two-hybrid screen. It shows high similarity to SIR2 (Silent Information Regulator 2) like proteins. SIR2 enzymes exhibit NAD+-dependent deacetylase activity by catalyzing the deacetylation of post-translationally acetylated lysine residue in histones as well as non-histone proteins. SABP2 is critical for inducing systemic acquired resistance (SAR) and local resistance in tobacco and other plants. By its esterase activity, SABP2 catalyzes the conversion of methyl salicylate to salicylic acid (SA), a plant hormone which plays an important role in plant defense system. Arabidopsis SRT2 (AtSRT2) negatively regulates the basal defenses by inhibiting the biosynthesis of SA with no visible effect in growth phenotype, indicating its possible role in plant immunity. A large number of proteins/enzymes implicated in various metabolic pathways present in cytoplasm and various organelles in plants and other organisms have been found to be lysine acetylated. SBIP-428 was cloned from tobacco leaves and heterologously expressed in *E. coli*. Interestingly the lyse residue of recombinant SBIP-428 expressed in *E.coli* was found to be in acetylated, which opens up the possibility of its post-translational regulatory role in the expression of the various non-histone protein present in plant cell including defense related proteins. Expression of SIR2 is influenced by various biotic and abiotic stress factors which implicates SIR2 plant stress signaling. Transgenic tobacco plant with altered expression (RNAi-mediated silencing and inducible overexpression of SBIP-428) is being developed which will be used to study its role in stress signaling in tobacco plants. Understanding the role of SBIP-428 is likely to implicate SABP2/SA in regulating various plant stress signaling pathways via acetylation/deacetylation.

[P42] Probing the Volatile Terpenome of Switchgrass Roots in Interaction with Rhizobacteria

Presenter(s): Dorothea Tholl, Department of Biological Sciences, Virginia Tech

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Plant roots sustain intimate relationships with diverse microbiota that contribute to plant productivity and resilience. The plant traits that define the composition of root microbial communities by "habitat" filtering are poorly understood. Volatile organic compounds (VOCs) such as terpenoids affect colonization by epiphytic bacteria in aboveground tissues (Junker and Tholl, 2013). However, the role of VOCs in interaction of roots with epiphytic and endophytic microbes is largely unknown. We investigate the function of volatile terpenoids as host-specific chemo-selective factors in the rhizosphere by using the native prairie grass, switchgrass *(Panicum virgatum)*, as a model system. Root volatile profiling of multiple

switchgrass cultivars demonstrated qualitative and quantitative variation of several terpenoid compounds. By contrast, the monoterpenoids, camphor and borneol, were highly conserved among cultivars suggesting an important role for these VOCs in belowground interactions. Camphor is known for its antimicrobial and insect repellent effects but also serves as the exclusive carbon source of soil-borne microbes. The accumulation of camphor was stimulated by inoculation of switchgrass seedlings with nitrogen-fixing rhizobacteria, which supports the role of this compound as a potential chemical mediator in the rhizosphere. Following a root transcriptome analysis, we are currently identifying root-expressed terpene synthase genes to subsequently generate terpenoid (camphor) biosynthetic mutants and analyze associated changes of their root microbiomes. These studies will help define VOC-based niches of microbes in the rhizosphere.

Junker R. and D. Tholl (2013). Volatile Organic Compound Mediated Interactions at the Plant-Microbe Interface. Journal of Chemical Ecology 39: 810-825.

[P43] Identification and In-silico Analysis of Fatty Acid Amide Hydrolases in Tomato

Presenter(s): Vijay Tiwari, East Tennessee State University

Author(s): Vijay Tiwari, Derek Stuffle and Aruna Kilaru, East Tennessee State University

N-acylethanolamines (NAEs) are a family of signaling lipids derived from a minor membrane lipid constituent N-acylphosphatidylethanolamine (NAPE). In Arabidopsis, NAE mediates physiological functions such as seedling growth, flowering, and response to stress via abscisic acid (ABA) –dependent and –independent signaling pathways. The function of NAEs is terminated by a highly conserved fatty acid amide hydrolase (FAAH). Studies in model plant Arabidopsis showed the significant role of NAEs that makes it relevant to elucidate the conserved metabolic pathway of NAEs in crop species such as tomato. It is hypothesized that there is a functional FAAH in tomato that hydrolyzes NAEs. To test this hypothesis, AtFAAH was used as a template to identify putative FAAH sequences in tomato, using BLASTX. Six SlFAAH sequences with the conserved amidase signature sequence and the catalytic triad, formed by Lys205, Ser281, and Ser305 in AtFAAH, were identified. Phylogenetic analysis of putative SlFAAH homologs and other FAAH family proteins (*Arabidopsis*, rice and moss), using CLUSTALW, revealed the two sequences that are closely related to the functionally characterized AtFAAH1. Using molecular visualization system (PyMOL), protein structures of putative SlFAAH1and 2 were predicted and compared with AtFAAH; both sequences showed similar domain structure to AtFAAH, with minor differences in spatial arrangement. For further biochemical characterization, full-length coding sequence of SlFAAH1 and SlFAAH2 were isolated and cloned into a heterologous expression system. The expressed protein will be characterized for its hydrolytic activity against radiolabelled NAE substrates. Furthermore, transcript levels for SlFAAH1 and SlFAAH2 will be quantified and correlated with the NAE levels in various tissues to predict their role in tissue-specific NAE hydrolysis. Together, these molecular and biochemical characterization studies in tomato are expected to further validate the conserved nature of NAE metabolic pathway in plants.

[P44] Biosynthesis Enhancement and Purification of Bioactive Prenylated Stilbenoids from Hairy Root Cultures of Peanut by High Performance Countercurrent Chromatography

Presenter(s): Christopher Aaron Tollett, Arkansas State University

Author(s): Carson Day, Arkansas State University Tianhong Yang, Arkansas State University Luis

Nopo-Olazabal, Arkansas State University and Fabricio Medina-Bolivar, Arkansas State University

Stilbenoids are polyphenolic compounds found in plants like peanuts and grapes which have a wide range of biological effects and potential benefits to human health. Among the peanut stilbenoids is arachidin-1, a prenylated analog of piceatannol which has shown higher antioxidant activity and cytotoxicity in different cancer cell lines and potentially higher metabolic stability than other stilbenoids. In order to produce this and other prenylated stilbenoids, peanut hairy root cultures were co-treated with multiple elicitors for different periods of time. Ethyl acetate extracts were prepared from the culture medium of the elicited cultures and analyzed by HPLC. In addition to previously described stilbenoids, potentially novel stilbenoids were also detected in the culture medium. To further study the biological activity of these compounds, they are being purified using high performance counter current chromatography (HPCCC). Initially, the distribution constant of the desired compounds is determined using a solvent system composed of different volumes of hexane, ethyl acetate, methanol and water. Pooled extracts from the culture medium are then separated by HPCCC and fractions corresponding to the various compounds are analyzed by HPLC to assess purity. Our preliminary studies have shown that the levels of prenylated stilbenoids can be increased several fold by co-treatments with different elicitors and that HPCCC can be used to obtain selected prenylated stilbenoids with high levels of purity.

[P45] *Calea urticifolia* lyophilized aqueous extract inhibits lipopolysaccharide-induced inflammation in RAW 264.7 macrophages.

Presenter(s): Maria Lucina Torres Rodriguez, University of San Luis Potosí

Author(s): Maria Lucina Torres Rodriguez, University of San Luis Potosí, Mark Berhow, United State Department Agriculture, Agricurtural Research Service, Elvira Gonzalez de Mejia, University of Illinois at Urbana-Champaign, Erika Garcia Chavez, University of San Luis Potosi, and Celia Aradillas Garcia, University of San Luis Potosi

Low-grade inflammation has been widely related with metabolic disorders and chronic diseases, such as insulin resistance, obesity, type 2 diabetes, cardiovascular diseases and cancer. The tea prepared with *Calea urticifolia* leaves is used traditionally like a remedy to treat gastric ulcers, diabetes and inflammatory processes by the Xi'uy ancient native community of San Luis Potosi, Mexico. The objective of this study was to characterize the phenolic compounds of *Calea urticifolia* lyophilized aqueous extract (CuAqE) and evaluate in vitro its anti-inflammatory and anti-oxidant properties in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Phenolic compounds of CuAqE were characterized by LC-ESI-MS. RAW 264.7 macrophages were treated with CuAqE (10, 25, 50, 75 and 100 mg/mL) and stimulated with LPS. Nitric oxide (NO) release, inducible nitric oxide synthase, prostaglandin E2 production, cyclooxygenasa-2, p65, p50 and reactive oxygen species (ROS) were measured. A mix of caffeoylquinic acid derivatives and flavonoid-glucoside was found in CuAqE. NO (5.3, 17.9, 57.4, 90.2 and 103.2% respectively) and ROS (94.4, 99.3, 108.1, 108.4 and 108.1% respectively) production were significantly ($P<0.05$) inhibited in a dose-response manner in LPS-stimulated RAW 264.7 macrophages. Therefore, phenolic compounds in CuAqE could be responsible to inhibit LPS-induced inflammation through ROS/NO reduction. In conclusion, results support the traditional knowledge of *Calea uritifolia* tea such as an anti-inflammatory agent and suggested antioxidant properties.

[P46] Sub-chronic oral toxicity of *Calea urticifolia* lyophilized aqueous extract in Wistar rats.

Presenter(s): Maria Lucina Torres Rodríguez, University of San Luis Potosi

Author(s): Maria Lucina Torres Rodríguez, University of San Luis Potosi and Erika Garcia Chavez, University of San Luis Potosi

Calea urticifolia is a shrub of the Asteraceae family and is used by the Xi'uy ethnic group of San Luis Potosi, Mexico. The tea prepared with the leaves is used such as remedy to treat gastric ulcers, diabetes and inflammatory processes. However, toxicity studies of aqueous extract have not been reported. Therefore, the objective of this research was to evaluate sub chronic toxicity in vivo of *Calea urticifolia* lyophilized aqueous extract (CuAqE) as part of its pre-clinical studies. Male and female young adult Wistar rats (180-200 g) were treated with 0.5, 50 and 500 mg/kg of CuAqE by gavage during 28 days. Mortality incidence, clinical signs, body-weight gain changes, food and water consumption were registered every day. Organ weights, biochemical (urea, creatinine, alanine transaminase and aspartate transaminase) and hematological (white blood cells, red blood cells, platelets, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin) parameters were measured at the end of study. Sub chronic oral administration of CuAqE during 28 days did not produce both mortality and clinical signs. Food and water consumption, organ weights, biochemical and hematological parameters showed not-significant changes in both male and female rats ($P > 0.05$). Body weight gain was significantly ($P < 0.05$) lower than control group (distilled water), when was administered 50 mg/kg at 21 and 28 day (28 ± 25 % and 44 ± 25 % respectively) and 500 mg/kg at 7, 14, 21 and 28 day(95 ± 66 %, 64 ± 45 %, 59 ± 31 %, 64 ± 19 % respectively) in female rats. Eventually, results of this study support that CuAqE has a low grade of toxicity. Therefore, the traditional dose (0.55 mg CuAqE/kg body weight) of *Calea urticifolia* tea can be safely consumed.

[P47] High-throughput Phenotyping of Rice Lines Within a Rice Diversity Panel to Determine Salinity Tolerance

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Rice is currently one of the most important crops for global food security. A significant portion of optimal rice producing areas are prone to climate change and rising sea levels. This is causing the soil in these rice producing areas to become increasingly saline over time. Eventually the lands become too saline to produce the crops and become unutilized. In order to keep meeting rice demand there is a need to develop new rice varieties that can thrive under saline conditions. This work is a collaborative effort between the Walia Laboratory at the University of Nebraska Lincoln and the Lorence Group at Arkansas State University. The Walia team has been studying the salt tolerance of a rice diversity panel at two developmental stages that are particularly susceptible to this stress in greenhouse experiments. Based on their results, a selected group of rice lines including salt tolerant and salt sensitive types as well as positive and negative controls were sent to the Lorence team to study their response to salt stress at the early vegetative growth stage in greenhouse experiments. We have utilized a powerful high throughput phenotyping system, the Scanalyzer HTS, in order to acquire digital images using visible and florescence cameras to quantify the tolerance to salt of over 85 rice accessions grown in hydroponic conditions. In this work we will present our progress on

the identification and characterization of salt tolerant rice lines.

[P48] Isolation of bioactive compounds of *Piper holdridgeanum* identified to cause inhibition of yeast cell growth

Presenter(s): Federico Urbano Muñoz, University of Nevada Reno

Author(s): F. Urbano-Muñoz, Christopher .S. Jeffrey, Lora .A. Richards, and Ian .S. Wallace, University of Nevada Reno

Natural products isolated from an array of organisms have served as templates for medicine and many other applications. Piper is a phytochemically diverse genus, which has not fully been chemically explored. One major challenge lies in identifying bioactive molecules from crude extracts. To identify possible bioactive compounds found in a species of *Piper*, a series of assays were performed to determine which species crude extract demonstrated growth inhibition on *Arabidopsis thaliana* Col-0 root length, and cells of *Saccharomyces cerevisiae* S288c,and Escherichia coli DH5α. We combined these screens with NMR based metabolomics analysis to determine possible candidates for further isolation of bioactive compounds. From the 31 species screened in triplicate we found high inhibition levels of cell growth of S. cerevisiae S288c grown in the presence of *Piper holdridgeanum* extract. In further investigations of these species we found that *P. holdridgeanum* produces two sesquiterpenes and two unknown phytol isomers. Future studies will focus on employing bioassay guided fractionation to identify active metabolites and more comprehensive bioassays of isolated compounds to determine if synergistic effects are resulting in enhanced bioactivity.

[P49] A novel emulsion-forming arabinogalactan gum from the stems of Frost grape (*Vitis riparia* Michx.)

Presenter(s): Steven F. Vaughn, USDA, ARS, NCAUR

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A novel arabinogalactan polysaccharide (FGP) is described that is produced in large quantities from the cut stems of Frost grape (*Vitis riparia* Michx.). The sugar composition consists of L-arabinofuranose (L-Araf, 55.2 %) and D-galactopyranose (D-Galp 30.1%), with smaller components of D-xylose (11.2 %), D-mannose (3.5%), and glucuronic acid (GlcA, ~2%), the latter linked via a galactosyl residue. Permethylation identified 3-linked Galp residues, some substituted at the 2-position with Galp and Manp, terminal Araf and Xylp, and internal 3-substituted Arap. NMR identified βGalp and three αAraf spin systems, in a Araf-α1,3-Araf-α1,2-Araf-α1,2-Galp structural motif. Diffusion-orientated NMR showed that the FGP has a molecular weight of 1 - 10 MDa. Unlike gum arabic, the FGP does not contain a hydroxyproline-rich protein. FGP forms stable gels at >15 % w/v, and solutions at lower concentrations (1 - 12% w/v) are viscous, and are excellent emulsifiiers of flavoring oils (grapefruit, clove, and lemongrass), giving stable emulsions for ≥72 hours. Lower concentrations (0.1% w/v) were less viscous, yet still gave stable grapefruit oil/water emulsions. These properties indicate that FGP is a β-1,3-linked arabinogalactan with potential as a gum arabic replacement in the food and beverage industries.

[P50] Evaluation of the Cytotoxic Activity of *Origanum Vulgare*

Presenter(s): Tibebe Woldemariam, California Northstate College of Pharmacy

Author(s): Tibebe Woldemariam and david Pearson, California Northstate College of Pharmacy

Herbs used for flavoring and scents have been shown to be a rich source of phytochemicals and are getting much attention as a potential source of cancer chemoprotective agents. Strong epidemiological evidence also suggests that regular ingestion of herbs and spices can reduce the risk of cancer. The culinary herb

oregano *Origanum vulgare* for example has been demonstrated to have antimicrobrial activity along anti-inflammatory and wound healing activity. Oregano was also reported to contain anti prostate cancer activity.

In the present study, Carvacrol derived from Oregano methanolic extract was isolated and assessed for possible in vitro cytotoxic activity against a panel of pancreatic cell lines and non-small cell lung cancer cell line A549 and PANC-10.

Bioactivity-guided fractionation of the methanol extract afforded several fractions and compounds which showed significant reductions in cell viability. The major compound, carvacrol, was found to be significantly active against both lung and pancreatic cell lines. The results of these studies including the bioassay-guided fractionation of the methanolic extracts, isolation of carvacrol and cytotoxic activity of the fractions and compounds will be presented.

These outcomes highlight the prominence of the use of herbs and spices as possible sources for prevention and treatment of cancer.

[P51] Anthocyanin biosynthesis in red-colored dark tobacco plants activated by PAP1/MYB75

Presenter(s): Xianzhi He, Department of Plant and Microbial Biology, North Carolina State University, NC 27695, USA,

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PAP1/MYB75 is master regulator controlling anthocyanin biosynthesis in Arabidopsis thaliana. Its regulatory function requires transcription factor partner, such as TT8/bHLH and TTG1/WD40. Although PAP1 can activate anthocyanin pathway, anthocyanin molecule diversities depend upon plant species. In this study, we introduce PAP1 into two dark tobacco varieties. The anthocyanin pathway is activated by PAP1 overexpression. Most of pathway genes are upregulated to form an engineered pathway toward anthocyanins. Eleven anthocyanin molecules are detected from extracts of engineered plants. One anthocyanin molecule accounts for approximately 98% of total anthocyanins. HPLC-qTOF-MS/MS analysis identifies that the dominant anthocyanin is cyanidin 3-O-rutinoside. This result implies a preferred effect of PAP1 regulation molecular dominance of anthocyanins in dark tobacco plants.

[P52] Metabolic diversity during ontogeny of *Piper* species

Presenter(s): Lydia Fumiko Yamaguchi, University of São Paulo

Author(s): Lydia Fumiko Yamaguchi, University of São Paulo; Anderson M. Gaia, University of São Paulo; Christopher S. Jeffrey, University of Reno; Massuo J. Kato, University of São Paulo

Tropical forests comprise almost 5% of Earth surface and with estimates of at least 3 million of organisms. Astonishingly only about a sixth of these are known to science. The *Piper* species have pantropical distribution and considered as model system to understand the intricate net of ecological interactions in tropical ecosystems. The investigation of the dynamics in secondary metabolism during ontogeny in plants would provide essential clues for understanding their interactions with herbivores. Ten species of *Piper* were analysed and three groups of chemical profile were observed; for species producing amides in seedling stages (*P. tuberculatum*, *P. amalago* and *P. reticulatum*) no differences in the composition were observed during the development. In the second group (*P. gaudichaudianum*, *P. solmsianum*, *P. regnellii*, *P. caldense* and *P. hemmendorfii*) allylphenols were detected as major compounds in the seedling leaves while adult leaves contained lignans, neolignans or prenylated benzoic acids. Additionally, in a third group (*P. richardiaefolium* and *P. permucronatum*) amides were observed as major compounds in the

seedling leaves instead of lignans or flavonoids found in the adult leaves (Fig. 1). Such disparity in the metabolic profile between different stages could be related to several different adaptive strategy to increase the survival rate of the seedlings.

[P53] Suspension culture of transgenic red tobacco cells to understand proanthocyanidin biosynthesis

Presenter(s): Seyit Yuzuak, North Carolina State University

Author(s): Seyit Yuzuak and De-Yu Xie, North Carolina State University

Proanthocyanidins (PAs) are synthesized in the fruits, leaves, barks and seeds of many plants. They are oligomers or polymers of flavonoids resulting from polymerization of flavan-3-ol units (e.g., epicatechin, catechin) and characterized by diverse structures. PAs can prevent plants from pathogens and radiation-caused damages. PAs and their monomers are also strong antioxidant nutrients having multiple beneficial effects to human health, such as protective activities against cardiovascular, cancer and Alzheimer's diseases. In food products, PAs give an astringent flavor. To date, the biosynthetic pathway of PAs has gained intensive understanding from phenylalanine through leucoanthocyanidins to flavan-3-ols. Two late pathways, the LAR (Leucoanthocyanidin reductase) and the ANR (Anthocyanidin reductase) pathways, have been identified from leucoanthocyanidins to flavan-3-ols. The ANR pathway has allowed the success of metabolic engineering of PAs in anthocyanin-producing cells of plants. However, mechanisms of PA polymerization remain completely unknown. In this report, we show establishment of suspension of red transgenic tobacco cells. Four to six liters of suspension culture were developed to obtain red cells with a high production of anthocyanins culture. Different methods are tested to understand enzymatic activities of red cells. Our preliminary experiments show that suspension cultured cells contain certain catalytic activities to convert catechin and epicatechin. We will continue red cell suspension culture to characterize whether the unknown catalytic activities are associated with PA biosynthesis.

[P54] Low-Temperature Conditioning of "Seed" Cloves Induces Changes in Protein Profile, Enhances Expression of Phenolic Metabolism Genes and Anthocyanin Content in 'Coreano' Garlic (*Allium sativum*) during Plant Development.

Presenter: Miguel David Dufoo-Hurtado, Universidad Autónoma de Querétaro, University of Illinois at Urbana-Champaign

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Low-temperature conditioning of garlic "seed" cloves at 5 °C for 5 weeks accelerated the development of the crop cycle, decreased plant growth, and increased the synthesis of phenolic compounds and anthocyanins in outer scale leaves of the bulbs at harvest time, leading to 3-fold content increase compared with those conditioned at room temperature. Cold conditioning of "seed" cloves also altered the anthocyanin profile during bulb development and at harvest. Two new anthocyanins are reported for the first time in garlic. The high phenolic and anthocyanin

contents in bulbs of cloves conditioned at 5 °C for 5 weeks were preceded by overexpression of putative genes of the phenolic metabolism [6-fold for phenylalanine ammonia lyase (PAL)] and anthocyanin synthesis [1-fold for UDP-sugar:flavonoid 3-O-glycosyltransferase (UFGT)] compared with those conditioned at room temperature. Results also revealed that low-temperature conditioning of garlic "seed" cloves causes alterations in the accumulation of proteins involved in cellular growth, antioxidative/oxidative state, macromolecules transport, protein folding and transcription regulation process. Thus, low-temperature conditioning affected protein biosynthesis and quality control system, photosynthesis, photorespiration, energy production, and carbohydrate and nucleotide metabolism. These processes can work cooperatively to establish a new cellular homeostasis that might be related with the physiological and biochemical changes observed during cold conditioning and plant development.

[P55] Phenolics in alcohol-free fermented blueberry-blackberry beverage caused differential gene expression in pancreatic tissue of C57BL/6J mice fed high-fat diet

Presenter: Diego Luna-Vital, University of Illinois at Urbana-Champaign

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The aim of this research was to determine the potential of phenolic compounds from a fermented blackberry-blueberry beverage to modulate the pancreatic gene expression in mice fed a 60% high fat diet for ten weeks. C57BL/6j mice were randomized into six groups and allowed to drink (ad libitum) an alcohol-free blackberry-blueberry beverage (AFFB), three doses of a phenolic extract (PAE) from AFFB at 1 (0.1 X), or 9 (1X) or 19 (2X) mg ANC/kg BW/d anthocyanin (ANC) concentrations, sitagliptin (hypoglycemic positive control), or water (negative control). Changes in expression of mice genes were analyzed using an Illumina MouseWG-6 v2.0 Expression BeadChip. As determined in the heatmaps, there was a trend to shift the differentially expressed genes (DEG) profile with the water and sitagliptin groups having similar patterns, but different from AFFB (1 DEG), 0.1X (29 DEGs), 1X (202 DEGs) and 2X (123 DEGs). The top up-regulated genes for 0.1X, 1X and 2X were Acot1 (FC=7.42), REEP1 (FC= 3.84) and REEP2 (FC=3.88) respectively, while the top down-regulated genes were GPER (FC=-1.90), GCNT1 (FC=-2.69) and RPRM (FC=-2.53) respectively. These genes are related to fatty acid oxidation, cell surface proteins expression and cell proliferation. The most significantly affected pathways caused by PAE included amino acid degradation and biosynthesis such as methionine, glycine and cysteine, amino acids known to have a negative impact in insulin secretion. On the other hand, some genes with important roles in carbohydrate metabolism such as ME1, ME2, PGD, GALK1, GPD1 and GPD2 were down regulated as a response to PAE treatment (FDR=0.3). Additionally, treatments caused a significant decrease of differential gene expression related to concentration of glucose, according to IPA analysis ($Z < -2$). Results suggested that in the pancreas, PAE contributed to the antidiabetic effect of the AFFB modulating genes related to glucose homeostasis and amino acid metabolism.

[P56] Effect of sulfur dioxide and lactic acid during corn steeping process on extraction of anthocyanins from purple corn pericarp

Presenter: Qian Li, Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign

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Purple corn is rich in natural pigments such as anthocyanins, which can potentially be used as an alternative to artificial colorants in food. Anthocyanins can be extracted during corn processing, which usually generates anthocyanin-rich coproducts. Our previous data shows that steeping water from wet-milling process contains 79.1% of total anthocyanins of the whole corn. Therefore, the objective of this study was to optimize the extraction of anthocyanin from purple corn pericarp steeped using common wet-milling chemicals, sodium metabisulfite ($Na_2S_2O_5$) and lactic acid (L.A.). Two thousand ppm SO_2 treated steeping water contained total anthocyanin (TA) equivalent to 20.5 ± 1.5 mg cyanidin-3-glucoside (C3G)/g pericarp, which was significantly higher than only water for steeping (7.1 ± 0.6 mg C3G/g pericarp, $p < 0.05$). Lactic acid did not change TA concentration. But the combination of SO_2 and lactic acid significantly increased TA up to 22.9 ± 0.2 mg C3G/g pericarp ($p < 0.05$). HPLC revealed that this greater extraction was associated with increased C3G and decreased condensed forms of anthocyanins as compared to only using water. Chroma was highly correlated to the concentration of anthocyanins ($r = 0.999$) and tannin ($r = 0.994$) and Hue angle and Saturation were not significantly correlated to any of the variables measured. This study proposes an economical and novel procedure to enhance total anthocyanin extraction from corn pericarp.

[P57] Phytohormones production during floral transition of soybean

Presenter: Prem L. Bhalla, The University of Melbourne

Authors: Prem L. Bhalla, Chui E. Wong, Mohan B. Singh, The University of Melbourne

Soybean is a major legume crop and a significant source of dietary proteins and edible oil. Flowering process governs seed set and hence affects crop productivity. Soybean is a short-day plant grown broadly across the latitude but with each cultivar having a narrow range of the north to south adaptation. This geographic adaptation of soybean is likely a result of diversity associated with a large number of genes regulating soybean flowering behavior. We used an integrated bioinformatic and experimental approach to understanding the molecular control of soybean flower initiation. RNA-Seq analysis of shoot apical meristem and leaf undergoing floral transition revealed phytohormones might play predominant roles in the short-day-induced floral initiation process. ABA level was measured using an immunoassay at the shoot apical meristem showed a significant increase in ABA during floral transition. Further, our data indicated a major reprogramming events in leaves and the shoot apical meristem that point toward phytohormones gibberellins and cytokinin as key regulators in the production of flowering signal(s) in leaves.

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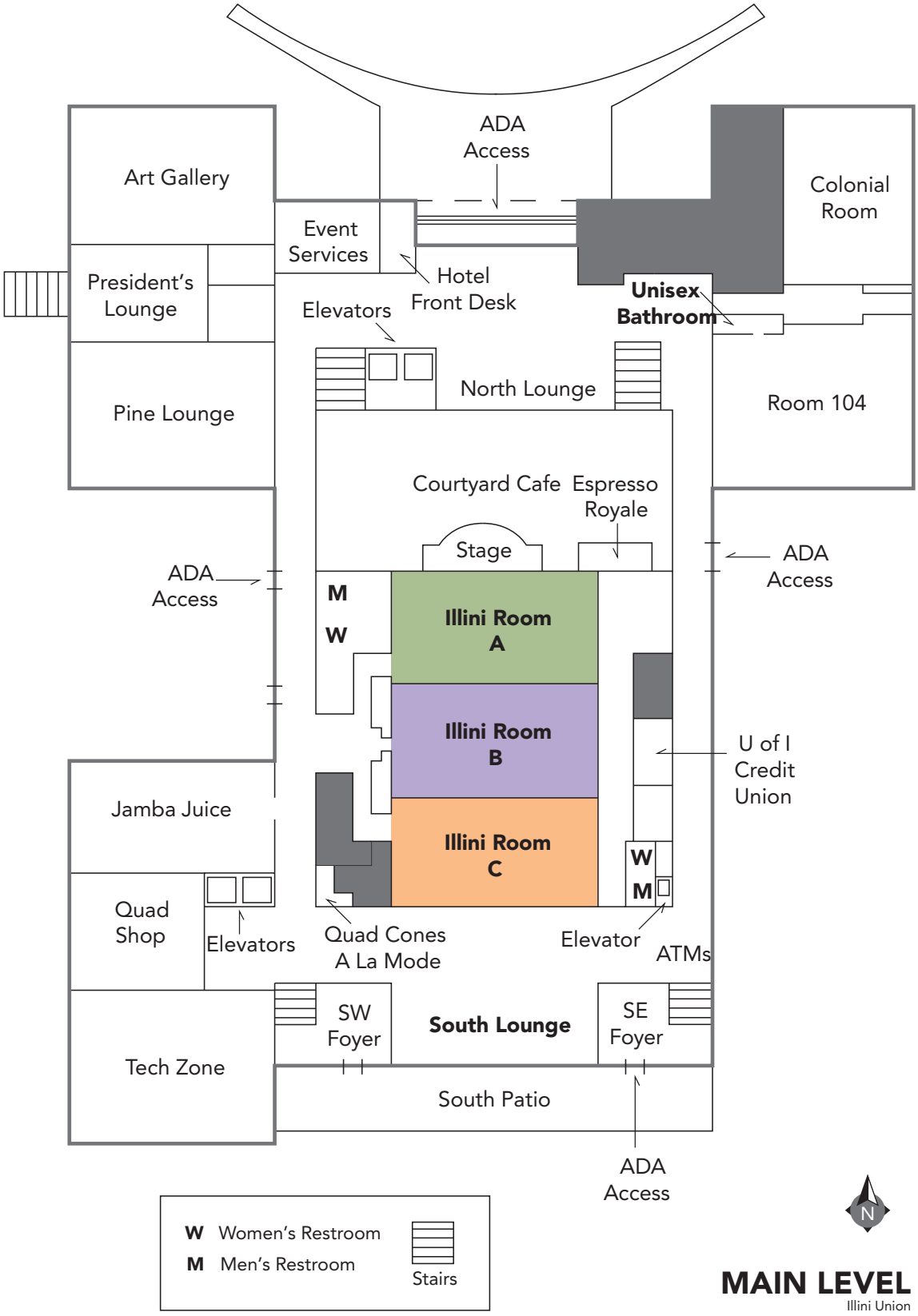
Illini Union



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PROGRAM ADDENDUM (as of 8/5/15)

The following posters have been added to the program (odd numbers present on Sunday, even numbers present on Monday):

[P58] Genistein reduced colon cancer metastasis to liver through regulating the expression of metastasis-related genes in a mouse xenograft model

Qian Li[1], Stephane Lezmi[2], Danni Chen[1], Hong Chen[1]. [1]Food Science and Human Nutrition, [2]Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL

This study aimed at investigating the effect of genistein (Gen) on liver metastases derived from colon cancer and its molecular mechanisms. Male athymic mice were randomized to three dietary groups, which were western diet (W; calories%, fat 39%, carb 50%), W diet supplemented with 100 ppm Gen (GL) or 500 ppm Gen (GH) . Animals were fed with assigned diets for 16 weeks before they were intra-splenically injected with metastatic human colon cancer cells SW620 and sacrificed 8 weeks later. GL and GH groups have much lower liver metastasis rate (MR), which were 36% and 17%, respectively, compared to W group with MR of 55%. Mean metastases size of W group (4.7 mm^2) is larger than GL (2.1 mm^2) and GH (2.0 mm^2) groups. To understand the molecular mechanism of the anti-metastatic function of Gen, the expression of multiple metastasis-related genes were determined by real-time PCR. Importantly, mRNA of NDRG1 gene was greatly decreased by 50% and 60% in GL ($p=0.005$) and GH group ($p=0.001$) compared to W group. As NDRG1 has been reported as a biomarker for metastasis and poor prognosis in hepatocellular carcinoma, the down-regulation of NDRG1 by Gen supplementation was corresponding to decreased liver metastasis. In summary, our study indicated that dietary supplementation of Gen suppressed colon cancer metastasis, which is associated with altered expression of metastasis-related genes by genistein.

[P59] Role of Acyl-lipid Desaturase 1 in Priming for Cold Acclimation in Arabidopsis

Mingjie Chen[1,2] and Jay J. Thelen[2]
[1]Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, P. R. China.
[2]Division of Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, Missouri 65211, USA

Membrane fluidity change has long been suggested as the primary mechanism plants adapt to cold stress. To further study this process, *acyl- lipid/CoA desaturase 1* (ADS1; EC 1.14.99) was characterized using genetic, cellular, and biochemical approaches. Plants containing T-DNA insertion alleles of *ads1* all appear similar to wild type (WT) under either standard growth conditions or low temperature, but displayed enhanced freezing tolerance after cold acclimation. Fatty acid composition analysis demonstrated that *ads1* mutant plants grown at 23°C have 20% lower 18:1 content compared to WT. Lipidomics analysis revealed that 34C- species of monogalactosyl diacylglycerol (MGDG) content in *ads1* mutants were 4.0% lower compared to WT. Lipid positional analysis identified that the sn-2 position of MGDG had higher 18:0 and 10% lower 18:1 fatty acid content compared to WT. Metabolomics profiling identified that the concentrations of some carbohydrates, amino acids and secondary metabolites were higher in *ads1* mutants than in WT post cold acclimation. The cytosolic calcium content in *ads1* mutant plants also was 1-fold higher than that of WT in response to cold shock. Subcellular localization of C- and N-terminal enhanced-fluorescence-fusion proteins indicated that ADS1 localizes exclusively to chloroplasts. These observations suggested that ADS1 encodes a plastid $18:1^{\Delta 9}$ desaturase specific to the sn-2 position of MGDG and is involved in cold perception and acclimation response.

[P60] Phytochemical characterization and antioxidant activity of different whole freeze dried berries and cherries with potential effect for type II diabetes.

Boris Nemzer[1,2], Jing Yuan Wang[1], Elvira de Mejia[2]
[1]*FutureCeuticals, Inc., Momence, IL 60954, USA*
[2]*Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

Type II diabetes is becoming more prevalent in the United States with about 8.3% of the population having this metabolic disorder. Berries are considered super fruits because of their natural antioxidants and have been investigated for their potential health benefit to manage diabetes.

Objective: This study aimed to provide phytochemical composition, antioxidant capacity (AC) and evaluation of freeze dried whole elderberry, bilberry, blueberry (wild and cultivated), blackberry, black currant, tart cherry, sweet cherry, raspberry, cranberry, and strawberry for future biomedical functions in chronic diseases such as type II diabetes.

Methods: Total anthocyanins (TA) content was determined by UV/Vis spectrophotometry by pH differential method and results were expressed as cyaniding 3-glucoside equivalent. The anthocyanins composition was provided by HPLC. The total polyphenols (TP) content was determined by spectrophotometry using Folin-C reagent and results were expressed as milligram of gallic acid equivalent per gram of dry sample. AC was measured by ORAC (oxygen radical absorbance capacity) assay. Results were expressed as micromoles of Trolox equivalent per gram of sample. α –Glucosidase, α-Amylase and DPP-IV methods for enzymatic inhibition activity were performed. Absorbance was read at 405 nm using Synergy2 Multi-well plate reader. Results were expressed as percent inhibition relative to diabetes drug acarbose as the positive control.

Results: Thirteen anthocyanins were identified by HPLC and the highest total anthocyanins content was found in bilberry (2.89%) followed by elderberry (1.63%). TP ranged from 50.2 ± 0.14 mg/g in elderberry to 12.2 ± 0.05 mg/g in sweet cherry. Blackberry and bilberry are other good sources of high TP content (44.1± 0.08 and 41.3± 0.05 mg/g respectively). Elderberry had the much highest level of AC (1399 umole TE/g) compared to all other berries and cherries due to the highest level of polyphenols. The ORAC values were positively correlated to the total anthocyanins and polyphenol content. α-Glucosidase varied from 20.84± 3.46% inhibition/mg for whole elderberry to 104.12 ± 0.49 % inhibition for whole raspberry; α-Amylase varied from 35.64± 7.72 % for whole elderberry to 99.05 ± 1.53% inhibition for whole raspberry; DPP-IV varied from 43.74± 8.86% inhibition/mg for whole strawberry to 102.08 ± 1.02 % inhibition for whole elderberry.

Conclusion: The results indicated that elderberry had the highest AC and TP content and higher DPP-IV inhibition activity. The formulation of berries blend with elderberry, raspberry and others have potential benefit for oxidative stress and diabetes prevention.

PROGRAM ADDENDUM (as of 8/7/15)

- **Cancellation:** [S6-4] Tuesday at 3:50 pm / Presenter: Foluso Oluwagbemiga Osunsanmi
- **Change:** [S7-3] will be presented Tuesday at 3:50 pm instead of Wednesday at 9:45 am / Presenter: Okiemute Rosa Johnson-Ajinwo
- **Cancellation:** [S7-4] Wednesday at 10:15 am / Presenter: Syeda Maryam Hussain
- **Change:** [S7-5] & [S7-6] will shift up to 9:45 am & 10:15 am on Wednesday / Presenters: Pavel Somavat & Richard Licayan

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Boris Nemzer[1,2], Jing Yuan Wang[1], Elvira de Mejia[2]
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[2]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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Objective: This study aimed to provide phytochemical composition, antioxidant capacity (AC) and evaluation of freeze dried whole elderberry, bilberry, blueberry (wild and cultivated), blackberry, black currant, tart cherry, sweet cherry, raspberry, cranberry, and strawberry for future biomedical functions in chronic diseases *s*uch as type II diabetes.
Methods: Total anthocyanins (TA) content was determined by UV/Vis spectrophotometry by pH differential method and results were expressed as cyaniding 3-glucoside equivalent. The anthocyanins composition was provided by HPLC. The total polyphenols (TP) content was determined by spectrophotometry using Folin-C reagent and results were expressed as milligram of gallic acid equivalent per gram of dry sample. AC was measured by ORAC (oxygen radical absorbance capacity) assay. Results were expressed as micromoles of Trolox equivalent per gram of sample. α –Glucosidase, α-Amylase and DPP-IV methods for enzymatic inhibition activity were performed. Absorbance was read at 405 nm using Synergy2 Multi-well plate reader. Results were expressed as percent inhibition relative to diabetes drug acarbose as the positive control.
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Conclusion: The results indicated that elderberry had the highest AC and TP content and higher DPP-IV inhibition activity. The formulation of berries blend with elderberry, raspberry and others have potential benefit for oxidative stress and diabetes prevention.

[P61] Facultative Mutualism Between the Navel Orangeworm (*Amyelois transitella*) and *Aspergillus flavus*

Daniel S. Bush[1], Joel P. Siegel[2], and May R. Berenbaum[1]
[1]University of Illinois at Urbana-Champaign, [2]USDA, Agricultural Research Service, Parlier, CA

The highly aflatoxigenic fungal species *Aspergillus flavus* is closely associated with the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), which attacks damaged or overripe tree nuts and fruits. Recent work suggests that this association is a facultative mutualism; *A. flavus* opportunistically infects nuts damaged by navel orangeworm, for example, and is phoretic on mobile life stages of the insect, while navel orangeworm larvae and adults are attracted to fungal volatiles. We now provide evidence of a nutritional component of this apparent mutualism, in which *A. flavus* detoxifies phytochemicals in host plants and increases the quality of the caterpillar's diet, while *A. transitella* provides additional nitrogen-rich substrate (in the form of frass) for *A. flavus*. Growth rates for *A. transitella* and *A. flavus* were measured separately and together on a diet containing almond meal, in the presence and absence of the furanocoumarin xanthotoxin (which occurs in some fruit hosts of *A. transitella*). In the absence of xanthotoxin, each species grows faster in the presence of the other than they do in isolation. *A. transitella* reaches adulthood 33% more quickly in the presence of *A. flavus*, while the fungus covers its medium 43% faster in the presence of *A. transitella*. In addition, xanthotoxin in the diet reduces the growth rate of *A. transitella*, but only when the fungus is absent. Ascomycete fungi, including *Aspergillus* species, are known to metabolize xanthotoxin and other furanocoumarins, and we suggest that this activity is highly beneficial to insect mutualist partners. These data are indicative of an ectosymbiotic mutualism between the fungus and the lepidopteran, an ecological relationship that may be more widespread than hitherto suspected and that has important implications for management of *A. transitella* in almonds and other economically important host plants.