PSNA 2013

52nd Annual Meeting of the Phytochemical Society of North America

Oregon State University • Corvallis, Oregon • August 3-7, 2013

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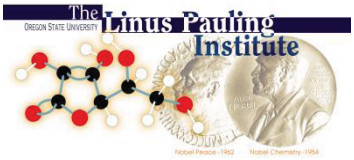

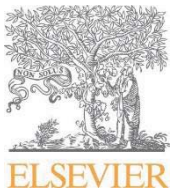

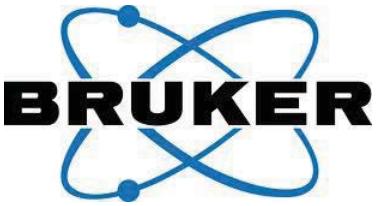

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August 3, 2013

WELCOME!

On behalf of Oregon State University and the 2013 Organizing Committee, welcome to our 52nd Annual Meeting of the Phytochemical Society! We are very pleased to have the meeting on the campus of Oregon State University in Corvallis. We hope that you will enjoy the cutting-edge science presented by national and international leaders in the field of phytochemistry and plant biochemistry. This meeting is a unique opportunity for you to present your work and to interact with scientific colleagues.

We have put together a program that reflects our society's mission, with symposia focused on natural product biosynthesis and plant metabolism, plant metabolomics, bioproducts and biofuels, botanicals and medicinals, and on the role of phytochemicals in the interaction between plants and their environment. Each symposium features two invited speakers who are leaders in their respective fields. Thanks to all of you, we are fortunate to offer a rich program with a total of 47 talks and 63 poster presentations. The enormous diversity at this meeting is exemplified by the 115 registrants who represent well over 25 academic and other research institutions from no less than 12 countries.

The meeting has special sessions dedicated to young scientists at the undergraduate, graduate and postdoctoral levels. On Sunday, August 4, Elsevier will host a lunch workshop on how to get published in the scientific literature. In addition to the more than 30 young PSNA members at the meeting, this workshop will be attended by about 20 Oregon State University students and postdocs. The Arthur Neish Young Investigator Award Symposium is scheduled for Monday morning, August 5. In this symposium, our society will honor three talented young scientists who will present their scholarly accomplishments. Last but not least, the 2012-2013 PSNA/Elsevier Award Lecture will be delivered by Professor Aimee Eggler on Tuesday just before the banquet.

We wish to gratefully acknowledge the very generous support by our various sponsors. Without their support, this meeting would not be possible. Thank you very much, sponsors!

Oregon is a beautiful state on the west coast. There are many extraordinary places to visit near Corvallis and in Oregon, such as Marys Peak, Silver Falls, Sea Lion Caves, Oregon Caves (a national monument), the Columbia River Gorge, the Cascade Mountains, Crater Lake (a national park featuring the deepest lake in the US), Hell's Canyon (the deepest canyon in the US), and the Avenue of the Giants just across the border with California.

Welcome to the 52nd PSNA meeting in Corvallis, Oregon!

Fred Stevens
Conference Chair and Local Host

Toni Kutchan
PSNA President

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2013 PSNA Meeting Program

Saturday, August 3	
Time	Session Activity
12:00 – 15:00	Weyerhauser Board Room
	PSNA Executive Meeting
17:30 – 19:30	Giustina Gallery
	Registration Desk Open
18:00 – 20:00	Evening Reception

Sunday, August 4	
Time	Session Activity
07:30	Giustina Gallery
	Registration Desk Open
08:15	Construction and Engineering Hall
	Opening Remarks
08:25	**Symposium I *Biosynthesis and Metabolism*** Session Chairs: Argelia Lorence and Deyu Xie
08:30	[S1-1] Jörg Bohlmann (University of British Columbia, Vancouver Canada) *A modular pathway of diterpene synthases and cytochrome P450s for diterpene resin acid biosynthesis in conifers*
09:10	[S1-2] Harro Bouwmeester (Wageningen University, The Netherlands) *Strigolactones, apocarotenoid signaling molecules with surprising in- and outdoor activities*
09:50	Giustina Gallery
	Morning Refreshments
10:20	Construction and Engineering Hall
	[S1-3] Susan Howat (The University of Edinburgh, UK) *Improving paclitaxel production in cultured cambial meristemic cells by exploring transcriptional regulation during methyl-jasmonate elicitation*
10:40	[S1-4] Zerihun A. Demissie (University of British Columbia, Kelowna BC, Canada) *The biosynthetic origin of irregular and regular monoterpenes in Lavandula: The role of "cis" isoprenyl diphosphate synthases*
11:00	[S1-5] Marianna Galata (University of British Columbia, Kelowna BC, Canada) *Illumina-led transcriptome profiling and molecular cloning and functional characterization of a monoterpene synthase from the seeds of Coriandrum sativum (Coriander)*
11:20	[S1-6] Matthew Kilgore (Donald Danforth Plant Sciences Center, St. Louis, MO) *Identification of a 4'-O-methyltransferase in the galanthamine biosynthetic pathway*
11:40	[S1-7] Toshiaki Umezawa (Kyoto University, Japan) O-Methyltransferases involved in lignan biosynthesis
12:00	Giustina Gallery
	Lunch Young Members Meeting: Publishing Connect Workshop presented by Elsevier Students, Post-docs and new faculty are encouraged to attend

13:25	**Construction and Engineering Hall**
	Symposium II *Interaction of Phytochemicals and Insects* Session Chairs: Toni Kutchan and Mark Bernards
13:30	[S2-1] James Tumlinson (Pennsylvania State University, University Park PA) *Dynamic nature of plant volatile release in response to insect herbivory*
14:10	[S2-2] Vojislava Grbic (University of Western Ontario, London ON, Canada) *Interaction between Arabidopsis and spider mite Tetranychus urticae*
14:50	Giustina Gallery
	Afternoon Refreshments
15:20	Construction and Engineering Hall
	[S2-3] Eric T. Johnson (USDA, Peoria IL) *Elevated levels of two transgenic proteins in maize enhances resistance to fall armyworm and corn earworm*
15:40	[S2-4] Matthew C. Crispin (Iowa State University) *Metabolism of acylphloroglucinols in the medicinal species, Hypericum gentianoides*
16:00	[S2-5] Kathryn Storey (University of British Columbia, Vancouver BC, Canada) *Functional characterization and genomic organization of genes involved in gibberellin and diterpene resin acid biosynthesis in white spruce, Picea glauca*
16:20	[S2-6] Lucas Busta (The University of British Columbia, Vancouver BC, Canada) *Cuticular waxes from the gametophyte, sporophyte, and calyptra of the moss Funaria hygrometrica*
16:40	Giustina Gallery
	Late Afternoon Break
16:55	Construction and Engineering Hall
	[S2-7] Shivakumar Devaiah (East Tennessee State University, Johnson City) *Substrate specificity and kinetic properties of flavonol-3-O-glucosyltransferase from Citrus paradise*
17:15	[S2-8] Mehran Dastmalchi (University of Western Ontario, Agriculture and Agri-Food Canada)
17:35 - 19:45	Giustina Gallery
	Poster Session and Reception

Monday, August 5	
Time	Session Activity
08:00	Giustina Gallery
	Registration Desk Open
	Construction and Engineering Hall
08:25	**Symposium III *Plant Metabolomics*** Session Chairs: Claudia Maier and David Gang
08:30	[M3-1] Wolfram Weckwerth (University of Vienna, Austria) *Plant metabolomics – Digging in the 1001 metabolome*
09:10	[M3-2] Vladimir Shulaev (University of North Texas, Denton TX) *Harvesting the strawberry genome: From genes to metabolic networks*
09:50	Giustina Gallery
	Morning Refreshments
10:20	Construction and Engineering Hall
	[M3-3] Bernd Markus Lange (Washington State University, Pullman WA) *Integrating metabolomics and transcriptomics to unravel natural product biosynthesis in medicinal plants*
10:40	[M3-4] Reinhard Jetter (University of British Columbia, Vancouver BC, Canada) *Direct mass spectrometric mapping of surface composition: TOF-SIMS investigations of epidermal cell types on Arabidopsis organs*
11:00	[M3-5] Doralyn S. Dalisay (Washington State University, Pullman WA) *Visualizing 'phytochemical factories' in situ*
11:20	[M3-6] Mark W. Sumarah (Agriculture and Agri-Food Canada, London ON) *GC-MS Metabolomic profiling of soybean plants for identification of four common root diseases*
11:40	[M3-7] Michael Qian (Oregon State University, Corvallis OR) *Understanding grape maturity and wine flavor–A flavoromics approach*
12:00	Giustina Gallery
	Lunch PSNA Society Member's Meeting (Construction and Engeering Hall) All PSNA members are welcome and encouraged to attend
13:30	Construction and Engineering Hall
	Arthur Neish Young Investigator Award Symposium Session Chair: Toni Kutchan
13:40	Diana Roopchand (Rutgers University, New Brunswick NJ) *Concord grape pomace polyphenols complexed to soy protein isolate are stable and hypoglycemic in diabetic mice*
14:00	Dejan Nikolic (University of Illinois at Chicago) *The nitrogen-containing secondary metabolites from Black cohosh*
14:20	Daniel Vassão (Max Planck Institute for Chemical Ecology, Jena, Germany) *Toxicity and detoxification of plant chemical defenses in insect pests*

15:00 – 22:15	**Trip to the Coast**
	15:00 – Bus leaves for the coast 16:00 – Arrival at Yaquina Head Lightouse 17:45 – Bus leaves for Historic Bay Front, dinner on own 21:00 – Bus leaves for Corvallis 22:15 – Bus arrives at the Hilton Garden Inn

Tuesday, August 6	
Time	Session Activity
08:00	Giustina Gallery
	Registration Desk Open
08:25	Construction and Engineering Hall
	Symposium IV *Bioproducts and Biofuels* Session Chairs: Mark Berhow and Diana Roopchand
08:30	[T4-1] Steven Vaughn (USDA, Peoria IL) *Biobased products research at the National Center for Agricultural Utilization Research*
09:10	[T4-2] Eric Lam (Rutgers University, New Jersey) *Aquatic Agronomy: Integrating Plant Biology and Engineering for Sustainable Production of Duckweed Biomass on Wastewater*
09:50	Giustina Gallery
	Morning Refreshments
10:20	Construction and Engineering Hall
	[T4-3] De-Yu Xie (North Carolina State University, Raleigh NC) *Spatial differentiation of volatile complexity in camelina, an oil crop for biofuel production*
10:40	[T4-4] Sung-Jin Kim (Washington State University, Pullman WA) *Modified poplar producing allyl/propenyl phenols*
11:00	[T4-5] Toshiya Muranaka (Osaka University, Japan) *Production of licorice triterpenoids in both transgenic plants and cultured yeast cells*
11:20	[T4-6] Daniel Owens (USDA, University MS) *Impact of physico-chemical properties on the uptake, translocation, and metabolism of the herbicidal compound leptospermone*
11:40	[T4-7] Joaquim Marques (Washington State University, Pullman WA) *Poplar as a bio-factory for the production of specialty chemicals*
12:00	Guistina Gallery
	Lunch
13:25	Construction and Engineering Hall
	Symposium V (HSB 240) *Botanicals and Medicinals* Session Chairs: Fred Stevens and John Thor Arnason
13:30	[T5-1] Nadja Cech (University of North Carolina, Greensboro NC) *Addressing complexity and synergy in botanical medicines*
14:10	[T5-2] Claus Schneider (Vanderbilt University, Nashville TN) *Biochemical pharmacology of curcumin*

14:50	**Giustina Gallery**
	Afternoon Refreshments
	Construction and Engineering Hall
15:20	[T5-3] Fatima Rivas (St. Jude Children's Research Hospital, Memphis TN) *Evaluation of Jatropha isabelli natural products and their synthetic analogs as potential antimalarial therapeutic agents*
15:40	[T5-4] Yoshiyasu Fukuyama (Tokushima Bunri University, Tokushima, Japan) *Neurotrophic compounds of Javanese ginger, Zingiber purpurenum*
16:00	[T5-5] Jay S. Kirkwood (Oregon State University, Corvallis) *A metabolomics driven elucidation of the anti-obesity mechanisms of xanthohumol*
16:20	[T5-6] Chieu Anh Ta (University of Ottawa, Ottawa ON, Canada) *Antifungal saponins from the maya medicinal plant Ik che (Solanaceae)*
16:40	Giustina Gallery
	Late Afternoon Break
16:55	Construction and Engineering Hall
	[T5-7] Jeremy J. Johnson (University of Illinois at Chicago) *Deconstructing the Mediterranean herb Rosemary to characterize its anticancer activity*
17:15	[T5-8] **2012-2013 PSNA/Elsevier Award Lecture** Aimee Eggler (Villanova University) *Sensing electrophilic phytochemicals: A model for how to Keap1 C151 modification leads to decreased ubiquitination of Nrf2, a key target in disease prevention*
18:30	CH2M HILL Alumni Center Ballroom
	Banquet

Wednesday, August 7	
Time	Session Activity
08:00	Giustina Gallery
	Registration Desk Open
08:25	Construction and Engineering Hall
	Symposium VI *Phytochemicals in the interaction between plants and their environment* Session Chairs: Reinhard Jetter and Soledade Petras
08:30	[W6-1] Peter Constabel (University of Victoria, Vancouver Island, Canada) *Complex ecological roles of tannins – Beyond plant defense*
09:10	[W6-2] Nicole Kho Clay (Yale University, New Haven CT) *The chemical outputs of the plant innate immune system*
09:50	Giustina Gallery
	Morning Refreshments
10:20	Construction and Engineering Hall
	[W6-3] Mark A. Bernards (University of Western Ontario, London, Canada) *Hairy root as a model to investigate the role of suberin in soybean resistance to Phytophtora sojae*
10:40	[W6-4] Keyvan Dastmalchi (The City College of New York and CUNY Institute for Macromolecular Assemblies) *Metabolite profiling of extracts of wound healing potato cultivars*
11:00	[W6-5] Dhirendra Kumar (East Tennessee State University) *Lipid mediated salicylic acid signaling is mediated by SABP2*
11:20	[W6-6] Soledade Pedras (University of Saskatchewan, Saskatoon SK, Canada) *Interaction of cruciferous phytoalexins and glucosinolates with fungal pathogens*
11:40	[W6-7] Katherine A. Lisko (Arkansas State University) *Engineering elevated vitamin C content in rice to improve abiotic stress tolerance*
12:00	Closing Remarks and Adjournment
12:15 -	RAP Editorial Board Meeting & Incoming Executive Meeting

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How to Get Published
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Sunday, August 4th 2013
12:00 pm
Construction and Engineering Hall
LaSells Stewart Center
Lunch meeting for young Members

Presenter: Natalie Steffen,
Publisher Inorganic and Organic Chemistry, Elsevier

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Symposium I: Biosynthesis and Metabolism

Joerg Bohlmann, Ph.D.
Professor and Distinguished University Scholar
Michael Smith Laboratories
University of British Columbia
Vancouver, Canada

Dr. Jöerg Bohlmann is a Professor and Distinguished University Scholar in the Michael Smith Laboratories at the University of British Columbia, Vancouver, Canada (www.michaelsmith.ubc.ca/faculty/bohlmann/). He received his PhD from the Technical University Braunschweig, Germany (1995), was a Feodor Lynen Fellow of the Alexander von Humboldt Foundation at Washington State University, USA (1995-1998) and research associate at the Max Planck Institute for Chemical Ecology, Germany (1998-2000). He holds academic appointments in the Departments of Botany and Forest Sciences, and is an associate of the UBC Wine Research Centre. His research deals with the molecular biochemistry and biology of terpenoids, genomics of conifers, grapevines, and various medicinal plants, and plant defense against insects. His research is funded by the Natural Sciences and Engineering Research Council of Canada (NSERC), and other sources. Jöerg has been the project leader of five large-scale, Genome Canada funded genomics projects He has received several national and international awards and distinctions including a Feodor Lynen Fellowship of the Alexander von Humboldt Foundation, the C.D. Nelson Award of the Canadian Society of Plant Physiologists, the Charles A. McDowell Award for Excellence in Research awarded by UBC, the E.W.R. Steacie Memorial Fellowship of the Natural Sciences and Engineering Research Council of Canada; he is an elected Fellow of the American Association for the Advancement of Science.

[S1-1] **A Modular Pathway of Diterpene Synthases and Cytochrome P450s for Diterpene Resin acid Biosynthesis in Conifers**

Joerg Bohlmann

University of British Columbia, Michael Smith Laboratories, Vancouver, BC Canada V6T1Z4

Conifer trees produce large amounts of oleoresin defenses for their protection against insects (e.g. bark beetles) and insect-associated fungal pathogens. Using a combination of conifer genome and transcriptome sequencing, proteomics, and biochemical approaches, we identified and functionally characterized families of terpenoid synthases (TPS-d family) and cytochrome P450 dependent monoxygenases (CYP720B family) of conifer oleoresin biosynthesis. Both bifunctional as well as monofunctional diterpene synthases are active in conifers and together with members of the CYP720B family form a modular pathway system for diterpene resin acid biosynthesis. The many functions of members of the TPS-d and CYP720B gene families are critical for the plasticity and diversity of secondary metabolism in conifer defense and the successful evolution of long-lived conifer trees, which often survive for several hundred years in the same location defeating many generations of faster evolving insect pests and pathogens.

Symposium I: Biosynthesis and Metabolism

Harro Bouwmeester, Ph.D.
Profesor and Chair
Laboratory of Plant Physiology
Wageningen University
Netherlands

Harro Bouwmeester is chair of the Laboratory of Plant Physiology of Wageningen University, the Netherlands. The work in his group is centered around the elucidation of the biosynthesis of isoprenoids in plants and their role in plant-environment interaction. In this work a number of different expertises are integrated, such as: metabolomics to quantify metabolites, products of enzyme assays and the consequences of plant metabolic engineering; plant physiology to study the regulation of secondary metabolite and signalling molecule formation and their importance in plant growth and development; biochemistry to characterise isolated and heterologously produced enzymes; molecular biology for the isolation of genes encoding key-steps in pathways and the modification of these pathways in planta; biology: studies on the importance of these metabolites in the interaction of plants with parasitic plants, insects and plant pathogens. The Laboratory of Plant Physiology is part of the Plant Sciences Group of Wageningen UR that is one of the largest institutes in plant science in the world with about 800 staff. These have a strong expertise in molecular genetics, plant development, plant physiology, entomology, biochemistry, cell biology and the -omics technologies (genomics, proteomics, metabolomics). The research facilities are among the most modern in the world including ultramodern new climate rooms, greenhouse facilities, several GC-MSes, LC-QTOF-MS, Q-PCR.
The research in the Bouwmeester lab has resulted in an internationally recognised research group in the area of terpenoid biosynthesis. His work has resulted in many publications in international peer-reviewed journals and an international position and extensive network in the field of terpenoid biosynthesis. To his top achievements belong papers in Science, Nature, Nature Genetics, Nature Biotechnology and reviews in Trends in Plant Science and Current Opinion in Plant Biology. At the end of 2005, he was awarded a Vici-grant of 1.2 M€ for his work on underground chemical communication of plants with parasitic plants and arbsucular mycorrhizal fungi and in 2007 a professorship in The Physiology of Plant Communication. From June 2008 he is full chair of the Laboratory of Plant Physiology of Wageningen University. He is a referee for the main international journals in plant science and for many funding agencies. He published over 130 papers in international journals that were cited over 2500 times and has an H index of 30 (ISI Web of Science). He co-authored 10 book chapters and is inventor on 7 patent (applications).

[S1-2] **Strigolactones: Apocarotenoid Signalling Molecules with Surprising In- and Outdoor Activities**

Bouwmeester, HJ

The newly identified group of plant hormones strigolactones (SLs) plays an important role in the regulation of shoot branching/tillering and root architecture in plants. In addition to this internal plant signalling function SLs are germination stimulants for root parasitic plant species of the Orobanchaceae, and chemical signals stimulating plant root colonization by symbiotic arbuscular mycorrhizal (AM) fungi. The biosynthetic pathway of SLs has been partially elucidated, using highly branched/tillered mutants of Arabidopsis (*max)*, rice (*dwarf* or *htd*), Petunia (*dad*) and pea (*rms*). Using these mutants it was shown that the carotenoid isomerase D27, the carotenoid cleavage dioxygenases CCD7 and CCD8 (in Arabidopsis called MAX3 and MAX4) and a cytochrome P450 (MAX1 in Arabidopsis) are involved in strigolactone biosynthesis. An F-box protein MAX2 and an α/β hydrolase D14 seem to be involved in strigolactone perception/downstream signaling. The role of strigolactones in the regulation of several biological processes, the regulation of their biosynthesis and the current knowledge of their biosynthetic pathway will be discussed.

Symposium I: Biosynthesis and Metabolism

[S1-3] **Improving Paclitaxel Production in Cultured Cambial Meristematic Cells by Exploring Transcriptional Regulation during Methyl-Jasmonate Elicitation**

Susan Howat[1], Rabia Amir[1], Eunjung Kwon[1], Zejun Yan[1], Young-Woo Jin[2], Eun-Kyong Lee[2] and Gary Loake[1]

[1]Institute for Molecular Plant Sciences, The University of Edinburgh, Edinburgh, UK, EH9 3JR

[2]Unhwa Corp., 176-1, 1Ga, Hosung-Dong, Dukjin-gu, Jeonju, Korea.

Paclitaxel is a key anti-cancer drug which is isolated from the bark of *Taxus spp*. Demand for paclitaxel is high and plant cell culture is an attractive option for producing this drug. Elicitors, such as methyl-jasmonate (Me-JA), can upregulate paclitaxel production in plant cell culture however the effect is only transient.

We isolated and cultured innately undifferentiated cambial meristematic cells (CMCs) from *Taxus cuspidata*. These possess stem-cell-like properties and have superior growth on an industrial scale compared to typical dedifferentiated cell cultures. Roche454 sequencing was employed to establish the transcriptome of CMCs which was utilized as a reference to observe the transcriptional profile of CMCs elicited by Me-JA. Subsequent analysis identified 19 transcription factors (TFs) from five distinct families as candidate regulators of paclitaxel biosynthesis.

The function of these 19 TFs was explored by investigating their interaction with five promoters of key paclitaxel biosynthetic genes, which were found to be rich in the cognate binding sites for these TFs. Interaction was investigated *in vitro* using electromobility shift assays and *in vivo* by transient assays. To date every promoter interacts with at least one of the TFs and two TFs interacted with four out of five promoters tested.

[S1-4] **The Biosynthetic Origin of Irregular and Regular Monoterpenes in *Lavandula*: The Role Of "*CIS*" Isoprenyl Diphosphate Synthases**

Zerihun A. Demissie[1], Mark R. Rheault[1], Soheil S. Mahmoud[1]

[1]*Department of Biology, University of British Columbia, 3333 University Way, Kelowna, British Columbia V1V 1V7, Canada*

Monoterpenes (C_{10}) derived from head–to–tail condensed isopentenyl diphosphate (IPP; C_5) and dimethylallyl diphosphate (DMAPP; C_5) are known as regular monoterpenes while those derived from the non–head–to–tail condensed precursors are called irregular monoterpenes. In lavenders, two isoprenyl diphosphate synthases called geranyl diphosphate synthases (GPPSs) and lavandulyl diphosphate synthases (LPPSs) catalyze the head–to–tail and non–head–to–tail condensation reactions, respectively. cDNAs encoding for GPPSs and other enzymes involved in regular monoterpene biosynthesis have been described from lavenders and related plants. Despite the presence of irregular monoterpenes (e.g. lavandulol) in lavender essential oils (EOs), however, enzymes involved in their biosynthesis are yet to be identified. We recently described the pathway leading to the biosynthesis of irregular monoterpene constituents of lavender EOs by identifying and functionally characterizing a novel cDNA encoding for a *'cis'* family member enzyme called *L. x intermedia* lavandulyl diphosphate synthase (LiLPPS). LiLPPS couples two DMAPP units through a unique head–to–middle condensation reaction to generate lavandulyl diphosphate (LPP; C_{10}), the precursor molecule of the irregular monoterpene lavandulol. LiLPPS is the first cloned *'cis'* family member enzyme involved in irregular monoterpene biosynthesis.

Symposium I: Biosynthesis and Metabolism

[S1-5] **Illumina-Led Transcriptome Profiling, Molecular Cloning, and Functional Characterization of a Monoterpene Synthase from the Seeds of *Coriandrum Sativum* (Coriander)**

Galata[1], M., Mahmoud[1], S.

[1]Department of Biology, University of British Columbia Okanagan Campus, Kelowna, BC, Canada V1V 1V7

Plant terpenes are a large and diverse class of naturally-derived compounds, valuable in the medicinal, perfume and culinary industries, for example. The seeds of *Coriandrum sativum* (coriander) produce essential oil (EO) rich in monoterpenes, volatile C_{10} terpenes. In this study, the transcriptome of coriander seeds at three developmental stages (early, mid, late) was sequenced via Illumina technology. Analysis of the differential expression of terpene biosynthetic genes between these stages revealed that two EO production pathways are constitutively active in the seeds, with slight upregulation in the mid-developmental stage. To validate the usability of the transcriptome sequence data, several terpene synthase (TPS) candidate genes were identified. One of these candidates, *CsγTRPS*, was cloned and functionally characterized in an effort to identify TPS genes that describe coriander seed EO. *CsγTRPS* was expressed in bacteria and the recombinant protein purified by affinity chromatography. Enzymatic assays with geranyl diphosphate (GPP), the precursor to monoterpenes, revealed that *CsγTRPS* catalyzes the conversion of GPP to a monoterpene constituent of coriander EO. Knowledge gained from these experiments will facilitate future studies concerning essential and fatty acid oil production in coriander. They also enable efforts to improve the EO of coriander through metabolic engineering or plant breeding.

[S1-6] **Identification of a 4'-*O*-Methyltransferase in the Galanthamine Biosynthetic Pathway**

Kilgore MB, Rolf M, Kutchan TM

Donald Danforth Plant Science Center, St. Louis, MO, USA, 63132

Galanthamine is a plant alkaloid used to treat the symptoms of Alzheimer's disease. This compound is produced by members of the family Amaryllidaceae and is primarily isolated from *Narcissus spp.*, *Galanthus spp*., and *Leucojum aestivum*. Despite its importance as a medicine, genes involved in the biosynthetic pathway of galanthamine have not yet been identified. Using BLAST to find sequences in a *Narcissus* 'Carlton' transcriptome that contain signatures for plant *O*-methyltransferases, a collection of candidate genes was obtained for the methylation of norbelladine to 4'-*O*-methylnorbelladine in the galanthamine biosynthetic pathway. Using HAYSTACK, expression profiles of the methyltransferase genes were compared to the pattern of galanthamine accumulation in *Narcissus* 'Carlton' leaf, flower, and bulb tissues. One of the candidate methyltransferase genes fit the HAYSTACK model. This methyltransferase cDNA was expressed in *E. coli* and found to convert norbelladine to 4'O-methylnorbelladine. The further characterization of this methyltransferase and identification of other genes involved in this biosynthetic pathway are in progress.

Symposium I: Biosynthesis and Metabolism

[S1-7] *O*-Methyltransferases Involved in Lignan Biosynthesis

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Lignans are a group of plant phenolic compounds with various biological activities, including antitumor and antioxidant properties. *O*-Methylation is a critical step in biosynthesis of these compounds. However, little is known about the *O*-methyltransferase (OMT) enzymes that catalyze lignan *O*-methylation. We discovered a highly regioselective OMT activity in safflower (*Carthamus tinctorius*) seeds that catalyzed the methylation of matairesinol, a dibenzylbutyrolactone lignan, into 4'-*O*-methylmatairesinol (arctigenin) but not 4-*O*-methylmatairesinol (isoarctigenin). We named this OMT as *C. tinctorius* matairesinol OMT (CtMROMT). By correlating OMT activities with *OMT* transcript abundances during seed development, we cloned a cDNA encoding the matairesinol methylating OMT. Using *CtMROMT* as a probe, we next isolated and characterized a cDNA encoding an OMT involved in the biosynthesis of antitumor podophyllotoxin-related lignans in cow parsley (*Anthtriscus sylvestris*). A recombinant protein of this OMT converted thujaplicatin into 5-methylthujaplicatin, and was named AsTJOMT. Transcript abundances of the OMTs in different organs show that *AsTJOMT* has the highest expression levels in rhizomes, which is in line with previous reports indicating high amount of lignan in this organ. To our knowledge, this is the first report of the isolation and characterization of plant OMTs involved in lignan biosynthesis.

Symposium II: Interaction of Phytochemicals and Insects

James H. Tumlinson, Ph.D.
Professor
Department of Entomology,
Penn State University
University Park, Pennsylvania

James H. ('Jim') Tumlinson received his BS in Chemistry from Virginia Military Institute in 1960, and his MS in 1966 and PhD in 1969 in Organic Chemistry from Mississippi State University. He was a Post Doctoral student with Robert M. Silverstein at the New York State College of Forestry, Syracuse, in 1969-1970. Subsequently, in 1970 he joined the USDA, ARS, Insect Attractants, Behavior and Basic Biology Lab in Gainesville, FL, as a Research Chemist, and in 1972 became the Research Leader of the Insect Chemistry Research Unit, a position he held until 2003. In 2003 he joined the faculty of the Department of Entomology at Penn State University, as The Ralph O. Mumma Professor of Chemical Ecology and in 2006 became the Director of the Center for Chemical Ecology at Penn State. He has authored or coauthored over 240 articles in peer-reviewed journals. His research has included studies of insect chemical communication and chemical ecology: defining chemical communication systems, including pheromones and other semiochemicals that mediate insect-insect and plant-insect interactions; biosynthesis of pheromones and plant chemical signals; insect behavior, including learning, mediated by semiochemicals. Emphasis is on developing fundamental knowledge and principles that can be applied in environmentally safe, ecologically sound, sustainable pest management programs. Presently, his research is focused on the mechanisms by which insect herbivore-produced elicitors induce plants to produce and emit volatile organic compounds that attract natural enemies of the herbivores

[S2-1] **Dynamic Nature of Plant Volatile Release in Response to Insect Herbivory**

James H. Tumlinson, Irmgard Seidl-Adams

Plants emit volatile organic compounds (voc) in response to insect herbivory, or mechanical wounding and application of insect herbivore-produced elicitors like linolenoyl-L-glutamine (LG). Parasitoids and predators use these voc as cues to locate hosts or prey. The biosynthesis and release of induced voc are affected by numerous factors including light and temperature. Typically, maximum voc emission occurs around midday when light intensity is greatest. In maize seedlings emission of induced sesquiterpenes is modulated by factors affecting stomatal aperture. Sesquiterpenes are synthesized in internal cells of the maize leaf, even in the dark phase, but emitted only when stomata are open, which normally occurs during the diurnal period.

Symposium II: Interaction of Phytochemicals and Insects

Vojislava (Vava) Grbic. Ph.D.
Associate Professor
Faculty of Science
The University of Western Ontario

Vojislava (Vava) Grbic received her MSc in Plant Genetics (1989) from University of Novi Sad, Serbia and PhD in Genetics (1994) from University of Wisconsin, Madison, USA. She received postdoctoral training at University of Wisconsin (1994-1996), and was an EMBO postdoctoral fellow (1996-1998) at University of Cambridge, UK. In 1998, she joined the Faculty of Science at The University of Western Ontario, where she is now an Associate Professor. Her research is aimed at understanding the molecular mechanisms that govern diversity of plant shoot forms. By using the reference plant *Arabidopsis thaliana* and its wild inbred strains with altered shoot morphology (e.g. Sy-0), her group identified changes in the expression of flowering time genes FLC, FRI and HUA2, as required for the establishment of the altered shoot phenotype in the Sy-0 accession. Her lab is now focused on understanding the functions of the *HUA2* gene, a putative pre-mRNA processing factor. The other research project in her lab is focused on development of alternative pest control strategies for sustainable agriculture. Using *Arabidopsis thaliana* and the newly established chelicerate model *Tetranychus urticae* (spider mite), the group aims to uncover genomic responses of both organisms during plant-herbivore interaction in order to understand the interaction between the plant and its herbivore.

[S2-2] **Interaction between *Arabidopsis* and Spider Mite *Tetranychus Urticae***

Vladimir Zhurov[a], Marie Navarro[a,b], Maria E. Santamaria[a,c], Kristie A. Bruinsma[a], Vicente Arbona[d], Marc Cazaux[a,b], Nicky Wybouw[e], Edward J. Osborne[f], Cherise Ens[a], Isabel Diaz[c], Markus Schmid[h], Miodrag Grbic[a,b], Richard M. Clark[f,g], Thomas Van Leeuwen[e], Vojislava Grbic[a,b]

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While pathogen-induced immunity is well characterized, plant defense responses induced by arthropod herbivores are less studied due to the lack of tractable interacting model organisms. The two-spotted spider mite, *Tetranychus urticae*, is a polyphagous chericerate herbivore able to both detoxify diverse plant compounds and rapidly develop pesticide resistance, which makes it a major pest worldwide. Using a combination of genomic and genetic resources in the model plant *Arabidopsis thaliana,* and newly developed genomic tools for the spider mite, we dissected reciprocal *A. thaliana* - *T. urticae* responses. We found that mites feed on single leaf cells, and that defense programs acting downstream of the recognition of mite feeding are mediated by jasmonic acid (JA) biosynthesis and signaling, leading to the accumulation of indole glucosinolates that markedly increase both mite mortality and developmental times. As revealed by genome-wide transcriptome profiling of spider mite responses to increasing levels of indole glucosinolates, a diverse set of genes associated with detoxification of xenobiotics was upregulated in a dose-dependent manner, demonstrating that mites are sensitive to induced plant responses. Our findings establish transcriptional responses of both herbivore and plant involved in the interaction.

Symposium II: Interaction of Phytochemicals and Insects

[S2-3] **Elevated Levels of Two Transgenic Proteins in Maize Enhances Resistance to Fall Armyworm and Corn Earworm**

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Transgenic expression of combinations of resistance molecules in plants is limited. We developed transgenic maize lines capable of overproducing maize ribosome-inactivating protein (MRIP) and wheat germ agglutinin (WGA). Many transgenic plants produced both MRIP and WGA in leaves. Mature leaves expressing elevated levels of MRIP and WGA were more resistant to feeding by first-instar larvae of fall armyworm (*Spodoptera frugiperda*) and corn earworm (*Helicoverpa zea*). Insect resistance was correlated to levels of the transgenic proteins. Statistical analysis indicated that there was no antagonism or synergy between MRIP and WGA. In addition, a slight increase in resistance to *Fusarium verticillioides* was noted in the transgenic leaves. This study indicates that a combination of food derived proteins can enhance insect resistance in maize leaves.

[S2-4] **Metabolism of Acylphloroglucinols in the Medicinal Species, *Hypericum Gentianoides***

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[5]Department of Biochemistry/Biophysics & Molecular Biology, Iowa State University

Hypericum gentianoides (Pineweed) is an herbaceous annual plant related to the medicinal plant St. John's Wort (*H. perforatum*). Similar to *H. perforatum*, *H. gentianoides* extracts have medicinal properties, but contain a very different set of bioactive compounds, three of which are diacylphloroglucinols. To identify the range of acylphloroglucinols accumulating in *H. gentianoides* and to gain an understanding of the biosynthetic pathway, we used a combination of high pressure liquid chromatography (HPLC) with UV and mass detector, hybrid quadrupole-time of flight-mass spectroscopy (Q-TOF-MS), and fourier transform/ion cyclotron resonance-mass spectroscopy (FT/ICR-MS). These analyses revealed nine prevalent acylphloroglucinols, as well as phlorisobutyrophenone and eight methylated and prenylated derivatives. Q-TOF spectrometry was used to identify phlorisobutyrophenone and the eight additional PIB derivatives. Laser desorption ionization (LDI) reveals that the diacylphloroglucinols as well as their precursors are located in the translucent subdermal glands that cover the leaves and sepals. Combined, these finding indicate the diacylphloroglucinols of *H. gentianoides* are biosynthesized via decoration of phlorisobutyrophenone precursor, followed by dimerization, and that this process occurs in the translucent glands.

Symposium II: Interaction of Phytochemicals and Insects

[S2-5] **Functional Characterization and Genomic Organization of Genes Involved in Gibberellin and Diterpene Resin Acid Biosynthesis in White Spruce (*Picea glauca*)**

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Diterpenoids have important functions in both general and specialized metabolism of conifers. Diterpene synthases (diTPSs), and to some degree cytochromes P450 (CYPs), are quite well characterized in conifer specialized metabolism, but less is known about their roles in general metabolism. This study compares two pathways in white spruce that provide examples of similar enzymatic steps in biosynthetic pathways of general (gibberellin biosynthesis; GA) and specialized (diterpene resin acid biosynthesis; DRA) metabolism. The genomic organization/content and enzymatic activities of six CYPs and diTPSs mediating committed steps in the similar but contrasting pathways of GA and DRA biosynthesis were analyzed. Gene expression and resin metabolite changes were measured over a year long time course of vegetative bud growth, as well as from several tissue types of a methyl jasmonate treatment (to induce a defense response) time trial. Over the bud time course GA-associated gene transcript levels were highest during periods of active growth in spring and early summer, with minimal levels in late summer to autumn, coinciding with bud dormancy. DRA-associated gene transcripts were low during the period of active bud growth but highest in months of peak insect attack when defenses and resin are needed most.

[S2-6] **Cuticular Waxes from the Gametophyte, Sporophyte, and Calyptra of the Moss *Funaria Hygrometrica***

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The cuticle is an external, complex layered structure that protects plant surfaces from desiccation. It is a long-standing question whether moss tissues may be coated by a cuticle similar to that of vascular plants, pointing to the evolution of ancestral structures enabling the adaptation to plant life outside aqueous environments. In this context, it has previously been shown that a multilayered cuticle is present on aerial organs, including the calyptra, of the moss species *Funaria hygrometrica*. Herein, we provide further insight into the cuticular properties of mosses through comparative wax analysis of the calyptra, mature sporophyte, and leafy gametophyte. All three organs had very low wax coverage ranging from 1 to 8 ng/cm^2, with mixtures consisting mostly (≥50%) of very-long-chain (VLC) hydroxy alkyl esters, but also of fatty acids, n-alcohols, aldehydes and n-alkanes. An abundant (30% of the total wax load) and previously unidentified series of compounds was observed in the mature sporophyte wax. Mass spectral analysis led to the tentative identification of this series as alkyl esters, 42 to 50 carbons in length, with a hydroxyl functionality on C-6, C-7, or C-8 of the alcohol chain. An authentic 7-hydroxytriacontyl tetradecanoate standard is currently being synthesized to verify these assignments.

Symposium II: Interaction of Phytochemicals and Insects

[S2-7] **Substrate Specificity and Kinetic Properties of Flavonol-3-O-Glucosyltransferase From *Citrus Paradisi***

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Glucosyltransferases (GTs) are enzymes that expedite the incorporation of UDP-activated glucose to a corresponding acceptor molecule. This enzymatic reaction stabilizes structures and affects solubility, transport, and bioavailability of flavonoids for other metabolic processes. Flavonoid glycosides affect taste characteristics in citrus making the associated glucosyltransferases particularly interesting targets for biotechnology applications. Custom design of enzymes requires understanding of structure/function of the protein. The present study focuses on creating mutant flavonol-3-O-glucosyltransferase (F-3-O-GT) proteins using site-directed mutagenesis and testing the effect of each mutation on substrate specificity, regiospecificity and kinetic properties of the enzyme. Mutations were selected on the basis of sequence similarity between grapefruit F-3-O-GT, an uncharacterized GT gene in blood orange (98%), and grape F3GT (82%). Grapefruit F-3-O-GT prefers flavonol as a substrate whereas the blood orange sequence is annotated to be a flavonoid 3GT and the grape GTs could glucosylate both flavonols and anthocyanidins. Mutants of F-3-O-GT were generated by substituting N242K, E296K and N242K+E296K and proteins were expressed in *Pichia pastoris* using the pPICZA vector. Analysis of these mF-3-O-GTs showed that all of them preferred flavonols over flavanone, flavone, isoflavones, or anthocyanidin substrates and showed decrease in enzyme activity of 16 to 51% relative to the wild type F-3-O-GT.

[S2-8] ***Chalcone Isomerase*** **and the Soybean Isoflavonoid Biosynthesis Machinery – A Genetic and Functional Characterization**

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Isoflavonoids are plant natural products, almost exclusive to legumes, synthesized by the phenylpropanoid pathway. They are actors in symbiosis with nitrogen-fixing bacteria and plant stress response. Isoflavonoids are noted for their human health benefits. Chalcone Isomerase (CHI) catalyzes the reaction producing flavanones, the backbone for isoflavonoids. There are eight soybean *CHI* genes, including the novel *CHI3B.* We identified *CHI3B* and confirmed expression in five soybean cultivars. *CHI* gene family members showed different temporal and spatial expression across tissues. CHI isozymes, with previously established catalytic capability to produce flavanones, localized to the nucleus, cytoplasm, and in some cases, the ER. These subcellular localizations coincided with Isoflavone Synthase, the key pathway enzyme, suggesting intra-cellular compartmentalization of isoflavonoid machinery and/or nuclear regulatory functions. To identify isoflavonoid-specific *CHI*(s)*,* quantitative gene expression analysis in soybean roots, with different isoflavonoid levels was performed. Expression of *CHI2* corresponded with higher isoflavonoid content. Function of CHI2 *in planta* was further studied through silencing of *CHI2*, and subsequent analysis of isoflavonoid levels. Our results suggest that *CHI2* is the isoflavonoid-specific member of the family. Identification of factors regulating CHI and further investigation of metabolon formation will help our understanding of the genetic and molecular basis of isoflavonoid biosynthesis.

Symposium III: Plant Metabolomics

Wolfram Weckwerth, Ph.D.

Professor at the University of Vienna
Head of the Department of Ecogenomics and Systems Biology, Austria

Working in the field of metabolomics since 2000, Wolfram Weckwerth has established metabolomic and proteomic platforms at the Max-Planck-Institute of Molecular Plant Physiology in Potsdam, Germany, for the GOFORSYS program of the Federal Ministry of Education and Research (BMBF), Germany, and recently at the University of Vienna, Austria. In 2008 Wolfram Weckwerth moved as a full professor to the University of Vienna and founded the Department of Molecular Systems Biology (MOSYS). Since 2013 he is the head of the newly founded Department of Ecogenomics and Systems Biology combining ecosystemic approaches with high throughput technologies such as genomics, proteomics and metabolomics. The Weckwerth lab develops genome-wide metabolomics and proteomics/phosphoproteomics technologies as elementary systems biology techniques. Further research comprises data integration strategies by combining experimental approaches with multivariate statistics, pattern recognition and modeling of metabolism. The Weckwerth lab has solved the biochemical Jacobian from metabolomics data using an inverse modeling approach. This approach combines statistical features of metabolomics data with metabolic reconstruction and prediction from genome sequences and variable genotypes and therefore establishes the systematic analysis of the genotype-phenotype-relationship. The Weckwerth lab applies these concepts in ecology, evolution and development as well as biotechnology within the framework of “Green Systems Biology”.

[M3-1] **Plant metabolomics – Digging in the 1001 Metabolome**

Wolfram Weckwerth

The plant metabolome is one of the largest resources for chemical diversity and phytochemicals. Plant primary and secondary metabolism are a result of millions of years of evolutionary chemical adaptation. This information should be available in the plant genome. However, there is a big gap between the availability of full genome sequences due to next-generation-sequencing and their functional interpretation, especially with respect to the diversity of the metabolome and the plastic physiome of the plant. Thus, linking genomic information and genome-scale molecular and physiological studies of plants will provide the basis for predictive models of the genotype-phenotype relationship. Most of the molecular phenotypic plasticity is found in the natural environment and will provide the basis for the functional interpretation of the ecophysiology of plant. However, it is a big challenge to apply metabolomics technologies – typically used in highly controlled experiments - in the field. In this context, we applied integrated GC-MS and LC-MS metabolomic techniques to a large grassland study called the Jena-experiment and revealed metabolic signatures for biodiversity and physiological adaptation of individual plant species in their “quasi-natural” environment. The interpretation of these high-dimensional molecular data requires theoretical genome-scale metabolic models which can be used to compare predictions of the genome model and real experimental data to finally develop an iterative and predictive approach linking technologies such as metabolomics, proteomics and genomics to ecosystems studies. To link these reference datasets systematically to the genotype information we have developed a metabolomics toolbox combining multivariate statistical and biomathematical approaches for the interpretation of complex metabolomics datasets at the interface of primary and secondary metabolism. Furthermore an integrated platform will be presented for the automated identification of novel structures from these highly complex metabolomics datasets. Knowledge exchange of ecosystems research and plant biotechnology is anticipated based on the principles of natural variation, biodiversity and the genotype–phenotype environment relationship.

Symposium III: Plant Metabolomics

Vladimir Shulaev, Ph.D.
Professor
Department of Biological Sciences
University of North Texas
Denton, Texas

Professor Vladimir Shulaev is a faculty member of the Plant Signaling Cluster in the Department of Biological Sciences at UNT at UNT. The Signaling Mechanisms in Plants research cluster draws upon existing expertise and emerging research strengths in cell biology, biochemistry, genetics, metabolomics and informatics to study how plants use cellular communication - a complex network of molecular signals - in their growth, development and defense responses to stress. Manipulating signaling mechanisms in plants also will lead to new technologies in agriculture, human nutrition, phytoremediation of environmental toxicants and sustainable energy. Prof. Shulaev holds dual Ph.Ds. in Biological Sciences and Plant Biology and has over 30 years research experience working at academia and industry. At UNT he established the state of the art Metabolomics facility and heads the Center for Metabolic Signaling Research and Biochemical Profiling Group. Before joining UNT Prof. Shulaev was a faculty at the Virginia Bioinformatics Institute at the Virginia Polytechnic Institute and State University and led research programs at several biotechnology companies. Prof. Shulaev research is focusing on metabolomics technology, bioinformatics for metabolomics and application of metabolomics to solve important biological questions. His research group develop novel analytical techniques for both targeted and non-targeted metabolomics using mass spectrometry platforms and applied metabolomics platform to systems biology, fruit functional genomics, gene function elucidation, cancer development and progression, malaria and mode of action of antimalarial drugs, modeling and simulation of biological networks, and yeast systems biology.

[M3-2] **Harvesting the Strawberry Genome: From Genes to Metabolic Networks**

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Strawberry, genus *Fragaria*, is an attractive model to study flavonoid biosynthesis due to localization of flavonoid pigments throughout the plant in different tissue and organs, i.e., fruits, achenes, petiole and stolon tissue, petals and leaf margins under different environmental conditions. Sequencing the genome of the woodland strawberry, *F. vesca*, have led to the identification of thousands of new genes; the function of most remains however unknown. The development of tools for high-throughput gene discovery and functional validation is therefore critical for harvesting the fruits of the genome sequencing efforts. Our group is working on discovering biosynthetic and regulatory genes involved in the biosynthesis and metabolism of flavonoids and anthocyanins in woodland strawberry, *Fragaria vesca*, and to validate their function. We use a combination of bioinformatics, metabolomics, structural biology, and functional genomics approaches to reconstruct flavonoids metabolic networks in strawberry using fully sequenced genome to identify a set of key genes coding for metabolic enzymes and transcription factors involved in these networks. Several examples of using this approach will be presented.

Symposium III: Plant Metabolomics

[M3-3] **Integrating Metabolomics and Transcriptomics to Unravel Natural Product Biosynthesis in Medicinal Plants**

B. Markus Lange[1], Daniel J. Cuthbertson[1], Sean R. Johnson[1], Jasenka Piljac-Žegarac[1], Julia Kappel[1,2], Sarah Schäfer[1,2], Matthias Wüst[2], Joaquim V. Marques[1], Laurence B. Davin[1], Norman G. Lewis[1], Raymond E. B. Ketchum[1], Rodney B. Croteau[1], Megan Rolf[3], Toni M. Kutchan[3], D. Doel Soejarto[4]

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Plant natural products are the direct source or structural inspiration for approximately 25 % of contemporary pharmaceuticals. Many natural products are synthesized in specialized cell types (so-called phytochemical factories), so that the bioactive plant metabolites are not toxic to the plant itself. Natural products can be structurally complex and thus cannot be obtained synthetically in an economically viable manner. Alternative approaches to the production of natural products suffer from an incomplete knowledge of the pathways involved in their biosynthesis. Spatially resolved metabolomics efforts offer the potential to learn more about the accumulation and distribution of natural products in medicinal plants. However, tools for the fast and reliable characterization of natural products and their biosynthetic intermediates are only beginning to emerge. We have developed an accurate mass – time (AMT) tag library for the LC/MS-based identification of natural products in complex plant extracts. LC/MS and MS/MS data sets were integrated into online spectral search tools and repositories (Spektraris and MassBank), thus allowing users to interrogate their own data sets for the potential presence of PNPs. By integrating medicinal plant metabolomics and transcriptomics data sets, we are speeding up gene discovery. The characterization of novel genes involved in the biosynthesis of the anticancer diterpene taxol will serve as an example to illustrate the power of this approach.

[M3-4] **Direct Mass Spectrometric Mapping of Surface Composition: ToF-SIMS Investigations of Epidermal Cell Types on *Arabidopsis* Organs**

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To protect their vast surfaces, plants have evolved protective skins consisting of waxes embedded into a polyester matrix. The chemical composition and spatial arrangement of these highly lipophilic compounds vary widely between plant species and organs, resulting in specific overall surface properties. However, the exact chemical composition of the outermost layer of compounds and their μm-scale patterning on plant surfaces are not known to date. We have now established Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) as a new tool to map wax compounds with sub-micron resolution across the surface of various plant organs. As a first model system, we investigated leaf and inflorescence stem surfaces of *Arabidopsis thaliana* wild type, since its wax composition had been determined in great detail by GC-MS before. ToF-SIMS spectra of authentic standards were acquired for all known constituent classes in the positive and negative ion modes. Most of the compound classes were thus identified in the ToF-SIMS spectra of adaxial and abaxial leaf surfaces, with homologous fatty acids appearing to be prevalent. The distribution of these compounds is currently being mapped across trichome, guard cell and pavement cell surfaces.

Symposium III: Plant Metabolomics

[M3-5] **Visualizing 'Phytochemical Factories' *in Situ***

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Many plant secondary metabolites are widely utilized by humanity due to their highly valued medicinal benefits. Some are efficacious in treating various diseases (e.g., cancer, pain, malaria, glaucoma), as well as functioning as chemical scaffold leads for drug discovery efforts. Medicinals are often structurally complex, present in minute quantity, and cannot be synthesized economically. Yet frequently little is known about the cell types where they are biosynthesized. Accordingly, identification of the highly specialized cell types ('phytochemical factories') that produce these medicinals would assist in establishing unknown biochemical pathway steps (intermediates, enzymes, genes). We describe herein the use of MALDI-TOF tissue metabolite imaging mass spectrometry to identify and characterize the specialized cells/tissues accumulating podophyllotoxin and its congeners in *Podophyllum peltatum* and *P. hexandrum* rhizomes. This enabling technology allows us to now rapidly identify their spatial (cell types involved) and temporal distributions, as well as the range of metabolites that co-exist in these specific cell types. In addition, application of laser micro-dissection enables the facile isolation of specific cell types, and investigation of the nature of the biochemical machinery (genes, proteins) present.

[M3-6] **GC-MS Metabolomic Profiling of Soybean Plants for Identification of Four Common Root Diseases**

Weihuan Niu[1], Mark Gijzen[1], Tim McDowell[1], Blake Green[1], Mark W. Sumarah[1]
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Soybean [Glycine max (L.) Merr.] plants are susceptible to a number of root pathogens including: *Phytophthora sojae, Pythium ultimum, Fusarium solani f.sp.* and *Rhizoctonia solani*. These diseases are responsible for significant crop losses in Canada and currently there are no reliable methods for their identification in field samples. The ability to accurately identify the soybean plant pathogen is critical, allowing farmers to implement the most effective pest management treatment. A GC-MS metabolomics method was developed to differentiate soybean plants infected with each of these four diseases. Metabolite profile differences for the infected root or hypocotyl with each of the diseases were differentiated using PCA. Individual metabolite biomarkers for each disease were also used for identification. The GC-MS method was validated using a "double blind study" that consisted of plants infected with all four diseases and controls. Control plants were easily differentiated by the absence of glyceollins. The two fungal diseases were differentiated from the oomycetes by analysis of the PCA plots and by the presence of an unidentified phytoalexin (MW 498). *P. sojae* was further isolated from *P. ultimum* by the 10x fold increase in glyceollin I and the presence of α-D-glucopyranoside and 3,9-Dioxa-2,10-disilaundecane for *P. ultimum*. The two fungal diseases were further separated from each other by significant increases in asparagine for *R. solani* and citric acid in *F. solani*.

Symposium III: Plant Metabolomics

[M3-7] **Understanding Grape Maturity and Wine Flavor–A Flavoromics Approach**

Michael Qian

Department of Food Science and Technology, Oregon State University, Corvallis, U.S.A.

Terpene alcohols and C_{13}-norisoprenoids are very important secondary metabolites of wine grapes. Terpene alcohols are mainly present in the grape as glycoside precursors, and the amount varies tremendously with grape varieties. Terpene alcohol glycosides have no aroma impact to wine flavor, however, they can be hydrolyzed during wine-making process to release free terpene alcohols. Many terpene alcohols such as linalool and geraniol, can contribute floral, fruit, citrus aroma to wine. C_{13}-norisoprenoids are the breakdown products of carotenoids, many of the C_{13}-norisoprenoids, such as β-damascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and Vitispirane, have very low sensory thresholds, and they can contribute to wine aroma at very low concentration. Grape varieties, clones, as well as climate and viticultural practices all can affect the biosynthetic pathways of these compounds, resulting in unique flavor profile in the final wines. It was demonstrated that grape maturity greatly affected the concentration of free and bound terpene alcohols and C_{13}-norisoprenoids. Viticultural practices, such as basal leaf removal, also affect the metabolism of these compounds. For instance, basal leaf removal increased the concentration of terpenoids, including glycosidically-linked cis-linalool oxide, trans-linalool oxide, linalool, α-terpineol, and geraniol, as well as β-damascenone and the total C13-norisoprenoids in Pinot noir grapes.

Arthur Neish Young Investigator Award Symposium

Diana Roopchand, Ph.D.
Faculty Research Assistant
Rutgers University

Diana Roopchand earned her PhD in Biochemistry from McGill University (2005) and recently completed a NIH T32 Postdoctoral Fellowship with the Pennington-LSU-Rutgers NIH Center on Botanical Approaches to Combat Metabolic Syndrome (2009-2012) prior to being promoted to her current position as faculty Research Assistant. In addition, she has four and a half years of experience in the pharmaceutical and dietary supplement industries. Dr. Roopchand's cross-disciplinary academic research experience spans the areas of metabolic syndrome, type-two diabetes, cell cycle and cancer biology. Her research at Rutgers University is focused on dietary phytochemicals and addresses both fundamental and applied research questions relevant to nutrition, food and human health.

[MN-1] **Concord Grape Pomace Polyphenols Complexed to Soy Protein Isolate Are Stable and Hypoglycemic in Diabetic Mice**

Diana E. Roopchand[1], Peter Kuhn[1], Christian G. Krueger[2, 3], Kristin Moskal[4] and Ilya Raskin[1]

[1] Rutgers, The State University of New Jersey, School of Environmental and Biological Sciences, Foran Hall, 59 Dudley Road, New Brunswick, NJ 08901, USA
[2] Complete Phytochemical Solutions, LLC, 317 South Street, Cambridge, WI, 53523, USA
[3] University of Wisconsin-Madison, Reed Research Group, Dept. of Animal Sciences, 1675 Observatory Drive, Madison, WI, 53706 USA
[4] Nutrasorb LLC, 675 US Highway 1, North Brunswick, NJ, 08902, USA

Grape pomace is a byproduct of the grape juice and wine industries comprising the seeds, skins and stems of the grapes. Grape pomace contains beneficial polyphenols, such proanthocyanidins and anthocyanins; however, it is not a palatable source of these compounds. In this study we have developed and optimized a method to extract the polyphenols from grape pomace using food-compatible solvents. Complexation of grape pomace polyphenols to a protein-rich food matrix, such as soy protein isolate (SPI), was found to confer greater stability to polyphenols at 37 ºC. Grape pomace polyphenol-SPI complex (GP-SPI) showed hypoglycemic activity in obese and hyperglycemic C57BL/6 mice after single dose administration. Compared to SPI alone, C57BL/6 mice fed a high fat diet containing GP-SPI was showed decreased body weight gain, improved oral glucose tolerance and lower body fat mass. GP-SPI allows capture of grape pomace polyphenols in a food ingredient matrix that may be useful for management of metabolic syndrome/diabetes.

Arthur Neish Young Investigator Award Symposium

Dejan Nikolić, Ph.D.
Research Assistant Professor
Department of Medicinal Chemistry and Pharmacognosy
University of Illinois at Chicago

Dejan Nikolic received his BS in Pharmacy from University of Belgrade, Serbia and PhD degree in Medicinal Chemistry from University of Illinois at Chicago working on the development of new technique for screening combinatorial libraries and natural products extracts for ligands to target receptors. He then joined the UIC/NIH Center for Botanical Dietary Supplements Research where he is currently in charge of daily operations of the Analytical Core that provides analytical support for all Center Projects. In his research he uses modern LC-MS and LC-MS-MS approaches to address challenging problems in phytochemical research. His interests include structure elucidation of natural products, determination of ADME properties of active plant ingredients as well as development of new assays for drug discovery from plant sources. He is also interested in the development and validation of modern UHPLC MS-MS methods for quantitative analysis of active ingredients both in plant extracts and in clinical specimens in support of Phase I and Phase II clinical trials. His research is currently focused on the structure elucidation and biological activities of alkaloids and other nitrogenous compounds from black cohosh and on the development of new analytical methods for identification and quantitative analysis of pyrrolizidine alkaloids in various matrices. He has authored and co-authored more than 70 publications and is currently an Associated Editor of an open-access journal Natural Products Against Cancer.

[MN-2] **The Nitrogen-Containing Secondary Metabolites from Black Cohosh**

Dejan Nikolić, Tamara Cisowska, Tanja Gödecke, Shao-Nong Chen, David C. Lankin, Guido F. Pauli, Richard B. van Breemen[1]

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

Black cohosh (*Cimicifuga racemosa L.*) remains one of the most popular dietary supplements for relief of menopausal symptoms. In terms of the chemical composition, triterpene glycosides and phenolic acids represent the major constituents and interest in them has dominated the phytochemical and biomedical research on this plant for decades. In this talk I will discuss the most recent progress in identification and characterization of the third group of constituents that consist of alkaloids and other nitrogen-containing secondary metabolites. Utilizing modern mass spectrometry-based metabolomic approaches in combination with chemical synthesis, we have identified close to a hundred compounds, many of which are new natural products. Among the alkaloids, several classes such as guanidine alkaloids, isoquinolies, β-carbolines and pyrrolizidines were identified. Another large group of compounds are cinnamides of hydroxycinnamic acid with various biogenic amines and amino acids. Many of the cinnamides are present in the mono and di-glycosydated forms. Given that alkaloids are a well-known group of bioactive natural products, investigation of biological activities of these compounds provides a new direction in the research of this popular plant and offers a new, and perhaps more relevant compound class for future chemical standardization.

Arthur Neish Young Investigator Award Symposium

Daniel G. Vassão, Ph.D.

Project Leader (Group: Detoxification & Mode of Action)
Department of Biochemistry
Max Planck Institute for Chemical Ecology

Daniel G. Vassão received his B.Sc. in Chemistry (2001) from the University of São Paulo, and Ph.D. in Biochemistry (2008) from Washington State University. As an undergraduate in Brazil, he gained his first contact with Natural Products Chemistry while spending 3 years in the laboratory of Prof. Massuo J. Kato. During his doctoral studies under the supervision of Prof. Norman G. Lewis, Daniel studied the Chemistry and Enzymology of the biosynthesis of plant phenylpropanoids. After a short post-doctoral stay at WSU, in 2010 Daniel joined the group of Prof. Jonathan Gershenzon at the Max Planck Institute for Chemical Ecology in Jena, Germany, where in 2011 he became leader of a research group.
The current research in his group is focused on the interaction between plant defensive compounds and insect herbivores, specifically on chemical and biochemical aspects of metabolism, detoxification, and toxicology of these plant chemicals. His group uses isotope labeling, enzymology, in vivo bioassays, transcriptomic and proteomic techniques to examine how insects biochemically process compounds such as glucosinolates and benzoxazinoids, and how these phytochemical defenses exert their toxic effects.

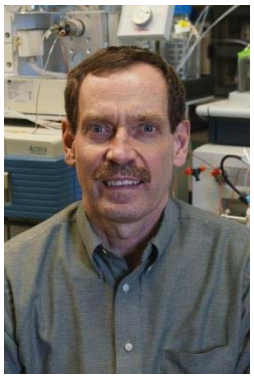
[MN-3] **Toxicity and Detoxification of Plant Chemical Defenses in Insect Pests**

Vassão, Daniel G.

Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, TH, Germany 07745

Many larvae from lepidopteran generalists are major destructive agricultural pests despite encountering a multitude of powerful plant chemical defenses in their diet. In order to succeed as herbivores, these insects employ exquisite biochemical detoxification strategies to circumvent or alleviate the toxicity of these phytochemicals. We have examined how some of these insects process chemicals from two defensive classes after ingestion: the glucosinolates present in plants of the order Brassicales, and the benzoxazinoids produced by grasses such as corn. Compounds from both of these classes are stored in plants as non-toxic glucosides, and are activated by glucosidases upon tissue damage forming reactive electrophiles. Using stable- and radio-isotope labeling, we found that all tested generalists from different orders process glucosinolates using the same conserved pathway, via conjugation of the corresponding isothiocyanate to glutathione. However, they differ remarkably in the extent to which they metabolize these compounds, with deep implications to their growth and fitness. On the other hand, however, a restricted subset of these same generalist species can metabolize benzoxazinoid aglucones via stereospecific re-glycosylation to render them harmless and facilitate excretion. We are now examining what role the enzymes involved may play for the fitness of both plants and insects.

Symposium IV: Bioproducts and Biofuels



Steven F. Vaughn
Plant Physiologist
Functional Foods Research Unit
USDA, ARS, National Center for Agricultural Utilization Research (NCAUR)

Steven F. Vaughn is a Plant Physiologist with the Functional Foods Research Unit at the USDA, ARS, National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois. Dr. Vaughn also holds faculty appointments in the Department of Crop Sciences at the University of Illinois at Urbana-Champaign and in the Department of Biology at Bradley University in Peoria. Dr. Vaughn obtained a B.S. in Botany from the University of Massachusetts-Amherst in 1981, a M.S. in Plant Breeding from Texas A&M University, College Station, Texas in 1983 and a Ph.D. in Agronomy from Texas A&M in 1987. He was a Plant Physiologist with the ARS from 1987 to 1989 at the Red River Valley Potato Research Laboratory in East Grand Forks, Minnesota, and since 1989 he has been a Plant Physiologist at the NCAUR where he has conducted research on a number of topics, including the postharvest physiology/pathology of horticultural crops, the value-added utilization of crop coproducts, and the isolation, identification and utilization of plant biochemicals with biological activity. He is the author of approximately one hundred publications on this research, and has been awarded four U.S. patents. Much of his research during the past ten years has been concerned with the development of novel bioproducts derived from low value crop processing coproducts such as biochars, dried distillers grains, and oilseed seedmeals/presscakes.

[T4-1] **Biobased Products Research At The National Center For Agricultural Utilization Research.**

Steven F. Vaughn, Neil P. Price, Steven C. Peterson, Robert W. Behle, Fred J. Eller, Jill K. Moser, Erica L. Bakota and Mark A. Berhow

USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, USA 61604.

Recent research by our group at the NCAUR has concerned the research and development of biobased products, most of which are derived from the residues produced during agricultural processing.. These include: novel sophorolipids from yeast as natural emulsifiers and surfactants for certified organic pesticides; biochars produced from various agricultural residues as replacements for peat moss in greenhouse substrates; seedmeals from biofuel processing as organic fertilizers; novel bioabsorbents from modified dried distillers grains; and plant extracts enriched in phytochemicals as nutraceuticals in processed foods.

Symposium IV: Bioproducts and Biofuels

Eric Lam, Ph.D.
Distinguished Professor
Department of Plant Biology and Pathology at Rutgers
State University of New Jersey

Dr. Lam is currently a Distinguished Professor in the Department of Plant Biology and Pathology at Rutgers, the State University of New Jersey. He joined the faculty at Rutgers in 1989 after 4 years of postdoc research at The Rockefeller University in New York. He has also served concurrently for three years as the Chair Professor of Botany in the University of Hong Kong between 2001 and 2004. More recently, he served for two years as the Director for the Biotechnology Center for Agriculture and the Environment at Rutgers. He also serves on the advisory board for the Rutgers Energy Institute. His research interests include the study of mechanisms that control programmed cell death and stress tolerance in plants, the regulation of global gene expression and chromatin structure, and more recently, the application of genetic engineering approaches in higher plants to facilitate and optimize plant biomass production for renewables. Dr. Lam is author of over 150 publications in journals including *Science* and *Nature*, and has been awarded 5 patents relating to biotechnology methods.

[T4-2] **Aquatic Agronomy: Integrating Plant Biology and Engineering for Sustainable Production of Duckweed Biomass on Wastewater**

Eric Lam

Rutgers the State University of New Jersey, USA

The quest for sustainable production of biomass and renewable fuels with low carbon footprints has become a global priority, with the expected decline in fossil fuel production and the need to curb climate change urgent issues facing humanity in this decade. In our consideration of alternative sources of renewable biomass that can be “domesticated” for food and energy production, the Lemnaceae family of aquatic plants, commonly called duckweed, holds great potential as a commercially viable feedstock for fuel and feed production driven by sunlight. Their rapid growth rate and ability to grow directly on existing wastewater sites are key advantages. Importantly, their natural growth characteristics also provide simple harvestiing strategies. This new “Aquatic Agronomy” holds great promise to complement current agriculture by its use of non-arable land and its ability to remediate wastewater from agriculture or municipalities. However, as an aquatic plant, creating a new production platform that is environmentally and economically sustainable will pose a formidable challenge to scientists, engineers and entrepreneurs. In this talk, I will discuss this vision and some of our recent efforts in establishing this new agriculture platform – from strain collection and characterization, to harvesting and post-harvest processing technology development.

Symposium IV: Bioproducts and Biofuels

[T4-3] **Spatial Differentiation of Volatile Complexity in Camelina: An Oil Crop for Biofuel Production**

Ming-Zhu Shi[1], Xiuli Lin[1], De-Yu Xie[1]

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

Camelina sativa is a traditional oil crop native to Europe and Central Asia areas. Its tolerance to arid environment allows its wide growth in marginal area as a new renewable crop for biofuel. Conversion of vegetable oil into biofuel products such as aviation oil requires a certain amount of volatile metabolites. To enhance the development of camelina oil into biofuel, we utilize GC-MS based metabolomics coupled with different isolation approaches to understand volatile complexities in different tissues and seed oil products. Both headspace SPME and solvent extractions coupled with GC-MS analysis determine differentiation of volatile complexities in flowers, leaves and seed oils. Our data particularly indicate biosynthetic differentiation of monoterpenes and sesquiterpenes between inflorescence and leaf tissues. In addition, our data show that profiles and levels of volatiles vary not only in different cultivar seed oil products but also differ in the same cultivar grown in different seasons. These results provide indicative information for field growth of camelina plants to produce necessary volatiles. This research is funded by ARPA-E.

[T4-4] **Modified Poplar Producing Allyl/Propenyl Phenols**

Kim, S.-J., Marques, J.V., Lu, D., Davin, L.B., Lewis, N.G.

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

Monomeric allylphenols and propenylphenols are important phenylpropanoid constituents of essential oils/flavours of several herbs, spices and flowers. They are presumed to serve in defense against herbivores and parasites and, in specific cases, as pollinator attractants, e.g. methyl chavicol. Monomeric allyl- and propenyl-phenols can also potentially be used for petrochemical substitutes, such as monomers for polymer production, including substituted polystyrenes and polyethylenes. The creosote bush (*Larrea tridentata*) harbors monolignol acyltransferase (*LtCAAT*) and allylphenol (*LtAPS*) and propenylphenol (*LtPPS*) synthase gene families, with the corresponding enzymes being substrate versatile. In order to partially redirect carbon flow away from lignin formation to instead produce allyl/propenylphenols, *LtCAAT1*/*LtAPS1* and *LtCAAT1*/*LtPPS1* double constructs harboring CaMV 35S promoter were individually assembled, and used to transform hybrid poplar. Transcript levels in leaves of transgenic poplars were measured by real-time PCR, with trees with highest expression levels selected for field trial. Transformed plants accumulated chavicol, eugenol and isoeugenol, as well as glycosylated derivatives of these compounds. Glycosylation not only decreases products toxicity, but also increases carbon flow through the pathway and result in a stable sink for the products that can be easily recovered later by means of chemical or enzymatic hydrolysis.

Symposium IV: Bioproducts and Biofuels

[T4-5] **Production of Licorice Triterpenoids in Both Transgenic Plants and Yeast Cultured Cells**

Toshiya Muranaka[1,2], Hikaru Seki[1], Kazuki Saito[2]
[1]Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka 565-0871, Japan,
[2]RIKEN CSRS, Yokohama 230-0045, Japan

Triterpenoid saponins are a diverse group of specialized metabolites with many biological properties. Among these chemicals, glycyrrhizin derived from *Glycyrrhiza* (licorice) plants, is one the most important crude drugs in the world. Their production largely depends on the collection of wild licorice plants, and this has caused a decrease in licorice reserves and an increase in desertification where it is harvested. Glycyrrhizin is synthesized from β-amyrin, a commonly occurring triterpenoid in plants, by series of site-specific oxidation and glycosylation. Our group isolated two genes encoding cytochrome P450 monooxygenases (CYP88D6 and CYP72A154), these perform subsequent oxidations of β-amyrin at positions C-11 and C-30, respectively. Recently we also cloned strong candidates for UDP-glucronidases for the glycosylation. Based on these results, two research projects are now on going in Japan. One is production of glycyrrhetinic acid in yeast and the other is production of glycyrrhizin in soybean. Both are based on the idea of "re-direct" of common precursors, 2, 3-oxidosqualen (yeast) and β-amyrin (soybean) to the heterologous glycyrrhizin pathway by recruiting glycyrrhizin biosynthetic genes (CYPs/UGTs) not only from licorice but also appropriated plant species. In the meeting, recent progress of both projects is shown and prospects of the studies will be discussed.

[T4-6] **Impact Of Physico-Chemical Properties On The Uptake, Translocation, And Metabolism Of The Herbicidal Compound Leptospermone**

Daniel K. Owens[1], Franck E. Dayan[1]

[1]United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University, MS, 38677

Leptospermone is a naturally occurring herbicidal β-triketone whose mode of action has been established as inhibition of *p*-hydroxyphenylpyruvate dioxygenase (HPPD). The compound is an herbicidal component of the essential oil of *Leptospermum scoparium* which has both pre- and post-emergent activity resulting in bleaching and dramatic growth reduction in grass and broadleaf weed species. Although great effort has been put forth in understanding the herbicidal activity and molecular target site of leptospermone, much remains to be learned of its *in planta* activity. In this study, ^{14}C labeled leptospermone was synthesized and used to examine uptake and translocation after root or foliar application in large crabgrass (*Digitaria sanguinalis*). Very little movement was observed after foliar application suggesting poor phloem mobility. However, root exposure resulted in approximately 50% of the absorbed leptospermone transported to the foliage suggesting rapid acropetal movement. The physico-chemical properties of leptospermone including the molecular mass, number of hydrogen donors and acceptors, and number of rotatable bonds were investigated while the *logP* and *pKa* values were experimentally determined. All parameters were shown to be consistent with the observed uptake and translocation data. Initial steps were taken to examine the *in planta* metabolism of leptospermone after uptake by crabgrass.

Symposium IV: Bioproducts and Biofuels

[T4-7] **Poplar as a Bio-Factory for the Production of Specialty Chemicals**

Marques, J.V., Costa, M.A., Dalisay, D.S., Davin, L.B., Lewis, N.G.

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

Poplar is a fast growing hardwood tree grown commercially through the world and considered an important platform for development of biotechnological technologies in plants. We have hybrid *Populus tremula × P. alba* capable of producing phenylethanol, a colorless chemical widely used as flavoring/fragrance agent in food and cosmetics. Transgenic poplar were successfully transformed using *Agrobacterium tumefaciens* with pKGW vector harboring phenylacetaldehyde synthase (PAAS) cloned either from *Rosa hybrida* cv. Fragrant Cloud or *Petunia hybrida* cv. Mitchell, and phenylacetaldehyde reductase (PAR) from *Lycopersicum esculentum*. Genes expressed under the 35S promoter were capable of converting endogenous phenylalanine into phenylacetaldehyde and phenylethanol, respectively. Plants not only accumulated free phenylethanol in leaves and stems, but also accumulated several derivatives of phenylethanol, including phenylethanol-glucoside. Localization of these metabolites in the vasculature was established via MALDI-TOF tissue imaging. The PAAS from rose was more effective in producing 2-phenylethanol than the petunia gene.

Symposium V: Botanicals and Medicinals

Nadja Cech, Ph.D.
Associate Professor of Chemistry
The University of North Carolina Greensboro

Nadja Cech earned her BS degree in chemistry from Southern Oregon University in 1997, and her PhD in Analytical Chemistry from the University of New Mexico in 2001. Her PhD training is in the area of mass spectrometry, and for the last 13 years she has worked to apply this expertise to solve challenging problems in natural products research. In particular, she has developed strategies to address synergy and complexity in the biological activity of complex botanical extracts. Dr. Cech's interests in this area stem from a long history of involvement in alternative medicine; her family owns and operates one of the largest medicinal herb seed companies in the country, and she spent her childhood working on their farm. As a faculty member at the University of North Carolina Greensboro, Dr. Cech supervises a research group of twelve students and postdoctoral research associates. She is the recipient of the 2011 Jack L. Beal Award for Best Paper in the Journal of Natural Products by a Young Investigator, and the 2010 University of North Carolina Greensboro Junior Research Excellence Award. Dr. Cech is funded by the National Institutes of Health on several projects that involve identification of botanical products effective against inflammation and infection.

[T5-1] **Addressing Complexity and Synergy in Botanical Medicines**

Nadja B. Cech[1]

[1]Department of Chemistry and Biochemistry, The University of North Carolina Greensboro, Greensboro, NC, 27402 USA

Investigators who study botanical medicines are faced with a formidable challenge. Bioactive compounds are present at varying abundance in extremely complex mixtures, and the overall activity of these mixtures is often due to the combined (additive, synergistic, or antagonistic) action of more than one component. Our research group is engaged in the development of mass spectrometric strategies to study combination effects in botanical extracts. This talk will focus on goldenseal *(Hydrastis canadensis*) as a case study for this problem. We observed synergy with *Hydrastis canadensis* on several different levels. First, using an approach we termed "synergy-directed fractionation", several flavonoids were identified that contribute to the overall antimicrobial activity of goldenseal but possess no direct antimicrobial activity of their own. Such compounds would be missed with traditional bioactivity-directed fractionation approaches. Secondly, we have demonstrated that complex goldenseal preparations can act against bacteria via at least three different mechanisms, antimicrobial, efflux pump inhibition, and anti-virulence. Of particular interest, different compound classes are responsible for each of these different activities. Overall, our investigations demonstrate the complexity of the interactions that occur between complex botanical extracts and biological systems, and provide insights into strategies that can be employed to address this complexity.

Symposium V: Botanicals and Medicinals

Claus Schneider, Ph.D.
Associate Professor of Pharmacology
Vanderbilt University

Claus Schneider received his M.Sc. (Staatsexamen, 1992) and Ph.D. (1997) in food chemistry from the University of Würzburg, Germany. He did postdoctoral training at the Department of Pharmacology, Vanderbilt University Medical School, Nashville, TN, U.S.A., where he joined the faculty first on the research (2001) and then on the tenure track (2006). He is currently an Assistant Professor of Pharmacology at Vanderbilt and affiliated with the Vanderbilt Institute of Chemical Biology. He has authored or co-authored 65 articles in peer-reviewed journals, and is currently principal investigator of two NIH R01 awards. Dr. Schneider's research interests are in the biochemical transformation of curcumin and in lipid biochemistry. His studies on curcumin transformation have led to the discovery of an oxidative pathway of degradation of curcumin that yields a number of previously unrecognized metabolites that have been identified using HPLC-, UV-, MS-, and NMR-based methods. The lab is currently investigating the oxidative transformation pathway in humans and mice and its physiological relevance in mediating biological effects of curcumin. Dr. Schneider's interest in lipid biochemistry has led to the discovery of a novel biosynthetic link of the leukotriene (5-lipoxygenase) and prostaglandin (cyclooxygenase-2) biosynthetic pathways. Convergent oxygenation of arachidonic acid by 5-LOX and COX-2 results in the formation of hemiketal eicosanoids that are the second major research focus in the Schneider lab.

[T5-2] **Biochemical Pharmacology of Curcumin**

Odaine N. Gordon, Claus Schneider

Department of Pharmacology, Division of Clinical Pharmacology, and Vanderbilt Institute of Chemical Biology, Vanderbilt University Medical School, Nashville, TN 37232, U.S.A.

The diphenol curcumin is recognized for its antioxidant, anti-inflammatory, and anti-tumorigenic bioactivities. It is currently tested in more than 70 clinical trials for the prevention or treatment of a broad range of diseases, including intestinal cancers as well as inflammatory and neurodegenerative diseases. A large number of *in vitro* cellular targets of curcumin have been identified but the precise chemical-molecular mechanisms by which curcumin affects its biological targets have not been conclusively elucidated. We have discovered a novel, previously unrecognized transformation of curcumin: a spontaneous, rapid, and prominent autoxidation reaction gives rise to a dioxygenated cyclopentadione derivative of curcumin as the major product. Autoxidation of [$^{14}C_2$]curcumin as a radiotracer has enabled us to isolate and identify additional products and reaction intermediates. We are testing the hypothesis that oxidative transformation is the biochemical basis underlying some of the biological effects of curcumin. To that end, we have shown that an early reactive intermediate of oxidative transformation of curcumin is responsible for poisoning of human topoisomerase, a major mechanism of the anti-tumorigenic activity of curcumin. The detection of oxidative metabolites in plasma and intestinal mucosa of mice implicate that oxidative transformation is a physiologically relevant route of curcumin metabolism. (Supported by NIH awards CA159382 and AT006896)

Symposium V: Botanicals and Medicinals

[T5-3] **Evaluation of *Jatropha isabelli* Natural Products and Their Synthetic Analogs as Potential Antimalarial Therapeutic Agents**

Victor Hadi[a], Taotao Ling[a], Yandira G. Salinas[a], Fatima Rivas[a]

[a] *Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, Tennessee 38105-3678 USA.*

Protozoal diseases such as malaria are a leading world health concern. We screened a library of fractionated South American natural products to identify new potential therapeutic leads and discovered that jatrophone (a product of *Jatropha isabelli*) exerts significant activity against *Plasmodium falciparum* strains 3D7 and K1. A focused jatrophone-scaffold library was synthesized to evaluate jatrophone's mode of action and identify more selective analogs. Two derivatives of this natural product–inspired compound library exhibited micromolar EC_{50} values against strains 3D7 and K1, thus providing a new antimalarial molecular scaffold. Our report describes an efficient derivatization approach used to evaluate the structure-activity relationship of jatrophone analogs in search of potential new antimalarial agents.

[T5-4] **Neurotrophic Compounds of Javanese Ginger, *Zingiber purpurenum***

Yoshiyasu Fukuyama[1], Miwa Kubo[1], Megumi Nakai[2], Kenichi Harada[1], Nobuaki Matsui[1], Masaaki Akagi[1]

[1]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan;

[2]Department of Pharmacy, Kochi Medical School, Kochi 783-8505, Japan

Javanese ginger Bangle, *Zingiber purpurenum,* has been used as a spice as well as an important component of traditional medicine "Jamu" in Indonesia. As part of our efforts to discover natural products with neurotrophic properties, we investigated the EtOH extract of the roots of Bangle (*Zingiber purpurenum*) that exhibited neuritogenesis activity in PC12 cells, resulting in the isolation of neurotrophic phenylbutenoid dimers **1** and **2**, and a new compound **3**. The structure of **3** was elucidated by analysis of spectroscopic data and comparing the NMR data with cussumunarin A, and its absolute configuration was determined by CD spectrum. Compounds **1** and **2** were found not only to significantly induce neurite sprouting of PC12 cells, but also to increase the neurite length and number of neurites in primary cultured rat cortical neurons, and also showed protective activity against cell death caused by deprivation of serum. Furthermore, chronic treatment of these compounds enhanced hippocampal neurogenesis in dementia model OBX mice. These results suggest that compounds **1** and **2** have both neurotrophic effects and neurogenesis, and thus Bangle may be developed as a valuable functional food for potentially protecting neurodegenerative diseases such as Alzheimer disease.

Symposium V: Botanicals and Medicinals

[T5-5] **A Metabolomics Driven Elucidation of the Anti-Obesity Mechanisms of Xanthohumol**

Jay S. Kirkwood[1], LeeCole L. Legette[1], Cristobal L. Miranda[1], Yuan Jiang[2], and Jan F. Stevens[1]

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Xanthohumol (XN) is a flavonoid from hops (*H. lupulus*) which appears to be beneficial for obesity and related conditions, but the mechanisms are largely unknown. To elucidate the potential mechanisms of action, we used untargeted metabolomics to explore the effects of chronic XN treatment on Zucker fatty rats. Analysis of fasting plasma revealed that in XN treated rats, common markers of dysfunctional fatty acid oxidation (DFAO) and ROS were markedly and coherently decreased. Since skeletal muscle is the major consumer of fatty acids during fasting, we hypothesized that XN was improving DFAO in this tissue. Cell culture experiments with myocytes revealed that XN is a mitochondrial uncoupler, increasing uncoupled respiration 100% at 5 μM. Tetrahydroxanthohumol, a hydrogenated product of XN which lacks electrophilicity, retained uncoupling activity and in addition, XN uncoupled respiration in several cell types, suggesting that XN is a nonspecific protonophore. Time course metabolomics of XN treated myocytes revealed markers of stress, such as electrophilic adduction, as well as an adaptive stress response, including an increase in glutathione recycling and synthesis as well as protein degradation. Together, these experiments suggest that XN may improve DFAO by uncoupling mitochondria and reduce ROS by enhancing glutathione synthesis.

[T5-6] **Antifungal Saponins from the Maya Medicinal Plant Ik Che (Solanaceae)**

Chieu Anh Ta[1], Jose A. Guerrero-Analco[1], Elizabeth Roberts[1,2], Rui Liu[1], Christopher Mogg[3], Ammar Saleem[1], Marco Otarola[4], Luis Poveda[4], Pablo Sanchez-Vindas[4], Victor Cal[5], Federico Caal[5], Francisco Caal[5], Rajagopal Subramaniam[3], Myron L. Smith[2], and John T. Arnason[1]

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Bioassay-guided fractionation of the crude extract (80% EtOH) of the leaves of Ik che, a plant used by Q'eqchi' Maya healers, resulted in the isolation and identification of two spirostan saponins (**S1** and **S2**). Structure elucidation by HRESIMS, 1D and 2D-NMR spectroscopic methods identified them to be the known saponin (25R)-1β,2α-dihydroxy-5α-spirostan-3-β-yl-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (**S1**) and new saponin (25R)-1β,2α-dihydroxy-5α-spirostan-3-β-yl-*O*-β-D-galactopyranoside **(S2)**. While **S2** showed little or no antifungal activity at the highest concentration tested, **S1** showed promising activity against *Saccharomyces cerevisiae* strains S288C, BY4741, and BY4743 (MIC of 16.5 μM), *Candida albicans*, *Cryptococcus neoformans,* and *Fusarium graminearum* (MIC of 132 μM). This is the first phytochemical report of Ik che as well as the antifungal activity of its constituents. Possible mechanisms of action are also explored.

Symposium V: Botanicals and Medicinals

[T5-7] **Deconstructing the Mediterranean Herb Rosemary to Characterize its Anticancer Activity**

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University of Illinois at Chicago College of Pharmacy, Department of Pharmacy Practice [1]
and University of Illinois Cancer Center [2]

The Mediterranean diet has long been attributed with a variety of health promoting properties for cardiovascular disease, diabetes, and even cancer. One aspect that has not received enough attention is the benefits of Mediterranean herbs. Specifically, rosemary and its polyphenolic diterpenes (carnosic acid and carnosol) are known to possess anti-oxidant activity that is beneficial as a food preservative. In fact, the European Union has even adopted the use of standardized rosemary extracts as food preservatives into its legislation. Another potential benefit of these diterpenes is that they may be beneficial in cancer control. Herein, we describe the *in vitro* and *in vivo* studies carried out towards understanding the molecular mechanisms of carnosic acid and carnosol leading to inhibition of prostate cancer. The reported findings suggest that these polyphenols target multiple signaling pathways involved in cell cycle modulation and apoptosis. Using a xenograft tumor model we have observed individual phytochemicals to suppress tumor growth compared to mice receiving placebo. These results are especially significant as it is becoming more likely that individuals will be consuming standardized rosemary extracts that are a part of a natural preservative system in various food preparations.

2012-2013 PSNA/Elsevier Award Lecture

Aimee Eggler, Ph.D.
Assistant Professor
Department of Chemistry
Villanova University
Villanova, Pennsylvania

Aimee Eggler received her B.S. in Chemistry with highest honors from the University of California at Santa Cruz in 1996 and her Ph.D. in Biochemistry from the University of Wisconsin, Madison in 2002. Dr. Eggler began studies on phytochemical activation of the Nrf2 transcription factor in 2003 during postdoctoral training with Dr. Andrew Mesecar in the Department of Medicinal Chemistry and Pharmacognosy at the University of Illinois-Chicago. Nrf2 activation leads to increased levels of numerous proteins that defend cells against oxidative and electrophilic stresses, resulting in protection of higher organisms from chronic diseases such as cancer and neurodegenerative diseases. Dr. Eggler continued studies of the mechanisms of Nrf2 activation as an Assistant Research Professor first at UIC and subsequently at Purdue University, and in 2012 she joined the Chemistry Department at Villanova University as an Assistant Professor. Her group's current research interests are in both the molecular and cellular mechanisms of Nrf2 activation by phytochemicals, including synergistic activation of Nrf2 by combinations of phytochemicals and the role of reactive oxygen species.

[T5-8] **Sensing Electrophilic Phytochemicals: A Model for How to Keap1 C151 Modification Leads to Decreased Ubiquitination Of Nrf2, A Key Target in Disease Prevention**

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Many phytochemicals, including sulforaphane from Brassicaceae, activate the transcription factor Nrf2. A number of cytoprotective genes are upregulated by Nrf2, comprising a promising therapeutic strategy for the prevention of cancer and other diseases. At basal conditions, Nrf2 is repressed by the cysteine-rich Keap1 protein. Keap1 targets Nrf2 for ubiquitination by forming a bridge between Nrf2 and the Cul3 protein, part of a ubiquitination complex, leading to subsequent Nrf2 degradation by the 26S proteasome. Nrf2 is activated upon modification of Keap1 cysteines by sulforaphane and other electrophilic compounds, and this modification downregulates Nrf2 ubiquitination and degradation. We find C151 to be readily modified by sulforaphane and other electrophilic phytochemicals promising for disease prevention, and their activation of Nrf2 is largely dependent on the presence of this cysteine. We sought to understand the structural and functional changes that occur upon modification of Keap1 C151, primarily by using a mimic of electrophilic modification, *i.e.* mutation of C151 to tryptophan. Interestingly, we find that when Keap1 C151 is modified, there is a significant conformational change in the Keap1-Cul3 complex. We present a structural model in which this conformational change restricts the ability of the Keap1-Cul3 complex to target Nrf2 lysines for ubiquitination.

Symposium VI: Phytochemicals in the Interaction between Plants and Their Environment

Peter Constabel, Ph.D.
Professor of Biology
Director of the Centre for Forest Biology
University of Victoria

C. Peter Constabel first became interested in ecological biochemistry during his MSc studies with G.H. Neil Towers at the University of British Columbia. He obtained his doctorate in molecular biology from the Université de Montréal in 1993, and received post-doctoral training in the laboratory of Bud Ryan at the Institute for Biological Chemistry at Washington State University. He joined the University of Alberta as an Assistant Professor in 1997, where he began his current research program on the molecular biology of induced defense in *Populus*. Since 2002 he has been at the University of Victoria, British Columbia, where he is currently Professor of Biology and Director of the Centre for Forest Biology. His current research is focused on condensed tannins and other bioactive phenolics in poplar, in particular on the complex regulation of tannin synthesis. He is also very interested in the ecological functions of the tannins, which he studies using genetically engineered poplar trees.

[W6-1] **Complex Ecological Roles of Tannins - Beyond Plant Defense**

Constabel, C. P.

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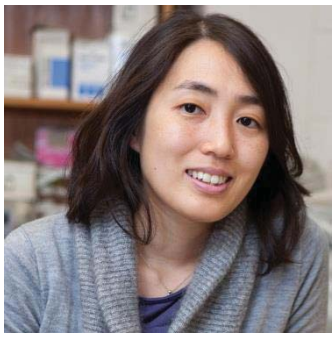
The plant tannins are high molecular weight polyphenolic phytochemicals. They are well-known for their health-promoting properties and are found in significant levels in nuts, fruit, some whole grains, and red wine. Tannins are defined by their ability to precipitate proteins, and for this reason are often believed to function in herbivore-defense, but they can play diverse biological roles. The most common tannins are the condensed tannins (CTs), polymeric flavan-3-ols also known as proanthocyanidins. In *Populus tremuloides*, CTs are induced by herbivory, UV exposure, pathogens, and nutrient stress, suggesting multiple stress-related functions. Direct evidence for the role(s) of tannins is generally lacking, however. We have recently identified tannin regulatory genes in poplar, which has allowed us to generate transgenic poplar trees with substantially enhanced levels of tannins. These plants have led to the identification of a network of regulatory genes underlying tannin synthesis in poplar, and also provide a platform for testing the ecological functions of tannins. In other systems, for example fruits and grains, studies on the regulation, localization, and developmental profiles of tannins are also providing hints as to their enigmatic biological functions.

Symposium VI: Phytochemicals in the Interaction between Plants and Their Environment

Nicole Clay, Ph.D.
Assistant Professor
Molecular, Cellular & Developmental Biology
Yale University



Nicole K. Clay received her B.S. in Biology (1996) from Massachusetts Institute of Technology, and Ph.D. in Biology (2005) from Yale University. She received her postdoctoral training in the field of plant-microbe interactions at the Massachusetts General Hospital, an affiliate of the Harvard Medical School (2005-2010). 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 the molecular basis for the adaptive diversification of the plant innate immune system, which is based on the perception and production of small molecules, and rivals the mammalian innate immune system in combating pathogenic infections. In particular, we are interested in understanding how pathogen discrimination is achieved through a molecular pattern recognition system, how glycan modifications on immune sensors regulate pathogen perception, and how the chemical outcomes of secondary metabolic pathways are diversified and functionalized. The first two strategies are conserved in eukaryotic innate immune systems while the latter is a hallmark response of the plant innate immune system, which relies more heavily on diversified chemical defenses than those of motile organisms. To elucidate the full "metabolic potential" of any plant family to synthesize a family of closely related molecules through paralogous enzymes, her lab is using a functional genomics approach in conjunction with targeted metabolic profiling strategies to mine the phylogenetic diversity of defensive genes and metabolites. Dr. Clay is currently studying the use of phylogenetically conserved transcription factors to identify phylogenetically restricted antimicrobials and/or anti-infectives in unrelated plant species, as well as the viral recognition system to identify antiviral compounds.

[W6-2] **The Chemical Outputs of the Plant Innate Immune System**

Nicole K. Clay, Assistant Professor of Molecular, Cellular & Developmental Biology

The plant innate immune system relies more heavily on diversified chemical defenses than those of motile organisms. More specifically, the plant innate immune system uses transcription factors and paralogous enzymes to trigger the production and activation of defense-related secondary metabolites. The receptor-mediated perception of broadly conserved pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs) triggers a basal defense response that is defined partly by the deposition of the glucan polymer callose and the phenolic polymer lignin at the cell wall at sites of pathogen contact. Callose deposition at the cell wall is a hallmark response of MAMP-activated immunity, and is widespread among species spanning the eudicot phylogeny. By contrast, the MAMP-activated production of specific defense-related secondary metabolites is phylogenetically restricted to a subclade of closely-related plant species. In *Arabidopsis thaliana*, the MAMP-activated callose response requires the production of the secondary metabolite 4-methoxy-indol-3-ylmethylglucosinolate, which is restricted to mustard plants. This result suggest that other defense-related secondary metabolites with similar bioactivities and/or chemical structures are responsible for activating the conserved callose immune response in unrelated plant species, and that general microbial elicitors can be used to interrogate the chemical diversity of defensive metabolites in most plant species. Here, we will share our results regarding the phylogenetic conservation and diversity of MAMP-induced indole and phenylpropanoid genes and metabolites, respectively, in *Arabidopsis.*

Symposium VI: Phytochemicals in the Interaction between Plants and Their Environment

[W6-3] **Hairy Roots as a Model to Investigate the Role of Suberin in Soybean Resistance to *Phytophthora sojae***

Trish L.A. Tully[1], Jessica Koteles[1], Pooja K. Sharma[1], Mark A. Bernards[1]
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Current disease management recommendations for control of *Phytophthora sojae* in soybean (*Glycine max* [L.] Merr.) emphasize the use of partial resistance, in which pathogen colonization of plant tissue is restricted, but not completely inhibited. One potential significant contributor to partial resistance is pre-formed root suberin, since this cell wall reinforcing polymer is located in both epidermal and endodermal tissues; in order to successfully infect soybean plants, *P. sojae* has to traverse both of these dermal layers. We have previously shown that there is a strong negative correlation between the amount of suberin and soybean mortality in *P. sojae*-infested fields. This correlation is linked to the modified fatty acids that make up part of the suberin polymer. Oxidation of the terminal (omega) carbon of fatty acids is a critical step in the biosynthesis of suberin, since it introduces a second functional group that allows three-dimensional cross-linking within the polymer. We have identified six fatty acid omega hydroxylase genes (*GmFAwH*) from soybean and have now established their expression patterns in soybean tissues, including *Agrobacterium rhizogenes*-induced hairy roots. The latter can now be used to manipulate suberin levels to test the hypothesis that suberin contributes to soybean resistance to *P. sojae.*

[W6-4] **Metabolite Profiling of Extracts of Wound Healing Potato Cultivars**

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Potato (*Solanum tuberosum* L.) is a staple food crop in many parts of the world. However, there is a considerable amount of waste during the collection and cultivation of this crop due to wounding and subsequent unfavorable healing conditions. The economic and nutritional importance of wound healing motivates our metabolite profiling studies to understand better the process by which suberin is formed in wound healing periderms of potato tubers. The biopolymer suberin offers protection against microbial infection and water loss, but its biosynthesis and assembly may vary among different plant species and cultivars. In the current investigation, extracts of the healing tissue from four different potato cultivars (Atlantic, Chipeta, Norkotah Russet, and Yukon Gold) were prepared at three and seven days after wounding. The extracts were analyzed using LC-MS, TOF-MS, and NMR spectroscopic methods. Multivariate analyses of the data revealed that distinctions among the cultivars' metabolite profiles decreased during the period following wounding. The biomarkers that discriminate between the cultivars at each wound healing stage were tentatively identified based on their mass and retention data. Kukoamines and kaempferol glycosides were identified as markers for Atlantic and Chipeta, respectively, at both stages, days 3 and 7, post wound induction.

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[W6-5] **Lipid Mediated Salicylic Acid Signaling Is Mediated By SABP2**

Dhirendra Kumar, Phillip Dean, Ferdous Amin and David Donald

Department of Biological Sciences, East Tennessee State University, Johnson City, TN

Salicylic acid (SA) mediated defense pathway has been shown to be one of the major defense tactics used by plants to defend themselves against microbial pathogens. Following pathogen attack high levels of methyl salicylate (MeSA) are produced which can be converted to SA by the enzyme salicylic acid binding protein 2 (SABP2). The accumulation of SA has been shown to lead to the activation of several defense mechanisms leading to systemic acquired resistance (SAR). Transgenic plants lacking SA or SABP2 demonstrate a dramatic decrease in pathogen resistance. A yeast two-hybrid screening was performed to identify protein interacting with SABP2. Several interesting putative SABP2 interacting proteins (SBIP) were identified. SBIP-436 shows high homology to phospholipases. SBIP-470 is a putative lipid transfer protein while SBIP-5 and 24 are fatty acid desaturases. Phospholipases, desaturases and lipid transfer proteins has been previously implicated in various plant processes including defense response. Interaction of these putative SBIPs with SABP2 makes them very interesting and suggests a direct role in SA-signaling in plants. Several of these SBIPs have been cloned and expressed in *E. coli* and purified SBIP is being used for their biochemical characterization. Arabidopsis mutants corresponding to each of these SBIPs are being characterized and would be used for complementation studies. Understanding these SBIPs and their role in innate defense and other pathways can play vital role in developing strategies that give plants a leg up in their ongoing evolutionary arms race against pathogen attackers.

[W6-6] **Interaction of Cruciferous Phytoalexins and Glucosinolates with Fungal Pathogens**

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Crucifers, as for example canola, mustard, and cauliflower, respond to stress employing metabolic pathways that involve the biosynthesis of numerous and structurally diverse natural products, including phytoalexins and phytoanticipins. These metabolites are involved in plant responses to stress inflicted by pathogens, metal salts, UV radiation, etc. Tryptophan is the primary biosynthetic precursor of cruciferous phytoalexins, whereas phytoanticipins derive from a variety of precursors. Glucosinolates are phytoanticipins derived from different amino acids including phenylalanine and tryptophan. The interactions of the phytoalexins brassinin and camalexin and indolyl and benzyl glucosinolates with three fungal pathogens were analyzed: *Alternaria brassicicola* that infects stems, leaves and pods of *Brassica* species, *Rhizoctonia solani* that infects mainly below ground plant organs in a wide range of species, and *Sclerotinia sclerotiorum* that infects above ground organs of different plant species. It was discovered that both phytoalexins were detoxified by all fungal species, while only *A. brassicicola* metabolized benzyl glucosinolate, but indolyl glucosinolates were not transformed by any of the pathogens. Details of this work will be presented, pathways will be proposed and implications in plant disease resistance will be discussed.

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[W6-7] **Engineering Elevated Vitamin C Content in Rice to Improve Abiotic Stress Tolerance**

Katherine A Lisko[1], Gregory C. Phillips[1], Anna McClung[2], Jamie Underwood[3], Vibha Srivastava[3], and Argelia Lorence[1]

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Vitamin C (L-ascorbic acid, AsA) is a vital antioxidant for both plants and animals. In plants AsA is also a modulator of cell division and growth, flowering, senescence and photosynthesis. Ascorbate is used to protect lipids, proteins, and nucleic acids from reactive oxygen species formed in response to abiotic stresses. Rice (*Oryza sativa)* is a staple crop grown worldwide and the main abiotic stresses negatively impacting rice yields are soil salinity and drought. The need for food and feed is growing so it is essential that we improve the stress tolerance of our crops to meet this demand. In previous studies in *Arabidopsis thaliana* we have shown that increasing AsA content leads to faster growth, enhanced accumulation of aerial and root biomass, and broad tolerance to abiotic stresses. In this work, we have established the basal metabolism of AsA in rice for several varieties during ontogenesis. We have also developed rice with elevated AsA content via over-expression of AsA biosynthetic enzymes and we are currently testing the ability of these high-AsA lines to cope with the damage commonly seen during salt and drought stresses.

Poster Abstracts

[P01] **Selected Point Mutations of a Flavonoid 3-*O*-Glucosyltransferase from *Citrus paradisi* (Grapefruit) and Effect on Substrate and Regiospecificity**

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Flavonoids are secondary metabolites that are important in plant defense, protection, and human health. Most naturally-occurring flavonoids are found in glucosylated form. Glucosyltransferases (GTs) are enzymes that catalyze the transfer of glucose from a high energy sugar donor to an acceptor molecule. At this time, it is not possible to accurately predict putative GT activity from sequence alone; biochemical characterization is critical. A flavonol-specific 3-O-GT enzyme has been identified and cloned from the leaf tissues of grapefruit. The enzyme shows rigid substrate specificity and regiospecificity. F3GTs from grape and grapefruit were modeled against F7GTs from *Crocus sativus* and *Scrutellaria biacalensis*, and several non-conservative amino acid differences were identified that may impact regioselectivity. This research is designed to test the hypothesis that specific amino acid residues impart the regiospecificity of the grapefruit enzyme. Site-directed mutagenesis was performed on three potentially key amino acid residues within the grapefruit F3-GT that were identified through homology modeling. Enzyme activity of the mutant F3-GT proteins will be analyzed for a possible change in glucosylation pattern. Other flavonoid classes will also be tested with the mutant enzymes to test for change in substrate specificity.

[P02] **Functional Characterization of Myb115, a Putative Regulator of Tannin Biosynthesis in Poplar**

Amy M. Franklin[1], Kazuko Yoshida[1], and C. Peter Constabel[1]
[1]Centre for Forest Biology, Department of Biology, University of Victoria, Victoria, BC, Canada, V8P 5C2

Condensed tannins are wide-spread polyphenols with diverse ecological functions, including defense against herbivores and microbes. In poplar, condensed tannin synthesis is induced by a variety of stresses. The R2R3 MYB transcription factor, MYB134, was shown to be a key stress-responsive regulator of induced tannin synthesis in poplar (Mellway et al., Plant Physiol. 150: 924-941, 2009). A second putative regulator of tannin synthesis, MYB115, has recently been identified. In dual-luciferase promoter activation assays, MYB115 and MYB134 activated the promoter of a tannin-specific biosynthetic enzyme, anthocyanidin reductase. This suggests that both transcription factors act as direct regulators of tannin synthesis. Furthermore, MYB115-overexpressing transgenic poplar accumulate both condensed tannins and the tannin precursor, catechin, at higher levels than wild-type plants. HPLC analysis of MYB115 overexpressors showed additional changes to phenylpropanoid metabolism, including changes in levels of salicinoid phenolic glycosides. Our results indicate an important role of MYB115, in addition to MYB134, in the regulation of the condensed tannin pathway in poplar.

Poster Abstracts

[P03] **Poplar Repressor-like MYB Factors which Negatively Regulate Proanthocyanidin Biosynthesis**

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Proanthocyanidins (PAs, also known as condensed tannins) are common secondary plant metabolites important for protection of plants against diverse biotic and abiotic environmental stresses. In the last decade, molecular genetic studies have identified PA regulators in many plant species. In poplar, the UV light- and wound-inducible transcription factor MYB134 was characterized and shown to positively regulate PA synthesis. Constitutive expression of MYB134 in transgenic poplar resulted in the specific activation of PA pathway genes as well as previously uncharacterized MYB transcription factors with repressor motifs. To elucidate the roles of the repressor-like factors in the regulatory network of PA pathway, two parallel approaches were taken. First, transient promoter-reporter activation assays in plant cells revealed that these repressor-like poplar MYB factors had strong repressive effects on promoter activation by the MYB134/bHLH complex. In this assay, the repressors showed distinct regulation depending on the combination of the positive regulators and cofactors. Second, analysis of poplar hairy roots expressing one specific MYB repressor suggested it functions in negative regulation of the PA biosynthesis.

[P04] **Acyl-Activating Enzymes in Taxus: Key Enzymes in The Biosynthesis of Taxol and Its Derivatives**

Hartmann, M.B.[1], Li, Q. [1], Lange, I.[1], Ketchum R.E.B.[1], Croteau R.B.[1], May G.D.[2], Crow J.A.[2], Lange, B.M.[1,]*

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Paclitaxel (taxol®) is a diterpene used for the treatment of lung, ovarian, breast, head and neck cancers. Despite extensive efforts toward a better understanding of the complex biosynthetic pathway leading to taxol, to date, the enzymes catalyzing at least six out of approximately 20 predicted metabolic steps remain unknown. We obtained Illumina transcriptome data for a time course experiment performed with MeJA-induced *Taxus* x *media* cell suspension cultures. Bioinformatic processing indicated the presence of a large family of genes encoding acyl-activating enzymes (AAEs), some of which were found to be induced by the experimental treatment. The Taxus AAE genes present in our transcriptome data set were cloned and functionally expressed in *E. coli*. Some of the AAE candidates that we have functionally tested thus far have shown relatively broad substrate specificity *in vitro* and could theoretically provide a variety of activated substrates (short-chain aliphatic and aromatic CoA esters) for the final 3'-N-acylation of the phenylisoserine side chain, thereby contributing to the diverse taxoid composition of *Taxus* cell cultures. The implications of these findings for engineering taxol biosynthesis in plants or microbes will be discussed.

Poster Abstracts

[P05] **Wax Biosynthesis in *Arabidopsis thaliana*: Is SGNH1 Involved in Alkane Formation?**

Daniela Hegebarth[1], Fred Beisson[2], Reinhard Jetter[1,3]

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[3] Department of Chemistry, The University of British Columbia, Vancouver, Canada, BC V6T 1Z1

The compounds forming the plant cuticle (wax and cutin) are synthesized in epidermal cells of vascular plants before being transported to the organ surface. Many processes involved in biosynthesis and translocation of cuticular waxes have recently been elucidated in *Arabidopsis thaliana*. However, the central part of wax biosynthesis, alkane formation, still remains to be understood. Several genes potentially involved in alkane formation were identified by epidermis-specific microarray assays and based on mutant wax phenotypes in *A. thaliana*. The goal of our work is to characterize these candidate genes and determine the roles of their enzyme products in alkane formation. One gene in particular, *SGNH1*, was found highly up-regulated when induced by WIN1, a transcriptional activator of epidermal wax synthesis. A Toluidine Blue assay showed disruption of the cuticle in knock-out *sgnh1 A. thaliana* lines. The *sgnh1* wax composition of petals, leaves and stems was determined by GC-MS and GC-FID, revealing reduced levels of leaf alkanes. Further gene characterization is currently under way, including cutin analysis, quantification of gene expression levels in different plant organs, subcellular localization of the protein, as well as site-directed mutagenesis and heterologous yeast expression to determine enzyme activity as well as substrate and product specificities.

[P06] **Modeling of the Interface of Primary and Secondary Metabolism in Peppermint Glandular Trichomes**

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Peppermint is an economically important crop, as well as a model system for the study of terpenoid essential oil synthesis in specialized cell types. In peppermint, the pathways that directly synthesize terpenoids have been studied extensively, but the pathways from the uptake of sucrose to the formation of terpenoid precursors have not been examined in detail. A stoichiometric model of metabolic pathways in secretory phase peppermint glandular trichomes was developed by combining cell type-specific gene expression data with previously published genome scale plant models, specific enzyme assays, and data from the peppermint literature. Fluxes through these pathways were estimated based on reports from the literature and from feeding ^{14}C-sucrose to isolated glandular trichomes in the presence or absence of metabolic inhibitors. We also assessed the contribution of respiration and fermentation to power metabolite transport and cofactor regeneration. This work serves as an example of how large scale stoichiometric models can be used as a framework for organizing data and generating testable hypotheses about metabolism in specific tissues.

Poster Abstracts

[P07] **Rice OS9BGLU31 Transglucosidase Activity Changed in A Single Point Position around the Active Site Mutation**

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Glycosylation is a mechanism by which plants regulate the functionality of various compounds, including the reactivities and bioactivities of plant secondary metabolites and phytohormones. Rice Os9BGlu 31 is a glycoside hydrolase family GH1 enzyme that acts as a transglucosidase that can transfer a glucose moiety to phenolic acids, flavonoids, and phytohormones. The Os9BGlu31 gene is most highly expressed in senscing flag leaf and developing seed. Site directed mutagenesis of Os9BGlu31 residues around active site (Asn243, Gln172, and Gln183) was done to determine the role of these residues in determining transglycosylation versus hydrolysis activity. The relative rates of transglycosylation and hydrolysis activity of enzyme, with 4-nitrophenyl-β-D-glucopyranoside as the donor substrate in reactions with or without ferulic acid or 4-hydroxybenzoic acid acceptor substrates, respectively. The mechanism of Os9BGlu31 was also probed with 2-fluoroglucoside inhibitors of GH1 β-glucosidases, including 2,4-dinitrophenyl-β-D-2-deoxy-2-fluoroglucoside. The wild type enzyme neither showed any inhibition by nor utilized these mechanism-based inhibitors as substraes, despite the fact that 2,4-dinitrophenyl-β-D-glucoside served as a good donor substrate. These results suggest that GH1 transglycosidases have unique mechanistic properties that distinguish them from GH1 hydrolases.

[P08] **Identification of Caffeic Acid *O*-methyltransferase Gene Family Involved in Lignin Biosynthetic Pathway in *Liriodendron tulipifera***

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Liriodendron tulipifera is one of the early branching angiosperm lineages, referred to as “basal angiosperm”, and is a member of the Magnoliaceae family in the order Magnoliales. It has many common names such as tulip tree, tulip poplar, yellow poplar, and white poplar. As it is a quickly growing tree species with relatively high stress resistance to both abiotic and biotic stresses, it is regarded as a future renewable bio-material in Korea, not only for use in the bioenergy industry, but also as a useful substitute for petroleum products. In our previous study, we demonstrated that the treatment with (+)-*epi*-brassinolide (BR) induces a significant reduction of lignin deposition with alteration of caffeic acid *O*-methyltransferases (*COMT*) expression at transcription level in two-year-old yellow poplar stem. In order to understand BR-induced modification of lignin biosynthesis, we isolated the full-length cDNAs of five putative *COMT* from the yellow poplar stem, and constructed vector for overexpression of these genes using gateway system. In addition, promoter region of two COMTs, whose transcripts were highly increased following BR treatment, were isolated. In order to investigate the enzyme activity of these recombinant COMTs, we have constructed expression vectors of these recombinant proteins and transformed into *E. coli*.

Poster Abstracts

[P09] **Development of Poplar Superclones for Lignocellulosic Biomass via Construction of Expression Libraries of Poplar NAC Transcription Factor Family**

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NAC (NAM, ATAF1/2 and CUC2) domain proteins are plant-specific transcriptional factors known to play wide range of diverse roles in various plant developmental processes. N-terminal NAC domain has been implicated in nuclear localization, DNA binding, and the formation of homodimers or heterodimers with other NAC domain proteins. In contrast, the C-terminal regions of NAC proteins are highly divergent. NAC transcription factors comprise of a large gene family represented by more than 100 members in Arabidopsis (~105 genes), rice (~140 genes), soybean (~101 genes) and poplar (~185 genes). A comprehensive analysis was reported for NAC gene family in poplar, i.e., phylogeny, chromosomal location, gene structure, conserved motifs, and expression profiling analysis. Recently accumulating evidences indicated that a considerable portion of NAC domain proteins play crucial roles in the processes of xylogenesis, fiber development, and wood formation in vascular plants. As an effort to understand roles of NAC domain protein in poplar and to develop superclones for lignocellulosic biomass, the entire NAC family members of *Populus trichocarpa* were cloned and transformed into hybrid poplar (*Populus alba* x *Populus tremula* var. *glandulosa*) for functional characterization. Construction of transgenic poplar library either overexpressing or suppressing each NAC members is in progress.

[P10] **Biosynthesis and Accumulation of Very-Long-Chain Alkylresorcinols in Cuticular Waxes of *Secale cereale* and *Brachypodium distachyon***

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5-Alkylresorcinols are a class of phenolic lipids, which have been identified, in the cuticular wax of various cereal crops. Due to their antifungal and antibacterial properties, ARs have potential applications as nutraceuticals. They are biosynthesized by type-III polyketide synthases (PKSs). Two candidate PKS genes were previously isolated from the two model grass species rye and *Brachypodium distachyon*, and were shown to encode alkyl resorcinol synthases (*ScARS* and *BdARS*, respectively). Here we report the further characterization of these two enzymes, with the goal to test whether they are involved in the formation of cuticular wax alkylresorcinols. The accumulation of ARs was monitored in waxes on various organs of etiolated and normal plants, and the product amounts found to correlate with gene expression patterns. Two new series of alkylresorcinols were identified and quantified, the first containing ARs with C19 – 27 alkyl chains, and the second methyl-branched alkylresorcinols with C19 – 25 chains. Subcellular localization using GFP fusions showed that the ARSs proteins are associated with ER membranes of epidermal cells, where very-long-chain acyl CoA substrates of ARSs are known to accumulate. Overall, our data indicate that both enzymes are indeed involved in the biosynthesis of grass surface alkylresorcinols.

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[P11] **'Rational Metabolic-Flow Switching' for the Production of Exogenous Secondary Metabolites in Plant Suspension Cultured Cells – A Proof-Of-Concept Study Using Bamboo Cells**

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Synthetic biology using microbial hosts has been attracting attention to produce useful plant secondary metabolites despite various difficulties, such as inclusion body formation and need for substrate supply. In contrast, plant cultured cells are hardly used for such purpose due to their slow proliferation, time-consuming transformation procedures, and low productivity. Here, we developed a novel procedure named 'rational metabolic-flow switching', to efficiently produce exogenous secondary metabolites using suspension cultured cells of bamboo (*Phyllostachys nigra*) as host. Firstly, we surveyed secondary metabolites occurring mainly in bamboo cells and identified feruloylputrescine and *p*-coumaroylputrescine, suggesting that phenylpropanoid and polyamine biosynthetic pathways are highly active, and the bamboo cells are suitable for the production of alternative secondary metabolites derived from those pathways. Thus, secondly, we generated stable transformant of bamboo cells expressing *agmatine coumaroyltransferase* gene of barley in the expectation of metabolic-flow switching from hydroxycinnamoylputrescines to hydroxycinnamoylagmatines. As a result, in the recombinant cells, the putrescine amides content decreased and instead high content of agmatine amides was newly produced as expected, where the content of major product *p*-coumaroylagmatine reached approximately 350 mg per liter of culture. The results provide proof-of-concept to the usefulness of 'rational metabolic-flow switching' in synthetic biology using plant cell hosts.

[P12] **Lignan Biosynthesis in *Sesamum indicum***

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(+)-Sesamin, a furofuran class lignan, is widespread in vascular plants and represented by *Sesamum* spp. Here we present a *Sesamum* P450, CYP81Q1, that alone catalyzes (+)-sesamin biosynthesis from (+)-pinoresinol via (+)-piperitol by forming two methylenedioxy bridges. The CYP81Q1 gene expression profile was temporally consistent with the accumulation pattern of (+)-sesamin during seed development. I would like to discuss on evolutional and biochemical aspects of lignan biosynthesis in sesame seed. References: Ono, E. *et al* (2006) *PNAS* 103, 10116-10121; Noguchi, A. *et al* (2008), *Plant J*. 54, 417-427; Kim, H-J. *et al* (2010) *Plant Cell Physiol*. 50, 2200-2209.

Poster Abstracts

[P13] **Chemical Composition of Cuticular Waxes of *Aloe arborescens* Leaves: Identification and Distribution of Novel 1,3-Bifunctional Compounds**

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As part of our survey of plant species in search for cuticular wax biosynthetic pathway intermediates, surface lipids from leaves of *Aloe arborescens* were analyzed by gas chromatography with mass spectrometric and flame ionization detection. Very-long-chain (VLC) alkanes were most abundant, followed by aliphatic esters, fatty acids, alcohols, and a novel class of VLC 3-hydroxy fatty acid methyl esters (3OH-FAMEs), whose identity was confirmed using synthetic standards. This finding is of special interest since beta-functionalized fatty acids represent a missing link between elongation intermediates and previously described 2-ketones and 2-alcohols. VLC aliphatic ester isomer profiles were obtained from the mass spectrum of each homologue and showed good correlation with the distribution of corresponding fatty alcohol biosynthetic precursors. The partitioning of wax constituents between epicuticular and intracuticular layers was also studied to locate compounds either available at the outermost surface for direct defense against pathogens and herbivores, or accumulating inside the cuticle to form a transpiration barrier. Trace amounts of cyclic triterpenoids were detected only in intracuticular wax, in agreement with reports on other species. Hentriacontane clearly dominated epicuticular wax, which is consistent with surface crystal formation and thus explains glaucous leaf appearance. The partitioning of 3OH-FAMEs is currently under investigation.

[P14] **Analysis of the Jasmonic Acid Effect on Primary and Specialized Metabolism of *Hamelia patens* Plants by NMR-Based Metabolomics**

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Hamelia patens Jacq. is a perennial bush known as Mexican firebush, hummingbird bush or *canchoc*, from the Rubiaceae family and native to Mexico and Central America. It has been used in the folk medicine to treat chronic wounds and tumors. Monoterpenoid indole alkaloids and monoterpenoid oxindole alkaloids are specialized metabolites isolated from this bush. In order to study the biosynthesis and production of these alkaloids, 5-month-old *H. patens* plants were elicited with different jasmonic acid (JA) concentrations. At 1 mM JA, gene expression and alkaloid accumulation were determined. Results from the reduction by partial least square-discriminant analysis (PLS-DA) of the ^{1}H-NMR data showed a separation based on the JA treatment applied. Analysis of the loading plot of this PLS-DA model allowed identification of the metabolites involved in the discrimination between control and JA-treated plants. Sucrose, chloragenic acid, glutamine, glutamic acid and the alkaloids pteropodine, isopteropodine and palmerine were the predominant metabolites in JA-treated plants. A time course of the MIA and MOA contents was followed. Maximum accumulation of MOAs and MIAs was observed at days 40 and 10 in JA-treated plants, respectively.

Poster Abstracts

[P15] **Proteomic Analysis of *Uncaria Tomentosa* Root Cultures Grown Under Oxidative Stress**

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Uncaria tomentosa (cat's claw), an indigenous plant from the Amazon rainforest, is the source of monoterpenoid oxindole alkaloids (MOA) mainly mitraphylline, rhynchophylline and their stereoisomers with immune modulatory, cytotoxic, anti-AIDS and antileukemic activities. In cell and root cultures, these highly oxidized alkaloids are produced and stimulated by oxidative stress. In secondary metabolism, besides the enzymes, transport and regulatory proteins are also involved, which makes the proteome an essential topic for their studies. In order to investigate the regulation of biosynthetic pathway leading to MOA, the differential proteome profile of *U. tomentosa* roots culture under oxidative stress was performed. 13 days-old root cultures were elicited with a combination of buthionine sulfoximine and jasmonic acid (BSO-JA). Proteomic analysis showing that the elicitors up regulated the expression of strictosidine synthase (STR), triosephosphate isomerase, protease subunit alpha type, cytosolic ascorbate peroxidase, ribulose 1,5-biphosphate carboxylase oxygenase. RT-PCR expression analysis of the main enzymes involved in the MOA biosynthesis, STR and strictosidine beta glucosidase, also exhibited an increase in the mRNA levels after elicitation. A correlation between induced alkaloid related proteins and alkaloid production was also observed.

[P16] **Evidence of Dimeric Plant Polyphenolic Products Produced By a Bacterial Laccase**

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Plant-derived polyphenolics are a natural source of potent antioxidant activity. Potential exists to increase the potency of these chemicals through coupling of the same compound or synergistically acting phytochemicals. The objectives of this research are to: 1) use enzymes to couple natural polyphenolics, 2) characterize changes in antioxidant activity of the altered phenolics, and 3) identify the change to chemical structure of the polyphenolic. Laccases are multicopper oxidase enzymes that can oxidize phenolic groups to produce reactive radicals that undergo further non-enzymatic radical coupling reactions. A bacterial laccase (SCO6712) was characterized for potential to act on polyphenolic flavonols and hydroxycinnamic acids. SCO6712 showed activity on 10 different phenolic compounds including the monolignol ferulic acid, the stilbene resveratrol, and the flavonol quercetin. UPLC-MS analysis indicated the enzymatic production of dimeric compounds from quercetin, morin, and myricetin. However, the DPPH scavenging activity assay also showed reduced antioxidant activity of the reaction products compared to initial substrates. Future experiments will characterize product formation and bioactivity over time, and will use MS-MS to further investigate the structure of reaction products. Moreover, additional antioxidant assays will be performed to assess the antioxidant activity of laccase-treated phenolics in lipophilic environments.

Poster Abstracts

[P17] **Filling in The Gaps - Deep Transcriptome Sequencing Data-Based Identification and Characterization of Candidate Genes for the Galanthamine Biosynthetic Pathway in *Narcissus pseudonarcissus***

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The Amaryllidaceae alkaloid galanthamine possesses therapeutic utility as a treatment for Alzheimer's disease. Its biosynthesis has been outlined in the literature but functional characterization of the biosynthetic genes/enzymes is missing. In the proposed pathway, the precursors, 3,4-dihydroxybenzaldehyde and tyramine, arise from phenylalanine and tyrosine, respectively. They combine to form norbelladine, which is then *O*-methylated to form 4'-*O*-methylnorbelladine. 4'-*O*-methylnorbelladine undergoes intramolecular *para-ortho'* oxidative coupling followed by spontaneous oxide bridge formation to yield *N*-demethylnarwedine. *N*-demethylnarwedine is reduced to *N*-demethylgalanthamine, which in turn is *N*-methylated to yield galanthamine. Here, the focus was on the amine aldehyde condensation leading to norbelladine, which represents a junction between primary and secondary metabolism. It is analogous to strictosidine synthase (STR)- and norcoclaurine synthase (NCS)-catalyzed steps in terpenoid indole alkaloid and benzylisoquinoline alkaloid biosynthetic pathways, respectively. A BLAST search for STR and NCS using the *Narcissus pseudonarcissus* transcriptome (National Center for Genome Resources-assembled) yielded some promising candidates. One particular STR-like candidate was cloned full-length but its bacterial expression was sub-optimal. Subsequently it is being used for optimization of expression of STR-like proteins in Baculovirus-based Sf9 insect cell expression system and seed-specific expression in *Camelina sativa*. This will also be followed up by analysis of other candidates in hand.

[P18] **Investigating the Biosynthesis of Cuticular Alkanes in *Arabidopsis thaliana*: Characterization of Scd2**

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Plant cuticular lipids are synthesized in the endoplasmic reticulum (ER) of epidermal cells for coating the surfaces of aerial organs against various stresses. A new gene, *SCD2* (Susceptible to Coronatine-Deficient Pst DC3118-2), was recently discovered that is likely involved in formation of *Arabidopsis* leaf surface wax. Here, we report the characterization of *SCD2* and its protein product. Two independent *scd2* mutant lines had drastically increased levels of very long chain aldehydes and decreased levels of alkanes in their leaf wax. The wax of mutants was restored to wild type composition by complementation with the native gene. Quantitative RT-PCR revealed highest transcript abundance in rosette and cauline leaves, whereas in stems and roots it was present at low levels. Finally, the protein localized to the ER in tobacco and *Arabidopsis* lines expressing SCD2-GFP fusions. All these findings are consistent with an enzymatic role of SCD2 in wax biosynthesis, leading us to hypothesize that this protein is a wax aldehyde decarbonylase. However, the promoter was active in the phloem, a tissue not apparently involved in cuticle formation. To reconcile all previous results and further test the role of SCD2 in wax formation, we are currently conducting *in vitro* enzyme assays.

Poster Abstracts

[P19] **Understanding the Product Profile of a Model Monoterpene Synthase**

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The tens of thousands of terpenoids found in nature are all derived from acyclic prenyl diphosphate precursors by the activity of terpene synthases. Many of the known terpene synthases produce multiple, structurally diverse products due to variations in the electrophilic reaction mechanism. Limonene synthase, a model monoterpene synthase for which a crystal structure has been reported, produces (-)-limonene as the predominant product (> 95 %), with myrcene, α-pinene and β-pinene as minor products. We used site directed and site saturation mutagenesis, in combination with computational modeling, to better understand the roles of all amino acid residues that form the active site of this enzyme. Based on experimentally determined mutant product profiles and quantum mechanical calculations, we propose a model for the catalytic determinants of product specificity in limonene synthase.

[P20] **Identification of Acyltransferases Associated with Oil Accumulation in Avocado Fruit**

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In avocado, an economically important crop, fruits can store up to 70 % oil in the form of triacylglycerols (TAGs). While TAG synthesis in seed tissues mostly depends on an acyl CoA-dependent enzyme, diacylglycerol (DAG) acyltransferase (DGAT) to catalyze the conversion of DAG to TAG, the enzymes involved in non-seed tissues remains to be elucidated. Recent studies on oil palm suggested participation of an acyl-CoA-independent enzyme, phospholipid:diacylglycerol acyltransferase (PDAT), in TAG synthesis. Our research focuses on identifying acyltransferases involved in oil accumulation in mesocarp tissues of avocado. Furthermore, in 'Hass' avocado, where 20-60 % of the fruit are phenotypically small, even under favorable conditions, we are interested in determining the association between oil accumulation and fruit size. To this extent, we quantified gene expression levels for DGAT 1 and 2 and PDAT and the rate of oil accumulation in developing mesocarp (oil-rich) and seed (non-oil rich) tissues of phenotypically 'small' and 'normal' fruits, using real-time PCR and gas chromatography, respectively. Candidate acyltransferase genes, highly expressed in mesocarp but not in seed, will be cloned and characterized. Understanding TAG synthesis in non-seed tissues will allow us to develop genetic tools necessary for generating bioenergy-rich crops.

Poster Abstracts

[P21] **The Effects of Nitrogen Supplementation on Phytohormone Metabolism in *Vitis vinifera* Using Multiple Reaction Monitoring LC-Mass Spectrometry**

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Nitrogen is an important nutrient that supports vine vegetative and reproductive growth. Phytohormones can act antagonistically or synergistically with one another and accumulate at specific times to mediate diverse processes including berry ripening and shoot elongation. Measuring multiple hormone concentrations simultaneously will help clarify our understanding of their role in grapevine development and their dynamics with respect to nitrogen supply. The objective of this study is to compare the metabolism of hormones in expanding shoots and berries of grapevines during the first phase of berry development in vines with or without nitrogen fertilizer. An extraction protocol and LC-MS-based analytical method were adapted for simultaneous analysis of 34 species of cytokinin, auxin, gibberellic acid, and abscisic acid analytes. We observed diverse responses in both shoots and berries to nitrogen treatment. The effects of nitrogen were specific to the hormone class (auxin and cytokinin). We also observed differences between the two populations of plants depending on the stage and tissue analyzed. Cytokinins were found to accumulate rapidly in the berries of fertilized plants during the early stages and at véraison while auxin accumulated in berries of fertilized plants only at véraison. In shoots, only auxin levels were found higher in fertilized plants during the early phase of the berry formation. Acknowledgments: The authors thank the Oregon Wine Board and Oregon State University for financial support.

[P22] **Isolation and Characterization of a Suspected Phytoalexin from *Acer rubrum* L.**

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Wilted red maple leaves are toxic to horses, causing death by oxidation of hemoglobin and inducing anemia. Gallic acid derivatives have been identified as the main oxidants present in the leaves. However, we have identified a previously unknown and suspected phytoalexin that is produced by wilting red maple leaves. The water-soluble compound fluoresces blue in certain TLC systems and was initially observed in leaf extracts only after wilting. Wilted leaves were collected, dried, and extracted with methanol. Leaf extracts were purified through repeated thin layer and column chromatography, and finally by preparative HPLC. The isolated fraction has been analyzed by spectrofluorimetry, high field NMR and HPLC-MS. Initial mass spectral data indicate a larger molecule, with a molecular mass greater than 1000 Da. Work is continuing to characterize the compound and assess its toxicity. This research may provide insight regarding the known toxicity of wilted red maple leaves to horses.

Poster Abstracts

[P23] **Carotenoid and C13-Norisoprenoid Composition during Pinot Noir Grape Development**

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Grape secondary metabolites such as C_{13}-norisoprenoids make important contribution to the wine varietal flavor characteristics. In this study, carotenoids and C_{13}-norisoprenoids composition during Pinot noir grape development were investigated. Grapes were sampled every week from pea size to harvest during the 2011–2012 season. Carotenoids were extracted by ethyl acetate and analyzed using HPLC. Free C_{13}-norisoprenoids were analyzed by headspace-solid-phase micro-extraction-gas chromatography–mass spectrometry (HS-SPME-GC-MS), and C_{13}-norisoprenoids potential was analyzed by HS-SPME-GC-MS after acid hydrolysis. Most carotenoids decreased during berry development although neochrome *b* and violaxanthin showed an accumulation before véraison. The concentration of most C13-norisoprenoids was low, but the potentials were much higher. Both the free volatile and potential aroma compounds were affected by grape maturity, and in different patterns. The β-damascenone potential increased dramatically after véraison, correlating with the decrease of some carotenoids, but total α-ionone and β-ionone had the opposite trend. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) and Vitispirane were presented in Pinot noir grape as bound form only, and their concentration decreased at the early stage of grape development but increased during the final stages of ripening.

[P24] **Integrative Study of Nutrition and Metabolomics on Flavonoid Metabolism of *Rubus idaeus***

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Optimization of mineral nutrition in growth media is a challenge of micropropagation of red raspberries. In an initial study modeling of mineral contents on Murashige and Skoog (MS) medium, mesos ($CaCl_2$, $MgSO_4$ and KH_2PO_4) salts affected the quality and growth of red raspberry shoots. For fine-tune of mineral contents for micropropagation of red raspberry, we examined the effects of extents of mesos on plant flavonoids because biosyntheses and metabolisms of flavonoids are regulated by various environmental factors. A red raspberry cultivar, Indian Summer (*Rubus idaeus*) was grown on the media containing 1x or 1.5x mesos. LC-MS-based metabolomics in conjunction with multivariate analysis was applied for determination of metabolic change in the plant flavonoids. The plant group on 1.5x mesos seems to have high contents of flavonoids and some glucuronidated or glycosylated forms of flavonoids. In the 1.5x mesos group, the plant shoots showed improved overall quality and multiplication and increased uptake of Ca, S, and Mg in the plant shoots detected using ICPs-OES. These results indicate correlation of flavonoid metabolism and concentration of mesos salts on the plant growth, which provides an aspect of optimization of the growth medium for micropropagation of red raspberries.

Poster Abstracts

[P25] **Collision-Induced Fragmentation Accurate Mass Spectrometric Analysis Methods to Rapidly Characterize Plant Extracts**

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The rapid advances in analytical chromatography equipment have made the reliable and reproducible measurement of a wide range of plant chemical components possible. Full chemical characterization of a given plant material is possible with the new mass spectrometers currently available. However, the software needed to automate the identification process is yet up to the job. New methods are being developed to enhance this software process. For phytochemicals, we have used the latest accurate mass LC-MS analysis to quickly characterize the potential chemical formulas for a series of unknown compounds in a variety of seed and leaf extracts using a standardized CID/HCD mass fragmentation experiment in which interpretation of daughter ions produced allowed for the identification of a variety of phytochemical aglycones and their various substituents. These results – coupled with DEPT NMR analysis as needed – resulted in the identification of a series of phytochemicals in camelina and soy seed extracts. Accurate LC-MS^N screening and characterization analyses can be coupled to more rapid non-destructive spectrophotometric analytical methods such as pulsed NMR and near infrared (NIR) spectrometry, which will allow for the rapid and non-destructive analysis of thousands of samples for a wide range of chemical composition parameters.

[P26] **Construction of A Taxane LC-MS And NMR Database**

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Members of the genus Taxus generally contain hundreds of taxane-type diterpenes. One of these diterpenes, paclitaxel (Taxol®, Bristol-Myers Squibb), is a valuable anti-cancer drug originally isolated from the bark of the Pacific Yew tree, *Taxus brevifolia*. The low yields of taxol from *T. brevifolia* prompted considerable efforts to develop a more sustainable and cost effective approach for the production of taxol. Such approaches included total synthesis of taxol, semi-synthesis from more abundant taxanes, production in *Taxus* cell cultures, and attempts to engineer the biosynthetic pathway of taxol into microorganisms. Despite extensive research efforts, many gaps still remain in understanding the biosynthetic pathway of taxol. These gaps are roadblocks to successful metabolic engineering of *Taxus* cell cultures or microbial hosts. Here we present the development of a web-based, searchable, accurate mass-time tag (AMT) library for LC-MS analysis of taxanes. This library combines high-resolution mass spectrometric data, fragmentation patterns, and relative retention times. Furthermore, we built a web-based NMR database for rapid de-replication of unknown taxane peaks. Examples for the utility of the combined LC-MS/NMR database for identifying taxanes in biological samples are presented.

Poster Abstracts

[P27] **Constructing the Information Pipeline to A Natural Compound Library by Integrated Screening Methods for Elucidating the Various Effects of Natural Product Extracts**

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The Nagoya Protocol on Access and Benefit-sharing is an innovative multilateral environmental agreement, which considerable implications for the rights of indigenous peoples and local communities, for research and commercial development activities in various sectors, as well as for food security, health, trade, oceans, and development cooperation. Therefore, National Institute of Biological Resources in Korea (NIBR) is preserving the native flora as valuable resources in future and also the medical effect. Especially, NIBR is collecting many wild plants with the exact collection sites, and making a library to find the application of the crude extracts for medicinal purposes. The crude extract preserved in NIBR would be useful for future medicinal or practical application; therefore, five different areas were selected for further characterization for the effectiveness simultaneously. The effects of the compounds were screened simultaneously on herpes virus suppression, proteasome inhibition, antibacterial effect for preventing cavity, cosmetic application and agrochemical for preventing plant diseases. Surprisingly, the crude compounds showed multiple functions for the usage. Based on the advantages of the approach, NIBR is developing a strategy to maximize the usage of the compounds by integrating many screening methods for various functions. The information will be used to construct information along with the real natural products library.

[P28] **Metabolomics of Down-Regulated (RNAi) Arogenate Dehydratase in *Populus tremula × P. alba***

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As part of our effort to develop poplar as a platform for biofuels and specialty chemicals, we have generated RNA interference transgenic lines for the silencing/down-regulation of different *arogenate dehydratase* (*ADT*) genes found in this important fast-growing biomass tree. Arogenate dehydratases catalyze conversion of arogenate into phenylalanine, and this amino acid is also a precursor for the biosynthesis of the important biopolymer lignin, several small secondary metabolites (e.g., flavonoids, coumarins, etc.) besides its use in protein formation. In *Arabidopsis thaliana*, the knock-out of *ADT* genes caused reductions in lignin content amongst other metabolic changes. We have employed an UPLC-ESI-qTOF to profile methanol/water extracts that contain important poplar metabolites previously described in the literature (e.g., flavonoids and phenolic glycosides). Data generated was analyzed with metabolomics specific programs that performed retention time alignment of all chromatographic runs and pairwise comparison with corresponding statistical analysis (XCMS) and mass spectrum profile annotation (CAMERA). For non-targeted metabolite analysis, differentially accumulated metabolites (with at least 2 fold change and P=0.05) were annotated based on standards, literature reports and general fragmentation rules. Within these metabolites, ADT down-regulated poplar was found to accumulate significant lower amounts of metabolites downstream from phenylalanine (e.g., flavonoids, phenylpropanoids) with a concomitant increased accumulation of compounds upstream of phenylalanine (e.g., salicortin, tremuloidin, benzoic acid).

Poster Abstracts

[P29] **Isolation and Structural Determination of Novel Anthocyanin and Pyranoanthocyanins from Staghorn Sumac (*Rhus typhina*) via UPLC-ESI-MS, ^{1}H, ^{13}C and 2D NMR Spectroscopy**

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Anthocyanins were isolated from the burgundy coloured fruits of Staghorn Sumac, a shrub native to eastern North America, and the structures of four novel compounds were determined by NMR spectroscopic methods as being 7-*O*-methyl-delphinidin-3-*O*-(2''galloyl)-β-D-galactopyranoside, 7-*O*-methyl-cyanidin-3-*O*-(2''galloyl)-β-D-galactopyranoside, 7-*O*-methyl-delphinidin-3-*O*-(2'''galloyl)- β-D-galactopyranoside-4-vinyl-catechol-3''-*O*-β-D-glucopyranoside, and 7-*O*-methyl-cyanidin-3-*O*-(2'''galloyl)- β-D-galactopyranoside-4-vinyl-catechol-3''-*O*-β-D-glucopyranoside. The identities of an additional six new anthocyanins, reported here for the first time, were assigned by analogy using UPLC-MS and spectrophotometric methods as being delphinidin-3-*O*-(2''galloyl)- β-D-galactopyranoside, 7-O-methyl-delphinidin-3-O-galactopyranoside, delphinidin-3-O-(2'''galloyl)- β-D-galactopyranoside-4-vinyl-catechol-3''-O-β-D-glucopyranoside, cyanidin-3-O-(2'''galloyl)- β-D-galactopyranoside-4-vinyl-catechol-3''-O-β-D-glucopyranoside, 7-O-methyl-delphinidin-3-O-β-D-galactopyranoside-4-vinyl-catechol-3''-O-β-D-glucopyranoside, and 7-O-methyl-cyanidin-3-O-β-D-galactopyranoside-4-vinyl-catechol-3'''-O-β-D-glucopyranoside. A putative biosynthetic pathway leading to the *in planta* production of these highly unusual 4-vinyl-catechol pyranoanthocyanins in sumac is discussed.

[P30] **Diversity of Metabolites from Cultured Mycobionts of Vietnamese Lichens**

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Lichens are symbiotic organisms of fungi (mycobionts) and photoautotrophic algal partners, namely, green algae and/or cyanobacteria. About 18,500 different lichen taxa have been described worldwide. Vietnam has a tropical monsoon climate that is favorable for diverse tropical lichens. Lichens produce many unique compounds, which are considered to have important biological and ecological functions, such as antimicrobial activity. Most of these metabolites are produced by the fungal partner, in symbiosis or in the aposymbiotic state. Cultures of isolated lichen mycobionts, however, often exhibit the ability under osmotically stressed conditions to produce substances that have never been detected in the lichenized state. From our interest in metabolic capability of lichen micobionts, we collected crustose lichens *Graphis vestitoides* (Fink) Staiger*, Graphis* sp.*, Trypethelium* sp.*,* and *Sarcographa tricosa* (Ach.) Müll. Arg. in Vietnam and cultivated their spore-derived mycobionts on conventional malt-yeast extract medium supplemented with 10% sucrose at 18˚C in the dark. Purification of their metabolites afforded diverse novel compounds: a 14-membered macrolide and an isocoumarin (*G. vestitoides)*; *a* 2*H,*5*H*-pyrano[3,2-c][1]benzopyran-10-carboxylic acid derivative (*Graphis* sp.); three sesquiterpenes (*S. tricosa*); a naphthoquinone and a phenalenone (*Trypethelium* sp.) together with known compounds. Their structures were determined by spectroscopic and chemical means.

Poster Abstracts

[P31] **Bioengineering the Mint Trichome System for Improving Essential Oil Yield and Composition**

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Mint species are cultivated widely for their essential oils. The most abundant constituent of spearmint oil is the monoterpene (-)-carvone. Peppermint, a hybrid between spearmint and watermint, produces an oil rich in (-)-menthone and (-)-menthol. The selfing of spearmint resulted in a progeny from which one line, termed Erospicata, was selected that contained high amounts of (-)-menthone but was essentially devoid of (-)-menthol. This variety, unlike peppermint, is resistant to Verticillium wilt. To study the causes for the differences in oil composition among cultivars, we performed next-generation transcriptome analyses with glandular trichomes, the specialized anatomical structures responsible for the biosynthesis of essential oils. Promoter sequences were obtained for the most highly expressed genes in these specialized cells from peppermint. These promoters were fused to the gene encoding menthone:menthol reductase, and constructs were transformed into Erospicata. In certain transgenic Erospicata lines (-)-menthone was converted to (-)-menthol. However, the isolated promoters were not equally effective in driving the expression of transgenes in a glandular trichome-specific fashion. Additionally, our RNA-seq. data revealed the main regions of sequence variations between active L6OH (limonene-6-hydroxylase) in spearmint and seemingly inactive L6OH-like proteins in peppermint/watermint which were in the substrate recognition sites and heme binding region.

[P32] **Mineral Nutrient Modification Results in Increased Antioxidants in Micropropagated Red Raspberries**

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Mineral nutrition directly involves with plant metabolism affecting growth and development. Micropropagated red raspberries grown on commonly used Murashige and Skoog medium (MS) display growth disorders, caused by non-optimum important minerals. An initial study modeling MS mineral components revealed that the quality of red raspberry shoot cultures was significantly affected by mesos ($CaCl_2$, $MgSO_4$ and KH_2PO_4) components of MS medium. This study investigated the effect of increased mesos on shoot mineral content and metabolism. The cultivar Indian Summer was grown on standard MS medium or on medium with increased mesos (1.5xMS). After 3 weeks, shoots were evaluated for quality, shoot multiplication, shoot length, mineral content and metabolic changes. Mineral content was determined by inductively-coupled plasma spectrometer (ICPs) and CNS-2000 Macro Analyzer. Metabolic changes were determined by LC-MS/MS (ESI). Shoots grown on increased mesos had improved quality (51.51%), shoot length (113.3%) and multiplication (40%) compared to shoots grown on MS medium. Increased mesos altered mineral uptake resulting in > 40% increases in Ca, S and Mg in shoots, and Fe, Cu and Zn decreased slightly (<10%). Metabolic analysis indicated that increased mesos shoots had increased proanthocyanidin (epi-catechin), quercetin, and ellagic acid derivatives functioning as antioxidant molecules for scavenging reactive oxygen species.

Poster Abstracts

[P33] **Anti-Proliferative Effects of Synthetic 8-*O*-4′ Neolignans**

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Lignans and neolignans have a broad range of biological effects including antifungal, antileishmanial, and anti-PAF activities. In contrast to the lignans, few studies have reported biological activity of 8-*O*-4′ neolignans on human health, including studies of virolin and surinamensin (9, 9′-deoxy-8-*O*-4′ neolignans). Recently, Katayama *et al*. found that incubation of sinapyl alcohol and coniferyl alcohol with enzyme preparation from young shoots of *Eucommia ulmoides* gave optically active *erythro*- and *threo*-syringylglycerol-8-*O*-4′-(sinapyl alcohol) ethers (SGSE) and guaiacylglycerol-8-*O*-4′-(coniferyl alcohol) ethers (GGCE), respectively. Very recently, Alam *et al*. has been clarified the absolute configuration of four stereoisomers of SGSEs. Therefore, we have interest to investigate the anti-cancer activity of the two 8-*O*-4′ neolignans. The cancer preventive activities of SGSE and GGCE were investigated using different cancer cell lines [human breast adenocarcinoma (MCF-7), human liver hepatoblastoma (HepG-2) and human colon adenocarcinoma (HT-29)]. Their antiproliferative effects on the three cancer cell lines were investigated using the microculture tetrazolium (MTT) assay. TUNEL assay was performed with the Apoptosis Detection System, Fluorescein Kit. Among the three cell lines, inhibitory effect of SGSE on HT-29 and HepG-2 & GGCE on MCF-7, cells proliferation was dose- and incubation time-dependent was observed even at 50 µmol/L concentration.

[P34] **Evaluation of Hypotensive Effect of *Acorus calamus* through Inhibition of Angiotensin Converting Enzyme (ACE)**

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In Ayurvedic medicine, *Acorus calamus* belongs to the family Araceae is an important herb and is valued as a "rejuvenator" for the brain and nervous system, also as a medication for hypertension. The present study was aimed to investigate the hypotensive effect of standardized extract of *A. calamus*. The ACE inhibition activity was investigated by UV method. In ACE inhibition method, the test sample was allowed to react with substrate hippuryl-L-histidyl-L-leucine and resultant hippuric acid was analyzed by UV method. A HPTLC method was developed to standardize and quantify major bioactive components α-asarone, by using the solvent system toluene: ethyl acetate (8:3 v/v). Methanolic extract and ethyl acetate fractions showed the maximum inhibitory activity in concentration-dependent manner having the IC_{50} value 80.37± 1.48 and 113.21 ± 3.51 µg/ml respectively. The Rf value of α-asarone in the plant extract was found to be 0.71. The amount of α-asarone present in the test extract was found to be 2.13 % (w/w). These results exhibited that *A. calamus* extract showed concentration-dependent ACE inhibitory activity. The present work demonstrated that *A. calamus* has a potent hypotensive effect.

Poster Abstracts

[P35] *Butea frondosa*, a Rho Kinase 2 and Phosphodiesterase 5 Enzyme Inhibitor, Increases Sexual Function in Young And Aged Rats

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The study was designed to evaluate the effect of *Butea frondosa*, a ROCK-II and PDE5 inhibitor, on the sexual behavior of young (5 months) and aged (24 months) male Wistar rats in presence of female rats and compare the effect with the rats treated with vehicle and sildenafil, a well known medicine for management of erectile dysfunction (ED).

Dosing with 100 mg/kg body weight of Butea extract for 28 days increased sexual behavior of young rats significantly ($p < 0.05$) that is evident by decrease in mount latency, intromission latency and post ejaculatory interval and increase in mount frequency, intromission frequency and ejaculation latency. In aged rats with reduced sexual function, treatment with extract increased sexual function significantly ($p < 0.05$). The treatment increased smooth muscle level and decreased collagen level in the penile tissue of rats when compared with control group receiving vehicle.

Butea frondosa increased sexual function in young and aged rat and might be helpful in the management of ED in human.

[P36] *Cinnamomum cassia*, an Arginase and Rho Kinase Inhibitor Increases Sexual Function in Male Rats

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Methanol extract of *Cinnamomum cassia* was found to inhibit Arginase and Rho Kinase 2, enzymes implicated in the development of Erectile Dysfunction, a male sexual dysfunction. This study was designed to validate the effect of *Cinnamomum cassia* extract on sexual behavior of male rats. Sexually active male rats were divided into three groups containing 6 rats each. Group I, treated with vehicle; Group II, treated with 100 mg/kg of methanol extract of *Cinnamomum cassia*; Group III, treated with 5 mg/kg of sildenafil citrate. Sexual behavior of male rats in presence of estrous female rats, of group II and group III were compared with group I after 28 days of treatment. The significant difference between the mean value of control and experimental groups for *in vivo* study was determined by one-way analysis of variance (*ANOVA*) with Dunnet's test using statistical software SPSS version 17. P value < 0.05 was considered as statistically significant. Data is presented as mean ± standard error of mean (SEM). Treatment with methanol extract of *Cinnamomum cassia* and sildenafil significantly increased the sexual behavior of male rats. Both the treatments decreased mount latency, intromission latency and post ejaculatory interval whereas increased mount frequency, intromission frequency and ejaculation latency. The methanol extract of *Cinnamomum cassia* increases sexual function in male rats and may be helpful in the management of erectile dysfunction.

Poster Abstracts

[P37] **Induction of Classic and Alternate Cell Death Pathways by the Cyanobacterial Metabolite Coibamide A**

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Coibamide A is an unusual *N*-methyl-stabilized depsipeptide that was isolated from a marine cyanobacterium collected from the Coiba National Park, Panama. Previous testing of coibamide A in the National Cancer Institute *in vitro* 60 cancer cell line panel revealed a potent anti-proliferative response and "COMPARE-negative" profile indicative of a unique mechanism of action. We report that coibamide A induces biochemically and morphologically distinct forms of cell death according to cell type. Human SF-295 glioblastoma cells, MDA-MB-231 breast cancer cells and mouse embryonic fibroblasts (MEFs) showed caspase-3 activation and evidence of apoptotic cell death. In contrast, cell death in human U87-MG glioblastoma cells was characterized by extensive cytoplasmic vacuolization and a lack of apoptotic features. Cell death was attenuated, but still triggered, in Apaf-1-null MEFs lacking a functional mitochondria-mediated apoptotic pathway. With increasing recognition of alternate cell death pathways, the inherent ability of a small molecule to induce more than one mode of cell death may be a particularly useful pharmacological property in cancer therapeutics. As a historically rich source of cytotoxins, cyanobacterial metabolites may hold great potential as molecular tools for the study of alternate cell death pathways in apoptotic-resistant cancers.

[P38] **Morphoanatomical and Physicochemical Standardization of *Drynaria Quercifolia* (L.) J. Smith**

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Drynaria quercifolia (L.) J. Smith (Polypodiaceae), a parasitic fern, is well-known crude drug used by tribal communities of Tamil Nadu and Kerala in India for diverse ailments like jaundice, typhoid fever, rheumatic pain, dyspepsia, etc. The present study was conducted to establish the detailed pharmacognostical parameters for the histological and physico-chemical standardization of *D. quercifolia*. The physico-chemical parameters were performed as per standard procedures of WHO guidelines on quality control methods for medicinal plant materials. Morphoanatomy, venation pattern, trichomes distribution, stomatal morphology, paradermal sections and powder microscopical examination of leaf were studied by employing bright field light for the normal observations and polarized light for the study of crystals, starch grains, and lignified cells. Microscopical descriptions of tissues were supplemented with photographs of different magnifications employing Motic DMBA 300 microscopic unit. Additionally, reliable quantitative HPTLC and HPLC methods were successfully developed and validated as per ICH guidelines for the determination of marker phytochemical (naringin; a flavanone glycoside) in the plant material. The physicochemical, anatomical parameters and HPTLC & HPLC standardization may be proposed as parameters to establish the authenticity of *D. quercifolia* and further assist in standardization of plant *viz*., quality, purity, and sample identification.

Poster Abstracts

[P39] **Synergistic Nrf2/Antioxidant Response Element Pathway Activation by Sulforaphane and Zerumbone through Addition of Redox Catalyst**

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Activation of the Nrf2 transcription factor by phytochemicals is promising for prevention of numerous pathological conditions, including cancer and cardiovascular disease. Nrf2 upregulates cytoprotective enzymes by binding an antioxidant response element (ARE) upstream of their respective genes. Phytochemicals that activate Nrf2 overwhelmingly have an electrophilic moiety important for this activation. Interestingly, reactive oxygen species (ROS) also contribute to Nrf2/ARE pathway activation by phytochemicals, including celastrol ("Thunder-of-God" vine). For example, diphenolic compounds generate ROS via redox-cycling. Here, using $CuCl_2$ to catalyze this cycling, we explore the contribution of ROS to phytochemical Nrf2/ARE activation. We find Nrf2/ARE activation by celastrol in Hepa1c1c7 cells is markedly increased with $CuCl_2$, which hints that ROS synergistically contribute to Nrf2/ARE activation. We hypothesized that, as a general phenomenon, ROS from redox-cycling could synergistically increase Nrf2/ARE activation. Thus, two phytochemicals structurally incapable of redox-cycling, sulforaphane (cruciferous vegetables) and zerumbone (tropical ginger), were evaluated. While a low level of ARE activation was observed in the presence of $CuCl_2$ alone, ARE activation by sulforaphane or zerumbone was substantially enhanced by $CuCl_2$, presumably by ROS generated through redox cycling of media components. This effect was more than additive, suggesting synergistic activation of the ARE by ROS and electrophilic compounds.

[P40] **Measurement of the Efficacy of the Extracts of Natural Products on the Proteasome Activity**

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Proteasomes located at nucleus and cytoplasm is the major machinery for degrading unneeded or damaged protein by proteolysis. In mammals, the proteasome is the cytosolic 26S proteasome, which contains one 20S protein subunit and two 19S regulatory cap subunits. Recently, the proteasome has been validated as a therapeutic target, with proteasome inhibitors showing particular efficacy in the treatment of multiple myeloma. Therefore, there have been increasing numbers of studies to verify the effective natural compounds to develop a new anticancer drug. In our experiment, we purchased a commercial 20S proteasome activity assay kit, and tested the newly extracted 16 natural crude compounds. The Multiple Myeloma, interestingly total 10 natural compounds decreased the proteasomic activities. Further fractions of the natural compounds also exerted proteasome inhibiting activities, therefore, all our data strongly indicate that we are in success of identifying pure compounds inhibiting the proteasome activity. Further experiments are requested to identify the pure compounds by applying the techniques of purification, proteasome kit assay, and the real cancer cell culture.

Poster Abstracts

[P41] **Activiral Activites of the Natural Products against Gamma Herpesviruses**

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Human gammaherpesviruses, including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), are common opportunistic pathogens and are associated with a number of tumors. EBV and KSHV establish life-long persistent infection in the host and can be reactivated when the host's immune system becomes compromised. There is no vaccine available against gammaherpesviruses and the efficacy of antiviral treatments using traditional nucleoside analogs is limited. Therefore, there are emerging needs for the discovery of a new anti-herpesviral drug. Here, we tested antiviral activities of a dozen of selected natural plant extracts against gammaherpesviruses using marine gammaherpesvirus 68 (MHV-68) and found that 2 crude extracts effectively inhibit viral lytic replication. Furthermore, these extracts were also effective in inhibiting EBV and KSHV, confirming their antiviral activities. Antiviral activities of additional fractions of the extracts were tested and shown to be highly effective against KSHV and EBV. Among them, hexane fractions exhibited a strong inhibitory effect without any cytotoxicity in a low dose. Although further experiments are needed to investigate detailed mechanisms by which the hexane fractions inhibit gammaherpesvirus infection, we expect that our results will open up a new venue to develop a novel anti-gammaherpesviral drug.

[P42] **Artemisinin Decreases PSA Induced by Tgfβ1+DHEA in Prostate Cancer Epithelial and Stromal Cell Co-Culture**

Renee Y. Choi, Jade Stefano, Cynthia A. Wenner, Mark R. Martzen

Artemisinin, a sesquiterpene lactone in *Artemisia annua,* has been shown to have anti-cancer properties through heme-catalyzed excess of reactive oxygen species induced apoptosis. In previous studies, addition of TGF-β1 (transforming growth factor beta-1) to DHEA (dehydroepiandrosterone)-treated prostate stromal-epithelial cell co-cultures reproduced a reactive stromal microenvironment and significantly increased the androgenicity of both cell types, as measured by increased PSA and testosterone production. This study aimed to investigate if artemisinin can reverse this effect. LAPC-4 prostate cancer epithelial cells were grown in co-culture with 6S prostate stromal cells and treated with DHEA + TGFβ1 +/- varying doses of artemisinin. The effects of artemisinin on PSA and testosterone expressions were determined using real time PCR and ELISA. Artemisinin treatment led to a dose-dependent decrease in DHEA+TGFβ1 induced PSA protein and gene expression and dose-dependent inhibition of testosterone metabolism. In this *in-vitro* model of endocrine (DHEA)-immune (TGF-β1) –paracrine (co-culture) interactions in the prostate, artemisinin appears to be effective in decreasing PSA and testosterone production induced by DHEA + TGFβ1. Further research to assess the potential clinical benefit of *Artemisia annua* extracts for prostate cancer is warranted.

Poster Abstracts

[P43] **Probing the Human FXR LBD – Prenylflavonoid Interactions by Hydrogen/Deuterium Exchange Mass Spectrometry**

Liping Yang[1], David Broderick[1], Yuan Jiang[2], Yan Campbell[3,5], Adrian F. Gombart[3,5], Jan F. Stevens[3,4], Victor Hsu[5] and Claudia S. Maier[1]

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Xanthohumol (XN), the principal prenylflavonoid in hops (*Humulus lupulus L.*), has been linked to modulate lipid metabolism by at least partly acting on the farnesoid–X receptor (FXR). In this study, we tested the hypothesis that XN and related prenylflavonoids, isoxanthohumol (IX) and 8-prenylnaringenin (8-PN), may function as selective bile acid receptor modulators (SBARMs). Our biological results shown that XN induced a) FXR target gene BSEP promoter activity in transfected cells and b) the endogenous expression of FXR target genes in biliary carcinoma cell lines. To obtain the molecular description of the interaction of prenylflavonoids with FXR, we studied the interaction of the prenylflavonoids with the ligand binding domain (LBD) of FXR by hydrogen/deuterium exchange (HDX) Mass Spectrometry (MS). HDX-MS data indicated that XN, IX, 8-PN indeed interact with the FXR-LBD and induced significant protection in the following regions: 288-298 (helix 3), 320-336 (helix 5), and 368-375 (helix 7) as indicated by the low deuterium levels. Most intriguingly was the finding that these prenylflavonoids seem to have a stronger conformational stabilizing on the region 386-396 (the C-terminal part of helix 8), compared to CDCA, the endogenous ligand on FXR. In conclusion, our biochemical and HDX-MS studies supported above hypothesis.

[P44] **Characterization of Xanthohumol Conjugates Using High Resolution Mass Spectrometry**

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Emerging evidence of the therapeutic potential of flavonoids for several chronic diseases (i.e. cardiovascular disease, cancer, obesity and type II diabetes) has led to increased interest in flavonoid metabolism, specifically the role of glucuronide and sulfate conjugation. In particular, xanthohumol (XN), a prenylflavonoid derived from hops, has emerged as a flavonoid of note since recent findings have shown consumption of XN lowers body weight and plasma glucose in obese rats. XN glucuronides and sulfates were characterized using high resolution mass spectrometry through the use of a quadrupole-time of flight mass spectrometer (ABSCIEX TripleTOF 5600, AB Sciex, Foster City, CA). Plasma samples used for characterization of XN conjugates were obtained from a clinical pharmacokinetic study of XN in healthy men and women. Although XN glucuronides were observed in plasma samples, XN sulfates were not detected; therefore an XN monosulfate standard was synthesized for characterization. For XN glucuronide, ($C_{27}H_{30}O_{11}$), $[M+H]^+$ was calculated for 531.1861 and observed at 531.1873. For XN monosulfate ($C_{21}H_{22}O_8S$), $[M+H]^+$ was calculated for 435.1114 and observed at 435.1104. Analyzing XN sulfates in positive ion mode enabled us to observe a distinct fragmentation pattern which showed attachment of the sulfate group to the A ring of the flavonoid.

Poster Abstracts

[P45] **Chemo-Enzymatic Synthesis of Physiologically Modified Avenanthramides**

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Avenanthramides are a group of phenolic alkaloids produced, among food crops, uniquely by oats. These metabolites function as phytoalexins in vegetative tissue and they are produced in the grain where their function is unknown. *In vitro* the avenanthramides inhibit the activation of nuclear factor kappa beta (NFκB). Thus, avenanthramides also demonstrate anti-inflammatory properties and are the active component in oat-meal salves used in folk medicine to relieve symptoms of skin irritation. They might also retard development of atherosclerosis and other inflammatory diseases. Like many phytonutrients, avenanthramides undergo hepatic modification in mammals. Plasma samples from rats gavaged with avenanthramides 2c, 2f and 2p show much higher avenanthramide content when treated with a mix of glucuronidase/sulfonase. The experiments reported here were undertaken to determine the extent to which the avenanthramides are modified by either glucuronidation or sulfonation and to determine the chemical structures of these modified compounds. Thus the synthesis of the three principal avenanthramides, 2c, 2f and 2p is described as well as the effect of treating these pure synthetic avenanthramides with rat liver extracts to yield the glucuronidated and the sulfonated forms. Recombinant human glucuronate- and sulfotransferases were also employed to modify these natural products.

[P46] **Anti-Inflammatory Effects of Meadowfoam Isothiocyanates in Human Keratinocytes**

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Keratinocytes have been used as in vitro models to examine the protective effects of phytochemicals against skin inflammation induced by chemical irritants, cytokines, and UV exposure. Flavonoids, triterpenes, curcumin, and isothiocyanates (ITCs) have been shown to suppress chemically-induced and UVB-induced inflammation in the skin. The aim of this study was to examine the anti-inflammatory activity of meadowfoam isothiocyanates in TNF-α/IFN-γ-treated human HACAT keratinocytes, an in vitro model of skin inflammation. Treatment of HACAT cells with a combination of TNF-α and IFN-γ (10 ng/ml each) produced a marked increase in IL-6 and MCP-1 levels measured by ELISA in the culture media. However, MCP-1 levels were significantly decreased by co-treatment with the meadowfoam compounds, benzyl isothiocyanate (BITC), 3-methoxybenzyl isothiocyanate (MBITC), and 3-methoxyphenyl-acetonitrile (MPACN) in a dose-dependent manner in the low micromolar range. The cytokine-induced production of IL-6 was not altered by the meadowfoam coumpounds. MBITC was more potent than BITC, and MPACN was the least effective in suppressing the induction of MCP-1 in HACAT cells treated with TNF-α/IFN-γ. These findings suggest that the ITCs found in meadowfoam have specific anti-inflammatory targets (e.g., MCP-1) in keratinocytes and that replacing the isothiocyanate group with a nitrile group results in reduced anti-inflammatory activity.

Poster Abstracts

[P47] **Bioavailability and Biological Functions of Sulforaphane in Humans**

Atwell, L.L.[1,2], Clarke, J.D.[1], Hsu, A.[1], Bella, D.[1], Pereira, C.B.[3], Stevens, J.F.[2,4], Dashwood, R.H.[2,5], Williams, D.E.[2,5], Ho, E.[1,2].

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Sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables, has many potential health benefits. Little is known regarding SFN bioavailability from dietary sources and its biological functions in humans. Thus, selecting appropriate dietary sources and outcome measures for clinical trials remains challenging. Crucifers contain glucoraphanin (GFN), which is hydrolyzed by myrosinase to yield SFN. Myrosinase is present within plants. We previously demonstrated a 7-fold decrease in SFN bioavailability from GFN supplements (lacking myrosinase) than from fresh broccoli sprouts, suggesting the lack of myrosinase impacts SFN bioavailability. Study goals were to evaluate SFN bioavailability from a SFN-rich, broccoli sprout extract (BSE) supplement and fresh broccoli sprouts and to assess biological effects of consuming dietary SFN in humans. More SFN was absorbed from myrosinase-treated BSEs compared to myrosinase-inactivated GFN supplements, but SFN bioavailability from BSEs was 3 times lower than from sprouts. Alternatively, molecular and epigenetic analyses revealed similar impacts of BSEs and sprouts on hemeoxygenase-1 protein and HDAC activity. High-throughput metabolomic analysis revealed changes in metabolic profiles with SFN consumption with some effects dependent on the source consumed. Overall, this research will inform strategies for SFN supplement use in clinical trials and increase understanding how consuming dietary SFN can promote optimal health.
Funding: R01CA122906 & P01CA090890

[P48] **Matrix Metalloproteinase, Hyaluronidase and Elastase Inhibitory Potential of Standardized *Curcuma longa* L. Extract**

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Traditionally *Curcuma longa* L. belongs to the family Zingiberaceae is used for beatifying the skin and related problems. The objective of this study was to screen the anti wrinkle activity of standardized extract of *C. longa* through hyaluronidase, elastase and matrix metalloproteinase inhibition assays. Powdered rhizome of *C. longa* was extracted with methanol and fractionated with n-butanol, ethyl acetate and aqueous solvents. Extract and fractions at the concentration of 1.56-50 µg/ml together with standard ursolic acid were used to screen the *in-vitro* enzymetic assay. Methanolic extract and n-butanol fraction were chosen for further HPTLC study with reference to Curcumin as a standard. The extract exhibited the strongest anti-hyaluronidase and anti-elastase activity with 50% inhibition (IC_{50}) at 20.35±0.47 and 14.45±0.29 µg/ml respectively. The n-butanol fraction was found to have the most effective amongst all fractions, with IC_{50} at 32.00±0.38 and 35.22±0.33 µg/ml respectively and good MMP-1 inhibition. The content of Curcumin in extract and n-butanol fraction was found to be 31.81 and 40.54% w/w respectively. It can be concluded that Curcumin may be responsible compound for the enzyme inhibition activity and suggest that *C. longa* methanolic extract and n-butanol fraction have potential as an anti-wrinkle agent for use in cosmetic products.

[P49] **Activities of V*ernonia amygdalina* on the Fate of Metformin in Rabbits**

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The use of herbal preparations in parallel with conventional drugs is on the increase, thus a great concern has arisen. The leaf of *Vernonia amygdalina* (VA) is one of the most widely used herbal remedies in Nigeria by diabetic patient with an encouraging safety profile. It has been claimed to increase the efficacy of metformin. This study investigated the effect of the extract of VA on the pharmacokinetics of metformin (MET) in rabbits. The Pharmacokinetics of MET (100 mg/kg b. wt., o.r.) was studied in rabbits with or without co-administration of aqueous extract of VA (500 mg/kg b.wt., o.r.). Blood samples were drawn at 0 h and at 0.5 to 24 h post dosing from the retro orbital plexus into sodium heparin bottles and centrifuged to separate plasma. The animals were allowed food after 4 h post dose. The plasma samples were assayed for MET levels using validated HPLC method. SPSS 15 statistical package with P set at 0.05 was used to analyze data. Following VA co-administration, MET showed significantly higher K_a, F, C_{max}, $AUC_{0-\infty}$ and T_{max} than the control group. No significant difference was seen in $t_{1/2el}$ between the groups. These results indicated that VA enhanced absorption constant K_a thus increasing C_{max}. Therefore patients on MET therapy should consume VA with caution as life threatening interaction may occur.

[P50] **Mixture of *Artemisia iwayomogi* and *Curcuma longa* Exerts Anti-Hyperlipidemic Effects**

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Both *Artemisia iwayomogi* and *Curcuma longa* have been widely used in traditional Korean medicine. We evaluate anti-hyperlipidemic effects of extract mixture of Artemisia iwayomogi and Curcuma longa (ACE) using high fat diet (HFD)-induced hyperlipidemia mice model. Mice were fed with HFD with/without ACE (50, 100 and 200 mg/kg, mixed with HFD). Ten-week of HFD considerably altered the serum lipid profiles including total cholesterol (TC), low density lipoprotein (LDL), triglyceride, TC/HDL ratio, glucose, and body weight. However, ACE treatment significantly ameliorated these alterations compared with control group ($P < 0.01$). The hepatic antioxidant enzymes such as superoxide dismutase and catalase were reduced significantly in the HFD fed group, and these levels were significantly increased ($P < 0.01$) by the administration of ACE (100 and 200 mg/kg). The malondialdehyde of serum and liver was significantly increased in HFD fed group ($P < 0.01$) and was significantly reduced by ACE ($P < 0.05$). HFD feeding remarkably up-regulated the hepatic gene expressions of fatty acid synthase (FAS), peroxisome proliferator-activated receptor (PPAR)-γ, and sterol regulatory element-binding protein (SREBP)-1c while as PPAR-α was down-regulated respectively. Those results strongly suggest the anti-hyperlipidemic properties of mixture of *Artemisia iwayomogi* and *Curcuma longa.*

Poster Abstracts

[P51] **Bioactive Polyketides from Cultured Lichen Mycobionts of *Pyrenula* sp.**

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Lichens, symbiotic associations between fungi (mycobionts) and photoautotrophic algae or cyanobacteria (photobionts), produce characteristic secondary metabolites, namely, lichen substances, some of which are potentially useful and biologically active compounds. Most of these metabolites are produced by the fungus, in symbiosis or in the aposymbiotic state. However, previous studies demonstrated that the secondary metabolites in cultured mycobionts are often structurally unique and similar to fungal metabolites. These findings suggested that laboratory cultures of lichen mycobionts could provide a potential source of novel secondary metabolites. In continuing our chemical studies on the cultured lichen mycobionts, we have cultivated the spore-derived mycobionts of *Pyrenula* sp. collected in Vietnam and isolated from their cultures eight new compounds along with chrysophanol, emodin and 1,5,8-trihydroxy-3-methylxanthone. The novel compounds were polyketides closely related to cladobotric acids A and C isolated from fermentation broth of fungus *Cladobotryum* species. Their structures were elucidated by spectroscopic and chemical means. Administration of sodium [1-^{13}C]-acetate and sodium [1,2-$^{13}C_2$]-acetate showed that the assembly pattern of acetate units in their biosynthesis was similar to that of cladobotric acids A and C. The inhibitory activities of new polyketides against mammalian DNA polymerases were also evaluated.

[P52] **Evaluation of the Cytotoxic Activity of *Rosmarinus officinalis***

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Rosmarinus officinalis, commonly known as Rosemary, is a common household perennial shrub, best known for its strongly aromatic, needle-like evergreen leaves. The plant is widely used in traditional medicine for its tonic, carminative, and antispasmodic properties. The essential oil extracted from the seeds and leaves of Rosemary have been demonstrated to possess antioxidant, antibacterial, antifungal, and antiviral activities. In the present study, several compounds derived from a Rosemary methanolic extract were 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 extracts afforded several fractions and compounds which showed significant reductions in cell viability were observed. The major compounds, rosmarinic and ursolic acids, were found to be significantly active against both lung and pancreatic cell lines with the strongest inhibitory effect being observed against pancreatic cell lines. The results of these studies including chemical and physical data for the active compounds, cytotoxic activity and the bioassay-guided fractionation of the methanolic extracts will be presented. These findings highlight the importance of the use of herbs and spices as potential lead sources for prevention and treatment of cancer.

Poster Abstracts

[P53] **Characterizing the Active Compounds of *Centella asiatica***

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Centella asiatica has been used for centuries in Ayurvedic medicine to enhance cognition. Previous research in our lab has shown that the water extract of *Centella asiatica* (CAW) reverses β-amyloid (Aβ)-induced cognitive deficits in a transgenic model of Alzheimer's Disease and prevents intracellular Aβ toxicity *in vitr,* yet the active compounds in CAW remain unknown. The biological activities of *Centella asiatica* have been attributed to the triterpene compounds in the plant: asiatic acid, madecassic acid, asiaticoside and madecassoside. Using thin layer chromatography coupled to high-resolution mass spectrometry (TLC-HRMS) we determined that asiatic and madecassic acid were absent from CAW, although low levels of asiaticoside and madicassicoside were present. However, the triterpenes showed no protective activity in the in vitro model of intracellular Aβ toxicity, suggesting they are not the active compounds in CAW. We also identified several caffeoylquinic acids in CAW using high performance liquid chromatography coupled to HRMS. These compounds are reported to be neuroprotective against exogenous Aβ administration *in vitro*. Studies are currently underway to determine whether these compounds are responsible for CAW's protective effects against intracellular Aβ toxicity. The role of these compounds in the biological activity of *Centella asiatica* has not been widely studied.

[P54] **Polyphenols from Alaska Cedar Inner Bark with Antioxidant Properties**

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Alaska Cedar *(Chamaecyparis nootkatensis*), also known as yellow cedar or Nootka cypress, is an important timber and ecological species of the coastal Pacific Northwest. The bark, an underutilized forest by-product, has received relatively little attention. The condensed tannins from Alaska Cedar inner bark were investigated for their structure and antioxidant activity. The methanol extract purified by column chromatography (Sephadex LH-20) and analyzed by ^{13}C NMR and MALDI-TOF MS. Antioxidant activities were measured using 1,1'-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging, ferric reducing/antioxidant power (FRAP), and β-carotene-linoleic acid model system (β-CLAMS) assays. Results showed that the condensed tannins consisted of both homogeneous and heterogeneous oligomers of procyanidin (catechin/epicatechin) and prodelphinidin (gallocatechin/epigallocatechin) flavan-3-ol units as oligomers from trimmers to heptamers with dominant interflavan linkages of the B-type as it is most common in proanthocyanidins. These polyphenols showed significant antioxidant activity as the median inhibition capacity IC_{50} is comparable to the catechin control response. Alaska Cedar inner bark oligomers show high antioxidant capacity, evaluated by both methods based on electron transfer mechanisms and hydrogen atom transfer reactions. This bark may be potentially considered as a new source of natural antioxidants for nutraceutical ingredients.

Poster Abstracts

[P55] **Flavonoids from Leaves and Stem of *Cedrela odorata* L.**

Martha Rosales-Castro[1]*, Samantha Mariscal-Lucero[1], Vicente Sánchez-Monsalvo[2], Jeffrey Morré[3], and Joseph J. Karchesy[3]

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Cedrela odorata L. is a highly valued forest species, because of the high quality of its wood. However plantations of this species are difficult to establish because of severe attacks by the shoot borer *Hypsipyla grandella*. Samples were collected from plantations establish in southeastern Mexico, and analyzed for their flavonoids. Methanol extracts were obtained from leaves and stems, and partitioned with ethyl acetate. The ethyl acetate fraction was purified by column chromatography (Sephadex LH-20) eluted with methanol. Fractions were obtained and analyzed using HPLC-DAD and LC-MS. Flavan-3-ols such as catechin were found in the stems. In leaves, compound 1 with UV bands at 266 and 350 nm, [M+H]+ m/z 595, MS/MS (m/z) 449 and 286, corresponding to luteolin/kaempferol-di glucoside and compound 2 with UV bands at 256 and 354 nm, [M+H]+ m/z 611, MS/MS (m/z) 465 and 303, corresponding to quercetin-di-glycoside (rutin) were found. Some flavonoids such as quercetin 3-rutinoside and luteolin 7-0-glucoside have been reported as feeding attractants to insects and some as feeding deterrents to larvae. Some may also act as a contact oviposition stimulant to some butterflies for laying eggs on leaves. The flavonoids found in this study need further investigation in relation to the shoot borer.

[P56] **A Molecular Switch Reprograms Phenylpropanoid Metabolism in Pathogen Defense**

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The hydroxycinnamate/monolignol branch of the phenylpropanoid pathway is responsible for the production of the cell wall polymer lignin as well as antimicrobial small molecules such as coumarins and lignans, and is dramatically reprogrammed in response to pathogens. However, the molecular switches responsible and the resulting diversity in defensive phenylpropanoid metabolism are unknown. Using a functional genomics approach and pathogen-associated molecular patterns as elicitors, we have identified a novel transcription factor that is necessary for the production of defensive lignin in non-vascular tissue in response to pathogen perception, as opposed to infection. Genome-wide transcriptional profiling and transactivation assays also revealed that this transcription factor directly activates genes involved in the biosynthesis of coumarins and lignans. In addition, comparative HPLC-fluorescence-MS profiling of genetic knockouts and overexpression transgenics found that ferulate esters were the predominant precursor to defensive metabolites. Finally, phylogenetic analysis suggests that this transcription factor is highly conserved among vascular plants, and is present in both monocots and dicots. Transcription factors have been shown to function as metabolic switches in response to stressors, and our transcription factor could potentially be used to engineer plants for increased production of agriculturally or pharmalogically relevant defensive phenylpropanoids through a general mechanism, producing species-specific metabolites.

Poster Abstracts

[P57] **Meadowfoam Seed Meal as a Soil Amendment with Pre-Emergent Herbicidal Activity**

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Meadowfoam (*Limnanthes alba*) is an oilseed crop grown in western Oregon. After oil extraction, meadowfoam seed meal (MSM) retains 2 to 4% glucosinolate, glucolimnanthin (GLN). Myrosinase enzymes present in soil microbes and meadowfoam seeds can convert GLN to glucosinolate breakdown products (GBPs), which have herbicidal activity and potential use as bioherbicides. Studies were conducted to determine the fate and persistance of GLN and GBPs in soil and to determine their effects on the germination of lettuce. When MSM was incubated in soil at 3% with ground meadowfoam seeds, inhibition of lettuce germination was observed for six days. GLN was rapidly converted into 3-methoxybenzyl isothiocyanate (isothiocyanate), within 24 hours. By day six, the isothiocyanate was degraded. 3-Methoxyphenylacetonitrile (nitrile) persisted for at least one week. 3-Methoxyphenylacetic acid (MPAA), a previously unknown metabolite of GLN, appeared at day six. Its identity was confirmed by LC-UV and high resolution LC-MS/MS comparisons with an authentic standard. MPAA was derived from the nitrile because incubations of the nitrile in soil produced MPAA. All GBPs inhibited lettuce germination. Isothiocyanate was about 10 times more effective than nitrile and MPAA. Biodegradation of GLN and GBPs in the soil suggests the potential of MSM as a pre-emergence herbicide.

[P58] **Meadowfoam (*Limnanthes Alba*) Seed Meal as a Soil Amendment with Fungicidal and Nematicidal Activity**

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Meadowfoam (*Limnanthes alba*) is a commercial oilseed crop grown in Oregon. The seed meal remaining after oil extraction is rich in the glucosinolate glucolimnanthin, which is converted to 3-methoxybenzyl isothiocyanate (ITC) and 3-methoxyphenylacetonitrile (nitrile) in the presence of the enzyme myrosinase from unprocessed seed. Previously, we demonstrated that ITC and nitrile are toxic to the plant-pathogenic oomycete *Pythium irregulare* and the plant-parasitic nematode *Meloidogyne hapla*. In this study, we evaluated factors that influence the implementation of meadowfoam seed meal into agricultural production systems for disease control. Rate-finding experiments demonstrated that a soil amendment rate of 1% seed meal formulated with 1% unprocessed, ground seed kills both organisms within 2 hours after exposure, but this rate was consistently phytotoxic to wheat, cucumber, and tomato. Phytotoxic effects were decreased to negligible levels by delayed planting into amended soil. Evaluation of the concentration of nitrile and ITC produced in amended soils found that nitrile levels remained relatively constant for 6 days whereas ITC production peaked at 12-24 hours, but was no longer detectable by the sixth day.

Poster Abstracts

[P59] **Involvement of a Putative Lipid Transfer Protein, SBIP-470 in SA-Mediated Signaling**

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Salicylic Acid (SA) plays key role in pathogen resistance through gene expression of Pathogen Related (PR) proteins. During this process, plant produces high levels of SA which is converted to Methyl Salicylate (MeSA), a mobile signal for inducing SAR (Systemic Acquired Resistance). Salicylic Acid Binding Protein 2 (SABP2) catalyzes conversion of MeSA into SA to induce resistance. Yeast two-hybrid screening using SABP2 as a bait and in vitro pull down assay, have shown a tobacco protein (SBIP-470) interacts with tobacco SABP2. To further characterize putative SBIP-470 as a true Lipid Transfer Protein, it is being expressed in E. coli and purified for biochemical analysis. Arabidopsis knock out mutant showing most homology to SBIP-470 has been obtained from SALK is being used for complementation studies using SBIP-470. To investigate the role of SBIP-470 in SA mediated pathway, pathogen response assay is being performed using TMV and bacterial pant pathogens. Composition and quantification of cuticular wax and cutin of various plant parts will be analyzed using GC and GC-MS. Understanding the role of the SBIP-470 in SA mediated pathway can lead to development the pathogen resistant plant and abridging the use of expensive pesticide and fungicide for healthy world.

[P60] **Does Lysine Acetylation Have a Role in the SABP2-Mediated Salicylic Acid Signaling Pathway?**

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Recently, lysine acetylation posttranscriptional modification has been shown to play an important role in signaling pathways of both prokaryotic and eukaryotic organisms. However, the exact function of lysine modification in salicylic acid (SA) mediated pathway in plants is not clear. Salicylic Acid Binding Protein2 (SABP2) is an enzyme which is known to play an important role in SA-mediated pathway by catalyzing the conversion of methyl salicylate (MeSA) to SA. Accumulation of SA, leads to activation of heightened resistance in systemic parts of plants referred to as systemic acquired resistance (SAR). A yeast-two hybrid screening was performed to identify the proteins which interacted with SABP2. Several putative SABP2 Interacting Proteins (SBIP)s were identified. SBIP-428 is one of the interacting proteins which show high homology to deacetylase enzymes. Full length SBIP-428 was cloned and expressed in *E. coli* as a His tag on its N-terminus and purified protein is being used for biochemical characterization. Expression of SBIP-428 in response to TMV and other microbial pathogen is being studied using RT-PCR. Interactions of a deacetylase (SBIP-428) with SABP2 also raised the possibility of SABP2 itself being acetylated. Both native and recombinant SABP2 were used for western blot analysis using anti-lysine antibodies. Deciphering the role of SABP2/SA in regulating lysine acetylation and its role in plant defense signaling would help in better understanding of these pathways.

Poster Abstracts

[P61] **Characterization of a SA-Methyl Esterase from Tomato**

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Salicylic Acid Binding Protein 2 (SABP2) is a known tobacco esterase that enzymatically converts methyl salicylate (MeSA) into the product salicylic acid (SA). Tobacco plants resisting pathogen infection synthesize and accumulate high levels of MeSA. SA produced by the activity of SABP2 results in induction of resistance in plants. Previous study has determined that the tomato plant contains an enzyme (LeSABP2) with high genetic similarity to tobacco methyl salicylate esterase (SABP2). To determine if they share the same function, a variety of gene expression and biochemical analysis were performed. Recombinant purified 6xHis tagged LeSABP2 protein was used with para-nitrophenyl acetate, a known artificial substrate of SABP2. Our data suggests that LeSABP2 exhibits higher Km value compared to tobacco SABP2. To further characterize LeSABP2, modulation of its activity by various defense signals, e.g. salicylic acid, jasmonic acid, and methyl salicylic acid. Temporal expression of LeSABP2 is being studied in tomato plants subjected to a variety of biotic and abiotic stress. For biotic stress, tomato plants will be infected with *Pseudomonas syringae* pv. tomato DC3000, leaf samples collected at various time points and used for gene expression analysis. Understanding this defense signaling pathway will lead to development of more resistant tomato plants.

[P62] **Use of Emulsifiers for Control of Blast and Rice Sheath Blight in Organic Rice Cultivation**

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Currently, methods for controlling disease in organically produced rice crops have not been as effective as conventional methods. The objective of this research was to determine the effect of various organic emulsifiers (natural emulsifier A and B, loess sulfur, brown rice vinegar, and insecticidal soap) on the suppression of blast (*Pyricularia oryzae*) and rice sheath blight (*Corticium sasaki*) and rice growth. Blast in Petri dish assay trials was 100% suppressed by 50,000 ppm of loess sulfur or natural emulsifier B. The order of effects on suppression of blast is loess sulfur > natural emulsifier B > insecticidal soap > natural emulsifier A > brown rice vinegar. Rice sheath blight was 100% suppressed by 5,000 ppm of loess sulfur, 30,000 ppm of brown rice vinegar or insecticidal soap. The order of effects on suppression of rice sheath blight is loess sulfur > insecticidal soap > brown rice vinegar > natural emulsifier B > natural emulsifier A. Injury was reduced in blast infected greenhouse grown rice plants 38~75% by the organic emulsifiers (5,000-100,000 ppm) when compared to non-treated control groups. In this research, organic emulsifiers showed an effective control of blast and rice sheath blight without negatively affecting rice growth.

[P63] **Heterologous Expression and Characterization of Putative Secondary Product Glucosyltransferase (PGT) Clones 4 and 11 Isolated from *Citrus paradisi***

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Plant secondary products such as flavonoids have a variety of roles in plants including UV protection, anti-feedant activity, pollinator attraction, stress response, flavor, and many more. These compounds also have effects on human physiology. Glucosylation is an important modification of many flavonoids and other plant secondary products. In grapefruit, glucosylation is important in the synthesis of the bitter compound naringin and several flavonoid glucosyltransferase (GT) enzymes have been characterized from young grapefruit leaf tissue. To study structure and function of flavonoid GTs, it is necessary to isolate cDNA's that can be cloned and manipulated. In prior work, the plant secondary product glucosyltransferase (PSPG) box was used to identify putative GT clones. We report on results from experiments to test the hypothesis that PGT clones 4 and 11 are plant secondary product GTs, specifically flavonoid GTs. Previously, PGT 4 was cloned into a bacterial expression system, however all protein was localized into inclusion bodies and GT activity could not be tested. For this work, recombinant PGT 4 and PGT 11 were transformed into yeast and the proteins expressed and screened for glucosyltransferase activity with a variety of flavonoid substrates including flavanones, flavones, and flavonols.

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